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Effects of *Verticillium dahliae* on Gas Exchange of Potato

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ABSTRACT

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Growth-chamber and field experiments were conducted to determine the effects of *Verticillium dahliae* on gas exchange of potato. Infection caused lower stomatal conductance, lower transpiration, and higher leaf temperature in leaves of all ages. Infection reduced carbon assimilation rate in high light, but reductions were not always observed in low light. Infection did not affect dark respiration. Intercellular CO_2 concentration was decreased and leaf water use efficiency was increased in diseased nonsymptomatic leaves. However, diseased chlorotic leaves often did not show this effect. The rate of stomatal opening in the morning was slower for diseased leaves compared with nondiseased leaves. Infection did not affect the relationship between assimilation rate and stomatal conductance in high light. Changes in gas exchange caused by infection with *V. dahliae* matched previously reported effects of drought on potato gas exchange.

Additional keywords: photosynthesis, *Solanum tuberosum*, Verticillium wilt.

Verticillium dahliae Kleb. is the primary component of the potato (*Solanum tuberosum* L.) early dying syndrome in Wisconsin (15). Potato early dying is characterized by gradual leaf chlorosis, necrosis, and defoliation beginning at the base of the plant. Wilting of leaves or parts of leaves sometimes is observed. In indeterminate cultivars like Russet Burbank, the first symptoms usually are observed in mid-July. By the end of August, foliage loss may approach 100% in severe cases. Yield losses due to potato early dying range from insignificant to 50%, depending on soil inoculum levels, weather, cultivar, fertility, irrigation, and presence of other members of the disease complex (23,24).

To integrate the various factors that determine yield loss, several research groups have coupled *V. dahliae* modules to crop growth simulation models (1,10,14). Each of these models includes postulated effects of infection on host photosynthesis. However, there are few reports of experiments that demonstrate such effects. Mathre (18) found reduced photosynthetic rates in leaf disks from cotton infected with the defoliating strain of *V. dahliae*. The decrease occurred before visible chlorosis or wilting of the sampled leaves. In a preliminary report, Pennypacker et al (21) found reduced photosynthesis in alfalfa infected with *V. albo-atrum*. More data are required, especially data from intact leaves, from the field, and using other hosts or strains of *Verticillium*.

Information about the effects of infection by *V. dahliae* on host transpiration rate is more abundant. Reductions in transpiration rates were reported in chrysanthemum (11,17), cotton (29), potato (12), and tomato (28). The reports from potato and tomato were based on weight loss of excised leaves. This method allows significant changes in leaf water potential during measurement and therefore is biased. More accurate measurements of the effect of infection by *Verticillium* on potato transpiration are needed.

Another reason for studying gas exchange characteristics of diseased leaves is for possible use in epidemiological studies as a nondestructive means of determining incidence or severity of infection by *V. dahliae*. Of course, detection of infection would require the pathogen to produce a characteristic gas exchange

"signature." Relationships between gas exchange parameters also could provide information about the mechanisms involved in pathogenesis at the organ or tissue level.

The objectives of this research were to describe the qualitative effects of infection by *V. dahliae* on the net photosynthesis, stomatal conductance, transpiration, leaf temperature, and intercellular CO_2 of leaves of potato cultivar Russet Burbank, and to investigate the relationships among these variables in infected versus healthy plants. A preliminary report has been published (4).

MATERIALS AND METHODS

Five experiments (C1 through C5) were conducted in walk-in growth chambers (2.6 × 3.6 m) at the University of Wisconsin Biotron in Madison, WI. Experiments C1 through C3 were designed to test the physiological effects of infection by *V. dahliae* on leaves of different ages. The purposes of experiments C4 and C5 were to obtain gas exchange diurnal curves and light response curves, respectively.

Experiment C1. Eight pathogenic isolates of *V. dahliae* from potato were grown on 10% strength potato-dextrose agar (Difco Laboratories, Detroit, MI) at 20 C for 2 wk. The agar containing the fungal colonies was removed from petri dishes and homogenized in a blender at low speed. It then was mixed thoroughly by hand with peat-vermiculite (1:1 v:v; bulk density 0.2 g/ml) potting medium at the rate of 1 ml/L of soil mix. Control potting mix received no inoculum. Soil samples were collected 3 wk after planting, dried at room temperature for 2 wk, then assayed by plating soil dilutions on a polypectate selective medium as described by Nicot and Rouse (20). Estimated inoculum level was 1,100 propagules per gram (ppg) of soil (i.e., 220 propagules per milliliter).

Plants were obtained from axenically propagated, virus-free cuttings of potato (cultivar Russet Burbank) and were transplanted to 20-L plastic pots containing infested or noninfested peat-vermiculite potting medium. There were 11 infested pots and nine control pots. Treatments were completely randomized in the growth chamber.

Light was supplied by cool white fluorescent (1,270 W m^{-2} electric power at the top of the chamber) and incandescent (474 W m^{-2}) lamps. Lights were turned on at 0600, and the photoperiod was 16 hr. Incident photosynthetically active radiation (PAR) at canopy height started near 420 μmol m^{-2} s^{-1} and increased due to plant growth to around 550 μmol m^{-2} s^{-1} by the end of the experiment. Day temperature was 25 ± 0.5 C, and night temperature was 15 ± 0.5 C. Relative humidity was maintained at 50 ± 5%. All plants automatically were watered to excess four times per day with one-quarter strength Hoagland's solution.

Each plant produced a single main stem, which was staked to prevent lodging. Axillary branches were pruned to three basal branches per main stem. When a stem terminated in an inflorescence, the first subapical branch was considered the new main stem. Individual main stem leaves were tagged every 4 days beginning 31 days after planting (referred to simply as days). The youngest leaf in which the terminal leaflet was greater than 3 cm long was chosen for tagging. The set of leaves tagged on a particular day will be referred to as a cohort. Leaf age was defined as the number of days since tagging. Plants had tuberized and produced 35–40 leaves by the time the experiment was terminated at 62 days.

Disease incidence based on visible symptoms was sometimes difficult to determine because symptoms were often indistinct or transitory. However, each inoculated plant had expressed typical symptoms of Verticillium wilt (leaf wilting or unilateral necrosis) on at least one leaf by the end of the experiment. Controls remained symptomless. *V. dahliae* was reisolated on polypectate medium from stem bases of all inoculated plants, but not from controls.

