Net Carbon Gain and Growth of Bell Peppers, *Capsicum annuum* 'Cubico', Following Root Infection by *Pythium aphanidermatum*

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ABSTRACT

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The first characterization of alterations in whole-plant photosynthetic rate and carbon assimilation of bell peppers associated with infection by *Pythium aphanidermatum* is described. Relationships of root disease caused by *P. aphanidermatum* to whole-plant net carbon exchange rate (NCER), total carbon accumulation, dark respiration rates, water loss, and destructive growth parameters were quantified in vegetative, hydroponically grown pepper plants (*Capsicum annuum* 'Cubico'). Inoculated plants displayed lower whole-plant NCER. This translated into a loss of

28% in cumulative C gain during 7 days after inoculation and occurred before visible shoot symptoms developed. Leaf area and dry weight of shoots and roots were significantly decreased and the shoot/root ratio was higher in inoculated plants than in noninoculated plants. We propose that reduced NCER in inoculated plants was mainly due to restricted development of leaf area, because no differences in NCER and evapotranspiration were observed between control and inoculated plants when expressed based on leaf area and root dry mass, respectively. These findings indicate that *Pythium* infection did not affect the photosynthetic apparatus directly and that the reductions in photosynthesis and growth were not caused by inefficient water transport by diseased roots. These results enlarge on the understanding of physiological responses of host plants to early stages of root disease.

Pythium aphanidermatum (Edson) Fitzp. is a principal pathogen contributing to root rot of bell pepper (*Capsicum annuum* L.) and other hydroponic greenhouse crops (4,28,39,45). *P. aphanidermatum* is worldwide in distribution and can enter water supplies and nutrient solutions by many means, including dust deposited on greenhouse roofs (35). In hydroponic systems, roots, root exudates, and circulating nutrient solution provide a favorable environment for growth, reproduction, and dispersal of *P. aphanidermatum*, which is dispersed as zoospores or as hyphae on fragments of diseased roots (29,45). Infected roots develop tip necrosis, expansive browning, and decay. Relationships of root rot epidemics to shoot growth and fruit development, however, are poorly understood.

Epidemics of root rot usually progress rapidly in hydroponic pepper crops and increase in severity over several months (28). Symptoms on aerial portions of the crop often include stunted shoots and smaller, fewer fruits. When root rot is severe, and especially when air and/or nutrient solution temperatures are high, the foliage may wilt and the plants may die within a few days (20,28). The leaf canopy generally remains green until advanced stages of wilting. Root rot affects host growth and reproduction long before visible symptoms such as wilting or chlorosis on aerial portions of the plants become noticeable.

The relationship of root disease caused by *P. aphanidermatum* and growth in hydroponic peppers is currently understood chiefly from investigations in small-scale hydroponic systems. When applied as zoospore suspensions to roots of vegetative-stage pepper plants grown in single-plant hydroponic containers or in trough systems containing five to seven plants, the pathogen reduced the

volume, fresh mass, total length and surface area of the roots, as well as total leaf area, and shoot height and fresh mass over 2 to 3 weeks following inoculation (23,28). Reductions in root and shoot dry mass were observed in plants grown in trough systems (23). No significant change in chlorophyll content in leaves was encountered in these investigations. *P. aphanidermatum* rapidly colonized a majority of the roots, but incidence of root browning increased at widely differing rates that were related to temperature and other environmental variables (29). While plant growth is generally reduced after roots are infected and colonized by root-rotting pathogens including *P. aphanidermatum*, the changes in physiological processes that mediate yield reduction are not well understood, whether in hydroponic systems or soils.

