Fungi recovered from diseased roots and crowns of alfalfa in north central Alberta and the relationship between disease severity and soil nutrient levels

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A survey of alfalfa fields in north central Alberta was undertaken in 1980-81 in order to determine which soilborne pathogens were most prevalent. Fields with "alfalfa sickness" symptoms were generally luvisolic and sulfur-deficient. Root and crown damage tended to be more severe on sulfur-deficient soils. Cylindrocarpon gracile and Fusarium *roseum* were the fungi recovered most frequently from diseased tissue. Soil type, sampling date, and symptom type all affected frequency of recovery of fungal genera. Symptoms of Plenodomus *meliloti* were frequently observed but the fungus was only rarely recovered. Phytophrhora megasperma var. megasperma was not recovered from "alfalfa sickness" affected plants or field soils. There appeared to be a relationship between disease severity and the concentration of soil nutrients.

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En 1980-81 un inventaire des champs de luzerne dans le nord de la region centrale de l'Alberta fut entrepris afin d'y determiner l'identite des principaux pathogenes du sol. Les champs presentant des symptômes de «maladie de la luzerne» etaient generalement luvisoliques et pauvres en soufre. Les dommages aux racines et au collet tendaient à être plus severes dans les sols pauvres en soufre. Cylindrocarpon gracile et *Fusarium* roseum, deux champignons, ont ete isolés le plus frequemment à partir de tissus malades. Le type de sol, la date d'ehantillonnage et le type de symptômes ont influence la frequence d'isolation des differents genres de champignons. Les symptômes causes par Plenodomus meliloti ont souvent ete observes mais celui-ci a rarement été isolé. Phytophthora megasperma var. megasperma n'a pas ete isolé à partir de plants presentant des symptômes de amaladie de la luzerne» ni à partir des échantillons de sol. Il semble exister une relation entre la severite de la maladie et la concentration en elements nutritifs du sol.

Introduction

Poor growth of alfalfa in north central Alberta, characterized by yellowing, stunted plants, has been referred to as "alfalfa sickness" (17). Several attempts have been made to identify the cause of this problem and, most recently, *Phytophthora megasperma* has been implicated (3). Other researchers have presented conflicting results (6, 13) and further evaluation of the problem appeared necessary. In this regard, a disease survey was carried out in 1980-81 in order to further assess the prevalence of various root and crown pathogens in north central Alberta alfalfa fields. This report summarizes the results of that survey.

Materials and methods

Alfalfa fields were selected randomly from lists of contract fields obtained from alfalfa dehydration plants. Fifty-five fields were sampled between May 5 and June 4, 1980. The fields were located within the counties of Lac Ste. Anne, Barrhead, Smoky Lake and Athabasca, and the Municipal District of Sturgeon. Within each field, four sites representative of the terrain were selected and several plants (4-10) were collected from each site. Usually, plants were dug in such a way that a block of soil containing the top 6-12 inches of the taproot was removed and placed in a plastic bag. This insured that most lateral and tertiary roots would be retained. Fields were classified as either "alfalfa sick" or "healthy" on the basis of the presence or absence of characteristic symptoms (17). Samples were kept cool until examination and, generally, diseased tissue was selected and plated out within 48 hr of collection.

Root and crown damage was assessed by dividing the symptoms into several groups, each with its own severity scale (Table 1, Figs. 11 - 14). Tissue from each type of symptom was then surface sterilized in 1% NaOCl for 3 min. or 30 sec., rinsed 3X in sterile distilled H_2O, and blotted dry before plating. Most isolations were made on acidified potato dextrose agar (PDA), acidified Malt agar, and PDA amended with 300 ppm streptomycin. Corn meal agar, amended with 200 ppm vancomycin and 5 ppm penicillin, and VYS-PBNC agar (12) were used to a lesser extent. Plates were incubated in the dark at 2° or 15°C for up to 2 weeks and examined regularly for the appearance of fungal colonies. Cultures were retained for identification.

