Effect of *Verticillium dahliae* on Photosynthesis, Leaf Expansion and Senescence of Field-grown Sunflower

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On the basis of known sunflower (*Helianthus annuus* L.) responses to soil water deficit, it is proposed that the effect of the fungus *Verticillium dahliae* Klebahn on plant leaf area precedes and is greater than its effect on leaf photosynthesis and stomatal conductance. To test this hypothesis, we measured shoot and leaf area growth, leaf photosynthetic rate, stomatal conductance and disease symptoms in a field experiment including hybrids of high (Sankol) and low (Dekasol 3900) susceptibility to *V. dahliae*. Plants inoculated with *V. dahliae* and controls were compared. We also investigated the effect of *V. dahliae* on key components of plant leaf area, leaf expansion and senescence, in inoculated and control plants of Sankol and Toba, a hybrid of intermediate susceptibility to *V. dahliae*. Reduction in plant leaf area caused by *V. dahliae* was first detected 31 d after inoculation (DAI), when visual symptoms of disease in inoculated plants were slight (Sankol) or absent (Dekasol 3900). Reduction in leaf photosynthesis was first observed 66 DAI; stomatal conductance and leaf dark respiration were both unaffected by *V. dahliae* during the whole experiment. In comparison with controls, *V. dahliae* reduced seasonal duration of plant leaf area by 25 % in Dekalb 3900 and by 55 % in Sankol, whereas the average reduction in leaf photosynthetic rate was 9 %. In correspondence with the reduction in leaf area duration, inoculation reduced shoot dry matter of mature Sankol by 50 %. In both experiments, less leaf expansion accounted for most of the early reduction in plant leaf area; as the disease progressed, increasing senescence also contributed to reduced plant leaf area. It is concluded that the response of sunflower to *V. dahliae* resembled the response of the plant to soil water deficit: (1) plant leaf area, rather than leaf photosynthetic rate, accounted for the reduction in growth in mass; and (2) reduced leaf expansion early in the season and faster leaf senescence in older plants accounted for the decrease in plant leaf area.   © 2000 Annals of Botany Company

Key words: *Helianthus annuus*, *Verticillium dahliae*, allometry, apical dominance, drought, leaf expansion, leaf senescence, photosynthesis, stomatal conductance, growth.

INTRODUCTION

Biotic and abiotic stresses contribute to a gap—usually large—between potential and attainable crop yield (Boyer, 1982). Of these stresses, fungal diseases, including verticillium wilt (*Verticillium dahliae* Klebahn), are critical for sunflower (*Helianthus annuus* L.) (Seiler, 1992; Bertero de Romano *et al.*, 1994; Sadras and Villalobos, 1994). Studies on the physiological effects of *V. dahliae* on sunflower have focused on ultrastructural changes and symptom expression (Robb *et al.*, 1979a and literature cited therein); much less attention has been paid to the whole-plant physiology of diseased sunflower.

Research with potato (*Solanum tuberosum* L.) demonstrated a positive association between yield loss and reduction in leaf area caused by *V. dahliae* (Johnson *et al.*, 1987; Johnson, 1988). In the framework of Johnson (1987), *V. dahliae* could therefore be considered as a reducer of light interception. In other studies, *V. dahliae* also reduced radiation-use efficiency (Bowden and Rouse, 1991). Further work with potato showed that *V. dahliae* reduced leaf water potential but did not change osmotic potential (Bowden *et al.*, 1990). This supports the classification of *V. dahliae* as a 'turgor reducer' (Boote *et al.*, 1983). Associated with the decline in leaf water potential, reduced stomatal conductance led to a slower rate of carbon assimilation in symptomless leaves of infected potato plants (Bowden and Rouse, 1991). Bowden and co-workers (Bowden *et al.*, 1990; Bowden and Rouse, 1991) concluded that the effect of *V. dahliae* on potato gas exchange is comparable to the effect of drought. Reduction in stomatal conductance attributable to *V. dahliae* was also reported in cotton (*Gossypium hirsutum* L.) (Hampton *et al.*, 1990) and cauliflower (*Brassica oleracea* L. *botrytis* L.) (Xiao and Subbarao, 1998).

Plant responses to drought include reductions in both stomatal conductance and rate of leaf expansion. In general, tissue expansion is much more sensitive to soil water deficit than stomatal conductance and gas exchange (Hsiao *et al.*, 1976; Sadras and Milroy, 1996). Under water stress, isohydric plant species tend to close stomata and maintain leaf water potential, whereas anisohydric species are able to maintain large stomatal conductance at very low leaf water potential (Tardieu *et al.*, 1996). In expanding canopies of droughted sunflower—a typical anisohydric species—leaf expansion rather than stomatal conductance controls plant water loss (Sadras *et al.*, 1991; Sadras *et al.*, 1993b). Importantly, stomatal conductance is unnaturally sensitive to soil water deficit when sunflower is grown in pots in controlled environments (Zhang and Davies, 1989; Masia *et al.*, 1994), hence the relevance of field studies involving water deficit in this species (Sadras *et al.*, 1993a).