Experiment C2. Experiment C2 was similar to C1 with the following modifications. To promote tuberization, the photoperiod was changed to 14 hr. To achieve a more realistic diurnal pattern, lights came on at 10% intensity at 0600, then

went to full intensity at 0700. At 1900 they were switched to 10%, and at 2000 they were switched off. Pots were watered with one-eighth strength Hoagland's solution to decrease the possibility of salt accumulation. Leaf tagging began earlier (17 days); the experiment lasted longer (92 days); and leaves were tagged only every 8 days. The same infested peat-vermiculite stock soil was used as in the first experiment. The stock soil had been dried and stored for 5 mo at room temperature. This treatment killed most of the short-lived propagules. Estimated inoculum level was 120 ppg based on soil assay. There were eight replications of the two treatments.

By the end of the experiment, seven of eight inoculated plants had expressed typical Verticillium wilt symptoms on at least one leaf. Controls remained symptomless. *V. dahliae* was reisolated from all inoculated plants, but not from controls.

Experiment C3. Growth-chamber experiment C3 was similar to C2 with the following modifications. Night temperature was increased to 20 C to promote disease development. To more closely match the field situation, plants were grown in Plainfield loamy sand (bulk density of 1.4 g/ml). The soil was pasteurized at 65 C for 30 min to eliminate pathogenic microorganisms. The inoculum was modified to improve the shelf life and to reduce the number of short-lived conidia. Three isolates of *V. dahliae* from potato were grown on autoclaved rye grains at 20 C for 4 wk. The infested grain was dried for 2 wk at room temperature, then ground in a Wiley mill. Dried, ground rye grain inoculum containing microsclerotia of *V. dahliae* was added to pasteurized Plainfield loamy sand at the rate of approximately 18 g/L of soil 2 mo before the start of the experiment to stabilize populations before planting. This stock inoculum was mixed with the potting soil at 1:9 and 1:99 to produce a high dose (179 ppg by soil assay) and low dose (18 ppg by calculation), respectively. There were six replications of the three treatments. The experiment was terminated at 104 days.

By the end of the experiment, all inoculated plants had expressed typical Verticillium wilt symptoms on at least one leaf. Controls remained symptomless. *V. dahliae* was reisolated from lower, middle, and upper stem segments from all inoculated plants. *V. dahliae* also was reisolated from one of the six controls. The fungus was detected in the stem base but was not isolated from the middle or upper stem or from any petioles. The infection appeared to be in the latent phase as defined in the companion paper (5). The source of contamination is not known.

Experiment C4. The fourth growth-chamber experiment was dedicated to generating gas exchange diurnal curves. Methods were similar to those used in experiment C3. Inoculum levels were 486 (assayed) and 49 (calculated) ppg for the high and low dose, respectively. At the time of measurement, inoculated plants were each expressing symptoms of infection, and controls were symptomless. The experiment was terminated at 107 days.

Experiment C5. Growth-chamber experiment C5 was designed to obtain gas exchange light response curves. Methods were similar to those used in experiment C3 with the following modifications. To save preparation time, plants were grown in peat-vermiculite rather than Plainfield loamy sand. Dried rye grain inoculum was added to the potting mix at 0.1 g/L (high dose) or 0.025 g/L (low dose) 2 days before planting. There were six replications of the low dose, six replications of the high dose, and 12 controls. Estimated inoculum levels (by soil assay) were 450 ppg for the low treatment and 1,565 ppg for the high treatment. The experiment was terminated at 88 days. At the time of measurement, inoculated plants were each expressing symptoms of infection, and controls were symptomless.

Gas exchange measurements. In experiments C1 through C3, net photosynthesis and transpiration of all tagged leaves were measured every 4 days between 0800 and 1500 with a Li-Cor LI-6200 portable photosynthesis system (Li-Cor, Inc., Lincoln, NE). The CO_2 analyzer was calibrated daily with a known CO_2 concentration. All CO_2 concentrations are given in parts per million (ppm). Elevation was 265 m above sea level, and atmospheric pressure averaged 990 mb. Gas exchange measurements were done on terminal leaflets in situ, and care was taken to

disturb the leaf as little as possible. After a leaf was enclosed in the chamber, 8–10 sec were allowed for the system to stabilize, then a 10-sec reading was taken. Measurements were done under ambient light conditions, which were always below saturating levels. Investigators wore gas masks connected to a vacuum line to prevent breathing on the plants during measurements. Leaves that had noticeable chlorosis or loss of turgor were noted. Leaves with severe turgor loss were considered to have senesced.

Photosynthesis calculations were based only on living leaf area. Photosynthesis, stomatal conductance, transpiration, leaf temperature, air temperature, PAR, ambient CO_2 concentration (C_a), and intercellular CO_2 concentration (C_i) were calculated by means of the LI-6200 software. Equation B18 of von Caemmerer and Farquhar (30) was used to calculate C_i. Because bias in the C_i calculation caused by stomatal patchiness (7,27) was not routinely tested, the statistic will be referred to as apparent C_i. Photosynthesis, stomatal conductance, and transpiration values represent the sum from both sides of the leaf. Leaf boundary layer conductance was estimated using an empirical function of leaf area. Transpiration rates measured in the cuvette were not necessarily the same as the normal leaf transpiration in ambient conditions due to changes in the boundary layer conductance and vapor-pressure deficit. The difference between air temperature and leaf temperature was determined from the chamber air temperature thermistor and the leaf thermocouple measurements.

Analysis of variance. Data from growth-chamber experiments C1 to C3 were analyzed as a repeated measures design with plants as the main experimental unit and leaf cohorts representing repeated measurements within the plants. Because of defoliation and normal senescence, all data sets were unbalanced with respect to cohorts by the end of each experiment. Therefore, a subset of the oldest leaf cohorts was deleted to obtain a balanced data set. This reduced data set was analyzed with SAS procedure GLM using the REPEATED statement, and the Greenhouse-Geisser correction for autocorrelation was applied to degrees of freedom for F-tests involving cohorts (25). Infection by *V. dahliae* tended to increase the standard error of gas exchange measurements in some cases. However, the heterogeneity of variance across treatments and leaf ages was low. Therefore, the analysis of variance (ANOVA) was carried out without transforming the data.

Diurnal curves. The diurnal gas exchange curves of four replicates of inoculated plants and controls were compared at 103 days in experiment C4. Gas exchange of symptomless, unshaded leaves 12–20 days old was measured approximately every hour beginning 40 min after lights came on at 0600. The initial reading was under the "dawn" conditions of 10% light. The penultimate reading was under "dusk" conditions of 10% light, and the last reading was 1 hr after dark.

