

Incidence and severity of verticillium wilt of alfalfa in Prince Edward Island (1988-89) and New Brunswick (1988)

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In 1988, verticillium wilt of alfalfa was diagnosed on 12 farms in New Brunswick. A survey on Prince Edward Island in the same year found that 20% of alfalfa fields surveyed had some symptoms of verticillium wilt with approximately 5% of the fields severely infested. No severely infested fields were found on P.E.I. in 1989, although approximately 15% of sampled fields were found to be suffering from verticillium wilt. There was circumstantial evidence from the survey data to indicate that the pathogen responsible for verticillium wilt of alfalfa, *Verticillium albo-atrum*, was introduced by seed at various locations in the two provinces, possibly in 1984.

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En 1988, on a identifié des cas de flétrissure verticillienne de la luzerne dans 12 fermes du Nouveau-Brunswick. Une enquête effectuée dans l'Île-du-Prince-Édouard la même année a révélé que 20 % des luzernières échantillonnées montraient quelques symptômes de flétrissure et qu'environ 5 % étaient gravement atteintes. On n'a pas rencontré de luzernières gravement infectées dans l'Île-du-Prince-Édouard en 1989, même si environ 15 % des champs échantillonnés se sont avérés atteints par la flétrissure. Il semblerait, d'après les données d'enquêtes, que l'agent pathogène responsable de la flétrissure verticillienne de la luzerne, *Verticillium albo-atrum*, ait été introduit par la semence à divers endroits dans les deux provinces, peut-être en 1984.

Introduction

Verticillium wilt of alfalfa (*Medicago sativa* L.), as incited by *Verticillium albo-atrum* Reinke & Berthold, was first identified in North America in Quebec in 1962, although it did not become established at that time (3). The next report of its occurrence in North America was in 1976, from the north western United States (4). In 1977, verticillium wilt was found again in Canada in south central British Columbia (9). Since its detection in the United States and Canada it has spread rapidly and has been reported in New York State, in 1981 (5) and by 1983 in Alberta, Saskatchewan, Ontario and Nova Scotia (1,2). In 1986, verticillium wilt again was identified in scattered locations in Quebec (7,8).

Until 1988, verticillium wilt had not been identified in either New Brunswick or Prince Edward Island. In late 1988, alfalfa fields in each province were identified as being positive for verticillium wilt. The current study was undertaken to determine the incidence and severity of verticillium wilt of alfalfa in the two provinces.

Materials and methods

New Brunswick (1988): A total of 150 alfalfa fields were assessed for the presence of field symptoms of verticillium wilt. Fields were surveyed from August through to October and classified into one of the follow-

ing three groups: (1) not having sufficient regrowth for symptom expression, (2) not having suspect plants present, or (3) having chlorotic plants displaying symptoms of verticillium wilt. Lower stem samples from plants from suspect fields were randomly collected for laboratory confirmation.

Stem sections 1.5 cm long from the base of the plants were dipped in 95% ethanol and surface sterilized in 0.5% sodium hypochlorite for three minutes. Sections were rinsed in sterile water, aseptically split lengthwise and placed cut surface upwards on potato dextrose agar (PDA). Approximately 40 stem pieces, representing 20 plants, were evaluated for verticillium infection from each of the suspect fields. Plated stem sections were maintained under U.V. light, 12 hour photoperiod, and periodically examined for the presence of *Verticillium*. Sub-cultures were transferred to water agar for *V. albo-atrum* identification.

Prince Edward Island (1988): After the initial identification of a verticillium wilt infected alfalfa on Prince Edward Island, a survey was initiated on alfalfa fields across the province. The survey was conducted during September and October in 58 randomly selected fields. Each field was inspected for plants showing symptoms of verticillium wilt by walking the entire field in an "W" pattern, usually twice by two different people. Each field was assessed for verticillium wilt based on symptom expression. Fields were rated as not infected, slight infection, moderate infection (10-20% of plants showing symptoms), or severely infected. Plants from all moderately and severely infected fields were collected for laboratory confirmation of *V. albo-atrum* infection.

