Ecology and Epidemiology

# Chronology of Gas Exchange Effects and Growth Effects of Infection by *Verticillium dahliae* in Potato

R. L. Bowden and D. I. Rouse

## ABSTRACT

Bowden, R. L., and Rouse, D. I. 1991. Chronology of gas exchange effects and growth effects of infection by *Verticillium dahliae* in potato. Phytopathology 81:301-310.

Growth-chamber and field experiments were conducted to study the chronology of effects on gas exchange, visible symptoms, defoliation, and stunting caused by *Verticillium dahliae* in potato. Use of gas exchange measurements for detecting individual diseased leaves was successful in growth chambers and the field but was sometimes confounded by water stress. Gas exchange effects often were detectable before visible symptoms for individual leaves. Defoliation was roughly concurrent with these effects, but stunting occurred later, if at all. Disease development could be divided into three phases. In the latent phase, the fungus was present in stems, but no symptoms or gas exchange effects were detectable. In the local phase, there was an acropetal procession of wilting, chlorosis, or senescence of leaves or parts of leaves, but young leaves had normal gas exchange and no visible symptoms. Acropetal defoliation continued in the systemic phase, and young leaves had reduced gas exchange and often were stunted. Reduced radiation use efficiency accounted for about one third and reduced radiation interception accounted for about two thirds of the yield loss caused by *V. dahliae* in two field seasons.

Additional keywords: carbon assimilation rate, disease progress, photosynthesis, *Solanum tuberosum*, stomatal conductance, transpiration.

*Verticillium dahliae* Kleb. is an important component of the potato (*Solanum tuberosum* L.) early dying syndrome (23). Isaac and Harrison (16) described the characteristic leaf symptoms of infection by *V. dahliae* in potato as recoverable true wilting, unilateral permanent wilting, and unilateral chlorosis and necrosis. *V. dahliae* reduced tuber, stem, and leaf growth rates, increased leaf senescence rate, and caused fluctuating specific leaf weights (14,15). Leaf appearance rate was reduced by *V. albo-atrum* but not by *V. dahliae*. *V. dahliae* often was isolated from plants before development of visible symptoms (16).

In the companion paper (4), the gas exchange effects of infection by *V. dahliae* in potato were shown to be decreased carbon assimilation rate, stomatal conductance, transpiration, apparent intercellular $CO_2$ concentration, and increased leaf temperature. Effects on gas exchange were detectable in both chlorotic and nonsymptomatic leaves. This suggests that gas exchange may provide a more sensitive measure of disease than visible symptoms.

Gas exchange could be used in two ways to quantify disease. First, population average disease severity could be estimated by the reduction in population average gas exchange rates. Alternatively, the proportion of the population of leaves that is diseased (i.e., disease incidence) could be estimated if the disease status of individual leaves could be determined.

Gas exchange effects of infection by *V. dahliae* on populations of leaves at the end of several experiments were presented in

the companion paper (4). This provided a snapshot of disease effects at their most severe stage. An examination of the time course of events may provide a better understanding of disease effects. The objectives of this paper were to develop a leaf health classification (discrimination) system based on nondestructive gas exchange measurements on individual leaves, to use this system to examine disease progress within individual leaves and plants, to compare disease progress based on gas exchange with other measures of disease progress such as visible symptoms, defoliation, and stem or leaf growth rates, and to assess the relative importance of the effects *V. dahliae* on potato canopy radiation use efficiency (RUE) and canopy radiation interception (RI) over the course of the season. A preliminary report of portions of this research has been published (3).

## MATERIALS AND METHODS

**Growth-chamber experiments.** Disease progress was studied in growth-chamber experiments C1 through C3, which were described previously (4). Leaves were tagged every 4 days in experiment C1 and every 8 days in experiments C2 and C3. Because new leaves were produced approximately once every 2 days, between 25 and 50% of leaves were tagged. The youngest leaf in which the terminal leaflet was greater than 3 cm long was chosen for tagging each day. The set of leaves tagged on a particular day will be referred to as a cohort. Cohorts were labeled A, B, C, etc., from oldest to youngest. Gas exchange was measured on all tagged leaves every 4 days with a Li-Cor

LI-6200 photosynthesis system (Li-Cor, Inc., Lincoln, NE) as described (4).

The length and width of the terminal leaflet of each leaf cohort were measured 1 day after tagging and subsequently every 4 days. Length and width growth were highly correlated and approximately linear over the first week after tagging. The top leaf growth rate was operationally defined as the average length increase per day over the first 4 days after tagging for each cohort. Stem length was measured from the soil line to the tip of the main stem meristem every 4 days. Main stems were staked upright to facilitate measurements. Stem growth rate was defined as the average length increase per day over each 4-day period. Leaf insertion number of the top leaf was determined every 4 days. Leaf production rate was defined as the average number of leaves produced per day over each 4-day period. Proportion defoliated leaves was calculated for each plant as the number of surviving tagged leaves divided by the cumulative total number of tagged leaves. Proportion diseased leaves as determined by visible symptoms was calculated for each plant as the proportion of surviving tagged leaves with some degree of turgor loss on any part of the leaf. Proportion diseased leaves as determined by gas exchange was calculated for each plant as the proportion of surviving tagged leaves classified as diseased.

**Field experiments.** The 1988 and 1989 field experiments at the University of Wisconsin Hancock Research Station in Hancock, WI, were described in the preceding paper (4). Gas exchange measurements of top leaves in full sunlight were taken approximately every 2 wk.

Canopy interception of photosynthetically active radiation (PAR) was measured with a Li-Cor LI-191SB 1-m line quantum sensor attached to a Li-Cor LI-185B photometer. Approximately once per week, measurements of PAR were made above the canopy and below the canopy perpendicular to the hills. Dead stems were removed if necessary before taking readings. Canopy proportion radiation interception (PRI) was calculated as measurements (above − below)/above the canopy. Two replicate sets of measurements were taken per plot and averaged.

**Disease classification by gas exchange.** The characteristic gas exchange "signature" of potato infection by *V. dahliae* (4) was used to develop nondestructive methods of determining whether individual leaves were healthy or diseased. Variables used in classification were leaf carbon assimilation rate, stomatal conductance, transpiration rate, temperature difference between air and leaf, ratio of leaf intercellular $CO_2$ ($C_i$) to ambient $CO_2$ ($C_a$), water use efficiency (WUE, mol $CO_2$ fixed per mol $H_2O$ transpired), and light use efficiency (LUE, mol $CO_2$ fixed per mol PAR). The $C_i/C_a$ ratio was used to minimize the effects of differences in $C_a$ between measurements.

Observations were classified as either healthy or diseased. An observation consisted of one gas exchange measurement on a particular leaf on a particular day. Classification algorithms were parameterized with a calibration data set and then evaluated with an independent validation data set. Calibration data for the classification systems were selected from data sets that showed strong disease effects. The independent validation data included all time periods to avoid possible plant age-related bias. Growth-chamber data and field data were analyzed separately.

For the growth-chamber experiments, data from experiment C1 at 62 days after planting (hereafter referred to simply as days) were selected as the calibration data set ($n = 89$). The independent validation data consisted of all other observations from growth-chamber experiments C1, C2, and C3 ($n = 3,878$). Growth-chamber data sets included leaves in different light environments and of different ages.

For field experiments, calibration data were pooled from 99 and 104 days in 1988 and from 118 days in 1989 ($n = 158$). All other field observations from 1988 and 1989 were pooled for validation ($n = 622$). Field data sets included only young leaves in full sunlight.

Four classification systems were evaluated. In the first two systems, stepwise discriminant analysis (24) was used to select a subset of variables that contained significant ($P \leq 0.05$)

information for classification of the calibration data. In the first classification method, this subset was used in a standard discriminant analysis (24). The second method used nearest neighbor discriminant analysis with 1, 2, or 3 neighbors (24).

In the third method, canonical discriminant analysis using all variables was used to derive a single canonical variable that best described the differences between classes (24). The division between classes along the axis defined by the canonical variable was chosen so that less than 5% of uninoculated leaves were classified as diseased in the calibration data set.