Light response curves. The photosynthetic response of leaves to incident light was tested on plants from experiment C5 at 80–82 days. Leaves were 10–12 days old. Each plant was moved from the original growth chamber to a darkened growth chamber, and a portion of the terminal leaflet of a symptomless leaf was placed in the LI-6200 leaf cuvette. The leaf was illuminated by a Sylvania 300-W ELH bulb in a Kodak slide projector. Light level at the leaf was adjusted by changing the distance between projector and leaf or by interposing layers of 2-mm-mesh wire screen. The initial PAR level was $1,500 \pm 50 \mu$mol m^{-2} s^{-1}, and the leaf was allowed to equilibrate at that level for 30 min. During equilibration, the LI-6200 was operated in the open mode, and C_a was 340 ± 5 ppm. Leaf temperature was held at 25 ± 1.5 C by changing the temperature of the growth chamber. The vapor-pressure deficit was maintained at 15 ± 1 mb by changing the humidity of the growth chamber and by the proportion of air flow passed through magnesium perchlorate desiccant. Photosynthetic rates were obtained by switching to the closed mode and measuring CO_2 depletion. Photosynthetic rates were obtained at a mean ambient CO_2 of 300 ± 2 ppm. PAR levels of approximately 1,500, 1,000, 600, 400, 200, 70, and 0 μmol m^{-2} s^{-1} were tested in that order with a 5-min equilibration time between each level.

Field experiments. Wisconsin certified potato seed (cultivar

Russet Burbank) were planted on 5 May 1988 on Plainfield loamy sand (bulk density of 1.4 g/ml) at the University of Wisconsin Hancock Research Station in Hancock, WI. The field previously had been fumigated with different rates (370 to 560 L/ha) of metham sodium (32.7% a.i.). Ground, dried rye grain inoculum of three isolates of *V. dahliae* was rototilled into some plots 3 days before planting. The low dose received 11.8 g m^{-2}; the high dose received 58.9 g m^{-2}; and the control received no inoculum. Soil assays estimated inoculum levels of 0 for the control, 9.3 ppg for the low treatment, and 39.3 ppg for the high treatment at 8 wk after planting.

Isolations were made on polypectate medium from stem bases of 20 stems per plot at 105 days. Incidence of infection by *V. dahliae* was 19, 79, and 64% for the control, low dose, and high dose, respectively. Contamination of controls may have been from a small number of soilborne microsclerotia that escaped soil fumigation or from tuber-borne inoculum.

Fifty percent emergence was achieved on 22 May. Plots were watered by center-pivot irrigation three times per week. Standard best management practices according to University of Wisconsin recommendations were followed for fertilizer application, weed control, foliar disease control, and insect control. Vines were killed on 14 September.

Gas exchange was measured as described above with the following modifications. Gas masks were not worn in the field. Measurements were taken by block between 0900 and 1500 approximately every 2 wk. Data were taken only on sunny days after irrigation or rain to assure saturating light and adequate soil moisture. Three stems were sampled systematically in each plot to avoid selection bias. A 3.7-m transect was placed across the rows in an undisturbed portion of the plot. Stems were sampled at 0.9-m intervals along the transect. The uppermost unshaded terminal leaflet with a leaf area of at least 7 cm^2 was chosen for measurement on each stem. Leaves with necrotic areas or severe turgor loss were not sampled. Plants with visible symptoms of blackleg caused by *Erwinia carotovora* were not included in the data set. Data were averaged by plot before performing the analysis of variance using SAS procedure GLM (25). There were three treatments and four blocks in the randomized block design.

At 99 and 104 days, special data sets were collected with increased subsample sizes to increase statistical precision. Eight stems per plot were sampled from the high inoculum dose and control, but the low inoculum dose was not sampled.

The experiment was repeated in 1989. The field was fumigated in the fall of 1988 and again in the spring of 1989 with 470 L/ha of metham sodium. The low inoculum dose received 8.1 g m^{-2} of ground rye grain inoculum plus 32.3 g m^{-2} of ground noninfested rye; the high dose received 40.4 g m^{-2} of ground rye inoculum; and the control plots received 40.4 g m^{-2} of ground noninfested rye. Soil inoculum levels were 14.2 ppg for the high *Verticillium* treatment, 4.4 ppg for the low *Verticillium* treatment, and 0.6 ppg for the uninoculated controls at 16 wk after planting. At 98 days, incidence of infection by *V. dahliae* was 42, 92, and 95% for the control, low dose, and high dose, respectively.

The planting date was 10 May, and 50% emergence was achieved on 29 May. The number of stems sampled per plot was increased to four or five. On 23 June (44 days), an application of a tank mix of a tin fungicide and pyrethroid insecticide proved phytotoxic, and about 10% of the leaf area was damaged in all plots. Growing points were not injured, and new growth was unaffected. Vines were killed on 14 September.

RESULTS

The companion paper (5) describes the complete time course of gas exchange effects for each of these experiments. The following data are from near the end of each experiment when disease effects were most distinct. The effects of infection by *V. dahliae* on gas exchange at earlier dates were smaller but qualitatively similar.

Gas exchange effects. In experiment C1 at 62 days, top leaves of inoculated plants averaged 36% lower net photosynthesis, 55%

lower transpiration, 67% lower stomatal conductance, 1.0 C higher leaf temperature, and 18% lower apparent intercellular CO_2 concentration, relative to controls (Fig. 1). Inoculum treatment main effects were statistically significant for each variable at $P \leq 0.005$. Cohort main effects were significant for photosynthesis, stomatal conductance, and transpiration but not for apparent C_i or the difference between air temperature and leaf temperature at $P \leq 0.05$. Youngest leaves showed highest rates of photosynthesis and transpiration. There was no interaction of inoculum treatment and cohort for any of the responses. In

other words, all cohorts showed treatment effects of equal magnitude. However, on a percentage basis, effects were greatest for the lower leaves for photosynthesis, stomatal conductance, and transpiration because controls were lower for these leaves.

