Verticillium Wilt Resistance in Pistachio Rootstock Cultivars: Assays and an Assessment of Two Interspecific Hybrids

D. P. MORGAN and L. EPSTEIN, Department of Plant Pathology, University of California, Berkeley 94720, and L. FERGUSON, Kearney Agricultural Center, Parlier, CA 93648

ABSTRACT

Morgan, D. P., Epstein, L., and Ferguson, L. 1992. Verticillium wilt resistance in pistachio rootstock cultivars: Assays and an assessment of two interspecific hybrids. Plant Dis. 76:310-313.

Pistacia integerrima (rootstock cultivar Pioneer Gold) is resistant to *Verticillium dahliae*, and *P. atlantica* (rootstock cultivar Standard Atlantica) is susceptible in the field. We developed greenhouse and laboratory assays that differentiate between the resistant and the susceptible cultivars. After injection of the stems of seedlings with conidia, the cultivars showed differential symptoms. After infusion of conidia into stems, *V. dahliae* colonized significantly fewer xylem vessels in the resistant than in the susceptible cultivar. We used the assays and a field test to assess Verticillium wilt resistance in two interspecific hybrid pistachio rootstock cultivars, Pioneer Gold II (PGII) and University of California at Berkeley I (UCBI). We conclude that UCBI is moderately resistant to resistant and that PGII is moderately susceptible to susceptible in comparison with Pioneer Gold and Standard Atlantica.

Verticillium wilt, caused by *Verticillium dahliae* Kleb., is the most serious disease of pistachio nut trees in California (2). The pistachio scion, *Pistacia vera* L. (cv. Kerman), is susceptible to Verticillium wilt (13). In California, all rootstocks are propagated by seed; scions are budded onto rootstocks. Disease control currently depends on the use of a resistant rootstock of *P. integerrima* Stewart (cv. Pioneer Gold), rather than a susceptible *P. atlantica* Desf. Recently, two new interspecific hybrid rootstock cultivars were introduced in California—Pioneer Gold II (PGII) and University of California at Berkeley I (UCBI). The goals of this project were to develop relatively rapid, quantitative assays to evaluate the response of pistachio rootstocks to *V. dahliae* and to test the resistance of the two new hybrids to Verticillium wilt.

All correspondence should be addressed to the second author.

This research was partly supported by grants from the California Pistachio Commission and the University of California Pistachio Liaison Committee.

Accepted for publication 25 October 1991 (submitted for electronic processing).

MATERIALS AND METHODS

V. dahliae isolate LH6-1 was obtained from J. MacDonald, University of California at Davis, who isolated it from a pistachio tree with Verticillium wilt symptoms. To produce conidia, *V. dahliae* was grown at 21 C in the dark on potato-dextrose agar. Conidia were collected from 10-day-old cultures with sterile deionized water. The names, sources, and parental species of the tested cultivars are indicated in Table 1.

Inoculation of seedlings with *V. dahliae*. For the laboratory and greenhouse experiments, seedlings were grown in pasteurized U.C. mix in a greenhouse for 8 mo. Seedlings were maintained at approximately 23 C and fertilized once per month. Supplemental lighting was used when the day length was shorter than 14 hr. Before inoculation, each seedling was punctured in the basal internode on opposite sides of the stem with a dissecting needle. Fifty microliters of a conidial suspension containing 10^7 conidia per milliliter was injected into each of the two puncture wounds per seedling with a syringe and a 22-gauge needle. To enhance transpiration and, thus, uptake of the conidial suspension, the plants were incubated at 23 C under wide-spectrum fluorescent lights and placed near a fan which created a very gentle breeze (3). After inoculation, plants were incubated in the greenhouse for 6 wk. There were 30 replicate seedlings per cultivar.

After 2, 4, and 6 wk, symptom severity was rated on a scale of 0–4 where 0 = asymptomatic; 1 = mild wilt, leaf reddening, or chlorosis affecting any leaves; 2 = moderate wilt, leaf necrosis, or defoliation; 3 = severe wilt, leaf necrosis, or defoliation; 4 = dead. The data were analyzed with the Kruskal-Wallis test, a nonparametric procedure.

After 6 wk, the extent of fungal colonization also was determined. From

each seedling, one stem section, 3 mm long, was excised 5, 15, and 30 cm above the injection site. For surface-sterilization, sections were dipped in 70% ethanol and then immersed in 0.5% NaOCl for 2–3 min. From each section, 10 subsamples, each 2 mm^3, of tissue containing xylem were placed on pectate agar (5) amended with 0.2 g L^{-1} of penicillin G sodium salt (pectate-penicillin agar) (7). After 7 days, the plates were rated for the presence or absence of *V. dahliae*.

