Incidence of External Seedborne *Verticillium albo-atrum* in Commercial Seed Lots of Alfalfa

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ABSTRACT

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Verticillium albo-atrum was detected on up to 2% of alfalfa (*Medicago sativa*) seed in commercial seed lots. The pathogen was found in 5 of 20 seed lots assayed from the Columbia Basin of Washington in 1979 and in 2 of 20 seed lots in 1980, respectively. The occurrence of external inoculum could be a factor in disease spread.

Verticillium wilt of alfalfa (*Medicago sativa* L.), a destructive disease of alfalfa, has been known in the Pacific Northwest since 1976 (4). This disease, caused by a strain of *Verticillium albo-atrum* Reinke & Berth., could spread across the nation with seed or in hay, at least in some areas (3). In 1980, the disease was found in Wisconsin (5). *V. albo-atrum* has been reported from alfalfa seed (6–9) and was recently shown to be borne within the seed coat (2). Pathogenic *Verticillium* isolates were isolated from seed and debris in alfalfa seed lots (8). The incidence and prevalence of external seedborne *V. albo-atrum* have remained unknown. Because alfalfa seed produced in Washington might originate from stands diseased with *V. albo-atrum*, this study reports the incidence of external seedborne *V. albo-atrum* in commercial seed lots and the method used to detect seedborne (internal and external) *V. albo-atrum*.

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MATERIALS AND METHODS

Twenty lots of alfalfa seed produced in 1979 and 1980 in the Columbia Basin of Washington were obtained through the Seed Branch, Washington State Department of Agriculture, Yakima. A total of 300 seeds per lot was plated, 25 per petri dish, onto a selective-agar medium (1,2) and incubated at room temperature (20–25 C) for 10–20 days. In the 1979 assay, plates were examined microscopically, and *Verticillium*-like colonies were transferred to prune-lactose-yeast agar (10) for identification. In 1980, a direct assay was used in which plated seeds were rolled over twice onto a previously unoccupied area of the agar at 2- to 4-day intervals to allow colony growth to develop on the agar with minimal competition from other organisms. Colonies of *V. albo-atrum* were identified without transfer within 1–2 wk.

Pathogenicity of *V. albo-atrum* isolates to alfalfa was tested by inoculating alfalfa plants using a root-soak method (3). To determine whether seedborne propagules found in this study were located internally or externally, a sample of 10,000 seeds that passed through a 14.1-mm (1/18 in.) but not through a 12.7-mm (1/20 in.) screen were plated from one seed lot. These seeds were from the 1980 seed lot, which had a 1.3% incidence of *V. albo-atrum*. They were surface-sterilized with sodium hypochlorite and 70% ethanol (1:9, v/v) plus Tergitol as described earlier (2) and were plated (100 seeds per dish) with a vacuum head designed to fit a petri dish.

RESULTS

V. albo-atrum was isolated from up to

Table 1. Incidence of all seedborne *Verticillium albo-atrum* propagules in unsterilized alfalfa seed lots from the Columbia Basin of Washington

Year	Incidence[a] (%)	Location
1979	0.3	Othello
	0.7	Othello
	2.0	Othello
	1.0	Mesa
	1.3	Mesa
1980	0.3	Pasco
	1.3	Pasco

[a] A single sample of 300 alfalfa seeds was assayed for each incidence reported.

2% of the alfalfa seeds in five of the 20 seed lots sampled in 1979 (Table 1). Fewer propagules were obtained with seed lots assayed in 1980 (Table 1). All *V. albo-atrum* cultures recovered from seed were pathogenic to alfalfa. Surface-sterilization of the 10,000 seeds from the 1980 seed lot eliminated the fungus from the seed.

DISCUSSION

V. albo-atrum was found on seed in 25% of the seed lots assayed in 1979 and in 10% assayed in 1980. The fungus might have been detected in more of the seed lots if a larger number of seeds had been sampled. A sample size of 10,000 is used to detect *Phoma lingam* (Tode ex. Fr.) Desm. in cabbage (*Brassica oleracea* var. *capitata* L.) seed (11). Christen (2) found internal infection in three seeds from 40 racemes having Verticillium wilt symptoms in a field plot and estimated that internal infection would occur in a very low percentage of seed in commercial alfalfa seed lots from diseased stands. The higher level of seedborne *V. albo-atrum* found herein was demonstrated to be external inoculum. The external inoculum was probably acquired before seed harvest while in contact with infected plant parts or during seed harvest and cleaning operations.

Incidence of external seedborne *V. albo-atrum* is an economic concern. At a seeding rate of 11.2 kg/ha, 18,000 seeds per hectare could harbor *V. albo-atrum* externally. The incidence of external seedborne *V. albo-atrum* at any level might provide the primary inoculum necessary to cause a stand decline after 3–4 yr of secondary inoculum spread.

LITERATURE CITED

1. Christen, A. A. 1982. A selective medium for isolating *Verticillium albo-atrum* from soil. Phytopathology 72:47-49.
2. Christen, A. A. 1982. Demonstration of *Verticillium albo-atrum* within alfalfa seed. Phytopathology 72:412-414.
3. Christen, A. A., and Peaden, R. N. 1981. Verticillium wilt in alfalfa. Plant Dis. 65:319-321.
4. 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.
5. Grau, C. R., Delwiche, P. A., Norgren, R. L., O'Connell, T. E., and Maxwell, D. P. 1981. Verticillium wilt in alfalfa in Wisconsin. Plant Dis. 65:843-844.
6. Isaac, I. I. 1957. Wilt of lucerne caused by species of *Verticillium*. Ann. Appl. Biol. 45:550-558.
7. Isaac, I., and Heale, J. B. 1961. Wilt of lucerne caused by species of *Verticillium*. III. Viability of *V. albo-atrum* carried with lucerne seed; effects of seed dressings and fumigants. Ann. Appl. Biol. 49:675-691.
8. Richardson, J. J. 1979. An Annotated List of Seedborne Diseases. 3rd ed. Commonw. Mycol. Inst., Kew, Surrey, England. 320 pp.
9. Sheppard, J. W., and Needham, S. N. 1980. Verticillium wilt of alfalfa in Canada: Occurrence of seed-borne inoculum. Can. J. Plant Pathol. 2:159-162.
10. Talboys, P. W. 1960. A culture-medium aiding the identification of *Verticillium albo-atrum* and *V. dahliae*. Plant Pathol. 9:57-58.
11. Williams, P. H. 1980. Black rot: A continuing threat to world crucifers. Plant Dis. 64:736-742.