Prince Edward Island (1989): Two separate samplings were used in the survey of Prince Edward Island fields in 1989. The first samples were collected in late May and

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early June from a total of 90 fields. The second samples were collected from 60 fields in early August just prior to the second cut of alfalfa. In each field 100 lower stem cuttings were taken randomly while walking a "W" pattern through the field.

Stem sections collected in each field were observed for sporulation by *V. albo-atrum* in the laboratory. A 1 cm long section was cut from each stem and split longitudinally. The sections were surface sterilized for ten minutes in 20% Javex® (6% sodium hypochlorite), containing 0.01% Tween 20, rinsed in distilled water and plated on water agar containing 125 ppm chlortetracycline and 125 ppm dihydrostreptomycin sulphate. Plates were then incubated at room temperature for 3 to 4 days and examined microscopically for the presence of *Verticillium*. Sub-cultures of *Verticillium* were plated on PDA for confirming identification.

Results

In New Brunswick samples from a total of 92 fields from 47 different farms were examined in the laboratory for *V. albo-atrum*. Based on plant symptoms and laboratory identification verticillium wilt was confirmed for fields of 12 of the 47 farms with another 9 farms being suspect (Fig. 1). Cultivars in 45 *Verticillium* infested fields were Iroquois (35.5%), Apica (15.5%) and Minto (13.3%) with the remaining 35.5% being other cultivars, an unknown cultivar or mixed plantings. Infested fields were evenly distributed between plantings made from 1983 to 1988 and scattered throughout the alfalfa growing region.

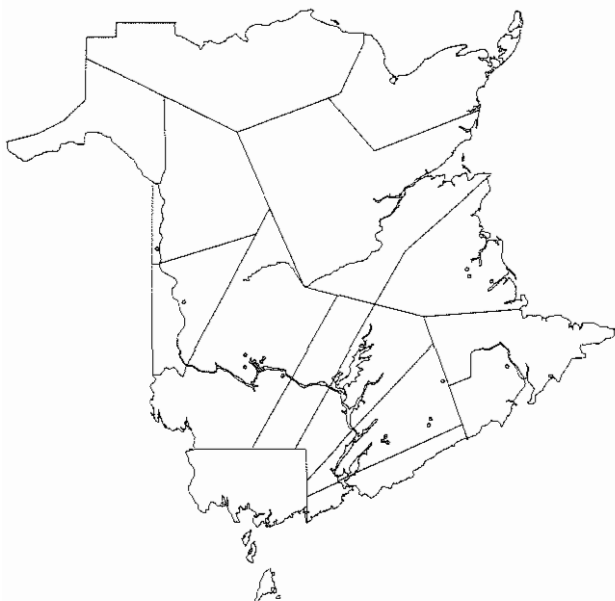


Fig. 1. Distribution of verticillium wilt of alfalfa in New Brunswick in 1988. ● farms with verticillium wilt, ○ farms suspected as having verticillium wilt.

Approximately 30% of the fields surveyed in New Brunswick were positive for *V. albo-atrum* infestation, however, the actual level may have been higher given that only fields showing characteristic verticillium wilt symptoms were sampled for laboratory confirmation. The number of fields with *V. albo-atrum* infested plants may have been higher if plants were symptomless, or had insufficient regrowth after cutting to identify disease symptoms.

Plants in eleven of the 56 fields surveyed in Prince Edward Island in 1988 demonstrated symptoms of verticillium wilt (Fig. 2). Of these fields, three were severely infested and one was moderately infested, with symptoms spread uniformly across the fields. The infections in the remaining positive fields were restricted to isolated plants. Infested fields were at least three years old and the most common cultivars were Apica and Iroquois.



Fig. 2. Distribution of verticillium wilt of alfalfa on Prince Edward Island in 1988. ▲ alfalfa fields surveyed but without verticillium wilt symptoms, △ fields with occasional infect plant, ◇ fields moderately infested (10-20% of plants) and ◆ fields severely infested (50% of plants infested).



Fig. 3. Distribution of verticillium wilt of alfalfa on Prince Edward Island in 1989. ▲ farms surveyed but no infested alfalfa fields, ◆ farms surveyed with fields positive for verticillium wilt and alfalfa ◇ fields positive for verticillium wilt but not part of main survey.

