Verticillium Wilt of Alfalfa in California

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ABSTRACT

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Verticillium wilt of alfalfa, caused by *Verticillium albo-atrum*, has been identified in three coastal counties of central and northern California. The disease appears to be well established in these areas. In contrast, a survey of alfalfa fields in 11 counties of the San Joaquin and Sacramento valleys did not identify any Verticillium-wilt infected plants. Twenty cultivars and experimental lines were tested for their reactions to a California isolate of *V. albo-atrum* at two inoculum concentrations by a root dip assay conducted in a greenhouse. Rankings of the entries at the two inoculum concentrations based on a visual symptom severity index were highly correlated (r = 0.866). Rankings of resistant and susceptible control entries were similar to reactions previously reported for isolates from other states. Several semidormant and nondormant entries for which disease reactions had not been reported previously showed a high level of resistance to Verticillium wilt. On the basis of these results and recently published information on host range and vegetative compatibility, the California isolate was indistinguishable from those previously reported in North America.

Verticillium wilt of alfalfa (*Medicago sativa* L.), caused by *Verticillium albo-atrum* Reinke & Berth., has long been a serious disease in northern Europe; it was first reported in the United States in 1977 (6). Since that time, Verticillium wilt has become widely distributed in North America, imposing a significant limitation on alfalfa production in many areas (1,7). Although most common in cool northerly growing regions, Verticillium wilt has been found recently in alfalfa fields as far south as Kansas, below 38° latitude (10). This disease also is known to occur in Oregon and Washington but has not been reported previously from California. This report describes the occurrence of Verticillium wilt in the central and north coast regions of California and the reaction of semidormant and nondormant alfalfa cultivars to a California isolate of *V. albo-atrum*.

MATERIALS AND METHODS

Initial field samples from Humboldt and Monterey counties, which led to the first identification of *V. albo-atrum*, were received from cooperative extension personnel who had been looking for evidence of the disease since it was first identified in the Pacific Northwest. Subsequent survey samples were collected from at least two fields in each of the counties indicated in Figure 1 in the fall of 1985. The plants were removed from stands that were at least 2 yr old and were in the late-bud growth stage. All plants sampled showed symptoms of dieback or stunting. Samples were transported to the laboratory under refrigeration.

Isolations were made from surface-disinfected (0.25% sodium hypochlorite, 5 min) crown tissue on water agar; stem pieces or petioles from wilt-affected plants were placed directly on water agar without sodium hypochlorite treatment. Isolation plates were incubated under cool-white fluorescent light at room temperature (21–24 C). Sporulation was apparent on infected tissue pieces within 3–5 days. Single conidial heads were removed with a glass needle and spread on water agar supplemented with 150 μg ml^{-1} streptomycin and 100 μg ml^{-1} penicillin. Single germinated spores were transferred to cornmeal agar (CMA) 24 hr later. Pathogen identification was based on the presence of verticillately branched conidiophores with darkened bases on host tissue and the production of dauer (dark resting) mycelium and the absence of microsclerotia in culture.

Pathogenicity of all isolates was initially established on the basis of tests conducted at Prosser, WA, by R. G. Gilbert (*personal communication*). These results were confirmed later through tests carried out in Berkeley, using 3- to 4-wk-old alfalfa seedlings of the cultivar Moapa 69 in a root dip assay (2). Plant roots were immersed in water and the lower one-quarter of the root system was cut off. Plants then were transferred to an aqueous suspension containing 2×10^7 spores per milliliter for 5 min, repotted in U.C. mix (8), and maintained in a greenhouse for 2–3 wk before evaluation. Inoculum for all pathogenicity tests was grown on CMA at 21–24 C.

A somewhat different procedure was used to evaluate the reaction of 20 alfalfa cultivars to a California isolate of *V. albo-atrum*. The isolate used in these tests, designated CA4, was originally obtained from diseased alfalfa in Monterey County and verified to be pathogenic as described above. Isolate CA4 expressed relative virulence equal to isolate 40-3, which had been used as a standard check isolate in the original confirming pathogenicity tests conducted at Prosser, WA (R. G. Gilbert, *personal communication*). Plants to be inoculated were grown from seed in peat transplant plugs (Castle & Cook Techniculture, Salinas, CA). A single seedling was grown in each plug.

When seedlings were 3 wk old, the bottom 1 cm of each plug was cut off, effectively severing 5–10 roots. The seedlings were placed briefly in tap water, to eliminate any differences in water content that would affect the amount of spore suspension retained by the peat plug, and then dipped in a spore suspension containing either 2×10^6 or 2×10^7 spores per milliliter for 5 min. Six seedlings of a single cultivar were inoculated simultaneously in this manner, after which they were replanted in a 15-cm-diameter clay pot containing U.C. mix (8) and maintained in a greenhouse.

