Occurrence of verticillium wilt of alfalfa in southern Alberta, 1980-86

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Surveys from 1980-86 revealed that verticillium wilt, caused by *Verticillium albo-atrum*, was a widespread and often serious disease of alfalfa in southern Alberta. It was found in 156 (10.1%) of 1537 fields surveyed during this period. The disease occurred most frequently in irrigated alfalfa fields used for hay, pasture and dehydrated products. It was rarely observed in seed and dryland fields. The highest incidence and severity of wilt were seen in the Lethbridge-Taber area. The disease was more extensive and destructive in stands four years of age and older. Although it was positively diagnosed for the first time in Alberta in 1980, alfalfa seed assay data suggest that the disease was present in southern Alberta prior to this date. Many producers plowed down wilt-infected alfalfa crops because of stand debilitation and to minimize the risk of disease spread to nearby fields.

Can. Plant Dis. Surv. 71:1, 21-27, 1991.

Des enquêtes effectuées de 1980 à 1986 ont révélé que la flétrissure verticilienne causée par *Verticillium albo-atrum* était une maladie répandue et souvent grave de la luzerne dans le sud de l'Alberta. On l'a rencontrée dans 156 (10,1 %) des 1 537 luzernières qui ont fait l'objet des enquêtes au cours de cette période. La maladie se rencontre plus fréquemment dans les luzernières irriguées utilisées pour le foin, la paissance et les produits déshydratés. On l'a rarement observée dans les luzernières de semences et en l'absence d'irrigation. La plus forte fréquence et gravité de la maladie a été observée dans la région de Lethbridge-Taber. La maladie était plus étendue et dévastatrice dans les peuplements âgés d'au moins quatre ans. Même si la maladie a été positivement identifiée pour la première fois en Alberta en 1980, les données sur les essais de semence de luzerne donnent à penser qu'elle était présente dans le sud de l'Alberta avant cette époque. De nombreux producteurs ont labouré et enfoui les cultures de luzerne infectées à cause de la détérioration des peuplements et pour minimiser le risque de propagation de la maladie aux luzernières voisines.

Introduction

During the past decade, verticillium wilt (VW), caused by *Verticillium albo-atrum* Reinke & Berth., became a widespread and economically important disease of alfalfa (*Medicago sativa* L.) in several areas of Canada and the United States (2, 7, 10). Although the first records of VW in North America were from Québec (3) and British Columbia (1), the disease was not confirmed in commercial alfalfa fields in Canada until 1977 (7). Because VW represented a potentially serious threat to Alberta's alfalfa industry (1, 18), a preliminary survey of 124 alfalfa fields across the province was carried out in 1979 (11). Although VW was not found, it was concluded that a more comprehensive annual survey was warranted. As a result, systematic field surveys were carried out through all or part of the province between 1980-88. These activities were coordinated by Alberta Agriculture and involved staff from Alberta Agriculture, Agriculture Canada, Alberta Environment and the University of Alberta. This report describes the results of surveys for VW and other diseases of alfalfa in southern Alberta, the most intensive alfalfa-producing area of the province, from 1980-86. Survey reports for 1987-88 have already been published (14, 16).

Materials and methods

Field Surveys. Approximately 1537 alfalfa fields in southern Alberta were surveyed for VW from 1980-86. A quota of fields was established for each Census Division (CD) (Fig. 1) based on the alfalfa hectarage in these areas. Within each CD, alfalfa fields were selected at random for surveying. The presence of distinctive VW symptoms, such as wilting, V-shaped yellow or pinkish-brown sections on leaf tips and twisting and curling of younger leaves, was taken as evidence that the disease was present. Two types of survey procedure were used. In one, surveyors entered each field at a corner, walked 200 paces toward the center, then exited at 90° to the closest edge of the field. On the exit transect, the number of VW-suspect plants seen within one meter on each side of the line was estimated. In the second type, which also was started at one corner of each field, surveyors walked an M-shaped transect of approximately 800 paces, stopping at 10 equally-spaced spots along the way. At each spot, the number of VW-infected plants in a 1 m area was determined. The type of survey procedure used was left

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Accepted for publication September 5, 1990.

to the discretion of individual survey teams. In some instances, the survey procedure was expedited by simply noting the presence or absence of VW-infected plants without estimating disease incidence. If suspect plants were found, samples were taken to a laboratory for confirmation. Surveys were generally carried out in July, August or September, when disease symptoms were the most apparent.