Eighteen farms were sampled on Prince Edward Island in 1989 and alfalfa fields on 5 farms were identified as positive for verticillium wilt (Fig. 3). Two fields from one farm were identified as being positive for verticillium wilt during the first of the two sample periods in 1989. A second sample indicated that two other fields on the same farm were also positive for verticillium wilt. In addition, four other fields on separate farms, were also identified as being infested by *V. albo-atrum* by the second sampling. In all cases the incidence on each farm was less than 4%. Severity was in general low, with no field exhibiting infestation levels to match 1988, when some fields had nearly 100% of the plants showing symptoms. In two fields, approximately 10% of plants showed symptoms, while in the remaining fields symptom expression was confined to less than 5% of plants. Two fields showed symptoms in the field but there was no confirmation of *Verticillium* spp. infection in the laboratory. All fields which were positive for *Verticillium* spp. in the laboratory showed verticillium wilt symptoms in the field. In each case where stem section platings were positive for *Verticillium* infection the age of the alfalfa stand was five years or older. One three year old stand had symptoms but no positive identification from plating. This field was adjacent to an older contaminated field and both were managed by the same grower.

There was *Verticillium* infestation at moderate to trace levels in other fields in 1989, which were not part of the 1989 general survey. One of these was the most severely infested field found in 1988. In this field, disease progress had been rapid (6) and plant survival during the winter of 1988-89 was very poor with approximately 10% or less surviving the winter.

Discussion

The survey indicated that verticillium wilt of alfalfa is firmly established in both New Brunswick and Prince Edward Island. While the origin of the primary infection is unknown, the disease pattern in the severely infested fields and the wide geographic distribution of verticillium wilt in the two provinces indicates that the initial introduction into the region was probably from contaminated seed. A number of the fields did exhibit

infection patterns which indicated that the introduction of the pathogen was via contaminated farm equipment or movement of contaminated plant residue.

The geographic distribution of the disease in New Brunswick and Prince Edward Island, makes it unlikely that the disease can be eradicated. While some containment may be possible the methods of pathogen spread between fields is such that this would likely not prevent further infestations but only reduce its rate of progression through New Brunswick and Prince Edward Island. The identification of cultivars which are resistant to verticillium wilt and adapted to the Atlantic Region was not in the past a regional priority. Greater emphasis is now being placed on the selection of regionally adapted and verticillium wilt resistant cultivars to minimize the impact of the disease.

Literature cited

1. Atkinson, T.G. 1981. Verticillium wilt of alfalfa: challenge and opportunity. *Can. J. Plant Path.* 3:266-272.
2. Atkinson, T.G., M.R. Hanna, A.M. Harvey, R.J. Howard, H.C. Huang, J.P. Miska, J.W. Sheppard and J.M. Yorston. 1983. Verticillium wilt of alfalfa. Agriculture Canada, Research Branch, Ottawa. 24 pp. (Revised contribution 1982-8E).
3. Aube, C. and W.E. Sackston. 1964. Verticillium wilt of forage legumes in Canada. *Can. J. Plant Sci.* 44:427-432.
4. Graham, J.H., R.N. Peaden and D.W. Evans. 1977. Verticillium wilt of alfalfa found in the United States. *Plant Dis. Rep.* 61:337-340.
5. Kalb, D.W. and R.L. Millar. 1986. Dispersal of *Verticillium albo-atrum* by the fungus gnat (*Bradysia impatiens*). *Plant Dis.* 70:752-753.
6. Martin, R.A. and P. Boswall. 1989. First occurrence report of verticillium wilt of alfalfa on Prince Edward Island. *Can. Plant Dis. Surv.* 69:51.
7. Nicholls, H., C. Richard and J.-G. Martin. 1987. Verticillium wilt of alfalfa in Quebec. *Can. Plant Dis. Surv.* 67:17-20.
8. Richard, C. and H. Nicholls. 1988. Survey of verticillium wilt of alfalfa in Quebec, 1986-87. *Can. Plant Dis. Surv.* 68:72-73.
9. Sheppard, J.W. 1979. Verticillium wilt, a potentially dangerous disease of alfalfa in Canada. *Can. Plant Dis. Surv.* 59:60.

