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Host, pathogen and environmental effects on incomplete resistance to anthracnose (*Colletotrichum truncatum*) in lentil (*Lens culinaris*)

A Thesis
Submitted to the Faculty of Graduate Studies

by

Godfrey Chongo

in Partial Fulfilment of the
Requirements for the Degree

of

Doctor of Philosophy

Department of Plant Science
The University of Manitoba
Winnipeg, MB, CA

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**Host, pathogen and environmental effects on incomplete resistance to
anthracnose (*Colletotrichum truncatum*) in lentil (*Lens culinaris*)**

BY

GODFREY CHONGO

**A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University
of Manitoba in partial fulfillment of the requirements of the degree**

of

DOCTOR OF PHILOSOPHY

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*To my wife Chishala and my
daughter Chisha and...*

ABSTRACT

Chongo, Godfrey, Ph.D. The University of Manitoba. March, 1998. Host, pathogen and environmental effects on incomplete resistance to anthracnose (*Colletotrichum truncatum*) in lentil (*Lens culinaris*). Major Professor: Dr. C.C. Bernier.

Incomplete resistance has recently been identified in a few lentil (*Lens culinaris*) lines/cultivars to anthracnose (*Colletotrichum truncatum*). The effectiveness of this incomplete resistance in controlling anthracnose, the responses of incompletely resistant lines/cultivars to the pathogen under various environmental factors favourable to anthracnose development and the components of incomplete resistance were investigated in one or more of five incompletely resistant lines/cultivars Indianhead, 458-57, PI 299331, PI 320937, PI 345629 and the susceptible cultivar Eston. Line 458-57 was evaluated for control of anthracnose with and without applications of the fungicide chlorothalonil in fields with low, moderate and high anthracnose levels. Yield losses in untreated plots across disease pressures ranged from 26-57% in Eston and 20-28% in 458-57. Line 458-57 with and without chlorothalonil was most effective in reducing disease severity (DS) and yield losses over those of Eston at high disease pressure where the effectiveness of incomplete resistance was better or equal to a single application of 1 or 2 kg a.i./ha chlorothalonil to Eston. In a study with Indianhead, 458-57 and Eston inoculated with a single isolate, DS was found to increase with temperature from 16 to 28°C, inoculum density (up to 1×10^5 conidia/ml) and longer leaf wetness period (up to 48 h), and plants were more susceptible at early flowering. In all the tests, DS was reduced on the incompletely resistant lines/cultivars compared to Eston. Analysis of the components of resistance in the six lines/cultivars in the field and growth chamber using one isolate of *C. truncatum* indicated that incomplete resistance was characterized by longer incubation and latent periods, fewer and smaller lesions and reduced sporulation. Disease severity and the area under the disease progress curve values were also lower on incompletely resistant lines/cultivars than on Eston.

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List of Abbreviations

Abbreviation	Definition
AUDPC	Area under the disease progress curve
FS	Field study
GC	Growth chamber
IP	Incubation period
LN	Lesion number
LP	Latent period
LS	Lesion size
SLS	Sporulating lesions per stem

Foreword

This thesis is written in a manuscript style approved by the Faculty of Graduate studies and the Department of Plant Science at the University of Manitoba. Three manuscripts are presented each including an abstract, introduction, materials and methods, results and discussion. A general introduction and literature review precede the manuscripts. A general discussion, literature cited and appendices terminate the thesis. All manuscripts are formatted to conform with the requirements of the Canadian Journal of Plant Pathology in which some of them are intended to be published.

1.0. INTRODUCTION

The lentil (*Lens culinaris* Medik.), is a leguminous plant species which is cultivated all over the world mainly as a pulse crop. In Canada lentil production began in 1969, and increased to over 300 000 ha annually by 1994 with over 80% produced in Saskatchewan (Slinkard and Vandenberg 1995). Production has usually been profitable (Morrall 1997). Canadian produced lentils are exported mainly to Europe, the middle east and south America (Smatt 1990, Slinkard and Blain 1988, Buchwaldt et al. 1995).

More recently production has been affected by disease problems. In 1987 anthracnose was first discovered on lentil in Manitoba (Morrall 1988) and the causal pathogen was identified as *Colletotrichum truncatum* (Schw.) Andrus & W.D. Moore. Subsequently anthracnose was also reported in Saskatchewan (Morrall and Pedersen 1991), but to date the disease is more prevalent in Manitoba. Anthracnose causes necrotic lesions on stems, leaves, and pods, and may cause plant death (Gibson 1994, Buchwaldt et al. 1996). Yield losses may be as high as 60-70% (Morrall et al. 1990, Morrall and Pedersen 1991).

Although a number of high yielding lentil cultivars with good agronomic characters have been developed for western Canada none of these cultivars have resistance to anthracnose (Slinkard and Vandenberg 1995). Therefore anthracnose in lentil has been controlled by using crop rotations and a protective foliar fungicide. A few lines with incomplete resistance were identified at the University of Manitoba after screening over 1300 accessions (Bernier et al. 1992). In the absence of cultivars with complete resistance, incomplete resistance could provide an acceptable option for disease control. In differentiating between complete and incomplete resistance, Parlevliet (1979) indicated that complete resistance prevents multiplication of the pathogen and inhibits spore production. The term incomplete resistance refers to all resistances in which spore production is reduced even though the host plants are

susceptible to infection. Partial resistance and slow rusting are forms of incomplete resistance. A characteristic of partial resistance is that although lesions may be reduced in number, they are similar in size to that on a susceptible host (Parlevliet 1979). The term slow rusting is used in rust pathogen systems to denote a reduced rate of epidemic development. Partial resistance and slow rusting are both assumed to be race-non-specific in nature (Parlevliet 1985). Any form of incomplete resistance can result in slow rusting but does not necessarily provide the evidence that the resistance is race-non-specific (Parlevliet 1985).

Incomplete resistance has been attributed to components such as longer incubation and latent periods and reduced frequency of lesions and sporulation (Aquino et al. 1995, Nutter and Pederson 1985). Thus, incomplete resistance can be studied and selected for by looking at these components (Green and Wynne 1986, Rashid 1991, Aquino et al. 1995). One or more of the resistance components may also reduce the rate of disease development in the field. Therefore, incomplete resistance may also be identified by comparing parameters of disease progress in the field such as the area under the disease progress curves or the apparent infection rate (Wilcoxson 1981, Conner and Bernier 1982).

Resistance to *Colletotrichum* spp. has been identified in crops such as bean (*Phaseolus vulgaris* L.) to *C. lindemuthianum* Sacc. Magnus. (Tu 1992), Sorghum (*Sorghum bicolor* [L.] Muench.) and maize (*Zea mays* L.) to *C. graminicola* [Ces] G.W. Wils. (Warren 1981, Duncan et al. 1991, Casela et al. 1993). However, in most crops including cucumber (*Cucumis sativus* L.) to *C. lagenarium* [Pass.] Ell. & Halst., tomato (*Lycopersicon esculentum* Miller) to *C. Coccodes* Wallr. and sorghum to *C. graminicola*, the resistance is incomplete and usually polygenic in nature (Waller 1992). Incomplete resistance to other pathogens has also been reported in other crops including soft red winter wheat (*Triticum aestivum* L.) to powdery mildew (*Erysiphe graminis* DC. f.sp. *tritici* Em. Marchal.) (Pearce et al. 1996), peanut (*Arachis hypogaea* L.) to early leaf spot (*Cercosporidium arachidicola* Hori.) (Green and Wynne 1986), barley (*Hordeum vulgare* L.) to leaf rust (*Puccinia*

hordei Oth.) (Parlevliet and Van Ommeren 1975) and to net blotch (*Pyrenophora tritici* Drechs. f.sp. *teres* Smedeg.) (Steffenson and Webster 1992).

Combining incomplete resistance with fungicides to control diseases has shown that the amount of fungicides used could be reduced in comparison to fungicides applied to susceptible cultivars. In common rust (*Puccinia sorghi* Scwein.) of maize (Pataky and Eastburn 1993), cucumber anthracnose (Thompson and Jenkins 1985a,b) and tomato anthracnose (Precheur et al. 1990, Latin 1991) susceptible cultivars were found to require higher rates and additional applications of fungicides. Fungicidal control of *C. truncatum* in susceptible lentil cultivars has been demonstrated before (Gibson et al. 1991, Morrall and Pedersen 1991, Gibson 1994), but the effectiveness of incomplete resistance to *C. truncatum* in controlling anthracnose alone or in combination with fungicide applications has never been reported.

Disease development can be affected by many variables that relate to the environment, the host or the pathogen. Normally, these variables affect disease development indirectly by affecting components of resistance. Incubation and latent periods are affected by temperature and tissue age (Tomerlin et al. 1983, Pedersen and Morrall 1994). Lesion size and number may also be influenced by inoculum density, temperature and leaf wetness period (Nutter and Pederson 1985, Pedersen and Morrall 1994, Zheng and Sutton 1994) and the virulence of pathogen isolates. Among these factors, only the effects of leaf wetness period and temperature on the development of anthracnose have been reported in lentil (Gibson 1994). Development of anthracnose was found to be most severe at moderate temperatures of about 20-25°C and leaf wetness periods of at least 16 h (Gibson 1994). Variability in the virulence of *C. truncatum* isolates on a number of lentil lines has also been observed (B.D. McCallum and C.C. Bernier, unpublished).

The objectives were [1] to determine the effectiveness of incomplete resistance in controlling lentil anthracnose in the field, with and without fungicide applications [2] to investigate the responses

of lentil lines/cultivars with incomplete resistance to various host, pathogen and environmental factors [3] to determine the basis of incomplete resistance by analysis of the components of resistance in lentil.

2.0. LITERATURE REVIEW

2.1. The host (*Lens culinaris*)

2.1.1. Origin, taxonomy and domestication

Lentils (*Lens culinaris* Medik.) are believed to have originated in the areas between Turkey and Iraq (Cubero 1984), Asia minor and the surrounding regions (Kay 1979). Other areas of archeological interest on the origins of lentil have been centered in the near eastern and southern Europe. Barulina (1930) proposed the eastern border of southwestern Asia as a possible center of origin for cultivated lentil based on the occurrence of the highest proportion of endemic varieties of cultivated species in the region around Afghanistan, India and Turkestan. The strongest evidence suggest that lentils were first cultivated in the 'Fertile Crescent' in the middle east and southern Turkey, and later spread to Egypt, Greece and central Europe along the river Danube (Cubero 1984). However, Ladizinsky (1979) has disagreed with the suggestion that lentils were ever domesticated in the southern part of the 'Fertile Crescent' because the species (*L. orientalis*) he used differed from the cultivated type in one reciprocal chromosome translocation. Cubero (1984) argued that chromosome translocation was a common phenomenon even in cultivated species. It is, therefore, difficult to pinpoint the exact location where lentils were first domesticated. Nevertheless, a wide distribution and diversification of lentil occurred in the areas around the Mediterranean region, central and southern Europe, India and north Africa (Hansen and Renfrew 1978, Erskine 1984). Lentils were also successfully introduced into the New World and are cultivated in the north and south America (Kay 1979, Slinkard and Blain 1988).

The genus *Lens*, contains five species including *L. montbrettii* (Fisch & Mey), *L. ervoides* (Brign.) Grande., *L. nigricans* (M. Bieb) Godron., *L. orientalis* (Boiss.) Handel-Mazzetti. and *L. culinaris*. Each of the five species, has at least four synonyms indicating the confusions that

taxonomists have had with the genus (Cubero 1981). Cubero (1984) suggested that the domesticated species of *L. culinaris*, probably originated from *L. orientalis* populations or from intermediate forms between *L. nigricans* and *L. orientalis* or mixed populations. The *L. ervoides* species has yet to be domesticated together with *L. montbrettii*, a doubtful lentil but related to lentil.

2.1.2. Biology

Within the cultivated species, two types of lentil have been described based on geographical location as macrosperma and microsperma (Kay 1979, Cubero 1984). The former contains large flattened yellow lentil seeds about 6-9 mm in diameter and is also referred to as the Chilean type while the latter is described as lens shaped, often varying in color, small to medium of about 3-6 mm in diameter also known as the Persian type.

Cubero (1984) described lentil as an annual vetch-like legume with pinnate leaves bearing about 8-10 leaflets and a terminal tendrils. The stem is herbaceous when young but becomes woody at the basal ends towards maturity. Branching varies with genotype and plant density. The flowers may be white, pink, red, violet or blue-violet, depending on genotype and are largely self-fertilized, but cross pollination can occur (Cubero 1981, Kay 1979). The pods are smooth and contain 2 small lens-like seeds which may vary from 3-9 mm in diameter. The seed coat color varies from light green, tan, red, brown, black to purple or mottled black. Plant height varies between 15 to 75 cm Cubero (1981). The roots may be shallow and highly branched, slender and deep tap-rooted or intermediate (Kay 1979).

2.1.3. Major uses

Lentil has high nutritional value (Slinkard and Bhatta 1979) and very low levels of anti-nutritional factors. In traditional lentil producing areas such as the middle east, lentil is widely popular.

The seed is used to make soups, ground into flour and used to make cakes or bread and in the preparation of baby foods (Kay 1979) or the seed can be cooked or fried and eaten whole. Lentil seeds vary considerably in the range of protein content but are deficient in two essential amino acids; methionine and cystine. Williams et al. (1975) reported a range of 15-30% protein content, while a range of 20.6-33.4% with a mean of 28.1% from 1863 accessions was reported by (Solh and Erskine 1984).

Lentil is also high in carbohydrates and provides a good feed supplement to animals. The seeds are sometimes used in commercial starch for use in the textile industry (Kay 1979). Lentils are occasionally grown as fodder for animal feed with plants cut and fed to animals or grazed from fields. The straw and pod debris from the threshing have a high feed value. The seed coat left after decortication is also a valuable feed which may contain up to 13% protein (Nygaard and Hawtin 1981). Like many other legumes, lentil is capable of fixing its own nitrogen in the soil, especially when inoculated with the appropriate *Rhizobium* bacteria (Smatt 1990). Lentils are also useful as a green manure (Chopra and Swamy 1975), providing a rich source of nitrogen to other crops when used in rotations.

2.1.4. World production

Lentil production is most suited to temperate climatic conditions and maturation occurs well under dry conditions (Kay 1979). The largest lentil producer in the world is India. Other countries in Africa (mainly, Algeria, Egypt, Ethiopia, Libya, Morocco, Somalia, Sudan and Tunisia) in north and central America (Canada, USA, Guatemala, Mexico and Costa Rica) in south America (Argentina, Chile, Columbia, Peru and others) in Asia (Bangladesh, Turkey, Syria, Pakistan, Iran and Iraq) and in Europe (notably, Spain, Greece, France, Russia and Italy) also produce lentils (Solh and Erskine 1984). Most lentils found in international trade are either green or red. Black seed coat lentils are

popular in India and Pakistan whereas in Arabic countries yellow lentils are preferred. In countries such as India and Pakistan lentils are cultivated during the dry season.

In Canada, lentil production began in 1969 when about 600 ha were cultivated in Saskatchewan (Slinkard and Vandenberg 1995). Canada is the world's largest exporter of green lentil and countries in the middle east, Africa, Europe and south America are the primary markets (Buchwaldt et al. 1995). The two main types of lentils grown in western Canada are the Chilean type (large seeded) and the Persian type (small seeded). The Chilean cultivar, Laird registered in 1978 (Slinkard 1978) and Eston a Persian-type, early maturing cultivar (Slinkard 1981), are the most widely grown lentils in western Canada. Both Laird and Eston are yellow cotyledoned cultivars with greenish-yellow seed coats. Other types of lentil cultivars grown in western Canada include the Common Chilean, CDC Richlea, CDC Gold, CDC Redwing, CDC Matador, French green, Spanish brown and the red cotyledon cultivar Rose (Slinkard and Vandenberg 1995).

The area of lentil production in the Canadian Prairies has increased steadily since 1989. Total lentil production rose from 103 000 ha in 1989 to 331 000 ha in 1996 with a farm gate income of about \$200 million. The 1996 average yields were estimated at 1.32 tonnes per hectare. Canada has become one of the major lentil producers in the world, with over 80% of the crop produced in Saskatchewan and the rest in Manitoba and Alberta (Slinkard and Vandenberg 1995, Buchwaldt et al. 1995).

2.1.5. Major diseases

Most diseases reported on lentil around the world are fungal diseases. These include vascular wilt caused by a number of *Fusarium* species, root rots caused by *Fusarium* and *Pythium* species, rust (*Uromyces fabae* Pers.), stem rot (*Sclerotinia sclerotiorum* (Lib.) de Bary), ascochyta blight (*Ascochyta fabae* Speg. f.sp. *lentis* Gossen et. al.), powdery mildew (*Erysiphe polygoni* DC), down

mildew (*Peronospora lentis* [Berk.] Casp.) and anthracnose (*Colletotrichum truncatum*) (Khare 1981).

In western Canada, increased production resulted in considerable increase in the occurrence of a number of diseases including ascochyta blight *Sclerotinia*, *Fusarium* and *Rhizoctinia* root rots, *Botrytis* stem and pod rots (*Botrytis cinerea* Pers.) and anthracnose. Of these the most important are ascochyta blight, *Botrytis* stem and pod rot, and anthracnose (Morrall 1997). Ascochyta blight occurs most commonly in Saskatchewan while anthracnose is most common in Manitoba. Anthracnose was first reported in Manitoba in 1987 (Morrall 1988) and since then it has become increasingly important in major lentil producing areas of Manitoba.

2.2. The Anthracnose pathogen (*Colletotrichum truncatum*)

Colletotrichum truncatum (Schwein.) Andrus & W.D. Moore is characterized by falcate conidia and was formerly regarded as a forma specialis of *Colletotrichum dematium* (Pers. Fr.) Grove by von Arx (1957). However, Sutton (1980) distinguished *C. dematium* from *C. truncatum* on the basis of the latter having broader conidia. In addition, *Colletotrichum pisi* Pat. from pea (*Pisum sativum* L.) which was earlier considered to be synonymous with *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz. by von Arx (1957) has recently been suggested to be synonymous with *C. truncatum* (Weidemann et al. 1988) and not *C. gloeosporioides*.

2.2.1. Classification

Colletotrichum species remain a challenging taxonomic problem. While the sexual state (teleomorph) of this fungus has been classified into the ascomycete genus *Glomerella*, the asexual state (anamorph) belongs to the coelomycete genus *Colletotrichum* (Skipp et al. 1995). At present, not all

Colletotrichum species have a recognized *Glomerella* state. A teleomorph state of *C. truncatum* has not been reported. The classification of *Colletotrichum* species is largely based on anamorphic characteristics such as size and shape of conidia, presence or absence of setae and shape of appressoria, coupled with knowledge of the host plant (Bailey et al. 1995). In a broad sense *Colletotrichum* species can be divided into two broad groups based on straight or falcate conidia (Skipp et al. 1995).

