ETIOLOGY OF SEEDLING BLIGHT AND ROOT ROT OF

FABA BEAN (VICIA FABA) IN MANITOBA

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by

Lakhdar Lamari

In Partial Fulfillment of the

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of

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ETIOLOGY OF SEEDLING BLIGHT AND ROOT ROT OF

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BY

LAKHDAR LAMARI

A thesis submitted to the Faculty of Graduate Studies of
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FOREWORD

The materials, methods and results in this thesis are presented in the form of three manuscripts intended for publication in the Canadian Journal of Plant Pathology. The style as well as the preparation of tables and figures comply with the requirements of the journal. A general discussion of the results is included after the manuscripts.

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GENERAL ABSTRACT

Lamari, Lakhdar. M.Sc., The University of Manitoba, March, 1982.

Etiology of Seedling Blight and Root Rot of Faba Beans (Vicia faba)

in Manitoba. Major Professor; C.C. Bernier.

A severe seedling blight and root rot of faba bean (Vicia faba L.)

has been observed since 1978 in experimental plots at the University

of Manitoba (U of M) and in some commercial faba bean fields in Eastern

Manitoba. Aphanomyces euteiches Drechs. was found to be the main agent

of the disease at the U of M Campus farm, where faba bean root rot was

consistently severe. Rhizoctonia solani Kuehn. was isolated from plants

of faba bean and other crops infected at different locations. The

pathogenic isolates of R. solani caused damping-off of faba bean plants

except for one isolate recovered in Eastern Manitoba. The latter

caused a soft rot of the stem and roots and was more aggressive than

any of the other isolates. A Fusarium tentatively identified as F.

avenaceum (Fr.) Sacc. was recovered from mature faba bean plants in

Eastern Manitoba and found to be pathogenic to faba bean and peas in

the greenhouse.

In addition to V. faba, 12 leguminous species were infected by A.

euteiches. The following species were infected and do not appear to have

been previously reported as hosts of this fungus: Lathyrus ochroleucus

Hook., L. sativus L., Lens culinaris Medic., Vicia calcarata Desf.,

V. cracca L., V. disperma D.C., V. narbonensis L. and V. tetrasperma

Schreber. The faba bean type isolate (AE1) differed in pathogenicity

from a Wisconsin pea isolate (P14). The latter was avirulent on faba bean. Three pathotypes were identified among the Manitoba isolates of A. euteiches. Pathotypes AE1, AE2 and AE3 were pathogenic to peas; pathotypes AE1 and AE2 were distinguished by a pea cultivar and AE3 was not virulent on faba bean and lentils.

Tolerance to Aphanomyces root rot was found in the licensed cultivars Ackerperle and Herz Freya but not in Diana and Erfordia. Variability in tolerance to root rot was also found in some 350 faba bean accessions. The following accessions were considered tolerant after two seasons of field testing: 2N19, 2N21, 2N37, 2N66, 2N94, 2N104, 2N112, 2N114, 2N116, 2N134, 2N140, 2N218, 2N236, 2N296, DN75-38 (ERF) and DN76-8. Partial control of Aphanomyces root rot was achieved for 5 weeks by seed treatment with the fungicide Dowco 444 only.

GENERAL INTRODUCTION

Root diseases of faba beans (<u>Vicia</u> <u>faba</u> L.) have been reported in many parts of the world where the crop is grown. Wilts were found to be caused by at least three highly specialized species of <u>Fusarium</u> (Yu, 1944; Yu and Fang, 1948; Schneider and Dalchow, 1975) and root rots involved a wide variety of organisms. These include <u>Rhizoctonia</u> <u>solani</u> Kuehn., Pythium spp., Fusarium spp. (Salt and Hornby, 1971a,b; Platford and Bernier, 1973; Bernier, 1975; Lu and Thibodeau, 1978). <u>Fusarium</u> <u>solani</u> f. sp. <u>fabae</u> (Yu and Fang) was found to be specialized on <u>V</u>. <u>faba</u> and was reported to occur in China (Yu and Fang, 1948), Japan (Yamamoto <u>et</u> <u>al</u>., 1955) and the Sudan (Ibrahim and Hussein, 1974). In Rothamsted, England, root rot and wilt were also caused by <u>Phytophthora</u> <u>megasperma</u> Drechs. (Salt and Hornby, 1971a,b).

Early surveys in Manitoba indicated that root rot incidence was low (Bernier, 1975). However, root rot and seedling blight were severe from 1977 to 1981 in experimental field plots at the University of Manitoba (U of M). Root rot was also found to occur in some commercial fields in Eastern Manitoba in 1978 and 1979 (G. Platford, personal communication).

This study was initiated to provide information on the organisms causing the disease in Manitoba. The first section deals with the etiology of the disease and includes the isolation, identification and pathogenicity tests of the organisms associated with root rot. The second section deals with Aphanomyces euteiches, the pathogen found to

cause root rot of faba beans in the field at the U of M. The host range of the isolate AE1 and the pathogenic variability among the isolates recovered in Manitoba were investigated. The third section reports on the screening in the field of some 350 faba bean accessions for tolerance to A. euteiches, as well as on the evaluation of several seed treatment fungicides for the control of seed rot and seedling blight.

REVIEW OF LITERATURE

1. The Host

1.1 Origin and History

Faba bean (Vicia faba L.) is an important pulse crop and ranks
fourth only to dry beans, dry peas and chickpeas on a worldwide basis
(Hawtin and Stewart, 1979). Faba bean is thought to have been first
cultivated in the late neolithic period (Schultze-Motel, 1973; Zohary
and Hopf, 1973). From its near eastern centre of origin, Vicia faba
spread to all the Mediterranean Basin, Abyssinia, Europe and Asia
(Cubero, 1974). Most of the world's faba beans are now grown in China,
Northern Africa, the Middle East and, to a lesser extent, in Southern
Europe (Hawtin and Stewart, 1979) and Western North America (Evans and
Slinkard, 1975).

The division of V. faba into three subspecies, i.e. major, equina
and minor is based on the seed size. All three subspecies are cross
compatible. They are used for human and animal consumption because
of their high protein content.

Faba beans have been found to be well adapted to the moister por-
tions of the cereal growing areas in Western Canada (Evans et al., 1972).
Their ability to fix nitrogen when inoculated with suitable Rhizobium
bacteria makes them a potentially attractive high protein crop for
Manitoba (Candlish and Clark, 1975).

Small seeded types can be handled with standard cereal machinery

and are, therefore, preferred to the larger-seeded types, particularly in mechanized agriculture.

The development of faba beans in Western Canada for animal feed is likely to be influenced by the prices of high protein animal meals imported from the USA. Faba bean acreage has been significantly reduced in the United Kingdom (Bond, 1979) and Spain (Cubero, 1979) partly because of the import of cheap high protein animal feed.

Several diseases of faba bean occurring in the Middle East and Europe have also been reported to occur in Canada. These include Ascochyta leaf and pod spot, chocolate spot caused by _Botrytis_ spp., rust, powdery mildew and several virus diseases (Bernier, 1975; Lu and Thibodeau, 1978). Seedling blight and root rots were generally low in Manitoba in 1974, but it was thought that faba beans might be as prone as peas to root diseases and long rotations were recommended to prevent diseases from building up (Bernier, 1975).

1.2 Seedling Blight and Root Rot of Faba Beans

Several pathogens have been associated with faba bean root diseases in many parts of the world. Along with other diseases, root rots and wilts are thought to be a major problem in West Asia and North Africa, especially those caused by _Fusarium_ spp., _Rhizoctonia solani_ Kuehn and the _Sclerotinia_ spp. (Hawtin and Stewart, 1979).

Faba beans have been found to be susceptible to wilt caused by several _Fusarium_ spp. _Fusarium vasinfectum_ Atk. was possibly the first species reported to cause wilt of _Vicia faba_ in Belgium (Marchal and Verplancke, 1926). _Fusarium oxysporum_ Schl. f. sp. _fabae_ Yu and Fang has been reported to cause wilt in China (Yu and Fang, 1948), Japan (Ikata, 1951; Yamamoto _et al._ 1955), Canada (Coulombe, 1957), Russia (Dunin, 1962)

and Egypt (Ibrahim and Abdel Rehim, 1965). Another wilt was caused by
F. avenaceum (Fr.) Sacc. f. sp. fabae Yu in China (Yu, 1944) and Japan
(Yamamoto et al., 1955) and by a new species, Fusarium inflexum Schneider
in Germany (Schneider and Dalchow, 1975). F. inflexum did not infect
Vicia sativa L., V. villosa Roth., Pisum sativum L., Lupinus albus L.,
L. luteus L., L. angustifolius L., Phaseolus vulgaris L., Cajanus indicus
Spreng. and Crotalaria juncea L. and was, therefore, thought to be
specialized on V. faba.

Wilt Fusaria are usually restricted to their hosts but may cause
disease on related host species. F. oxysporum f. sp. pisi (Linford) Snyd.
and Hans. race 1 has been reported to mildly infect V. faba (Linford,
1928) but such infection could not be reproduced by Schneider and Dalchow
(1975).

Root rot is usually caused by pathogens with a wider host range
than the wilt organisms, with the exception of Fusarium solani f. sp.
fabae (Yu and Fang, 1948; Yamamoto et al., 1955; Ibrahim and Hussein,
1974).

In Rothamsted, England, several fungal pathogens and nematodes
have been observed in faba bean infected roots. These include the stem
eelworm (Ditylenchus dipsaci Kuehn.), species of Pythium, Fusarium,
Rhizoctonia and Chaetomium, arbuscules of a mycorrhizal fungus and
oospores of Olpidium brassicae (Woron.) Dang. and Phytophthora megasperma
Drechs. Only P. megasperma actually caused wilt and root decay of faba
bean when tested in pot experiments (Salt and Hornby, 1971a,b). McEwen
et al. (1973) reported the isolation of Cylindrocarpon spp., bacteria,
F. oxysporum, F. avenaceum, Pythium spp. and Phoma sp. from discolored
lesions on stem bases of faba beans.

In Egypt, species of <u>Fusarium</u> and <u>Rhizoctonia</u> solani were reported to be associated with root diseases of faba beans (Aly, 1967; Abdallah, 1969). Abd El Rahim (1962) found that root rot and wilt were caused by <u>F</u>. <u>oxysporum</u>, <u>F</u>. <u>solani</u>, <u>Erwinia</u> <u>carotovora</u> (Jones and Holland) and <u>Pseudomonas</u> <u>polygoni</u> (Thornberry and Anderson).

In Manitoba and Quebec, root rot was generally low and was caused by several <u>Fusarium</u> spp., <u>R</u>. <u>solani</u>, <u>Sclerotinia</u> <u>sclerotiorum</u> (Lib.) de Bary and <u>Pythum</u> spp. (Platford and Bernier, 1973; Bernier, 1975; Lu and (Thibodeau, 1978).

Faba bean roots turn black in response to infection by pathogens, mechanical damage or just by exposure to air, making symptoms caused by different pathogens appear similar. McEwen <u>et</u> <u>al</u>. (1981), however, recognized two types of black roots: (i) a wet rot of the root cortex caused mainly by oomyceteous pathogens and (ii) a dry rot in which the cortex becomes black or dark brown but does not disintegrate, which is caused by several members of the Fungi Imperfecti. This latter type of symptom was found to precede normal senescence but could be detected earlier when host resistance was decreased by stress from other causes.

2. <u>Aphanomyces euteiches</u>

2.1 <u>History</u>

The genus <u>Aphanomyces</u> was established by de Bary (1860) and was included in the Saprolegniales. Most species in this genus have some parasitic tendencies on fish, algae, fungi and on phanerogamous plants (Coker, 1923). A detailed taxonomic study of the Saprolegniales including some species of <u>Aphanomyces</u> was published (Coker, 1923). The list of <u>Aphanomyces</u> species was updated by Sparrow (1960) and a monograph

of this genus was published by Scott (1961).

Aphanomyces euteiches was first described by Jones and Drechsler
(1925) and recognized as the causal agent of the common root rot of peas.
Surveys indicated that Aphanomyces euteiches was present in
nearly all pea-growing areas in the USA, where it was and is still con-
sidered to be a limiting factor for pea cultivation (Papavizas and
Ayers, 1974). It has also been reported to occur in various parts of
the world where peas are cultivated. It was found in France, England
and Wales, Norway, Sweden, Denmark, the USSR, Jamaica and Australia
(Papavizas and Ayers, 1974). It has also caused pea root rot in Eastern
Canada (Ontario and Quebec), but was considered to be an unimportant
pathogen in Ontario (Conners, 1967).

The disease symptoms on peas have been thoroughly described (Jones
and Drechsler, 1925). Above ground symptoms are not necessarily unique
to A. euteiches. Stunting, wilting of the lower leaves, sudden wilting
of the whole plant or weak adult plants are found to be caused by this
pathogen. The disease can be better diagnosed through observation of
the underground parts. This includes a pale yellow discoloration of
early infected basal stem and root tissues, followed by a soft decay of
the cortical tissue. If such infected plants are pulled, the stem fails
to break at the seed and the vascular cylinder is very often pulled out
with the plants. Microscopic observation of the root and basal stem
always reveals the presence of typical oospores.

2.2 Isolation of Aphanomyces euteiches from Peas

The isolation of Aphanomyces spp. and of A. euteiches in particular
has been difficult. Pea root rot was solely attributed to Pythium
when it was a complex of Pythium sp. and A. euteiches (Jones and Drechsler,

1925). Two major difficulties are encountered: i) bacterial contamination to which _Aphanomyces_ spp. are very sensitive and ii) contamination by other fungi associated in the root rot complex, such as _Pythium_ spp., _Fusarium_ spp. and _R. solani_. Several methods have been used to secure pure cultures of the fungus. Jones and Drechsler (1925) used segments of freshly infected root tissue and made several transfers of the ones where they could microscopically detect hyphae full of granular material. Sherwood (1958) used the ability of _Aphanomyces_ to produce zoospores and infect sterile corn kernels in petri dishes containing sterile water. Streptomycin was also used to secure bacteria-free cultures (Sundheim, 1972). No techniques have been developed to separate _Aphanomyces_ spp. from fungal contaminants.

In their descriptive paper, Jones and Drechsler (1925) noted that the mycelium transferred its content into oospores which are formed upon the destruction of the host cells. After this stage it was almost impossible to secure growth on culture media. Thus, recovery of _A. euteiches_ is only possible if isolation attempts are done using newly infected seedlings.

2.3 Host Range

Since its isolation and description (Jones and Drechsler, 1925), _A. euteiches_ has been tested against several plant species. More than 80 species from 19 families have been reported to be hosts of _A. euteiches_. An extensive list of these hosts has been compiled (Papavizas and Ayers, 1974). One of the earliest work on the host range of this species has been done by Linford (1927), who showed that _Medicago sativa_ L., _Melilotus alba_ Desr., _Lathyrus odoratus_ L., _L. latifolius_ L., _Lathyrus_ sp., _Vicia sativa_ L., _V. panonica_ Crantz., _V. monantha_ Retz.,

$\underline{V}$. $\underline{gigantea}$ Hook., $\underline{V}$. $\underline{fulgens}$ Batt., $\underline{V}$. $\underline{ervilia}$ Willd., $\underline{V}$. $\underline{dasycarpa}$ Ten. and $\underline{V}$. $\underline{angustifolia}$ L. could be parasitized in naturally infested soil. In later studies, often by pure cultures of the pathogen in sterile sand or soil (Sherwood and Hagedorn, 1962) or by the excised root tip method (Carlson, 1965), species of many families were found to be parasitized by $\underline{A}$. $\underline{euteiches}$. The families involved were the Alismataceae (Ridings and Zettler, 1973), Amaranthaceae (Sherwood and Hagedorn, 1962; Carlson, 1965), Caryophyllaceae (Carlson, 1965), Chenopodiaceae (Sherwood and Hagedorn, 1962; Carlson, 1965), Compositae, Cruciferae, Cucurbitaceae (Carlson, 1965), Gramineae (Geach, 1936; Carlson, 1965; Carley, 1969; Haensler, 1976), Leguminosae (Haensler, 1926; Linford, 1927; Geach, 1936; Sherwood and Hagedorn, 1962; Schmitthener, 1964; Carlson, 1965; Carley, 1969; Pfender and Hagedorn, 1980), Liliaceae (Carlson, 1965), Malvaceae (Carlson, 1965); Pinaceae (Eliason, 1928), Polygonaceae, Portulacaceae, Scrophulariaceae, Solanaceae, Umbelliferae and Violaceae (Carlson, 1965).

Some discrepancies have been noted in the list published by Papavizas and Ayers (1974); these include infection of non-leguminous plants by $\underline{A}$. $\underline{euteiches}$ in non-sterile soil. Tomato was infected by Aphanomyces $\underline{cladogamous}$ Drechs. (McKeen, 1952) and not by $\underline{A}$. $\underline{euteiches}$ Drechs. $\underline{A}$. $\underline{cladogamous}$ was isolated and described by Drechsler (1929) and was found later in roots of spinach and flax (Drechsler, 1935).

Field infections of lettuce, pepper and eggplant were caused by an $\underline{Aphanomyces}$ sp. which Mix (1945) did not identify at the species level. Because of its host range, $\underline{A}$. $\underline{cladogamus}$ would be a better candidate than $\underline{A}$. $\underline{euteiches}$, which has been mainly reported on leguminous species. The severity of the disease described by Mix (1945) led

McKeen (1952) to the conclusion that Mix's isolate did not represent the same species as the one isolated from tomato and which did not cause severe damage. Pathogenic variability has been found to exist in A. euteiches (Beute and Lockwood, 1967; Sundheim, 1973) and in A. cochlioides (Buchholtz and Meredith, 1944). It is likely that such variability exists in A. cladogamous.

In the case of the Dutch isolate, "A. euteiches P.F. 2", isolated from pansies, Arabis and spinach (Meurs, 1929), it appears that A. cladogamous was probably the pathogen involved and that Meur's isolate was not A. euteiches, the pathogen isolated from peas (Drechsler, 1954). Later work showed that "A. euteiches P.F. 2" was only slightly pathogenic to pansies in Holland, whereas Brevilegnia gracilis and B. macrospora caused severe root rot (Van Eek, 1938).

The infection of celery by A. euteiches in naturally infested soil, reported by Doran et al. (1942), was questioned by Drechsler (1954) in view of the fact that no detailed evidence had been given.

It would then appear that there is very little or only poor evidence for the infection of non-leguminous plants by A. euteiches in naturally infested fields or in non-sterile soils. This does not prove that A. euteiches has a strong tendency towards leguminous species, but it does suggest it. Reports on the host range of this pathogen still contain contradictory information (Papavizas and Ayers, 1974). This may be partly due to different inoculation techniques and to different patho-types of the fungus used, the inoculation technique probably being the most important factor leading to contradictory results; these ranged from planting the host in naturally infested soil (Linford, 1927) to inoculation of whole plants in sterile sand (Sherwood and Hagedorn,

1962) and inoculation of excised root tips (Carlson, 1965).

2.4 Pathogenic Variability and Host Specialization

Whether isolates of A. euteiches are restricted to a certain number of hosts remains an unanswered question. The first level of specialization that has been reported is the ability of A. euteiches to infect differentially at the species level. Sherwood and Hagedorn (1962) found that one of their isolates infected Lotus corniculatus L., Onobrychia viciifolia Scop., Trifolium hybridum L. and V. pannonica, whereas another isolate parasitized only Trifolium pratense L. and Amaranthus retroflexus L. Both isolates were pathogenic to peas (Pisum sativum L.). The Amazon sword plant isolate (Ridings and Zettler, 1973) was able to infect pea (Pisum sativum), broadbean (Vicia faba), beets (Beta vulgaris L.), cowpea (Vigna sinensis L.) and radish (Raphanus sativus L.), whereas the pea isolates AE6 and AE7 to which they compared their isolate, were restricted to peas. Physiologic specialization has also been found by Carlson (1965) in inoculations of non-leguminous species by the excised root tip method.

