Leaf Gas Exchange and Chlorophyll *a* Fluorescence in Maize Leaves Infected with *Stenocarpella macrospora*

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ABSTRACT

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This study investigated the effect of macrospora leaf spot (MLS), caused by *Stenocarpella macrospora*, on photosynthetic gas exchange parameters and chlorophyll *a* fluorescence parameters determined in leaves of plants from two maize cultivars ('ECVSCS155' and 'HIB 32R48H') susceptible and highly susceptible, respectively, to *S. macrospora*. MLS severity was significantly lower in the leaves of plants from ECVSCS155 relative to the leaves of plants from HIB 32R48H. In both cultivars, net CO₂ assimilation rate, stomatal conductance, and transpiration rate significantly decreased, while the internal to ambient CO₂ concentration ratio increased in inoculated plants relative to noninoculated plants. The initial fluorescence and nonphotochemical quenching

significantly increased in inoculated plants of ECVSCS155 and HIB 32R48H, respectively, relative to noninoculated plants. The maximum fluorescence, maximum PSII quantum efficiency, coefficient for photochemical quenching, and electron transport rate significantly decreased in inoculated plants relative to noninoculated plants. For both cultivars, concentrations of total chlorophyll (Chl) (*a* + *b*) and carotenoids and the Chl *a/b* ratio significantly decreased in inoculated plants relative to noninoculated plants. In conclusion, the results from the present study demonstrate, for the first time, that photosynthesis in the leaves of maize plants is dramatically affected during the infection process of *S. macrospora*, and impacts are primarily associated with limitations of a diffusive and biochemical nature.

Additional keywords: *Zea mays*.

Maize (*Zea mays* L.) is one of the world's most important and widely grown cereal crops and serves as a staple human food, feed for livestock, and raw material for many industrial products (22,34). Macrospora leaf spot (MLS), also known as Diplodia leaf streak or spot, caused by the necrotrophic fungus *Stenocarpella macrospora* (Earle) Sutton (syn. *Diplodia macrospora* Earle) (19,33,61), is one of the major diseases affecting maize yield worldwide (2,18,23,40), mainly when maize is grown under warm and humid conditions in tropical and subtropical regions (25,61). On leaves, MLS symptoms appear as small, water-soaked lesions (18,23). As the elliptical lesions expand, they become brown in color, with yellow or reddish edges that may have darker concentric rings and contain black structures called pycnidia (2,18). The mycelia and pycnidia of *S. macrospora* can overwinter on maize debris and on seed (16,50). Under warm and moist conditions, conidia are extruded from pycnidia in long cirri and are dispersal by wind, rain, and insects, favoring severe MLS epidemics and great yield losses (2,15,17,18,33,43). The major strategies to control MLS are maize residue management, use of healthy seed, and crop rotation (18). To date, there are no fungicides registered for the control of MLS, and information about the resistance of commercial hybrids is scarce (3,6,42,45).

Pathogens can directly and indirectly affect several physiological processes in their hosts (44). The alteration in the rate of physiological processes in asymptomatic leaf tissue may be pro-

portional to, proportionally greater than, or proportionally smaller than the corresponding infected leaf tissue caused by a certain disease (44,57). The physiological state of plants infected by pathogens can be investigated in a noninvasive way by the simultaneous measurement of leaf gas exchange and chlorophyll *a* fluorescence parameters (12). A decrease in the photosynthetic efficiency and stomatal and mesophyll conductance limitations as well as biochemical alterations are the primary effects caused by pathogen infection (7,12). Fungal infection may reduce photosynthesis rates through a number of potential mechanisms: impairment of the functional leaf area and reduction in the photosynthetic efficiency of the remaining green leaf area, as reported for interactions between barley and *Rhynchosporium secalis* (36) and bean and *Colletotrichum lindemuthianum* (35); alterations in chloroplasts and a reduction in chlorophyll concentration, as reported in barley leaves infected with *Puccinia hordei* (44); stomatal closure, as reported in potato leaves infected with *Verticillium dahlia* (14); and impairment of the photosynthetic apparatus or disruption in photosynthetic metabolic pathways, as reported in poplar leaves infected with *Marsonia brunnea* f. sp. *brunnea* (24). Measurement of chlorophyll *a* fluorescence is an important tool for assessing the photosynthetic performance of the leaves of plants submitted to many types of abiotic and biotic stresses (5,11,53,56). Analysis of chlorophyll *a* fluorescence is a quantitative measure of both photochemical and nonphotochemical energy dissipation processes occurring in leaves (30,52). Changes in the intensity of chlorophyll *a* fluorescence in the chloroplasts reflect its functional state (31) and provides important information related to the composition of the pigment systems, excitation energy transfer, physical changes in pigment-protein complexes, primary photochemistry and kinetics, and rates of electron

