Impact of Pecan Leaf Blotch on Gas Exchange of Pecan Leaves

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ABSTRACT

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The influence of pecan leaf blotch infection (caused by *Mycosphaerella dendroides*) on net CO_2 assimilation rate, conductance, transpiration rate, intercellular CO_2 concentration, water use efficiency, and chlorophyll concentration of Cape Fear and Choctaw pecan (*Carya illinoensis*) leaves was assessed. Physiological variables were related to disease severity in a linear or curvilinear manner. A leaf blotch disease severity rating of 40% resulted in declines in net CO_2 assimilation rate of 63 and 47% for spring- and summer-flush Cape Fear leaves, respectively, and 72 and 79% for spring- and summer-flush Choctaw leaves, respectively. Smaller percentage reductions occurred in leaf conductance, transpiration rate, and leaf chlorophyll concentration. Leaf intercellular CO_2 concentration increased substantially with leaf blotch infection. Plots of net CO_2 assimilation rate versus leaf conductance to CO_2 and intercellular CO_2 concentration of leaf blotch-infected and control leaves revealed that net CO_2 assimilation approached zero before complete stomatal closure and that the degree of stomatal closure was not sufficient to prevent an increase in intercellular CO_2 concentration. These data indicate that leaf blotch may reduce leaf efficiency by adversely affecting the photosynthetic apparatus.

Leaf fungal diseases contribute greatly to reductions in photosynthetic efficiency and often induce premature defoliation of deciduous fruit crops in the fall. Downy spot (caused by *Mycosphaerella caryigena* Demaree & J. R. Cole) (11), powdery mildew (caused by *Microsphaera penicillata* (Wallr.:Fr.) Lév.) (8), and pecan scab (caused by *Cladosporium caryigenum* (Ellis & Langl.) Gottwald [= *Fusicladium effusum* G. Wint.]) (7) infections have been shown to reduce gas exchange of pecan (*Carya illinoensis* (F. A. Wagenheim) K. Koch) leaves. Net CO_2 assimilation of greenhouse-grown and greenhouse-inoculated pecan seedlings declined in a 1:1 ratio to the proportion of leaf surface infected with powdery mildew (8) or scab (7). By contrast, natural infection by downy spot in the field (11) resulted in reductions in net CO_2 assimilation rate of two or more times the percentage of infected leaf surface.

The maintenance of high photosynthetic rates in the fall can be important to the current and the subsequent season's nut production (12,16,24,25). Pecan leaves retain high levels of chlorophyll and net CO_2 assimilation until leaf abscission (1), which occurs after the first hard freeze if the trees have received proper insect and disease control management. Pecan nutlets are strong assimilate sinks during kernel development (August–October) (3,22). A single nutlet may require assimilates produced by eight to 20 leaves (2), although the leaf surface area required to manufacture sufficient photosynthates depends on the cultivar and on photosynthesis (13,15). Late-season photosynthetic capacity of pecan leaves during kernel development also influences the tendency of the trees to bear light and heavy crops in alternate years (16,24,25).

This study was initiated to determine the impact of pecan leaf blotch (caused by *Mycosphaerella dendroides* (Cooke) Demaree & J. R. Cole) on gas exchange and chlorophyll content of leaves of different ages. Specific variables measured on fully expanded spring- and summer-flush leaves included net CO_2 assimilation rate, conductance to water vapor, transpiration rate, intercellular CO_2 concentration, total leaf chlorophyll concentration, and efficiency of water use. Our objectives were first to quantify the impact of leaf blotch on these physiological variables and second to determine whether changes in leaf gas exchange were mediated by effects on stomata or on the photosynthetic apparatus.

MATERIALS AND METHODS

Plant material. The planting was a 10-ha mixed block of Cape Fear and Choctaw pecan at the University of Florida Agricultural Research and Education Center in Monticello. Trees were in the second-leaf stage and received standard cultural and management practices, although fungicides were not applied.

