Effect of sowing date and initial inoculum of *Alternaria helianthi* on sunflower in the south region of Brazil

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ABSTRACT

Three field experiments were carried out in Londrina, PR, south region of Brazil, in 1997/1998, 1998/1999 and 1999/2000 growing seasons, to investigate Alternaria leaf spot severity in sunflower sown at different dates and the effect of inoculum of the first sowing dates on subsequent ones. Four sowing dates (October, November, December and January) and two sowing types (contiguous and isolated) were used to simulate different levels of initial inoculum. Disease severity, under natural conditions in the field, was evaluated weekly, with reference to a diagrammatic scale of this disease, used to calculate the value of the area under disease progress curve (*AUDPC*). Marked plants were harvested individually, for evaluation of grain yield. Disease severity was highest when plants were sown in December regardless of the year. Sowing sunflower in October resulted in high yield and low disease severity. Sanitation measures to reduce initial inoculum concentration delayed the onset of the disease by 11 days.

Key words: *Alternaria* leaf spot – epidemiology – *Helianthus annuus* – primary inoculum.

INTRODUCTION

The potential for increasing sunflower (*Helianthus annuus* L.) cultivated area in Brazil can be limited by leaf blight and stem spot diseases, caused by *Alternaria helianthi* (Hansf.) Tubaki and Nishihara. The disease is reported to affect leaves, stem and sunflower heads, causing necrotic brown to black lesions, with a round or angular shape. Due to the frequent occurrence of climate conditions favorable for disease epidemics, i.e. high relative humidity and temperatures between 25°C and 30°C, Alternaria leaf blight is the most important sunflower disease in Brazil, occurring in all regions and sowing dates (Leite, 2005).

For many foliar diseases, once an epidemic has been initiated, infectious lesions within the crop are the predominant source of initial inoculum for newly planted tissues (Vanderplank, 1963; Jeger, 1982). Two main factors can influence disease severity: sowing date and presence of fungal inoculum in the production area. Preliminary studies about the development of Alternaria leaf spot during one growing season showed that lowest disease severities were observed in sunflower sown in October and November in the south region of Brazil and the fungal inoculum from the first sowing plots was important for pathogen dissemination to the last sowing plants (Carvalho et al., 1995).

Methods to reduce initial inoculum, i.e. Vanderplank's sanitation measures, can be used to decrease disease severity. Vanderplank (1963) used simple exponential equations to describe how sanitation measures could delay the onset of the disease, by reducing the amount of initial inoculum from which an epidemic starts. He argued that, assuming that the disease progress rate (r) is not affected by sanitation, if initial inoculum was reduced by sanitation from x_0 to x_{0s}, then the epidemic would be delayed by the time taken for disease to increase from x_{0s} to x_0. The relationship between the inoculum ratio (x_0 / x_{0s}) and the time delay in the epidemic (Δt) could be described by $\Delta t = \ln (x_0 / x_{0s}) / r$.

The main objective of this study was to investigate Alternaria leaf spot severity in sunflower sown on different dates, in the south region of Brazil, and the effect of inoculum from the first sowing plots on the last ones.

MATERIALS AND METHODS

Field experiments

Three field experiments were carried out in Londrina, State of Paraná, in the south region of Brazil (latitude 23º11'50" south; altitude 585 m), in three growing seasons: 1997/1998, 1998/1999 and 1999/2000. The experimental sunflower hybrid SE02, developed by the Embrapa Soja genetic breeding program, was used. All experiments followed the randomised complete block design, with four sowing dates, two sowing types and four replications. Each plot consisted of four 5m rows, with between-row spacing of 0.90 m; three sunflower plants were left per linear meter. The trials received the conventional cultural practices of commercial fields, including sowing and top-dressing fertilisation, spraying against insects, and sprinkle irrigation, when necessary (Castro et al., 1996). The trials were planted in an area intensively used for sunflower experimentation.

To establish several levels of disease severity, sowing was carried out in the months of October, November, December and January of each year, in two sowing types: contiguous and isolated plots. The first type had all the plots of the different sowing dates located side by side, while, in the other type, plots of the different sowing dates were separated by six rows of corn, a non-host barrier to the fungus. No artificial inoculation of *A. helianthi* was performed; disease occurred by natural infection of the plants. The pathogen was identified by isolation in laboratory and plant inoculation in glasshouse.

Assessments of disease severity, area under disease progress curve and yield

The evaluation of disease severity and yield was made on the two central rows of each plot, disregarding 0.5 m at each row end. The single plant approach was adopted (Kranz and Jörg, 1989), in which 10 or 8 plants of each plot were marked, thus a total of 320 plants for the first trial and 256 for the second and third trials were obtained. Plants were marked after the appearance of the fourth true leaf (V4 growth stage) (Schneiter and Miller, 1981), and an attempt was made to select individuals of the same development stage, height and vigour.