Isolation of *V. albo-atrum*. One cm long pieces were cut from the lower stems of infected plants. These were dipped in 70% ethanol, placed in 1% sodium hypochlorite for three minutes, rinsed in sterile water, split in half longitudinally, and placed onto Czapek's agar amended with 200 ppm of streptomycin or onto Christen's selective medium (4). Isolation plates were incubated at ca. 20°C for 5-7 days in a dark incubator or occasionally under natural or cool-white fluorescent light at room temperature (21-24°C). *V. albo-atrum* was confirmed based on the presence of verticilliate conidiophores with darkened bases on the host tissue, the presence of dark resting mycelium, the absence of microsclerotia and the occurrence of numerous, hyaline, aseptate, ellipsoidal to sub-cylindrical conidia (9).

Pathogenicity Testing. From 1980-82, the pathogenicity of a representative number of *V. albo-atrum* isolates was determined using the root dip technique (19). Alfalfa plants (cv. Anchor) were grown in a greenhouse for at least 8 weeks prior to inoculation. *Verticillium* cultures were grown on Czapek's agar for at least two weeks prior to use. A concentrated conidial suspension (10^6-10^8 condia/mL) was prepared by flooding a 9 cm diameter Petri plate with 10 mL of sterile water, then rubbing the colony surface with a blunt instrument to dislodge the conidia. The trimmed roots of alfalfa seedlings were then swirled and soaked in the conidial suspension for ca. 3 minutes, and the inoculated seeedlings were transplanted into individual pots containing a steam-pasteurized potting medium. The plants were maintained in a greenhouse for 3-5 weeks before examination for wilt symptoms. At least two seedlings were inoculated with each isolate.

Results and discussion

Field Surveys. From 1980-86, 1537 alfalfa fields in southern Alberta were surveyed for VW (Table 1). Of these, 156 (10.1%) had the disease. The majority of surveyed fields were irrigated (1136) and 13.5% of these were infested with VW. Less than 1.0% of the dryland fields examined had the disease. The highest concentration of VW was in CD 2 (Fig. 1). The majority of alfalfa fields in this area were irrigated. Overall, 118 of the 883 irrigated fields (13.4%) surveyed in CD 2 between 1980-86 had the disease. Lesser amounts of VW were found in CD's 1, 3, 5 and 6; none was found in CD 4, a predominantly dryland region.

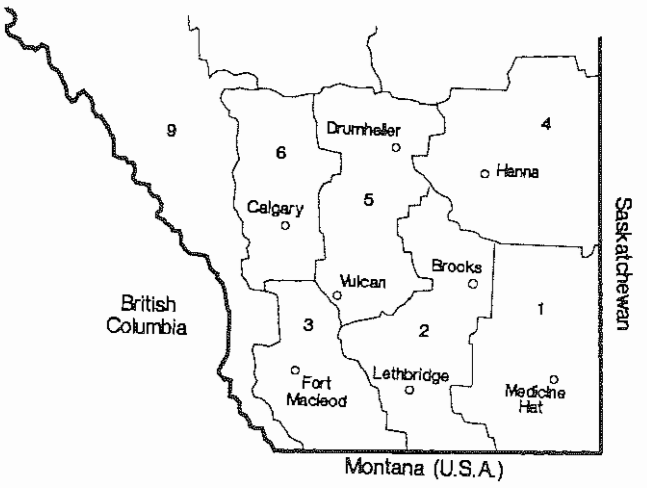

Fig. 1. Census divisions of southern Alberta.

