

Gas Exchange Studies on the Transpiration and Photosynthesis of Tomato Leaves Affected by *Fusarium oxysporum* f. sp. *lycopersici*

J. M. Duniway and R. O. Slatyer

National Science Foundation Postdoctoral Fellow and Professor, respectively, Research School of Biological Sciences, Australian National University, Box 475, Canberra, A.C.T. 2601, Australia. Present address of senior author: Department of Plant Pathology, University of California, Davis 95616.

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ABSTRACT

Leaves of Bonny Best tomato plants were used for gas exchange measurements at various times after root inoculation with *Fusarium oxysporum* f. sp. *lycopersici* race 1. Simultaneous measurements of transpiration and photosynthesis, under conditions of CO₂ limitation and light saturation, allowed the calculation of stomatal and intracellular resistances to CO₂ diffusion. Photosynthesis and transpiration were unaffected by the host-pathogen interaction until 15 days after inoculation, when there were marked reductions in both. The reduction in photosynthesis was caused in part by an increase in stomatal resistance and in part by a large increase in

intracellular resistance. Measurements of leaf water content and water potential indicated that the increases in stomatal and intracellular resistances preceded the onset of water stress in a given leaf. After the host-pathogen interaction had caused some water stress to occur in a leaf, the intracellular resistance became nearly infinite. Comparable levels of water stress had little effect on the intracellular resistance of healthy leaves. The increase in intracellular resistance of infected leaves was associated with reductions in both dark respiration and photorespiration, but was not associated with a decrease in the activity of ribulose diphosphate carboxylase. Phytopathology 61: 1377-1381.

Additional key words: vascular wilt, physiology of diseased plants, *Lycopersicon esculentum*.

The most recent study on photosynthesis in plants with vascular wilt disease was done by Mathre (15). He used manometric and ¹⁴C₂O₂ incorporation techniques and found an inhibition of photosynthesis in cotton leaves with visual symptoms of Verticillium wilt. Mathre (15) also found chloroplasts from the same leaves to be less efficient than those from healthy leaves in carrying out the Hill reaction. Beckman et al. (1) did gas exchange measurements on banana leaves affected by *Pseudomonas solanacearum*, and found that marked oscillations and reductions in both transpiration and photosynthesis occurred as wilt symptoms developed.

Measurements of the rate of photosynthesis are frequently of limited value unless the rate limiting factors are also evaluated. Under light-saturated conditions, these factors are generally either CO₂ supply, which is primarily mediated by stomatal resistance, or the carboxylation efficiency of the photosynthetic apparatus. The studies on vascular wilt disease cited here and, with one recent exception (11), those on other diseases have not evaluated both of these factors. Although Beckman et al. (1) concluded that the effects of water stress on stomata were responsible for the observed alterations in photosynthesis, their data are not complete enough for one to calculate stomatal resistance. It is interesting to note that foliar pathogens may also alter stomatal and cuticular resistances to gas diffusion (8, 9) and that such alterations are expected to affect the apparent rate of photosynthesis.

This paper gives the results of gas exchange measurements of transpiration, photosynthesis, and respiration in leaves of Bonny Best tomato plants (*Lycopersicon esculentum* Mill.) infected with *Fusarium oxysporum* Schlecht. f. sp. *lycopersici* (Sacc.) Snyder & Hans. race 1.

MATERIALS AND METHODS.—*Biological materials.*—Tomato plants were grown in sterilized soil at 25 C in a greenhouse and inoculated 4 weeks after seeding by the root dip technique (17). Control plants were treated in the same manner with sterile water. After treatment, plants were placed in the shade for 24 hr, then in a controlled environment chamber. The conditions in the chamber (85 w m⁻² of light in the 300-700 nm wave band for 16 hr/day at 30 ± 1 C and 60 ± 5% relative humidity) were such that leaf water stress was the major visible symptom of disease development. The youngest fully expanded leaf of a plant was used for gas-exchange measurements. This was the fourth adult leaf up to 9 days after inoculation and the fifth adult leaf after 9 days.