A second level of variability is the ability of A. euteiches to differentially infect a set of pea cultivars. Although the first report is attributable to King and Bissonnette (1954), the most extensive report is due to Beute and Lockwood (1967) who used six pea lines as differential series and tested 15 single spore isolates from several locations. The authors were able to identify two races (1 and 2). Both races were pathogenic on peas since they were differentiated by the percentage of pea seedlings killed each day, for nearly 2 weeks. The same method was used by Sundheim (1972) in Norway. He identified four races including race 1 of Beute and Lockwood. The other races were designated

3, 4 and 5.

Determination of races with a bean series (<u>Phaseolus</u> spp.) has been attempted (Carley, 1969). Seven races were detected but the results of the experiment were highly variable due to the use of unknown inoculum levels. The race concept of <u>A</u>. <u>euteiches</u> has not been assessed for validity and no further work has been reported in this area since 1972. In addition to the reports on races, several studies showed that variability in aggressiveness on peas existed in this pathogen (Lockwood, 1960a; Scharen, 1960; Haglund and King, 1961; Carlson, 1965; Shehata <u>et</u> <u>al</u>., 1976).

The existence of pathogenic variability raises the question of the mechanisms of variation in this pathogen. There are so far no reported studies on this subject, nor on any other genetical aspect in this fungus. As reported for other oomycetes (Sansome, 1961, 1962, 1965; Barksdale, 1966, 1968; Bryan and Howard, 1969), <u>Aphanomyces</u> spp. may be diploid in its vegetative phase. Cytological evidence of gametangial meiosis has been provided by Traquair and McKeen (1980).

2.5 Survival and Epidemiology of <u>A</u>. <u>euteiches</u>

Long rotations up to 10 years without peas, have decreased disease severity but did not eliminate Aphanomyces root rot (Jones and Linford, 1925; Temp, 1966). Laboratory studies showed that oospores were able to survive 2 years of alternate or continuous freezing without losing their pathogenicity (Sherwood and Hagedorn, 1962). This confirms the belief that the thick-walled oospore of <u>A</u>. <u>euteiches</u> represents the survival unit of this pathogen. Previous work by Scharen (1960) showed that oospores embedded in plant debris could be induced to germinate if buried next to the roots of several plant species and that germina-

tion was highest next to pea roots. Zoospores which were produced were used to infect pea plants and proved pathogenic. Although zoospores were used in almost all infection studies, this was the first report which demonstrated the infection role of the zoospores and confirmed the role of the oospore in the survival of A. euteiches.

Sherwood and Hagedorn (1958) designed a method for indexing soil for disease prior to planting. Soil was sampled from commercial fields, planted with a susceptible pea cultivar in the greenhouse and scored for disease. Three types of fields were distinguished on the basis of the greenhouse disease severity index (DSI): i) safe for planting (DSI = 0-50%), ii) questionable (DSI = 51-69%) and iii) hazardous (DSI = 70-100%). The indexing system was elaborated earlier for sugar beets (Fink, 1948; Fink and Buchholtz, 1954) and later adapted for peas (Johnson, 1953, 1957). Good correlations were obtained between DSI's obtained in the greenhouse and those obtained in the fields from which the samples were taken (Reiling et al., 1960). Furthermore, the number of debris containing oospores/100 g of soil has been shown to be positively correlated to the DSI observed in the greenhouse and field (Boosalis and Scharen, 1959).

Another approach to the quantitative estimation of A. euteiches in soil used by Mitchell et al. (1969) is thought to be sensitive in detecting Aphanomyces in plant debris previously separated from soil by a sieving method (Boosalis and Scharen, 1960). This method consisted in putting susceptible pea seedlings in contact with the plant debris in moist paper towels. Observation and/or isolation of A. euteiches is thus rendered easier.

Despite these findings, little is known about the dynamics of inoculum in situ mainly because there are no consistent methods of

recovering and enumerating propagules of A. euteiches directly from soil without baiting with a susceptible host.

The survival of A. euteiches in soil for long periods in the absence of peas has been a subject of speculation. Several workers consider that the wide host range of this pathogen is likely to play a role in its persistence in soil despite long rotations. Thus, the concept of alternative hosts has been suggested (Linford, 1927; Sherwood and Hagedorn, 1962; Carlson, 1965; Carley, 1969). The contradictory information on the host range of A. euteiches is consequently reflected in the alternate host concept. For instance, Sherwood and Hagedorn (1962) thought that "parasitization of legumes other than peas might play a role in the perpetuation of A. euteiches and that non-leguminous plants apparently are not suitable hosts". Carlson (1965), on the other hand, believed that even non-leguminous hosts might play a role. This apparent disagreement seems to result from the inoculation techniques used in each case. The excised root tip method (Carlson, 1965) indicated that many host families could be parasitized. Infections of whole plants grown in sterile sand (Sherwood and Hagedorn, 1962) were positive mainly on leguminous species and very few non-leguminous species were infected. Parasitization under non-sterile conditions were found to include fewer species than with other methods (Carley, 1969). The question of the alternate host for survival of A. euteiches is only partly answered and whether non-leguminous species help maintain, increase or rejuvenate propagules of this pathogen in the absence of peas needs more clarification. In addition, the mycelium of A. euteiches was found very susceptible to lysis in non-sterile soil (Lockwood, 1960c), making this species a poor saprophyte under natural conditions.

3. Factors Affecting the Development of Disease
Caused by A. euteiches

3.1 Inoculum Potential

Most of the inoculations in laboratory and greenhouse experiments were made using zoospore suspensions which i) are easy to obtain free of other propagules, ii) can be easily counted and iii) can be applied as uniform inoculum (Papavizas and Ayers, 1974). The zoospore has been shown to be the infective unit, as oospores germinated only by zoosporangia when buried in special devices, next to roots of peas (Scharen, 1960). Infectivity of the zoospore of A. euteiches has been found to be quite high. Bhalla (1968) found that three motile or two non-motile zoospores per seedling could initiate infection in 50% pea seedlings. The ED_{50} values were 16 and 282 motile zoospores in sterile and non-sterile soils, respectively. Concentrations as low as one zoospore/ml could produce oospores in excised root tips of certain hosts (Carlson, 1965).

For practical purposes, concentrations of more than 10^4 zoospores/ ml are used to produce a consistently high level of disease. Johnson (1953) obtained maximum root rot of pea by applying 1.6 X 10^4 zoospores per square inch of soil. Lockwood and Ballard (1959) found that the application of 10 ml of a 1.5 X 10^5 zoospores/ml suspension per 10 inch row of seedling was needed. Under field conditions natural inoculum consists of oospores embedded in plant debris. The amount of debris containing oospores was positively correlated to DSI (Boosalis and Scharen, 1959). Inoculation of pea seedlings with artificially in- duced oospores (Beute and Lockwood, 1967) indicated no difference in infection patterns from those obtained by zoospore inoculations.

3.2 <u>Temperature</u>

Aphanomyces root rot of peas has been found to develop at temperatures ranging from 15 to 30-35° C (Haensler, 1925; Jones and Drechsler, 1925; Jones and Linford, 1925). There is almost general agreement that no symptoms develop at temperatures below 14° C (Smith and Walker, 1941; Burke <u>et al</u>., 1969). Lockwood and Ballard (1959) found no significant differences between root rot indices at 20, 24 and 28° C. Oospore formation has also been influenced by temperature. Optimal temperature for oospores was around 25° C (Cho and King, 1963; Carley, 1969). The experiment carried out by Burke <u>et al</u>. (1969) showed an interesting aspect of the relation of temperature and infection. They found that disease incidence at 16° C was as high as that for 20, 24 or 28° C but symptoms were delayed. If the plants infected at 16° C were washed and transferred to 28° C, rapid development of symptoms occurred. In naturally infested soil, <u>A</u>. <u>euteiches</u> had a selective advantage at 16° C over other root rot fungi (<u>Thielaviopsis</u> <u>basiola</u> (Berk. and Br.) Ferraris and <u>Fusarium</u> sp.) which are less active at low temperatures.

3.3 <u>Age of Seedlings at Inoculation</u>

There seems to be agreement in the results obtained by different workers, that the age of the seedlings at inoculation affects the severity of Aphanomyces root rot of peas (Lockwood and Ballard, 1959; Lockwood, 1960) and sugar beets (Schneider, 1956). Inoculation with <u>A</u>. <u>euteiches</u> has generally been performed on 7- to 8-day-old seedlings (Beute and Lockwood, 1967; Sundheim, 1972; Shehata <u>et al</u>., 1976). This would indicate that root rot of peas caused by <u>A</u>. <u>euteiches</u> is particularly important at the seedling stage, although symptoms are often observed on mature plants due to the fact that the seedlings may sur-

vive early infection.

3.4 Soil Moisture

Being a "water mold", A. euteiches depends to a very great extent
on high soil moisture to generate disease. Haensler (1927) found a
minimum level of disease when soil was at 30% saturation. High soil
moisture favored the development of the disease. Smith and Walker
(1941) did not observe disease below 45% water holding capacity (WHC)
while severe infection (72% plants infected) was obtained at 75% WHC.
In the field, root rot was severe in wet seasons and low or negligible
in dry years (Reinking, 1942; Reinking and Newhall, 1950).

4. Control of Aphanomyces Root Rot of Peas

4.1 Search for Tolerance to A. euteiches in Peas

Little work was done in the 1920's and 30's on the screening
for tolerance in peas to Aphanomyces root rot. The period was charac-
terized by the identification (Jones and Drechsler, 1925), surveys of
pea fields and studies of the biology of the fungus (Linford, 1927).
However, some studies showed that certain pea cultivars were more
tolerant than others (Haensler, 1925; Jones, 1926; Jones and Drechsler,
1925), but no immunity was reported.

Johnson (1953) compared the susceptibility of 22 pea introductions
to A. euteiches. Twelve of these lines had greater tolerance to
root rot than the commercial pea cultivars. Screenings for tolerance
to A. euteiches in the greenhouse required the development of inocula-
tion techniques. Johnson (1953) used known zoospore concentrations to
infect peas growing in sterilized soil. Lockwood and Ballard (1959)

performed their tests with sterilized sand instead of soil. Another

technique was developed by Haglund and King (1961) whereby peas were

grown in vermiculite until 1-week-old. They were then removed, their

roots washed and dipped for 24 hours in a 10^5 zoosp/ml suspension and

replanted in steam-treated soil in the greenhouse. A laboratory

screening technique, based on the formation of oospores of A. euteiches

in excised root tips of peas, was developed and used (King and Cho,

1962; Cho and King, 1963; Carlson, 1965; Morrison et al., 1971). Root

tips are usually cut and placed in petri dishes containing a zoospore

suspension. After a period of incubation, the root tips are observed

under a microscope and the number of oospores that have formed are

counted. The area immediately behind the root cap was found to attract

zoospores, while the root cap and the root hair zone did not (Cunningham

and Hagedorn, 1962a). This region of the root tip was used for oo-

spore counting (Morrison et al., 1971). More oospores formed in suscep-

tible than in tolerant lines. A study on the penetration and infection

of pea roots by zoospores of A. euteiches (Cunningham and Hagedorn,

1962b) showed no differences between a susceptible and a tolerant line

at 24 hours of incubation with regard to depth of penetration. The

only "clear cut correlation was in the relative abundance and maturity

of oogonia in the roots after periods of 59-65 hours". The "excised

root tip technique" was not always precise,enough to detect "small

amounts" of tolerance (Carlson, 1965) and is only suggested for preli-

minary screening of large numbers of pea introductions in the laboratory,

followed by additional testing of the best lines under greenhouse or

field conditions.

More recently, a new testing approach was used which utilized con-

trolled environment chambers and greenhouses (Shehata et al., 1976).

Seedlings were "submerged up to the hypocotyl area" in 100 ml of a

1.5×10^5 zoospores/ml suspension for 16 hours, then planted in pots con-

taining autoclaved sand. The pots of sand were saturated and placed

in plastic bags to maintain 100% relative humidity for 7 days. In the

second phase, the inoculated seedlings were transplanted to a non-

autoclaved soil-mix bed in the greenhouse and kept at 10-13° C (soil

temperature) to favor "plant growth more than pathogen growth". The

authors claim that this technique reduced resistance breakdown at an

early stage of host development and gave a good separation between

susceptible and tolerant lines. Attempts to incorporate tolerance into

commercial types of peas were not always successful, and only a limited

number of lines were released (King et al., 1957; Lockwood, 1960b;

Shehata et al., 1976).

Studies on the inheritance of tolerance of peas to A. euteiches are

rare. The only reported attempt revealed that tolerance was associated

with undesirable dominant wild type alleles at three unlinked marker

loci, i.e. Le (tall), A (colored flowers) and PL (yellow cotyledons).

Substitution of the recessive alleles lead to a decrease in tolerance

(Marx et al., 1972).

4.2 Control of A. euteiches with Fungicides

There is little information on control of A. euteiches by fungi-

cide seed treatment. Most of the work reported employed soil treatments.

Chloronitropropane (Lanstan) was effective as an in-furrow application

(Haglund, 1968), but was not recommended because it was found to be

lacrymatory and highly toxic (Papavizas and Ayers, 1974). Some fumi-

gants have also been found effective in greenhouse tests and in some

cases in the field. According to Papavizas and Ayers (1974), these fungicides have not been adequately tested in the field.

Among the non-volatile fungicides, Dexon appeared to be effective, but not economically feasible (Mitchell and Hagedorn, 1971). This fungicide has been found to persist in soil for long periods of time and to reduce propagules of A. euteiches. Approximately 1 μg of residual Dexon per gram of soil was needed to inhibit zoospore formation. An amount of 30 lb/acre would be required to meet this residual quantity (1 μg/g soil).

4.3 Control of A. euteiches by Herbicide Treatments

Effects of herbicides on disease incidence under field conditions have been recently reviewed by Altman and Campbell (1977). Some negative effects of herbicide applications have been observed. The application of PCA and pebulate to soil in greenhouse experiments resulted in increased damping-off of sugar beet by Rhizoctonia solani (Altman and Ross, 1967; Altman, 1972). Similar results were observed by Antonopoulos (1969). Trifluralin and dinoseb also increased disease due to R. solani on snapbean (Roming and Sasser, 1972) and studies in vitro showed that these herbicides reduced the amount of phytoalexin in bean plants, thus altering the biochemical defences of the host. Trifluralin and dinitroaniline herbicides, however, have been found to reduce pea root rot in the field. Carlson and Hopen (1971) found that plots treated with trifluralin (Treflan) for weed control had less disease and similar effects were reported by other workers (Harvey et al., 1975; Grau and Reiling, 1977; Sacher et al., 1978). Grau (1977) showed that these herbicides had a direct effect on the pathogen in vitro and in vivo. The mechanism of root rot suppression was first

explained by Teasdale <u>et</u> <u>al</u> (1979). These authors demonstrated that suppression was not due to the effect of the herbicide on the plant but to direct effect on the pathogen, as shown by Grau (1977). The most sensitive stage in the life cycle of <u>A</u>. <u>euteiches</u> was found to be the production of motile zoospores which could be completely inhibited by 0.01 ppm of all dinitroaniline herbicides tested. This was confirmed by a more recent study (Jacobsen and Hopen, 1981). Dinoseb was found effective in pea root rot control. In <u>in</u> <u>vitro</u> studies, trifluralin was found to enhance oospore production by the pathogen, while Dinoseb did not (Jacobsen and Hopen, 1981). It is not known whether this enhancement occurs under field conditions.

RESULTS OF RESEARCH

1. Etiology of Seedling Blight and Root Rot of Faba Bean
(Vicia faba) in Manitoba

ABSTRACT

An Aphanomyces sp., Rhizoctonia solani Kuehn and a Fusarium sp. were associated with seedling blight and root rot of faba bean (Vicia faba L.) in Manitoba. Pathogenicity tests indicated that the Aphanomyces sp. was the main pathogen at the Campus farm of the University of Manitoba. In addition to faba bean, the Aphanomyces sp. infected lentils (Lens culinaris L.), Lathyrus sp., garden peas (Pisum sativum L.) and field peas (P. sativum subsp. arvense L.). The Aphanomyces sp. from faba bean was morphologically and culturally similar to Aphanomyces euteiches Drechs. It would appear that this is the first report on the association of A. euteiches with root rot of faba bean and lentils in the field.

The 10 isolates of R. solani from faba bean, pea and rapeseed tested, varied in pathogenicity to faba bean. The pathogenic isolates of R. solani caused damping-off on faba bean plants, except for one isolate from Eastern Manitoba. The latter caused a soft rot of the stem and roots and had more pathogenic capabilities than any of the other isolates.

The Fusarium sp. recovered from mature faba bean plants in a commercial field in Eastern Manitoba caused severe root rot when inoculated on faba bean and pea seedlings. This fungus was tentatively identified as Fusarium avenaceum (Fr.) Sacc.

INTRODUCTION

Faba beans (<u>Vicia faba</u> L.) have been grown commercially on a limited acreage in Manitoba for the last decade. In 1980, the area under cultivation was estimated to be 8,800 hectares (Anonymous, 1981). The crop was found to be well adapted to the moister cereal growing areas in Western Canada and is grown as a high protein crop for animal feed and export (Evans <u>et al</u>., 1972).

Very little is known about pathogens that may cause root diseases on faba beans in Manitoba. Early surveys indicated that <u>Fusarium</u> spp., <u>Rhizoctonia solani</u> Kuehn and <u>Sclerotinia sclerotiorum</u> (Lib.) de Bary were associated with root rot of faba beans in the province (Platford and Bernier, 1973) and that root diseases were generally of low incidence. However, severe root rot and seedling blight have been observed in experimental field plots at the University of Manitoba (U of M) since 1978. Young seedlings were often blighted and older plants were stunted and yellow. The root systems of virtually all plants in the disease nursery were blackened and plants of more susceptible cultivars were completely destroyed. Root rot was also found to be common and severe in some commercial fields of faba bean in Eastern Manitoba in 1978 and 1979 (G. Platford, personal communication).

Studies were initiated to determine the etiology of seedling blight and root rot of this crop in Manitoba. This paper reports on the isolation, identification and pathogenicity of the organisms involved and compares the symptoms induced in the greenhouse by the fungi isolated, with those of field-infected plants.

MATERIALS AND METHODS

Fungal Isolation

Infected seedlings uprooted during field surveys were brought to the laboratory in plastic bags and stored in a refrigerator at 5° C when not used immediately. The roots were thoroughly washed by hand under running tap water, cut into 5-10 mm pieces and surface sterilized in 1% sodium hypochlorite for 30-90 seconds. Pieces of roots of each seedling were rinsed in sterile distilled water, blotted dry on sterile filter paper and plated on 2% water agar (WA), potato dextrose agar (PDA) and acidified PDA (APDA). Other media were used to isolate specific pathogens, namely Nash and Snyder's medium (1962) for _Fusarium_ spp. and Ko and Hora's medium (1971) for _R. solani_. When specific media were used, the roots were usually not surface sterilized prior to planting. The two selective media are described in Appendices 1 and 2.

Inoculum Production

Isolates of all _Fusarium_ spp. were cultured in potato dextrose broth in 100 ml flasks and incubated at room temperature (ca 22° C) on a shaker for 4 days. The content of the flasks was filtered through cheesecloth to remove the mycelium. The spores were washed, suspended and adjusted to 10^6 conidia/ml with distilled water. Spore concentrations were determined with a haemocytometer.

Zoospores of the _Aphanomyces_ sp. were produced by the method of Llanos and Lockwood (1960) with a slight modification. Water agar plates

were used instead of flasks, thereby requiring no aeration to enhance sporulation. The zoospores suspensions were adjusted to 10^5 zoospores/ml (zsp/ml) with distilled water (Appendix 3).

Mycelium of all isolates of R. solani and the Aphanomyces sp. was produced by culturing on PDA plates and incubating for 1 week at room temperature.