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Hsiao *et al.* (1976) emphasized the importance of knowing the relative sensitivity of different plant processes in the interpretation of plant responses to water stress. Likewise, it is of interest to establish the relative sensitivity of key plant processes to verticillium wilt. On the basis of known sunflower responses to soil water deficit, it is proposed that the effect of *V. dahliae* on leaf area precedes and is greater than its effect on stomatal conductance and gas exchange. To test this hypothesis under field conditions, we measured shoot and leaf area growth, leaf photosynthesis, and disease symptoms in sunflower hybrids differing in their susceptibility to *V. dahliae*; plants inoculated with *V. dahliae* and controls were compared.

MATERIALS AND METHODS

Two field experiments were carried out on Typic Argiudols at Balcarce, Argentina (37° 45′S). In expt 1, the sensitivity of plant leaf area and photosynthesis per unit leaf area to verticillium wilt were compared. The effects of *V. dahliae* on key components of plant leaf area, *viz.* leaf expansion and senescence, were also assessed (expt 2).

In both experiments, weeds were controlled with herbicide before planting ($1 \cdot 3$ l trifluralin ha^{-1}) and manually thereafter. Plants in expt 1 were treated with commercial foliar fertilizer providing N, P, K and micronutrients (Samppi No. 3), and diammonium phosphate (60 kg ha^{-1}) 2 weeks after planting. No infestation of damaging insects occurred during the experiment. Sprinkler irrigation was applied during the growing season as required to supplement rainfall. Table 1 summarizes meteorological conditions during the experiments.

Plant and pathogen material

Sunflower hybrids Dekasol 3900 and Sankol were used in expt 1, and Toba and Sankol in expt 2. Ranking of susceptibility to *V. dahliae* based on visual symptoms in field-grown plants is Sankol > Toba > Dekasol 3900

(V.R. Pereyra and A. Escande, 1998, unpubl. res.). The inoculum was isolated from stems of diseased plants collected at Orense (SE of Buenos Aires province) using the method described by Dhingra and Sincalir (1985) with slight modifications. Briefly, diseased tissue was cultured in potato-dextrose agar (PDA, 2 %) at 20 °C, *V. dahliae* was isolated in PDA, multiplied in rice : water ($1 \cdot 5 : 1$), and tested for pathogenicity before use.

Experiment 1

Treatments and experimental design. Two sunflower hybrids (Sankol, Dekasol 3900) and two treatments [inoculated (+V) and control (−V)] were combined factorially in a randomized design with four replicates. Each replicate included four rows ($0 \cdot 7$ m apart) and 40 plants per row. Seedlings were raised in a glasshouse in trays filled with vermiculite. Treatments were established 4 weeks after sowing, when seedling trays were placed in larger trays with tap water (−V) or a suspension of *V. dahliae* spores (10^5 ml^{-1}) for 5 min (+V). Seedlings were planted on wet soil immediately after treatment on 22 Nov. 1999. To help plant establishment while avoiding washing out of spores before plant infection, experimental plots were sprinkler irrigated 24 h after planting.

Measurements. At weekly intervals, the percentage of the leaf population with visible disease symptoms—as described by Kolte (1985)—was determined in 30 randomly selected plants per replicate. The percentage of plants with microsclerotia—an indication of the effectiveness of inoculation—was assessed by observation with a magnifying glass (×6) of 20 stems per replicate that were allowed to dry out in the field until 123 d after inoculation (DAI). After observation of microsclerotia, stem samples were incubated (72 h at room temperature, 100 % relative humidity), and *V. dahliae* conidia and conidiophores observed with a light microscope (×125). The incidence of *V. dahliae* quantified by this method was significantly

TABLE 1. *Meteorological conditions during the experiments*

Week after inoculation	Maximum temperature (°C)		Minimum temperature (°C)		Solar radiation (MJ m^{-2} d^{-1})		Rainfall (mm)	
	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2	Expt. 1	Expt. 2
1	25·2	29·4	14·0	15·8	19·4	22·2	1	3
2	23·2	29·9	14·1	14·8	17·9	23·6	16	13
3	27·0	29·5	10·2	14·7	22·1	21·7	5	75
4	29·1	28·6	15·2	16·1	22·0	21·4	34	41
5	27·4	25·3	11·9	14·9	22·3	18·4	11	107
6	29·9	25·6	14·4	13·9	23·4	21·7	2	22
7	30·6	26·1	15·2	15·9	22·2	17·5	31	55
8	29·7	26·0	16·7	13·5	20·8	16·3	3	10
9	30·2	25·1	14·2	14·5	25·6	13·8	13	24
10	28·9	24·5	14·5	12·4	20·8	13·3	75	39
11	28·7		16·0		21·0		31	
12	26·4		16·1		18·7		117	
13	24·7		13·6		21·4		22	
14	27·1		16·2		17·4		55	

Temperature and radiation are weekly averages, rainfall is weekly total.

associated with the incidence derived from observation of microsclerotia ($r^2 = 0.60$; $P < 0.0005$). This association reinforces the reliability of microsclerotia as diagnostic criteria, as proposed by Kolte (1985).

