Growth and Physiological Response of Resistant Alfalfa Clones Infected with *Verticillium albo-atrum*

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ABSTRACT

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Verticillium albo-atrum previously was reported to reduce the growth and flowering of resistant alfalfa cultivars. However, attempts to determine whether the pathogen directly affected the growth of individual resistant plants were thwarted by the fact that each alfalfa cultivar is a heterogeneous population of plants encompassing all levels of resistance to *V. albo-atrum*. Inoculation of an alfalfa cultivar and the subsequent death of susceptible plants resulted in the emergence of an inoculated-resistant subpopulation—a subpopulation that could not be duplicated in the absence of the pathogen. All growth measurements were taken on the resistant subpopulation, and the absence of an identical uninoculated subpopulation prevented researchers from unequivocally concluding that *V. albo-atrum* affected the growth of resistant plants. The present experiments were conducted on two resistant alfalfa clones to resolve this point. After a 6-wk establishment period, half the clones were inoculated with *V. albo-atrum* by placing a 20-μl drop of inoculum (3.65×10^6 spores/ml) on each freshly cut stem stub, and half were treated with sterile water. Growth and physiological parameters were measured 12 wk after inoculation and then weekly for an additional 6 wks. The experiment was repeated, and the data pooled. *Verticillium albo-atrum* caused significant reduction in height, percentage of plants flowering, and stem, leaf, and aerial biomass. Stomatal conductance of infected plants was significantly reduced during the afternoons of the last growth period. The significant reductions in these parameters are evidence of a host-pathogen interaction. Thus, the previously reported reduction in growth and flowering in alfalfa cultivars infected with *V. albo-atrum* was due, in part, to a host-pathogen interaction.

Additional keywords: Medicago sativa L., lucerne, Verticillium wilt.

Verticillium wilt of alfalfa (*Medicago sativa* L.), caused by *Verticillium albo-atrum* Reinke & Berthold, was first noted in the United States in 1976 (9), and has since spread through the entire northern alfalfa-growing region of the U.S. and as far south as southern California (5). Field studies with either naturally infected (10) or inoculated cultivars (31) have documented yield losses in susceptible cultivars ranging from 0.9 to 2.5 Mg/ha during the third production year. Similar results were reported in England on naturally infected alfalfa cultivars, although reductions in the yield of susceptible cultivars were not significant until the fourth harvest year (2).

Alfalfa is an open-pollinated autotetraploid. Consequently, alfalfa cultivars are extremely heterogeneous (4). Numerous studies (7,11,13,18,22,32) have demonstrated that the seedling population comprising a resistant alfalfa cultivar includes plants representing the spectrum of response to *V. albo-atrum* ranging from very susceptible to highly resistant. Panton (19) concluded that resistance to this pathogen was multigenic with either additive or multiplicative effects. Nielsen and Andreasen (17) and Viands (30) later confirmed the additive nature of resistance in commercial cultivars.

Resistant cultivars of alfalfa include plants that remain asymptomatic when inoculated with *V. albo-atrum* (22). The pathogen was recovered from these plants 7 mo after inoculation, and it was pathogenic on susceptible alfalfa. In a recent greenhouse study using resistant alfalfa cultivars (23), we attempted to determine whether the pathogen was active or merely quiescent in the infected, symptomless plants. We documented a significant reduction in height, dry weight, and flowering in the inoculated populations when compared to the uninoculated populations. The significant reduction in height and flowering persisted when only data from plants lacking foliar symptoms were analyzed. Similar results were reported by Hawthorne (11), working with New Zealand cultivars of alfalfa. However, it was not possible to determine whether the alterations in growth were due to the energy demands of a host-pathogen interaction (26) or were simply a consequence of the genetic heterogeneity of alfalfa cultivars. *V. albo-atrum* killed the susceptible members of the inoculated populations, and the surviving plants comprised resistant subpopulations that may have been genetically shorter and of lower biomass than the original population. It was impossible to select an identical subpopulation to serve as a control; therefore, experiments on alfalfa cultivars could not resolve this point.

The present study, conducted on resistant clonal plants to allow identical control and inoculated populations, was undertaken to determine whether *V. albo-atrum* is capable of altering the growth and physiological responses of resistant alfalfa plants or whether the previously reported growth alterations were due entirely to selection pressure on a heterogeneous population.

MATERIALS AND METHODS

Selection of resistant clones. Based on its performance in previous studies (22,23), alfalfa cultivar WL 316 was used for the selection of resistant clones. A population of 195 plants of this cultivar was established from seed. Following 3 mo of growth, 48 plants were selected for vigor and screened for resistance to *V. albo-atrum*. Forty-eight plants, while insufficient to characterize cultivar resistance, was a large enough population to allow detection of resistant plants. Ten cuttings were removed from each plant and were placed in sand for 3 wk to allow root development. The rooted cuttings were planted in commercial potting mix (CPM) (Terra-Lite, Redi-earth, Peat-Lite Mix, W. R. Grace & Co., Cambridge, MA) in multicompartmented plant containers. Duplicate sets of five clonal plants per mother plant were grown to ensure that a representation of the clonal plants would remain free of the pathogen.