The experiment was arranged in a complete randomized design and was repeated with similar results. The data were analyzed with the Kruskal-Wallis test. Results from a single experiment are shown.

Microscopic quantification of fungal colonization. This procedure was adapted from Newcombe and Robb (10). Terminal stem cuttings were excised from healthy seedlings. The stems were recut under sterile water to a final length of 12–20 cm; there was a minimum of 2 cm between the excised surface and the next node. The stems were infused with an aqueous suspension of 1.5×10^7 conidia of *V. dahliae* and 7.5×10^6 3-μm-diameter red vinyl beads per milliliter (Polysciences, Warrington, PA) (4) by immersing the cut ends in the suspension. Again, to increase uptake of the suspension, the plants were placed under lights near a fan, as explained above. After 60 min, the infused stems were washed in a stream of nonsterile water to remove conidia from the stem surface. Stems then were placed in 250-ml beakers with sterile water approximately 5 mm deep and incubated for 7 days in a humid chamber. The chamber was maintained at 21 C and illuminated by wide-spectrum fluorescent lights for 16 hr daily. There were 12 replicate seedlings per cultivar.

After 7 days, freehand cross sections were taken 9 mm above the excision site and stained with hot (90 C) Chlorazol Black E in lactoglycerol (10); the Chlorazol Black E was filtered with Whatman No. 2V paper immediately before use. The sections were destained with 0.3% (v/v) 0.1 N NaOH in 100% ethanol, mounted in 50% glycerol, and then examined microscopically at ×400.

Using one section per stem, we recorded the number of fungus-containing vessels with red beads (primary sites) and the number of fungus-containing vessels without red beads (secondary sites). We also recorded the number of vessels in which *V. dahliae* penetrated from a primary site into a contiguous vessel (a lateral penetration site). The lateral penetration ratio is the number of lateral penetration sites divided by the number of primary sites. The colonization ratio is the number of secondary sites divided by the number of primary sites. The colonization ratio was analyzed by an analysis of variance and Duncan's multiple range test with $\alpha = 0.05$.

The experiment was arranged in a complete randomized design and was repeated once with similar results. Results from a single experiment are shown.

Field trial. A field trial was conducted in a 1-ha plot with Panoche sandy clay loam at the Westside Field Station (WSFS), University of California, Five Points. The field had a history of cotton and tomato cultivation and Verticillium wilt. The microsclerotial concentration in soil was determined. Twenty regions, 5 × 6 m were selected which were uniformly spaced throughout the plot. From each site, 10 soil cores, 2 cm in diameter and 30 cm deep, were collected along a grid pattern. The soil from each site was bulked, air-dried, ground, and passed through a 2-mm sieve (1). The concentration of microsclerotia per gram of air-dried soil was determined by plating diluted soil (12) from two determinations per site onto pectate-penicillin agar.

Seedlings of Standard Atlantica, Pioneer Gold, PGII, and UCBI were grown in pasteurized soil. One-year-old seedlings were planted on 8 March 1990 at the WSFS. Twenty trees per cultivar were placed into each of four completely randomized blocks. The trees were spaced 5 m apart in 6-m-wide rows. A row of completely randomized rootstocks was planted along the plot border and was not included in data analyses.

Trees were rated on 19 June, 12 September, and 20 November 1990 using the 0–4 scale indicated above. Trees rated 1 (mild wilt, leaf reddening, or chlorosis) were examined for xylem discoloration in the affected region. Trees were classified as symptomatic if they were either rated 1 and had discolored xylem or were rated 2 or above. On each sampling date, the data on percentage of symptomatic and dead trees were analyzed separately by an analysis of variance and a Duncan's multiple range test with $\alpha = 0.05$. Disease ratings were analyzed by the Kruskal-Wallis test.

To determine if symptomatic trees were infected with *V. dahliae*, on 19 June, stem tissue was excised from the affected area of either every symptomatic tree or 20 symptomatic trees per cultivar. Ten pieces of xylem from the discolored region from each sample were plated on pectate-penicillin agar as described above.

