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The influence of Verticillium dahliae and drought on potato crop growth. 1. Effects on gas exchange and stomatal behaviour of individual leaves and crop canopies

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Abstract

Potato (*Solanum tuberosum* L.) plants cv. *Saturna* were subjected to infection with *Verticillium dahliae* and drought stress. At the early stages of growth, stomatal conductance, transpiration and net photosynthesis were measured at light saturation (PAR > 300 W m^{-2}) on individual leaves and with mobile field equipment with the aid of field enclosures. No significant changes in stomatal conductance and gas exchange characteristics occurred as a result of *V. dahliae* inoculation until a month after emergence. From then on *V. dahliae* infection led to decreased stomatal conductance, transpiration and and photosynthetic rates, especially on older leaves and on plants exposed to direct sunlight for a longer period of time. In combination with drought, *V. dahliae* only occasionally showed interaction; their effects being less than additive. High values of coefficients of variation necessitated a high number of measurements per treatment; the more so in the inoculated plants which shows that *V. dahliae* seems to affect certain leaves while not affecting others early in growth. Crop photosynthesis was less reduced by *V. dahliae* than individual leaf photosynthesis due to the levelling effect of integration over the whole canopy and possibly through a stimulation of the top leaves. The upper non-affected leaves are responsible for the bulk of photosynthetic crop activity. The results indicate that following an infection with *V. dahliae* photosynthesis is reduced early in growth as a result of drought stress in the leaves.

Additional keywords: Canopy enclosure, stomatal conductance, light response curve, light use efficiency, photosynthesis, *Solanum tuberosum* L., transpiration, water use efficiency.

Introduction

In recent years it has become apparent that in areas with frequent cropping of potatoes, the length of rotation with other crops can have a significant effect on potato yields. Haverkort et al. (1989) demonstrated that in seasons with lack of rainfall in July and August such losses on a young marine soil were over 25% when comparing a six year to a three year rotation. In years with more rain the three year rotation led to only a 5-10% reduction in yield compared with the six year rotation. These losses may be due to several factors including the development of a complex of deleterious soilborne microorganisms following short rotations. Schippers et al. (1985) and Scholte et al. (1985) have shown that a complex of fungi, actinomycetes and bacteria may be responsible for a portion of these yield reductions. A major contributing factor is the soil borne wilt fungus *Verticillium dahliae* Kleb. (Bollen et al., 1989). Synergistic effects of *V. dahliae* with nematodes have been shown by e.g. Scholte and s'Jacob (1989).

Verticillium dahliae is a primary cause of the potato early dying syndrome (Rowe et al., 1987). Typical symptoms of *V. dahliae*, such as the asymmetric yellowing of leaves are not often observed in the Netherlands, although wilting of some leaves may occur on days with a high evaporative demand. Yield losses are often associated with premature dying of the crop, but under the conditions of low evaporative demand yields may also be reduced by a slower growth beginning early in the season (Scholte and s'Jacob 1989). It is not clear how growth reduction by *V. dahliae* is brought about. The role of crop growth parameters such as leaf expansion rate, dry matter distribution, stomatal conductance, transpiration rate and photosynthesis are not known.

Harrison (1971) found that potato plants infected with *V. dahliae* or *V. albo-atrum* had reduced transpiration rates during the day. Bowden and Rouse (1990a) found lower stomatal conductance, transpiration and photosynthesis rates and higher leaf temperature in leaves of *V. dahliae* infected potato plants then in noninfected controls. They showed that these effects can occur before visual symptoms occur and progress in magnitude with time from older leaves to younger leaves (Bowden and Rouse, 1990b). It was shown that the initial decrease in photosynthesis was caused by stomatal closure (Bowden et al., 1990). Several previous reports on the effect of wilt causing pathogens on stomatal behaviour and gas exchange characteristics have dealt with detached leaves of other crops. Zazzerini (1975) found that pepper plants infected with *V. dahliae* had reduced net photosynthesis during the day and increased respiration at night. Mathre (1968) observed reduced photosynthetic rates of cotton leaf disks infected with a defoliating strain of *V. dahliae* before visual symptoms appeared.

Gas exchange characteristics of potato crop canopies adequately provided with water and minerals have been described in literature by Bodlaender (1977), Leach et al. (1982), and by Vos and Groenwold (1989a). Leach et al. (1982) reached a good agreement between the estimates of dry matter accumulation derived from plant harvests and from CO_2-exchange measurements. The highest rates of CO_2-uptake of whole plants reported by these authors were approximately 6 g h^{-1} per m^2 ground area. This value is comparable to those found by Alberda et al. (1977) and by Bodlaender et al. (1986). The influence of drought on canopy photosynthesis and related characteristics was described by Bodlaender et al. (1986) and by Vos and Groenwold (1989a). With increased soil water depletion, stomatal conductance of the canopy was reduced earlier and to a greater extent than net photosynthesis. A relatively larger effect of drought on stomatal conductance than on photosynthesis was also shown on individual leaves by Vos and Oyarzun (1987). This phenomenon, as well as an increased water use efficiency (Vos & Groenwold, 1989b), are features typical of drought.

This paper is the first in a series which describes the influence of *V. dahliae* and drought on potato crop growth. The project is a joint effort of the Centre for Agrobiological Research (CABO) and the Research Institute for Plant Protection (IPO), both in Wageningen, the Netherlands. The objectives of the whole project are to study the interaction between stresses caused by biotic and abiotic soil related factors. The objectives of the work described here are to study both the influence of drought and *V. dahliae* on stomatal conductance, transpiration, net photosynthesis and dark respiration under controlled and field conditions. Interaction between the effects on these characteristics caused by drought and by the fungus is studied, as well as the influence of leaf position, plant age and the location of the plant with regard to exposure to the sun.

