An Investigation into the Epidemiology and Control of Anthracnose (*Colletotrichum truncatum*) of lentil in Manitoba.

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RICHARD J. GIBSON

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AN INVESTIGATION INTO THE EPIDEMIOLOGY AND CONTROL OF ANTHRACNOSE (*Colletotrichum truncatum*) OF LENTIL IN MANITOBA

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General Abstract

Repeated foliar application of chlorothalonil significantly increased lentil (*Lens culinaris*) seed yield in field plots infested with anthracnose caused by *Colletotrichum truncatum*. Two to four fold increases over the yields of non-treated plots occurred when conditions for disease development were favourable. Propiconazole significantly increased seed yield at an irrigated test site at the University of Manitoba Campus Farm but did not significantly improve seed yield at other sites, probably due to dry conditions. Levels of infestation in harvested seed were generally low, ranging from 2.25% in seed from 16 commercial lentil fields, to below 1.42% from untreated plots and less than 0.25% from plots sprayed with chlorothalonil. No evidence of seed to seedling transmission was obtained when seed lots with 6.4%, 3.4% and 2.3% infected seed were sown in isolated plots at the Campus Farm. Propiconazole reduced disease severity on lentil when applied up to 48 hours after plants were inoculated with *C. truncatum* in the greenhouse. Chlorothalonil did not reduce disease severity when applied to plants 24 or 48 hours after inoculation. In growth cabinet studies, disease severity on inoculated lentil plants increased with increasing temperature and length of leaf wetness periods. Symptomless infection of lentil and faba bean was demonstrated in field and greenhouse studies. The ability of *C. truncatum* to overwinter on lentil stubble for up to two years in a commercial field was determined using a bioassay. In a three-year field study, lentil cultivars and breeding lines were ranked for disease severity from lowest to highest as follows; Indianhead, Laird, Laird-cross La x 17310-8, Eston, followed by the landraces French Green, Chilean and Spanish Brown. Greenhouse studies showed that lentil, faba bean (*Vicia faba*), field pea (*Pisum sativum*), flat pea (*Lathyrus sp.*) and vetch (*Vicia sativa*) were susceptible to *C. truncatum* from lentil. Several cultivars of soybean (*Glycine max*) and field bean (*Phaseolus vulgaris*) were resistant to *C. truncatum* from lentil.

General Introduction

Lentil (*Lens culinaris* Medik.) is a moderately drought tolerant crop adapted to brown and dark brown soils. Lentil seed is high in protein and is used primarily for human consumption. Lentil production in Manitoba began in 1970 and has increased from approximately 930 ha, sown in 1972, to over 24,300 ha sown in 1987 (Manitoba Department of Agriculture 1990). Domestic consumption is less than 1% with the majority of the annual production exported to Europe and South America. Canada is the world's second largest exporter of lentil, with about 150,000 ha grown in Manitoba and Saskatchewan with a value of approximately $100 million per year (Slinkard and Blain 1988). For economic reasons producers have grown the crop under much shorter rotations than recommended for areas of high precipitation and humidity, thereby greatly favouring the development and build up of foliar diseases.

Until recently ascochyta blight (*Ascochyta fabae* Speg. f.sp. *lentis*) had been the only foliage and pod disease of lentil reported in western Canada (Morrall and Sheppard 1981, Gossen *et al.* 1986) but in 1987 an anthracnose disease was discovered in Manitoba in areas with a long history of lentil production (Morrall 1988). Four years later, anthracnose of lentil was identified for the first time in Saskatchewan (Morrall and Pedersen 1991). The pathogen infected stems and foliage and was identified as *Colletotrichum truncatum* (Schw.) Andrus and W.D. Moore. The identification of the fungus was made by the Biosystematics Research Institute, Ottawa, Canada. However, previous cultures sent to the Commonwealth Mycological Institute, Kew, U.K. have been identified as *C. destructivum* O'Gara. In a disease survey in Manitoba Morrall *et al.* (1989) reported recovering a fungus identical in morphology to *C. truncatum* from faba bean (*Vicia faba* L.) and wild vetch (*Vicia sativa* L.). *Colletotrichum truncatum* from lentil was able to infect lentil, faba bean and pea (*Pisum sativum* L.) in greenhouse tests (Morrall *et al.* 1989).

Anthracnose on lentil first appears on the lower stems and branches and new lesions appear rapidly once the crop canopy closes. Lesions develop on the leaves, stems and pods causing extensive leaflet abscission, reduced pod filling, stem collapse and lodging. Anecdotal evidence from producers indicates yield losses in lentil range from 12 to 70% (Morrall and Pedersen 1991).

Further research is required to determine the agronomic factors which lead to severe disease. The source of primary inoculum and the longevity of the pathogen in soil or stubble is not known. Although *C. truncatum* has been observed in lentil and faba bean fields and on native vetch, the extent of the host range on other legumes in Manitoba is unknown. *Colletotrichum truncatum* on soybean (*Glycine max* L.) can remain latent in infected tissues prior to development of symptoms, but it is not known to what extent latent infection occurs in lentil. Crop protection measures utilizing fungicides, cultural control practices or selection of resistant lentil genotypes should be investigated.

Review of Literature

1.0 The Host

1.1 Taxonomy The genus *Lens* Miller is one of the five genera of the tribe *Viciaceae*, which includes *Pisum*, *Lathyrus*, *Vavilovia* and *Vicia*. The tribe *Viciaceae* is one of the thirty-two tribes included under the sub-family *Papilionoideae* within the *Leguminosae*. The genus *Lens* currently contains only 6 recognized species (Smartt 1990); these are, *L. montbretii* (Fisch. & Mey) Davis & Plitmann, *L. ervoides* (Brign.) Grande, *L. nigricans* (Bieb.) Godron, *L. orientalis* (Boiss.) Handel-Mazzeti, *L. culinaris* Medikus and *L. odomensis* Ladizinsky.

The cultivated lentil, *L. culinaris* Medik was first studied by Alefeld (1866) who used the specific name *L. esculenta* Moench and included eight subspecies (Cubero 1984). Later, Barulina (1930) conducted a detailed study of lentil taxonomy and proposed that *L. orientalis* represents the wild ancestral type of *L. culinaris*, a hypothesis which was later confirmed by hybridization studies (Ladizinsky 1979). Barulina constructed a classification system based on differences between pod, seed and flower morphology, growth habit and geographical location.

Two races of cultivated lentil were immediately recognized based on seed size. The race *macrosperma* contains large flattened seeds 6-8mm in diameter with yellow or orange cotyledons and the race *microsperma* which contains small to medium (3-6mm in diameter) lens-shaped seeds which can vary in colour (Cubero 1984). On the basis of morphology and geographic location, Barulina included six groups under the race *microsperma*: *europeae*, *asiaticae, intermediae*, *subspontanae*, *aethiopicae* and *pilosae*. Cubero (1984) reports that recent expeditions to collect germplasm have recovered all but the *subspontaneae* form.

The vegetative morphology of the cultivated lentil is vetch-like with pinnate leaves commonly bearing 10-20 leaflets with a terminal tendril. The stem is thin, square

and generally herbaceous and weak. The basal portion of the stem often becomes woody and supports several basal branches (Saxena and Hawtin 1981). Lentil branching patterns often vary with genotype and plant density. Plant height can reach between 15 and 35cm. The lentil is an annual legume that is primarily self-fertile although cross pollination can occur. The seed pods are smooth, compressed, approximately 1.25-2.0cm long and contain two smooth lens-shaped seeds. Germination is hypogeal. The roots are capable of nodulation and nitrogen fixation when inoculated with appropriate *Rhizobium* bacteria. The optimal climate for the crop is temperate and maturation occurs ideally under dry or arid conditions (Cubero 1984, Smartt 1990).

1.2 Origin and History Archaeological evidence indicates that the lentil crop was distributed throughout the Mediterranean region, central and southern Europe and India following the adoption of dry land agricultural practices (Jolly and Plog 1987, Hansen and Renfrew 1978). The cultivated lentil is believed to have originated in the 'Fertile Crescent' region near southern Turkey and northern Iraq. It is hypothesized that populations of *L. orientalis* and possibly *L. nigricans* were unconsciously selected by man which eventually culminated in the appearance of *L. culinaris* (Cubero 1984).

Historically, lentil has been cropped on marginal agricultural land (Cubero 1984). The lentil seed has a high nutritional value (Bhatty and Slinkard 1979) with low levels of anti-metabolites. The seed is used in soups, ground into flour, eaten whole or used as a source of starch for textile and printing industries. The immature pods can also be eaten as a vegetable. Lentil crops are sometimes planted as a green manure crop or as forage (Chopra and Swamy 1975).

1.3 Lentil Breeding The lentil has become adapted to dry land farming over many centuries of natural selection and possesses a primary gene pool containing both

wild and domestic components (Cubero 1984). Secondary and tertiary gene pools are comprised of *L. nigricans* and the remaining species within the genus *Lens* (Ladizinsky 1979). Adaptive changes to specific habitats have been reported to occur not only through changes in allelic frequency but also through allelic reorganization (Allard 1988). Lentil flowers are primarily self-pollinated, however, outcrossing by insect vectors can occur and lead to problems of maintaining purity in seed stocks. In order to utilize the collected lentil germplasm more effectively in breeding programs, the degree of outcrossing of lentil landraces from Chile, Greece and Turkey was determined by Erskine and Muehlbauer (1991) and found to be 6.6%, 2.9% and 2.2% respectively. Erskine and Muehlbauer (1991) report that the surveyed germplasm contained a complex multilocus structure where the genotypic variability at one locus is linked to the genotypic states of other loci. Successful breeding of new lentil genotypes or cultivars will require knowledge of the relationship between multilocus organization, genomic diversity and adaptation.

Grain legumes, such as lentil, have proven to be less amenable to genetic transformation than most other dicotyledonous crops. However, the successful transfer of a T-DNA construct containing a beta-glucuronidase gene (GUS) by *Agrobacterium tumefaciens* to lentil has proven that transformation is possible (Warkentin and McHughen 1991, 1992). Regeneration of lentil from shoot tip culture, organogenesis, somatic embryogenesis and the production of calli from lentil protoplasts has also been reported (Williams and McHughen 1988). Transgenic lentil plants may allow novel combinations of genes to be inserted which would normally not be possible in a conventional breeding program. For instance, introduction of a herbicide resistance gene into the lentil genome may be useful because lentil is not competitive with weeds and very few herbicides are available for use on lentil.

1.4 Status of Lentil Production Most of the world's crop is consumed in the major areas of production with over two thirds of the crop being grown in Asia. The production of lentil in Northern Europe, former USSR, USA and Canada appears to be commercially motivated (Smartt 1990). Manitoba produces approximately 15% of the total Canadian crop, while the majority of lentil production is located in Saskatchewan (Manitoba Department of Agriculture 1990). Domestic consumption in Canada is less than 1% of total production with a majority of the exported lentil going to Europe and South America (Slinkard and Blain 1988). Lentil production in Canada has been expanding since 1977 and has made Canada the second largest exporter of lentil in the world next to Turkey.

Lentil production in Manitoba began in 1970 and increased from approximately 930 ha, sown in 1972, to over 24,300 ha sown in 1987. In 1989 there were 10,120 ha of lentil in production with an average yield of 1200 kg/ha (Manitoba Department of Agriculture 1990). The first crops grown were primarily the large seeded common Chilean landrace grown under contract but not licensed in Canada. Currently, there are primarily two landraces grown under contract for export to specialty markets, the 'dark speckled' or 'French Green' lentil and the common Chilean type.

The increasing area of lentil production in the Prairie Provinces warranted the development of adapted cultivars. Three cultivars have been licensed from the Crop Development Centre, University of Saskatchewan, Saskatoon, Canada. The cultivar, Laird, was licensed in Canada in 1978 and was developed from a number of lines from Russia selected at the U.S. Department of Agriculture, Plant Introduction Station, Pullman, Washington in 1972 (Slinkard and Bhatty 1979). Laird is a large seeded Chilean lentil with yellow cotyledons; it is higher yielding, taller and later maturing than the common Chilean lentil. The second cultivar to be registered in Canada was Eston, a small seeded Persian type with yellow cotyledons; it represents the majority of seed sown in Manitoba (Slinkard 1981).

Eston was developed from a line selection from the U.S.D.A. Plant Introduction Station obtained from Turkey and is higher yielding than either the Laird or the Chilean lentil. The third cultivar registered, Rose, is similar in many characteristics to Eston but has a slightly higher seed weight and red cotyledons.

1.5 Important Fungal Diseases of Lentil in the World Several fungal diseases cause economically important losses in lentil production worldwide including ascochyta blight, rust, sclerotinia stem rot, seedling blight, root rot, fusarium wilt and botrytis stem and pod rot.

Ascochyta blight of lentil was first discovered in the former USSR, where the pathogen was described as a new species, *Ascochyta lentis* Vassilievsky. The disease can now be found in many lentil producing regions including South America, the Middle East, Russia and India (Nene *et al.* 1988). The disease was observed on lentil in Canada in 1978 (Morrall and Sheppard 1981). The pathogen has been renamed *Ascochyta fabae* Speg. f. sp. *lentis* Gossen *et al.* (Gossen *et al.* 1986) to reflect the similarities of cultural and morphological characters, but separate host specificities, to the faba bean pathogen *A. fabae* f.sp. *fabae*. The pathogen affects all aerial portions of the plant producing small, round, grey to tan-coloured lesions surrounded by a darker margin in the case of leaf lesions. Spores develop within pycnidia and are disseminated by splashing rain. Destruction of photosynthetic area by defoliation and lesion development induced by *A. fabae* f.sp. *lentis* (Gossen and Morrall 1983) decreases the availability of photosynthates during seed formation and reduces yield. Yield loss in lentil plots inoculated with *A. fabae* f.sp. *lentis* compared to fungicide-protected, noninoculated plots ranged from 25 to 40% for Eston and Chilean lentil and from 8 to 13% for Laird lentil (Gossen and Morrall 1983). Foliar application of fungicide (Beauchamp *et al.* 1986a,b) increased seed yield and reduced seed infection over non-treated check plots. Ascochyta blight of lentil rapidly became established throughout the Prairie

Provinces in the late 1970's and is believed to have been introduced by the use of infected seed. Infected plant residue has also been recognized as a highly effective source of inoculum (Morrall and Sheppard 1981, Gossen and Morrall 1986, Yu 1947, Nene *et al.* 1988)

Rust caused by *Uromyces fabae* (Pers.) de Bary is reported to be a major limiting factor for lentil production in many European and Mediterranean regions (Nene *et al.* 1988, Sinha and Yadav 1989). Initial symptoms appear as yellow-white pycnia and aecia on the abaxial surfaces of leaflets and pods. Uredopustules are formed later and are present on both surfaces of the leaflets followed by the production of dark brown to black teleutopustules on stems and petioles late in the season (Nene *et al.* 1988). Identification of resistant biotypes is underway (Erskine 1984).

Sclerotinia stem rot caused by *Sclerotinia sclerotiorum* (Lib). de Bary and botrytis pod and stem rot caused by *Botrytis cinerea* (Pers.) ex Fr. occur in most lentil producing regions. Both diseases can occur in Western Canada and develop primarily in dense vegetative stands where prolonged periods of high humidity at the soil surface exist (Nene *et al.* 1988). Symptoms include wilting and premature ripening of some lentil stems and leaves in mid to late summer, which adversely affects pod filling.

Root rot, seedling blight and wilt of lentil can be caused by a number of fungi which may contribute to the disease, including *Fusarium* spp., *Rhizoctonia* spp. and *Pythium* spp. (Nene *et al.* 1988). In Western Canada, increased levels of pathogenic fungi in the soil due to intensive lentil cultivation may increase yield loss. Plants may become stunted, turn yellow and die prematurely at any stage. Roots may appear rotten and separate easily from the above ground portions of the plant.