Growth and productivity at the whole plant level depend on photosynthesis and respiration (22). To date, most studies of these variables in diseased plants have focused on gas exchange in single leaves of infected plants compared with control plants. Changes in leaf photosynthesis and transpiration associated with stomatal closure were recognized as early foliar responses to root-infecting pathogens (3,31). In bell peppers inoculated at the stem with *Phytophthora capsici*, leaf photosynthesis and conductance were significantly reduced 4 days after inoculation (DAI), coupled to a rise in dark respiration and ethylene production (1). Net CO₂ assimilation in leaves of young avocado plants was reduced 7 days after the plants were transplanted into soil infested with *Phytophthora cinnamomi*, and by 75% at 14 days (31). Reductions in assimilation, stomatal conductance, and transpiration preceded development of visible shoot symptoms, chiefly wilting and defoliation, by several days. Similar responses and reductions in leaf photosynthesis were reported for the vascular wilt pathogens *Verticillium* or *Fusarium* in tomato (10,26), potato (5-7,18), pepper (16), chrysanthemum (17,27), and alfalfa (30). One study of the relationship between photosynthesis and disease, however, examined eggplants infected by *V. dahliae* on a whole-plant basis.

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Plants grown in *Verticillium*-infested soil had a lower whole-plant photosynthetic rate than did the controls (14). The reduced canopy photosynthesis was attributed to the reduced leaf area due to defoliation of infected plants. Defoliation is a clear and severe loss of photosynthetic capacity of a plant canopy. In more sublethal situations, the canopy does not show such visible changes, but photosynthetic capacity may be altered by drooping of the leaves, presentation of the leaves to the light source, or by actual alterations in the photosynthetic rate due to chemical signals transported to the leaves. Regardless of the mechanism, it is of fundamental importance to develop an understanding of how root disease affects, both directly and indirectly, processes that influence net carbon gain and thus productivity in whole plants.

In the present study, whole-plant development was studied in vegetative-stage pepper plants grown in single-plant hydroponic units by quantifying growth of the leaf canopy and roots. Concomitantly, water loss and net carbon exchange rates (NCER) throughout photoperiods and dark cycles were measured continuously. Total daily carbon accumulation and host growth parameters were quantified after plants were inoculated with *P. aphanidermatum*. There were three main objectives of these experiments. The first was to determine the relationships between root disease caused by *P. aphanidermatum* and changes in host plant productivity. The second was to use day and night (diel) gas exchange measurements of the whole plants as a means for noninvasive measurement of crop growth during the onset and progression of root rot. The third was to test whether gas exchange (CO_2 and/or H_2O) could be used to predict the onset of infection and disease. Taken together, these data would allow use of the plant as a primary sensor of its health status in early stages of root rot epidemics.

MATERIALS AND METHODS

Plant material and growth conditions. Seeds of *C. annuum* cv. Cubico (De Ruiter Seed Co., Columbus, OH) were germinated on filter paper for 5 days and transferred to small rockwool cubes (Grodan Group, Roermond, The Netherlands). After 4 weeks, the seedlings were transplanted to 475-ml plastic containers (Fisher Scientific, Ottawa, ON) filled with nutrient solution (0.83 g of Plant Prod 7-11-27 N:P:K supplemented with 0.57 g of CaNO_3 per liter, Plant Products Ltd., Brampton, ON), pH 5.8 and electrical conductivity (EC) 1.8 mS/cm, and aerated with aquarium pumps (Maxima, Rolf C. Hagen Inc., Montreal, QC). The solution was topped up manually at intervals of 1 to 2 days, when conductivity was also checked. Plants were grown in growth chambers (Convion, Winnipeg, MB) at $27 \pm 0.1^\circ\text{C}$ (day) and $24 \pm 0.1^\circ\text{C}$ (night), with an ambient CO_2 concentration of 365 $\mu\text{l/liter}$ and relative humidity (RH) of $50 \pm 5\%$ at an initial planting density of 58 plants per m^2 . The light source was a combination of fluorescent lamps (Cool White 115 W, GTE Sylvania Canada Ltd.) and incandescent bulbs (Cool White 60 W, GTE Sylvania Canada Ltd.) that provided approximately 400 $\mu\text{mol/m}^2/\text{s}$ of photosynthetically active radiation (PAR) at canopy height during 16-h photoperiods.