Three randomly selected shoots per replicate were harvested at 31, 37, 50, 64, 79 and 94 DAI. Leaf area was measured immediately after harvest with a leaf area meter (LI-3100; LICOR, Lincoln, Nebraska, USA). Green leaves, senescent leaves ($< 50\%$ green), stems + petioles, branches and capitula were separated and weighed after drying to constant weight (forced draft at $70°C$). In plants sampled 50 DAI, the number of nodes and green leaves ($> 50\%$ green) were counted and the fraction of senescent leaves (i.e. number of senescent leaves/number of nodes) calculated. At maturity, plant height and diameter of capitula in ten plants per replicate were measured.

While handling the plants sampled at 37 DAI it was noticed that many plants had abnormal apical dominance, as indicated by activation of axillary buds (uncommon in cultivated sunflower). To assess the effect of hybrid and inoculation treatment on apical dominance, the number of plants with active axillary buds (branches > 2 cm) in samples of 30 plants per replicate was determined 45 DAI.

Photosynthetic rate was measured weekly in expanded, fully exposed leaves at the fourth to sixth node from the top in two plants per replicate. Measurements were taken on cloudless days at solar noon (± 1 h) using a portable photosynthesis system (LICOR 6200; LICOR, Lincoln, Nebraska, USA). Leaf dark respiration was also measured using the method of Hampton *et al.* (1990).

Experiment 2

Two sunflower hybrids (Sankol, Toba) and two treatments [inoculated ($+V$) and control ($-V$)] were factorially combined in a randomized design with four replicates. Experimental design and procedures were similar to those described for expt 1, except for the later planting date (11 Jan. 2000). Leaf area measurements were made on four tagged plants per replicate. At 36 and 44 DAI (when plants reached first anthesis) green and dead leaves were counted, and the area of individual green leaves was measured non-destructively (Pereyra *et al.*, 1982). After anthesis, progression of leaf senescence was assessed by counts of senescent leaves ($< 50\%$ green). At 36 and 44 DAI, the percentage of the leaf population with visible symptoms was determined in the four plants used for leaf area measurements and in larger samples including 20 plants per replicate.

Data analysis

ANOVA was used to assess the effect of hybrid, inoculation treatment, and hybrid $\times$ treatment interaction on plant response variables. Response variables defined as proportions were arcsine transformed for statistical analyses but percentages or proportions are presented in the tables and figures for easier interpretation of data. Using values of organ (stem, leaf, capitulum) dry matter (y) and shoot dry matter (x), allometric relationships were investigated with

least-squares regression of $\log_e$-transformed variables (Coleman *et al.*, 1994). In expt 1, quadratic polynomials were fitted to describe the changes in leaf area per plant with time, and leaf area duration between 31 and 94 DAI was calculated as the integral of the fitted function. In expt 2, the rate of leaf senescence after anthesis was calculated using normalized values, as in Sadras and Hall (1988), to account for differences in plant leaf area at anthesis.

RESULTS

Experiment 1

Development of symptoms. In control plots, visual symptoms of disease were slight (Sankol) or absent (Dekasol 3900) (Fig. 1). Symptoms developed fast in inoculated Sankol, and were negligible in their Dekasol 3900 counterparts (Fig. 1). Microsclerotia were used as diagnostic criteria to identify the disease (Kolte, 1985); the percentage of plants with microsclerotia in inoculated plots (i.e. 53 ± 2.2 in Dekasol 3900 *vs.* 62 ± 7.5 in Sankol), indicated that the effectiveness of inoculation was similar in both hybrids. Differences in symptoms between hybrids (Fig. 1) were therefore unrelated to degree of infection.

Plant growth. *V. dahliae* caused a statistically significant but small reduction in shoot dry matter in both hybrids between 37 and 64 DAI (Fig. 2). At maturity, inoculated plants were 50% lighter than controls in Sankol, whereas inoculated and control plants of Dekasol 3900 had a similar
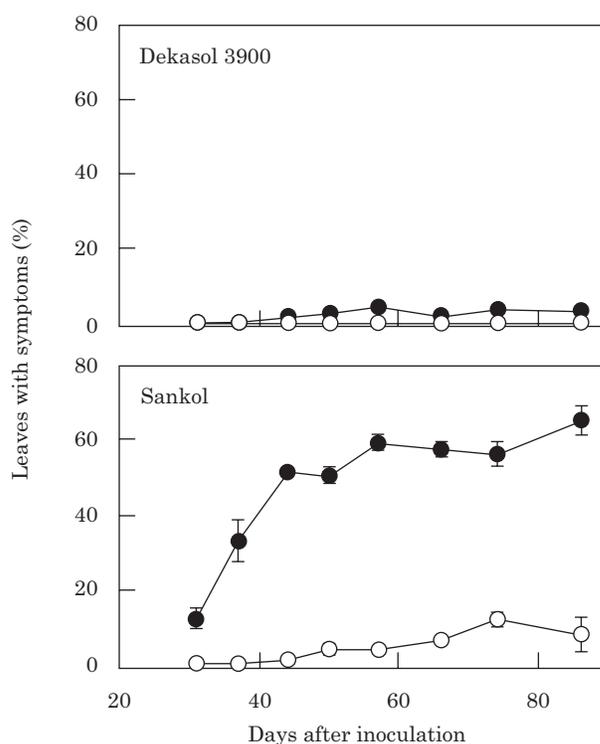