Pathogenicity Tests

Seeds of the cultivars used were surface sterilized in 2.5% sodium hypochlorite for 5 min, rinsed twice in sterile water and planted in a standard steamed soil:sand:peat mix (1:1:1 v/v), or in vermiculite if the seedlings were to be uprooted for inoculation. Plastic flats (52 cm X 26 cm X 5.5 cm) were used in all experiments.

For isolates of the Fusarium spp., 7-day-old seedlings of the cultivars Ackerperle (faba bean), Trapper (field pea) and Maple Presto (soybean) were uprooted, the roots washed in tap water, dipped in a 10^6 spores/ml suspension for 10 min and replanted in the soil mix. At least 40 plants were inoculated with each isolate.

For the Aphanomyces sp., 10 ml of a 10^5 zsp/ml suspension were pipetted onto each row (10 to 12 seedlings) close to the stems of 7-day-old seedlings. Inoculation with mycelium was performed on seedlings grown in vermiculite. A 3 mm disc of PDA with mycelium was applied against the tap root of each seedling, 1 cm below the seed and covered.

For R. solani, a 5 mm PDA disc with mycelium was placed 2 cm deep in the soil near the stem of each seedling and covered with soil.

Evaluation of Selected Cultivars in Naturally Infested Soil

Several cultivars of leguminous and non-leguminous species were

evaluated at the U of M in a field with a history of seedling blight and root rot (Table 2). Each cultivar was planted in a single row plot, 3 m long and replicated three times.

Disease Rating

Faba bean plants were scored for disease on a 0-4 scale adapted from the scale described for peas by Smith and Walker (1941), where 0 = healthy white roots, 1 = cortical black discoloration of tap root, 2 = black discoloration on the tap and lateral roots and epicotyl but no decay, 3 = black areas soft, epicotyls and roots water soaked and 4 = tissues disintegrated. Dead plants were rated 4. A disease severity index (DSI) was computed as follows (Sherwood and Hagedorn, 1958):

$$\text{DSI (\%)} = (\sum_{i=0}^{4} n_i \, X_i / \sum_{i=0}^{4} n_i) \times 25$$

where X_i = class value (0, 1, 2, 3, 4) and n_i = number of plants in class X_i.

For microscopic observation, blackened infected faba bean tissue was dipped in 1% sodium hypochlorite solution until cleared (15 to 20 mn), rinsed in water and mounted on slides after staining with aniline or trypan blue in lactophenol.

RESULTS

Seedling blight and root rot were generally severe in 1979, 1980 and 1981 in the disease nursery and some other fields at the Campus farm (U of M). The fungi isolated during the three seasons are listed in Table 1, along with their frequency of isolation.

In 1979, only *Fusarium* spp. and *R. solani* were isolated from faba bean seedlings sampled in the middle and the end of the season. In 1980 and 1981, an *Aphanomyces* sp. was isolated along with the two previous fungi. Other fungi, bacteria and saprophytic nematodes were commonly recovered during the three seasons. Microscopic observations of the roots of almost every plant sampled in 1980 and 1981 from the disease nursery revealed the presence of numerous oospores similar to those of *Aphanomyces* (Fig. 1). The increase in percentage of recovery of the *Aphanomyces* sp. in 1980 and 1981 is attributed to the use of newly infected seedlings for fungal isolation and also to the inclusion of several susceptible faba bean and pea cultivars in the tests (Table 2). *R. solani* and *Fusarium* spp. were isolated from decayed roots of faba bean as in 1979.

The *Aphanomyces* sp. was isolated more frequently on 2% WA and PDA, but it was difficult to transfer mycelium free of bacteria from PDA cultures. The *Fusarium* spp. and *R. solani* were isolated on their respective selective media as well as on PDA, APDA and WA. Bacterial contamination was reduced if the roots of newly infected faba bean seedlings were washed by hand and left overnight under running tap water.

TABLE 1. Fungal species isolated from
   diseased faba bean seedlings in
   experimental plots at the University
   of Manitoba.

| Fungal species | % of Seedlings[+] | | |
	1979	1980	1981
Aphanomyces sp.	0	40	72
Fusarium spp.	75	72	55
Rhizoctonia _solani_	6.2	20	35
Other fungi[++]		10	10

Fungal isolations were made from 80 plants
in 1979 and from approximately 200 plants
in both 1980 and 1981.

+ Values represent the % of seedlings from
  which a given fungus was isolated. More
  than one fungal species may be isolated
  from the same seedling.

++ These include _Alteraria_ spp.,
   Cylindrocarpon spp., _Rhizopus_ spp.,
   Aspergillus spp. and a few non-identified
   fungi.
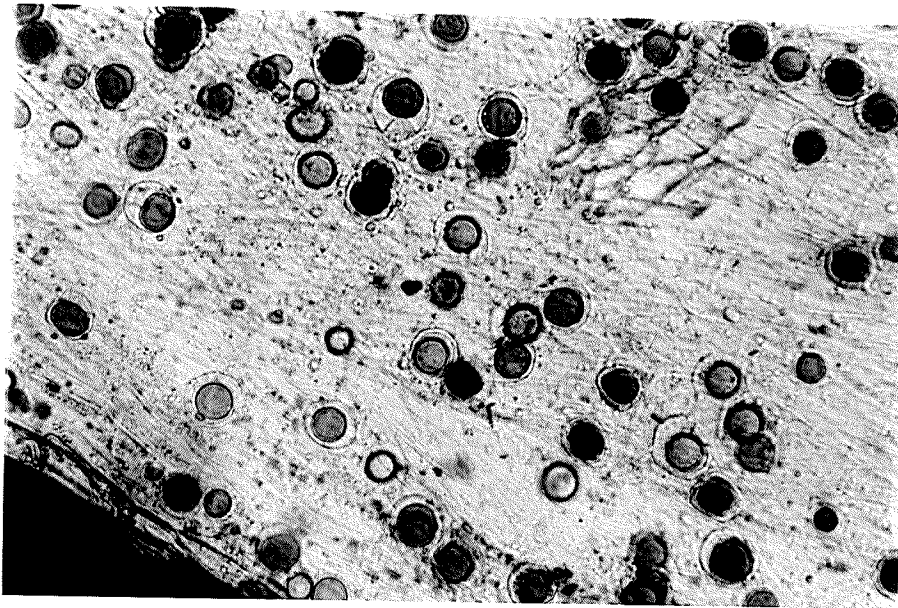
Figure 1. Oospores of <u>Aphanomyces</u> <u>sp.</u> in an
      infected root of the faba bean cultivar
      PI 222128.

TABLE 2. Response of leguminous and non-leguminous species and
cultivars to Aphanomyces root rot in a naturally infested
field at the University of Manitoba.

	Infection[*]	Oospores[**]	Isolates of Aphanomyces sp.
Peas (garden)			
Little Progress	++	P	I
Thomas Laxton	++	P	I
Homesteader	+	P	I
Little Marvel	+++	P	I
Green Arrow	++	P	I
Peas (field)			
Trapper	+	P	I
Century	++	P	I
Triumph	+	P	I
Tara	+	P	I
Faba bean			
Ackerperle	+	P	I
PI 222128	+++	P	I
Triple White	+++	P	I
Mikko	++	P	I
Erfordia	++	P	I
Herz Freya	+	P	I
Diana	+++	P	I
Sugar beet	-	A	NI
Lentils			
White lentil	++	P	I
Dark lentil	++	P	I
Alfalfa			
Pioneer	-	A	NI
Soybean			
Mc All	-	A	NI
Maple Amber	-	A	NI
Maple Presto	-	A	NI
Lathyrus sp.	++	P	I
Snap beans			
Black beans	-	A	NI
Sea Fearer	-	A	NI
Mung beans	-	A	NI
Rapeseed			
Candle	-	A	NI
Regent	-	A	NI
Potato		A	NI
Flax	-	A	NI

[*] -, +, ++, +++ = respectively: no symptoms, slight, moderate
and severe infections.

[**] Microscopic observations of oospores were done on at least
10 plants/cultivar or species; P = Present; A = Absent.

I = Isolated; NI = Not isolated

Recovery of Aphanomyces sp. was improved by this washing schedule.

R. solani and a Fusarium sp. were isolated from four mature faba
bean plants with severe root rot in 1979. The plants were from a
commercial field in Eastern Manitoba and were received through the
Manitoba Department of Agriculture. In 1980, a survey of some commer-
cial fields of faba beans in Eastern Manitoba indicated that root rot
incidence was low (2-3%). Fungal isolations were made from 50 diseased
seedlings and yielded R. solani (100% of seedlings) and an isolate of
the Fusarium sp. recovered in 1979 and tentatively identified as
Fusarium avenaceum (Fr.) Sacc. according to the description of Booth
(1977).

Evaluation of Selected Cultivars in Naturally Infested Field

All faba bean, lentil and pea cultivars, as well as Lathyrus sp.
planted in the disease nursery at the U of M were infected by the
Aphanomyces sp. Parasitization was confirmed by the presence of oospores
typical of the genus (Fig. 1) and also by the isolation of the fungus in
pure culture (Table 2). Oospores of Aphanomyces were not found in roots
of alfalfa, snap beans, rapeseed, potato, flax, sugar beet, volunteer
wheat and pigweed. R. solani was isolated from snap bean plants early
in the season and later from peas, faba beans, potato and Lathyrus sp.,
while Fusarium spp. were commonly isolated from infected roots and stems
of all cultivars used for fungal isolation. Damage due to R. solani on
snap beans was not important because the seedlings from which the fungus
was isolated appeared healthy and the lesions were superficial on the
below-ground part of the stem. Several seedlings from faba bean and pea
cultivars infected by the Aphanomyces sp. were severely affected, there-
fore the contribution of R. solani and Fusarium spp. to the disease

development could not be assessed.

Pathogenicity Tests

Twelve isolates of the _Fusarium_ spp. isolated from faba bean in 1979 at the U of M and four isolates from Eastern Manitoba tested along with _F. graminearum_, _F. oxysporum pisi_ race 1 and _F. solani_ (supplied by Dr. Reid, Department of Botany, U of M) were found to be non-pathogenic to Ackerperle, Trapper and Maple Presto.

The _Fusarium_ tentatively identified as _F. avenaceum_ recovered in 1979 caused severe root rot on faba bean and peas but not on soybean. Initially, it induced black lesions of the lateral roots and later on the tap root of faba bean with the whole seedling eventually collapsing. Symptoms induced by this species of _Fusarium_ could not be ompared with field symptoms because the fungus was isolated from mature plants.

The identification of _R. solani_ was based solely on the asexual stage. All isolates tested for pathogenicity were found to be multi-nucleate when stained by the method described by Burpee _et al_. (1978) (Appendix 4). In addition to their nuclear condition, the isolates had all the characteristics attributed to _R. solani_ Kuehn by Parmeter and Whitney (1970).

Eight isolates of R. solani recovered from infected faba bean plants as well as one isolate each from peas and rapeseed were tested for pathogenicity (Table 3). Isolates 3, 12 and 13 from the disease nursery, as well as isolates 1 and 20, respectively from Eastern Manitoba and the greenhouse (U of M) were pathogenic to faba beans on the basis of their ability to infect the stem of the two cultivars tested. Isolate 3 was the most virulent isolate on both pea cultivars. Isolates 1, 12 and 13 were mildly pathogenic to Trapper, but not to Century (Table 3).

TABLE 3. Stem and seed infection of two faba bean and two field pea cultivars inoculated with 10 isolates from <u>Rhizoctonia</u> <u>solani</u>.

| Isolate # | Origin | Host | Faba Bean | | | | Field Peas | | | |
| | | | Ackerperle | | PI 222128 | | Century | | Trapper | |
			Stem*(%)	Seed**(%)	Stem	Seed	Stem	Seed	Stem	Seed
1	E. Man.+	FB++	76.0	72.0	96.7	80.6	4.2	79.2	18.5	62.9
2	U of M 18	Pea	62.5	37.5	32.3	38.2	57.1	91.4	44.4	92.5
5	U of M 18+++	FB	0.0	3.6	2.8	2.8	0.0	40.0	0.0	38.8
7	U of M 5	FB	0.0	7.4	0.0	0.0	3.1	56.7	0.0	32.1
8	U of M 5	FB	3.3	6.6	0.0	6.1	0.0	45.7	0.0	20.7
10	Aboretum	Rapeseed	0.0	19.4	0.0	0.0	3.2	54.8	0.0	55.2
12	U of M 18	FB	44.0	60.0	15.6	68.7	8.8	67.6	24.2	100.0
13	U of M 18	FB	40.0	88.0	0.0	65.6	12.1	100.0	44.1	94.1
14	E. Man.	FB	4.8	19.0	0.0	0.0	0.0	63.9	3.4	31.0
20	Greenhouse	FB	43.3	76.7	16.2	77.4	21.9	93.8	23.5	97.1
Noninoculated			0.0	5.5	0.0	10.3	0.0	5.8	0.0	6.6

+ E. Man. = Eastern Manitoba, commercial fields.

++ FB = faba bean.

+++ U of M 18 = University of Manitoba, Campus farm, number indicates the field number.

* % of plants with lesions extending at least 3/4 of the stem.

** % of seeds decayed, after germination. Data represent averages on 30-36 plants/isolate.

The pathogenic isolates of R. solani induced damping-off with the exception of isolate #1 which produced a black soft rot of stems and part of the roots of faba beans (Fig. 2). Isolate #1 produced sclerotia on artificial media 4-5 days earlier than the other isolates and had a longer incubation period when inoculated on faba bean. Symptoms induced by isolate #1 were visible 10 days after inoculation, whereas damping-off by the other isolates occurred 4-5 days after inoculation of 7-day-old faba bean seedlings.

Isolates 5, 7, 8, 10 and 14 were not pathogenic on either faba bean or peas, although they caused some seed rot on peas, but not on faba bean (Table 3).

Plants inoculated with zoospores or mycelium of the Aphanomyces sp. were all infected and showed above ground symptoms approximately 1 week after inoculation (Fig. 3). The entire root system and the above ground stem turned black in response to fungal invasion. The discoloration was at first cortical but affected all the vascular system as disease progressed. A decay of roots and epicotyls followed and seedlings collapsed.

The extent of the black discoloration was measured for the plants inoculated with mycelium and was found to range from 3-8 cm for the roots and from 1.8-4.4 cm for the stems of different cultivars (Table 4). Numerous oospores were observed in discolored tissues of each cultivar.

Of the fungi isolated from the U of M disease nursery, only Aphanomyces sp. reproduced the symptoms observed on field infected seedlings (Fig. 3a, b, c).

The Aphanomyces sp. was recognized by its typical arachnoid type of growth on artificial media. Before evacuation, the zoospores were arranged