The clonal plants grew for 6 wk, the time required to reach physiological maturity, before being cut to 4 cm in height and inoculated with *V. albo-atrum*. Half of the plants were inoculated by spraying a spore suspension (3.4×10^6 spores/ml) of the pathogen on the freshly cut stubble, and the remainder were sprayed with sterile water. Inoculum was a mixture of spores from three isolates of *V. albo-atrum* grown on prune-yeast extract agar (PYA) (27) for 1 wk. The *V. albo-atrum* isolates came from field-grown alfalfa plants collected in Centre County, PA, and were used in previous studies (22,23). Inoculated plants were placed in a mist chamber at 20 C for 24 hr and then returned to the greenhouse. Plants were scored for symptoms of Verticillium wilt at 6-wk intervals over a period of 18 wks. A five-point rating scale was used with 1 = no symptoms, 2 = chlorotic leaves, 3 = dead leaves, 4 = dead stems, and 5 = dead plant. Plants were cut to 4-cm in height after each scoring and allowed to regrow. Presence of the pathogen in plants scored as resistant on the basis of lack of foliar symptoms was verified by isolation. The basal portion of each stem was surface sterilized for 5 min in 0.525% sodium hypochlorite, then placed on 2% water agar and incubated at 25 C for 10 days before being examined for the presence of conidiophores of *V. albo-atrum*.

The clones of WL-316 were screened for resistance to *V. albo-atrum* between March and July 1987. Due to the wide temperature fluctuations common in Pennsylvania in the spring, greenhouse temperatures ranged from 18 C at night to 25 C on cloudy days and 30–40 C on sunny days.

Experiments in plant growth containers. The effect of *V. albo-atrum* on the growth of mature, resistant alfalfa clones was determined on plants grown in specialized growth containers that allowed the development of large root systems. The containers were constructed from 20×90 cm sections of polyvinyl chloride (PVC) schedule 40 sewer pipe, fitted with perforated plywood bases. Holes were drilled to allow the insertion of a soil moisture tensiometer (Soilmoisture Equipment Co., Santa Barbara, CA) 30 cm from the top of the container. Containers were filled with a 2:1, v/v, mixture of CPM and coarse sand. The mix in each container was amended with 160 g of 13%N-13%P-13%K slow-release fertilizer (Osmocote, Mallinckrodt, Inc., St. Louis, MO), 90 g of gypsum and 67 g of Esmigran slow-release micronutrients (Sierra Chemical Co., Milpitas, CA), to ensure adequate mineral nutrition for long-term growth. Nitrogen was used rather than nodulation with *Rhizobium meliloti* to ensure a uniform level of nitrogen fertility between experimental units. When at least one tensiometer read −0.04 MPa, water was added to all containers until it drained from their bottoms. The growth containers, growth medium, and watering method have been described (24). After inoculation with the pathogen, each growth container was fitted with a 75-cm tall cage constructed from 16-gauge steel-wire utility fencing. The cages minimized the possibility of contact between plants in adjacent experimental units and facilitated data collection by keeping the plants upright. Each plant growth container was planted in a circular pattern, with seven 1-month-old rooted cuttings of either clone WL-5 or clone 1079 and was considered to be one experimental unit. Clones were not mixed within experimental units. Clone 1079, selected from cultivar Agate, was received from R. N. Peaden (USDA-ARS, Irrigated Agriculture Research and Extension Center, Prosser, WA), and clone WL-5 was selected as previously described from cultivar WL 316. The plants were grown for 6 wk before being inoculated with the pathogen.

Inoculation method. Following the 6-wk establishment period, the plants to be inoculated were placed in a mist chamber at 20 C. Plants were cut to 4-cm height and inoculated by placing a 20-μl drop of spore suspension on the end of each freshly cut stub. Inoculum was dispensed with a micropipette. The spore suspension (3.65×10^6 spores/ml) was prepared from 2-wk-old PYA cultures of one of the previously mentioned isolates of *V. albo-atrum*. Plants in one experimental unit were cut and inoculated before proceeding to the next experimental unit to ensure similarly aged infection courts. Inoculated plants were kept in the mist chamber for 24 hr before return to the greenhouse. Control plants were treated similarly with sterile water but were not placed in the mist chamber, to minimize chance infection by *V. albo-atrum*. Seven plants of a susceptible alfalfa clone (selected from cultivar DK-131) also were inoculated, solely to verify pathogenicity of the isolate of *V. albo-atrum*.

