



Symptoms of *Verticillium* wilt of alfalfa include bunched top, short internodes, and upward curling of leaves on stem compared with healthy stem (far left). Leaves may be wilted and pinkish to yellow on one side of stem (left). Compared with healthy leaf (right), diseased leaves show marginal and veinal yellowing.

Verticillium wilt found in southern California alfalfa

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The disease has been found in alfalfa south of the Tehachapi Mountains, but not yet in the Central Valley of California. High summer temperatures do not seem to be a barrier to the fungus, and several alfalfa cultivars as well as cowpea were susceptible in greenhouse tests.

Verticillium wilt disease of alfalfa is relatively new to California, having been found in isolated fields in Humboldt and Monterey counties in 1985. It is a vascular wilt disease caused by *Verticillium albo-atrum* Rke et Bert., alfalfa strain. Field inspections by plant pathologists suggest that Verticillium wilt does not yet occur in northern California or in the Sacramento and San Joaquin Valleys. In 1987, however, we found Verticillium wilt in 28 of 52 fields sampled in Riverside and San Bernardino counties.

Verticillium wilt of alfalfa was first reported in the United States in 1977. Since then, nearly all of the northern alfalfa-growing states have reported the disease. It has become prevalent in all alfalfa-growing areas of Washington state, Oregon, Idaho, and northern Utah. (U.S. Department of Agriculture, Agricultural Research Service Bulletin 456, 1984, *A Guide for Identification of Verticillium Wilt of Alfalfa*, by R. N. Peaden and A. A. Christen, describes the disease in detail.)

Verticillium albo-atrum is systemic in alfalfa, colonizing the water-conducting tissue (xylem) and producing a toxin that causes yellowing of leaves and death of stems. Since the fungus colonizes the xylem tissue, it continues to live in a dormant state in dry hay. In Canada, it has been shown to live for 3 years in dry stems. A small percentage (less than 1%) of seeds harvested

from naturally infected plants can become infected. At this time there is no evidence of Verticillium wilt in California's major seed-producing areas (west side of the San Joaquin Valley, Palo Verde Valley or Imperial Valley).

Verticillium albo-atrum, alfalfa strain, should be distinguished from the strain of *V. albo-atrum* reported to cause a vascular wilt of potato and tomato in the northeastern United States. The tomato and potato strains have a lower temperature optimum (70°F) than the alfalfa strain (75°F). This may have been the reason that California Pest Exclusionary Advisory No. 221 (Nov. 9, 1985) forecast that *V. albo-atrum*, alfalfa strain, "... if introduced would not survive in the central valleys of California." Since we show here that the alfalfa strain causes vascular wilt in Riverside, Redlands, Lakewood-Nuevo, Antelope Valley, and Mojave Desert (fig. 1), which have temperatures as high as or higher than those in the Central Valley, it is likely that *V. albo-atrum* could survive in the Central Valley if introduced.

Verticillium albo-atrum from alfalfa is distinguishable from *V. dahliae*, the cause of

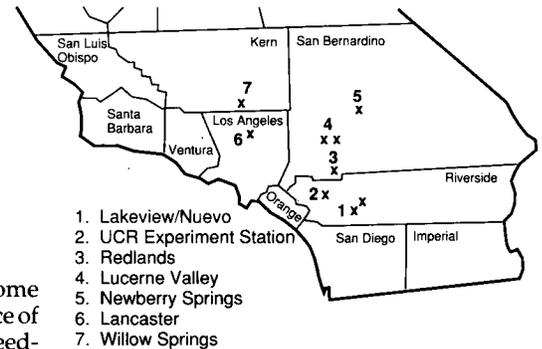


Fig. 1. Distribution of Verticillium wilt caused by *Verticillium albo-atrum*, alfalfa strain. The disease was identified in 28 of 52 fields sampled.

Verticillium wilt on cotton in the San Joaquin Valley. Both species have similar optimum temperatures for growth, but *V. dahliae* produces microsclerotia (small, thick-walled resting bodies) in infected dead stem tissue and can thus persist in dry soil for many years. *Verticillium albo-atrum*, which does not produce microsclerotia, cannot persist as long in soil.