Figure 2. Symptoms induced by <u>Rhizoctonia</u> <u>solani</u> in
    plants of <u>Vicia</u> <u>faba</u> in the greenhouse. a) faba bean
    seedlings 6 days after inoculation with <u>R.</u> <u>solani</u> ,
    isolate # 1. b) seedlings in fig. 2a, 3 days later,
    c) stem and some root discoloration induced by isolate
    #1, d) damage to a seedling by a damping-off isolate
    of <u>R.</u> <u>solani</u>.
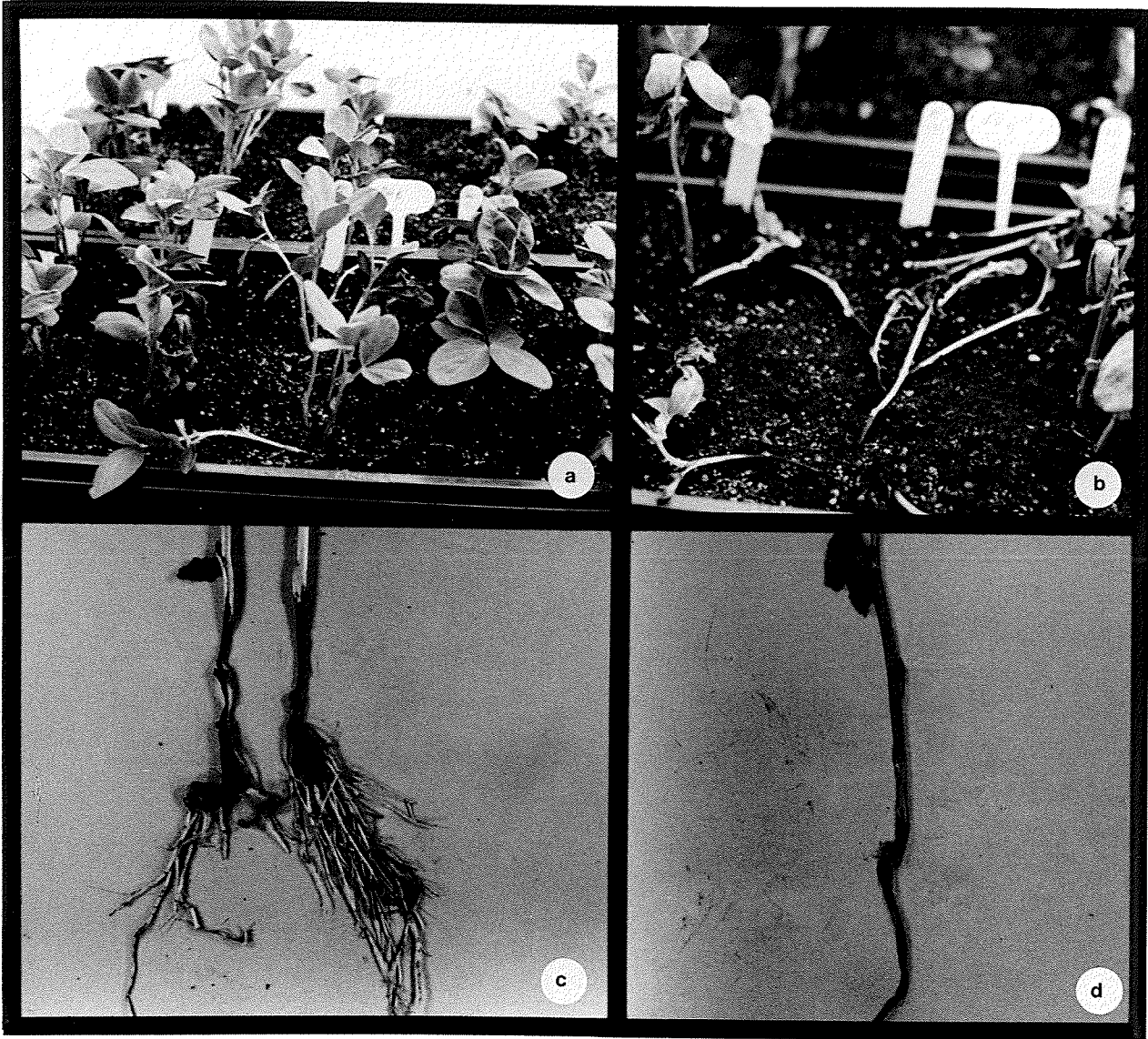
Figure 3.   Symptoms induced by Aphanomyces euteiches in
   plants of Vicia faba in the field and in the greenhouse.
   a) seedling blight observed in the field. Arrow indicates
   a dead seedling.   b) and c) decayed and discolored roots
   of faba bean in the field,   d) and e) greenhouse inoculated
   faba bean seedling. Note black discoloration of roots and
   stems.
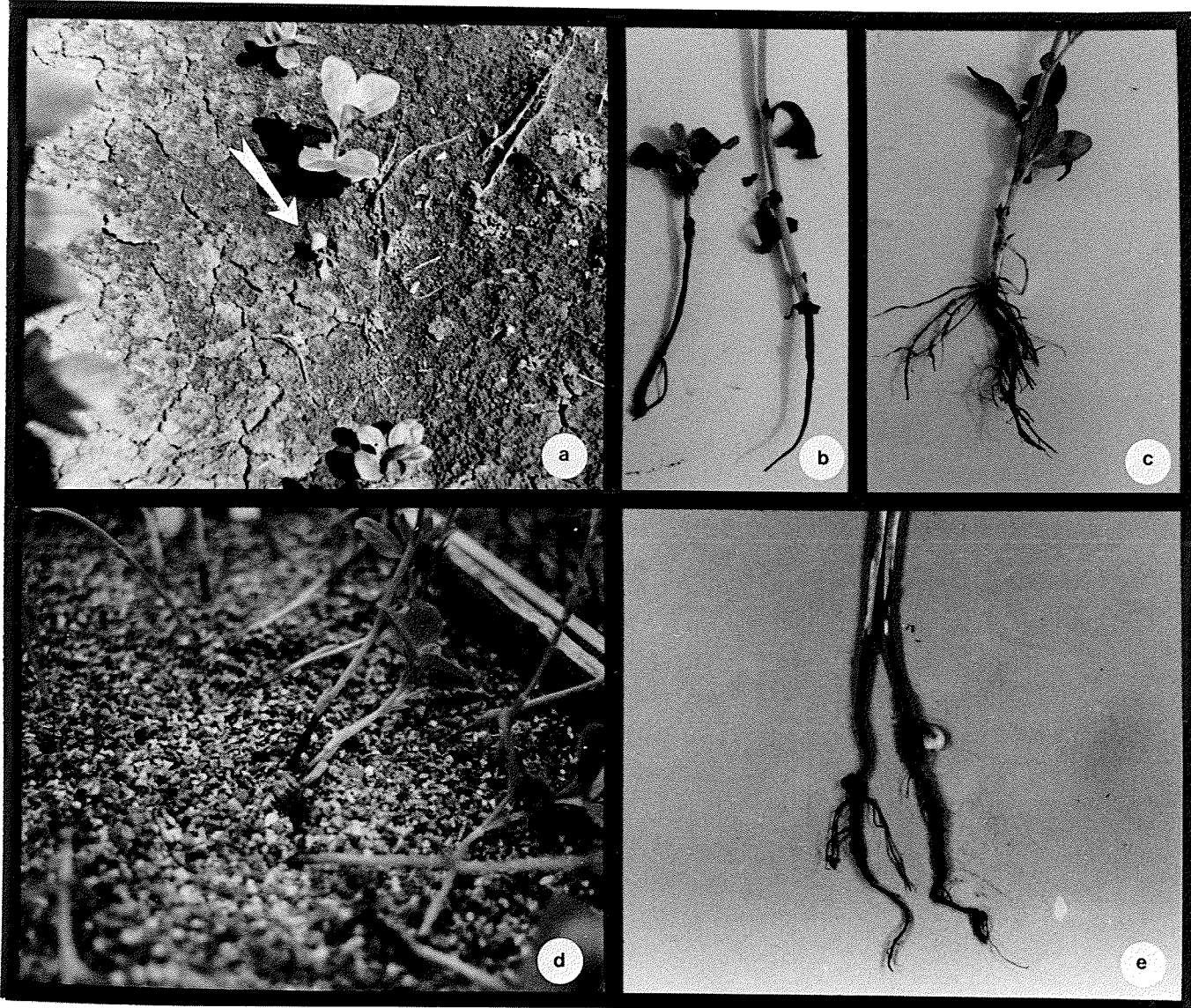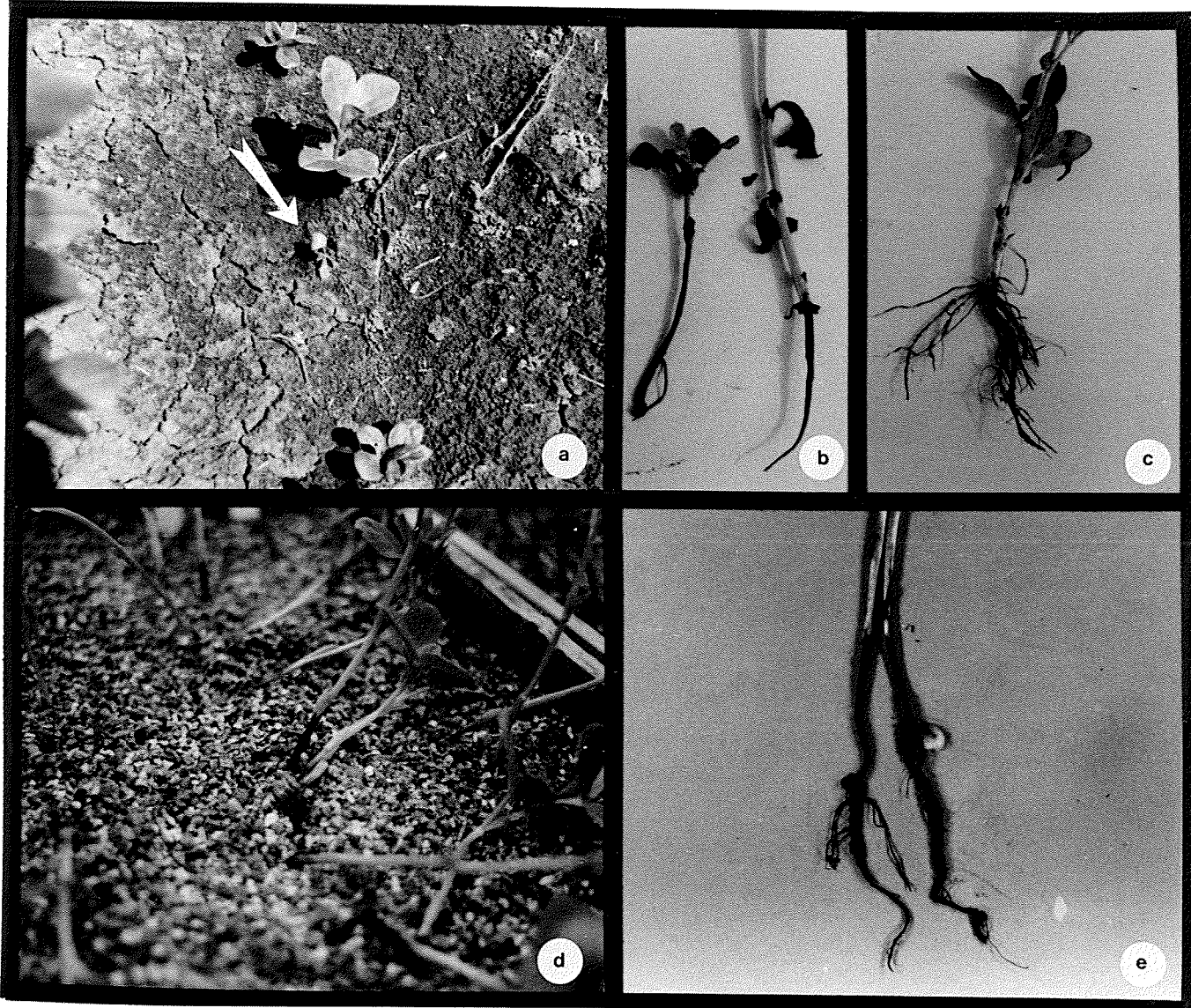
TABLE 4. Response of three faba bean and one field pea cultivars to inoculation with mycelium of an _Aphanomyces_ sp. from faba bean.

	Plants inoculated (no.)	Plants infected (no.)	DSI (%)	Root discoloration (cm)	Stem discoloration (cm)
Faba bean					
PI 222128	20	20	92.5	8.0	4.4
Diana	19	19	92.1	7.2	4.0
Ackerperle	20	20	87.5	5.2	2.1
Field pea					
Trapper	15	15	65.3	3.0	1.8

*The extent of discoloration was measured from the seed downward (root) and upward (stem). Values represent means from at least 15 plants/cultivar.

Figure 4. Vegetative and sexual structures of Aphanomyces
euteiches isolated from Vicia faba. a) zoospores typically
arranged in a single row. b) a cluster of primary non-
motile zoospores. c) an oogonium and antheridium before
oospore formation. d) a fully mature oospore.
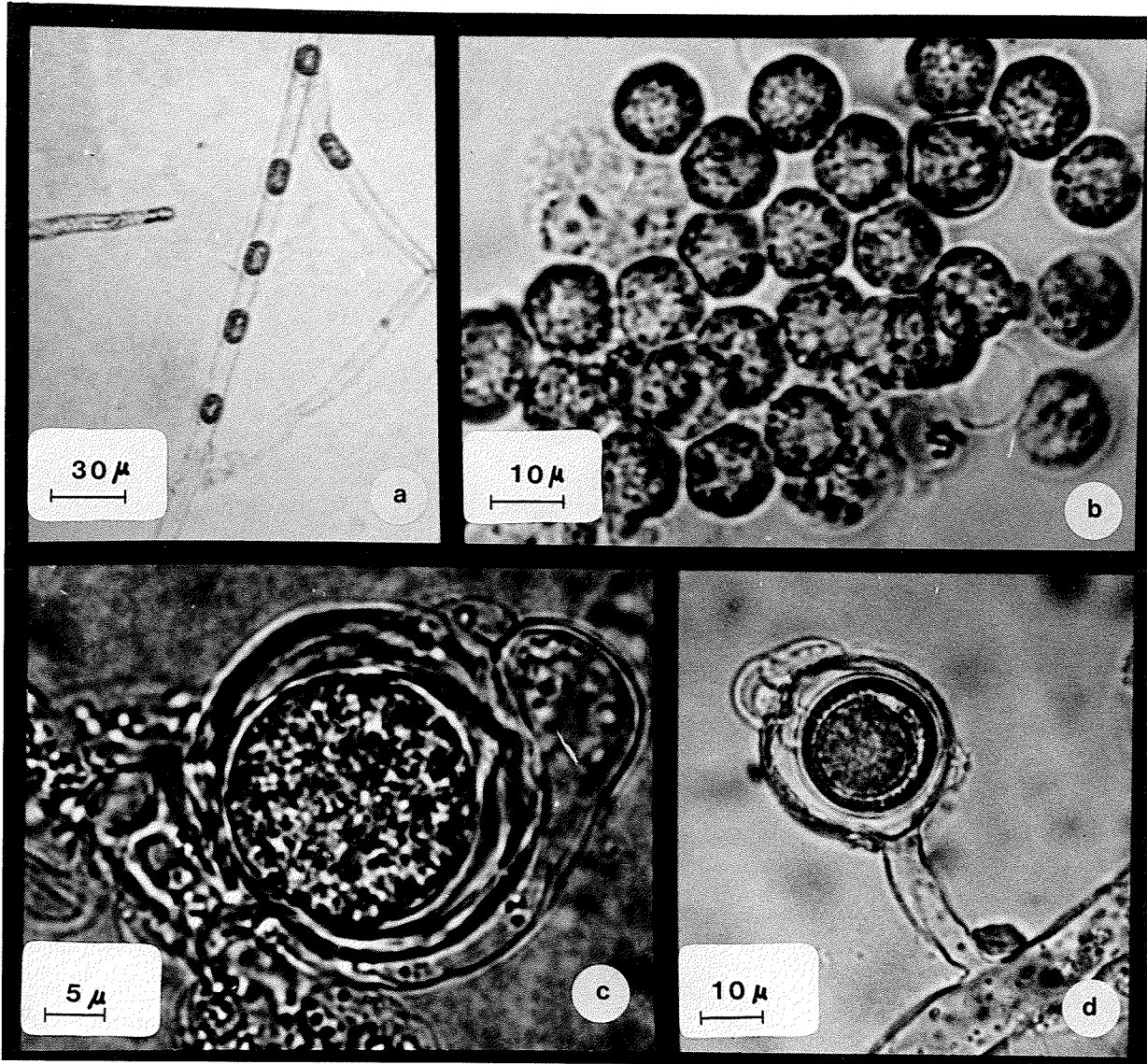
in a single row within an undifferentiated zoosporangium (Fig. 4a).

The primary zoospores rounded off and clustered at the mouth of the

evacuation tube (Fig. 4b), then gave rise to laterally biflagellate

zoospores. Oogonia were terminal on short lateral branches and were

delimited from the stalk on which they were formed by a septum (Fig.

4c, d) with the oogonium containing a single thick-walled oospore (Fig.

4d). The measurements of the sexual structures and other characteris-

tics, along with the pathogenicity to peas and other leguminous plants,

place the isolates recovered from faba bean at the U of M closest to

A. euteiches Drechs., the pathogen of the common root rot of peas

(Jones and Drechsler, 1925). The identification was confirmed by the

Biosystematics Research Institute, Ottawa (# B413).

DISCUSSION

Seedling blight and root rot of faba bean have been found in this study to be caused by A. euteiches at the U of M Campus farm and by R. solani in commercial fields in Eastern Manitoba. The Fusarium avenaceum (?) isolated from mature plants with severe root rot in Eastern Manitoba was found to be pathogenic to faba bean as well as to peas. However, attempts to evaluate the importance of this fungus in the field in 1980 failed.

The increased frequency of recovery of A. euteiches from infected plants of several legumes in the seedling stage at the U of M experimental field plots suggests that disease was primarily due to A. euteiches and that R. solani and Fusarium spp. acted as secondary invaders. A similar situation was reported by Salt and Hornby (1971a), who isolated Fusarium and Pythium spp., R. solani and Phytophthora megasperma from infected faba bean plants. The latter was the least frequently isolated, but was found to be the only fungus able to cause root rot and wilt in greenhouse experiments. It was thought that P. megasperma was possibly replaced by the other fungi once the symptoms were advanced (Salt and Hornby, 1971b).

All damping-off isolates of R. solani tested in this study induced discrete brown or black lesions on the stems of the seedlings at ground level, whereas R. solani isolate #1 and A. euteiches induced a soft, black rot of the entire root system and part of the stems. McEwen et al. (1981) also recognized two types of symptoms: i) a wet root rot

caused by oomycetous fungi and ii) a dry rot of the root cortex and stem bases associated with Fusarium spp., R. solani and other Fungi Imperfecti. The association of several fungi in root diseases, in addition to the black discoloration of faba bean roots in response to any injury, make it difficult to visually characterize the symptoms caused by different pathogens in the field. Isolation of the organisms in pure cultures and tests for pathogenicity, although time-consuming, remain the only reliable method of ascertaining the cause of root diseases of faba bean.

Vicia faba has been reported to be a host of A. euteiches in inoculation experiments with pure cultures of the fungus (Carlson, 1965; Ridings and Zettler, 1973), but not in non-sterile soil (Haensler, 1926). This would appear to be the first report on the association of A. euteiches with root rot of faba bean in the field. This pathogen has been found to cause pea root rot in Eastern Canada, but was apparently an unimportant pathogen in Ontario (Conners, 1967). However, in many pea growing areas of the USA, this pathogen is still a limiting factor for pea cultivation (Papavizas and Ayers, 1974).

The isolates of R. solani tested in this study were more invasive to faba bean stems than to those of peas. The latter crop appeared to be more susceptible to seed rot.

The evaluation of several cultivars in the disease nursery (U of M) revealed the existence of variability in tolerance to Aphanomyces root rot among, as well as within, the leguminous species tested. Lentils (Lens culinaris L.) appeared to be very susceptible to A. euteiches. Root rot of lentils does not appear to have been previously found associated with A. euteiches in the field.

The susceptibility of some licensed faba bean cultivars to the three pathogens involved in this study suggest that root diseases should be added to the list of other potentially destructive diseases of faba bean in Manitoba (Bernier, 1975).

2. Host Range and Pathogenic Variability of Aphanomyces euteiches Isolates From Faba Bean

ABSTRACT

The host range of Aphanomyces euteiches Drechs. isolated from naturally infected seedlings of faba bean (Vicia faba L.) at the University of Manitoba was investigated. Only leguminous species became infected in the field and the greenhouse. Twelve species were infected, of which the following do not appear to have been previously reported as hosts of A. euteiches: Lathyrus ochroleucus Hook., L. sativus L., Lens culinaris Medic., Vicia calcarata Desf., V. cracca L., V. disperma DC., V. narbonensis L. and V. tetrasperma (L.) Schreber.

The faba bean isolate AE1 differed in pathogenicity from a Wisconsin pea isolate P14 , which was avirulent on faba bean, whereas AE1 was virulent on both. Three pathotypes were identified among the Manitoba isolates of A. euteiches. Pathotypes AE1, AE2 and AE3 were pathogenic to peas; pathotypes AE1 and AE3 were distinguished on a pea cultivar to which AE1 was not aggressive and AE3 was avirulent on faba bean and lentil.

Inoculum concentration, age of seedlings at time of inoculation and temperature influenced disease development. Tolerance of the cultivars Ackerperle (faba bean) and Trapper (field pea) was overcome at 30° C (air temperature).

INTRODUCTION

A severe seedling blight and root rot had been observed in faba
bean experimental field plots at the University of Manitoba (U of M)
since 1978. Infected seedlings were stunted and had extensive black
discoloration of the root system and epicotyls. Under high moisture
conditions, the disease incidence and severity were high and entire
plots of faba bean were affected. Fusarium spp. and Rhizoctonia solani
Kuehn were isolated in 1979, but typical symptoms could not be repro-
duced in faba bean seedlings inoculated with recovered isolates of the
two fungi.

In 1980, the etiology of seedling blight was investigated more
thoroughly. Aphanomyces euteiches Drechs. was isolated from field-
infected seedlings, along with the two previous fungi and shown to be
the main agent of the disease at the U of M Campus farm (Thesis Section
I). Early surveys in Manitoba indicated that root rot was associated
with Sclerotinia sclerotiorum (Lib.) de Bary, Fusarium spp. and R. solani
(Platford and Bernier, 1973), but A. euteiches does not appear to have
been associated with root rot of faba beans in the field. In the USA,
A. euteiches and Aphanomyces cochlioides Drechs. still cause serious diseases
of peas and sugar beets, respectively, and an extensive review on these
pathogens has been published (Papavizas and Ayers, 1974). In addition to
peas, A. euteiches has been reported to infect several leguminous species
(Linford, 1927; Sherwood and Hagedorn, 1962), as well as non-
leguminous species (Carlson, 1965). Pathogenic variability has been

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found in <u>A</u>. <u>euteiches</u> and races identified (Beute and Lockwood, 1967;
Carley, 1969; Sundheim, 1972).

This study was initiated to provide further information on the
host range of the faba b n   isolate recovered in Manitoba and to
compare it to a Wisconsin pea isolate for pathogenicity. Pathogenic
variability among Manitoba isolates of <u>A</u>. <u>euteiches</u> and some factors
affecting disease development in the greenhouse were also evaluated.

MATERIALS AND METHODS

Roots and epicotyls from plants infected in the field and in the greenhouse were processed as described previously (Thesis Section I) and planted on 2% water agar (WA) for fungal isolation.

Inoculum was prepared and 7-day-old seedlings inoculated as previously described (Thesis Section I). Greenhouse experiments were carried out under continuous light at a temperature of 20-24° C. When controlled environment rooms were used, the photoperiod was adjusted to 18/6 hours and the temperature at 25/20° C (day/night).

Host Range and Pathogenic Variability

Twenty-nine plant species, including 11 species previously reported to be hosts of A. euteiches (Papavizas and Ayers, 1974) were evaluated in the greenhouse using mycelial and zoospore inoculum of the Aphanomyces isolate AE1 from faba bean as well as by planting in naturally infested field soil. Some plant species were also evaluated in a field at the U of M, previously found to be naturally infested with the pathogen (Thesis Section I) and hereafter referred to as the disease nursery.

Tests to differentiate between isolates recovered at the U of M and to compare the faba bean isolate AE1 to the Wisconsin pea isolate P14 (supplied by Dr. Pfender, Wisconsin, USA) were carried out in controlled environment rooms. The test cultivars were grown in a pasteurized soil:sand:peat mix (1:1:1; v/v/v) in plastic flats. The flats contained one row (10-12 seedlings) of each cultivar. Ten ml of a 10^5 zoospores/ml suspension were poured per row, next to the stem of

7-day-old seedlings. All isolates tested originated from hyphal tips.

Single zoospore cultures from each isolate were also tested.