The lack of distinctive characters has resulted in giving different names to morphologically indistinguishable isolates simply because they were obtained from different hosts, without conducting host range tests (Bailey et al. 1995). For example, von Arx (1957) considered that the host origin was not important in defining species and concluded that out of 1000 *Colletotrichum* species originally described separately about 500 were synonymous to *C. gloeosporioides*. Subsequently, this number was reduced to 11. Sutton (1980) listed 25 species which were later increased to 37 (Sutton 1992), because of an increased reliance on host origin.

2.2.2. Infection strategies of *Colletotrichum* species

The infection process exhibited by many *Colletotrichum* species is described as two-phase or hemibiotrophic (Leach 1922, Luttrell 1974, Bailey et al. 1995). The initial phase is symptomless. During this phase, the pathogen is confined to one or a few host cells without killing them. This is followed by a second phase in which the pathogen invades many more cells, may kill host cells and result in lesion formation (Bailey et al. 1995). Initially the pathogen may be intracellular and may grow in several epidermal and cortical cells, e.g. *C. lindemuthianum* (Sacc. & Magnus) Briosi & Cav. infection of bean (Mercer et al. 1974, O'Connell et al. 1985), or it may be confined to a single cell, e.g. *C. lindemuthianum* infection of cowpea (*Vigna unguiculata* L.) (Bailey et al. 1990) and *C. truncatum* infection of pea (O'Connell et al. 1993) or it may initially grow subcuticularly as in *C.*

capsici (Syd.) infection of cowpea (Pring et al. 1995) and *C. truncatum* in soybean (Manandhar et al. 1985). In certain cases however, some species have initially exhibited intracellular and subcuticular e.g. *C. gloeosporioides* of citrus (Brown 1977).

Penetration can occur through natural openings such as stomata or wounds, although direct penetration through the cuticle is the most common route (Bailey et al. 1992). Penetration usually occurs after appressorium formation (Bailey et al. 1992, 1995) but penetration without appressoria has been reported (Manandhar et al. 1985, Porto et al. 1988). Three penetration mechanisms including force alone, cell degrading enzymes or both have been a subject of much debate (Bailey et al. 1992).

2.2.3. Importance and disease cycle

Anthrachnose of lentil is capable of causing yield losses of up to 70% in western Canada (Morrall and Pedersen 1991, Gibson 1994) particularly when temperature and moisture are high (Gibson 1994). The symptoms first appear as water-soaked greenish lesions on all above ground plant parts. The lesions eventually become necrotic leading to extensive leaf defoliation, stem collapse and plant death (Gibson 1994). In the field, symptoms are first visible usually at the beginning of flowering as small necrotic lesions on lower leaves and stems (Gibson 1994, Buchwaldt et al. 1996). The pathogen then spreads to upper parts of the foliage and eventually to the pods. Although the seed may become infected, seed-to-seedling transmission has not been confirmed (Gibson 1994).

The fungus survives on lentil debris as small black sclerotia. In a survival study of *C. truncatum*, Buchwaldt et al. (1996) showed that when lentil debris is left on the soil surface, the infectivity of the fungus in the infested debris declined during the first 12 months, while buried debris declined after 48 months. A new lentil crop becomes infected by the fungus from germinating sclerotia in debris of previous crop. It has also been shown that sclerotia may be transported from one field to

another by blowing wind or rain (Buchwaldt et al. 1996). Conidia and sclerotia produced on lesions during the current season may be disseminated by splashing rain to uninfected plant tissues.

Sinclair (1991) and Khan and Sinclair (1991) reported that *C. truncatum* in soybeans can initiate infections without producing any symptoms at the beginning of the season but that symptoms begin to show up during the early reproductive stage as necrotic lesions on leaves and as acervuli and sclerotia on dead plant parts (Sinclair 1982). This phenomenon has also been observed in lentils where symptoms first appear at early flowering (Gibson 1994, Buchwaldt et al. 1996) as sunken necrotic lesions on leaves, stems and pods followed by extensive leaf defoliation. Quiescent or latent infection (symptomless period shortly after infection) has been demonstrated in lentil. Gibson (1994) indicated that infection may occur as early as two weeks from emergence (about 3 weeks after seeding) although symptoms always appear at early flower or about 4-5 weeks from seeding. It therefore, seems that the quiescent or latent period lasts for about 2 weeks in lentil.

2.2.4. Pathogen variability and host range

Colletotrichum truncatum is known to infect several genera of the *Leguminosae* including; *Lens*, *Vicia*, *Pisum*, *Lathyrus*, *Cicer*, *Lupinus* and *Indigofera* (Weidemann et al. 1988). Morrall et al. (1989) reported that *C. truncatum* from lentil is capable of infecting other legume species, notably faba beans (*Vicia faba* L.) and peas. *Colletotrichum truncatum* has been found to have varying levels of virulence on different lentil lines/cultivars. Isolates of *C. truncatum* collected from fields in Manitoba were shown to have different levels of virulence on lentil cultivars (B..D. McCallum and C.C. Bernier, unpublished).

Gibson (1994) reported that cultivars and land races of lentil, faba bean, field pea, *Lathyrus* (*L. sativa* L., and *L. tingitanus* L.) and yellow vetch (*Vicia sativa* L.) were all susceptible to two isolates of *C. truncatum* which had been isolated from infected lentils in the field. However, soybean

(*Glycine max* [L.] Merr.), field bean (*Phaseolus vulgaris* L.), alfalfa (*Medicago sativa* L.), lupin (*Lupinus angustifolius* L.), round leaved mallow (*Malva pusilla* Smith) and chickpea (*Cicer arietinum* L.) were found to be resistant. In contrast, Weidemann et al. (1988) reported that *Cicer* and *Lupinus* inoculated with *C. truncatum* were among the susceptible genera. This raises a question as to whether *C. truncatum* reported on soybean (Manandhar et al. 1985, Kunwar et al. 1985, Khan and Sinclair 1991, 1992) can also infect lentils. *Colletotrichum truncatum* has also been described on the weed species, hemp (*Sesbania exaltata* (Raf.) Cory) (Van Dyke and Mims 1991, Boyette 1991). In the study by Boyette (1991), out of representative plants from seven families inoculated with *C. truncatum* (*Compositae*, *Convolvulaceae*, *Cucurbitaceae*, *Graminaceae*, *Leguminosae*, *Malvaceae* and *Solanaceae*), only some species from the *Leguminosae* including soybean were susceptible. However, within soybean, only three of nine cultivars were susceptible and six were highly resistant. Therefore, *C. truncatum* has a narrow host range which is restricted to the *Leguminosae* family and within a species, certain cultivars may be resistant or susceptible (Boyette 1991). It is not clear whether *C. truncatum* is specialized on specific legume species as forma speciales. In the case of soybean, other *Colletotrichum* species are known to also infect the crop (Sinclair 1982), although the primary pathogen is *C. truncatum*. It is not known whether other *Colletotrichum* species can also infect lentils.

2.3. Control of anthracnose diseases

Many *Colletotrichum* diseases on various hosts are usually controlled or managed by a combination of measures mainly including chemical, cultural and resistant cultivars (Waller 1992). The use of resistant cultivars whenever available has been a major strategy in controlling many plant diseases. It is one of the most effective means of controlling plant diseases that is also environmentally sound.

Colletotrichum truncatum in lentil is a recent problem and very little research has been conducted on controlling the disease. Presently all commercial cultivars are susceptible and control measures used in western Canada based on 3-4 year crop rotations and 1-2 applications of a foliar fungicide are not always adequate and the later is costly. The logistics of applying foliar fungicides at an appropriate time in wet years reduces their effectiveness and in some cases disease control is very poor.

Anthracnose diseases are common and destructive on other crops. In cases where the control of anthracnose diseases have been more extensively researched than in lentil, control is based on the use of fungicides and crop rotations and for some diseases, resistance has also been used (Lenné 1992). In anthracnose caused by *C. truncatum* in soybean resistant cultivars are not available (Manandhar et al. 1988), so that control strategies including the use of fungicidal seed treatment, crop rotations and incorporation of residues are practiced (Sinclair 1982). In cucumber, anthracnose (*C. lagenarium*) control measures are based on the use of cultivars with high levels of incomplete resistance (Sitterly 1972) and frequent applications of fungicides (Thompson and Jenkins 1985a,b). However, in commercial production it has been found that the effectiveness of incomplete resistance in cucumber may be reduced under environments that are highly favorable to disease development. Similarly, the recommended measures for controlling tomato (*Lycopersicon esculentum* Miller) anthracnose (*C. Coccodes* (Wallr.) Hughes are based on the use of resistant cultivars (Barksdale and Stoner 1981), applications of fungicides throughout the growing season and crop rotations of 3-4 years (Dillard 1987, 1990, Dillard et al. 1991), elimination of weed hosts (Andersen and Walker 1985) and harvesting on time (Sherf and MacNab 1986). The failure to control anthracnose on tomato is often attributed to the poor timing of fungicide applications (Dillard and Rutkowski 1985, Dillard 1986), while the effectiveness of fungicides is also influenced by levels of resistance (Latin 1991).

In the control of bean anthracnose (*C. lindemuthianum*), resistant cultivars are used worldwide and have been found to be effective but cultural and fungicidal control are also used (Pastor-Corrales and Tu 1989). Cultural practices have also helped to reduce the importance of bean anthracnose in north America, Europe and Australia (Pastor-Corrales and Tu 1989). Because of extensive variation in races of *C. lindemuthianum*, single gene resistant cultivars have been reported to lose resistance to new races of the pathogen (Hubbeling 1976). In view of this, emphasis has been placed on an integrated control program including quarantine, seed treatment, seed inspection and resistant cultivars in Ontario, Canada (Tu 1992).

In general resistance to *Colletotrichum* spp. in most crops has been found to be incomplete and polygenic in nature (Lenné 1992) and is greatly influenced by environmental conditions that are favorable to anthracnose. The timing of fungicide applications to control anthracnoses has been a problem and fungicide themselves are also costly. An integrated approach involving the use of several measures to control anthracnose diseases seems to be the best strategy currently being used in most crops.

2.3.1. Host resistance

Plant resistance to a particular pathogen has been defined as the ability of a host to reduce the amount of disease compared to a susceptible host by limiting the growth and development of a pathogen (Nelson 1973). This definition implies that there are many degrees of resistance, some of which allow reproduction of the pathogen. In a broad sense resistance can be categorized into two types; non-host resistance which is expressed by all genotypes of a plant species to an organism pathogenic to another plant species and host resistance which is expressed by certain genotypes of an otherwise susceptible host (Heath 1987). Pathogen attack stimulates plants to respond with a variety of specific and non specific defense mechanisms.

Resistance to *Colletotrichum* species varies considerably. While bean can exhibit resistance as a hypersensitive reaction (O'Connell et al. 1985, Mould et al. 1991), in some cases only lesions may be restricted (Mercer et al. 1974). Vanderplank (1963) described two types of resistances, vertical resistance (VR) and horizontal resistance (HR). Vertical resistance is classified on the basis of differential interactions among isolates of a pathogen and host cultivars (Vanderplank 1963) and this resistance often demonstrates a gene-for-gene relationship (Flor 1956). Vanderplank (1968) redefined VR and HR in statistical terms, using analysis of variance. The presence of significant interactions between host and pathogen genotypes indicated VR and the presence of significant main effects because of pathogen and host genotypes indicated HR. However, these two definitions soon became obsolete as further study revealed that even VR could produce significant main effects.

Vanderplank (1978) later stated that resistance was horizontal if host cultivars produced constant ranking when tested with different isolates of the pathogen. However, constant ranking can also be achieved with VR. Because of these problems, the differences between the two resistances were renamed and redefined as race specific resistance (RSR) and race non specific resistance (RNR). Race-specific resistance is characterized by the presence of genetic differential interactions between host and pathogen genotypes (Parlevliet 1985). Race non specific (RNR) resistance is only recognized by the lack of host-pathogen differential interactions (Sawhney 1995). Scott et al. (1980) defined RNR as resistance that has not shown any specificity after prolonged testing. This however, is difficult to prove as it requires demonstration that the pathogen is incapable of evolution towards increased pathogenicity specific for that resistance (Johnson 1984).

2.3.2. Incomplete resistance

Sometimes the terms complete and incomplete resistance are used to denote host resistance. Complete resistance prevents multiplication of the pathogen and there is no spore production whereas

in incomplete resistance there is some spore production (Parlevliet 1985). Differences in resistance to *Colletotrichum* diseases have been demonstrated among cultivars of most crops but resistance is often incomplete and is greatly influenced by other host and environmental factors (Waller 1992). Incomplete resistance to anthracnose diseases occurs in tomato to *C. coccodes* (Miller et al. 1984), in coffee (*Coffea arabica* L.) to coffee berry disease (*C. coffeanum* Noack *sensu* Hindorf) (Masaba and Waller 1992), in cucumber to *C. lagenarium* (Thompson and Jenkins 1985a,b), in *Stylosanthes* spp. to *C. gloeosporioides* (Chakraborty et al. 1988, Chakraborty 1990, Iamasupisit et al. 1991) and in lentil to *C. truncatum* (Bernier et al. 1992). In three lentil lines/cultivars with incomplete resistance, a single dominant gene was found to confer resistance (L. Buchwaldt and C.C. Bernier, unpublished) and in each line the gene was different from those in the other two lines.

Other forms of incomplete resistance have been described. Partial resistance, is a form of incomplete resistance that is largely race-non-specific and polygenic in nature. It is characterized by fewer lesions of susceptible type and longer latent periods compared to susceptible cultivars (Parlevliet 1985, Burdon 1993). Partial resistance and slow rusting are often used to indicate the same type of resistance (Parlevliet 1985). Since simply inherited race specific resistance can also be expressed as slow rusting, Parlevliet and Van Ommeren (1975) also used the infection types to distinguish between RSR and RNR. Cultivars which had high infection types and also rusted slowly were considered to carry RNR which they also called partial resistance.

Partial resistance has been reported in many crops including wheat to stem rust (Wilcoxson et al. 1975), wheat to leaf rust (Caldwell et al. 1970, Shaner and Finney 1980); barley (*Hordeum vulgare* L.) to leaf rust (*Puccinia hordei* Otth.) (Parlevliet and Van Ommeren 1975) and powdery mildew (*Erysiphe graminis* D.C. f.sp. *hordei* Em. Marchal.) (Knudsen et al. 1986), maize to *Puccinia sorghi* Schwein. (Kim and Brewbaker 1977), oat to crown rust (*Puccinia coronata* Corda. f.sp. *avenae*) (Luke et al. 1972, Brière et al. 1994), rye (*Secale cereale* L.) to leaf rust (*Puccinia recondita*

Rob. ex Des.) (Parlevliet 1977), faba bean to rust (*Uromyces vicia-fabae* [Pers.] Schroet.) (Conner and Bernier 1982, Rashid and Bernier 1986), peanut (*Arachis hypogaea* L.) to early leaf spot (*Cercospora arachidicola* Hori.) (Green and Wynne 1986) and late leaf spot (*Cercospora personatum* [Berk. & M.A. Curtis] Deighton) (Aquino et al. 1995), flax to flax rust (Rashid 1991), winter wheat to *Septoria nodorum* Berk., (Griffiths and Jones 1987) and in sorghum (*Sorghum bicolor* [L.] Moench.) to *C. graminicola* (Ces.) G.W. Wils. (Casela et al. 1993).

2.3.2.1. Assessment of incomplete resistance

The assessment of resistance in the field is based on either measuring disease severity on plant parts at one particular period which is assumed to be the peak, or frequent measurements of the disease at several time intervals over the growing season or during the epidemic (Parlevliet 1985). In the former, comparisons between cultivars are made based on the amount of disease occurring on the different cultivars, and this measurement is assumed to represent the cumulative result of all resistance components (Parlevliet and Van Ommeren 1975). Frequent disease measurements allow the calculation of the apparent infection rate (r), Vanderplank (1963, 1968) or area under the disease progress curve (AUDPC) values (Wilcoxson et al. 1975) which are then used to compare different cultivars.

The r -value is the least suitable parameter for describing slow rusting or partial resistance (Rees et al. 1979) as it depends not only on resistance but also on growth stage (Shaner and Finney 1977). Although the r -value affects the slope of the disease progress curve, the amount of disease occurring depends on both the rate and duration of the epidemic and the subsequent yield losses are proportional to the epidemic duration. The AUDPC takes into account the rate and duration of the epidemic and has therefore, been found to be a better parameter for assessing resistance and is the most frequently used (Wilcoxson et al. 1975, Shaner and Finney 1977, Johnson and Wilcoxson 1978, Skovmand et al. 1978, Conner and Bernier 1982, Rashid and Bernier 1986, Knudsen et al. 1986, Xi

et al. 1991, Casela et al. 1993). Wilcoxson et al. (1975) found that the AUDPC was more reliable and convenient than the *r*-value as the differences between wheat cultivars infected with *Puccinia graminis* f.sp. *tritici* were consistent from one trial to another.

2.3.2.2. Components of resistance

The factors that affect the rate of development of an epidemic may operate before or after host penetration and formation of infection hyphae although most factors influencing the pre-penetration phase are not well known (Parlevliet 1989). Most rate reducing factors, however, occur after penetration and the formation of infection hyphae. The rate of the epidemic development is reduced when for example, a given initial inoculum concentration, produces fewer lesions and/or lesions that sporulate later, and/or sporulate less (Parlevliet 1985). In most studies, the components of resistance evaluated include one or more of the following; incubation period, latent period, receptivity, sporulation and the infectious period.

2.3.2.2.1. Incubation and latent periods

Incubation and latent periods have often been defined as the time in days from deposition of inoculum to the development of symptoms and to 50% sporulation (or to first sporulating lesion), respectively (Rashid 1991, Aquino et al. 1995). Incubation and latent periods are closely correlated (Parlevliet 1979) and the main factors influencing these two components are temperature and age of the host tissues (Tomerlin et al. 1983, Nutter and Pederson 1982, Thompson and Jenkins 1985c) and leaf wetness duration (Pedersen and Morrall 1994). Parlevliet and van Ommeren (1975) found that barley cultivars infected with leaf rust showed the greatest differences in latent period at heading when the flag leaf had the longest latent period but decreased on lower leaves. In lentil, infected with *Ascochyta fabae* Speg. f.sp. *lentis*, incubation and latent periods decreased with increasing temperature

between 10-20°C (Pedersen and Morrall 1994). Since both components measure and reflect the differences in the growth rate of the pathogen, and are also strongly correlated, only one of these components need be measured.