Anthracnose of lentil has been reported only recently and literature on this disease is limited. The disease was been observed in Syria (Bellar and Kebabeh 1983), Brazil (Manara and Manara 1983) and Islamabad, Pakistan (R.A.A. Morrall, unpublished). However, the pathogen was identified only as a *Colletotrichum* species. Anthracnose was first reported on lentil in Canada in 1987 (Morrall 1988) and the pathogen has been shown to be identical in morphology to *Colletotrichum truncatum* of soybean. High rainfall and temperatures in the major lentil growing areas of Manitoba and Saskatchewan favour the development of the disease. Yield losses due to lentil anthracnose in Canada have been estimated by producers to range from 12 to 70% (Morrall and Pedersen 1991).

2.0 The Pathogen

2.1 Taxonomy and Biology Anthracnose of lentil was first identified in Manitoba in 1987 (Morrall 1988) and is present in all major areas of lentil production. The identity of the pathogen was confirmed by the Biosystematics Research Institute, Ottawa, Canada as *Colletotrichum truncatum* (Schw.) Andrus and W.D. Moore. However, the Commonwealth Mycological Institute, Kew, U.K. identified similar isolates as *C. destructivum* O'Gara (Platford 1988).

Considerable confusion has arisen with regard to the identification of *Colletotrichum* spp. because of their ubiquitous conidia, variable morphology in culture and often overlapping host ranges. The taxonomy of *Colletotrichum* has been based on classical descriptive criteria concerning conidial shape and size, presence or absence and morphology of setae, host range and symptoms (Sutton 1980).

Andrus and Moore (1935) identified *C. truncatum* as the causal organism of an unidentified anthracnose disease of lima beans (*Phaseolus lunata*) and garden beans (*P. vulgaris*). The pathogen had curved conidia rather than straight conidia and different growth characteristics in culture from the common bean anthracnose pathogen, *C. lindemuthianum*. Tiffany and Gilman (1954) identified *C. truncatum* as the causal organism of soybean anthracnose, based on conidial shape, host range and cultural morphology defined previously (Andrus and Moore 1935). Tiffany and Gilman collected numerous isolates of *Colletotrichum* and divided the isolates into those producing falcate and those producing straight conidia. The falcate group included *C. truncatum*, *C. pisi* Pat., *C. capsici* (Syd.) Butl. & Bisby, and *C. villosum* Weimer. The straight spore group contained *C. trifolii* Bain & Essary, *C. graminicolum* (Cesati) G.W. Wilson, *C. destructivum* O'Gara and *C. lindemuthianum*. The practice of naming morphologically similar fungi according to their hosts has persisted and several hundred 'new' taxa of Coelomycetes have

been created (Nag Raj 1981, Sutton 1980). Taxonomic keys developed by Sutton (1980) and Arx (1957) are commonly used for the identification of *Colletotrichum* species.

Arx (1957) considered *Colletotrichum* species pathogenic on legumes to be the forma specialis *truncatum* of *C. dematium* while Sutton's key, based on conidial ontogeny, retains *C. truncatum* under Andrus and Moore's (1935) morphological description. Weidemann *et al.* (1988) suggests that *C. pisi* should be considered synonymous with *C. truncatum* and not with *C. gloeosporioides* as classified by Arx. This manuscript recognizes *C. truncatum* as described by Sutton (1980); possessing falcate conidia with obtuse apices, conidial dimensions of variable length from 15.5-24 μm by 3.5-4 μm, borne within an acervulus.

2.2 Symptomatology and Epidemiology *Colletotrichum truncatum* is a relatively unspecialized pathogen which has a wide host range and a wide geographical distribution (Weidemann *et al. 1988*, Allen 1983). Since anthracnose of lentil caused by *C. truncatum* has only been recently identified as a problem for lentil production, much of the literature concentrates on reports of disease occurrence (Bellar and Kebabeh 1983, Gibson *et al.* 1991, Morrall 1988, Morrall *et al.* 1989,1990, Morrall and Pedersen 1991).

Literature on the epidemiology of *C. truncatum* on soybean (*Glycine max* (L.) Merr.), however, is extensive. Soybean anthracnose is favoured by prolonged periods of high rainfall, humidity and warm temperatures. Soybean anthracnose can involve several *Colletotrichum* species such as *C. destructivum* O'Gara and *C. gloeosporioides* (Penz.) Sacc. However *C. truncatum* is generally accepted to be the primary pathogen (Sinclair 1982). Soybean plants are susceptible to *C. truncatum* at all growth stages and field studies have shown the development of infection points near the base of the plant giving rise to new lesions which appear

on the upper portions of the plant (Abney and Richards 1982, Khan and Sinclair 1991).

Conidial germination and development of appressoria on above ground plant parts tend to occur near trichomes, leaf veins and petioles where free water remains for extended periods of time. Cellular damage and hypertrophy were associated with appressorium and infection peg formation, indicating that toxic materials or enzymes may be produced during penetration of leaf cells (Manandhar *et al.* 1985). Direct penetration by germ tubes and production of hyphae between the cuticle and epidermis of the host has been observed (Chau and Alveraz 1983, Manandhar *et al.* 1985). *Colletotrichum truncatum* is able to initiate a symptomless latent infection which typically leads to symptom development early during the reproductive stages of the crop (Sinclair 1991). Techniques for early detection of latent infection have been developed which utilize desiccant-type herbicides (Cerkauskas and Sinclair 1980, Cerkauskas *et al.* 1983) to increase membrane permeability and nutrient availability for the liberation of latent fungi.

Development of anthracnose symptoms on soybean includes discrete veinal necrosis on leaves with production of acervuli, sclerotia and stromatic bodies on the dead plant parts at the end of the season (Sinclair 1982, Khan and Sinclair 1991). On lentil, typical lesions on leaves are sunken, tan coloured, necrotic, and spreading. Leaflet abscission may be excessive. Lentil stem lesions become oval shaped, tan coloured and necrotic, ranging in depth from superficial to invasive, often resulting in stem collapse.

Colletotrichum truncatum had been considered to be a foliar pathogen until sclerotia from some soybean isolates (Khan and Sinclair 1990, 1991) were shown to be pathogenic to soybean roots and hypocotyls of cultivars differentially resistant to foliar anthracnose. The source of primary inoculum for above-ground parts of

soybean plants include infected soybean seed, infected crop stubble and alternative weed hosts (Sinclair 1991). The pathogen was recovered from 14 genera of weed hosts and overwintered soybean samples (Hartman *et al.* 1986).

2.3 Yield loss and Control Yield loss in lentil due to *C. truncatum* has been reported to be a major limiting factor for lentil production in the Rio Grande do Sol area of Brazil (Manara and Manara 1983). In Saskatchewan lentil yield losses due to anthracnose have been estimated to range from 12 to 70% in 1990 (Morrall and Pederson 1991). The agronomic factors which lead to severe disease in lentil are unknown. However, environmental factors which favour soybean anthracnose and lead to yield losses include high temperatures (above 25C) and prolonged periods of leaf wetness during the growing season (Sinclair 1982). Seed loss in soybean due to infection by *C. truncatum* is related to the severity of anthracnose and is a primary component in determining seed yield (Backman *et al.* 1982).

For soybean anthracnose, reducing the source of primary inoculum by using disease-free seed (Roy 1982, Hepperly *et al.* 1983, Kunwar *et al.* 1985), controlling alternative weed hosts (McLean and Roy 1988, Hepperly *et al.* 1980) and utilizing crop rotations to avoid contact with infected crop stubble may minimize the risk of severe outbreaks (Sinclair 1991). Many *Colletotrichum* spp. can survive and overwinter on host debris at the soil surface but lose viability when buried. Minimal tillage practices may lead to an increase in inoculum levels over those with conventional tillage practices which bury crop residues (TeBeest 1982, Lipps 1988).

Considerable research has been conducted on controlling the pathogen by application of foliar fungicides such as; benomyl, thiophanate-methyl, propiconazole, and chlorothalonil (Sinclair 1980, Walters 1980, Hovermale and Sciumbato 1981, Whitney 1983, 1985). Screening of resistant soybean genotypes

has identified a number of anthracnose resistant soybean lines (Backman *et al.* 1982, Sinclair 1982).

Benomyl and thiophanate-methyl are benzimidazole compounds used as systemic foliar fungicides. The benzimidazoles interrupt the mitotic process by the specific binding of the active agent, carbendazim, to the tubulin subunits of the fungal cell resulting in a reduced rate of growth (Sisler 1988). Application of benomyl has been reported to significantly increase soybean seed yield when conditions for high yield and disease exist (Heatherly and Sciumbato 1986).

Propiconazole is a triazole fungicide that has protective, curative and systemic activity (Sisler 1988). Propiconazole is a sterol biosynthesis inhibitor and belongs to a class of compounds known as demethylation inhibitors (DMI's). Propiconazole blocks the demethylation of an intermediate compound in the synthesis of ergosterol from lanosterol. The production of ergosterol is interrupted and fungal cell membranes function and integrity is affected (Koller 1988, 1992).

Chlorothalonil is a broad spectrum, multisite inhibitor of low biochemical specificity (Thomson 1988). Chlorothalonil is biologically unselective but functions as a protectant fungicide reacting with the nucleophilic sites of the fungal cell wall but not penetrating past the leaf cuticle to the susceptible plant cells within the leaf (Eckert 1988).

There is a lack of information regarding the development of anthracnose resistant lentil lines or studies on the effectiveness of fungicide applications for the control of anthracnose of lentil.

Evaluation of Chlorothalonil and Propiconazole for the Control of Anthracnose of Lentil Caused by *Colletotrichum truncatum*

3.0 Abstract

Field experiments were conducted on the effectiveness of increasing lentil yields using foliar fungicides and to examine the possibility of seed-to-seedling transmission of anthracnose. Greenhouse tests were conducted to determine the effects of fungicide application prior to, or after infection by *C. truncatum*. Chlorothalonil applied repeatedly throughout the season was effective in reducing yield loss when conditions for anthracnose were favourable. Yield losses due to anthracnose appear to be dependent on the environment. Anthracnose reduced the yield of untreated plots to 22-56% of yield obtained from chlorothalonil treated plots in 1989 and 1991. Propiconazole significantly reduced yield loss at the Campus Farm in 1991. However, results from Rosenort in 1990 and Bagot in 1991 were inconclusive probably due to dry weather during the study. Seed infection levels in field plots treated with chlorothalonil were less than 0.25%. The highest level of seed infection (1.42%) was obtained in untreated plots at Portage in 1989. The levels of anthracnose-infected seed were below 2.25% in sixteen seed samples obtained from commercial lentil fields in 1991. No evidence of seed-to- seedling transmission was observed when seed lots with 6.4%, 3.4% and 2.3% infection were sown in isolated plots at the Campus Farm. In greenhouse studies, pre-application of chlorothalonil or propiconazole to lentil plants inoculated with *C. truncatum* reduced disease severity over that of inoculated and non-protected plants. Application of propiconazole up to 48 hours after lentil plants were inoculated with *C. truncatum* and incubated caused slight lesions to develop compared with moderate to severe lesion production that occurred with post-application of chlorothalonil or in non-protected plants.

3.1 Introduction

Anthracnose of lentil was identified in Manitoba in 1987 (Morrall 1988). In subsequent surveys anthracnose was shown to be present in all major areas of lentil production in Manitoba (Morrall *et al.* 1989,1990, Gibson *et al.* 1991). In 1990, the disease was reported for the first time on lentil in Saskatchewan where the crop had been intensively grown for 15-20 years (Morrall and Pedersen 1991). The source of primary inoculum for anthracnose in Manitoba is not known but appears to be associated with lentil stubble. The disease is usually first observed at early flowering and spreads rapidly within lentil fields in rotations of one to four or more years.

The causal organism of lentil anthracnose according to Sutton's nomenclature (1980) is *Colletotrichum truncatum* (Schw.) Andrus and W.D. Moore. This pathogen is also known in Arx's key (1970) as *C. dematium* (Pers. ex Fr.) Grove var. *truncata*. In addition, Weidemann *et al.* (1988) report that the species *C. pisi* should be considered synonymous with *C. truncatum*.

In Manitoba, lentil plants infected by *C. truncatum* develop lesions on stems, leaves, petioles and pods. On foliage, lesions appear first as water soaked spots which become sunken, circular, tan coloured and necrotic causing a marked increase in leaflet abscission. Stem infections appear as diamond shaped, tan coloured, necrotic lesions which may cause girdling and tissue collapse, resulting in the death of distal portions of the plant. Lesions first appear at the base of the plant and new lesions develop as the conidia progress upward by splashing rain. Rapid and severe disease development by *Colletotrichum* species is favoured by relatively high temperatures and precipitation (Sinclair 1982). High yield losses in lentil have been reported when symptoms on stems, foliage and pods are severe (Morrall and Pedersen 1991, Gibson *et al.* 1991). *Colletotrichum truncatum* is reported to be seed-borne in soybean (*Glycine max*) and other legumes (Roy 1982,

Hepperly et. al 1983, Kunwar *et al.* 1985). However, the extent of seed infection by *C. truncatum* in lentil and the role of infected seed as a source of inoculum are not known.

Because all of the present lentil cultivars are susceptible to *C. truncatum* and crop rotations do not appear to be very effective in reducing disease incidence, the present study was initiated to: 1) determine the effectiveness of the fungicides chlorothalonil and propiconazole in reducing disease severity and yield losses in lentil crops, 2) establish the magnitude of yield losses and 3) determine the occurrence of seed infection and its role as a potential source of inoculum. Chlorothalonil and propiconazole were selected for this study because both have been reported to have some activity against *Colletotrichum* species (Thomson 1988, Sinclair 1980) and because both are registered for use on other crops in Canada. The latter should facilitate registration of either product should tests prove them to be effective. The ability of both fungicides to control anthracnose before and after infection was also investigated in the greenhouse.

3.2 Materials and Methods

3.2.1 Field evaluation The experimental sites were located in regions with a long history of lentil production. The chlorothalonil trials were located at Rosenort (approximately 50 kilometers south of Winnipeg) and Portage La Prairie, Manitoba in 1989, 1990 and 1991 and also at the University of Manitoba Campus Farm in 1991. The propiconazole trials were located at Bagot (approximately 10 kilometers west of Portage La Prairie) in 1990 and at Rosenort and the Campus Farm in 1991. Field trials were sown with a small self-propelled seeder during the last week in May into lentil stubble naturally infested with anthracnose, with the exception of the Campus Farm site where anthracnose infested stubble was introduced. Seed of the lentil cultivar 'Eston' was sown at a rate of 40 seeds/m with a row width of 30 cm. The treatments were replicated 6 times in a randomized complete block design.

Rhizobium inoculum (*Rhizobium leguminosarum*, Nitragin Company, Milwaukee Wisc. U.S.A.) was added in the furrow at approximately 1.0 g/m. Lentil plots were 4.9 m x 2.4 m and separated from adjacent lentil plots in a block by a 4.9 m x 1.2 m plot of the faba bean cultivar 'Aladin' to reduce drifting during fungicide applications. Replicates were separated from each other by 1 m walkways. The Campus Farm plots were identical with the exception that they were 3.0 m rather than 4.9 m long. Weeding was done by hand in 1989 and 1990 while a herbicide treatment (Sencor 500F, Chemagro Ltd.) was used when necessary in 1991.

Chlorothalonil (Bravo 720, ISK Biotech, Painesville, Ohio) was applied as follows: one application at flowering of either 1.2 or 2 kg a.i./ha, two applications (one at flowering and one 8-9 days later) of 1.2 or 2 kg a.i./ha; or three applications (one at flowering, one 8-9 and one 17-19 days later) of 1.2 kg a.i./ha.

Propiconazole (Tilt 250E, Ciba-Geigy Ltd. Mississauga, Ontario) was applied as follows: one application at flowering of either 200 g a.i./ha, 125 g a.i./ha or 75 g a.i./ha; two applications (one at flowering and one 14 days later) of either 125 g a.i./ha or 75 g a.i./ha, three applications (once at flowering, one 14 and one 28 days later) of 75 g a.i./ha.

To assess the impact of anthracnose on yield, a non-sprayed check and a 'positive check' was included in both the chlorothalonil and propiconazole plot designs. The positive check was treated with chlorothalonil at 2 kg a.i./ha every 8-9 days, starting 2 weeks after emergence until the end of the season.