Pairs of variables chosen by the stepwise discriminant procedure also were plotted in a simple graphical analysis. Class divisions were drawn arbitrarily so that less than 5% of uninoculated leaves were misclassified in the calibration data set.

**Analysis of variance.** Growth-chamber data were analyzed as a balanced repeated measures design with plants as the main experimental unit and time as the repeated measure within plants. Allocation of inoculum treatments to plants was completely randomized. All proportion data were transformed using an arcsine square root transformation before analysis. Analysis of variance was performed with SAS procedure GLM using the REPEATED statement; the Greenhouse-Geisser correction for autocorrelation was applied to degrees of freedom for $F$-tests involving time (24). Approximate least significant differences (LSD, $P = 0.05$) for comparing treatments within the same time were calculated according to Milliken and Johnson (20). LSDs were calculated only for variables that had significant $F$-tests for inoculum treatment or the interaction of treatment and time.

Field data were analyzed as a balanced repeated measures design with plots as the main experimental unit and time as the repeated measure within plots. Allocation of inoculum treatments to plots was done as randomized complete blocks. Analysis of variance was as described above except for the analysis of stomatal conductance in the 1988 experiment. In that case, a subjective judgment was made that variances were heterogeneous across time points. Therefore, separate randomized complete block analyses of variance were performed for each time point. The experiment-wise type I error rate was maintained at $P \leq 0.05$ using the Bonferroni method by requiring each $P$-value to be less than or equal to 0.05/8 because there were eight time points (26).

**Importance of gas exchange effects.** Pathogen effects on yield loss can be partitioned into effects on RUE versus RI if a few assumptions are made. The season was assumed to start at 50% emergence with a canopy PRI of zero and was assumed to end when vines were killed on 14 September. Daily values of PRI and carbon assimilation rate were interpolated from the appropriate pair of measurements. The value of PRI for the last day of the season was assumed to be equal to the last measured values. Values for assimilation rate for the first and last days of the season were assumed equal to the first and last measured values, respectively. Daily total RI was calculated as PRI times the daily total incident radiation (MJ m$^{-2}$ day$^{-1}$) as determined by a solar radiometer stationed about 1 km from the field plots. Proportion radiation use efficiency (PRUE) was calculated as mean leaf assimilation rate divided by the maximum leaf assimilation rate (25 $\mu$mol m$^{-2}$ s$^{-1}$). The assumption is that top leaf photosynthetic efficiency is a good estimator of canopy photosynthetic efficiency. Daily canopy RUE was calculated as PRUE times 1.4 g dry weight MJ$^{-1}$, which is an empirical value derived by Monteith (21). Daily dry weight gain was calculated as the product of RI and RUE. Cumulative total dry weight gain was calculated for the low and high inoculum treatments and the uninoculated controls for 1988 and 1989.

## RESULTS

**Disease classification by gas exchange.** Four methods for determining the health of individual leaves were compared. The best system was chosen based on the highest ratio of sensitivity to error. Sensitivity was defined as the probability of classifying a leaf from an inoculated plant as diseased. Error was defined as the probability of classifying a leaf from an uninoculated plant

as diseased.

For the growth-chamber data, variables chosen by the stepwise discriminant procedure were WUE, LUE, and the difference between air temperature and leaf temperature. The standard discriminant analysis, nearest neighbor discriminant analysis, canonical discriminant analysis, and graphical analysis all gave similar results. However, the best system was a simple graphical approach based on WUE and LUE. Leaves with an LUE greater than 0.02 mol $CO_2$ per mol photons and a WUE less than or equal to 0.0025 mol $CO_2$ per mol $H_2O$ were classified as healthy. Leaves with an LUE less than or equal to 0.02 and a WUE less than or equal to 0.001 were classified as senescent. All other leaves were classified as diseased. This system is depicted in Figure 7A of (4). When applied to the validation data set, the system classified 20.4% of the leaves from inoculated plants as diseased and 3.0% of the leaves from uninoculated plants as diseased. The chi-square statistic for the hypothesis of no treatment effect was significant at $P \le 0.0001$.

Although only 20.4% of the leaves from the inoculated treatments were classified as diseased, this does not imply low sensitivity. Many observations were from leaves that were not yet diseased because the validation data consisted of all observations from three entire experiments (except the calibration data).

The adequacy of the classification system is best shown in data sets from the ends of the experiments when disease effects were greatest. For instance, 71 and 69% of the leaves on inoculated plants were classified as diseased at the end of experiments C1 and C3, respectively. In addition, 13 and 2% of the leaves on inoculated plants were classified as senescent in experiments C1 and C3, respectively.

For the field data, the variables chosen by the stepwise procedure were the $C_i/C_a$ ratio, assimilation rate, and temperature difference. All classification systems except the canonical discriminant analysis had error rates over 11% for leaves from uninoculated plots when applied to the validation data set. Canonical discriminant analysis was also the best system according to the sensitivity-to-error ratio. Each variable was standardized with the mean and standard deviation from the calibration data set, then multiplied by weighting coefficients to obtain a single canonical variable (Table 1). Assimilation rate, the $C_i/C_a$ ratio, and the difference between air temperature and leaf temperature were most heavily weighted. When applied to the validation data, the system classified 23.5% of the leaves from plots infested with V. dahliae as diseased and 4.2% of the leaves from uninfested plots as diseased. The chi-square statistic for the hypothesis of no treatment effect was significant at $P \le 0.0001$. The explanation for the apparently low sensitivity is the same as discussed for the growth-chamber experiments.

**Disease progress within individual plants.** The classification system was used to examine disease progress in individual leaves and plants in experiments C1, C2, and C3. Representative examples of three inoculated plants were contrasted with three control plants from experiment C1 (Fig. 1). In control plant 1, all the leaves were consistently rated as healthy. In the other

two controls, one leaf at one time was classified as diseased. This reflects the 3% error rate of the classification system. Cohort A on control plant 3 started healthy, then became chlorotic, then was classified as senescent by gas exchange, then died in a normal senescence process.

Plants from the inoculated treatment exhibited the typical acropetal development of disease symptoms and defoliation. Leaves frequently were classified as diseased based on gas exchange a week or more before visible symptoms appeared. Most diseased leaves were classified as senescent just before they died.
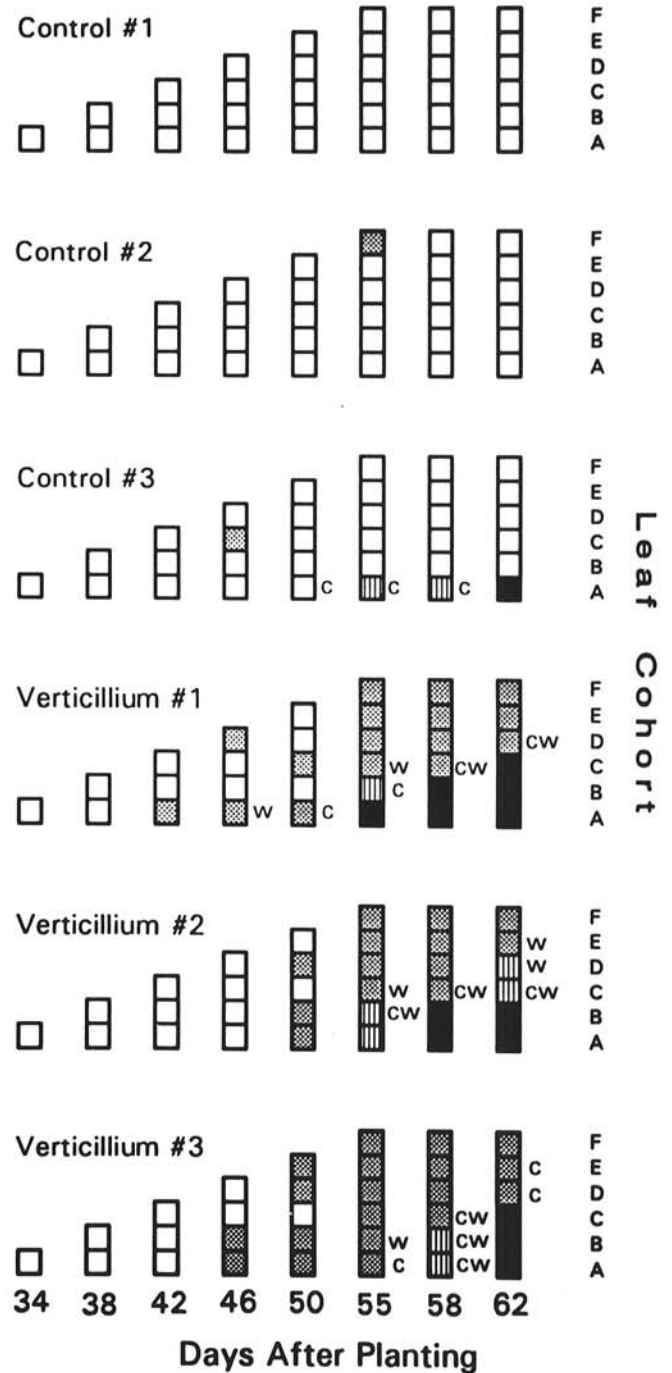