Latent period is difficult to measure in large plots and in some cases it is unreliable when moisture is limiting especially in pathogens like *Pyrenophora tritici* Drechs. f.sp. *teres* Smedeg. (anamorph *Drechslera teres* [Sacc.] Shoemaker f.sp. *teres*) the cause of net blotch of barley that require moisture periods of sufficient durations for sporulation to occur (Nutter and Pederson 1985). In such cases incubation period may provide a measurement comparable to latent period. Latent period was found to be the most important and reliable component of resistance in barley leaf rust (Parlevliet and Van Ommeren 1975) and was correlated to AUDPC (Johnson and Wilcoxson 1978, Aquino et al. 1995). Casela et al. (1993) evaluated the length of the latent period to development of anthracnose in several cultivars of sorghum under greenhouse conditions and found that cultivars which were most susceptible had the shortest latent periods. Among the components of rate-reducing resistance to *Uromyces vicia-fabae* on faba beans, latent period was one of the components yielding consistent results in both greenhouse and field evaluations (Bhalla 1988). Ali (1985) found that incubation period in faba bean to ascochyta blight (*Ascochyta fabae* Speg.) was negatively correlated with other components studied in the growth room but not in the field. It was concluded that incubation period was a reliable measurement for evaluating resistance with little variation between inoculation dates in the field and growth room.

2.3.2.2.2. Receptivity

Receptivity is evaluated by counting pustules or lesions per plant or per unit area and has been previously defined as the number of pustules or lesions resulting in sporulation (Parlevliet 1979). Receptivity, also known as infection frequency, infection density, infection efficiency or simply lesion

number varies with host line and age, and environmental conditions (Johnson and Wilcoxson 1979). The differences in receptivity in barley cultivars to leaf rust varied with the development stage (Parlevliet and Kuiper 1977) and with leaf wetness duration (Nutter and Pederson 1985). Zheng and Sutton (1994) found that receptivity to *Diplocarpon earlianum* (Ell. & Ev.) Wolf (anamorph *Marssonina fragariae* [Lib.] Klebahn) on strawberry (*Fragaria x ananassa* Duchesne) leaves increased with leaf age, leaf wetness duration and with increases in temperature from 10-30°C. Lesions on soybean were found to increase with temperature in response to infection by *C. truncatum* (Khan and Sinclair 1991). Other studies have also reported receptivity to increase with leaf wetness duration and temperature (Evans et al. 1992, Mathieu and Kushalappa 1993). In the study of Pedersen and Morrall (1994), the number of lesions/plant in lentil increased with increasing temperature between 10-15°C and thereafter decreased at higher temperatures in response to infection by *Ascochyta fabae* f.sp. *lentis* .

2.3.2.2.3. Lesion size

Lesion size also reflects the growth rate of the pathogen and has previously been evaluated by measuring lesion length or width as in net blotch of barley (Nutter and Pederson 1985) and ascochyta blight of lentil (Pedersen and Morrall 1994); lesion diameter as in late leaf spot of peanut (Aquino et al. 1995); lesion area as in early leaf spot of peanut (Green and Wynne 1986), *Septoria nodorum* in wheat (Griffiths and Jones 1987) and alternaria leaf blight in muskmelon Evans et al. 1992); or infection type as in net blotch of barley (Steffenson and Webster 1992) and flax rust (Rashid 1991). Lesion size or length was suggested to be an appropriate measure of cultivar resistance in maize anthracnose (*C. graminicola*) (Nicholson and Warren 1976) but was also found to be significantly influenced by light intensity and temperature (Poneleit and et al. 1972, Leonard and Thompson 1976, Schall et al. 1980). Lesion size on cucumber anthracnose (*C. Lagenarium*) was also significantly

affected by temperature and leaf age (Thompson and Jenkins 1985c). Lesion size in soybean anthracnose (*C. truncatum*) increased with temperature up to 30°C and thereafter declined (Khan and Sinclair 1991).

Green and Wynne (1986) found all components involved in peanut to early leaf spot measured in the greenhouse, including lesion size to be correlated with those measured in the field and concluded that it was possible to evaluate and select for resistance in the greenhouse to develop resistant lines for the field. Nutter and Pederson (1985) suggested that the level of resistance in barley genotypes to *Pyrenophora tritici* f.sp. *teres* could be increased by selecting genotypes that restricted lesion size and receptivity. Griffiths and Jones (1987) however, found that lesion size was of no value in predicting cultivar resistance to *Septoria nodorum* in spring and winter wheat. In peanut, the incompletely resistant peanut cultivars reacted by producing significantly smaller lesions in response to infection by late leaf spot compared to the susceptible cultivars (Chiteka et al 1988).

2.3.2.2.4. Sporulation

Sporulation is expressed in various ways including spore production per unit leaf area (Green and Wynne 1986, Rashid 1991), spore production per unit lesion area or spore production per unit area of sporulating surface (Lancashire and Jones 1985, Griffiths and Jones 1987), or number of lesions sporulating (Ali 1985, Aquino et al. 1995) which can then be expressed as a percentage of the total number of lesions per plant or unit area.

Sporulation is affected by other components of resistance such as receptivity, latent period and the length of sporulation and their interactions (Parlevliet 1979, 1989, Neervoort and Parlevliet 1978) and by host line, plant part and age (Kochman and Brown 1975, Tomerlin et al. 1983), temperature and moisture (King et al. 1997, Thompson and Jenkins 1985c). Sporulation in cucumber anthracnose (*C. lagenarium*) increased with increasing free moisture while older leaves sporulated more than

younger leaves (Thompson and Jenkins 1985c). Sporulation was found to increase with increasing temperature above 15°C in strawberry in response to three *Colletotrichum* species *C. gloeosporioides*, *C. acutatum* Simmonds and *C. fragariae* Brooks (King et al. 1997). Wheat and oat cultivars inoculated with *P. recondita* f.sp. *tritici* and *P. graminis* f.sp. *avenae*, respectively, had greater total spore production at heading than at seedling stages while sporulation was higher at lower temperatures (Tomerlin et al. 1983).

Since measurements of sporulation can extend over long periods of time, precise measurements are often difficult. In some cases the difficulties encountered in measuring sporulation require that this component be estimated by lesion size assuming a close correlation of sporulation to lesion size (Parlevliet 1985). Slow mildewing in wheat was attributed to low receptivity and low spore production under low inoculum showing that low spore production limited disease development (Shaner 1973). Among the components measured, Griffiths and Jones (1987) found that average sporulation per lesion could be used with more confidence for predicting resistance in the field to *S. nodorum* in winter and spring wheat cultivars. Partially resistant flax cultivars to flax rust had reduced sporulation and receptivity compared to susceptible cultivars (Rashid 1991).

2.3.2.2.5. Infectious period

The infectious period is the period during which new infectious propagules are produced by the pathogen. Infectious period or the duration of sporulation is also affected by other resistance components, host genotypes and age. Tomerlin et al. (1983) found that spore production per day on wheat infected with *P. recondita* was higher on seedlings than on adult plants although total spore production was greater on adult plants due to a longer infectious period. Uredinia of the cereal rusts sporulate over extended periods although most spores are produced in the early phase of the infectious period (Neevoort and Parlevliet 1978). The infectious period, similar to sporulation, shows a negative

correlation with receptivity. Parlevliet (1985) stated that the influence of shorter infectious periods on partial resistance is probably small compared with the effect of other components because the period over which the epidemic develops is often not much longer than the infectious period of an individual pustule.

2.3.2.2.6. Correlations among components

Many studies have shown that incomplete resistance is characterized by one or more of the following; reduced receptivity, longer incubation and/or latent periods and less sporulation (Johnson and Wilcoxson 1978, Neevoort and Parlevliet 1978, Nutter and Pederson 1985, Rashid 1991, Casela et al. 1993, Aquino et al. 1995). These components have been shown to vary in association with other components and to be affected by environmental factors. Some components show strong positive or negative correlations among themselves. Johnson and Taylor (1976) suggested that the total spore production was a measure of the total effect of all components of host resistance, but Parlevliet (1979) stated that latent period was a better component for evaluating resistance since a long latent period is usually correlated with low receptivity, short infectious period and reduced sporulation. Variations in partial resistance were largely explained by variations in latent period in barley to leaf rust (Parlevliet and Van Ommeren 1975, Neevoort and Parlevliet 1978).

In sorghum anthracnose (*C. graminicola*), latent period in the greenhouse was significantly correlated to partial resistance in the field (Casela et al. 1993). Latent period and the maximum number of lesions that sporulated were the components that were correlated to late leaf spot development in peanut (Aquino et al. 1995). Green and Wynne (1986) found that sporulation per leaf in early leaf spot of peanut was significantly and negatively correlated with the predicted time required to reach a standard lesion number or receptivity in the field. Also, necrotic area per leaf was correlated to sporulation per leaf. Aquino et al. (1995), showed that the maximum number of lesions sporulating

and latent period were the components most highly correlated to resistance of late leaf spot in peanut. It is difficult to determine the contribution of individual components of resistance (Parlevliet 1985) because components tend to vary in association. If a component acts independently, it is still difficult to evaluate it as other components may still have to be evaluated (Johnson and Taylor 1976).

2.3.2.3. Inheritance and uses of incomplete resistance

In many *Colletotrichum* spp. resistance has been found to be incomplete e.g. in cucumber to *C. lagenarium* (Thompson and Jenkins 1985a,b), in tomato to *C. coccodes* (Miller et al. 1984) and in lentil to *C. truncatum* (Bernier et al. 1992). The inheritance of this type of resistance to *Colletotrichum* spp. has been found to be quantitative or controlled by several genes in some cases such as tomato to *C. coccodes* (Miller et al. 1984) or controlled by single genes in other cases such as lentil to *C. truncatum* (L. Buchwaldt and C.C. Bernier, unpublished). The inheritance of forms of incomplete resistance that are described as partial resistance or slow rusting is quantitative in nature (Parlevliet 1985). The number of genes for partial resistance has varied from a few in the maize-*P. sorghi* pathosystem (Kim and Brewbaker 1977), the oat-*P. coronata* pathosystem (Luke et al. 1975), the wheat-*P. graminis* f.sp. *tritici* pathosystem (Skovmand et al. 1978) to many in the barley-*P. hordei* pathosystem (Johnson and Wilcoxson 1979). Generally, partial resistance in various hosts appears to be controlled by a number of genes which act additively and resistance appears more durable (Parlevliet 1981). This type of resistance which is quantitative and controlled by several genes is more difficult to work with than qualitative, monogenic resistance.

Although forms of incomplete resistance have been reported in several pathosystems, the effectiveness of this resistance to control disease under field conditions has been demonstrated in very few cases. Incomplete resistance occurring in most barley cultivars presently grown in western Europe reduced the incidence of barley leaf rust (Parlevliet 1979). Incomplete resistance is also a major

component in the control of common rust of sweet corn (Pataky and Eastburn 1993), late leaf spot of peanut (Aquino et al. 1995), anthracnose of cucumber (Thompson and Jenkins 1985a), tomato anthracnose (Latin 1991, Dillard et al. 1991) and powdery mildew in soft red winter wheat (Pearce et al. 1996).

2.3.2.4. Summary

The problem of anthracnose in lentil is relatively recent in western Canada and little is known about the epidemiology, genetics of resistance and control of this disease. Presently, the resistance identified in a few lentil lines to *C. truncatum* is incomplete as it is in many other diseases caused by *Colletotrichum* species. Whether this resistance will be effective in controlling anthracnose in lentil fields remains to be demonstrated. The current control measures based on the use of crop rotations of 3-4 years and fungicide applications are not adequate and frequent fungicide applications are costly. Information on forecasting anthracnose occurrence in lentil fields and timing of fungicide applications could help to improve the use of fungicides in a more effective and efficient manner and reduce costs but very limited research has been conducted on these aspects. Also, little or no information is available on the effects of various environment variables such as temperature and leaf wetness on anthracnose development and on components of incomplete resistance. Information on these variables could play a significant role in disease forecasting. If lentil cultivars with incomplete resistance become available for controlling anthracnose in the field, they could prove useful in programs that integrate several control measures. The continued search for new sources of resistance with a view of developing lentil cultivars with complete or higher levels of incomplete resistance would benefit lentil production in western Canada.

3.0. Integrated control of anthracnose of lentil using incomplete resistance and fungicide applications.

3.1. Abstract

Incomplete resistance and fungicide applications were evaluated in 1995 and 1996 for control of anthracnose in lentil (*Lens culinaris*) caused by *Colletotrichum truncatum*. Line 458-57 (resistant) and cv. Eston (susceptible) were planted in fields with low, moderate and high anthracnose pressures. Unsprayed plots were compared to plots receiving a single application of either 1 or 2 kg a.i./ha of chlorothalonil at the 10-12 node stage, and to two applications of 1 kg a.i./ha at the 10-12 node stage and 10 days later. Anthracnose severity in plots not protected with the fungicide was highest (78% for Eston and 62% for 458-57) at high, intermediate (62% for Eston and 46% for 458-57) at moderate and lowest (26% for Eston and 15% for 458-57) at low disease pressure. Yield losses in unsprayed control plots across disease pressures ranged from 26-57% in Eston and 20-28% in 458-57 with the most significant losses occurring in Eston at high disease pressure. Applications of chlorothalonil reduced anthracnose severity and yield losses of both 458-57 and Eston. However, at high disease pressure, anthracnose severity and yield losses in unsprayed plots of 458-57 were lower or similar to those of Eston with a single application of 1 or 2 kg a.i./ha chlorothalonil. Also, under high disease pressure, yield losses of Eston receiving one application of 1 or 2 kg a.i./ha chlorothalonil were significantly reduced as compared to the control whereas those of 458-57 were not. The results suggest that incomplete resistance in 458-57 was effective in reducing disease severity and yield loss when anthracnose level was high and that the effectiveness of incomplete resistance was enhanced by applying a fungicide.

3.2. Introduction

Anthracnose of lentil (*Lens culinaris* Medik.) caused by *Colletotrichum truncatum* (Schw.) Andrus & W.D. Moore has become an important disease in Manitoba (Morrall et al. 1989, 1990). First reported in 1987 (Morrall 1988), anthracnose can cause seed yield losses ranging from 12% to 70% (Morrall and Pedersen 1991, Gibson et al. 1991).

In the field, symptoms are first visible at the beginning of flowering as necrotic lesions on leaves, stems, and later on pods (Gibson 1994, Buchwaldt et al. 1996). Most of the lesions are first confined to the stem bases and lower branches but gradually appear on the upper foliage leading to extensive defoliation, reduced pod filling, stem collapse and death (Gibson 1994). Wet and humid conditions favor high and rapid disease development (Buchwaldt et al. 1992). Unlike *C. truncatum* of soybean (*Glycine max* [L.] Merr.), which is transmitted by seed (Schneider et al. 1984, Sinclair 1991), seed borne transmission in lentil has not been confirmed, although seed may become infected (Morrall and Pedersen 1991, Gibson 1994). The source of inoculum in lentil is mainly conidia and sclerotia from stubble, which are dispersed by wind and rain help to disperse the inocula around fields (Buchwaldt et al. 1996)

The most desirable control measure would be use the of resistant cultivars, but none are available at present. Indianhead, a small black seeded cultivar is incompletely resistant, but the seed color and size are undesirable. Cultivars with incomplete resistance can be infected but compared to susceptible cultivars, disease development and spread is slower and initially lesions are fewer and perhaps smaller than those on the susceptible cultivars. By the end of the season disease severity can be as high as in the susceptible cultivar.

Some measure of control can be achieved by the use of a protective foliar fungicide chlorothalonil (Bravo 500, ISK Biosciences, London, Ontario) in combination with crop rotations. Lower yield losses were reported in fungicide treated cultivars Laird and Eston compared to unsprayed

plots (Morrall et al. 1990). Combining incomplete resistance with fungicide applications in other crops has shown that the amount of fungicide applied may be substantially reduced (Thompson and Jenkins 1985a, Pataky and Eastburn 1993) without subsequent yield loss. The discovery of incomplete resistance to anthracnose in lentil (Bernier et al. 1992) raised the question of whether it would be adequate to reduce disease severity and whether incompletely resistant lines would benefit from fungicide application.

The objectives of this study were to evaluate the combined use of incomplete resistance with applications of chlorothalonil for control of anthracnose and to assess the yield losses resulting from anthracnose at different disease pressures in the field.

3.3. Materials and methods

Site selection. Field studies were conducted in five and four sites in 1995 and 1996, respectively, in Manitoba. Sites were established in fields considered likely to have low, moderate and high anthracnose on the basis of the years between lentil crops in the rotations, and the occurrence of anthracnose in previous crops. Thus, fields never cropped to lentils were selected as sites with low anthracnose pressure since it was expected that they would have little or no inoculum. Sites expected to have moderate anthracnose pressure were fields which had a lentil crop with anthracnose 3-4 years before the trial. Sites expected to have high anthracnose pressure had a lentil crop with anthracnose 1-2 years before the trial. The inclusion in the study of sites with low anthracnose pressure would provide information about yield loss differences between a susceptible and an incompletely resistant line in the presence of little or no anthracnose. All the sites were established in fields which were considered not likely to have other diseases that would affect lentil such as root rots, ascochyta blight (*Ascochyta fabae* Speg f.sp. *lentis* Gossen et al.) and *Sclerotinia* (*Sclerotinia sclerotiorum* [Lib.] de Bary).

Plant materials and experimental design. Line 458-57 (Indianhead x Eston) previously identified to have incomplete resistance to anthracnose (Bernier et al. 1992) and the susceptible cv. Eston (Appendix 2.0) were sown in five locations in 1995. In 1996 sowing was repeated at three of the locations used in 1995 and one new location was included. In each of the two years, Eston and line 458-57 were planted in a split-plot design with four replicates at each site. Line 458-57 and Eston were main-plots while fungicide treatments with chlorothalonil were sub-plot factors. The sub-plot treatments were as follows: no fungicide, one application of 1 kg a.i./ha, one application of 2 kg a.i./ha and two applications of 1 kg a.i./ha. The initial fungicide treatments were applied when plants were at the 10-12 node stage, and second applications of the split application were applied 10-14 days later.

Planting. Seeding was done using a six row seeder at seeding rates of 40 seeds/m row at 2-5 cm depth. Each plot consisted of eighteen 5 m rows with 18 cm spacing between rows and 1-1.5 m between plots. In 1995, the sites were sown on the following dates: May 11 at Carman, May 18 at Rosenort, May 15 at Bagot, May 17 and 18 at different locations west of Portage la Prairie. In 1996 the sites were sown on the following dates: May 13 at Carman, May 16 at Miami, May 28 at Bagot and May 30 at Rosenort (planting at the last two locations was delayed because of excessive soil moisture). Seed was treated with *Rhizobium leguminosarum* (Nitragin Co. Milwaukee, Wisc.) at planting.

