

Microclimate and ramulosis occurrence in a cotton crop under three plant population densities in Southern Brazil

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SUMMARY

This study aimed to evaluate crop microclimate and its influence on ramulosis disease in a cotton crop conducted in three population densities. The experiment was carried out in Piracicaba, state of São Paulo, Brazil, where the genotypes IAC 23 and Coodetec 401 were sowed with the following plant population densities: 55,000; 111,000; and 166,000 plants per hectare. To start the epidemic process, a conidia suspension of *Colletotrichum gossypii* var. *cephalosporioides* was inoculated in the crop at 30 and 45 days after sowing. The weather variables, air temperature, relative humidity and leaf wetness duration, were recorded with an automatic weather station located at the experimental area and with six micro-stations located within the crop canopy (three in each genotype). Results showed that plant density had low effect on air temperature, but differences were found on relative humidity and leaf wetness duration. These differences were observed until the canopy became a continuous surface. The microclimate promoted by plant densities showed to have little influence on disease progress, since macroclimate during the experiment was favorable to disease development. Genotype IAC 23 was more resistant to ramulosis than Coodetec 401. The area under disease progress curve presented a define relationship with yield ($R^2 = 0.70$ for all treatments and $R^2 = 0.93$ for averages), being a potential parameter for evaluating the impact of ramulosis disease on cotton production in southern Brazil.

Key words: air temperature, leaf wetness duration, *Colletotrichum gossypii* var. *cephalosporioides*, yield, disease intensity

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RESUMEN

El objetivo de este trabajo fue evaluar el microclima en el cultivo de algodón a tres densidades poblacionales y su efecto sobre la intensidad de la ramulosis. El experimento fue conducido en Piracicaba, SP, Brasil. Los genotipos IAC 23 y Coodetec 401 fueron sembrados a las densidades de 55.000, 111.000 y 166.000

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plantas por hectárea. El cultivo fue inoculado con una suspensión de conidios de *Colletotrichum gossypii* var. *cephalosporioides* a los 30 y a los 45 días después de la siembra. Las variables temperatura del aire, humedad relativa y duración del follaje mojado fueron registradas a través de una estación automática instalada en el sitio experimental y de seis microestaciones ubicadas dentro de la canopia del cultivo (tres en cada genotipo). Los resultados mostraron que la densidad de plantas tuvo poco efecto sobre la temperatura del aire, aunque se registraron diferencias en la humedad relativa y la duración de follaje mojado. Estas diferencias fueron observadas hasta el momento en el cuál la cobertura de la canopia fue total. El microclima generado por las tres densidades de plantas tuvo poco efecto en el progreso de la enfermedad, ya que el macroclima durante el experimento fue siempre favorable para el desarrollo de la ramulosis. El genotipo IAC 23 fue más resistente a la ramulosis. El área bajo la curva de progreso de la enfermedad presentó buena asociación con el rendimiento ($R^2 = 0,7$ en todos los tratamientos y $R^2 = 0,93$ en los promedios), manifestándose como un parámetro potencial para la evaluación del impacto de la ramulosis en la producción de algodón en el sudeste de Brasil.

Palabras clave – temperatura del aire, duración de follaje mojado, *Colletotrichum gossypii* var. *cephalosporioides*, rendimiento, intensidad de la enfermedad.

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INTRODUCTION

More than 250 different diseases can attack cotton crop. Many of these diseases do not present any economical importance. However, some of them can cause severe yield losses when in favorable climatic conditions (Cia & Fuzatto, 1999). Among these diseases, ramulosis (*Colletotrichum gossypii* var. *cephalosporioides*) stands out as the most dangerous to Brazilian cotton production, mainly in the Central region of the country, known as Cerrado.

The most rational method for diseases control is the use of resistant genotypes (Hammerschmidt & Kuác, 1995). However, for some pathogens there are no cotton genotypes with this characteristic (Cia & Salgado, 1995), and, even when the use of resistant genotypes is possible, the use of other measures, as fungicide sprays, is recommended in an integrated disease management system.

