HELIA, **28**, Nr. 42, p.p. 89-100, (2005) UDC 633.854.78:632.1

VIRTUAL LESION OF ALTERNARIA BLIGHT ON SUNFLOWER

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Received: December 26, 2003
Accepted: May 22, 2005

SUMMARY

Alternaria blight in sunflower, caused by the fungus *Alternaria helianthi*, is one of the major diseases that affects sunflower in Brazil. The effect of this organism on net photosynthetic rate of four sunflower genotypes was measured under field experimental conditions. Through a mathematical approach the diseased leaf area and net photosynthetic rate were related and used to analyze the pathosystem sunflower/*Alternaria*. An average decrease in the photosynthetic rate was observed in diseased leaves for all analyzed genotypes in comparison with healthy leaves. Alternaria blight reduces photosynthesis not only through a reduction in green leaf area, but also through an effect on photosynthesis of the remaining green leaf tissue. Leaves free of the pathogen lesions in diseased plants present also a photosynthetic reduction.

Key words: Alternaria blight, disease quantification, net photosynthetic rate

INTRODUCTION

Alternaria blight, one of the major diseases that affects sunflower in Brazil, is caused by the necrotrophic fungus *A. helianthi* Tub. Nish. According to Leite and Pascholati (1995), Agrios (1997) and Lucas (1998), the first symptom caused by necrotrophic fungi on leaves is a chlorosis, which enlarges, coalesces and can cause organelle destruction through an enzymatic reaction or by toxic metabolites produced by the fungus during the infection development. Goodman *et al.* (1986), discuss that this fungus should negatively influence the photosynthetic process, starting with chloroplast alterations, which led to a chlorophyll degeneration and reduction in CO_2 fixation.

Alternaria helianthi can cause leaf and stem lesions, seedling blight and head rot (Sackston, 1981; Allen *et al.*, 1983a). It is reported to reduce seed and oil yield by 27 to 80% and 17 to 33%, respectively, and can cause germination losses (Reddy

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and Gupta, 1977; Balasubrahmanyam and Kolte, 1980). It is more dangerous in tropical and subtropical regions since the combination of high temperatures (25 to 30ºC) and air humidity over 70% helps disease development (Prasad and Singh, 1983; Dudienas *et al.*, 1998).

Moraes *et al.* (1983) found different susceptibility reactions among sunflower genotypes. For the genotypes IAC-Uruguai and IAC-Anhandy, the spots were located mostly in the lower leaves while in VNIIMK the disease caused death in all the inoculated plants. Besides, the fungus produces a secondary metabolite, named deoxiradicinin (Tal *et al.*, 1985), which is toxic to the plant and is able to affect important cell processes, leading to a less effective photosynthesis and lower crop yield (Paul *et al.*, 1990).

For various pathosystems, the effect of the pathogen on photosynthesis of the remaining green leaf area has been reported. Goto (1965) showed that yield loss due to leaf blast exceeded yield loss caused by cutting off a percentage of leaf area equal to the percentage of leaf covered by the fungus. This extra reduction means that the disease influenced the host plant more than the visual lesion would do. For various pathosystems, the effect of the pathogen on photosynthesis of the remaining leaf area has been reported, such as *Alternaria alternata* in cotton (Ephrath *et al.*, 1989). On the other hand, some pathosystems did not cause photosynthesis reduction of the healthy leaf area (Spitters *et al.*, 1990; e van Oigen, 1990).

In 1991, Bastiaans working with *P. oryzae* derived a model to relate the net photosynthetic rate of diseased leaf area to the photosynthesis of comparable leaf area and disease severity. This function is based on the assumption that the visual lesion is part of a virtual lesion in which photosynthesis is negligible.

Reliable estimates of the damage caused by plant pathogens are the first step for the development of a well-established integrated control program of diseases.

In the present study, the effect of *A. helianthi* on leaf photosynthesis was measured and the Bastiaans's model was used to describe this effect in different sunflower genotypes.

MATERIALS AND METHODS

Inocula

The fungus *A. helianthi* was obtained from Soybean National Research Center-Embrapa Soja. It was cultured on oat-dextrose-agar which, according to Srinivas *et al.* (1997), favors mycelium growth and sporulation. The mycelia were maintained in the dark, at 25ºC during 15 days. After this period, they were replicated and transferred to essay tubes containing the same substrate and went to sporulation under fluorescent light of 40 W, at 25ºC during 15 days. The resulted colonies were dark green and presented enough development and sporulation. The inoculation

was done with a suspension of distilled water, 1×10^4 conidia ml^{-1} and 0.05% of Tween 80.