Weed control was achieved by applying Sencor (Metribuzin, Chemagro Ltd.) and Poast (Sethoxydim, BASF) at the recommended rates to control broad leaved and grassy weeds, respectively. Hand weeding was done to control persistent weeds. Fertilizer was applied at each site following recommendations made from soil tests before planting.

Disease rating. Four weeks after planting, ten plants from each plot were randomly sampled and rated for frequency, size and depth of lesions on the stems. Three additional samples were taken bi-weekly approximately at early flower, full flower, and pod filling stages. Infections on stems were rated using a 0-3 scale modified from that of Graham et al. (1976) as follows; 0 = no lesions, 1 = few small superficial lesions, 2 = deep lesions not girdling the stem and 3 = several extending lesions girdling the stem. Percent disease severity (DS) per plot was then calculated using the following formula:

$$\%DS = \frac{\Sigma[\text{No. of plants/scale} \times \text{Scale value}]}{[\text{Highest scale} \times \text{Total no. of plants}]} \times 100$$

[1]

The area under the disease progress curve (AUDPC) values per plot were also calculated from the disease severity data by using the formula by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^n [(Y_{i-1} + Y_i) \times 0.5] [T_i - T_{i-1}]$$

[2]

Where Y_i = anthracnose severity at the i^{th} observation, T_i = time in days of the i^{th} observation, and n = total number of observations.

Harvesting. When lower pods turned brown, plants were desiccated with Reglone (diquat, ICI Chipman) applied at the recommended rate. One week after desiccation, plots were harvested using a Hege combine (Model 125 B, Germany). Each plot was harvested separately, and after cleaning, seed was weighed at an average of 10.5% moisture content (Moisture Meter Model 919/3.5, Canadian Aviation Electronics). Seed yield per plot was converted to kg/ha. Yield losses were expressed as the

percent reduction in weight of unsprayed and plots treated with a single application of 1 or 2 kg a.i./ha chlorothalonil compared to the highest yields obtained from plots protected with two applications of 1 kg a.i./ha of chlorothalonil.

Analysis. Analysis of co-variance (ANCOVA) was performed on seed yield and final disease severity using the general linear models (GLM) to adjust yield for disease severity and to reduce the error variances. Analysis of variance (ANOVA) was used on anthracnose severity and AUDPC values. Treatment mean comparisons for seed yield, disease severity and AUDPC values were performed using the least significant difference (LSD). All analyses were done using the Statistical Analysis System (SAS Institute Inc., Carry N.C.).

Initially each site was analyzed separately. Then Bartlett's test was performed on site error variances for each variable (yield, disease, AUDPC) to determine whether they were homogenous and so allow pooling of data over sites and years. The nine sites were combined in groups of three reflecting the three disease pressure groups. Each disease pressure group included one site in 1995 and two sites in 1996 or vice versa.

Linear regressions were performed on yield and final disease severity means of combined sites and years for each disease pressure category and for line 458-57 and Eston at each disease sampling time. Regressions of yield on final disease severity produced the best relationships between yield and anthracnose severity. Inspection of residuals and their scatter plots as well as standardized residuals showed normal and homogeneous variances for yield.

3.4. Results

Anthracnose severity. All the interactions involving the line/cultivar were not significant for disease severity at each level of anthracnose pressure but the line/cultivar and fungicide main effects were all

significant at all disease pressures (Appendix 3.0.). The disease co-variate was not significant either but was retained in the model for the purpose of reducing yield error variances. Anthracnose was initially recorded at the second sampling date at all sites in line 458-57 and Eston (Fig. 3.0), but at this time, significant differences in disease severity were present only at high disease pressure. The differences in severities at moderate and low disease pressures became apparent, respectively at the eighth and tenth week after seeding. Lesions were more numerous, larger and deeper on Eston than on 458-57. Although the low disease pressure sites had no previous cases of anthracnose or lentil production, up to 26% disease severity occurred in Eston and 15% in line 458-57 in the unsprayed plots (Table 3.0). At moderate disease pressure the maximum anthracnose severities were 46% in 458-57 and 62% in Eston (Table 3.1) while maximum anthracnose severities at high disease pressure were 62% in line 458-57 and 78% in Eston (Table 3.2). Disease severity values in the unsprayed plots of 458-57 were therefore, lower than those of Eston, showing a positive effect of incomplete resistance in reducing anthracnose severity on its own (Table 3.0-3.2). The resulting disease progress curves of line 458-57 were lower than those for Eston at each fungicide rate and at all three disease pressures (Fig 3.0). Maximum AUDPC values at each site for both Eston and 458-57, occurred in unsprayed plots and were lowest in the split fungicide application (Table 3.0-3.2).

Anthracnose severity was significantly lower in both 458-57 and Eston when the fungicide was applied except in the case of line 458-57 at low disease pressure. The lowest disease severities occurred when two applications of 1 kg a.i./ha of chlorothalonil were used. There were no significant differences in severities when a single application of either 1 or 2 kg a.i./ha was used within 458-57 and Eston at all three disease pressures except in Eston at high disease pressure (Table 3.2).

Seed yield. The line/cultivar x fungicide interaction for yield was significant ($p=0.001$) only at high disease pressure (Appendix 3.0.). This indicated that there was a major difference between the 458-57

and Eston and that this difference was affected by levels of fungicide treatments used at high disease pressure.

The highest yields for both Eston and 458-57 were obtained under low disease pressure and ranged from 1861-2513 kg/ha for Eston and 2039-2559 kg/ha for 458-57 (Table 3.0). At moderate disease pressure the yields were intermediate and ranged from 1429-2018 kg/ha in Eston and 1584-2202 kg/ha in line 458-57 (Table 3.1). The lowest yields occurred at high disease pressure and ranged from 715-1651 kg/ha in Eston and 1290-1700 kg/ha in line 458-57 (Table 3.2). Within Eston or 458-57 the yields in fungicide treated plots were significantly different from those in untreated plots, except those from plots receiving 1 kg a.i./ha chlorothalonil in line 458-57 at low disease pressure (Table 3.0-3.2). Regardless of the disease pressure level, the highest yields in 458-57 and Eston were obtained in plots treated with two applications of 1 kg a.i./ha of chlorothalonil and were significantly different from all other treatments (Table 3.0-3.2). The yields of Eston and 458-57 treated with a single application of 1 or 2 kg a.i./ha chlorothalonil were not significantly different except in 458-57 at low disease pressure (Table 3.0-3.2).

For both 458-57 and Eston, yield losses were highest in plots which were not protected with the fungicide and were 20, 28 and 24% for 458-57 and 26, 29 and 57% for Eston at low, moderate and high disease pressure, respectively (Table 3.0-3.2). Compared to unsprayed plots, yield losses significantly decreased with fungicide applications at all three disease pressures for both 458-57 and Eston (Table 3.0-3.2). At low and moderate disease pressure, yield losses of 458-57 and Eston in plots protected with 1 kg a.i./ha chlorothalonil were significantly reduced. At high disease pressure the losses in unprotected plots of both Eston and 458-57 were significantly reduced but those of 458-57 (24%) were lower than Eston (57%) showing a positive effect of incomplete resistance in reducing yield loss (Table 3.2). In addition, yield losses in plots of Eston protected with a single application of 1 or 2 kg a.i./ha chlorothalonil were significantly reduced whereas those for 458-57 were not (Table

3.2), showing the combined effect of the incomplete resistance in 458-57 and fungicide in reducing anthracnose severity and yield losses. The magnitude of yield loss in the untreated plots of 458-57 (24%) was about equal to the yield loss of Eston (19%) receiving a single application of 2 kg a.i./ha and much lower than Eston (39%) receiving one application of 1 kg a.i./ha chlorothalonil (Table 3.2).

Table 3.0. Anthracnose severity and seed yield in the lentil cultivar Eston and line 458-57 in field plots with no history of anthracnose (low disease pressure).

Fungicide (Bravo 500)	Eston		458-57		Eston		458-57	
	DS (%)	AUDPC	DS (%)	AUDPC	Yield (kg/ha)	Yield [‡] loss(%)	Yield (kg/ha)	Yield [‡] loss (%)
Control	26 a	285 a	15 a	162 a	1861 a	26 **	2039 a	20 **
[†] 1 kg a.i./ha	18 b	206 b	12 a	131 b	2254 b	10 *	2191 a	14 *
[†] 2 kg a.i./ha	17 b	201 b	12 a	111 b	2402 bc	4 ns	2373 b	7 *
[†] 1+ [‡] 1 kg a.i./ha	8 c	102 c	6 b	59 c	2513 c	0	2559 c	0

Means with the same letter within each column are not significantly different, LSD=0.05.

[†]Applied at 10-12 node stage and [‡]10-14 days later.

[‡]Percent yield difference of the split application.

*, ** significant at p= 0.05, p=0.01. ns=non significant.

Table 3.1. Anthracnose severity and seed yield in the lentil cultivar Eston and line 458-57 in field plots with a 3-4 year lentil rotation with a history of anthracnose (moderate disease pressure).

Fungicide (Bravo 500)	Eston		458-57		Eston		458-57	
	DS (%)	AUDPC	DS (%)	AUDPC	Yield (kg/ha)	Yield loss (%)	Yield [‡] (kg/ha)	Yield [‡] loss (%)
Control	62 a	1112 a	46 a	706 a	1426 a	29 *	1584 a	28 **
[†] 1 kg a.i./ha	52 b	957 b	37 b	629 b	1745 b	14 **	1969 b	11 **
[†] 2 kg a.i./ha	50 b	913 b	35 b	514 c	1856 b	8 ns	1989 b	10 ns
[†] 1+ [‡] 1 kg a.i./ha	43 c	660 c	25 c	380 d	2018 c	0	2202 c	0

Means with the same letter within each column are not significantly different, LSD=0.05.

[†]Applied at 10-12 node stage and [‡]10-14 days later.

[‡]Percent yield difference of the split application.

*, ** significant at p= 0.05, p=0.01. ns=non significant.

Table 3.2. Anthracnose severity and seed yield in the lentil cultivar Eston and line 458-57 in field plots with a 1-2 year lentil rotation with a history of anthracnose (high disease pressure).

Fungicide (Bravo 500)	Eston		458-57		Eston		458-57	
	DS (%)	AUDPC	DS (%)	AUDPC	Yield (kg/ha)	Yield [‡] loss (%)	Yield [‡] (kg/ha)	Yield [‡] loss (%)
Control	78 a	1527 a	62 a	1005 a	715 a	57 **	1290 a	24 *
[†] 1 kg a.i./ha	72 b	1424 b	49 b	832 b	1014 b	39 **	1565 b	8 ns
[‡] 2 kg a.i./ha	67 c	1215 c	46 b	776 b	1345 c	19 **	1521 b	11 ns
[†] 1+ [§] 1 kg a.i./ha	54 d	913 d	38 c	534 c	1651 d	0	1700 c	0

Means with the same letter within each column are not significantly different, LSD=0.05.

[†]Applied at 10-12 node stage and [§]10-14 days later.

[‡]Percent yield difference of the split application.

*, ** significant at p= 0.05, p=0.01. ns=non significant.

Regression of yield on final disease severity. Linear relationships were established between yield and final anthracnose severities (Fig 3.1.). The coefficients of determination (r^2) between 458-57 and Eston representing the amount of variability in yield attributed to anthracnose severity across the disease pressures ranged from 0.87 to 0.98 (Fig 3.1.). All the intercepts and slopes were significantly different from 0 at $p < 0.05$ when disease pressure was moderate or high in both 458-57 and Eston. However, at low disease pressure, only the intercepts were significantly different from 0 ($p < 0.01$) in both 458-57 and Eston while the slopes were not. Therefore, there was a significant negative effect of anthracnose on yield of both 458-57 and Eston under moderate or high disease pressure which affected Eston more than 458-57.

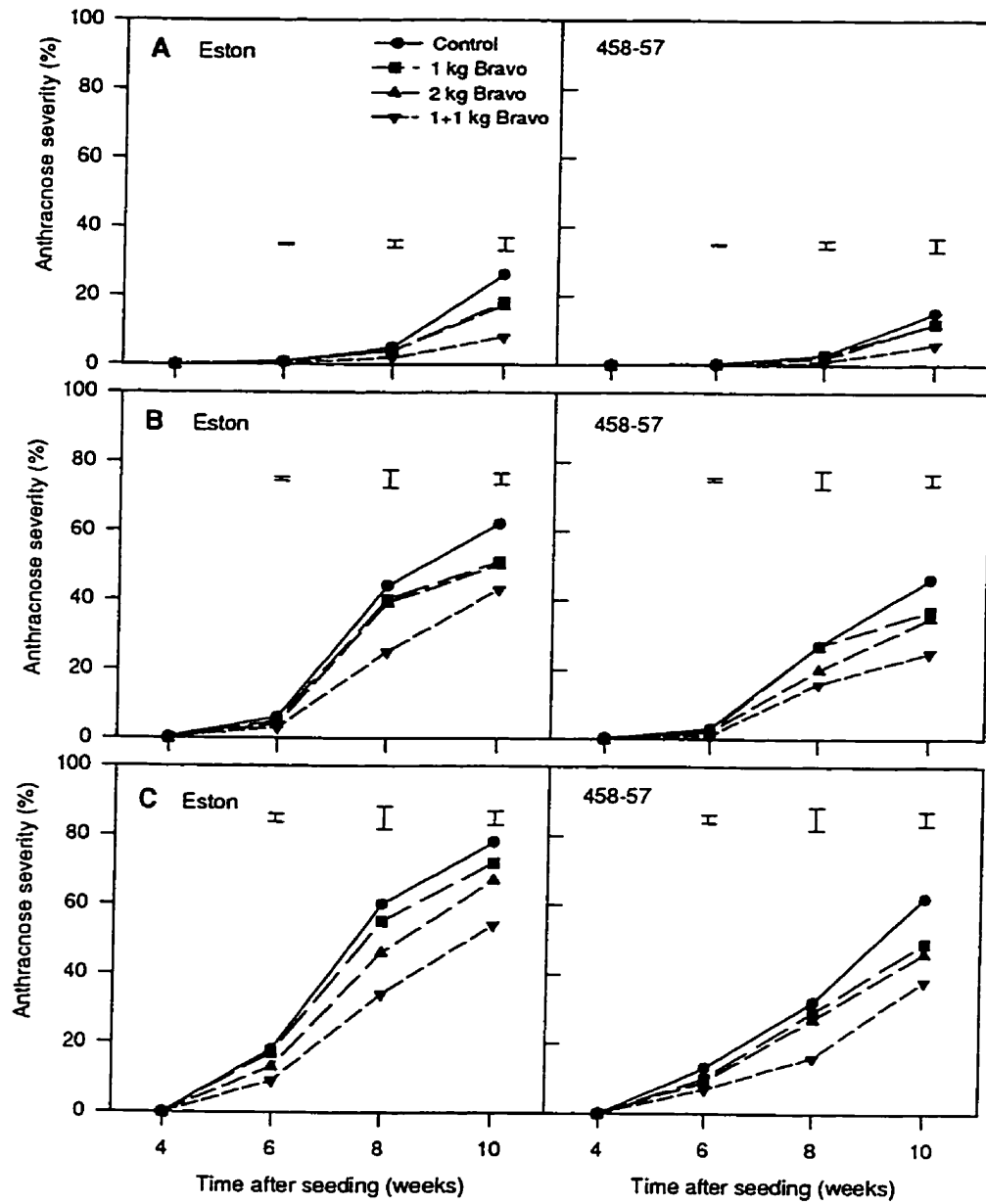


Fig. 3.0. Disease progress curves for (A) low (B) moderate and (C) high anthracnose pressure in the lentil cultivar Eston and line 458-57. Bar=LSD, $p=0.05$.

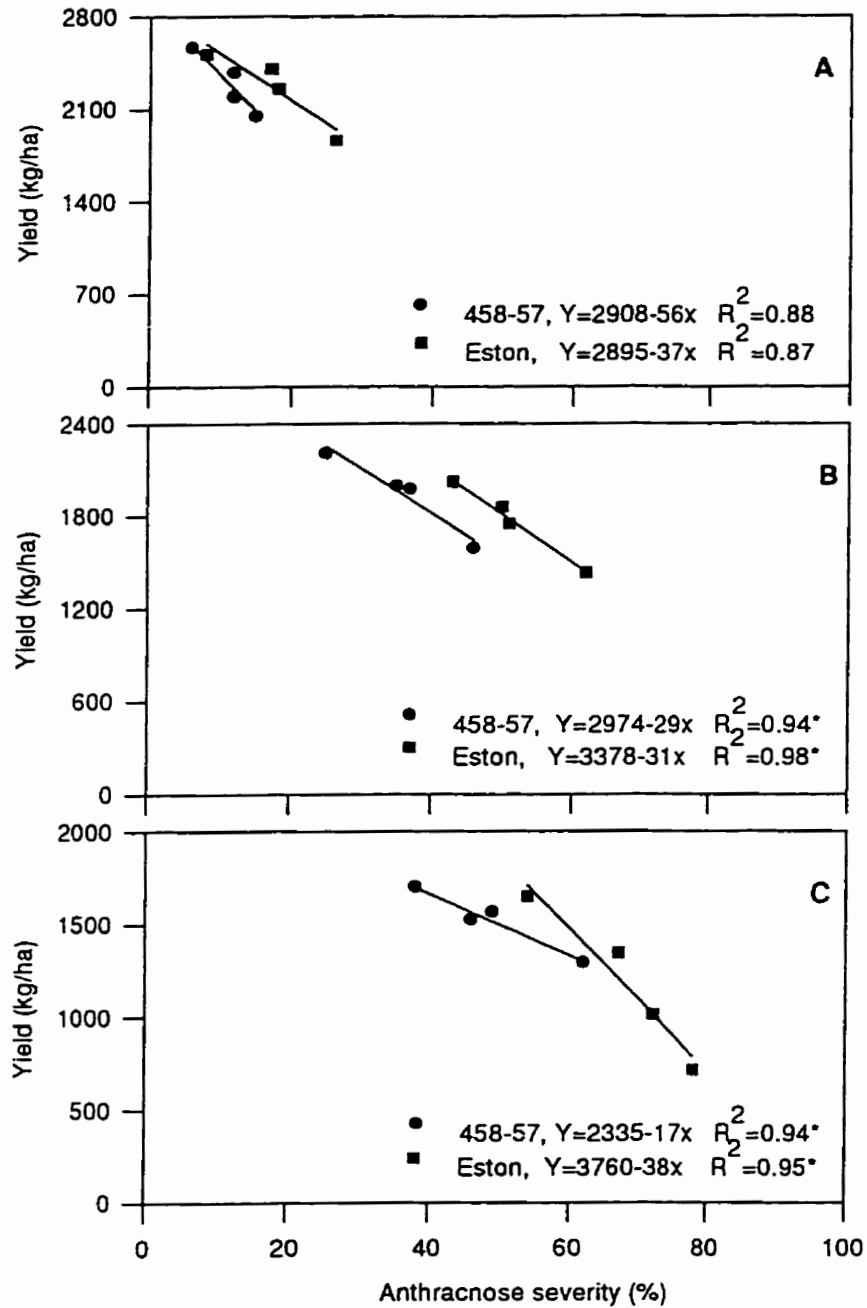


Fig. 3.1. Relationship between seed yield and anthracnose severity in the lentil cultivar Eston and line 458-57 under (A) low (B) moderate and (C) high disease pressure. *Significant at $p=0.05$.