A selected group of the fields examined in May was sampled again in July and November, 1980, and in March, 1981. The same procedure was followed except that for the latter two sampling dates plants were not rated for disease severity.

Samples of root tissue from plants collected in July were placed in a Baerman funnel apparatus (14). After 24 hr of incubation, suspensions were examined for the presence of nematodes possessing stylets (7). Soil samples collected in July, 1980, were analyzed by the Provincial Soil and Feed Testing Laboratory, Alberta Agriculture. Linear regression equations were derived to show relationships between root disease severities and levels of soil minerals. The regressions were examined with the analysis of variance (ANOVA) procedure.

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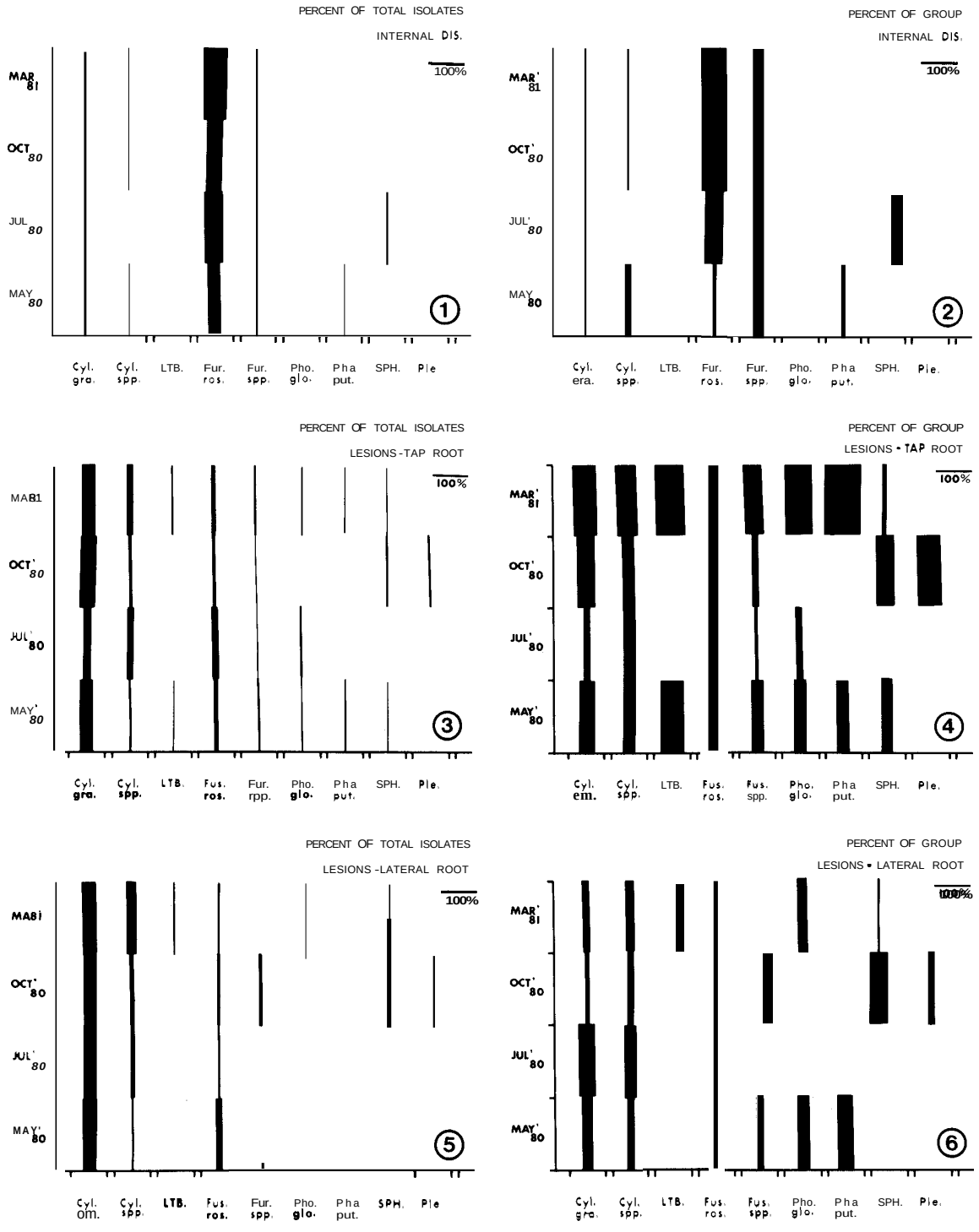