Factors Affecting Disease Development

To assess the relationship between seedling age and susceptibility

of faba bean, the cultivars Ackerperle and PI 222128 were planted at

weekly intervals for 3 consecutive weeks and then inoculated with mycelium

of A. euteiches.

The effect of inoculum concentration on disease development was

evaluated by inoculating plants of the susceptible cultivar PI 222128

with 0, 10^3, 10^4, 2×10^4, 4×10^4 and 10^5 zoospores/plant (zsp/plant)

poured in 2 ml aliquots next to the stem of each seedling.

Four constant air temperature regimes, ranging from 10 to 30° C,

were also tested for their effect on disease development. Soil temp-

erature was usually 2 to 4° C lower than air temperature at 25 and

30° C.

Isolation and Distribution of A. euteiches

The isolation of A. euteiches from field infected plants was found

to be difficult because of fungal and bacterial contaminations. The

use of newly infected seedlings appeared to improve the frequency of

isolation of Aphanomyces (Thesis Section I), therefore a test was

conducted to determine the optimal time after planting for isolation

of the fungus. The cultivars Ackerperle, Diana and PI 222128 were

planted in five 3 m-rows in a naturally infested field. Plants of the

cultivars were uprooted when they were 11-, 23-, 40- and 49-day-old and

used for fungal isolation.

To detect the presence of A. euteiches in experimental fields at

the Campus farm (U of M), soil was sampled and tested according to the method of Sherwood and Hagedorn (1958) as described in Appendix 5.

Disease rating. Plants were scored for disease on a scale ranging from 0-4 adapted from Smith and Walker's (1941) and previously described (Thesis Section I) where 0 = healthy white roots, 1 = cortical black discoloration of tap root, 2 = black discoloration of the tap and lateral roots and epicotyl, but no decay, 3 = black areas soft, epicotyls water soaked and 4 = tissues disintegrated. Dead plants were rated 4. A disease severity index (DSI) was computed as follows (Sherwood and Hagedorn, 1958):

$$\text{DSI } (\%) = (\sum_{i=0}^{4} n_i X_i / \sum_{i=0}^{4} n_i) \times 25$$

where X_i = class value (0, 1, 2, 3, 4) and n_i = number of plants in class X_i.

RESULTS

Host Range

Field and greenhouse studies on the host specificity of the A. euteiches isolate AE1 are summarized in Table 1. Amongst the leguminous species tested, alfalfa (Medicago sativa L.), chickpea (Cicer arietinum L.), soybean (Glycine max L.) were immune; Vicia americana Muhl. and V. lutea L. showed a brown discoloration, without decay, at the site where mycelial inoculum was applied but were not affected when inoculated with zoospores or planted in naturally infested soil. Snap beans (Phaseolus vulgaris L.) were mildly infected when inoculated with mycelium, but not by the other methods. V. cracca L. and Lathyrus ochroleucus Hook. were moderately susceptible; the fungus grew 2-3 down the roots but did not infect the stem. The other leguminous species were susceptible to very susceptible (large portions of the roots and part of the stems discolored, stunting and death). Among the susceptible species, Lathyrus ochroleucus, L. sativum L., lentils (Lens culinaris Medic.), V. calcarata Desf., V. cracca L., V. disperma DC., V. narbonensis L. and V. tetrasperma (L.) Schreber do not appear to have been previously reported as hosts of A. euteiches. None of the nonleguminous species tested became infected with any inoculation method (Table 1). A. euteiches was isolated from plants of all species infected in naturally infested soil and re-isolated from plants infected in the greenhouse.

TABLE 1. Response of leguminous and non-leguminous species to infection by <u>Aphanomyces</u> <u>euteiches</u> from <u>Vicia</u> <u>faba</u>.

	Mycelium[*]	Zoospores[*]	Field soil[*] in greenhouse	Field[**]
<u>Leguminosae</u>				
<u>Cicer</u> <u>arietinum</u> L.	-	-	-	-
<u>Lathyrus</u> <u>sativus</u> L.	+	+	+	nt
L. <u>ochroleucus</u> Hook.	+	+	+	nt
<u>Lathyrus</u> sp.	+	+	+	+
<u>Lens</u> <u>culinaris</u> Medic.	+	+	+	+
<u>Medicago</u> <u>sativa</u> L.	-	-	-	-
<u>Phaseolus</u> <u>aureus</u> L.	-	-	nt	-
<u>Phaseolus</u> <u>vulgaris</u> L.	+/-	-	-	-
<u>Pisum</u> <u>sativum</u> L.	+	+	+	+
<u>Pisum</u> <u>sativum</u> <u>arvense</u> L.	+	+	+	+
<u>Vicia</u> <u>americana</u> Muhl.	-	-	-	nt
<u>V</u>. <u>angustifolia</u> Reich.	+	+	+	nt
<u>Vicia</u> <u>calcarata</u> Desf.	+	+	nt	nt
<u>V</u>. <u>cracca</u> L.	+	+	nt	nt
<u>V</u>. <u>disperma</u> DC.	+	+	+	nt
<u>V</u>. <u>faba</u> L.	+	+	+	+
<u>V</u>. <u>lutea</u> L.	-	-	-	nt
<u>V</u>. <u>monantha</u> Retz.	+	+	+	nt
<u>V</u>. <u>narbonensis</u> L.	+	+	+	nt
<u>V</u>. <u>tetrasperma</u> (L.) Schreber	+	+	+	nt
<u>Glycine</u> <u>max</u> L.	-	-	-	-
<u>Chenopodiaceae</u>				
<u>Beta</u> <u>vulgaris</u> L.	-	-	-	-
<u>Liniaceae</u>				
<u>Linum</u> <u>usitatissimun</u> L.	nt	-	-	-
<u>Cruciferae</u>				
<u>Brassica</u> <u>campestris</u> L.	-	-	-	-
<u>B</u>. <u>napus</u> L.	-	-	-	-
<u>Raphanus</u> <u>sativus</u> L.	-	-	-	nt
<u>Solanaceae</u>				
<u>Lycopersicum</u> <u>esculentum</u> L.	-	-	-	
<u>Solanum</u> <u>tuberosum</u> L.	nt	nt	nt	-

- = no infection; + = infected; +/- = infection restricted to infection site (decay) in inoculations with mycelium; nt = not tested.

[*]Observations based on at least 15 plants. All inoculation experiments were repeated at least twice.

[**]Cultivars planted in a naturally infested soil. Three 3-m-rows were planted for each cultivar tested in the field.

Comparative Virulence of a Faba Bean Isolate and a Wisconsin Pea Isolate

The virulence patterns of the two isolates differed greatly when three pea and two faba bean cultivars were inoculated with a 2 X 10^5 zsp/ml suspension (Fig. 1). The Wisconsin pea isolate (P14) killed plants of the pea cultivars faster than the faba bean isolate, but was not pathogenic to faba bean. Only a few oospores were formed by P14 in the root cortex of plants of the faba bean cultivar PI 222128, but were not found in plants of Ackerperle. The faba bean isolate AE1 heavily infected plants of the Little Marvel pea and PI 222128 (Fig. 1).

In a second test including additional cultivars and a lower inoculum concentration (10^5 zsp/ml), peas were killed faster by isolate P14, whereas Lathyrus sp. and lentils were more susceptible to the faba bean isolate (Table 2). Plants of Ackerperle and PI 222128 were only infected by the faba bean isolate. Neither isolate infected snap beans.

Pathogenic Variability Among Manitoba Isolates of A. euteiches

Preliminary tests with seven isolates recovered from different experimental fields at the U of M Campus farm resulted in the identification of three pathotypes based on the virulence patterns produced by inoculating faba bean, pea and lentil cultivars. Five isolates were similar to the type isolate (AE1), whereas each of the two other isolates had a particular virulence pattern and were, therefore designated AE2 and AE3. Further testing of AE1, AE2 and AE3 resulted in the same virulence patterns observed in the previous test (Fig. 2). All three pathotypes were virulent on peas; pathotypes AE1 and AE2 were differentiated by the pea cultivar Homesteader to which AE1 was not aggressive, whereas AE3 was avirulent on the faba bean and lentil cultivars. Pathotypes AE1 and AE3 were isolated from plants of faba bean (PI 222128)
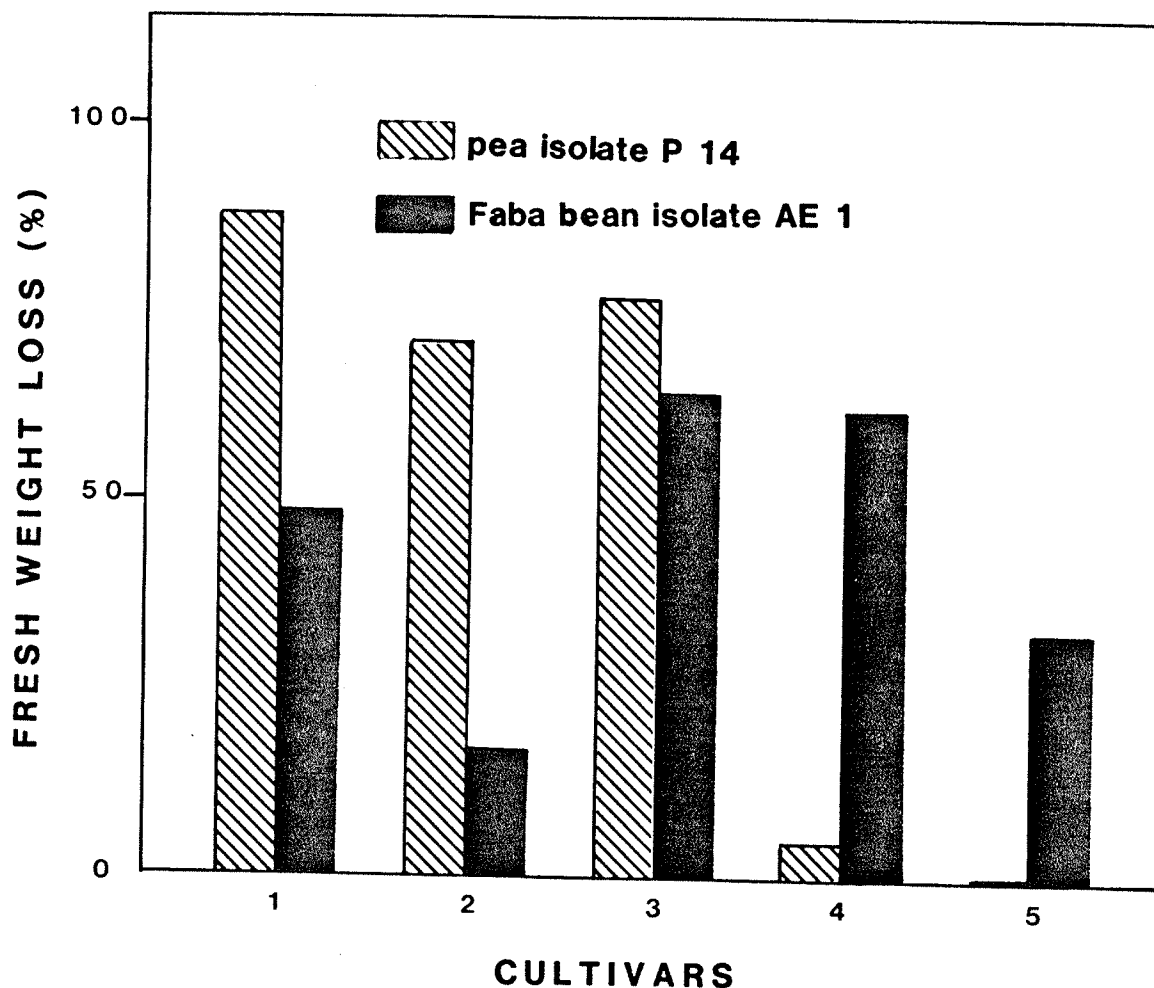
Figure 1. Virulence patterns of a faba bean isolate (AE 1) and a
Wisconsin pea isolate (P 14) of <u>Aphanomyces</u> <u>euteiches</u> on five
legume cultivars. The test cultivars were inoculated with
10 ml of a 2×10^5 zoospores/ml suspension per row (10-12 seed-
lings). 1- Miragreen, 2- Homesteader, 3- Little Marvel, 4-
Ackerperle, 5- PI 222128. Cultivars # 1, 2, and 3 are peas
and cultivars # 4, 5 are faba beans.

TABLE 2. Response of 12 legume cultivars to inoculation
with a faba bean isolate (AE1) and a Wisconsin pea
isolate (P14) of $\underline{A}$. $\underline{euteiches}$.

Host	Cultivar	P14[*]	AE1[**]
Faba bean	PI 222128	-	+++
	Ackerperle	-	++
Snap bean	Kentucky Wonder	-	-
	Bountifull	-	-
Lentil	Brown lentil	+	+++
	White lentil	++	+++
Pea	Century	+++	++
	Tara	++	+
	Trapper	+++	++
	Triumph	++	+
	Little Marvel	+++	+++
$\underline{Lathyrus}$ sp.		++	+++

+, ++, +++, respectively = slight, moderate and
severe infection.
- = no infection.
[*]Observations made on 50-60 plants/cultivar.
[**]Observations made on 15-20 plants/cultivar.
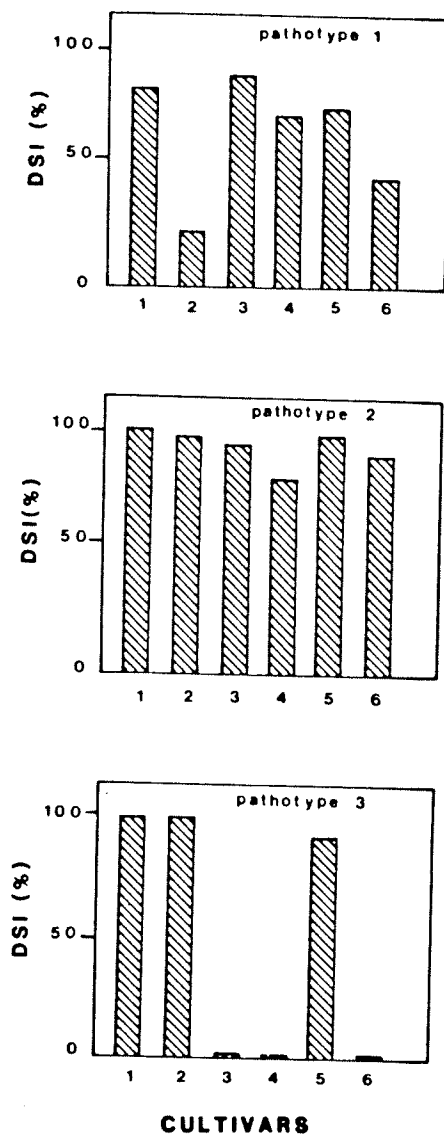
Figure 2. Virulence patterns of isolates of Aphanomyces
euteiches from the University of Manitoba in six
legume cultivars. The test cultivars were inoculated
with 10 ml of a 10^5 zoospores/ml suspension per row (10-
12 seedlings). 1 - Little Marvel (pea), 2- Homesteader
(pea), 3- PI 222128 (faba bean), 4- Ackerperle (faba
bean), 5- Trapper (field pea), 6- Tekoa (lentil).

and field pea (Tara), respectively, infected in the disease nursery.
Pathotype AE2 was isolated from plants of PI 222128 infected in a
different field than the two previous pathotypes. Differences in
virulence patterns were not observed between cultures started from
hyphal tips or single zoospores.

Factors Affecting Disease Development

Results of studies to determine the influence of seedling age at
the time of inoculation on disease development are shown by Figure
3a and b. Inoculation of 7-day-old seedlings with mycelium resulted
in a high percentage of plants killed and fresh shoot weight loss for
both PI 222128 and Ackerperle. On the other hand, inoculation of 3-
week-old seedlings did not cause significant losses in either cultivar.
Significant differences between Ackerperle and PI 222128, both in terms
of the percentage of plants killed and fresh weight loss, were observed
when 2-week-old seedlings were inoculated.

Increasing inoculum concentrations had a significant effect on the
DSI, percentage of plants killed and fresh shoot weight loss in plants
of the cultivar PI 222128 (Fig. 4). Significant differences with res-
pect to the non-inoculated control were not observed below 10^4 zsp/plant
for DSI and below $2X10^4$ zsp/plant for the percentage of plants killed
and fresh weight loss. Maximum DSI was observed with $2X10^4$ zsp/plant
and 60% of the seedlings were killed and fresh weight was reduced by 70%
at 10^5 zsp/plant.

