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Impact of leaf age on common fig susceptibility to rust caused by *Cerotelium fici*[1]

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ABSTRACT

Rust caused by *Cerotelium fici* leads to significant defoliation in common fig trees (*Ficus carica*). However, studies on its epidemiology remain scarce. The aim of the present study is to investigate common fig rust progress in leaves inoculated at different ages over two growing seasons (dry and rainy) in an experimental fig orchard. Artificially inoculated leaves and those exposed to natural infection showed more severe rust symptoms when they were inoculated earlier - up to 45 days after leaf emergence. Disease severity and leaf longevity (in days) were inversely and directly proportional to leaf age at inoculation time, respectively. Leaves inoculated at earlier presented significantly higher disease progression rates based on number of pustules per cm², broader area under the disease progress curve (AUDPC) recorded for pustule density and higher maximum severity, which was visually estimated as the percentage of lesioned leaf area. Inoculated young leaves abscised 40 to 60 days earlier than the uninoculated leaves, which were protected from natural infection by plastic bags. The uninoculated leaves remained attached to the plants from 90 to 100 days. These findings highlight apical sprouts as primary targets for protective fungicide applications, which must be more intense at early growing season in order control rust in common fig crops.

Keywords: *Ficus caricae*; rust epidemic; epidemiology, infection and latent period; leaf abscission.

INTRODUCTION

Common fig *(Ficus caricai* L.) is a vigorous and productive tree. Its parthenocarpic fruits can be harvested still green for sweets or jam production purpose, whereas ripe fruits are sold fresh for human consumption. This species is native to arid and semi-desert regions so it can tolerate high temperatures, water shortage, and soil and water salinity.[1] Irrigated ficiculture is a good alternative crop for regions presenting mild, warm and semi-arid climate.[1,2] Brazil's ranking in the international rank of ripe fig production for fresh consumption ranges from the second to the fourth position. During the Turkish off-season, most of its production is exported to the European Union and to the USA. Brazilian fig production is concentrated in its Southeastern and Southern States, mainly in São Paulo, Rio Grande do Sul and Minas Gerais states. Altogether, these states account for more than 90% of its national production. Back in 2023, the Brazilian fig production reached 20,881 t of fruits produced in a total cultivated area of 2006 ha, which corresponded to total product value of R$128 million.[3] Warm winters (dry season) in the states of Rio de Janeiro and Espírito Santo are expected to anticipate both pruning and the out-of-season production in order to meet the regional demand of the fig fruits market by Christmas time.[4]

Cultivar 'Roxo de Valinhos' can be assumed as the only commercially cultivated variety in Brazil. Despite its productive advantages, this cultivar is vulnerable to pest attacks and diseases, mainly to leaf rust, which is caused by fungus *Cerotelium fici* (Butler) Arth. Rust is the main leaf fig disease in Brazil and it compromises fruit yield and quality.[5] *C. fici* is widely distributed in tropic and subtropic regions, and its damage to fig crops is more severe in lower lands, in the subtropics.[6] Severe defoliation is the main damage caused by leaf rust because fig trees can be fully defoliated under high rainfall, within 20 to 30 days.[7] Although reports of this pathogen in Brazil date back to more than one century, only few studies have been published on common fig rust epidemics; yet control strategies described in them are only based on fungicide spraying.[8-11] The higher susceptibility of mature leaves to infections is highlighted by the fact that older leaves often present more severe rust symptons.[7] However, ineffective weekly fungicide spraying to control fig rust has been previously justified by chemicals' fail in protecting shoots during continuous fig tree sprouting on summer (rainy season).[12] The aim of the present study was to investigate leaf age effect on fig susceptibility to rust and to subsequent defoliation. In order to do so, rust progression was assessed in leaves inoculated at different ages. These assessments were conducted for two seasons (dry and rainy) in an experimental field at Campos dos Goytacazes, Rio de Janeiro State.

MATERIALS AND METHODS

Two experimental trials were conducted from April to June 2000 (dry season) and from September 2000 to January 2001 (rainy season) at the Experimental Station of PESAGRO-RJ, Campos dos Goytacazes County, Rio de Janeiro State, in a field with 500 common fig trees of the cultivar 'Roxo de Valinhos'. Trees were 3-5 years old and were planted at 3 x 1.5 m spacing. According to Köppen's classification, climate in the region is tropical-humid (Awi) with seasonal water shortage.[13] Yet, it is featured by two well-defined weather seasons: rainy summer, from October to March (rainy season); and dry winter, from April to September (dry season). The coldest time of the year extendeds from June to July, with mild temperature close to 18 °C. Crop site was treated with liming, organic fertilization and minerals (NPK 4: 14: 8) on plant pits before planting besides microsprinkling irrigation on a weekly basis, whenever necessary.