Figs. 1 - 6 Effect of sampling date on recovery of fungi. "Percent of total" indicates percent that each fungal group represents out of all fungal isolates recovered from that symptom type for a given sampling date. "Percent of group" indicates percent of all isolates of a fungal group which were recovered from that symptom type for a given sampling date. Fungal groups: Cyl. gra. = *Cylindrocarpon* gracile, Cyl. spp. = *Cylindrocarpon* spp.. LTB = Low temperature basidomycete, Fus. ros. = *Fusarium roseum*, Fus. spp. = *Fusarium* spp., Pho. glo. = *Phoma* glomerata, Pho. put. = P. *putaminum*, SPH = unidentified Sphaeropsidales, Ple. = *Plenodomus meliloti*.

July soil samples were also subjected to baiting tests designed to detect *Phytophthora megasperma* var. *megasperma* (10).

Results

Several different species of fungi (Table 2) were recovered during this survey but *Cylindrocarpon gracile* Bugn. and *Fusarium roseum* (LK.) emend. Snyder and Hansen were the fungi most frequently isolated from diseased plant tissue. Symptoms of brown root rot *[Plenodomus meliloti* Mark.-Let.] were commonly observed but *P. meliloti* was only rarely recovered from the lesions. Recovery of *P. meliloti* could be improved by plating pycnidia on agar media, however, this was not done routinely. *Phytophthora megasperma* var. *megasperma* was not recovered from diseased plants nor was it detected during the baiting tests. The effect of sampling date on the recovery of fungi from various types of symptoms soil types is shown in Figs. **1-10.** Only the most frequently recovered fungi and others of interest are shown. Incubation temperature and soil type (luvisols vs. chernozems) had little effect on the recovery of most fungi listed in Table **2.** Certain groups, however, were markedly affected. For example, **72.2** percent of the isolates of *Phoma glomerata* were recovered from plants growing in chernozemic soils whereas those recovered from luvisolic soils represented only **27.8** percent. Most *Phoma putaminum* isolates, on the other hand, came from plants growing in luvisolic soils **(90.5** percent).

Symptoms observed were generally similar to those previously described *(2,* 8, **9,** 11). The "vascular streaking" symptom has not been previously described on alfalfa by workers in this area. It typically consists of narrow reddish brown streaks which appear to be located in the vascular tissue of the taproot (Fig. 14). The streaking, which originates in the crown but is not necessarily associated with significant crown damage, may extend down the entire taproot or only partway. Survey results indicated that this symptom was more widespread in M.D. Sturgeon fields (chernozemic soils) than in Lac Ste. Anne county fields (luvisolic soils). The fungi recovered most frequently from tissue with this symptom were *Phoma medicaginis* Malbr. and Roum. **(44%)** and *Fusarium roseum* **(31%).** Similar symptoms have been described elsewhere (4, **16).**

Table 1. Alfalfa root and crown symptom severity scales.

Symptom*	Code	Disease Severity Scale**
Crown Rot	CR	0 - 4
Lateral Root Damage	LR	**0 - 5**
Tap Root Damage	TR	0 - 5
Internal Discolouration	ID	0 - 6
Vascular Streaking		%§

* Discoloured fine roots were plated out but the degree of damage was not assessed.

** "0" indicates the absence of disease and the maximum value indicates that either 100 percent of the tissue was discoloured (CR, LR, TR) or, in the case of ID, that the discolouration extended down the taproot for a distance greater than **12** cm.