RESULTS

Four cultivars (Standard Atlantica, Pioneer Gold, UCBI, and PGII) were inoculated with *V. dahliae* by injecting conidia into the stems. After 2 wk, 97% of the Standard Atlantica seedlings were symptomatic and the disease rating was relatively high (Table 2). The disease ratings remained high throughout the experiment, and after 6 wk, 7% of the Standard Atlantica seedlings were dead. Whereas 73% of the Pioneer Gold seedlings had symptoms after 2 wk, the disease rating was lower than in the Standard Atlantica seedlings. Moreover, the disease rating of the Pioneer Gold seedlings decreased from 1.2 at 2 wk to 0.5 at 4 wk and then 0 at 6 wk. The

Table 1. Pistachio rootstock cultivars used in 1990 study

Cultivar	Source[x]	Female parent[y]	Male parent[y]
Standard Atlantica	S & J	*Pistacia atlantica*	*P. atlantica*
KAC-Atlantica	KAC	*P. atlantica**	*P. atlantica*
Pioneer Gold	PN	*P. integerrima***	*P. integerrima*
KAC-Integerrima	KAC	*P. integerrima*	*P. integerrima****
Pioneer Gold II (PGII)	PN	*P. integerrima***	*P. atlantica*
UCBI[z]	KAC	*P. atlantica**	*P. integerrima****

[x] S & J = S & J Ranch, Inc., Pinedale, CA; KAC = Kearney Agricultural Center, University of California, Parlier, CA; PN = Pioneer Nursery, Visalia, CA.
[y] The same superscript denotes the same tree.
[z] UCBI was selected by L. J. Ashworth, Jr., and D. P. Morgan.

Table 2. Development of Verticillium wilt symptoms in pistachio rootstock cultivar seedlings 2, 4, or 6 wk after being inoculated by injecting conidia into the stems[x]

	Percent plants rated 1–4[y]			Disease rating[y]		
Cultivar	2 wk	4 wk	6 wk	2 wk	4 wk	6 wk
Standard Atlantica	97[z]	100	100	2.4	2.7	2.6
Pioneer Gold	73	40	3	1.2	0.5	0.03
PGII	57	83	23	1.0	1.5	0.4
UCBI	53	80	3	0.9	1.2	0.03

[x] Thirty replicate seedlings per cultivar.
[y] Disease rating scale: 0 = asymptomatic; 1 = mild wilt, leaf reddening, or chlorosis; 2 = moderate wilt, leaf necrosis, or defoliation; 3 = severe wilt, leaf necrosis, or defoliation; and 4 = dead.
[z] Data were analyzed by the Kruskal-Wallis test. For each column, the chi-square probability is <0.001.

ratings decrease if either the affected leaves abscise or the plant recovers. After 6 wk, the hybrid UCBI had disease ratings and percentages of symptomatic plants comparable to the field-resistant Pioneer Gold. The hybrid PGII had a higher disease rating and percentage of symptomatic plants than Pioneer Gold after 6 wk but had lower values than the Standard Atlantica.

Six weeks after *V. dahliae* conidia were inoculated into the stems, the fungus was isolated from the pistachio tissue (Table 3). The fungus was uniformly recovered from the four cultivars 5 and 15 cm above the inoculation site. At 30 cm above the inoculation site, *V. dahliae* was isolated from 42–43% of both the Pioneer Gold and UCBI seedlings, 60% of the PGII seedlings, and 84% of the Standard Atlantica seedlings.

We used a microscopic procedure (8,10) to determine the spread of *V. dahliae* from xylem vessels which were infused with conidia and colored vinyl beads; the beads were present in the infused vessels but absent from the newly colonized vessels (4,10). As a control treatment, stems were infused with only the colored beads; there was no apparent plant response to the beads alone. Vessels of all cultivars colonized with *V. dahliae* generally contained amorphous brown material. The colonization ratios, which are measurements of fungal invasion into noninfused xylem vessels, were significantly ($P < 0.05$) lower in Pioneer Gold (0.2) and KAC-Integerrima (0.5) than in any of the other cultivars tested (Table 4). The colonization ratios of KAC-Atlantica (0.7) and UCBI (0.8) were significantly higher than Pioneer Gold but indistinguishable from KAC-Integerrima. PGII (1.9) had a significantly greater colonization ratio than these cultivars, and the Standard Atlantica (2.6) had the highest colonization ratio. In Pioneer Gold, *V. dahliae* penetrated laterally from only 3% of the infused vessels (primary sites) into adjacent vessels. In contrast, *V. dahliae* penetrated laterally from 43% of the infused vessels into adjacent vessels of the Standard Atlantica. While there is a statistically ($P < 0.05$) higher lateral penetration ratio in stems of Standard Atlantica than in stems of Pioneer Gold, we did not use the lateral penetration ratio further because most of the cultivars had too few lateral penetration sites for a meaningful comparison.