Verticillium wilt in 1980-81. In 1980, 5 of 73 alfalfa fields in southern Alberta were found to have the disease (Table 1). These infested fields (ca. 120 ha) were irrigated and used for hay production. The average disease incidence ranged from a trace to slight (1-5%). The following year, 22 of 114 fields surveyed (ca. 565 ha) had VW, and disease incidence ranged from a trace to very high (1->50%). The distribution of diseased fields in 1981 extended beyond the area where it had been found in 1980 (Fig. 2). A dramatic increase in the number of diseased fields occurred in the Lethbridge area where, among others, eight dairy farms were found to be infested. All of the VW-infested alfalfa fields were irrigated and used for hay, silage, or pasture. This finding coincided with the observations of Christen and Peaden (5) that VW was primarily a problem on irrigated alfalfa.

VW was found in pure stands of alfalfa as well as in alfalfa-grass mixtures during the 1980-81 surveys. No VW was found in fields used for seed production or dehydrated products. The seed used to plant several of the diseased fields was imported from Washington State where VW was known to occur (7). It appeared likely that VW was introduced to southern Alberta on Washington-grown alfalfa seed infested with *V. albo-atrum* based on this circumstantial evidence and the results of previous seed tests (19). Thor appeared to be the most susceptible of several cultivars examined. The majority of *V. albo-atrum* isolates were pathogenic to alfalfa seedlings in greenhouse tests.

Growers on whose farms VW was found were informed of its presence and given control advice. A key point in the disease management strategy was the recommendation that they attempt to eradicate VW by plowing down infested alfalfa crops. In response to this advice, ca. 80% of the 1980 VW-infested hectarage and 47% of the field area infested in 1981 was plowed by the end of November, 1981.

Table 1. Incidence of verticillium wilt in alfalfa fields in southern Alberta, 1980-86.

Year	Census division	No. of irrigated fields		No. of dryland fields		Total fields	
		Surveyed	With VW	Surveyed	With VW	Surveyed	With VW
1980	1	4	0	2	0	6	0
	2	44	5	7	0	51	5
	3	1	0	11	0	12	0
	4	0	0	4	0	4	0
		49	5	24	0	73	5
1981	1	1	1	0	0	1	1
	2	74	21	19	0	93	21
	3	8	0	4	0	12	0
	4	3	0	5	0	8	0
		86	22	28	0	114	22
1982	1	56	6	17	0	73	6
	2	639	58	39	1	678	59
	3	31	5	50	0	81	5
	4	1	0	27	0	28	0
	5	64	2	36	0	100	2
	6	0	0	78	0	78	0
		791	71	247	1	1038	72
1983	1	5	0	0	0	5	0
	2	32	9	2	0	34	9
	3	7	1	4	0	11	1
	4	1	0	4	0	5	0
	5	7	2	2	0	9	2
	6	4	0	12	0	16	0
		56	12	24	0	80	12
1984	1	4	1	3	0	7	1
	2	37	9	1	0	38	9
	3	7	0	4	0	11	0
	4	0	0	4	0	4	0
	5	5	0	5	0	10	0
	6	4	0	15	0	16	0
		54	10	32	0	86	10
1985	1	6	4	0	0	6	4
	2	16	8	1	0	17	8
	3	8	2	4	0	12	2
	4	1	0	8	0	9	0
	5	3	2	0	0	3	2
	6	1	0	5	2	6	2
		35	16	18	2	53	18
1986	1	8	2	0	0	8	2
	2	41	8	0	0	41	8
	3	7	3	5	0	12	3
	5	9	4	4	0	13	4
	6	0	0	19	0	19	0
		65	17	28	0	93	17
Grand Total		1136	153	401	3	1537	156