Fig. 1. Disease progress (as determined by gas exchange) in representative individual potato plants inoculated with *Verticillium dahliae* and controls in growth-chamber experiment C1. Each small square represents a single tagged leaf that was sampled repeatedly over time. Each stack of squares represents a single stem. Leaves were tagged every 4 days. (A set of leaves tagged on a particular day is termed a "cohort.") Leaf cohort A was the oldest and cohort F was the youngest. Leaves were classified as healthy (open square), senescent (square with vertical lines), diseased (square with cross hatching), or dead (solid square). Leaves with visible symptoms were noted (c = chlorosis, w = some degree of wilt).

TABLE 1. Mean, standard deviation (SD), and standardized weighting coefficients used to calculate the canonical variable for classification of potato leaves as healthy or infected by *Verticillium dahliae* in the field[a]

| Variable | Mean | SD | Coefficient |
|---|---|---|---|
| Assimilation rate | 13.38 | 5.71 | 0.756 |
| Stomatal conductance | 0.28 | 0.21 | 0.074 |
| $C_i/C_a$ ratio | 0.63 | 0.12 | 0.604 |
| Temperature difference | −0.59 | 1.28 | 0.378 |
| Transpiration rate | 6.02 | 3.08 | −0.080 |
| Light use efficiency | 0.008 | 0.003 | −0.112 |
| Water use efficiency | 0.002 | 0.001 | −0.021 |

[a]Variables for individual leaves were standardized to the mean and standard deviation, then multiplied by the weighting coefficient and summed to obtain the value of the canonical variable. Leaves were classified as diseased when the value of the canonical variable was less than −0.4. Leaves with higher values were classified as healthy.

In the early stages of disease expression, diseased leaves were interspersed with healthy leaves along the stem axis. This phase of disease lasted at least 9 days for inoculated plant 1 but lasted only a few days for plant 2. In experiment C3, several plants remained in this phase for more than 1 mo. Top leaves usually were not affected during this local phase. After the local phase, all leaves were affected.

**Chronology of disease effects in growth chambers.** Various measures of disease progress were compared in growth-chamber experiments C1 to C3 (Figs. 2–4). In experiment C1, the interaction of inoculum treatment and time was statistically significant for proportion diseased leaves, as determined by gas exchange, and proportion defoliated leaves ($P \leq 0.001$). Inoculation had significant main effects on assimilation rate ($P \leq 0.001$), stomatal conductance ($P \leq 0.001$), and proportion diseased leaves as determined by visible symptoms ($P = 0.01$). Inoculation had no statistically significant effect on top leaf growth rate.

Detection of an interaction of inoculum treatment and time depends on when it is sought. For experiment C1, especially, the first measurements were taken just before the disease started to cause effects. If measurements had been started earlier, an interaction probably would have been found. Therefore, LSDs were used to detect significant inoculum treatment differences within times whenever inoculum main effects or interactions of inoculum treatment and time were significant.

Differences were first detected in stomatal conductance at 38 days. At 50 days, proportion diseased leaves as determined by gas exchange and proportion defoliated leaves were both significant. At 55 days, the first significant difference in visible symptoms was detected. At 58 days, assimilation rate was significantly affected. The large peak in proportion diseased leaves of uninoculated plants at 55 days was associated with accidental disconnection of the watering system on that day and probably reflects water stress (Fig. 2).

In experiment C2, the interaction of inoculum treatment and time was significant for proportion diseased leaves as determined
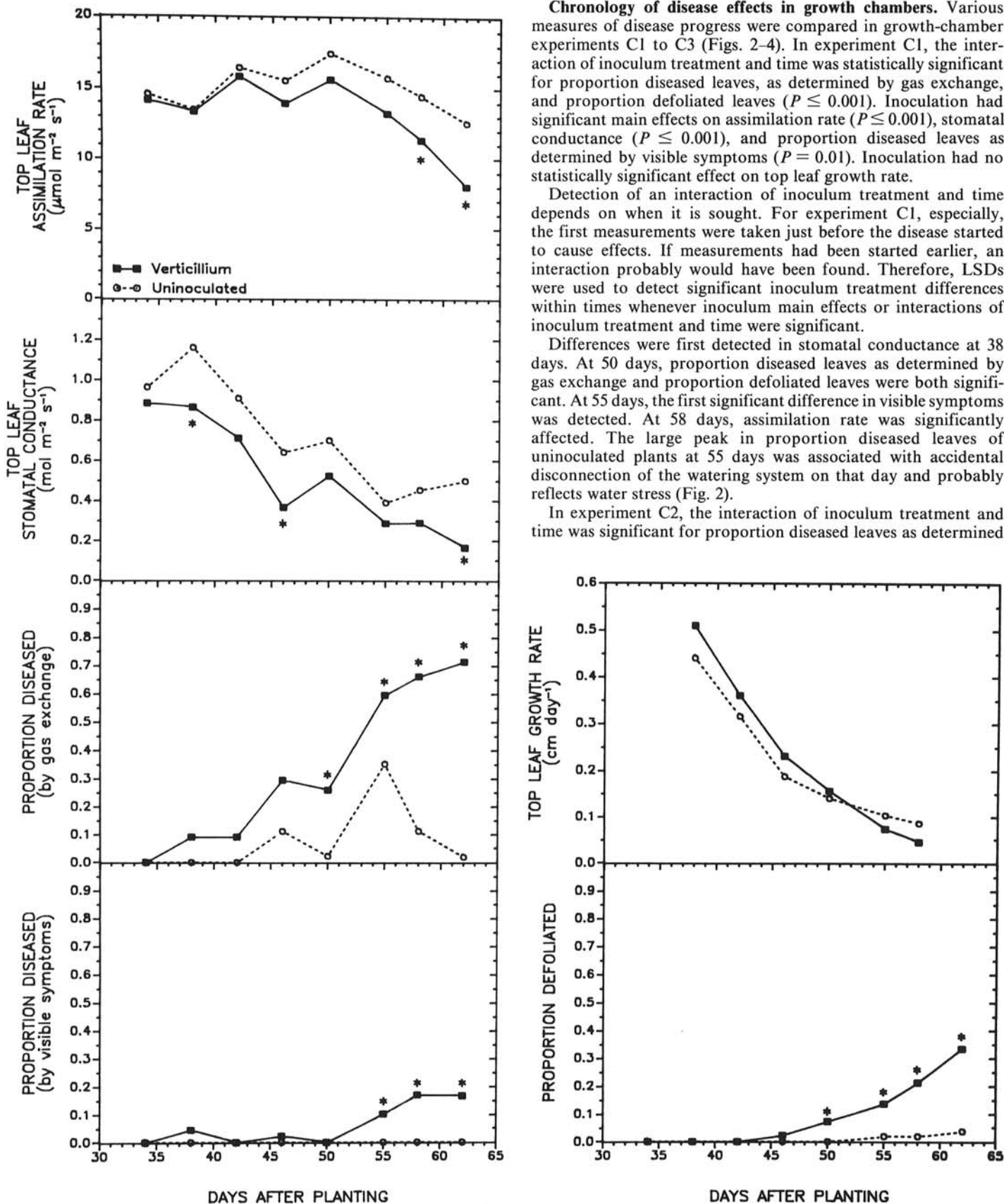


**Fig. 2.** Various measures of disease progress for potato infected by *Verticillium dahliae* in growth-chamber experiment C1. Asterisks mark significant differences (least significant difference, $P = 0.05$) between inoculated and uninoculated plants. Proportion data were transformed before analysis with an arcsine square root transformation, then back-transformed for presentation.

by gas exchange ($P = 0.01$), proportion diseased leaves as determined by visible symptoms ($P = 0.01$), stem growth rate ($P = 0.04$), leaf growth rate ($P = 0.003$), and proportion defoliated leaves ($P = 0.001$). The main effect of inoculation treatment was significant for assimilation rate ($P = 0.04$). There were no signifi-

cant differences for stomatal conductance or leaf production rate.

