Verticillium Wilt in Alfalfa

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ABSTRACT

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Verticillium wilt of alfalfa, caused by *Verticillium albo-atrum*, is extensively distributed in the Pacific Northwest. Diseased alfalfa occurs on humid coastal acid soils of Washington and Oregon. It is common in irrigated neutral to alkaline soils of the Columbia Basin of Washington, north-central and eastern Oregon, southwestern Idaho, and southern British Columbia, Canada. The disease was absent in dryland fields, even those adjacent to irrigated fields. Field symptoms, which are distinct when plants are in the early bud stage, typically consist of V-shaped pinkish orange brown necrotic areas on the leaflets. Leaflets on severely affected shoots are usually necrotic and twisted, forming spirals. Diseased stems remain erect but do not become chlorotic until all leaves have lost chlorophyll. New shoots on infected plants appear normal at first but show typical symptoms as they approach physiologic maturity. Symptoms were produced in controlled temperature chambers at 15–27 C. Isolates grown in the laboratory produced dark resting mycelia. Dimensions of conidia and conidiophores correspond with those described for *V. albo-atrum*. Thirty-eight geographic isolates produced similar symptoms and death in susceptible alfalfa plants.

Verticillium wilt of alfalfa (*Medicago sativa* L.), caused by *Verticillium albo-atrum* Reinke and Berth., which forms dark mycelia, was first found in the United States in 1976 (3).

Verticillium wilt of alfalfa was found in Sweden in 1918 (5) and throughout Europe by the 1940s (9). Verticillium wilt reportedly was found in eastern Canada in 1962 but not the following year (1). Although several reports discuss the general wilting of infected plants (6–8,10,13), a detailed description of the disease is lacking.

We describe the symptoms of Verticillium wilt of alfalfa in the Pacific Northwest, the occurrence of the disease, and the methods and procedures for recovering and identifying the causal organism.

MATERIALS AND METHODS

Collection and isolation of *V. albo-atrum*. Alfalfa plants with symptoms of Verticillium wilt were sampled by excising stems above the crown. Infected foliage was placed in plastic bags and stored at 10 C until isolation of fungus was attempted (1 or 2 days after sampling).

Stem segments (~1.5 cm long) were surface sterilized in a solution of Clorox and 95% ethanol (1:1, v/v) for 15–20 sec, rinsed in sterile distilled water, and blotted dry on sterile filter paper. Four to six segments were then aseptically plated in each petri dish containing 2% water agar and incubated at 25 C for several days. Hyphal tips of the resulting fungi were transferred to prune lactose yeast agar (12) for identification. Single-spore cultures were made from each of 38 isolates. Conidiophores and conidia were measured in an isolate from diseased stem material plated on water agar and incubated under Westinghouse daylight fluorescent light at about 2,400 lux at 22–25 C for 3 or 4 days.

Testing of Koch's postulates. Difco Czapek Dox broth cultures were grown at 20–25 C and filtered, and conidia were centrifuged (15 min, at 2700 *g*) twice. The conidial suspension was adjusted to a final inoculum concentration of 8×10^6 conidia per milliliter by use of a hemocytometer. Seed of a susceptible Washington alfalfa line (WQS1) was sown in metal flats (34 × 49.5 cm) containing Ritzville silt loam soil and peat (4:1) that had been steamed for 2 hr at 20.7 kg/cm^2 (20 psi).

Five-week-old plants were dug and their roots were rinsed, trimmed, and soaked for 20 min in a conidial suspension. Inoculated plants were transplanted into steam-sterilized soil, five plants per 10-cm pot, three pots per replication, and incubated at 21 C under fluorescent light at 8,400 lux with a 14-hr day in a growth chamber for 4–6 wk. Four-week-old plants were inoculated and incubated in growth chambers at 15, 27, 31, 33, and 35 C to determine the disease temperature range. Plants 5- and 9-wk-old were inoculated and then sampled for the fungus at daily intervals for 1, 2, 3, 4, and 6 days.