§ This symptom was recorded as the percent of plants in the sample which had vascular streaking.

Table **2.** Fungi recovered from roots and crowns of diseased alfalfa plants, **1980-81.**

Fungi Recovered	Percent of Total Isolates	Fungi Recovered	Percent of Total Isolates
Acremonium	**0.2**	*Phoma glomerata*	1.0
Aspergillus	**0.4**	*Phoma putaminum*	1.1
Low Temp. Basidiomycete	**0.9**	*Phytophthora*	**0.2**
Cylindrocarpon gracile	**24.6**	*Pythium*	**0.6**
Cylindrocarpon spp. *	**9.1**	*Rhizoctonia*	**1.3**
Fusarium roseum	**19.7**	*Plenodomus meliloti*	**0.3**
Fusarium spp. **	4.6	*Trichoderma*	*0.5*
Geotrichum	*0.2*	DHB§	**7.5**
Gliocladium	**0.9**	Mucorales	**1.3**
Papulaspora	**0.3**	Sphaeropsidales	**1.8**
Penicillium	**0.3**	Misc. identified	**0.5**
Phialophora	**3.2**	Misc. unidentified	**12.9**
Phoma medicaginis	**6.7**		

* ***Cylindrocarpon*** isolates belonging to species other than C. ***gracile.***

** ***Fusarium*** isolates belonging to species other than ***F. roseum.***

§ Non-sporulating isolates with dark-grey mycelium and compact growth habit.

Table 3. Relationship of root disease severity values to soil nutrient levels.

	Equation	R^2	Age of Stand (Years)
May+	ID§ = 0.798 + 0.082 (P)	0.86""	4 - 6
	ID = 0.027 (K) - 4.250	0.92""	4 - 6
	ID = 1.635 (N) -0.167	0.65"	2 - 3
	TR = 1.576 + 0.071 (P)	0.67 *	4 - 6
	LR = 1.093 + 0.030 (P)	0.77 *	4 - 6
	LR = 0.10 (K) -0.833	0.87 **	4 - 6
	LR = 1.364 + 0.024 (P) - 0.046 (S)	0.94"	4 - 6
July+	LR = 1.480 - 0.095 (S) - 0.079 (N)	0.88"	4 - 6
	LR = 0.110 + 0.055 (N) - 0.001 (K)	0.99 **	1

+ Equations were derived from severity values (Table 1) obtained from plants collected in May or July, 1981.

§ ID = internal discolouration, TR = exterior damage to tap root, LR = exterior damage to lateral root, N = nitrogen (kg/ha), P = phosphorous (kg/ha), K = potassium (kg/ha), S = sulfur (ppm).

* The regression is significant at P = .05 (ANOVA).

"* The regression is significant at P = .01 (ANOVA).

Significant relationships were found to exist between disease severity values and the nutrient status of field soils (Table 3). The results of soil analyses indicated that fields with the symptoms of "alfalfa sickness" had soils that were either deficient in sulfur (0.55-2.10 ppm), acid (pH $<$ 6.0), or both. Fields with such symptoms generally occurred on luvisolic soils.

Disease severity values recorded for plants collected in M. D. Sturgeon fields (chernozemic) tended to be lower than those recorded for plants originating in Lac Ste. Anne Cty. (luvosolic). These differences were usually not statistically significant, however (Table 4).

Nematodes possessing stylets were detected in root samples from most fields surveyed. ***Tylenchus*** sp., found in 10 out of 15 fields, was the genus most frequently recovered. ***faratylenchus*** sp. was observed in samples from 5 out of 15 fields.