***Pythium* inoculation protocol.** Zoospores of *P. aphanidermatum* isolate P6 (29) were produced by a method similar to that of Rahimian and Banihashemi (32). The isolate was grown on V8-juice agar medium in petri dishes at 25°C for 48 h, after which the medium was cut into six 1-cm-wide strips. Alternate strips in each dish were transferred to an empty petri dish, and all dishes were flooded with 25 ml of sterile distilled water. The plates were incubated at 25°C under fluorescent lights, and after 48 h, the water was replaced with another 25 ml of sterile distilled water. After incubation at 20°C under fluorescent lighting for 4 h to stimulate zoospore release, zoospore suspensions were collected and zoospore density was estimated with a hemacytometer as described previously (29). Plants were inoculated 14 days after trans-

planting by immersing the root systems in suspensions of 6.25×10^3 zoospores per ml of sterile water for 30 min. Roots of control plants were immersed in sterile water for 30 min.

Evaluation of colonization and disease. Roots were visually assessed for percent incidence of discolored roots and percent incidence of brown root tips at 24 h after inoculation, and at 48-h intervals thereafter using separate incremental scales as follows: 0 = 0%, 1 = 1 to 10%, 2 = 11 to 20%..., and 10 = 91 to 100%. The incidence of roots colonized by *P. aphanidermatum* from inoculated and control plants was estimated at 24 h after inoculation, and at 48 h intervals thereafter. At each time of sampling, 10-cm-long distal portions of each of three primary roots per plant were cut off and the laterals were removed with a sterile scalpel. For surface disinfections, roots were immersed in 50% ethanol for 10 s and in 1% NaOCl for 30 s, rinsed three times in sterile distilled water, and then blotted dry. Each root was cut into 10 1-cm-long segments and the segments were placed on a *Pythium*-selective medium (P_5ARP agar medium [21]) in petri dishes. Incidence of root pieces with *Pythium* colonies was estimated after 24 h and the plates were examined at 72 h for incidental fungi.

Destructive plant growth analysis. Six plants of each treatment were randomly selected for destructive harvest at 48-h intervals starting 1 day prior to inoculation until 7 DAI. Leaf area was measured with a belt meter (LI-3000, LI-COR, Lincoln, NE). Fresh mass of leaves, stems, and roots was measured immediately after harvesting. These tissues were then dried at 75°C for 48 h prior to dry weight determination.

Nondestructive plant growth analysis. Biomass gain was determined from analysis of daytime and nighttime whole-plant net CO_2 gas exchange, measured as described by Dutton et al. (11) and modified by Leonardos et al. (25). Ten days after transplanting, pepper plants were transferred from the growth cabinet to sealed gas analysis chambers with computer-controlled temperature (27°C day/ 24°C night), humidity (45% RH), and CO_2 concentration (365 $\mu\text{l/liter}$). Four plants were positioned so as to minimize mutual shading in each of four separate chambers. High pressure sodium lamps (1,000 W, Lumalux LU 1,000; GTE Sylvania Canada Ltd.) provided PAR (400 to 700 nm) at 400 $\mu\text{mol/m}^2/\text{s}$ as measured by quantum sensors (Q3991-4; LI-COR) at the top of the plant canopy.

Transpiration of the whole plants was also determined continuously. Inside the sealed chambers, pots were placed on an analytical balance (PG8001-S, Mettler Toledo, Columbus, OH) with measurements recorded at 4-min intervals (BalanceTalk XL, Labtronics Inc., Guelph, ON) to estimate whole-plant water loss by evapotranspiration. Nutrient solution was added daily as needed, without opening the chambers, with a gravity-controlled line feed from an external overhead reservoir. The nutrient solution in each container was aerated with air from within the gas analysis chamber. Evaporation from the nutrient solution during aeration was accounted for by subtracting values for control runs (i.e., containers with no plants) from the cumulative water loss data of those with plants.

NCER was measured continuously for 10 days commencing with the daylight period following an overnight equilibration of 12 h; chambers were opened and the plants were removed for inoculation on the third full day, allowing 7 days of postinoculation measurements. NCER was calculated from initial and final CO_2 concentrations in the chamber atmosphere and pure CO_2 injection measurements every 6 min in a manner described previously (25). Carbon accumulation was calculated by integrating NCER over the measurement period. Statistical significance of total carbon accumulation was assessed by one-way analysis of variance (S-Plus 2000, Insightful Corporation, Seattle, WA).