FIG. 1. Percentage of the leaf population with symptoms of verticillium wilt in inoculated (●) and control (○) plants of two sunflower hybrids. Bars are two s.e.m. and are not plotted when smaller than symbol.
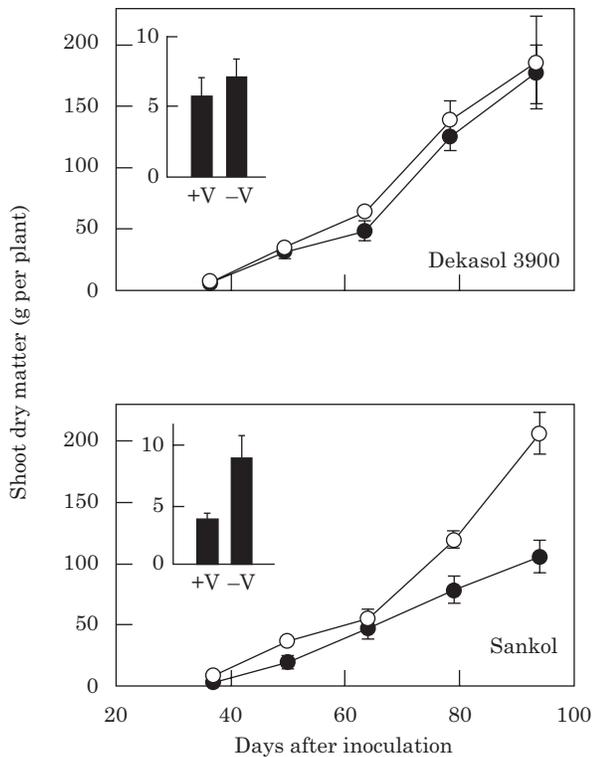
F IG. 2. Dynamics of shoot dry matter in inoculated (+V, ●) and control (−V, ○) plants of two sunflower hybrids. Bars are two s.e.m. and are not plotted when smaller than symbol. Insets show shoot dry matter (g per plant) at 37 d after inoculation; error bar is one s.e.m.

dry mass (inoculation effect: $P < 0.05$; hybrid effect: $P > 0.31$; interaction: $P = 0.09$). In comparison with controls, mature inoculated plants were shorter, (65 ± 1.4 cm *vs.* 79 ± 2.8 cm; $P < 0.001$) and had smaller capitula (13 ± 0.9 cm *vs.* 15 ± 0.8 cm; $P = 0.10$) in both hybrids.

Plant leaf area. At the first sampling date, inoculation with *V. dahliae* caused a 27 % reduction in leaf area in both hybrids (inoculation effect: $P = 0.059$; hybrid effect: $P > 0.35$; interaction: $P > 0.29$) (Fig. 3A and B, insets). Reduction in leaf area of inoculated plants increased as the season progressed (Fig. 3A and B). Analysis of variance including all harvests indicated that plant leaf area of both

hybrids was similarly reduced by verticillium wilt (inoculation effect: $P < 0.0001$; interaction hybrid × inoculation treatment: $P > 0.21$). Consistently, leaf area duration of both hybrids was similarly reduced by verticillium wilt (inoculation effect: $P < 0.0001$; interaction hybrid × inoculation treatment: $P > 0.44$). In comparison with controls, however, reduction in leaf area duration in inoculated plants was 55 % in Sankol and 25 % in Dekasol 3900 (Fig. 3C and D). Verticillium wilt did not affect specific leaf area (data not shown).

Reduction in leaf area could have been caused by reduction in leaf expansion, accelerated leaf senescence, or both. Measurements at 50 DAI showed that verticillium wilt did not affect the fraction of senescent leaves, yet caused a 30 % reduction in leaf area of Dekasol 3900 (Table 2). The reduction in leaf area was therefore caused by reduced leaf expansion. In Sankol, verticillium wilt increased leaf senescence by 53 %, and reduced leaf area by 43 %.

Leaf photosynthesis. Reduction in leaf photosynthesis attributable to *V. dahliae* was first detected 66 DAI ($P = 0.10$) and was similar in both hybrids (interaction hybrid × inoculation treatment: $P > 0.95$). On average, during the period from 66 to 86 DAI, leaf photosynthetic rate was reduced by 9 % in inoculated plants compared to controls (inoculation effect: $P < 0.05$; interaction hybrid × inoculation treatment: $P > 0.85$) (Fig. 4). Related variables, including stomatal conductance, leaf temperature and leaf dark respiration were unaffected during the whole experiment.

Dry matter partitioning. Reduced allocation to reproductive organs can contribute to reduction in yield caused by verticillium wilt. Allometric coefficients indicate that verticillium wilt hardly affected dry matter partitioning with the exception of a small reduction in allocation to leaves in inoculated Sankol, which was consistent with the faster senescence in this treatment (Table 3).