Increasing temperatures (from 10 to 30° C) resulted in increased
disease development as measured by the reduction in fresh shoot weights
(Fig. 5). Plants of PI 222128 were significantly more affected than
plants of Ackerperle and Trapper at 15 and 25° C (air temperature).
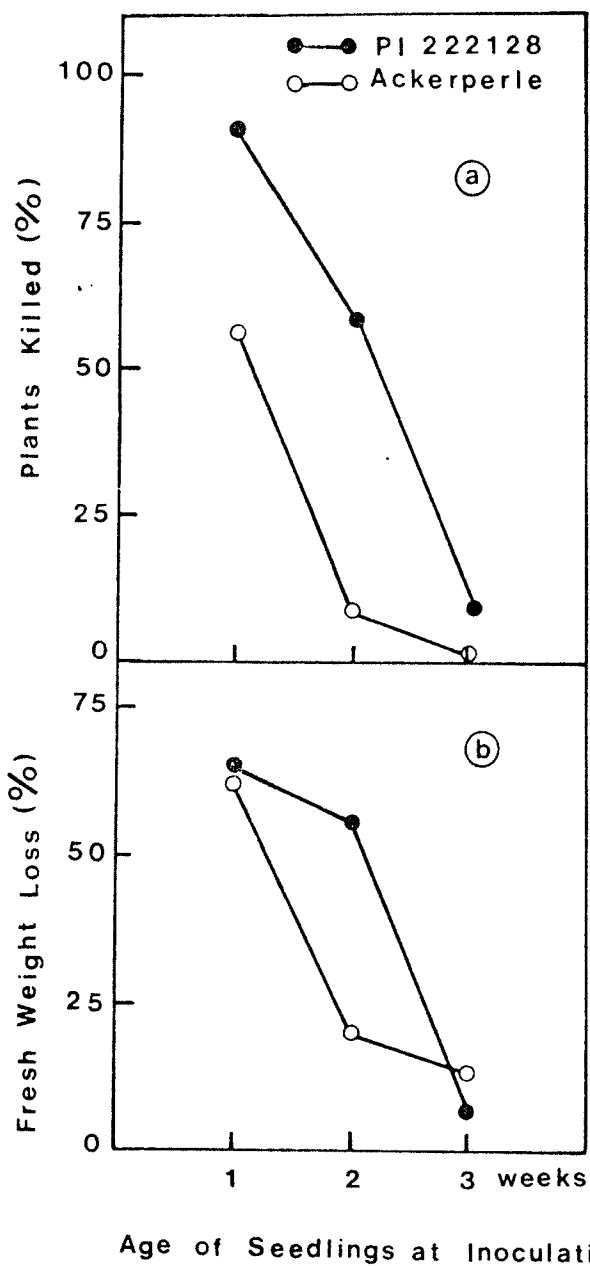
Figure 3. Effect of seedling age at time of inoculation
on disease development. Disease expressed as   a)
% plants killed, and b) fresh weight loss/plant (%).
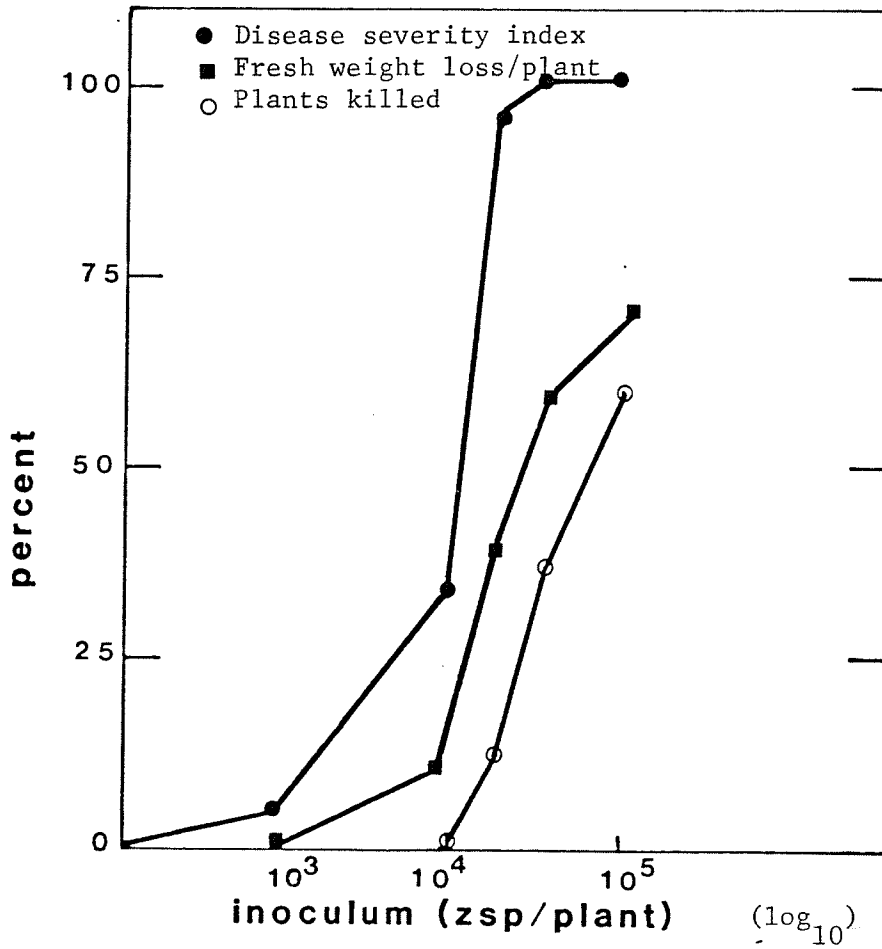
Figure 4. Effect of inoculum concentration on disease development. Treatments were replicated 5 times (12-16 plants/replication).
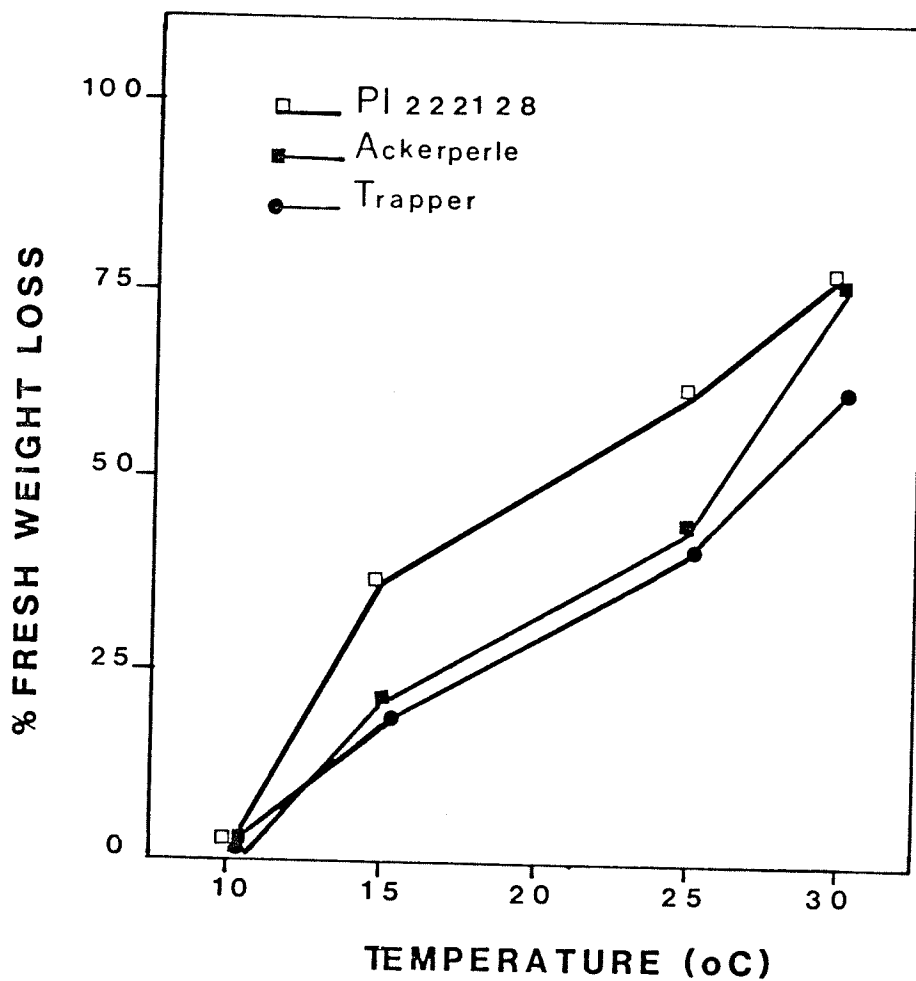
Figure 5. Effect of temperature on disease development.
    Soil temperatures were 2-4°C lower than air temperatures.
    Treatments were replicated 3 times (20-24 plants/replication).

At 30° C, fresh shoot weight losses ranged from 60-75% and plants of all cultivars were dead 10 days after inoculation. Disease symptoms were not observed at 10° C with any of the cultivars tested.

Recovery of A. euteiches from Field-Infected Seedlings of Faba Beans

Recovery of A. euteiches on 2% WA from field-infected faba bean plants was highest 11 days after planting (92%) and lowest (2.3%) after 49 days (Fig. 6). Fusarium spp. and other fungi were recovered more frequently from dead roots and epicotyls at the end of the test period. The best sampling time for isolation of A. euteiches from faba bean plants appears to be between 1 and 3 weeks after germination in naturally infested fields.

Distribution of A. euteiches at the U of M Experimental Fields

A. euteiches was detected in five of eight fields assayed (Table 3). The infested soil samples contained enough propagules of the pathogen to induce root and epicotyl discoloration in plants of PI 222128. Even though a limited number of fields were sampled, the results suggest a positive relationship between the number of previous pea and/or faba crops and the infestation with A. euteiches.

The assay used in this study did not detect the fungus in fields #7 and 11, where peas and faba beans had not been cropped for 3 and 6 years, respectively.
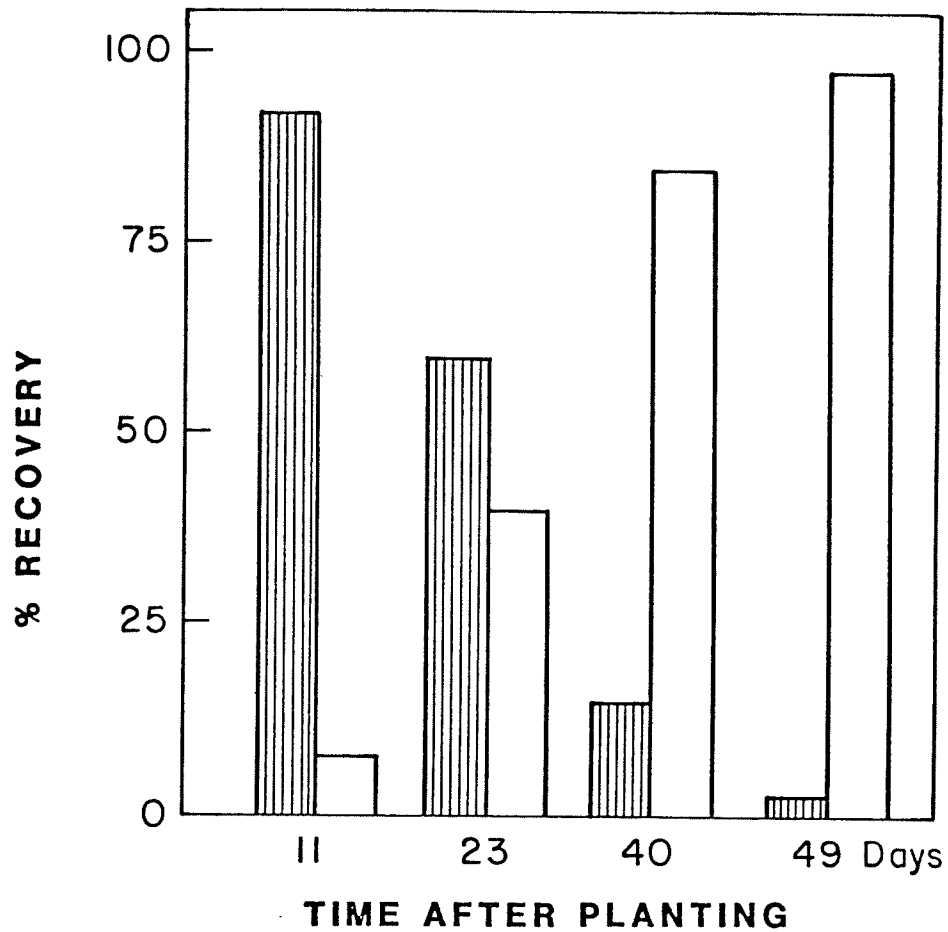
Figure 6. Effect of the growth stage of naturally infected
faba bean plants on the frequency of isolation of Apha-
nomyces euteiches. Fungal isolations were made on 2%
water agar from at least 30 plants of the cultivars Acker-
perle, Diana and PI 222128. Values represent means of
recovery from the three cultivars. ▥▥ A. euteiches ,
▢ Fusarium sp. and other fungi.

TABLE 3. Isolation of <u>Aphanomyces</u> <u>euteiches</u> from infested soil sampled at the University of Manitoba and assayed in the greenhouse.

Field No	Crops in Past 12 Years		Years without pea & faba bean	Isolation[*] of A. euteiches	DSI (%)
	Pea	Faba bean			
1	4	1	1	+	45.8
2	4	1	1	+	85.4
5	5	2	1	+[**]	na
6	3	1	1	+	87.5
7	2	1	3	-	0.0
8	2	2	2	+	65.6
11	4	0	6	-	0.0
18	3	3	1	+	87.5

[*]<u>A</u>. <u>euteiches</u> was isolated on 2% water agar from 2-week-old seedlings.

[**]<u>A</u>. <u>euteiches</u> isolated from field-infected seedlings.

DSI represents average of 4 pots, each planted with 4 seeds of the faba bean cultivar PI 222128.

na = not available.

of tolerant plants (rating 1-2). Plants of the tolerant accessions

also had many new white lateral roots.

Seedling blight and root rot were generally more severe in 1981

because of a relatively wet period early in the season and more

accessions were blighted than in 1980. Only 17% of the accessions

were rated 1 (no apparent symptoms) as shown in Figure 1b. A.

euteiches was isolated from the plants uprooted to observe root systems.

As in 1980, immunity was not observed. The following accessions were

identified as tolerant: 2N22, 2N26, 2N32, 2N37, 2N54, 2N58, 2N62,

2N71, 2N74, 2N83, 2N97, 2N106, 2N196, 2N217, 2N229, 2N355, 2N371,

2N475, 2N478, 2N480, 2N486, 2N487, 2N509, 2N511, 2N517, 2N518, 2N519,

2N520, 2N522, 2N523 and 2N534.

The accessions selected during the 1980 season for tolerance to

root rot again expressed tolerance in 1981 (Fig. 1c), with the exception

of nine accessions. The reactions of the selections in the three repli-

cations were not always consistent due to a non-uniform distribution of

the pathogen in the field. The use of PI 222128 as a susceptible control

allowed the detection of non-infested areas in the field and thus reduced

the possibility of selecting material that had escaped infection

(Appendix 3).

The following selections exhibited tolerance in each of the three

replications and were reselected: 2N19, 2N21, 2N37, 2N66, 2N68, 2N94,

2N104, 2N112, 2N114, 2N116, 2N134, 2N140, 2N218, 2N236, 2N296, DN75-38

(ERF) and DN76-8. Two to three bagged single plants from each selec-

tion were harvested separately.

In addition to their tolerance to root rot in the field, the selec-

tions 2N19, 2N94, 2N112 and 2N134 appeared to outyield all the

DISCUSSION

The pathotypes of A. euteiches recovered at the University of
Manitoba appear to be restricted to leguminous species, since non-
leguminous species were not infected by this fungus under greenhouse
and field conditions. Lathyrus ochroleucus, L. sativus, Lens culinaris,
V. calcarata, V. cracca, V. disperma, V. narbonensis and V. tetrasperma
were infected by the fungus in this study and do not appear to have been
previously reported as hosts of A. euteiches. The results of the host
range study generally agree with previous findings that several legumes
may serve as alternative hosts for A. euteiches in the absence of peas,
whereas non-leguminous species are not suitable hosts (Sherwood and
Hagedorn, 1962).

The faba bean isolate AE1 and the Wisconsin pea isolate P14 ,
although morphologically similar, had different virulence patterns
when compared on five legume cultivars (Fig. 1). Thus, they represent
distinct pathotypes of A. euteiches, isolate P14 lacking the ability to
cause disease on faba bean.

Three pathotypes were identified among the Manitoba isolates of
A. euteiches. The use of faba bean, pea and lentil cultivars as a
differential series in this study revealed the existence of a patho-
type avirulent to faba bean (AE3). This pathotype would not have been
detected had the test been carried out with the same pea differentials
used in race identification elsewhere (Beute and Lockwood, 1967). The
results of this study confirm previous findings regarding the physio-

logic specialization in A. euteiches (Beute and Lockwood, 1967;
Carley, 1970; Sundheim, 1972). The presence of different pathotypes
such as AE1 and AE3 in the same field may give A. euteiches the possi-
bility to recombine and evolve new pathotypes.

The origin of the infestation in the experimental fields at the
U of M with A. euteiches is not known. However, it is possible that
inoculum built up on field peas which were frequently grown as a green
manure crop over the past 20 years. This is supported by the fact
that the faba bean crop is of recent introduction and that the field
pea cultivars were only found to be moderately susceptible to this
pathogen in the field. Disease on field peas may have gone unnoticed
because, as revealed in the present study, symptoms were not observed
on aerial parts of field infected plants.

In addition to field #18 used as a source of naturally-produced
inoculum throughout the present study, several other fields at the U
of M Campus farm were found to be infested with A. euteiches, when
assayed for the presence of this pathogen. The root rot potential,
as expressed by the DSI, appears to be positively related to the
number of previous pea and/or faba crops. This generally agrees with
the findings of Temp (1966) that the number of pea crops, prior to the
assay, was the largest single factor affecting the root rot potential
of Wisconsin pea fields.

The tolerance of the cultivars Trapper and Ackerperle was over-
come at high air temperature (30° C). Whether this effect was due to
an increased activity of the fungus and/or temperature stress on the
seedlings, cannot be inferred from this study. These findings are not
in agreement with those of Lockwood (1960a) who found that soil temper-

ature of 28° C did not alter pea tolerance to A. euteiches.

Resistance of faba bean seedlings to A. euteiches was found to increase with age but not to the same extent in all cultivars. Seedlings of PI 222128 appear to have a longer period of susceptibility than those of Ackerperle (Fig. 3a, b). Significant differences in both fresh shoot weight losses and percentage of plants killed between PI 222128 and Ackerperle were only observed on 2-week-old inoculated seedlings. Similar results were obtained by Schneider (1956) for the black root disease of sugar beets, induced by A. cochlioides.

In this study, inoculum concentrations were found to influence disease development, as expressed by the DSI, percentage of plants killed or fresh shoot weight loss. Furthermore, typical symptoms were not induced on plants of the faba cultivar at inoculum levels of 2 X 10^4 zsp/plant or lower, although root infection was observed to occur with inoculum as low as 10^3 zsp/plant. These results suggest that a threshold in inoculum concentration was required for induction of typical symptoms as previously reported for A. cochlioides (MacWithey, 1965). However, studies by Carlson (1965) and Bhalla (1968) indicated that a threshold of inoculum concentration was not needed for infection of pea roots by zoospores of A. euteiches.

The percentage recovery of A. euteiches from field-infected faba bean seedlings appears to be strongly influenced by the age at which the seedlings are sampled for fungal isolation (Fig. 6), confirming previous observations (Thesis Section I).

The results of this study provide a better understanding on the seedling blight and root rot of faba bean in Manitoba and help in developing more effective control measures.

3. Screening Faba Bean (<u>Vicia faba</u>) for Tolerance to Root
Rot Caused by <u>Aphanomyces euteiches</u>

ABSTRACT

Yield losses of five licensed faba bean (<u>Vicia faba</u> L.) cultivars were found to be very high when planted in a field naturally infested with <u>Aphanomyces euteiches</u> Drechs. The cultivars Ackerperle and Herz Freya expressed tolerance to root rot, whereas Erfordia and Diana suffered 64 and 72% yield losses, respectively. Variability in tolerance to Aphanomyces root rot was also found in 350 accessions tested in this study. Selection among and within the accessions resulted in the identification of tolerant accessions. The following faba bean accessions were considered tolerant after two seasons of testing: 2N19, 2N21, 2N37, 2N66, 2N68, 2N94, 2N104, 2N112, 2N114, 2N116, 2N134, 2N140, 2N218, 2N236, 2N296, DN75-38 (ERF) and DN76-8.

Partial control of Aphanomyces root rot was achieved by seed treatment with Dowco 444 only and then for up to 5 weeks after planting. Seed rot and pre-emergence damping-off were not important during the two seasons of testing and the fungicides could not be evaluated for control of seed rot.

INTRODUCTION

Seedling blight and root rot of faba bean (<u>Vicia</u> <u>faba</u> L.) has been associated with <u>Aphanomyces</u> <u>euteiches</u> Drechs., <u>Rhizoctonia</u> <u>solani</u> Kuehn and <u>Fusarium</u> spp. in the experimental field plots at the University of Manitoba. Greenhouse tests showed that only <u>A</u>. <u>euteiches</u> was able to reproduce the symptoms observed in the field (Thesis Section I).

Field infected plants were either killed in the seedling stage or severely stunted with premature yellowing of the foliage. Many cultivars of faba bean and other legumes were found to be very susceptible to <u>A</u>. <u>euteiches</u> in the field and greenhouse studies (Thesis Sections I and II).

This long-lived soil-borne fungus is known for its pathogenicity to peas, in which it causes very heavy losses (Jones and Drechsler, 1925). Very few attempts have been made to control the common root rot of pea with fungicide seed treatments and effective control has not been achieved by this method (Papavizas and Ayers, 1974). However, soil treatments with Dexon at a rate of 30 lb per acre effectively controlled the disease but the rate of application was not economically feasible (Mitchell and Hagedorn, 1971). The lack of success and the prohibitive cost of chemical control made the search for genetic resistance in peas an attractive alternative. Partial resistance has been reported by several workers (Johnson, 1953; King and Cho, 1962; Cho and King, 1963; Lockwood, 1960a, b; Shehata <u>et</u> <u>al</u>., 1976). However, immune or highly

resistant pea cultivars have not been released from the screening or breeding programs.

In view of the potential economic importance of the disease, five licensed cultivars and some 350 faba bean accessions from different regions of the world were evaluated for their reaction to A. euteiches in the field. The effectiveness of selected fungicide seed treatments was also investigated.

MATERIALS AND METHODS

An experimental field with a history of seedling blight and root rot of faba bean was used throughout this study and referred to as the disease nursery. The disease in the field was shown to be caused by a complex of root pathogens, including pathogenic R. solani and A. euteiches (Thesis Section I). The field had been cropped with faba bean since 1978. Fertilizer was not applied during this period but crop residues were plown under at the end of each season. Plots were sown with a mechanical seeder and the seed was inoculated with a commercial culture of Rhizobium leguminosarum (Frank) at the time of planting.