3.5. Discussion

Favorable weather conditions for anthracnose development occurred in both 1995 and 1996. Anthracnose symptoms were first visible on leaves and stems six weeks after planting or at early flowering. Disease progress from the sixth week onwards was faster on Eston where stem lesions were deeper and larger compared to 458-57 which had fewer deep lesions that generally developed much later in the season, and had many superficial lesions. This resulted in much lower disease progress curves in 458-57 compared to the susceptible cultivar Eston. No symptoms were observed at an earlier sampling done four weeks after planting. However, plants may still have been infected but remained symptomless. Gibson (1994) showed that although symptoms were visible at early flowering stage in the field, plants may be infected as early as two weeks from emergence or about three weeks after seeding. Symptomless infections by *Colletotrichum* spp. are common such as for *C. truncatum* in soybean (Sinclair 1991) and *C. coccodes* Wallr. in tomato (*Lycopersicon esculentum* Miller) (Fulton 1948, Cerkauskas 1988).

Chlorothalonil reduced disease severity and decreased yield losses of both Eston and 458-57. However, at any level of fungicide applied, anthracnose severity values on 458-57 were much lower than on Eston due to additional control provided by resistance. Similar results have been reported for common rust (*Puccinia sorghi* Schwein.) in sweet corn (*Zea mays* L.) (Pataky and Estburn 1993) anthracnose (*C. lagenarium* [Pass.] Ell. & Halst.) in cucumber (*Cucumis sativus* L.) (Thompson and Jenkins 1985a) and tomato (Precheur 1990, Latin 1991). In these studies, moderate levels of resistance combined with fungicide applications were found to be more effective in reducing disease development than fungicides applied to the susceptible cultivars and yield losses were proportional to cultivar susceptibility (Thompson and Jenkins 1985b). In the present study, yield loss was directly related to anthracnose severity but the susceptible cultivar Eston was affected more severely, especially at high disease pressure. The yield losses ranging from 20 to 57% obtained in unsprayed

control plots of Eston and 458-57 across the three disease levels are similar to the range of losses reported previously (Morrall et al. 1990, Morrall and Pedersen 1991, Gibson (1994).

Fields with no previous history of anthracnose showed severities of up to 26 % in the susceptible cultivar Eston and 15 % in 458-57. However, the rate of anthracnose progress was much slower than at moderate or high disease pressure. The inoculum for the observed anthracnose at low disease pressure could have been introduced from surrounding lentil fields either through conidia or sclerotia on crop debris dispersed by wind and/or rain before or during the growing season (Buchwaldt et al. 1996). Therefore, when lentils are sown in fields where they have not been grown before it cannot be assumed that anthracnose will not be present. Very likely there will be some inoculum dispersed from surrounding areas, especially in regions of lentil production. However, resulting anthracnose severity and yield losses in such fields are likely to be low in the first year of cropping so that even susceptible cultivars may perform well.

This study showed that under low to moderate anthracnose pressure there was no beneficial effect of incomplete resistance since the yield losses of the 458-57 were equal to those of Eston. This assumes that other diseases were not present, although chlorothalonil does have an added benefit of controlling ascochyta blight (Beauchamp et al. 1986), another important disease of lentil. Under high anthracnose pressure, the effectiveness of incomplete resistance in reducing anthracnose severity and yield losses was better or equal to a single application of 1 or 2 kg a.i./ha chlorothalonil to the susceptible cultivar. The benefit of incomplete resistance is further illustrated in comparing yield losses in plots receiving 1 and 2 kg a.i./ha chlorothalonil at high disease pressure. Yield losses in 458-57 were not significantly reduced whereas those for Eston were significantly reduced and were much higher than losses of 458-57. Therefore, fungicide applications to 458-57 enhanced the incomplete resistance of 458-57 in reducing disease severity and yield losses. This demonstrates for the first time

the usefulness of incomplete resistance to control anthracnose in lentil. The results are encouraging and indicate that efforts to improve the levels of resistance in lentil should continue.

4.0. Effects of host and growth stage, inoculum concentration, leaf wetness duration and temperature on anthracnose of lentil.

4.1. Abstract

The effects of host and growth stage, concentration of conidia, leaf wetness duration and temperature on the development of anthracnose (*Colletotrichum truncatum*) on lentil (*Lens culinaris*) were assessed in growth chamber/greenhouse studies using the cv. Indianhead and line 458-57 with incomplete resistance and a susceptible cv. Eston. Each line/cultivar was assessed for incubation period (IP), latent period (LP), number of lesions (LN) per stem and disease severity (DS). Incubation period and LP decreased linearly with increasing conidial concentration, leaf wetness duration and temperature. Both components became progressively shorter as the plants aged between 2 to 6 weeks and increased between 6 to 8 weeks after seeding. Lesion number and DS increased linearly with inoculum concentration leaf wetness duration and temperature. Lesion number and DS also increased with plant age between 2 to 4 weeks and decreased between 4 to 8 weeks. The optimum growth stage of lentil and temperature required for severe disease development ranged, respectively, from 4 to 6 weeks and 20 to 24°C, when plants were inoculated at a concentration of 4×10^4 conidia/ml and provided with a leaf wetness period of 24 h. Generally, Indianhead and 458-57 had significantly longer IP and LP and lower LN per stem and subsequently DS was lower than that of Eston for each factor.

4.2. Introduction

Anthracoze caused by *Colletotrichum truncatum* (Schwein.) Andrus & W.D. Moore is one of the major diseases of lentil (*Lens culinaris* Medik.) in western Canada. Infection results in lesions on all above ground plant parts usually becoming visible in the field at early flowering and as the disease progresses, plants may wilt and die (Buchwaldt et al. 1996). The occurrence of the disease in Manitoba lentil fields has been on the increase in the past few years mainly due to increasing levels of inocula left in the field which then spread to previously uninfested fields (Buchwaldt et al. 1996).

Anthracoze on lentil can be very severe and result in significant yield reductions (Morrall and Pedersen 1991, Gibson et al. 1991), particularly during high rainfall and warmer temperatures (Buchwaldt et al. 1993, Gibson 1994). Several environmental factors such as temperature, the duration of leaf wetness and inoculum density in addition to host and pathogen genotypes, affect infection and the development of anthracnose diseases. In many *Colletotrichum* spp. disease severity (Makowski 1993) and incidence (Tu 1992) have been shown to increase with inoculum concentration. Disease severity may also increase with increasing temperature (Chakraborty 1990, Khan and Sinclair 1991, Makowski 1993, Gibson 1994) and leaf wetness period (Thompson and Jenkins 1985c).

Lesion size (Thompson and Jenkins 1985c, Khan and Sinclair 1991) and conidial production (Thompson and Jenkins 1985c, King et al. 1997) were reported to increase with temperature. Temperature was found to have a significant effect on lesion number in lentil in response to ascochyta blight while older plant tissues were more resistant than younger tissues (*Ascochyta fabae* f.sp. *lentis* Gossen et. al.) (Pedersen and Morrall 1994). In cucumber (*Cucumis sativus* L.) anthracnose (*C. lagenarium* [Pass.] Eil. & Halst. lesion size and conidial production were found to be greater on older than on younger leaves (Thompson and Jenkins 1985c).

Studies on the effect of environmental factors on anthracnose of lentil are necessary to provide a better understanding of their impact on disease development. Such studies, however, are lacking at

present. Greenhouse and growth chamber conditions offer more accurate alternatives to measuring environmental factors in the field, where monitoring is difficult.

The objective of this study was to investigate the effects of host lines/cultivars and growth stage, inoculum concentration, leaf wetness period and temperature on the development of anthracnose on lentil in the growth chamber/greenhouse.

4.3. Materials and methods

Plant Material. Line 458-57 and cv. Indianhead (incompletely resistant) and the susceptible cv. Eston were used (Appendix 2.0). Seeds of each line/cultivar were planted in 0.5 L plastic pots containing soil, sand and peat in a 2:1:1 (v/v/v) mixture for the studies involving inoculum concentration, leaf wetness duration and growth stage. In the temperature study, Metro-Mix (W.R. Grace and Co. Canada Ltd. Ajax, Ontario) was used as the planting medium in the same type of pots. Eight seeds were planted per pot and seedlings were thinned to five per pot after germination. The pots were kept in the growth chamber, maintained at 20/16°C (day/night) temperatures, 16 h photoperiod and 250 - 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ photon flux density provided by a mixture of cool white fluorescent and Vita-Lite fluorescent lamps (Philips Lighting Co. Somerset NJ., and Duro-test Corp. North Bergen, NJ, respectively). The plants were fertilized weekly with a 20:20:20 (N:P:K) liquid fertilizer .

Inoculum preparation. An isolate of *C. truncatum* (JPPTNL 882) obtained from Portage la Prairie, Manitoba was used in all experiments. For storage purposes a single spore culture was grown on sodium-chloride yeast agar sucrose (SYAS) medium containing 5% sodium chloride, 3 g yeast extract, 20 g agar, 10 g sucrose, (Manandhar et al. 1986), and 10 ml each of the antibiotics chlortetracycline and streptomycin sulphate were added per liter. As the colony expanded on a Petri dish small sterile

5 mm diameter paper discs were placed around the fungal colony. When the colony grew over the discs the discs were peeled and placed in plastic vials, dried under vacuum and stored at -10°C .

Whenever inoculum was required cultures of the isolate from paper discs were raised on SYAS medium at room temperature under continuous white fluorescent light. Conidia from seven day old cultures were scraped from the Petri dishes using a sterile glass slide and suspended in sterile distilled water. The conidial suspension was then filtered through a layer of Mira-Cloth (Calbiochem-Behring Corp., La Jolla, CA.) and conidial concentration determined using a haemocytometer. The conidial suspension was diluted with sterile distilled water to obtain the final concentrations required for each experiment.

Plants were inoculated with 5-6 ml of conidial suspension/pot using a DeVilbiss Atomizer (Somerset, PA). One drop of Tween 20 was added as a wetting agent per 100 ml of conidial suspension before inoculation. Plants were inoculated four weeks after planting (8-10 node stage) except for the growth stage study (see below).

Inoculum concentration study. The effect of inoculum concentration was investigated by inoculating plants with conidial concentrations of 2×10^4 , 4×10^4 , 6×10^4 , 8×10^4 and 1×10^5 conidia/ml. Control plants were inoculated with distilled water to which Tween 20 was added (1 drop/ml). Inoculated plants were placed in an incubation chamber that ensured continuous leaf wetness for 24 h. The incubation chamber was made of a steel frame covered with a clear polyethylene sheet kept at a temperature of approximately 20°C and 16 h photoperiod. Continuous leaf wetness was provided by misting plants using a humidifier. After incubation, plants were kept in the greenhouse at $20/16^{\circ}\text{C}$ (day/night) temperature and 16 h photo period. Plants were arranged on a greenhouse bench as a 3 x 6 factorial in a randomized complete block design (RCBD) with four replicates and three pots of each line/cultivar per replicate. The experiment was repeated twice.

Leaf wetness duration study. Plants were inoculated with a conidial suspension of 4×10^4 conidia/ml and incubated at a continuous leaf wetness for 6, 12, 24, 36 and 48 h as described for the inoculum concentration study. Control plants were not incubated, but were immediately transferred to the greenhouse after inoculation. Incubated plants were kept in the greenhouse under conditions described above and arranged together with control plants as a 3 x 6 factorial in RCBD with four replicates and two pots of each line per replicate. The experiment was repeated once.

Growth stage study. Four growth stages were used: the seedling, 8-10 node, flowering and early pod stage (or 2, 4, 6 and 8 weeks after seeding, respectively). Seeding dates were staggered to inoculate plants of different growth stages at the same time.

At each seeding date, Indianhead was sown first, followed by 458-57 and Eston three and five days later, respectively to synchronize their growth stages based on differences in the time to first flowering. Plants were inoculated with a concentration of 4×10^4 conidia/ml, incubated for 24 h under a continuous leaf wetness and then transferred to the greenhouse where pots were placed as 3 x 4 factorial in a RCBD with four replicates consisting of two pots of each line/cultivar per replicate. The experiment was repeated once.

Temperature study. Plants were inoculated with a concentration of 4×10^4 conidia/ml and incubated for 24 h under a continuous leaf wetness period. Following incubation the plants were kept in different growth chambers at the following temperatures; 16/12, 20/16, 24/20 and 28/24 °C (day/night) each with a 16 h photoperiod and $250-300 \mu\text{mol.m}^{-2}.\text{s}^{-1}$ photon flux density. The experiment was set up as a split-plot design, with temperature as main-plot and line/cultivars as sub-plot factors. There were two pots of each line/cultivar at each temperature. To provide three more replicates the experiment was repeated three times.

Anthracnose rating. In all experiments, stem tissue was used to assess incubation period (IP), latent period (LP), lesion number (LN) and disease severity (DS). Incubation period was defined as the time in days from inoculation to the appearance of lesions on stems and LP as the time in days from inoculation to the formation of acervuli in the lesions. A 10-20X hand lens was used to observe acervuli. Observations for IP and LP were done every day from the third day after inoculation. The number of lesions per stem was determined by counting all visible lesions four weeks after inoculation on the main stem. Disease severity was also determined four weeks after inoculation using a 0-3 rating scale modified from Graham et al. (1976), where 0=stems with no lesions, 1=stems with few superficial lesions, 2=stems with many deep lesions not girdling the stem and 3=stems with many deep coalescing lesions girdling the stem. Percent DS was then calculated using the following formula:

$$\%DS = \frac{\Sigma[\text{No. of plants/scale} \times \text{Scale value}]}{[\text{Highest scale} \times \text{Total no. of plants}]} \times 100$$

[1]

Analysis. The data for IP, LP, LN/stem and DS in the conidial concentration and leaf wetness duration studies were analyzed by regression. Concentration was transformed to the log scale before analysis. The data of the effects of temperature on IP and LP were also analyzed by regression. Scatter plots of residuals with predicted values from regression lines and standardized residuals computed by dividing residuals by their respective standard errors, were visually inspected for possible violations of the assumptions of normal distribution but were found to be normally distributed.

The data for the effects of temperature on LN/stem and DS were subjected to analysis of variance (ANOVA) with orthogonal polynomials and contrasts using the general linear model procedures. Analysis of the effects of growth stage on IP, LP, LN and DS were performed by using ANOVA and mean comparisons were done by using the least significant difference (LSD, $p=0.05$).

Bartlett's homogeneity tests for experimental variances performed on all variables indicated homogenous error variances and therefore, allowed pooling of data over experiments. All analyses were done using the Statistical Analysis System (SAS, Institute Inc., Carry, N.C.).

4.4. Results

Inoculum concentration. The effects of inoculum concentration on anthracnose development are presented as regression of means of incubation period (IP), latent period (LP), lesions number (LN)/stem and disease severity (DS) on log conidial concentration (Fig 4.0.).

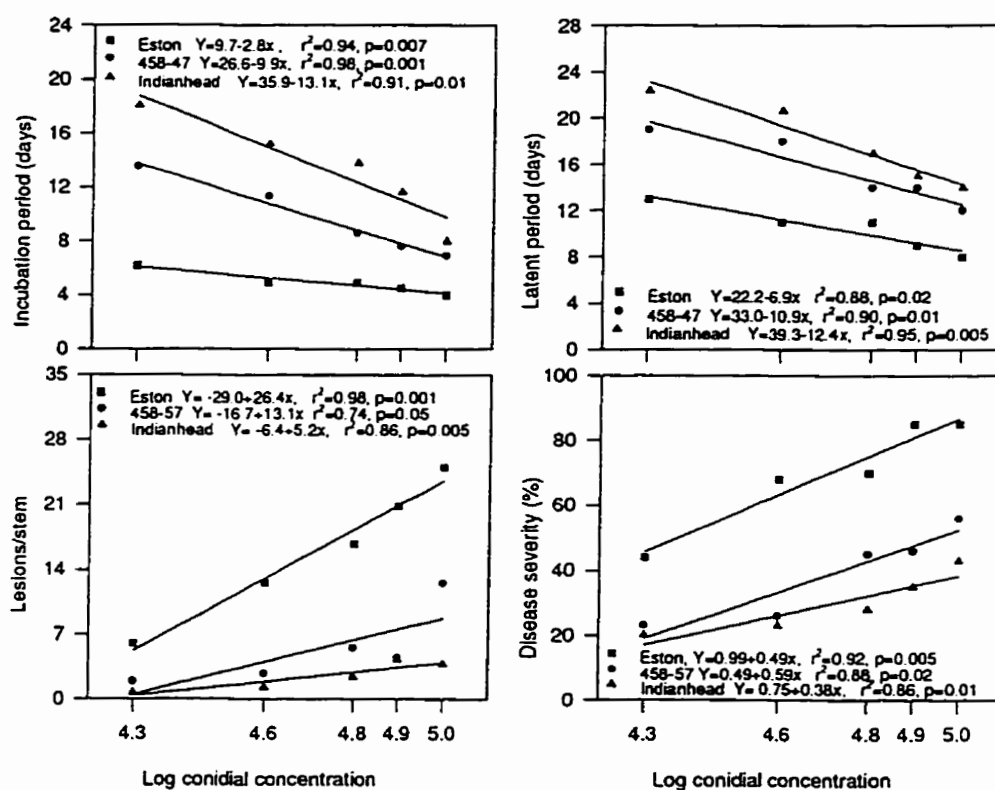


Fig. 4.0. Effect of log conidial concentration on incubation period, latent period, lesion number per stem and disease severity in lentil lines/cultivars inoculated with isolate JPPTNL 882 of *Colletotrichum truncatum*.

The duration of IP and LP decreased linearly with log conidial concentration in Indianhead, 458-57 and Eston but were shortest in Eston, intermediate in 458-57 and longest in Indianhead at each log conidial concentration rate. The coefficients of determination (r^2) ranged from 0.94-0.98 for IP and 0.88-0.95 for LP in Indianhead, 458-57 and Eston and the models were significant ($p \leq 0.05$).