Apical dominance. Figure 5 A shows the proportion of plants with active axillary buds 45 DAI. In the control treatment, 84 % of Sankol plants had active axillary buds compared with 23 % of Dekasol 3900. Inoculation with *V. dahliae* decreased the proportion of plants with active

TABLE 2. *Effect of* V. dahliae *on sunflower plant leaf area and leaf senescence at 50 days after inoculation (expt 1)*

| | Hybrid | | | |
| | Dekasol 3900 | | Sankol | |
Treatment	Leaf area (cm² per plant)	Senescent leaves (fraction)*	Leaf area (cm² per plant)	Senescent leaves (fraction)
Control	2087 ± 94.6†	0.16 ± 0.024	1509 ± 19.7	0.22 ± 0.075
Inoculated	1481 ± 106.1	0.17 ± 0.063	859 ± 280.7	0.47 ± 0.010

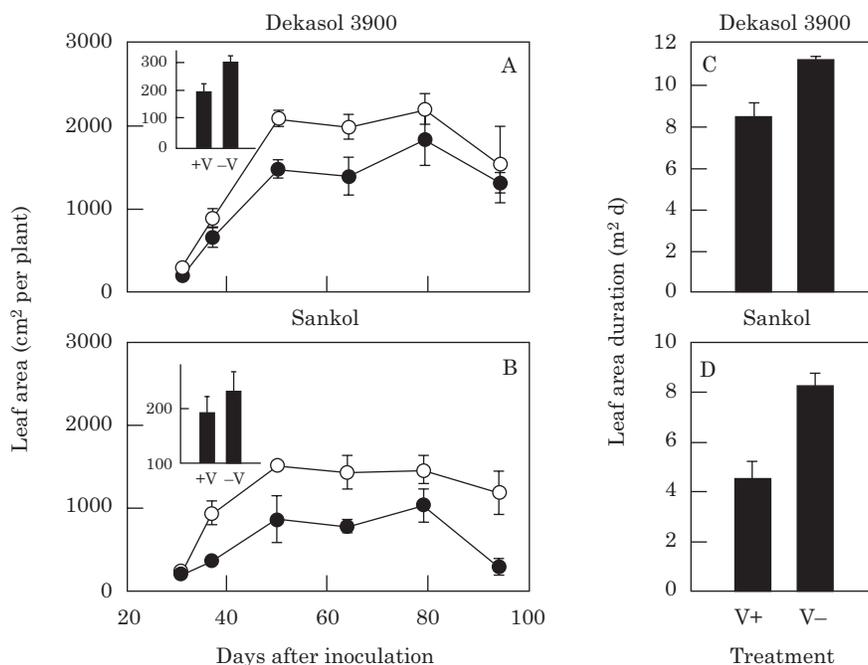* Number of senescent leaves/number of nodes.
† s.e.m.

FIG. 3. A and B, Dynamics of plant leaf area in inoculated (+V, ●) and control (−V, ○) plants of two sunflower hybrids. Bars are two s.e.m. and are not plotted when smaller than symbol. Insets show leaf area (cm² per plant) 31 d after inoculation. C and D, Leaf area duration between 31 and 94 d after inoculation; error bar is one s.e.m.

axillary buds in Sankol and increased the proportion in Dekasol 3900 (hybrid × treatment interaction: $P < 0.05$). *V. dahliae* consistently decreased branch dry matter in Sankol and increased it in Dekasol 3900 (Fig. 5B and C).
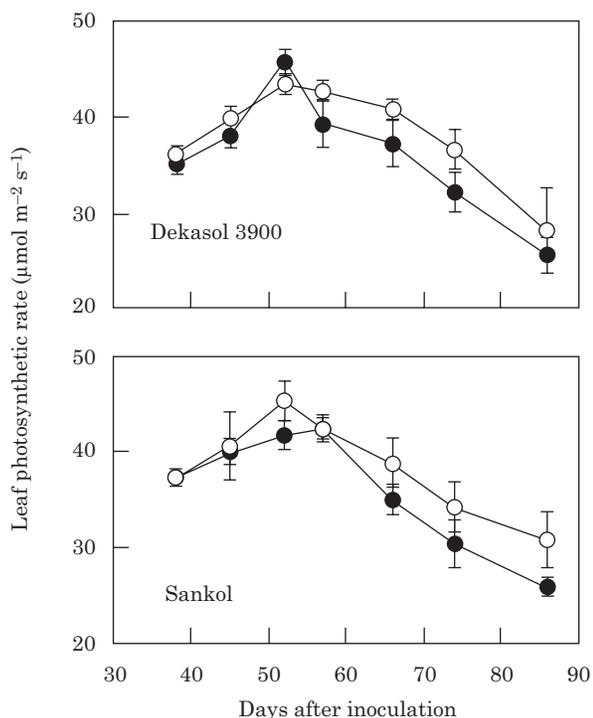

FIG. 4. Photosynthetic rate in leaves of inoculated (●) and control (○) plants of two sunflower hybrids. Bars are two s.e.m. and are not plotted when smaller than symbol.

