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A STUDY OF A FUSARIUM ROOT ROT OF LENTILS

by

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A thesis submitted to the School of Graduate Studies
in partial fulfillment of the requirements for the degree of
Master of Science

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TO, MY PARENTS

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ABSTRACT

Fungal isolates were recovered from a field of diseased lentils (*Lens culinaris* Medik.) in 1979, using the soil dilution plate method. *Fusarium* spp. were the most frequent fungi isolated. In subsequent pathogenicity tests *F. oxysporum* Isolate 82A was found to be highly virulent to the lentil variety, Laird.

In the summer of 1980, four varieties of lentils were used in field experiments in Ottawa. In naturally infested soil, *F. oxysporum* caused a large reduction in seed yield, particularly in the two large seeded varieties. Treatment with the systemic fungicide benomyl did not significantly improve yield of the variety Laird; however, seed yield was significantly greater in earlier than in later sowing dates.

A time course study on symptom development by *F. oxysporum* Isolate 82A showed that it invaded the roots preferentially at the sites of emergence of the secondary roots and caused extensive necrosis of the root tissues. Comparative studies with this isolate and other *Fusarium* spp. including other isolates of *F. oxysporum* recovered from diseased lentils confirmed that Isolate 82A was the most virulent and that the rate of symptom development in seedlings was greatest for this isolate. Lentil seedlings inoculated simultaneously with spore suspensions from *F. oxysporum* Isolate 82A and *F. solani* Isolate 196A developed less severe symptoms than plants infected with the most virulent isolate alone. The symptoms expressed by these plants were intermediate to those produced by either isolate alone.

Cytological studies on the penetration and subsequent development of *F. oxysporum* Isolate 82A showed that the hyphae penetrated the

epidermal cell layer directly at the zone of elongation. Penetration was not observed through the root apex or root hairs during the initial stages of infection. Prior to invasion, the cytoplasm of many epidermal cells appeared plasmolyzed suggesting that cell disorganization occurred in advance of hyphal penetration. Initially the pathogen grew only intercellularly in the cortex; however, after the tissue became injured or degraded due to the consequences of intercellular invasion, hyphal strands were also observed to have penetrated into the cells. Within 24 hours following inoculation the fungus had invaded the entire cortex; however, hyphae were not present in the vascular tissue. Observations 72 hours after inoculation revealed the presence of large cavities in the xylem parenchyma but there was no evidence of fungal invasion into the stele.

Cytological evidence in addition to the symptoms produced by Isolate 82A on lentil seedlings grown in aseptic culture, in pasteurized soil, and under field conditions suggests that this isolate is primarily a cortical root rot pathogen of lentils. This study is the first report of the occurrence of F. oxysporum as a pathogen of lentils in Canada.

RÉSUMÉ

En 1979, utilisant la méthode de dilution du sol sur boite de Pétri, des champignons ont eté isolés á partir d'échantillons récoltés dans un champs de lentilles malades: (*Lens culinaris* Medik.). L'espéce *Fusarium* s'est revelée étre la plus frequemment isolée. D'autre part, les tests de pathogénicité realisés sur l'isolat 82A de l'espéce *F. oxysporum* ont montré que ce champignon était trés fortement virulent pour la varieté de lentilles, Laird.

Au cours de l'été 1980, quatre varietés de lentilles ont été cultivées expérimentalement dans un champs de la région d'Ottawa. Sur un sol naturellement infesté, *F. oxysporum* s'est revélé étre la cause d'une importante diminution dans la production des graines et ceci particuliérement dans le cas des deux varietés produisant de grosses graines. Un traitement réalisé avec le fongicide systémique, benomyl n'a pas amélioré de façon significative le rendement de la varieté Laird; cependant, la production des graines s'est averée meilleure dans le cas d'une récolte précoce que dans celui d'une récolte tardive.

Une étude dans le temps, faite sur le developpement des symptômes causés par le champignon *F. oxysporum* Isolat 82A, a révelé un envahissement des racines, préferentiellement au niveau des sites d'émergence des racines secondaires; on a pu noter d'autre part une importante nécrose des tissus racinaires. Des études comparatives realisées entre cet isolat et d'autres *Fusarium* incluant d'autres isolats de *F. oxysporum* prelevés sur des lentilles malades ont confirmé que l'isolat 82A était le plus virulent et que le degré d'expression des

symptômes sur les plants de lentilles était le plus élevé. Des lentilles inoculeés simultanément avec des suspensions de spores provenant de *F. oxysporum* Isolat 82A et de *F. solani* Isolat 196A ont présenté des symptômes moins sevéres que les plantes uniquement infecteés avec l'espéce la plus virulente.

Des études cytologiques realisées sur la pénetration et le developpement de *F. oxysporum* Isolate 82A ont montré que les hyphes pénétraient la couche cellulaire épidermique directement au niveau de la zone d'élongation. Au cours des premiéres étapes de l'infection, la pénetration n'a jamais été observée au travers de l'apex ou des poils racinaires. Avant même l'invasion, le cytoplasme d'un grand nombre de cellules épidermiques est apparu plasmolysé ainsi qu'une désorganisation cellulaire précéde la pénétration des hyphes. Initiallement, le pathogéne ne se develloppe qu'entre les cellules du cortex, mais cependant, lorsqu'une degenerescence et une dégradation apparaissent dans le tissu eu raison de l'invasion intercellulaire, des fibres hyphales peuvent pénétrer à l'intérieur des cellules. Au cours des 24 heures suivant l'inoculation, le champignon envahit le cortex tout entier mais les hyphes ne sont jamais présentes dans le systéme vasculaire. Des observations réalisées 72 heures aprés l'inoculation ont revelé l'existence de grandes cavités dans le parenchyme xylémique mais aucune evidence d'invasion fongique dans la stele n'a pu être démontrée.

L'effect cytopathogénique ainsi que les symptômes produits par l'isolat 82A sur des lentilles cultivées en milieu aseptique,

sur sol pasteurisé et dans des conditions naturelles, suggèrent que cet isolat est l'agent pathogène de la pourriture racinaire corticale des lentilles. Cette étude met en évidence pour la première fois au Canada, l'existence de _F._ _oxysporum_ en tant qu'agent pathogène de lentilles.

INTRODUCTION

Lentils (*Lens culinaris* Medik.) are an Old World pulse cultivated extensively throughout the world. They are exceeded only by wheat, corn, rice and barley in harvested acreage (Robinson, 1975). One of the factors limiting their production is their susceptibility to a few important diseases and those incited by members of the genus *Fusarium* are considered to be the most serious, often resulting in considerable yield losses. Although various species of this ubiquitous genus have been implicated, *F. oxysporum* Schlecht. is the most widespread and serious pathogen. It has been reported as a pathogen of lentils in India, U.S.S.R., Czechoslovakia, Hungary, Argentina, Uruguay and the United States of America.

1.1 *Lens culinaris* Medik.

The genus *Lens* Miller, belongs to the family Leguminosae and to the subfamily Papilionoideae (Purseglove, 1968). Many taxonomists (Lawrence, 1966) consider this subfamily to be an independent family termed Papilionaceae (syn. Fabaceae). *Lens*, comprising five annual species (Ladizinsky, 1979a) is usually placed in the tribe Vicieae and is most closely affiliated with the section Ervum (Zohary, 1976). *L. culinaris* Medik. is the only cultivated species and is thought to have originated from the wild lentil *L. orientalis* (Boiss) M. Popov. (Barulina, 1930) although the possibility of gene flow from *L. nigricans* (Bieb.) Godr. cannot be minimized (Smartt,1976).

There is evidence to indicate that lentils were one of the first plants brought under cultivation by man (Pakistan, 1963). The remains of lentil seeds have been found in archeological digs throughout the Middle East suggesting that neolithic man was already familiar with this plant as early as 8000 B.C. (Ladizinsky, 1979b). The place of domestication of lentils is uncertain. Renfrew (1973), on the assumption that L. nigricans is the progenitor of the cultivated lentils concludes that southern Europe is the place where lentils evolved whereas other authors have suggested northern India (Barulina, 1930), the Near East (Zohary, 1972), and the Mediterranean (Vavilov, 1949).

Lentils are slender plants with an erect growth habit (Fig. 1). Germination is hypogeal; the flowers are predominantly self-fertilized (Smartt, 1976). Seeds, which are lens-shaped, are produced in broadly oblong pods, usually 1-2 per pod. There are two distinct subspecies based on seed size. Subspecies microspermae includes those cultivars with small pods and small seed (3-6 mm; 100 seed weight of 1.8 g); the large seeded lentils (6-9 mm; 100 seed weight of 5.4 g) are grouped in the subspecies macrospermae (Papp, 1980).

Lentils are grown extensively throughout the world although they are best adapted to the cooler temperate zones. Recent F.A.O. statistics indicate that lentils are cultivated on about 1.9 million hectares (ha) annually and that total production is greater than 1.2 million tonnes (F.A.O., 1978). India and Turkey are the largest producers of lentils followed by Syria, Spain, and then the United States of America, the largest lentil producer in the Western Hemisphere. Ninety-five percent of the U.S.A. production is centered in the Palouse area of eastern Washington State and northern Idaho (Youngman, 1968) where over 50,000 ha

Figure 1. Mature lentil plants var. Laird with flowers (white arrow) and mature seed pods (black arrow)

FIG. 1

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are sown to this crop annually. Commercial production of lentils in Canada was initiated in 1970 when 600 ha were grown in the Eatonia district of west-central Saskatchewan (Slinkard and Drew, 1980). In 1980, production increased to 44,000 ha with most of this acreage in Saskatchewan but with some acreage contributed by Manitoba and Alberta (Slinkard and Drew, 1981). Most of the Canadian lentils are grown under contract and yields average about 1,000 kg/ha with a reported high of 2,018 kg/ha (Manitoba, 1975).

There are two varieties of lentils licensed for use in Canada. Lentil variety Laird was developed at the Crop Development Centre, University of Saskatchewan and licensed in November 1978, following unsuccessful field trials with the varieties Commercial Chilean and Tekoa both of which were grown extensively throughout the Palouse area (Wilson _et_ _al_., 1971). Laird is a taller, later maturing, higher yielding and larger seeded lentil than Commercial Chilean (Slinkard and Bhatty, 1979) and is well adapted to Saskatchewan growing conditions.

Recently, a second lentil cultivar, designated Eston, was licensed in Canada. Eston is a small-seeded variety, which is shorter than Commercial Chilean, but produces more seed (Slinkard, 1981).

Lentil seeds are used primarily as a source of vegetable protein for human consumption. The percentage of protein in lentil seeds varies considerably and a value of 25% (dry weight basis) is considered representative (Smartt, 1976). Although lentil proteins are deficient in the sulphur containing amino acids, methionine and cystine, they contain more lysine and threonine than wheat and rice. (Bhatty and Slinkard, 1979; Saint-Clair, 1972). In addition to the use of the seeds for human food, lentil plants are often employed as a green manure (Pakistan, 1963) and in India, lentil husk, bran and straw are utilized as feed for livestock (India, 1962). The use of lentil plants as a forage crop in Canada is increasing (Slinkard, personal communication).

1.2 PREVALENCE AND DISTRIBUTION OF LENTIL DISEASES

Although lentil is a valuable pulse crop, there are few reports of diseases, especially from North America. Barulina (1930) noted that lentils were susceptible to mildew caused by *Erysiphe communes* ~~Grev~~. and *Peronospora lenti* Gaumann and to a rust produced by *Uromyces ervi* Winter. The first record of a soil-borne disease of lentils originated in the U.S.S.R. when in 1931, Prissyajnyuk described a seedling blight of lentils incited by *Fusarium oxysporum* Schlecht. f. sp. *lentis* (Vasudeva and Srinivasan) Gordon. Three years later the occurrence of this disease was noted from India (Anonymous, 1934). Subsequent descriptions of this disease have come from other parts of the world, including Argentina (Carrera, 1940), Czechoslovakia (Ujevic *et al*., 1965), Hungary (Fleischmann, 1937), United States of America (Wilson and Brandsberg, 1965), and Uruguay (Carrera and Noll, 1941).

The severity of the disease has been variable, with the extent of the mortality of plants ranging from 25 percent in the seedling stage (Kannaiyan and Nene, 1972) to 50 percent at the flowering and fruiting stage (Khare *et al*., 1972). In addition to *F. oxysporum*, Carrera and Noll (1941) reported that *Fusarium avenaceum* (Corda ex Fr.) Sacc. (= *Fusarium roseum* Lk. emend Snyd. & Hans. 'Avenaceum'), *Fusarium scirpi* Lamb. & Fautr. var. *acuminatum* (Ell. & Ev.) Wollenw. and *Fusarium culmorum* (W.G. Smith) Sacc. were associated with lentil wilt in Uruguay.

Fusarium roseum 'Avenaceum' causes a root rot of lentils grown in eastern Washington State, and although it is not considered severe under field conditions it may become damaging when the lentil crop follows bluegrass in rotation or when the soil has first been fumigated (Lin and Cook, 1979). Its lack of virulence under natural soil conditions is thought to be due to suppression associated with antagonistic soil microflora. McKenzie and Morrall (1975) reported that a root rot of lentil seedlings caused by *F. roseum* occurred in the lentil growing areas of Saskatchewan. This disease was not serious, affecting a mean of only 2.7% of the plants just prior to swathing.

Fusarium solani (Mart.) Appel & Wr. produces a very severe root rot of lentils in Egypt and may, in conjunction with other root pathogens, result in up to 83% pre-emergence damping-off. *F. solani* gave the highest percentage of post-emergence damping off (Shatla *et al*., 1975) under similar conditions. *F. solani* f. sp. *pisi* has also been isolated from lentils in Washington State (Lin and Cook, 1977) and tests showed that this isolate produced a black root rot, although it was not widespread.

In Iran, Kaiser and Horner (1980) isolated *F. oxysporum*, *F. roseum*, and *F. solani* from diseased lentil roots but in subsequent greenhouse pathogenicity tests they found that these three *Fusarium* spp. were nonpathogens. However, they observed that isolates of *Phythium ultimum* Trow and *P. aphaniderumatum* (Edson) Fitzp. recovered from lentil roots were highly pathogenic resulting in over 50% plant mortality. In contrast, *P. ultimum* failed to produce root rot symptoms in pathogenicity tests conducted by Lin and Cook (1977).

A collar rot, caused by *Sclerotium rolfsii* Sacc. has been reported on lentils by Pavgi and Upadhyay (1967) and Mathur and Deshpande (1968). Pavgi and Upadhyay (1967) noted a 25 percent or more loss in yield of lentils grown in northern India. Claudius and Mehrotra (1973) reported that the root rot incited by *S. rolfsii* was less common but more destructive than the wilt disease caused by *F. oxysporum*.

A species of *Rhizoctonia* has been reported on lentils in the U.S.A. by Wilson and Brandsberg (1965) and in Canada by McKenzie and Morrall (1975). A root rot of lentils, produced by *R. solani* Kuhn. was described by Shukla *et al*. (1972) and Kannaiyan and Nene (1975) from India.

Other reports of root diseases include a seedling blight of lentils caused by *Alternaria alternata* (Fr.) Keissler (Mishra and Rath, 1975) and root rots caused by *Macrophomina phaseolina* (Tassi) Goid. and *Helminthosporium* sp. Link (Khare *et al*., 1971).

McKenzie and Morrall (1975) have observed stem rots of lentils caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. and *Botrytis cinerea* Pers. ex. Fr. The latter pathogen, along with *B. parasitica* cav., *F. oxysporum*, and *Uromyces viciae-fabae* (Pers.) Schroet. is listed as being seed-borne on lentils by Richardson (1979). A recent report by Morrall and Sheppard (1981) describes a seedling blight caused by seed-borne *Ascochyta lentis* Bondartzeva-Monteverde & Vassilievsky. They suggest that this is a potentially serious disease in western Canada affecting seed quality more so than seed yield.

1.3 EPIDEMIOLOGY AND CONTROL OF *FUSARIUM* DISEASES OF LENTILS

Khare *et al*. (1971) found that disease incidence was inversely proportional to the percentage of soil moisture. In greenhouse studies, maximum infection was associated with 25 percent moisture in the soil and no disease was observed at saturation levels. Lin (1975), working with F. roseum 'Avenaceum' also found that root rot of lentils was more severe in dry soil than in wet soil. These results are in contrast to the observations of Kaiser and Horner (1980) who noted that disease severity was greater with varieties that were irrigated most frequently. Also, the root rot of soybean caused by F. oxysporum was found to be most destructive when soil was water-saturated soon after seeds were sown (Dunleavy, 1961).

Soil type was also found to have an influence on disease incidence, as evidenced by the work of Vasudeva and Srinivasan (1952) and Khare et al. (1971). The former workers showed that infection was greater in sand (100%) and least in clay soils (10%). Khare et al. (1971) observed a higher percentage of disease in sandy loam type soil than in clay. Soil having a pH range of 7.6 to 8.0 favoured disease development on lentils by F. oxysporum (Khare et al., 1971); however, Fleischmann (1937) seasenoted that lentils grown in acidic soils were more diseased than those in alkaline soils.

Soil temperature was also found to be an important factor in disease development. Carrera and Noll (1941) found that Fusarium disease of lentils was more widespread in hot weather than at cooler temperatures. Other studies have shown that high temperatures favour the incidence of disease in lentils (Claudius and Mehrotra, 1973; Shatla et al., 1975; Vasudeva and Srinirvsan 1952). In contrast, Lin (1975) observed that lower temperatures facilitated disease development. He reported that lower temperatures retarded growth of lentil seedlings allowing a greater period of time for exposure to F. roseum 'Avenaceum'.

Sowing of the lentil crop late in the year in India and the Middle East has been recommended to improve seed yields (Hussein et al., 1977; Kannaiyan and Nene, 1975; Khare et al., 1972; Kyalova, 1973; Shatla et al., 1975). Since lentils are relatively frost hardy, early sowing in North America was recommended by Slinkard (1980). Presumably the lower soil temperatures inhibit growth of the fungal pathogens (Kannaiyan and Nene, 1975).

The first attempt at chemical control of F. oxysporum infection in lentils was made by Kovacilova (1970) who obtained effective control of the disease by treating lentil seeds with a mixture of captan and thiram. Khare et al. (1971) reported that when Benlate treated seeds (1:400 w/w) were sown in soil previously treated with Benlate (8 mg/pot), they achieved a decrease in mortality of almost 40% over check plants. In the same experiment, the percentage of disease was also reduced when seeds were treated with chloroneb and thiram. Kannaiyan and Nene (1972) obtained effective control, under field conditions, by treating seed with triarimol (0.05% active ingredient (a.i.) w/w) and benomyl (0.15% a.i. w/w). Shatla et al. (1975) in their investigations of the chemical control of lentil root rot in Egypt found that benomyl, when applied at the rate of 9 kg/feddan (= 23.8 kg/ha) as a drench to soil in pots, gave 69% disease control. In Canada, captan was applied as a seed treatment at 2.8 g a.i. per kg seed for the control of a seedling blight caused by Fusarium spp. and Rhizoctonia solani. However, this treatment did not significantly increase seed yields over non-treated controls (Duczek and Buchan, 1978). Attempts at controlling Fusarium disease of lentils by the use of biological control measures was investigated by Mehrotra and Claudius (1972). They obtained good results against F. oxysporum f. sp. lentis by the use of such antagonists as Trichoderma viride, Streptomyces gougeroti as well as other species of Streptomyces and two unidentified bacterial isolates.

1.4 THE PATHOGEN

The genus Fusarium is world-wide in distribution and comprises a number of species that are soil-borne pathogens of many agriculturally important crops. F. oxysporum is considered to be the most economically important species, inciting severe wilts in many host plants (Booth, 1971). Snyder and Hansen (1940) subdivided this species into a number of strains or forms based upon their pathogenicity.

Fusaria in general and F. oxysporum in particular are quite variable morphologically as well as physiologically. They can only be identified with certainty if the cultures are maintained under standardized conditions (Toussoun and Nelson, 1968). Due to the use of non-standardized conditions by some researchers, there is much uncertainty in the literature concerning the nomenclature of fusarial isolates. An example of the confusion that can arise in the identification of different species of Fusarium is in the identity of F. oxysporum.

Prissyajnyuk (1931) and Fleischmann (1937) identified a species of Fusarium as the organism responsible for yield losses in the U.S.S.R. and Hungary, respectively. Somewhat later, Carrera (1940) reported that F. avenaceum and F. orthoceras were the casual agents. In addition, a species of Fusarium, different from F. orthoceras was isolated by Padwick (1941) and was reported to be the cause of disease in both lentils and gram. Altogether two new species of Fusarium (F. scirpi var. acuminatum and F. culmorum), along with F. avenaceum were found associated with the disease. Vasudeva and Srinivasan (1952) identified the pathogen and named it as F. orthoceras Schlecht. var. lentis Vasudeva and Srinivasan, adopting the Fusarium taxonomic system of Wollenweber and Reinking (1935). Claudius (1968), using the key proposed by Synder and Hansen (1940), changed the name to F. oxysporum Schl. f. sp. lentis Vasudeva & Srinivasan.

In their description of the pathogen, Vasudeva and Srinivasan (1952) noted that the medium used for growing the fungus turned pink within a few days and that it produced microconidia, macroconidia and chlamydospores in culture. Microconidia have been reported to be single-celled, hyaline, ovoid, and measure 6.7 x 3.7 microns (Khare et al., 1971); whereas Vasudeva and Srinivasan (1952) measured the size of microconidia as 8.5 x 2.7 microns. They described macroconidia as being pointed at the tip and notched at the base, fusaroid, with 1-6 septa and 10-50 x 5-6.7 microns in size. Chlamydospores are unicellular and terminal or intercalary with thick walls (Khare et al., 1971).

The pathogen grows best in the range of 27^{o} to 30^{o}C and prefers a pH of 5.0 in the medium although it is capable of tolerating a wide range (Vasudeva and Srinivasan, 1952). Sharma and Agnihotri (1971), found that the organism grew well on natural and synthetic media and that the fungus could successfully utilize a wide range of carbon compounds.

It is generally assumed that the fusaria exist in soil predominantly as chlamydospores; single-celled, thick-walled asexual spores (Cook, 1968; French and Neilson, 1966; Nash et al., 1961; Trujillo and Snyder, 1963). Toussoun and Snyder (1961) noted that conidia from

F. solani f. sp. phaseoli germinated readily in soil but that chlamydospores from the same strain germinated only under conditions conducive to infection. Park (1965) concluded that this infers a greater adaptability of chlamydospores for survival in soil. Chlamydospores may arise by the modification of conidia added to soil (Park, 1955) or conidia may germinate briefly to give rise to chlamydospores (Nash, Christou, and Snyder, 1961). French and Neilson (1966) observed that macroconidia of F. oxysporum f. sp. batatas, which were formed profusely at soil surface were rapidly converted to chlamydospores when washed into the soil. Caldwell (1958) showed by direct microscopic observation that conidia were not as transitory as first assumed and that conidia could survive for up to 1 year in soil. Chlamydospores of F. oxysporum f. sp. cubense may survive upwards of 40 years as shown by Stover and Waite (1954) while still retaining their capacity to infect banana plants. Houston and Knowles (1949) have observed a 50-year survival period for F. oxysporum f. sp. lini. The actual number of survivors after this period is probably rather small because most propagules of F. oxysporum were shown to lyse soon after the host crop was removed (Smith, 1970). Concurrently, there appears to be a loss in the degree of pathogenicity Wensley and McKeen (1963) with the time of host removal. They studied the muskmelon wilt organism F. oxysporum f. sp. melonis and found that at harvest 70% of the F. oxysporum isolates were pathogenic whereas a few months later only 12-15% of the isolates were pathogenic.

Armstrong and Armstrong (1948) and Hendrix and Nielson (1958) observed that pathogenic forms of F. oxysporum could infect roots of non-host plants without pathogenesis and that this was crucial to the survival of soil-borne pathogens in soil. The importance of these symptomless carriers lies in the activation of germination of the fungal propagules and their establishment in roots (Banihashemi and de Zeeuw, 1975) enabling the fungus to reproduce and to increase its numbers without exposure to antagonistic soil saprophytes (Katan, 1971). Chlamydospores of F. solani f. sp. phaseoli were observed to germinate when placed adjacent to seeds of nonsusceptible bean plants. Within 3-5 days, newly formed chlamydospores were visible around the germinating seeds. No germination of chlamydospores in the original inoculum was observed in non-rhizosphere soil (Schroth and Hendrix, 1962). These results indicated that the pathogen was able to perpetuate itself in a host-free environment. Davis (1963) however, showed that Fusarium species infecting noncompatible hosts were unable to produce toxins and Mussell and Green (1970), studying F. oxysporum f. sp. lycopersici, found that this strain produced polygalacturonase, a wall-hydrolyzing enzyme, in susceptible tomato plants but not in resistant cultivars.

Several workers have reported that germination of Fusarium chlamydospores is stimulated by nutrients exuded from plant roots (Kommedahl, 1966; Schroth and Snyder, 1961; Schroth, Toussoun and Snyder, 1963). Fusarium chlamydospores are able to germinate when exposed to certain amino acids and sugars to form a mycelium that will infect a host plant or give rise to replacement chlamydospores.

1.5 PENETRATION AND ESTABLISHMENT OF THE PATHOGEN IN HOST ROOT TISSUES

As previously discussed, Fusarium is capable of existing in soil for long periods of time while still maintaining its capacity to infect host root tissues when spore germination is stimulated by the appropriate root exudates. The interval between the appearance of the germ tube in the vicinity of the host tissues and the establishment of pathogenesis in lentil roots was investigated in this study. The penetration and proliferation of F. oxysporum in lentil roots has not previously been elucidated although there are a number of reports describing the penetration of this species into other hosts (Abawi and Lorbeer, 1971; Alconero, 1968; Anderson and Walker, 1935; Chambers and Corden, 1963; Chi et al., 1964; Hepple, 1963; Maitra et al., 1976; McClure, 1950; Pennypacker and Nelson, 1972). Kamel et al., (1973) have described the infection of lentil hypocotyls by Fusarium solani.

In their descriptions of the mode of penetration into host tissues, some authors have observed that Fusarium species penetrate their hosts primarily through wounds caused by nematodes, insects or other agents (Alconero, 1968; Garofalo, 1960; Kreitlow and Hanson, 1950). In their studies with F. conglutinans Wollenw., Anderson and Walker (1935) and Reid (1958) showed that penetration was intercellular whereas Chi et al. (1964) noted that Fusarium spp. penetrated the host epidermis intracellularly as well.

It is generally agreed upon that the root is differentially susceptible to fungal invasion. Alconero (1968) and Chi et al., (1964) found that most fungi invaded through the meristematic regions of the root tips but that areas further up could also be penetrated. Tisdale (1917) found that F. conglutinans penetrated the root hairs of cabbage plants and for many years this was considered the chief avenue of entry into the host. Smith and Walker (1930) found invasion to occur chiefly through the root cap and the zone of elongation, and only in-frequently through the root hairs. Tippett et al. (1976) noted that germ tubes of Phytophthora cinnamomi Rands penetrated the roots of Eucalytus obliqua preferentially in a zone 1 cm in length behind the root cap. Zentmyer (1965) observed that zoospores of the same pathogen clustered preferentially at the zone of elongation on roots of avocado.