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transfer reactions in photosystem II (29). Several studies reported that the infection of plants by pathogens often leads to complex alterations in chlorophyll *a* fluorescence that can be related to changes in the efficiency of photosynthetic processes (53,56). Measurements of chlorophyll *a* fluorescence have shown that the maximum fluorescence (F_m), maximum photochemical efficiency of PSII (F_v/F_m) of dark-adapted leaves, and electron transport rate (ETR) are often decreased during the infection process of pathogens, as described for *Phaeoisariopsis griseola* and *Uromyces appendiculatus* in bean (9) and *Bremia lactucae* in lettuce (49). The fraction of absorbed light energy that was thermally dissipated (nonphotochemical quenching [NPQ]) increased in tomato leaves infected with *Oidium neolycopersici* (48).

Due to the importance of MLS in decreasing maize yield, and whereas the physiological responses of plants to fungal infection is almost unknown, this study was designed to examine how the infection process of *S. macrospora* could affect the photosynthetic performance of plants using a combination of gas exchange and chlorophyll *a* fluorescence measurements along with an analysis of chlorophyll pools.

MATERIALS AND METHODS

Plant cultivation. Maize seeds from 'ECVSCS155' and 'HIB 32R48H', susceptible and highly susceptible, respectively, to *S. macrospora*, were sown in plastic pots containing 2 kg of Tropstrato (Vida Verde, Mogi Mirim, São Paulo, Brazil) substrate composed of a 1:1:1 mixture of pine bark, peat, and expanded vermiculite. In order to provide phosphorus to the plants, a total of 1.63 g of calcium phosphate monobasic was added to each plastic pot. In total, five seeds were sown per pot, and each pot was thinned to three seedlings 5 days after seedling emergence. Plants were kept in a greenhouse during the experiments (temperature $28 \pm 2^\circ\text{C}$ during the day and $12 \pm 4^\circ\text{C}$ at night, relative humidity $70 \pm 5\%$) and were fertilized weekly with 50 ml of a nutrient solution composed of 2.6 mM KCl, 0.6 mM K_2SO_4 , 1.2 mM MgSO_4 , 1.0 mM $\text{CH}_4\text{N}_2\text{O}$, 1.2 mM NH_4NO_3 , 0.0002 mM $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 0.03 mM H_3BO_3 , 0.04 mM ZnSO_4 , 0.01 mM CuSO_4 , and 0.03 mM MnCl_2 . The nutrient solution was prepared using deionized water. Plants were watered as needed.

Inoculum production and inoculation procedure. Plants were inoculated with a monospore isolate of *S. macrospora* (UFV-DFP *Sm* 01) obtained from maize leaves (hybrid HIB 32R48H) with MLS symptoms. The isolate of *S. macrospora* was grown in petri dishes containing oat-agar medium and incubated for 35 days in an incubator (22°C , photoperiod of 12 h of light and 12 h of darkness). All of the leaves on each plant of the inoculated treatment were sprayed with a conidial suspension of *S. macrospora* (6×10^4 conidia/ml) at 30 days after emergence (growth stage V5) (10) using a VL Airbrush atomizer (Poasche Airbrush Co, Chicago). Gelatin (1% wt vol $^{-1}$) was added to the suspension to aid conidial adhesion to the leaf blades. The plants of noninoculated controls were sprayed with a solution containing 1% gelatin without *S. macrospora* conidia. Immediately after application of treatments, all of the plants (both noninoculated and inoculated) were transferred to a growth chamber at $25 \pm 2^\circ\text{C}$, $90 \pm 5\%$ relative humidity, with a photoperiod of 12 h of light and 12 h of darkness for 30 h. After this period, the plants were transferred to a plastic mist growth chamber (MGC) inside a greenhouse for the duration of the experiments. The MGC was made of wood (2 m wide, 1.5 m high, and 5 m long) and covered with 100- μm thick transparent plastic. The temperature inside the MGC ranged from $25 \pm 2^\circ\text{C}$ (day) to $20 \pm 2^\circ\text{C}$ (night). The relative humidity was maintained at $90 \pm 5\%$ using a misting system in which nozzles (model NEB-100; KGF Company, São Paulo, Brazil) sprayed mist every 30 min above the plant canopies. The relative humidity and temperature were measured with a thermo-hygrograph (TH-508, Impac, São Paulo, Brazil).

