Survey of eastern Ontario alfalfa fields to determine common fungal diseases and predominant soil-borne species of *Pythium* and *Fusarium*[1]

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One hundred alfalfa ***Medicago sativa)*** fields in **12** counties of eastern Ontario were surveyed in **1981.** Based on symptoms and isolations from host tissues and soil samples, the following pathogens were detected. ***Phoma medicaginis*** (spring black stem), ***Colletotrichum trifolii*** (anthracnose), ***Phytophthora megasperma*** f. sp. ***medicaginis*** (root rot), and ***Sclerotinia trifoliorum*** (sclerotinia crown and stem rot) were found in **19, 10,** 9, and **1** fields, respectively. ***Pseudopezize mediceginis*** (common leaf spot) and ***Stemphylium botryosum*** (Stemphylium leaf spot) were present in most fields while ***Asronospora trifoliorum*** (downy mildew) occurred sporadically. ***Verticilliumalbo-atrum*** (wilt) was not found. ***Pythium irregulare, Fusarium oxysporum*** and ***F. equiseti*** were widely distributed in soil, but their population densities seemed to bear no significant relationship to fields visually rated as being in good, fair or poor condition; however, in laboratory tests, certain isolates of each of the latter three fungi were pathogenic to alfalfa seedlings.

Can. Plant Dis. Surv. 63:2, 5 1-54, 1983.

Cent champs de luzerne ***Medicago sativa)*** dans **12** comtés de l'est de l'Ontario furent inventories en **1981.** En **se basant** sur **les** symptômes **et les** isolations faites à partir des tissues des plantes hates et des échantillons de sol, **les** pathogènes suivants ont **été détectés.** ***Phoma medicaginis*** (tige noire printanière), ***Colletotrichumtrifolii*** (anthracnose), ***Phytophthora megasperma*** f. **sp.** ***mediceginis*** (pourridié phytophthoréen) et ***Sclerotinia trifoliorum*** (flétrissure sclérotique) ont **été** identifies respectivement dans **19, 10,** 9 **et 1** champs. ***Pseudopeziza medicaginis*** (tache commune) **et** ***Stemphylium botryosum*** (tache stemphylienne) **étaient** presents dans la plupart des champs tandis que ***Asronospora trifoliorum*** (mildiou) ne l'était que sporadiquement. ***Verticillium albo-atrum*** (flétrissure verticillienne) n'a pas **été** isolé. ***Pythium irregulare, Fusarium oxysporum*** et ***F. equiseti*** **sont** largement distribués dans **le** sol **mais il** ne semble pas exister une relation significative entre leurs **densités** de population et **les** champs visuellement classes bons, passables ou pauvres.

Introduction

In conjunction with recent surveys for Verticillium wilt (***Verticillium albo-atrum*** Rienke & Berth.) of alfalfa (***Medicago sativa*** L.) across Canada (2), an effort was made to obtain information on the occurrence of other common fungal diseases (9) and soil-borne pathogens (7, 8) in eastern Ontario. Although winterkill is believed to be one of the major factors for alfalfa decline (4), the importance of root and crown rot pathogens, such as, species of ***Phytophthora, Pythium, Fusarium. Aphanornyces,*** and other fungi is well documented (6, 7, 10, 12, 14, 15, 17). The incidence of foliage diseases (3, 5) also cannot be ignored.

Materials and methods

Based on the production acreage of alfalfa and its mixtures (1), 100 alfalfa fields were chosen in a stratified-random manner (18) from the 12 counties of eastern Ontario (Fig. 1). Plants, within a **2** m wide W-shape path (3) in each field, were examined for disease symptoms (9) and pathogens were isolated from host tissues to confirm diagnosis when required. Soil samples (500g) near plant roots were taken from each of 10 approximately equally spaced sites along the path; these were bulked, mixed, sieved, and tested for the presence of ***Phytophthora, Pythium.*** and ***Fusarium*** species using selective media (13, 17, 19). ***Phytophothora megasperma*** Drechs. f. sp. ***mediceginis*** Kuan & Erwin (11) was detected by the alfalfa seedling baiting method (13); species of ***Pythium*** and ***Fusarium*** were isolated from dilution plates as described by Singh and Mitchell (17), and Wensley and McKeen (19), respectively. The pathogenicity of representative isolates was tested by the inoculum layer technique (16), using **40** alfalfa seedlings per treatment in duplicate pots. The names of alfalfa cultivars and field size were noted, and the condition of each field was rated visually as good, fair or poor, depending upon the crop growth and stand. Fields were visited a few days before the first, second or third cut. Paired *t* tests (18) were performed to determine if there were significant differences in the population densities of the fungi in good, fair, and poor fields.