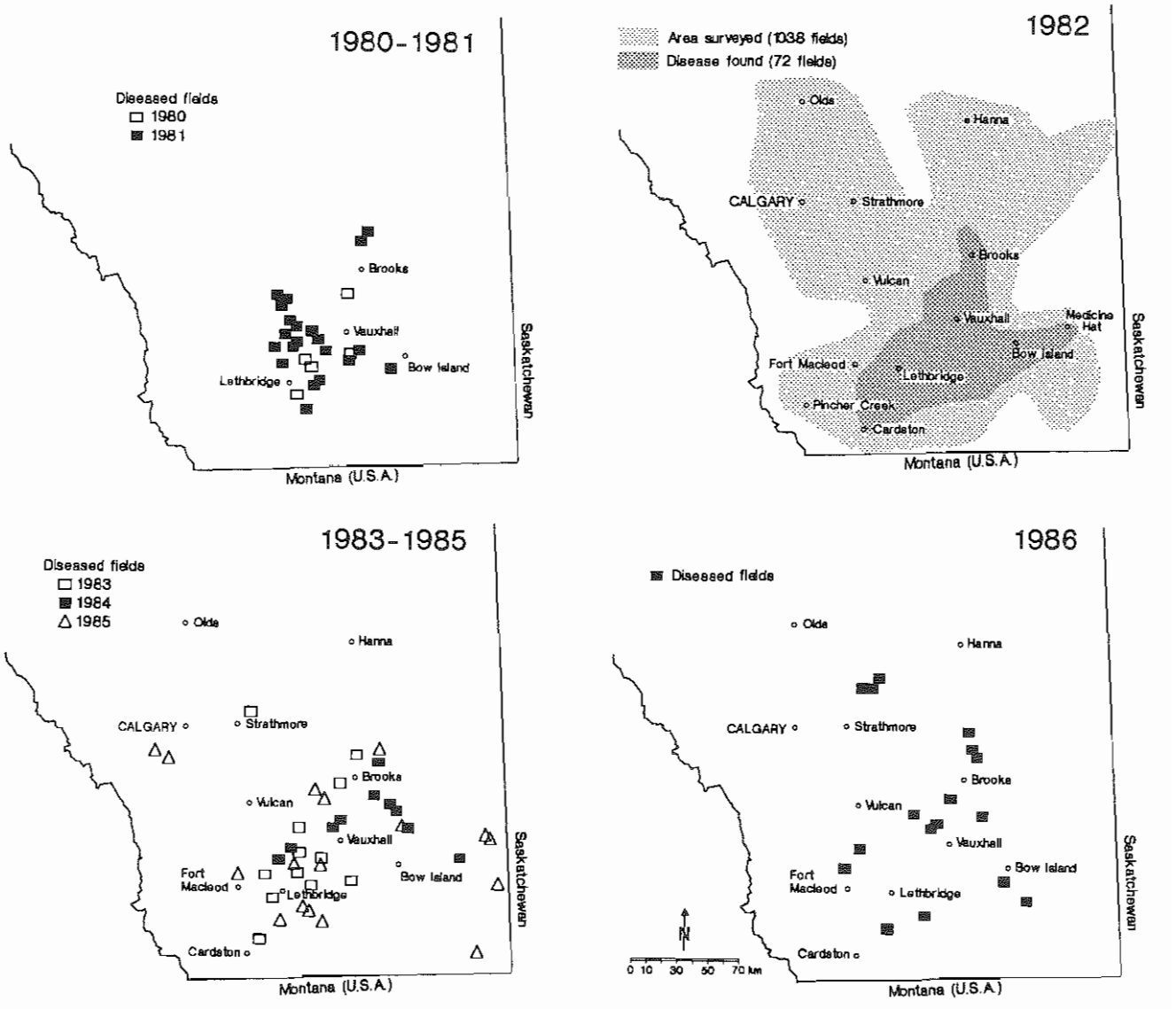

Fig. 2. Distribution of verticillium wilt of alfalfa in southern Alberta from 1980-86.

Verticillium wilt in 1982. The most extensive survey was carried out in 1982 when 1038 fields in southern Alberta were examined (Table 1). The disease was most concentrated in the irrigated districts in CD 2 (Fig. 2), especially in the County of Lethbridge where 41.6% of the fields and 42.5% of the hectarage examined had VW. The disease was largely restricted to fields of irrigated alfalfa grown for hay and dehydrated products. Disease incidence typically ranged from a trace to high (1-35%), but several fields were devastated by the disease (>50% of the plants infected). Only 1 of 247 dryland fields surveyed was infested. No VW was found in any seed fields. All *V. albo-atrum* isolates obtained during the 1982 survey produced typical wilt symptoms when inoculated onto alfalfa seedlings.