The maximum natural photon flux density at plant canopy height was $\approx 900 \mu\text{mol m}^{-2} \text{s}^{-1}$.

Assessment of MLS severity. The fourth leaf (counted from the base to the top) of each plant per replication of each treatment (18 leaves per treatment, 360 leaves per each evaluation time, 180 leaves total) were marked and collected to evaluate MLS severity at 48, 72, 96, 120, and 168 h after inoculation (hai). The collected leaves were scanned at 300 dpi resolution and the obtained images were processed using QUANT software (58) to obtain severity (chlorosis and necrosis symptoms) values. The area under disease progress curve (AUDPC) for each leaf in each plant was computed using trapezoidal integration of the MLS progress curves over time using the formula proposed by Shaner and Finney (55).

Photosynthetic measurements. The leaf gas exchange parameters were simultaneously determined by conducting the measurements of chlorophyll *a* fluorescence using a portable open-flow gas exchange system (LI-6400XT; LI-COR Inc., Lincoln, NE) equipped with an integrated fluorescence chamber head (LI-6400-40; LI-COR Inc.). The net carbon assimilation rate (A), stomatal conductance to water vapor (g_s), transpiration rate (E), and internal (C_i) to ambient (C_a) CO_2 concentration ratio (C_i/C_a) were measured for the fourth leaf of each plant per replication of each treatment at 48, 72, 96, 120, and 168 hai from 10:00 to 13:00 h (solar time), which is when A was at its maximum under artificial PAR (i.e., $1,200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ at leaf level and $400 \mu\text{mol atmospheric CO}_2 \text{mol}^{-1}$). All of the measurements were performed at 25°C and the vapor pressure deficit was maintained at ≈ 1.0 kPa, while the amount of blue light was set to 10% of the photosynthetic photon flux density to optimize the stomatal aperture. Previously dark-adapted leaves (30 min) were illuminated with weak modulated measuring beams ($0.03 \mu\text{mol m}^{-2} \text{s}^{-1}$) to obtain the initial fluorescence (F_0). Saturating white light pulses of $8,000 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ were applied for 0.8 s to ensure maximum fluorescence emissions (F_m), from which the variable to maximum chlorophyll fluorescence ratio, $F_v/F_m = [(F_m - F_0)/F_m]$, was calculated. In light-adapted leaves, the steady state fluorescence yield (F_s) was measured following a saturating white light pulse ($8,000 \mu\text{mol m}^{-2} \text{s}^{-1}$, 0.8 s) that was applied to achieve the light-adapted maximum fluorescence (F_m'). The actinic light was then turned off and far-red illumination was applied ($2 \mu\text{mol m}^{-2} \text{s}^{-1}$) to measure the light-adapted initial fluorescence (F_0'). Using these parameters, the capture efficiency of the excitation energy by the open PSII reaction centers (F_v'/F_m') was estimated as $F_v'/F_m' = (F_m' - F_0')/F_m'$. The coefficient for photochemical quenching (q_p) was calculated as $q_p = (F_m' - F_s)/(F_m' - F_0')$, while that for NPQ was calculated as $\text{NPQ} = (F_m'/F_m') - 1$. The actual quantum yield of PSII electron transport (Φ_{PSII}) was computed as $\Phi_{\text{PSII}} = (F_m' - F_s)/F_m'$, from which the ETR was calculated as $\text{ETR} = \Phi_{\text{PSII}} \cdot \text{PPFD} \cdot f \cdot \alpha$, where f is a factor that accounts for the partitioning of energy between PSII and PSI and is assumed to be 0.5, which indicates that the excitation energy is distributed equally between the two photosystems, and α is the absorbance by the leaf photosynthetic tissues and is assumed to be 0.84 (37).

Determination of the concentration of photosynthetic pigments. The concentrations of chlorophylls (Chl) *a* and *b* and carotenoids were determined using dimethyl sulfoxide (DMSO) as an extractor (54). Five leaf disks (10-mm in diameter) were punched from the fourth leaves at 48, 72, 96, 120, and 168 hai. The collected disks were immersed in glass tubes containing 5 ml of saturated DMSO solution and calcium carbonate (CaCO_3) (5 g liter^{-1}) (60) and kept in the dark at room temperature for 24 h. The absorbances of the extracts were read at 480, 649.1, and 665.1 nm using a saturated solution of DMSO and CaCO_3 as a blank.