The alfalfa strain produces conidiophores (stalks) on which two or three short branches emerge in a verticillate (whorled) manner from a single node on the main conidiophore. The mycelium (fungal threads) eventually becomes black because of melanin produced in the cell walls.

Verticillium nigrescens also can be isolated from alfalfa but differs from *V. albo-atrum* and *V. dahliae* in producing black chlamydospores (thick-walled resting spores)

TABLE 1. Pathogenicity of six isolates of *Verticillium albo-atrum* on CUF101 alfalfa tested by bare root inoculation

Isolate	Source	Foliar disease index*	Vascular discoloration index ^o
1393 N-41	Newberry Springs	4.6 a	4.6 a
1385-3A	Lakeview	4.7 a	4.9 a
1289-4	San Jacinto	4.4 a	4.9 a
VA-1	San Jacinto	4.4 a	4.3 a
1292-17	Redlands	5.0 a	5.0 a
1383-12D	Redlands	4.6 a	4.7 a
Check	—	1.0 b	1.0 b

NOTE: Six-week-old plants were root dipped in a spore suspension (10⁶/ml) and transplanted to steamed UC mix (50% peat moss:50% fine sand). Data, taken 6 weeks later, followed by different letters differ significantly (P = 0.01) by Duncan's multiple range test.

* Foliar disease index on scale of 1 to 5: 1 = no symptoms; 2 = slight necrosis of lower leaves; 3 = chlorosis of all leaves, some defoliation; 4 = total defoliation; 5 = plant killed.

^o Vascular discoloration index. 1 = no symptoms; 2 = trace of discoloration; 3 = 25%-50% of xylem discolored; 4 = 50%-75% discolored; 5 = all of xylem discolored; 5 = plant killed.



in a 10-day-old culture. We isolated this species three times from alfalfa and once from cowpea, but none of the cultures were pathogenic on alfalfa or cowpea.

The first symptom of *Verticillium* wilt in an infested alfalfa field is usually scattered dead stems on which the leaves continue to cling. This symptom alone is not conclusive, since other pathogens such as

Colletotrichum trifolii also cause individual stems to die in a similar fashion. Close observation of *Verticillium*-infected plants reveals a shortening of internodes near the top of the stems, which causes a bunched top. Leaves often wilt on only one side of the plant. Affected leaves become yellow at the tip, often in a V-shaped pattern. Some of the affected leaves take on a pink cast. When the root of an infected plant is removed from the soil, a cross-section shows that the xylem tissue is usually tan in color compared with the white color of a healthy root.

We successfully isolated *V. albo-atrum* from surface-sterilized (10% commercial chlorine bleach containing 5.25% sodium hypochlorite) tissue of stems and roots on water agar-S (S = amended with streptomycin at 30 ppm) or on potato dextrose-S agar. Isolation is usually more successful from stems than roots because there are fewer contaminating organisms. Since the disease is systemic and *V. albo-atrum* colonizes the stem tissue, it is generally accepted that mowing will spread the disease from a small focus of infection to an entire field. Freshly cut stubble is highly susceptible when inoculated with spores.

We isolated *V. albo-atrum* from 28 of 52 fields inspected in the Riverside-San Jacinto area and in the Antelope Valley and Mojave Desert. Isolations were positive from April

until November. Thus, despite the high summer temperatures (about 100°F) well above the maximum for growth of *V. albo-atrum* (86°F), the fungus remained in a viable state in infected stems.

Greenhouse study

To determine the disease-causing ability of the alfalfa strain, we grew plants in steamed UC mix (50% river silt, 50% peat moss) fertilized once with Osmocote (30% NPK), a slow-release fertilizer, and daily with a solution of a complete liquid fertilizer (modified Hoagland's solution). Alfalfa and cowpea plants were grown from *Rhizobium*-inoculated seed. Since fungal gnats (*Bradysia* sp.) have been shown to transmit *V. albo-atrum* from pot to pot, the surface of each pot was treated with 1/4 teaspoon of the insecticide Lorsban (chlorpyrifos) granules (15% active ingredient). We determined that this dosage did not affect growth of *V. albo-atrum*. Greenhouse temperatures ranged from 68°F at night to 77° to 80°F during the day.