Plants from all fungicide field trials were harvested when the lower pods were light brown and the seeds were loose in the pods. Lentil plants were hand harvested on August 1, 1989 and August 12-14, 1990 from the six inner rows, placed into burlap bags and air dried at the Campus Farm until the pods shattered easily. A stationary

thresher (Vogel) was used to collect and clean the seed. In 1991 a plot combine was used to harvest and thresh seeds simultaneously from the 6 inner rows August 20th. Plot yields were determined by weighing harvested seed at a moisture content of approximately 14%.

Heavy spring rains in 1990 forced the abandonment of the Rosenort and Bagot chlorothalonil trials due to extensive flooding. To avoid losing a season of data, new plots were established in moderately diseased commercial Eston lentil fields at the mid-flowering stage at Rosenort and Bagot. Plots were 3.9 m x 2.4 m and arranged into a randomized complete block design consisting of four replicates of 6 treatments. Each treatment within a replicate was separated from the others by a 1.2 m strip of lentil to reduce the effects of fungicide drift. The chlorothalonil treatments were applied at the rates stated previously and the inner 1.5 m x 3 m of each plot was harvested.

3.2.2 Preparation of Artificial Inoculum The inoculum was prepared by growing isolate ALRNLN88 of *C. truncatum* (obtained from lentil near Rosenort in 1988) on potato dextrose agar (PDA) plates under 24-hour fluorescent illumination for 7-10 days. A small amount of sterile distilled water was added to the growing thallus in each plate to release and spread large numbers of conidia across the entire surface. The plates were then re-incubated for a minimum of 3 days until prolific acervulus development was observed. The conidia were collected after flooding each plate with sterile water and scraping the acervuli with a microscope slide. The conidial suspension was filtered through a folded coarse cloth to remove mycelial fragments and then agitated in a blender. A haemocytometer and sterile distilled water were used to obtain a working concentration of $5.5x10^5$ conidia/ml.

Inoculum was introduced into each of the Campus Farm plots June 15, 1991 in three ways to ensure effective inoculation; 1) by adding naturally infected lentil

stubble, 2) by adding dried infected lentil and faba bean plants artificially inoculated in the greenhouse, and 3) by inoculating the faba bean guard rows in the evening with a suspension of $5x10^5$ conidia/ml of *C. truncatum*, using a hand held sprinkler.

3.2.3 Plating Assay for Anthracnose Seed Infection Four hundred randomly collected lentil seeds from each chlorothalonil treatment in 1989 and 1990 were surface sterilized in 0.6% NaOCl for 10 minutes (Gossen & Morrall 1983), and removed without rinsing (Sauer & Burroughs 1986), placed on autoclaved paper towels in a laminar flow hood and plated directly onto PDA plates. The plates were incubated for a minimum of 10 days under continuous fluorescent lighting and visually rated for seed infection before the 14th day by counting the number of seeds which produced *C. truncatum* mycelium. *Colletotrichum truncatum* was identified by characteristic growth in culture and by microscopic examination of questionable thalli, when necessary. Seed infection percentages were determined by dividing the number of infected seeds detected by 400 and multiplying by 100.

Samples of lentil seed obtained from sixteen diseased commercial fields were assayed for seed-borne infection by *C. truncatum* and *Ascochyta fabae* Speg. f.sp. *lentis* Gossen *et al.*, as described above, and the percentage of seed infected by each pathogen was recorded .

3.2.4 Seed-to-Seedling Transmission in the Field Anthracnose infected lentil seeds were sown into non-replicated 4-row plots 20 m long on land that had never been sown to lentil before at the Campus Farm. In 1989 the percent seed infections determined by seed assay prior to planting were 0%, 3.4% and 6.4%. In 1991 a single non-replicated 8- row plot 30 m long was sown with lentil seed (cv. Eston) infected with *C. truncatum* (2.3%) at approximately 35 kg seed/ha. The plots were observed periodically throughout the season for disease symptoms.

3.2.5 Pre- and Post Application of Chlorothalonil and Propiconazole on Lentil Inoculated with *C. truncatum*

Eston lentil plants were sown and grown for three weeks in clay pots containing a 2:1:1 (v/v/v) mix of soil, sand and peat moss. Each fungicide treatment consisted of four replicates with four plants per replicate. The experiment was repeated three times. Fungicide treatments were applied either 24, 48 or 72 hours before or after inoculation of the plants with a conidial suspension of *C. truncatum* (1×10^5 conidial/ml). Inoculated and non-sprayed plants were included as a control. The fungicide treatments were applied to the potted plants with a backpack sprayer calibrated to apply chlorothalonil at 2.0 kg a.i./ha and propiconazole at 125 g a.i./ha. Inoculated plants were incubated in a humidity chamber at approximately 22C for 24 hours and then maintained on a greenhouse bench. Disease severity was assessed after 14 days according to the following scale; 0=no disease, 1=trace, 2=slight, 3=moderate, 4=severe, 5=plant death.

3.2.6 Method of Data Analyses Yield losses were obtained by comparing yields of treated plots to that of the 'positive check' plots where disease levels were low due to repeated fungicide application. Analysis of the data for yield, percent seed infection, and disease severity were performed using SAS (Statistical Analysis System Institute Inc. Box 8000, Cary, North Carolina).

3.3 Results

3.3.1 Foliar Sprays with Chlorothalonil Seed yields from each of the treatments at Portage in 1989 were significantly greater than the non-treated check (Table 3.0). However, seed yields of plots treated one to three times and the positive check were not significantly different from each other ($p = 0.05$). The seed yield of the untreated check at the Portage site was approximately 56% of the yield obtained for the positive check. Seed yields from untreated plots or plots treated one to many times at Rosenort were not significantly different from each other (p =

0.05). Severe weed infestation may have reduced the yield potential of the sprayed plots. However, disease severity was reduced at both the Rosenort and Portage sites in 1989 by all the fungicide treatments.

At Portage, chlorothalonil significantly reduced ($p = 0.05$) the percentage of infected seed (0%) as compared to the non-treated plots (1.42%). There were no significant differences in seed infection percentages at the Rosenort site. Infected seeds were symptomless and were not distinguishable from uninfected seeds prior to plating in the seed assay.

Seed yields from chlorothalonil treatments in 1990 (Table 3.1) were not significantly different from each other or the non-treated plots ($p=0.05$) at either the Portage site (original, seeded plots) or at Rosenort and Bagot (replacement plots). At Rosenort and Bagot, there were also no significant differences ($p = 0.05$) in the level of seed infection among treatments (Table 3.1). Infection assays were not performed on seed from the Portage site due to loss of stored seed to rodents.

Seed yields for the positive check and the single 2.0 kg a.i./ha application of chlorothalonil at Rosenort in 1991 were significantly higher ($p = 0.05$) than in the non-treated check (Table 3.2) but not significantly different from the remaining fungicide treatments. At the Campus Farm, none of the chlorothalonil treatments significantly increased seed yield over the non-treated check ($p = 0.05$) with the exception of the positive check.

3.3.2 Foliar Sprays with Propiconazole Seed yields and disease severity in plots treated with propiconazole at Bagot in 1990 or Rosenort 1991 (Table 3.3), whether applied one or several times at 75, 125 or 200g a.i./ha, were not significantly different from each other ($p = 0.05$). At the Campus Farm in 1991, only the single application of propiconazole at 200g a.i./ha increased seed yield over that of the non-treated plots. The treatments had very little effect on disease severity.

3.3.3 Effectiveness of Chlorothalonil and Propiconazole Applied to Lentil Pre- and Post- Inoculation with *C. truncatum* Lentil plants inoculated with conidia of *C. truncatum* in the growth chamber remained symptomless when chlorothalonil at 2.0 kg a.i./ha or propiconazole at 125 g a.i./ha was applied either 72, 48, 24 or 0 hours before inoculation (Table 3.4). In contrast, lentil plants sprayed with 2.0 kg a.i./ha of chlorothalonil at 24, 48 and 72 hours after inoculation developed moderate to severe symptoms (Table 3.4). Plants sprayed with propiconazole at 125g a.i./ha at 24, 48 and 72 hours after inoculation developed trace, slight and moderate levels of disease, respectively. Inoculated control plants developed severe symptoms in all trials.

3.3.4 Seed to Seedling Transmission 1989,1991 No symptoms of anthracnose were detected in 1989 at the Campus Farm in plots sown with seeds containing 0%, 3.2% and 6.4% anthracnose-infected seeds. In 1991, the plot sown with 3.2% anthracnose-infected seed remained free of symptoms until late in the season when slight to moderate levels of infection developed along one edge of the plot. The symptoms were present in an area adjacent to a windbreak of wheat which separated the test plot from the anthracnose-inoculated fungicide test at the Campus Farm. The presence of disease symptoms may have been due to the dispersal of infected and abscised leaflets from the adjacent inoculated site.

3.3.5 Percent Seed Infection in Commercial Seed Lots 1990 Five of 16 seed samples assayed had detectable levels of *C. truncatum*. The levels of anthracnose infection were 2.25%, 1.75%, 1.50%, 0.25% and 0.25%. *Ascochyta fabae f.sp. lentis* was present in 9 of the 16 samples assayed, with the highest percent seed infection being 22%, followed by 13.5%, 6.25%, 1.5%, 1.5%, 1.25%, 1.25%, 0.5% and three samples at 0.25% each.

3.4 Discussion

Lentil production in Manitoba is possible because of adequate soil moisture and number of temperature degree days in the growing season (McKenzie and Hill 1989). However, prolonged periods of high humidity and temperature can occur which favour the development of anthracnose of soybean (Hepperly 1985). In the present study two fungicides were selected for the control of anthracnose of lentil. Chlorothalonil is an alkylating agent which reacts with cellular thiols and prevents conidial germination. It is resistant to ultraviolet photodegradation and has a relatively long residual activity but requires repeated application to protect new plant growth (Thomson 1988). Effective control of *C. truncatum* (Backman 1985) on soybean by chlorothalonil was reported to be related to the retention of the fungicide on the plant surface. Propiconazole is a systemic fungicide which is absorbed by leaves and stems and is transported acropetally into areas of new growth (Agriculture Canada 1987). Propiconazole belongs to a class of demethylation inhibitor (DMI) fungicides that disrupt normal metabolic function by preventing ergosterol synthesis in higher fungi (Koller 1992).

At the Portage site in 1989, precipitation late in the summer may have favoured the development of disease, resulting in significantly greater yield in the chlorothalonil-sprayed plots than the non-treated plots. However, the Rosenort site experienced

dryer conditions near the end of the season, possibly preventing the realization of full yield potential from the chlorothalonil-sprayed plots.

Disease severity was high in 1990. However, dry conditions prevailed at Portage, Rosenort and Bagot and there were no significant differences between chlorothalonil-treated and non-treated plots at the three sites. In 1991 the positive check and the 2.0 kg a.i./ha treatment at the Rosenort site were significantly different from the non-treated checks, but not from the remaining fungicide treatments.

Propiconazole at 200 g a.i./ha was effective in improving seed yield over the untreated check at the Campus Farm in 1991. The dry conditions late in the summer of 1990 and the flooding damage at Rosenort in 1991 may have prevented the yield potential of treated plots from being realized. Although propiconazole reduced disease severity in greenhouse tests the formulation may not provide adequate protection of lentil in the field and further refinement of timing and application rates is warranted.

Manandhar *et al.* (1985) studied conidial germination and appressorium formation in soybean inoculated with *C. truncatum* and showed that conidia germinated within 4 hours on the host tissue. After the formation of appressoria, hyphae were observed between the cuticle and epidermis, followed by mesophyll penetration within 48 hours of inoculation. In the present greenhouse study, the application of chlorothalonil, 24 or 48 hours after the inoculation of lentil plants with *C. truncatum* conidia, had minimal effect on the development of primary infection sites, probably due to the physical separation of the fungicide from the fungus after the formation of appressoria and penetration of the epidermal cells (Sisler 1986, Manandhar *et al.* 1985). Foliar sprays of propiconazole 24 and 48 hours after inoculation reduced

disease severity over that of non-protected plants indicating some eradicant activity in lentil even after fungal penetration.

Soybean seed is generally infected during and after physiological maturity when local lesions on the outer surface of the pod can lead to seed infection (Hepperly 1985). Sinclair (1991) reports that *C. truncatum* within infected soybean seed is generally latent and asymptomatic but Schneider *et al.* (1974) observed fruiting structures on harvested seed. The fungus is found primarily in the three layers of the soybean seed coat (Kunwar *et al.* 1985) and rarely within the cotyledonary tissue. The incidence of anthracnose-infected soybean seed is increased when the crop is grown in humid tropical or sub-tropical environments or harvested under wet conditions (Hepperly 1985, Schneider *et al.* 1974, Rodriguez-Marcano and Sinclair 1978). In the present study, *C. truncatum* infection of lentil seed was also symptomless and the amount of anthracnose-infected seed was detected only by a seed plating assay. Assays of 16 commercial seed samples in 1990 showed less than 2.25% anthracnose infected seed. In addition, assays conducted on seed samples from Saskatchewan in 1990 indicated that levels of seed infection were less than 5% (Morrall and Pedersen 1991) although samples taken from areas within one infected field showed levels as high as 30% (R.A.A. Morrall, personal communication). Seed infection levels were less than 0.25% from chlorothalonil trials in 1989 and 1990. Beauchamp *et al.* (1986a) report that lentil seed infection by *A. fabae* f. sp. *lentis* is also reduced with increasing numbers of chlorothalonil applications.

There have been no published studies on the frequency of transmission of *C. truncatum* from lentil seed to seedlings in the field. Seed-to-seedling transmission was investigated to determine if infected seed was capable of introducing anthracnose in fields which had never been sown to lentil. No evidence of seed-to-seedling transmission was observed during a two year study at the isolated Campus

Farm using infected seed containing 6.4% and 3.4% anthracnose in 1990, and 2.3% anthracnose in 1991. Anthracnose does not appear to be highly seed borne, but environmental factors appear to influence the incidence of seed infection and may influence the potential for seed to seedling transmission.

In general, chlorothalonil had non-significant effects on seed yield during dry weather but increased seed yield when conditions were favourable for disease progression. Early and frequent applications of the fungicide reduced disease severity when disease pressure was high. Propiconazole reduced disease severity in the greenhouse, although field trials were not conclusive, perhaps due to the dry conditions during the study. The role of seed-borne inoculum and seed-to-seedling transmission should be studied further to determine the epidemiological consequences of introducing anthracnose-infected seed into uninfected areas.