Leaf gas-exchange measurements.—The remainder of MATERIALS AND METHODS, with the exception of the section on enzyme assays and some minor modifications in leaf gas exchange procedures, is taken from work of Slatyer (20). On the evening prior to the day of measurement, the test plant was brought to a constant temperature (25 C) laboratory and set up in a leaf chamber ready for measurement. The leaf chamber was basically that described by Jarvis & Slatyer (13), with the addition of a β-gauge for the measurement of leaf water content. The β-gauge consisted of a source of β particles (0.6 mc ¹⁴⁷Pm) and a Geiger-Muller tube which were placed on opposite sides of the leaf blade. The geometry of the system was constant, and the observed count rate was converted to relative water content by the method of Jarvis & Slatyer (14). Individual attached leaflets were set up in the inner compartment of the chamber, the environment of which was rigorously controlled at desired gas concentrations, air transfer rates, air and leaf temperatures, and radiation flux density. The light source was a mercury vapor lamp

which provided a radiation flux density of 330 w m^{-2} (300-700 nm) for measurement purposes.

Leaf temperature was measured with a copper-constantan thermocouple threaded through a side vein of the leaf blade, and was maintained at $32 \pm 1 \text{ C}$ in the light and $24 \pm 2 \text{ C}$ in the dark. Transpiration was determined from measurements made with differential psychrometers (21) and CO_2 exchange was measured with an infrared gas analyzer calibrated with gas mixing pumps. Capillaries and micromanometers were used to measure the flow rates of air, and the output from all sensors was displayed on an integrating digital voltmeter.

On the day of measurement, net photosynthesis and transpiration were measured at various levels of CO_2 and dark respiration was measured at one level of CO_2 . Individual measurements were done under steady-state conditions. The desired CO_2 level was obtained by partial removal of CO_2 from air prior to reaching the leaf chamber.

Water vapor exchange parameters.—The equation for transpiration is generally written in the following form:

$$E = (c'_w - c'_a)/(r'_a + r'_1) \quad (\text{I})$$

where E ($\text{g cm}^{-2} \text{ sec}^{-1}$) is the rate of water vapor loss, c'_w and c'_a (g cm^{-3}) are, respectively, the water vapor concentrations at the surfaces of the mesophyll cell walls and in the bulk air, r'_a (sec cm^{-1}) is the diffusive resistance to water vapor transfer across the boundary layer which sheathes the leaf, and r'_1 is the diffusive resistance in the leaf. Strictly speaking, r'_1 includes cuticular and stomatal diffusive resistances (19), but under open stomata conditions, as occurred in these experiments, the cuticular resistance to water vapor transfer is so high that there is little transfer via the cuticle, and it can be neglected with acceptable error.

Since c'_w was assumed to be the saturation vapor concentration at leaf temp, $(r'_a + r'_1)$ was obtained from measurements of transpiration rate, leaf temperature, and ambient water vapor concentration (20). The level of r'_a was made constant and relatively small by standardized ventilation procedures. Its value was found to be 0.27 sec cm^{-1} , using wet blotting paper ($r'_1 = 0$) instead of a real leaf. For any set of measurements of $(r'_a + r'_1)$, r'_1 was then obtained by subtraction of r'_a .

CO_2 exchange parameters.—The equation for photosynthesis in the steady state can be written in the following form:

$$F = (c_a - c_w)/(r_a + r_1) = (c_w - c_c)/r_{\text{int}} \quad (\text{II})$$

where F ($\text{g cm}^{-2} \text{ sec}^{-1}$) is the rate of CO_2 uptake, c_c (g cm^{-3}) is the effective CO_2 concentration at the site of carboxylation, and r_{int} is the intracellular resistance to CO_2 transport (20). The other symbols refer to CO_2 concentrations and resistances in the same manner as used for water vapor in equation I.

The key parameters affecting photosynthesis are those which affect gaseous CO_2 supply ($r_a + r_1$) and those which affect the intracellular resistance to CO_2 transport (r_{int}). The intracellular resistance is a

parameter which describes the effective "resistance" arising within the mesophyll cells themselves. It includes not only resistance to liquid phase transfer from the surface of the mesophyll cells to the sites of carboxylation and decarboxylation, but also resistance associated with the biochemical and biophysical processes involved in photosynthesis (20). It is therefore a measure of the photosynthetic efficiency of the tissue under study.