Twelve commercial alfalfa cultivars were identified amongst the fields that had VW in 1982 (Table 2). Thor was a constituent, in whole or in part, of at least 21 (29%) of the 72 VW-positive fields. This widely grown cultivar was a favourite amongst hay and dehy producers because of its rapid regrowth after cutting. This characteristic often permitted three cuts per season. The disease was found in stands aged 1 to 25 years, averaging 6.3 years over the 67 infested fields where such data were available. The relatively old age of many of these fields raised the possibility that VW may have been present at low levels for several years or had otherwise gone undetected by the producers until it was identified during this survey.

Table 2. Cultivars and age of stands in alfalfa fields with verticillium wilt in southern Alberta, 1982.

Cultivar*	No. fields with VW	Average age of stands (yr)	Range of stand ages (yr)
Anchor	1	4.0	4
Beaver	8	7.5	4-12
Canada #1	5	3.6	1-5
Chimo	1	10.0	10
Gemini	1	5.0	5
Ladak	1	5.0	5
Thor	10	3.6	2-5
Vernal	6	5.8	4-10
919 Brand	4	6.3	5-8
Mixtures	14	5.4	3-15
Unknown	21	8.6	3-25
	72 avg. =	6.3	

* The nine named cultivars were pure stands. Mixtures: Anchor (1), Beaver (3), Canada #1 (2), Grimm (1), Ladak (1), Rambler (1), Thor (11), Trek (1), Vernal (2), and 919 Brand (5); figures in parenthesis represent the number of fields containing that cultivar where mixed stands occurred. Unknown: producers had no record of the cultivar(s) planted.

Machinery usage may have been a factor in the spread of VW in southern Alberta. At least 20 of the 72 producers with VW-positive fields reported that machinery other than their own was used to harvest their alfalfa crops (Table 3). Significant amounts of diseased alfalfa debris were seen adhering to swathers, hay conditioners, balers and bale wagons harvesting infested fields in 1982. Although no firm conclusions could be drawn concerning the extent to which machinery spread of VW had occurred in southern Alberta, research in Washington State (6) had shown that *V. albo-atrum* infected plant debris spread onto an alfalfa field immediately after cutting could initiate VW, as could cutting a healthy stand with an infested mower.

Table 3. Machinery use patterns on farms with verticillium wilt of alfalfa in southern Alberta, 1982.

Use pattern	No. fields
Used own machinery	40
Used own machinery and occasionally cut fields for relatives or neighbors	9
Used own machinery and occasionally hired a custom operator to cut hay	12
Hired a custom operator to cut hay	8
Unknown	3
	72

Although two cuts per season was the most common practice amongst alfalfa producers in southern Alberta, some took a third cut if sufficient regrowth occurred after the second harvest. VW was found in fields subjected to both two and three cuts/season (Table 4); however, a greater proportion of the two-cut fields were infested. Although most producers claimed that the alfalfa was used on their own farms, a significant number sold all or part of their crop to cattle feeders, dairies or dehydration plants. Hence, the movement of infested hay may have served to spread VW to locations where the disease was not previously present. Over 80% of the alfalfa fields with VW in 1982 were hay crops, with the remainder being used for pasture or dehydration.

Thirty-one (43%) of the VW-infested fields identified in 1982 were plowed, in whole or in part, by the fall in an effort to reduce the risk of disease spread (Table 5). An additional 18 fields (25%) were scheduled to be taken out of alfalfa production by fall, 1983. The remainder, most of which had a light incidence of the disease, were designated to be rotated out of alfalfa within the following 3-5 years.