Experimental design. The effect of *V. albo-atrum* on the growth and flowering of resistant alfalfa clones was tested in a 2×2 factorial experiment arranged in a randomized complete block design. Two levels of pathogen, inoculated and uninoculated, and two resistant alfalfa clones were used. There were five replications of the four treatments giving a total of 20 experimental units.

The plants underwent three consecutive 6-wk growth periods. No growth data were collected during the first growth period; however, isolations for the presence of *V. albo-atrum* were conducted on the basal 4 cm of the stems at harvest. The effect of *V. albo-atrum* was assessed at the end of the second growth period. Plants were scored for disease symptoms using the Graham et al scale (9) with 1 = no symptoms, 2 = one or two chlorotic leaflets, 3 = leaflets on more than one shoot chlorotic, 4 = most of leaflets chlorotic, 5 = dead plant. The height of the tallest stem on each plant was measured, the plant was cut at 4-cm height, and the the number of internodes on the tallest stem, number of stems, and flowering were recorded. Leaves were separated from stems, and the separated plant material was dried for 48 hr at 70 C before leaf and stem dry weight and aerial biomass (leaf + stem dry weight) were determined.

During the third growth period, one plant was removed weekly from each experimental unit and the previously mentioned growth parameters were measured, thus permitting statistical analysis of changes in growth over time. Regrowth of the plant was prevented by severing the aerial portion of the plant below the crown. The experiment was conducted in 1988 and repeated in 1989.

Statistical analysis. Growth parameter data for individual years were subjected to an analysis of variance with the General Linear Models program of SAS (SAS Institute, Inc., Cary, NC). Following the individual analyses, the data were analyzed over years. Orthogonal contrasts were used in the analysis of variance of the combined data to determine the effect of year on the treatments. In the absence of a significant year × treatment interaction, the pooled data were subjected to multiple regression analysis

TABLE 1. Schedule of stomatal conductance measurements during the third growth period of Experiment 2[a]

Duration (wks)	Daily sampling times								
	0800	0900	1000	1100	1200	1300	1400	1500	1600
1	X		S		X		X	S	X
2	X		X		S		X	S	X
3	X		X		X		S	X	X
4		X	X		X		X	S	X
5		X	X		X		X	X	X
6		X	X				X	S	X

[a]Sampling times are indicated with an X, and sampling times when significant differences ($P = 0.10$) were detected are indicated with an S.

with orthogonal contrasts, and used to construct predicted growth parameter curves. When the year × treatment interaction was significant, the pooled data underwent an additional analysis of variance with all possible single degree contrasts to determine the factor causing the significant interaction.

Physiological measurements. A LI-1600 Steady State Porometer (LI-COR, Lincoln, NE) was used to measure stomatal conductance. Measurements were made on similarly aged leaves whenever possible. During Experiment 2, stomatal conductance was measured during weeks three and four of growth period two and six times a day on a weekly basis (Table 1) in growth period three. Stomatal conductance was measured less frequently during Experiment 1, due to the limited availability of the porometer.

Leaf water potential (ψ) measurements were taken with a pressure bomb (PMS, Corvallis, OR) after first covering the leaf to be measured with cellophane tape to prevent loss of moisture when the leaf was excised from the plant (16). Twice during growth period two (weeks three and four), leaf ψ measurements were taken seven times between 0600 and 2200 hr to allow determination of diurnal leaf ψ curves. Measurements were made between 1300 and 1500 hr during the third through sixth weeks of the last growth period. Data from the physiological tests were not combined over experiments due to the limited amount of data available from the first experiment. The data were subjected to an analysis of variance. The diurnal leaf ψ curves were analyzed by multiple regression analysis with orthogonal contrasts.

Environmental conditions. The experiments were conducted during the winter months from November to March in 1988 and 1989 to avoid the confounding influence of high temperature on the expression of resistance (14,15). Natural day length was extended to 14 hr with 400-watt metal-halide lamps and was monitored with two LICOR Quantum Line Sensors (LI-COR). The metal-halide lamps provided an additional mean of 116 (range 60–155) μmol m^{-2} s^{-1} of photosynthetically active radiation (PAR) at soil level and an additional mean of 1842 (range 590–3370) μmol m^{-2} s^{-1} of PAR at 100 cm above soil level.

Greenhouse temperatures were measured with a hygrothermograph. During the first experiment, temperatures ranged between 19 and 25 C for 54.5% (424 of 778 hr) of the second growth period and 50% (414 of 790 hr) of the third growth period. In 1989, temperatures were in this range for 52% (386 of 772 hr) and 44% (354 of 798 hr) of the respective growing periods. In the second experiment, the plants were exposed to temperatures above 25 C for 6.5 and 9% more hours during the second and third growth periods, respectively, than in Experiment 1.