About 10 CUF101 as well as other cultivars of alfalfa plants were grown in each 6-inch square plastic pot for 3 to 6 weeks before inoculation and cut back to 1.5 to 2 inches at monthly intervals. Cowpea and cotton were planted at two plants per pot and were not cut back.

Inoculum of *V. albo-atrum*, alfalfa strain isolates, was grown in potato dextrose broth; the fungus reproduced by continuous sporulation. We applied the spore suspension (a million spores/ml) in some experiments as a root drench, pouring 200 ml of inoculum in holes or trenches in the soil beside the plants, then covering the holes with soil. In some tests, bare roots of alfalfa plants were clipped and soaked in the inoculum for 5 to 20 minutes before transplanting to soil. In other experiments, we inoculated freshly cut stubble of potted alfalfa plants by spraying it with the spore suspension (5 million spores/ml) (stubble inoculation).

Results

Verticillium albo-atrum isolates caused disease symptoms in nondormant alfalfa germplasms and the CUF101 cultivar by all three inoculation methods (tables 1, 2 and 3). The bare root method caused a more severe disease expression in a shorter time than did the other methods. However, any influence on disease severity due to the physiological stress of bare-rooting was negligible, since the reactions were similar with other methods that did not cause plant stress (tables 2 and 3). In addition to the cultivars listed, nondormant UCCibola and Moapa 69 were also susceptible in other experiments. The symptoms of disease on alfalfa caused in greenhouse tests were similar to those seen in the field. In all cases,

TABLE 2. Pathogenicity of *Verticillium albo-atrum* (isolate 1289-1 from San Jacinto) on six cultivars of alfalfa tested by stubble inoculation

Cultivar or germplasm	Foliar disease index*	
	11	36
Germplasm:		
UC 302 (Lehman)	1.2	2.8 a
UC 329 (Lehman)	1.4	2.5 a
UC 340 (Lehman)	1.5	2.3 a
Cultivar:		
CUF101 (California)	0.1	3.0 a
Vernema (Peaden)	1.1	1.4 b
Vertus (France)	0.1	1.0 b
Control (all of above)	0.0	0.0 c

NOTE: Freshly clipped plants were sprayed three times with a spore suspension (5×10^6 /ml). Data in columns followed by different letters differ significantly (Duncan's multiple range test, $P = 0.01$).

* Foliar disease index on scale of 1 to 5. See table 1 footnote (*).

TABLE 3. Pathogenicity of five isolates of *Verticillium albo-atrum* inoculated on alfalfa (CUF 101) and cowpea (CB5) by soil drench

Isolate	Source	Foliar disease index*		Vascular discoloration index [§]	
		Alfalfa	Cowpea	Alfalfa	Cowpea
1393 D-10	Lucerne Valley	3.9 a	2.7 ab	3.5 ab	3.0 a
1384-N41	Lucerne Valley	4.6 a	3.2 ab	4.3 a	3.2 a
1389-19	UCR	3.5 a	3.0 ab	3.1 b	3.0 a
Va1	San Jacinto	3.9 a	4.0 a	3.6 ab	3.0 a
Va2	Redlands	4.6 a	2.3 b	4.3 a	3.0 a
Noninoc.	—	1.0 b	1.0 c	1.0 c	1.0 b

NOTE: Cowpea plants inoculated in 15x15x15-cm square pots containing UC Mix soil, by soil-drenching with 200 ml of a spore suspension (10^6 /ml) in two holes impressed in each pot beside the plant. Alfalfa plants inoculated by soil drench and stubble inoculation. Data in columns followed by different letters differed significantly (Duncan's multiple range test, $P = 0.01$).

* Foliar disease index on scale of 1 to 5. See table 1 footnote (*).

[§] Vascular discoloration index on scale of 1 to 5. See table 1 footnote (*).