Experiment 2

At 36 and 44 DAI, visual symptoms of verticillium wilt were similar in both hybrids and significantly more marked in inoculated plants (inoculation effect: $P < 0.01$; hybrid effect: $P > 0.22$; interaction: $P > 0.31$; Fig. 6). At both dates, *V. dahliae* reduced plant leaf area ($P < 0.05$) and increased the proportion of senescent leaves ($P < 0.002$) in both cultivars (Table 4). At 36 DAI, however, the contribution of leaf senescence to plant leaf area was small because it involved the oldest, smallest leaves at the bottom of the plant (Fig. 6). The profiles of leaf area in Fig. 6 clearly show that reduced leaf expansion, chiefly of the larger leaves at mid-position nodes, accounted for most of the reduction in plant leaf area, whereas the importance of leaf senescence increased as plant leaf area approached its maximum at anthesis (44 DAI). Verticillium wilt did not affect the rate of leaf senescence after anthesis (data not shown).

DISCUSSION

Mechanisms of host-plant response to Verticillium dahliae

From the well established effects of water deficit on sunflower physiology (Connor and Sadras, 1992; Connor and Hall, 1997; Sadras and Trápani, 1999), it was hypothesized that leaf area should respond to *V. dahliae* more intensely and earlier than stomatal conductance and leaf photosynthesis. Our field study supported this proposal. The effect of *V. dahliae* on plant leaf area was first detected at 31 DAI whereas the first effect on leaf photosynthesis was detected at 66 DAI (Figs 3 and 4). Importantly, effects of verticillium

TABLE 3. *Effect of* Verticillium dahliae *on the allometric coefficients of sunflower hybrids*

Hybrid	Treatment	Capitulum	Leaf	Stem
Dekasol 3900	Control	1.79 ± 0.056	0.55 ± 0.041	0.91 ± 0.036
Dekasol 3900	Inoculated	1.67 ± 0.058	0.57 ± 0.038	0.81 ± 0.036
Sankol	Control	1.75 ± 0.100	0.60 ± 0.052	0.76 ± 0.038
Sankol	Inoculated	1.72 ± 0.053	0.50 ± 0.035	0.82 ± 0.038

Coefficients are the slope ($\pm$s.e.) of the regression between organ dry matter (g) and shoot dry matter (g) (both variables $\log_e$ transformed); $0.85 > r^2 > 0.99$, $P < 0.0001$.
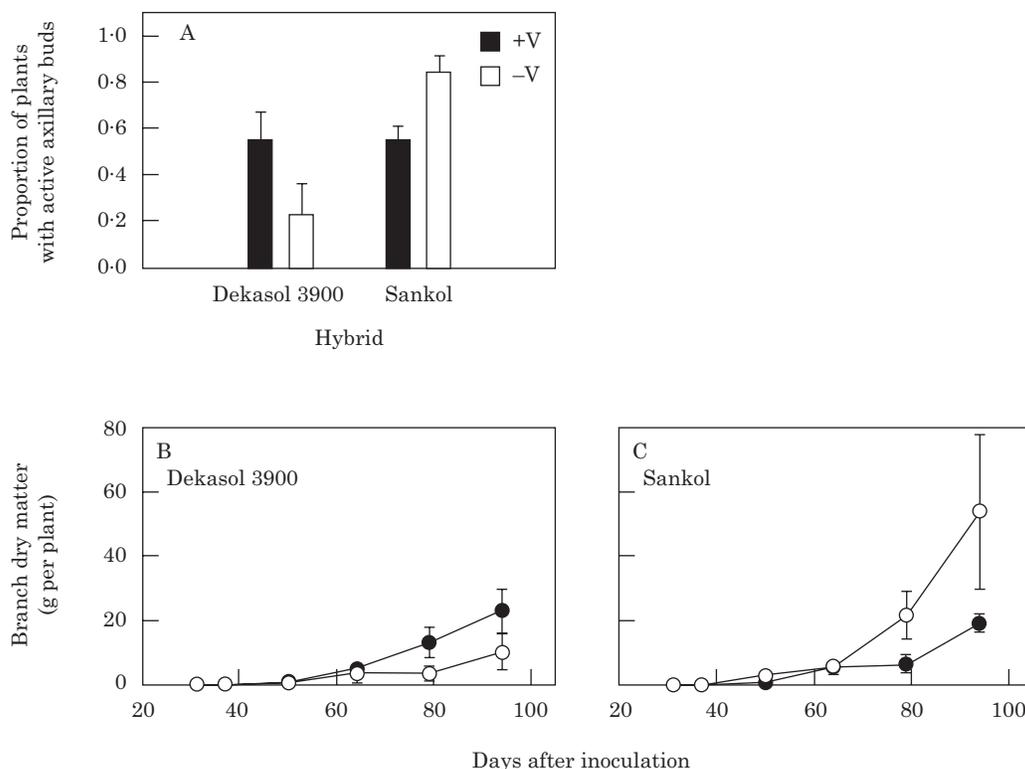

FIG. 5. Effect of *Verticillium dahliae* on the apical dominance of two sunflower hybrids. A, Proportion of plants with active axillary buds (branches > 2 cm) 45 d after inoculation. Error bars are one s.e.m. B and C, Dynamics of dry matter accumulation in branches of inoculated (●) and control (○) plants. Bars are two s.e.m.