Common fig rust was assessed under natural and artificial inoculation conditions over the two assessed seasons. Pruning was carried out at late March and August. According to recommendations by Simão[14], pruning was drastic, it only left 3-4 thicker branches (diameter > 5 cm; 10 cm long); 20 to 25 vegetative branches remained per plant. Plant shoot without expanded apical leaves (first unfolded leaves presenting <3 cm lenght) were lambelled and protected at different time intervals to achieve healthy leaves at different leaf ages. Leaf labeling day was the adopted time zero (day zero) to set the leaf age for each subsequent inoculation day. Labeled shoots were covered with transparent plastic bags, except at day zero. These bags (20 x 30 cm) presented 1mm holes on their bottom to allow gas exchange and, consequently to protect the shoots from natural inoculation. At least ten shoots of ten different plants were covered and protected from natural inoculation at each leaf protection day. Labeling started at late April (1999) in the first experiment, 20 days after the drastic pruning performed in March. It was carried out from late September to late October in the second experimental trial, 21 days after the drastic pruning conducted in August. Leaves without rust symptoms were inoculated at labeling (day 0), in the following day (day 1) and, subsequently, at days 7, 14, 20, 30, 45 and 60, in both trials. Inoculation on two subsequent days (0 and 1st days) was adopted to assess the humid chamber effect on younger leaves' infectivity. It was done because inoculation was carried out without placing the plastic bags to protect plants from natural and artificial inoculation, just as at the first inoculation day. One additional (extra) treatment was applied to the leaves that remained covered for 90 days (without inoculation) after day zero (labeling date).

New and fully expanded leaves accounting for many sporulated lesions were selected for inoculum collection based on the artificial inoculation method. Urediniospores were collected through vacuum suction, conditioned in open test tubes previously filled with saturated Calcium Chloride solution ($CaCl_2.6H_2O$) inside hermetically sealed dry chamber and stored in refrigerator (4±2°C) at relative air humidity ranging from 38% to 40%.[15] The plastic bags were removed from all shoots in the following morning, after 8-10h nocturnal incubation. Urediniospores germination rate reached 45% after inoculation in the first trial (dry season) and 50%, in the second one (water season), in 2% agar-water medium, in the dark, at 25 °C. All inoculations were carried out at late afternoon. The labeled leaves were abaxially sprayed with suspension comprising 2.10^4 urediniospores. mL^{-1} in tween-20 (0.05%) water solution. Inoculated leaves were covered with plastic bags (10 L) filled with moistened cotton wicks after inoculation and tied at the bottom.

Incubation time (from inoculation to sporulation) estimates were based on number of days between inoculations or on inoculated leaves' exposure and on the day when initial rust pustules had emerged. Subsequently, leaf rust was quantified on a weekly basis by counting the number of $pustules.cm^{-2}$ under magnifying glass (10X). The counting was carried out in four leaf surface areas with the aid of a cardboard template with four square holes (1 cm^2 each) in it. Disease severity in labeled leaves was also visually estimated with the aid of a disease scale diagram based on images of infected fig leaves at 4 classes of leaf lesioned area: 1%, 5%, 7% and 13%.[16] Leaf duration (in days) was calculated by taking into consideration the time elapsed from labeling to the day of the last assessment when it was possible observing lack of labeled leaves in plants. The total number of leaves per branch in the labeled branches was quantified on a weekly basis, and it was used to estimate vegetative plant growth rate.