Following penetration, the fungal hyphae advanced into the cortical area where initial growth was intercellular but later became intracellular as well (Chi et al., 1964). Christou and Snyder (1962) working with F. solani f. sp. phaseoli found that in the initial stages of invasion, the pathogen avoided internal penetration of living tissue. It preferred to grow between and around cells rather than in them. After the cells became degraded or senescent, the fungus entered the interior of the cells and tissue decay followed. Chi et al. (1964) observed that invasion of the vascular tissues was mostly through the meristematic regions and that hyphae colonized the xylem vessels and parenchyma cells causing occlusion with a darkly staining substance. Christou and Snyder (1962) noted that the pathogen remained restricted to the cortex until the final stages of cortical decay. They concluded that this was perhaps due to the intercellular nature of the pathogen and its relative inability to invade the suberized walls of the endodermis. This observation is in agreement with Conant (1927) who reported that the endodermis of tobacco was of some protective value against Thielaviopsis basicola (Berk. & Br.).

Many pathogenic forms of F. oxysporum cause a vascular wilt in which the fungus rapidly colonizes the xylem elements of the infected plants (Armstrong and Armstrong, 1973). However, there are a number of reports in the literature which indicate that F. oxysporum may instead produce symptoms of a cortical decay in which vascular discolouration is limited to the basal regions of the plant and reisolation of the fungus is successful from only 1-2 cm beyond the discoloured zone (Alconero, 1968; Jarvis _et al_., 1975; Smith, 1975; Weimer, 1944). In the root rot of soybeans caused by a number of undetermined _Fusarium_ spp. the pathogens are confined to the cortical tissues except in the very advanced stages of disease, when the vascular elements also become invaded (Sinclair and Shurtleff, 1975).

The present investigation is a study of the aetiology of the root rot disease of lentils caused by _F_. _oxysporum_ in eastern Canada. It represents the first report of the occurrence of this fungus as a pathogen of lentils in Canada.

MATERIALS AND METHODS

2.1 ISOLATION OF FUNGI FROM FIELD-GROWN LENTILS

Diseased lentil seedlings were collected from a plot located on the Central Experimental Farm, Ottawa, Ontario, during the month of September, 1979. Four 1.0 cm segments were removed from the discoloured tap root and surface-disinfected by immersion in a 0.6% sodium hypochlorite solution for 1 minute followed by three rinses in sterile distilled water. The central 0.5 cm portion of each piece was excised and incubated on plates of nutrient agar at 21°C on a laboratory bench. After three weeks of incubation an inoculating loop was used to transfer a small portion of the mycelial mat to a microscope slide. Fungal structures were stained with methylene blue and observed with a Wild compound microscope.

2.2 ISOLATION OF SOIL-BORNE FUNGI FROM A DISEASED LENTIL FIELD

2.2.1 Collection Of Soil Samples

The Central Experimental Farm plot sown to lentils in 1979 was divided into 32 sample areas, each approximately 5 x 10 m. A standard soil extractor was used to remove a 2.4 x 25 cm core from the centre of each site. Prior to the removal of each soil core, the extractor was swabbed with paper towelling soaked in 70% ethanol, rinsed in sterile distilled water and dried with sterile paper towelling. Cores were carefully withdrawn with sterile tongue depressors and placed into sterile 530 ml Whirl-Pak (Fisher Scientific) plastic bags. Each bag was labelled and sealed. In addition, 8 core samples were randomly selected from the non-cultivated area adjacent to the diseased lentil field, in order to compare inoculum densities between these two areas.

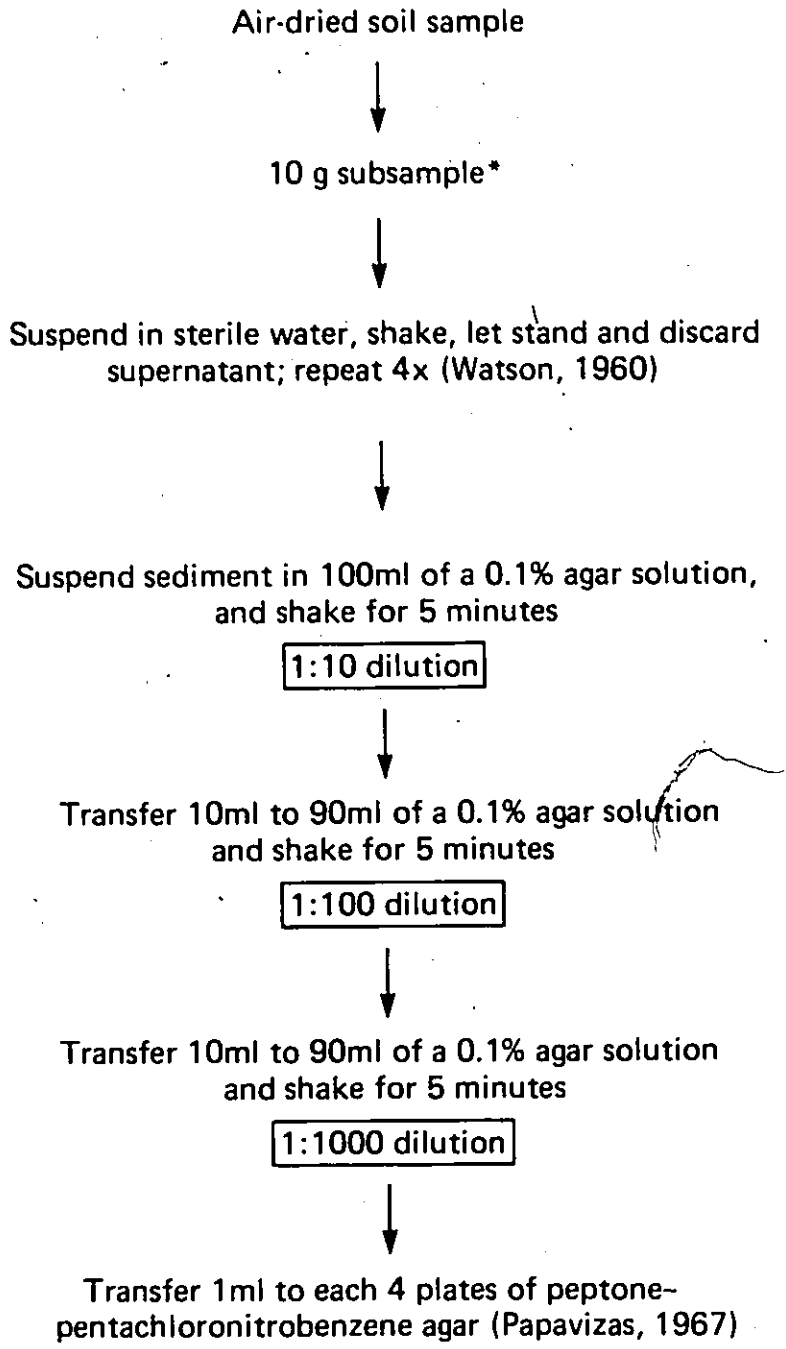
2.2.2 Preparation Of Soil Dilution Plates

On return to the laboratory each bag of soil was agitated to obtain a uniform mixture and large soil particles were pulverized by hand. Under aseptic conditions two fifteen gram subsamples were removed from each bag with a flamed spatula and placed into separate sterile 9 cm plastic Petri dishes. The subsamples were designated as either A or B. This procedure was carried out for all 40 samples, resulting in a total of 80 subsamples. The dishes were placed into a cool oven at 25^{o}C, with the fan in operation to promote drying of the samples. The oven door was left slightly open to facilitate the inflow of air from the room. The soil samples were allowed to dry for four days and then the moisture content of the soil was determined as a measure of the water lost during drying using 10 randomly selected dishes. Following the drying regimen, the soil samples were passed individually through a 2 mm sieve to remove stones and large soil particles.

The procedure used in the preparation of the soil dilution plates is outlined in figure 2. Incorporated in this technique is Watson's soil washing modification to increase the percentage recovery of propagules of _Fusarium_ spp. A 10.0 g sample of the air-dried soil was put into a sterile 500 ml Erlenmeyer flask containing approximately 40-2 mm glass beads. Following the addition of 200 ml of sterile distilled water, the flask was agitated for 5 minutes on a gyrorotary

Figure 2. Procedure used for the isolation of <u>Fusarium</u> spp. from soil using Watson's (1960) modification of the soil dilution plate technique.

Isolation of Fusarium spp. From Soil Using Watson's Modification of the Soil Dilution Plate Technique



Air-dried soil sample

↓

10 g subsample*

↓

Suspend in sterile water, shake, let stand and discard supernatant; repeat 4x (Watson, 1960)

↓

Suspend sediment in 100ml of a 0.1% agar solution, and shake for 5 minutes

1:10 dilution

↓

Transfer 10ml to 90ml of a 0.1% agar solution and shake for 5 minutes

1:100 dilution

↓

Transfer 10ml to 90ml of a 0.1% agar solution and shake for 5 minutes

1:1000 dilution

↓

Transfer 1ml to each 4 plates of peptone-pentachloronitrobenzene agar (Papavizas, 1967)

* This procedure was performed in duplicate, using a second 10 g. subsample.

FIG. 2

shaker at 140 rpm to facilitate the breakdown of soil aggregates and then allowed to settle for 1 min at a 45^{0} angle before the supernatant was discarded. This soil washing technique was repeated an additional three times in order to dilute the number of spores of Penicillium, Aspergillus, bacteria and actinomycetes. These organisms are usually more abundant in soil and tend to overpopulate the plates, thereby suppressing the growth of other genera of fungi (Watson, 1960). After the final decantation, the sediment was suspended in 100 ml of a 0.1% agar solution and shaken for 5 minutes. The agar solution was selected as the diluting medium because it maintained the dispersed particles in suspension while the transfers were made. Ten ml of the soil suspension was transferred to a 250 ml Erlenmeyer flask containing 90 ml of the agar suspension. While being agitated continuously, 10 ml was removed and made up to 100 ml with the diluting agent which resulted in a final dilution of 1:1000. Preliminary tests with dilution factors ranging from 1:100 to 1:100,000 had revealed that this dilution was optimal to ensure good separation of colonies on the soil dilution plates. Four 1 ml aliquots of this suspension were removed and pipetted onto the surface of four separate 3 day-old plates of a modified peptone-pentachloronitrobenzene agar recommended by Papavizas (1967) for the selective isolation of Fusarium species from soil.

The medium was composed of 15 g peptone (Difco), 1 g potassium dihydrogen phosphate (KH_2PO_4), 0.5 g magnesium sulfate ($MgSO_4 \cdot 7H_2O$), 0.5 g pentachloronitrobenzene (75% wettable powder, Terraclor), 0.5 g Bacto-oxgall and 20 g of agar in 1 litre of distilled water. The medium was adjusted to pH 6.0 with glacial acetic acid, then autoclaved at $121^{o}C$ for 15 min, and cooled to $45^{o}C$ in a water bath.

Chlorotetracycline HCl (50 mg/l) and streptomycin sulfate (100 mg/l) were added to the cooled medium and aliquots of approximately 20 ml were poured into sterile Petri dishes. These were left in a darkened room at 21°C for 3 days because initial drying of the agar encourages absorption of liquid from the soil suspensions, thereby reducing the spread of bacterial and yeast contaminants (Nash and Snyder, 1962).

Dilution plates were inverted and incubated at room temperature in a laboratory cabinet enclosed with brown paper for 3 days prior to removal of the paper and incubation for an additional 4 days on a laboratory bench (Wensley and McKeen, 1962). Diffuse daylight for the first few days of incubation was necessary for sporulation of the fusaria and to inhibit growth of phycomycetes (Nash and Snyder, 1962). Plates were examined initially on the 4th day of incubation and daily thereafter.

2.2.3 Identification Of Field Isolates

After one week of incubation, the dilution plates were examined under a stereoscopic microscope at 50x magnification. A flamed broach (size XX Fine, Union Broach Corp.) was used to isolate a small piece of agar containing a single hyphal tip from each morphologically distinct fungal colony. The agar block was transferred upside down onto the surface of plates of potato-sucrose-agar (PSA; Booth, 1977; modified by reduction of the sucrose concentration to 15 g/l) and incubated for 7 days under continuous cool white fluorescent illumination (10,760 lux) at 22°C to encourage sporulation. Those colonies which failed to produce spores under these conditions were placed under near-ultraviolet light on a 12 hr on/off cycle or small blocks of agar were removed from the colony edge and placed into sterile water to stimulate spore production.

Five drops of sterile distilled water were spread onto the surface of each sporulating colony with a flamed loop. One loop of the spore suspension was transferred and dispersed in 20 drops of sterile water in a Petri dish. A loopful of the resulting spore suspension was transferred and mixed with 20 drops of water in another Petri dish and the process repeated until a dilute spore suspension was obtained. This was spread onto the surface of a plate of 2% water-agar which was left uncovered in a laminar flow hood for 2 hours or until all of the water had been absorbed into the agar. A broach was used to isolate and transfer single conidia to plates of PSA. Thirteen plates were prepared, each with a single conidium at its centre, for each fungal isolate. Twelve of these were incubated at 25°C, 30 cm below 4 cool-white fluorescent tubes (4,950 lux) for exactly 96 hours. A growth rate was calculated for each isolate based upon the average colony diameter attained by the first 8 monoconidial colonies (Gordon, 1952). The thirteenth plate was incubated 30 cm below two daylight fluorescent tubes (2,800 lux) at 22°C for 2 weeks for the determination of culture pigmentation by comparison with Rayner's (1970) mycological colour charts.

In addition to culture pigmentation and colony growth rate, each isolate was examined both macro- and microscopically and notes made on the following features: colour and texture of the aerial mycelium; shape, size and septation of the macroconidia and microconidia if present; and the presence or absence of chlamydospores in culture.

These morphological characteristics were compared with the descriptions of Booth (1971) to determine the identification of each fusarial isolate. In addition, monoconidial cultures, or in the absence of spores, colonies arising from single hyphal apices, growing on PSA were sent to the Biosystematics Research Institute, Agriculture Canada, for verification of the identifications.

2.3 PATHOGENICITY OF FIELD ISOLATES TO LENTILS

2.3.1 Source Of Plant Material

Lentil seeds, (_Lens culinaris_ Medik.) var. Laird were purchased from Neufield Seed Ltd., Nipawin, Saskatchewan. The varieties Commercial Chilean and Eston as well as a Plant Introduction (P.I.) 179313 were kindly provided by Dr. A.E. Slinkard, Crop Development Centre, University of Saskatchewan, Saskatcon. All seeds were stored in darkness, at -20°C, until use. To ensure uniformity among samples, only seeds with green coats as designated by reference to the colour terminology of the Inter-Society Colour Council - National Bureau of Standard Colour - Name Charts (corresponding to centroid colour chips 91 and 105) were selected for each experiment. In addition, individual seeds were examined with a stereoscopic microscope and only those free from cracks and other seedcoat abnormalities were included as part of the experimental material.

2.3.2. Seed Surface Sterilization

Several treatments to surface sterilize lentil seed were tested (Table 3). The method selected for routine use consisted of the preliminary soaking of lots of 150 seeds for 5 hours in 200 ml of distilled water amended with 0.04 g of powdered detergent (Sparkleen, Fisher Scientific). The seeds were then soaked in a 0.6% solution of sodium hypochlorite for 17 minutes and rinsed three times in sterile distilled water, each for 10 minutes. Seed was germinated on plates of water-agar (Fig. 3) incubated in darkness at 22°C. This surface sterilization procedure was selected because it yielded a high percentage of micro-organism-free seed without adversely affecting germination or subsequent seedling growth. In addition, any contaminants that survived the seed treatment were readily observed on the agar surface and the host seed was culled.

2.3.3 Pathogenicity Testing Under Aseptic Conditions

Uniform appearing, surface-disinfected 2 day-old seedlings having a radicle length of approximately 1 cm were selected for use in the pathogenicity tests. These were transferred aseptically to 3.5 x 30 cm glass culture tubes containing 75 g of 2 mm glass beads (Fig. 4) to which a 5% Hoagland's solution (Hoagland and Arnon, 1938) was added to cover all but the top two layers of beads. The bottom of each tube was fitted with a black plastic sleeve, 6 cm in height, to restrict entry of light into the root zone. The sleeve could be removed to observe the root system during symptom development. Culture tubes were incubated in a growth cabinet set to provide an 18 h photoperiod with day/night temperatures of 22 and 16°C, respectively. Illumination (7,540 lux) was provided by both 40 watt incandescent bulbs and cool-white fluorescent tubes.

Seedlings were inoculated when 7-days old with 1 ml of a spore suspension, prepared from 7-day old cultures grown on PSA. The suspension was adjusted to 100,000 conidia/ml with water as determined

Figure 3. Two day-old surface-sterilized lentil seed germinated in petri plates containing sterile 2% agar.

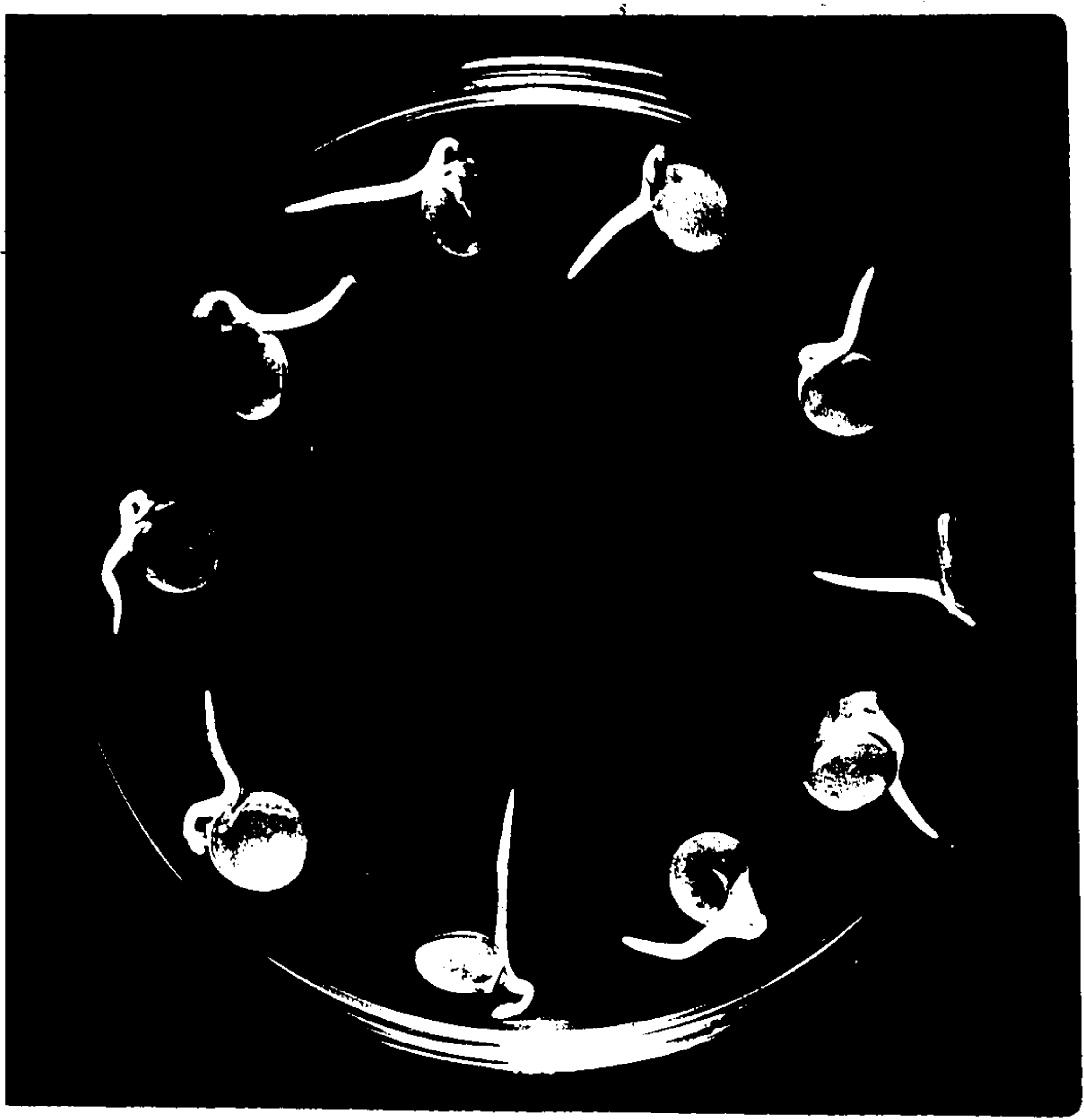


FIG. 3

Figure 4. Drawing of the culture tubes used in the aseptic pathogenicity tests. The glass tubes were 3.5 x 30 cm and the seedlings were supported on a bed of 2mm glass beads. An external black plastic sleeve was used to shade the root zone from light.

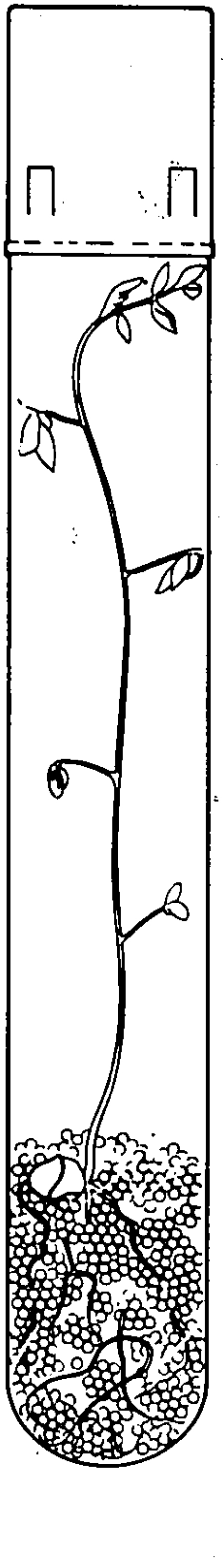


FIG. 4

in an American Optical hemacytometer counting chamber. The glass beads were pushed aside with a sterile 23 cm pipette, the inoculum was administered to the stem at the point of attachment of the cotyledons, and the glass beads were replaced. The tube was agitated to distribute the spores throughout the root zone. Control seedlings received 1 ml of sterile distilled water. Inoculated and control seedlings were returned to the incubator, and examined every other day for the presence of disease symptoms. Each treatment consisted of 6 plants and all treatments were performed in duplicate or triplicate.

2.3.5 Disease Assessment

When plants grown in aseptic culture were examined, individual root systems were scored 0,1,2,3, or 4 on the basis of the degree of root discolouration, lesion development and extent of killing as follows:

0 = healthy root system without disease symptoms
1 = slight discolouration of roots confined to lateral root junctions and/or lateral root tips
2 = isolated lesion formation on tap root and up to 50% discolouration
3 = coalescence of lesions with 50-75% discolouration of tap root
4 = complete discolouration of root system

Foliar symptoms were evaluated similarly:

0 = healthy seedling - no symptoms of disease
1 = chlorosis of first true leaves
2 = leaf chlorosis extending half way up the plant

3 = chlorosis of all leaf tissue with necrosis of lowermost leaves

4 = complete necrosis.

The same evaluation scheme was used for the assessment of foliar symptoms in seedlings grown in pasteurized soil.

In addition, a disease index was devised so that the rate of disease development in lentils could be evaluated graphically (Fig. 13). Using a modified version of the symptom and senility index described by Isaac and Harrison (1968) and McLean and Walker (1941), root and shoot systems of aseptically grown seedlings were divided into 4 equal regions and class values were assigned to each based upon the disease assessment scheme described above. The number of root or shoot segments in each class was multiplied by its respective class weight, the products then added, and the total multiplied by 100. This value was divided by 4 (the maximum value of symptoms) times the maximum segment total to yield the disease index for that segment. The 4 segment indices were then added and an average disease index calculated for each isolate at four day intervals. The disease index is therefore a measure which expresses both the percentage and degree of infection (McLean and Walker, 1941).

2.3.6 Reisolations From Diseased Plants

Twenty-one days following inoculation, plants were removed from the growth medium and four segments were removed, 0.5 cm in length, beginning with the tip of the tap root and extending upwards to the shoot apex (Fig. 5). The excised pieces of tissue were then surface-sterilized by immersion in a 0.6% solution of sodium hypochlorite for 1 minute prior to three 10 min rinses in sterile distilled water. The centre 2 mm of each segment was cut-out and plated onto PSA in

Figure 5. Drawing of a lentil seedling showing the sites selected for the reisolation of fungal pathogens from diseased seedlings: A - tap root apex; B - hypocotyl; C - lower stem; D - shoot apex.

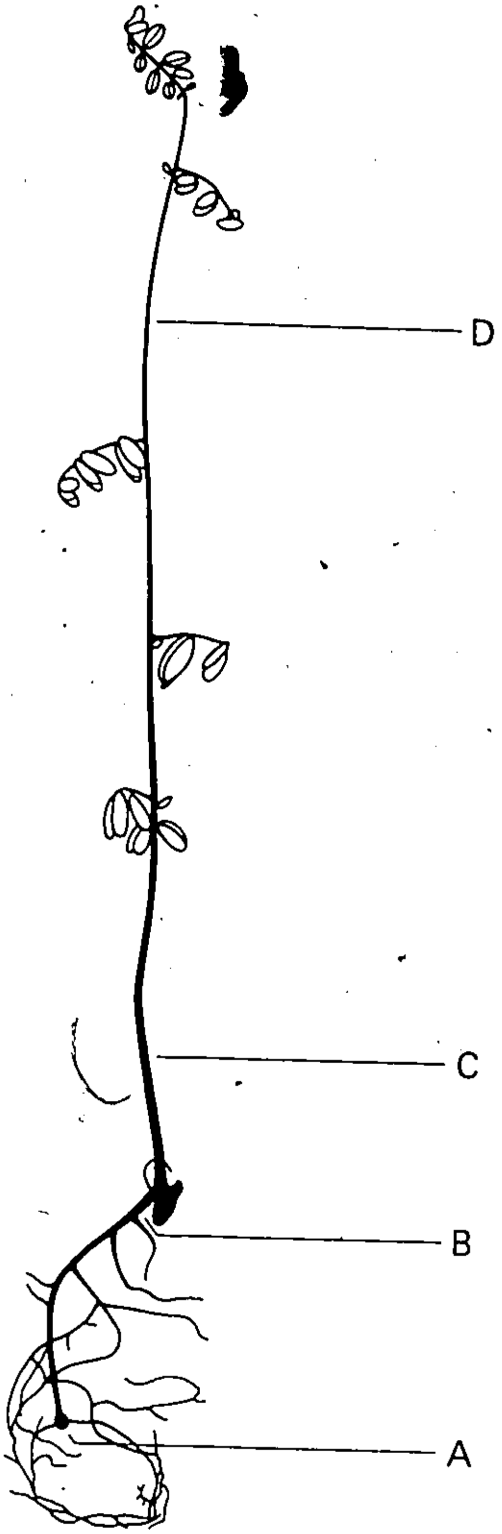


FIG. 5

Petri dishes. The dishes were incubated in a 22°C room under fluorescent illumination (10,760 lux). After 4 days, the plates were examined and monospore cultures were prepared for identification purposes, as previously described (Sec. 2.2.3).