RESULTS

Evaluation of clones from 48 seed-grown plants of cultivar WL 316 yielded one clone (disease rating = 1.0) that did not show foliar symptoms of Verticillium wilt at 18 wk after inoculation. The clone was designated WL-5. Seven clones were rated resistant (2.0–2.9), and 23 were rated moderately resistant (3.0–3.9). Seven clones were susceptible (4.0–4.9) to *V. albo-atrum*, and 10 were highly susceptible (5.0). *V. albo-atrum* was isolated from all inoculated plants of the resistant clone WL-5 at the end of the evaluation period.

Two wk after inoculation of plants in the factorial experiment, the susceptible-control clone showed severe symptoms of

TABLE 2. Results of analysis of variance showing the main effect means of treatment (*Verticillium albo-atrum* and no *V. albo-atrum*) and clone (clone 1079 and clone WL-5) on the growth of resistant alfalfa, 12 wk after inoculation[a]

Parameter	Control	Inoc.	CL 1079	CL WL-5
Disease rating	1.1	1.4[b]	1.5	1.0[d]
Height (cm)	89.8	82.5[c]	87.2	85.3
Flowering (%)	70.0	58.0	95.0	33.0[d]
Stems (#)	8.4	8.9	10.7	6.6[d]
Leaf dry wt (g)	1.5	1.4	1.4	1.5
Stem dry wt (g)	2.7	2.4	2.7	2.4
Aerial biomass (g)	4.2	3.8	4.1	3.9

[a]Means represent the average of 40 values.
[b]Treatment significant at $P = 0.001$.
[c]Treatment significant at $P = 0.01$.
[d]Clones significant at = 0.0001.

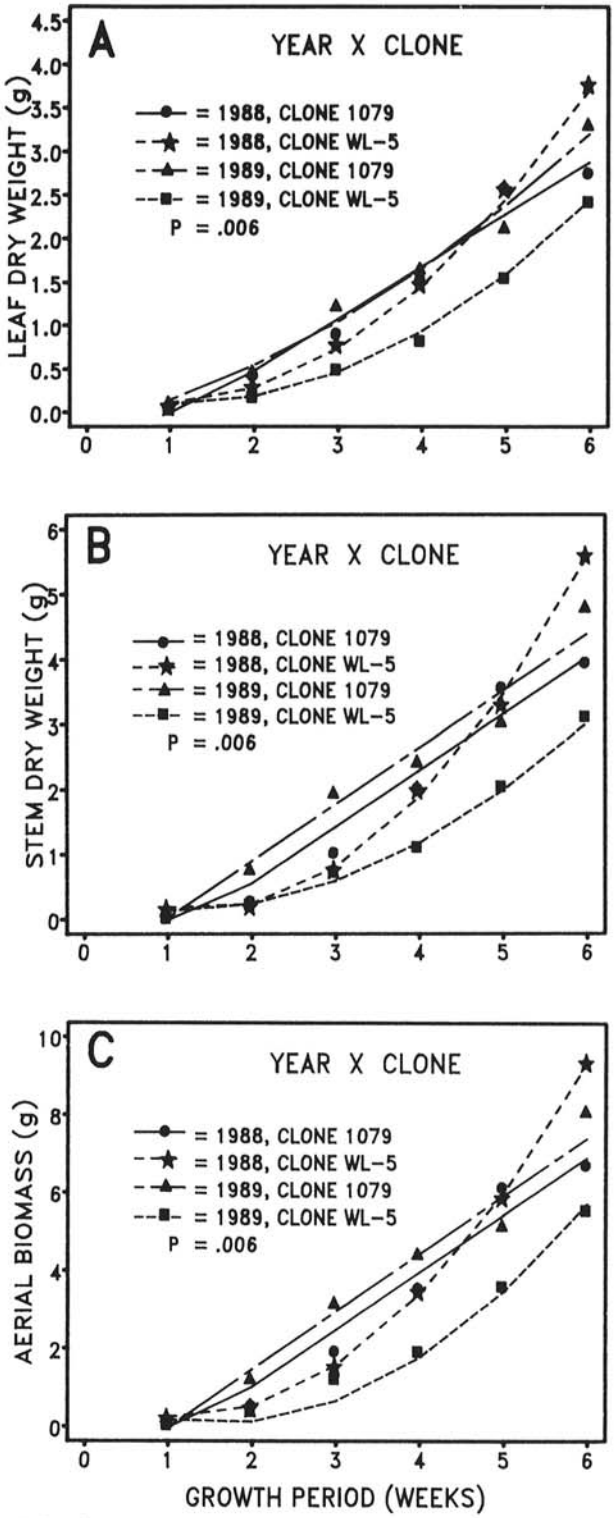

Fig. 1. Predicted growth curves of alfalfa showing the effect of the year × clone interaction during the third 6-wk growth period on **A**, leaf dry weight; **B**, stem dry weight; and **C**, aerial biomass. Symbols represent the mean of 10 replications, and lines represent the predicted growth curves based on multiple regression analysis. Note that in all cases the interaction was due to the response of clone WL-5 to the different environmental conditions between the two experiments. Clone 1079 showed no response to the different environments.