The leaf areas (*LA*) (cm^2) of all leaves of each marked plant were estimated weekly, starting from the appearance of the fourth true leaf (V4 growth stage). For this, the maximum width (cm) of each leaf (*L*) was measured with a ruler. Leaf area was calculated using the equation $LA = -155.86 + 22.40\ L$ (R^2=0.90) (Leite and Amorim, 2002). Assessment of the severity of Alternaria leaf spot was simultaneous with the evaluation of leaf area, with the aid of a diagrammatic scale, which was previously elaborated and validated (Leite and Amorim, 2002). Marked plants affected by other diseases or showing any problems in their development were discarded.

The area under disease progress curve (*AUDPC*) for each plant was calculated by trapezoidal integration using the formula (Bergamin Filho et al., 1997):

$$AUDPC = \sum_{i=1}^{n-1} ((S_i + S_{i+1})/2)(t_{i+1} - t_i)$$

where $S_i = S(t_i)$, n was the number of assessments, S was disease severity (in percentage) and $(t_{i+1} - t_i)$ was the interval (days) between two consecutive assessments.

Marked plants were harvested individually, after physiological maturity (R9) (Schneiter and Miller, 1981), for evaluation of grain yield (kg ha^{-1}).

Data analysis

Data of *AUDPC* and grain yield were submitted to ANOVA, using the factorial design (sowing dates x sowing types), with four replications. Duncan's multiple range test (p=0.05) was performed to detect the significant differences for *AUDPC* and yield means among sowing dates and sowing types, using SAS software (SAS Institute, USA).

Data were also analysed by non-linear regression, using the software STATISTICA 5.0 (Statsoft, Tulsa, USA). Data were fitted individually by negative exponential model, $Y=B_1 \exp(-B_2 X)$, where Y represents the yield component, X represents *AUDPC*, B_1 represents the intercept and B_2 represents the slope.

To calculate the initial inoculum, Alternaria leaf spot severity data for each sowing date and sowing type were individually fitted by logistic model (Berger, 1981), $Y=1/(1+B_1 \exp(-B_2 X))$, where Y is the disease severity, X is the number of days after sowing, B_1 is the parameter related to initial inoculum and B_2 is the disease progress rate, for the three consecutive years (1997/1998, 1998/1999 and 1999/2000).

The time delay in the epidemic was calculated based on the theory of Vanderplank (1963), which assumes that disease progress rate (r) is not affected by any measure of reducing initial inoculum. In the present work, the sanitation measure used for reducing initial inoculum was the isolated sowing type, where the different sowing dates were separated by six rows of corn, compared with the contiguous sowing type, where the different sowing-date plots were located side by side.

Using the logistic model, a constant disease progress rate (r) for each sowing date was calculated with the disease severity means of contiguous and isolated plots, for the three consecutive years. The initial inoculum for both contiguous (x_{0c}) and isolated (x_{0i}) sowing types was calculated keeping the rate constant. The time delay in the epidemic (Δt) (days) as a function of reducing initial inoculum by the sanitation method was calculated by:

$$\Delta t = \ln (x_{0c} / x_{0i}) / r$$

where: x_{0c} was the initial inoculum present in contiguous sowing plots, x_{0i} was the initial inoculum present in isolated sowing plot and r was the constant disease progress rate for each sowing date.

RESULTS AND DISCUSSION

Sowing sunflower in four different months each year proved to be effective for obtaining a wide range of Alternaria leaf spot severity. A significant higher *AUDPC* mean was observed in plants sown in December of the three years, for both sowing types (Table 1). This variable decreased for plants sown in January, since many leaves were senescent due to the disease and were not considered for disease severity assessment. Healthy plants (*AUDPC*=0) were only observed in plants sown in October and November 1999; for the first month, considering both sowing types (contiguous and isolated plots), all plants remained disease-free. Comparing sowing types, *AUDPC* was significantly lower in isolated plots sown in January 1998 and in December 1999 (Table 1). Sunflower plants sown in January 1999 did not produce grains, as well as plants sown in contiguous plots in December 1998 (Table 1).