One-year-old seedlings were transplanted into a field estimated by soil dilution to contain 40 microsclerotia per gram of soil (SEM = 1.3). Significantly ($P < 0.05$) fewer plants of Pioneer Gold and UCBI transplants were killed in comparison with Standard Atlantica and PGII (Table 5). At the end of the first year, none of the Pioneer Gold or the UCBI plants were dead, whereas 12 and 17% of the Standard Atlantica and PGII, respectively, were dead. Thus, as predicted, Pioneer Gold was resistant and Standard Atlantica was susceptible to Verticillium wilt in our field trial. Moreover, UCBI appears resistant and the PGII appears susceptible.

In the field trial, Verticillium wilt-like symptoms were observed in all cultivars; by the end of the season, 36–92% of each cultivar were symptomatic. However, the percentages of affected trees varied with the cultivar; significantly ($P<0.05$) fewer

Table 3. Recovery (%)[x] of *Verticillium dahliae* from pistachio rootstock cultivars after seedlings were inoculated by injecting conidia into the stems[y]

Cultivar	Distance above injection point (cm)		
	5	15	30
Standard Atlantica	100	97	84
Pioneer Gold	97	87	43
PGII	100	90	60
UCBI	100	88	42
Chi-square probability[z]	0.39	0.60	0.02

[x]Ten pieces of xylem approximately 2 mm^3 from each replicate were plated onto pectate agar and rated for the presence or absence of *V. dahliae*. The replicate was scored positively for *V. dahliae* if the fungus was recovered from any of the subsamples.
[y]Thirty replicate seedlings per cultivar.
[z]Data in each column were analyzed separately by the Kruskal-Wallis test.

Table 4. Microscopic assessment of fungal colonization of xylem of pistachio rootstock cultivars after excised stems were infused with *Verticillium dahliae* conidia[u]

Cultivar	1° sites[v] (no.)	Lateral peneration sites[w] (no.)	2° sites[x] (no.)	Lateral peneration ratio[y]	Colonization ratio[z]
Standard Atlantica	15 ± 1	6 ± 1	30 ± 5	0.4 ± 0.1	2.6 ± 0.4 a
KAC-Atlantica	14 ± 1	1 ± 0.4	9 ± 1	0.1 ± 0.03	0.7 ± 0.1 c
KAC-Integerrima	14 ± 0.7	3 ± 0.5	7 ± 0.6	0.2 ± 0.04	0.5 ± 0.04 cd
Pioneer Gold	16 ± 1	1 ± 0.2	2 ± 0.7	0 ± 0.01	0.2 ± 0.04 d
PGII	11 ± 0.4	1 ± 0.3	21 ± 1	0.1 ± 0.03	1.9 ± 0.1 b
UCBI	15 ± 1	2 ± 0.4	13 ± 2	0.2 ± 0.03	0.8 ± 0.07 c

[u]Excised stems were infused with a mixture of conidia and red vinyl particles. After a 7-day incubation, the vascular tissue 9 mm above the excision point was examined microscopically.
[v]The number of infused vessels, i.e., containing fungus and red beads.
[w]The number of newly colonized vessels adjacent to primary sites.
[x]The total number of newly colonized vessels, i.e., containing fungus and no red beads.
[y]The number of lateral penetration sites divided by the number of primary sites.
[z]The number of secondary sites divided by the number of primary sites. Values followed by the same letter are not significantly different by the Duncan's multiple range test with $\alpha = 0.05$.

Table 5. Morbidity and mortality of pistachio rootstock cultivars planted in a field with soil infested with *Verticillium dahliae*[w] on three sampling dates in 1990

Cultivar	Dead ± SEM (%)			Symptomatic ± SEM[x] (%)			Disease rating[y]		
	19 June	12 Sept.	20 Nov.	19 June	12 Sept.	20 Nov.	19 June	12 Sept.	20 Nov.
Standard Atlantica	0 ± 0 a[z]	11 ± 4 b	12 ± 5 b	17 ± 2 b	56 ± 8 b	58 ± 7 b	0.4	1.5	1.6
Pioneer Gold	0 ± 0 a	0 ± 0 a	0 ± 0 a	27 ± 3 c	44 ± 3 ab	43 ± 5 ab	0.5	0.8	0.7
PGII	6 ± 3 b	15 ± 3 b	17 ± 2 b	53 ± 4 d	90 ± 4 c	92 ± 3 c	1.3	2.2	2.3
UCBI	0 ± 0 a	0 ± 0 a	0 ± 0 a	6 ± 3 a	36 ± 5 a	36 ± 5 a	0.1	0.7	0.7