Leaf gas exchange measurements. CO_2 and H_2O vapor exchanges were measured in the field under ambient conditions of light, temperature, and relative humidity. Air temperature, leaf temperature, relative humidity, and photosynthetic photon flux varied between 19 and 27 C, 20 and 28 C, 16 and 49%, and 1,400 and 1,800 μmol m^{-2} sec^{-1}, respectively. Ambient and leaf chamber CO_2 and H_2O vapor concentration, ambient air and leaf chamber temperature, and photosynthetic photon flux were measured with an ADC Model LCA-2 infrared CO_2 analyzer, an air supply unit (flow rate maintained at 400 cm^3 min^{-1}), and a Parkinson broadleaf leaf chamber (Analytical Development Corp., Hoddesdon, United Kingdom). Net CO_2 assimilation rate, leaf conductance to water vapor, transpiration rate, and intercellular CO_2 concentration were calculated with a DL2 Data-logger, GW-Basic software, and an IBM-compatible personal computer. Boundary layer conductance was estimated according to Parkinson (14), and the infrared gas analyzer response to water vapor (E_{max}) was calculated according to von Caemmerer and Farquhar (18). Leaf conductance to CO_2 was calculated as leaf conductance to water vapor $\times$ 0.625, based on the ratio of water vapor and CO_2 diffusion coefficients in air. Water use efficiency was calculated by mole fraction (i.e., net CO_2 assimilation rate divided by transpiration rate and multiplied by 1,000). Leaf chlorophyll (a + b) was extracted by placing 6.25 cm^2 of leaf tissue in *N,N*-dimethylformamide in darkness for 24 hr (9). Leaf chlorophyll (chl) was calculated from absorbance at 646 and 664 nm by the following equation: $\mu g\ chl/ml = 17.90\ A_{646} + 8.08\ A_{664}$.

Leaves were categorized on the basis of age as expanded summer-flush leaves or expanded spring-flush leaves. Leaves were selected for measurement on the basis of age and the presence or absence of leaf blotch. Gas exchange was measured on a 6.25-cm^2 area on two to 10 sun-exposed leaflets per tree. Approximately 10–20 trees were measured per day between 10 a.m. and 2 p.m. on 29 September and 1, 7, 8, 12, and 13 October 1987. All measurements were made with leaflets oriented perpendicular to sunlight. The portion of the leaflet used for gas exchange measurements was delineated with a felt-tip pen. Leaflets were then detached and transported to the laboratory in a cooler with ice for assessment of disease severity.