Descriptive analyses applied to epidemics was based on plotting a chart for each assessed period, as well as on regression analysis by using a linear model applied to the number of $pustules.cm^{-2}$ based on leaf age (in days) at inoculation day. The area under de disease progress curve (AUDPC) was also calculed based on the number of $pustules.cm^{-2}$, as recommended by Campbell & Madden.[17] Disease rates (slope or angular coefficient recorded through linear equations) were estimated for each plot and inoculation leaf age to find disease severity expressed in percentage of lesioned leaf area. Variance homogeneity and the normality of other variables were assessed. ANOVA followed by Tukey's test was performed in Sigma-Plot software version 12.5. Variation sources (factors) were: (1) seasons (dry or rainy), (2) inoculation method (natural or artificial) and, (3) leaf age at inoculation day (days). Means were analyzed and plotted based on the maximum and minimum amplitude levels to inoculation leaf age (days). The two other variation sources did not have significant effects based on ANOVA results. Regression analyses allowed applying curvilinear models to Leaf Duration -LD (in days) and Maximum Severity - SEVmax (% of estimated injured leaf area) as leaf age function expressed in days, from labeling day to inoculation day. In addition, linear regression analysis, based on degree-3 polynomial model for LD; and non-linear regression, based on inversed-logistic model for SEVmax, were perfomed by taking into account leaf age (days). The SAEG Statistical package[18] was adopted for non-linear regression analyses.

RESULTS AND DISCUSSIONS

Artificially inoculated and naturally inoculated leaves presented severe and cumulative symptoms at both seasons. They reached maximum severity (AFL% = lesioned leaf area percentage) when leaves were inoculated at younger ages (Figures 1, 2 and 3, Table 1). There was IP (incubation period) increase and AACPD decrease at both trial periods, for the two inoculation methods (natural and artificial). Leaf rust progression rate decreased as leaves were inoculated at older ages (Figures 1, 2 and 3, Table 1).

Young leaves inoculated at the age of 0 or 1, 7 and 14 days presented oscillating initial rust symptoms from 5 to 10 days after inoculation (DAI) - mean incubation period (IP) was 7 DAI. Leaves inoculated at the age of 20 and 30 days (after labeling date) presented IP ranging from 7 to 13 DAI - mean incubation period was 8 and 11 DAI, respectively (Figure 3). On the other hand, leaves inoculated at the age of 45 days, or older, recorded higher IP variation depending on inoculation day and trial. This finding suggests likely secondary infections. Symptoms in leaves inoculated at the age of 45 days in the rainy season began at 10 DAI. Symptons in leaves inoculated at the age of 45 days in the dry season started at 30 DAI (Figures 1, 2 and 3). Mean IP when leaves were inoculated at 0-30 DAI did not significantly differ from each other at 5% level. Values ranged from 5 to 11.3 DAI, respectively, when means recorded for both trail periods were taken into account regardless of inoculation method (Figure 2, Table 1). However, the onset of the first pustules changed depending on trial, at 45 DAI (Table 1). The emergence of such rust symptoms in leaves inoculated at the age of 45 days was observed from 10 DAI onwards, in the rainy season, and from 30 DAI at the coldest (dry) time of the year, regardless of inoculation method (Figures 1, 2 and 3). They remained practically healthy until the end of the warmest season (rainy season). The first pustules were observed from 18 to 32 DAI in leaves inoculated at the age of 60 days in the dry season – 25.5 DAI, on average (Figures 1, 2 and 3, Table 1).

Based on the results, common fig rust has varying incubation period depending on both leaf-age and the season. This outcome can change depending on leaf age and on time of the year - 5 to 13 days under favorable conditions to the disease. IP ranged from 5 to 10 days in the rainy season and from 7 to 13 days in the dry season. These findings corroborate studies based on inoculating detached and non-detached leaves (in plants) under controlled conditions.[5] These authors determinated 7-day incubation time and latent period ranging from 8 to 9 days after inoculation with *C. fici* urediniospores on leaf discs under mild temperatures (22 and 24 ºC)**.** Shorther IP in non-detached leaves in the present experiments can be justified by the fact that leaf-inoculation age was not taken into consideration by Czaja *et al*.[5] Shoot, tender organs and young leaves' highest susceptibility to infections is corroborated by studies on phytopathogenic biotrophic fungi, mainly rust.[19,20]

The herein observed IP longer than 15 days, and up to 32 days, after older leaves' inoculation can be artifacts. Leaf duration remained longer when they were exposed to natural inoculation. Therefore, the methodology adopted in the present study did not allow specifying the incubation period in leaves inoculated at older ages because of secondary infections' interference. According to Czaja *et al*.[5], the longest *C. fici* incubation time in fig plant leaves was 15 days, at 18 °C and 30 ºC.