The experiment was set up as a

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randomized block design with a total of five replicates; different benches in the same greenhouse served as blocking units. The following cultivars and experimental selections were tested: Pierce, Amador, 79176 BV1, and 82506, supplied by Northrup King & Co.; WL-316, WL-320, WL-Southern Special, WL-516, and WL-605, supplied by W-L Research, Inc.; Apollo II, GT-13R Plus, and Diamond, supplied by AgriPro; 5929 and 581, supplied by Pioneer Hi-Bred International, Inc.; Armona, Maricopa, and Yolo, supplied by Plant Genetics, Inc.; Vernema, obtained from R. G. Gilbert; and Moapa 69 and Lahontan, donated by V. L. Marble. Four weeks after inoculation, plants were evaluated and rated on the following scale: 1 = symptomless, 2 = stunted with some chlorosis, 3 = extensive chlorosis and up to 50% of the leaf area necrotic, and 4 = killed by the pathogen.

RESULTS AND DISCUSSION

Locations of diseased fields. During the spring and summer of 1985, plants affected with Verticillium wilt were found in two coastal counties in California: Humboldt County, less than 160 km south of the Oregon border on the north coast, and Monterey County, on the central coast (Fig. 1). The disease was identified in five widely separated fields comprising over 40 ha in Humboldt County. The incidence and distribution of the disease were similar in Monterey County, with the disease confirmed to be present in five fields. The fields spanned the length of the intermontane region of Monterey County, extending approximately 120 km from north to south. All these fields fall within the relatively cool coastal growing region of California, consistent with the historical prevalence of Verticillium wilt in cool growing regions of Europe and North America (3,6).

In contrast, diseased plants were also found in two fields of San Luis Obispo County in a more inland location than any of the Monterey County sites. Furthermore, the San Luis Obispo County locations are below 36° latitude and represent a warmer climate than regions where the disease has traditionally been a problem worldwide.

The latter finding suggested that the pathogen may be adapted to warmer environments and could therefore represent a threat to the major forage and seed production areas in the Central Valley of California. For this reason, a survey of eight counties in the San Joaquin Valley, representing over 50% of the alfalfa production hectarage in California, was conducted in the fall of 1985. Plants that were wilted, flagged, or generally unthrifty were removed from 16 fields (Fig. 1) throughout this area. *V. albo-atrum* was not isolated from any of the samples collected in the San Joaquin Valley. Three counties in the Sacramento Valley (Sacramento, Solano, and Yolo) also were surveyed. Eight fields were sampled without finding *V. albo-atrum*. Visual examination of the crowns indicated varying degrees of crown decay or crown rot. The predominant symptom observed was diagnostic for Stagonospora crown rot, incited by *Stagonospora meliloti* (Lasch) Petr., although nonspecific crown rot also was frequently noted. The pathogens most commonly isolated from the sampled plants were *S. meliloti* and *Phytophthora megasperma* Drechs.

For historical accuracy, we should note that the disease was detected once at a single site in the Sacramento Valley in 1982. This site was an experimental plot on the University of California at Davis campus. The source of the localized field infestation was bare-rooted seedlings obtained from the University of Minnesota, St. Paul. The infected plant material was destroyed by on-site burning, and the field was fumigated twice with 560 kg ha^{-1} methyl bromide and chloropicrin before being replanted with an alfalfa trap crop. The trap crop remained free from the disease for 3 yr, and the pathogen was declared to have been eradicated from the research plots. Thus, although Verticillium wilt does appear to be established in some of the coastal alfalfa-growing areas of California, there is, as yet, no evidence that the pathogen exists or can persist in the Central Valley.

Cultivar evaluation. The reaction of 20 alfalfa cultivars to *V. albo-atrum* isolate CA4, at two inoculum concentrations, is reported as average severity indices (ASI) in Table 1. Control plants remained healthy throughout the period of observation. The ranking of the cultivars was somewhat different at the two inoculum levels tested, but the five most resistant cultivars were the same in each case and there is a good correlation between the two sets of ASI values (r = 0.866). At both inoculum levels the disease ratings suggest a virtual continuum in terms of cultivar reaction to the pathogen without discrete classes of host reaction type.