Materials and methods

Seed potatoes of the cultivar Saturna, class E, 40-45 mm, were presprouted at 11 °C in continuous light. About two weeks prior to planting, the seed potatoes were transferred to trays and covered with moist perlite grains or moist sand to allow roots to form. Meanwhile a suspension of microsclerotia and spores was cultured on a liquid potato dextrose medium. On the day of planting into the experimental unit, the perlite or sand was rinsed away and the roots of half of the plants were soaked in the *V. dahliae* medium, the other half in water. This methodology was developed at the Research Institute for Plant Protection (IPO). In total four different experiments were carried out.

Experiment 1 was planted in 70 cm × 90 cm containers which were 40 cm deep. These were placed one meter apart under a rainshelter with open sides which altered ambient conditions such as rainfall (absent) and incident solar radiation (−25%). Six plants were planted per container. The surface of the soil was covered with a plastic sheet through which 3 stems per plant were allowed to protrude; excess was trimmed. The containers were placed in four rows (replicates) running from North to South, 16 in a row to allow periodic harvests. The plants were watered twice a week to replenish transpired water by weight. In this experiment, an early drought stress (Treatment 1) was created by witholding water from emergence until the crop showed a slight wilting in the afternoon and some growth retardance. This occurred about one month after planting. A late drought stress (Treatment 3) in different containers was applied by witholding water from the end of the first drought stress period onward.

Experiment 2 was planted in 1988 in 26 cm diameter, 25 cm deep pots. Pots were chosen here to allow the plants to be placed in the assembly for routine measurements of gas exchange of intact plants under controlled conditions (Louwerse and Oorschot, 1969). The drought treatment consisted of witholding water for 4 days prior to measurement. On the day that photosynthesis was measured, no drought symptoms were visible. That *V. dahliae* inoculation was successful was deduced from the proportion of leaves of all leaves present that were yellow: 7% in the untreated plants, 18% in the inoculated plants. Four series of measurements were taken per treatment.

Experiment 3 was carried out in 1989 similarly to experiment 1 but the presprouted seed tubers were planted directly into the container. Inoculation with *V. dahliae* consisted of placing 4 g of perlite covered with microsclerotia under the seed tuber. This inoculum was prepared by moistening perlite in the liquid inoculum and allowing a few weeks time at 25 °C for *V. dahliae* to further develop and cover the perlite with microsclerotia. The late drought treatment consisted in this case of supplying only half the amount of water transpired by the control plants.

Experiment 4 was carried out in 1989 in the field. Its main purpose was to measure crop gas exchange with the enclosure method as influenced by *V. dahliae* infection. The plots were irrigated frequently by sprinkling to make up for evapotranspiration. To arrive at full ground cover with green foliage as rapidly as possible, planting density was high (30 cm × 30 cm). The plot size was 3 by 3 meters. On the day of planting square frames of 2 m^2 used to support the canopy enclosures, were dug 20 cm deep in the centre of each plot with the top just protruding. The 1.8 m^3 enclosure chambers consisted of transparent acrylic. This was sealed onto the metal frame in which the plants were grown. The enclosure material reflected somewhat over 10% of the incoming radiation. In the calculations of the light use efficiencies this was not accounted for. The air flow containing ambient CO_2 concentration (340 ppm) through the whole system was 68 m^3 per hour corresponding with an average residence time of about 95 seconds. The canopy enclosure operated as an open system with overpressure to avoid effects of leakage, contribution of soil respiration and evaporation. For this reason too, at the beginning of each week of measurement, the soil between the plants was covered with about 3 cm of dry fine gravel. The measurements of stomatal conductance, transpiration and net photosynthesis were carried out with mobile equipment provided with infrared gas analyzers, to determine the CO_2-concentrations and humidity of incoming and outgoing air of the crop chambers. The equipment and methods are described by Louwerse and Eikhoudt (1975). Two crop enclosures operated simultaneously, one in the control plot and one in the inoculated plot. With the crop enclosure method, total crop response is measured rather than individual leaf response. The conductance for diffusion of water vapour through the stomates (stomatal conductance) was derived from transpiration and from the net photosynthesis as given by Louwerse (1980). Thus it accounts for the crop boundary CO_2-conductance which is a function of the leaf area index (LAI). The crop enclosures were put in place on Monday June 12. The measurements took place continuously until Friday noon on June 16. Then the LAI of the green and yellow leaves was determined with an area meter (LiCor Inc. Lincoln Nebraska Type 3100) and tests for the presence of *V. dahliae* were carried out with plating techniques described at the end of this section. The night temperature (from 21:00 h until 7:00 h) was maintained at 15 °C. The day temperature was 22 °C on Tuesday and 29 °C on Wednesday. Gas exchange measurements were also taken with the ADC-equipment on plants of the border rows of the plots where field photosynthesis was measured. In the first week of measurement, individual leaf photosynthesis was measured on Tuesday June 13 and on Thursday June 15. A week later the same procedures were repeated. Substomatal CO_2 concentrations (Ci) were derived from net photosynthesis (PH), stomatal conductance (SC) and the substomatal CO_2 concentration (Ca) as given by Schapendonk et al. (1989): $PH = SC \times (Ca - Ci)$.

An overview of the characteristics of all four experiments is given in Table 1.