Light saturation

Light saturation curves were done to determinate the light intensity responsible for high photosynthetic efficiency, with the purpose of a constant maintenance of light intensity during field evaluations with IRGA.

Net photosynthesis was measured at 10-min intervals during 1 h, for each light intensity. The following sunflower genotypes were used:

- IAC-Uruguai – long season variety that presented some resistance to Alternaria in previous research.
- IAC-Iarama –selected for low sensibility to Alternaria disease; short season variety.
- Rumbos 91 - sensible to Alternaria disease; long season hybrid.
- M 742 - less sensible to Alternaria; short-medium season hybrid.
- BRS-191 - substituted M 742 in the field experiments. With exception to Alternaria disease, it showed good oil and grain yield in field trials. Short season hybrid.

IRGA light source was determined and adjusted to 1600 μmol quanta m^{-2} s^{-1}.

Net photosynthetic rate (*A*)

In order to determine the effect of leaf blight caused by *A. helianthi* on photosynthesis of sunflower leaves, field experiments were conducted at the Central Experimental Center of Agronomic Institute of Campinas, state of São Paulo, Brazil, in a complete block design, with a 4-2-4 factorial including 4 genotypes, 2 treatments - with and without disease inoculation - and 4 replications. Each block had 4 rows with 30 plants each, in a 90 × 20 cm plant density. The 2 central rows were considered for the photosynthesis evaluation. The blocks received nutrients according to Quaggio and Ungaro (1996). To avoid contamination between diseased and disease free blocks, *Crotalaria junceae* was sown between blocks. The sowing dates were selected according to the results obtained by Sentelhas *et al.* (1996) and Dudienas *et al.* (1998), which found that the fungus development is strongly influenced by rainfall and high air temperature, and that October/ November sowings are most favorable for the disease development while sowings between May and August are least favorable.

Net photosynthesis was measured with a portable photosynthesis meter LI 6400 (IRGA/Li-Cor).

Favorable disease period

The experiment was sown in September 2000. The first photosynthesis evaluation was done at V_n stage, for all genotypes. The second one, at $R_{5.1}$ (10% of the capitula with open flowers) for IAC-Uruguai and Rumbosol, and at $R_{5.8}$ (80% of the

capitula with open flowers) for BRS 191 and IAC-Iarama. Measurements were done at the border of a well-developed leaf lamina, in order not to damage the petiole and to avoid the main veins.

The inoculation of *A. helianthi* in the diseased blocks was done 30 days after emergency. First symptoms appeared 12 h after inoculation. One week later, the lesion diameters were between 0.3 and 0.5 cm, which was adequate to start the photosynthesis evaluations.

Four competitive and well-developed plants were selected in the two central rows of each plot. The leaf area in the IRGA analyzer chamber was demarcated by a water pencil, and measured 6 cm^2.

IRGA was programmed to measure ***A*** at one-minute intervals during 1 h. Data were collected from 8:30 AM to 5 PM. Under the given conditions it was possible to evaluate 8 plots per day.

Unfavorable disease period

The experiment was similar to the previous one. It was sown in June 2001. The second evaluation was done at $R_{5.2}$ (20% of opened flowers) for IAC-Iarama and BRS 191, and at R_3 (floral budding) for IAC-Uruguai and Rumbos 91. Well-developed leaves located in the middle portion of the plant were used since the lower leaves were infected by oidia and received chemicals for disease control.

IRGA measurements were done only between 9 AM and 12 AM since the data obtained in the September sowing showed high amplitude of variation between measurements done after 12 AM.

The photosynthetic activity was taken in 8 plants per plot, totaling 256 observations per genotype. Yield evaluations could not be done due to a storm that destroyed the experiment at grain filling period.

Photosynthetic performance of healthy leaves in diseased plants

In the diseased plots of the experiment sown in June 2001, photosynthetic activity was evaluated in leaves that showed disease spots and leaves free of *Alternaria* spots, both belonging to the same plant. The leaves were all located in the medium part of the plant. Light intensity was 1600 μmol m^{-2} s^{-1}. Eight plants were evaluated in each plot. This experiment had the objective of verifying the photosynthetic performance of healthy leaves in plants with diseased leaves in order to determinate the influence of the pathogen on the whole plant.

Disease severity

After each photosynthesis evaluation a picture of the leaf was taken with a digital camera Casio QV-10, at the distance of 20 cm. Measurements of leaf width and length were done in order to adjust the leaf size before the analysis by the WinDIAS program, following the rules described in Webb and Jenkins (2000).