Lesion number per stem and DS on the other hand, linearly increased with log conidial concentration. Eston had the highest LN/stem and was more diseased compared to 458-57 or Indianhead (Fig 4.0.). The r^2 values among the three lines/cultivars ranged from 0.74-0.98 and 0.86-0.92 for LN/stem and DS, respectively, and all the models were significant at $p \leq 0.05$. Therefore, log conidial concentration rate explained most of the variability in IP and LP, LN per stem and DS. Control plants inoculated with distilled water and Tween 20 only showed no symptoms.

Leaf wetness duration. Leaf wetness duration had a significant effect on anthracnose development (Fig 4.1). Control plants not given a wetness period were not infected. When the leaf wetness period was increased from 6 to 48 h, the length of IP for Eston linearly decreased while that for Indianhead and line 458-57 also decreased but were well described by quadratic functions ($p < 0.009$). Latent period for Indianhead, 458-57 and Eston linearly decreased with increase in wetness duration ($p < 0.01$) (Fig. 4.1).

Lesion number per stem was smallest and DS was lowest for all three hosts when leaf wetness was 6 h but both increased linearly as wetness period increased to 48 h ($p < 0.05$) (Fig.4.1). The r^2 values among Indianhead, 458-57 and Eston ranged from 0.92-0.99, 0.79-0.93, 0.92-0.98 and 0.91-0.96 for IP, LP, LN/stem and DS, respectively and were significant ($p < 0.05$). LN per stem was not significantly different among the three hosts when wetness period was 6 or 12 h. However, at each wetness period, Eston had the highest DS.

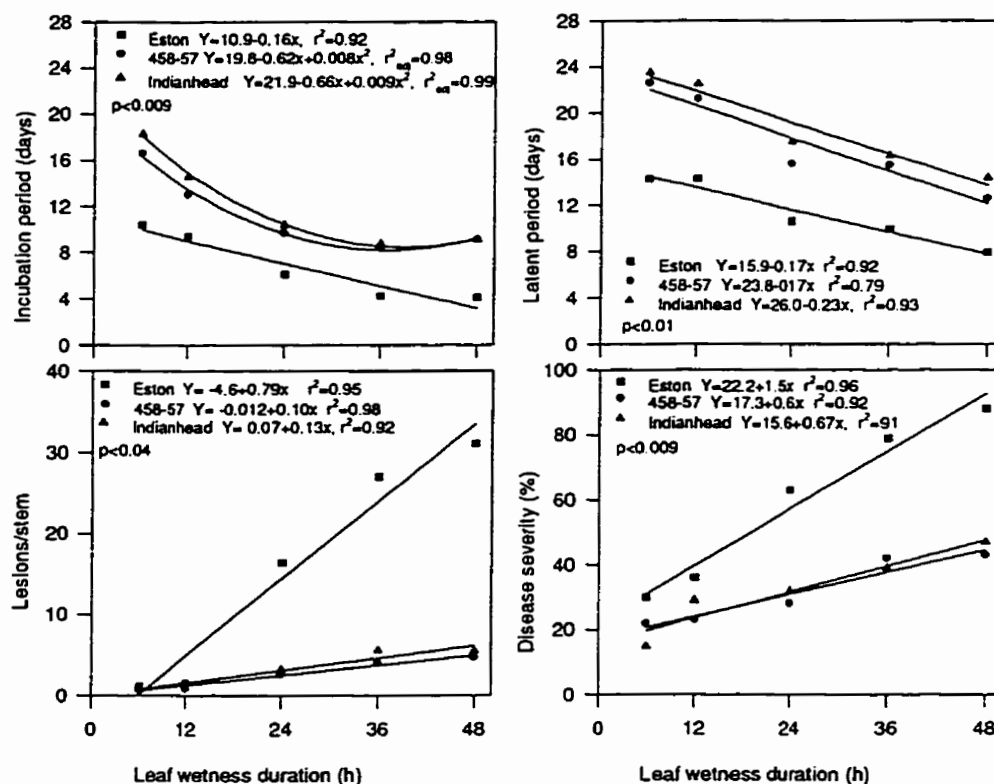


Fig. 4.1. Effect of leaf wetness duration on incubation period, latent period, lesion number per stem and disease severity in three lentil lines/cultivars inoculated with isolate JPPTNL 882 of *Colletotrichum truncatum*.

Growth stage. Growth stages and lines had significant effects on IP, LP, LN per stem and DS ($p=0.0001$) (Appendix 4.0, Fig 4.2). The interactions between lines and growth stages were significant for IP, LP, LN per stem and DS ($p=0.0001$) (Appendix 4.0.), showing that the differences among the host line/cultivars for these factors were significantly dependent on growth stage. Incubation period was longest when plants were 2 weeks old (7.6, 15.8 and 19.9 days for Eston, 458-57 and Indianhead, respectively) (Fig 4.2.).

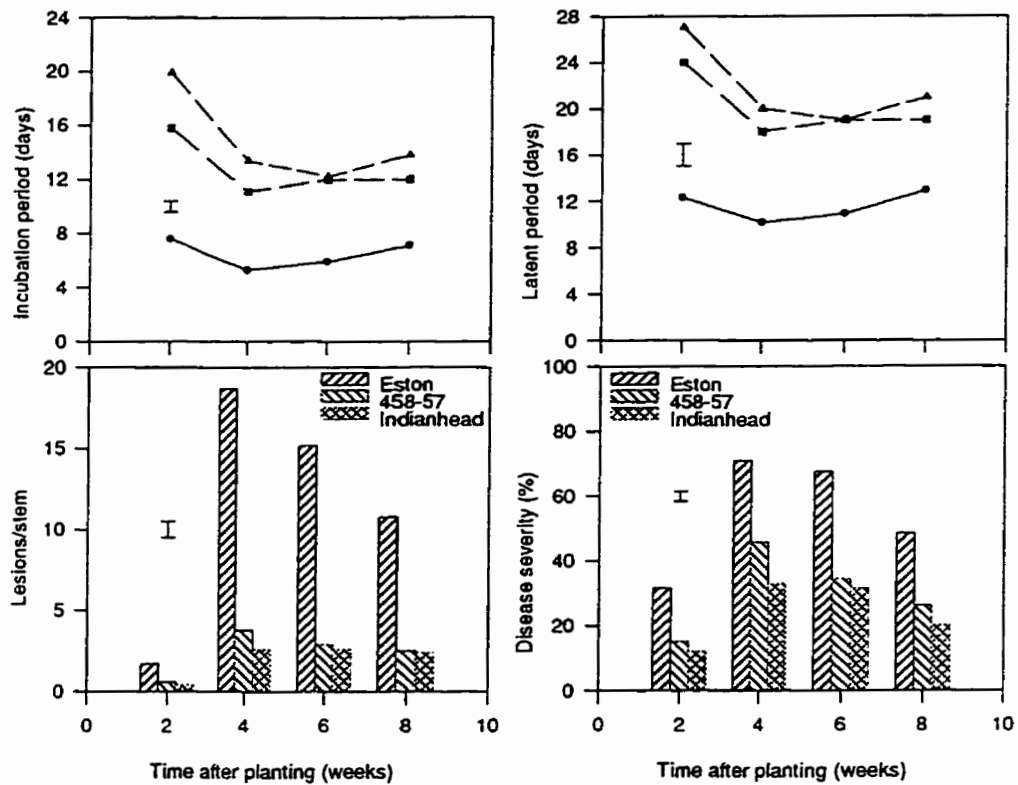


Fig. 4.2. Effect of host growth stage on incubation period, latent period, lesion number per stem and disease severity in three lentil lines/cultivars inoculated with isolate JPPTNL 882 of *Colletotrichum truncatum*. Bar=standard error of the difference.

However, as plants became older, IP decreased reaching the shortest periods at 4 weeks in Eston and 458-57 (5.3 and 11.1 days, respectively) and at 6 weeks in Indianhead (12.2 days). At 8 weeks, IP slightly increased to 7.1, 12 and 13.8 days in Eston, 458-57 and Indianhead, respectively. Similarly, LP initially decreased with plant age from a high of 27, 24 and 13.9 days in Indianhead, 458-57 and Eston, respectively, at 2 weeks to a low of 20 days at 6 weeks in Indianhead, 18 days at 4 weeks in 458-57 and 10.2 days at 4 weeks in Eston. Slight increases of about 2 days in IP and LP between 4-8 weeks in Indianhead and Eston and of only 1 day in line 458-57 were observed at 8 weeks. There were

significant differences for IP and LP at each growth stage ($p=0.05$) especially between the susceptible cv. Eston and the incompletely resistant cv. Indianhead and line 458-57 (Fig 4.2.).

Two week old plants had the smallest LN per stem, but lesions increased in number with plant age reaching the highest levels of 18.7, 3.8 and 2.6 at 4 weeks in Eston, 458-57 and Indianhead, respectively, and then slightly decreased after four weeks (Fig. 4.2.). Disease severity was lowest on 2 week old plants i.e. 31.6% in Eston, 15.1% in 458-57 and 12.4% in Indianhead. Disease severity increased with plant age with maximum levels of 71, 45.8 and 33% occurring at 4 weeks in Eston, 458-57 and Indianhead, respectively, and thereafter decreased (Fig. 4.2.). Eston had significantly higher LN per stem and DS than Indianhead and 458-57 at all growth stages. However, in Indianhead and 458-57 the LN per stem and DS were not significantly different at any growth stage except at 4 weeks.

Temperature. Incubation period and LP were longest at 16°C for Indianhead, 458-57 and Eston but decreased linearly with increasing temperature (Fig 4.3.). The regression of means for IP ($r^2=0.89-0.99$, $p\leq 0.05$) and LP ($r^2=0.82-0.99$, $p\leq 0.05$) on temperature indicated that most of the variability in Indianhead, 458-57 and Eston was explained by the regression lines.

ANOVA and orthogonal polynomials and contrasts showed that temperature (T) and host line/cultivar (L/C) had significant effects ($p\leq 0.001$) on LN per stem and DS (Fig. 4.3, Appendix 4.1.). The linear and quadratic polynomials for LN per stem and only the linear polynomial for DS were significant (Appendix 4.1). For LN per stem, the linear component sum of squares contributed much of the variation indicating that LN per stem and DS also increased linearly with temperature. Since the interaction between T x L/C was also significant ($p\leq 0.01$) for the LN per stem, the sum of squares for T and T x L/C interaction were partitioned into single degree contrast sum of squares (Appendix 4.1) to determine the important polynomials.

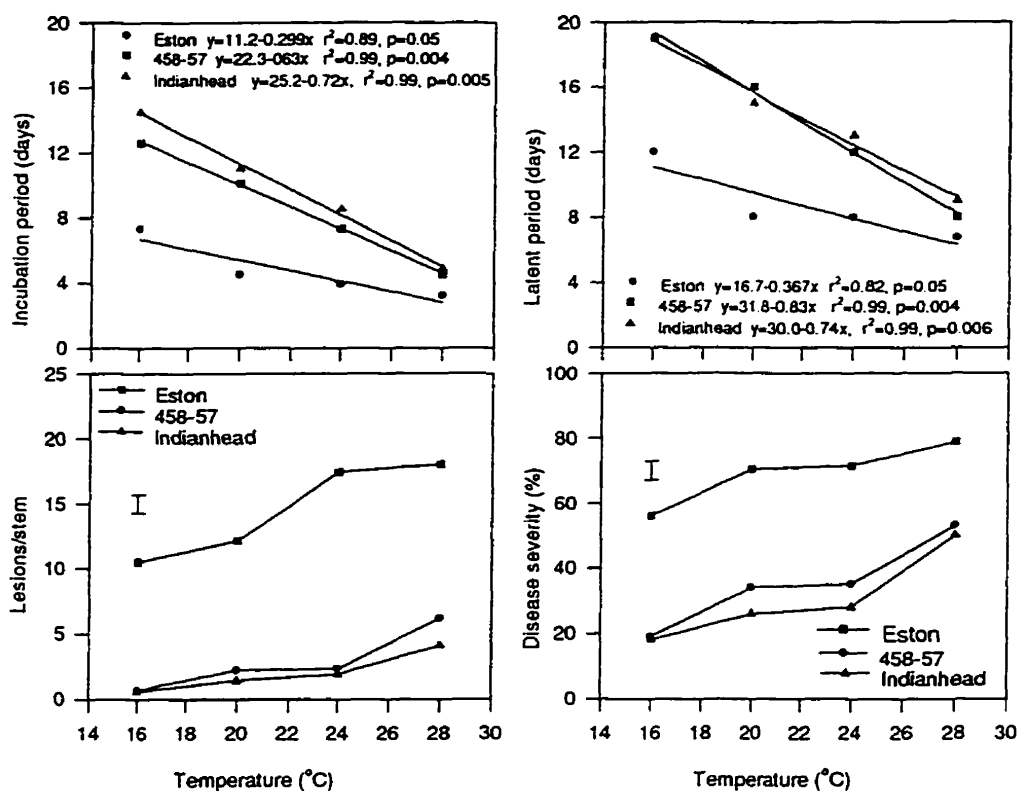


Fig. 4.3. Effect of temperature on incubation period, latent period, lesion number per stem and disease severity in lentil lines/cultivars inoculated with isolate JPPTNL 882 of *Colletotrichum truncatum*. Bar=standard error of the difference.

Although the $T_{\text{quadratic}} \times L/C$ or the $T_{\text{cubic}} \times L/C$ were significant in some cases, it was mostly the $T_{\text{linear}} \times L/C$ that accounted for most of the variability for each line/cultivar (Appendix 4.1), confirming that the number of lesion/stem linearly increased with temperature in the line and the two cultivars.

The LN per stem was lowest at 16°C but increased as temperature was increased to 28°C in all three lines (Fig 4.3.). Eston had significantly higher LN/stem across the temperature range (10.5-18) compared to Indianhead and 458-57 (0.6-6.2). The number of lesions per stem was similar at each temperature for Indianhead and 458-57 except at 28°C where LN was significantly higher on 458-57.

Disease severity was also lowest at 16°C but increased, as temperature was increased reaching the highest levels at 28°C in Indianhead, 458-57 and Eston (Fig 4.3). Disease severity was always highest on Eston and was significantly different from the DS values of Indianhead and 458-57 at each temperature. Disease severity was however, not significantly different between Indianhead and 458-57-57 at each temperature.

4.5. Discussion

Anthracoze development on lentil can be influenced by inoculum concentration, the duration of leaf wetness period, growth stage and temperature. This study demonstrated that all these factors had significant effects on IP, LP, LN and DS but more so on Eston than on Indianhead and 458-57.

Increasing conidial concentrations from 2×10^4 to 1×10^5 conidia/ml linearly decreased the time required for lesions to develop on stems and the time to first sporulation and increased LN and DS on Eston, Indianhead and 458-57. Although the rates of change in IP and LP as affected by log conidial concentration were higher in Indianhead and line 458-57, anthracnose severity was greater on Eston due to a higher infection frequency. When log conidial concentration was higher than 6×10^4 conidia/ml, most plants of the susceptible cultivar died, with anthracnose severity reaching about 85% within 1-2 weeks after inoculation. However, the same concentration only resulted in severities ranging from 46-56% on line 458-57 and 35-43% on Indianhead, and did not result in plant death. In the study by Chakraborty (1990) DS in *Stylosanthes* in response to *C. gloeosporioides* increased with conidia concentration to an optimum level and thereafter decreased at higher concentrations. Makowski (1993) reported increased DS and high plant mortalities in round leaved mallow (*Malva pusilla* Smith) with increase in spore concentrations *C. gloeosporioides*. Since plant death in the susceptible lentil cultivar occurred at inoculum concentrations longer than 6×10^4 conidia/ml, for all studies involving leaf wetness period, growth stage and temperature a concentration of 4×10^4 conidia/ml was used in order

to allow assessment of LPs in the susceptible cultivar as well as the incompletely resistant lines/cultivars.

Leaf wetness period had a significant effect on anthracnose development. Inoculated plants given a 6-12 h wetness period took much longer to show symptoms and only 0.8-1.1 lesions/stem on Indianhead and 458-57 and 1.1-1.5 lesions/stem on Eston were on average observed. Severe disease was only observed when plants were provided with at least a 24 h wetness period. It also took much longer for stem lesions to develop and to sporulate when plants were provided with a 6 h wetness period. While the IP for Indianhead, 458-57 and Eston decreased as the wetness period was extended to 48 h, the reductions in the IP for Indianhead and 458-57 were less responsive to increasing leaf wetness period beyond 24 h. Eston was most affected by longer wetness periods as the infection frequency increased from 1 to 31 lesions/stem when the wetness period was prolonged from 6 to 48 h compared to an increase of 0.75 to 6 lesions/stem for Indianhead and 458-57. Furthermore, when the wetness period was at least 36 h, a DS of about 80-88% resulted in death of most Eston plants within 1-2 weeks of inoculation. A 24 h leaf wetness period would appear to be the most effective for comparisons between incompletely resistant and susceptible lentil lines/cultivars.

The optimum growth stage for infection occurred when plants were inoculated 4-6 weeks after seeding. Plants inoculated at 2 or 8 weeks after seeding were more resistant than 4-6 week old plants, including the susceptible cultivar. The period 4-6 weeks after seeding was the time when plants were most susceptible, and this coincided with the time of early flowering (5-6 weeks from seeding). This is also the time at which symptoms normally first appear in lentil fields (Gibson 1994, Buchwaldt et al. 1996) even though inoculum may be present in the field before flowering. This effect of growth stage may explain why symptoms in the field first appear at early flowering. Most lesions on 2-6 week old plants were confined to stem bases. However, 8 week old plants tended to have most lesions on upper portions. A similar finding was also reported by Pedersen and Morrall (1994) in lentils inoculated with

Ascochyta fabae f. sp *lentis* Gossen et al., the cause of ascochyta blight. They suggested that this resistance was related to tissue age since inoculation of older plants affected younger tissues. In this study, the growth stage-related resistance increased IP and LP and reduced LN/stem and DS more on the incompletely resistant Indianhead and 458-57 than on the susceptible cultivar Eston.

When temperature was increased incrementally from 16 to 28°C, IP and LP decreased linearly while DS and the LN/stem increased. The decreases in IP or LP with increase in temperature have also been reported in lentil infected with ascochyta (Pedersen and Morrall 1994) and in strawberry (*Fragaria x ananassa* Duchesne) in response to three *Colletotrichum* spp. (King et al. 1997). Similarly, DS in round leaved mallow inoculated with *C. gloeosporioides* (Makowski 1993) and in soybean (*Glycine max* [L.] Merr.) anthracnose (*C. truncatum*) (Khan and Sinclair 1991) increased with temperature between 10-25°C and decreased at 30°C. In a study by Tu (1992), disease severity in beans (*Phaseolus vulgaris* L.) inoculated with *C. lindemuthianum* Sacc. & Magn. was greater at temperatures ranging from 20 to 24°C than at lower or higher temperatures. While LN/stem increased with temperature from 16-28°C in the present study, lentils infected with ascochyta blight showed a decrease in LN/plant with increase in temperatures from 15 to 25°C (Pedersen and Morrall 1994). In this study, temperature affected Eston more than Indianhead and 458-57. At a temperature of 16°C, lesions appeared later and were fewer than at temperatures between 20-28°C. The highest number of lesions per stem occurred when temperature was 28°C in all the three hosts but lesions were also much smaller than at any other temperature. Khan and Sinclair (1991) also reported an increase in lesion number with increasing temperature up to 30°C in soybean anthracnose. In the present study, plants grown at 28°C were also affected by heat scorching and appeared stunted, suggesting that precise disease ratings may have been affected at 28°C. Therefore, the optimum temperature was considered to be between 20-24°C. The slower rate of symptom development at the lowest temperature might also help explain the delay in appearance of symptoms until early flowering observed in the field. In some

years, temperatures during early spring when plants are young are likely to be low, especially at night. These conditions may be less favorable for germination of sclerotia.