Verticillium wilt, indication that the isolate of *V. albo-atrum* was pathogenic. Isolations were conducted 6 wk after inoculation of the resistant plants as a further check on the inoculation technique. *V. albo-atrum* was isolated from 86% (60:70) of the plants in 1988 and from 84% (59:70) of the plants in 1989. The pathogen was not isolated from any uninoculated plants in either year.

Growth analysis. *Growth Period Two*. The growth parameter data, collected after 6 wk of growth, were pooled and analyzed over years. One year × treatment interaction was detected in the data from this harvest. During 1988, clone 1079 had a disease rating of 2.1 at harvest, whereas the disease rating of the clone WL-5 was 1.1. In 1989, clone 1079 scored 1.4 and clone WL-

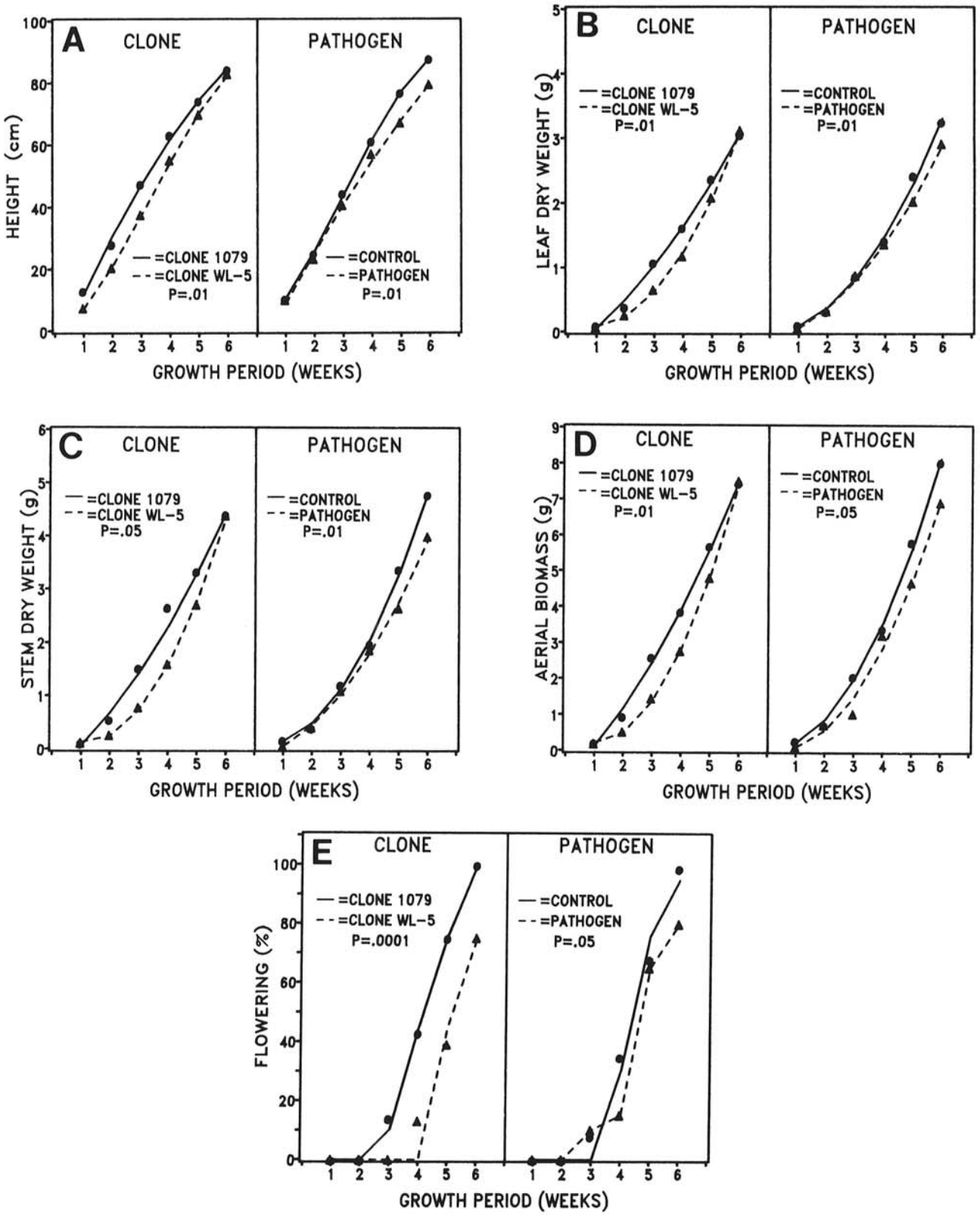

Fig. 2. Predicted curves of alfalfa infected with *Verticillium albo-atrum* showing the effect of clone and pathogen on **A,** height; **B,** leaf dry weight; **C,** stem dry weight; **D,** aerial biomass; and **E,** percent plants flowering. Symbols represent the mean of 10 replications, and lines represent the predicted growth curves. Plants infected with *V. albo-atrum* showed significant reductions over time in all parameters measured. Clones differed in rate of dry matter accumulation, although they showed no differences in these parameters at time of final harvest. The percent of plants flowering indicates that the clones differed in time of flowering. No year × pathogen interactions were detected.

5 scored 1.02. Plant height was the only parameter other than disease rating to be affected significantly by the pathogen, with infected-resistant plants being significantly ($P = .009$) shorter than the uninoculated plants (Table 2).

The two clones differed significantly in stem number, flowering, and stem dry weight (Table 2). A significant interaction was detected between clone and pathogen for stem number. Clone 1079 plants had a mean of 10.05 stems in the uninoculated population versus 11.16 in the inoculated plants; while uninoculated plants of clone WL-5 had a mean of 6.68 stems, and the corresponding inoculated plants had 6.49 stems. Thus, clone 1079 responded to *V. albo-atrum* by producing slightly more stems during the second growth period, and clone WL-5 showed essentially no difference in stem number between inoculated and uninoculated plants.