Table 3.0 Effect of Chlorothalonil on Seed Yield, % Seed Infection and Disease Severity of Eston Lentil in 1989

	Portage[1]			Rosenort		
Treatment	Yield (g/plot)	% Seed Infected	Disease Severity	Yield (g/plot)	% Seed Infected	Disease Severity
Check (No Fungicide)	755 b	1.4 b	3.0 a	655 a	0.7 a	3.6 a
2.0 kg a.i.ha/ one application	1250 a	0.0 a	1.7 b	765 a	0.0 a	2.1 b
2.0 kg a.i. ha/ two applications	1210 a	0.0 a	1.8 b	595 a	0.0 a	2.3 b
2.0 kg a.i. ha/ three applications	1255 a	0.0 a	2.0 b	--[3]	--	--
Positive Check	1340 a	0.0 a	1.5 b	770 a	0.0 a	1.9 b

1. Portage and Rosenort plots sown on infected lentil stubble
2. Disease severity ratings; 0 = No disease, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, 5 = plant death
3. Final fungicide application not applied, Plots harvested August 1/1989

Values in a column followed by the same letter are not significantly different from each other according to Tukey's studentized range test (p = 0.05)

Table 3.1 Effect of Chlorothalonil on Seed Yield and % Seed Infection of Eston Lentil in 1990

Treatment	Portage[1]			Rosenort[2]			Bagot[2]		
	Yield (g/plot)	% Infected Seed	Disease Severity	Yield (g/plot)	% Infected Seed	Disease Severity	Yield (g/plot)	% Infected Seed	Disease Severity
Check (No fungicide)	900 a	nt[3]	3.1 a[4]	2300 a	0.06 a	3.0 a	1220 a	0.13 a	3.5 a
1.2 kg a.i./ha one application	1080 a	nt	2.8 a	2440 a	0.18 a	3.0 a	1090 a	0.06 a	3.3 a
1.2 kg a.i./ha two applications	1030 a	nt	2.5 a	2160 a	0.00 a	3.0 a	1470 a	0.00 a	3.3 a
1.2 kg a.i./ha three applications	850 a	nt	2.5 a	2370 a	0.00 a	3.0 a	---	---	---
2.0 kg a.i./ha one application	940 a	nt	2.4 a	2280 a	0.06 a	3.0 a	1530 a	0.06 a	3.5 a
2.0 kg a.i./ha two applications	1030 a	nt	2.7 a	2240 a	0.06 a	3.0 a	1540 a	0.25 a	3.5 a
Positive Check	960 a	nt	2.9 a	---[5]	---	---	---	---	---

1. Portage plots sown on infected lentil stubble
2. Plots were established on naturally infected commercial lentils fields at early flowering
3. Not tested due to loss of stored seed to rodents
4. Disease severity ratings; 0 = No disease, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, 5 = plant death
5. Treatments not included

Values in a column followed by the same letter are not significantly different from each other according to Tukey's studentized range test (p = 0.05)

Table 3.2 Effect of Chlorothalonil on Seed Yield and Disease Severity of Eston Lentil in 1991

Treatment	Rosenort[1]		Campus Farm[2]	
	Yield (g/plot)	Disease Severity	Yield (g/plot)	Disease Severity
Check (No fungicide)	170 b	2.8[3] a	470 b	3.8 a
1.2 kg a.i./ha one application	480 ab	2.8 a	550 ab	3.5 a
1.2 kg a.i./ha two applications	530 ab	2.7 a	590 ab	3.8 a
1.2 kg a.i./ha three applications	420 ab	3.0 a	540 ab	3.3 ab
2.0 kg a.i./ha one application	680 a	2.8 a	590 ab	3.3 ab
2.0 kg a.i./ha two applications	600 ab	2.8 a	470 ab	3.8 a
2.0 kg a.i./ha three applications	560 ab	3.0 a	550 ab	3.5 a
Positive Check	780 a	1.5 b	815 a	2.7 b

1. Rosenort plots sown on infected lentil stubble
2. Anthracnose infected stubble introduced. Plots were irrigated by sprinkler during dry weather
3. Disease severity ratings; 0 = No disease, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, 5 = plant death

Values in a column followed by the same letter are not significantly different from each other according to Tukey's studentized range test (p = 0.05)

Table 3.3 Effect of Propiconazole on Seed Yield and Disease Severity of Eston Lentil in 1990 and 1991

Treatment	Bagot 1990		Rosenort 1991		Campus Farm 1991[1]	
	Yield (g/plot)	Disease Severity	Yield (g/plot)	Disease Severity	Yield (g/plot)	Disease Severity
Check (No fungicide)	680 a	3.6[2]a	150 a	3.5 a	270 b	4.0 b
75 g a.i./ha one application	910 a	3.0 a	170 a	3.5 a	430 ab	3.3 ab
75 g a.i./ha two applications	780 a	3.0 a	180 a	3.5 a	310 ab	3.8 ab
75 g a.i./ha three applications	770 a	3.4 a	130 a	3.5 a	300 ab	3.8 ab
125 g a.i./ha one application	800 a	3.4 a	210 a	3.7 a	400 ab	3.5 ab
125 g a.i./ha two applications	780 a	3.0 a	210 a	3.4 a	380 ab	3.3 ab
200 g a.i./ha one application	900 a	3.4 a	190 a	3.2 a	530 a	3.0 a

1. Campus Farm site irrigated by sprinkler system during dry weather
2. Anthracnose infected stubble introduced at the Campus Farm site which was irrigated by sprinkler system during dry weather
3. Disease severity ratings; 0 = No disease, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, 5 = plant death

Values in a column followed by the same letter are not significantly different from each other according to Tukey's studentized range test (p = 0.05)

Table 3.4 Effectiveness of Pre- and Post-Inoculation Applications of Chlorothalonil and Propiconazole on Disease Severity of Lentil Plants Inoculated with Colletotrichum truncatum in the Greenhouse

	Mean Disease Severity Rating[1]					
	Fungicide applied before inoculation with C. truncatum[2]			Fungicide applied after inoculation with C. truncatum		
Treatment	72 hours	48 Hours	24 Hours	24 Hours	48 Hours	72 Hours
Chlorothalonil (2.0 kg a.i./ha)	0.0	0.0	0.0	4.2	3.3	3.7
Propiconazole (125 g a.i./ha)	0.0	0.0	0.0	0.7	1.8	3.0
Check (No fungicide)	4.3	4.3	4.0	4.3	4.0	4.0

1. Disease severity ratings; 0 = No disease, 1 = trace, 2 = slight, 3 = moderate, 4 = severe, 5 = plant death
2. Inoculum applied at 10^5 conidia/ml until runoff and incubated in a humidity chamber for 24 hours, values in the table are the means from 3 experiments with 4 replicates each.

Reaction of Lentil Cultivars, Landraces, Breeding Lines and other Cultivated and Native Legumes to Anthracnose caused by *Colletotrihum truncatum*

4.0 Abstract

A three-year field study was conducted to determine the reaction of lentil cultivars, landraces and breeding lines to anthracnose of lentil caused by *Colletotrichum truncatum*. Field plots were established on lentil stubble naturally infected with anthracnose. In each year of testing, the landraces Common Chilean and Spanish Brown were the most susceptible. The cultivar Laird and Laird-cross La x17310-8 had significantly lower disease severity ratings than the cultivar Eston or the landrace French Green. The cultivar Indianhead had the lowest disease severity ratings overall but has small black seeds and is utilized primarily as a green manure crop. Two *C. truncatum* isolates were recovered from naturally infected lentil and used to inoculate potential host plants in the greenhouse. Three cultivars and three landraces of lentil, five cultivars of faba bean (*Vicia faba*), nine cultivars of field pea (*Pisum sativum*), two Lathyrus species (*L. sativa, L. tingitanus*) and one species of yellow vetch (*Vicia sativa*) were susceptible to *C. truncatum*. Three soybean (*Glycine max*) cultivars, two field bean (*Phaseolus vulgaris*) cultivars, one alfalfa cultivar (*Medicago sativa*) and one species each of blue lupin (*Lupinus angustifolius*), round-leaved mallow (*Malva pusilla*), and chickpea (*Cicer arietinum*) were symptomless after inoculation and considered resistant to *C. truncatum*.

4.1 Introduction

Anthracnose caused by *C. truncatum* (Schw.) Andrus and Moore is well established in the major lentil (*Lens culinaris* Medik.) growing areas of Manitoba and Saskatchewan where the crop has been intensively cultivated since the early 1970's. Currently, the two commercially grown cultivars Eston and Laird are susceptible to anthracnose. Yield losses due to the disease have been reported to be 60% or greater when conditions are favourable for disease development (Morrall

et al. 1990). Field surveys in Manitoba and Saskatchewan (Morrall 1988, Morrall and Pedersen 1991, Morrall et al. 1990, 1991 and Gibson et al. 1991) have shown that anthracnose may be as severe in fields where legume crops were grown in rotations of three to four years as in fields where lentil crops were grown the previous year.

The host range of *C. truncatum* is comprised of several genera of the Leguminosae including Lens, Vicia and Pisum (Weidemann, TeBeest and Cartwright 1988). Although *C. truncatum* has been isolated from faba bean (Vicia faba) growing adjacent to infected lentil and from a commercial faba bean crop (Gibson et al. 1991), it has not been successfully isolated from other commercial legume crops in Manitoba and the role of pea (Pisum sativum), bean (Phaseolus vulgaris), soybean (Glycine max (L.) Merr.) and native legumes as potential sources of inoculum is not known.

The objectives of this study were to evaluate a number of lentil cultivars, breeding lines and landraces for their reaction to anthracnose in the field and to establish whether other cultivated or native legumes are hosts of *C. truncatum*. In the latter study, which was done in the greenhouse, the degree of susceptibility of infected plants was also determined and reactions obtained indoors were compared with those in field tests.

4.2 Materials and Methods

4.2.1 Evaluation of lentil cultivars and breeding lines in the field A number of lentil genotpes including the registered cultivars Eston, Laird, and Rose, and breeding lines and landraces provided by Prof. R.A.A. Morrall (University of Saskatchewan) were evaluated for their reaction to *C. truncatum*. The cultivars and lines were part of a yearly lentil evaluation trial whereby some lines were removed each year based on poor agronomic performance, and new lines added. Test plots

were established at Portage La Prairie in 1989 and 1990 on stubble naturally infected with anthracnose. An additional test site was established at the Campus Farm, University of Manitoba in 1991 on land which had never been sown to lentil. To provide inoculum, stubble from a lentil crop infected with anthracnose was spread between the rows two weeks after seedling emergence.

Plots were sown and maintained as described previously (Section 3). At Portage La Prairie plots consisted of four rows 4.9m long, 30cm apart replicated six times. The plots at the Campus Farm site consisted of 4 rows 2.4m long, 30cm apart replicated four times.

The plants were rated for disease severity after flowering (last week in July) each year on a scale of 1-5 where 0=no disease and 5=extensive plant death (Table 4.0). Analysis of the disease severity data was performed using the Statistical Analysis System (SAS Institute Inc. Box 8000 Cary, North Carolina). The means of the disease severity values were compared using Duncan's multiple range test at the $p = 0.05$ level of significance.

4.2.2 Host Range of *C. truncatum* in the Greenhouse The lentil cultivars Eston, Laird, Indianhead, and the unregistered landraces Chilean, Spanish Brown, and French Green were selected for study. In addition, five cultivars of faba bean (Ackerperle, Herz Freya, Orion, Pegasus, Aladin), nine cultivars of pea (Tara, Fortune, Tipu, Titan, Victoria, Century, Princess, Express, Triumph), three cultivars of soybean (McCall, Maple Amber, Maple Ridge) and two cultivars of field bean (Loop, Seafarer) were selected. Additional species tested included; grass pea (*Lathyrus sativa*), tangier pea (*Lathyrus tingitanus*), yellow vetch (*Vicia sativa*), blue lupin (*Lupinus angustifolius*), Alfalfa cv. Rambler (*Medicago sativa*) and chickpea (*Cicer arietinum*) breeding line P502. Round-leaved mallow (*Malva pusilla*), a common weed of lentil crops, was also selected for testing.

Ten seeds of each selection were sown into each of six 15cm clay pots containing a 2:1:1 (v/v/v) mix of soil, sand and peat moss. Seedlings were thinned to five per pot and placed in the greenhouse and watered as required until two weeks old at which time four pots were inoculated with a conidial suspension of *C. truncatum* and two were sprayed with water as controls. The isolates JPRNLN88 (obtained from an infected lentil plant in the Portage La Prairie region) and ALRNLN88 (obtained from an infected lentil plant at Rosenort) used in this study were identified as *C. truncatum* on the basis of conidial and cultural morphology. The isolate ALRNLN88 was confirmed by the Biosystematics Research Institute, Ottawa, Canada as *C. truncatum* (Schw.) Andrus and W.D. Moore. A conidial suspension was prepared by growing each isolate on potato dextrose agar plates as described in Section 3. Conidial concentrations were adjusted to $1.5x10^6$, $5x10^5$ and $1.5x10^5$ conidia/ml using a haemocytometer and each was applied to plants with an air brush until run-off. The experiment was repeated twice with each isolate.

In preliminary experiments conidial concentrations of $1.5x10^6$ and $5.5x10^5$ conidia/ml were found to cause severe to lethal symptoms on all the lentil, faba bean, vetch, lathyrus and pea cultivars tested. However, at $1.5x10^5$ conidia/ml, differential symptom development between species and cultivars was obtained. Thus, all the cultivars in this study were evaluated with each isolate at $1.5x10^5$ conidia/ml.

Inoculated and non-inoculated pots were placed in a humidity chamber for 24 hours to maintain leaf wetness, after which they were returned to the greenhouse bench and arranged in a completely randomized design. Plants were assessed 7 and 14 days after inoculation for resistance or susceptibility by the presence or absence of lesions. The degree of susceptibility was determined by estimating the percent area of leaf and stems affected by lesions as follows; 0=no disease, 1=1-10%, 2=11-25%, 3=26-50%, 4=51-75%, 5=76-100%. Lesion morphology was noted.

To determine whether the observed symptoms were caused by *C. truncatum* several infected plants from each susceptible host were excised at the base of the stem, washed under running tap water and incubated in a humidity chamber for several days under fluorescent light. Leaves and stems were observed under the binocular microscope for the presence of acervuli and typical setae of *C. truncatum.* Diseased tissue was excised from selected lesions and observed under the light microscope for the presence of conidia typical of *C. truncatum.*

4.3 Results

4.3.1 Evaluation of Lentil Cultivars, Breeding Lines and Landraces in the Field

Anthracnose was prevalent each year in all plots. However, disease severity was greater in 1991 than in the previous two years due to above average precipitation which favoured secondary infection and rapid disease progression. The high precipitation during the study may have promoted the development of root rot at the Campus Farm site which complicated the disease ratings.

Among the selections evaluated in each of the three years (Table 4.1), mean disease severity values of 2.5 for Laird and line La x17310-8 were significantly lower than that for French Green and Eston (Disease severity values 2.9-3.0) but not from that for line 179310 x La25 (disease severity value 2.7). The landraces Chilean and Spanish Brown were the most susceptible (disease severity values 3.3 - 4.8) and the cultivar Indianhead (disease severity values 1.0-1.8) the least susceptible to anthracnose.

4.3.2 Host Range of *C. truncatum* in the Greenhouse All selections of lentil (6), of field pea (9), of faba bean (5), of vetch (1) and of Lathyrus species (2) were susceptible to *C. truncatum* when inoculated with either isolate JPRNLN88 or ALRNLN88 (Table 4.2). All cultivars of soybean (3), of field bean (2) and the single

genotypes of blue lupin, alfalfa, chickpea and round-leaved mallow had incompatible reactions and were resistant. All non-inoculated control plants from each cultivar or line were symptomless after 14 days.

The amount of infected leaf area for the lentil genotypes ranged from less than 10% for Indianhead, 11-25% for Laird, 26-50% for Eston and French Green and 76-100% for both Chilean and Spanish Brown. Typical lesions on leaves were sunken, tan coloured, necrotic, and spreading. Stem lesions became oval shaped, tan coloured and necrotic and ranged in depth from superficial to invasive, often resulting in stem collapse. Leaflet abscission, coalescing stem lesions and stem collapse characterized the more severe lentil reactions.

All pea cultivars were susceptible and when inoculated developed well defined, tan-coloured necrotic lesions on stems and leaves which remained superficial, restricted and non-spreading. Affected leaf and stem areas ranged from 26-50% for Tara, Fortune and Tipu to 51-75% for Titan, Victoria, Century, Princess, Express, and Triumph. However, pea plants which developed leaf and stem lesions covering as much as 51-75% of the plants continued to grow with little or no apparent detrimental effect.

The faba bean cultivars Ackerperle, Herz Freya, Orion, Pegasus and Aladin were all susceptible to *C. truncatum*. Anthracnose lesions covered approximately 51-75% of the leaves and became grey and sunken with raised rims surrounded by necrotic regions. Infected leaves often developed extensive necrosis leading to leaf collapse and abscission. Stem lesions became invasive and necrotic with diamond-shaped areas in both leaf axils and internode regions. Plant death often occurred due to stem collapse at lesion sites. Grass pea, tangier pea and yellow vetch were highly susceptible to *C. truncatum* with plant death usually occurring within 7 days after inoculation.

The soybean cultivars, McCall, Maple Amber and Maple Ridge and the field bean cultivars, Loop and Seafarer, were all resistant to *C. truncatum* and remained symptomless. Additional genotypes tested and found to be resistant included blue lupin, round-leaved mallow, alfalfa (cv. Rambler) and Chickpea. To establish whether *C. truncatum* was present or not in the symptomless tissue, several plants from each species were washed under running tap water and leaves and stems placed in a humidity chamber for several days under fluorescent light. No evidence of fungal growth or sporulation typical of *C. truncatum* was observed.