The first significant effect was detected by proportion diseased leaves according to gas exchange at 60 days, followed by proportion diseased leaves according to visible symptoms at 64 days. At 80 days, a significant difference was found in proportion
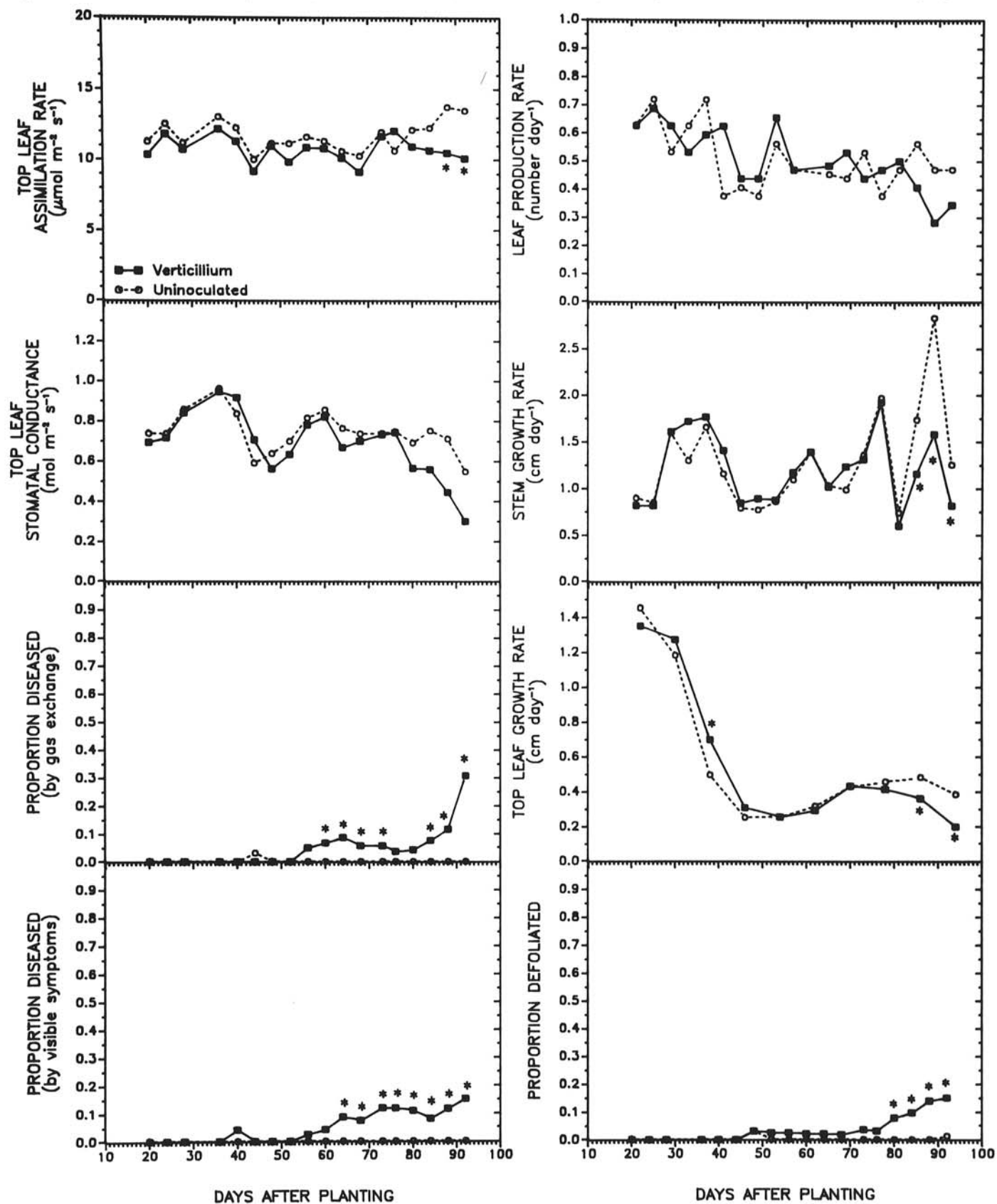


**Fig. 3.** Various measures of disease progress for potato infected by *Verticillium dahliae* in growth-chamber experiment C2. Asterisks mark significant differences (least significant difference, $P = 0.05$) between inoculated and uninoculated plants. Proportion data were transformed before analysis with an arcsine square root transformation, then back-transformed for presentation.

defoliated leaves. At 86 days, both top leaf growth rate and stem growth rate differences were significant. At 88 days, assimilation rate showed a significant treatment effect.

In experiment C3, the interaction of inoculum treatment and time was significant for stomatal conductance ($P \leq 0.001$), proportion diseased leaves according to gas exchange ($P \leq 0.001$), proportion diseased leaves according to visible symptoms ($P = 0.003$), and proportion defoliated leaves ($P = 0.03$). There were no signifi-
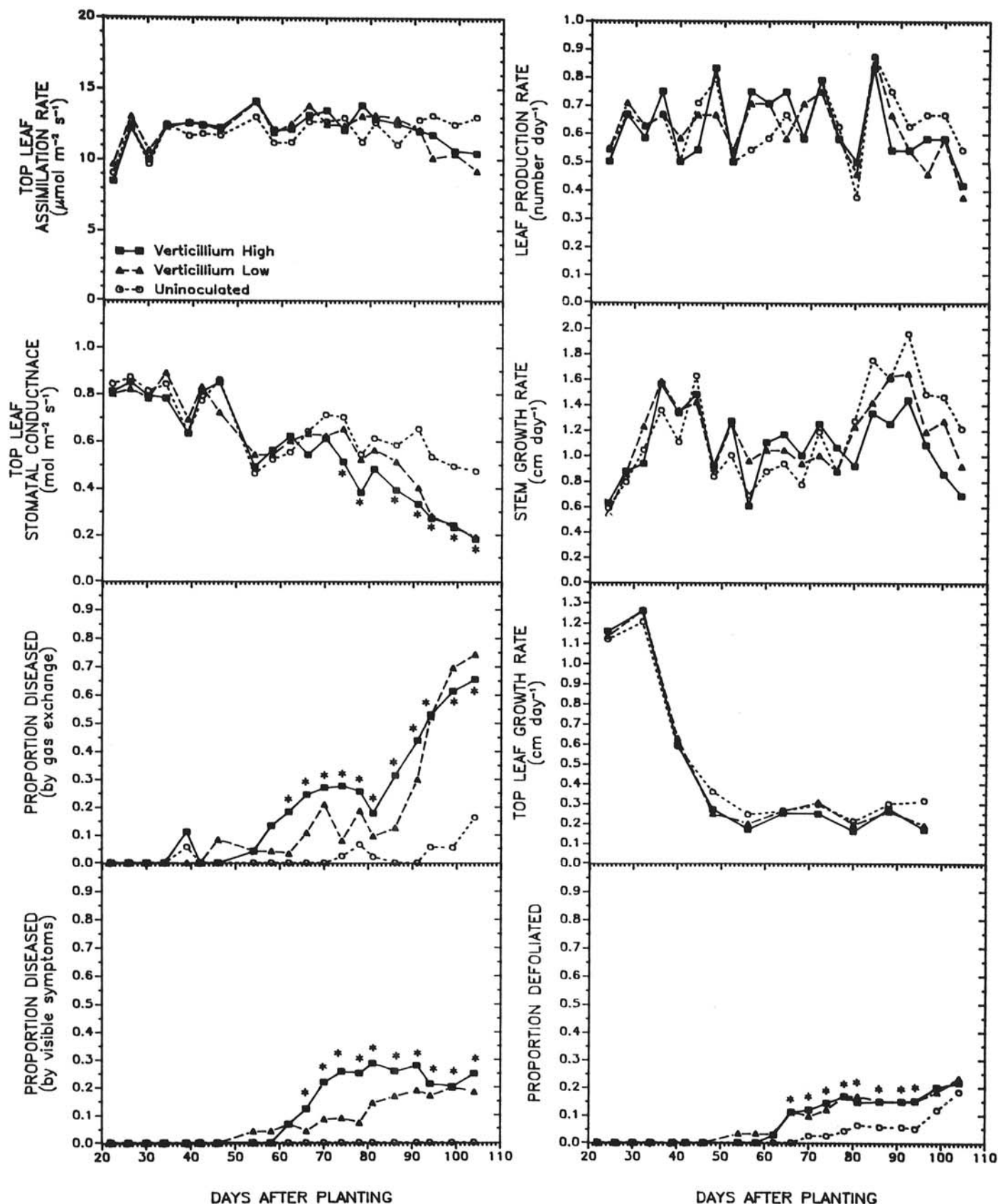