RESULTS

Field symptoms. Symptoms may first appear as a yellow blotchiness on leaflets of a single stem on a plant (Fig. 1D,H), as a V-shaped yellow (Fig. 1D, I) or pinkish-orange brown (Fig. 1C,D,E,J) chlorotic segment of the leaf tip, or as yellow streaks along midrib and veins (Fig. 1C, D,I). Younger leaflets tend to curl upward and inward from the leaf tip or to twist along the midrib, either loosely or more tightly, to form a spiral (Fig. 1F,G); older leaflets tend to remain open (Fig. 1D,J,K). Stems do not wilt but remain green until all leaves are dead (Fig. 1G).

Infected plants are usually distributed throughout the field, with one or more shoots on any given plant showing evidence of the disease (Fig. 1A). Under the canopy more severely affected plants are stunted, with most shoots having severe symptoms (Fig. 1B). Chlorotic areas of leaflets are pinkish-orange brown that may bleach out under sprinkler irrigation, rainfall, or heavy dew.

Symptoms generally are not observed until late in the first year, but by the end of the second or early in the third year, many plants throughout a field have symptoms (Fig. 1A). Symptoms in affected fields may appear as early as the third week of April and then in each successive cutting by the time plants reach the floral bud stage. Once infected, plants become progressively weaker and eventually die, even though regrowth after each cutting initially appears healthy. The taproot of affected plants may contain yellow to orange vascular discoloration; it is not distinctive, however, and cannot be used as a reliable diagnostic symptom.

Disease occurrence. The disease was prevalent in 1978 in irrigated alkaline (pH >7) desert land (<10 cm of rain per year) in Adams, Benton, Douglas, Franklin,

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Grant, Okanogan, Stevens, Walla Walla, and Yakima counties of Washington; Malhuer and Umatilla counties of Oregon; Ada and Canyon counties of Idaho; and Creston, Midway, and Okanagan Falls areas of British Columbia, Canada. The disease was also found in acid (pH 5–6), high-rainfall coastal areas (>100 cm of rain per year) in Clark, Pierce, and Whatcom counties of Washington and in Marion County south to Douglas County, Oregon. The disease was not found in dryland alfalfa sampled in Klickitat, Spokane, Stevens, and Whitman counties of Washington; in Bonner and Boundary counties of Idaho; and in irrigated fields in an isolated valley in Kittitas County, Washington.

Isolation and identification of *V. albo-atrum*. *V. albo-atrum* was easily recovered from fresh green stems bearing leaves with symptoms. Verticillate conidiophores were produced at the ends of cut stems 24–36 hr after incubation on water agar. On prune lactose yeast agar, the fungus produced conidia and dark resting mycelia typical of *V. albo-atrum*

Fig. 1. Symptoms of Verticillium wilt of alfalfa: **(A)** Scattered diseased plants. **(B)** Stunted plant in advanced stage of disease. **(C)** Trifoliolate leaf with V-shaped tip necrosis following chlorosis. **(D)** Plant in early stage of disease. **(E)** Trifoliolate leaf as in C. **(F and G)** Stem in advanced stage of disease. Trifoliolate leaf with **(H)** initial chlorosis, **(I)** chlorotic areas and V-shaped chlorosis, and **(J)** typical pinkish orange-brown V-shaped necrosis. **(K)** Leaflet with necrosis over greater area on one side of leaflet.

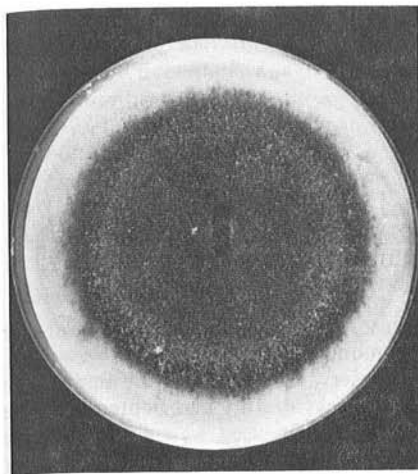

Fig. 2. Colony of *Verticillium albo-atrum* on prune lactose yeast agar.

(Fig. 2); colonies at 25 C were gray because of the resting mycelia and pruinose with irregular margins. Hyaline mycelia produced conidiophores on which singly produced conidia coalesced into hardened slime balls. In young colonies the center was gray to yellowish gray-green and the outer 4-mm margin of the colony was white.