Other alfalfa diseases encountered during the 1982 survey were alfalfa mosaic [Alfalfa Mosaic Virus], anthracnose [*Colletotrichum destructivum* O'Gara], blackstem and leaf spot [*Phoma medicaginis* Malbr. & Roum.], crown and root rot [*Fusarium* spp., *Pythium* spp. and bacteria], downy mildew [*Peronospora trifoliorum* de Bary], yellow leaf blotch [*Leptotrichila medicaginis* (Fckl.) Schuepp.] and miscellaneous abiotic disorders. Crown and root rot, incited mainly by species of *Pythium* and *Fusarium* (17), was the most destructive of these diseases and it occurred in nearly every field over two years of age. In many cases, it was even more destructive than VW and reduced stand productivity to the point where it was no longer economical to maintain some fields. Crown rot was prevalent in both irrigated and dryland fields and seemed to be worse in stands that were under drought stress, too frequently cut, or suffering from winter injury.

Verticillium wilt in 1983. VW was the most serious in irrigated hay fields in the Lethbridge-Taber area (Table 1; Fig. 2). It was confirmed for the first time in three alfalfa seed fields in CD 2, one near Vauxhall and two near Brooks, but disease incidence was very low (<1%). A new northern limit for VW was established with the confirmation of one lightly infested field near Strathmore (Fig. 2).

Verticillium wilt in 1984. VW remained confined to irrigated alfalfa fields used for hay and dehydrated products, and it continued to be the most widespread in the Lethbridge-Taber area (Table 1; Fig. 2). Disease incidence ranged from a trace to high (1-30%).

Verticillium wilt in 1985. Fifty-three fields were surveyed and VW was found in 18 (34%) (Table 1; Fig. 2). Sixteen of these fields were irrigated and two were dryland. The dryland fields were located in CD 6, southwest of Calgary. These locations represented the most westerly distribution ever noted for VW in southern Alberta. VW occurred in dryland fields at trace levels (<1%), while in irrigated fields the incidence ranged from 1-25%.

Table 4. Cuts per season, disposition and end use of alfalfa crops on farms with verticillium wilt in southern Alberta, 1982.

Cuts per season		Disposition of crop		End use of crop	
Type	No. fields	Type	No. fields	Type	No. fields
Two cuts	43	Own use	42	Hay	57
	16	Sold	13	Pasture	3
Three cuts	8	Own use and sold	13	Pasture & hay	4
				Dehydration	4
Undetermined	21	Undetermined	4	Dehy & hay	4
	72		72		72

Verticillium wilt in 1986. Ninety-three fields were surveyed and VW was found in 17 (18.3%) (Table 1; Fig. 2). All of the infested fields were irrigated. Three were located 33 km northeast of Strathmore, which represented the most northerly occurrence of the disease to that date. Between 1980-86, over 600 alfalfa fields in central and northern Alberta also were surveyed for VW by staff of various agencies, and none was found (I.R. Evans, Alberta Agriculture, personal communication). An isolated outbreak of VW occurred in research plots at the University of Alberta, Edmonton, in 1987, although it was likely that the disease had become established one or two years earlier (J.P. Tewari, University of Alberta, personal communication). The disease had been eradicated from this location by 1988.

Table 5. Status of alfalfa fields with verticillium wilt in southern Alberta, 1982.

Status	No. fields
Plowed by fall '82	28
Plowed worst areas of field by fall '82	3
Anticipate plowing by spring '83	6
Anticipate plowing by fall '83	12
Keeping crop	16
Unknown	7
	72

Conclusions

During the period 1980-86, VW became a major new disease of alfalfa in the irrigated region of southern Alberta, and it was often destructive enough to cause fields to be taken out of production. It is certain that the disease was established in Alberta prior to 1980 because Sheppard and Needham (19) confirmed the alfalfa strain of *V. albo-atrum* in 2 of 90 lots of alfalfa seed from the crop of 1978. The infested lots orginated from fields in the Brooks area. Efforts to confirm the presence of VW in the source fields in 1979 were unsuccessful (13) and it was concluded that the infected plants had died or were so few as to escape visual detection.

Although it appears that VW was introduced into southern Alberta on infested seed, it seems likely that the disease was spread locally mainly by machinery, infested plant material, insects and livestock (8, 12, 15). Considering the many means by which dissemination can occur, it is probable that VW will continue to spread northward in Alberta. The destruction caused by the disease in alfalfa plots at the University of Alberta in 1987 proved that the disease has the potential to develop in central Alberta. The preference of the pathogen for moist, cool climatic conditions may mean that many of the alfalfa-producing areas of central and northern Alberta are at risk from VW. The fact that it has not yet been detected in commercial fields in these areas may be due to less intensive production practices relative to southern Alberta and to subtle differences in environmental conditions that influence the survival, spread and/or pathogenicity of *V. albo-atrum*.