In experiment C2 at 92 days, the response of each variable showed the same trends as in experiment C1, and P-values ranged from 0.0571 to 0.1685 for treatment differences. The inoculum density (24 propagules per milliliter) in this experiment was about one tenth of the density in the other experiments. This might explain the small size of treatment differences. Because no differ-
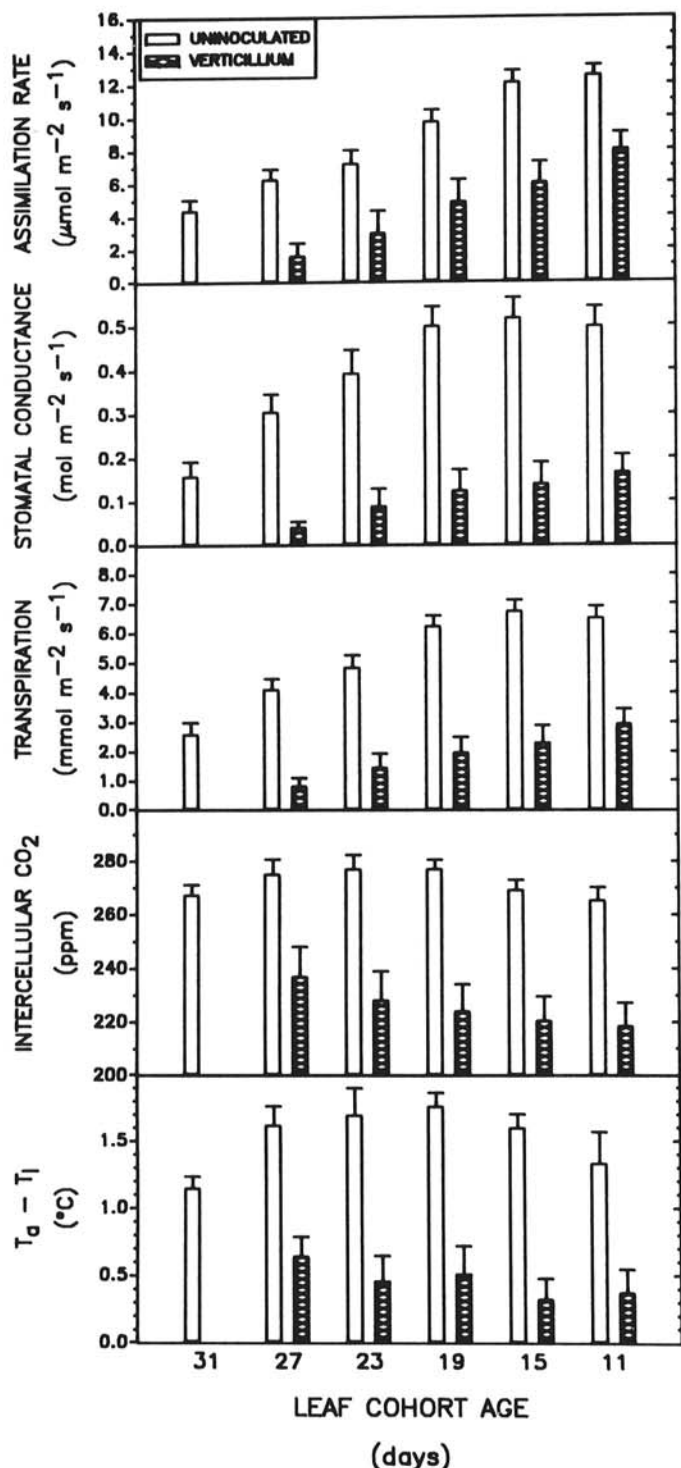

Fig. 1. Effects of infection by *Verticillium dahliae* on potato leaf gas exchange variables and air temperature (T_a) minus leaf temperature (T_l) at 62 days in growth-chamber experiment C1. Data are from 11 inoculated plants and nine controls. The oldest cohort (set of leaves tagged) of every inoculated plant senesced. Bars represent one standard error.
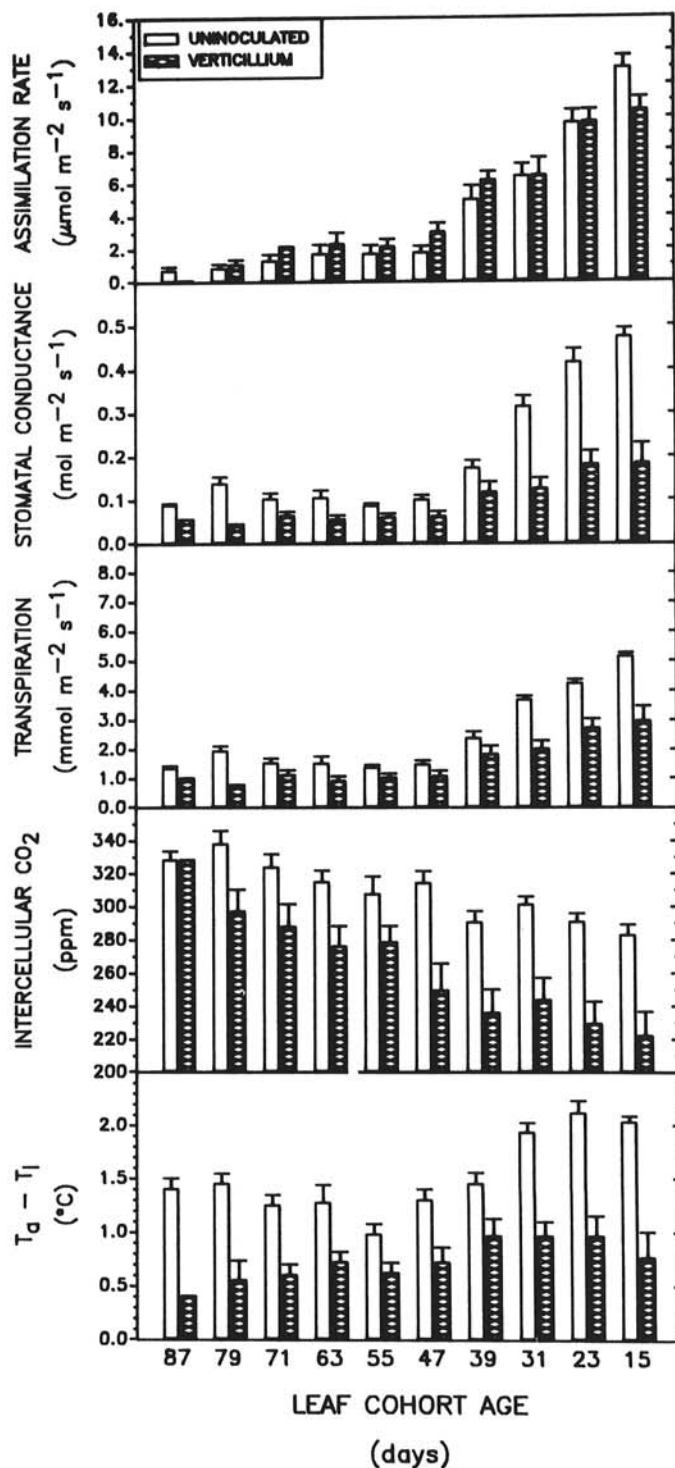
Fig. 2. Effects of infection by *Verticillium dahliae* on potato leaf gas exchange variables and air temperature (T_a) minus leaf temperature (T_l) at 104 days in growth-chamber experiment C3. Data are from six inoculated plants and six controls. Bars represent one standard error.

ences were significant at $P = 0.05$, the results will not be presented.