Experimental design and data analysis. A two-by-two factorial experiment, consisting of two maize cultivars (ECVSCS155 and HIB 32R48H) and noninoculated or inoculated plants was arranged in a completely randomized design with six replications. The experiment was repeated three times. Each experimental unit

corresponded to a plastic pot containing three plants. In total, 120 plastic pots were used in each experiment (30 plastic pots per treatment at all evaluation times), which equals 18 plants per treatment at each evaluation time. All variables were subjected to an analysis of variance (ANOVA) and the treatment means were compared by Tukey's test ($P \leq 0.05$) using SAS software (SAS Institute Inc., Cary, NC). For MLS severity, the ANOVA was considered to be a two-by-five factorial experiment, consisting of two maize cultivars and five evaluation times. For the photosynthetic measurements, the concentration of total Chl ($a + b$), concentration of carotenoids, and the Chl a/b ratio, ANOVA was considered to be a two-by-two-by-five factorial experiment, consisting of two maize cultivars, noninoculated or inoculated plants, and five evaluation times. For each cultivar, the Pearson correlation was used to determine relationships among the photosynthetic measurements and MLS severity, as well as among the concentration of total Chl ($a + b$), concentration of carotenoids, and the Chl a/b ratio and MLS severity.

RESULTS

MLS severity and AUDPC. The factors cultivar and evaluation time as well as their interaction had significant effects ($P \leq 0.05$) on MLS severity. Cultivar was the only significant factor ($P \leq 0.05$) for AUDPC (Table 1). MLS severity was significantly reduced on the leaves of plants from ECVSCS155 relative to the leaves of plants from HIB 32R48H (Fig. 1A). From 48 to 168 hai, MLS severity increased from 0.5 to 5.1% on the leaves of plants from ECVSCS155 and from 1.4 to 8.0% on the leaves of plants from HIB 32R48H. For plants of ECVSCS155, AUDPC was significantly reduced by 34.5% compared with plants from HIB 32R48H (Fig. 1B).

Photosynthetic parameters. At least one of the evaluated factors (cultivar, plant inoculation, and evaluation time) as well as some of their interactions were significant ($P \leq 0.05$) for A , g_s , E , and C_i/C_a . Plant inoculation was the most important factor due to its higher F values, and it explained the variation in all variables

TABLE 1. Analysis of variance on the effects of cultivar (C), plant inoculation (PI), and evaluation time (ET) and their interactions for the variables macrospora leaf spot severity (Sev), area under the disease progress curve (AUDPC), photosynthetic gas exchange parameters (Photosynthetic: net carbon assimilate rate [A], stomatal conductance to water vapor [g_s], transpiration rate [E], internal to ambient CO_2 concentration ratio [C_i/C_a]), chlorophyll a fluorescence parameters (Chlorophyll: initial fluorescence [F_0], maximum fluorescence [F_m], maximum PSII quantum efficiency [F_v/F_m], capture efficiency of excitation energy by the open PSII reaction centers [F_v'/F_m'], coefficient for photochemical quenching [q_p], nonphotochemical quenching [NPQ], and electron transport rate [ETR]), concentrations of total chlorophyll $a + b$ (Chl) and carotenoids (Car), and Chl a/b ratio^a

Variables	C	PI	ET	C $\times$ PI	C $\times$ ET	PI $\times$ ET	C $\times$ PI $\times$ ET
Disease data							
Sev	<0.001	–	<0.001	–	<0.001	–	–
AUDPC	<0.001	–	–	–	–	–	–
Photosynthetic							
A	0.011	<0.001	<0.001	0.472	0.001	<0.001	0.002
g_s	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.001
E	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
C_i/C_a	<0.001	<0.001	<0.001	0.066	0.003	<0.001	0.076
Chlorophyll							
F_0	0.243	<0.001	<0.001	0.020	0.270	<0.001	0.035
F_m	0.413	<0.001	0.002	0.398	0.458	<0.001	0.771
F_v/F_m	0.001	<0.001	<0.001	<0.001	0.008	<0.001	<0.001
F_v'/F_m'	<0.001	<0.001	0.070	0.981	0.135	0.461	0.273
q_p	0.169	<0.001	<0.001	0.874	0.809	0.001	0.174
NPQ	0.002	<0.001	0.779	0.358	0.041	<0.001	0.304
ETR	0.130	<0.001	<0.001	0.445	0.698	0.282	0.181
Chl	0.293	<0.001	<0.001	0.272	0.671	<0.001	0.823
Car	0.028	<0.001	<0.001	0.069	0.042	<0.001	<0.001
Chl a/b	0.006	<0.001	<0.001	0.523	<0.001	<0.001	0.014

^a F -based P values.