Severity progress rate ("r") was expressed by linear variation in the percentage of lesioned leaf area (% AFL) (dependent variable) based on leaf age (independent variable). This variable best discriminated the leaf age factor in the mean comparison test, regardless of inoculation method and trial / season (Table 1). Higher epidemics progression rates set for severity (% AFL) were recorded when younger leaves were inoculated. These rates decreased as leaves remained protected (uninoculated) for longer periods-of-time (Table 1, Figure 3). Lesioned leaf area progression rates (r) lower than 2 (% AFL.d^{-1}) were observed when leaves were inoculated at the age of 30 days. The lowest "r" rate (lower than 0.5% AFL.d^{-1}) was observed in leaves inoculated at the age of 45 days, or older (Table 1, Figure 3). Leaf age ranging from 45 to 55 day partially corresponded to the end of flowering phase (69) and to the beginning of the fruiting phase (growth stage 7: syconium development), according to developmental stages defined by Singh *et al*.[2] These authors assessed the phenology of common fig, var. "Diana", under field conditions and semi-arid weather in India, and observed bud, leaf and shoot growth overlapping at early growing seasons. Vegetative branches' physiological maturity tends to take place after 6–7 weeks. Reproductive bud development stages prevail after this time. However, the current results cannot be fully extrapolated to other regions and situations, because variations in the physiological and phenological stage of common fig tree development depend on factors such as genetic background, nutritional and cultural management and, seasonal and climatic variations.[2]

Results in the present study are in compliance with field observations and with the literature, according to which, common fig rust is often observed in mature fig leaves, at fruit development or reproductive phase. Its symptoms worsen during the growing season and the disease reduces leaf area and, consequently, increases tree defoliation.[4] The number of lesions on leaves grows as inoculum rates increase over the growing season. This process gets worse due to critical defoliation from the beginning to the end of harvest time.[21] Although the disease seems to be more severe in older leaves[7], it is an illusion caused by infections' accumulation due to the polycyclic nature of rust epidemics.

Leaf life duration in plants (days to abscission) was proportional to inoculation age and inversely proportional to leaf rust severity (Figure 4). Leaf abscission was anticipated from the age of 40 to 60 days in leaves inoculated at the age of 0 to 20 days in comparison to leaves inoculated at the age of 60 days, or older. Leaves inoculated at the age of 60 days and uninoculated leaves (kept covered) remained in plants at the age of 90 to 100 days, or longer (Figure 4). Maximum rust severity (% AFL) was observed at final assessments applied to leaves inoculated at the youngest ages (Figures 1 and 2).