The development of a given disease is the result of the interaction of a susceptible plant, a pathogenic

agent and a favorable environment. The environment, therefore, is an important component in this interaction (Campbell & Norman, 1998), since several diseases can cause no losses under unfavorable environmental conditions (Agrios, 1997). A very important component of the environment is crop microclimate, which is related to macroclimate and crop characteristics, like plant architecture, leaf area, and plant population. Among the crop characteristics that affect microclimate, plant population is one of the most important (Campbell & Norman, 1998), mainly when ultra-narrow-row is used as an alternative to reduce costs with weed control (Vories *et al.*, 2001). Plant population is a factor that can change radiation interception and balance, which determine temperature, humidity, and wind regimes inside the crop canopy. Therefore, they control wetness duration, allowing various portions of leaves and canopies to become wet and dry at different times (Huber & Gillespie, 1992).

Wetness is a very important factor in plant disease

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epidemiology and its duration, named as leaf wetness duration (LWD), together with air temperature are the two most important micrometeorological parameters influencing many phytopathosystems. Consequently, they are used as inputs in many disease-warning systems which advise growers when fungicide sprays are really necessary (Huber & Gillespie, 1992).

Few papers are available in the literature discussing the effect of the microclimate on ramulosis disease in cotton crop. According to Cia & Salgado (1995), the favorable environmental conditions to ramulosis in cotton crop are: intense rainfall, good soil fertility, and temperature ranging from 25 °C to 30 °C. However, this general information, without quantify the relationship between disease and weather variables, provides few practical results. Therefore, more studies about the relationship between meteorological conditions and ramulosis disease are required, especially when the purpose is to develop a warning systems to guide fungicide sprays.

Based on above discussion, it is important to know better the interactions among ramulosis disease, crop management, and microclimate to identify the required conditions for the occurrence and development of this disease. Thus, the main goal of this study was to evaluate the effect of different plant populations of two cotton genotypes on crop microclimate, ramulosis occurrence, and cotton yield.

MATERIAL AND METHODS

Location

The field experiment was carried out at the University of São Paulo, in Piracicaba, State of São Paulo, Brazil, (22° 42' S, 47° 37' W, altitude 546 m.a.s.l.), from November 2001 to April 2002. According Köppen's classification, the climate of this region is Cwa (humid tropical with dry season during the winter).

Experimental design

Two cotton crop genotypes were used: IAC 23 –which presents yield stability and resistance to diseases, and Coodetec 401– which presents high yield and fiber quality but without resistance to diseases.

The experimental plots had four lines of 5 m length, where only the two central lines were considered to evaluations. The inter-rows spacing was 0.9 m. The three different densities were set with 5, 10 and 15 plants per meter in rows, which corre-

spond approximately to 55,000, 111,000, and 166,000 plants per hectare.

Sprays of inoculums suspensions over the plants were required to standardize inoculums amount through the experimental area. The isolations of *Colletotrichum gossypii* var. *cephalosporioides* were prepared in an agar and nutrients based culture substrate. The pathogen colony was removed from Petri's dishes and mixed with distilled water. The resulting inoculum suspension was introduced in a manual sprayer with final concentration of 10^5 conidium mL⁻¹. The inoculation was applied at 30 days after seedling (DAS), after sunset, to promote the infection with favorable temperature and humidity. The inoculum suspension was sprayed over all the plants of all plots. A second inoculation was performed at 45 DAS, in order to reinforce the presence of inoculums, repeating the same procedures of the first spraying. Additional treatments without inoculation where also installed, with the objective of evaluating the occurrence of ramulosis in natural conditions.

The final experimental design had twelve treatments combining: two genotypes in three densities, inoculated and not inoculated, with six replications each. The statistical design was simple plots randomly distributed.