4.4 Discussion

The present evaluation of lentil genotypes indicates that none of the lentil breeding lines, cultivars or landraces currently grown in western Canada are resistant to *C. truncatum*. The Indianhead lentil had the lowest disease severity of all genotypes tested over the three year study. However, Indianhead is used as a green manure crop which does not have a high value. The registered cultivar Laird, and the Laird cross La x179310-8, had significantly lower disease severity than either the Persian-type cultivar Eston, or the speckled French Green landrace. Although the Laird cultivar appears to have a higher tolerance for anthracnose than Eston, the Eston lentil is shorter in plant height, earlier maturing, and slightly higher yielding than Laird and is better suited to Manitoba's climate.

Lentil plants are primarily self-pollinated and the cultivars, breeding lines and landraces are composed largely of homozygous genotypes (Solh et al. 1984). The results from both the field and greenhouse studies indicate that the rankings of the various cultivars, breeding-lines and landraces to infection by *C. truncatum* are relatively unaffected by changes in environment. These results suggests narrow genetic diversity with respect to resistance to anthracnose (Iqbal et al. 1990).

The host range study indicates that field pea cultivars registered for use in Manitoba are susceptible but appear to be tolerant to *C. truncatum* in the greenhouse. Field surveys (Gibson et al. 1991, Zimmer and Platford 1991) conducted in 1990 identified lesions which were symptomatic of *C. truncatum* in several commercial crops and research plots of field pea. *Colletotrichum truncatum* was isolated (Gibson et al. 1991) from field pea grown adjacent to infected lentil test plots but attempts at isolation of *C. truncatum* from commercial field pea crops were not successful. Anthracnose has not been recognized as a disease problem on pea and it appears that pea may be only slightly susceptible or tolerant. The extent to which infected field pea serves as a source of inoculum for anthracnose of lentil is unclear, but would appear to be limited based on this study. However, because lentil production in Manitoba is located in areas with a long history of both lentil and field pea cultivation the ways whereby inoculum is dispersed are not fully understood.

The host range of *C. truncatum* as reported by Sinclair (1982) and Weidemann et al. (1988) includes pathogenesis on soybean and severe symptoms on pea. These reactions were not induced on either host in this study with the isolates obtained from lentil. Thus, it would appear that the lentil isolates either belong to a separate species or are a pathotype of *C. truncatum*. A greater number of isolates need to be tested to identify subspecific taxa within the genus *Colletotrichum* which may vary in pathogenicity and host range.

Table 4.0 Scale for Rating the Reactions of Lentil Cultivars and Breeding Lines to *Colletotrichum truncatum* in the Field

Disease Rating	Disease Intensity	Description
0	No Disease	
1	Trace	Occasional leaf and stem lesions
2	Slight	Scattered lesions on leaves, stems and occasionally on pods
3	Moderate	Lesions as #2 but with increasing frequency; includes some defoliation and shoot dieback
4	Severe	Extensive lesions, defoliation and shoot dieback
5	Lethal	Plant death

Table 4.1 Reaction of Lentil Cultivars and Breeding Lines to Infection by *C. truncatum* in field tests from 1989-1992

	Disease Severity			
Lentil Genotype	1989[1]	1990	1991	Mean 1989-1991
Indianhead	1.0 a[2]	1.0 a	1.8 a	1.3 a
Rose	2.2 bc	2.5 bc	-[3]	
TB406M	2.3 cd	-	-	
Laird	1.8 b	2.3 bc	3.5 bcde	2.5 b
La x 179310-8	2.2 bc	2.1 bc	3.3 bcde	2.5 b
179310 x RC16	2.5 bcd	2.8 c	-	
179310 x La25	2.2 bc	2.4 bc	3.5 bcde	2.7 bc
179310 x La24	2.5 bcd	2.9 c	-	
PR86-78	-	-	2.8 b	
French Green	2.7 bcd	2.5 bc	3.6 cde	2.9 c
89LPR-122	-	-	2.9 bc	
Eston	2.7 bcd	2.5 bc	3.8 de	3.0 c
179310 x La33	3.0 cd	-	-	
ZT-4	-	-	3.1 bcd	
179310 x La7	2.7 bcd	-	3.8 de	
VLT-15	-	-	3.5 bcde	
Chilean	3.3 de	3.8 d	4.0 e	3.7 d
La x 179310-14	-	-	4.0 de	
Spanish Brown	4.0 e	4.2 d	4.8 f	4.3 e

1. Field plots established each year on lentil stubble naturally infested with *C. truncatum*
2. Rating scale, 0=no disease, 1=trace, 2=slight, 3=moderate, 4=severe, 5=lethal Entries followed by the same letter are not significantly different according to Duncan's multiple range test (p=0.05).
3. '-' indicates not tested

Table 4.2 Host Range of *Colletotrichum truncatum* in the Greenhouse

Host		Compatibility Reaction[1]	Degree of Susceptibility[2]
Lentil (6 cvs.)	*Lens culinaris*	+	3.2[3]
Pea (9 cvs.)	*Pisum sativum*	+	3.7[4]
Faba bean (5 cvs.)	*Vicia faba*	+	4.0[5]
Soybean (3 cvs.)	*Glycine max*	-	
Field bean (2 cvs.)	*Phaseolus vulgaris*	-	
Grass pea	*Lathyrus sativa*	+	5.0[3]
Tangier pea	*Lathyrus tingitanus*	+	5.0[3]
Blue lupin	*Lupinus angustifolius*	-	
Round-leaved mallow	*Malva pusilla*	-	
Yellow vetch	*Vicia sativa*	+	5.0[3]
Alfalfa cv Rambler	*Medicago sativa*	-	
Chickpea	*Cicer arietinum*	-	

1. '+' indicates a compatible reaction
2. Degree of susceptibility based on % leaf area infected, 0=no disease, 1=1-10%, 2=11-25%, 3=26-50%, 4=51-75%, 5=76-100% Ratings are averages when more than one cultivar tested.
3. Coalescing tan coloured lesions with leaf abscission
4. Restricted non-spreading tan coloured lesions
5. Coalescing necrotic invasive lesions of stem and leaves

Effect of Temperature and Leaf Wetness Duration on the Growth and Development of *Colletotrichum truncatum*

5.0 Abstract

In greenhouse studies, disease severity on lentil increased with increasing temperature and length of leaf wetness periods. Disease severity was greatest when leaf wetness periods were 16 hours or longer. Lesions were most numerous when plants were held at 25C with an 8 hour leaf wetness period. Lesion development was inhibited indefinitely when plants were inoculated and held at 15C and resumed when temperatures were increased to at least 25C. Permissive incubation conditions of 25C for 24 hours prior to transfer to 15C resulted in severe lesions. Symptomless infection of lentil and faba bean was demonstrated in field and greenhouse studies. Evidence of infection by *C. truncatum* was detected in lentil at least 3 weeks prior to the appearance of symptoms in the field. The ability of *C. truncatum* to overwinter on lentil stubble for up to two years in a commercial field was shown using a bioassay.

5.1 Introduction

Anthracnose, a recently identified disease of lentil (*Lens culinaris Medik.*) caused by *C. truncatum* Schw. Andrus and Moore has been recognized as a potential threat to economic production of the crop in Manitoba and to a lesser extent in Saskatchewan (Morrall *et al.* 1989, Gibson *et al.* 1991). Symptoms usually appear at early flowering when the crop canopy closes in, and the disease then spreads rapidly (Morrall *et al.* 1989). The effects of the environment on infection and development of *C. truncatum* are not known. The objectives of this study were to determine the effect of temperature and duration of the leaf wetness period on infection and development of the disease (McRae and Auld 1988). Since other *Colletotrichum* species are known to cause symptomless infection (Sinclair 1991),

the ability of *C. truncatum* to remain latent and asymptomatic in lentil was also investigated indoors and in the field.

The primary source of inoculum for *C. truncatum* on lentil is believed to be anthracnose infested stubble, although the role of infected seed requires further investigation (Section 3, Morrall and Pedersen 1991). Naturally occurring infested lentil stubble has been used to successfully introduce anthracnose into lentil plots on the Campus Farm in 1991 (Section 2). Thus, the survival of *C. truncatum* on overwintered lentil stubble was investigated utilizing a bioassay to assess saprophytic growth and conidial production on crop debris.

5.2.0 Materials and Methods

5.2.1 Effects of Temperature on Growth of *C. truncatum* in Culture

Colletotrichum truncatum isolates A (RFRNVT901), B(LLRNLN901), and C(NNSJFB901) were each grown from a single conidium on potato dextrose agar (PDA) for 14 days at room temperature under continuous fluorescent light. A 3mm agar plug (containing *C. truncatum)* from each culture was transferred to fresh petri plates containing PDA. Six replicates of each isolate were then incubated in the dark at either 12.5, 18.5, 22.5, 25.5, 29.5, 31.5, 33.0 or 39.5C. The experiment was repeated once. The diameter of the mycelial growth was recorded daily for 8 days. The data were analyzed using the Statistical Analysis System (SAS Institute Inc. Box 8000 Cary, North Carolina) by ANOVA and Duncan's multiple range test at the $p = 0.05$ level of significance.

5.2.2 Effect of Temperature and Leaf Wetness Duration on Lesion Development of *C. truncatum* on lentil

Lentil seeds (cv. Eston) were sown into 15cm clay pots as described previously (Section 3) and thinned to 5 plants per pot. Plants were grown in the greenhouse

for 21 days and transferred to growth cabinets at either 15, 20, 25 or 30C for two days prior to application of treatments. Each treatment consisted of 4 pots of lentil inoculated with $1x10^5$ conidia/ml of *C. truncatum* isolate MDRNLN90 (as described previously in Section 3). Control pots were sprayed with sterile water. To provide a range of leaf wetness periods, inoculated and control pots were enclosed in polyethylene bags and returned to the growth chambers at the temperatures specified above. The polyethylene bags were removed from each treatment at 0, 8, 16 or 24 hours after inoculation. The experiment was repeated once. Plants were observed daily and disease severity based on the amount of affected leaf area was estimated after 2 weeks.

5.2.3 Effect of low temperature and leaf wetness period on lesion development by *C. truncatum* on lentil

Each treatment consisted of six 15cm pots containing 5 lentil plants prepared and placed in growth chambers at 15 or 20C (as outlined in 5.2.2) . Leaf wetness periods were maintained by enclosing the pots in polyethylene bags for 0, 8, 16 or 24 hours after which all treatments were returned to a single growth chamber at 15C. Controls consisted of a single pot of lentil plants sprayed with sterile water. Plants which were incubated at 20C and then placed into the 15C chamber will be referred to as permissive treatments.

Both inoculated and non-inoculated plants were observed for 21 days then removed from the growth chamber and inspected under a dissecting microscope for lesions characteristic of *C. truncatum*. If lesions were not observed, plants were sealed in plastic bags containing wet paper towels and placed under continuous fluorescent lighting at 20-22C for 3 days to encourage lesion development. If *C. truncatum* lesions were still not observed, the plants were cut into sections, surface

sterilized for 2 minutes in 1% sodium hypochlorite (NaOCl), placed into petri plate humidity chambers, and examined daily for *C. truncatum* lesions.

5.2.4 Assessment of Symptomless Infection in the Field

Field trials were sown May 22, 1991 at Rosenort, Manitoba on lentil stubble naturally infested with anthracnose, as described previously in section 3. Samples consisting of approximately 24 whole plants were collected on each of June 14th, June 18th, July 4th and July 11th. Each plant was examined under a dissecting microscope for characteristic lesions and fruiting structures of *C. truncatum* before being placed in humidity chambers (as described previously in section 4) and observed daily for *C. truncatum* lesions.

5.2.5 Assay of Commercial Lentil Stubble for the Presence of *C. truncatum*

Stubble samples was obtained by sampling four $1m^2$ sites from the two Rosenort, Manitoba plot sites in the spring of 1991. Both fields had been cropped to lentil which became infected with anthracnose, one in 1990, the other in 1989. The latter field was sown to a cereal crop in 1990.

The stubble samples were obtained from the top 5cm of soil outlined in the $1m^2$ sample area. The soil was screened to remove cereal straw, soil clumps and rocks. The organic material which largely consisted of lentil residue was then separated from the remaining soil by flotation in water. Plant debris was skimmed from the surface to fill a 1L beaker and immersed in an antibiotic solution for 24 hours to inhibit bacterial growth (100,000 units/L each of tetracycline and streptomycin). The antibiotic solution was decanted and the stubble incubated in a growth cabinet at 25C for 5 days. The stubble was washed with 100 ml of sterile distilled water to collect fungal conidia. The washing fluid suspension was filtered through several folds of coarse cloth to remove fine granular dirt and debris and used to inoculate test plants.

Three-week old faba bean plants (cv Aladin) and lentil plants (cv Eston) were used as indicator plants to ascertain the presence of pathogenic fungi in the suspension. Faba bean plants have the advantage of being highly susceptible to *C. truncatum* and developing large necrotic lesions. Six plants of each cultivar were inoculated with washing fluid by an air brush until run-off, placed in a humidity chamber for 24 hours, and incubated in the greenhouse for 2 weeks. Control plants were sprayed with washing fluid obtained from disease free lentil stubble grown at the Campus Farm in 1989.

The plants were examined 2 weeks after inoculation for the presence of lesions. Stem and leaf sections with suspected anthracnose lesions were excised and washed under running tap water, placed in petri plate humidity chambers and examined for characteristic setae, acervuli and conidia of *C. truncatum*. Conidia were isolated from suspected *C. truncatum* lesions and examined under the microscope or cultured on PDA for identification.

5.3 Results

5.3.1 Growth of *C. truncatum* in Culture

Mycelial growth on PDA for all three isolates combined was greatest at 22.5 C and 25.5 C (Figure 5.0). Growth at 29.5C was less than that observed for 22.5 C and 25.5C but significantly greater than the growth recorded from the remaining temperature treatments. Growth was reduced at temperatures either greater than 31.5C or lower than 18.5 C. Growth of isolate A was greater than either isolate B or C at 22.5, 25.5 or 29.5 C.

5.3.2 Effect of Temperature and Leaf Wetness Duration on Lesion Development of *C. truncatum* on lentil

Disease development was severe when plants were given leaf wetness periods greater than 8 hours and most severe when leaf wetness periods were longer than

16 hours at 20, 25 and 30 C (Figure 5.1). No symptoms were induced on lentil plants incubated at 15 C with either 0, 8, 16 or 24 hours of leaf wetness.

5.3.3 Effect of low temperatures on lesion development of *C. truncatum* on lentil

Lentil plants that were inoculated at 20 C and given a leaf wetness period of 16 or 24 hours prior to incubation at 15 C developed severe symptoms. Plants that were inoculated and given leaf wetness periods of 0, 8, 16 or 24 hours at 15 C did not develop symptoms after 14 days (Figure 5.1). However, excised and incubated stem pieces placed in humidity chambers (prepared as described in section 4) from symptomless plants which received 16 or 24 hours of leaf wetness developed acervuli and setae characteristic of *C. truncatum*. In addition, several symptomless plants which had received 16-24 hours of leaf wetness at 15 C produced discrete anthracnose lesions after the termination of the experiment when the plants were placed in a greenhouse at temperatures of 25 C or higher.

5.3.4 Detection of symptomless infection of lentil by *C. truncatum* in the field

Colletotrichum truncatum lesions were not observed on lentil at the Rosenort site during the first 2 weeks after emergence, at which time 24 intact plants were collected and verified to be symptomless when examined under a dissecting microscope. However, after several plants were incubated for several days in humidity chambers, setae, acervuli and conidia characteristic of *C. truncatum* were observed on the stems. Plants collected approximately 5 weeks after emergence, after a period of intermittent rain and high humidity had trace levels of anthracnose lesions (as described previously in section 3). Six weeks after emergence leaf and stem lesions were readily observed on many plants throughout the field and disease severity was judged to be moderate.

5.3.5 Ability of *C. truncatum* to Survive on Commercial Lentil Stubble

Lentil stubble collected in the spring of 1991 from an anthracnose infected lentil crop grown in 1990 was infectious: washing fluid from each of the four incubated stubble samples produced discrete anthracnose lesions on inoculated faba bean and lentil indicator plants. Conidia from characteristic anthracnose lesions were isolated, grown on PDA and exhibited morphological and cultural characteristics indistinguishable from known *C. truncatum* isolates from lentil. A conidial suspension was obtained from the agar plates and sprayed onto lentil plants as described in Section 4. The inoculated plants developed typical anthracnose lesions containing conidia identical to those of known isolates of *C. truncatum*.