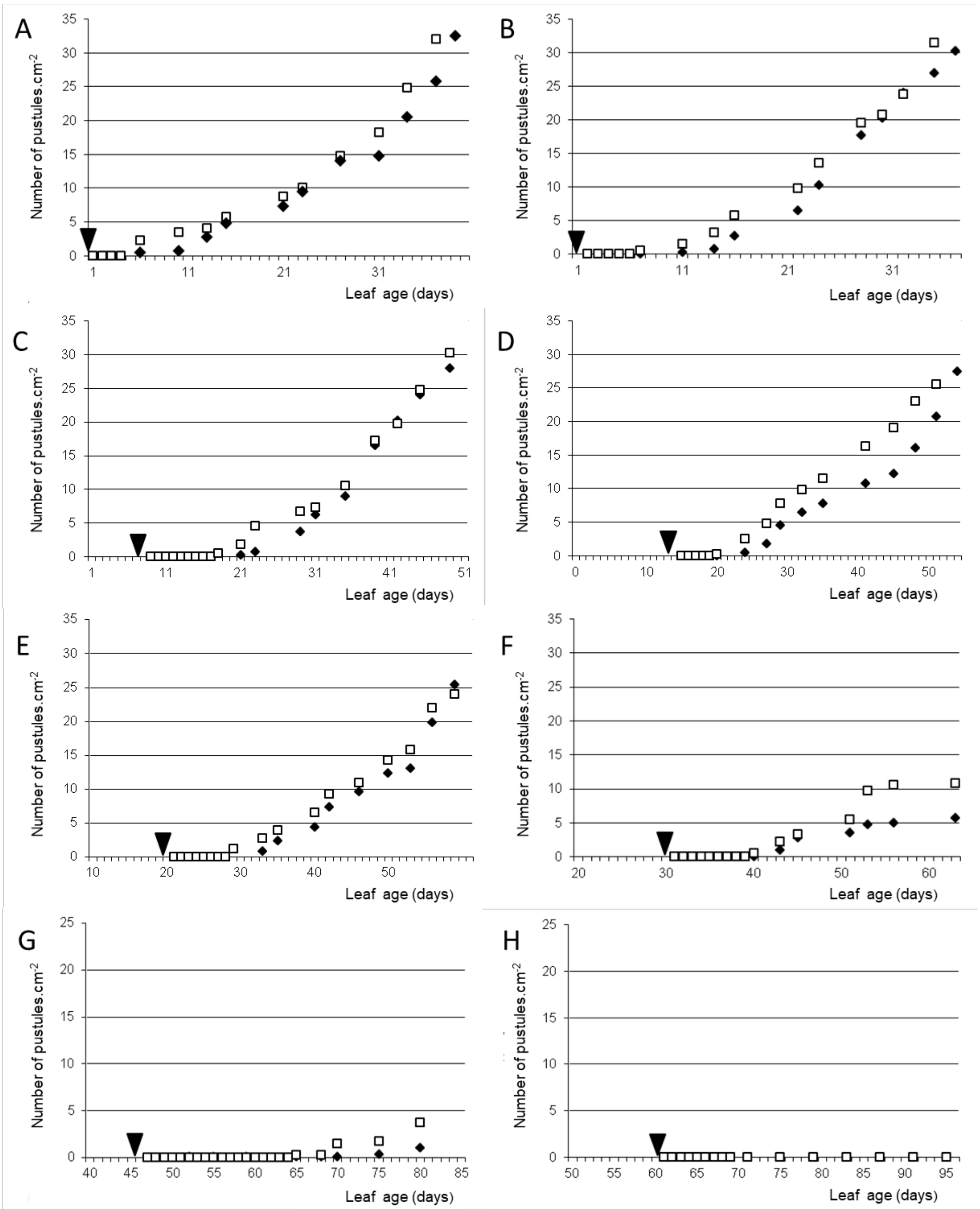

Figure 1: Progress of number of pustules.cm^{-2} in common fig rust (*Cerotelium fici*) on inoculated leaves (□) and on leaf exposed to natural inoculation (♦) at different leaf ages* (A = 0, B = 1, C = 7, D = 14, E = 20, F = 30, G = 45 and H = 60 days) at the dry season (April to September 2000) in Campos dos Goytacazes, RJ, Brazil. The horizontal axis corresponds to leaf age (days) counted after leaf labeling (day zero). * Inoculation leaf age was defined based on days after labeling day when shoots had their first leaves unfolded, since it corresponds to phase 19, according to Singh *et al*. [2] Caption: Inoculations were made and leaves were unprotected (▼).