2.4 EFFECT OF Fusarium oxysporum CULTURE FILTRATES ON SEEDLING GROWTH

Filtered extracts from many fungal cultures have been shown to be important in disease development, (Pringle and Scheffer, 1964). Preliminary tests with 4- and 12-day old culture filtrates of Fusarium oxysporum Isolate 82A were collected to determine the role of extracellular metabolites on symptom expression. Erlenmeyer flasks, each containing 250 ml of Czapek - Dox liquid medium (Tuite, 1969) were inoculated with a spore suspension prepared from a 1 week-old monoconidial culture of isolate 82A. The flasks were incubated for either 4 or 12 days on a gyrorotary water bath shaker (120 rpm) at 26°C. Culture filtrates were harvested by passing them through 2 layers of cheesecloth to remove the mycelial mass prior to filtration through Whatman No. 3 filter paper. The extract was then Millipore-filtered (0.22 μm) and passed through a 0.20 μm Nalgene Filter Unit to render it aseptic. The sterile filtrate was transferred to sterile Erlenmeyer flasks and stored at 5°C until use.

Sterile lentil seedlings with a radicle length of 4 cm were grown for 1 week in the undiluted filtrate and in a series of dilutions ranging from 1:1 - 1:100 in sterile distilled water. The experimental set-up is shown in figure 6. Disposable 25 ml scintillation vials were wrapped with aluminum foil and autoclaved at 121°C for 20 minutes. Seedlings were positioned in 20 ml of the appropriately diluted extract

Figure 6. Vial used to study the effect of culture filtrates on lentil seedlings. Disposable 25 ml scintillation vials were wrapped in aluminum foil and sterilized. Aseptically grown seedlings were positioned in 20 ml of the treatment solution.

FIG. 6

(treated plants) or in sterile distilled water (control plants). Each treatment was replicated 4 times in a completely randomized experimental design and all experiments were performed in duplicate. Seedlings were incubated for 7 days in a growth chamber with day/night temperatures of 22 and 16^{0}C, respectively. Illumination was provided by incandescent bulbs (40 w) and by cool-white fluorescent tubes (5,380 lux). The photoperiod was 18 h.

Following the 7 day incubation period, the seedlings were examined and observations recorded on tap root length, the presence or absence of secondary roots and the number of dead seedlings. In addition, root discolouration and foliar chlorosis were scored 0,1,2,3, or 4 on the basis of discolouration or chlorosis of 0, 1-25, 26-50, 51-75, and 76-100%, of the tissues, respectively.

2.5 SCREENING FOR THE PRESENCE OF Fusarium oxysporum AS A SEED-BORNE INOCULUM SOURCE

In many economically important crops seed-borne inoculum may serve as an important source to initiate primary infections and to introduce the pathogen into new areas (Nath, et al., 1970). In order to investigate this possibility, seed from the varieties Laird, Eston, Commercial Chilean, and P.I. 179313 were screened for the presence of fungal contaminants. All seed was surface-sterilized as previously described (Sec. 2.3.2) and germinated on PSA, malt-extract-agar and on V-8 juice-agar, (Tuite, 1969). One hundred seeds of each variety were plated on each culture medium. After 7 days of incubation at 22^{0}C under cool-white fluorescent illumination (10,760 lux), observations on the contaminating fungi were made initially under the stereoscopic microscope and then subsequently under the compound microscope. Identification of the seed-borne fungi was determined as described earlier (Sec. 2.2.3).

2.6 CYTOLOGY OF LENTIL ROOTS INFECTED WITH Fusarium oxysporum

2.6.1 Inoculum Source

In all experiments, one week-old 'wild-type' monoconidial cultures of Fusarium oxysporum, Isolate 82A, grown on PSA were used as the inoculum source. Cultures were incubated under continuous fluorescent illumination with a light intensity of 10,760 lux at plate level, in a room maintained at 22°C. Agar plugs, 3 mm in diameter, were removed from the growing edge of the colonies for use in the cytological studies.

2.6.2 Plant Material

'Green' lentil seeds (var. Laird) free from surface imperfections were surface-disinfected and germinated on 2% water agar, in darkness, at 22°C. Two day-old seedlings were examined with a stereoscopic microscope and those with a uniformly appearing tap root without fungal contamination were selected for use.

2.6.3 Inoculation Techniques

Selected seedlings were transferred aseptically to 2% water agar in 9 cm plastic Petri dishes from which three 5 mm discs of agar had previously been removed with a cork borer. The seedlings were positioned with the cotyledons in the holes created in the agar and the tap root on the agar surface. To secure the seedlings in this position, 1-2 drops of a slightly warm 2% agar solution were dispensed into the wells, around the cotyledons. Three such seedlings were established per plate as is shown in figure 7. An agar plug containing the actively growing fungus was placed alongside the root, at the zone of elongation. Check seedlings were inoculated with an agar disc from a non-inoculated PSA plate. Four dishes containing inoculated seedlings were secured with a rubber band and incubated, on edge, with the roots directed downwards, 30 cm below continuous cool-white fluorescent illumination (2,800 lux). Seedlings were incubated for 2,4,8,16,24,48 and 72 hours following inoculation.

2.6.4 Sectioning Of Fresh Material

Following the appropriate periods of incubation, roots were harvested and transverse free-hand sections approximately 10-50 μm in thickness were cut using Gillette Blue blades. The razor blades were first cleaned in acetone to remove surface film and then moistened with distilled water, prior to use.

2.6.5 Fixation and Embedding Procedures

Root tissue, adjacent to the inoculum zone was cut into 5 mm pieces and fixed in formalin-acetic acid-alcohol (FAA) for at least 24 h at 5°C, (Johansen, 1940). The fixed specimens were washed in 50% ethanol and then in 70% ethanol prior to hand-sectioning.

Alternatively, sections of root, 2-4 mm in length were cut into 0.1 M sodium cacodylate buffer at pH 7.2 and then fixed for 1 h in a cacodylate-buffered mixture of 2% glutaraldehyde and 4% acrolein. The fixative was removed with three changes of buffer, until no aldehyde fumes could be detected. Root pieces were placed first under

Figure 7. The procedure used to inoculate aseptically grown lentil seedlings for the cytological studies. A 5 mm diameter plug from 1 week-old fungal cultures was placed on the root at the zone of elongation.

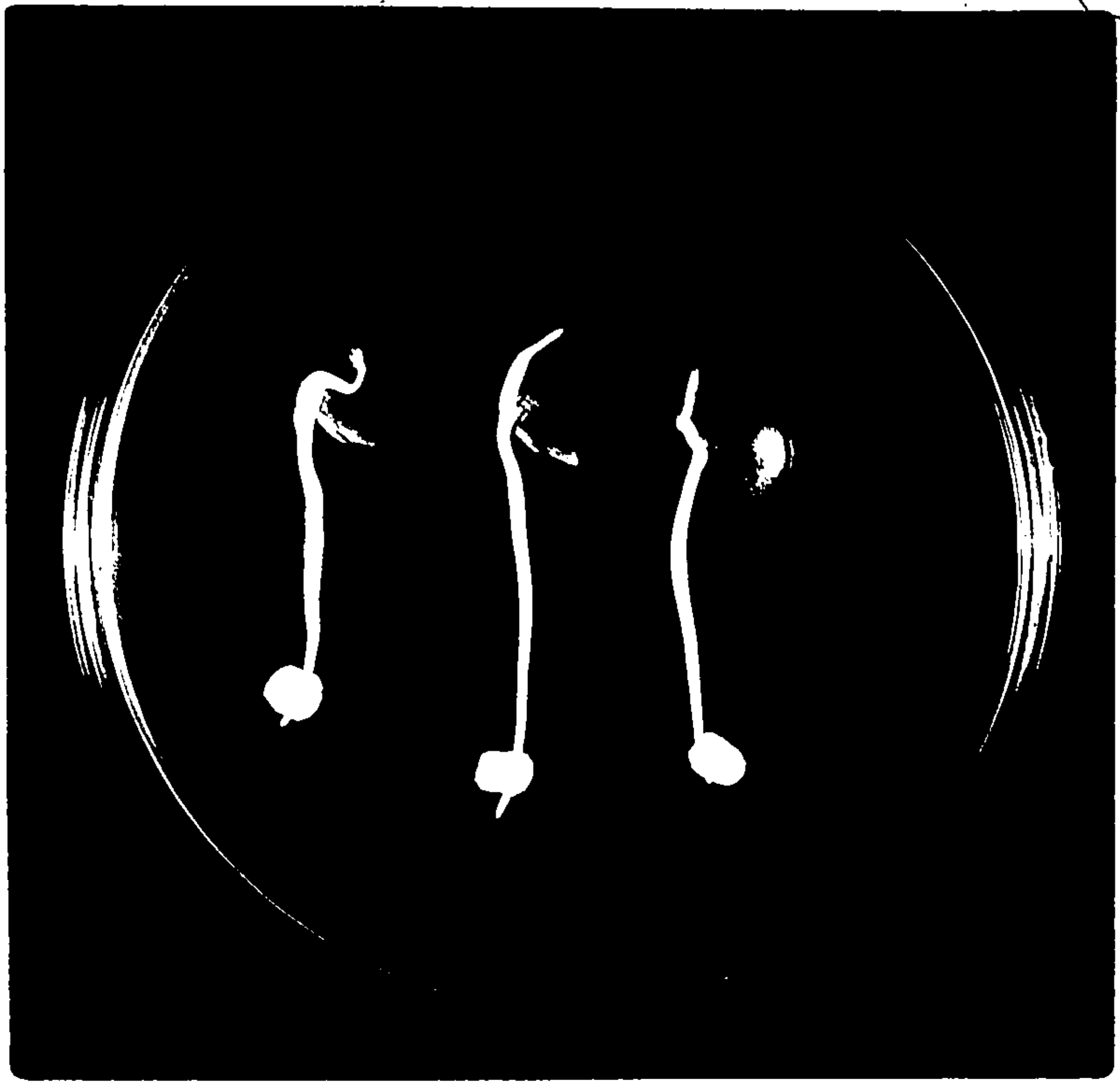


FIG. 7

low vacuum on a water aspirator and then onto a high vacuum apparatus (.1 mμ Hg) for 1½ hours to eliminate air from tissues. Roots were post-fixed in 2% osmium tetroxide in cacodylate buffer for 1 h and washed three times in the same buffer solution. Specimens were dehydrated through a graded ethanol series and then in a graded series of ethanol/propylene oxide to pure propylene oxide. All solutions used in the fixation and subsequent dehydration procedures were maintained at 1-4°C. Root segments in propylene oxide were brought to room temperature and this solution was replaced with a 50% mixture of Epon 812 resin (5.5 parts Mixture A: 4.5 parts Mixture B; Luft, 1961) in propylene oxide. The propylene oxide was evaporated slowly over a 24 h period after which time the Epon remaining was replaced with fresh undiluted resin. Two changes of resin were made daily during the three day infiltration period. After infiltration, the specimens were oriented with the longitudinal axis of the root perpendicular to the cutting edge in Beem capsules (size 00; J.B. EM Services Inc., Dorval, Quebec) and polymerized in an oven at 35,50,65 and 75°C, on successive days.

2.6.6 Sectioning Of Embedded Material

Sections 1.5 - 10 μm in thickness were cut with glass knives mounted on a Reichert Om U2 ultramicrotome. The sections were floated off onto Millipore-filtered (0.22 μm) water and transferred to water droplets on acid-washed glass slides. Excess liquid was drained off and the slide placed on a 60°C warming plate. Sections handled in this fashion were in serial order and adhered to the slide without adhesive. They were observed unstained or after appropriate staining.

2.6.7 Staining Procedures

Hand-sections were stained in 0.05% Toluidine Blue O in benzoate buffer at pH 4.4 for 30 seconds, washed briefly with distilled water and mounted in either 15% glycerine or in clear lactophenol. Epon embedded sections were stained for 2 minutes on an 80°C warming tray in 0.1% Toluidine Blue O in sodium cacodylate buffer at pH 9.0. Slides so stained were rinsed in distilled water for 1 min, dried on the warming tray, and mounted in immersion oil.

2.6.8 Microscopy And Photomicrography

All material was examined using a Reichert microscope equipped with both bright-field and phase contrast optics. Black and white and colour photomicrographs were recorded on Kodak Plus X Pan and high speed Ektachrome film, respectively.

2.7 FIELD EXPERIMENTS

Experiments were designed and plantings made on two fields at the Central Experimental Farm, Agriculture Canada, Ottawa, in 1980. The general climate of this area is warm and humid with mean high and low atmospheric temperatures during the growing season of 24 and 11°C, respectively, and an average precipitation of 81 mm (for a more detailed weather summary, refer to the Appendix, Table 1).

One field (Merivale) was situated on land assigned to the Plant Products and Quarantine Division, Agriculture Canada. The previous cropping history of the Merivale Field included the cultivation of lentils as well as various other leguminous crops during the 1979 growing season. In 1978, this area lay fallow. A second field

(Neatby) was located adjacent to the K.W. Neatby Building, on land allocated to the Ottawa Research Station, Agriculture Canada. During the previous two growing seasons, the Neatby plot was left uncultivated.

Both locations were ploughed to a depth of 25 cm in the fall of 1979 and in the following spring the seedbed was disc-harrowed twice, and levelled.

Six randomly-selected soil samples were extracted from each field with a standard soil sampling tube, sealed in plastic bags and mailed to the Ontario Ministry of Agriculture and Food Soil Testing Laboratory, at the University of Guelph, Guelph, Ontario, for mineral analysis. The results from this analysis are summarized in the Appendix (Table 2).

2.7.1 Seeding Techniques

Except where indicated, the large-seeded lentil variety, Laird, was selected, as previously described (Sec. 2.3.1) for use in all field studies.

Seeds were sown by hand to a depth of 3 cm, using a seeding rate of about 40 seeds per m, in rows 30 cm apart. Each row constituted a replication and all treatments were replicated six times in a completely randomized design. Each treatment row was surrounded by a border row of untreated Laird seed.

2.7.2 Harvesting

Lentils were harvested for yield determination approximately three months after sowing, when the earliest-formed pods were yellow. The lentil swaths were packed into cotton gunnysacks and dried in a forced-air grain dryer at 37°C for four days prior to storage in a room exposed to the external environment for 1-2 weeks. The dried plants were threshed in a Almaco plot thresher (Allan Machine Co.) and the chaff separated from the seed by use of an air-column seed cleaner designed and manufactured by the Engineering and Statistical Research Institute, Agriculture Canada. Yield from each plot was estimated by weighing the recovered seed. Data were interpreted by an analysis of variance and significance was determined by Duncan's New multiple range test (Waller and Duncan, 1968).

2.7.3 Evaluation Of Benomyl Seed And Soil Treatments For The Control Of Fusarium Root And Collar Rot

On the Merivale plot, an experiment was designed to investigate the effects of benomyl (Benlate) (methyl 1-(butylcarbamoyl)-2-benzimidazole carbamate), a systemic fungicide, on disease control and seed yield in lentils. The treatments incorporated in this experiment were:

A - check
B - benomyl treated seed
C - benomyl soil treatment
D - benomyl seed and soil treatment

Laird lentil seeds were divided into replicate lots of 200 and placed into 8 x 12 cm manila envelopes. Thirty milligrams of Benlate 50 W.P. (duPont) at a rate equivalent to 1.0 g active ingredient (a.i.) per kg seed was added to randomly selected envelopes receiving the seed treatment. Following the addition of the fungicide, the envelopes were sealed and shaken to uniformly coat the seeds. For treatments C and D, 5.2 g (21.5 kg/ha) of Benlate was suspended in 2 l of distilled water and applied to each row at the time of seeding.

This rate of application was calculated based upon investigations by Shatla et al. (1975) on the chemical control of lentil root rot in Egypt. They observed that benomyl when applied at the rate of 9 kg/feddan resulted in 69% disease control over untreated check plots. Treatment rows sown on May 14 were 4 m in length and this trial was repeated on June 4. Plant stands were determined 1,2,3,4 and 6 weeks after planting. The central 100 plants from each plot were selected for harvest on August 12 for the first planting and on September 2 for the second planting.

2.7.4 The Effect Of Benomyl Seed Treatment On Emergence, Rhizobium Nodulation And Yield Of Lentils

This experiment was conducted on the Neatby field and it was designed to determine the effects of benomyl on the rate of seedling emergence, root nodulation and seed yield of lentils. Treatments in this experiment consisted of (A) an untreated check; (B) seedlings inoculated with Rhizobium leguminosarum; (C) seed treated with benomyl; and (D) seed treatment and Rhizobium inoculation. Seed was selected and treated with the fungicide, as described above and sown in plots 4 m in length on May 13.

Czapek-Dox liquid medium (150 ml) was inoculated with 14 g of a commercial preparation of Rhizobium leguminosarum 'c' (Nitragin Co.) and incubated at 25°C. Forty-eight hours later this was used to inoculate an additional 900 ml of the medium which was incubated on a rotary shaker (100 rpm) set at 31°C for 72 h. Following incubation the culture was filtered through 2 layers of cheesecloth prior to centrifugation in a Sorvall refrigerated centrifuge (10 min at 4000 rpm).

The pellet was washed three times in distilled-water to remove residual nutrient medium and then resuspended in 500 ml of distilled water. Treatments received 10 ml of the concentrated culture in 1 L of distilled water, 2 weeks after planting. Treatments A and C received 1 L of distilled water without the added Rhizobium. All treatments received an additional 1 L to facilitate penetration of the bacteria into the soil. This trial was repeated on June 5 and seedling emergence data was estimated 6 weeks after planting. One week prior to harvest, 2 plants were removed from each treatment and the number of nodules on each root system recorded. These were oven-dried at 40^0C for 4 days and then weighed. Nodulation data was expressed in terms of number of nodules per g dry weight of roots. The central 100 plants from each planting date were selected for yield estimation on August 13 for the early seeding and on September 3 for the later planting.

2.7.5 Evaluation Of Benomyl, PCNB, And Zineb Seed Treatment On Emergence And Seed Yield Of Four Lentil Cultivars

The three fungicides selected for use in these field studies were applied as powders at the rate of 1 g a.i. per kg seed. These were applied 24 hours prior to seeding to 6 replicates of 50 seeds of 4 different varieties (Laird, Commercial Chilean, Eston and P.I. 179313), using the method previously described (Sec. 2.7.3). The chemicals chosen for use were Benlate (duPont) 50 W.P.; Parzate Zineb (duPont) 75% W.P.; and Quintozene (Plantco) 75% W.P. Plots were seeded on the Neatby field on May 23 in rows 1 m in length. Seedling emergence counts were made 1,2,3 and 4 weeks following planning and on August 16, all plants in each plot were harvested for yield determination.

2.7.6 Varietal Trials

This experiment was performed to test the degree of field resistance in some lentil varieties and the effect of seed coat characteristics on seedling emergence and yield. The varieties used in these studies were the same as those used in the fungicide experiment. Seeds of each variety were subdivided into six replicate lots of 50 seeds each of green, yellow and except for Laird mottled seedcoats. In the Laird variety, spotting of the seedcoat is uncommon but a large proportion possesses cracks in the thin testa; therefore, seeds with cracked testa were selected instead. The seeds for this experiment were sown on June 5 on the Merivale field at a rate of 50 seeds/m and seedling emergence data was collected 1,2,3 and 4 weeks after planting. On September 3, all plants in each plot were harvested for yield determination expressed as seed weight/50 plants.

EXPERIMENTAL RESULTS

3.1 ISOLATIONS FROM FIELD-GROWN LENTILS

Diseased lentil seedlings were collected from a plot on the Experimental Farm in the summer of 1979 and preliminary examination of the fungi recovered from the surface-disinfected plants suggested that they were all Fusarium spp. Subsequently, using Booth's (1971) growth rates and morphological criteria the isolates were identified as F. equiseti, F. oxysporum, and F. solani. F. equiseti and F. oxysporum were the most common fungi isolated from the diseased lentils. These results in addition to the observations made by Mr. J. Sheppard, Laboratory Services Division, Agriculture Canada (personal communication), indicated that the pathogen(s) responsible for the disease symptoms observed in the field were probably members of the genus Fusarium. In order to investigate this possibility further, soil from the diseased field plot was sampled to provide a source of mycoflora.

3.2 ISOLATIONS FROM FIELD SOILS

3.2.1 Fungi Recovered From Soil Dilution Plates

From the soil dilution plates, 15 different fungal isolates were recovered, 10 of which were Fusarium spp. based upon Booth's criteria (Tables 1 and 2). Four of the isolates were identified as

Table 1. Growth Rates and Morphological Characteristics of *Fusarium* spp. Recovered From Lentil-Sown Field Soil

Isolate	Growth Rate[a] (cm)	Substrate Pigmentation[bc]	Colour of Mycelium[c]	Nature of Aerial Mycelium
F. oxysporum				
1B4	4.5	Vinaceous Purple→Salmon	white→pink	cottony
43C	5.2	Vinaceous Purple→Buff	white→pink	cottony
82A	3.6	Vinaceous Purple→Buff	white→purple	felted
117A	6.0	Vinaceous Purple→Saffron	pink→white	felted
F. solani				
1A1	3.6	Honey	white	floccose
8A3	4.3	Honey	white	floccose
196A	4.0	Buff	white	floccose
F. equiseti				
11C1	4.3	Salmon	pink	felted
12B1	4.6	Salmon	pink	felted
F. melanochlorum				
17D	1.0	Olivaceous Buff	white	floccose

[a] mean colony diameter of eight monoconidial colonies incubated for 96 hours.

[b] colours determined by comparison with Rayner's (1970) mycological colour chart.

[c] (→) indicates change in pigmentation from colony centre to colony edge.

Table 2. Size and Septation of Conidia and the Presence or Absence of Chlamydospores in the Culture of *Fusarium* spp. Recovered from Lentil-Sown Field Soil

Isolate	Microconidia[a]		Macroconidia[a]		Chlamydospores Present (+) or Absent (-)
	Size in µm (ranges)	Septation	Size in µm (ranges)	Septation	
F. oxysporum					
1B4	6.5x1.9 (4-13x1-4)	0	36.5x3.6 (25-46x3-5)	3	+
43C	7.7x5.1 (4-15x1-6)	0	35.7x2.8 (25-51x2-5)	3	+
82A	3.9x3.3 (3-14x3-4)	0	30.2x4.5 (22-37x4-8)	3	+
117A	10.2x2.8 (4-15x3-4)	0	34.4x4.5 (19-51x4-6)	5	+
F. solani					
1A1	8.8x2.4 (2-14x2-3)	0	46.0x4.5 (38-56x3-6)	4	-
8A3	10.6x3.8 (8-14x3-6)	0	33.2x7.7 (20-42x6-9)	3	-
196A	18.4x3.2 (11-20x3-5)	1 1	43.8x5.3 (33-52x4-6)	4	-
F. equiseti					
11C1	-	-	24.7x4.4 (16-28x4-5)	2	+
12B1	-	-	19.7x4.3 (15-25x3-5)	1	+
F. melanochlorum					
17D	-	-	53.6x5.7 (44-65x4-8)	2	-

[a] means of 100 observations.

Fusarium oxysporum Schlecht., three as *F. solani* (Mart.) Sacc., two as *F. equiseti* (Corda) Sacc., and one as *F. melanochlorum* (Casp.) Sacc. Other fungi isolated from the soil included *Acremonium strictum* W. Gams, an unidentified *Acremonium* sp., *Microdochium bolleyi* (Sprague) de Hoog & Hermanides-Nijhof, as well as unidentified species of *Alternaria* Nees, and *Cylindrocarpon* Wollenw. The non-fusarial isolates were much less common on the soil dilution plates than were the *Fusarium* spp. and identification of these fungi was completed by the Biosystematics Research Institute, Agriculture Canada.

3.2.2 Inoculum Density

Inoculum density was determined by multiplying the average number of colonies of *Fusarium* spp. per dilution plate by the dilution factor to obtain the total count per gram of the initial soil sample. This number was then divided by the grams of dry matter per gram of moist soil. The dry matter content of the soil was estimated as 0.8 g.

The results indicated that the levels of the *Fusarium* population in oven-dry soil ranged from 160-2,000 propagules/g with a mean count of 930, and, of these, 330 were identified as propagules of *F. oxysporum* Isolate 82A. On a typical soil dilution plate 7 days after incubation Isolate 82A could readily be distinguished on the basis of differences in colony morphology such as the regular nature of the colony border, the abundance of macroconidia at the colony edge, the radial appearance of the aerial mycelium, and the presence of a purple discolouration of the substrate at the colony centre. Soil samples extracted from a non-cultivated area adjacent to the lentil field had a *Fusarium* population estimated to be about 765 propagules/g dry soil and the density of Isolate 82A was 112 propagules/g.

3.3 ASSESSMENT OF SEED SURFACE DISINFECTION PROCEDURES

Table 3 lists the results obtained with various surface-sterilization treatments on "Laird' lentil seed. Germination of untreated seed plated directly onto water-agar was 99% and germination of seed hydrated before plating was 98%. Imbibing the seed prior to plating resulted in a 50% reduction in the numbers of contaminated seed (Table 3). The treatment selected for use throughout this study involved the disinfection of imbibed seed in sodium hypochlorite. These seeds were essentially disease-free and had a high rate of germination with vigorous, relatively straight growth of the radicle. Although no contaminants were recovered from seed treated with formaldehyde, germination was reduced to 50%. The emerging radicle coiled as it elongated and frequently it was discoloured and constricted in the vicinity of the root apex. This abnormal growth was also observed with seeds exposed to an atmosphere of either propylene oxide or gaseous formaldehyde, but germination in both of these cases was unaffected. Hydrogen peroxide, mercuric chloride and sulphuric acid did not substantially reduce the percentage of contamination but the numbers of germinated seed was reduced by the latter two chemicals.

3.4 PATHOGENICITY TESTS ON LENTILS GROWN IN ASEPTIC CULTURE

The following descriptions of disease symptoms induced in lentil seedlings by the field isolates were compiled from observations on

Table 3. The Effect Of Sterilization Treatments On Percent Germination And Contamination Of Lentil Seeds[a]

Sterilant	Concentration	Time	Seed Pretreatment[b]	% Germination	% Contamination[c]
Check (nontreated)	-	-	Dry	99	22
Check (nontreated)	-	-	Imbibed	98	11
Sodium Hypochlorite	0.6%	17 min	Imbibed	98	4
Formaldehyde	37%	10 min	Dry	50	0
Formaldehyde[d]	37%	4.5 h	Dry	100	0
Propylene Oxide[d]	98%	2 h	Dry	100	0
Hydrogen Peroxide	10%	5 min	Imbibed	100	15
Mercuric Chloride	0.001% in 70% ETOH	2 min	Dry	87	29
Sulphuric Acid	96%	10 min	Dry	67	11

[a] treatments listed represent the best results obtained from 60 different combinations of sterilant, concentration and treatment duration.