The interactions between environmental factors were not investigated. It is expected that the level of infection and subsequent disease development could also depend on these interactions as reported in other studies (Evans et al. 1992, Zheng and Sutton 1994). In this study, plants at all growth stages were infected by *C. truncatum* but the period between 4-6 weeks after seeding was optimal for infection in Indianhead, 458-57 and Eston. Plants inoculated with 4×10^4 conidia/ml required a minimum of 6 h leaf wetness period for infection to occur while severe anthracnose occurred with at least 24 h wetness duration and temperatures of at least 20°C.

5.0 Field and growth chamber evaluation of components of incomplete resistance to *Colletotrichum truncatum* in lentil.

5.1. Abstract

The components of incomplete resistance to anthracnose (*Colletotrichum truncatum*) were evaluated in lentil (*Lens culinaris*) in the growth chamber and in the field. Lines PI 299331, PI 320937, PI 345629, 458-57 and cv. Indianhead with different levels of incomplete resistance and the susceptible cultivar Eston, were inoculated in a growth chamber and in the field using a single virulent isolate of *C. truncatum*. Each study was repeated once. Incubation and latent periods and percent sporulating lesions per stem were measured on stems. A computer imaging system was used to measure lesion number, lesion size and percent necrotic area per square centimeter on stem samples. The area under the disease progress curve (AUDPC) values were calculated using disease severity from the field samples. Lines PI 320937, PI 345629, 458-57 and cv. Indianhead were found to have high levels of incomplete resistance as indicated by significantly smaller and fewer lesions and longer incubation and latent periods than Eston. For each component, PI 299331 was intermediate except for lesion size, which was larger than that of Eston. The data for all the components obtained in the growth chamber were significantly correlated to those measured in the field ($r=0.86-99$, $p\leq 0.01$) and to disease severity and AUDPC. Therefore, evaluation of incomplete resistance in lentil can be conducted under controlled conditions and in the field.

5.2. Introduction

One of the most important diseases of lentil (*Lens culinaris* Medik.) in western Canada is anthracnose caused by *Colletotrichum truncatum* (Schwein.) Andrus and W.D. Moore. Anthracnose is most severe in seasons with high rainfall and humid conditions (Buchwaldt et al. 1993) and can cause significant reductions in yield of up to 70% (Morrall and Pedersen 1991, Gibson et al. 1991, Gibson 1994). Infected seed may also reduce seed quality (Morrall and Pedersen 1991).

All lentil cultivars grown in western Canada are susceptible to anthracnose. Available control measures include the use of fungicides (Morrall et al. 1990) and crop rotations. Applications of fungicides to susceptible cultivars may require large amounts or several applications of the fungicide (Thompson and Jenkins 1985a,b, Precheur et al. 1990, Latin 1991). This could be costly in terms of economic and environmental considerations. Resistant cultivars could prove more effective in disease control and reduce fungicide use. Recently, a few lentil lines with incomplete resistance were identified at the University of Manitoba, but lines with complete resistance were not recovered (Bernier et al. 1992). Incomplete resistance may involve one or more of the following components: longer latent period, reduced infection frequency, or reduced spore production compared to susceptible cultivars (Johnson and Wilcoxson 1978, Palevliet 1979).

Effective development of cultivars with this type of resistance requires an understanding of the components of incomplete resistance. Components identified could then be used as criteria for selection of lines in breeding programs and in genetic studies of individual components. In many studies, components of incomplete resistance evaluated include incubation and latent periods, infection frequency and sporulation (Griffiths and Jones 1987, Rashid 1991, Aquino et al. 1995). Parameters of disease progress such as area under disease progress curve (AUDPC) values have also been used to compare different lines with incomplete resistance in the field (Wilcoxson 1981, Conner and Bernier 1982, Rashid and Bernier 1986, Casela et al. 1993, Brière et al. 1994).

The objectives of this study were to characterize the components of incomplete resistance in lentil to anthracnose including incubation period, latent period, lesion number per unit stem area, lesion size and percent sporulating lesions per stem under controlled and field conditions and to relate the components of each study amongst themselves as well as to disease severity (DS) and to AUDPC values obtained in the field.

5.3. Materials and methods

Plant material. Growth chamber studies were conducted using five lines/cultivars with different levels of incomplete resistance (Appendix 2.0). The cv. Eston was included as a susceptible check. Among the incompletely resistant lines, PI 299331 has an intermediate level of incomplete resistance. Lines PI 320937, PI 345629, 458-57 and cv. Indianhead have high levels of incomplete resistance. Line PI 320937 and Indianhead each has a different single dominant gene for incomplete resistance (Buchwaldt and Bernier, unpublished). The genetics of incomplete resistance in the rest of the incompletely resistant lines have not been determined. Eight seeds of each line/cultivar were sown in 0.5 liter plastic pots filled with Metro-mix (W.R. Grace and Co. Canada Ltd. Ajax, Ontario). The pots were kept in the growth chamber maintained at 20/16°C (day/night) temperatures, 16 h photo period, and 250-300 $\mu\text{mol.m}^{-2}\text{s}^{-1}$ photon flux density provided by a mixture of cool white fluorescent and Vita-Lite fluorescent lights (Philips Lighting Co., Somerset NJ., and Duro-test Corp. North Bergen, NJ., respectively). Plants were thinned to five per pot and fertilized with 20:20:20 (N:P:K) liquid fertilizer once a week.

The field study consisted of one experiment conducted in each of 1995 and 1996. Each line/cultivar was sown in a 2 m² plot containing eight one meter rows with 40 seeds/m row. The experimental design was a randomized complete block design (RCBD) with four replicates. The plots were separated by a 1.5 m space.

Inoculum preparation. Isolate JPPTNL 882 of *C. truncatum* initially obtained from a single spore was used in all growth chamber and field experiments. Cultures of this isolate were raised on sodium-chloride yeast agar sucrose (SYAS) medium containing 5% sodium chloride, 3 g yeast extract, 20 g agar and 10 g sucrose per liter of water (Manandhar et al. 1986) supplemented with the antibiotics chlortetracycline and streptomycin sulphate, each at 10 ml/L. The cultures were kept at room temperature under continuous fluorescent light. Conidia from one week old cultures were harvested by adding sterile distilled water and gently dislodging the conidia with a sterile glass slide. The suspension was filtered through a Mira-cloth layer (Calbiochem-Behring Corp., La Jolla, CA.) and adjusted to a final concentration of 4×10^4 conidia/ml as determined with a haemocytometer.

Inoculation. Four week old plants (8-10 node stage) were inoculated with 5-6 ml of the conidial suspension per pot using an atomizer (DeVilbis, Somerset, PA). Tween 20 (polyoxyethelene sorbitan monolaurate) was added at a rate of one drop per 100 ml of the suspension as a wetting agent. Control plants were inoculated with water and drops of Tween 20 only. Inoculated plants were kept in the humidity chamber with a continuous leaf wetness for 24 h. Incubated plants were arranged in the growth chamber as a RCBD with four replicates and one pot of each line/cultivar per replicate. The experiment was repeated once.

In the field plants were also inoculated four weeks after planting with the same isolate and conidial concentration. Each plot was inoculated with 400 ml of the spore suspension using a hand sprayer. Wooden frames were placed on inoculated plots and covered with clear polyethylene sheets for 24 h. The soil in each plot was saturated with water before inoculation to create high relative humidity during incubation. Inoculation was done on a cool evening to favor dew deposition and enhance leaf wetness (Ali 1985, Rashid and Bernier 1986).

Anthracnose rating and measurements. Stem tissue was rated daily from the third day after inoculation for incubation and latent periods both in the growth chamber and in the field. The Incubation period was determined as the time in days from inoculation to the first appearance of lesions and the latent period as the time from inoculation to first appearance of acervuli in lesions observed with a 10-20X hand lens. The acervuli were repeatedly observed until the formation of sclerotia. The presence of conidia on the lesions was confirmed by scraping lesions in distilled water and observing conidia under the microscope at 100X. Recovery of conidia from lesions bearing sclerotia was not successful.

Growth chamber plants were harvested four weeks after inoculation. The first 10 cm of stems from the base (5 per pot) were cut and leaves removed. Measurements of total stem area, total necrotic area and lesion size (area) in square millimeters, and lesion number were done by using a true-color image processing system built around the DT2871 HSI-color frame grabber (Data Translation, Marlborough, MA.) and a Sony CCD/RGB color video camera (Model DXC 151, No. 10633, Japan) and the ImageX software developed by Dr. L. Lamari (Dept. of Plant Science, University of Manitoba, Winnipeg, MB.). Measurements were taken on each sample by randomly positioning the stems under the camera connected to a computer. After taking the measurements, the stems were removed and then repositioned again to take another set of measurements. In all, four sets of measurements per sample for each replicate were taken. Control plants inoculated with water were not infected.

Percent disease severity was calculated as total necrotic lesion area divided by total stem area, multiplied by 100. Percent sporulating lesions per stem or disease efficiency, was determined by dividing number of sporulating lesions per stem by total number of lesions per stem, multiplied by 100. Lesion number, disease severity and AUDPC values were calculated on a per square centimeter basis. In the field, sampling was done four times at weekly intervals starting one week after inoculation. Ten plants were randomly sampled from each plot and measurements were taken as described for the

growth chamber. The AUDPC values were also calculated from the disease severity data obtained from the field using the formula by Shaner and Finney (1977):

$$AUDPC = \sum_{i=1}^n [(Y_{i-1} + Y_i) \times 0.5][T_i - T_{i-1}]$$

[2]

Where Y_i = anthracnose severity at the i^{th} observation, T_i = time in days of the i^{th} observation, and n = total number of observations.

Analysis. Analysis of variance (ANOVA) was used to determine differences among lines for incubation and latent periods, lesion number, lesion size, number of sporulating lesions per stem, disease severity and AUDPC values. Mean comparisons were done by using the least significant difference (LSD, $p=0.05$). Bartlett's variance homogeneity tests were performed for each variable before combining data over experiments or years. Pearson correlation analysis was used to compare the components amongst themselves and also to relate them to disease severity and AUDPC values. All analyses were performed using the Statistical Analysis System (SAS Institute, Carry, N.C.).

5.4. Results

Significant differences ($p=0.0001$) were detected for all measured components and for disease severity and AUDPC values in the growth chamber and in the field among the lines/cultivars (Tables 5.0-5.1, Appendices 5.0-5.1). The line/cultivar (L/C) x experiment interaction was significant only for lesion size ($p=0.001$) in the growth chamber (Appendix 5.0). The L/C x year interactions were significant for incubation and latent periods and lesion size ($p \leq 0.05$) in the field indicating that the

differences observed among the lines/cultivars were significantly affected by environmental effects in the field (Appendix 5.1).

Incubation period. The range in incubation period (IP) among all lines/cultivars was 6.1-15.1 days in the growth chamber (Table 5.0) and 6.9-18 days in the field (Table 5.1). In both the growth chamber (GC) and the field study (FS), IP was shortest (6.1/6.9 days, GC/FS) for Eston and was longest for PI 320937 (15.1/18 days, GC/FS). Incubation period for PI 299331 was not significantly different from that of Eston in the growth chamber but was significantly longer in the field by 5.9 days (Tables 5.0-5.1). For each line/cultivar IP was longer in the field than in the growth chamber and a significant change in lines/cultivars ranking between the growth chamber and the field, involving PI 345629 and 458-57 was observed.

Latent period. Latent period (LP), determined as the time from inoculation to the appearance of acervuli on stem lesions varied from 11 days on Eston to 24.4 days on PI 320937 in the growth chamber (Table 5.0), and from 13.1 days on Eston to 31.5 days on PI 320937 in the field (Table 5.1). Latent period was longer in the field than in the growth chamber for all lines and Indianhead and the ranking of lines/cultivar for this component was consistent between the growth chamber and the field.

Lesion number. Lesion number (LN) on the resistant lines varied from 1.9-4.0 cm⁻² in the growth chamber (Table 5.0) and from 1.8-3.6 cm⁻² in the field (Table 5.1). The number of lesions on Eston was 10.3 and 8.8 cm⁻² in the growth chamber and in the field, respectively (Table 5.0-5.1). The ranking of lines between the growth chamber and the field for LN did not significantly change.

Lesion size. Stem lesions were largest (7.2 mm^2) on PI 299331 intermediate (5.4 mm^2) on Eston and smallest ($3.5\text{--}4.0 \text{ mm}^2$) on Indianhead, 458-57, PI 345629 and PI 320937 in the growth chamber (Table 5.0). The same trend was apparent in the field where lesions were largest (6.1 mm^2) on PI 299331 intermediate (4.5 mm^2) on Eston and smallest ($3.4\text{--}4.4 \text{ mm}^2$) on the remaining lines (Table 5.1). The change in the ranking of the lines/cultivars between the growth chamber and the field for lesion size (LS) involved only Indianhead, 458-57 and PI 345629 and was not significant .

Number of sporulating lesions per stem. The number of sporulating lesions per stem (SLS) ranged from 26-44% and 8-45% on the incompletely resistant lines/cultivar in the growth chamber and in the field, respectively (Table 5.0-5.1). However, up to 87% of the lesions sporulated on Eston in the growth chamber and 76% in the field. On average, incompletely resistant lines had lower SLS in the field (29.8%) than in the growth chamber (35.6%) and the ranking of the lines/cultivar for this component remained consistent between the growth chamber and the field.

Disease severity and AUDPC. The final disease severity (DS) values ranged from 6.8-55.3% in the growth chamber (Table 5.0) and from 6.3-38.3% in the field (Table 5.1). Eston had the highest DS values while PI 299331 was intermediate (21.3-28.9%). The rest of the lines had low disease severities (6.3-11.2%) in the growth chamber and the field. Disease severity was generally higher in the growth chamber than in the field.

In both 1995 and 1996, progress of anthracnose in the field was much slower on the resistant lines/cultivar compared to Eston (Fig 5.0). The resulting AUDPC values calculated from disease severity data over the growing season were highest in Eston (287), intermediate for PI 299331 (140) and lowest for the remaining lines (30-70) (Table 5.1).

Table 5.0. Components of incomplete resistance and disease severity in six lentil lines/cultivars tested with isolate JPPTNL 882 of *Colletotrichum truncatum* in the growth chamber.

Line/cultivar	Component [†]					
	IP (days)	LP (days)	LN (cm ⁻²) [‡]	LS (mm ²) [‡]	SLS (%)	DS (%.cm ⁻²) [‡]
PI 320937	15.1 a	24.4 a	1.9 d	3.5 d	26 c	6.8 d
Indianhead	11.8 b	19.1 b	2.7 c	4.0 c	44 b	10.7 c
PI 345629	12.5 b	18.0 bc	2.4 c	4.0 c	33 bc	10.3 c
458-57	13.5 ab	17.0 bc	2.4 c	3.9 c	37 bc	9.5 c
PI 299331	7.5 c	15.5 c	4.0 b	7.2 a	38 bc	28.9 b
Eston	6.1 c	11.0 d	10.3 a	5.4 b	87 a	55.3 a

IP=incubation period, LP=latent period, LN=lesion number, LS=lesion size, SLS=sporulating lesions per stem, DS=disease severity.

[†]All components were measured on stems only and means were compared using LSD at 0.05 level.

[‡]Measured with computer imaging system.

Table 5.1. Components of incomplete resistance, disease severity and area under the disease progress values in six lentil lines/cultivars tested with isolate JPPTNL 882 of *Colletotrichum truncatum* in the field.

Host	Component [†]						AUDPC
	IP (days)	LP (days)	LN (cm ⁻²) [‡]	LS (mm ²) [‡]	SLS (%)	DS (%.cm ⁻²) [‡]	
PI 320937	18.0 a	31.5 a	1.8 d	3.4 d	8 c	6.3 d	30 d
Indianhead	16.0 b	28.9 ab	3.1 bc	3.6 cd	39 b	11.0 c	67 c
PI 345629	16.5 b	28.1 b	2.1 d	4.1 bc	16 c	8.5 cd	52 c
458-57	14.2 c	27.6 b	2.5 cd	4.4 b	41 b	11.2 c	70 c
PI 299331	12.8 d	19.3 c	3.6 b	6.1 a	45 b	21.3 b	140 b
Eston	6.9 e	13.1 d	8.8 a	4.5 b	76 a	38.3 a	287 a

IP=incubation period, LP=latent period, LN=lesion number, LS=lesion size, SLS=sporulating lesions per stem, DS=disease severity, AUDPC=area under the disease progress curve.

[†]All components were measured on stems only and means were compared using LSD at 0.05 level.

[‡]Measured with computer imaging system.

Correlations. Incubation period and LP were significantly and positively correlated in the growth chamber ($r=0.86$, $p=0.01$) (Table 5.2) and in the field ($r=0.96$, $p=0.001$) (Table 5.3). Incubation period and LP were also significantly and negatively correlated to other components in the growth chamber and in the field ($r=-0.80$ to -0.98 , $p\leq 0.01$) except LS, and were also significantly correlated to DS in the growth chamber ($r=-0.85$ to -0.89 , $p=0.01$) and in the field ($r=-0.98$, $p=0.001$), and to AUDPC in

the field ($r=-0.97$ to -0.98 , $p=0.001$) (Table 5.2 & 5.3). Lesion number and SLS were significantly correlated to DS and AUDPC ($r=0.91-0.92$, $p=0.001$) and to all other components except LS (Table 5.2 & 5.3). Lesion size was the only component not significantly correlated to any other component. Each of the components measured in the growth chamber was significantly correlated with the same component measured in the field ($r=0.86-0.99$, $p\leq 0.01$) without exception (Table 5.4). This showed that components measured under controlled conditions were representative of the same components in the field.

Table 5.2. Pearson correlation coefficients for components of incomplete resistance and disease severity in six lentil lines/cultivars tested with isolate JPPTNL 882 of *Colletotrichum truncatum* in the growth chamber.