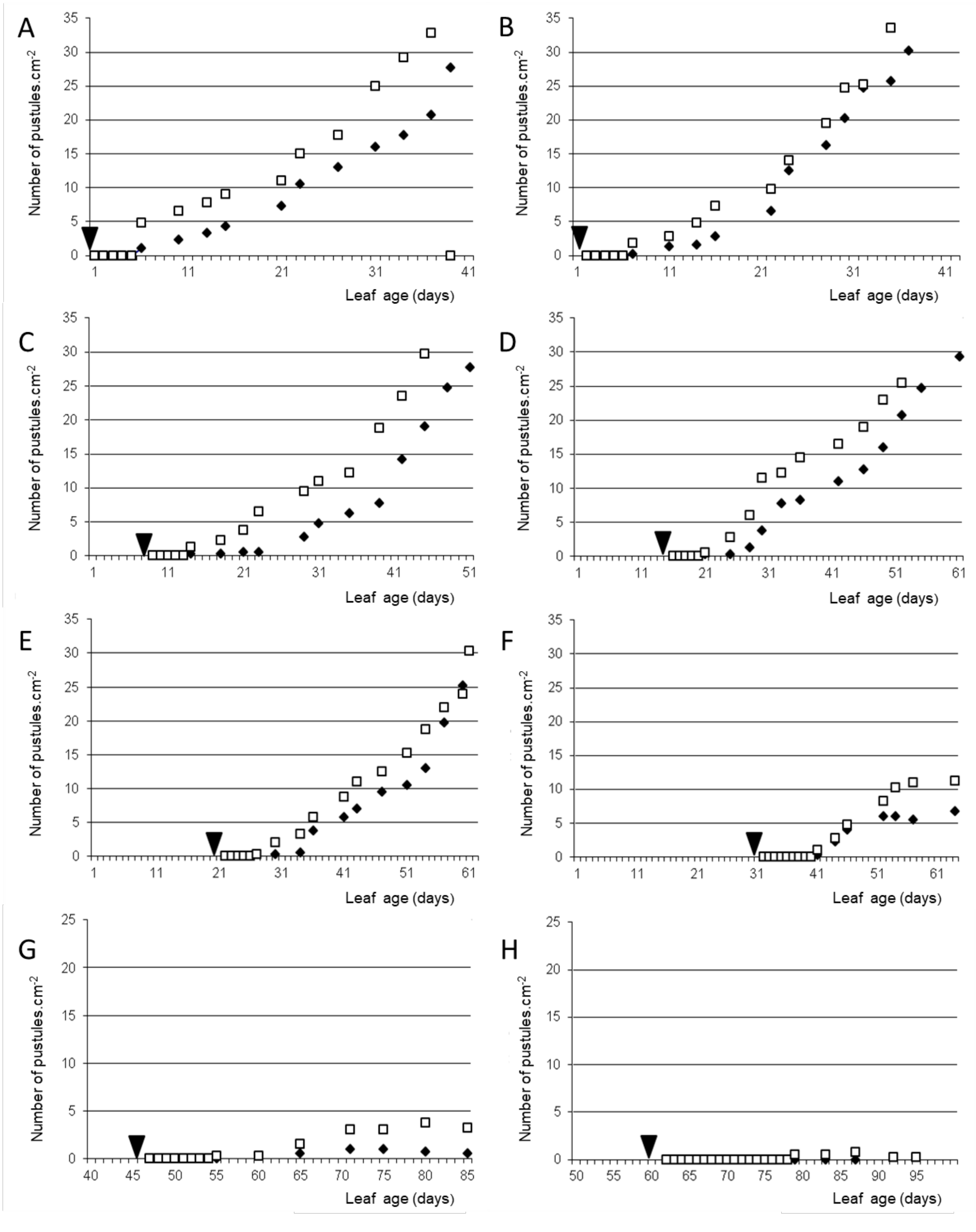

Figure 2: Progress of number of pustules.cm-2 in common fig rust (*Cerotelium fici*) on inoculated leaves (□) and on leaf exposed to natural inoculation (♦) at different leaf ages* (A = 0, B = 1, C = 7, D = 14, E = 20, F = 30, G = 45 and H = 60 days) at the rainy season (April to September 2000) in Campos dos Goytacazes, RJ, Brazil. The horizontal axis corresponds to leaf age (days) counted after leaf labeling day (day zero). * Innoculation leaf age was defined on days after labeling day when shoots had their first leaves unfolded, since it corresponds to phase 19, according to Singh et al. (2) Caption: Inoculations were made and leaves were unprotected (▼).