In order to express plant CO_2 and H_2O gas exchange on a unit biomass basis, the leaf area of the plant canopy in each chamber was measured destructively at the end of the 10-day CO_2 measurements. Dry weights of leaf, stem, and root tissues were

also determined as described. Leaf area and dry matter changes of plants in the NCER chambers were approximated from predicted values generated from regressions fitted to data from destructive harvests of plants grown concurrently under similar environmental conditions in growth cabinets. Percent roots discolored and percent colonized by *P. aphanidermatum* were also estimated.

Apparent quantum yield. Apparent quantum yield (AQY) was defined as the ratio between mole CO₂ fixed/mole photons incident on the canopy within the whole-plant gas exchange chambers (42). This determination of canopy photosynthetic efficiency utilizing the total leaf area within the chamber is a modification to the QY method, as discussed by Bugbee and Monje (8). While Bugbee and Monje (8) used the QY approach when discussing the theoretical limits to crop productivity, they presented an empirical function of absorbed photon flux for a wheat canopy. In the ab-

sence of empirical measurements of light absorption by the plants in each chamber, a more conservative estimate is proposed as the AQY. The term apparent is applied here to distinguish between determinations of photosynthetic efficiency based on incident (AQY) or absorbed (QY) radiation.

RESULTS

Growth inhibition accompanying root discoloration. Root discoloration (chiefly browning) increased rapidly and exceeded 80% by 7 DAI in the inoculated plants, whereas all roots of non-inoculated control plants remained white (Fig. 1A). Colonization incidence by *P. aphanidermatum* of root segments of inoculated plants was high (95 to 100%) at all sampling times, but the pathogen was not recovered from the controls. Following conventional destructive harvesting, dry mass estimates of the shoots and roots were significantly lower in the inoculated plants compared with those in the control plants at 5 DAI (*t* test, *P* = 0.0001), and were 32 and 69% lower, respectively, at 7 DAI (Fig. 1B). The progressive suppression of shoot and root dry mass in the inoculated plants roughly paralleled the increase in percent discolored roots. Roots of control plants grew continuously during the experiment (Table 1), whereas in the inoculated plants, the length of the longest root decreased over time. Root regeneration was not observed in the inoculated plants.

Data of the destructive harvests indicated that by 5 DAI, shoot/root ratios were significantly greater, and shoot fresh mass, length of the longest root, and total leaf area were significantly lower in the inoculated plants compared with the control plants (Table 1). These differences increased by 7 DAI. At 7 DAI, total leaf area was 41% lower in the inoculated plants than in non-inoculated controls. The changes in both root growth and leaf canopy development following inoculation with *P. aphanidermatum* are important in understanding changes in the primary physiological behavior of the host plants.

Nondestructive estimations of whole-plant water loss. On a per plant basis, transpiration rates of control plants were not significantly different from those of inoculated plants for at least 5 days, at which time the larger control plants lost more water (Fig. 2A). However, when water loss from the canopy was expressed on a leaf area basis, there was no difference between control and inoculated plants throughout the entire experiment (Fig. 2B). Taken together, Figure 2A and B clearly indicate that any differences in the absolute amount of water lost by inoculated and control plants were probably related to the larger, more rapidly expanding leaf canopy of the control plants (Table 1).

To provide a crude estimate of the effect of any disease in the root systems inoculated with *P. aphanidermatum* on the capacity of the host plants for water uptake via the transpiration stream, transpiration rates were also expressed on a root mass basis (Fig. 2C). According to these calculations, the inoculated plants tran-

Fig. 1. A, Disease progress in roots and B, dry mass measurements of shoots and roots of hydroponic pepper plants following inoculation with *Pythium aphanidermatum*. Each data point and histogram represents the mean $\pm$ SE for a minimum of six plants. The experiment was repeated four times with similar results for similar inoculum density and vigor; results from one representative experiment are shown.