Table 1. Area under disease progress curve (*AUDPC*) of Alternaria leaf spot and yield of sunflower, sown on four months and two sowing types, in three consecutive years

Sowing date	*AUDPC*[1]		Yield (kg/ha)[1]	
	Sowing type		Sowing type	
	Contiguous	Isolated	Contiguous	Isolated
1997/1998				
Oct	336.75 dB	504.19 cA	2450.54 aA	1395.04 aB
Nov	572.94 CA	485.13 cA	649.34 bA	622.3 bA
Dec	907.35 aA	867.59 aA	5.14 cA	137.76 cA
Jan	778.25 bA	630.68 bB	100.11 cA	348.65 cA
Mean	625.44		713.61	
CV (%)	10.41		24.83	
1998/1999				
Oct	163.67 dA	285.51 cA	2898.44 aA	2182.82 aB
Nov	305.26 cA	308.01 cA	920.41 bB	1214.78 bA
Dec	844.32 aA	811.82 aA	0 cB	304.82 cA
Jan	505.93 bB	667.67 bA	0 cA	0 dA
Mean	485.52		940.15	
CV (%)	17.47		15.22	
1999/2000				
Oct	0 cA	0 bA	2062.23 aA	2428.96 aA
Nov	45.92 cA	71.69 bA	1900.83 aB	2462.43 aA
Dec	771.99 aA	452.05 aB	673 bB	1886.73 bA
Jan	481.42 bA	409.77 aA	892.22 bB	1459.84 cA
Mean	279.10		1673.60	
CV (%)	19.38		19.28	

[1]For each variable and growing season, means followed by the same letter (capital letters in columns and minuscule letters in line) are not different by Duncan´s multiple range test (5%).

Data of yield and disease severity observed in this work indicate that sowing sunflower in October resulted in high grain yields and low or no disease severity. This corroborates the fact that the recommended sowing date for sunflower in the State of Paraná is from August to October (Castro et al., 1996). Silveira et al. (1993), studying sowing dates for sunflower in this State, also observed higher yields for sunflower sown in August and lower yields for sunflower sown in December.

Vanderplank´s sanitation ratio theory was used to account for the time delay in the epidemic (Δt) as a function of reducing initial inoculum by a sanitation measure. This theory was also used by Young et al. (2003), for predicting epidemics of yellow rust (*Puccinia striiformis*) on the upper canopy of wheat, compared to disease severity on lower leaves. Vanderplank (1963) considered that the effectiveness of sanitation should be linearly related to the delay in reaching any given level of the disease. This delay (Δt) is the additional time required to reach a given severity in a crop with sanitation measures, as compared to the crop without sanitation measures (Plaut and Berger, 1981).

In this study, the time delay in the epidemic in terms of reducing the initial inoculum by the sanitation method varied from 0.75 day, on November 1998 sowing date, to 11.56 days, on December 1998 sowing date (Table 2). This confirms that sowing sunflower separated by rows of corn was enough to decrease the primary inoculum, compared with the contiguous sowing, and cause a delay in disease epidemics. The delay of 11 days on the onset of the disease is important considering the early crop cycle of 100 days. As disease is delayed, the damage to sunflower yield becomes lower.

Vanderplank (1963) considered some factors that could limit plant disease development when he first discussed the sanitation theory. He was cautious in recommending sanitation measures as a disease control strategy, particularly for diseases with high infection rates and for epidemics of a long duration. Management of Alternaria leaf spot of sunflower should not be seen as an isolated measure. The reduction in initial inoculum, which was used to simulate the effect of sanitation, may not be identical to benefits derived from actual sanitation measures (Plaut and Berger, 1981). Farmers of the same region should concentrate sunflower sowing on the same date, in order to decrease pathogen dissemination from one area to another. Low initial disease was apparently compensated for by accelerated rates of disease increase. Thus, sanitation measures in the management of compound interest diseases may be less effective than previously theorized (Plaut and Berger, 1981).

We concluded that sowing sunflower in October resulted in a high yield and low Alternaria leaf spot severity in the State of Paraná, Brazil. Sanitation measures to reduce initial inoculum concentration delayed the onset of the disease by 11 days.

Table 2. Time delay in the epidemic (Δt) and parameters of logistic function, Y=1/(1+(1/x_0)-1) exp(-rX)), where Y is disease severity, X is days after sowing, x_0 is initial inoculum and r is constant disease progress rate for each sowing date, for Alternaria leaf spot of sunflower, in three consecutive years. Plants sown on four different dates and two sowing types (contiguous and isolated) were used for regression analysis.

Sowing date	r	Sowing type		Δt (days)
		Contiguous	Isolated	
		x_0	x_0	
1997/1998				
Oct	0.0601	0.0019	0.0025	-
Nov	0.0797	0.0010	0.0009	1.14
Dec	0.0620	0.0077	0.0061	3.77
Jan	0.0492	0.0105	0.0080	5.58
1998/1999				
Oct	0.1467	5.39E-07	1.02E-06	-
Nov	0.1128	5.36E-05	5.83E-05	0.75
Dec	0.0756	0.0061	0.0026	11.56
Jan	0.0563	0.0037	0.0030	3.73
1999/2000				
Oct	-	-	-	-
Nov	0.2474	9.9E-12	1.61E-11	1.97
Dec	0.0555	0.0036	0.0022	9.32
Jan	0.0226	0.0214	0.0175	8.78

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