**Fig. 4.** Various measures of disease progress for potato infected by *Verticillium dahliae* in growth-chamber experiment C3. Asterisks mark significant differences (least significant difference, $P = 0.05$) between inoculated and uninoculated plants for the high inoculum dose only. Proportion data were transformed before analysis with an arcsine square root transformation, then back-transformed for presentation.

cant differences for the other variables.

Proportion diseased leaves according to gas exchange showed the first significant effect at 62 days. At 66 days, both proportion defoliated leaves and proportion diseased leaves according to visible symptoms showed statistical differences. At 74 days, stomatal conductance was significantly affected.

The three growth-chamber experiments differed in the rate of disease development, presumably mostly because of differences in inoculum dose. However, because the inoculum was prepared differently in each of these experiments, comparison of doses between experiments is not appropriate. Within experiment C3, the effect of lower inoculum dose was primarily a delay in disease progress compared with the high dose. Final disease severity was not affected according to the various measures of disease.

**Chronology of disease effects in the field.** Proportion diseased leaves in growth-chamber experiments included leaves of various ages. In contrast, proportion diseased leaves in the field was based on young, brightly lit leaves from the top of the leaf canopy. Proportion diseased leaves as determined by gas exchange was compared to assimilation rate and stomatal conductance of top leaves and to canopy RI in 1988 and 1989 (Figs. 5 and 6).

In 1988, the interaction of inoculum treatment and time was significant for the proportion diseased leaves determined by gas exchange ($P = 0.05$) and PRI ($P \leq 0.001$). The main effect of inoculum treatment was significant for assimilation rate ($P = 0.03$). The Bonferroni method detected a significant effect for stomatal conductance at the last time period. Because gas exchange measurements were taken only approximately every 2 wk, fine distinctions in timing could not be made. In general, effects on all variables occurred at about the same time.

The suspiciously high disease proportion according to gas exchange on the first three sample dates in 1988 correlated with unusually low stomatal conductances in all three treatments and was associated with a period of extremely hot, dry weather. Estimated evapotranspiration obtained from the Wisconsin state climatologist was in excess of applied irrigation plus precipitation during this period. It is probable that spurious results were caused by water stress on these sample dates.

In 1989, the interaction of inoculum treatment and time was significant for assimilation rate ($P = 0.03$) and the PRI ($P \leq 0.001$). The main effect of inoculum treatment was significant for stomatal conductance ($P = 0.001$) and the proportion diseased leaves determined by gas exchange ($P = 0.01$). The first statistically significant differences were detected at 72 days by PRI followed at 93 days by assimilation rate and stomatal conductance. The proportion diseased leaves as determined by gas exchange was significant at 98 days.

Very clear inoculum dose effects were seen in both years. A fourfold difference in inoculum resulted in a 10- to 15-day delay in disease progress. Disease progress in the field was similar to that in growth-chamber experiments with respect to timing and magnitude of effects on gas exchange. However, defoliation was more severe in the field than in the growth chamber.

**Importance of gas exchange effects.** Measured values of RI and RUE were used to estimate the total dry weight growth curves for 1988 and 1989 (Fig. 7). Estimated cumulative dry weight was highly correlated with final tuber dry weight ($R^2 = 0.75$, $P = 0.0001$, $n = 24$). Estimated losses in total dry weight were 14.4 and 25.1% for the low and high inoculum doses, respectively, in 1988. This corresponded closely to the 16.4 and 23.2% losses in tuber dry weight that were actually measured at final harvest. Estimated losses in total dry weight were 14.8 and 30.0% for the low and high doses, respectively, in 1989. Actual losses in tuber dry weight were 10.8 and 30.4%, respectively.

Between 59 and 69% of the loss in estimated cumulative dry weight was accounted for by losses in RI (Fig. 7). Decreased RUE accounted for 31–41% (average 35%) of the loss in cumulative total dry weight.

## DISCUSSION

Development of disease classification systems based on gas

exchange characteristics represents a novel technology in plant disease epidemiology. Gas exchange has been used to measure disease in populations of leaves before, but this is the first time it has been used to rate individual leaves and produce disease progress curves (Figs. 2–6). The method is objective, nondestruc-
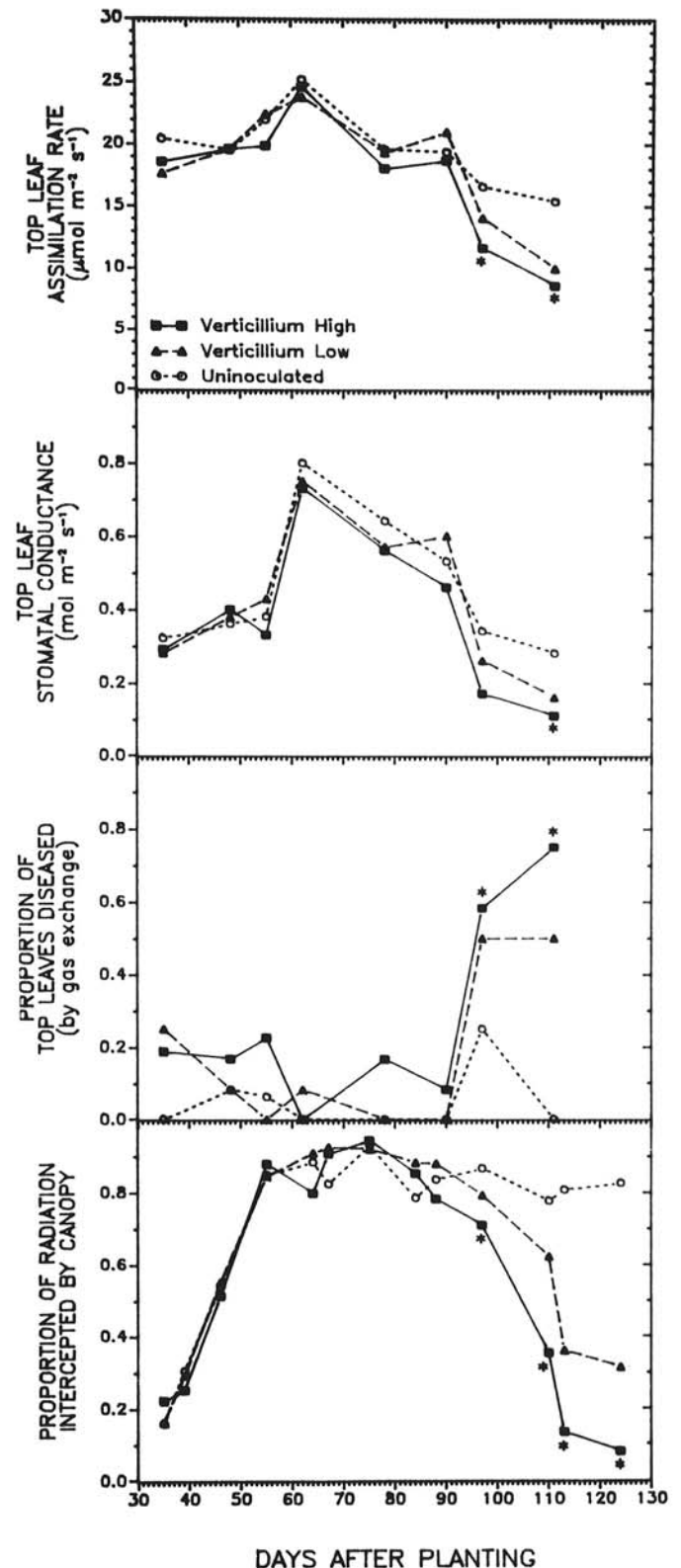