Disease evaluation

Disease evaluation was done every 14 days, from the first inoculation until the end of the crop cycle. Disease intensity was evaluated using a descriptive key (Cia & Salgado, 1995), considering the following scores: (1) disease absence; (2) just necrotic lesions in the leaves or branches; (3) apical region affected, with meristem death, (4) over-shooting at the apical region; (5) over-shooting at the apical region and significant plant size reduction in comparison to healthy plants.

The average scores of each treatment were obtained by arithmetic average of the scores of 60 evaluated plants (10 plants per plot). The evaluated plants were always the same during the crop cycle. These results were used to calculate the area under disease progress curve (AUDPC), which is the product between disease intensity and the corresponding period of time to the final score of disease intensity.

Microclimate measurements

An automatic weather station was installed at the center of the experimental area for recording the following data: air temperature (T), relative humidity

(RH), rainfall (P), leaf wetness duration (LWD), wind speed (U) and incoming solar radiation (SR). Additionally, automatic micro-stations, which recorded T, RH, and LWD, were installed in the center of one plot of each treatment, just below the top of the cotton canopy, with the height of the sensors adjusted every week to follow the plants growth and development.

All the sensors were connected to a data-logger (model CR23X, Campbell Scientific, Logan, UT) programmed to measure each sensor every 5 seconds and record data every 15 min by the average for T, RH, LWD, U, and SR, and by the total for P, during 90 days.

Crop growing and yield measurements

Cotton leaf area was determined at 30, 60, 90, 120 e 150 DAS (from phenological growth stages V6 to R8), to characterize crop growing. Leaf area (LA) was based on length and width of leaves of two

plants used as samples, according to the method presented by Monteiro *et al.* (2005). Leaf area index (LAI) was determined as the relationship between LA and land area for each plant, which varied for each plant population.

Crop yield was measured in the end of the crop cycle, from 136 to 166 DAS, being considered the harvest of the total mass of cotton (linter and seeds) of all balls in the two central useful lines of each plot.

Data analysis

Leaf area index (LAI) was plotted against time to show crop canopy growing along the cycle and the differences between treatments. LAI was also used to determine LAI duration (LAID), which is the integration of the leaf area index over time. Tukey's test was applied to compare LAID among treatments.

Microclimate analysis gave emphasis to T and LWD since they are the two most important meteo-