Growth Period Three. The data from growth period three were collected weekly and analyzed over weeks as well as years. As a consequence of the increased degrees of freedom in the analysis of variance, the level of precision was greater for this portion of the experiment. Analysis of variance over years detected a significant year × treatment interaction in leaf dry weight, stem dry weight, and aerial biomass. Further statistical analysis indicated that the interactions were due entirely to a difference in the response of the clones to growing conditions during the two experiments (Fig. 1). Clone WL-5 experienced a significant reduction in each of the parameters during the second experiment, while clone 1079 responded similarly both years. No fungus × year interactions were detected, indicating that the effect of the pathogen on plant growth was similar during both experiments.

Infected plants were significantly shorter and had significantly lower leaf dry weights, stem dry weights, and aerial biomass as well as reduced flowering when compared to the uninoculated plants (Fig. 2A–E). A significant pathogen-by-clone interaction was detected in disease rating (Fig. 3), with clone 1079 having a higher disease score than clone WL-5. All disease scores, however, were <3, within the range considered to be resistant.

For each of the growth parameters, a significant clonal effect also was detected (Fig. 2A–E). Resistant clone WL-5 was significantly shorter than clone 1079 (Fig. 2A) and initiated flowering later than did clone 1079 (Fig. 2E). The two clones also differed in their growth rates as reflected in leaf, stem, and aerial biomass (Fig. 2B–D).

Physiological parameters. No significant differences in stomatal conductance were detected during growth period two. Stomatal conductance of inoculated plants was significantly ($P = 0.1$) less than that of control plants in growth period three at 1500 hr during weeks 1, 2, 4, 6 (Fig. 4A), and at 1400 hr during week 3. Similar reductions in stomatal conductance were noted at 1000 hr during week 1 and at 1200 hr during week 2. Fungus × clone interactions were present at 1500 hr during weeks 4 and 6 (Fig. 4B) and indicated a difference in sensitivity to the pathogen. In one instance, data from the two experiments corresponded closely enough in time of measurement to allow an analysis of variance over experiments. The data were taken at 1500 hr during week 4 of the final growth period, and the combined analysis of variance indicated a significant reduction in stomatal conductance in the inoculated plants and no pathogen × experiment interaction.

No significant differences were detected in leaf ψ measurements taken during growth period two (Fig. 5) or three. Leaf ψ between 1300 and 1500 hr, during weeks 3, 4, 5, and 6 of growth period 3 for the inoculated clones, was −0.60 MPa, −0.67 MPa, −0.87 MPa, and −1.30 MPa, respectively, and that of the uninoculated clones was −0.71 MPa, −0.75 MPa, −0.86 MPa, and −1.18 MPa.