Washing fluid from 3 of the 4 samples obtained from anthracnose infected lentil stubble which had overwintered for two years produced several discrete anthracnose lesions on inoculated faba bean and lentil indicator plants. Conidia from characteristic anthracnose lesions were isolated, grown on PDA and then used to inoculate lentil plants as described previously in section 4. The cultural characteristics and lesion morphology of the isolates recovered from the inoculated plants were indistinguishable from known *C. truncatum* isolates of lentil. The results suggest that *C. truncatum* can survive on lentil stubble for 2 years in the field and that infected lentil stem and pod fragments may be a potential source of inoculum.

5.4 Discussion

Anthracnose diseases are favoured by high temperatures and periods of high humidity and leaf wetness (Sinclair and Backman 1989). This study indicates that growth of *C. truncatum* isolates from lentil in culture is also greater at high temperatures (29.5C) than at low temperatures (18.5C), and greatest at moderate temperatures (22-25.5C).

On lentil, penetration and infection of tissues by *C. truncatum* produces lesions which may cause tissue collapse and death of the upper portions of the plant which significantly reduces yield (section 3). Manandhar *et al.* (1985) observed that damage to soybean tissue by *C. truncatum* was associated with infection peg formation on plant surfaces where free water and conidia may be retained, such as near trichomes, or in leaf axils. The extent and duration of favourable environmental factors influences conidial germination, appressorial development and infection peg penetration of the host tissues.

Lesion production on inoculated lentil test plants in the current study was found to increase with leaf wetness periods of 16 or more hours at 20C, 25C and 30C. No infection or disease developed at 15C unless the plants were inoculated and incubated first at 20C (a permissive temperature for conidial germination and penetration) for 16 or more hours before being returned to a 15C environment. Similar results were obtained by Littrell and Epps (1965) studying bean anthracnose caused by *C. lagenarium*. Parbery (1981) reports that, in general, conidial germination of most *Colletotrichum* spp. can occur between 5 and 35C and optimally at temperatures around 21-26C.

In the field, environmental conditions in southern Manitoba are generally conducive for pathogen development from early May through to harvest because adequate temperatures and leaf wetness periods can be attained. Lentil disease surveys (Morrall *et al.* 1989) in Manitoba suggest that disease severity is related to overall temperature and precipitation.

In general, anthracnose diseases caused by *Colletotrichum* spp. are initiated from small amounts of infected surface residue producing localized infected plants from which conidia are disseminated by splashing, blowing rain or physical contact. In the present study, anthracnose infected stubble produced inoculum after one and

two years in the field. The specific mechanism or structure by which *C. truncatum* overwinters in lentil stubble in Manitoba and becomes a primary source of inoculum is unknown. *Colletotrichum truncatum* of soybean may overwinter as mycelium in infected debris or in melanized acervuli as microsclerotia (Khan and Sinclair 1991). Parbery and Emmett (1977) also observed that appressoria of some *Colletotrichum* spp. may become melanized and thick-walled and serve as survival structures similar to chlamydospores or sclerotia.

Colletotrichum species are able to remain latent in many hosts once infection has taken place (Tiffany 1951, Cerkauskas 1988). At the Rosenort evaluation site lentil infection by *C. truncatum* was detected approximately 3 weeks before observation of anthracnose lesions. The cause of the latent period in the field may be related to temperature, as demonstrated in the growth chamber study (section 5.3.3). However, the mechanism involved in latency was not determined.

Latent infections by *Colletotrichum* spp. are believed to be caused by a biotrophic interaction of the fungi with their hosts whereby after appressorium formation and penetration, the infection hyphae remain latent within the upper cell layers of the epidermis (Cerkauskas 1988). Dormancy is broken with changes in environment, physiological development, wounding, membrane permeability, or release of soluble nutrients from the host which may stimulate a resumption in growth (Cerkauskas 1988).

Appressoria have also been reported to attach to plant surfaces and produce one or more infection threads which are a key feature in latency of many *Colletotrichum* spp. of fruit and possibly of leaves, stems and fruits of other plants (Muirhead 1981, Hepperly *et al.* 1980, Cerkauskas 1988). Production of hemicellulose products by the enzymatic degradation of the host cuticle may produce a partial or total

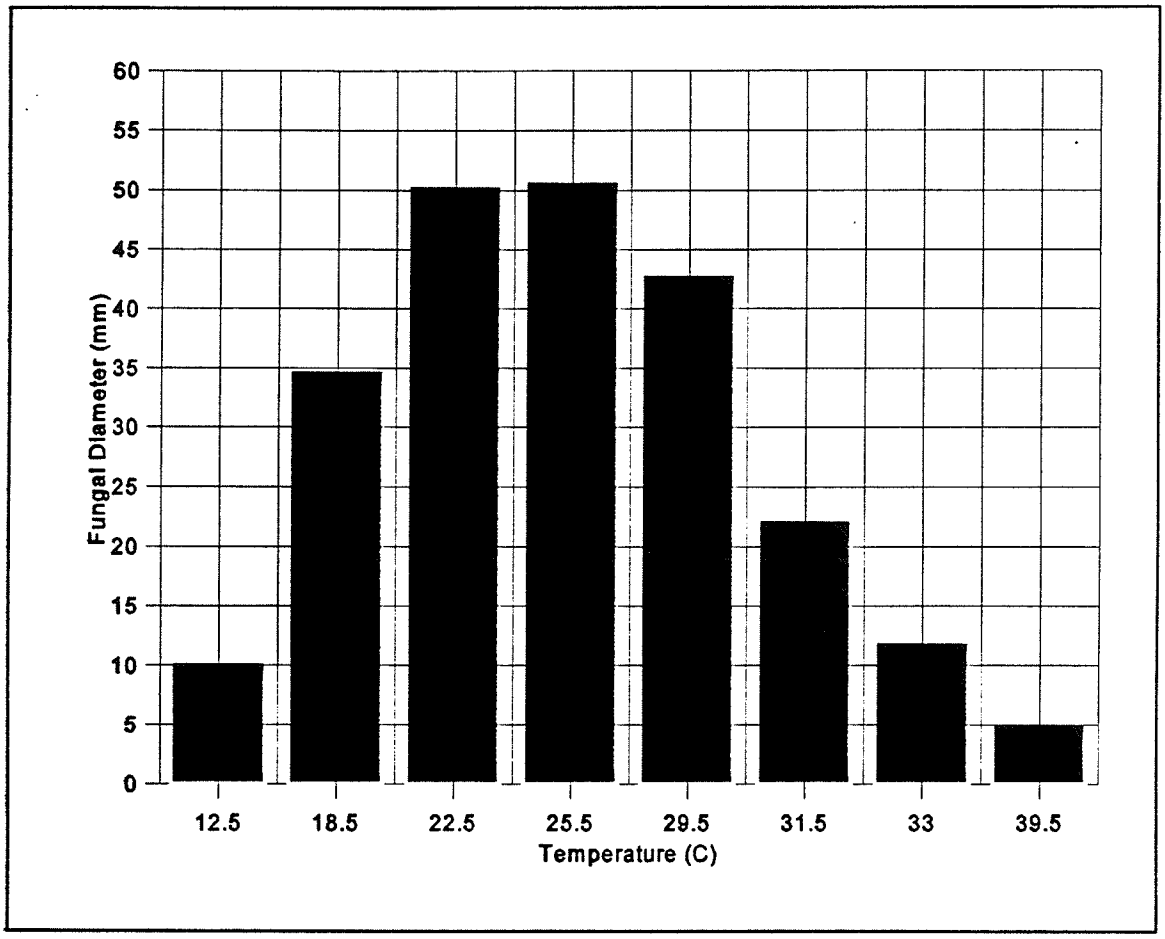
envelope of cuticular wax allowing appressoria to remain dormant for extended periods of time on the host surface (Parbery and Emmett 1977).

Anthracnose has been observed in lentil fields where crop rotations to legumes were at intervals of least four years or more, suggesting that inoculum either survives on crop residues, is introduced from infected seed (Section 3), or arrives from wind-blown infected crop debris. Infected lentil stubble produced *C. truncatum* inoculum after overwintering two years in commercial fields. However, conservation tillage practices may result in the incomplete burial of crop residues (Lipps 1988) which might facilitate pathogen survival. The amount of viable mycelium in overwintered stubble from several crops infected with *Colletotrichum* diseases may be reduced by burying crop residues after harvest (Lipps 1988, Naylor and Leonard 1977).

The dissemination of conidia in anthracnose fungi is considered to be primarily by splash dispersal. However, studies with *C. graminicola* by Nicholson and Moraes (1980) indicated that conidia could remain viable during storage at humidity levels as low as 45%. It was hypothesized that the water-soluble conidial matrix protects the viability of the conidia even during periodic desiccation and that these desiccated spore masses may be wind dispersed without loss of viability.

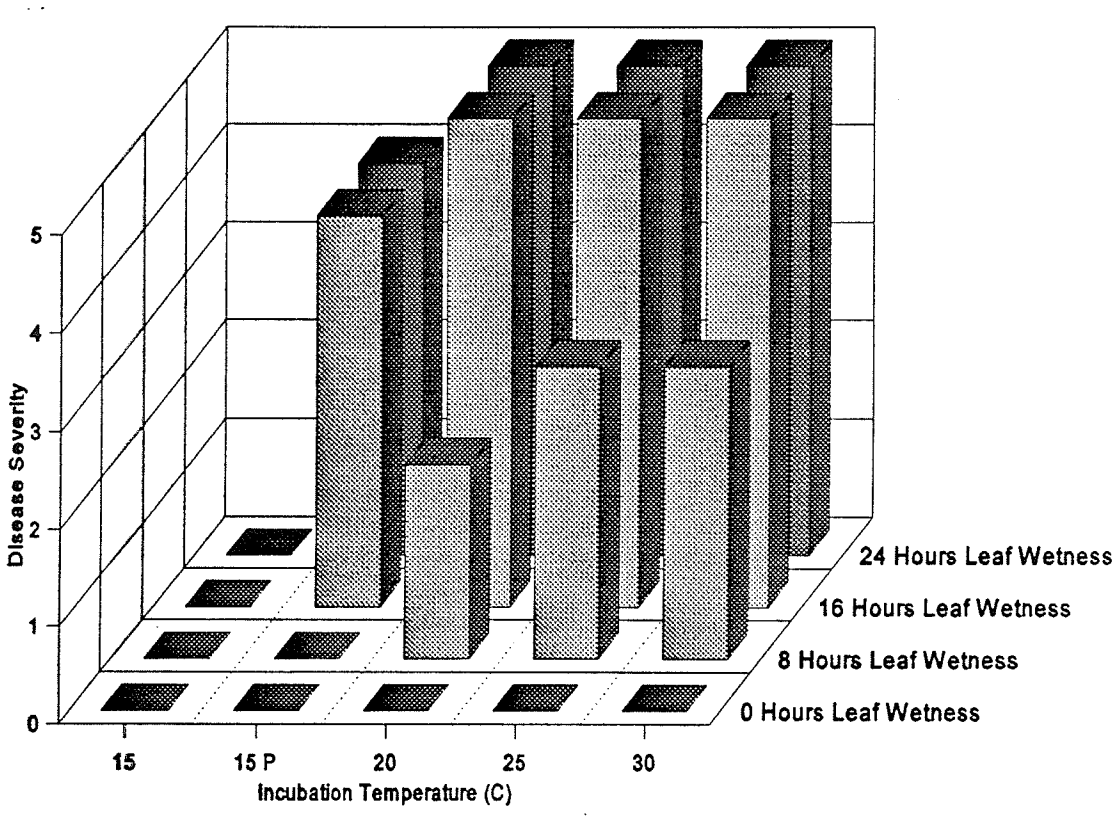
Further study into the mechanics of long distance dissemination of *C. truncatum* by infected seed, infected stubble or air-borne spores is warranted. In addition, the fungal structures and environmental factors responsible for the survival of the fungus on stubble from year to year should be determined.

Figure 5.0 Mycelial Growth of *Colletotrichum truncatum* on Potato Dextrose Agar at Various Temperatures



Bars represent average diameters of 3 isolates after 8 days of incubation on PDA plates in the dark; each isolate was replicated six times at each temperature.

Figure 5.1 Effect of Temperature and Duration of Leaf Wetness on Severity of Symptoms caused by *C. truncatum* on Lentil



1. Scale: 0=No Disease, 1=Trace, 2=Slight, 3=Moderate, 4=Severe, 5=Lethal. Plants transferred to greenhouse after specified incubation and rated 14 days later.

2. 15p: Plants inoculated at 20C and returned to 15C chamber after 0, 8, 16, or 24 hours of leaf wetness.

General Discussion

Lentil production in Manitoba has been increasing since 1970. However, economic conditions have caused many producers to sow lentil in much shorter rotations than recomended for areas of high precipitation and humidity thereby greatly favouring the development and buildup of foliar diseases. Prior to the discovery of anthracnose, ascochyta blight was the only predominant foliar disease of lentil in Canada. Anthracnose of lentil caused by *C. truncatum* (Schw.) Andrus and Moore has only recently been recognized and much of the literature concentrates on disease incidence (Morrall and Pedersen 1991, Morrall *et al.* 1990, Baldanzi *et al.* 1988, Bellar and Kebabeh 1983, Manara and Manara 1983).

Chlorothalonil was selected as a possible control of lentil anthracnose because it has been shown to reduce disease severity and increase yield of soybean infected with *C. truncatum* (Backman 1986). Similar results with ascochyta blight of lentil have been reported (Beauchamp *et al.* 1986ab, Gossen and Morrall 1983). In the present study it was observed that chlorothalonil reduced the incidence of seed infection and increased seed yield up to four fold over that obtained from untreated plots when conditions were conducive for the development of *C. truncatum*.

Application of systemic fungicides to control anthracnose of lentil has not been previously reported but was investigated in the present study. Benzimidazole fungicides such as benomyl have been reported to have systemic and antipenetrant activity on many fungi, including *Colletotrichum* spp. but offer only limited control of *A. fabae* on lentil and faba bean and are prone to mutational resistance (Beauchamp *et al.* 1986a, Kharbanda and Bernier 1979, Sisler 1986, Ishii 1992). Ergosterol biosynthesis inhibitors have a moderately broad disease control spectrum and eradicant activity. In addition numerous variations in chemical structure are available to impede the appearance of resistant fungi (Ishii 1992). The

effectiveness of propiconazole in reducing severity of lentil anthracnose in the greenhouse was demonstrated, but field tests were inconclusive.

A chemical related to propiconazole called tricyclazole has been shown to adversely affect the melanization of appressoria of *Colletotrichum* spp. (Sisler 1986). Non-melanized appressoria produced by chemical inhibition of melanin synthesis or by genetic mutation in *C. lindemuthianum* or *C. lagenarium* are non-pathogenic (Kubo *et al.* 1982,1985, Wolkow *et al.* 1983). The non-melanized appressoria are believed to lack the architecture to support the mechanical forces required to penetrate plant epidermal walls (Wolkow *et al.* 1983, Woloshuk *et al.* 1983). An antipenetrant compound such as tricyclazole combined with an ergosterol biosynthesis inhibitor such as propiconazole may provide enhanced activity on anthracnose of lentil.

Infection of lentil seed by *C. truncatum* most likely occurs from lesions on the pod wall similar to that observed for *A. fabae* f.sp. *lentis*. Seed plating experiments in the present study indicate that *C. truncatum*-infected seeds are symptomless prior to plating and *Colletotrichum truncatum* is not extensively seed borne in lentil, unlike *A. fabae* f.sp. *lentis* which can colonize the seed coat, cotyledons and embyro. Histopathological studies of soybean seeds infected with *C. truncatum* showed that the fungus colonizes the seed coat tissues but only rarely infects the cotyledons (Kunwar *et al.* 1985). Schneider *et al.* (1974) observed *C. truncatum* mycelia in injured and exposed "hourglass" tissue located between the palisade and parenchyma cells of the seed coat. The fungus is believed to penetrate the seed coat through epidermal pores and cracks in soybean seed (Kunwar *et al.* 1985, Wolf and Baker 1972). Anthracnose of soybean can cause preemergence and postemergence killing or blighting of soybean seedlings (Roy 1982). A similar histopathological investigation of *C. truncatum* of lentil is warranted to determine the extent of fungal colonization within the seed coat.