Assessment of Yield Loss

The licensed cultivars Ackerperle, Aladin, Diana, Erfordia and Herz Freya were planted in the Aphanomyces infested field and in a non-infested field at the University of Manitoba during the first week of May 1981. The plots consisted of four rows, 3-m-long and 30-cm apart and planted with 50 seeds per row. Nine replications of each cultivar were planted at each location in a randomized complete block design. Fresh shoot weights of plants from 2-m of a middle row of each plot were taken 3-months after planting. At this stage, the plants were still green but had formed pods. To estimate yield losses, two 2-m-rows were harvested at maturity, air dried for 3 weeks and threshed. Seed yield was recorded for each plot.

Screening and Selection for Tolerance

In 1980, a total of 155 accessions (Appendix 6) and in 1981, 196 (Appendix 7) were planted in single 1-m-row plots in an Aphanomyces infested field. Two months after planting, the accessions were rated for disease on a visual scale ranging from 1-4, where 1 = no apparent symptoms, 2 = normal growth but premature yellowing of the lower leaves, 3 = stunting and extensive yellowing of the foliage and 4 = very pronounced stunting and most plants dead. The limited number of seeds planted did not allow uprooting of large numbers of plants for scoring root systems, particularly in the tolerant accessions which were to be saved for seed production. However, a few seedlings were uprooted to assess the presence of A. euteiches. Most of the accessions were not uniform, possibly due to the partially-outcrossing nature of faba beans (Poulsen, 1975). Susceptible plants within a generally tolerant accession were discarded prior to harvest and only outstanding plants were harvested.

Thirty-eight selections (Appendix 3) from the 1980 accessions were tested in 1981. Each selection was sown in a 3-m-row (25 to 30 seeds) flanked on each side by one row of the susceptible PI 222128 to verify the presence of the pathogen and to detect potential disease escape. Three replications of each accession were planted in a randomized complete block design. Rating for disease was done as described for the accessions. Furthermore, plants from accessions without symptoms (i.e. class 1) located between severely infected plants of PI 222128 within each selection were enclosed with a nylon mesh bag to prevent cross pollination by bees.

Seed Treatments with Fungicides

In 1980, seven fungicides were applied as a slurry to the seeds of the cultivar Ackerperle at the rates specified in Table 3. The plots consisted of three rows, 3-m-long and 30 cm apart. Two sets of the same experiment were planted at 1-month intervals in the disease nursery and a third set near Beausejour in Eastern Manitoba on land where root rot had been reported in the crop the previous year (Thesis Section I).

In 1981, the fungicides were used to treat seeds of the cultivar Diana instead of Ackerperle, which was found to have some field tolerance to A. euteiches. In the water-soaked treatments, the fungicides were added to a given amount of water and the seed soaked in the solution until all the liquid was absorbed. The seed was then dried and packaged. Acetone was used as a carrier in seed treatment with Terrazole. The fungicide was added to the acetone and the seeds soaked in the solution for 2 hours. The acetone was then allowed to evaporate in a fume hood. Plot sizes and design were similar to the 1980 experiment.

Plants from each plot were rated for disease and fresh shoot weights taken 5 and 8 weeks after planting. The disease was rated on a scale of 0-4 adapted from the scale described for pea by Smith and Walker (1941), where 0 = white healthy roots, 1 = cortical black discoloration on tap root, 2 = black discoloration on the tap and lateral roots and epicotyl, but no decay, 3 = black areas soft, epicotyls water-soaked and 4 = tissues disintegrated. A disease severity index (DSI) was computed according to the method of Sherwood and Hagedorn (1958):

$$DSI\ (\%) = (\sum_{i=0}^{4} ni\ Xi / \sum_{i=0}^{4} ni) \times 25$$

where Xi = class value (0, 1, 2, 3, 4) and ni = number of plants in class Xi.

RESULTS

Effect of Disease on Yield and Fresh Shoot Weights

Yields of the five cultivars were not significantly different in the non-infested field (Table 1). This was not the case, however, in the infested field where yields of Ackerperle, Herz Freya and Aladin were significantly superior to those of Erfordia and Diana. The percentage reduction in yield computed for each cultivar showed that Diana and Erfordia suffered significantly more losses than the three other cultivars. Fresh shoot weights, as well as the percentage reduction in fresh shoot weights, showed similar trends (Table 2).

Evaluation of Faba Bean Accessions and Selections

The season was particularly dry in 1980 and faba bean root rot was not severe on cultivars such as Ackerperle. However, 46% of the accessions were rated 3 and 4 (Fig. 1a) (i.e. extensive stunting, yellowing and death). Several accessions were entirely blighted during the first month after planting. The following accessions did not show yellowing nor stunting and were considered tolerant: 2N43, 2N296, 2N101, 2N23, 2N15, 2N19, 2N20, 2N98, 2N342, 2N10, 2N134, 2N240, 2N37, 2N428, 2N26, 2N109, 2N121, 2N96, 2N63, 2N242, 2N21, 2N236, 2N411, 2N18, 2N94, 2N112, 2N113, 2N114, 2N239, 2N123, 2N2, 2N18 and 2N11.

Immunity to infection by A. euteiches was not observed in plants of any of the accessions. Uprooted plants showed a black discoloration of the root system. The discoloration was extensive and deep in roots of susceptible accessions (categories 3 and 4) and superficial on roots

TABLE 1. Effect of Aphanomyces root rot on the yield
of five licensed faba bean cultivars.

	Non-infested field (g)	Infested field (g)	% Reduction[+]
Ackerperle	406.1 a	218.3 a[*]	46.2 a[*]
Herz Freya	388.9 a	220.6 a	43.2 a
Erfordia	441.0 a	156.1 b	64.6 b
Aladin	455.2 a	212.0 a	53.2 a
Diana	389.0 a	107.7 c	72.3 b
LSD .01		14.6	11.4

Mean values of 8 replications for dry grain from two,
2-m-rows.

[+] % Reduction = (1- infested/non-infested) X 100.

[*] Treatments with same letter in the same column are not
significantly different at p = .01 (Duncan's Multiple
Range Test).

TABLE 2.   Effect of Aphanomyces root rot on the fresh
     shoot weight of five licensed faba bean cultivars.

	Infested field (g) [+]	Non-infested field (g) [+]	% Reduction [++]
Ackerperle	1298.8 a[*]	2201.3 ab[*]	41.0 a[*]
Herz Freya	1190.0 a	2058.6 bc	42.2 a
Erfordia	1193.6 a	2475.0 a	51.8 a
Aladin	1073.6 a	2350.0 ab	54.3 ab
Diana	717.5 b	1816.3 c	60.5 b
LSD .01	323.6	323.6	14.6

+ Fresh shoot weight of plants in 2 m row/plot data
  represent averages of 9 replications.

++ % reduction in fresh shoot weight = (1- infested/
  non-infested) X 100.

*Treatments with same letters, within the same column,
  are not significantly different at p = .01 (Duncan's
  Multiple Range Test).
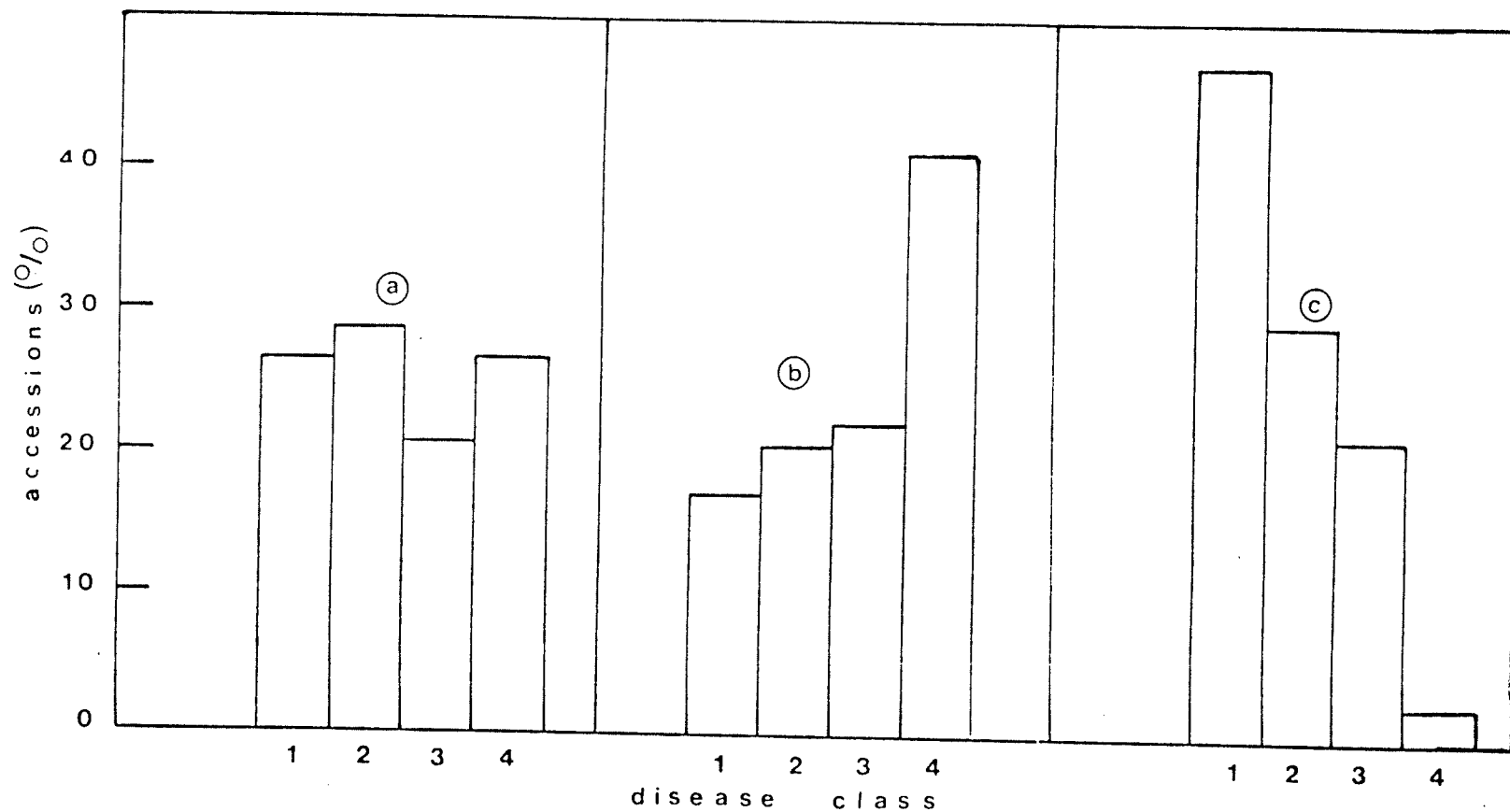
Figure 1. Frequency distribution of faba bean accessions in response to Aphanomyces root rot. a) 155 accessions tested in 1980, b) 196 accessions tested in 1981 and c) 38 selections from the 1980 program re-tested in 1981. Classes 1 and 2 represent tolerance and classes 3 and 4 represent susceptibility.

licensed cultivars tested in 1981 in the same infested field (Table 3).

Effect of Fungicide Seed Treatments on Disease Development

Root rot was only moderately severe on the cultivar Ackerperle in the plots at the U of M in 1980 and that damage was not great was probably due to the fact that the cultivar Ackerperle had some tolerance to A. euteiches under field conditions (Appendices 6 and 8; Tables 1 and 2). Significant differences in seedling emergence (stands) were not observed at the U of M for both dates of planting and also in the commercial field near Beausejour (Table 4).

In 1981, differences between stands were again not significant (Table 5). The experiment revealed that Ridomil had some phytotoxic effects when applied as a water soak treatment at the rate specified in Table 5. This property had not been observed in 1980 when the fungicide was applied as a slurry. Other treatments were not phytotoxic.

The differences between treatments both in terms of disease severity index (DSI) and fresh shoot weight/plant were significant at the first date of disease rating (5 weeks after planting) for both applications of Dowco 444 (Table 5). The fungicide was effective in restricting the disease, although the roots had the typical black discoloration caused by A. euteiches as indicated by the isolation of the fungus from infected roots. The differences between the Dowco 444 treatments and the remaining treatments were significant for the DSI at the 5 and 1% levels, but not for the fresh shoot weight/plant (Table 5). At the flowering stage and later, the plants of all plots were stunted and had a premature yellow foliage. The plots were not harvested because seed set was very low or nil in all treatments including Dowco 444.

TABLE 3. Comparative yield of four selections and five licensed faba
    bean cultivars in an Aphanomyces infested field.

	Yield (g)[*]		
	Infested		Non-infested
	Plot mean	Highest plot	
Tolerant selections			
2N134	327.4		
2N112	317.4		
2N19	293.8		
2N94	267.0		
2N140	251.8		
Cultivars			
H. Freya	220.5	320.0	388.9
Ackerperle	218.3	297.0	406.1
Aladin	212.0	289.0	455.2
Erfordia	156.1	174.0	441.0
Diana	107.7	182.0	389.0

*Values for 1 replication (20-25 plants) for the selections and the
mean of 9 replication (35-40 plants/replication) for the licensed
cultivars

TABLE 4. Effect of fungicide seed treatments on the emergence of the faba bean cultivar Ackerperle in soil naturally infested with _Aphanomyces_ _euteiches_ and _Rhizoctonia_ _solani_.

Fungicide	Rate % ai	University of Manitoba[+]		East Manitoba
		May 15	June 15	May 16
Control	0	100.0	100.0	100.0
Delsene 40 F	.2	95.0	91.0	112.9
Terrachlor 75 W	.1	104.9	100.4	116.6
Demosan 65 W	.1	100.9	102.7	111.7
Delsene 40 F + Terrachlor 75 W	.1	87.4	98.2	110.5
Delsene 40 F + Demosan 65 W	.1	92.8	99.9	121.6
Ridomil 25 WP	.2	94.5	98.3	108.7
Ridomil 25 WP + CGA 64251	.02	94.4	101.4	121.4
DPX 1015 F	.1	96.5	94.6	112.1
Vitaflo 80 W	.1	98.6	101.5	116.3
		NS	NS	NS

[+]Values represent the average of 9 replications.

NS = non-significant.

Values were converted into % of non-inoculated control.

TABLE 5. Effect of fungicide seed treatments on the stand, disease severity
index and fresh shoot weight of the cultivar Diana in soil naturally
infested with _Aphanomyces_ _euteiches_ and _Rhizoctonia_ _solani_.

Fungicide	Rate (ai) (%)	Emergence[*] (% control)	1st Rating[+]		2nd Rating	
			DSI (%)	Fresh W[++]	DSI (%)	Fresh W
Control	0	100.0 a	58.45 b	4.7 c[**]	61.47 c	10.6 a
Delsene 40 F	.2	109.8 a	57.30 b	5.2 bc	60.50 c	10.7 a
Vitaflo 80 W	.1	116.8 a	53.66 b	5.6 bc	56.74 bc	10.4 a
Terrazole Sl.	.1	110.4 a	53.48 b	5.4 bc	57.29 c	11.2 a
Captan	.1	99.1 a	57.59 b	4.9 bc	57.96 c	10.1 a
Terrazole acetone	.1	112.6 a	54.68 b	4.7 c	59.55 c	10.4 a
Dowco 444 Sl.	.6 ml/10 g seed	103.9 a	40.28 a	7.6 a	50.62 ab	13.4 a
Dowco 444 W.S.	"	118.6 a	40.45 a	6.8 ab	44.72 a	11.7 a
Ridomil	.2	53.7	-	-	-	-
LSD .01		NS	8.31	1.5	7.55	NS

+   1st date of rating, 5 weeks after planting; 2nd date of rating, 8 weeks after planting.

+ +Fresh shoot weight/plant.

[*] Data represent average of 6 replications.

[**] Treatments with same letters, in the same column, are not significantly different
at p = 0.01 (Duncan's Multiple Range Test).

DISCUSSION

Yield losses, as well as losses in fresh shoot weight, were very
high for all commercial cultivars tested, although losses in Herz
Freya, Ackerperle and Aladin were significantly less than in Erfordia
and Diana (Table 1). It is difficult to ascribe the losses to the
pathogen only because soil analysis at the end of the season indicated
that the soils of the infested and non-infested fields differed substan-
tially in nitrogen content (29.0 vs 63.0 kg/ha, respectively). Growth
of faba bean is not likely to be greatly influenced by soil nitrogen since
plants from seeds inoculated with Rhizobium bacteria were found to
nodulate well and fix large amounts of atmospheric nitrogen (Candlish
and Clark, 1975). However, the root systems of infected plants were
likely impaired or not functional. The yield and fresh shoot weight
losses induced on the commercial cultivars in the field, in this study,
do not appear to be excessively high compared to the fresh shoot weight
losses in plants infected under greenhouse conditions (Thesis Section II)
or to losses caused by A. euteiches on peas in commercial fields
(Papavizas and Ayers, 1974).

The variability in reaction to infection by A. euteiches observed
among the licensed cultivars (Table 1) was also found in the 350 faba
bean accessions evaluated in the field. Although immunity was not
observed, the tolerance and susceptibility of the plants were clearly
expressed in the 1981 season, which was particularly favorable for
Aphanomyces root rot because of high precipitation. Selection against

root rot in 1980 was effective in identifying tolerance among the faba

bean accessions. This is supported by the fact that 76% of the acces-

sions selected in 1980 expressed tolerance to root rot in 1981 (Fig. 1c),

whereas only 37% of the accessions tested for the first time were found

to be tolerant (Fig. 1). Furthermore, the yielding ability of four

selected accessions was apparently not seriously affected by Aphanomyces

root rot since they outyielded all the licensed cultivars (Table 5).

The potential usefulness of the four tolerant accessions to a breeding

program requires further evaluation.

The lack of significant differences between stands of all fungi-

cides seed treatments and non-treated plots over two seasons of testing

indicates that seed rot activity and pre-emergence damping-off were

not important in spite of the fact that R. solani was present in the

experimental field plots at the U of M. It is likely that the inoculum

density of R. solani in the field was low and/or that the most prevalent

forms of this fungus were not pathogenic to faba bean seeds. This is

supported by the fact that some isolates of the fungus were not able

to infect and rot seeds of faba bean when tested in the greenhouse

(Thesis Section I).

Partial control of Aphanomyces root rot was achieved for up to 5

weeks when seeds of the cultivar Diana were treated with Dowco 444. It

is likely that the fungicide restricted the spread of the fungus within

the host plant but did not prevent infection as indicated by the typical

root discoloration and a DSI of 40% in the first rating (Table 5).

Because Dowco 444 provided temporary control of Aphanomyces root rot in

the field, the combined effect of the fungicide and host tolerance should

be evaluated further under greenhouse and field conditions.

GENERAL DISCUSSION

In this study, seedling blight and root rot of faba bean were found to be associated with Aphanomyces euteiches, Rhizoctonia solani and Fusarium sp. Pathogenicity tests showed that isolates of each fungal species were able to infect faba bean seedlings. Furthermore, symptoms induced singly by A. euteiches and R. solani may be similar, indicating that the disease cannot be attributed to either one of the pathogens on a visual basis only. It is, therefore, suggested that infected root tissue be observed microscopically for fungal structures to help select the appropriate method(s) for fungal isolation.

Interaction between the organisms associated with the root rot complex, in this study, has not been investigated. The very common presence of non- or low-pathogenic Fusarium spp. in infected roots in U of M experimental plots suggests that the relationship of these Fusarium sp. to faba bean root rot is more than coincidental and requires, therefore, further investigations. Available information seem to indicate that "minor" pathogens (i.e. unable to cause disease when inoculated alone) may be responsible for yield losses, and may also interact with other "minor" and "major" pathogens to influence disease development (Salt, 1979). A better understanding of the interactions of A. euteiches, R. solani and Fusarium sp. in the U of M fields is likely to facilitate the making of control measures for faba bean root rot.

Several faba bean accessions were identified for their tolerance to the root rot complex in a field where A. euteiches was the most important pathogen (Thesis Sections I and III). The scale used to score the aerial part of a whole plot of faba bean for disease has not been standardized with the scale used for rating roots, therefore, the tolerant selections should be evaluated further to ascertain the nature of the reaction. It would be desirable to be able to distinguish between low and high levels of tolerance based on the extent of root rot.