In experiment C3 at 104 days, top leaves of plants which received the high inoculum dose averaged 20% lower net photosynthesis, 43% lower transpiration, 61% lower stomatal conductance, 1.3 C higher leaf temperature, and 21% lower apparent C_i, relative to controls (Fig. 2). The effects of the low inoculum dose were intermediate between the high dose and control (data not shown). The interaction of inoculum treatment and cohort was significant for stomatal conductance, transpiration, and leaf temperature difference ($P \leq 0.003$). In each case, the effect of inoculation was greater in magnitude in the youngest cohorts. Inoculum treatment had a significant main effect on C_i ($P = 0.002$) but not on assimilation rate ($P = 0.982$). As in experiment C1, youngest cohorts showed the highest rates of photosynthesis and transpiration.

Field data at 104 days in 1988 corroborated the growth-chamber data (Fig. 3). Top leaves of inoculated plants averaged 40% lower net photosynthesis, 49% lower transpiration, 68% lower stomatal conductance, 1.6 C higher leaf temperature, and 22% lower apparent C_i, relative to controls. All differences were significant at $P \leq 0.03$.
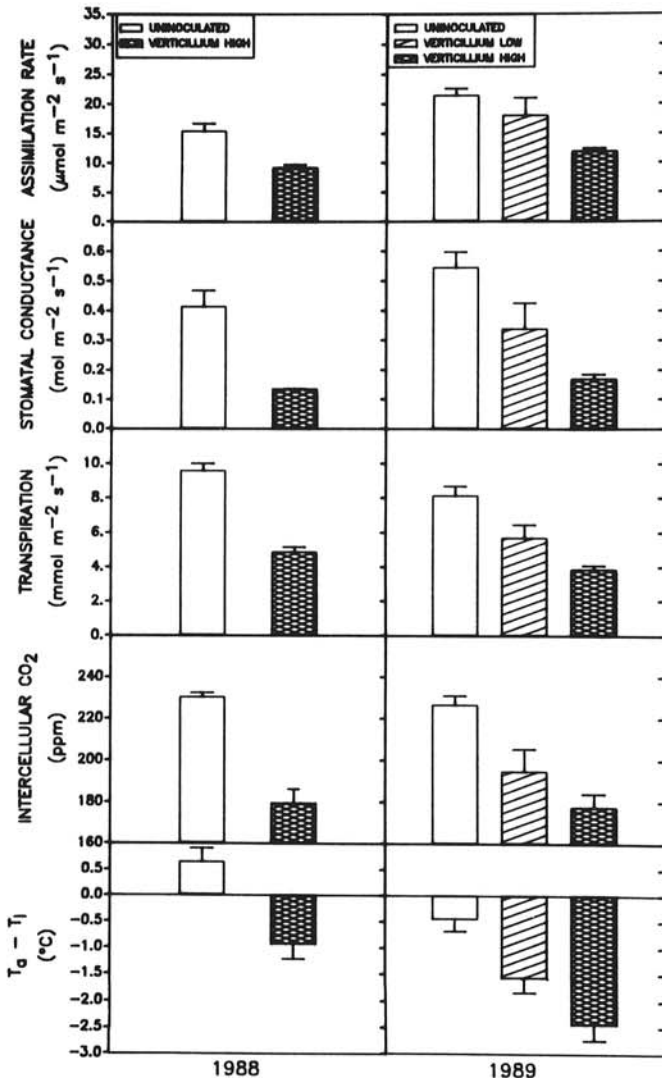
At 98 days in 1989, top leaves of plants in the high inoculum treatment averaged 45% lower net photosynthesis, 53% lower transpiration, 69% lower stomatal conductance, 2.0 C higher leaf temperature, and 22% lower apparent C_i. The low inoculum dose was consistently intermediate between the high dose and the control with respect to each variable (Fig. 3). Inoculum dose had a significant effect on all variables at $P \leq 0.02$. Infection by *V. dahliae* had little or no effect on the relationship between assimilation rate and stomatal conductance in the field (Fig. 4A and B).

Diurnal curves. Infection had no effect on the diurnal pattern of assimilation rate in experiment C4 except in the most severely diseased leaf (Fig. 5). In contrast, the diurnal curve of stomatal conductance was depressed in all infected leaves, especially in the morning. Both stomatal conductance and assimilation rate showed an afternoon decrease beginning about 1400. Leaves from both treatments exhibited almost complete stomatal closure in the dark (after 2000). Dark respiration was not affected.

Assimilation versus light. The relationship between assimilation rate and incident PAR was examined for a population of leaves of different ages and in different light environments in experiment C1 at 62 days. Infection by *V. dahliae* tended to reduce the light use efficiency (LUE, mol CO_2 fixed per mol PAR) (Fig. 6A). Some severely affected chlorotic leaves had negative carbon assimilation rates.

The responses of carbon assimilation rate to incident PAR of three leaves from control plants and three nonsymptomatic leaves from inoculated plants were studied in experiment C5 (Fig. 6B). Infection had no significant effect on dark respiration or the initial slope of the response curve, but above 100 μmol m^{-2} s^{-1} of light, two of the inoculated leaves showed a pronounced flattening of the response curve compared with the controls. The