wilt on leaf area preceded any reduction in shoot growth, while the decline in photosynthesis was small and occurred when substantial reduction in growth was already evident. Leaf dark respiration, leaf temperature and stomatal conductance were all unaffected by inoculation during the whole experimental period, further supporting the low importance of assimilation per unit leaf area as a factor affecting the growth of diseased sunflower. Photosynthesis was measured in relatively young leaves at the top of the plant; hence earlier or more marked effects on photosynthesis in older leaves cannot be excluded. It should be noted, however, that expansion of leaves at the fourth to sixth node from the top was severely reduced by *V. dahliae* in contrast to their small and late reduction in photosynthesis. Comparison of shoot dry matter of mature plants (Fig. 2) and leaf area duration (Fig. 3C and D) helped in the understanding of the effects of *V. dahliae* on whole-plant assimilation. In Sankol, the 51 % reduction in growth

caused by *V. dahliae* was close to the 55 % reduction in leaf area duration; this indicated no substantial change in whole-plant assimilation. In Dekasol 3900, verticillium wilt caused a 25 % reduction in leaf area duration whereas shoot dry matter of mature plants was unaffected. This indicated some degree of compensatory photosynthesis (Rosenthal and Kotanen, 1994), a conclusion that is further supported by an average increase in unit leaf rate of 27 %, from 1.5 ± 0.24 g m^{-2} d^{-1} in controls to 2.1 ± 0.45 g m^{-2} d^{-1} in diseased plants.

The lack of stomatal response to *V. dahliae* in sunflower contrasted with findings in other species (Hampton *et al.*, 1990; Bowden and Rouse, 1991; Xiao and Subbarao, 1998) and was consistent with the limited stomatal responsiveness to water deficit in field-grown sunflower (Sadras *et al.*, 1993b). Plant strategies in response to water deficit (i.e. anisohydric *vs.* isohydric; Tardieu *et al.*, 1996) can partially account for these contrasting responses to verticillium wilt;

TABLE 4. *Effect of* V. dahliae *on plant leaf area and leaf senescence (expt. 2)*

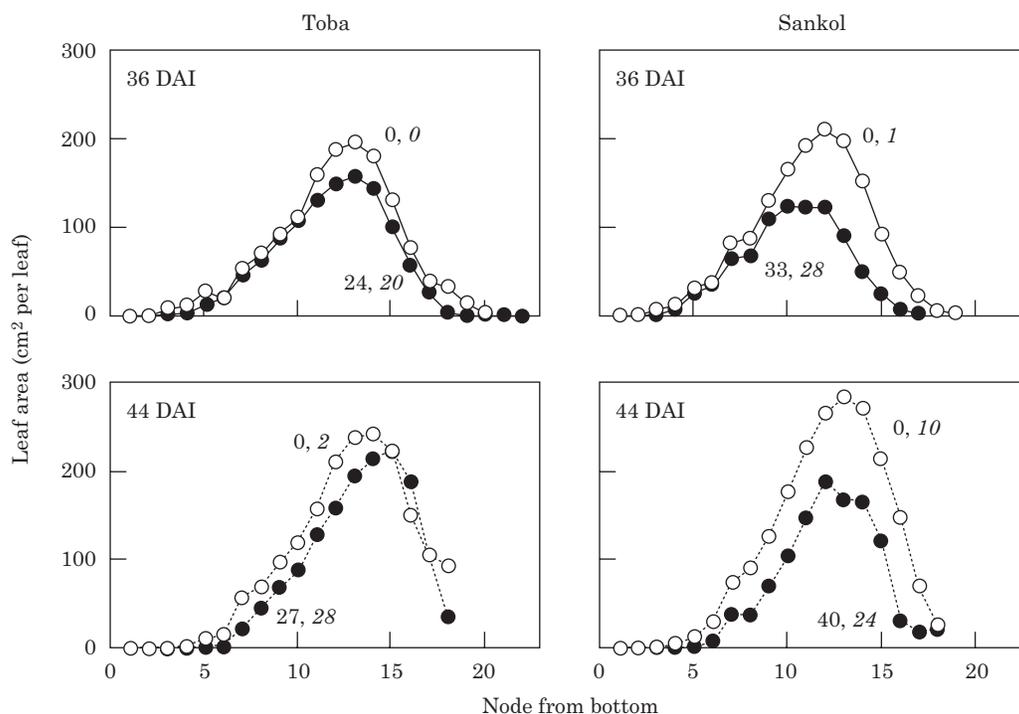|		Hybrid			
		Toba		Sankol	
Days after inoculation	Treatment	Leaf area (cm² per plant)	Senescent leaves (fraction)*	Leaf area (cm² per plant)	Senescent leaves (fraction)
36	Control	1406 ± 351·1†	0·10 ± 0·032	1473 ± 192·5	0·11 ± 0·022
	Inoculated	1128 ± 136·1	0·16 ± 0·022	850 ± 111·1	0·21 ± 0·020
44	Control	1783 ± 428·6	0·21 ± 0·040	1998 ± 239·2	0·24 ± 0·036
	Inoculated	1484 ± 148·2	0·31 ± 0·007	1092 ± 161·7	0·35 ± 0·021

* Number of senescent leaves/number of nodes.
† s.e.m.