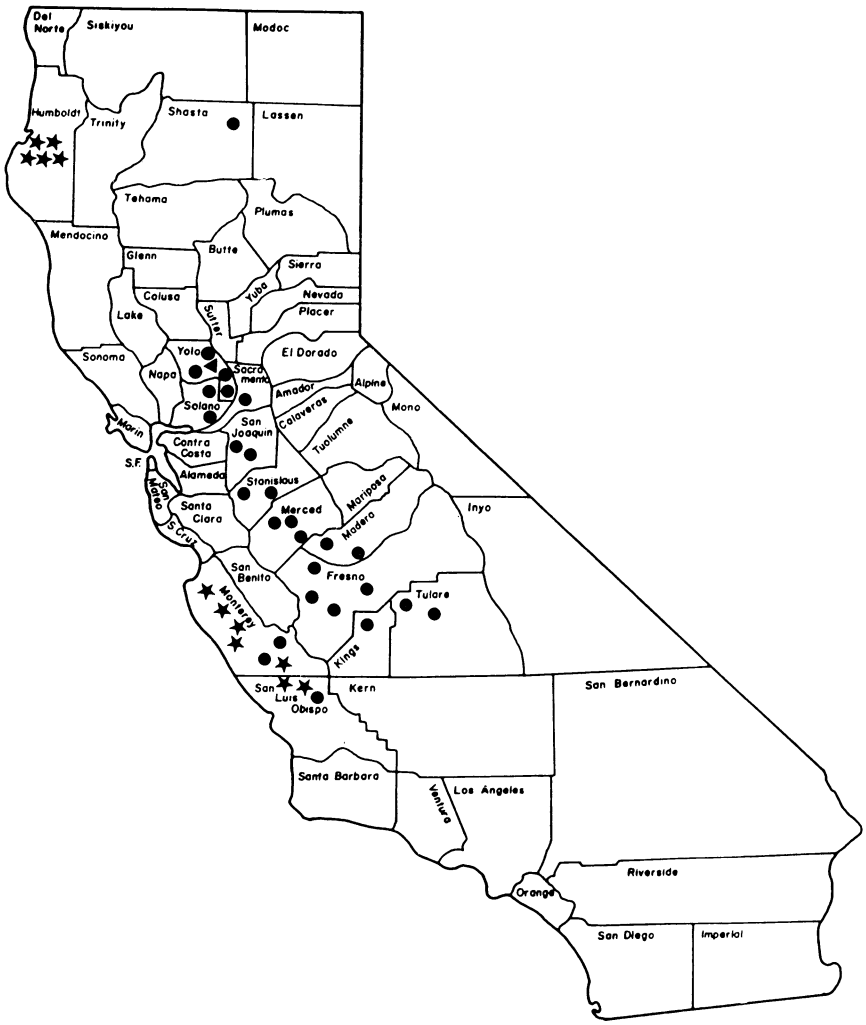

Fig. 1. Sites in the coastal and central valley alfalfa hay production areas in California surveyed for Verticillium wilt. Sites were recorded as positive (★) or negative (•) for the pathogen following standard isolation procedures. The pathogen was introduced on infected bare-root transplants at one site (▲) in Yolo County and subsequently eradicated, with no further incidence.

Table 1. Mean disease rating of selected alfalfa cultivars of different dormancy classifications inoculated at two spore concentrations with a California isolate of *Verticillium albo-atrum*

			Inoculum concentration			
			2×10^7 Spores ml^{-1}		2×10^6 Spores ml^{-1}	
Cultivar	DOR[w]	PRR[x]	ASI[y]	% Killed[z]	ASI	% Killed
NK82506	...	*	1.7 a	3	0.8 a	3
Apollo II	4	MR	1.8 ab	7	1.4 bc	3
NK79176 BV1	...	*	2.0 abc	13	1.3 ab	7
WL-316	4	R	2.3 abcd	10	1.3 ab	3
Vernema	4	MR	2.4 bcd	20	1.3 ab	0
Armona	7	*	2.5 cde	30	2.4 def	20
Pierce	7	*	2.6 cdef	33	1.9 bcde	3
WL-Southern Special	6	LR	2.6 cdef	23	2.0 cde	7
WL-516	7	*	2.7 cdef	27	1.9 bcde	7
Moapa 69	7	*	2.8 def	23	1.7 bcd	7
WL-320	5	MR	2.8 def	23	1.8 bcde	7
Yolo	7	*	2.9 defg	27	2.1 cdef	13
Diamond	6	*	2.9 defg	30	2.4 def	20
WL-605	8	*	2.9 defg	43	2.5 ef	30
Maricopa	7	*	2.9 defg	33	2.5 ef	30
Pioneer-Brand 5929	8	*	3.0 defg	40	2.3 def	10
Amador	6	*	3.1 efg	50	2.1 cdef	13
GT-13R Plus	7	*	3.2 fg	31	2.4 def	13
Pioneer-Brand 581	6	*	3.3 fg	53	2.5 def	10
Lahonton	6	*	3.5 g	50	2.8 f	20

[w] Dormancy rating (DOR) is on a 1–8 scale, with 1 = most dormant and 8 = least dormant, and was obtained from the publication of the Certified Alfalfa Seed Council (Woodland, CA) entitled *1987 Alfalfa Varieties: Currently Marketed Varieties Approved for Certification by an Agency of the Association of Official Seed Certifying Agencies.*

[x] Previously reported reactions (PRR) (obtained from the same publication as the dormancy ratings) are given according to the percentage of plants resistant to Verticillium wilt: S (0–5%), LR (6–14%), MR (15–30%), R (31–50%), and HR (>50%); * = no reaction reported.