TABLE 1. Destructive harvest measurements of hydroponic pepper plants following inoculation with *Pythium aphanidermatum*^a

Days after inoculation	Treatment	Shoot/root ratio ^a	Shoot fresh mass (g)	Length of longest root (cm)	Leaf area (cm ²)
-1	Control	9.7	40.9 $\pm$ 1.4	27.8 $\pm$ 1.6	948.9 $\pm$ 17.2
1	Control	10.0 a	47.7 $\pm$ 2.6 a	26.8 $\pm$ 2.0 a	1,106.3 $\pm$ 65.0 a
	<i>Pythium</i>	12.8 a	41.8 $\pm$ 1.7 a	26.8 $\pm$ 3.2 a	988.2 $\pm$ 32.2 a
3	Control	9.7 a	52.5 $\pm$ 4.2 a	27.8 $\pm$ 2.1 a	1,212.0 $\pm$ 94.1 a
	<i>Pythium</i>	11.3 a	44.2 $\pm$ 1.2 a	26.1 $\pm$ 2.1 a	1,051.5 $\pm$ 24.8 a
5	Control	9.2 a	61.5 $\pm$ 5.4 a	27.7 $\pm$ 1.9 a	1,400.7 $\pm$ 139.4 a
	<i>Pythium</i>	14.9 b	48.7 $\pm$ 4.0 b	21.0 $\pm$ 1.6 b	1,128.2 $\pm$ 90.6 b
7	Control	7.1 a	88.9 $\pm$ 4.4 a	30.3 $\pm$ 0.4 a	1,926.3 $\pm$ 86.1 a
	<i>Pythium</i>	16.7 b	50.1 $\pm$ 5.3 b	20.8 $\pm$ 2.8 b	1,131.3 $\pm$ 121.2 b

^a Each value represents the mean $\pm$ SE of six plants per treatment. The experiment was repeated four times with similar results for similar inoculum density and vigor; results from one representative experiment are shown. Values followed by different letters within each day indicate statistical significance using general linear models least square means at α = 0.05.

^b Shoot/root ratio values were calculated from dry mass measurements presented in Figure 1.

spired more water than did the healthy controls (Fig. 2C). This trend became apparent as early as 2 DAI, indicating that despite any root decay associated with discoloration (Fig. 1A) and reduced root length (Table 1), roots and xylem columns of the inoculated pepper plants remained functional with respect to water uptake and movement of water from roots to shoots.

Nondestructive estimations of whole-plant productivity following inoculation with *P. aphanidermatum*. On a per plant basis, within 3 DAI, photosynthetic rates (NCER in the light) of inoculated plants were lower than those of the controls (t test, $P = 0.0001$) (Fig. 3A). No significant difference was observed (t test, $P = 0.05$) in whole-plant dark respiration rate on a plant basis until 7 DAI (t test, $P = 0.0001$).

When the CO_2 exchange rates were recalculated to show total C accumulation with time (Fig. 3B), it was clear that inoculated plants were growing more slowly than the controls largely due to the differences in the photosynthetic uptake of C during the photoperiod. By 7 DAI, the healthy control plants accumulated 28% more biomass than the inoculated plants did (Fig. 3B), which was consistent with data obtained following destructive harvest and measurement of dry mass (Table 1).

Expression of CO_2 exchange rates on a leaf area basis provides an estimate of photosynthesis per unit surface area of leaf canopy available for light interception. No differences in NCER were observed between control and inoculated plants, indicating that the photosynthetic machinery of the infected plants was as efficient on a leaf area basis as that of control plants (Fig. 3C). There was no difference in whole-plant dark respiration rate on a leaf area basis until 7 DAI (t test, $P = 0.0001$). When C accumulation was calculated on a leaf area basis, there was no difference in the diel pattern of C gain and loss during the light and dark periods (t test, $P = 0.05$) (Fig. 3D).

Growth of plants is often expressed on a dry weight basis. When NCER and daily C gain were expressed on a shoot dry mass basis (data not shown), there were no significant differences between the photosynthetic rates and relative growth rates of control and inoculated plants. The pattern of growth on a mass basis was essentially that described previously when calculated on a leaf area basis (t test, $P = 0.0001$).

Predicting changes in plant productivity determined non-invasively. Figure 4 shows correlations between root discoloration with NCER (Fig. 4A), whole-plant dark respiration rate (Fig. 4B), and carbon accumulation (Fig. 4C) expressed on a leaf area and per plant basis. Whole-plant dark respiration was not correlated with root discoloration by *P. aphanidermatum*, but photosynthesis and daily C gain were correlated with root discoloration (t test, $P = 0.0001$).