[b] imbibed seeds were soaked for 5 hours in 200 ml of distilled water amended with 0.04 g of powdered detergent (Sparkleen, Fisher Scientific).

[c] percentage of seed yielding bacterial and/or fungal contaminants.

[d] the seeds were suspended on glass beads above the disinfectant, in capped vials.

infected plants grown in aseptic culture. After the 21-day incubation period, reisolations were made from each seedling using surface-sterilized plant material excised from four portions of the plant in accordance with figure 5 (Sec. 2.3.6).

3.4.1 *Fusarium oxysporum*, Isolate 82A

Initial disease symptoms produced by this isolate were usually observed within 48 h following inoculation. The first distinguishable symptoms appeared as rings of discoloured tissue at the sites of emergence of older lateral roots and as mycelial development on the hypocotyl and cotyledons. Hyphal proliferation continued to develop on these structures and after 4 days the transitional zone was completely covered with mycelial growth. Tissues beneath the mycelium exhibited a brown soft rot especially on the hypocotyl. The root system of many infected plants was noticeably reduced when compared with non-inoculated seedlings and foliar symptoms, comprising a yellowing of the lowermost leaflets, appeared. After 5 days in culture, tissue discolouration at lateral root junctions had spread throughout the root zone and chlorosis had progressed acropetally from the lowermost leaflets, followed rapidly by necrosis. Frequently, the fungus was observed to sporulate on the stem axis, at soil level, just above the point of attachment of the cotyledons. Seedling death often occurred as early as 6 days after inoculation as evidenced by killing of the tap root and complete necrosis of stem and leaves (Fig. 8, 9-B and 10-B). Extensive disintegration of the root in which the cortical tissues were readily detachable from the stele, was frequently observed as was the premature fall of the necrotic leaves, resulting in defoliation of the lower portion of the

Figure 8. Control seedling (left) and one inoculated with a spore suspension of *F. oxysporum*, Isolate 82A (right). Note aerial hyphae (*) on stem and discolouration of roots (†) on insert.

FIG. 8

Figure 9. Symptoms produced on lentil seedlings, 21 days following inoculation with the most virulent isolates of _F_. _oxysporum_, _F_. _solani_, and _F_. _equiseti_.

A. Control

B. _F_. _oxysporum_, Isolate 82A

C. _F_. _solani_, Isolate 196A

D. _F_. _equiseti_, Isolate 11C1.

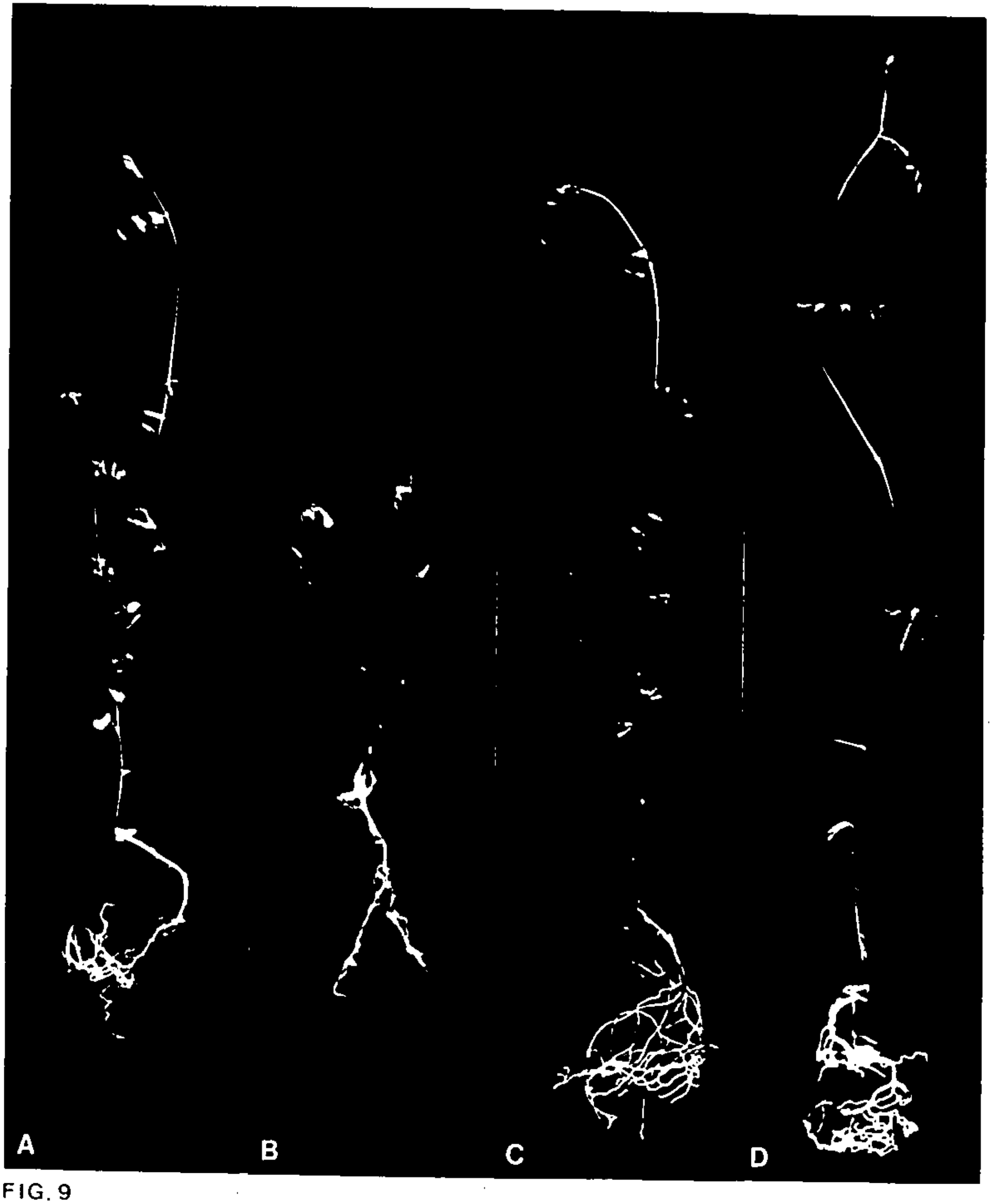


FIG. 9

Figure 10. Symptoms produced on lentil roots, 21 days following inoculation with the most virulent isolates of _F. oxysporum_, _F. solani_, and _F. equiseti_.

A. Non-inoculated check

B. _F. oxysporum_, Isolate 82A

C. _F. solani_, Isolate 196A; note brown soft rot (↑)

D. _F. equiseti_, Isolate 11C1; note small necrotic lesion (↑).

FIG. 10

stem. At the termination of the experiment, Isolate 82A could consistently be recovered from all portions of severely diseased plants.

3.4.2 *F. oxysporum*; Isolate 43C

Within 4 days of inoculation, areas of discolouration were observed on older portions of the tap root and often at lateral root junctions. Also, there was extensive mycelial proliferation on the seed coat and along the root-shoot axis. After 1 week, discolouration at the sites of emergence of the lateral roots was evident throughout the root zone and in addition, some lateral root apices became brown. Chlorosis, although initially confined to the lowermost leaflets was later observed to progress in an acropetal direction until at the conclusion of the experiment, at 21 days, the terminal leaflet was chlorotic and leaf tissue at the base of the stem had necrosed. Reisolation of this fungus was possible from the tap root apex, hypocotyl and lower stem.

3.4.3 *F. oxysporum*, Isolate 117A

Sparse mycelial development on the hypocotyl and on the seedcoat was first observed 1 week after inoculation. Also, at this time, zones of discolouration at older lateral root junctions were visible. Chlorosis was restricted to the lowermost leaflets. Towards the completion of the experiment, there was an increasing amount of fungal proliferation although it was confined to the area just above and below the point of attachment of the cotyledons. Only isolated lesions were visible on the tap root and in most seedlings these were restricted to areas of lateral root attachment. Chlorosis spread acropetally until all leaf tissue became chlorotic and in addition, the lowermost leaflets became necrotic. This isolate was recovered from tissue pieces removed from the hypocotyl and lower stem, and in the case of one severely diseased plant, also from the shoot apex.

3.4.4 F. oxysporum, Isolate 1B4

The first observable symptoms, visible 1 week following inoculation, were limited to discolouration of the hypocotyl and the proximal portions of the adventitious roots which emerged from it. More specifically, the tissue darkening was confined to the sites of emergence of these roots and occasionally to their apices. Within 2 weeks, mycelial development on and about the point of attachment of the cotyledons was observed as was initial sporulation of the fungus on the seedcoat. Chlorosis of the leaf tissue was restricted to the lower half of the plant and throughout the experiment the tap root and distal portions of the secondary roots remained disease-free. This isolate was recoverable only from tissue excised from the hypocotyl and lower stem.

3.4.5 F. solani, Isolate 196A

Following 3 days of incubation, tissue discolouration was observed at the apices of most secondary roots. Mycelial development, if present, was sparse and restricted to the seedcoat. As the infection progressed, discolouration of the root system became more pronounced and at the end of 9 days, a brown soft rot on the distal portions of the secondary roots was visible (Fig. 9-C and 10-C). In addition, dark-brown-to-black lesions formed at the sites of emergence of younger lateral roots, while older portions remained essentially disease-free. The leaf tissue became progressively chlorotic and at 2 weeks following inoculation, the shoot in severely diseased plants was completely chlorotic with necrosis of the lowermost leaflets. Usually stunting occurred before chlorosis and necrosis but often this symptom did not appear in mildly diseased plants. At the end of the 21-day incubation period, this isolate was recovered from all portions of severely diseased plants.

3.4. 6 F. solani, Isolate 1A1

Initial signs of infection involved the appearance of fungal mycelium on the seedcoat and along the hypocotyl. Within 9 days, a brown discolouration of the tissues at lateral root junctions and tips was visible and as the seedlings matured, these areas turned a dark brown and eventually coalesced to form large lesions on older portions of the root system. Although the seedlings became progressively chlorotic, necrosis was confined to the lowermost leaflets. This fungus was recovered from the tap root apex, hypocotyl, and the lower portion of the stem.

3.4.7 F. solani, Isolate 8A3

Sparse hyphal development was evident on the seedcoat 3 days after inoculation. In addition, there were signs of discolouration at the sites of emergence of the lateral roots and at the lateral root tips. Tissue browning remained confined to these areas during the duration of the experiment and further symptom development was restricted to the appearance of a chlorosis of the lowermost leaflets. This isolate was found only in tissue removed from the tap root apex and from the hypocotyl.

3.4.8 F. equiseti, Isolates 11C1 and 12B1

Within 2 days of inoculation, mycelial development was evident on the root-stem axis at the point of attachment of the cotyledons. At 5 days, there was a chlorosis of the lowermost leaflets. Further symptom development was restricted to an increase in mycelial growth and the progressive increase acropetally in the number of chlorotic leaflets (Fig. 9-D and 10-D). In some seedlings, the terminal leaflet became chlorotic and there was necrosis of the lowermost leaflets. Throughout the experiment, the root systems of seedlings inoculated with Isolate 12B1 remained free of discolouration whereas those infected with Isolate 11C1 occasionally developed small lesions on the secondary roots. Both of these isolates were recovered from tissues removed from the hypocotyl, lower stem and shoot apex.

3.4.9 F. melanochlorum, Isolate 17D

The initial disease symptoms observed 6 days after inoculation included a slight discolouration at the lateral root junctions and sparse mycelial development on the seedcoat. Two days later, there was an increase in hyphal mass but it remained confined to the seedcoat. After 11 days in culture, there were signs of chlorosis at the lowermost leaflets and just prior to the termination of the experiment, the chlorosis spread upward to include leaflets at nodes 3,4, and 5. No further lesion development was observed on the root system (Fig. 11-B and 12-B). This Fusarium species was recovered from the tap root apex and the hypocotyl.

Figure 11. Symptoms produced on lentil seedlings, 21 days following inoculation with *Fusarium melanochlorum*, *Microdochium bolleyi*, and *Acremonium strictum*.

A. Non-inoculated check

B. *F. melanochlorum*, Isolate 17D

C. *M. bolleyi*, Isolate 89A

D. *A. strictum*, Isolate 38B.

FIG. 11

Figure 12. Symptoms produced on lentil roots, 21 days following inoculation with *Fusarium melanochlorum*, *Microdochium bolleyi*, and *Acremonium strictum*.

A. Non-inoculated check

B. *F. melanochlorum*, Isolate 17D

C. *M. bolleyi*, Isolate 89A; note small lesion on secondary root (↑)

D. *A. strictum*, Isolate 38B; note lesion on older secondary root (↑).

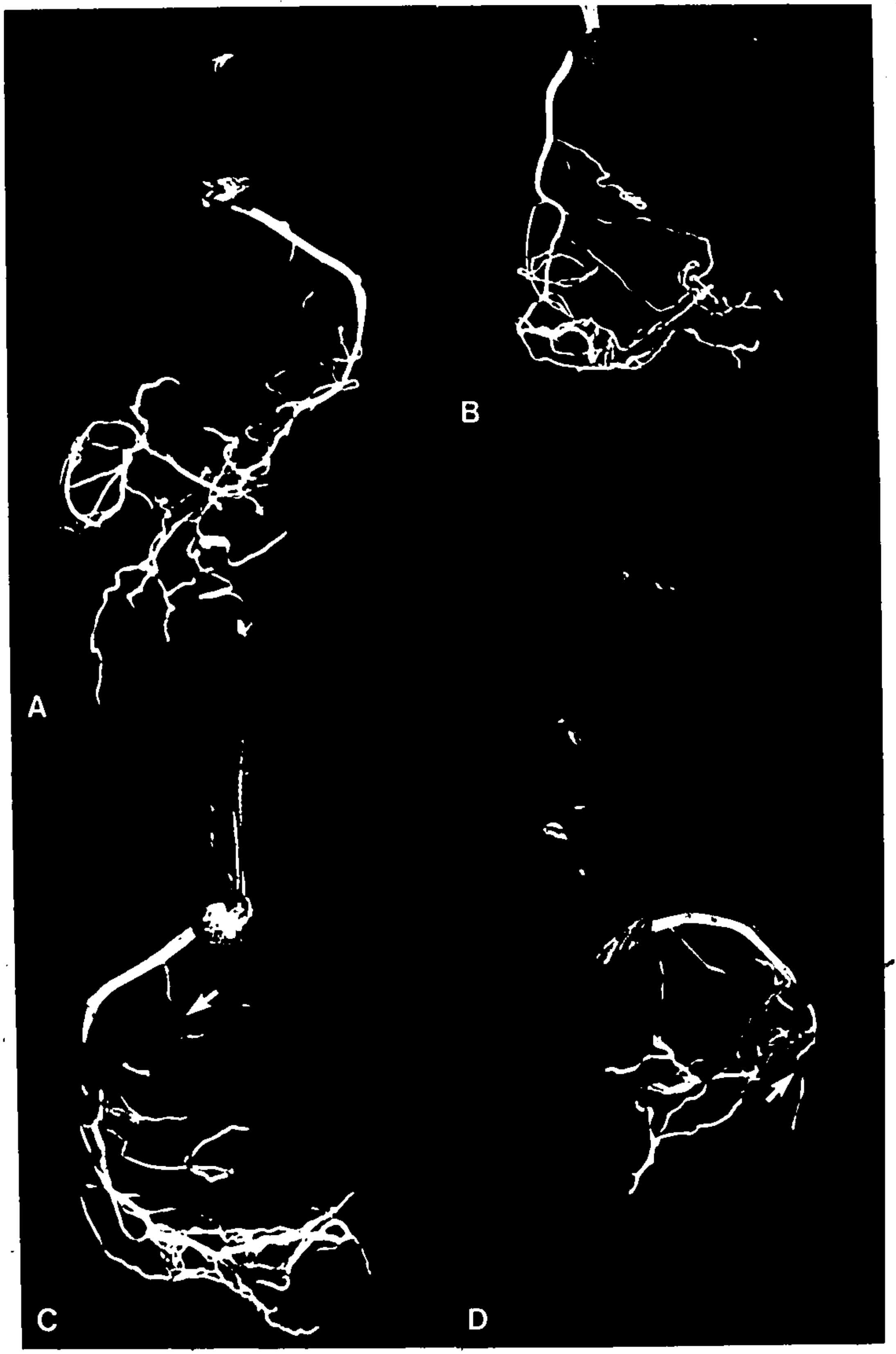


FIG. 12

3.4.10 Microdochium bolleyi, Isolate 89A

One week following inoculation, an overall chlorosis of some seedlings was observed. Within 2 weeks, many of the leaflets had numerous small pinkish lesions giving a mottled appearance to the leaf tissue. Frequently, leaf drop was observed at the lower nodes causing defoliation of the lower stem. Root discolouration was limited to dark brown lesions on the secondary roots (Fig. 11-C and 12-C). This pathogen was successfully reisolated from the hypocotyl and lower stem.

3.4.11 Acremonium strictum, Isolate 38B and Acremonium sp., Isolate 9C

Lentil seedlings incubated with this isolate for 21 days were stunted with a V-shaped chlorosis of the lowermost leaflets. Sparse mycelial development was visible on the seedcoat and root discolouration was confined to the appearance of dark brown lesions on older secondary roots (Fig. 11-D and 12-D). This isolate was recovered only from hypocotyl tissue.

3.4.12 Cylindrocarpon sp., Isolate 76C

No symptoms were observed following 21 days of incubation with the isolate and it could not be recovered from inoculated seedlings.

3.4.13 Alternaria sp., Isolate 18D

No symptoms were observed following 21 days of incubation with this isolate and it could not be recovered from inoculated seedlings.

3.4.14 Disease symptom index

Figure 13 is a graphical representation of the onset of disease symptoms and their rate of progress. The rate of development of symptoms varied markedly for the individual isolates; however, there was a trend for disease development to reach a maximum within the twenty-one day incubation period. The rate of symptom evolvement was greatest for F. oxysporum Isolate 82A, particularly during the initial stages (Fig. 13-B). Within the incubation period, seedlings infected with this isolate attained a disease index rating of 94 while the next most virulent isolate of F. oxysporum, 43C, produced a maximum rating of 52. Other isolates of F. oxysporum did not vary substantially from Isolate 43C and these results are compatible with the data listed in Table 4.

Symptom expression in lentils inoculated with Isolate 196A of F. solani was comparatively more severe than with Isolates 1A1 and 8A3, although rates of disease development were similiar initially with all three isolates (Fig. 13-A).

The remaining isolates were less pathogenic than the F. solani or F. oxysporum isolates. The 2 isolates of Acremonium were only slightly different in the course of disease development (Fig. 13-C) and these results are reflected in Table 4, indicating that perhaps both are isolates of the same species.

Isolate 11C1, resulted in a uniform rate of increase in the disease index (Fig. 13-D) which reached a maximum value at 18 days following inoculation. Isolate 12B1 was somewhat less virulent although this was not evident from Table 4.

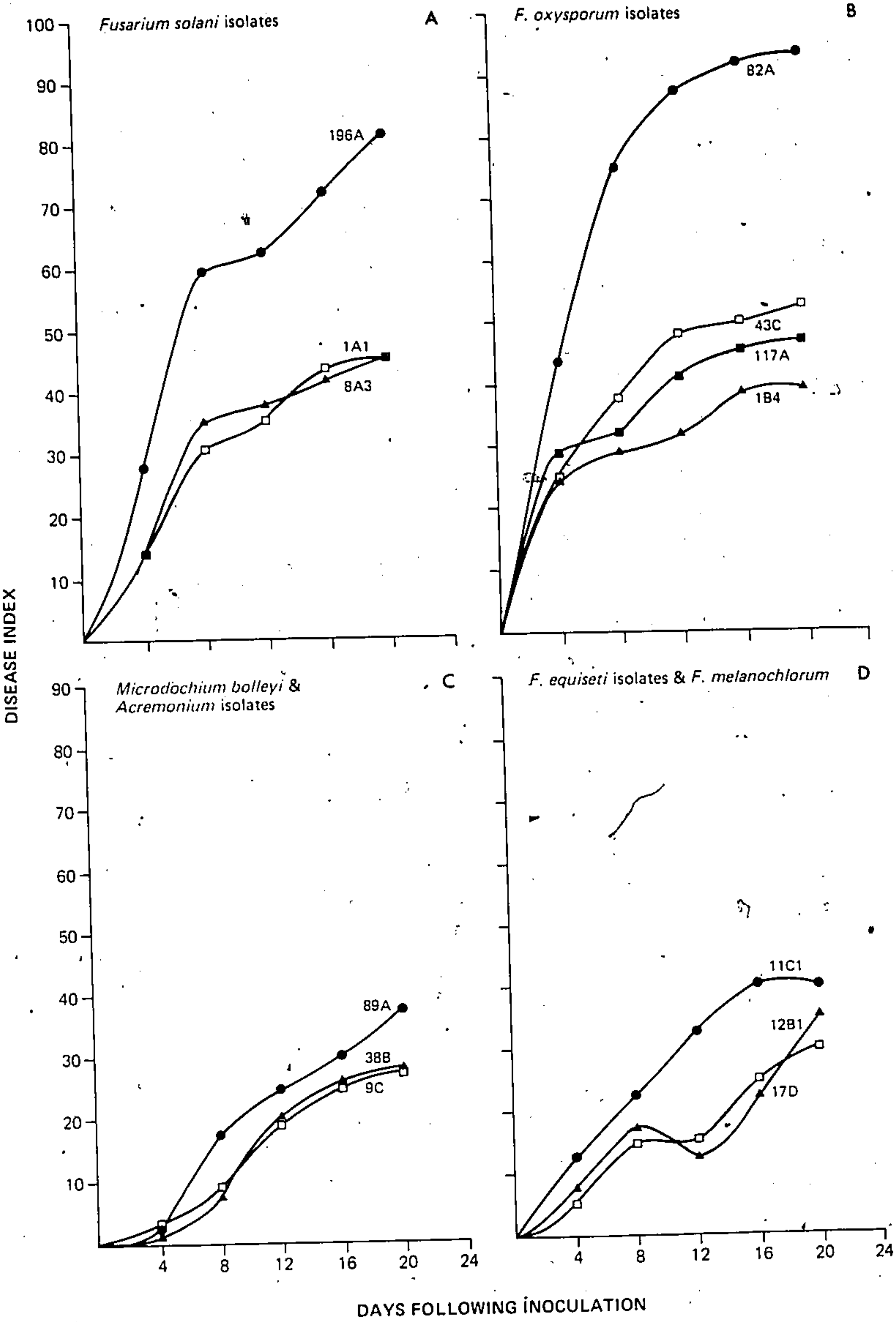
Figure 13. The relative infectivity (disease index) of field soil isolates at 4 day intervals following inoculation.

A. Fusarium solani, Isolates 1A1, 8A3, and 196A.

B. F. oxysporum, Isolates 1B4, 43C, 82A, and 117A.

C. M. bolleyi, Isolate 89A and Acremonium Isolates 9C and 38B

D. F. equiseti, Isolates 11C1 and 12B1 and F. melanochlorum, Isolate 17D.



Fusarium solani isolates
A
F. oxysporum isolates
B
Microdochium bolleyi & Acremonium isolates
C
F. equiseti isolates & F. melanochlorum
D
DISEASE INDEX
100
90
80
70
60
50
40
30
20
10
196A
1A1
8A3
82A
43C
117A
1B4
89A
38B
9C
11C1
12B1
17D
4
8
12
16
20
24
DAYS FOLLOWING INOCULATION

FIG. 13

Table 4. Comparative Pathogenicity of Soil-Borne Fungal Isolates to Lentils, 21 Days Following Inoculation

Isolate	Root Disease Index[a]	Foliar Disease Index[b]	Reisolation[c]			
			A	B	C	D
Fusarium oxysporum						
82A	4	4	+	+	+	+
43C	3	3	+	+	+	-
117A	2	3	-	+	+	-
1B4	2	2	-	+	+	-
Fusarium solani						
196A	4	3	+	+	+	+
1A1	3	3	+	+	+	-
8A3	2	2	+	+	-	-
Fusarium equiseti						
11C1	1	3	-	+	+	+
12B1	1	3	-	+	+	+
Fusarium melanochlorum						
17D	1	2	+	+	-	-
Microdochium bolleyi						
89A	1	3	-	+	-	-
Acremonium strictum						
38B	1	2	-	+	-	-
Acremonium sp.						
9C	1	2	-	+	-	-
Cylindrocarpon sp.						
76C	0	0	-	-	-	-
Alternaria sp.						
18D	0	0	-	-	-	-

[a] 0 = healthy seedlings; 1 = slight discolouration of roots confined to lateral root junctions and/or lateral root tips; 2 = isolated lesions on tap root with up to 50% discolouration; 3 = coalescence of lesions with 50-75% discolouration of tap root; 4 = tap root killed.

[b] 0 = healthy seedlings; 1 = chlorosis of first true leaves; 2 = leaf chlorosis extending half way up the seedling; 3 = chlorosis of all leaf tissue with necrosis of lowermost leaflets; 4 = complete necrosis of the seedling.

[c] presence (+) or absence (-) of the fungal isolate in surface-disinfected host tissue pieces excised from A - tap root apex; B - hypocotyl; C - lower stem; and D - shoot apex.

3.5 PATHOGENICITY TESTS ON LENTILS GROWN IN PASTEURIZED SOIL

The symptomatology of lentil plants infected with the field isolates is described in the following sections. Only the foliar disease symptoms produced by the most virulent isolate of each species were assessed. After yield determination, reisolations were made from each seedling using surface-disinfected plant material excised from four portions of the plant as described in Sec. 2.3.6. Results of these tests are summarized in Table 5.

3.5.1 F. oxysporum, Isolate 82A

In severely infected seedlings, this isolate caused a chlorosis of all leaf tissue within 5 days of inoculation. In addition, many of the younger leaflets were curled inward from the leaf tip or twisted spirally about the mid-rib. More typically however, initial disease symptoms were not observed until 10 days following inoculation. The first sign of infection was a V-shaped chlorosis of the lowermost leaflet tips which spread acropetally to the leaves half way up the stem by the end of three weeks. During that period the stems remained disease-free. The plants were noticeably stunted due to a shortening of the internodes, particularly towards the shoot apex (Fig. 14), and the leaflets were much reduced in size. Chlorosis spread up the stem and after 7 weeks many of the plants were completely chlorotic with necrotic lesions on the lowermost leaflets, especially at the leaf margins. A few plants set seed in pods which initially appeared healthy but later developed brown necrotic lesions. The seed recovered from the pods was often considerably reduced in size, paper-thin, and

Figure 14. Symptoms produced on lentil seedlings grown in pasteurized soil, 10 days following inoculation with F. oxysporum, Isolate 82A.

left: non-inoculated check

right: seedling inoculated with F. oxysporum, Isolate 82A; note the infected seedling is stunted with small leaflets (+).