Determination of disease severity. Disease severity, based on the percentage

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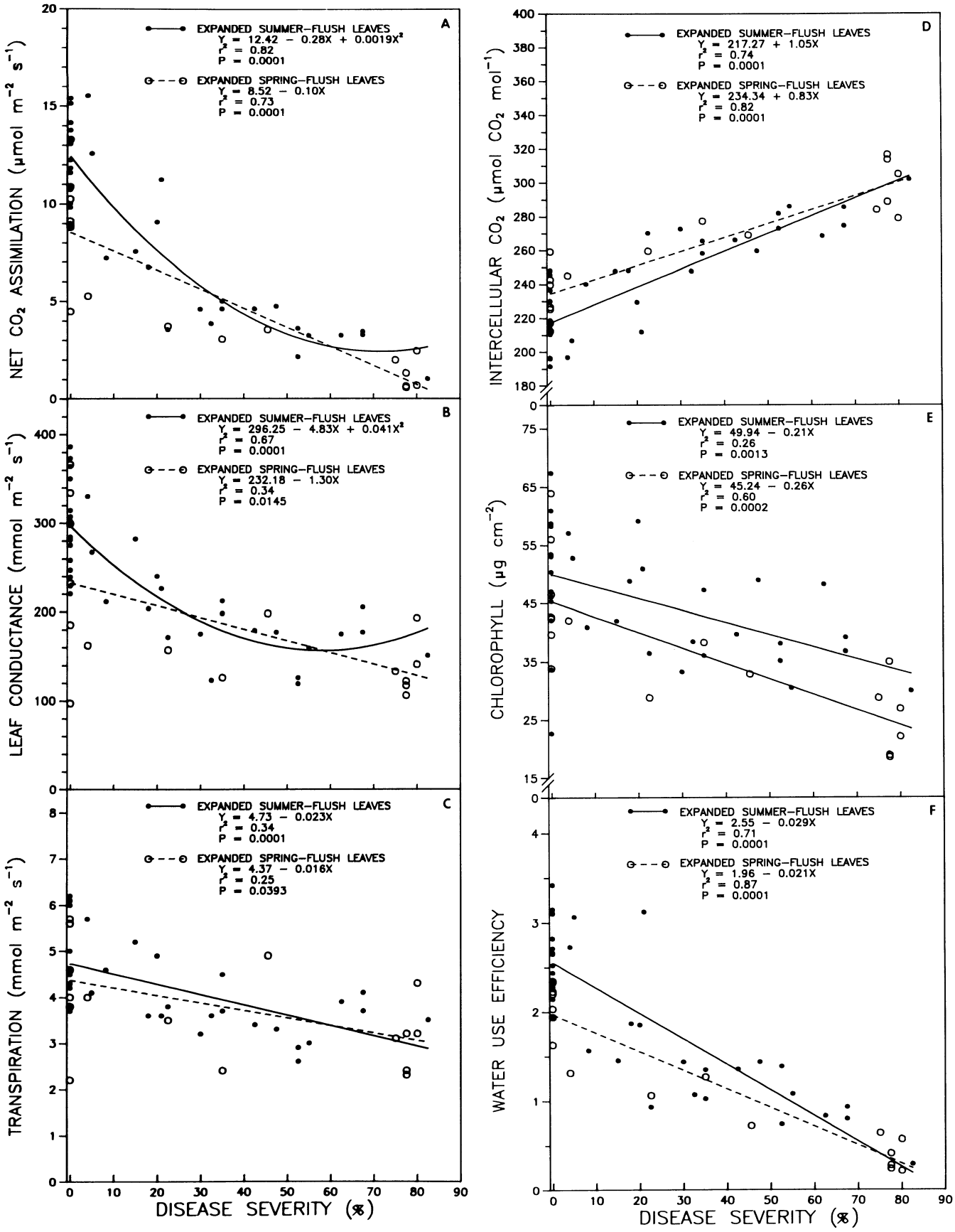

Fig. 1. Influence of leaf blotch disease severity on **(A)** net CO_2 assimilation, **(B)** leaf conductance to water vapor, **(C)** transpiration rate, **(D)** intercellular CO_2 concentration, **(E)** leaf chlorophyll concentration, and **(F)** water use efficiency of expanded summer-flush ($n = 37$) and expanded spring-flush ($n = 17$) Cape Fear pecan leaves.

of leaf surface area occupied by a lesion, was determined on the portion of each leaflet previously measured for gas exchange. Care was taken to eliminate leaflets that exhibited symptoms of diseases other than leaf blotch. Leaflets showing uniform distribution of the disease were selected whenever possible, although no attempt was made to quantify disease incidence outside this 6.25-cm^2 sector. Tissue affected by leaf blotch was irregular in shape, and the amount of disease varied between the abaxial and adaxial leaflet surfaces. To account for this disparity, two evaluators independently estimated the percentage of disease on both sides of each leaflet, and the estimates for both surfaces were averaged. The difference in the disease estimates of the two evaluators was generally within 5%.

Statistical analysis. Data were analyzed by regression, following general linear model procedures. For each cultivar, regression analysis was performed by treating disease severity as the independent variable and measured physiological variables as dependent variables. Dependent variables were analyzed in terms of a first-, second-, or third-degree polynomial as appropriate. Cape Fear and Choctaw were analyzed separately because the slopes of regression lines for the two cultivars were often significantly different (from analysis of the heterogeneity of slopes). Relationships between net CO_2 assimilation and leaf conductance to CO_2 or intercellular CO_2 concentration were plotted by disease severity and by leaf age; in this case, data for both cultivars were analyzed collectively because slopes did not differ significantly ($P < 0.05$).

RESULTS

Leaf tissue infected by *M. dendroides* was delineated by olive green to dark brown tufts of fungal hyphae in various stages of coalescence on summer-flush leaves. Chlorotic patches were also apparent. Infected spring-flush leaves had extensive dark brown necrotic areas.