The resistances to CO_2 transport (r_1 and r_{int}) were obtained from sets of measurements relating the rate of CO_2 uptake (F) to the ambient CO_2 concentration (c_a), collected simultaneously with measurements of the water vapor parameters in equation I under conditions of CO_2 limitation and light saturation (20). Since the concentration of CO_2 at the site of carboxylation (c_c) was not known, a straight line relating the rate of CO_2 uptake (F) to the concentration of CO_2 at the mesophyll cell walls (c_w) was drawn, and the intracellular resistance (r_{int}) was obtained as the reciprocal of the slope; i.e., as the change in c_w divided by the corresponding change in F . Estimates of c_w were obtained from equation II rewritten in the following form:

$$c_w = c_a - F(r_a + r_1) \quad (\text{III})$$

assuming that $r_a = 1.35 r'_a$ and $r_1 = 1.56 r'_1$ (10). Photorespiration was estimated from the F intercept of the curve relating F to c_w ; i.e., F at $c_w = 0$, and the compensation point was estimated from the c_w intercept, i.e., c_w at $F = 0$.

Enzyme assays.—At the conclusion of each set of gas exchange measurements, the experimental leaf was used for assays of the enzymes ribulose diphosphate carboxylase and glycolate oxidase. These enzymes were examined because ribulose diphosphate carboxylase is the primary carboxylating enzyme in species like tomato, which show typical Calvin cycle photosynthesis, and because of the likely role of glycolate oxidase in photorespiration (12). The assay procedures used have been described in detail by Downton & Slatyer (5).

RESULTS.—The transpiration and photosynthesis rates measured in infected plants 8 and 13 days after inoculation were similar to those measured in healthy plants 7, 12, and 14 days after inoculation (Fig. 1). The rates were calculated for conditions which approximate those outdoors (50% relative humidity, $r_a = 0.2 \text{ sec cm}^{-1}$ and $c_a = 552 \times 10^{-9} \text{ g cm}^{-3}$). The predominate visual symptom of the disease at 8 days was a slight epinasty of the leaves, and at 13 days there was epinasty plus slight wilting of the oldest leaves. In the infected plant used 15 days after inoculation, both the transpiration and photosynthesis rates were very much less than those observed on previous days (Fig. 1). The leaf used this day had severe epinasty but appeared turgid, whereas the older leaves on the plant appeared moderately wilted. Reference to Fig. 1 also shows that the reduction in transpiration at 15 days after inoculation resulted from an increase in stomatal resistance (r'_1). Although the reduction in photosynthesis at 15 days was partially due to stomatal closure, there was also a

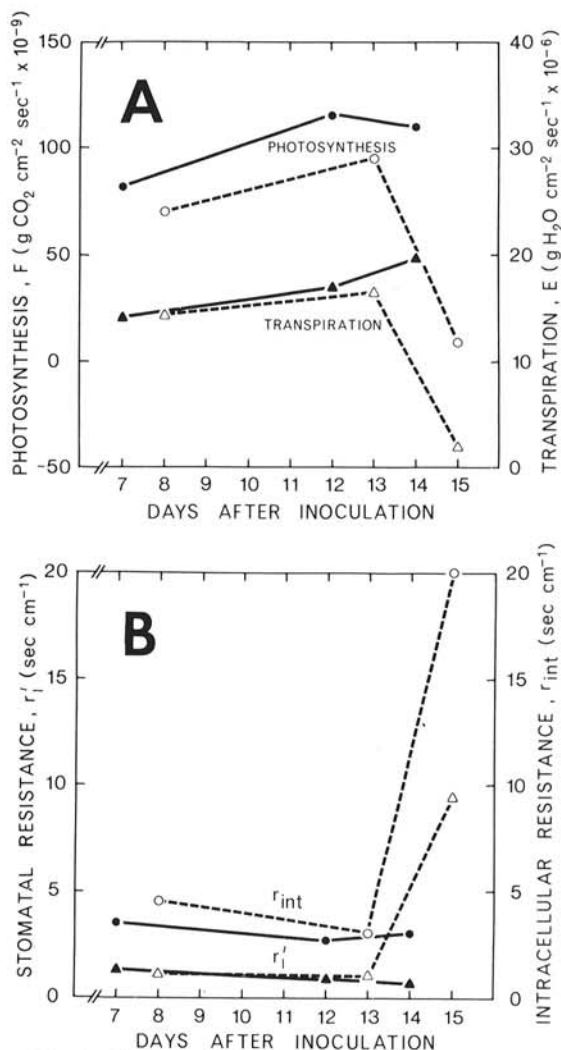


Fig. 1. A) Transpiration and photosynthesis rates with B) the corresponding stomatal and intracellular resistances calculated from gas exchange measurements on healthy and *Fusarium*-infected tomato plants. Closed symbols and solid lines refer to healthy plants, whereas open symbols and broken lines refer to infected plants. Triangles and circles indicate water vapor and CO₂ exchange parameters, respectively.

marked increase in intracellular resistance, indicating a major reduction in the photosynthetic capacity of the mesophyll.