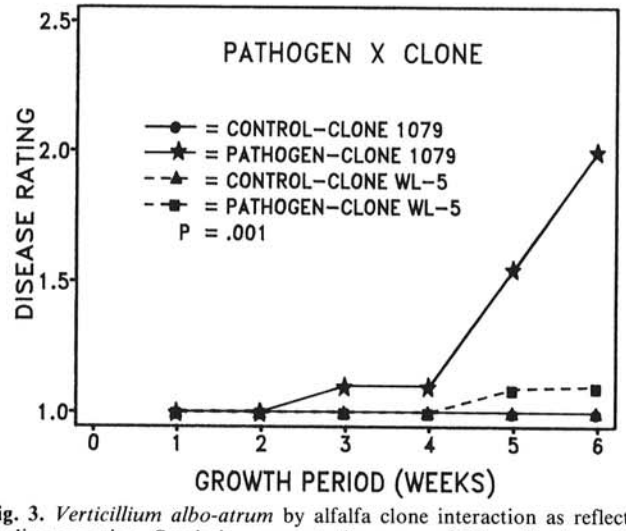

Fig. 3. *Verticillium albo-atrum* by alfalfa clone interaction as reflected in disease rating. Symbols represent the mean of 10 replications. The lines representing the uninoculated treatments are superimposed on each other. Symbols represent the mean of 10 replications. Note that clone 1079 was more sensitive to the pathogen than was clone WL-5; however, in both cases, the disease rating scores were in the range considered resistant.

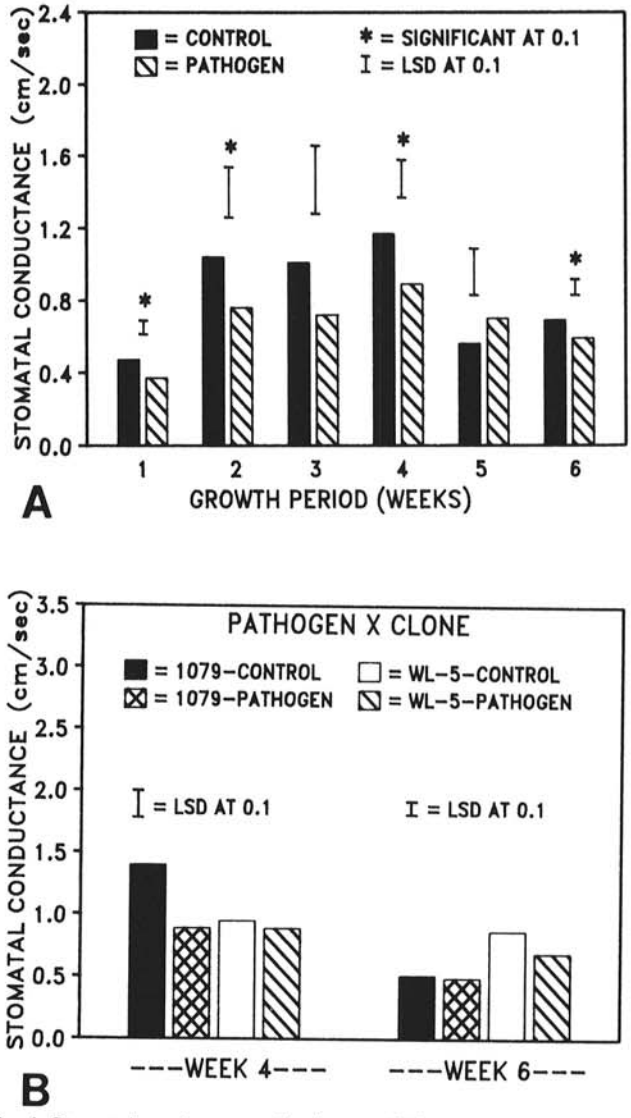

Fig. 4. Stomatal conductance of resistant alfalfa plants during the third 6-wk growth period of experiment 2. **A,** weekly stomatal conductance of *Verticillium albo-atrum* infected and uninfected plants at 1500 hrs. Significant differences were detected at 1, 2, 4, and 6 wks as indicated by the asterisks. The LSD is indicated for each bar. **B,** pathogen × clone interactions were detected during weeks 4 and 6. Note that during week 4, there was a significant difference between the stomatal conductance of the *V. albo-atrum* inoculated and control plants of clone 1079 and no difference between the inoculated and control plants of clone WL-5, while the opposite response was true during week 6.

DISCUSSION

Levels of resistance to *V. albo-atrum* vary from plant to plant within alfalfa cultivars due to the genetic heterogeneity of the crop (4). Commercial methods of screening for resistance to this pathogen are aimed at identifying maximum levels of resistance. The current commercial-screening method involves immersing wounded root systems of 4–12-wk-old plants in massive amounts of inoculum for a minimum of 5 min (20). Uptake of such large amounts of inoculum can cause severe symptoms in plants with levels of resistance that would be practical under field conditions (7). The present study addressed the question of whether growth alterations previously detected in *V. albo-atrum*-infected, symptom-free alfalfa cultivars (23) were the consequence of a host-pathogen interaction. In an attempt to challenge the host with a level of inoculum comparable to that occurring in field situations, we chose an inoculation method that would mimic mowing, an important mode of spread of *V. albo-atrum* in the field (12). Inoculation of freshly cut stems with a small quantity of inoculum allowed the host maximum opportunity to express resistance; consequently, our resistant clones probably represent the practical level of resistance referred to by Fyfe (7).