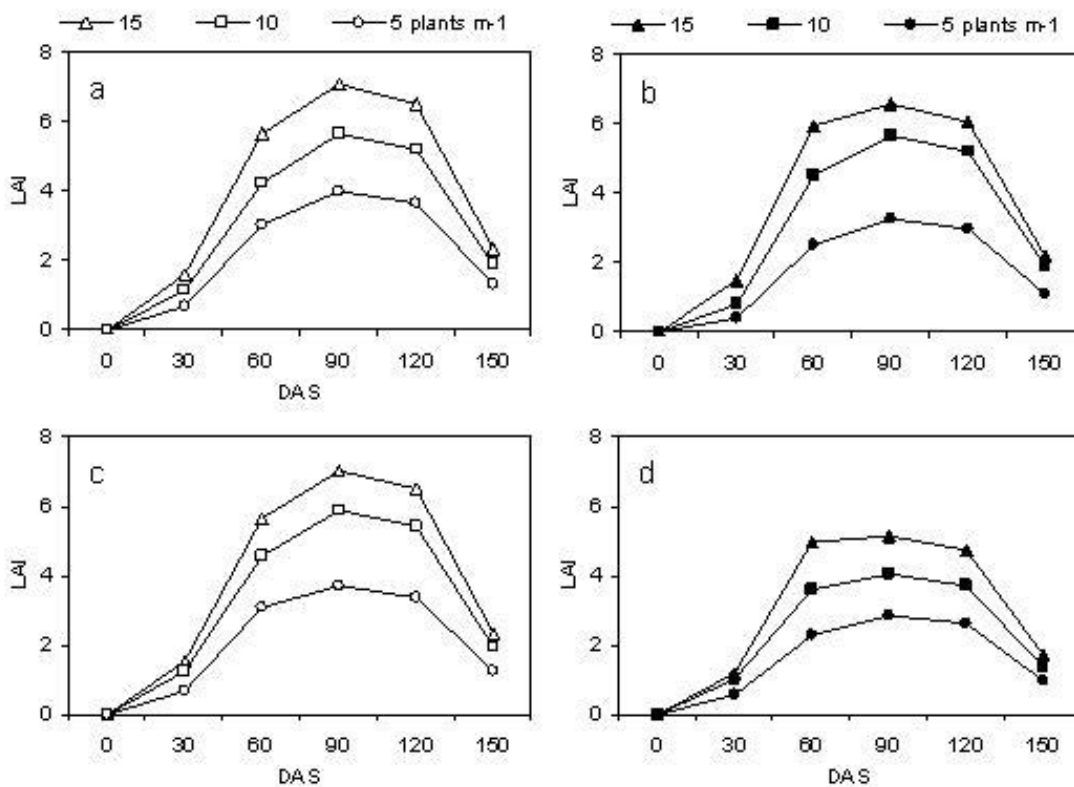


Figure 1. Cotton leaf area index (LAI) from 0 to 150 days after seeding (DAS) for genotype IAC 23 not inoculated (a) and inoculated (b), and for genotype Coodetec 401 not inoculated (c) and inoculated (d), with plant densities of 5, 10 and 15 plants per meter, in Piracicaba, SP, Brazil, 2001/2002.

Table 1. Leaf area index duration (LAID) and area under disease (ramulosis) progress curve (AUDPC), in cotton crop, genotypes IAC 23 and Coodetec 401, with 5, 10 and 15 plants per meter (pl m^{-1}), at inoculated and non inoculated plots, in Piracicaba, Brazil, 2001/2002.

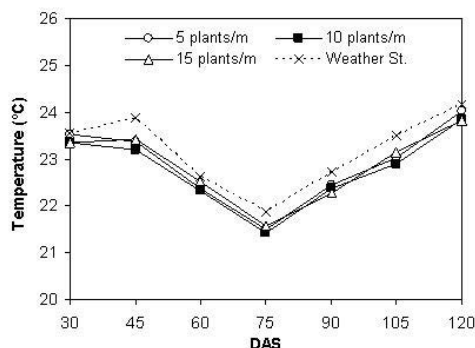
Genotype	IAC 23			Coodetec 401		
	5 pl m^{-1}	10 pl m^{-1}	15 pl m^{-1}	5 pl m^{-1}	10 pl m^{-1}	15 pl m^{-1}
	LAID ($\text{m}^2 \text{ day}$)					
Inoculated plots	339cd	509b	642a	252de	375c	485b
Not inoculated	351cd	502b	645a	279d	482b	606a
	AUDPC (units)					
Inoculated plots	106b	115b	101b	241a	228a	246a
Not inoculated	49c	53c	53c	54c	61c	67c

* Values followed by same letter in the lines or columns do not differ statistically from each other by Tukey Test, with confidence range of 95%.

rological parameters to the majority of fungal diseases. Daily crop LWD was obtained considering 24-h periods beginning at noon (12:00) and ending at 11:45 for 15-minute data sets. Daily average T was determined for the same period of time. Micrometeorological data were grouped in seven-day-period averages in different phases of the crop cycle. This procedure was adopted to reduce data variability.

The epidemiology of ramulosis was described by the disease progress curve for all treatments. To compare disease intensity among treatments, AUDPC was determined and submitted to Tukey's test.

Average crop yield was obtained considering all replications of each treatment and differences among treatments were tested by Tukey's test with a confidence of 95%. The relationship between AUDPC and yield was determined by regression analysis for all replications of each treatment and for treatment averages.