Conidiophores and conidia from inoculated alfalfa stems were measured. The length of the terminal phialide (30–49 μm), the tapering width of the phialide (2.2–4.4 × 1.25 μm), the length of the phialide (22–27 μm), the distance between whorls (29–46 μm), the tapering of conidiophores (3.8–5.6 × 2.2–3.1 μm), the length of conidiophores (55–163 μm), and the dimensions of the conidia (1.3–3.1 [avg. 2.2] × 2.8–11.3 [avg. 5.3] μm) were similar to those given by Smith (11) for *V. albo-atrum* on potato stems. The dark color and the shape of basal cells, the conidiophore morphology, and the conidial shape (all nonseptate) were consistent with those illustrated by Smith (11) for *V. albo-atrum*.

Pathogenicity. All *V. albo-atrum* isolates caused symptoms and subsequent death in 5-wk-old WQS1 alfalfa plants inoculated by soaking roots. Symptoms were typical in plants incubated at 15–27 C and were less pronounced at 31 C. The fungus could be reisolated from an occasional plant at 33 C but not at 35 C.

Initial symptoms appeared as early as 8 days after inoculation in 5-wk-old plants incubated at 21 C, and the plants were dead by 24 days after inoculation. Severe and uniform disease symptoms in 4- or 5-wk-old plants consisted of yellowed and orange to pinkish necrotic leaves curling inward. Fewer V-shaped or unilateral leaf symptoms were present than in older plants. Diseased plants were shorter and contained fewer nodes (three to five) than healthy plants (seven or eight). The initial distribution of the fungus was different in younger and older plants; the fungus was recoverable from the entire stem of each 5-wk-old plant by 6 days after inoculation but from only one or two of the two to four stems on 9-wk-old plants.

DISCUSSION

Verticillium wilt has been found in both the irrigated desert lands and the humid, high rainfall coastal lands, affecting about 202,000 ha (500,000 acres). Its absence in Washington's dryland alfalfa suggests that dry soil and less dense canopy are unfavorable for establishment of the fungus. This is particularly evident in Stevens County, Washington, where irrigated and dryland fields are side by side and the disease occurs only in the irrigated fields.

The lack of a preference for soil type, evidenced by the presence of the disease in alfalfa grown on both alkaline and acid soils, makes it likely that the disease can spread with seed or within hay to other parts of the United States.

Verticillium wilt was found in virtually every alfalfa stand older than 1 yr in the Columbia Basin of Washington; diseased fields were less frequent in the Willamette Valley of Oregon. Some 3-yr-old stands were reduced to uneconomic levels in 1978, although stands should last 6 or 7 yr. The disease was also present in most seed fields surveyed in the Columbia Basin but affected a smaller proportion of the plants. The low prevalence of the disease in seed fields may be related to less frequent irrigation, less active plant growth, and less traffic, which reduces the risk of spreading inoculum.

We estimate the disease to have been present in the Pacific Northwest 3 or 4 yr before 1976. The disease can be spread through infected plant debris carried with the seed (6); the fungus may have initially been introduced in this manner. Disease spread within and between fields may be attributable to machinery movement (6), airborne conidia (2), and root contact (4).

When viewed during the floral bud stage, foliar symptoms in Pacific Northwest fields are distinct from those of other alfalfa diseases. Frost, drought, and boron deficiency may mask or alter leaf coloration caused by *V. albo-atrum*, as these same conditions can produce leaf symptoms similar to but not as distinctive as those of Verticillium wilt. Other fungal diseases cause wilting of the stems; in contrast, stems affected by Verticillium wilt eventually become chlorotic but do not wilt. Leaf hopper feeding produces a V-shaped yellowing on alfalfa leaflets, which is different from that caused by *V. albo-atrum*. A yellowish-orange discoloration of the xylem in the taproot and crown aids in diagnosis when the foliage cannot be examined, but this discoloration is not limited to Verticillium wilt. Isolation and identification of the pathogen from fresh diseased stems, petioles, and leaflets easily verifies field determinations. It is difficult to isolate from crowns or roots.

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