Verticillium was not isolated from 14–16% of the inoculated plants in the factorial experiment. However, stem regrowth rather than the entire basal portion of the plant was used for these isolations. Stem regrowth in clones originates from adventitious and axillary buds (6), and its xylem is not continuous with the entire vascular region of the basal portion of the plant. *V. albo-atrum* does not move between vascular bundles in stems of susceptible alfalfa (21), and the lack of lateral mobility may mean that portions of the xylem of the plant's basal region are pathogen free. Stems originating from these areas would not be infected with the pathogen.

Growth reduction was noted previously in symptom-free alfalfa plants infected with *V. albo-atrum* (11,23,25), but, in all cases, observations were made on inoculated alfalfa cultivars without the benefit of identical uninoculated control populations. The possibility of a genetic shift in the heterogeneous cultivars, due to the death of susceptible genotypes, prevented researchers from concluding beyond doubt that the growth reductions were the direct consequence of a host-pathogen interaction (18,23). The occurrence of similar growth reductions in infected clonal plants measured during this study allows us to conclude that the growth reductions previously reported were due, at least in part, to the interaction of *V. albo-atrum* and the resistant host, rather than entirely to the effect of selection pressure on a diverse population.

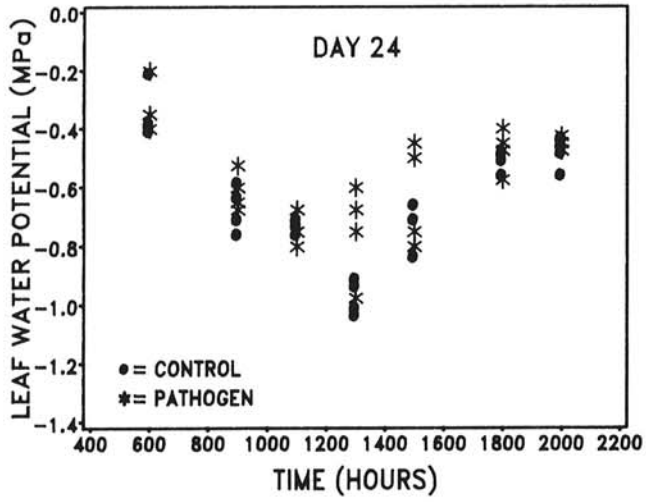

Fig. 5. Diurnal leaf water potential of alfalfa clones inoculated with *Verticillium albo-atrum* as compared to uninoculated clones. Measurements were made during the fourth week of the second 6-wk growth period. Each symbol represents one measurement. No significant differences were detected when the data were analyzed over time.

The mechanisms causing the reduction in growth noted in symptom-free plants infected with *V. albo-atrum* are not clear. Smedegaard-Petersen (26) stated that defense reactions influence the energy balance of the plant, exacting an energy cost from the host. Similarly, Van Loon (29) noted that plants infected with fungi produce a variety of stress proteins that are involved in phytoalexin synthesis. These proteins, products of alternate biosynthetic pathways, represent a redirection of photoassimilates.

Stomatal conductance measurements during this study detected a significant reduction in the stomatal conductance of infected plants. This reduction, detected only in the afternoon between 1400 and 1500 hr, was present throughout the third growth period. The afternoon detection of significant differences in stomatal conductance is consistent with the literature concerning the behavior of stressed plants (3). A reduction in leaf ψ did not accompany the reduced stomatal conductance. Similar findings were reported in several plant species subjected to water stress (1,8,28) and appear to indicate the existence of a "feed-forward" mechanism, a mechanism that allows stomates to react to a drying root environment before leaf ψ is affected and thus before there is a hydraulic stimulus for the reduction of stomatal aperature. The reduced stomatal conductance we detected may be indicative of such a feed-forward stomatal response to water stress. The use of soil tensiometers to schedule watering ensured that the plants in this study were not under abiotically induced water stress. The stomatal response of infected plants, however, indicates that pathogen-induced water stress may have occurred.

Although it was not possible to ascertain the mechanisms causing the growth reductions and physiological responses we detected in the infected plants in this study, there is no doubt that they occurred in response to *V. albo-atrum*. The detection of the growth reductions over several growth periods is additional evidence of an ongoing host-pathogen interaction. Similarly, the reduced stomatal conductance of infected plants during periods of peak evapotranspiration demand indicates that the plants were in a stress situation precipitated by the presence of the pathogen. We conclude, therefore, that the growth reductions previously detected in alfalfa cultivars infected with *V. albo-atrum* (23) were due, in part, to pathogen-induced host responses.

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