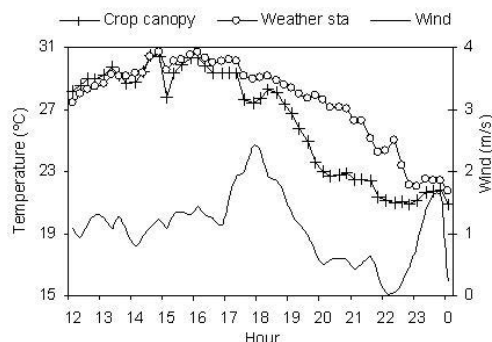
**Figure 2.** Daily average air temperature during the cotton crop cycle in three plant population densities and at the weather station, in Piracicaba, SP, Brazil, 2001/2002.

RESULTS

Crop growing

The pattern of LAI variation throughout the growing season was very similar for all treatments, with LAI increasing up to 90 DAS and decreasing after that (Fig. 1). Between 30 and 60 DAS, genotype Coodetec 401 showed a faster growing rate, 10.5% per day, than IAC 23, with 9.5% per day. After that, IAC 23 kept growing with a rate of 4.5% per day between 60 and 90 DAS, while Coodetec 401 presented a rate of only 2.5% per day.

No statistical differences on LAI were observed between not inoculated and inoculated plots for IAC 23. On the other hand, not inoculated plots of Coodetec 401 had greater LAI than inoculated ones. Also a significant difference was observed among the three plant densities for both genotypes, with LAI increasing, in general, from 5 plants m^{-1} , with LAI ranging from 3 to 4, to density of 15 plants m^{-1} , with LAI ranging from 5 to more than 7.

**Figure 3.** Wind speed and air temperature measured at the weather station and average air temperature measured by micro-stations, from 12:00 h to 0:00 h in Jan 2nd, 2002, in Piracicaba, SP, Brazil.

LAI presented the same pattern observed for LAI (Table 1). For IAC 23 no difference was observed between not inoculated and inoculated plots. However, differences between not inoculated and inoculated plots were observed for Coodetec 401, especially for densest plots (10 and 15 plants m^{-1}). Considering the genotypes, significant differences were observed only for inoculated and denser plots, showing stronger disease effect on Coodetec 401 genotype.

Microclimate

On average, the difference of temperature between weather station and micro-stations inside the crop canopy, at different plant densities, was smaller than 0.4 °C (Fig. 2). For the plots with different densities, average temperatures stayed close to each other, with differences not greater than 0.2 °C. However, short periods of 3 to 6 hours, after sun set, presented higher differences between temperatures measured in the weather station and at the crop canopy level (Fig. 3). Generally, during late afternoon and early evening with low wind speed, the temperature decreased faster at the crop level reaching around 4.5 °C less than at the weather station. Even with these differences had occurred for only 3 to 6 hours in some days, and apparently showing low importance on the average, it should be considered once it affects directly the dew deposition on the canopy, since dew point temperature is achieved earlier. An example is Jan 2, 2002, when LWD started at 21:30 in the crop canopy and only 3.5 hours later at the weather station in the experimental area.

In contrast to average temperature, greater differences were observed for relative humidity and LWD among plant densities, as well as among them and data recorded by the weather station in experimental area (Fig. 4). According to Geisler *et al.* (1996), in general, differences in micrometeorological conditions within the same crop tend to be subtle, except for leaf wetness and relative humidity which exhibit larger spatial variability. The LWD differences between crop canopy at different plant populations and weather station ranged from 2 to 5 hours, which is significant considering that they were in the same area.

Besides the microclimate variation in relation to the macroclimatic condition, LWD increased with the density of the plots, reaching 1 hour between 5 and 10 plants m^{-1} , and 1.5 hours between 10 and 15 plants m^{-1} . However, after crop canopy became a continuous surface (from 45 to 60 DAS), LWD differences among densities decreased gradually,

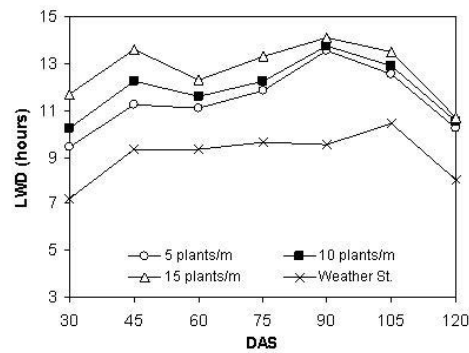


Figure 4. Daily average leaf wetness duration (LWD) during the cotton crop cycle, obtained with the micro-station in the plots with 5, 10 and 15 plants per meter and at the weather station, in Piracicaba, SP, Brazil, 2001/2002.