Leaf blotch affected all measured variables of expanded spring- and summer-flush leaves in a linear or curvilinear manner (Fig. 1 and Table 1). Net CO_2 assimilation rate was less than 5 μmol m^{-2} sec^{-1} at a disease severity rating of 40% or greater (Fig. 1A). Leaf conductance to water vapor and transpiration rate were reduced considerably less than net CO_2 assimilation rate, which resulted in low water use efficiency (Fig. 1 and Table 2). A 40% disease severity rating of leaf blotch was associated with 55 and 76% decreases in net CO_2 assimilation rate, 33 and 56% reductions in leaf conductance to water vapor, 17 and 38% reductions in transpiration rate, and 20 and 36% reductions in leaf chlorophyll concentration for Cape Fear and Choctaw leaves, respectively, when values for both leaf ages were averaged (Table 2).

Plots of net CO_2 assimilation versus leaf conductance to CO_2 showed that net CO_2 assimilation rate was reduced slightly more than leaf conductance for both leaf ages (i.e., the ratio of the decrease in net CO_2 assimilation rate to the decrease in leaf conductance to CO_2 was 1.2:1) (Fig. 2A). Data from leaf blotch-infected leaves are generally represented by net CO_2 assimilation rates less than 12 and 7 μmol m^{-2} sec^{-1} for expanded summer-flush and expanded spring-flush leaves, respectively; intercellular CO_2 concentration of infected summer- and spring-flush leaves was generally greater than 210 and 240 μmol mol^{-1}, respectively. The negative slope of equations of net CO_2 assimilation rate versus intercellular CO_2 for expanded summer-flush and expanded spring-flush leaves (Fig. 2B) shows that leaf blotch infection was associated with an increase in intercellular CO_2 concentration.

DISCUSSION

Pecan leaves affected by leaf blotch often had a ratio of percentage of diseased surface area to percentage decrease in net CO_2 assimilation rate greater than 1:2. Low disease severity often resulted in greatly reduced leaf gas exchange (Fig. 1A and Table 1).

Expanded spring-flush Choctaw leaves were particularly affected by leaf blotch, as evidenced by the great decline in net CO_2 assimilation rate and leaf chlorophyll concentration (Tables 1 and 2). Leaf blotch-infected leaves contributed little to whole-tree carbon gain, although they continued to use water at a moderate rate. For example, a 40% leaf blotch disease severity rating translated into 55 and 76% reductions in net CO_2 assimilation rate for the two cultivars (Table 2). Other physiological variables were affected much less (Fig. 1 and Tables 1 and 2), and consequently water use efficiency was extremely low.

Stomatal aperture (i.e., proportional to leaf conductance to water vapor or CO_2) and the tendency of mesophyll cells to fix CO_2 (net CO_2 assimilation rate) were closely linked in this study. This phenomenon has been well documented for many plant species (5,17,19–21). Abscisic acid treatment (4), alteration in nitrogen or phosphorus nutrition (20), and short-term changes in photon flux density (21) often result in concomitant and similar percentage reductions in net CO_2 assimilation rate and leaf conductance to CO_2 such that intercellular CO_2 concentration remains essentially constant. Similarly, at moderate levels of plant moisture stress, intercellular CO_2 concentration remains unaffected; however, under severe moisture stress conditions, the mesophyll capacity to fix

Table 1. The relationship of physiological variables to severity[a] of leaf blotch for Choctaw pecan

Dependent variable	Leaf age[b]	Equation	r^2	P[c]
Net CO_2 assimilation	1	$13.29 - 0.64X + 0.013X^2 - 0.000075X^3$	0.77	0.0001
	2	$8.44 - 0.25X + 0.0021X^2$	0.77	0.0001
Leaf conductance	1	$295.95 - 12.97X + 0.29X^2 - 0.0018X^3$	0.67	0.0001
	2	$224.37 - 5.08X + 0.051X^2$	0.63	0.0006
Transpiration rate	1	$4.66 - 0.036X$	0.40	0.0001
	2	$4.40 - 0.092X + 0.0011X^2$	0.51	0.0041
Intercellular CO_2	1	$215.03 + 2.08X - 0.019X^2$	0.59	0.0001
	2	$233.72 + 1.90X - 0.014X^2$	0.79	0.0001
Leaf chlorophyll	1	$51.43 - 0.26X$	0.24	0.0004
	2	$55.05 - 1.01X + 0.0079X^2$	0.87	0.0001
Water use efficiency	1	$2.79 - 0.066X + 0.00065X^2$	0.56	0.0001
	2	$1.90 - 0.023X$	0.68	0.0001

[a]Percentage of infected leaf area.
[b]1 = Expanded summer-flush leaves (n = 48); 2 = expanded spring-flush leaves (n = 18).
[c]Probability of significant slope.