[§] *Verticillium albo-atrum* was reisolated from stems of 100% of the inoculated plants.

V. albo-atrum was reisolated from inoculated plants with symptoms.

Inoculated plants that did not show either foliar or vascular browning symptoms (less than 1% of those inoculated) were reinoculated by root dip, and survivors were reselected. Seed from random cross-pollination by hand was collected, replanted and inoculated by root dip. A high percentage (over 50%) of the progeny were symptomless compared with 0% symptomless for the unselected CUF101 control.

Cowpea (California Blackeye 5) was highly susceptible following inoculation of soil with spores (table 3). About 10 to 14 days after inoculation, lower leaves became flaccid and growth was suppressed. The effect of the high degree of susceptibility of cowpea to Verticillium wilt is not known, since the disease has not been seen in the field. We isolated *V. albo-atrum*, alfalfa strain, in routine samples from cowpea plants at the UC Riverside experimental farm; the plants were growing near a plot of naturally infected alfalfa plants but did not show active symptoms. In selection of rotation crops for control of Verticillium wilt, cowpea would not be an advisable choice. Cotton (SJ-2) was susceptible following root drench inoculation, but the expression of disease was not as severe as that caused by inoculation with a defoliating isolate of *V. dahliae*.

Conclusions

Since Verticillium wilt appears to be established in two areas of southern California, high temperature will not limit its spread in California alfalfa-growing areas as had been earlier believed.

The most effective control of Verticillium wilt in other areas where dormant and semidormant alfalfa is grown is the use of resistant cultivars, some of which have been selected from existing cultivars. Unfortunately, resistant nondormant alfalfa cultivars are not yet available. Since we were able to select plants that were resistant to *V. albo-atrum*, and seed progeny from random crosses was also resistant, it appears that obtaining resistance will be the most effective approach to control of the disease in nondormant alfalfa.

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Survey detects viruses in almond, prune, and sweet cherry orchards

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Prunus necrotic ringspot and/or prune dwarf viruses were found in young California orchards, averaging 20% infection in almond and prune and 4% in sweet cherry. Nursery stock was implicated as the primary source, and efforts are now under way to propagate disease-free trees.

Prune dwarf and Prunus necrotic ringspot viruses have plagued California's tree fruit and nut orchards ever since these crops were established. Their effect on orchard trees was uncertain, however, until research in the 1940s and 1950s demonstrated that virus infections were responsible for reduced tree growth and fruit yield. During this same period, rapid diagnostic and virus elimination procedures were also being developed. The accumulated findings were put into commercial practice from mid-1960 to late 1970, resulting in the production and planting of virus-free fruit and nut orchards.

In 1987, however, we found that one in four cling peach (*Prunus persica*) orchards contained a high incidence of prune dwarf virus, Prunus necrotic ringspot virus, or both. The cling peach industry was back in a disease situation similar to that of earlier times. Economic difficulties had apparently led to increased demand for less expensive, noncertified cling peach trees in late 1970 to early 1980. The relatively high disease incidence in cling peach orchards included first-leaf trees, suggesting the use of infected budwood and/or seed or seedling rootstocks in propagation. During 1988, we expanded the surveys to determine virus incidence in almond (*P. dulcis*), sweet cherry (*P. avium*), and prune (*P. domestica*).

Economic importance

Prune dwarf virus (PDV) and Prunus necrotic ringspot virus (PNRSV) are serologically unrelated but share similar biologic

properties. For example, they are pollen- and seed-borne, and they are spread to healthy trees through pollination with virus-infected pollen and by grafting.

In the nursery, grafting diseased scions onto healthy understocks or healthy scions onto diseased stocks can result in a low percentage of bud take and retarded growth of the budling shoot.

In orchards, virus-infected trees may show leaf symptoms ranging from scattered or clustered chlorotic spots (caused, for example, by almond calico, a strain of PNRSV) to an initial chlorosis that turns into brown (necrotic) rings or areas that fall out (caused by strains of PDV and PNRSV). The latter results in a shredded-leaf appearance. The calico and rugose mosaic strains of

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