Figure 15. Seed harvested from lentils grown in pasteurized soil.

top: seed recovered from non-inoculated check plants

bottom: seed recovered from plants inoculated with F. oxysporum, Isolate 82A.

FIG. 14

FIG. 15

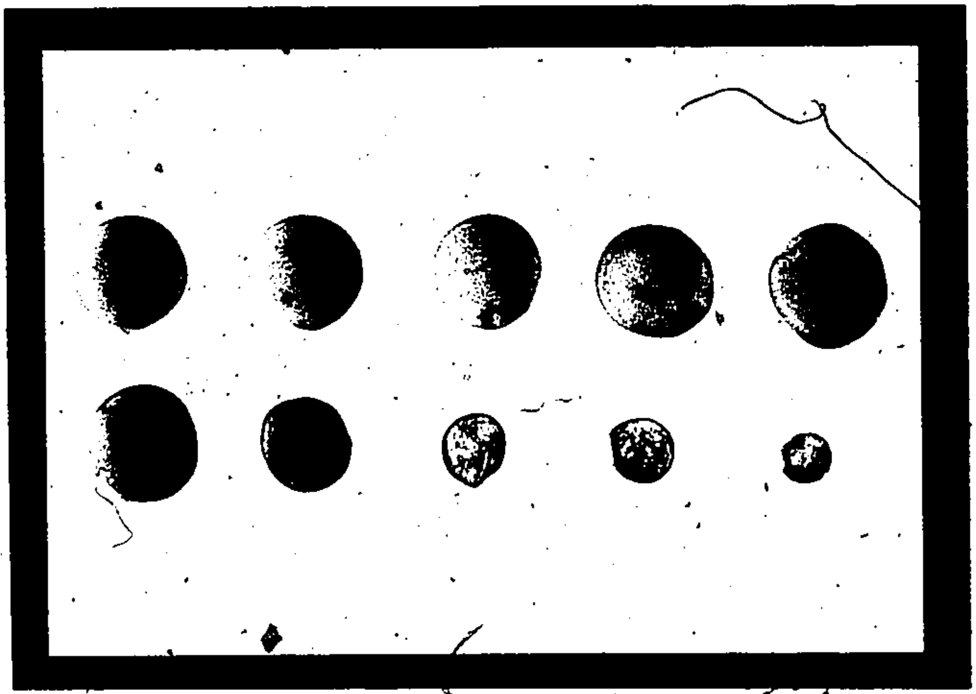


Table 5. Pathogenicity of Soil-Borne Fungal Isolates to Lentils Grown in Pasteurized Soil and their Reisolation from Diseased Host Tissues

Pathogen	Foliar Disease[1] Index	Yield[2] (grams)	Shoot[3] Length (cm)	Reisolation[4] A B C D
Non-inoculated Control	0	1.93^{a}	64.4^{ad}	- - - -
F. oxysporum, isolate 82A	3	0.05^{b}	42.5^{bc}	+ + + -
F. solani, isolate 196A	2	0.68^{c}	39.8^{c}	- + + -
F. melanochlorum, isolate 17D	2	0.71^{cd}	57.4^{bd}	- + + -
F. equiseti, isolate 11C1	1	1.56^{e}	56.1^{bd}	- + - -
A. strictum, isolate 38B	1	1.63^{ef}	59.4^{ad}	- + - -
M. bolleyi, isolate 89A	1	1.69^{f}	54.3^{bcd}	- + - -

[1] 0 = healthy plants; 1 = chlorosis of first true leaves; 2 = leaf chlorosis extending half-way up the plant; 3 = chlorosis of all leaf tissue with necrosis of lowermost leaflets; 4 = complete necrosis of the plant.

[2] average weight of seeds harvested from 6 plants; means followed by a common letter within a column are not significantly different ($P = 0.05$) according to Duncan's New Multiple Range Test.

[3] average of 6 plants; means followed by a common letter within a column are not significantly different ($P = 0.05$) according to Duncan's New Multiple Range Test.

[4] presence(+) or absence (-) of the fungus in surface-disinfected host tissue pieces excised from A - tap root apex; B - hypocotyl; C - lower stem; and D - shoot apex.

dark-brown (Fig. 15). Seed yield from inoculated lentil plants was 0.05 g/plant as compared with a yield of 1.93 g/plant from non-inoculated control plants (Table 5). Isolate 82A was reisolated from the tap root apex, hypocotyl, and lower stem of all diseased plants and, in addition, from the shoot apex of severely stunted plants.

3.5.2 F. solani, Isolate 196A

Within 2 weeks of inoculation the leaves on the lower half of the seedlings became chlorotic. Four weeks later, the chlorotic leaves were shrivelled and the plants severely stunted. Only a few of the infected plants flowered and seed yield was 0.68 g/plant. This isolate was recovered only from the hypocotyl and lower stem tissues.

3.5.3 F. melanochlorum, Isolate 17D

A chlorosis of the oldest true leaves and a reduction in leaflet size were the first symptoms seen on plants infected with this isolate. Within 4 weeks of inoculation, the chlorosis spread acropetally to the leaves mid-way up the stem. There was little difference in the average height of infected plants compared to that of the controls. The average seed yield from the diseased plants was about the same as that from plants infected with F. solani. Recovery of this isolate was made from the hypocotyl and the base of the stem (Table 5).

3.5.4 F. equiseti, Isolate 11C1

There was only a slight reduction in seed yield in plants infected with this Fusarium sp. Foliar symptoms remained confined to a chlorosis of the lowermost leaflets and reisolation was successful only from host hypocotyl tissue.

3.5.5 A. strictum, Isolate 38B

Foliar disease symptoms were restricted to a slight chlorosis of the lowermost leaflets. This symptom did not become apparent until the completion of the experiment and seed yield was only marginally reduced as compared to that from the control. A. strictum was recovered from hypocotyl tissues.

3.5.6 M. bolleyi, Isolate 89A

Foliar disease symtoms appeared as a chlorosis of the lowermost leaflets at the termination of the experiment. As with the previous isolate, yield was slightly reduced and this pathogen was recovered from the hypocotyl area.

3.6 PATHOGENICITY OF THREE ISOLATES OF Fusarium oxysporum TO LENTILS

An experiment was designed to compare the course of disease development of two other F. oxysporum cultures isolated from field grown lentils with that of Isolate 82A. An isolate of F. oxysporum (O-670), courtesy of Dr. P.E. Nelson, Fusarium Research Centre, The Pennsylvania State University was originally isolated from diseased lentils grown in Columbia, South America. A second isolate (W-2, my code), from lentil roots in Fairfield, Washington was supplied by Dr. R.J. Cook, Regional Cereal Disease Laboratory, U.S.D.A., Washington State University. Neither of these scientists had tested their isolates for pathogenicity to lentil seedlings and together with Isolate 82A they were cultured separately onto PSA, passed once through a lentil host and single-spored for the determination of cultural characteristics, spore morphology and growth rate. Their pathogenicity to lentils was determined under aseptic and controlled environmental conditions.

3.6.1 A Cultural Comparison Of Three Fusarium oxysporum Isolates Grown On Potato-Sucrose-Agar

Isolates 82A and 0-670 were similar, differing only in the size of their macroconidia (Table 6). Isolate W-2 differed from the other two isolates in substrate pigmentation, mycelium colour, and in the cottony growth of the aerial mycelium (Table 6). The average size of the W-2 macroconidia was the same as that of Isolate 82A which was smaller than that of Isolate 0-670.

3.6.2 Pathogenicity To Lentils Grown In Aseptic Culture

Within the 21-day incubation period, Isolate 82A caused extensive necrosis of the tap root which became discoloured a dark-brown-to-black. There was widespread disintegration of the cortical cells of the tap root, tissue necrosis, and proliferation of this isolate along the entire length of the hypocotyl and at the bases of the secondary roots which emerged from it. Frequently, necrotic lesions on these secondary roots were up to 1.5 cm in length; however, on each one, the apex remained free of discolouration. Numerous adventitious roots arose from the base of the stem as necrosis spread throughout the root zone (Fig. 16-A and 17-A). Chlorosis and some deformation of the leaflet tips was visible to mid-stem. Isolate 82A was found in host tissues removed from the tap root apex, hypocotyl and stem base but not however in tissues from the shoot apex.

Table 6. Characterization of Three Monosporic Isolates Of <u>Fusarium oxysporum</u> On Potato-Sucrose-Agar Medium

	<u>Fusarium oxysporum</u> Isolates		
	82A	0-670	W-2
Growth Rate[a]	3.4	3.3	3.4
Substrate Pigmentation[b]	Vinaceous purple → Buff	Vinaceous purple → Buff	Saffron
Colour of Mycelium	Pink	Pink	White
Nature of Aerial Mycelium	felted	felted	cottony
Microconidia[c]-size in μm	10.1 x 2.5	10.1 x 2.5	10.1 x 2.5
- (ranges)	(5-13 x 2-4)	(8-15 x 2-5)	(8-15 x 2-5)
- septation	0	0	0
Macroconidia[c]-size in μm	32.9 x 5.1	40.5 x 4.0	32.9 x 3.8
- (ranges)	(23-46 x 4-8)	(25-68 x 2-5)	(29-43 x 2-5)
- septation	3	3	3
Chlamydospores present (+) or absent (-)	+	+	+

[a] mean colony diameter (cm) of eight monoconidial colonies incubated for 96 hours.

[b] pigmentation determination by comparison with Rayner's (1970) mycological colour chart; (→) indicates change in pigmentation from colony centre to colony edge.

[c] means of 100 observations.

Figure 16. Symptoms produced on lentil seedlings, 21 days following inoculation with three different isolates of F. oxysporum.

A. Isolate 82A, recovered from field soil, Ottawa, Canada

B. Isolate 0-670, recovered from lentil roots, Columbia, South America

C. Isolate W-2, recovered from lentil roots, Washington State, United States of America.

FIG. 16

Figure 17. Symptoms produced on lentil roots, 21 days following inoculation with three different isolates of F. oxysporum.

A. Isolate 82A

B. Isolate 0-670

C. Isolate W-2

Note that in A and B, older portions of the root system, near the seed are more discoloured than those in C.

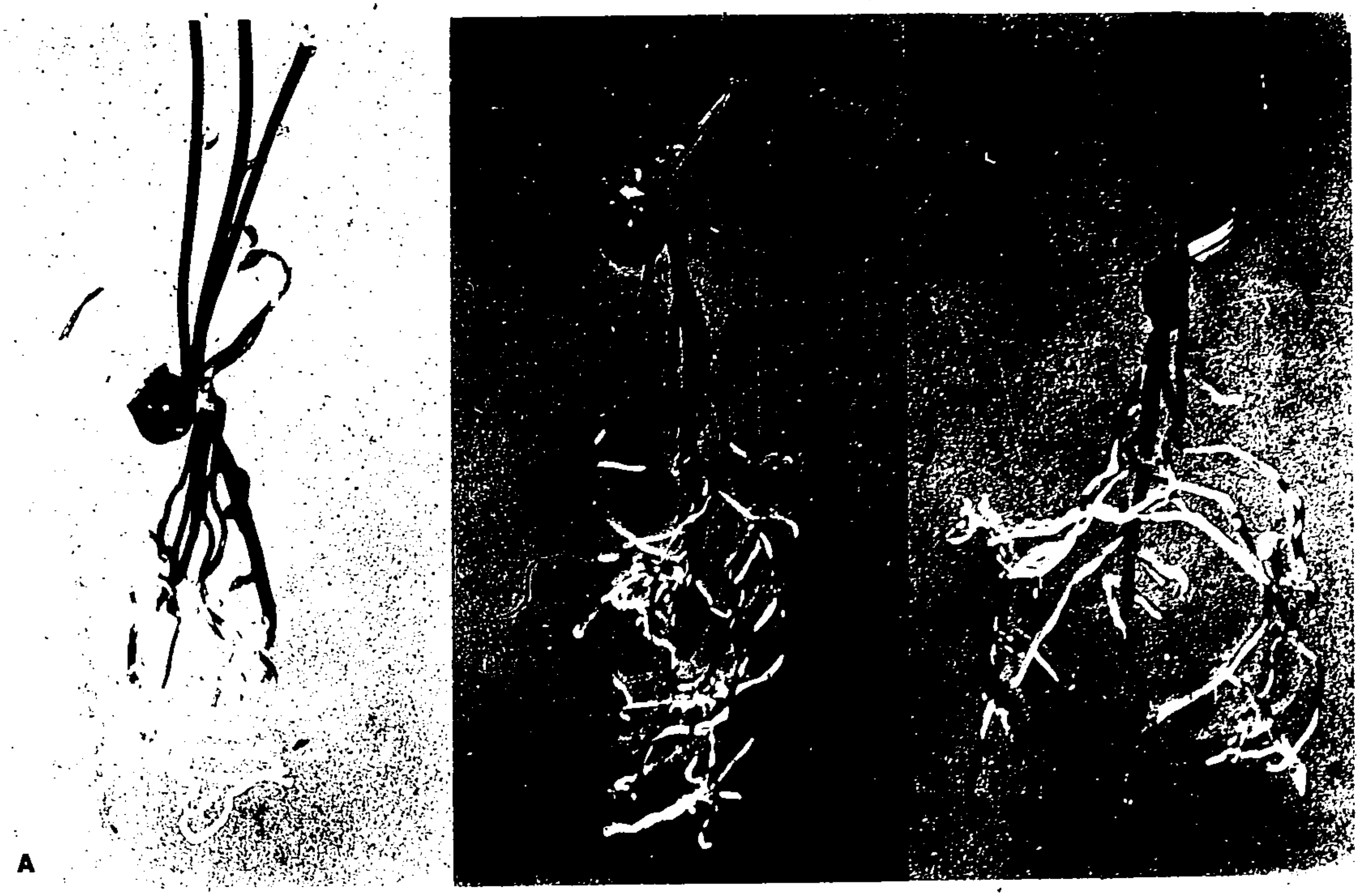


FIG.17

Disease symptoms produced by Isolate 0-670 were similar in some respects to those described for Isolate 82A but disimiliar in others. The tap root was discoloured a dark brown but many of the necrotic lesions on the secondary roots had coalesced rather than remaining discrete. Further, unlike Isolate 82A, there was a darkening of the tissues at the lateral root apices and no mycelial development was observed on the infected seedlings (Fig. 17-B). Foliar symptoms were limited to a chlorosis of the lowermost leaflets.

Isolate W-2 caused symptoms that were quite distinct from those produced by either Isolates 82A or 0-670. The tap root and hypocotyl remained free of disease and there were rarely signs of discolouration at the sites of emergence of the secondary roots. Discolouration of the root tissues was limited to the apices of the lateral roots and occasionally also included the points of emergence of the younger laterals (Fig. 17-C). There was premature abscission of leaves at the lowermost node (Fig. 16-C) and a V-shaped chlorosis of the leaflets at the third node.

In this experiment, the average shoot lengths of plants inoculated with Isolate 82A were significantly less than those inoculated with Isolates 0-670 or W-2 (Table 7).

3.6.3 Pathogenicity To Lentils Grown In Pasteurized Soil

Isolate 82A was the most virulent when tested on lentils grown in pasteurized soil and the symptoms produced were as described in Sec. 3.5.1. In contrast, other than an overall chlorosis of the leaf tissue of the lower half of the plants inoculated with Isolate W-2, no additional foliar symptoms were observed. Isolate 0-670 caused a reduction in shoot growth in infected seedlings and in many of the older leaflets,

Table 7. Comparative Pathogenicity of Three isolates of *F. oxysporum* To Lentils 21 Days Following Inoculation In Aseptic Culture

F. oxysporum Isolate	Root Disease Index[a]	Foliar Disease Index[b]	Shoot Length[c] (cm)	Reisolation[d] A B C D
82A	4	2	17.1^{a}	+ + + -
0.670	3	1	19.7^{ab}	+ + + -
W-2	1	1	21.3^{b}	- - + -
Check	0	0	22.4^{b}	- - - -

[a] 0 = healthy seedlings; 1 = slight discolouration of roots confined to lateral root junctions and/or lateral root tips; 2 = isolated lesions on the tap root (< 50% discolouration); 3 = coalescence of lesions with 50-75% discolouration of tap root; 4 = tap root killed.

[b] 0 = healthy seedlings; 1 = chlorosis of first true leaves; 2 = leaf chlorosis extending half way up the seedling; 3 = chlorosis of all leaf tissue with necrosis of lowermost leaflets; 4 = complete necrosis of the seedling.

[c] mean of 5 seedlings; means followed by a common letter within a column are not significantly different ($P = 0.05$) according to Duncan's New Multiple Range Test.

[d] presence (+) or absence(-) of the isolate in surface-sterilized host tissue segments excised from A - tap root apex; B - hypocotyl; C - lower stem and D - shoot apex.

a chlorosis and curling inwards from the tip. However, the leaf size was similar to that of the control plants instead of being smaller as those on 82A infected seedlings.

3.7 COMPARATIVE PATHOGENICITY OF Fusarium oxysporum, ISOLATE 82A AND F. roseum 'Avenaceum' TO LENTIL SEEDLINGS

A culture of Fusarium roseum 'Avenaceum' which has been reported to be a serious pathogen of lentils in Eastern Washington State (Lin, 1975), was supplied by Dr. R.J. Cook, Regional Cereal Disease Laboratory, U.S.D.A., Washington State University. The pathogenicity of this organism to lentil seedlings was compared with that of F. oxysporum Isolate 82A under both aseptic and controlled environmental conditions.

3.7.1 Pathogenicity Of F. roseum 'Avenaceum' To Lentils Grown Aseptically

Seedling death occurred within 7 days of inoculation with F. roseum 'Avenaceum'. The stem and all of the leaflets were necrotic and there was shrivelling and rolling of the leaflet margins inwards towards the mid-rib (Fig. 18-B). The hypocotyl and seedcoat were covered with a pinkish mass of fungal mycelium which frequently extended onto the base of the stem (Fig. 18-D). The tap root and proximal portions of the older secondary roots had necrosed and displayed a brown soft rot while the apices of these and younger laterals were free of discolouration. The time-course of disease development in lentils infected with F. roseum 'Avenaceum' as well as the overall symptomatology were similar to those obtained with F. oxysporum Isolate 82A (Section 3.4.1). However, F. roseum 'Avenaceum' produced more severe foliar symptoms but less severe root damage than F. oxysporum, Isolate 82A.

Figure 18. Disease symptoms produced by F. oxysporum Isolate 82A (A,C) and F. roseum 'Avenaceum' (B,D) on lentils, 21 days after inoculation. For a comparison of symptoms see Sec. 3.7.1.

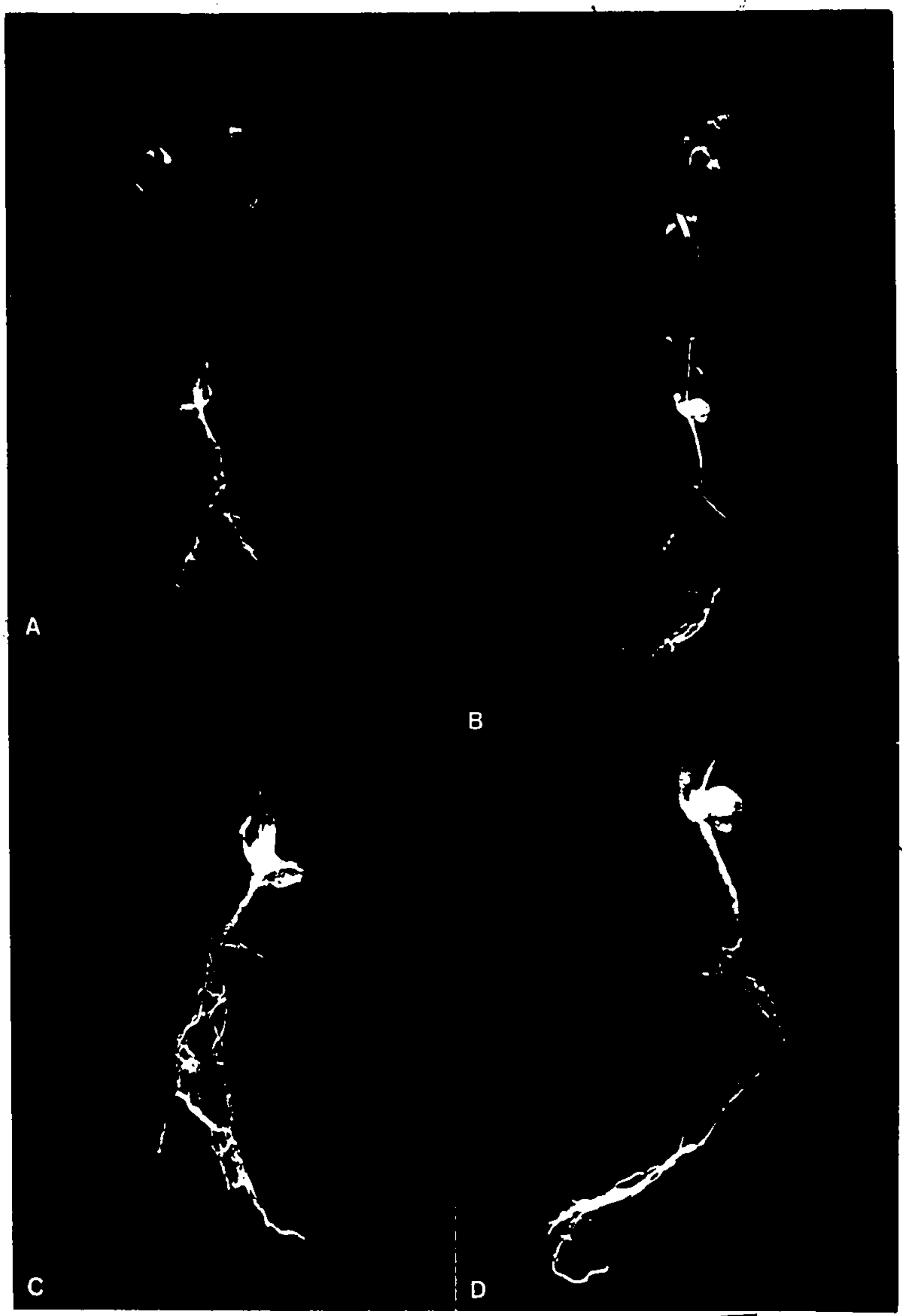


FIG. 18

3.7.2 Pathogenicity Of *F. roseum* 'Avenaceum' To Lentils Grown In Pasteurized Soil

Seedling death occurred within 12 days after inoculation with this *Fusarium* species. At 5 days following inoculation, a chocolate-brown lesion formed at soil level. This lesion expanded causing girdling and subsequent collapse of the stem (Fig. 19-A and 20-D). The foliar symptoms consisted of green but shrivelled leaflets with tan-coloured necrotic lesions (Fig. 19-C). The root system of inoculated plants was only slightly affected and differed only marginally from the roots of non-inoculated check plants (Fig. 20-A,B). The healthy appearance of the root system contrasts markedly with the severe root damage that resulted in plants inoculated with *F. oxysporum*, Isolate 82A. Because the seedlings inoculated with *F. roseum* 'Avenaceum' died so rapidly, seed production was nil but plants infected with *F. oxysporum*, Isolate 82A did produce some seed even when severely damaged.

3.8 PATHOGENICITY OF A SIMULTANEOUS INOCULATION OF *F. oxysporum* AND *F. solani* TO LENTILS

Årsvoll (1976), reported that simultaneous inoculation of *Phleum pratense* plants with isolates of two *Typhula* species resulted in less severe disease than if plants were inoculated with individual isolates of the most virulent species. In this experiment, inoculum from the two most virulent pathogens recovered from field soil, *F. oxysporum*, Isolate 82A and *F. solani*, Isolate 196A (Table 1), was mixed and used to inoculate lentil seedlings grown in aseptic culture and in pasteurized soil. In general, the symptoms produced by the mixture were less severe than those generated by Isolate 82A and only minimally more severe than those produced by Isolate 196A (Fig. 21 and 22). Some of the differences between the

Figure 19. Symptom expression on lentil seedlings grown in soil and inoculated with a spore suspension of F. roseum 'Avenaceum'. The inoculated seedling (A, right) with a crook-neck shoot, a typical wilt symptom. Leaves of the non-inoculated check plant (B) appear normal while those on the infected seedling (C) are shrivelled with necrotic lesions (↑).

FIG. 19

Figure 20. Root system of an *F. roseum* '*Avenaceum*' inoculated plant (B) lacks lesions and is smaller than the control root system (A). The base of the inoculated plant stem (D) is constricted (arrow) while that of the control plant is uniform (C).