Table 2. Percentage change in physiological variables associated with a pecan leaf blotch disease severity rating of 40% on leaves of pecan cultivars Cape Fear and Choctaw[a]

	Cape Fear		Choctaw	
Variable	Expanded summer-flush leaves (%)	Expanded spring-flush leaves (%)	Expanded summer-flush leaves (%)	Expanded spring-flush leaves (%)
Net CO_2 assimilation	−63	−47	−72	−79
Leaf conductance	−43	−23	−58	−54
Transpiration rate	−20	−15	−31	−44
Intercellular CO_2	19	14	25	23
Leaf chlorophyll	−17	−23	−20	−51
Water use efficiency	−45	−43	−57	−48

[a]Percentage change was determined from the regression equations in Table 1 and Fig. 1. The disease severity rating of 40% is about half the maximum disease severity rating.

CO_2 (i.e., mesophyll conductance) may be reduced more than stomatal conductance, and hence intercellular CO_2 would tend to rise (10). The negative y-intercept of the plot of net CO_2 assimilation rate versus leaf conductance to CO_2 (Fig. 2A) indicates that net CO_2 assimilation rate was reduced to zero before complete stomatal closure. Intercellular CO_2 concentration was often elevated to ambient CO_2 partial pressure (Fig. 2B) with leaf blotch infection. Our data show that a reduction in leaf conductance to CO_2 cannot completely account for the observed reductions in net CO_2 assimilation rate. Because intercellular CO_2 was elevated despite partial stomatal closure (Fig. 1D and Table 2) and because leaf chlorophyll concentration was substantially reduced (Fig. 1E and Table 2), our data support the hypothesis that the photosynthetic apparatus was damaged in leaf blotch-infected leaves. We must invoke a residual, nonstomatal component to be responsible for the disproportionate decrease in net CO_2 assimilation rate. Interestingly, our previous work with scab-infected leaves showed that zero net CO_2 assimilation and complete stomatal closure occurred concomitantly (*data not reported*).

Direct damage to the photosynthetic apparatus may only partially explain the disproportionate decline in net CO_2 assimilation rate. Leaf blotch tended to cover leaf surfaces to the extent that light penetration into the leaf interior was certainly reduced. Net CO_2 assimilation of pecan leaves approaches a maximum at relatively high photon flux densities (i.e., 1,200–1,500 μmol m^{-2} sec^{-1}) (P. C. Andersen, *unpublished*). Because photosynthetic photon flux was 1,400-1,800 μmol m^{-2} sec^{-1} during leaf gas exchange measurements, it is likely that leaf blotch on the cuticle reduced light penetration into the chloroplasts of mesophyll and palisade cells to levels below light saturation. Similarly, Wood et al (23) reported that sooty mold (caused by *Capnodium* sp.) on pecan leaves inhibited net CO_2 assimilation by up to 70% by preventing light penetration into the leaf. Respiration of the leaf blotch pathogen itself would also contribute to a decrease in the reduction in net CO_2 assimilation relative to the reduction in leaf conductance to water vapor.

Pecan trees have an exceptionally high late-season energy demand. Maintaining a high level of photosynthetic activity in the fall is important to facilitate kernel filling and to provide assimilate reserves necessary for future nut production. A premature decline in photosynthetic efficiency or leaf defoliation is also known to enhance the process of alternate bearing (16,24,25). We have shown that leaf blotch has a profound effect on all physiological variables investigated when measured under field conditions. Leaf blotch frequently covered more than 50% of the total leaf surface, and photosynthetic efficiency declined greatly. Retention of leaf blotch-infected pecan leaves with a disease severity rating above about 40% may be undesirable given that they contribute little to whole-tree carbon gain yet continue to use water at a moderate rate.