For WinDIAS, the disease is the portion of the leaf that presents dark brown spots plus the chlorotic portion around them. WinDIAS program calculated the diseased area (visual lesion) in relation to 6 cm^2 leaf area. Each evaluation was done 3 times. The percentage of diseased area was the disease severity and was used in Bastiaans's equation:

$Px/Po=(1-x)^{\beta}$

in which Px is the photosynthesis of a leaf with disease severity; Po is the photosynthesis of a health leaf; x is the visible diseased fraction of the leaf area.

Data analyses

CO_2 assimilation in individual sunflower leaves was used to estimate ***A*** and quantify the influence of *A. helianthi* on the ***A***. Net photosynthetic rate was related between genotypes and disease severity using Bastiaans's equation. The β parameter was obtained through regression analysis, using MiniTab program. To verify the effect of the treatments, analyses of variance and Tukey test were performed on ***A***, β parameter, and grain yield.

RESULTS AND DISCUSSION

Net photosynthesis

In the two field experiments the extent of reduction in ***A*** of infected in comparison with healthy leaves, expressed by β parameter, exceeded disease severity (Table 1).

Table 1: Net photosynthesis of health leaves (Px) and infected leaves (Po), reduction of *A* (%), disease severity (% necrotic leaf area -NLA) and for β parameter in the two evaluation dates. Sowing of September 2000.

Genotype	1st evaluation				
	Px	Po	Reduction of A (%)	% NLA (visual lesion)	b
BRS 191	15.3 a A	23.5	34.9	8.6	4.5
IAC – Iarama	11.0 ab A	19.5	43.6	12.7	4.7
IAC – Uruguai	7.7 b A	17.4	55.7	16.9	4.4
Rumbossol 91	9.0 b A	18.3	50.9	18.6	3.5
Genotype	2nd evaluation				
	Px	Po	Reduction of A (%)	% NLA (visual lesion)	b
BRS 191	3.2 a B	20.7	84.5	56	2.2
IAC – Iarama	2.8 ab B	20.5	86.3	60	2.2
IAC – Uruguai	0.5 b B	22.5	97.8	61	4
Rumbossol 91	1.3 ab B	19.9	93.5	76	2

Means followed by the same letter in the column are not statistically different by Tukey at 5%.
Means followed by the same capital letter in the column indicate no difference between genotypes, healthy and infected plants.

Disease reduced the amount of green leaf area and affected also the net photosynthesis of the remaining green leaf tissue. The percentage of leaf area covered by

Alternaria lesions was determined and it ranged from 8.6 to 18.6% in the first evaluation and from 56 to 76% in the second one, both in the first experiment.

In the first and second evaluations (Table 1) cv. BRS 191 showed the least visual lesion while Rumbos 91 showed the largest one. The β parameter expresses the relation between disease severity and net photosynthetic rate of infected leaves. The genotypes exhibited different responses at 5% statistical probability. Differences in β values between genotypes were reported by Bastiaans (1991) as associated to the mechanisms of plant-pathogen relationship, influencing leaf photosynthetic processes or associated to breeding and selection for some agricultural character.

In the second evaluation of ***A***, the plants were near physiological maturation (R_9) and the bottom leaves were senescent. The susceptibility of sunflower tissues to *Alternaria* disease increases with leaf age. When the flowering process begins and the photoassimilates start moving to the grain, and when oil filling process starts, the reduction in foliar area can cause up to 80% reduction in grain and oil yield (Allen *et al.*, 1981, 1982, 1983b; Potter and Breen, 1980; Connor and Hall, 1997). The effect of pollen on leaves should be pointed out as a factor of conidial germination on leaf surface, which can favor disease severity during and soon after flowering. It should also be taken in consideration that at the time the second evaluation, the plots showed some oidial spots that could further reduce the net photosynthesis. As a consequence, the β value found from the first evaluation seems to reflect better the pathogen x sunflower relationship.

Table 2: Net photosynthesis of health leaves (Px) and infected leaves (Po), reduction of *A* (%), disease severity (% Necrotic Leaf Area -NLA) and β parameter in the two evaluation dates. Sowing of June 2001.

Genotype	Po	Px	Reduction of ***A*** (%)	% NLA (visual lesion)	b
BRS 191	21.7 a A	7.3 a B	66.3	49.7	2.3
IAC – Iarama	21.0 ab A	6.4 b B	69.5	58.7	1.4
IAC – Uruguai	22.4 a A	7.0 a B	69.2	50.6	1.8
Rumbosol	20.4 b A	5.0 ab B	75.5	57.6	1.8

Means followed by the same letter in the column are not statistically different by Tukey at 5%.
Means followed by the same capital letter in the column indicate no difference between genotypes, healthy and infected plants.