[y] Each average severity index (ASI) value represents the mean of five replications; each replication included six plants. Means followed by a common letter are not significantly different at the 0.05 level according to Duncan's multiple range test. Correlation between ASI values at the two inoculum concentrations for all entries was $r = 0.866$.

[z] Values are based on assessment of 30 plants of each entry evaluated at the conclusion of the experiment.

Consequently, there is no clear point of separation between cultivars that are susceptible and those that are resistant. Nearly all plants given a disease rating of 2 recovered from the disease and were symptomless within 4 wk after the plants were rated. A cultivar with a mean disease rating of 2 or below might therefore be regarded as resistant. At the higher inoculum level (2×10^7), however, at least one dead plant from which *V. albo-atrum* was isolated occurred in each of the cultivars tested so that none can be considered completely resistant (Table 1). This likely reflects the heterogeneous condition of alfalfa populations such that even those cultivars considered to be resistant include some susceptible individuals (4).

Among the best performing entries were Vernema and Apollo II, which are dormant cultivars, and WL-316, a semidormant cultivar. All three have undergone selection for Verticillium wilt resistance and were included in this study as resistant checks. The Northrup King experimental selections 82506 and 79176, semidormant and nondormant, respectively, also performed well; both were field-selected for Verticillium wilt resistance. Other nondormant cultivars showing some evidence of resistance include Armona and Pierce. Lahontan, included as a susceptible check, was the most severely damaged by the disease. It has been reported recently that alfalfa isolates of *V. albo-atrum* from California were vegetatively compatible with isolates from Washington, Kansas, New York, Pennsylvania, and Wisconsin as well as with isolates from Yugoslavia, U.S.S.R., and France (5). Furthermore, all these isolates had an identical host range (5). Thus, in terms of the reaction of resistant and susceptible control cultivars in these tests and the aforementioned published criteria, the California isolate of *V. albo-atrum* used in this test appears to be similar to isolates from other alfalfa-growing areas in North America and Europe.

In summary, Verticillium wilt now seems to be established in some of the coastal alfalfa-growing areas in California but does not now occur in the Central Valley. Although the details of how the pathogen was introduced into the state are speculative, movement of infested alfalfa hay, cubes, and seed from areas outside California where the disease is established has undoubtedly resulted in repeated introductions, some few of which eventually became established in the coastal regions. Results of the pathogenicity tests indicate that a high level of resistance to Verticillium wilt is available in nondormant cultivars adapted to California growing conditions. If the disease develops here as it has elsewhere in North America, use of such cultivars will be an essential tool in disease management (9).

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LITERATURE CITED

1. Arny, D. C., and Grau, C. R. 1985. Importance of Verticillium wilt of alfalfa in North America. Can. J. Plant Pathol. 7:187-190.
2. Busch, L. V., Christie, B. R., Smith, E. A., and Boland G. 1985. Testing alfalfa cultivars for resistance to an alfalfa strain of *Verticillium albo-atrum*. Can. J. Plant Pathol. 7:203-205.
3. Christen, A. A., and French, R. C. 1982. Growth and pathogenicity of alfalfa strains of *Verticillium albo-atrum*. Plant Dis. 66:416-418.
4. Christie, B. R., Papadopoulos, Y. A., and Busch, L. V. 1985. Genetics and breeding for resistance to Verticillium wilt in alfalfa. Can. J. Plant Pathol. 7:206-210.
5. Correll, J. C., Gordon, T. R., and McCain, A. H. 1988. Vegetative compatibility and pathogenicity of *Verticillium albo-atrum*. Phytopathology 78:1017-1021.
6. Graham, J. H., Peaden, R. N., and Evans, D. W. 1977. Verticillium wilt of alfalfa found in the United States. Plant Dis. Rep. 61:337-340.
7. Heale, J. B. 1985. Verticillium wilt of alfalfa, background and current research. Can. J. Plant Pathol. 7:191-198.
8. Matkin, O. A., and Chandler, P. A. 1957. The U.C.-type soil mixes. In: The U.C. System for Producing Healthy Container-Grown Plants. K. F. Baker, ed. Calif. Agric. Exp. Stn. Serv. Man. 23.
9. Peaden, R. N., Gilbert, R. G., and Christen, A. A. 1985. Control of *Verticillium albo-atrum* on alfalfa. Can. J. Plant Pathol. 7:211-214.
10. Stuteville, D. L., Willis, W. G., Houfek Christensen, J. A., and Sim, T., IV. 1986. First report of Verticillium wilt of alfalfa in Kansas. Plant Dis. 70:475.