Fig. 1. A, Macrospora leaf spot progress curves and B, area under disease progress curve (AUDPC) on leaves of plants from ECVSCS155 and HIB 32R48H maize. Means from ECVSCS155 and HIB 32R48H followed by an asterisk (*) for each evaluation time are significantly different ($P \leq 0.05$) by Tukey's test. Bars represent standard errors of the means; $n = 6$. Three experiments were conducted with consistent results; results from one representative experiment are shown.

evaluated. The interaction cultivar–plant inoculation–evaluation time was significant only for A , g_s , and E (Table 1).

For both cultivars, A , g_s , and E significantly decreased from 48 to 168 hai for inoculated plants relative to their noninoculated counterparts (Fig. 2A to F). Relative to plants of ECVSCS155, reductions in the values of the above parameters were greater at

168 hai for plants of HIB 32R48H, with decreases of 52 and 56% for A , 41 and 49% for g_s and 41 and 46% for E in the inoculated plants relative to the noninoculated ones, respectively. For C_i/C_a , the highest values occurred in the inoculated plants, with significant differences between noninoculated and inoculated plants of ECVSCS155 occurring at 96 and 168 hai (Fig. 2G). For plants of

Fig. 2. A and B, Leaf gas exchange parameters, including net carbon assimilation rate (A); C and D, stomatal conductance to water vapor (g_s); E and F, transpiration rate (E); and G and H, internal to ambient CO_2 concentration ratio (C_i/C_a) determined in leaves of maize plants from ECVSCS155 (A, C, E, and G) and HIB 32R48H (B, D, F, and H) noninoculated (NI) or inoculated (I) with *Stenocarpella macrospora*. Means for the NI and I treatments for each cultivar followed by an asterisk (*) at each evaluation time are significantly different ($P \leq 0.05$) by Tukey's test. Symbols ▲ and ▼ indicate differences between ECVSCS155 and HIB 32R48H, respectively, for NI and I treatments at each evaluation time according to Tukey's test ($P \leq 0.05$). Bars represent standard errors of the means; $n = 6$. Three experiments were conducted with consistent results; results from one representative experiment are shown.

HIB 32R48H, significant differences occurred only at 168 hai (Fig. 2H). The C_i/C_a increased by 33 and 30% at 168 hai for the inoculated plants of ECVSCS155 and HIB 32R48H, respectively, compared with the noninoculated plants.

There were significant differences between noninoculated plants of ECVSCS155 and HIB 32R48H in A , g_s , E , and C_i/C_a . A was 7 and 6% higher for plants of HIB 32R48H than for plants of ECVSCS155 at 48 and 120 hai, respectively (Fig. 2A and B), and g_s was significantly higher for plants of HIB 32R48H than for plants of ECVSCS155 at 48 (18%), 72 (11%), 96 (20%), and 120 (16%) hai (Fig. 2C and D). E was significantly higher in plants of HIB 32R48H than for plants of ECVSCS155 by 9, 30, and 10% at 48, 96, and 120 hai, respectively (Fig. 2E and F). C_i/C_a was higher at 48 (27%) and 72 (33%) hai for plants of HIB 32R48H than for plants of ECVSCS155 (Fig. 2G and H); and g_s and C_i/C_a were significantly higher by 21 and 27%, respectively, at 48 hai (Fig. 2C, D, G, and H) for the inoculated plants of HIB 32R48H compared with the inoculated plants of ECVSCS155.

Chl a parameters. At least one of the evaluated factors (cultivar, plant inoculation, and evaluation time) as well as some of their interactions were significant ($P \leq 0.05$) for F_0 , F_m , F_v/F_m , F_v'/F_m' , q_p , NPQ, and ETR (Table 1). The interaction cultivar–