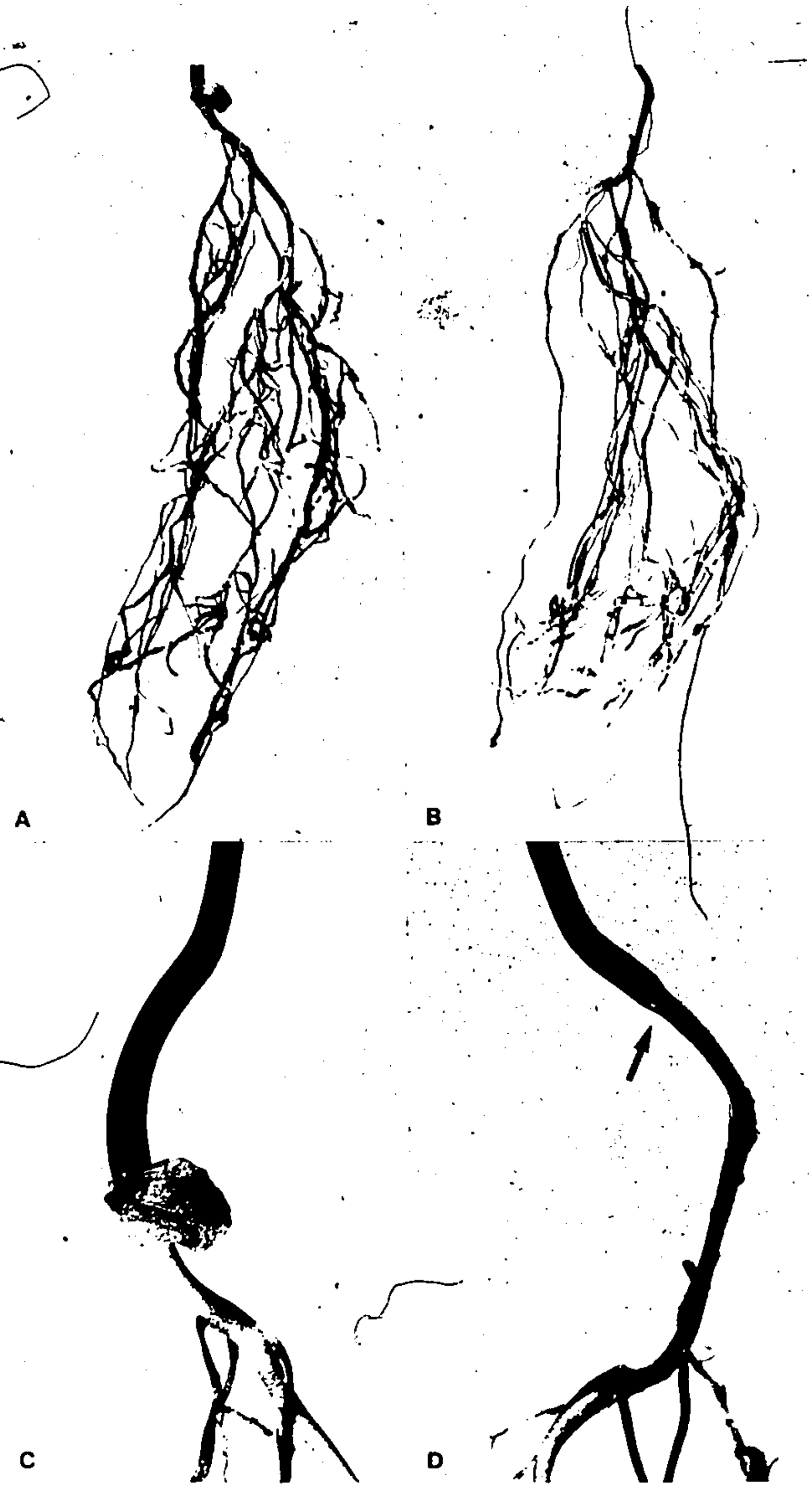


FIG. 20

Figure 21. Plants inoculated with F. oxysporum Isolate 82A (A), F. solani Isolate 196A (B) and with a mixture of both species; symptoms expressed by the mixture appear to be intermediate to those produced by either isolate alone.

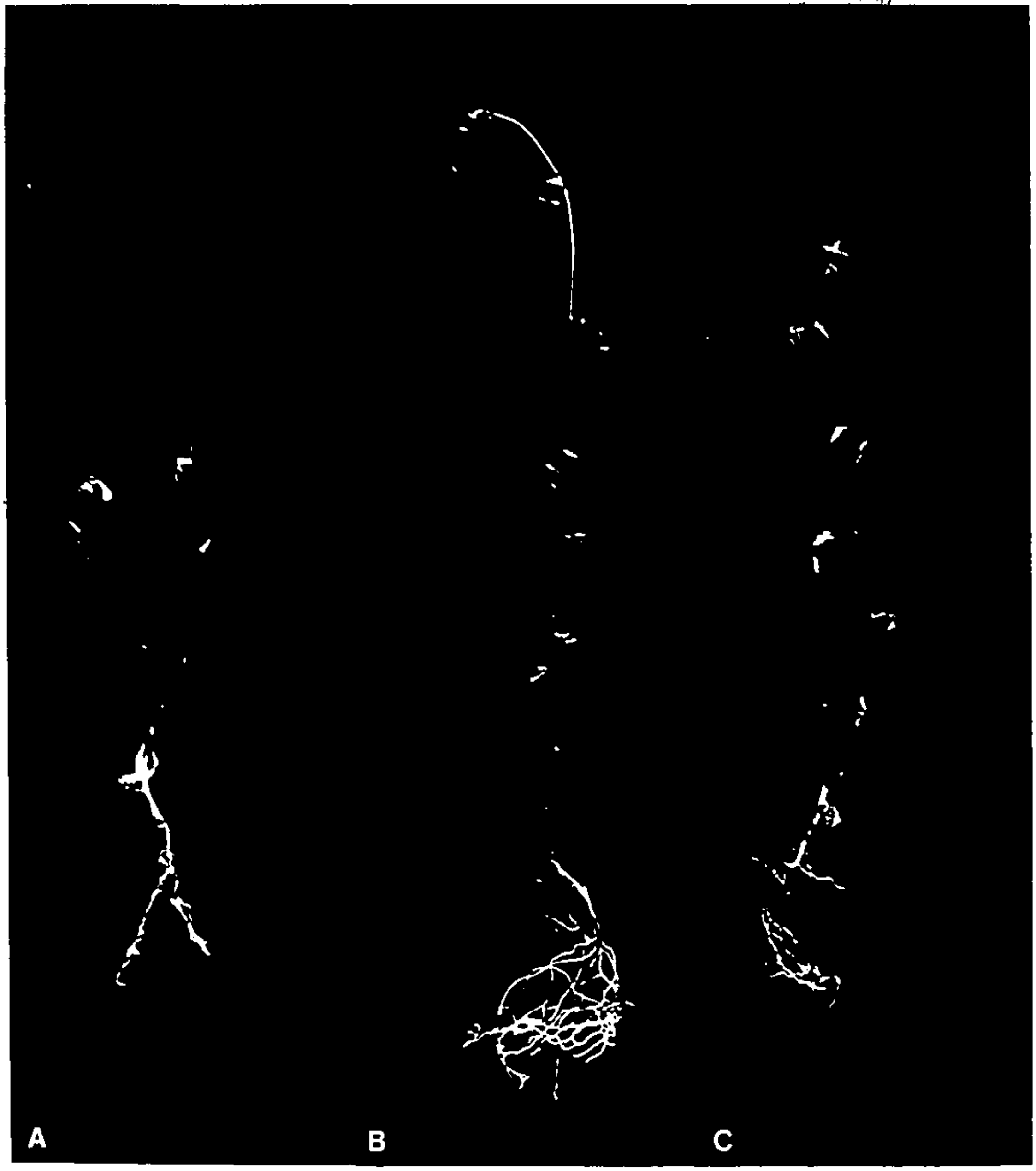


FIG. 21

Figure 22. Root systems of plants inoculated with two *Fusarium* isolates alone or in combination. The root system of the *F. oxysporum* Isolate 82A inoculated plant (A) has extensive lesions giving rise to an overall discolored appearance. The root system of a plant inoculated with *F. solani* Isolate 196A (B) is more extensive and has small lesions scattered throughout the root system (arrows). A plant inoculated with both isolates simultaneously has large lesions (arrows) on both old and young roots.

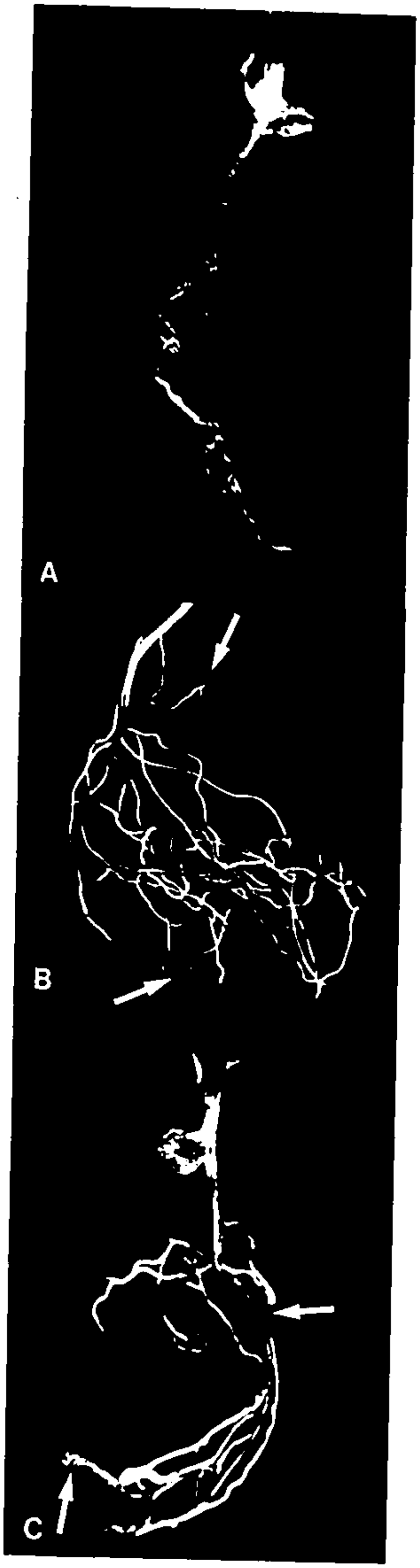


FIG. 22

inoculated plants are noteworthy: necrotic lesions on plants inoculated with the inter-specific mixture were often up to 1.5 cm in length and scattered throughout the root system, affecting both older and younger roots alike. When Isolate 82A was used, alone, lesion development was restricted to the tap root and to the distal protions of older secondary roots. Plants inoculated with the 196A isolate, alone, had lesions distributed throughout the root zone while the sites of emergence of the secondary roots remained disease-free. Also, mycelial proliferation was restricted almost entirely to the stem base with the mixed inoculum whereas with Isolate 82A, hyphal development extended along the hypocotyl and was present on the seedcoat. When inoculum from Isolate 196A was used to infect seedlings, external proliferation of the fungus on host tissues was not observed. Foliar symptoms produced by the two isolate inoculum included chlorosis of the leaflets at all nodes with necrosis of the lowermost ones. Isolate 82A, as noted previously (Sec. 3.4.1) caused necrosis of all leaf tissue and of large portions of the stem whereas Isolate 196A produced a chlorosis of leaflet tips extending to the shoot apex without chlorosis of the stem tissue.

3.9 THE EFFECT OF CULTURE FILTRATES FROM _F_. _oxysporum_, ISOLATE 82A ON LENTIL SEEDLINGS

Some of the lentil seedlings died within 48 hours after being placed into the undiluted culture filtrate, but others survived for up to 7 days. In the latter, the symptoms produced included a reduction in growth and a soft, wet rot of the tap root, suppression of lateral root formation, and necrosis of the shoot. Death of seedlings occurred after 7 days of incubation in a 1:1 dilution of the culture filtrate (Table 8). As the

Table 8. The Effects Of The Culture Filtrates From *F. oxysporum* (Isolate 82A) On Lentil Seedlings

Filtrate from a 4-day-old Culture	Root Discolouration[a]	Lateral Roots[b]	Foliar Chlorosis[c]	Seedling Death[d]
Check	0	+	0	0
Undiluted Filtrate	4	-	4	5
1:1 dilution	4	+	4	5
1:10 dilution	3	+	2	2
1:100 dilution	0	+	0	0

Filtrate from a 12-day-old Culture	Root Discolouration[a]	Lateral Roots[b]	Foliar Chlorosis[c]	Seedling Death[d]
Check	0	+	0	0
Undiluted Filtrate	4	-	4	5
1:1 dilution	4	-	4	5
1:10 dilution	2	+	2	0
1:100 dilution	0	+	0	0

[a] 0 = healthy, 1 = up to 25% of the root discoloured, 2 = up to 50% discolouration, 3 = up to 75% discolouration, 4 = 76-100%.

[b] presence (+) or absence (-) of lateral roots.

[c] 0 = healthy, 1 = 1-25% of the leaf tissue is chlorotic: 2 = 26-50%; 3 = 51-75%; 4 = 76-100%.

[d] number of necrotic seedlings from a total of 5.

filtrate was diluted further, disease symptoms became progressively less severe. Seedlings incubated in the 1:100 dilution were somewhat taller and as healthy appearing as the controls (Table 8). This experiment was repeated using a 12-day-old filtrate; similar results were obtained in that the most severe disease symptoms resulted from incubation in the undiluted filtrate. In addition, severe symptoms were expressed on plants in the 1:1 dilution.

3.10 RECOVERY OF SEED-BORNE MICROORGANISMS FROM LENTIL SEEDS

Seed-borne inoculum is often responsible for the transmission of diseases into new areas (Ainsworth, 1971). An experiment was conducted to determine the extent to which seed-transmitted pathogens may have co[illegible]ributed to the degree of field infestation. The lentil varieties Laird, Commercial Chilean, and Eston as well as P.I. 179313 were screened for the presence of seed-borne contaminants and Table 9 lists the results of this investigation.

Percent seed germination for all 4 lentil varieties tested was greatest on malt-extract-agar although P.I. 179313 germinated equally well on potato-sucrose-agar. V-8 juice-agar supported relatively poor germination of all varieties. The amount of seed-borne infection was low (2.3%) and the variety Commercial Chilean and P.I. 179313 were completely free of seed-borne contamination. The fungi tested in Table 10 were recovered from surface-disinfected seed of Laird and Eston. The frequency of isolation of each contaminant is also listed. _Septoria pisi_ was the most common fungus isolated from the seed. Two of the isolates were identical to those recovered from the soil, an unidentified

Table 9. Seed Germination And Incidence Of Fungal Disease On Four Lentil Varieties After 7 Days Incubation On Three Different Media

Lentil Varieties	Agar Medium					
	Potato Sucrose		Malt Extract		V-8 Juice	
	% Germination[a]	% Infection[b]	% Germination	% Infection	% Germination	% Infection
Laird	72	0	96	4	64	8
Commercial Chilean	60	0	88	0	44	0
Eston	52	8	96	4	84	4
P.I. 179313[c]	100	0	100	0	24	0

[a] Percentage of seeds with a radicle length of at least 1 cm; sample size = 100 seeds.

[b] Percentage of seeds from which hyphae emerged.

[c] P.I. = Plant introduction.

Table 10. Seed-Borne Fungi And The Frequency Of Their Isolation From Surface-Disinfected Seed

Seed-Borne Fungi	Isolation[a]
Septoria pisi West	5
Alternaria sp.	4
Fusarium equiseti (Corda) Sacc.	4
Penicillium sp.	4
Alternaria tenuissima (Kunze ex Pers.) Wiltshire	3
Cladosporium cladosporioides (Fresen.) de Vries	2
Fusarium acuminatum Ellis & Everhart	2
Ascochyta sp.	1
Aureobasidium pullulans (de Bary) Arn.	1
Epicoccum purpurascens Ehrenb. ex Schlect.	1
Geotrichum sp.	1

[a] Frequency of isolation from a sample size of 1200 seeds.

Alternaria species and Fusarium equiseti, F. acuminatum, isolated from Laird seed was the only other Fusarium species found as a seed-borne contaminant.

3.11 PENETRATION AND SUBSEQUENT DEVELOPMENT OF F. oxysporum ISOLATE 82A IN LENTIL ROOTS

These experiments were initiated to determine in detail the penetration and proliferation of Isolate 82A in lentil root tissues. The mechanism by which hyphae of F. oxysporum penetrate and establish infection in lentil roots is unknown and it is hoped that the observations made during the course of these studies will prove helpful in understanding the aetiology of this root-rot disease.

3.11.1 Cytology Of Non-inoculated Lentil Roots

Observations of toluidine blue-stained fresh and epon-embedded young root tissues revealed a cellular arrangement similar to that briefly described by Barulina (1930), in her monograph on the genus, Lens. The epidermis is composed of a single layer of closely-packed, nearly isodiametric cells with a relatively thin, non-uniform surface secretion (Fig. 23-A). The cortex, several cell layers in thickness consists of circular to ovoid parenchyma cells with large irregular intercellular spaces (Fig. 23-B). The innermost cortical layer is differentiated as an endodermis lacking casparian strips (Fig. 23-D). The central vascular cylinder is surrounded by the pericycle, which is uniseriate over the protophloem and 2-3 cell layers in thickness over the xylem poles. The vascular tissue consists of xylem in a triarch arrangement with trachery elements of varying diameters ensheathed by xylem parenchyma cells (Fig. 23-C). Located between the xylem ridges

Figure 23. Transverse sections through plastic-embedded non-inoculated check roots.

A. The cortex is surrounded by an epidermal layer of nearly isodiametric cells (epi) which have a thin non-uniform surface secretion (*).

B. The cortex consists of ovoid parenchyma cells and large intercellular spaces (↑).

C. The proto- (px) and metaxylem (mx) cells surrounded by parenchyma.

D. The phloem fibres with thick walls (↟); (*) indicates the location of the endodermal layer.

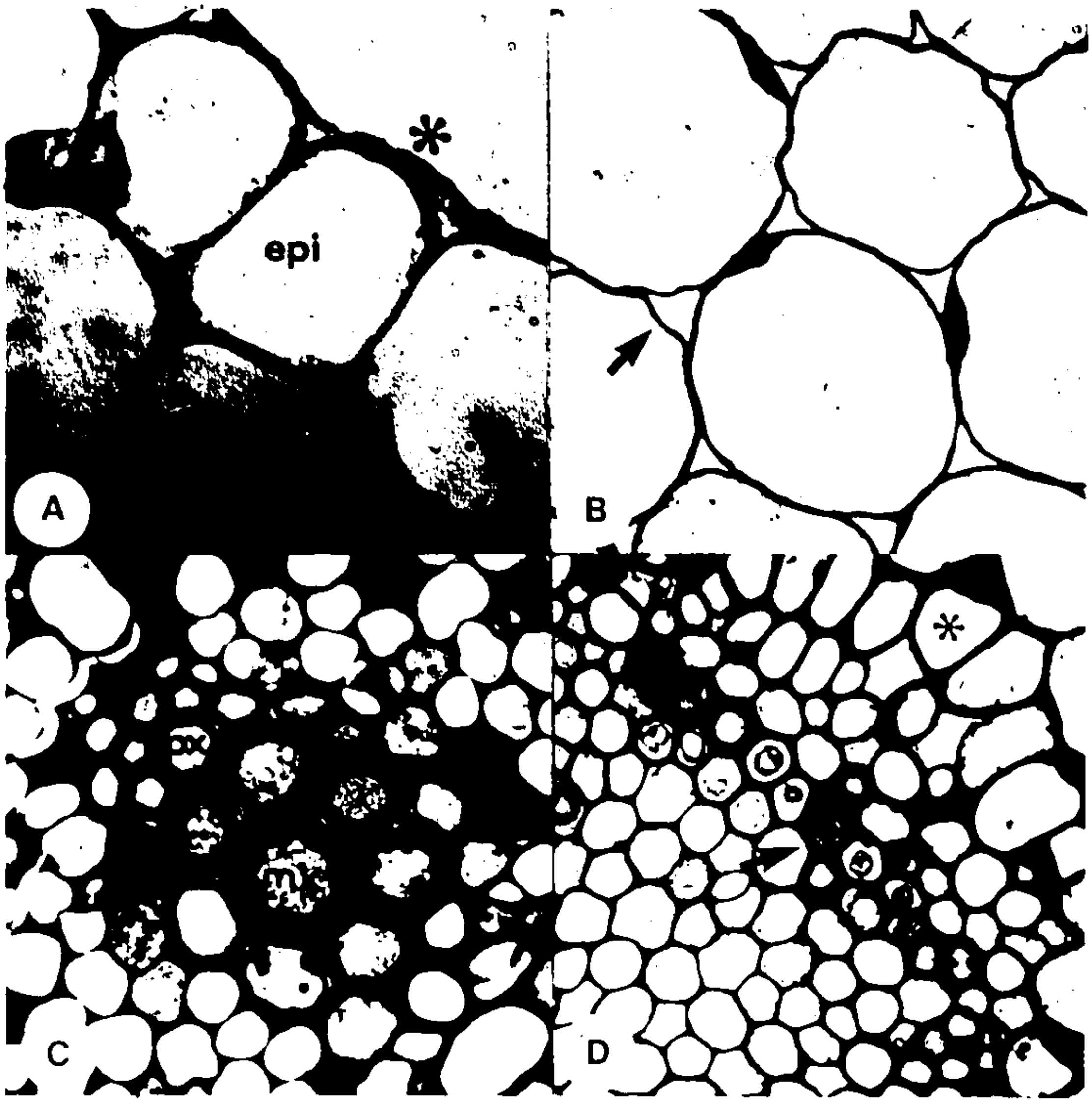


FIG. 23

is the phloem consisting of sieve elements, phloem parenchyma cells and bundles of fibers with thick secondary walls (Fig. 23-D).

3.11.2 Determination Of A Preferential Inoculation Site On Lentil Roots

To establish where on the root surface to place the inoculum discs an experiment was designed in which individual tap roots were inoculated at three sites and incubated for 16 hours prior to fixation in F.A.A. The discs were situated adjacent to the root apex, the zone of cell elongation, (in lentil roots, this zone extends from 0.5 to 7 mm behind the root tip), and the root hair zone. Examination of hand-sections stained with toluidine blue revealed that the area of elongation was preferentially susceptible to penetration by hyphae of F. oxysporum. Following the incubation period, hyphae had invaded the epidermis and up to 7 cell layers of the cortex (Fig. 24-B). Penetration into the cells of the root apex or root hair zone was not observed (Fig. 24-A,C) after 16 hours of incubation with the pathogen. In all subsequent inoculations, the agar discs were placed contiguous to the zone of cell elongation.

3.11.3 Cytology Of Inoculated Lentil Roots

Although embedded root tissue was examined 2 and 4 hours following inoculation with Isolate 82A, initial invasion of the epidermal cell layer was not observed until 8 hours after inoculation. The pathogen penetrated intercellularly and elongated along the junction between adjacent epidermal cells (Fig. 25-A). There was no evidence of formation of a true appressorial structure. Frequently, however, germ tubes were observed to swell at the point where they were contiguous with the host cell wall to form an appressorial-like structure. These structures were usually found associated with invaginations of the host wall (Fig. 25-B,C).

Figure 24. Transverse, free-hand sections through the root apex (A), the zone of cell elongation (B) and the root hair zone (C) 16 hours after inoculation with F. oxysporum, Isolate 82A; all subsequent figures show plastic embedded tissues inoculated with this isolate. Note, in (B) hyphae penetrated through the epidermis and into the cortex (↑) and the cells are disrupted but cellular penetration and cortical deterioration are not evident in (A) and (C).

FIG. 24

8

Figure 25. External growth of the pathogen and its effects on epidermal cells. A. Some hyphae (*) grew directly to the region between adjacent epidermal cells while others (B) became contiguous with the epidermal cell wall and formed an appressorial-like structure (*). C. Some epidermal cells had small invaginations at the point of contact with the pathogen (*). D. Epidermal cell (↑) with a granular cytoplasm; a hyphal strand is contiguous with this cell. E. Epidermal cell with an apparent plasmolyzed cytoplasm (*). F. A strand of hyphae which grew up to an epidermal cell then parallel with its wall (↑). Samples fixed 8 hours after inoculation; epi - epidermis; co - cortex.

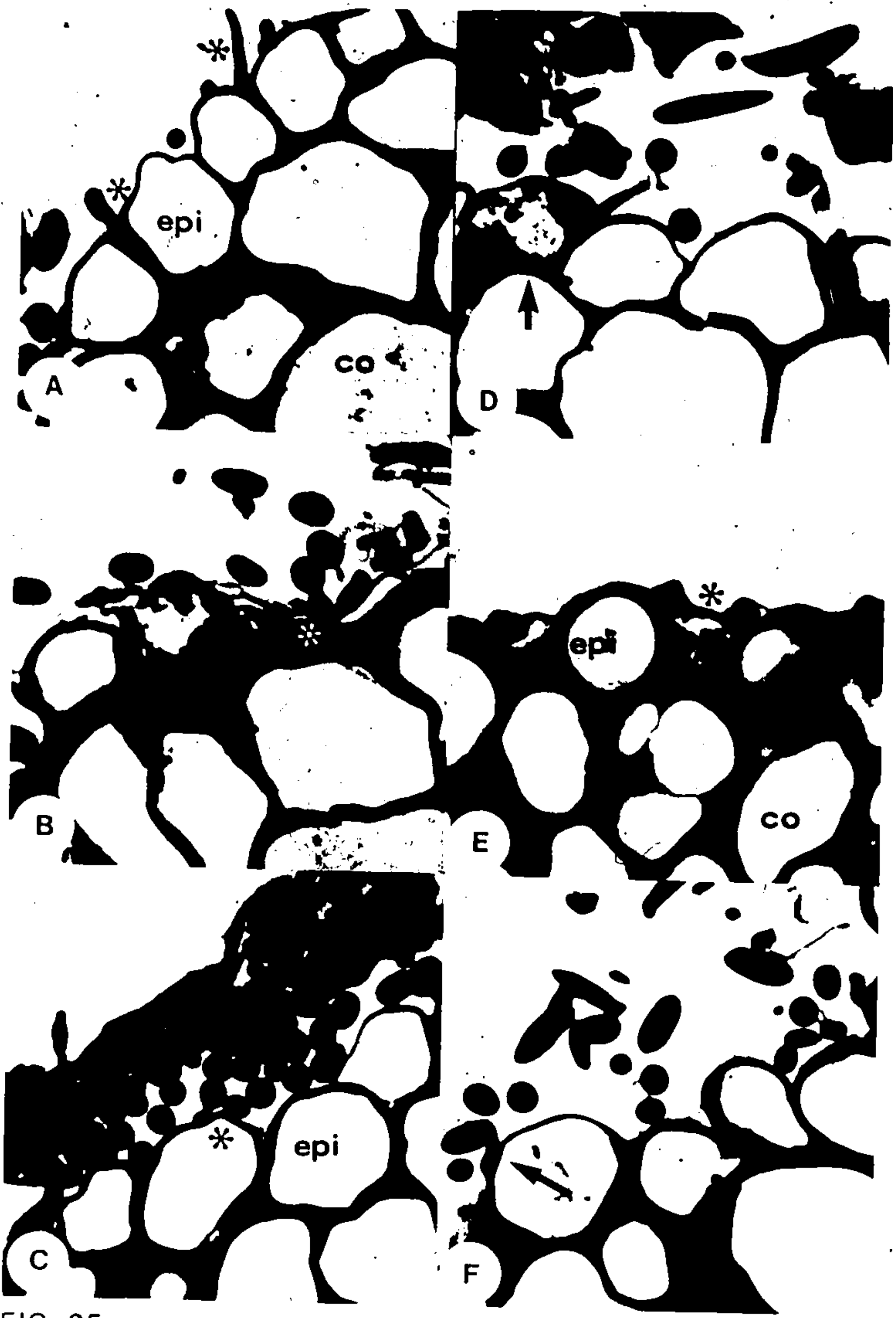


FIG. 25

Often the cytoplasm of cells adjacent to an infection hypha became granular in appearance (Fig. 25-D) and at times where the fungus was in contact with the host cell, the cytoplasm of that cell plasmolyzed (Fig. 25-E). When hyphae contacted an epidermal wall at a point away from the junction of two adjacent cells, the hyphae grew along the surface of the epidermal cell wall towards the junction (Fig. 25-F). Following penetration, hyphae grew intercellularly into the cortex where they proliferated in the intercellular spaces. In many instances, the outline of the cortical cells became irregular (Fig. 26-A) which was in marked contrast to the smooth, regular appearance of the non-inoculated cortical tissue (Fig. 26-B).