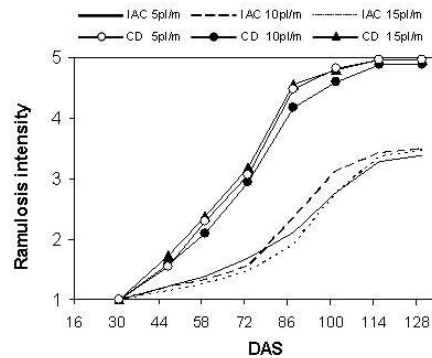


Figure 5. Ramulosis intensity in cotton crop, genotypes IAC 23 and Coodetec 401, under different plant densities, in Piracicaba, Brazil, 2001/2002 (Inoculated plots).

tending to similar values (Fig. 4).

Ramulosis occurrence

In the genotype IAC 23, the ramulosis intensity increased slowly at the beginning, having an increasing in the growth rate between 73 to 102 DAS. In the genotype Coodetec 401, ramulosis intensity increased faster, reaching maximum intensity after 100 DAS (Fig. 5). First lesions, from the initial inoculation (30 DAS), was source of inoculum for the secondary lesions, which allowed the pathogen to have a radial propagation, resulting in circular damaged areas.

Considering that the inoculation process was the same in both genotypes, the low disease intensity in genotype IAC 23 can be explained by its high resistance to the pathogen, which can be proved by the smaller number of lesions and, consequently, smaller intensity than observed in genotype Coodetec 401.

For treatments without inoculation, there was no

significant disease intensity difference between both genotypes, which was due to the fact that plants were only infected at their final height and with very slow vegetative growth, not allowing conditions for over-shooting and reduction of size caused by ramulosis - necessary conditions for scores greater than 3. In this case, the appearance of disease at the end of cycle originated the denomination "late ramulosis".

The differences between genotypes resistance to disease development was also compared by AUDPC (Table 1). On average, AUDPC was 2.2 times larger for genotype Coodetec 401 than IAC 23. No difference was observed for AUDPC among plant densities, even with differences in their microclimate in the first half of the cycle.

Cotton crop yield

There were no significant differences in cotton yield among plant densities (Fig. 6). For not inoculated plots, average cotton yields in the three population densities were close to 4,200 kg ha⁻¹ for IAC 23 and 4,400 kg ha⁻¹ for Coodetec 401. In the inoculated plots, average cotton yields were smaller, falling to 3,600 kg ha⁻¹ for IAC 23 and of 2,400 kg ha⁻¹ for Coodetec 401 (Fig. 6). These results showed that cotton yield was much more affected by ramulosis in Coodetec 401, even having this genotype a higher production potential than IAC 23.

Fig. 7 presents a linear regression analysis between AUDPC and cotton yield, considering all replicates of each treatment and the averages for each treatment. The relationship between cotton yield and AUDPC showed a define relationship between these two variables and the high dependence of cotton yield in relation to disease intensity

(AUDPC), with $R^2 = 0.70$, for all replicates of each treatment, and $R^2 = 0.93$, for averages, both highly significant. From linear regression analysis, considering averages of yield and AUDPC of both genotypes (Fig. 7b), we observed that for each 50 AUDPC units there was a yield reduction of 0.05 kg m⁻² or 500 kg ha⁻¹.