Figure 5 illustrates clearly that when AQY was plotted against time, the photosynthetic efficiency of the whole canopy of the control plants was greater than that of the infected plants. Based on the best line fit through the data of all experiments, differences in canopy efficiency between control and infected plants occurred 1 DAI (t test, $P = 0.0001$).

DISCUSSION

Monitoring responses of host plants inoculated with *P. aphanidermatum*. It is difficult to accurately detect the onset or monitor the progression of root diseases such as *Pythium* root rot in commercial environments, especially in crops with high plant densities, dense canopies, and reduced accessibility of roots to direct observation. Disease can go undetected when all plants in a cohort are similarly stunted and not exhibiting wilting, chlorosis, or noticeable defoliation (28,39). Disease symptoms such as visible root discoloration and inhibition of root growth in our small-scale hydroponic system (Fig. 1A) were similar to those previously published for hydroponic pepper plants (29). The expansive root browning caused by *P. aphanidermatum* in hydro-

ponically grown peppers agrees with earlier findings for this pathogen in pepper plants grown in sterilized soil (9).

Measuring changes in plant dry weight over time have traditionally provided the raw data to assess plant growth under different conditions, but these analyses require repetitive destructive sampling (12). Destructive analyses of plant growth and development can be enhanced greatly when additional parameters such as CO_2 gas exchange can be quantified noninvasively each day in whole plants (22). What is new from our studies on *P. aphanidermatum* are the results correlating changes in whole-plant transpiration, photosynthesis, respiration, and day and night growth patterns that were measured continuously during the first week following inoculation of the pepper plants. These data reveal changes in the primary plant productivity that were not previously documented in any host species infected with *P. aphanidermatum* or other *Pythium* spp.

Fig. 2. Cumulative water loss via evapotranspiration in hydroponic pepper plants inoculated with *Pythium aphanidermatum* compared with that of control plants expressed **A**, per plant, **B**, per square meter of leaf area driving transpiration, and **C**, per gram of dried roots driving uptake. Each value is a mean $\pm$ SE of four plants in each of two gas exchange chambers. The experiment was repeated four times with similar results for similar inoculum density and vigor; results from one representative experiment are shown.

The manner in which infection of roots by *P. aphanidermatum* alters gas exchange of pepper plants deserves consideration because the leaf canopy is the source of all assimilates for plant growth. However, the leaf canopy is a heterogeneous structure and clearly remote from the sites of infection and disease development in the roots. One explanation for the apparent drop in the photosynthetic capacity observed on a whole-plant basis (Fig. 3A and B), but not on the basis of leaf area (Fig. 3C and D) or biomass (data not shown), is the possibility that somehow *P. aphanidermatum* indirectly altered the architecture of the canopy. Alternatively, water stress may have occurred causing stomata to close. Toxins or phytohormones originating in the infected root systems might have affected leaf metabolism (respiration or photosynthesis) and altered leaf growth and expansion.

In the case of wilting or water stress, differences in stomatal behavior and in the presentation of the leaf canopy can occur, thereby changing the ability of leaves to trap light energy, assimilate CO₂, and partition photoassimilates to developing sinks. For example, a difference in orientation of leaves of infected plants can be subtle and not easily distinguished by destructive analyses alone, but the impact on canopy photosynthesis and on plant growth rate can be dramatic (43). However, in our studies with pepper plants inoculated with *P. aphanidermatum*, when the drop in NCER and C gain were first observed (Fig. 3A and B, respectively; Fig. 5), no early sign of wilting, epinasty, or other canopy symptoms were visible and no difference was found in chlorophyll a/b content of the infected plants compared with control plants (*unpublished data*). Interestingly, plant NCER (Fig. 3A) appeared to be altered before changes in

transpiration (Fig. 2A) were observed. While some stomatal limitations to CO₂ uptake were possible within certain leaves in the canopy, taken together, the whole-plant CO₂ (Fig. 3) and H₂O (Fig. 2) exchange data indicated that any cortical or vascular occlusions that might have resulted following inoculation with *P. aphanidermatum*, and reported in roots infected with other *Pythium* spp. (Stanghellini and Kronland [39] and references therein), were not a primary cause of reduced C fixation and growth inhibition.