Results and discussion

Since the major emphasis in this survey was to detect Verticillium wilt, plants showing wilting, yellowing or stunting were collected and cultured to isolate pathogens. None of the 64 plants with these symptoms gave any evidence of *V.*

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Accepted for publication May 3, 1983

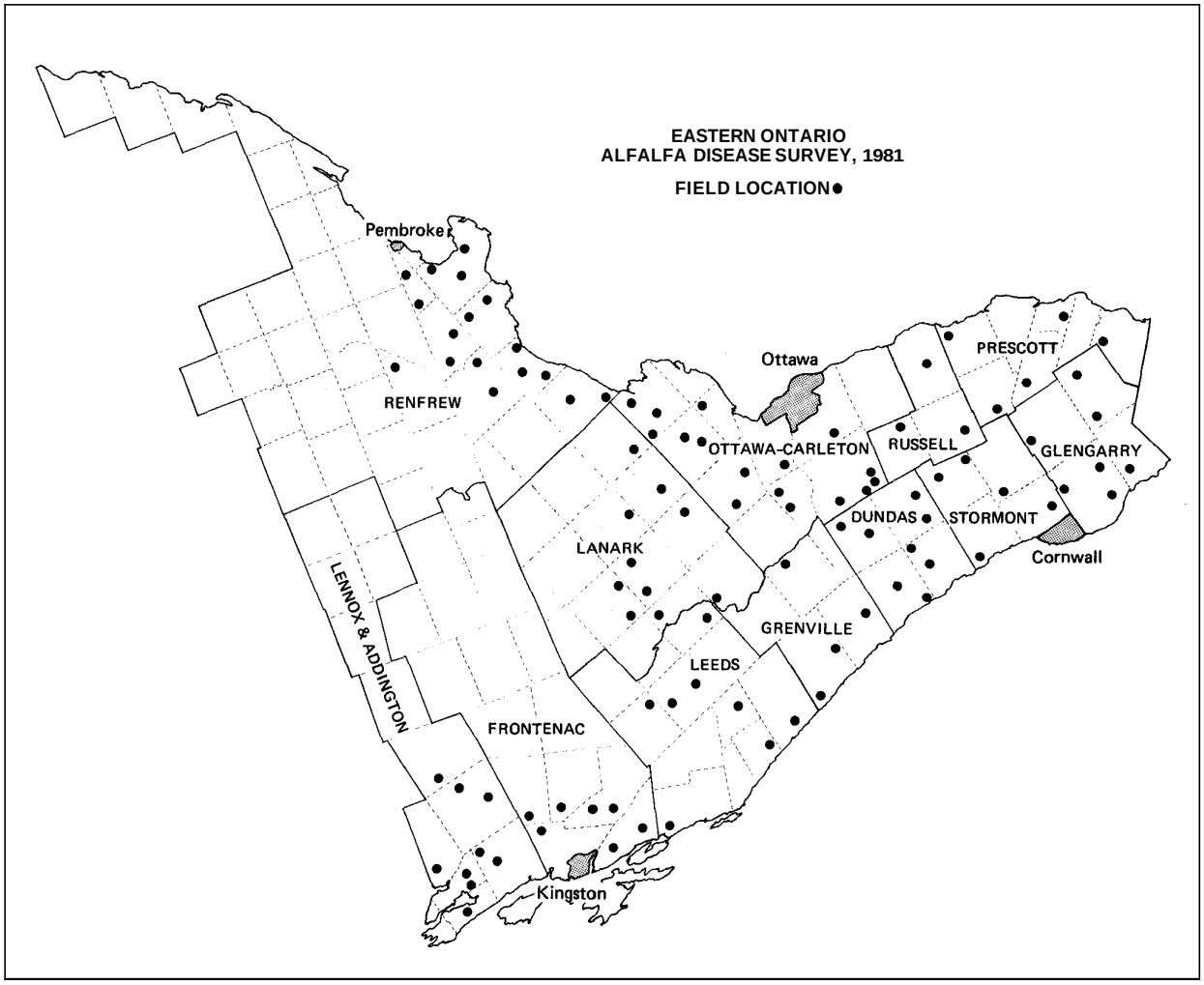

Figure 1. Showing approximate locations of alfalfa fields surveyed in eastern Ontario.

albo-atrum being present, indicating that the disease has not yet reached a detectable level in this area. Laboratory experiments at Ottawa showed that this pathogen can remain viable in alfalfa stems for at least 6 months through a temperature range from -5" to 35°C. and this is comparable to local outdoor temperatures (unpublished). Therefore, all suggested precautions (2) should be taken to prevent its introduction to this area through infected alfalfa stems.