DISCUSSION

According to Huber & Gillespie (1992), LAI is one of the main factors influencing the crop microclimate. LAI differences observed in this study were caused mainly by plant density and genotype, since inoculation of ramulosis only presented effect on Coodetec 401 (Fig. 1 and Table 1). Disease occurrence in the cotton crop did not affect the LAI development of the genotype IAC 23. This is better observed analyzing LAID data (Table 1), where we can see that ramulosis had effect only on Coodetec 401 growth, especially in the densities of 10 and 15 plants m⁻¹. It confirms that Coodetec 401 is much less resistant to ramulosis than IAC 23.

Even with the differences in LAI and LAID between genotypes and among plant densities, few differences were observed for average temperature (Fig. 2). However, LAI differences were enough to promote a significant difference in relative humidity and, consequently, in LWD (Fig. 4). Similar results were found by Giesler *et al.* (1996), studying microclimate and brown patch disease in *Festuca arundinacea*. The authors observed that LWD was 1.5 hours longer in the densest canopy. The effects of plant density and architecture on microclimate and also on disease have been shown in other cropping systems. For example, conditions within an open

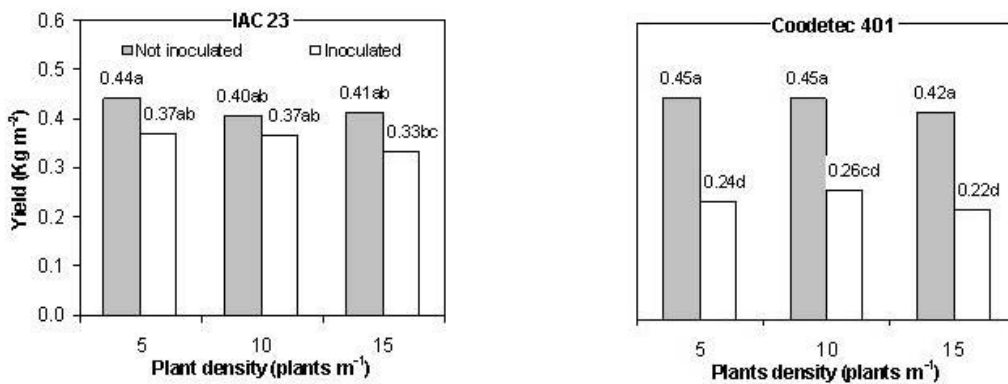


Figure 6. Cotton yield, for genotypes IAC 23 and Coodetec 401, sowed with 5, 10 and 15 plants per meter, for inoculated and non inoculated plots, in Piracicaba, Brazil, 2001/2002. Values followed by same letter do not differ statistically from each other at the level of 5% of probability, by Tukey test.

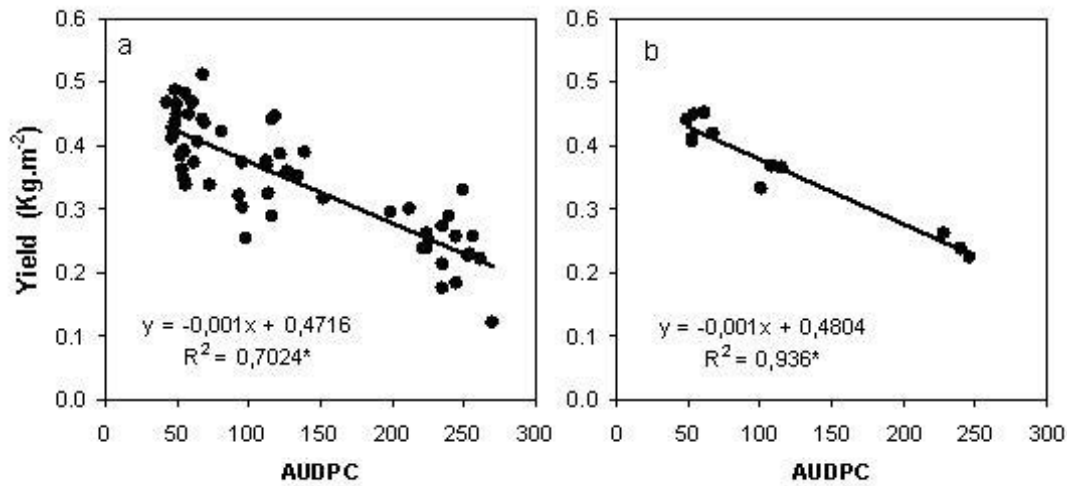


Figure 7. Relationships between area under disease progress curve (AUDPC) of ramulosis disease and cotton yield, considering all treatments (a) and averages (b). * Significant at 5% of probability.