Stomatal conductance and transpiration of 2 cm^2 leaf surface were measured with a LiCor 1600 steady state diffusion porometer (Lincoln NE, USA), attached to the abaxial side of the leaves. The CO_2-exchange was measured with a portable ADC instrument (Analytical Development Company, Hoddesdon, UK) attached to the abaxial side of the leaf. This instrument also measures stomatal conductance and transpiration which are represented in Tables 2 and 7. The light response curves in Exp. 3 were determined using the laboratory assembly for the measurement of photosynthesis and transpiration described by Louwerse and van Oorschot (1969). This method, which allows measurement at various temperatures which were set at 18 and 26 °C, uses whole leaves including rachis and leaflets for CO_2-exchange measurement. Metal screens with different sized openings were placed below the HPI/T 400 (Philips) lamps to achieve various light intensities and measurement started at the highest intensities. Photosynthesis measurements obtained with this equipment at light saturation were comparable to the

Table 1. Characteristics of the four experiments.

Detail	Exp. 1	Exp. 2	Exp. 3	Exp. 4
Presprouting time	5 weeks	4 weeks	8 weeks	4 weeks
Rooting medium	perlite	perlite	none	sand
Days root formation	13	15	none	17
Planting date	9-5-'88	4-8-'88	5-6-'89	9-5-'89
Location	rain shelter	greenhouse	rain shelter	open field
Experimental unit (cm)	container	pot	container	field plot
Area per unit (m^2)	0.9 × 0.7	13 cm radius	0.9 × 0.7	2
Soil type	white sand	humous soil	sandy humous	sandy humous
Plants per unit	6	1	6	40
Number of replicates	4	4	3	2
Drought treatments	1 none 2 early 3 late (withheld)	1 none 2 no water for 5 days	1 none 2 early 3 late (50%)	none
V. dahliae	1 none 2 treated	1 none 2 treated	1 none 2 treated	1 none 2 treated
Device used	LiCor 1600 and ADC	Laboratory assembly	ADC	ADC and Enclosure

ADC-readings.

All measurements, unless mentioned otherwise, were carried out on the youngest fully expanded leaf. These leaves did not show any *V. dahliae* symptoms such as (unilateral) chlorosis or wilting. For the measurements in Exps 1, 3 and 4, 2 cm^2 of the apical leaflet was exposed in the portable measuring device. Measurements were usually taken around noon at light saturation (unless mentioned otherwise) with photosynthetically active radiation PAR > 300 W m^{-2}. In Exps 1 and 4, the presence of *V. dahliae* was detected by plating discs of the basal part of the stem on an agar medium containing 10% strength Difco potato dextrose. *V. dahliae* was assessed visually from mycelium appearance and microsclerotial growth after 2 weeks at 20 °C.

Results

Photosynthesis in Exp. 1 was measured on June 13, 1988. The plants that were subjected to the drought treatment at that time had just started to show a visible growth retardation. That the *V. dahliae* inoculation was successful was shown in a periodic harvest, one month later: the mean level of infection of the stems of the inoculated plants was 87%, against only 7% in the non-inoculated plants. Photosynthesis, transpiration and stomatal conductance data are shown in Table 2. At this early stage of growth, their values were not reduced by the presence of *Verticillium dahliae.* The treatments that were subjected to drought, as expected, showed strongly reduced values. Interaction between *Verticillium* wilt and drought was evident, the effects were less than additive. A week later, transpiration and stomatal conductance values tended to decrease (with high variation coefficients) under the influence of *V. dahliae* (Table 3) and highly signifi-

Table 2. Photosynthesis (PH: mg CO_2 m^{-2} s^{-1}),transpiration (TR: mg H_2O m^{-2} s^{-1}) and stomatal conductance (SC: mm s^{-1}) of the top leaves in the container experiment (Exp. 1, drought treatment 1) ; June 13 1988, 4 observations per treatment (NS = not significant, * $P < 0.1$, ** $P < 0.05$, *** $P < 0.01$, CV = coefficient of variation).

Observation	*V. dahliae*	Drought treatment			Significances	
		control	droughted	mean		
PH	Control	0.697	0.590	0.643	*Vert.* effect	NS
	Inoculated	0.697	0.553	0.625	Drought effect	**
					Vert. × drought	NS
	Mean	0.697	0.572	0.634	CV	7.1%
TR	Control	82.6	59.1	70.8	*Vert.* effect	NS
	Inoculated	77.0	58.6	67.8	Drought effect	***
					Vert. × drought	NS
	Mean	79.8	58.8	69.3	CV	8.8%
SC	Control	6.01	3.40	4.71	*Vert.* effect	NS
	Inoculated	6.14	3.99	5.07	Drought effect	*
					Vert. × drought	NS
	Mean	6.08	3.69	4.89	CV	34.4%

Table 3. Transpiration (TR: mg H_2O m^{-2} s^{-1}) and stomatal conductance (SC: mm s^{-1}) of the top leaves in Exp. 1 drought treatment 1; June 20 1988, 9 observations per treatment (significances see Table 2).

V. dahliae	Control		Droughted		Significance	
	SC	TR	SC	TR		
Control	22.5	161	14.7	121	*Vert.* effect	NS
Inoculated	17.8	133	13.3	110	Drought effect	**
					Vert. × drought	**
					Coeff. var. SC	22.8%
Mean	20.2	147	14.0	116	Coeff. var TR	13.7%

cant interaction showed between the two treatments (indicative of *V. dahliae* and drought being less than additive with regard to stomatal conductance and transpiration).