[w]One-year-old pistachio seedlings were planted on 8 March 1990 in a field infested with 40 microsclerotia per gram of air-dried soil.
[x]A tree was rated symptomatic if there was either leaf necrosis, defoliation, or moderate to severe wilt. In addition, the tree was rated as symptomatic if the tree had mild wilt, leaf reddening, or chlorosis and the xylem in the affected area was discolored.
[y]Disease rating scale: 0 = asymptomatic; 1 = milt wilt, leaf reddening, or chlorosis; 2 = moderate wilt, leaf necrosis, or defoliation; 3 = severe wilt, leaf necrosis, or defoliation; and 4 = dead. Data were analyzed by the Kruskal-Wallis test. The chi-square probabilities for the disease ratings are 0.004, 0.015, and 0.008 for the disease ratings on 19 June, 12 September, and 20 November, respectively.
[z]Values in the same column followed by the same letter are not significantly ($P \geq 0.05$) different by the Duncan's multiple range test.

UCBI were symptomatic than PGII on all of the observed dates. On the four-point scale, the disease ratings at the end of the season for Pioneer Gold and UCBI were 0.7, whereas the disease ratings for Standard Atlantica and PGII were 1.6 and 2.3, respectively. Thus, in the field trial, the ranking of the cultivars for resistance to Verticillium wilt was similar regardless of whether the data was expressed as the percentage of dead or symptomatic plants or as a disease rating.

All of the symptomatic plants were infected by *V. dahliae* (*data not shown*). On 19 June, tissue was sampled from symptomatic plants rated 1 or above; the fungus was recovered from 100% of the plants, suggesting that the symptoms were caused by *V. dahliae*.

DISCUSSION

In areas in California where *V. dahliae* is present, scions on *P. atlantica* rootstocks have high mortality because of Verticillium wilt. The *P. integerrima* (Pioneer Gold) rootstock allows continued pistachio production in soil infested with *V. dahliae*. We developed a greenhouse assay which differentiates on the basis of symptoms between the susceptible *P. atlantica* (Standard Atlantica) and the resistant Pioneer Gold after a 6-wk incubation period. In this assay, the stem is injected with a discrete number of conidia. In another assay, we quantified microscopically the spread of *V. dahliae* from vessels that were infused with conidia; the infused vessels were marked with colored beads (4,10). Here, the rate of spread or the colonization ratio was 16 times greater in the Standard Atlantica than in the Pioneer Gold. In addition, the lateral spread from an infused vessel into an adjacent one was 13 times greater in the Standard Atlantica than in the Pioneer Gold.

Two assays were used to indicate the resistance to Verticillium wilt in two new hybrid rootstocks, PGII and UCBI. The hybrids were compared with Pioneer Gold and Standard Atlantica as the field-resistant and field-susceptible cultivars, respectively. Based on symptom development after inoculation of the stems, UCBI is resistant to moderately resistant and PGI is moderately susceptible. Gross colonization of stem tissue indicated that UCBI is resistant and PGII is moderately susceptible; however, the colonization ratio suggested that UCBI is moderately resistant and PGII is moderately susceptible. These results generally agree with our 1-yr field trial that showed that UCBI is resistant to Verticillium wilt and PGII is susceptible. However, data on horticultural performance and more data on long-term Verticillium wilt resistance is needed before UCBI can be recommended for planting in soil infested with *V. dahliae*.

Several experimental observations suggest that *V. dahliae* proliferates in pistachio vascular tissue to a greater extent in a susceptible than in a resistant host. After the same number of conidia were injected into the stems of pistachio seedlings, the fungus spread further more frequently in the susceptible than in the resistant cultivar. These results were corroborated by a significantly ($P < 0.05$) greater colonization ratio in xylem vessels in the susceptible than in the resistant cultivar. Thus, Verticillium wilt resistance in pistachios may be attributable, at least in part, to containment of the fungus within resistant vascular tissue. In tomatoes (*Lycopersicon esculentum* Mill.) and alfalfa (*Medicago sativa* L.), resistance to *V. albo-atrum* Reinke & Berthier is partly attributable to sequestering of the fungus in suberin-coated vessels (6,9,11,14).

