Resistant Alfalfa Plants as Symptomless Carriers of *Verticillium albo-atrum*

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ABSTRACT

Pennypacker, B. W., Leath, K. T., and Hill, R. R., Jr. 1985. Resistant alfalfa plants as symptomless carriers of *Verticillium albo-atrum*. Plant Disease 69:510-511.

Alfalfa cultivars resistant and susceptible to Verticillium wilt were grown in the greenhouse, stubble-inoculated with *Verticillium albo-atrum*, and evaluated for percentage of symptomless plants. Distribution of symptomless plants was: Vertus, 54%; NAPB 108, 54%; NAPB 110, 47.5%; Apollo II, 36%; CW8015, 36%; WL 316, 35%; Cimmaron, 22.5%; and Saranac AR, 10%. Symptomless plants in all cultivars except NAPB 108 (95%) were positive for *V. albo-atrum* when the stem bases were cultured 7 mo after inoculation. A high percentage of plants in resistant cultivars have the potential of serving as symptomless carriers of the pathogen.

Additional key words: *Medicago sativa*

Verticillium albo-atrum Reinke & Berth. causes a wilt in susceptible alfalfa (*Medicago sativa* L.) plants that is characterized by narrowing and longitudinal rolling of apical leaflets and V-shaped chlorosis of leaflet tips followed by death of leaves and petioles and defoliation (2,4,6). Since it was first reported in the Pacific Northwest in 1976 (3), Verticillium wilt has spread through much of the northern alfalfa-growing region of the United States (6). Resistant cultivars are presently the only feasible means of controlling this disease. Parent clones for resistant cultivars of alfalfa are selected on the basis of absence of typical symptoms or reduction in disease severity. Usually, no attempts are made to detect the presence of the pathogen in these symptomless plants. Consequently, we initiated a study of several resistant cultivars to determine whether these plants could act as symptomless carriers of *V. albo-atrum*.

MATERIALS AND METHODS

Alfalfa cultivars Saranac AR and Cimmaron (susceptible) and Vertus, NAPB 108, NAPB 110, CW8015, WL316, and Apollo II (resistant) were grown from seed in greenhouse carts (90 × 60 × 9 cm) filled with Terra-Lite Reddi-Earth Peat-Lite mix (W. R. Grace & Co., Cambridge, MA). Seeds were treated for 5 min in 0.5% sodium hypochlorite, rinsed in sterile H_2O for 5 min, and air-dried before planting. Seedlings were thinned to 10 per 60-cm row and allowed to grow for 4 wk before being cut to 10 cm to promote stem production. The experimental design was a randomized complete block with eight replicates of one row of each cultivar per replicate.

Inoculum was prepared by growing *V. albo-atrum* (freshly isolated from alfalfa) from single-spore transfers on prune-yeast-extract agar (1). A spore suspension with a concentration of 3.5×10^6 spores per milliliter was prepared by washing the spores into 1 L of sterile distilled water.

Three-month-old alfalfa plants were cut to 10 cm, and the resulting stubble was misted with the spore suspension. Each replicate received 125 ml of inoculum. After inoculation, the plants were kept in a mist chamber for 24 hr before being returned to the greenhouse. The natural light in the greenhouse was supplemented between 8:00 A.M. and 8:00 P.M. with metal-halide lamps.

Alfalfa plants were scored for disease reactions four times at 42-day intervals on the following scale: 1 = no symptoms; 2 = any symptom of Verticillium wilt, including stunt (plants less than 15 cm tall); and 3 = dead. Plants were then cut to 10-cm stubble height to approximate hay-crop practice. After the fourth cut, one stem from regrowth of each symptomless plant was excised and surface-sterilized in 0.5% sodium hypochlorite for 3 min. The basal 30 mm of the stem was then placed on water agar and examined microscopically after 7 days at about 22 C for the presence of *Verticillium* sp. Plants that were negative were reassayed using all stems.