plant inoculation–evaluation time was significant only for F_0 and F_v/F_m (Table 1). At 48 hai, F_0 significantly increased by 18 and 27% for inoculated plants of ECVSCS155 and HIB 32R48H, respectively, compared with their noninoculated counterparts (Fig. 3A and B). F_m , F_v/F_m , q_p , and ETR significantly decreased in the inoculated plants compared with their noninoculated counterparts (Figs. 3C to F and 4C, D, G, and H). However, the reductions in those parameters were larger at 168 hai for plants of HIB 32R48H than for plants of ECVSCS155, with decreases of 21 and 19% for F_m (Fig. 3C and D), 22 and 19% for q_p (Fig. 4C and D), and 31 and 22% for ETR (Fig. 4G and H). The reductions in F_v/F_m were greater at 96, 120, and 168 hai for plants of HIB 32R48H than for plants of ECVSCS155, with decreases of 4 and 2, 5 and 3, and 11 and 6%, respectively (Fig. 3E and F). NPQ increased for inoculated plants of both cultivars compared with their noninoculated counterparts (Fig. 4E and F). In plants of HIB 32R48H only, NPQ significantly increased at 120 and 168 hai by 11 and 17%, respectively. In the noninoculated plants, significant differences in F_v/F_m occurred between cultivars at 48 hai (Fig. 3E and F). For the inoculated plants, significant differences between cultivars occurred at 168 hai in F_v/F_m (Fig. 3E and F) and at 120 hai in F_v'/F_m' (Fig. 4A and B).

Fig. 3. A and B, Initial fluorescence (F_0); C and D, maximum fluorescence (F_m); and E and F, maximum PSII quantum efficiency (F_v/F_m) determined in leaves of maize plants from ECVSCS155 (A, C, and E) and HIB 32R48H (B, D, and F) noninoculated (NI) or inoculated (I) with *Stenocarpella macrospora*. Means for the NI and I treatments for each cultivar followed by an asterisk (*) at each evaluation time are significantly different ($P \leq 0.05$) by Tukey's test. Symbols ▲ and ▼ indicate differences between ECVSCS155 and HIB 32R48H, respectively, for NI and I treatments at each evaluation time according to Tukey's test ($P \leq 0.05$). Bars represent standard errors of the means; $n = 6$. Three experiments were conducted with consistent results; results from one representative experiment are shown.

Concentrations of leaf pigments. At least one of the factors examined (cultivar, plant inoculation, and evaluation time) as well as some of their interactions were significant ($P \leq 0.05$) for the concentrations of total Chl ($a + b$) and carotenoids and for the Chl a/b ratio (Table 1). For both cultivars, concentrations of total Chl ($a + b$) and carotenoids significantly decreased for the inoculated

plants relative to the noninoculated ones. The reduction in total Chl ($a + b$) concentration was higher for plants of ECVSCS155 than for plants of HIB 32R48H, with decreases of 58 and 49%, respectively. The reduction in the concentration of carotenoids was higher for plants of HIB 32R48H than for plants of ECVSCS155, with decreases of 40 and 17% at 168 hai, re-

Fig. 4. A and B, Capture efficiency of excitation energy by the open PSII reaction centers (F_v'/F_m'); C and D, coefficient for photochemical quenching (q_p); E and F, nonphotochemical quenching (NPQ); and G and H, electron transport rate (ETR) determined in leaves of maize plants from ECVSCS155 (A, C, E, and G) and HIB 32R48H (B, D, F, and H) noninoculated (NI) or inoculated (I) with *Stenocarpella macrospora*. Means for the NI and I treatments for each cultivar followed by an asterisk (*) at each evaluation time are significantly different ($P \leq 0.05$) by Tukey's test. Symbols ▲ and ▼ indicate differences between ECVSCS155 and HIB 32R48H, respectively, for NI and I treatments at each evaluation time according to Tukey's test ($P \leq 0.05$). Bars represent standard errors of the means; $n = 6$. Three experiments were conducted with consistent results; results from one representative experiment are shown.

spectively. The Chl *a/b* ratio significantly increased in inoculated plants compared with noninoculated plants of both cultivars.

Pearson correlations. For both cultivars, *A* was positively correlated with *g_s* and *E*, and *g_s* was positively correlated with *E*, but *A*, *g_s*, and *E* were negatively correlated with MLS severity (Table 2). In plants of HIB 32R48H, *C_i/C_a* was positively correlated with *g_s* whereas, in plants of ECVSCS155, *C_i/C_a* was positively correlated with MLS severity (Table 2). For both cultivars, there were significant positive correlations between *F_v/F_m* and NPQ, total chlorophyll, and carotenoids; between *q_P* and ETR; and between concentrations of total Chl (*a + b*) and carotenoids. For both cultivars, significant negative correlations occurred between *F_v/F_m* and Chl *a/b* ratio, *F_v'/F_m'*, and NPQ; between total Chl (*a + b*) and Chl *a/b* ratio; and between Chl *a/b* ratio and concentration of carotenoids. In both cultivars, *F_m*, *F_v/F_m*, and total Chl (*a + b*) concentration were negatively correlated with MLS severity (Table 3).