Our data suggest that *P. atlantica* varies in susceptibility to Verticillium wilt; KAC-Atlantica has significantly more resistance to Verticillium wilt than Standard Atlantica. Similarly, Raabe and Wilhelm (13) demonstrated variation in Verticillium wilt resistance in *P. atlantica* and *P. terebinthus* L. This variability is not surprising; *Pistacia* spp. are dioecious and can produce interspecific hybrids. One consequence of this variability is that classification of individuals can be problematic. Indeed, the female parent of UCBI was classified as *P. terebinthus* and *P. lentiscus* L. before classification as *P. atlantica* (D. Parfitt, *personal communication*). More importantly, the variability within the genus offers a large, currently unused gene pool for crop improvement. Despite the genetic variability within and among the *Pistacia* spp., only a few plant introductions are used in California. The colonization ratio could provide a method for screening additional *Pistacia* spp. selections for Verticillium wilt resistance. The procedure requires only a 7-day incubation period and the results are readily quantified. In addition to testing seedlings, the colonization ratio procedure also may be suitable for testing either young shoots from mature trees or suckers from budded rootstocks currently used for seed production.

ACKNOWLEDGMENTS

We thank J. MacDonald for the *Verticillium dahliae* isolate. J. Allen provided the Standard Atlantica seed, and C. Anderson and K. Puryear donated the Pioneer Gold and Pioneer Gold II seeds for the greenhouse and laboratory experiments. J. Allen produced the seedlings for the field trial. We thank J. MacDonald, J. Robb, J. Liebman, D. Parfitt, and T. Michailides for helpful discussions.

LITERATURE CITED

1. Ashworth, L. J., Jr., Waters, J. E., George, A. G., and McCutcheon, O. D. 1972. Assessment of microsclerotia of *Verticillium albo-atrum* in field soils. Phytopathology 62:715-719.
2. Ashworth, L. J., Jr., and Zimmerman, G. 1976. Verticillium wilt of the pistachio nut tree: Occurrence in California and control by soil fumigation. Phytopathology 66:1449-1451.
3. Beckman, C. H. 1968. An evaluation of possible resistance mechanisms in broccoli, cotton, and tomato to vascular infection by *Fusarium oxysporum*. Phytopathology 58:429-433.
4. Beckman, C. H., Halmos, S., and Mace, M. E. 1962. The interaction of host, pathogen, and soil temperature in relation to susceptibility to Fusarium wilt of bananas. Phytopathology 52:134-140.
5. Huisman, O. C., and Ashworth, L. J., Jr. 1974. Quantitative assessment of *Verticillium albo-atrum* in field soils: Procedural and substrate improvements. Phytopathology 64:1043-1044.
6. Mohan, R., and Kollattukudy, P. E. 1990. Differential activation of expression of a suberization-associated anionic peroxidase gene in near-isogenic resistant and susceptible tomato lines by elicitors of *Verticillium albo-atrum*. Plant Physiol. 921:276-280.
7. Morgan, D. P., Liebman, J. A., Epstein, L., and Jimenez, M. J. 1991. Solarizing soil planted with cherry tomatoes vs. solarizing fallow ground for control of Verticillium wilt. Plant Dis. 75:148-151.
8. Newcombe, A. G., Papadopoulos, Y. A., Robb. J., and Christie, B. R. 1989. The colonization ratio: A measure of pathogen invasiveness and host resistance in Verticillium wilt of alfalfa. Can. J. Bot. 67:365-370.
9. Newcombe, G., and Robb, J. 1988. The function and relative importance of the vascular coating response in highly resistant, moderately resistant and susceptible alfalfa infected by *Verticillium albo-atrum*. Physiol. Mol. Plant Pathol. 33:47-58.
10. Newcombe, G., and Robb, J. 1989. An improved method of determining the colonization ratio in *Verticillium*-infected alfalfa plants. Can. J. Plant Pathol. 11:60-64.
11. Newcombe, G., and Robb, J. 1989. The chronological development of a lipid-to-suberin response at *Verticillium* trapping sites in alfalfa. Physiol. Mol. Plant Pathol. 34:55-73.
12. Nicot, P. C., and Rouse, D. I. 1987. Precision and bias of three quantitative soil assays for *Verticillium dahliae*. Phytopathology 77:875-881.
13. Raabe, R. D., and Wilhelm, S. 1978. Susceptibility of several *Pistacia* spp. to *Verticillium albo-atrum*. Plant Dis. Rep. 62:672-673.
14. Robb, J., Powell, D. A., and Street, P. F. S. 1989. Vascular coating: A barrier to colonization by the pathogen in Verticillium wilt of tomato. Can. J. Bot. 67:600-607.