Fig. 3. Effects of infection by *Verticillium dahliae* on potato leaf gas exchange variables and air temperature (T_a) minus leaf temperature (T_l) for young fully sunlit leaves in field experiments at 104 and 94 days in 1988 and 1989, respectively. In 1988, data were collected from four inoculated (high inoculum dose) plots and four controls with eight plants per plot and one leaf per plant. In 1989, data were collected from four plots per inoculum treatment, and four to five plants were sampled per plot with one leaf per plant. Bars represent one standard error.
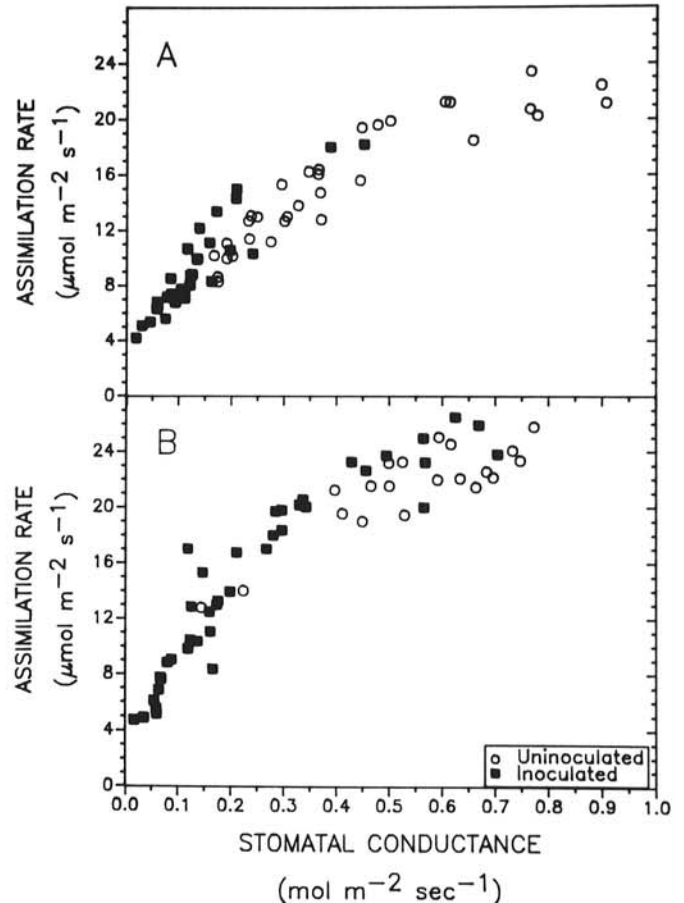

Fig. 4. Effects of infection by *Verticillium dahliae* on potato leaf carbon assimilation rate versus stomatal conductance for young, fully sunlit leaf populations in: **A**, 1988 field experiment at 104 days, and **B**, 1989 field experiment at 98 days.

third inoculated leaf was similar to the controls. Assimilation rate of all leaves was saturated at about 1,000 μmol m^{-2} s^{-1} of light. The two moderately diseased leaves had maximum assimilation rates of one half the control rates.

Water use efficiency versus light use efficiency. The graph of water use efficiency (WUE, mol CO_2 fixed per mol H_2O transpired) versus LUE at 62 days included leaves in different light environments and of various ages (Fig. 7A). The light response curve over the range of light levels used is approximately linear for healthy leaves (Fig. 6A). Therefore, LUE should be relatively constant for uninfected leaves. The graph has been divided arbitrarily into six sections. Leaves from control plants tended to fall into the sections labeled 1 or 2. Leaves from inoculated plants fell into sections 2–6. Most infected leaves in section 6 were chlorotic. Some controls occasionally fell into section 6 (not shown in this data set). Section 6 seemed to be a senescence region for both infected and control leaves. The relationship between WUE and LUE was reproducible among growth-chamber experiments and dates, although leaves rarely fell into section 6 until near the ends of the experiments.

Because light levels in the field were above saturation, LUE was calculated as assimilation rate divided by 1,000 μmol m^{-2} s^{-1} PAR (i.e., the saturation point). The curvature in the light response curve near saturating levels caused LUE from the field data to be lower than LUE from growth-chamber data. Nevertheless, field data showed essentially the same relationship between WUE and LUE (Fig. 7B). Note, however, that the positions of the points with respect to the axes were shifted. In fact, the positions of the points with respect to the axes were different on different days in the field. This probably reflected changes in transpiration caused by differences in ambient temperature and humidity.
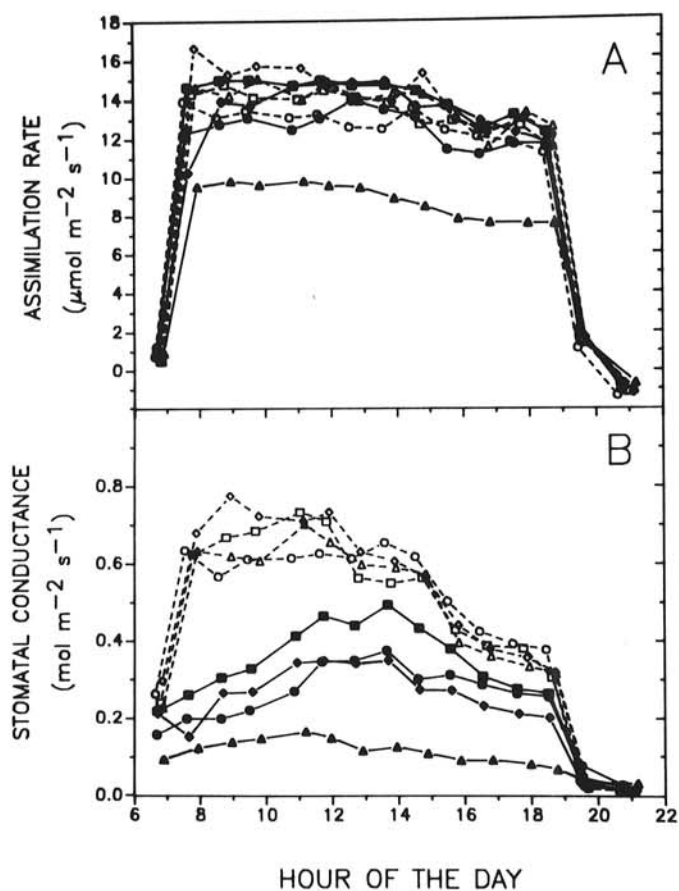
DISCUSSION

This study and a similar study conducted at the same time in the Netherlands (13) are the first reports of the effects of infection by *V. dahliae* on assimilation rate of intact leaves. They are also the first reports of data from the field and the first reports from potato. This research corroborated a previous report (18) that infection by *V. dahliae* decreased the carbon assimilation rate of cotton leaf disks in an in vitro assay. Duniway and Slatyer (8) also found reduced photosynthesis and transpiration in tomato infected with *Fusarium oxysporum*.

The reduction in photosynthesis was variable. In the field, statistically significant reductions of 40 and 45% were found in top leaves at the end of the season in 1988 and 1989, respectively. In growth-chamber experiment C1, there was a significant decrease in assimilation rate across all leaf cohorts, and a similar trend was observed in experiment C2. However, in experiment C3, despite significant changes in stomatal conductance, transpiration, C_i, and leaf temperature, there was no statistically significant effect on assimilation rate. Similarly, three of four infected leaves in the diurnal curve experiment exhibited reduced stomatal conductance, but no change in assimilation rate was detected.