The use of a pressure chamber (7) at the end of the gas exchange measurements showed that the minimum leaf water content observed with the β -gauge 15 days after inoculation corresponded to a leaf water potential of -5.0 bars. The minimum leaf water content on earlier days corresponded to water potentials between -3.5 and -4.8 bars. Frequent measurements with the pressure chamber under the conditions used for plant growth showed that leaflets comparable to those used for gas exchange measurements had water potentials higher than -6 bars. This was even the case in infected plants where older leaves had wilted, an observation consistent with the

estimated resistance to water movement in *Fusarium*-infected tomato plants (6). Leaf water potentials lower than -9 bars were associated with wilting in both healthy and infected plants.

Leaves which had wilted slightly during disease development were used for gas exchange measurements 16 and 17 days after inoculation; comparable leaves had water potentials between -9 and -10 bars under the conditions used for plant growth. The first leaf had a nearly constant water potential of -8.3 bars, and stomatal resistance (r_s') of 8.0 sec cm⁻¹ during gas exchange measurements. The second leaf wilted severely during the measurements, but the initial points were obtained when the water potential was higher than -8.0 bars (estimated from the relationship between leaf water content and water potential) and at stomatal resistances (r_s') less than 10 sec cm⁻¹. Neither of these leaves exhibited net photosynthesis, making intracellular resistance essentially infinite.

Although the foregoing results were obtained with only one plant for each day of measurement, a repeat of the gas exchange measurements 12 to 17 days after inoculation with plants grown on a separate occasion yielded almost identical results.

An additional experiment was done to examine the effect of low water potential on the photosynthetic capacity of healthy leaves. A short-term water stress was induced during gas exchange measurements by chilling the roots of a healthy plant grown in nutrient solution. A curve relating the rate of CO₂ uptake to the ambient CO₂ concentration was obtained at the start of the experiment; and then a curve relating intracellular resistance to leaf water content was obtained from measurements of CO₂ uptake at one ambient CO₂ concentration. It was assumed that the concentration of CO₂ at the site of fixation equaled the compensation point observed at the start of the experiment (20). Figure 2 shows that

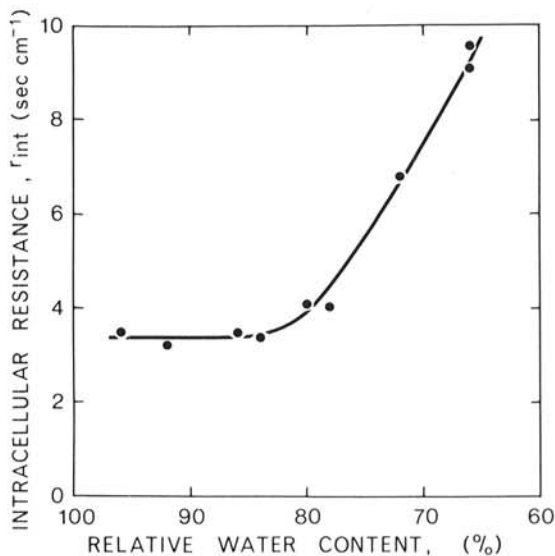


Fig. 2. Intracellular resistance of a healthy tomato leaf plotted as a function of decreasing relative water content.

TABLE 1. Dark respiration, photorespiration, glycolate oxidase, and ribulose diphosphate carboxylase activities per unit leaf area in healthy and *Fusarium*-infected tomato plants (the corresponding values for intracellular resistance to CO₂ uptake and total protein per unit leaf area are also given)

Days after inoculation	Treatment	Dark respiration	Photo-respiration	Glycolate oxidase	Ribulose diphosphate carboxylase	Intracellular resistance	Total protein
		$g\ CO_2\ cm^{-2}\ sec^{-1} \times 10^{-9}$	$\mu moles\ substrate\ min^{-1}\ cm^{-2}$	$\mu moles\ substrate\ min^{-1}\ cm^{-2}$	$sec\ cm^{-1}$	$mg\ cm^{-2}$	
7	Healthy	12	30	32.8	11.8	3.5	0.28
8	Infected	9	24	14.6	6.7	4.5	0.19
12	Healthy	8	35	37.7	12.6	2.7	0.29
13	Infected	7	34	26.1	18.3	3.0	0.25
14	Healthy	9	31	44.5	14.6	3.0	0.31
15	Infected	4	12	26.1	12.4	20.0	0.28

the intracellular resistance of a healthy leaf did not increase until the relative water content was less than 80%, which is equivalent to water potentials lower than -12 bars.