Porometer measurements in crops subjected to drought and *V. dahliae* were repeated in Exp. 3, two weeks after the end of the drought stress. In these measurements, taken on June 23 1989, a distinction was made between leaves of plants growing on the southern side and on the northern side of each container. Those on the South side received more light, were likely to transpire more water and to be more prone to stress than those on the North side. The influence of plant position, *V. dahliae* infection and drought on stomatal conductance and transpiration is shown in Table 4. The reduction of transpira-

Table 4. Transpiration (TR: mg H_2O m^{-2} s^{-1}) and stomatal conductance (SC: mm s^{-1}) of the top leaves in the container experiment (Exp. 3, drought treatment 3); June 23 1989, 6 observations per treatment (CV of SC: 44%, of TR: 17%; significances see Table 2).

Treatment		South		North		Significances	
drought	*V. dahliae*	SC	TR	SC	TR		
Control	Control	10.9	93.8	3.0	113.8	drought effect	**
Control	Treated	11.8	98.2	2.3	110.8	*Vert.* effect	NS
						South/North (S/N) eff.	***
Relieved	Control	11.1	103.8	13.3	117.3	drought × *Vert.*	NS
Relieved	Treated	12.2	107.7	13.3	116.7	drought × S/N	*
						Vert. × S/N	NS
Droughted	Control	8.5	86.5	12.7	111.3	drought × *Vert.* × S/N	NS
Droughted	Treated	5.6	78.3	12.8	111.0		

Table 5. Transpiration (TR: mg H_2O m^{-2} s^{-1}) and stomatal conductance (SC: mm s^{-1}) at different leaf positions in the container experiment (Exp 3, drought treatment 3); June 22 1989, 8 observations per treatment (significances see Table 2).

Leaf position	Drought	*V. dahliae*				Significance	
		control		treated			
		SC	TR	SC	TR		
High	Control	15.6	174	19.2	205	Drought	NS
Middle	Control	17.2	183	15.5	157	*V. dahliae* (*Vert.*)	*
Low	Control	11.9	139	2.1	55	Leaf position (lp)	*
						Drought × *Vert.*	NS
High	Droughted	11.1	173	12.5	149	Drought × lp	NS
Middle	Droughted	10.9	150	4.29	63	*Vert.* × lp	***
Low	Droughted	7.1	110	3.14	55	Drought × *Vert.* × lp	NS

tion from 86.5 to 78.3 mg H_2O m^{-2} s^{-1} due to *V. dahliae* in the droughted treatment was not significant but the drought effect as well as the South/North effect were marked. There was also an interaction between the drought and the South/North effect: leaves of the southern plants showed a stronger reduction of transpiration and stomatal conductance due to drought than the northern ones. The day before, the drought effect was not yet significant (Table 5) because in the morning all containers were watered, including the ones that only received half the transpired amount. Porometer measurements were taken at three leaf levels: the highest fully expanded leaves, leaves halfway up the stem and the lowest still green leaves. Here a *V. dahliae* effect did show, especially (P-interaction < 0.01) in the lower leaves. Transpiration and stomatal conductance were reduced when going down the stem in the control plants, but in the inoculated plants these parameters were much more strongly reduced.

The light response curves of plants with and without *V. dahliae* inoculation and drought stress revealed the following information. When measured at 26 °C, the maximum net photosynthesis (Amax = gros photosynthesis − respiration) was highest for the control plants (varying between 0.78 and 0.90 with a mean value of 0.87 mg CO_2 m^{-2} s^{-1}) followed by the inoculated plants (0.77 mg m^{-2} s^{-1}). At 18 °C, no significant difference was found between the *V. dahliae* treated plants and the controls (0.73 mg m^{-2} s^{-1}). The drought treatment still further reduced the Amax values; the values measured at 18 C of the *V. dahliae* treated and untreated plants are respectively 0.61 and 0.45 mg m^{-2} s^{-1}. The initial slopes of the light response curves are indicative of the relative increase in photosynthesis per W per m^2 increase of light intensity (mg J^{-1}). The values of this parameter decreased at 26 °C under the influence of *V. dahliae* from 0.018 to 0.016. Respiration in the dark at 26 °C was highest in the control plants: 0.081 versus 0.069 mg m^{-2} s^{-1} in the inoculated plants. Dark respiration measured at 18 °C was found to be 0.049 mg m^{-2} s^{-1} and was unaffected by *V. dahliae*. The observed reduction of maximum photosynthetic rates under the influence of *V. dahliae* cannot be explained by the slightly reduced nitrogen content of the dry matter in the leaves: 2.16% instead of 2.25% found in the controls in the 16 samples analyzed per treatment. The specific leaf area was not significantly affected and was 325 cm^2 per g dry leaf matter in the inoculated plants against 332 cm^2 g^{-1} in the controls.

In Exp. 4 the individual leaf photosynthesis measurements were taken on Tuesday June 13 and on Thursday June 15. The leaf area indices (total and green) are shown in Table 8. *V. dahliae* infection did not lead to lower LAI's. Table 6 shows the results of the *V. dahliae* tests of stem bases and higher plant parts of Exp. 4. The controls have partly become infected by natural infection processes through the soil and/or the seed tuber. The inoculated plants showed a high degree of basal stem infection as showed up through the growth of microsclerotia on the medium. At the end of the first week 32 vascular bundles across 18 out of 25 stems showed *V. dahliae* infection. None of the higher nodes were infected. A week later progress of infection was shown by the presence of the fungus in the higher nodal levels of the plant.