Fig. 5. Various measures of disease progress for potato infected by *Verticillium dahliae* in 1988 field experiment. Asterisks mark significant differences (least significant difference, $P = 0.05$) between inoculated and uninoculated plants for the high inoculum dose only. Proportion data were transformed before analysis with an arcsine square root transformation, then back-transformed for presentation.

tive, reasonably rapid (about 1 min per leaf), and physiologically based. This is especially useful for potato early dying because visible symptoms are often absent or ambiguous (16).

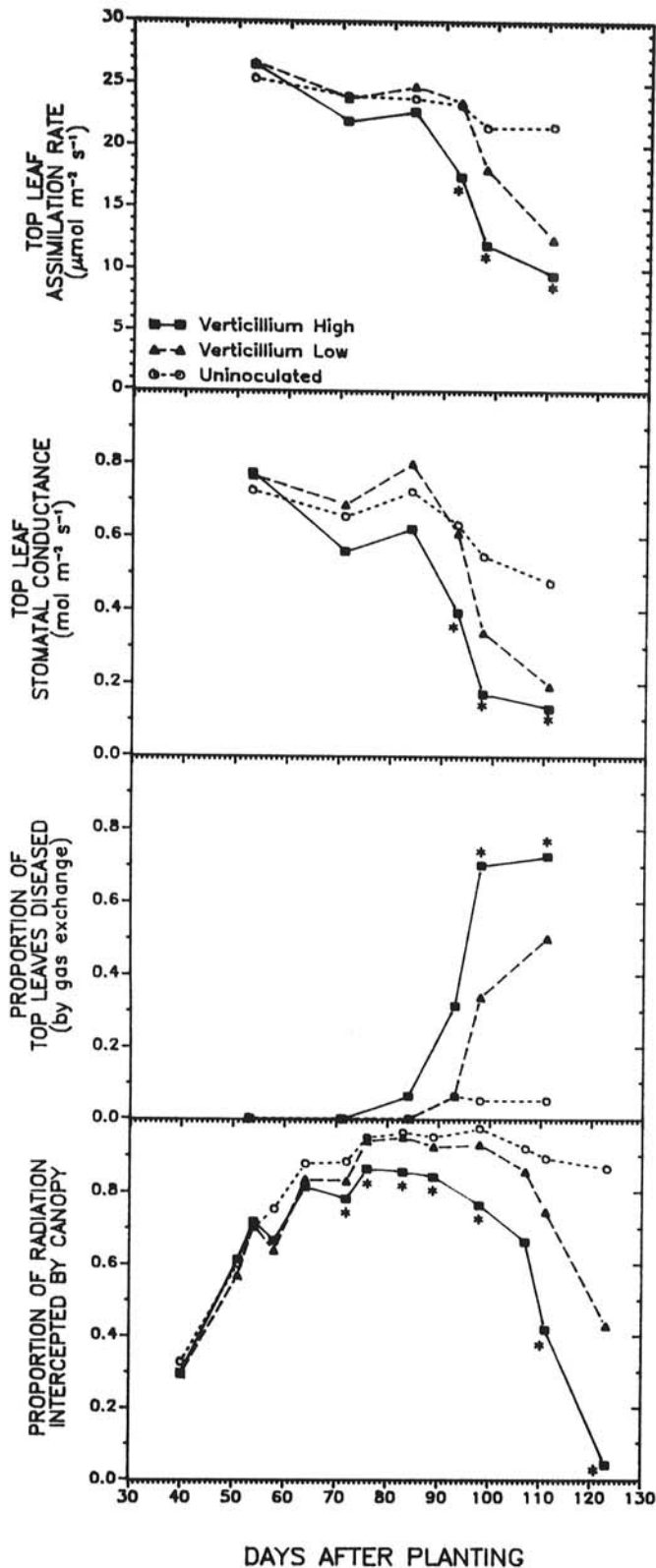The LUE-WUE system for classifying leaf health in controlled environment chambers was desirable for several reasons. First, the criteria had straightforward biological interpretations that related to plant productivity. Second, the ability to distinguish senescence as a distinct class was useful. In contrast, the canonical discriminant analysis tended to class senescent leaves as healthy. Third, the system was not sensitive to leaf age or light environment.

Use of this method in the field was hindered by several factors. LUE naturally decreases as light saturation is approached. This was not a problem in growth chambers where light responses were essentially linear. Also, WUE was variable depending on weather conditions, especially temperature and humidity.

The canonical discriminant analysis was the best classification system for the field. The function was based primarily on the $C_i/C_a$ ratio and assimilation rate. Interestingly, a decrease in the $C_i/C_a$ ratio is equivalent to an increase in WUE for a given atmospheric-pressure and vapor-pressure deficit (12). Also, assimilation rate under saturating light is analogous to LUE. Therefore, the field and growth-chamber systems were based on the same basic effects of the pathogen.

It was surprising that the differential between air temperature and leaf temperature was not more useful in discriminating diseased leaves in the field. The variable showed significant differences between treatments, but it varied greatly from day to day. The classification methods used calibration and validation data sets across days and years and therefore were biased toward effects that were environmentally insensitive.

The two major considerations for evaluating the classification system are sensitivity and error. Sensitivity is difficult to judge because there is no independent and nondestructive way to determine if a particular leaf is truly diseased. In Figure 1, repeatability of disease classification was shown to be very good for the same leaves over time. In Figures 2 through 6, the sensitivity seemed to be at least as good as any other measure of disease. Disease proportions of 0.70–0.80 were detected at the end of four of the five experiments.

Serious error problems for proportion of leaves classified as diseased by gas exchange were apparent in Figure 2 and Figure 5 for the uninoculated treatment. The error rate for misclassification of leaves from uninoculated plants reflected two problems. First, some of the uninoculated plants were infected in the field plots, presumably from microsclerotia of *V. dahliae* that escaped the soil fumigation treatments. Second, in both Figure 2 and Figure 5, errors were associated with periods of water stress. This ambiguity can be overcome only by careful irrigation. Another difficulty, noted in the preceding paper (4), is that other pathogens might cause gas exchange effects similar to infection by *V. dahliae*. Blackleg, caused by *Erwinia carotovora*, was present in field plots in both years, but all plants with external symptoms of blackleg were excluded from the data sets. There is no reason to suspect that *E. carotovora* caused a problem, but the possibility cannot be excluded.

The disease classification system was used for following disease progress in individual leaves and plants in growth-chamber studies (Fig. 1). Inoculated plants usually went through a phase where diseased and healthy leaves were interspersed along the lower part of the main stem axis. Affected leaves first exhibited reduced gas exchange with no symptoms, then unilateral or bilateral wilting or chlorosis. There usually followed a period when gas exchange was no longer distinguishable from normal senescence of uninoculated leaves. Finally, senescent leaves became necrotic and abscised. Upper leaves usually had normal gas exchange and no symptoms during this local disease phase.