In both the field and growth chambers, the supply of CO_2 inside the leaf (C_i) was reduced. The variable effect of reduced C_i on assimilation rate depended on whether light or CO_2 was the most limiting factor. In the field in full sunlight, this reduced supply of CO_2 was the limiting factor; therefore assimilation rate was decreased. However, in growth chambers under subsaturating light, the response of assimilation rate to decreased CO_2 supply
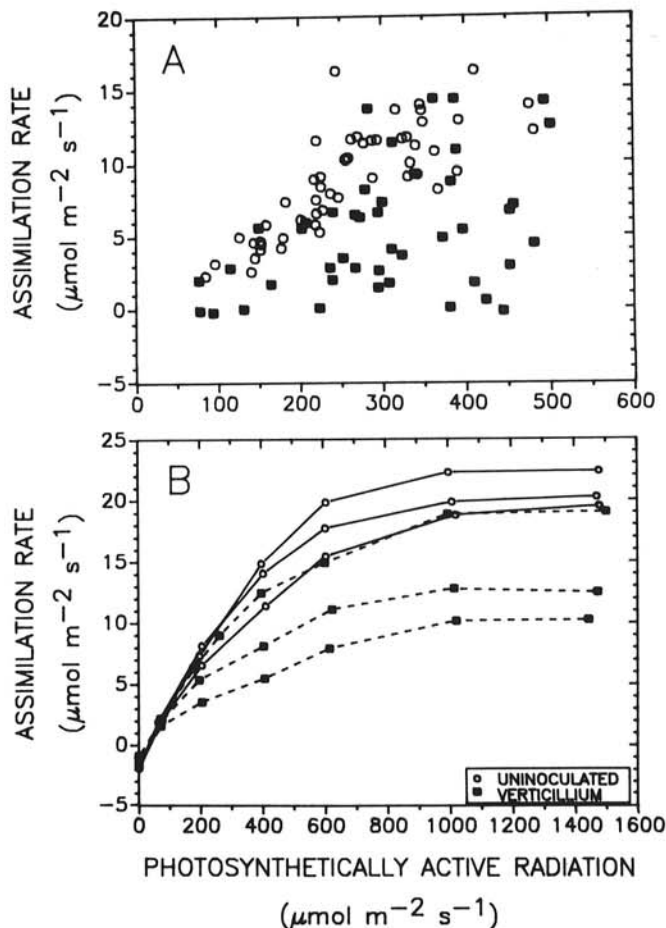

Fig. 5. Effects of infection by *Verticillium dahliae* on diurnal curves of assimilation rate **(A)** and stomatal conductance **(B)** for healthy (open symbols) and diseased (closed symbols) 12- to 20-day-old potato leaves from experiment C4 at 103 days. Each line represents repeated measurements on a single leaf, each on a different plant.

Fig. 6. Effects of potato infection by *Verticillium dahliae* on: **A,** carbon assimilation rate versus incident photosynthetically active radiation for a population of leaves of various ages from experiment C1 at 62 days, and **B,** light response of carbon assimilation rate for three control leaves and three symptomless leaves 10–12 days old from inoculated plants in experiment C5 at 80–82 days.

was often small or undetectable. Only large reductions in C_i would cause a noticeable reduction in assimilation rate. This concept accords well with the diurnal curve data, where only the leaf with the lowest stomatal conductance and the lowest C_i (data not shown) had a decreased assimilation rate.

Data in Figure 6B support the conclusion that the reduction in photosynthetic rate in infected leaves is a function of light level. At approximately 70 μmol m^{-2} s^{-1} PAR, the average reduction was 1%, whereas at 1,500 μmol m^{-2} s^{-1} PAR, the reduction was 33%. Similar light effects were reported in other host-pathogen combinations (19,22,26).

This explanation does not appear to account for the difference between growth-chamber experiments C1 and C3. In both experiments, the light levels were similar, and apparent C_i was decreased to the same level by infection. However, only experiment C1 exhibited significant decreases in assimilation rates. At least three factors may have contributed to the discrepancy. First, bias caused by patchy stomatal conductance would lead to overestimation of C_i (7,27). The true C_i for some leaves in experiment C1 may have been lower than the apparent C_i. In that case, CO_2 supply still could explain the differences between the experiments. Second, comparing population average C_is may be inappropriate when the relationship between assimilation rate and C_i is nonlinear. This might be called a bulking or pooling error. At the threshold between light limitation and CO_2 limitation, the relationship will be very nonlinear. This bias is actually the same as the previous bias, except at a higher hierarchical level (i.e., population versus leaf). Last, assimilation rate in some leaves may have been reduced by a third limiting factor. For instance, more leaves on infected plants had some chlorosis in experiment

C1 than in experiment C3 (27 versus 7%), and this may have reduced assimilation rate independently of either CO_2 supply or light supply.

The three photosynthesis light response curves for leaves on inoculated plants were each different (Fig. 6B). Obviously, there is no single characteristic light response curve for a leaf from an infected plant. In fact, to reconcile Figures 6A and B, there must be a whole family of curves that differ in their maximum assimilation rate (A_{max}) and the initial slope of the curve (quantum efficiency or maximum LUE). Some of the differences between curves could be caused by reduced CO_2 supply and some by damage to the photosynthetic mechanism. However, an important limitation in the analysis of light response curves is the confounding between stomatal effects and effects on the photosynthetic mechanism. The responses could be separated by obtaining joint light-CO_2 response curves (6).

The curvilinear relationship between WUE and LUE supports the hypothesis that there are two distinct effects of infection on leaf physiology (Fig. 7). Leaves on control plants generally maintained a constant LUE and WUE until they senesced (i.e., became chlorotic). Leaves on inoculated plants first exhibited a shift to higher WUE and lower LUE. This was associated with decreased stomatal conductance and C_i. Subsequently, they shifted to lower WUE and lower LUE, and this usually was associated with chlorosis and eventual abscission. Because it occurred later, this senescence effect was probably a secondary effect.

Compensation by unaffected leaves for photosynthetic loss in diseased leaves has been reported for barley infected by *Erysiphe graminis* (32) and bean infected with *Uromyces phaseoli* (16). If such compensation took place in plants infected by *V. dahliae*, it should have been seen as a shift toward higher LUE by some leaves on inoculated plants (Fig. 7). Although many leaves on inoculated plants had LUE equivalent to control plants, none had LUE that was significantly higher in growth-chamber experiments or in the field. Leaves of all ages were tested for compensation in growth chambers. However, because only young top leaves were tested in the field, compensation by lower leaves could have been missed. This is unlikely because symptoms of disease are usually most severe on lower leaves and because lower leaves are usually light limited.