No evidence of seed-to-seedling transmission was observed at the Campus Farm in the present study, suggesting that the transmission rate is low or that environmental conditions were not favourable for transmission. Seed-to-seedling transmission of *A. fabae* f.sp. *lentis* is favoured in seeds where the pathogen has either infected the embyro or is in close proximity to it prior to germination, allowing infection of the epicotyl during germination (Gossen and Morrall 1986). Temperatures at the time of germination may also affect transmission rates. Gossen and Morrall (1986) report that transmission of *A. fabae* f.sp. *lentis* occurs more frequently at low temperatures than at high temperatures because growth of the epicotyl is slow, which increases the chance of infection by the adjacent infected cotyledons. Cardwell *et al.* (1989) report that typical lesions of *C. graminicola* were observed on soybean seedlings grown from seeds with visible acervuli before sowing. Acervulus production on lentil seed was not observed in the present study and *C. truncatum* infection of lentil seed is believed to be restricted to the seed coat or, in rare circumstances, the cotyledons. Seed-to- seedling transmission may not be the primary means of dissemination although infected seed could introduce the disease into new areas at a low frequency. Beauchamp *et al.* (1986b) report that an epidemic of ascochyta blight of lentil was initiated from seeds with only 1.5% infection, suggesting that effective foliar fungicide application would have to reduce infection to almost zero before seed could be planted in new areas without risk. In addition, if pathogenic variation exists, special precautions in seed production and movement may be required to reduce the risk of introducing new and possibly more virulent isolates of *C. truncatum* into a region (Cardwell *et al.* 1989).

The reaction of lentil cultivars, landraces, breeding lines and other cultivated or native legumes to *C. truncatum* was investigated in (Section four). Weeden *et al.* (1992) report that significant portions of the lentil and pea genomes remains co-linear and additional linkages conserved between lentil and pea are likely to be conserved in faba bean and other closely related genera. In the present study,

susceptible genera to *C. truncatum* isolates obtained from lentil included *Lens*, *Vicia*, *Pisum*, and *Lathyrus*. Weidemann *et al.* (1988) report that *C. truncatum* obtained from pea was pathogenic on species from a number of genera; *Lupinus*, *Indigofera*, *Cicer*, *Lathyrus*, *Lens*, *Vicia* and *Pisum* but not from *Glycine*. In addition, Weidemann *et al.* (1988) report that *C. truncatum* obtained from pea was highly virulent on most cultivars of *P. sativum*, *Lathyrus*, and *Vicia*. The present study showed restricted non-spreading lesions on pea cultivars caused by *C. truncatum* obtained from lentil and no infection on three soybean cultivars. Van Dyke and Mims (1991) report that anthracnose of *Sesbania exaltata* (Raf.) Cory is caused by a host-specific isolate of *C. truncatum*. Further host range tests are required to determine the extent of pathenogenic variation of *C. truncatum* isolates obtained from lentil.

It would appear that there may be wide and overlapping host ranges within the genus *Colletotrichum*. Taxonomic practice has moved away from the use of host specificity as a taxonomic character in favour of morphological characteristics, and important physiological differences such as host specificity are indicated through the use of *formae speciales* (Gossen *et al.* 1986). The causal organisms in the above examples have been identified as *C. truncatum* (Schw.) Andrus and Moore but there are obvious pathogenic variations in both host range and virulence which are not easily reconciled. There is considerable confusion regarding the identification of *Colletotrichum* fungi based on cultural characteristics and conidial morphology. However, conidial dimorphism has been reported in *C. graminicola* during culturing and the presence of setae in many *Colletotrichum* species has been reported to be variable (Sutton 1980). Variability among *C. truncatum* isolates was not investigated in the present study and has not been previously documented on lentil. A more precise method of characterization is required to identify new pathogenic species and to define pathogenic variation within species. Random amplified polymorphic DNA (RAPD) may provide useful genetic markers for defining

fungal pathotypes of *C. truncatum*. RAPD markers are discrete fragments of DNA defined in the fungal genome by the annealing of specific primers and amplified by the polymerase chain reaction (PCR) (Williams *et al.* 1990, Mullis and Faloona 1987). Preliminary work defining the pathogenic variability of *C. graminicola* isolates by RAPD marker analysis has been conducted (Guthrie *et al.* 1992).

A number of temperatures and leaf wetness periods were used to determine the conditions favourable for *C. truncatum* growth and infection. Temperatures between 20 and 30C and leaf wetness periods of at least eight hours produced moderate levels of disease on lentil. In general, the conditions required for successful infection by the *C. truncatum* isolates obtained from lentil are typical of those obtained by other *Colletotrichum* spp. (Ishida and Akai 1969, Van Dyke and Mims 1991, Parbery 1981). Penetration of epidermal cells by appressoria producing infection pegs has been reported to be the primary method of entry. Indirect penetration through stomata or guard cells is rare (Manandhar *et al.* 1985, Van Dyke and Mims 1991). In the present study, temperatures of 30C were not inhibitory to successful infection, and no evidence of an infection-limiting effect of high heat was observed (Rahe and Kuc 1970). Wilson *et al.* (1990) constructed a regression model that predicts disease incidence in the field as a function of leaf wetness duration and temperature, for anthracnose of strawberry (*Fragaria* x *ananassa* Duch.), caused by *C. acutatum* Simmonds. A similar model may be useful in predicting disease incidence in anthracnose of lentil.

Colletotrichum coccodes (Wallr. Hughes), the causal organism of anthracnose of tomato (*Lycopersicon esculentum*) overwinters as sclerotial mycelium in infested plant debris and may survive for many years (Dillard 1988, Tu 1980). Unlike most sclerotium-forming fungi, *C. coccodes* overwinters as an acervulus derived from a stroma and in the spring produces conidia which serve as the source of primary inoculum. It was believed that *C. truncatum* of soybean was primarily a foliar

pathogen that infected seeds, crop and weed debris which serve as sources of inoculum (Hepperly 1985). However, Khan and Sinclair (1991) report of the discovery of sclerotium-forming isolates of *C. truncatum* which are capable of root and hypocotyl infection of soybean, even on cultivars possessing differentially resistant foliage. Infections of soybean seedling roots have also been observed to be latent and may serve as a primary source of inoculum.

Latent infection was also demonstrated in the greenhouse and observed in field studies. The definition of a "latent period" has been recently reviewed by Sinclair (1991). Sinclair's definition states that the latent period is considered a prolonged incubation period that begins at the time of infection and ends when the plant is under stress and macroscopic symptoms become visible. In lentil it would appear that the latent period may be related to temperature or stage of lentil growth. A more detailed investigation of latency is warranted to determine the timing of the initial infection and the extent of latency in susceptible crops and weed hosts. Cerkauskas and Sinclair (1980, 1982) report the use of paraquat to liberate latent fungi on soybean. In the present study, surface sterilization of lentil tissue with dilute sodium hypochlorite appeared to be sufficient to liberate latent *C. truncatum*. An understanding of latency and the mechanisms that trigger symptom development are important in the improvement of control measures (Sinclair 1991). Pathogen infection which occurs early in the season in a latent manner may not be controlled effectively by measures applied later in the season.

The discovery of anthracnose of lentil in Manitoba in 1987 and the economic losses caused by the disease prompted the present study. Fungicidal control of anthracnose of lentil has been demonstrated but the epidemiology of the disease and the biology of *C. truncatum* is poorly understood. Many opportunities for further investigation have been presented.

References

Abney, T.S. and T.L. Richards 1982. Fungal colonization of soybean stems. (Abstr.) Phytopathology 72: 995.

Agriculture Canada. 1987. Discussion Document: Propiconazole. Pesticides Directorate, Agriculture Canada, Ottawa, Ontario. pp.33.

Allard, R.W. 1988. Genetic changes associated with the evolution of adaptedness in cultivated plants and their wild progenitors. Journal of Heredity 79: 225-238.

Alefeld, F. 1866. Landwirtschaftliche Flora. Reprint 1966. ed. Otto Koeltz, Koenigstein-Taunus.

Allen, D.J. 1983. The pathology of tropical food legumes. Wiley-Interscience publication. Sussex; New York. pp.415.

Andrus, C.F. and W.D. Moore 1935. *Colletotrichum truncatum* (Schw.), N. Comb., on garden and lima beans. Phytopathology 25: 121-125.

Arx, J.A. von 1957. Die Arten der Gattung *Colletotrichum* Corda. Phytopathologische Zeitschrift 29:413-468.

Arx, J.A. von 1970. A revision of the fungi described as *Gloeosporium*. J. Cramer, ed. Lubrecht & Cramer, Lehre, Germany. pp.203.

Backman, P.A. 1985. Effect of spray adjuvants on depositions and retention of the fungicide chlorothalonil on soybean leaf surfaces. (Abstr.) Phytopathology 75: 1175

Backman, P.A., J.C. Williams and M.A. Crawford 1982. Yield losses in soybeans from anthracnose caused by *Colletotrichum truncatum*. Plant Disease 66: 1032-1034

Baldanzi, G., A.C. Baier, E.L. Floss, W. Manara, N.T.F. Manara, P.E. Veiga, and M.F.S. Tarrago, 1988. A lavouros de Inverno-2. Cevada, tremosis, Linis, Lentilha. Ed. Globo, Rio de Janiero. 184 pp.

Barulina, H. 1930. Lentils of the USSR and other countries. Suppl. 40 to the bulletin of Applied Botany, Genetics and Plant Breeding. Leningrad. (English summary). pp.265-304.

Baxter, A.P., G.C.A. Van Der Westhuizen and A. Eicker. 1985. A review of literature on the taxonomy , morphology and biology of the fungal genus *Colletotrichum*. Phytophylactica 17: 15-18.

Beauchamp, C.J., R.A.A. Morrall and A.E. Slinkard. 1986a. The potential for control of ascochyta blight of lentil with foliar applied fungicides. Canadian Journal of Plant Pathology. 8: 254-259.

Beauchamp, C.J., R.A.A. Morrall and A.E. Slinkard. 1986b. Effects of scheduling applications of benomyl, captafol and chlorothalonil on ascochyta blight of lentil. Canadian Journal of Plant Pathology 8: 260-268.

Bellar, M. and S. Kebabeh. 1983. A list of diseases, injuries and parasitic weeds in Syria (Survey 1979-80). Lens Newsletter 10: 30-31.

Bhatty, R.S. 1990. The cooking quality of lentils: The role of structure and composition of cell walls. Journal of Agriculture and Food Chemistry. 38:376-383

Cardwell, K.F., P.R. Hepperly, R.A. Frederiksen. 1989. Pathotypes of *Colletotrichum graminicola* and seed transmission of sorghum anthracnose. Plant Disease 73: 255-257.

Carroll, R.B., E.R. Jones and R.H Swain. 1977. Winter survival of *Colletotrichum trifolii* in Delaware. Plant Disease Reporter. 61: 12-15.

Cerkauskas, R.F., and J.B. Sinclair. 1980. Use of paraquat to aid detection of fungi in soybean tissues. Phytopathology 70: 1036-1038.

Cerkauskas, R.F., O.D. Dhingra and J.B. Sinclair. 1983. Effect of three desiccant-type herbicides on fruiting structures of *Colletotrichum truncatum* and *Phomopsis* spp. on soybean stems. Plant Disease 67: 620-622.

Cerkauskas, R.F. 1988. Latent colonization by *Colletotrichum* spp.: Epidemiological considerations and implications for mycoherbicides. Canadian Journal of Plant Pathology 10: 297-310.

Chau, K.F. and A.M. Alvarez. 1983. A histological study of anthracnose of *Carica papya*. Phytopathology 73: 1113-1115.

Chopra, K. and G. Swamy. 1975. Pulses: An analysis of demand and supply in India. Institute for social and economic change; Monograph 2. New Delhi: Sterling Publishers, PVT, Ltd. 132 pp.

Cubero, J. I. 1984. Taxonomy, distribution and evolution of the lentil and its wild relatives. In: Genetic resources and their exploitation-Chickpea, Faba Beans and Lentils. Witcombe, J.R. and Erskine, W. (Eds). The Hague: Martinus Nijhoff/Dr. W. Junk (for ICARDA & IBPGR). pp.187-203.

Dillard, H.R. 1988. Influence of temperature, pH, osmotic potential, and fungicide sensitivity on germination of conidia and growth of sclerotia of *Colletotrichum coccodes* in vitro. Phytopathology 78: 1357-1361.

Dillard, H.R. 1989. Effect of temperature, wetness duration, and inoculum density on infection and lesion development of *Colletotrichum coccodes* on tomato. Phytopathology 79: 1063-1066.

Erb, K. M.E. Gallegly, and J.G. Leach. 1973. Longevity of mycelium of *Colletotrichum lindemuthianum* in hypocotyl tissue of resistant and susceptible bean cultivars. Phytopathology 63: 1334-1335.

Erskine, W. 1984. Evaluation and utilization of lentil germplasm in an international breeding program In: Genetic resources and their exploitation-Chickpea, Faba Beans and Lentils. Witcombe, J.R. and Erskine, W. (Eds). The Hague: Martinus Nijhoff/Dr. W. Junk (for ICARDA & IBPGR). pp.225-237.

Erskine, W. and W.J. Goodrich. 1991. Variability in lentil growth habit. Crop Science 31: 1040-1044.

Erskine, W. and F.J. Muehlbauer. 1991. Allozyme and morphological variability, outcrossing rate and core collection formation in lentil germplasm. Theoretical and Applied Genetics. 83: 119-125.

Gibson, R.G, R.A.A. Morrall and C.C. Bernier. 1991. Anthracnose of lentil in Manitoba in 1990. Canadian Plant Disease Survey 71: 104.

Gossen, B.D. and R.A.A. Morrall. 1983. Effect of ascochyta blight on seed yield and quality of lentils. Canadian Journal of Plant Pathology. 5: 168-173.

Gossen, B.D. and R.A.A. Morrall. 1986. Transmission of *Ascochyta lentis* from infected lentil seed and plant residue. Canadian Journal of Plant Pathology. 8: 23-32.

Gossen, B.D., J.W. Sheard, C.J. Beauchamp and R.A.A. Morrall. 1986. *Ascochyta lentis* renamed *Ascochyta fabae* f.sp. *lentis*. Canadian Journal of Plant Pathology 8: 154-160.

Grant, N.T., E. Prusinkiewicz, R.M.D. Makowski, B. Holmstrom-Ruddick and K. Mortensen. 1990. Effect of selected pesticides on survival of *Colletotrichum gloeosporioides* f.sp. *malvae*, a bioherbicide for round-leaved mallow (*Malva pusilla*). Weed Technology 4: 701-715.

Guthrie, P.A.I., C.W. Magill, R.A. Frederiksen and G.N. Odvody. 1992. Random amplified polymorphic DNA markers: A system for identifying and differentiating isolates of *Colletotrichum graminicola*. Phytopathology 82: 832-835.

Hansen, J. and J.M. Renfrew. 1978. Paleolithic-neolithic seed remains at Franchthi Cave, Greece. Nature 271: 349-352.

Hartman, G.L., J.B. Manandhar and J.B. Sinclair. 1986a. Incidence of *Colletotrichum* spp. on soybeans and weeds in Illinois and pathenogenicity of *C. truncatum*. Plant Disease 70: 780-782.

Hepperly, P.R., B.L. Kirkpatrick and J.B. Sinclair. 1980. *Abutilon theophrasti*, wild host for three fungal parasites of soybean. Phytopathology 70: 307-310.

Hepperly, P.R., J.S. Mignucci, J.B. Sinclair and J.B. Mendoza. 1983. Soybean anthracnose and its seed assay in Puerto Rico. Seed Science and Technology 11: 371-380.

Hepperly, P.R. 1985. Soybean Anthracnose. In: Proc. World Soybean Research Conference III. R. Shibles (ed.), Westview Press, Boulder, CO. pp. 547-554

Ishida, N. and S. Akai. 1969. Relation of temperature to germination of conidia and appressorium formation in *Colletotrichum lagenarium*. Mycologia 61: 382-386.