Table 7 shows the observed photosynthesis and transpiration as well as the calculated stomatal conductance, light and water use efficiencies and the internal/external CO_2 concentrations of the various leaf levels on the different dates. The light use efficiency was calculated as the amount of CO_2 exchanged (g) per amount of incident photosyn-

Table 6. Results of *V. dahliae* tests in Exp. 4, 23 stem cross sections of the control and 23 of the inoculated plot were plated on June 16 (week 1) and on June 23 (week 2) and examined after 2 weeks.

Week	Treatment	Microsclerotial infection			Infected vascular bundles		
		stem base	3 nodes up	6 nodes up	stem base	3 nodes up	6 nodes up
1	Control	3	0	0	5	0	0
	Inoculated	18	0	0	32	0	0
2	Control	4	3	2	6	3	4
	Inoculated	12	9	4	23	22	10

Tabel 7. Field experiment 1989 (Exp. 4, significances see Table 2). Legend see Table 1 and Ci/Ca = ratio of internal and external stomatal CO_2 concentrations, LUE = light use efficiency, WUE = water use efficiency.
a: June 13, 6 observations per treatment, top leaves.
b: June 21, 10 observations, top leaves.

Observation	Dimension	*Verticillium dahliae*		Significance	Coefficient of var. (%)
		control	inoculated		
a					
Photosynthesis	mg CO_2 m^{-2} s^{-1}	0.519	0.513	NS	59
Transpiration	mg H_2O m^{-2} s^{-1}	47.4	33.7	NS	84
Conductance	mm s^{-1}	3.18	1.91	NS	115
LUE	g MJ^{-1}	2.76	2.43	NS	52
WUE	mg ml^{-1}	10.9	15.22	NS	61
Ci/Ca		0.565	0.455	NS	44
b					
Photosynthesis	mg CO_2 m^{-2} s^{-1}	0.725	0.474	**	40
Transpiration	mg H_2O m^{-2} s^{-1}	81.5	46.0	**	43
Conductance	mm s^{-1}	6.89	3.00	**	58
LUE	g MJ^{-1}	3.27	2.37	*	38
WUE	mg ml^{-1}	8.90	10.3	**	11
Ci/Ca		0.719	0.579	**	13

thetically active solar radiation between 400 and 700 nm. Similarly to the Haverkort and Bicamumpaka (1986) calculations for light use efficiency from intercepted solar radiation and dry matter accumulation. Early in the first week, on June 13, the top leaves did not show any significant difference between treatments. Two days later, a significant reduction of photosynthesis was observed only in the fourth leaf from the top. Early in the second week, the light use efficiency showed a significant decrease in the top leaves. When taking the fourth leaf from the top on the same day, photosynthesis, transpiration, stomatal conductivity and internal/external CO_2 ratio's all decreased significantly (while the water use efficiency increased), but due to fluctuating light conditions (CV = 90%), the decrease of the light use efficiency was not statistically significant. Two days later, on June 21, all the observed characteristics were significantly influenced, even in the top leaves. Table 7 shows the two extremes on June 13 and June 21, of tendency to a progressively increased statistical significance of the observed differences between the treatments with time. While measuring, it became clear that divergence from the average was greater in the inoculated plots than in the controls. Fig. 1 shows the frequency distribution of all 42 measurements on the inoculated and the control plots of Exp. 4. The control plots had photosynthesis rates ranging from 0.6 to 1.1 mg CO_2 m^{-2} s^{-1} while the readings from infected plots ranged from 0 to 1.1 mg CO_2 m^{-2} s^{-1}. Also reflective of the range of values were the coefficients of variation of the values found in the control and treated plots: the standard deviation divided by the mean value of the data (× 100 % = CV) for photosynthesis on June 15 was 15% in the control and 25% in the infected plot; for transpiration this was 21 and 46%

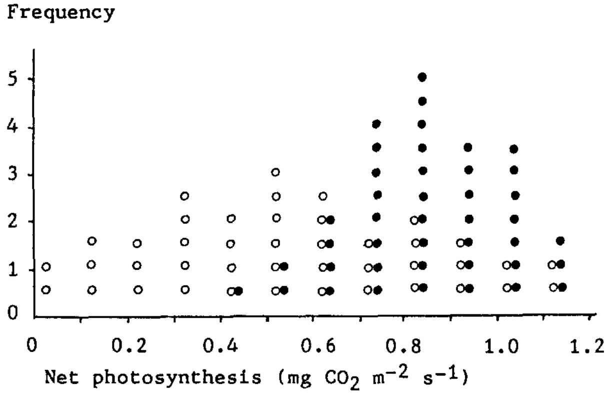

Fig. 1. Frequency distribution of all photosynthesis rates measured in Experiment 4. (●) uninoculated and (○) inoculated with *V. dahliae.*

respectively. On June 21 the CV of photosynthesis was 23% in the control plot and 62% in the inoculated plot; for transpiration this was 40 and 47% respectively. Although some leaves in the infected plots showed a large reduction in the value of the observed parameters, many did not.

The tendency towards an increased reduction of photosynthesis with time (Table 7) due to the presence of *V. dahliae* is associated with decreased transpiration rates and stomatal conductance. Meanwhile the water use efficiency increased and the internal/external CO_2 balance as well as the light use efficiency decreased. It is clear from these data (which corroborate those of Bowden and Rouse, 1990) that *V. dahliae* causes drought stress in the leaves leading to stomatal closure, a reduced substomatal CO_2-concentration and reduced photosynthetic rates as is also illustrated in reduced light use efficiency values. Reduced transpiration led to increased water use efficiency values, also typical of drought stress (e.g. Vos and Groenwold, 1989b).