The local phase of disease probably is best explained by local plugging of the vascular system either in the affected leaves, in the petioles, or in the narrow secondary vascular bundles in the stem just below the affected leaf. The main bundles in the stem are rather insensitive to small local plugs because the potato stem vascular system has extensive connections between the main vascular bundles (19). Petiole and leaf vein xylem bundles are much more susceptible to plugging than stem vascular tissue because they are narrow and there are few cross-connections that allow water to bypass the blockage (10). Nearly infinite resistance to water flow has been demonstrated in some petioles from tomato



Fig. 6. Various measures of disease progress for potato infected by *Verticillium dahliae* in 1989 field experiment. Asterisks mark significant differences (least significant difference, $P = 0.05$) between inoculated and uninoculated plants for the high inoculum dose only. Proportion data were transformed before analysis with an arcsine square root transformation, then back-transformed for presentation.

infected with *Fusarium oxysporum* (11) and tomato infected with *V. albo-atrum* (27). Discontinuous colonization of stems by *V. dahliae* was demonstrated in chrysanthemum (2) and in mint (8). The combination of discontinuous colonization of petiole or leaf vascular tissue plus the extreme susceptibility of these small vessels to plugging would explain the observed local phase of gas exchange effects.

The local phase was followed by a systemic phase where all the upper leaves showed a uniform decrease in photosynthesis and stomatal conductance. The lower leaves continued to show an acropetal progression of defoliation during this phase. The systemic phase sometimes began with a temporary color change of all the leaves to a darker green. This phenomenon also was noted by Garber (13) in cotton infected with *V. dahliae*. The dark green color typically disappeared in a few days, after which there were no obvious visible symptoms in the top leaves. Pathogen activities within individual leaves would not be expected to produce uniform systemic effects. Therefore, the systemic phase can best be explained by a critical resistance to water flow near the main stem base. Sometimes the bottom of the stem had a few healthy leaves, but the top of the stem was uniformly diseased. This pattern probably reflected a critical resistance to water flow near the middle of the main stem.

The systemic disease phase should not be confused with systemic colonization. In many cases, leaves that were classified as diseased did not contain detectable *V. dahliae* in the petioles (data not shown). Furthermore, the phases apply only to single stems because a single plant can have different stems in different disease phases. Logically, there must also be a disease phase between the time of infection and the first expression of symptoms or gas exchange effects. This was labeled the latent phase.

The different measures of disease progress in growth chambers gave different results. For instance, leaf production rate showed no significant treatment effects, whereas proportion diseased leaves as determined by gas exchange, proportion diseased leaves according to visible symptoms, and proportion defoliated leaves always showed significant effects. The other variables occasionally showed significant effects.

The earliest differences usually were detected with proportion diseased leaves according to gas exchange. Proportion with visible symptoms and proportion defoliated leaves also were among the early indicators. Top leaf assimilation rate, top leaf growth rate, and stem growth rate were late indicators if they were detected at all. The relative timing of reduced stomatal conductance of top leaves was variable in the different experiments.

Most of these differences among measures of disease progress can be explained by their different response to the disease phases. The proportion variables all included leaves of all ages and therefore were able to detect plants in the local disease phase. In contrast, top leaf assimilation rate, stomatal conductance, growth rate, and stem growth rate usually were not affected until the systemic phase.

In addition to being earliest, the proportion diseased leaves according to gas exchange consistently detected higher disease severity than the other proportion variables at the ends of the experiments. Unfortunately, classification by gas exchange was more susceptible to misclassifying leaves on uninoculated plants than was disease based on visible symptoms.

Proportion defoliation was sometimes also subject to difficulty as a measure of disease. At the end of experiment C3, older leaves on controls began to senesce naturally. This reduced the differences between treatments, and they were no longer statistically significant.

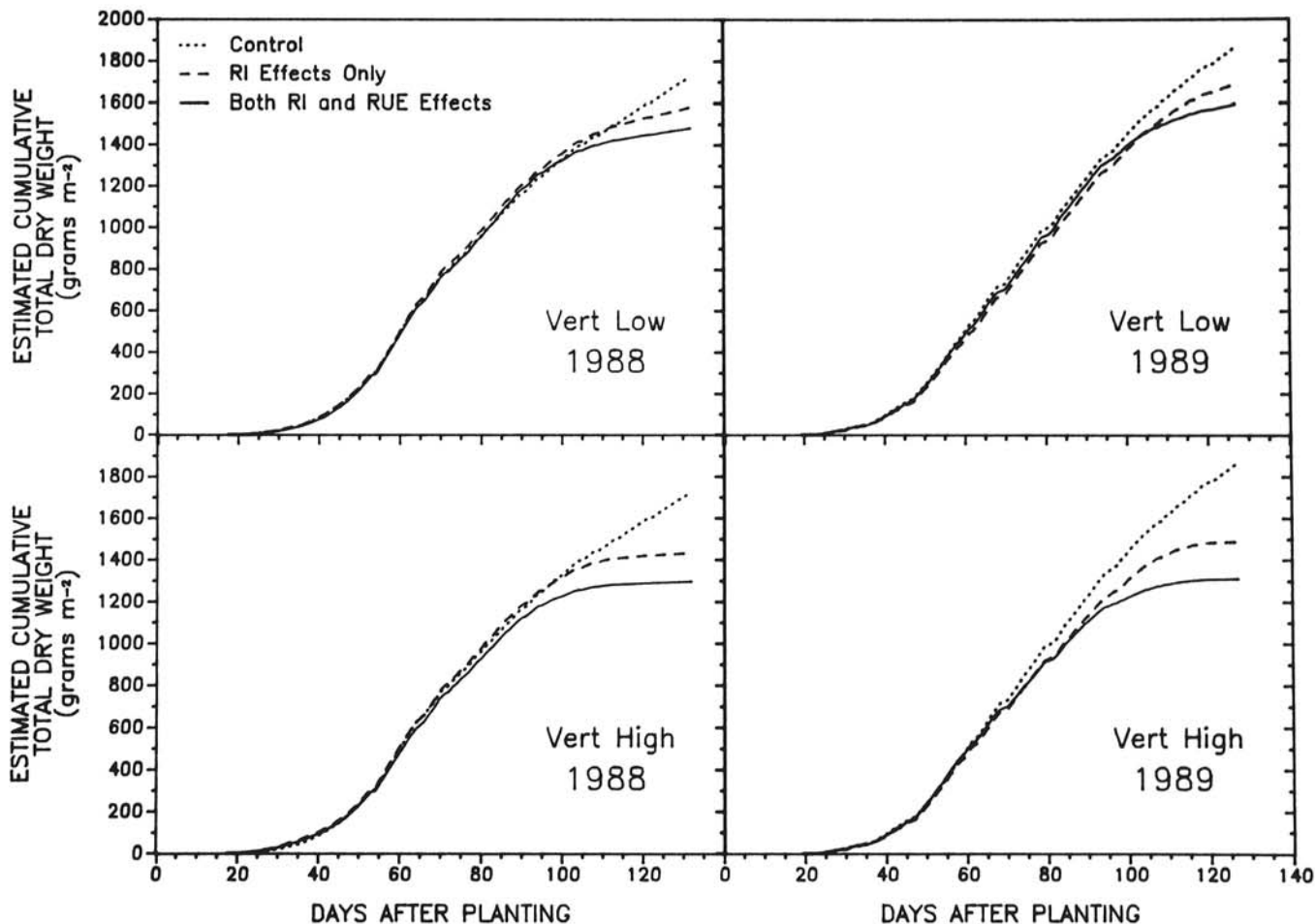In the field, the four different measures of disease progress



**Fig. 7.** Estimated potato plant dry weight growth curves for controls, plants infected by *Verticillium dahliae* but considering only the radiation interception (RI) effect, and infected plants considering both the radiation interception and radiation use efficiency (RUE) effects. Data are for the low and high inoculum doses of field experiments in 1988 and 1989.

gave similar results. In 1988, top leaf stomatal conductance and proportion diseased leaves according to gas exchange were impaired by excessive variability. However, in 1989, both variables gave better results. In both years, the most sensitive and least error-prone measure of disease progress was the proportion RI. Because it is directly related to defoliation, this variable detects plants in the local as well as systemic disease phases. Also, the line quantum sensor used to calculate RI effectively sampled several plants per plot, whereas the photosynthesis system sampled only four to five leaves per plot.