Following profuse intercellular growth of the fungus in the outer cortical layers, branches arose from the intercellular hyphae and penetrated the host cells intracellularly (Fig. 26-C). Cortical walls appeared thin in places and frequently there was disruption of the continuity of the wall indicating cell collapse (Fig. 26-D). When fungal hyphae penetrated through cortical walls, there was a constriction of the hyphal strand at the site of penetration (Fig. 26-E). As infection of host tissue proceeded, the pathogen grew inter- and intracellularly, ramifying in various directions throughout the cortex. Frequently cells containing fungal hyphae were stained darkly with toluidine blue (Fig. 26-F). There was complete disruption of the cellular structure of the cortex within 24 hours after inoculation and fragments of cell wall material could be seen interspersed with fungal hyphae (Fig. 27-A). In some cases, hyphal strands formed thick-walled swellings which resembled chlamydospores; these were either borne singly or formed in chains (Fig. 27-A). The pathogen, although well established in the cortex,

Figure 26. The presence of hyphae in the cortical tissues, 16 hours after inoculation. A. Hyphae penetrated intercellularly (↑); cortical cells (co) are irregular in shape (cf. B, a section from non-inoculated check roots). C. Some hyphae penetrated intracellularly (↑). Note cortical cells did not stain intensely (cf. B). D. An intracellularly penetrated cortical cell with a discontinuous cell wall (*). E. An apparent constriction in a hyphal strand where it passes through adjacent cell walls (*). F. Transverse section through a hyphal strand (*) in a uniformly stained cell. Other similarly stained cells have internal hyphae (lower right). Central cells are unstained and have discontinuous cell walls.

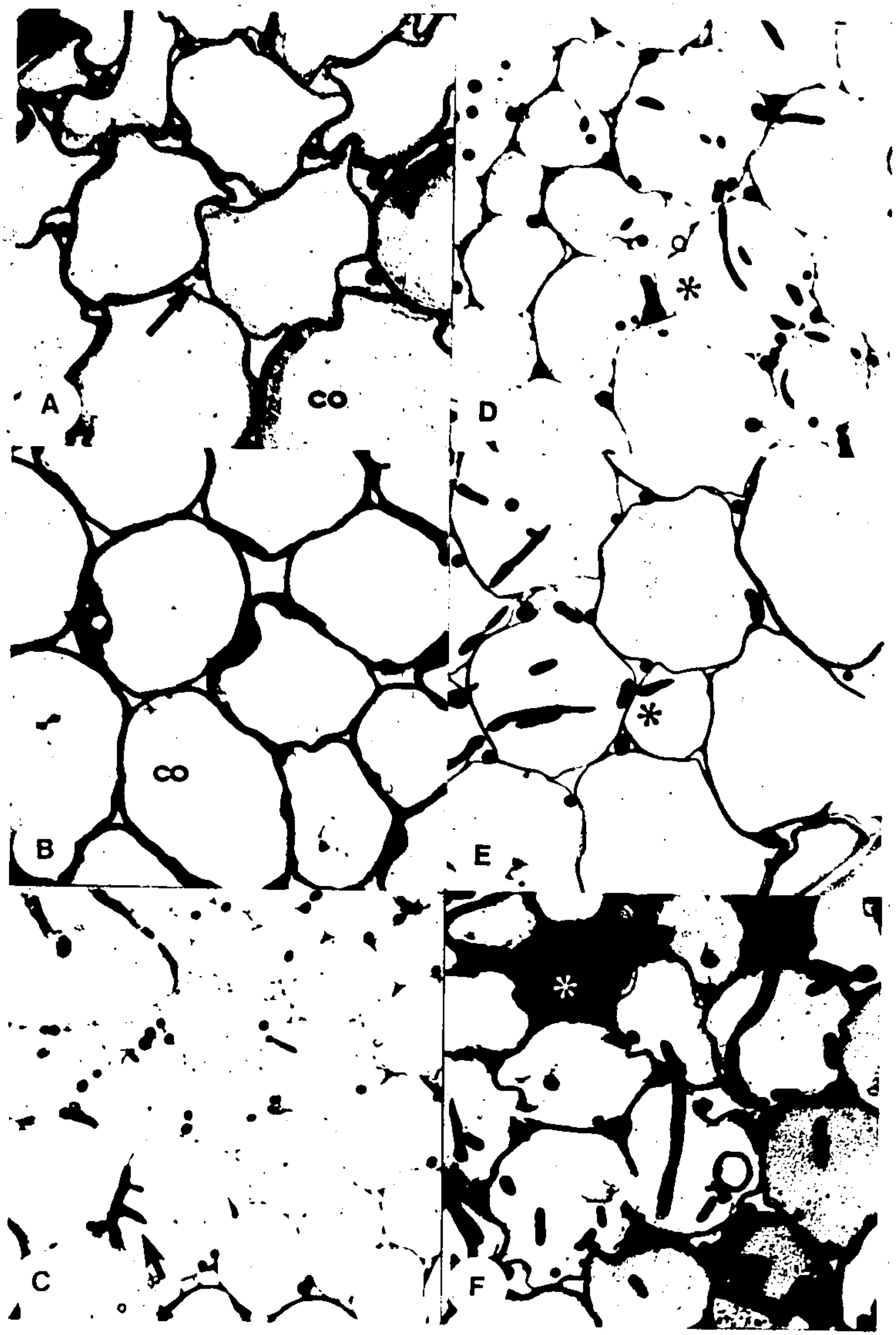


FIG. 26

Figure 27. Transverse sections through infected roots 24 (A) and 72 hours (B,C) after inoculation. A. Extensive proliferation of hyphae in the cortex; note lack of staining of the cell walls (↑) and an apparent chain of chlamydospores (*). B. The stele contains large cavities (*); X = thick walled xylem elements. Hyphal strands were not observed in the stele. C. In sections from roots inoculated in the root hair zone, hyphal penetration after 72 hours was limited to the outer three or four rows of cortical cells (*). Some hyphae penetrated into a root hair (↑).

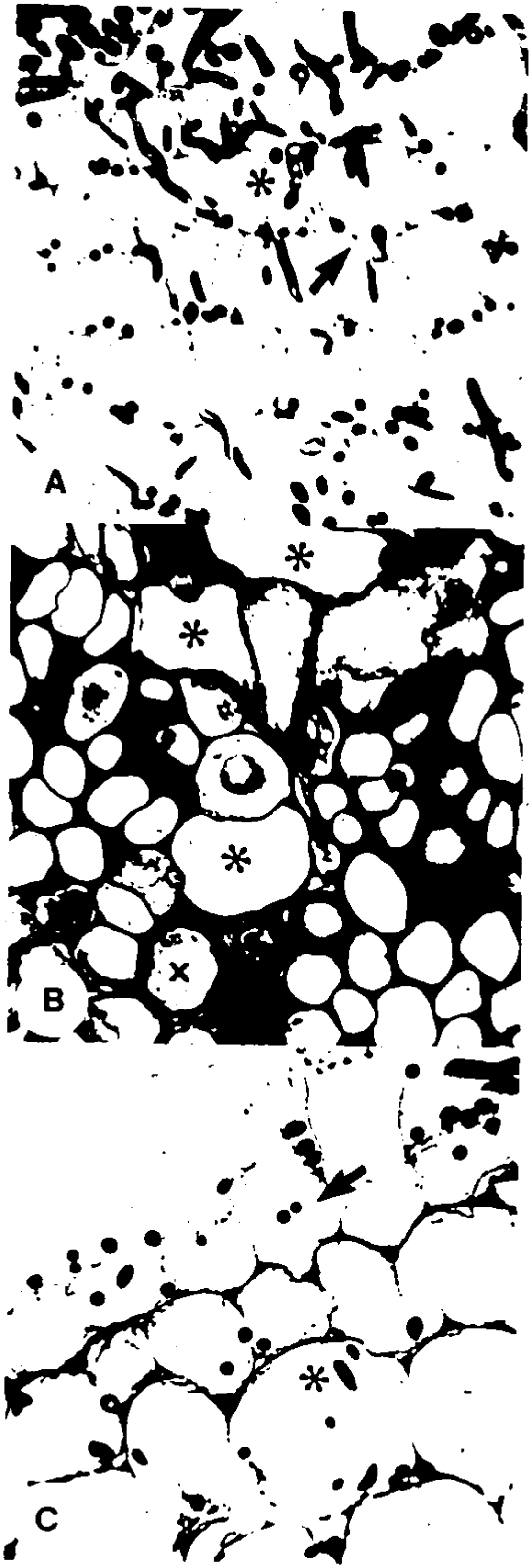


FIG. 27

did not enter into the vascular tissue. Even in specimens examined 72 hours following inoculation, fungal structures were not observed although large cavities were present within the stele (Fig. 27-B). It is possible that these cavities may have arisen from dissolution of parenchymal cells. Examination of the root hair zone, after 72 hours revealed that the pathogen was present in root hairs as well as in the first 3-4 cell layers (Fig. 27-C).

3.12 FIELD EXPERIMENTS

3.12.1 Field Symptoms

In the May plantings (Fig. 28), disease symptoms were first observed in mid-July, just after the lentil plants started to flower. Initial symptoms consisted of either a yellow blotchiness on leaflets or a v-shaped pinkish-brown discolouration of the leaflet tips. Older leaflets, generally the first affected, tended to curl upward and inward from the tip or to twist spirally along the mid-rib. In addition, a chocolate-brown lesion formed on the stem at soil level which enlarged to girdle the stem;subsequently the stem collapsed. When stems were dissected, vascular discolouration was observed to be confined to the girdled zone.

Initially individual diseased plants were scattered throughout the plot (Fig. 29); however, within two weeks, large portions of the field became chlorotic (Fig. 30-A) often with necrotic plants in the centre of these areas (Fig. 30-B). Many of the plants in the necrotic areas were completely detached from their root systems due to a rotting of the stem at soil level. Within the next 4 weeks all of the plants in the plots were necrotic (Fig. 31-A). Extensive defoliation and

Figure 28. A uniformly green field plot of lentils. Photographed July 4, 1980. This plot and ones in subsequent Figures were planted in mid-May.

Figure 29. An individual diseased plant in the field plot, many such plants were scattered throughout the plot. Photographed July 17, 1980.

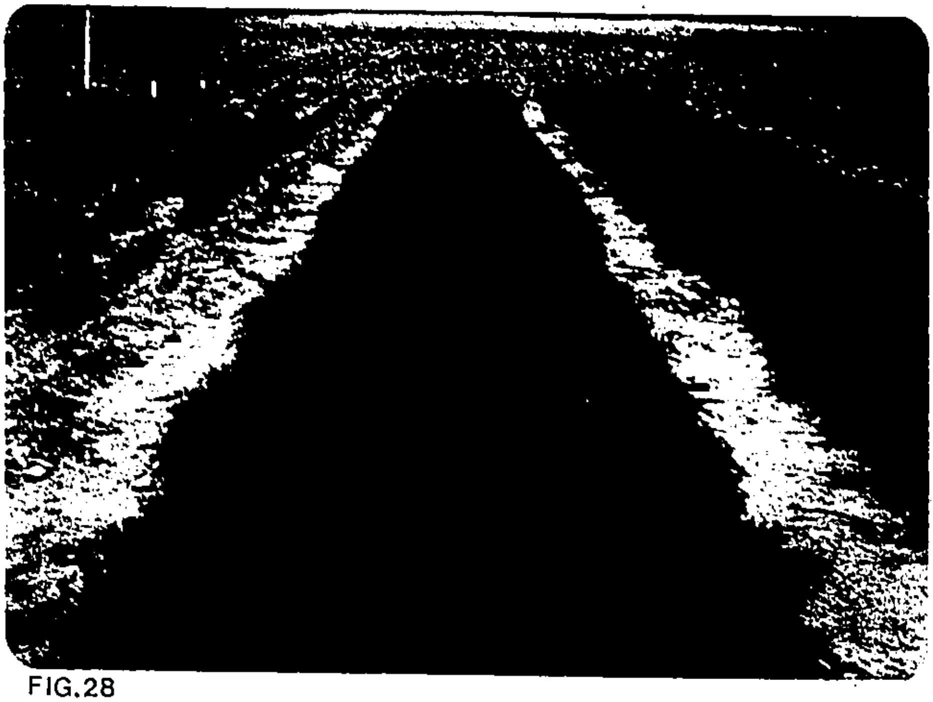


FIG.28

FIG.29

Figure 30. A. On July 30, 1980, large portions of the plot were chlorotic (cf. Figure 28). B. Necrotic plants in one of the more chlorotic areas in A.

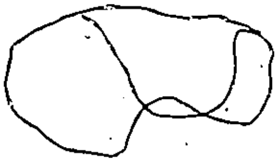


FIG.30-A

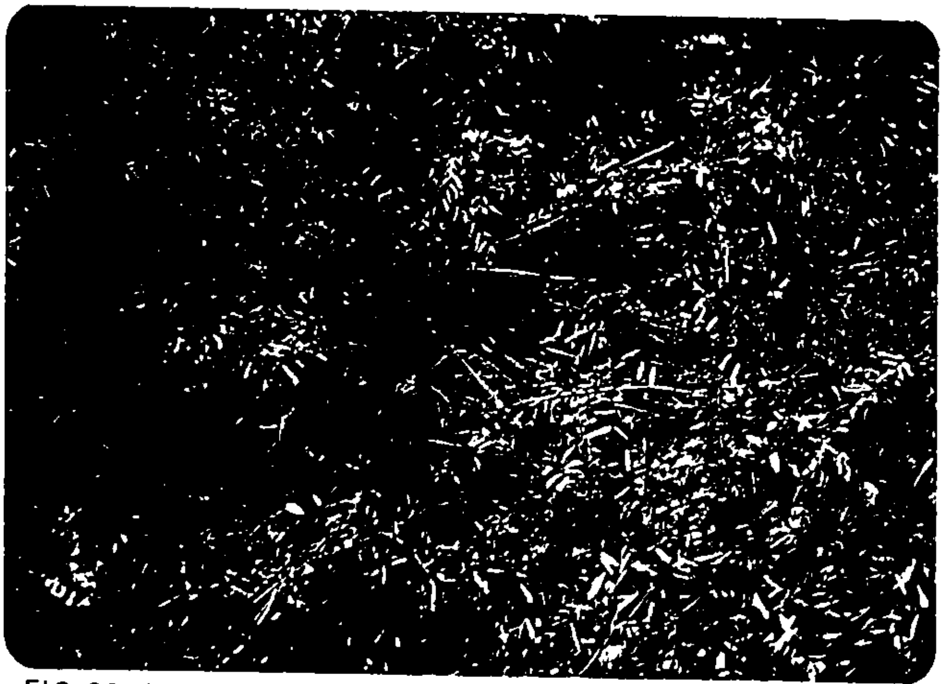


FIG.30-B

Figure 31. A. On August 10, 1980, almost the entire plot was necrotic with only small areas of upright, chlorotic plants. B. Prostrate plants in a necrotic area with surface fungal growth.

FIG 31-A

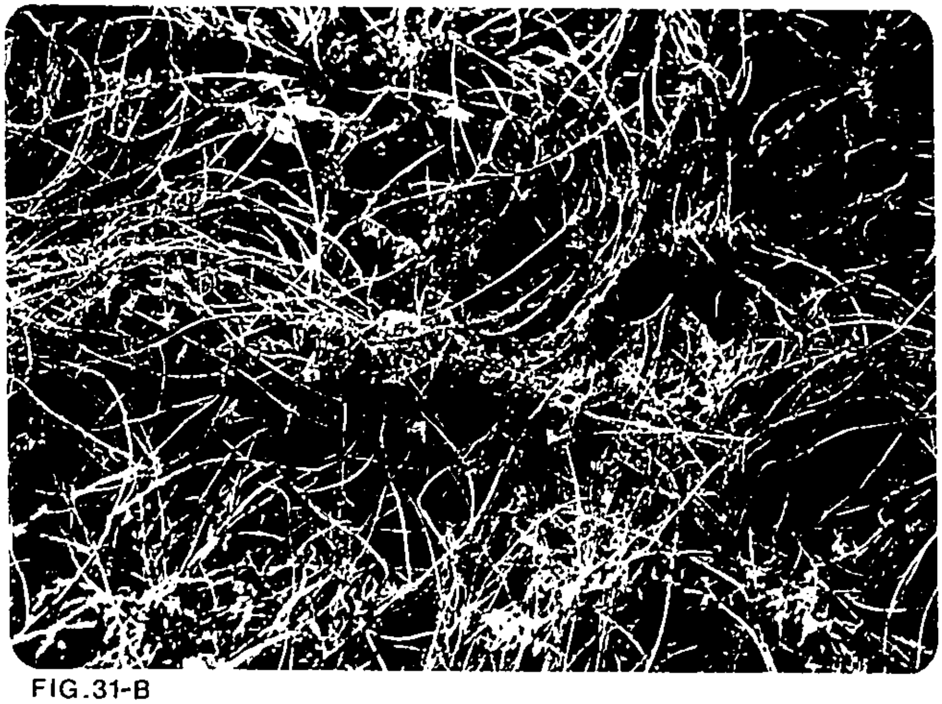


FIG.31-B

collapse of the stems occurred throughout the plot and saprophytic fungal development was observed on the decaying stems (Fig. 31-B). Disease progression in the June plantings followed a similar but accelerated course of development.

Only a few of the plants produced seed and these formed in dark-brown-to-black misshapen pods, on which fungal growth was often visible (Fig. 32-A). A representative selection of seed recovered from such pods is shown in the bottom row of figure 32-B. The top row is a sample of the seed used in the original seeding. The harvested seed is discoloured and shrivelled with areas of fungal development on the seedcoat.

3.12.2 Effect Of Benomyl Seed Treatment And Rhizobium Inoculation On Emergence, Nodulation, And Seed Yield Of Lentils.

In both the early and late sowings, none of the seed treatments significantly increased seedling emergence over that of the control (Table 11). Rhizobium inoculum applied to seedlings 2 weeks after planting increased root nodulation and significantly improved yields in the earlier planting. Treatment of seed with benomyl prior to planting reduced nodulation regardless of whether the seedlings were inoculated with Rhizobium. This decrease in nodulation was significant only when the seedlings were inoculated. All treatments produced higher yields over the controls in the earlier sowing but these increases were not significant with the exception of those plots inoculated with Rhizobium. In the later planting, the treatments did not have a significant effect on yield. Dates of sowing however, had a significant (P = 0.01) effect on yield with the earlier planting producing the higher yields.

Figure 32. A. Apical portion of a chlorotic stem harvested from the plot shown in Figure 31A. Seed pods varied in colour and size. B. Representative samples of seed from those originally planted (top row) and from the pods shown in Figure 32A (bottom row). Seeds from the latter varied in colour, size and shape; pinkish areas are fungal colonies.

FIG.32-A

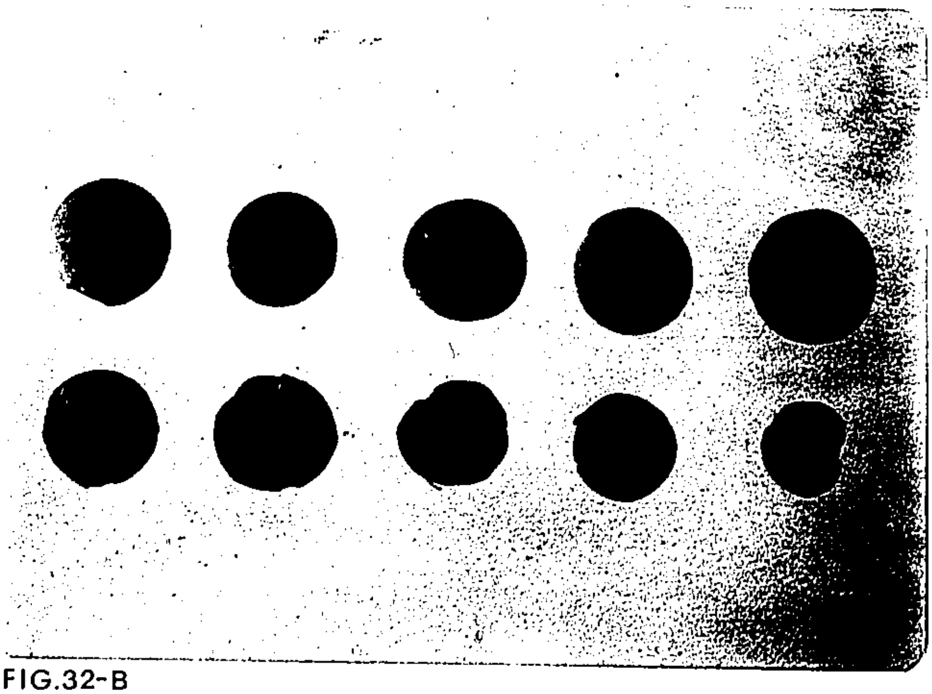


FIG.32-B

Table 11. Effect of Benomyl Seed Treatment And *Rhizobium* Inoculation on Emergence, Nodulation, And Seed Yield Of Lentils[1]

Treatments	May Planting[5]			June Planting[5]		
	Emergence[2]	Nodulation[3]	Yield[4]	Emergence[2]	Nodulation[3]	Yield[4]
Non-treated Check	85^a	110.7^{ab}	9.6^a	82^a	85.8^a	3.9^a
Benomyl seed treatment	80^a	99.7^{ab}	22.4^{ab}	87^a	100.7^a	2.6^a
Rhizobium inoculation	80^a	162.8^a	30.0^b	87^a	145.0^a	2.4^a
Benomyl seed treatment and *Rhizobium* inoculation	81^a	86.7^b	15.5^{ab}	89^a	127.7^a	2.9^a

[1] each value is the mean of six replicates.

[2] percentage of seedlings that emerged; data recorded six weeks after planting.

[3] nodules per gram dry weight of root tissue.

[4] seed yield in grams/100 plants.

[5] means followed by a common letter within a column are not significantly differen[illegible] (P = 0.05) according to Duncan's New Multiple Range Test.

3.12.3 Effect Of Benomyl Seed And Soil Treatments On Emergence And Seed Yield Of Lentils.

Seedling emergence in the June sowing was significantly higher in plots receiving both the benomyl seed and soil treatments than in those treated only with the benomyl soil drench (Table 12). A similar increase in May was not significant. None of the treatments in both the early or late sowings had a significant effect on yield. However, seed yield was significantly (P = 0.01) greater from the earlier than the later sowing date.

3.12.4 Effect Of Benomyl, PCNB, And Zineb Seed Treatments On Emergence And Seed Yield Of Four Lentil Varieties.

Seed of the lentil variety, Laird, germinated less well than the other varieties with all treatments tested, however, these results were significant only for Laird over P.I. 179313 (Table 13). The small seeded variety, Eston and P.I. 179313 were higher yielding although not significantly over the large seeded variety, Commercial Chilean. The extra-large seeded Laird produced the lowest yields and these differed significantly from both Eston and P.I. 179313. The seed treatments had no significant effect on seedling emergence or yield. In addition, there was little difference among fungicide treatments, on the rate of seedling emergence (Fig. 33).

3.12.5 Effect Of Seedcoat Characteristics On Emergence And Seed Yield Of Four Lentil Varieties.

Emergence of the variety Laird was significantly less than the other 3 varieties tested (Table 14). The small seeded variety Eston had the highest number of seedlings and produced the greatest seed yield. Yields of P.I. 179313 in addition to Eston were significantly higher than the

Table 12. Effect Of Benomyl Seed And Soil Treatments On Emergence And Seed Yield Of Lentils[1]

Treatments	May Planting[4]		June Planting[4]	
	Emergence[2]	Yield[3]	Emergence[2]	Yield[3]
Non-Treated Check	80^a	4.6^a	86^{ab}	3.1^a
Seed Treatment	81^a	2.1^a	82^{ab}	0.1^a
Soil Treatment	80^a	2.6^a	79^b	0.1^a
Seed and Soil Treatment	82^a	1.6^a	89^a	0.1^a

[1] each value is the mean of six replicates.

[2] percentage of seedlings that emerged; data recorded six weeks after planting.

[3] seed yield in grams/100 plants.

[4] means followed by a common letter within a column are not significantly different (P = 0.01) according to Duncan's New Multiple Range Test.

Table 13. Effect Of Benomyl, PCNB, And Zineb Seed Treatments On Emergence And Seed Yield Of Four Lentil Varieties[1]

Variety	Emergence[2]				Seed Yield[3]			
	Benomyl	PCNB	Zineb	Mean[4]	Benomyl	PCNB	Zineb	Mean[4]
Laird	42.5	42.3	40.5	41.8^{a}	6.3	3.7	4.7	4.9^{a}
Commercial Chilean	43.2	46.0	44.5	44.5^{ab}	8.8	8.8	12.7	10.1^{a}
Eston	43.8	46.3	41.8	44.0^{ab}	34.2	29.6	30.4	31.4^{b}
P.I. 179313	44.3	44.8	47.7	45.3^{b}	29.3	24.1	32.2	28.5^{b}

[1] each value is the mean of six replicates.

[2] plants that emerged from a total of 50 seeds per replicate; data recorded four weeks after planting.

[3] seed yield in grams/50 plants.

[4] means followed by a common letter within a column are not significantly different ($P = 0.05$ for emergence values; $P = 0.01$ for yield values) according to Duncan's New Multiple Range Test.

Figure 33. Effect of three fungicide seed treatments on the rate of seedling emergence in four field sown lentil varieties.