The large oscillations in gas exchange found by Beckman et al (2) in banana leaves of plants infected with *Pseudomonas solanacearum* were not detected in potato infected with *V. dahliae*. This was tested during the light response experiments where frequent repeated measurements of photosynthesis and transpiration were made and also during CO_2 response curve experiments (6). Oscillations in stomatal conductances sometimes were generated by changing light conditions but typically were damped within several minutes. Because Beckman also was able to induce oscillations in water-stressed banana, the effect is probably a peculiarity of the host.

Experimental results confirmed the report (12) that potatoes infected with *V. dahliae* have lower transpiration before wilting. They are also in agreement with reports from other host species infected with *Verticillium* spp. (11,17,28,29). The rise in leaf temperature of leaves from inoculated plants was consistent with the reduced transpirational cooling of the leaf. The percent reduction in transpiration was always less than the reduction in stomatal conductance to water. This can be explained by the increase in leaf temperature, which raised the vapor pressure inside the leaf.

Stomatal conductance was the most sensitive indicator of disease with respect to the percent change caused by infection by *V. dahliae*. As such, it is a convenient variable for comparing the effect of infection across different leaf ages. Visible symptoms of infection typically start on the lower leaves and progress acropetally. Therefore, we might expect stomatal conductance to be more severely affected in lower leaves than upper leaves on a percentage basis. This was found in experiment C1 but not in experiment C3. The relative effect on lower versus upper leaves is probably complex and depends on disease severity (5).

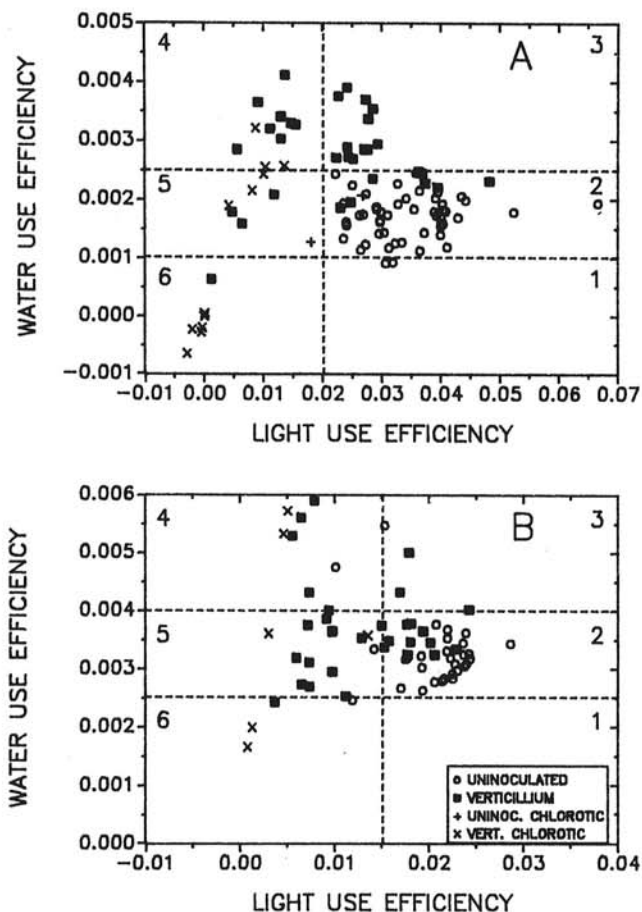Slow stomatal opening in the morning in the inoculated plants

Fig. 7. Effects of potato infection by *Verticillium dahliae* on water use efficiency (mol CO_2 fixed per mol H_2O transpired) versus light use efficiency (mol CO_2 fixed per mol photosynthetically active radiation) for: **A,** populations of leaves in different light environments and of various ages from experiment C1 at 62 days, and **B,** young leaves in full sunlight at 111 days in the 1989 field experiment.

was unexpected (Fig. 5B). Predawn water potentials were not measured, but because plants were growing in moist soil, predawn potentials should have been very high (i.e., near zero) in both infected and control plants. Slow stomatal opening could reflect a diurnal pattern in the hydraulic resistance of the vascular system. Alternatively, it could represent an aftereffect of the previous day's stress.

Infection did not affect the relationship between assimilation rate and stomatal conductance in high light (Fig. 4). However, WUE was increased in infected plants. This can be accounted for by the decrease in C_i in diseased leaves. Leaf WUE is related to C_i by the following equation (modified from Farquhar et al [9]), where C_a is the ambient CO_2 concentration and VPG is the vapor-pressure gradient (i.e., leaf vapor pressure − air vapor pressure):

$$\text{WUE} = \frac{(C_a - C_i)}{1.6\ \text{VPG}}.$$

As C_i decreases, WUE increases.

The effect of *Verticillium* infection on leaf WUE cannot be extrapolated easily to crop WUE because the defoliation effect of the disease would tend to increase evaporation from soil. Also, defoliation may result in the loss of fixed carbon before it can be mobilized from the leaf.

The data obtained in this study can be used to evaluate some of the assumptions of the mechanistic yield loss simulation models that have been developed. In Johnson's simulation model of infection of potato by *V. dahliae*, the fungus affected photosynthetic efficiency only through accelerated leaf aging (14). This would affect primarily older leaves. In the model of Gutierrez and DeVay (10), photosynthesis of infected leaves of all ages was decreased by the same proportion. In the model by Adams et al (1), both an accelerated leaf aging effect and a proportional decrease in photosynthetic efficiency were included. In the present study, infection by *V. dahliae* reduced the assimilation rate of both young and old leaves. Clearly, the data accord better with Gutierrez's and Adams' models.

Evidence has been presented in this paper that the effect of *Verticillium* infection on photosynthesis depends on light intensity. The proportional effect of *Verticillium* infection is greatest in high light. Photosynthetic rates in both the Gutierrez and Adams models respond to light intensity, but the proportional effect of *Verticillium* is not changed. This would lead to an error under low-light conditions.

The characteristic gas exchange "signature" of infection by *V. dahliae* in saturating light is decreased conductance, transpiration, C_i, and assimilation rate, and increased leaf temperature and WUE. The amount of independent information carried by these variables is less than it appears because most of these parameters are closely correlated. For example, leaf temperature, stomatal conductance, and transpiration are related. Also, C_i and WUE are related to the ratio of assimilation rate and transpiration.

Vos and Oyarzùn (31) reported that drought stress reduced assimilation rate, stomatal conductance, and C_i in potato. Therefore, the gas exchange patterns of *Verticillium* infection and drought stress are the same. Other pathogens that affect water relations in potato (e.g., *Corynebacterium sepedonicum* [3]) also could produce the same signature. Therefore, caution will be needed to avoid confounding factors when gas exchange is used as an epidemiological tool.

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