Discussion

Previous reports have indicated that ***fhytophthora megasperma*** is the cause of "alfalfa sickness" (3). Only two isolates of ***fhytophthora megasperma*** were recovered during this survey. Both isolates were nonpathogenic to alfalfa seedlings (Reeleder, unpublished data) and appear to belong to ***P. megasperma*** var. ***sojae.*** It is unlikely, therefore, that *P. megasperma* var. ***megasperma*** is the cause of "alfalfa sickness", although it has previously been detected in Alberta (15). In contrast, sulfur deficiency and soil acidity appear to be strongly associated with "alfalfa sickness". Hawn and Kozub (6) concluded that differences between "alfalfa sick" and healthy soils were due to differences in fertility and pH levels. Field experimentation is needed to verify these relationships.

Symptoms of brown root rot ***(flenodomus meliloti)*** were frequently observed during the survey. Most of the damage on tap and lateral roots could be attributed to brown root rot on the basis of the typical symptoms observed (11). ***Cylindrocarpon*** symptoms (2) were observed much less frequently than those of ***flenodomus meliloti*** but ***Cylindrocarpon gracile*** was nevertheless the fungus most often recovered during this survey. Cormack (2) attributed ***Cylindrocarpon*** root rot to ***C. erhenbergi*** **Wr.** but this species was rejected by Booth (1) in his revision of the genus. More work is needed to clarify the relationship of the various ***Cylindrocarpon*** species recovered to ***Cylindrocarpon*** root rot and other root diseases.

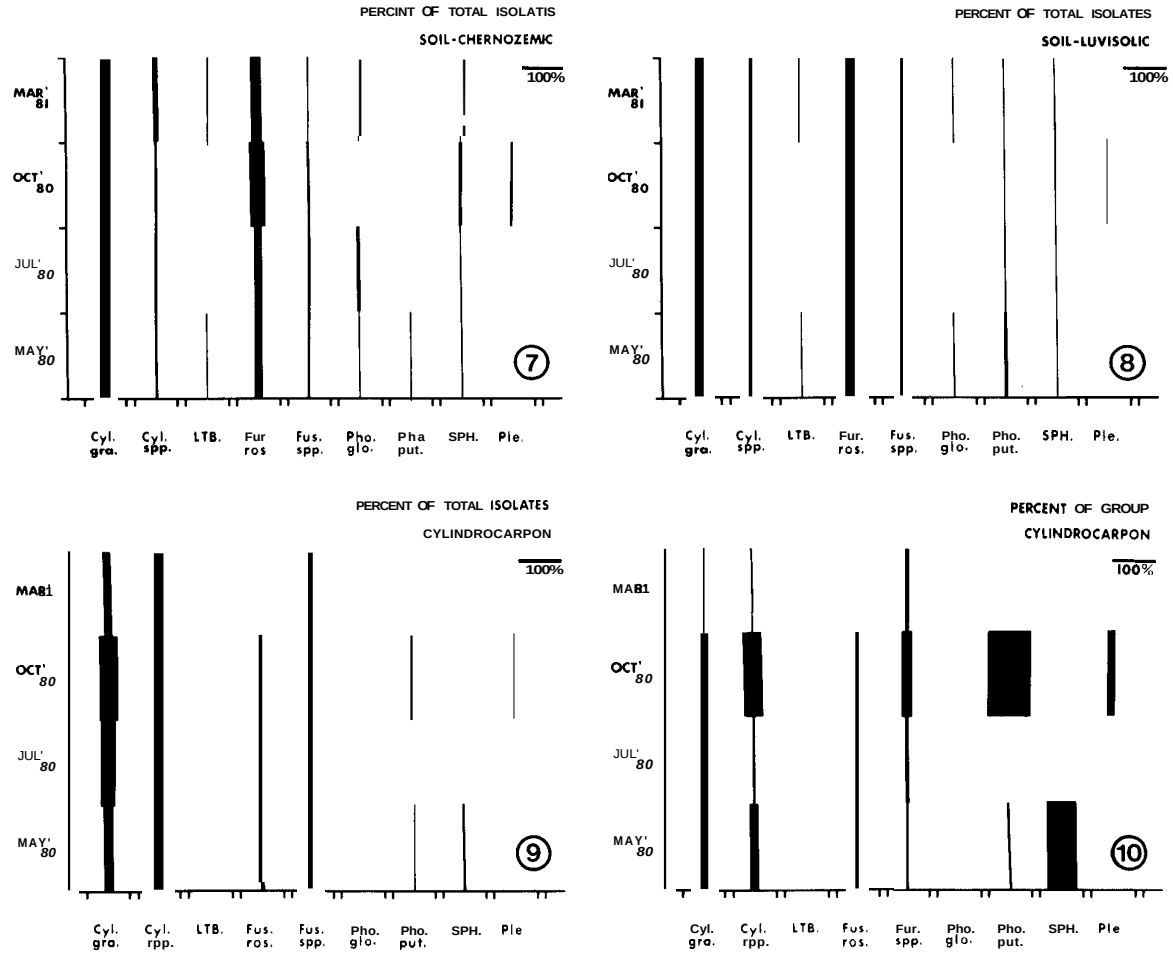
Table 4. Alfalfa root and crown disease severity values and soil sulfur levels in mature stands, May and July, 1980§