An abbreviated pecan disease control program limited to 10 wk after nut set (6) may be a viable management option under normal conditions but is definitely not advisable when there is a prevalent summer growth flush or when late-summer disease incidence is high. A summer growth flush is most common on young or rapidly growing trees or on bearing trees in an off-year in the alternate bearing cycle. The photosynthetic capacity of healthy summer-flush leaves remains high until leaf abscission (1). Thus, under these conditions, it is desirable to suppress leaf blotch and other pecan diseases during late summer.

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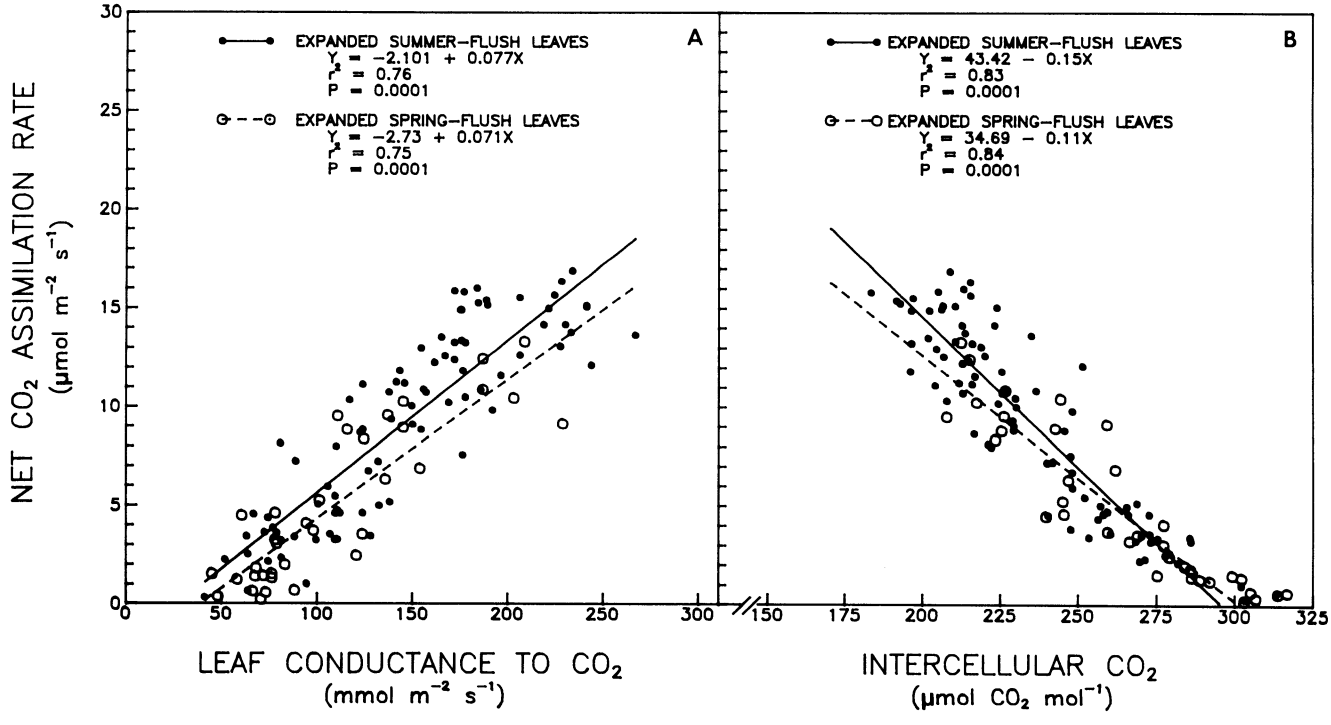

Fig. 2. The relationship of net CO_2 assimilation rate to **(A)** leaf conductance to CO_2 and **(B)** intercellular CO_2 concentration for expanded summer-flush ($n = 85$) and expanded spring-flush ($n = 35$) pecan (Cape Fear and Choctaw) leaves infected with leaf blotch.

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