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