Symptom	M. D. Sturgeon		Lac Ste. Anne Cty	
Code"*	May	July	May	July
CR	0.58"	1.79	1.65"	1.88
ID	1.42	1.79	2.88	2.21
TR	1.56	2.55	3.61	3.26
LR	1.34	1.33	1.96	2.03
Sulfur (ppm)	-	7.10	-	1.04

§ Fields were four to six years old. Severity values in newer fields were lower.

* Value for M. D. Sturgeon was significantly different (P = .05) than the value for Lac Ste. Anne Cty. (May).

** See Table 1.



Figs. 7 - 10 Effect of sampling date on recovery of fungi. "Percent of total" indicates percent that each fungal group represents out of all fungal isolates recovered from that symptom type for a given sampling date. "Percent of group" indicates percent of all isolates of a fungal group which were recovered from that symptom type for a given sampling date. Fungal groups: Cyl. gra. = Cylindrocarpon gracile, Cyl. spp. = Cylindrocarpon spp., LTB = Low temperature basidomycete, Fus. ros. = *Fusarium roseum,* Fus. spp. = *Fusarium* spp., Pho. glo. = *Phoma* glomerara, Pho. put. = *P. putaminum,* SPH = unidentified Sphaeropsidales, Ple. = *Plenodomus* ***meliloti.*** Figs. 9 - 10 illustrate fungal recovery from taproots with Cylindrocarpon root rot.

The significance of the presence of ***Tylenchus*** sp. and other plant parasitic nematodes is presently unknown. ***Tylenchus*** has been previously reported from Alberta (5) but little is known about its possible effects upon alfalfa roots. Hawn and Kozub **(6)** concluded that alfalfa was not attacked by ***Paratylenchus projectus*** Jenkins.

Although significant relationships appear to exist between soil nutrient levels and disease severity (Table 3), it should be noted that none of these relationships have as yet been confirmed with independent data. It is of interest, however, that higher concentrations of certain nutrients appear to increase the severity of some symptoms while other nutrients decrease severity. Also note that these relationships appear to vary with the sampling date (Table 3). Further research is required to determine whether soil nutrient levels can affect disease severity and thereby affect stand persistence and/or yield.

Work is presently underway to assess the pathogenicity of fungi recovered during this survey. Techniques to detect ***Medicago*** germplasm resistant to ***P. meliloti*** and ***Cylindrocarpon*** spp. are being developed.