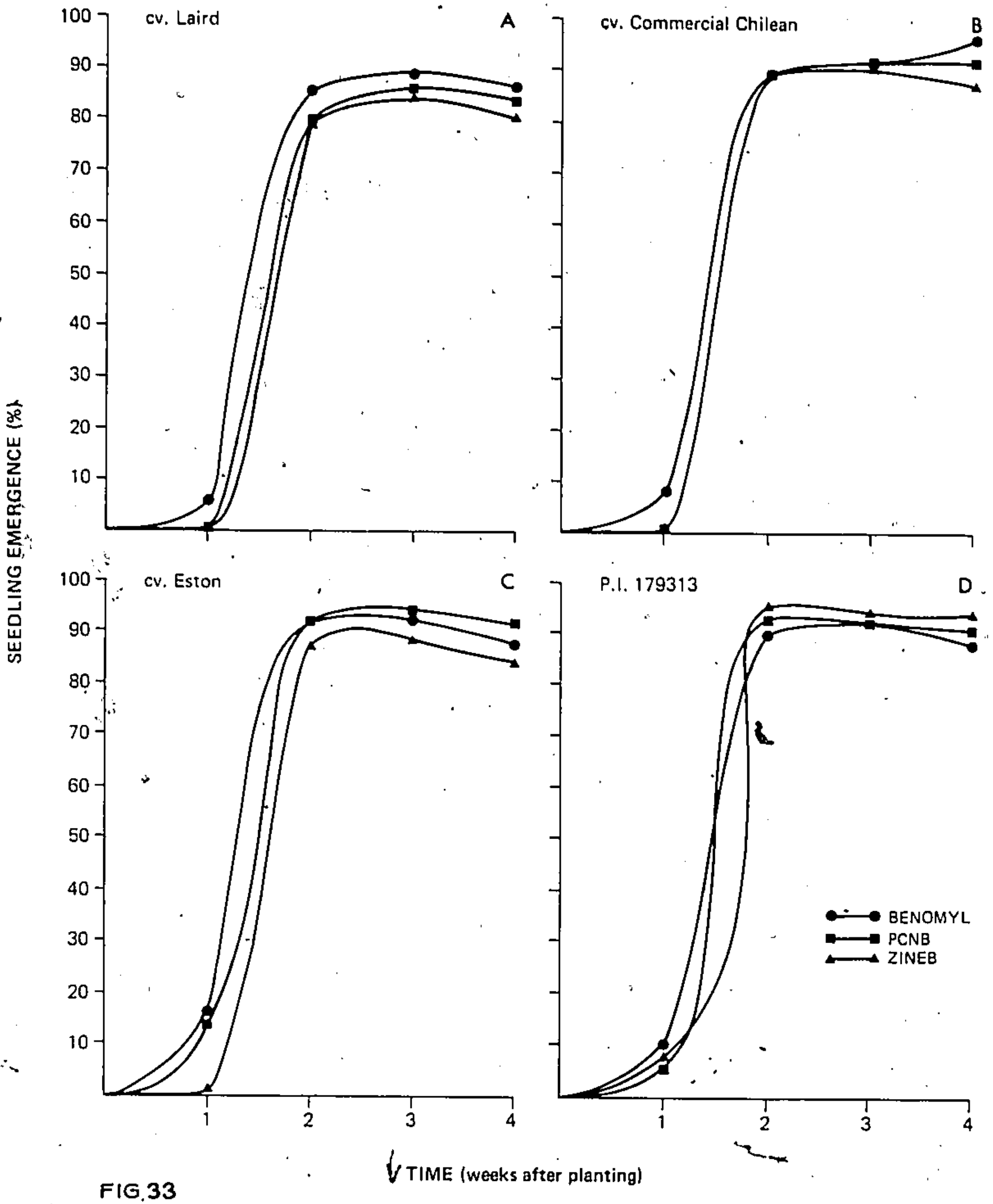

cv. Laird
A
cv. Commercial Chilean
B
cv. Eston
C
P.I. 179313
D
SEEDLING EMERGENCE (%)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
BENOMYL
PCNB
ZINEB
TIME (weeks after planting)

FIG.33

Table 14. Effect Of Seedcoat Characteristics On Emergence And Seed Yield Of Four Lentil Varieties[1]

Variety	Seedcoat Characteristics									
	Emergence[2]					Seed Yield[3]				
	Random	Green	Yellow	Mottled[4]	Mean[5]	Random	Green	Yellow	Mottled[4]	Mean[5]
Laird	29.2	31.5	30.7	27.5	29.7[a]	0.6	0.6	0.3	3.4	1.2[a]
Commercial Chilean	37.0	37.2	38.5	36.0	37.8[b]	3.0	1.0	2.8	2.4	2.3[a]
Eston	40.8	38.3	39.5	37.2	39.0[b]	13.0	12.0	12.4	11.7	12.3[b]
P.I. 179313	37.8	38.7	38.7	40.0	38.8[b]	11.1	6.2	10.2	5.1	8.1[c]

[1] each value is the mean of six replicates.

[2] plants that emerged from a total of 50 seeds per replication; data recorded four weeks after planting.

[3] seed yields in grams/50 plants.

[4] for variety Laird, seeds with cracked testa were selected instead of mottled seeds.

[5] means followed by a common letter within a column are not significantly different (P = 0.01) according to Duncan's New Multiple Range Test.

larger seeded varieties Laird and Commercial Chilean and Eston significantly out-yielded P.I. 179313. Seedcoat characteristics had no significant effect on the rate of seedling emergence (Fig. 34); the number of plants that emerged after four weeks; or yield.

Figure 34. Effects of seed coat characteristics on the rate of seedling emergence in four field sown lentil varieties.

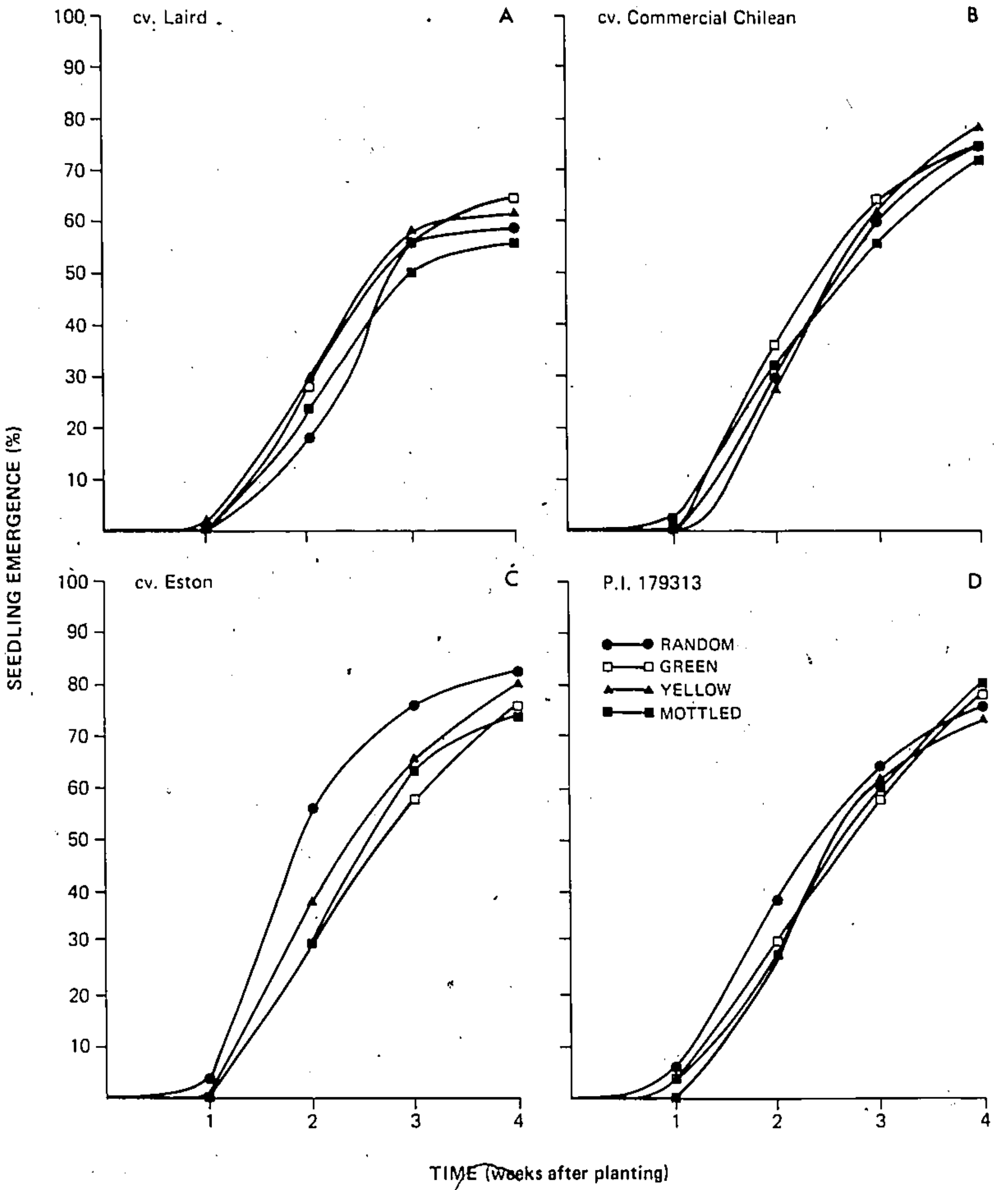

cv. Laird
A
cv. Commercial Chilean
B
cv. Eston
C
P.I. 179313
D
SEEDLING EMERGENCE (%)
100
90
80
70
60
50
40
30
20
10
1
2
3
4
RANDOM
GREEN
YELLOW
MOTTLED
TIME (weeks after planting)

FIG.34

DISCUSSION AND CONCLUSIONS

Fusarium oxysporum Schlecht. has been found to be a pathogen of lentils (Lens culinaris Medik.) in Asia (Anonymous, 1934), Europe (Fleischmann, 1937), South America (Carrera, 1940), and the United States of America (Wilson and Brandsberg,1965). This study represents the first report of the occurrence of this Fusarium sp. as a pathogen of lentils in Canada.

F. oxysporum was isolated from field-grown lentils, field soil on which lentils were cultivated, and from lentil seedlings grown in pasteurized soil and hydroponically, in aseptic culture. The latter two environments were artificially infested with a spore suspension of this fungus.

Isolations from diseased field-grown lentil plants in Ottawa yielded three different Fusarium species of which F. oxysporum and F. equiseti were the most common, and F. solani. These in addition to an isolate of F. melanochlorum were also recovered from lentil-sown field soil by use of the soil dilution plate method. This procedure was modified to preferentially isolate Fusarium spp. by incorporating Watson's (1960) soil washing technique and by using water agar (0.1%) as the diluting medium (Barron, 1971). In addition, peptone-pentachloronitrobenzene agar was used as a growth medium because it was reported to be especially suited for the isolation and enumeration of Fusarium spp. (Papavizas, 1967).

In addition to its qualitative use in the isolation of soil fungi the modified soil dilution method was used to determine the population levels of Fusarium spp. in the Ottawa lentil field soil. The use of this technique for estimating fungus populations in soil has been severely criticized (Barron, 1971; Clark, 1965; Johnson and Curl, 1972; Rodrizuez-Kabana, 1967). A limitation of this procedure is that those organisms which sporulate abundantly or which produce heavy propagules are favoured (Johnson and Curl, 1972). However, Tuite (1969) contends that with the use of the modifications described above, the accuracy of this technique compared to the unmodified version was greatly improved. Furthermore, fusaria are particularly well suited for isolation and enumeration using the soil dilution method because they produce many discrete, relatively uniform propagules (chlamydospores) in soil rather than predominantly existing as mycelia or conidia (Nash et al., 1961; Papavizas, 1967; Parkinson et al., 1971). Parkinson et al. (1971) concluded that assessments of Fusarium spp. per g dry weight of soil are underestimates of the actual population levels present in soil because clumps of cells may remain aggregated while in suspension or they may be adsorbed onto the walls of pipettes used in the preparation of dilutions. The relationship between inoculum density and the incidence of disease has been studied and it has been repeatedly shown that population estimates are important because inoculum density can be directly correlated with disease severity (Fleischmann, 1963; Kadow and Jones, 1932; Linford, 1931; Roberts and Kraft, 1973; Smith, 1970).

In this study, population levels of Fusarium spp. averaged 930 propagules per g of soil, three months after harvest. Of these, 330 were found to be propagules of F. oxysporum, Isolate 82A. Lim (1972) studied soils in Singapore and reported that the Fusarium population in cultivated soils was large and always greater than 1000 propagules per g dry weight of soil. Also, Nash and Snyder (1962) in their recovery of F. solani f. sp. phaseoli from bean fields at harvest, discovered that the population of this pathogen averaged 1,077 propagules/g soil which is considerably higher than the 330 propagules/g obtained for Isolate 82A. This variation in the numbers of propagules isolated from soil might be explained in part by the decline in soil inoculum with time as reported by Risbeth (1955) and Wensley and McKeen (1963). Risbeth (1955) showed that the number of propagules of F. oxysporum f. sp. cubense in the vicinity of banana plants rapidly decreased with time after the plants were removed from the soil. Wensley and McKeen (1963) found that populations of F. oxysporum f. sp. melonis immediately around diseased muskmelon plants ranged upwards to 3,300 propagules/g soil at harvest; however, another sampling of the field 200 days later revealed that the population had declined to approximately 800 propagules/g. The sharp increase in the number of Fusarium propagules in the presence of a susceptible host and the steady decline following harvest is compatible with the view that chlamydospores function as the chief survival form for many of the fusaria (McKeen and Wensley, 1961). Furthermore, this supports the contention that in a host-free environment, proliferation of the fungus occurs at low levels and only in sufficient amounts to facilitate replacement (Nash et al., 1961).

Wensley and McKeen (1963) reported that the muskmelon pathogen, F. oxysporum f. sp. melonis, composed a lower percentage of the total Fusarium population in soils extracted from inter-sites as compared with plant-sites. The percentage of the population of Isolate 82A in soil samples from a field not cropped to lentils was 14% while in an adjacent field where the host was present, this pathogen represented 35% of the Fusarium population and this is in agreement with Wensley and McKeen's (1963) conclusions.

Fungal isolates recovered from soil dilution plates were tested for pathogenicity to lentil seedlings. In order to establish proof of pathogenicity, completion of Koch's Postulates required that the suspected pathogen be isolated from the host and grown in pure culture and that subsequent pathogenicity experiments with the pure culture inoculum produce the characteristics of the disease. The validity of the inoculation tests was increased by using aseptic culturing techniques for growing the lentils thereby excluding other organisms from the experimental set-up. However, the use of these types of conditions eliminates the most important component of the soil environment - the soil microflora (Garrett, 1970). Lin (1975) reported that many fungi may become pathogenic under sterile conditions but that they may only rarely produce disease symptoms in less artificial environments. In agreement with Garrett (1970) and Lin (1975), F. melanochlorum as well as the two isolates of Acremonium recovered from lentil-sown field soil consistently produced disease symptoms on and were recoverable from lentils grown aseptically and in pasteurized soil. However, these isolates were not recovered from diseased lentil plants in the field. Lin (1975) reported that although F. roseum 'Avenaceum' was highly pathogenic to lentils in sterile soil it was rarely serious under field conditions in

Washington State. He attributed this suppression in natural soil to the inability of this fungus to establish a food base in the presence of fast-growing, saprophytic fungi such as Mucor spp. and Trichoderma viride. This observation may, in part, explain the variable pathogenicity obtained with F. melanochlorum and Acremonium, as both organisms were extremely slow-growing in culture (growth rates of 1.0 and 1.3 cm/4 days, respectively) compared with F. oxysporum, Isolate 82A (growth rate of 3.6 cm/4 days).

F. oxysporum, Isolate 82A was shown to be highly pathogenic to lentil seedlings in different cultural conditions. In aseptic culture, this isolate produced tissue discolouration at the sites of emergence of secondary roots within forty-eight hours after inoculation. Bolton and Nuttal (1968) working with F. poae found that disease severity in non-wounded tissues was equal to that observed in pea seedlings with artificially clipped root systems which also suggests that there were sufficient natural sites for entry. They considered then, that natural wounds caused by the emerging secondary roots were important as an avenue of entry into host tissues. Jarvis and Shoemaker (1978) likewise demonstrated the importance of such wounds for the penetration of F. oxysporum into tomato plants. Chatterjee (1958) and Christou and Snyder (1962) found that F. solani f. sp. phaseoli penetrated at the points of emergence of roots and that initial disease symptoms involved a discolouration of the tissues at these sites.

Root apices, in addition to the region of secondary root emergence have been shown to be frequent sites for hyphal penetration into host tissues (Scott, 1965). Christou and Snyder (1962) reported that the hypocotyl is penetrated easily due to the presence of stomata and Bywater's (1959) observations with F. solani f. sp. pisi indicated that stomatal entry was the most common mode of penetration. However, these reports are at variance with Chatterjee's (1958) observations of only occasional stomatal penetration at soil level.

Seedlings inoculated with Isolate 82A, in aseptic culture were severely stunted and the pathogen was successfully recovered from all of the sites chosen for reisolation. However, in reisolations from 82A infected seedlings growing in pasteurized soil, it was not possible to isolate the fungus from the shoot apex and similarly with field-grown seedlings, this isolate was recoverable only from diseased roots and from the lower portion of the stem. When stems were dissected, discolouration of the vascular tissue was observed but this extended only 1-2 cm beyond the root-stem transition zone. Jarvis and Shoemaker (1978) in their investigations of a foot and root rot disease of field and greenhouse tomatoes noted that F. oxysporum could not be isolated from beyond this zone of discolouration. Weimer (1944) described a similar response with F. oxysporum f. sp. radicis-lupini, the pathogen responsible for an observed foot and root rot of lupines in the United States of America.

Most pathogenic forms of F. oxysporum including all isolates previously recovered from diseased lentils produce a vascular wilt in host plants (Smith, 1975). A wilt disease, as defined by the Federation of British Plant Pathologists (1973) is characterized by a loss of turgidity and collapse of leaf tissue resulting from vascular infection by the pathogen. Vasudeva and Srinivasan (1952) in their description of a disease of lentils caused by F. oxysporum noted that initial symptoms involved curling of the leaf tissue at the lower end of the plant which then spread acropetally, followed by drooping of the crown and death of the plant and as is typical of most wilt diseases, the fungus was recoverable from all portions of the shoot including the apex. In addition, in greenhouse and field experiments, they observed that at the end of pathogenesis, the root system was poorly developed and discoloured with proliferation of secondary roots in the proximal portions of the tap root. Pennypacker and Nelson (1972) suggested that root discolouration in association with wilt disease caused by Fusarium spp. may be due to the presence of secondary organisms in the environment.

In the present investigation, root rot symptoms were produced by F. oxysporum, Isolate 82A, in aseptic culture. These differed only marginally from those produced by the root rot pathogen, F. solani (Isolate 196A) and often they were more severe. In pot trials, F. roseum 'Avenaceum' produced wilt symptoms in lentils and these differed from symptoms produced by Isolate 82A under the same conditions. Isolate 82A caused a progressive chlorosis of the leaf tissue and the root system was reduced in size and discoloured. Wilt symptoms were not produced by this isolate. In aseptic culture and in pasteurized soil the root damage caused by F. roseum 'Avenaceum' was less severe than that produced by Isolate 82A and more typical of a true vascular wilt disease (McLean and Walker, 1941).

A comparison of the pathogenicity of three isolates of _F_. _oxysporum_ to lentil seedlings showed that Isolate 82A was more virulent than Isolates 0-670 and W-2. However, Isolate 0-670 which was recovered from diseased lentils in South America produced similar symptoms in pot trials and in aseptic culture to those of Isolate 82A. A comparison of their cultural characteristics on PSA further substantiated the view that they were essentially identical although the range in size of the macroconidia produced in culture by Isolate 0-670 was greater than for Isolate 82A. It is probable that these two isolates are in fact the same and that this strain may have been introduced into the Ottawa area on seed of uncertain origin that was obtained from a local health food store and planted on the Central Experimental Farm in the summer of 1979 (C. Nozzolillo, personal communication). Kannaiyan and Nene (1972) and Ujevic _et al_. (1965) have reported that _F_. _oxysporum_ can be seed-borne on lentils and Nath _et al_. (1970) have emphasized the importance of seed-borne inoculum as a means of introducing a pathogen into new areas. Futrell (1972) has attributed the early spread of _Helminthosporium maydis_ into new areas to seed-borne inoculum.

A mixed inoculum obtained from the two most virulent isolates recovered from soil (_F_. _oxysporum_, Isolate 82A and _F_. _solani_, Isolate 196A) was tested for pathogenicity to lentil seedlings. Virulence of this interspecific mixture was intermediate between the results obtained with _F_. _oxysporum_, Isolate 82A, alone and with _F_. _solani_, Isolate 196A, alone. These results indicate that there is the possibility of mutual antagonism between these species. Årsvoll (1976) demonstrated a mutual antagonism between isolates of _Typhula ishikariensis_ and _T_. _incarnata_ which he attributed to the production of staling substances or metabolites which may be mutually inhibitive to the different isolates. Lebeau (1975) mixed pathogenic isolates of the 'low temperature basidiomycete' and noted that there was a reduction in the pathogenicity of this mixture to alfalfa. In addition, he observed that there was a decrease in the mycelial weight of the mixture when grown in culture together than if the isolates were grown individually.

The production of toxic metabolites by F. oxysporum as well as other fungi has been noted by Pringle and Scheffer (1964). Due to the rapid progression of disease symptoms in seedlings inoculated with Isolate 82A, the possible involvement of a phytotoxic compound was suspected. Seedlings incubated in 4-and 12-day-old culture filtrates of F. oxysporum, Isolate 82A produced disease symptoms that were similar to those produced by mycelial or conidial suspensions of this fungus. Differences in symptom development were not observed with the different ages of cultures used for the preparation of the filtrates. Stover and Waite (1954) however, noted that Fusarium produced low levels of fusaric acid during the first 16 days of incubation following which time, levels increased dramatically. In similar observations, Sharma and Agnihotri (1971) noted that the longer the fungus was grown in culture, the shorter the period required by the filtrate to produce wilt symptoms in lentils.

A cytological examination of the mode of penetration and subsequent development of this pathogen in lentil root tissues revealed that F. oxysporum, Isolate 82A was primarily an intercellular cortical parasite. Initial penetration of host tissues by the pathogen seen eight hours following inoculation was direct without prior formation of a true appressorial structure. This observation supports previous studies of Christou and Snyder (1962), Chi et al. (1964), Alconero (1968), Kamel et al., (1973), and Tippett et al. (1977). Frequently, however, germ tubes contiguous with an epidermal cell wall became swollen thus resembling appressoria although they were not delimited by a septum. Tippett et al. (1976) in their investigation of the prepenetration behavior of Phytophthora cinnamomi have implied that these swellings are formed when the outer epidermal cell wall impedes elongation of the germ tube. Her view is based upon the observation that no swellings are formed when a germ tube penetrates between epidermal cells, this being a less resistant mode of entry for the pathogen.

The zone of cellular elongation, in lentil roots located 0.5 - 7 mm behind the root apex, was the most susceptible to Isolate 82A and this observation is in agreement with the reports of Smith and Walker (1930), Pennypacker and Nelson (1972), and Tippett et al. (1976). The susceptibility of this area may be attributable to the low levels of pectin, cellulose, and other cell wall components present in the tissues of this zone (Scott, 1965). In addition, the amount of space available for proliferation of the pathogen and the availability of nutrients may be greater in this area than in the root apex or the root hair zone (Christou and Snyder, 1962).

The cytoplasm of cells adjacent to a fungal hypha often appeared granular and at times there was plasmolysis of the cellular contents prior to hyphal penetration. Tippett et al. (1977), in explaining the collapse of cells in roots of Eucalyptus obliqua, in advance of the invading hyphal front have suggested that production of a diffusible toxin by the pathogen may be responsible. In support of this view, Hanchey and Wheeler(1973) observed cell wall plasmolysis in oats treated with victorin, the toxin produced by Helminthosporium maydis.

Following direct penetration, hyphae of Isolate 82A grew through the middle lamella of adjacent epidermal cells and down into the cortex. The pathogen grew profusely in the first intercellular space encountered and from there it invaded the cortex intercellularly. After the cortical cells became degraded, intracellular growth of the fungus was also apparent. Christou and Snyder (1962), Chi et al., (1964), Kamel et al., (1973), and Tippett et al., (1976) have reported that cortical invasion of Fusarium is initially intercellular, only. Chi et al., (1964) in their comparative investigation of the penetration and development of F. solani, F. oxysporum, and F. avenaceum in alfalfa and red clover roots demonstrated that these three Fusarium spp. differed in the extent of the invasion of host tissues. They reported that F. solani was restricted primarily to the epidermal and cortical tissues whereas F. avenaceum and F. oxysporum were abundant in the stele. Alconero (1968) in his study of the root rot of vanilla caused by F. oxysporum f. sp. vanillae observed the presence of this fungus in the vascular tissues and similar results were reported by Pennypacker and Nelson (1972) with F. oxysporum f. sp. dianthi. Isolate 82A was not observed within the xylem tissue even though proliferation throughout the cortical tissues was extensive. In tissues examined 72 hours following inoculation, large cavities were visible in the xylem parenchyma and there is evidence that this cell disintegration prior to the presence of hyphae in the tissues may result from the production of pectinases by F. oxysporum (Albersheim et al., 1969;

Pennypacker and Nelson, 1972).

The causal agent of a root-rot of lentils observed under field conditions has been identified as Fusarium oxysporum. Although this Fusarium species is typically a vascular wilt pathogen, it has been observed to produce root rots of vanilla (Alconero, 1968), lupines (Weimer, 1944), tomato (Jarvis and Shoemaker, 1978), as well as of pine and douglas fir seedlings (Smith, 1975 and Bloomberg, 1976, respectively). In the present study, wilting of the leaf tissue was not observed in aseptic culture, in pasteurized soil, or under field conditions. However, the root systems of infected plants were poorly developed and discoloured. Cytological examination of the root tissues revealed extensive poliferation of this fungus in the cortical cells but failed to confirm its presence within the stele. It appears then, that this strain of F. oxysporum is different from F. oxysporum f. sp. lentis, the lentil wilt pathogen reported from Asia, Europe, South America and the United States of America.

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APPENDIX

Table 1. Weather summary, Central Experimental Farm, Ottawa. Data compiled and summarized by Agrometeorology section - Land Resource Research Institute, Research Branch, Canada Agriculture.

Month of May, 1980

Temperature (°C)

	1980	1979	Average*
Mean maximum	19.2	18.4	18.9
Monthly mean	13.7	13.4	12.7
Mean minimum	8.1	8.3	6.6

Precipitation

	1980	1979	Average*
Total precipitation (mm)	52.4	101.2	70.0
Days of measurable rain	12	14	11
Total bright sunshine (h)	273.5	208.8	231.5

Month of June

Temperature (°C)

	1980	1979	Average
Mean maximum	21.6	23.8	24.3
Monthly mean	16.1	18.0	18.1
Mean minimum	10.6	12.2	11.9

Precipitation

	1980	1979	Average
Total precipitation (mm)	60.8	97.8	82.8
Days of measurable rain	17	17	11
Total bright sunshine (h)	234.5	274.4	250.5

Month of July

Temperature (°C)

	1980	1979	Average
Mean maximum	25.9	27.7	26.7
Monthly mean	20.8	21.9	20.5
Mean minimum	15.7	16.0	14.3

Precipitation

	1980	1979	Average
Total precipitation (mm)	139.4	48.8	90.0
Days of measurable rain	22	13	11
Total bright sunshine (h)	256.6	299.4	275.6

Month of August

Temperature (°C)

	1980	1979	Average
Mean maximum	25.8	23.3	25.6
Monthly mean	20.8	18.5	19.2
Mean minimum	15.7	13.8	12.9

Precipitation

	1980	1979	Average
Total precipitation (mm)	45.2	103.2	81.2
Days of measurable rain	14	16	11
Total bright sunshine (h)	246.4	232.2	249.0

* Average = 1890-1965

Table 2. Soil Test Report.[1]

	Merivale Field	Neatby Field
soil texture	loam→silt loam	loam→silt loam
Phosphorous	excessive (77)	excessive (83)
Potassium	medium (120)	high (144)
Magnesium	medium (85)	high (135)
pH	7.2	7.3

[1] soil test values are enclosed in brackets. Soil test performed by the Department of Land Resource Science O.A.C.; University of Guelph, Ontario and Ontario Ministry of Agriculture & Food.