The rates of photorespiration and dark respiration observed 7-15 days after inoculation are shown in Table 1. Respiration in the leaves of infected plants was normal until 15 days after inoculation, when respiration decreased. Table 1 also shows that the increase in intracellular resistance 15 days after inoculation was not associated with a marked decrease in the activity of ribulose diphosphate carboxylase, and that infected plants consistently had lower levels of glycolate oxidase than did healthy plants.

DISCUSSION.—The marked reduction in the photosynthetic capacity of the leaf mesophyll, observed as an increase in intracellular resistance during the late stages of *Fusarium* infection, does not appear to be a consequence of leaf water stress. The gas exchange measurements 15 days after inoculation showed that a several-fold increase in intracellular resistance occurred before the onset of water stress in the experimental leaf of an infected plant. After the host-pathogen interaction had caused some water stress to occur in the experimental leaf, the effect of the interaction on the intracellular resistance of tomato was much more pronounced than the effect of a comparable water stress alone. Furthermore, other studies have shown that water potentials, which are considerably lower than those reported here for infected plants, are required for leaf water stress to markedly inhibit the photosynthetic activities of mesophyll cells (2) and chloroplasts (3).

Leaves on infected plants had stomatal resistances (r_1') between 8 and 10 $sec\ cm^{-1}$ and water potentials between -5.0 and -8.3 bars during the gas exchange measurements 15-17 days after inoculation, whereas healthy leaves at the same water potentials had stomatal resistances (r_1') less than 5 $sec\ cm^{-1}$. Thus, it can be argued that the initial effect of the pathogen on stomatal resistance is not mediated by water stress, even though water stress does cause stomatal closure (2, 9). This conclusion differs from that of Dimond & Waggoner (4) and that of Beckman et al. (1), but previous studies on the transpiration of plants with vascular wilt disease have not included

direct measurements of leaf water status. The oscillations found by Beckman et al. (1) were not evident in this study, probably because a low transpirational demand was imposed during gas exchange measurements.

The mechanism by which *Fusarium* infection increases intracellular resistance remains unknown. The increase was not associated with either chlorosis or a decrease in ribulose diphosphate carboxylase activity, as was found to be the case in virus-infected sugar beet leaves (11). The reduction in the Hill reaction of chloroplasts found by Mathre (15) does suggest that an increase in intracellular resistance also occurs in *Verticillium*-infected cotton, and that the increase in infected cotton plants would be associated with a basic alteration in the photosynthetic apparatus.

Increased respiration is sometimes regarded as a general host response to plant pathogens, and previous measurements of respiration in tomato plants infected with *Fusarium* have commonly supported this concept (16). Even though previous results have depended on the conditions used (16), it is difficult to explain the disparity between the results of manometric measurements in the dark and the results of gas exchange measurements reported here. The fact that both respiration and photosynthesis were reduced indicates that a cause and effect relationship between increased respiration and decreased photosynthesis, proposed for mildew-infected leaves (18), does not occur in *Fusarium*-infected tomato plants. In view of the proposed central role of glycolate oxidase in photorespiration (12), it is interesting to note that glycolate oxidase activity does not appear to regulate the rate of photorespiration in infected plants (Table 1).

This study has pointed out the importance of including stomatal resistance as a parameter in gas exchange measurements, and points out some major physiological changes which occur in leaves affected by *F. oxysporum* f. sp. *lycopersici*. Histological examination of the leaves on infected plants indicated that the pathogen was confined to the xylem of major veins, and thus, the occurrence of physiological changes in noninfected mesophyll tissue suggests that the infection process may produce toxic products which are translocated throughout the leaf blade.

However, it should be noted that there is no evidence that the action of toxic products in the leaf blade causes wilting (6).

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