We favor an alternative explanation for the differences in plant photosynthesis and biomass growth that were observed on a whole-plant basis (Figs. 2A and B, 3A and B, and 5), yet not seen on a leaf area (Fig. 3C and D) or biomass basis. The data are consistent with the conclusion that the lower rates of whole-plant photosynthesis in the infected plants are attributable to reduced development of the leaf canopy rather than to reduced photosynthesis per unit leaf area. The physically smaller leaf canopy of infected plants compared with control plants (Table 1) provided less leaf area to intercept light energy, and thus reduced C fixation to support growth (Fig. 5).

After assessment of numerous studies on other root diseases (1,5–7,15,16,18,26,30,31,34), it is concluded that our results with *P. aphanidermatum* in peppers are most similar to those for eggplant grown in *Verticillium*-infested soil (14). In eggplant, when photosynthetic and transpiration rates were expressed on a leaf area basis, there were no significant differences between treatments, supporting the conclusion that yield reductions associated with *V. dahliae* resulted from reduced leaf area instead of reduced photosynthetic efficiency per se.

Fig. 3. Effects of root inoculation with *Pythium aphanidermatum* on **A and C**, nondestructive net carbon exchange rate and **B and D**, total carbon accumulation in hydroponic pepper plants, expressed on the basis of **A and B**, the whole plant and **C and D**, the leaf area. Points represent the mean $\pm$ SE of two chambers each containing four plants. The experiment was repeated four times with similar results for similar inoculum density and vigor; results from one representative experiment are shown.

Why would leaf development be altered during root infection by *P. aphanidermatum*? An explanation for reduced leaf development can easily involve any one of the following factors or a combination of several factors including: (i) a limitation on the supply of water for leaf expansion, (ii) a chemical inhibition originating from the root zone, and (iii) an increased competition by the root zone for essential nutrients and photoassimilates that would deprive young developing leaves of essential nutrients for normal development.

The finding that transpiration expressed in absolute terms per plant was reduced only minimally in plants inoculated with *P. aphanidermatum* indicates that events in pathogenesis, such as possible cortical or vascular occlusion, were not a major factor limiting the availability of water for leaf expansion. When expressed per unit root mass, transpiration was actually greater in the inoculated plants compared with the controls (Fig. 2C). Thus, it appears that loss of root integrity associated with intense colonization by the pathogen and progressive discoloration did not significantly reduce water uptake by the plant. Because these observations did not support the view that the leaf canopy was

under sufficient water stress to limit leaf expansion, other mechanisms are likely involved. One alternative explanation is that leaf expansion was restricted through the effects of signals transferred from the root system to the leaves, such as via the transpiration stream. The strong transpiration in the inoculated peppers would be expected to allow continuous transfer of such signals.

The possibility that root infection can bring about shifts in acropetal transport of phytohormones or other chemical signals from the roots to the shoots, and alter developmental processes in the leaves, is consistent with several observations reported in the literature. In pepper plants grown in vermiculite and inoculated with *V. dahliae*, it was found that the concentration of ABA in the xylem sap declined during the first week after inoculation compared with that in noninoculated controls (15). At 20 DAI, the transpiration rates of the inoculated plants were comparable to those of the controls when expressed per unit area of leaf, but significantly lower on a per plant basis. In this study, infection of the roots by *V. dahliae* increased leaf senescence and chlorophyll degradation and decreased leaf conductance and photosynthesis relative to the controls. *P. ultimum* and *Pythium* group F were reported to produce indole-3-acetic acid (IAA) and tryptophol in culture (33), while cell enlargement and callus production in roots infected by *P. dissotocum* pointed to possible involvement of plant growth regulators in pathogenesis in pepper plants (29). In bentgrass, IAA content of the leaves was about 200 times greater in plants inoculated with *P. aphanidermatum* than in noninoculated plants, indicating that IAA is xylem mobile during pathogenesis (38). Root tips, apparently capable of synthesizing growth regulators, are commonly infected by *Pythium* spp. which might thus influence production of growth regulators at these sites. Aiken and Smucker (2) argued that root tips can transmit, from the root environment, signals capable of regulating plant growth. Farrar and Jones (13) reviewed evidence that roots exert some control over carbon acquisition from source leaves, thus limiting photosynthate available for new leaf expansion.