Acknowledgements

The authors are indebted to District Agriculturists and Agricultural Fieldmen in southern Alberta for their assistance in locating alfalfa fields for survey. The help received from Ms. C. Mueller, Ms. M. Schuler, Ms. P. Hawkings, Ms. K. Mitchell, Mr. D. Curry, Mr. B. Fujimoto, Ms. J. Hamilton, Ms. D. Schindler, Ms. J. Giles and Ms. S. Helmer in collecting data is also appreciated.

Literature cited

1. Atkinson, T.G. 1981. Verticillium wilt of alfalfa: challenge and opportunity. Can. J. Plant Pathol. 3:266-272.
2. Arny, D.C. and C.R. Grau. 1985. Importance of verticillium wilt of alfalfa in North America. Can. J. Plant Pathol. 7:187-190.
3. Aube, C. and W.E. Sackston. 1964. Verticillium wilt of forage legumes in Canada. Can. J. Plant Sci. 44:427-432.
4. Christen, A.A. 1982. A selective medium for isolating *Verticillium albo-atrum* from soil. Phytopathology 72:47-49.
5. Christen, A.A. and R.N. Peaden. 1981. Verticillium wilt in alfalfa. Plant Dis. 65:319-321.

6. Christen, A.A. and R.N. Peaden. 1982. Relative importance of sources of verticillium wilt infestation in alfalfa. Phytopathology 72:960 (Abstr.).
7. 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.
8. Harper, A.M. and H.C. Huang. 1984. Contamination of insects by the plant pathogen *Verticillium albo-atrum* in an alfalfa field. Environ. Entomol. 81:1799-1802.
9. Hawksworth, D.L. and P.W. Talboys. 1970. *Verticillium albo-atrum*. Descriptions of Pathogenic Fungi and Bacteria, No. 255. Commonw. Mycol. Inst., Kew, Surrey, England. 2pp.
10. Heale, J.B. 1985. Verticillium wilt of alfalfa, background and current research. Can. J. Plant Pathol. 7:191-198.
11. Horricks, J.S. 1980. Plant disease surveys: forage crops. Pg. 68 *in* 1979 Annual Report, Plant Industry Division, Alberta Agriculture, Edmonton.
12. Howard, R.J. 1985. Local and long-distance spread of *Verticillium* species causing wilt of alfalfa. Can. J. Plant Pathol. 7:199-202.
13. Howard, R.J. and E.R. Moskaluk. 1982. Verticillium wilt of alfalfa in southern Alberta. Can. J. Plant Pathol. 4:306 (Abstr).
14. Howard, R.J. and E.R. Moskaluk. 1989. Survey for verticillium wilt of alfalfa. Can. Plant Dis. Surv. 69:48.
15. Huang, H.C., R. Hironaka and R.J. Howard. 1986. Survival of *Verticillium albo-atrum* in alfalfa tissue buried in manure or fed to sheep. Plant Dis. 70:218-221.
16. Huang, H.C., L.M. Phillippe, R.J. Howard and E.R. Moskaluk. 1988. Survey of verticillium wilt of alfalfa in southern Alberta. Can. Plant Dis. Surv. 68:63-64.
17. Hwang, S.F., R.J. Howard and E.R. Moskaluk. 1989. Crown and root rot of alfalfa in southern Alberta. Can. Plant Dis. Surv. 69:9-11.
18. Sheppard, J.W. 1979. Verticillium wilt, a potentially dangerous disease of alfalfa in Canada. Can. Plant Dis. Surv. 59:60.
19. Sheppard, J.W. and S.N. Needham. 1980. Verticillium wilt of alfalfa in Canada: Occurrence of seed-borne inoculum. Can. J. Plant Pathol. 2:159-162.