Table 8 gives the experimental conditions, leaf area indices and selected photosynthesis data of the four selected days of crop photosynthesis measurement. These four days were selected because of their high solar input with only some cloudiness during parts of the day on June 14 and 21. The daily courses of the gas exchange measurements of the controls and the inoculated crops are shown in Fig. 2. Each represented value is the mean of 13 gas exchange observations per hour. The reduction of stomatal conductance around noon in the inoculated plots was more marked than the reductions of transpiration and photosynthesis. On June 14 transpiration was higher and the *V. dahliae* effect was greater than on June 13 because of the higher temperature in the enclosure which led to a higher evaporative demand. Stomatal conductivity and photosynthesis did not seem to be affected by the higher daytime temperature. The differences between the control and the inoculated plots were greater in the second week (on June 20 and 21) than in the first (on June 13 and 14). The increased difference between gas exchange values of the control and infected plots on the two days of the second week was associated with the increased presence of *V. dahliae* in stem tissue. Table 8 shows that on June 20, photosynthesis during the hours of daylight as a result of the presence of *V. dahliae* was reduced from 730 to 704 kg CO_2 ha^{-1} (−3.6%) and on

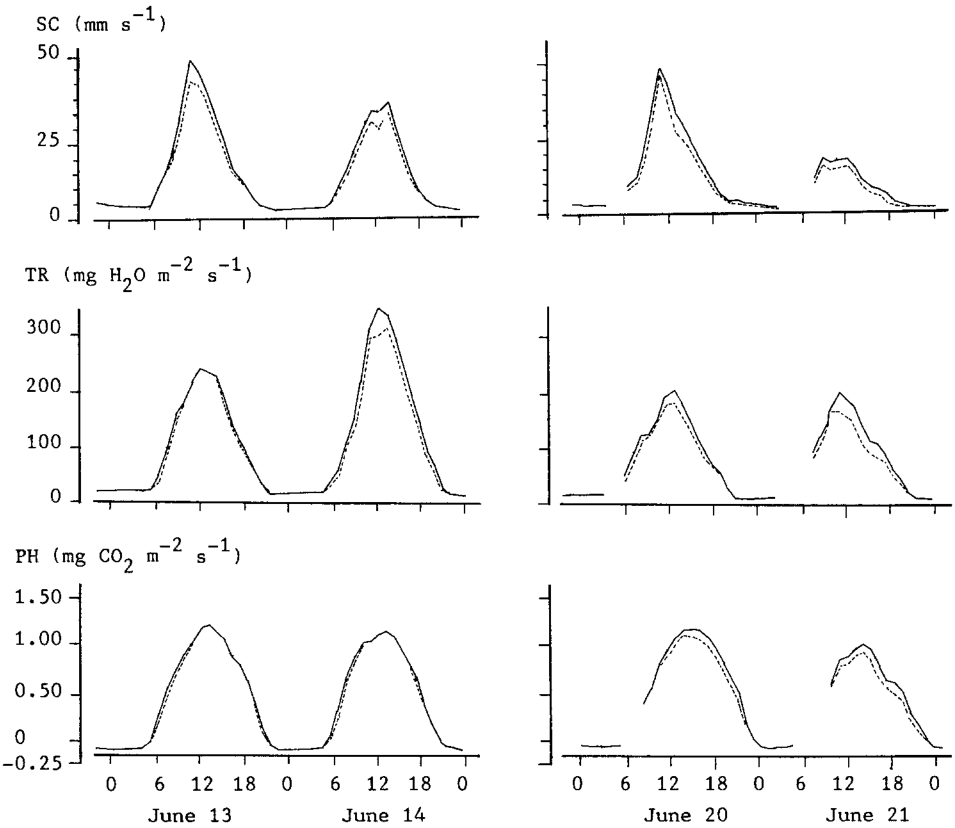

Fig. 2. Mean hourly values of stomatal conductance, transpiration and photosynthesis of plots inoculated with *V. dahliae* (broken lines) and controls (unbroken lines) on four selected days of measurement. Night temperature 15 °C, day temperature 22 °C on June 13 and 20, 29 °C on June 14 and 21

June 21 at higher temperatures but at a lower value of solar radiation from 538 to 495 kg CO_2 ha^{-1} day^{-1} (-8.0%). The effect of *V. dahliae* was not proportionally greater at light saturation which occurred around the solar noon ($>$ 400 W m^{-2}): stomatal conductivity, transpiration and photosynthesis were also noticeably reduced at light intensities lower than light saturation.

The increasing *V. dahliae* effect with time also becomes apparent from the light use efficiencies, calculated as net photosynthesis divided by total daily incident solar radiation (Table 8). On June 13 the daily mean values of the light use efficiencies were 2.69 g CO_2 MJ^{-1}, for both the inoculated plot and the control; on June 21, due to *V. dahliae* infection, they decreased from 2.42 to 2.23 g CO_2 MJ^{-1}. The values of about 2.5 g CO_2 per MJ intercepted radiation should be multiplied by 0.68 to convert to sugars (CH_2O) and by 0.55 to convert from sugars to dry matter to account for respiration (McCree, 1988), resulting in 0.935 g dry matter per MJ intercepted total incident solar radiation or 1.87 g dry matter per MJ intercepted photosynthetically active radiation. This value corresponds well with values derived from total dry matter production

Table 8. Experimental conditions and some selected results on four days of measurement (LUE = light use efficiency).