DISCUSSION

This study provides, to the best of the authors' knowledge, the first evidence of a physiological response associated with the infection by *S. macrospora* on maize leaves. A decrease in photosynthesis caused by pathogen infection has been reported to occur in several plant species (1,8,9,51,57). In the present study, there was a progressive decline in *A*, *g_s*, and *E* in leaves of inoculated plants compared with leaves of noninoculated plants of both cultivars tested, which was supported by the negative correlation between MLS severity and these parameters. Moreover, the reduction in *A* at the early stages of fungal infection was proportionally greater than the MLS severity; severity values of 5 to 8% reduced *A* by 52 and 56% at 168 hai for plants of ECVSCS155 and HIB 32R48H, respectively. This finding is in agreement with what was reported for interactions between maize and *Phaeosphaeria maydis* (28), bean and *Colletotrichum lindemuthianum* (39), and *Eucalyptus globules* and *Micosphaerella* spp. (46). The reduction in *A* was proportionally greater than was expected from the apparent reduction in green leaf tissue as a

result of the pathogen infection and, therefore, indicated that asymptomatic tissue was also affected by fungal infection. Concomitantly, as reported for other necrotrophic pathogens, the decrease in *A* in maize leaf tissue asymptomatic for MLS may be explained by the secretion of lytic enzymes or non-host selective toxins by *S. macrospora* that easily diffuse into uncolonized leaf tissue, causing maceration and compromising translocation of water and photoassimilates throughout the leaf tissue (32,38,57,59).

The concomitant decrease in both *g_s* and *E* during the infection process of *S. macrospora* in the leaf blades of plants of both cultivars may be associated with stomatal closure and is in accordance with reports on interactions between wheat and *Puccinia recondita* f. sp. *tritici* (38) and tomato and *O. neolyopersici* (48). Additionally, in the present study, desiccation of the leaf blades was observed as disease progressed, suggesting that the stomata of the infected leaves were closed to prevent excessive water loss and, therefore, contributed to the observed decrease in *E* values. Resende et al. (51) reported that *E* dramatically decreased in sorghum leaves infected by *C. sublineolum* as a method of keeping the stomata closed and maintaining a favorable water status within the leaf blades.

Reductions in *g_s* are usually associated with decreases in *C_i* and in *C_i/C_a* (21,26,27). By contrast, data from the present study showed that, despite a concomitant decrease in *A* and *g_s*, there was a progressive increase in *C_i/C_a*, which may suggest that the decrease in *A* cannot be explained solely by reduction in *g_s* but may also be due to a decrease in mesophyll conductance and impairment of the biochemical capacity of the leaves to adequately assimilate CO₂. Reductions in *A* and *g_s* related to an increase in *C_i/C_a* have been reported to occur during interactions between *E. urophylla* and *P. psidii* (1), bean and *Uromyces appendiculatus* (34), and barley and *R. secalis* (36). According to these authors, the reduction in *A* was unlikely to have been solely associated with less CO₂ entry into leaves but, rather, with some biochemical limitation to CO₂ fixation at the chloroplast level. Frequently, alterations in leaf photochemistry and carbon metabolism are related to reduced activity of ribulose biphosphate carboxylase and changes in the capacity for ribulose biphosphate regeneration (39,41).

TABLE 2. Pearson correlation coefficients among the net carbon assimilation rate (*A*), stomatal conductance to water vapor (*g_s*), transpiration rate (*E*), internal to ambient CO₂ concentration ratio (*C_i/C_a*), and macrospora leaf spot severity (Sev) on the leaves of plants from two maize cultivars^a

Variables	<i>A</i>	<i>g_s</i>	<i>E</i>	<i>C_i/C_a</i>	Sev
<i>A</i>	...	0.781**	0.729**	-0.391	-0.867**
<i>g_s</i>	0.753**	...	0.743**	0.255	-0.608**
<i>E</i>	0.762**	0.643**	...	-0.033	-0.551**
<i>C_i/C_a</i>	-0.239	0.425**	-0.106	...	0.462*
Sev	-0.870**	-0.617**	-0.571**	0.240	...

^a Values above and below the diagonal are the measurements made on plants from ECVSCS155 and HIB 32R48H maize, respectively. Levels of probability: * = 0.05 and ** = 0.01.