Growth of leaf area depends on division and enlargement of the leaf cells. Division of leaf cells is limited especially by the supply of photoassimilates and is not markedly affected by availability of water and nutrients (24). Cell expansion, however, is directly related to turgor pressure and dependent upon the availability of water and solutes but is relatively insensitive to the availability of photoassimilates. Given that water movement was not impaired in the inoculated pepper plants, the observed reduction in leaf area

Fig. 4. A, Whole plant photosynthesis, B, dark respiration rate, and C, daily carbon accumulation in hydroponic pepper plants in relation to estimates of percent root discoloration in pepper plants inoculated with *Pythium aphanidermatum*. Photosynthetic and respiratory rates were extracted from the data described in Figure 3A and B. Each point represents the mean of the gas exchange calculations of *Pythium aphanidermatum*-inoculated plants expressed as percentages of control values. Graphs include values from four experimental repetitions.

Fig. 5. Apparent quantum yield of hydroponic pepper plants following inoculation with *Pythium aphanidermatum*. Each point represents an hourly integral from one gas exchange chamber. The experiment was performed four times; results from one representative experiment are shown.

expansion was probably related to limited availability of photoassimilates. Because the rate of photosynthesis per unit leaf area in our peppers was generally unaffected in the inoculated plants until about 5 DAI (t test, $P = 0.0001$), a limited availability of photoassimilates to support division of leaf cells might be expected to have arisen from increased sink strength for photoassimilates elsewhere in our pepper plants. Patterns of relative sink strength and nutrient allocation are recognized as important factors influencing the rate of development and expansion of leaves (41).

A reduced availability of photoassimilates for leaf growth in the inoculated plants could have arisen from strong sink development in the infected roots. Factors contributing to increased sink strength of the infected roots could include demands by the pathogen and any other microbes associated with the roots, increased exudation of carbon compounds into the nutrient solution, energy demands for production of defense compounds by root cells (19), and overall increased metabolism. Observations in the inoculated peppers that whole-plant dark respiration rates were higher than those in the control plants, even though the infected plants were smaller than the controls, were consistent with the view that demand for photoassimilates in diseased roots was high.

We know of no published reports on demands of *Pythium* spp. for host carbohydrates, but other pathogens are known to utilize simple sugars from the host (36,40). *Phytophthora palmivora* hydrolyses sucrose extracellularly and takes up glucose and fructose in vitro (37). Increased partitioning of photoassimilates was observed in mycorrhizal clover roots (44). Given that infection by *P. aphanidermatum* involves a biotrophic phase, it is possible that initial colonization involves considerable sink strength. This possibility, and the intriguing prospect that new chemical signals may be transported acropetally from the diseased root systems to the competing shoot system via the xylem stream, remains to be investigated.

In summary, the extent to which changes in photosynthetic behavior of a leaf canopy can be used to monitor disease progress in plants depends greatly upon the parameters measured and the manner in which photosynthetic behavior is expressed. Although leaf photosynthesis may not be affected when expressed on a per area basis, there can be little doubt that reduced canopy photosynthesis in pepper plants infected by *P. aphanidermatum* is largely a consequence of reduced leaf area development. As pointed out by other studies on photosynthesis, to relate photosynthesis to growth, it is necessary to integrate CO₂ assimilation over the photoperiod (12). Our data clearly show that the control plants, in which leaf development continued throughout the experimental period, were more efficient in trapping available overhead radiation than the smaller leaf canopy of the inoculated plants. In addition, our data predict that photosynthetic signatures other than CO₂ uptake of the young or older leaves may provide signals of progression of *Pythium* infection; however, more research is required to test this hypothesis. Future research into leaf turnover, export, and partitioning of fixed C in plants infected with *P. aphanidermatum* is also warranted, as are investigations into the nature of signals moving from the roots to the shoots that might contribute to our understanding of reduced leaf development during infection.

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