Observation	June 13		June 14		June 20		June 21	
Enclosure night temp. (°C)	15		15		15		15	
Enclosure day temp. (°C)	22		29		22		29	
Global radiation (MJ m^{-2} day^{-1})	25.1		21.6		26.6		22.2	
Days after planting	39		40		46		47	
Day of the week	Tuesday		Wednesday		Tuesday		Wednesday	
	V. dahliae		*V. dahliae*		*V. dahliae*		*V. dahliae*	
	inoc.	contr.	inocul.	contr.	inocul.	contr.	inocul.	contr.
Total LAI on friday	5.05	5.06	5.05	5.06	4.64	5.06	4.64	5.06
LAI of green leaves on friday	4.41	4.48	4.41	4.48	4.18	4.64	4.18	4.41
Daily net photosynthesis (kg CO_2 ha^{-1} day^{-1})	678	677	596	593	704	730	495	538
Dark respiration (kg CO_2 m^{-2} s^{-1})*	0.159	0.161	0.157	0.160	0.111	0.120	0.069	0.077
Dark transpiration kg H_2O m^{-2} s^{-1})*	1.84	2.02	1.58	1.49	0.89	0.97	0.61	0.67
LUE in the morning (g CO_2 MJ^{-1})	2.64	2.65	2.63	2.66	2.42	2.53	2.28	2.42
LUE in the afternoon (g CO_2 MJ^{-1})	2.44	2.49	2.44	2.48	2.19	2.34	2.02	2.25
LUE daily mean value (g CO_2 MJ^{-1})	2.69	2.69	2.76	2.74	2.64	2.70	2.23	2.43

* Mean value of the first hour after midnight.

at periodic harvests and intercepted radiation data (e.g. Haverkort and Bicamumpaka, 1986). Due to hysteresis, caused by the inability of the roots of the plants to take up as much water as is transpired around noon, light use efficiencies were higher in the mornings than in the afternoons: about 2.65 versus 2.45 g CO_2 MJ^{-1} in the first week. In the first week, *V. dahliae* had little effect on light use efficiencies. In the second week of measurement differences became apparent, especially in the afternoons when light use efficiencies were reduced over 10 % by *V. dahliae* (Table 8). It should be noted here that the way they were calculated here for the mornings and afternoons, they slightly deviate from the maximum values because light saturation levelled off the response.

Discussion

This research corroborated the findings of Mathre (1968) and of Zazzerini (1975) who worked with *V. dahliae* infected cotton and peppers, respectively. Similar effects on CO_2-exchange were found by Duniway and Slayter (1971) caused by another wilt causing fungus (*Fusarium oxysporum*) on tomato. The results also corroborate those of Bowden and Rouse (1990a) for the northern American potato cultivar Russet Burbank. They found a similar relationship of gas exchange parameters with leaf position comparing *V. dahliae* infected plants with non-infected plants. The authors did not include the study of combined stress due to drought and *V. dahliae* in their research.

The main problem encountered when measuring stomatal conductance, transpiration and photosynthesis of individual leaves is the variability of the measurements within a treatment. Variation in leaf water characteristics (Vos and Oyarzun, 1987) seems to be mainly responsible for this variation. The authors reported the effects of leaf water potential and stomatal opening as influenced by leaf age, leaf position and illumination on photosynthesis of the different leaves of a stem. The variability this causes, proved to increase when measuring different plants within a container or within a plot. Differences in water supply from the soil to the plant and variation in transpiration as a result of exposure to sunlight then play a role as well. In the case of Table 4, both types of leaves, from plants growing up at the South and at the North side, were measured at the same light intensity, but the ones on the South had received more sunlight. Different leaf temperatures also play a role as at higher temperatures higher photosynthetic rates were recorded.

Application of both drought and infection with *V. dahliae* under our controlled conditions led to differences in crop behaviour not unlike those found under field conditions. Both inoculation methods (root soaking and supplying the planting hole with perlite overgrown with microsclerotia), gave a high proportion of stem infection, as became apparent from plating and observation of chlorosis. The degree of drought and infection could not be established with exactitude as they show great variability between plants and between stems of the same plant. For drought this was not a problem because we were more interested in the interaction with *V. dahliae* than in the effect of drought per se. But although a certain proportion of the basal stem parts and a number of vascular bundles was infected, with the methods that were used, it was not possible to indicate quantitatively the degree of infection or disease of each leaf that was measured.

A significant interaction between drought and *Verticillium* was shown in Table 3. The effects of *Verticillium* and drought on stomatal conductance and transpiration proved to be less than additive. This was probably not only caused by the fact that plants infected with *V. dahliae* transpire less water and hence will suffer less from drought. Such a phenomenon may have been responsible for the drought × plant position interaction in Table 4: the southern plants had transpired more water already and thus were susceptible to imposed lack of water. A more likely explanation for the *V. dahliae* × drought interaction of Table 3, is that the early drought made it more difficult for the fungus to infect the plants and the plants themselves produced a more extensive root system (future publication). This finding needs further research because of its potential importance for improved cultural practices.The significant negative effect of *V. dahliae* on stomatal conductance and transpiration in Table 5, was mainly due to the increased effect (significant interaction) at the lower leaf positions. In experiment 4 it was shown that lower nodes are infected earlier and to a higher degree than higher ones.

This is the first report of gas exchange characteristics of whole canopies of potato crops infected with *V. dahliae*. The results indicate a progressive negative influence of *V. dahliae* on stomatal conductance, transpiration and net photosynthesis. This is indicative of *V. dahliae*'s effect on the water relations of the potato crop. Vos and Oyarzun (1987) also found at early stages of drought stress that stomatal conductance of individual leaves declined faster than the photosynthetic capacity. Vos and Groenwold (1989a), measuring potato crop canopy gas exchange characteristics, found that during gradual soil water depletion, the stomatal conductance of the whole crop was reduced faster and to a greater extent than crop photosynthesis. The effect of *V. dahliae* on gas exchange rates were far less than that caused by a severe drought period as was shown by Vos and Groenwold (1989a) and Bodlaender et al. (1987). They reported reductions in the values of stomatal conductance and gas exchange rates of the whole canopy surpassing 30% during periods of the day with a high evaporative demand.