TABLE 3. Pearson correlation coefficients among the initial fluorescence (*F₀*), maximum fluorescence (*F_m*), maximum photochemical efficiency of PSII (*F_v/F_m*), capture efficiency of excitation energy by the open PSII reaction centers (*F_v'/F_m'*), coefficient for photochemical quenching (*q_P*), nonphotochemical quenching (NPQ), electron transport rate (ETR), concentrations of total chlorophyll *a + b* (Chl) and carotenoids (Car), chlorophyll *a/b* ratio, and macrospora leaf spot severity (Sev) on the leaves of plants from two maize cultivars^a

Variables	<i>F₀</i>	<i>F_m</i>	<i>F_v/F_m</i>	<i>F_v'/F_m'</i>	<i>q_P</i>	NPQ	ETR	Chl	Chl <i>a/b</i>	Car	Sev
<i>F₀</i>	...	0.757**	0.137	0.055	-0.267	0.455*	-0.165	-0.036	0.024	-0.022	0.110
<i>F_m</i>	0.205	...	0.747**	0.041	0.085	0.164	0.066	0.493*	-0.276	0.398	-0.460*
<i>F_v/F_m</i>	-0.615**	0.641**	...	-0.001	0.383	0.407*	0.247	0.778**	-0.430*	0.480*	-0.814**
<i>F_v'/F_m'</i>	-0.049	-0.141	0.043	...	0.021	-0.591**	0.689**	-0.248	0.251	-0.396	-0.140
<i>q_P</i>	-0.355	0.027	0.301	-0.456*	...	0.039	0.721**	0.420*	-0.165	0.349	-0.501*
NPQ	-0.273	0.353	0.665**	-0.447*	0.330	...	-0.484*	0.306	-0.289	0.360	0.006
ETR	-0.308	-0.083	0.209	0.273	0.692**	-0.069	...	0.119	0.059	-0.024	-0.451*
Chl	0.546**	0.248	0.625**	-0.188	0.294	0.129	0.212	...	-0.796**	0.855**	-0.718**
Chl <i>a/b</i>	0.565**	-0.402*	-0.763**	0.115	-0.148	-0.207	-0.071	-0.827**	...	-0.876**	0.361
Car	-0.477**	0.386	0.668**	-0.331	0.304	0.271	0.108	0.918**	-0.941**	...	0.396
Sev	0.401*	-0.615**	-0.814**	0.142	-0.371	-0.089	0.288	-0.591**	0.749**	-0.683**	...

^a Values above and below the diagonal are the measurements made on plants from ECVSCS155 and HIB 32R48H maize, respectively. Levels of probability: * = 0.05 and ** = 0.01.

Regardless of the maize cultivar, both F_0 and NPQ tended to be higher in the leaves of inoculated plants than in the leaves of noninoculated plants, suggesting that the PSII reaction centers were somehow damaged or that the transfer of excitation energy from the antenna to the reaction center was impaired (4,13,37). According to Plazek et al. (47), an increase in the F_0 values on barley leaves infected with *Bipolaris sorokimiana* was an indicator of antenna damage.

Despite the observed increases in NPQ values, the nonphotochemical dissipation was insufficient to avoid photoinhibitory damage, which was indicated by decreases in F_m and F_v/F_m values in the leaves of inoculated plants from both cultivars and was also supported by the negative correlations of F_m and F_v/F_m with MLS severity. ETR and q_p decreased in the leaves of inoculated plants of both cultivars, as indicated by the negative correlations of ETR and q_p with MLS severity. The observed decreases in q_p could be related to increases in the fraction of reduced Q_A (Q_A^-) in PSII, which indicates an increased possibility for photoinhibition to be affected (4) and, therefore, the electron transport chain to PSI via the cytochrome b_6/f complex and plastocyanin (13). Additionally, the decreases in F_v/F_m were accompanied by decreases in F_v'/F_m' , suggesting that the ability of the *S. macrospora*-infected leaves to capture and to transfer collected energy was dramatically compromised.

The concentration of pigments was negatively affected in the leaves of inoculated plants from both cultivars as the MLS progressed. The concentration of total Chl ($a + b$) decreased with the progress of MLS, which may have led to an increase in the Chl a/b ratio. The reduction in chlorophyll concentration could be associated with the action of lytic enzymes and non-host selective toxins released in the infected tissues by *S. macrospora*, in accordance with reports on maize leaves infected with *Exserohilum turcicum* (20). Additionally, the decrease in total Chl ($a + b$) concentration would lead to a reduction in the ability and the efficiency of the leaves to capture the energy required for the photochemical reactions in photosynthesis (20).

In conclusion, the results from the present study demonstrate, for the first time, that photosynthesis in the leaves of maize plants is dramatically affected during the infection process of *S. macrospora*, and impacts are primarily associated with limitations of a diffusive and biochemical nature.

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