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