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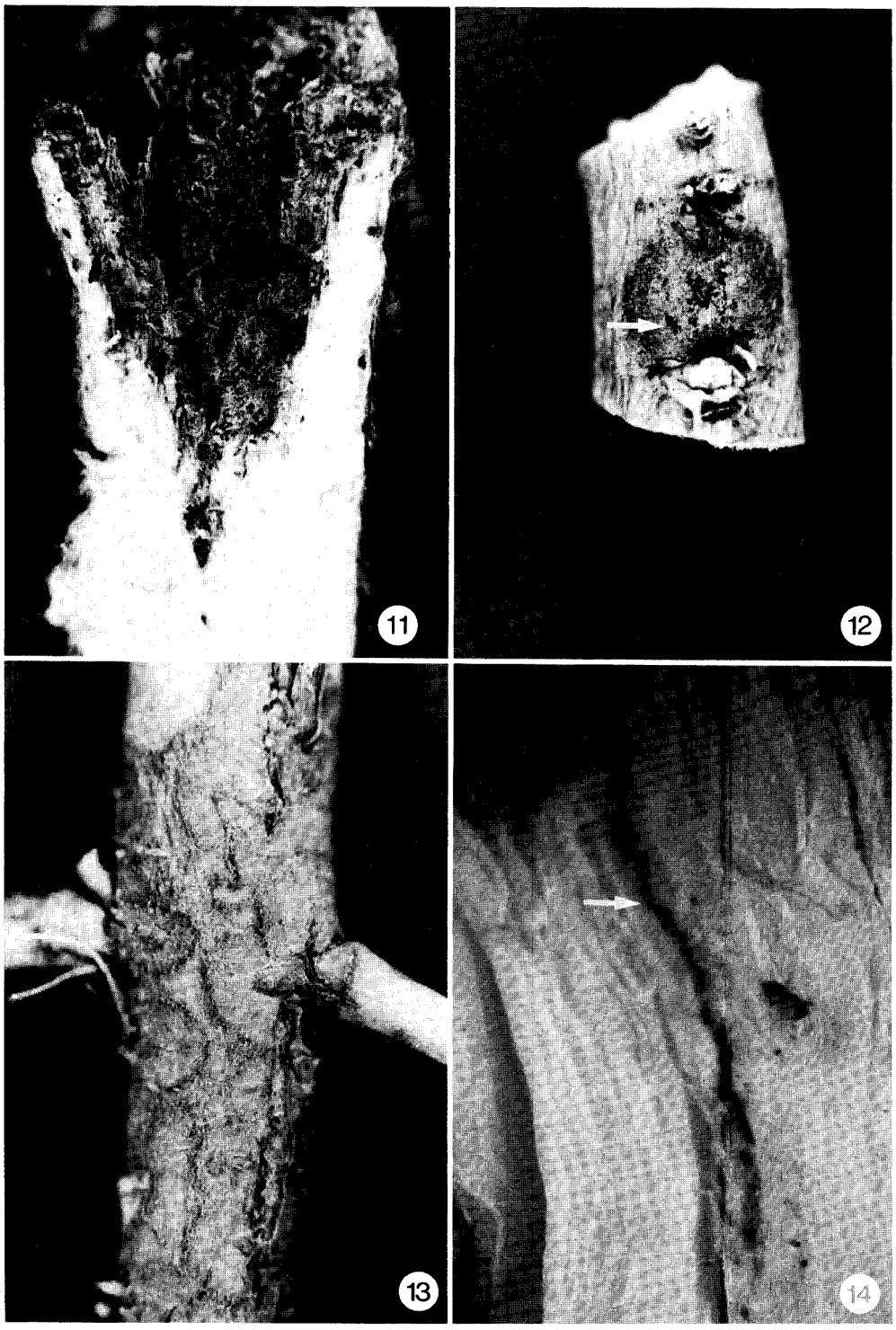

Fig. 11 Internal discolouration (ID), wedge-shaped rot extending from the crown into the taproot.

Fig. 12 Lateral root (LR) damage, caused by ***Plenodomus meliloti.*** Pycnidia are visible on surface of lesion (arrow)

Fig. 13 Taproot (TR) damage, caused by ***Plenodomus meliloti.*** Several lesions have coalesced together.

Fig. 14 Vascular streaking. Reddish-brown discolouration extending from the crown into the taproot.

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