Net photosynthetic rate and disease severity in the unfavorable disease period is presented in Table 2. The evaluations were done at $R_{5.2}$ (20% of open flowers) for the short season genotypes IAC-Iarama and BRS 191, and in R_3 for the long season Rumbos 91 and IAC-Uruguai. The data were adjusted to Bastiaans's (1991) models. The regression equations were significant at 5% level. The relation between disease severity and ***A*** of infected leaves was described by the β parameter. Similar to the favorable season experiment, all the genotypes showed a significant reduction of β, greater than the expected when considering only the reduction in leaf area. This confirms the presence of a virtual lesion in the pathosystem *Alternaria* × sunflower.

The level of disease severity in this experiment ranged between 0 and 84.2%, with the means presented in Table 2. Differences in disease severity did occur between the two experiments and they can be explained by the higher environmental humidity and temperature in the first experiment which would directly influence fungus development, as discussed by Sentelhas *et al.* (1996). These differences also lead to differences in the β parameter.

Table 2 presents differences between ***A*** from healthy and diseased leaves. The average reduction in net photosynthesis was around 70%. Rumbos 91 had the lowest ***A*** in both cases and also the greater reduction in net photosynthesis. The greatest β was from BRS 191.

The β values in this experiment were smaller than those in September experiment probably due to favorable environmental conditions in September, which intensified fungus sporulation and penetration.

Evaluation of *A* from healthy and diseased leaves in the same plant

This experiment was important for the understanding of *Alternaria* x sunflower relationship. One healthy and one diseased leaf of four plants per plot were evaluated, with a total of 64 leaves.

Table 3: Average results for *A* observed in healthy and diseased leaves of diseased plants

Genotype	Healthy leaves	Diseased leaves	Reduction 1 (%)	Reduction 2 (%)
BRS-191	17.25 a A	8.78 a A	49.1	20.5
IAC-larama	17.16 a A	8.01 ab A	53.3	18.3
IAC-Uruguai	16.92 a A	5.72 ab B	66.2	24.9
Rumbosol	16.56 a A	4.42 b B	73.3	19.9

CV%=25,7

Means followed by the same letter in the column are not statistically different by Tukey at 5%.
Means followed by the same capital letter in the column indicate no difference between healthy and infected plants between genotypes.
Reduction 1 reflects the difference of **A** between healthy and diseased leaves in diseased plants.
Reduction 2 means the comparison of ***A*** between healthy leaves in healthy and diseased plants.

Table 4: Average results for *A* observed in healthy leaves of control and infected plants

Genotype	***A*** of health leaves	***A*** of diseased leaves	Reduction (%)
BRS 191	22.07 a A	17.27 a B	21.74 a
IAC larama	20.97 a A	17.17 a B	18.12 a
IAC Uruguai	22.45 a A	16.92 a B	24.63 a
Rumbosol	20.42 a A	16.57 a B	18.85 a
Average	21.48 A	16.98	20.84
CV%	7.68		

Means followed by the same letter in the column are not statistically different by Tukey at 5%.
Means followed by the same capital letter in the column indicate no difference between healthy and infected plants between genotypes.

Table 3 shows the average results for ***A*** of healthy leaves of diseased plants. The genotypes showed around 20% reduction in leaf photosynthesis in comparison between ***A*** of healthy leaves in healthy plants and healthy leaves of diseased plants.

This ***A*** reduction indicates that the presence of the pathogen in the sunflower plant causes some limitations in the photosynthetical mechanism of the whole plant.

Bhaskaran and Kandaswamy (1979), found higher concentrations of phenolic compounds in the chlorotic halos of the lesions caused by *Alternaria* in sunflower, in the vegetative phase. The occurrence of such halos before flowering indicates a partial resistance mechanism due to phenolic compounds.

Livne and Daly (1966) and Pozsár and Király (1966), as mentioned by Stangarlin (1999), discussed that in fungal infections a carbohydrate source in the leaf colonized by the pathogen stimulates CO_2 fixation in healthy leaves of diseased plants. Nutrient translocation should go from healthy to the diseased tissues, while translocation from diseased leaves would be highly suppressed. There exists a competition for photoassimilates between healthy and diseased tissues. Besides that, leaves showing no disease symptoms are influenced by the pathogen as well as by toxic substances diffused in cell mesophyll which are associated with other factors related to carbon metabolism.

The reduced ***A*** observed in healthy leaves of diseased plants (Table 3) suggests a limitation in the photosynthetic mechanism of the whole plant, probably associated to the photoassimilates absorption, and the production of toxic secondary metabolites by the fungus.