The choice of disease measurement method will depend on the objectives of the study. Proportion RI is an excellent measure when large plots are the unit of interest. When individual plants or leaves are the unit of interest, the classification system based on gas exchange is a good choice. The major disadvantage of the method is the initial cost of gas exchange equipment. In the field, sunny weather conditions and adequate soil moisture also are required.

This study is in agreement with a previous report for *V. dahliae* on potato that stem stunting occurs late in disease development (14). However, stunting before foliar symptom development was reported for *V. albo-atrum* on tomato (25) and *V. dahliae* on cotton (22). Selman and Pegg (25) directly inoculated roots of tomato seedlings at the two-leaf stage with mycelial plugs. This probably subjected the immature vascular system to massive invasion and resulted in immediate systemic effects. The data of Pullman and DeVay (22) represent natural infection in the field and cannot be explained on this basis.

Leaf growth is more sensitive to drought stress than photosynthesis or stomatal conductance (6,7,9). If the systemic phase of disease is caused by xylem blockage which results in water stress, then why is leaf stunting difficult to detect? Boyer (5) showed that a minimum turgor is required for cell elongation and this minimum normally is not achieved during the day. Therefore, most growth occurs at night when reduced transpiration allows turgor recovery. In plants infected by *V. dahliae*, partial stem plugging would lead to daytime water stress and would delay nighttime turgor recovery but would not prevent complete turgor recovery. Therefore, cell elongation would be reduced only slightly until stem plugging was essentially complete. The growth-chamber experiments in this study were terminated before that point.

Adams and Rouse (1) reported that defoliation caused significant reductions in radiation absorption and was correlated with yield loss in potato inoculated with *V. dahliae*. An important question is whether the effect of *V. dahliae* on potato photosynthesis is significant compared with the defoliation effect. Selman and Pegg (25) used growth analysis to infer that *V. albo-atrum* reduced photosynthetic efficiency of tomato leaves. Isaac and Harrison (16), using similar methods, came to a similar conclusion for potato infected by *V. dahliae*. However, Johnson et al (18) used an analysis of foliage losses and yield to suggest that *V. dahliae* has little impact on photosynthetic efficiency of potato foliage.

The RI data combined with the assimilation rate data from the field provided an opportunity to gauge the relative importance of defoliation effects and reduced photosynthetic rate on yield loss. The percent loss in estimated cumulative dry weight predicted the tuber dry weight percent yield loss accurately. If the effect of reduced RUE was considered after accounting for defoliation, then about one third of the yield loss was attributable to reduced RUE.

There are two possible reasons why Johnson et al (18) did not detect an effect of *V. dahliae* on RUE. First, chlorotic leaves were considered to be defoliated. In the present study, only dead tissue was excluded from light interception measurements. Second, their integral "area under the proportion of green leaf area remaining" explained only 8–62% of the variability in yield in separate regressions using data from 2 yr and three cultivars (18). Because our data only attribute 35% of the yield loss to reduced RUE, the two studies may in fact be reconcilable.

Waggoner and Berger (28) argued that decreased canopy radiation interception was adequate to explain yield loss in most host-parasite combinations. Johnson (17) claimed that the potato leafhopper is an exception to that assertion. The present study showed that Verticillium wilt of potato is another exception.

## LITERATURE CITED

1. Adams, S. A., and Rouse, D. I. 1986. The effect of Verticillium wilt on PAR absorption and yield loss of potatoes. (Abstr.) Phytopathology 76:1068.
2. Alexander, S. J., and Hall, R. 1974. Verticillum wilt of chrysanthemum: Anatomical observations on colonization of roots, stem, and leaves. Can. J. Bot. 52:783-789.
3. Bowden, R. L., and Rouse, D. I. 1988. Chronology of gas exchange effects in Verticillium wilt of potato. (Abstr.) Phytopathology 78:1563.
4. Bowden, R. L., and Rouse, D. I. 1991. Effects of *Verticillium dahliae* on gas exchange of potato. Phytopathology 81:293-301.
5. Boyer, J. S. 1968. Relationship of water potential to growth of leaves. Plant Physiol. 43:1056-1062.
6. Boyer, J. S. 1970. Differing sensitivity of photosynthesis to low leaf water potentials in corn and soybean. Plant Physiol. 46:236-239.
7. Boyer, J. S. 1970. Leaf enlargement and metabolic rates in corn, soybean, and sunflower at low leaf water potentials. Plant Physiol. 46:233-235.
8. Brandt, W. H., Lacy, M. L., and Horner, C. E. 1984. Distribution of *Verticillium* in stems of resistant and susceptible species of mint. Phytopathology 74:587-591.
9. Dale, J. E. 1988. The control of leaf expansion. Annu. Rev. Plant Physiol. Plant Mol. Biol. 39:267-295.
10. Dimond, A. E. 1970. Biophysics and biochemistry of the vascular wilt syndrome. Annu. Rev. Phytopathol. 8:301-322.
11. Duniway, J. M. 1971. Resistance to water movement in tomato plants infected with *Fusarium*. Nature 230:252-253.
12. Farquhar, G. D., Ehleringer, J. R., and Hubick, K. T. 1989. Carbon isotope discrimination and photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 40:503-537.
13. Garber, R. H. 1973. Fungus penetration and development. Pages 69-77 in: Verticillium Wilt of Cotton. USDA-ARS-519. C. D. Ranney, ed. National Cotton Pathology Research Laboratory, College Station, TX. 134 pp.
14. Harrison, J. A. C., and Isaac, I. 1968. Leaf-area development in King Edward potato plants inoculated with *Verticillium albo-atrum* and *V. dahliae*. Ann. Appl. Biol. 61:217-230.
15. Harrison, J. A. C., and Isaac, I. 1969. Host/parasite relations up to the time of tuber initiation in potato plants infected with *Verticillium* spp. Ann. Appl. Biol. 64:469-482.
16. Isaac, I., and Harrison, J. A. C. 1968. The symptoms and causal agents of early-dying disease (Verticillium wilt) of potatoes. Ann. Appl. Biol. 61:231-244.
17. Johnson, K. B. 1987. Defoliation, disease, and growth: A reply. Phytopathology 77:1495-1497.
18. Johnson, K. B., Teng, P. S., and Radcliffe, E. B. 1987. Analysis of potato foliage losses caused by interacting infestations of early blight, Verticillium wilt, and potato leafhopper; and the relationship to yield. Z. Pflanzenkrankh. Pflanzenschutz 94:22-33.
19. McCauley, M. M., and Evert, R. F. 1988. Morphology and vasculature of the leaf of potato (*Solanum tuberosum*). Am. J. Bot. 75:377-390.
20. Milliken, G. A., and Johnson, D. E. 1984. Analysis of Messy Data. Vol. I. Designed Experiments. Van Nostrand Reinhold, New York. 473 pp.
21. Monteith, J. L. 1977. Climate and the efficiency of crop production in Britain. Philos. Trans. R. Soc. London B 281:277-294.
22. Pullman, G. S., and DeVay, J. E. 1982. Epidemiology of Verticillium wilt of cotton: Effects of disease development on plant phenology and yield. Phytopathology 72:554-559.
23. Rowe, R. C., Davis, J. R., Powelson, M.L., and Rouse, D. I. 1987. Potato early dying: causal agents and management strategies. Plant Disease 71:482-489.
24. SAS Institute. 1985. SAS/STAT User's Guide. Release 6.03 edition. SAS Institute, Inc., Cary, NC. 1028 pp.
25. Selman, I. W., and Pegg, G. F. 1957. An analysis of the growth response of young tomato plants to infection by *Verticillium albo-atrum*. Ann. Appl. Biol. 45:674-681.
26. Snedecor, G. W., and Cochran, W. G. 1980. Statistical Methods. 7th ed. Iowa State University Press, Ames, IA. 507 pp.
27. Street, P. F. S., and Cooper, R. M. 1984. Quantitative measurement of vascular flow in petioles of healthy and *Verticillium*-infected tomato. Plant Pathol. 33:483-492.
28. Waggoner, P. E., and Berger, R. D. 1987. Defoliation, disease, and growth. Phytopathology 77:393-398.