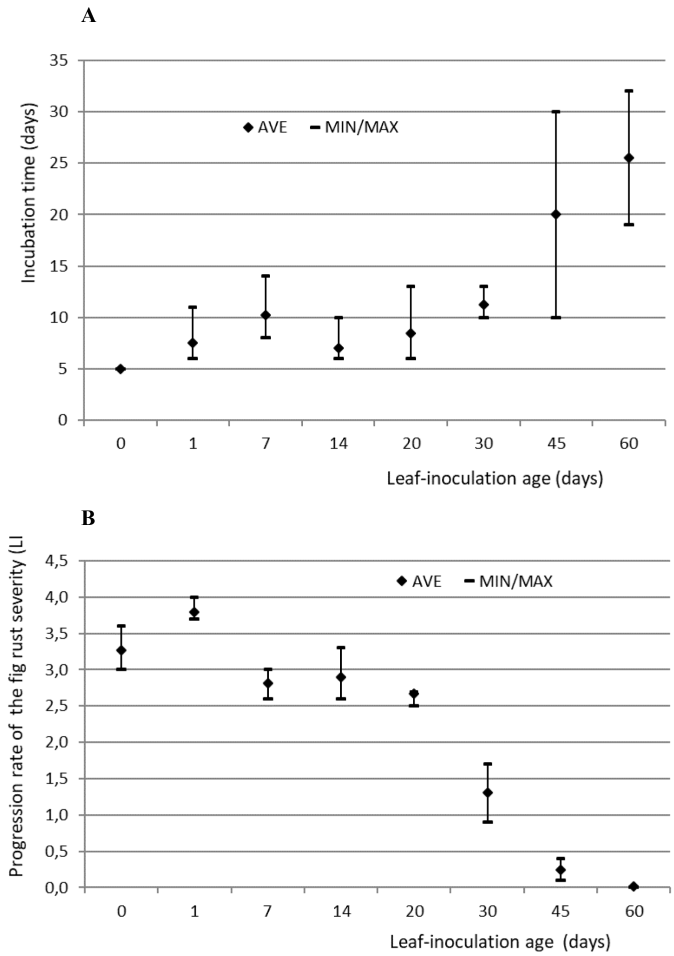

Figure 3: Common fig (*Ficus carica*, cv. Roxo de Valinhos) susceptibility to rust (*Cerotelium fici*) depending on leaf age*, expressed by the following variables: (A) Incubation time (days from inoculation to the emergence of the first pustules); (B) Disease Severity Progression Rate (r) base on the linear model: Y = Yo + rT, wherein Y = Disease severity (lesioned leaf area- LLA%), Yo = intercept, r = progress rate or slope, T = time (days). Marks point out minimum, mean and maximum values recorded for each leaf age. Average data of the two seasons (dry and raining) and of plants exposed to natural inoculation and to inoculation with urediniospore suspension. * Inoculation leaf age was defined on days after labeling day when shoots had their first leaves unfolded, which it corresponds to phase 19, according to Singh *et al*. [2]

Table 1: Common fig (*Ficus carica*, cv. Roxo de Valinhos) susceptibility to rust (*Cerotelium fici*) depending on inoculation leaf age* expressed by the following variables: incubation time (PI), area under the progression curve plotted for number of pustules.cm-2 (AACPD) and, lesioned leaf area rates (rAFL)

Inoculaion leaf age* (days)	**PI (days)**		**AACPD (n. pustules.cm^{-2}.d)**		**rAFL* (% AFL.d^{-1})**	
0	5.0	c	17.1	a b	3.300	a b
1	7.5	c	16.2	a b	3.800	a
7	10.3	c	15.6	a b	2.825	b c
14	7.0	c	21.0	a	2.875	b c
20	8.5	c	12.5	b	2.650	c
30	11.3	b c	11.6	b	1.325	d
45	20.0	a b	1.4	c	0.250	e
60	25.5	a	0.4	c	0.000	e

*Inoculation leaf age was defined on days after labeling day, when shoots had their first leaves unfolded, which correspondent to phase 19, based on the common fig development phases defined by Singh *et al*.[2] Means followed by the same letter did not differ from each other in the Tukey test ($p = 0.05$)

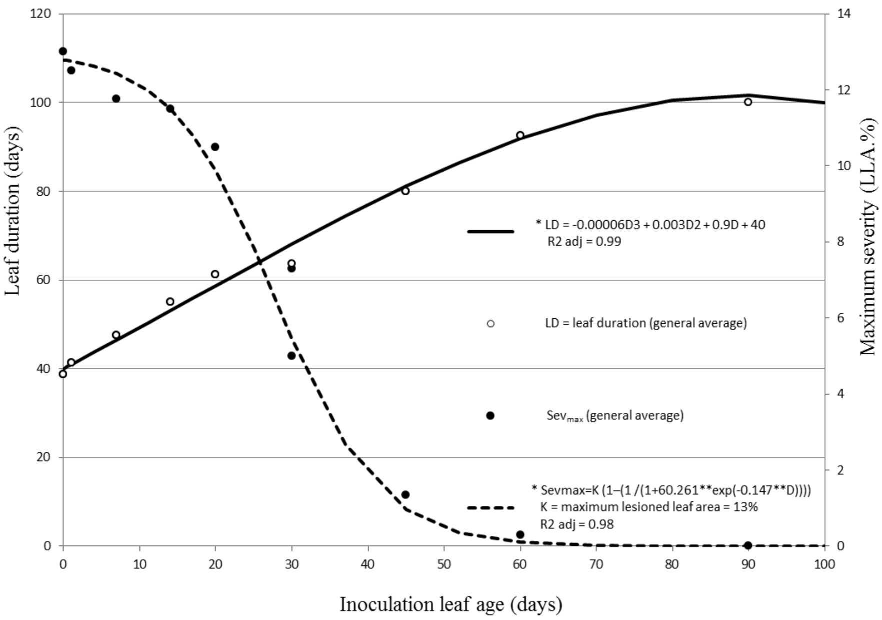

Figure 4: Common fig (*Ficus carica*, cv. Roxo de Valinhos) susceptibility to rust (*Cerotelium fici*) depending on inoculation leaf age* expressed by the following variables: Leaf Duration (days from leaf labeling to abscission) and Maximum Severity (maximum percentage of lesioned leaf area). Leaves were protected from natural inoculation, exposed to natural inoculation or inoculated at different times (ages on days) for two common fig-growing seasons, from April to June 2000 (dry season) and from September 2000 to January 2001 (rainy season) in Campos dos Goytacazes, RJ, Brazil. *Equations generated from the means recorded for the two seasons calculated for both inoculation methods. **Inoculation leaf age was defined on days after labeling day when shoots had their first leaves unfolded, which corresponds to phase 19.[2]