It was noteworthy that a number of the above 64 samples yielded the spring black stem and anthacnose pathogens, *Phoma medicaginis* Malbr. & Roum var. *medicaginis* Boerema and *Colletotrichum trifolii* Bain; these two diseases were found in 19 and 10 fields, respectively. Common leaf spot (*Pseudopeziza mediceginis* (Lib.) Sacc.) and Stemphylium leaf spot (*Stemphylium botryosum* Wallr.) were found in most fields while downy mildew (*Peronospora trifoliorum* de By.) occurred sporadically as observed in previous studies (3). Sclerotinia crown and stem rot (*Sclerotinia trifoliorum* Erikss.) was encountered in one field. *P. megasperma* f. sp. *medicaginis* was detected in soil samples from 9 fields that appeared in good condition with no distinct root rot patches evident.

Pythium irregulare Buisman, *Fusarium oxysporum* Schlecht, and *F. equiseti* (Corda) Sacc. were widely distributed in eastern Ontario alfalfa fields but their population densities varied greatly from field to field within each county as indicated by large standard error values (Table 1). There were 3 poor, 17 fair and 80 good fields. The population densities of each of these fungi (*P. irregulare, F. oxysporum* and *F. equiseti*) in poor and fair fields compared with 20 randomly chosen good fields did not differ significantly, as determined by paired *t* tests. Thus visual rating of fields as good, fair or poor showed no relationship with the population of fungi obtained. However, certain isolates of each of the three species were pathogenic to alfalfa seedling in laboratory tests (Table 2). Isolates of *P. irregulare* from Prescott, Leeds and Frontenac counties were virulent but those from other counties were not. Similarly, isolates of *F. oxysporum* also varied in virulence.

Table 1. Mean number of propagules of P. ***irregulare, F. oxysporum*** and ***F. equiseti*** per gram of soil from alfalfa fields in twelve counties of eastern Ontario.

County	No. of fields	P. *irregulare*	*F. oxysporum*	*F. equiseti*
Dundas	8	62.5 (13.9)"	1100.0 (280.3)	850.0 (219.6)
Frontenac	8	160.0 (40.5)	750.0 (244.2)	200.0 (151.2)
Glengarry	7	205.7 (49.1)	400.0 (174.6)	171.4 (119.0)
Grenville	4	65.0 (25.0)	900.0 (640.3)	100.0 (100.0)
Lanark	10	90.0 (23.3)	760.0 (300.8)	200.0 (89.3)
Leeds	8	192.5 (41.2)	800.0 (239.0)	0.0
Lennox & Addington	9	195.5 (37.5)	1688.9 (273.1)	133.3 (94.3)
Ottawa-car leton	16	265.0 (44.1)	900.0 (226.6)	350.0 (162.8)
Prescott	5	96.0 (38.7)	400.0 (219.1)	220.0 (80.0)
Renfrew	17	177.6 (19.5)	988.2 (191.3)	352.9 (136.7)
Russel	3	153.3 (6.7)	0.0	266.2 (133.3)
Stormont	5	304.0 (50.4)	1840.0 (449.0)	640.0 (240.0)

'Standard error in parenthesis

Table 2. Range of virulence* of representative isolates of ***Pythium irregulare, Fusarium oxysporum*** and ***F. equiseti*** from 10 countries of eastern Ontario.

County	*P. irregulare*	*F. oxysporum*	*F. equiseti*
Dundas	0.0	2.6	5.0
Frontenac	29.2	17.5	0.0
Grenville	0.0	5.6	0.0
Lanark	0.0	20.0	0.0
Leeds	50.0	17.1	0.0†
Lennox & Addington	0.0	0.0	0.0
Ottawa-Carleton	3.0	34.5	0.0
Prescott	68.2	5.7	0.0
Renfrew	0.0	0.0	7.5
Stormont	2.1	2.8	0.0

*Based on percentage of alfalfa seedlings showing brownish discoloration or lesions on roots.
† This isolate was from Glengarry to replace Leeds where *F. equiseti* was not found.

Most of the isolates of ***F. equiseti*** were non-pathogenic; only two from Dundas and Renfrew counties caused discoloration of alfalfa roots. It was clear, that different isolates of the same fungus varied in virulence; and this suggests the possible existence of races which cannot be verified until constancy of virulence can be demonstrated on specific alfalfa cultivars.

The various alfalfa cultivars encountered in this survey (such as, Saranac, Vernal, Iroquois, Pioneer, Anchor, Thor, Vista and others) were found to be representative of the common cultivars grown in Ontario. The incidence of diseases, however, did not appear to be restricted to specific cultivars or areas visited. The total field area surveyed was 650.8 ha representing about 0.6% of the production acreage of alfalfa and its mixtures (1).

Acknowledgements

The author wishes to thank Drs. J. D. S. Barr, G. A. Neish and K. Egger of the Biosystematics Research Institute, Agriculture Canada, Ottawa, for fungi identification, and N. J. Brown for his excellent technical assistance.

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