Ishii, H. 1992. Target sites of tubulin-binding fungicides. In: Target Sites of Fungicide Action. Wolfram Koller (ed.). CRC Press Inc. Boca Raton Florida. pp. 43

Iqbal, S.M., A. Bakhsh and R.A. Malik. 1990. Identification of resistant sources to ascochyta blight of lentil. Lens Newsletter 17: 26-27.

Jolly, C.J. and F. Plog. 1987. Physical Anthropology and Archeology. Alfred A. Knopf. New York, N.Y. pp. 386-387.

Kaiser, W.J. and R.M. Hannan 1986. Incidence of seedborne *Ascochyta lentis* in lentil germplasm. Phytopathology 76:355-360.

Kendrick, B. 1981. The history of conidial fungi. In: Biology of Conidial Fungi, Vol. 1. Academic Press. pp. 3-18.

Khan, M. and J.B. Sinclair. 1990. Effect of soil temperature on infection of soybean roots by sclerotia-forming isolates of *Colletotrichum truncatum*. (Abstr.) Phytopathology 80:1040.

Khan, M. and J.B. Sinclair. 1991. Effect of soil temperature on infection of soybean roots by sclerotia-forming isolates of *Colletotrichum truncatum*. Plant Disease 75: 1282-1285.

Kharbanda, P.D. and C.C. Bernier. 1979. Effectiveness of seed and foliar application of fungicides to control ascochyta blight of faba beans. Canadian Journal of Plant Science 59: 661-666.

Kollar, W. 1992. Antifungal agents with target sites in sterol functions and biosynthesis. In: Target Sites of Fungicide Action. Kollar, W. (Ed.), CRC Press Inc. Boca Raton, Florida. pp. 328.

Kubo, Y., K. Suzukui, I. Furusawa, and M. Yamamoto. 1982. Effect of tricyclazole on appressorial pigmentation and penetration of nitrocellulose membranes by *Colletotrichum lagenarium*. Phytopathology 72: 498-501.

Kubo, Y., K. Suzukui, I. Furusawa and M. Yamamoto. 1985. Melanin biosynthesis as a prerequisite for penetration of appressoria of *Colletotrichum lagenarium*: Site inhibition by melanin-inhibiting fungicides and their action of appressoria. Pesticide Biochemistry and Physiology 23: 47-55.

Kunwar, I.K., T. Singh, and J.B. Sinclair. 1985. Histopathology of mixed infections by *Colletotrichum truncatum* and *Phomopsis* spp. or *Cercospora sojina* in soybean seeds. Phytopathology 75: 489-492.

Ladizinsky, G. 1979. The origin of lentil and its wild gene pool. Euphytica 28: 179-187.

Lipps, P.E. 1985. Influence of inoculum from buried and surface corn residues on the incidence of corn anthracnose. Phytopathology 75: 1212-1216.

Lipps, P.E. 1988. Spread of corn anthracnose from surface residues in continuous corn and corn-soybean rotation plots. Phytopathology 78: 756-761.

Littrell, R.H. and Epps, W.M. 1965. Standardization of a procedure for artificial inoculation of cucumbers with *Colletotrichum lagenarium*. Plant Disease Reporter. 49: 649-653.

McKenzie, B.A. and G.D. Hill. 1989. Environmental control of lentil (*Lens culinaris*) crop development. Journal of Agricultural Science. 113: 67-72.

McLean, K.S. and K.W. Roy. 1988. Incidence of *Colletotrichum dematium* on prickly sida, spotted spurge, and smooth pigweed and pathogenicity to soybean. Plant Disease. 72: 390-393.

McRae, C.F and B.A. Auld. 1988. The influence of environmental factors on anthracnose of *Xanthium spinosum*. Phytopathology 78: 1182-1186.

Madden, L.V., J.K. Knoke and R. Louie. 1982. Considerations for the use of multiple comparison procedures in phytopathological investigations. Phytopathology 72: 1015-1017.

Manandhar. J.B., I.K. Kunwar, T. Singh., G.L. Hartman and J.B. Sinclair. 1985. Penetration and infection of soybean leaf tissues by *Colletotrichum truncatum* and *Glomerella glycines*. Phytopathology. 75: 704-708.

Manandhar, J.B., G.L. Hartman, and J.B. Sinclair. 1986. *Colletotrichum destructivum*, the anamorph of *Glomerella glycines*. Phytopathology 76: 282-285

Manandhar, J.B., G.L. Hartman and J.B. Sinclair. 1988. Soybean germ plasm evaluation for resistance to *Colletotrichum truncatum*. Plant Disease. 72: 56-59.

Manitoba Department of Agriculture, 1990. Lentil Production in Manitoba. 12pp.

Manara, N.T.F. and W. Manara. 1983. Some climatic elements affecting lentil yields, sown at several dates, at Santa Maria, central depression of the state of Rio Grande do Sol. Lens Newsletter 10: 22-24.

Menezes, J.R. and J.C. Dianese. 1988. Race characterization of Brazilian isolates of *Colletotrichum lindemuthianum* and detection of resistance to anthracnose in *Phaseolus vulgaris*. Phytopathology 78: 650-655.

Miehle, B.R. and Lukezic, F.L. 1972. Studies on conidial germination and appressorium formation by *Colletotrichum trifolii* Bain & Essary. Canadian Journal of Microbiology. 18: 1263-1269.

Morrall, R.A.A., and J.W. Sheppard. 1981. Ascochyta blight of lentils in western Canada: 1978 to 1980. Canadian Plant Disease Survey. 61:7-13

Morrall, R.A.A. 1988. A new disease of lentil induced by *Colletotrichum truncatum* in Manitoba. Plant Disease 72: 994

Morrall, R.A.A., B.D. McCallum and C.C. Bernier. 1989. Anthracnose and other diseases of lentil in Manitoba in 1988. Canadian Plant Disease Survey. 69:66.

Morrall, R.A.A., R.J. Gibson and C.C. Bernier. 1990. Anthracnose of lentil Manitoba. Canadian Plant Disease Survey. 70:79.

Morrall, R.A.A. and E.A. Pedersen. 1991. Discovery of lentil anthracnose in Saskatchewan in 1990. Canadian Plant Disease Survey. 71:105.

Mortensen, K. 1988. The potential of an endemic fungus *Colletotrichum gloeosporioides*, for biological control of round-leaved mallow (*Malva pusilla*) and velvetleaf (*Abutilon theophrasti*). Weed Science 36:473-478.

Muirhead, I.F. 1981. The role of appressorial dormancy in latent infection. In: Microbial Ecology of the Phylloplane. J.P. Blakeman (ed.). Academic Press, New York. pp.155-167.

Mullis, K.B. and F.A. Fallona. 1987. Specific synthesis of DNA *in vitro* via a polymerase-catalyzed chain reaction. Methods in Enzymology 155:335-350.

Nag Raj,T.R. 1981. Coleomycete Systematics; In: Biology of Conidial Fungi vol.1. Academic Press. pp.43-84.

Naylor, V.D. and K.J. Leonard. 1977. Survival of *Colletotrichum graminicola* in infected corn stalks in North Carolina. Plant Disease Reporter 61:382-383.

Nene, Y.L., Hanounik, S.B., Qureshi, S.H. and Sen, B. 1988. Fungal and bacterial foliar diseases of pea, lentil, faba bean and chickpea. In: World Crops: Cool Season Food Legumes. R.J. Summerfield (ed.). Kluwer Academic publishers. pp.577-588.

Nicholson, R.L. and W.B.C Moraes. 1980. Survival of *Colletotrichum graminicola*: Importance of the spore matrix. Phytopathology 70:255-261.

O'Gara, P.J. 1915. New species of *Colletotrichum* and *Phoma*. Mycologia 7:38-41.

Ostazeski, S.A., D.K. Barnes and C.H. Hanson. 1969. Laboratory selection of alfalfa for resistance to anthracnose, *Colletotrichum trifolii*. Crop Science 9:351-354.

Panaccione, D.G., L.J. Vaillancourt and R.M. Hanau. 1989. Conidial dimorphism in *Colletotrichum graminicola*. Mycologia 81: 876-883.

Parbery, D.G. 1981. Biology of anthracnoses on leaf surfaces. In Microbial ecology of the phylloplane. J.P. Blakeman (ed.) Academic Press, New York. pp.135-154.

Parbery, D.G. and Emmett, R.W. 1977. Hypothesis regarding appressoria, spores and survival and phylogeny in parasitic fungi. Revue Mycology. 41:429-447.

Picard, J.J.A. D.A. Bond,L. Monti and R. Steuckardt. 1988. Production of pea, lentil, faba bean and chickpea in Europe. In: World Crops: Cool Season Food Legumes. R.J. Summerfield (ed.). Kluwer Academic publishers. pp.1065-1080.

Platford, R.G. 1988. Incidence of plant diseases in lentils in Manitoba in 1987. Canadian Plant Disease Survey 68:121.

Porto, M.D.M., C.R. Grau,G.A. de Zoeten, and G. Gaard. 1988. Histopathology of *Colletotrichum trifolii* on alfalfa. Phytopathology 78:345-349.

Rahe, J.E. and J. Kuc. 1970. Metabolic nature of the infection-limiting effect of heat on bean anthracnose. Phytopathology 60:1005-1009.

Rouchaud, J. and P. Roucourt. 1988. Hydrolytic biodegradation of chlorothalonil in the soil and in cabbage crops. Toxicology and Environmental Chemistry 17:59-68

Roy, K.W. 1982. Seedling diseases caused in soybean by species of *Colletotrichum* and *Glomerella*. Phytopathology 72:1093-1096.

Sauer, D.B., and R. Burroughs. 1986. Disinfection of seed surfaces with sodium hypochlorite. Phytopathology 76:745-749.

Saxena, M.C., and G.C. Hawtin. 1981. Morphology and growth patterns. In: C. Webb and G.C. Hawtin (eds.). Lentils. Commonwealth Agricultural Bureau. Farnham, UK. pp.53-69.

Schneider, R.W., O.D. Dhingra, J.F. Nicholson and J.B. Sinclair. 1974. *Colletotrichum truncatum* Borne within the Seedcoat of Soybean. (Abstr.) Phytopathology 64:154-155

Sinclair, J.B. 1982. Compendium of Soybean Diseases. 2nd ed. American Phytopathological Society. St. Paul. MN. pp.104.

Sinclair, J.B. 1991. Latent infection of soybean plants and seeds by fungi. Plant Disease. 75: 220-224.

Sinclair, J.B. and P.A. Backman. 1989. Compendium of Soybean Diseases. 3rd ed. American Phytopathological Society, St. Paul, MN. pp. 106.

Sinha, R.P. and B.P. Yadav. 1989 Inheritence of resistance to rust in lentil. Lens Newsletter 16:16-17.

Sisler, H.D. 1986. Control of fungal diseases by compounds acting as antipenetrants. Crop Protection 5: 306-313.

Slinkard, A.E. 1981. Eston Lentil. Canadian Journal of Plant Science. 61:733-734.

Slinkard, A.E. and R.S. Bhatty. 1979. Laird lentil. Canadian Journal of Plant Science. 59: 503-504.

Slinkard, A.E., and H.L. Blain. 1988. Production of pea, lentil, faba bean and chickpea in North America. In: World Crops: Cool Season Food Legumes. R.J. Summerfield (ed.). Kluwer Academic publishers. pp. 1059-1063

Smartt, J. 1990. Grain Legumes: Evolution and Genetic Resources. Cambridge University Press. pp. 220-228

Solh, M.and W. Erskine. 1984. Genetic Resources of Lentils. in Genetic Resources and their Exploitation-Chickpeas, Faba beans and Lentils. J.R. Witcombe and W. Erskine (eds.). The Hague: Martinus Nijhoff/Dr. W. Junk (for ICARDA & IBPGR). pp. 205-221.

Sutton, B.C. 1980. The Coleomycetes. Commonwealth Mycological Institute, Kew, Surrey, England. pp. 696.

Tiffany, L. H. 1951. Delayed sporulation of *Colletotrichum* on soybean. Phytopathology 41:975-985.

Tiffany, L.H. and J.C. Gilman. 1954. Species of *Colletotrichum* from legumes. Mycologia 46:52-76.

TeBeest, D.O., C.W. Shilling, R.L. Hopkins and G.J. Weidemann. 1989. The number of nuclei in spores of three species of *Colletotrichum*. Mycologia 81: 147-149.

TeBeest, D.O. 1982. Survival of *Colletotrichum gloeosporioides* f. sp. *aeschynomene* in rice irrigation water and soil. Plant Disease 66:469-472.

Thompson D.C. and S.F. Jenkins. 1985. Effects of temperature, moisture, and cucumber cultivar resistance on lesion size increase and conidial production by *Colletotrichum lagenarium*. Phytopathology 75: 828-832.

Thomson, W.T. 1987. Agricultural Chemicals: Book IV Fungicides. Thomson Publications, Fresno CA. pp. 74-75.

Tu, J.C. 1980. Ontogeny of sclerotia of *Colletotrichum coccodes*. Canadian Journal of Botany 58:631-636.

Tu, J.C. 1983. Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean (*Phaseolus vulgaris*) in southern Ontario: Survival of the pathogen. Plant Disease 67: 402-404.

Van Dyke, G.C. and C.W. Mims. 1991. Ultrastructure of conidia, conidium germination, and appressorium development in the plant pathogenic fungus *Colletotrichum truncatum*. Canadian Journal of Botany 69: 2455-2467.

Warkentin, T.D. and A. McHughen. 1991. Crown gall transformation of lentil (*Lens culinaris* Medik.) with virulent strains of *Agrobacterium tumefaciens*. Plant Cell Reports 10:489-493.

Warkentin, T.D. and A. McHughen. 1992. *Agrobacterium tumefaciens*-mediated beta-glucuronidase (GUS) gene expression in lentil (*Lens culinaris* Medik.) tissues. Plant Cell Reports 11:274-278

Weeden, N.F., F.J. Muehlbauer and G. Ladizinsky. 1992. Extensive conservation of linkage relationships between pea and lentil gene maps. Journal of Heredity 83: 123-129.

Weidemann, G.J., D.O. TeBeest and R.D. Cartwright. 1988. Host specificity of *Colletotrichum gloesporioides* f.sp. *aeschynomene* and *C. truncatum* in the Leguminosae. Phytopathology 78:986-990.

Whitney, N.G. Evaluation of foliar fungicides on soybeans. Fungicide and Nematicide Tests. 41:118-120.

Williams, D.J. and A. McHughen. 1986. Plant regeneration of the legume Lens culinaris Medik. (lentil) in vitro. Plant Cell Tissue and Organ Culture.Dordrecht: Martinus Nijhoff publishers. 7:149-153.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA Polymorphisms amplified by arbitray primers are useful as genetic markers. Nucleic Acids Research 18: 6531-6535.

Wilson, L.L., L.V. Madden and M.A. Ellis. 1990. Influence of temperature and wetness duration on infection of immature and mature strawberry fruit by *Colletotrichum acutatum*. Phytopathology 80: 111-116.

Wolf, W.I. and F.L. Baker. 1972. Scanning electron microscopy of soybeans. Cereal Science Today 17: 125-130.

Wolkow, P.M., H.D. Sisler and E.L. Vigil. 1983. Effect of inhibitors of melanin biosynthesis on structure and function of appressoria of *Colletotrichum lindemuthianum*. Physiological Plant Pathology 23: 55-71.

Woloshuk, C.P., H.D. Sisler and E.L. Vigil. 1983. Action of the antipenetrant, tricyclazole, on appressoria of *Pyricularia oryzae*. Physiological Plant Pathology 22: 245-259.

Wrather, J.A. and J.M. Elrod. 1990. Apparent systemic effect of *Colletotrichum truncatum* and *C. lagenarium* on the interaction between soybean and *C. truncatum*. Phytopathology 80: 472-474.

Yu, T.F. 1947. Ascochyta blight and leaf pod spot of broad bean in China. Phytopathology 37:207-214.

Zimmer, R.C. and Platford, R.G. 1991. Diseases detected on field pea and field bean in southern Manitoba in 1990. Canadian Plant Disease Survey. 71:107.