The highest negative effect of *V. dahliae* on crop photosynthesis, in this experiment was at about solar noon on June 21, and did not exceed 15%. Based on measurements of stomatal conductance, transpiration and photosynthesis of individual leaves (e.g. Table 7 and Fig. 1), the effects occasionally exceeded 30%. The reasons for these differences may be that the individual leaf photosynthesis measurements were often carried out on the last formed fully expanded leaf, or at lower leaf levels. These leaves were likely to be more affected by *V. dahliae* (Table 6) than the very top leaves which were not yet fully expanded but which formed the upper leaf layers of the canopy and which lessened the overall *V. dahliae* effect. On individual leaves, stomatal conductivity and photosynthesis were measured on 2 cm^2 square sections of the abaxial side of leaflets; this may not have been representative of both sides of the whole leaflet, of the whole leaf or of the whole canopy. Another reason may be found in the observations of Dwelle et al. (1983). They found that removal of part of the leaves resulted in an increase of assimilation rates of the remaining leaves. Inhibition of assimilation in *V. dahliae* infected leaves may also have led to an increase of the highest leaves. The higher stomatal conductivity in the upper leaves (Tables 4 and 5) of the inoculated plants seem to point in this direction as well. Finally, it was demonstrated by Idso et al. (1989) that the chamber conditions of the porometer and leaf photosynthesis measurement apparatus may significantly perturb the determination of stomatal conductance and photosynthesis. This may also partly have been responsible for the observed differences.

Respiration and transpiration in the dark seemed not affected by the presence of *V.*

dahliae. These characteristics declined slightly (Table 8) but not more than proportional to the LAI. This is consistent with the hypothesis that *V. dahliae* in the early stages of growth reacts through water relations in the plant, which are not disturbed at the low evaporative demand during the night, but which are affected during times of higher evaporative demand during the hours of daylight.

In conclusion, *V. dahliae* negatively affects crop growth during the early stages of potato crop development. This effect occurs even before any visible symptoms appear, such as the yellowing of the lower leaves. The disease caused a reduction in the light use efficiency, but a stronger reduction of stomatal conductance, resulting in decreased external/internal CO_2 ratio's and in a higher net photosynthesis at similar values of stomatal conductance. *V. dahliae* caused effects which were similar to those of a mild drought stress in which stomatal closure is correlated with the reduction in photosynthesis and with an increase of the internal CO_2 concentration. The drought stress caused by *V. dahliae* was not sufficiently severe to allow mesophyll limitations (Schapendonk et al., 1989) to reduce photosynthetic capacity. Growth reduction associated with the presence of *V. dahliae* is partly caused by a reduced rate of net photosynthesis. This component of damage does not seem to be responsible for more than 10% reduction of dry matter production. In areas where the disease leads to an early and rapid decline of the canopy (potato early dying), reduction of photosynthesis may not be the most important component of damage, because reduction of intercepted radiation is then more important.

Interaction of the two stress factors seems to consist of *V. dahliae* reducing transpiration so that plants suffer less from drought early in the season. Stomatal conductivity, transpiration and photosynthesis showed a greater decrease when plants were more exposed to sunlight prior to measurements, when plants were older and when measurements were carried out on lower leaf positions.

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Samenvatting

De invloed van Verticillium dahliae en droogte op de groei van het aardappelgewas. 1. Effecten op gasuitwisseling en huidmondjesgedrag van individuele bladeren en bladerdekken

Aardappelplanten (*Solanum tuberosum* L.) cv. *Saturna* werden onderworpen aan stress als gevolg van *Verticillium dahliae* en droogte. In vroege stadia van de groei werden stomataire geleiding, transpiratie en netto fotosynthese bij lichtverzadiging (PAR $>$ 300 W m^{-2}) gemeten aan individuele bladeren en met een mobiel instrumentarium met behulp van gewaskappen . Er werden geen significante verschillen gevonden in de waarden van de stomataire geleiding en de gasuitwisslingskarakteristieken als gevolg van *V. dahliae*-besmetting tot een maand na opkomst. Daarna leidde infectie met *V. dahliae* tot een afname van de stomataire geleiding, transpiratie en netto fotosynthese, speciaal bij oudere bladeren en bij planten die meer aan zonlicht waren blootgesteld. Soms vertoonde *V. dahliae* interactie met droogte en bleken beide effecten minder dan optelbaar. De hoge waarden van de variatiecoëfficiënten maakten een groot aantal metingen per behandeling noodzakelijk; dit was vooral het geval bij met *V. dahliae* geïnfecteerde planten hetgeen aantoont dat *V. dahliae* vooral in het begin van de groei niet alle bladeren in gelijke mate aantast. Door de matigende invloed van de integratie van alle bladlagen en mogelijk doordat de bovenste bladeren werden gestimuleerd, werd de totale gewasfotosynthese in mindere mate beïnvloed door *V. dahliae* dan de individuele bladfotosynthese. De bovenste niet geïnfecteerde bladeren bleken verantwoordelijk voor het grootste gedeelte van de gewas-fotosynthese. De resultaten tonen aan, dat volgend op een infectie met *V. dahliae*, de fotosynthese reeds in een vroeg stadium van de groei wordt verminderd als een gevolg van droogtestress in de bladeren.

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