The genera *Alternaria* produce important phytotoxins that contribute to the increase in disease severity. The most common is tentoxin, produced by *A. tennuis*, which interferes with chloroplast development and restrains chlorophyll accumulation. The action mechanism includes the toxin connection to the link factor (CF1) in the chloroplasts, which causes the inhibition of CF_1 ATPase and electronic transport in the phosphorylation process (Pascholati, 1995). The same action can occur in the V complex (F_0 F_1 - ATPase) at mitochondrial bridge. According to Tal *et al.* (1985), the phytotoxins produced by *A. helianthi* are pironas derived from radicinin, with deoxyradicinin as the most important one relative the phytotoxic reaction. There are some speculations about this molecule acting in the place of tentoxin.

When pathogen reaches the host cell surface, a sequence of metabolic alterations begins. After the release of the enzymes cutinases and celulases for cell wall destruction, the penetration structure of the pathogen reaches the mesophyll tissues and the first visual necrosis appears. These necroses enlarge and coalesce leading to the death of leaves and other plant parts, reaching the capitulum and interfering with grain and oil yield.

Although respiration was not measured in this experiment, it can collapse due to diminished carbon reserves that are translocated from healthy to diseased tissues to support the metabolic activity of the pathogen. This can led the pentose-phosphate alternative way to produce phenolic compounds, which stay around the necrotic sites, in the chlorotic halos. This induces the hypersensitivity reaction that minimizes the pathogen penetration. If the infection occurs during the early stages

of development the pathogen will probably find a greater resistance against its penetration progress. This is due to the fact that those phenolic compounds were not found in mature leaves of sunflower.

The result of these complex physiological processes can influence yield results because a reduction in photosynthetic rate, associated with an increase in respiration rate, could indirectly influence crop yield.

CONCLUSIONS

In the genotypes used, leaves infected with *Alternaria helianthi* showed a reduced photosynthetic performance compared with healthy leaves.

Healthy leaves in diseased plants showed a photosynthetic reduction of around 20%.

Different genotypes exhibited different reductions in the photosynthetic rate.

The β values found in the experiments, that compare net photosynthesis of healthy and diseased leaves, were between 1.4 and 4.7, indicating that:

- there were differences in the b values between the genotypes;
- there were reductions in the photosynthetic rate in the remaining green tissue, confirming the existence of a virtual lesion for the pathosystem sunflower $\times$ *A. helianthi*.

ACKNOWLEDGEMENTS

The authors are grateful to the São Paulo State Research Foundation-FAPESP for financial support.

The senior author is highly thankful for the scholarship from University Student Research Improvement Council-CAPES

The second author is thankful for the research grant from National Council for Scientific and Technological Development-CNPq.

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LESIÓN VIRTUAL DE ALTERNARIA EN GIRASOL

RESUMEN

Las manchas de Alternaria en girasol, causadas por el hongo *Alternaria helianthi*, és una de las mayores enfermedades que afectan el girasol en Brasil. El efecto desto organismo en la tasa de fotosíntesis líquida de cuatro genotipos de girasol ha sido mesurado en condiciones experimentales. A través de una aproximación matemática, la área enferma de la hoja y la tasa de fotosíntesis líquida han sido relacionadas y empleadas para hacer el análisis del patosistema girasol/Alternaria. Una disminución media en la tasa de fotosíntesis líquida ha sido observada en las hojas infectadas para todos los genotipos analizados en comparación con las hojas sanas. Las manchas de Alternaria reducen la fotosíntesis no solo a través de la reducción de la área verde de las hojas, más también a través de uno efecto en la fotosíntesis del tejido verde restante de las hojas. En plantas enfermas, las hojas libres de lesiones del patógeno hán presentado también una reducción fotosintética.

DOMMAGE VIRTUEL D' ALTERNARIA SUR LE TOURNESOL

RÉSUMÉ

Les taches de l'Alternaria sur le tournesol, provoquées par le champignon *Alternaria helianthi*, est une des plus grandes maladies qui affectent le tournesol au Brésil. L'effet de cette organisme sur le taux de photosynthèse liquide de quatre génotypes de tournesol a été mesuré dans des conditions expérimentales. En utilisant une approche mathématique, la surface de feuille infectée et le taux de photosynthèse liquide ont été rapporté et utilisé pour analyser le pathosystème tournesol/Alternaria. Une décroissance moyenne dans le taux de photosynthèse liquide a été observé sur les feuilles malades pour tous les génotypes analysés en comparaison avec les feuilles saines. Les taches de l'Alternaria réduisent la photosynthèse non seulement à travers une diminution de la surface verte de la feuille mais aussi par un effet sur la photosynthèse des tissus verts restant de la feuille.