Greenhouse tests indicated that seedlings of the cultivars Ackerperle and PI 222128 became resistant to A. euteiches as they reached 2 and 3 weeks, respectively (Thesis Section II). This suggests that greenhouse inoculation may be performed on 2-week-old seedlings in order to eliminate the most susceptible accessions.

Physiologic specialization in A. euteiches was demonstrated in this study and has been previously reported (King and Bissonette, 1954; Sherwood and Hagedorn, 1962; Carlson, 1965; Beute and Lockwood, 1967; Sundheim, 1972; Reidings and Zettlers, 1973). The presence of different pathotypes (such as AE1 and AE3) in the same field suggest that other pathotypes might still be recovered through screening of isolates from faba bean and other leguminous species. Whether pathotypes of A. euteiches can anastomose and give rise to new recombinant forms is not known. As pointed out by Papavizas and Ayers (1974), this area of study is devoid of information, in spite of the fact that A. euteiches is known for more than 50 years and is still a limiting factor for pea cultivation in many regions.

Surveys of faba bean root pathogens in Manitoba, and particularly

of <u>A</u>. <u>euteiches</u>, are needed to obtain information about the distribution of the fungus in the field pea and faba bean growing areas of Manitoba. Such information would also be helpful in preventing inoculum from building up to the destructive level observed at the U of M (Thesis Section III) Since efficient methods of direct recovery of <u>A</u>. <u>euteiches</u> from soil have not been devised, it is suggested that plants of susceptible faba bean (PI 222128) and peas (Little Marvel) be used as baits in laboratory, greenhouse or field tests. The rapid black discoloration of the root of faba bean in response to infection facilitates the early detection of disease and the isolation of <u>A</u>. <u>euteiches</u>, as revealed in this study (Thesis Section II).

A partial control of Aphanomyces root rot of faba bean was achieved for up to 5 weeks through seed treatment with Dowco 444. The treatment did not, however, prevent infection of the seedlings by the fungus at an early stage. Since there are apparently no reports on the control of <u>A</u>. <u>euteiches</u> with fungicide seed treatments in peas, it is suggested that further testing be carried out with chemicals such as Dowco 444, particularly in combination with tolerance and dinitroaniline and trifluralin herbicides. These herbicides have been found to provide substantial control of the common root rot of pea by direct effect on the pathogen (Teasdale <u>et</u> <u>al</u>. 1979; Jacobsen and Hopen, 1981).

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* Other= BARDNER, R.,BRIGGS G.G., BROMILOW R.H., COCKBAIN A.J., DAY J.M., FLETCHER K.E., LEGG B.J., ROUGHLEY R.G., SALT S.A., SIMPSON H.R., WEBB R.M., WITTY J.F., and YEOMAN D.P.

APPENDIX 1. Peptone PCNB medium for isolation of _Fusarium_ sp.
        (Nash and Snyder, 1962)

Difco peptone	15.0 g
Agar	20.0 g
KH_2PO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Pentachloronitrobenzene (PCNB)	1.0 g
Streptomycin	300 ppm

APPENDIX 2. Selective Medium for _Rhizoctonia solani_
        (Ko and Hora, 1971).

K_2HPO_4	1.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
KCl	0.5 g
$FeSO_4 \cdot 7H_2O$	10.0 mg
$NaNO_2$	0.2 g
Gallic acid	0.4 g
Dexon	90.0 mg
Chloramphenicol	50.0 mg
Streptomycin	50.0 mg
Agar	20.0 g
Water (distilled)	1.0 l

APPENDIX 3. Procedure followed for the production of zoospores
   by <u>Aphanomyces</u> <u>euteiches</u>.

1- Inoculate 2 % water agar plate with mycelium of <u>A</u>. <u>euteiches</u>.

2- Flood the agar with maltose (3%) peptone (1%) broth.

3- Incubate at room temperature (22°C) for 4-5 days.

4- Remove broth and wash with tap water twice, then cover
   the mycelium with tap water for 1-2 hours.

5- Replace tap water with distilled water and check after 6 hours.

APPENDIX 4. Staining procedure used in the identification of Rhizoctonia
   <u>Rhizoctonia</u> <u>solani</u> (Burpee <u>et</u> <u>al</u>, 1978)

1- The fungus is grown on PDA for 4 days.

2- 7-mm mycelial plugs are cut from the edge and transfered to the
   center of Petri dishes containing 1.5 % water agar.

3- When culture at ca. 1cm from the edge of water agar plate, a
   drop of either .5 % aniline blue or trypan blue is placed
   directly on the mycelium midway between the center and the
   edge of the culture.

APPENDIX 5. Procedure followed for soil sampling and testing for
   the presence of <u>Aphanomyces</u> <u>euteiches</u> (Sherwood and Hagedorn, 1958).

1- Collecting soil samples.
       + walking in ziz-zag course
       + Every 55-75 paces a trowelful of soil is taken to a depth
         of ca. 15cm and placed in a polyethylene bag.

2- Testing in the greenhouse.
       + Soil dispensed in pots and planted with seeds of a susceptible
         pea (replaced by faba bean in this study).
       + Soil kept normally moist until 2 first leaves fully expanded,
         then soil held near saturation.

APPENDIX 6.   Reaction of faba bean accessions to Aphanomyces root
     rot in 1980 in field #18 at the U of M campus farm.

1 - No apparent symptoms

2N43, 2N296, 2N101, 2N23, 2N15, 2N19, 2N20, 2N98, 2N342, 2N10, 2N134,

2N240, 2N37, 2N428, 2N26, 2N109, 2N121, 2N96, 2N63, 2N242, 2N21, 2N236,

2N411, 2N18, 2N94, 2N112, 2N113, 2N114, 2N239, 2N123, 1, 12, 13, 16,

18, 2N2, 2N18, 2N11, Ackerperle, Herz Freya.

2 - Normal growth but yellowing of lower leaves

2N122, 2N396, 2N124, 2N297, 2N5, 2N138, FIDRIN, 2N73, 2N245, 2N3, 2N12,

2N41, 2N6, 2N119, 2N382, 2N304, 2N1, 2N398, 2N263, 2N235, 2N8, 2N109,

2N189, 2N333, 2N435, 2N436, 2N100, 2N340, 2N80, 2N382, 2N218, 5, 6, 7,

2N9, 2N69, 2N67, 2N121, 2N70.

3 - Stunting and extensive yellowing

2N246, 2N46, 2N402, 2N405, 2N313, 2N452, 2N42, 2N34, 2N133, 2N419, 2N238,

2N204, 2N432, 2N429, 2N396, 2N209, 2N424, 2N124, 2N408, 2N294, 2N320,

2N369, 2N412, 2N247, 2N407, 2N13, 2N38, 2N391, Diana, Erfordia, Triple
white.

4 - Very pronounced stunting and most plants dead

2N256*, 2N448, 2N410, 2N456*, 2N260, 2N262, 2N228, 2N358, 2N451, 2N447,

2N296, 2N455, 2N302, 2N424, 2N265, 2N264, 2N399, 2N328, 2N429, 2N257,

2N367, 2N406, 2N413, 2N423*, 2N402*, 2N411*, 2N266, 2N421*, 2N405, 2N246,

2N378, 2N250, 2N418, 2N452, 2N409, 2N426, 2N433, 2N440, 2N431, 2N450,

Kodrin, PI 222128.

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*Accessions which did not survive more than 4 weeks after planting.

APPENDIX 7.   Reaction of faba bean accessions to Aphanomyces root
    rot in 1981 in field #18 at the U of M campus farm.

1 - No apparent symptoms

2N22, 2N26, 2N32, 2N37, 2N54, 2N58, 2N62, 2N71, 2N74, 2N83, 2N97, 2N106,

2N138, 2N196, 2N217, 2N229, 2N346(?), 2N355, 2N371, 2N475, 2N478, 2N480,

2N486, 2N487, 2N509, 2N511, 2N517, 2N518, 2N519, 2N520, 2N522, 2N523,

2N534.

2 - Normal growth but premature yellowing of the lower leaves

2N44, 2N45, 2N53, 2N59, 2N65, 2N76, 2N82, 2N96, 2N110, 2N111, 2N134(?),

2N141, 2N148, 2N155, 2N195, 2N197, 2N218, 2N219, 2N227, 2N43, 2N295,

2N297, 2N321, 2N392, 2N425, 2N470, 2N477, 2N482, 2N483, 2N485, 2N489,

2N502, 2N506, 2N507, 2N508, 2N510, 2N516, 2N526, 2N527, 2N529.

3 - Stunting and extensive yellowing of the foliage

2N14, 2N34, 2N52, 2N72, 2N78, 2N89, 2N117, 2N200, 2N202, 2N203, 2N215,

2N220, 2N232, 2N237, 2N244, 2N252, 2N288, 2N291, 2N299, 2N307, 2N311,

2N312, 2N319, 2N331, 2N343, 2N368, 2N377, 2N404, 2N437, 2N467, 2N479,

2N488, 2N491, 2N493, 2N497, 2N499, 2N504, 2N514, 2N513, 2N524, 2N530,

2N531.

4 - Very pronounced stunting and most plants dead

2N4, 2N29*, 2N35*, 2N47, 2N50, 2N57, 2N67, 2N84, 2N85*, 2N86, 2N91*,

2N94, 2N156, 2N177, 2N206*, 2N211, 2N221, 2N222*, 2N223, 2N224*, 2N233*,

2N248, 2N250, 2N272*, 2N276, 2N277*, 2N278, 2N284*, 2N286*, 2N292*,

2N293, 2N303*, 2N316, 2N317, 2N318*, 2N322, 2N323, 2N326, 2N334, 2N335*,

2N337*, 2N345*, 2N348, 2N351*, 2N352*, 2N353, 2N354*, 2N356*, 2N357*,

2N370, 2N372*, 2N381, 2N384*, 2N390*, 2N394(?), 2N408, 2N412*, 2N414,

2N416, 2N422, 2N431, 2N434*, 2N444, 2N450*, 2N451, 2N465, 2N469, 2N471*,

2N472*, 2N473, 2N474, 2N484*, 2N490*, 2N492*, 2N494(?), 2N496(?), 2N503*,

2N505, 2N513, 2N521, 2N533*.

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*Accessions which did not survive more than 4 weeks after planting.

APPENDIX 8. Disease score of 38 accessions selected in 1980 and
tested in 1981.

	I	II	III	Final Score[**]
2N62	4/1	3-4/2	2-3/1	2
IZ (Diana 76-8)	4/1	4/2	4/1	2
IS (DN75-25 ERF)	4/3	3/1	4/3	3
B118	4/3	4/3	4/2 (Stunted)	3
*I4 (DN76-8 SPS)	1/1	4/1	4/ (3 plants)	1
*I12 (DN75-38 ERF)	4/1	3-4/1	-	1
Diana	4/3	3/1	-	3
*2N116	4/1	4/1	3/1	1
*2N68	4/1	4/1	-	1
2N120	4/2	4/3	-	3
*2N140	4/1	4/1	3/1	1
2N101	3/2	4/3	4/?	3
2N342	3/2	4/4	4/1-2	4
*2N66	4/1	4/1	4/1	1
2N113	4/1	4/2	-	2
Ackerperle	1/1	4/1	2-3/1	1
*2N114	2-3/1	3/1	-	1
*2N94	3/1	4/1	2/1	1
2N31	4/1	4/2	-	2
*2N236	4/1	2-3/1	1-2/1	1
2N63	4/1[+]	3-4/2	-	2
*2N296	4/1	3-4/1	-	1
*2N37	2-3/1	4/1	4/1	1
2N15	2/1	4/2	3-4/2	2
*2N218	3/1	3/1	4/1 (5 plants)	1
*2N112	4/1	4/1	3-4/1	1
*2N20	4/1	4/1	4/2 (Diana)	2
2N43	2/1	3/2	-	2
2N98	2/1	4/2	-	2
2N104	3/1	4/1	4/1	1
2N109	2/1	3-4/3	-	3
2N242	4/1	4/2	-	2

(Continued)

APPENDIX 8. (Continued)

	I	II	III	Final Score[**]
2N18	4/1	3-4/2		2
2N23	3/1	4/3	4/3	3
*2N19	2/1	3-4/11	3/1	1
2N340	3/1	4/2	4/2	2
2N96	4/3	4/2	3/3	3
2N121	4/2	4/2	3/11	2
*2N134	4/1	4/1	4/11	1
*2N21	2/1	4/-	4/1	1

[+]PI 222128/selection.

[*]Selection harvested in 1981

[**]The final score is the rating of the most severely infected
replication. Scale from 1-4.

APPENDIX 9. Analysis of variance.

Virulence patterns among Manitoba isolates of A. euteiches.

Source of variation	df	SS	MS	F
Replications	3	2.35	0.78	0.87 NS
Isolates	2	33.87	16.94	19.03 **
Error a	6	5.35	0.89	
Cultivars	5	35.65	7.13	47.53 **
Isolates x cultivars	10	70.35	7.03	46.86 **
Error b	30	4.60	.15	

CV main plot = 33.07%

CV sub plot = 13.73%

NS = non-significant at p = .05.
** = significant at p = .01.

APPENDIX 10. Analysis of variance.

Effect of the age of seedlings at inoculation time on disease.

1 - Fresh weight loss

Source of variation	df	SS	MS	F
Total				
Blocks	2	129.45	64.72	19.49**
Age	2	8566.86	4283.43	1267.28**
Error (a)	4	13.52	3.38	
Cultivars	1	518.31	518.31	20.29**
Age x cultivars	2	1454.07	727.31	28.45**
Error (b)	6	159.27	25.54	

CV (%) Main plot (age)   : 2.94
CV (%) Subplot (varieties): 8.09

2 - % plants killed

Source of variation	df	SS	MS	F
Total				
Blocks	2	89.48	44.74	.84 NS
Age	2	14585.54	7292.77	137.41**
Error (a)	4	212.28	53.07	
Cultivars	1	4255.95	4255.95	23.60**
Age x cultivars	2	1252.56	626.28	3.47 NS
Error (b)	6	1081.86	180.31	

CV (%) Main plot =
CV (%) Subplots =

NS = Non-significant at p = .05.
**Significant at .01.

APPENDIX 11. Analysis of variance.

Temperature effect on disease (Fresh weight loss).

Source of variation	df	MS	F
Replications	2	316.55	1.02 NS
Temperatures	3	18597.87	60.47**
Error a	6	307.53	
Cultivars	2	1076.50	15.20**
Temperatures x cultivars	6	262.03	3.70*
Error b	16	70.79	

% CV Main plot = 48.68

% CV Subplot   = 23.35

NS = Non-significant at p = .05.

*Significant at p = .05.

**Significant at p = .01.

APPENDIX 12. Inoculum density study - results.

	DSI (%)	% Plants killed	Fresh weight/ plant (g)	Fresh weight loss (%)
0	0.0 c[+]	0.0 d	3.46 ab	0.00
10^3	5.00 c	0.0 d	3.49 a	0.00
10^4	34.87 b	0.0 d	3.11 b	10.12
2.10^4	96.82 a	13.63 c	2.14 c	38.15
4.10^4	100.00 a	36.36 b	1.40 d	59.53
10^5	100.00 a	60.53 a	1.04 e	69.94
$\overline{X}$	56.11	18.42	2.44	
LSD .01	13.84	17.69	0.35	

Values represent the average of 5 replications.

[+]Treatments with same letters are not significantly different at p = .01 (Duncan's Multiple Range Test).

APPENDIX 13. Analysis of variance.

Inoculum density.

1 - (% plants killed)

Source of variation	df	SS	MS	F
Total	29	17739.78	611.72	
Treatments	5	15680.30	3136.06	32.44**
Blocks	4	126.27	31.57	0.32 NS
Error	20	1933.22	96.66	

CV (%) = 53.37

2 - Disease severity index (roots)

Source of variation	df	SS	MS	F
Total	29	60306.60	2079.54	
Treatments	5	58609.41	11721.88	197.97**
Blocks	4	512.90	128.22	2.16 NS
Error	20	1184.30	59.21	

CV (%) = 13.71

3 - Fresh shoot weight loss

Source of variation	df	SS	MS	F
Total	29	29.43	1.01	
Treatments	5	28.59		143.00**
Blocks	4	0.03	0.007	.18 NS
Error	20	0.80	0.04	

CV (%) = 8.19

NS = Non-significant.

**Significant at p = .01.

APPENDIX 14. Analysis of variance.

Assessment of yield loss.

1 - Yield in non-infested field

Source of variation	df	SS	MS	F
Total	44	200461.91		
Blocks	8	35599.91	4449.99	1.08 NS
Treatments	4	33526.58	8381.64	2.04 NS
Error	32	131335.42	4104.23	

CV (%) = 15.39

2 - Yield in infested field

Source of variation	df	SS	MS	F
Total	44	178756.44		
Blocks	8	46471.64	5808.96	4.35^{**}
Treatments	4	89552.44	22388.11	16.76^{**}
Error	32	42732.36	1335.39	

CV (%) = 19.95

3 - Yield loss

Source of variation	df	SS	MS	F
Total	44	10645.79		
Blocks	8	2711.76	338.97	4.42^{**}
Treatments	4	5479.54	1369.88	17.86^{**}
Error	32	2454.49		

CV (%) = 15.66

NS = Non-significant.
**Significant at p = .01.

APPENDIX 15. Fungicides 1980.

1 - University of Manitoba, 1st date

Source of variation	df	SS	MS	F
Total	89	13613.56		
Blocks	8	2162.16	270.27	2.17*
Treatments	9	2520.89	280.10	2.25*
Error	72	8930.51	124.03	

CV (%) = 10.14

2 - University of Manitoba, 2nd date

Source of variation	df	SS	MS	F
Total	89	6729.29		
Blocks	8	474.89	59.36	.90
Treatments	9	1543.73	171.53	2.62*
Error	72	4710.67	65.43	

CV (%) = 6.62

3 - Eastern Manitoba

Source of variation	df	SS	MS	F
Total	89			
Blocks	8		399.44	2.21*
Treatments	9		258.20	1.43 NS
Error	72		180.16	

CV (%) = 14.05

NS = Non-significant at p = .05.

*Significant at p = .05.

APPENDIX 16. Analysis of variance.

Fungicide experiment, 1981.

1 - Plant emergence

Source of variation	df	SS	MS	F
Total	47	11610.67		
Blocks	5	972.92	194.58	.88 NS
Treatments	7	2922.00	417.43	1.89 NS
Error	35	7715.75	220.45	

   CV (%) = 11.94

2 - Disease severity index, 1st rating

Source of variation	df	SS	MS	F
Total	47	3566.21		
Blocks	5	302.07	60.41	2.16 NS
Treatments	7	2287.12	326.73	11.70**
Error	35	977.02	27.91	

   CV (%) = 10.16

3 - Fresh shoot weights, 1st rating

Source of variation	df	SS	MS	F
Total	39	80.68		
Blocks	4	12.55	3.14	3.45*
Treatments	7	42.51	6.07	6.67**
Error	28	25.62	0.91	

   CV (%) = 17.83

NS = Non-significant at p = .05.
*Significant at p = .05.
**Significant at p = .01.

APPENDIX 17. Analysis of variance.

Fungicide experiment 1981, 2nd rating.

1 - Disease severity index

Source of variation	df	SS	MS	F
Total	47	2269.39		
Blocks	5	113.65	22.73	.98 NS
Treatments	7	1348.52	192.65	8.35**
Error	35	807.22	23.06	

CV (%) = 8.55

2 - Fresh shoot weights

Source of variation	df	SS	MS	F
Total	47	369.06		
Blocks	5	157.07	31.41	6.78**
Treatments	7	49.92	7.13	1.53 NS
Error	35	162.08	4.63	

CV (%) = 19.45

NS = Non-significant at p = .05.
**Significant at p = .01.