canopy of a common bean (*Phaseolus vulgaris* L.) with an upright habit were warmer and drier than within a denser variety (Blad *et al.*, 1978). Similar results were found by Sentelhas (1992) for wheat crop. However, this author observed that *Helminthosporium sativum* intensity on wheat was not affected by plant density, but by the macroclimate conditions during the experiments, which were very favorable to the disease during the growing season. Planting density presents influence on disease development only when macroclimate is marginal or not favorable to the pathogen development, as very well discussed by Rotem & Palti (1969). Therefore, the microclimate effect on disease development is limited by weather conditions of the growing season as well as by resistance of the crop to the pathogen.

There is no doubt that microclimate is a key factor influencing plant diseases and that LWD is the most important parameter for the infection phase. However, LWD is a difficult variable to measure and to estimate because it is related to different microclimate factors (Magarey *et al.*, 2001). Its variability within crop canopies is still a challenge in choosing a representative LWD measurement for operational purposes (Sentelhas *et al.*, 2005). However, in contrast with the most of plant diseases, this is not a problem for ramulosis-cotton pathosystem. Ramulosis occurs in plants in any stage but develops better on younger tissues, which makes the lesions be concentrated over apical parts. Additionally, the ramulosis main effect occurs when the apical meristem is affected, what causes lateral shootings,

vegetative growth, and a strong reduction on fructification. These characteristics make the top of the cotton canopy the best position to LWD monitoring. Even when the site specific measuring in the crop canopy is not possible, Sentelhas *et al.* (2005) showed that a standardized LWD measurement over turf grass is a very good estimator of LWD at the top of the crops. According to the findings of these authors, the mean absolute errors associated to such estimations are between 50 and 95 minutes which are small enough to allow use in many operational plant disease management schemes.

In this study, it is clear that the minimum LWD required to the ramulosis establishment was somewhat around 9-10 hours, which was the minimum average LWD observed at all crop densities during the days after inoculation (Fig. 4).

As no difference was observed in disease intensity among plant densities for both genotypes (Fig. 5 and Table 1), there was no significant difference on cotton yield for the same genotype (Fig. 6), which agrees with Staut & Lamas (1999) who analyzed experimental results of cotton yield from several regions of the world and not found an ideal plant density. This is due to the fact that cotton is a species with a very good morphologic plasticity, mainly when only quantitative parameters of the production are evaluated.

The comparison of damages caused by ramulosis in several cotton crop genotypes has been adequately studied (Gridi-Papp *et al.*, 1994). However,

little information is available about the relationship between disease intensity and yield. The relationship between ramulosis AUDPC and cotton yield obtained in this study (Fig. 7) is an important tool for control practices, because it is possible to know when the ramulosis intensity will cause such a yield loss higher than economic level.

CONCLUSIONS

Based on the results obtained in this study, we concluded that:

Plant density had low effect on temperature inside the canopy, but it promoted significant effect on relative humidity and leaf wetness duration, which increased in the densest populations;

The effect of plant density on microclimate occurred up to the limit when the canopy became a continuous surface. After that, the increase of the crop did not influence temperature and relative humidity within the canopy;

Favorable macroclimatic conditions for ramulosis development neutralized the effect of the different microclimates, caused by plant densities, on disease intensity;

The genotype IAC 23 showed higher resistance to ramulosis than Coodetec 401, as indicated by lower disease intensity and AUDPC;

The AUDPC showed a high negative correlation with yield and it could be a useful variable to estimate cotton yield loss when ramulosis occurs.

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