SEVmax (% AFL) values were statistically similar at the same inoculation ages, in both trials/seasons (Figure 4). Mean SEVmax values presented steep drop in leaves inoculated at at the age of 20 days. They were 50% lower than those recorded for leaves inoculated at the age of 30 days, regardless of season and inoculation method, as well as values lower than 2% AFL when leaves were inoculated at the age of 45 days (Figure 4).

Sigmoidal model (degrees 3 polynomial) was the best to explain LD (d) when inoculation leaf age (d) were taken into account, regardless of season and inoculation method. The inversed-logistic model was the best to estimate SEVmax (% AFL) based on inoculation leaf age. Determination coefficients (R^2adj) were higher than 98% based on the two selected models (Figure 4). Inflection was observed at the age of 17 days, after inoculation, when variations in leaf duration were taken into consideration based on inoculation leaf age. Leaf duration recorded increasing rates after the age of 17 days at inoculation day and decreasing rates after the same leaf age (Figure 4). SEVmax estimated inflection was observe at inoculation leaf age of 43 days and severity rates decreased after this leaf age (Figure 4), which corresponded to branches' vegetative maturity in the current trials. It precided the fruiting development phase (syconium phase).[2]

Plant phenology and management practices are relevant factors highlighting the importance of rust as defoliation cause in common fig crops. Sanity and number of leaves are factors closely associated with branches' vegetative development and length, as well as with the amount and quality of fruits.[21] The current results make it clear that rust infections are more severe on younger leaves and at early vegetative time. Young unprotected or inoculated leaves had their useful life (leaf duration in days) reduced. Therefore, defoliation is likely to be more intense and branch development to be impaired. Such reduction has negative effect on fruit amount and quality.[21] However, there are no specific studies associating leaf-rust severity with defoliation, and with fruit yield and quality in common fig crops. These studies could substantiate damage threshold determination to reduce fungicide spraying and costs with pest control in commercial orchards.

Leaf emittion ranged from 1 to 2 emitted leaves.branch^{-1}.week^{-1} in both seasons (dry and rainy), and it accounts for 50 to 80 leaves per plant, on average. Vegetative growth was more intense in the rainy season or at early summer when leaf emission grew from 4 to 6 leaves.branch^{-1}.week^{-1} – it led to the accumulation of 80 to 120 leaves per plant. Data recorded in the current study based on sesons' comparison did not show significant differences associated with rust intensity for most variables (progression in the rate of number of pustules.cm^{-2} and maximum severity). Accordingly, inoculation leaf age day and the phenological phase were factors mostly influencing rust severity in leaves and, consequently, leaves' life-time. Failing in controlling common fig rust at time intervals raging from 20 to 30 days between protective fungicide sprayings in the rainy season[12] can be associated with favorable environmental conditions for rust infection and epidemics, as well as with the effect of fungicide residue washing from leaves due to rain, rather than with the availability of susceptible host tissue. Further research on chemically controlling commong fig rust demands new strategies to achieve shoot and young leaves' fungicide coverage at the early growing season. It is also possible developing non-chemical protection methods, such as using of protective films or even physical protection by plastic bags or polymers in the field. It must be done to protect shoots from rust infection at the budding phase, at shoots' emission and leaf development until the fruiting phase takes place (when most leaves become mature) in order to reduce defoliation caused by the disease.

CONCLUSIONS

1. Young common fig leaves are highly susceptible to infection and the sooner the infection emerges in leaves, the shorter their lives (before leaf abscission).

2. Leaves become less susceptible to rust infection when they tend to reach maturity (approximately 20 days after sprouting). They become resistant from the age of 45 days onwards and immune to infection caused by *C. fici* from the age of 60 days or more.

3. Chemical protection should target the shoots and youngest leaves in order to best control fig rust. Spraying need to be more intense (applied at shorter intervals) at branches' early vegetative development.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interests.

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Writing – review & editing: José Roberto Vieira-Júnior, Silvaldo Felipe da Silveira, Luciana Aparecida Rodrigues.

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