Isolates of *Verticillium* sp. were obtained from representative symptomless plants in each cultivar 9 mo after inoculation. The 19 isolates were tested for pathogenicity on Saranac AR. The pathogenicity test was conducted as described previously (6), except inoculated plants were grown in a controlled-environment chamber (model CEL 25-7HL, Sherer-Gillett Co., Marshall, MI) with a constant temperature of 22 ± 1 C and a photoperiod of 16 hr at 200 $\mu E\ m^{-2}\ sec^{-1}$.

Data on the frequency of symptomless plants were subjected to an analysis of variance and *F* test followed by Duncan's modified (Bayesian) least significant difference test at $P = 0.05$ where appropriate.

Contribution 1470, Department of Plant Pathology, Pennsylvania Agricultural Experiment Station. Authorized for publication as Journal Series Paper 6985 and contribution 8409 of the U.S. Regional Pasture Research Laboratory, USDA-ARS, University Park, PA 16802.

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Accepted for publication 3 December 1984.

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Table 1. Percentage of symptomless plants infected with *Verticillium albo-atrum* in eight alfalfa cultivars 7 mo after inoculation[y]

Cultivar	Symptomless plants (%)	Infected symptomless plants based on isolations from one stem per plant (%)	Infected symptomless plants based on isolations from all stems of a plant (%)
Vertus	54.0 a[z]	74	100
NAPB 108	54.0 a	74	95
NAPB 110	47.5 ab	42	100
Apollo II	36.0 bc	29	100
CW8015	36.0 bc	55	100
WL316	35.0 bc	28	100
Cimmaron	22.5 cd	64	100
Saranac AR	10.0 d	61	100

[y] Based on examination of 80 plants of each cultivar.
[z] Entries followed by the same letter are not significantly different at $P = 0.05$.

RESULTS AND DISCUSSION

V. albo-atrum was inoculated into the alfalfa plants via a mist applied to the fresh stubble rather than a root soak to allow the plant the maximum opportunity to activate resistance mechanisms. Mist-inoculation approximates natural infection as described by Isaac (4) and should yield results that more closely reflect the field performance of resistant cultivars.

The incidence of symptomless plants in each cultivar 7 mo after inoculation is shown in Table 1. As expected, the susceptible cultivars Saranac AR and Cimmaron had the fewest symptomless plants, and Vertus, NAPB 108, and NAPB 110 had the highest level of resistance. Latunde-Dada and Lucas (5) reported 70% resistant plants in the cultivar Vertus under greenhouse conditions; however, when only symptomless plants (score = 0) were considered, resistance in Vertus dropped to 37% (5). The higher level of resistance that we found in Vertus may reflect the different inoculation method. Mist-inoculation is less rigorous, allowing more time for the expression of plant-initiated resistance.

V. albo-atrum was able to survive for a prolonged period in symptomless plants, and resistant cultivars included a large percentage of potential symptomless carriers (Table 1). The low fungal recovery rate after the first isolation compared with the almost complete recovery when all stems were cultured is an indication that colonization of the entire vascular cylinder of the crown by *V. albo-atrum* was delayed or inhibited in symptomless plants. The stem base was a reliable isolation site (5), and in addition, all stems must be cultured to optimize detection of symptomless carriers of *V. albo-atrum*.

All isolates of *V. albo-atrum* from symptomless plants were pathogenic on Saranac AR, a susceptible cultivar. Thus, regardless of the resistance of the host cultivar, the fungus retained its virulence during 9 mo in the symptomless plants.

This study establishes that symptomless, resistant plants can act as carriers of *V. albo-atrum* and raises the possibility that the presence of *V. albo-atrum* in symptomless plants could adversely affect the performance and long-term field survival of the resistant cultivars. Since symptomless alfalfa plants can harbor the pathogen, sanitation and crop rotation should be used with resistant cultivars to reduce inoculum and slow the spread of this pathogen.

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