FIG. 6. Profiles of leaf area in inoculated (●) and control (○) plants of two sunflower hybrids. Each pair of numbers indicates the percentage of leaves with visual symptoms of verticillium wilt in the four plants in which leaf area was measured, and in larger samples (20 plants, italics), respectively.

environmental conditions, ontogenetic stage of the host plant during infection and features of the pathogen could also contribute to differences in responses among species and experiments (Busch and Edgington, 1967; Busch and Schooley, 1970; Pegg, 1974; Sadras *et al.*, 1993*a*).

Early reduction in plant leaf area resulted primarily from a reduction in leaf expansion; as disease progressed, increasing senescence was more relevant (Tables 2 and 4, Figs 3 and 6). This sequence of responses in leaf area is similar to that described for droughted sunflower (Connor and Sadras, 1992). Profiles of leaf area measured in expt 2 are strong evidence of the extreme sensitivity of sunflower leaf expansion to damage caused by *V. dahliae* (Fig. 6).

The growth and activity of *V. dahliae* in the vascular tissue of host plants, including the occlusion of xylem vessels by tyloses and vessel coating materials (Robb *et al.*, 1979*b*), impairs water transport and may trigger physiological responses in the plant that resemble those commonly caused by drought, as found in this study and in previous work with potato (Bowden *et al.*, 1990; Bowden and Rouse, 1991). The mechanisms underlying host plant responses to *V. dahliae* probably include both hydraulic, i.e. loss of turgor (Bowden *et al.*, 1990), and chemical signals, i.e. fungal toxins (Gour and Dube, 1985; Buchner *et al.*, 1989; Nachmias *et al.*, 1990). Similarly, hydraulic signals interacting with chemical—chiefly hormonal but also ionic—signals are involved in shoot responses to soil drying (Gollan *et al.*, 1992; Munns *et al.*, 1993; Tardieu and Davies, 1993; Davies *et al.*, 1994; Tardieu *et al.*, 1996; Jackson, 1997). The similar plant responses associated with

V. dahliae and drought could therefore be partly related to common hydraulic signals. Hormone-like effects of fungal chemicals cannot be discounted, however. Experimental procedures, including split-root systems and balancing-pressure techniques, have been developed to isolate hydraulic and chemical signals in relation to drought (Blackman and Davies, 1985; Gollan et al., 1986; Passioura, 1988). It would be of interest to isolate the effects of *V. dahliae* that are mediated by a drop of tissue turgor from the putative effects of fungal toxins. The comparison of plant responses to fungal toxins and root-sourced plant hormones also warrants further research.

Apical dominance, i.e. the control exerted by the apical portions of the shoot over the outgrowth of the lateral buds (Cline, 1991, 1994), was dramatically affected by inoculation with *V. dahliae* (Fig. 5). In Sankol, with a low degree of apical dominance (i.e. profuse branching) in control plants, *V. dahliae* dramatically reduced branching. This may be related to the disruption of vascular connectivity and functionality required for the growth of axillary buds (Töpperwein, 1993; McIntyre, 1997). The reasons for the increase in branching in diseased Dekasol 3900, whose healthy plants showed high apical dominance typical of cultivated sunflower, remain unclear. The dramatic changes in apical dominance triggered by *V. dahliae* further support the concept of hormone-like signals—produced by the fungi, and/or by the infected plant—as an important component in the interaction between host plant and pathogen.

Implications for modelling and breeding

For modelling purposes, Johnson (1988) considered that (1) most of the effect of *V. dahliae* on potato crops was associated with loss of leaf area and (2) leaf loss was caused by premature senescence of old tissue rather than a difference in development of new tissue. In developing an interface to link *V. dahliae* and sunflower simulation models, our findings agree with the first assumption of Johnson (1988). Early effects of *V. dahliae* on leaf expansion, however, would also need to be considered in sunflower. Explicit modelling of the effect of verticillium wilt on dry matter partitioning does not seem necessary (Table 3).

Previous field studies based on visual assessment of host-plant symptoms showed that Sankol is more susceptible to *V. dahliae* than Dekasol 3900 (V.R. Pereyra and A. Escande, unpubl. res.). This was confirmed in our study with artificially inoculated plants (Fig. 1). Leaf area responses of both hybrids were, however, statistically indistinguishable. Importantly, reduction in plant leaf area preceded any visual symptom of verticillium wilt in Dekasol 3900 (Figs 1 and 3). In breeding programmes aimed at reducing susceptibility to *V. dahliae*, measurement of leaf area, using simple non-destructive methods, could complement the standard assessment of sunflower lines based on visual symptoms. Considering the sensitivity of leaf expansion to stresses in sunflower and the large variation in osmotic adjustment among hybrids (Chimenti and Hall, 1993), it would be of interest to assess the contribution of this trait to enhanced tolerance to *V. dahliae*.

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