Component	Component				
	IP	LP	SLS	LS	LN
Incubation period (days)	-				
Latent period (days)	0.86*	-			
Sporulating lesions stem ⁻¹ (%)	-0.85*	-0.85*	-		
Lesion size (mm ²)	-0.71	-0.61	0.39	-	
Lesion number (cm ⁻²)	-0.80*	-0.82*	0.96**	0.31	-
DS (%.cm ⁻²)	-0.89*	-0.85*	0.91*	0.63	0.98**

IP=incubation period, LP=latent period, LN=lesion number, LS=lesion size, SLS=sporulating lesions per stem, DS=disease severity measured at 4 weeks after inoculation.

*,** Significant at $p=0.05$, 0.01 , respectively.

Table 5.3. Pearson correlation coefficients for components of incomplete resistance, disease severity and area under the disease progress curve values in six lentil lines/cultivars tested with isolate JPPTNL 882 of *Colletotrichum truncatum* in the field.

Component	Component					
	IP	LP	SLS	LS	LN	DS
Incubation period (days)	-					
Latent period (days)	0.96**	-				
Sporulating lesions stem ⁻¹ (%)	-0.95**	-0.88*	-			
Lesion size (mm ²)	-0.44	-0.60	0.40	-		
Lesion number (cm ⁻²)	-0.96**	-0.91*	0.91*	0.21	-	
DS (%.cm ⁻²)	-0.98**	-0.98**	0.92**	0.44	0.97**	-
AUDPC	-0.98**	-0.97**	0.91**	0.39	0.98**	0.99**

IP=incubation period, LP=latent period, LN=lesion number, LS=lesion size, SLS=sporulating lesions per stem, DS=disease severity at final sampling measured at 4 weeks after inoculation.

AUDPC=area under the disease progress curve.

*,** Significant at p=0.05, 0.01, respectively.

Table 5.4. Pearson correlation coefficients among components of incomplete resistance, disease severity and area the disease progress curves in six lentil lines/cultivars tested with isolate JPPTNL 882 of *Colletotrichum truncatum* in the growth chamber and in the field.

Field component	Growth chamber component					
	IP	LP	SLS	LS	LN	DS
Incubation period (days)	0.86*	0.95**	-0.95**	-0.68	-0.80**	-0.90**
Latent period (days)	0.95**	0.90*	-0.85*	-0.56	-0.82**	-0.88**
Sporulating lesions stem ⁻¹ (%)	-0.96*	-0.85*	0.93*	0.19	0.96**	0.92*
Lesion size (mm ²)	-0.57	-0.53	0.18	0.92**	0.43	0.63
Lesion number (cm ⁻²)	-0.95**	-0.87*	0.95**	0.23	0.99**	0.97**
Disease severity (%.cm ⁻²)	-0.96**	-0.87*	0.89*	0.42	0.93**	0.99**
AUDPC	-0.88*	-0.88*	0.94**	0.58	0.98**	0.99**

IP=incubation period, LP=latent period, LN=lesion number, LS=lesion size, SLS=sporulating lesions per stem, DS=disease severity measured 4 weeks after inoculation; AUDPC=area under the disease progress curve.

*,** Significant at p= 0.05, 0.01, respectively.

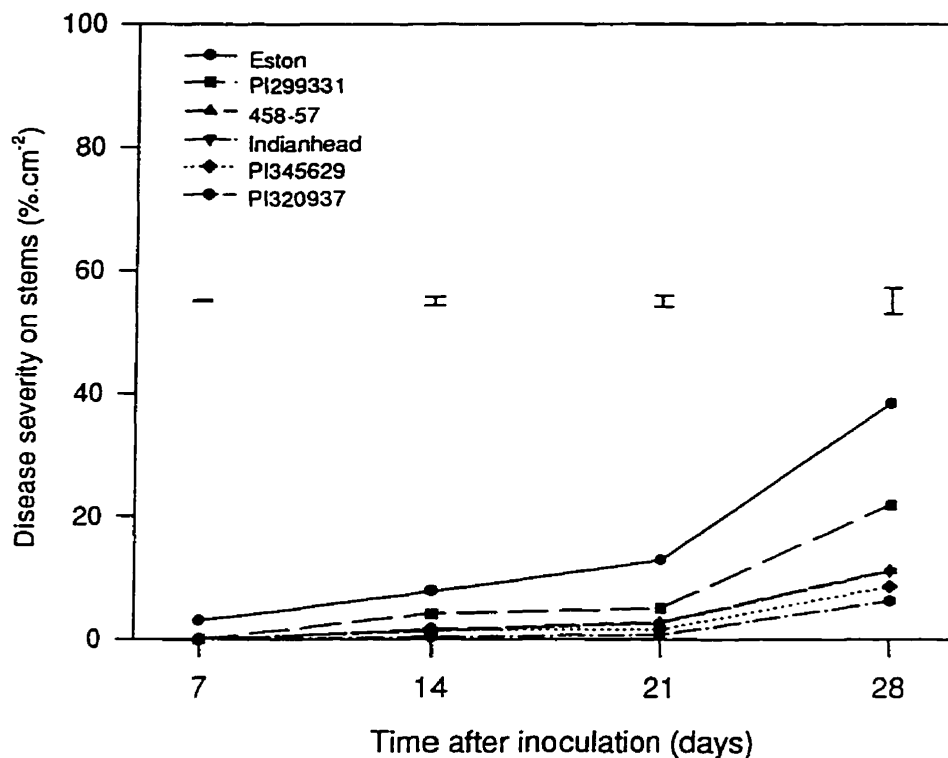


Fig. 5.0. Disease progress curves for six lentil lines/cultivars in small field plots inoculated with isolate JPPTNL 882 of *Colletotrichum truncatum* over 1995 and 1996. Bar=LSD, $p=0.05$.

5.5. Discussion

The host lines/cultivars tested were significantly different for all components of incomplete resistance measured in the growth chamber and in the field. Compared to Eston, the resistant lines had significantly longer IP and LP, fewer and smaller lesions and lower SLS. Disease severities and AUDPC values were lower on resistant lines than on Eston.

Line PI 299331 did not differ in IP from Eston in the growth chamber and had the largest lesions and second highest LN in the growth chamber and in the field. However, PI 299331 was

different from Eston by having significantly longer LP and fewer lesions. Although the lesions on stems were larger on PI 299331, they were not as deep as the lesions on the other lines/cultivars. This is another reason why DS values were lower on this line compared to Eston.

Among the resistant lines, PI 320937 ranked the highest while PI 299331 ranked the lowest for the components measured. The ranking of the lines for each component between the growth chamber and the field was consistent for LP, LN and SLS but for the other components, Indianhead, 458-57 and PI 345629 occasionally changed ranks. Based on most of the components, and also on DS and AUDPC values, PI 320937, PI 345629, 458-57 and Indianhead can be grouped together as having high levels of incomplete resistance with PI 299331 being intermediate between this group and Eston.

The significant line/cultivar by year interactions for IP, LP and LS in the field showed that these components were affected by environmental differences between years. The longer IP and LP observed in the field as compared to the growth chamber could be attributed to less favorable and more fluctuating environmental conditions in the field especially temperature (Nutter and Pederson 1982) and moisture which significantly influence these components. In cucumber (*Cucumis sativus* L.) anthracnose *C. lagenarium* (Pass) Ell. & Halst., lesion size in the field was mostly determined by the length of time from inoculation and was less affected by variations in environmental conditions (Thompson and Jenkins 1985c). In the present study, growth chamber experiments also showed a small environmental effect on LS. Such environmental effects on components of resistance in the growth chamber to flax rust (*Melampsora lini*) were attributed to differences in micro-climate arising during incubation and also age of plant tissues (Rashid 1991). Therefore, evaluation of components of incomplete resistance in the field may require more than 2 years of testing due to significant environmental effects on some of the components. Alternatively, incomplete resistance can concurrently be evaluated under controlled conditions where components were less affected by environmental effects.

The positive correlations between IP and LP as observed in this study are also common in other studies such as flax rust (Rashid 1991). Since IP and LP were significantly and negatively correlated to most of the components and to DS and AUDPC values, selection for longer IP or LP could play a significant role in increasing levels of resistance in lentil. The strong positive correlation between IP and LP suggests that selection for only one of the two components may be adequate and that the one to use may depend on relative ease of measurement. Using IP would be more appropriate as it was easier to measure compared to LP. However, the positive and negative correlations among the components indicated an interdependency of the components which has also been observed in other host pathosystems (Parlevliet 1979, Tomerlin et al. 1983, Rashid 1991). This means that selection for higher levels of incomplete resistance may sometimes require selecting for more than just one component (Griffiths and Jones 1987).

The AUDPC values from the field ranked the lines/cultivars for incomplete resistance in a manner comparable to the rankings produced by most of the resistance components. It is, therefore, possible to use AUDPC values to compare and select resistant lines. The correlation between AUDPC and final DS values was expected since final DS contributes in part to AUDPC. Conner and Bernier (1982) and Rashid and Bernier (1986) reported correlations between final DS and AUDPC in a study on slow rusting of faba bean (*Vicia faba* L.) to rust (*Uromyces viciae-fabae* [Pers.] Schroet.). They suggested that final DS could be used as a criterion for selecting lines with incomplete resistance in preliminary studies instead of AUDPC values which require sampling DS several times.

In conclusion, the results show that selection for incomplete resistance in lentil could be done by selecting for components under controlled or field conditions. The AUDPC or DS values could also be used for selection in the field. The strong and significant correlations between components measured in the growth chamber with the same components measured in the field indicate that incomplete resistance in lentil could quickly be evaluated under controlled conditions and that this would be

representative of the field situation. Selection for incomplete resistance by measuring as few components as possible could be done by using IP or LP as selection criteria thus speeding the process of developing cultivars with incomplete resistance, since these components were strongly correlated to each other and to most of the other components. Selected lines with incomplete resistance could then be used as parental lines in crosses to produce progeny with high levels of incomplete resistance or crossed to adapted susceptible cultivars with superior agronomic characters such as high yield, and seed quality to transfer resistance.

6.0. GENERAL DISCUSSION

Anthracoze, presently the most destructive disease of lentils in Manitoba, was first observed in eastern Manitoba in 1987 (Morrall 1988) and rapidly spread to all areas of production in Manitoba and Saskatchewan. In the absence of resistant lentil cultivars, effective control based on the use of crop rotations and foliar fungicides is difficult to achieve and costly.

Incomplete resistance to *C. truncatum*, identified in a few lentil lines at the University of Manitoba (Bernier et al. 1992) is an option for controlling anthracnose. Although cultivars with incomplete resistance may endure moderate disease levels during an epidemic, such levels of disease do not necessarily lead to significant yield reductions as demonstrated in the barley-leaf rust system (Johnson and Wilcoxson 1979). In the present study, incomplete resistance in line 458-57 tested under different disease levels in lentil, was found to be effective in reducing anthracnose severity and yield losses compared to a susceptible cv. Eston without the application of a fungicide and at high disease pressure.

Gibson (1994) reported that chlorothalonil had a non-significant effect on seed yield of lentil during dry weather but increased yields when conditions were favorable for anthracnose development. In the present study, yield losses were reduced to a greater extent in unsprayed plots of line 458-57 compared to those of Eston when disease pressure was high. Applications of chlorothalonil improved the yields of both Eston and 458-57 indicating that additional control of anthracnose was provided by the fungicide. Also, yield losses in of 458-57 in treated plots were not significantly reduced whereas those of Eston were significantly reduced when anthracnose pressure was high. Incomplete resistance to anthracnose caused by *C. coccodes* in tomato and *C. lagenarium* in cucumber was also reported to significantly reduce the fungicide requirements necessary to control the disease. Susceptible cucumber (Thompson and Jenkins 1985a,b) and tomato (Precheur et al 1990, Latin 1991) were found

to require higher rates and additional applications of fungicides to control the diseases than incompletely resistant cultivars. In the present study, the degree of protection against yield losses provided by the incompletely resistant line 458-57 was effective in fields with high anthracnose level, indicating that development of cultivars with incomplete resistance of this type could be beneficial to lentil production. In the event cultivars with incomplete resistance become available, effective control of anthracnose would still require complementary measures such as 3-4 year crop rotations and applications of foliar fungicides.

The occurrence of anthracnose in southern Manitoba is widespread and was found in high levels in the Rosenort area and at moderate to high levels in areas surrounding Portage la Prairie. Anthracnose was also detected at low levels in fields never cropped to lentils in Carman and Miami, suggesting that inoculum is brought in such fields from infested fields in surrounding areas. Buchwaldt et al. (1996) reported that the widespread dissemination of anthracnose and the high levels of inoculum present in many soils cropped to lentil may be due to the long term survival of sclerotia and to their finding that at harvest lentil crop debris infested with sclerotia can spread great distances by wind. Sclerotia were also shown to rapidly decline after one year when infested lentil debris were placed on the soil surface and a rapid decline occurred after two years when infested debris were buried (Buchwaldt et al. 1996). The ability of conidia and mycelia to overwinter, however, and contribute to inoculum levels is not known. Blakeman and Hornby (1966) reported that conidia of *C. coccodes* did not remain viable beyond three weeks whereas sclerotia remained viable for 83 weeks. It was concluded that sclerotia in free soil and on root debris were the primary inoculum and principal means of survival of *C. coccodes*.

Lentils in Manitoba are normally desiccated with chemicals such as paraquat or diquat to dry the plants and facilitate harvesting. Data from two locations (not presented) indicated that in desiccated plants sporulation was more abundant and sclerotia were more numerous than undesiccated

plants. This has also been reported in other *Colletotrichum* spp. in studies aimed at assessing latent infections. Cerkauskas (1988) developed a method for detecting latent infections of *C. coccodes* on tomato stems by surface sterilizing the tissue followed by treatment with paraquat. Treatment with paraquat resulted in significantly greater development of acervuli on the infected tissues. The use of paraquat as a field spray in soybean induced symptoms of *C. truncatum* two weeks before symptoms appeared on unsprayed plants (Sinclair 1991). Desiccation may therefore, be contributing to the inoculum levels in lentil fields in Manitoba. The extent to which this may be occurring also needs further investigation.

Environmental factors during the incubation of inoculated lentil plants under controlled conditions were found to have a major role on the development of anthracnose in the present study. Long leaf wetness periods were shown to be required for germination of conidia and infection. The interactions of the lines/cultivars with either temperature, leaf wetness period, inoculum density and host growth stage were all significant. Since plants were incubated under continuous leaf wetness, the effects of interrupted leaf wetness period which would probably simulate the field situation and also the interactions of various environmental factors on anthracnose development were not investigated.

Previous studies have shown that anthracnose severity in lentil increased with temperature between 20-25°C and with leaf wetness periods longer than 16 h (Gibson 1994). Results of the present study indicated that severe anthracnose development occurred at temperatures between 20-28°C, with leaf wetness periods of 24 to 48 h and inoculum concentrations of 4×10^4 to 1×10^5 conidia/ml. Parbery (1981) indicated that, in general, conidial germination of most *Colletotrichum* spp. can occur between 5-35°C and that the optimal temperature range is 20-26°C. In the present study, symptoms appeared much later when plants were kept at 16°C compared to plants kept between 20-28°C after inoculation. Gibson (1994) however, did not observe symptoms on lentil inoculated with *C. truncatum*

and incubated at 15°C whereas symptoms developed when such plants were transferred from 15°C to 25°C or at higher temperatures.

Anthracoze severity is also related to temperature and precipitation in lentil to *C. truncatum* (Morrall et al. 1989) and in bean to *C. lindemuthianum* (Tu 1992). In the present study, disease severity was significantly reduced by the incompletely resistant lines compared to Eston and in terms of growth stage, plants of both the susceptible cv. Eston and the incompletely resistant Indianhead and 458-57 were more susceptible at early flowering. The implication of these findings is that seasons with moderate temperatures accompanied by high rainfall or precipitation will be favorable to severe anthracnose development in lentil fields. The results of this study also provided standards with regards to temperature, leaf wetness period, inoculum concentration and growth stage which were later used to evaluate components of incomplete resistance. These standards will also prove useful for evaluation of lentil germ plasm to isolates of *C. truncatum* under controlled conditions.

Some of the findings from the study on environmental factors (thesis section 4.0) provided answers to questions related to observations made in the field such as to why symptoms appear at early flowering and not earlier and why anthracnose is more intense in Manitoba than Saskatchewan. The appearance of symptoms at early flowering in lentil fields even though infection may occur well before flowering was attributed to latent or quiescent infections and was thought to be related to temperature and growth stage (Gibson 1994). Latent infections are common in other *Colletotrichum* spp. including *C. truncatum* infections in soybean (Sinclair 1991) and *C. coccodes* infections in tomato (Fulton 1948, Cerkauskas 1988, Dillard 1992). In tomato anthracnose, the release of latent infections is related to the physiological maturity. When green fruits are infected, lesion development is delayed until ripening and this is affected by temperature (Fulton 1948). In the present study, younger plants took much longer to develop symptoms and appeared more resistant than plants which were inoculated at flowering (4-6 weeks old) and also at low temperature (16°C), symptoms developed later than on plants

growing at higher temperatures (20-28°C). These results then do provide answers to the questions above. The greater prevalence of anthracnose in southern Manitoba compared to Saskatchewan, where most of the lentils are grown, may be because lentils are grown in the semi-arid areas in Saskatchewan (Morrall 1997), which are drier areas than those where lentils are grown in Manitoba.

Incomplete resistance in lentil was demonstrated by evaluating components of resistance under controlled and field conditions. The components evaluated indicated that incomplete resistance could be selected for in either environment. In both environments, incomplete resistance was characterized by smaller and fewer lesions which developed much later in the growing season and had reduced sporulation compared to the susceptible cultivar. All the components were significantly correlated to each other except lesion size. Among the components evaluated, incubation period (IP), latent period (LP) and lesion number (LN) per stem appeared to be the most important with regards to the expression of incomplete resistance. Lesion size was the only component not correlated to incomplete resistance in the growth chamber and in the field. The fact that significant correlations were observed among the components indicated an interdependency of the components on each other as they affect disease development and are in agreement with previous findings (Parlevliet 1979, Tomerlin et al. 1983). Incubation period and LP were highly correlated and both were also correlated to all other components except lesion size. Since IP and LP are positively correlated and it was easier to measure IP than LP, incomplete resistance in lentil could more readily be evaluated by selecting lines with longer IP if only one component were to be used. The selected lines would be expected to have longer LP, reduced sporulation and fewer lesions.

The end-of-season disease severity and area under the disease progress curve (AUDPC) values also readily differentiated the lines tested and both were highly correlated. Thus, these parameters could also be used for screening resistance in the field. Conner and Bernier (1982) and Rashid and Bernier (1986) also found that final disease severity was strongly correlated to AUDPC values in faba bean

rust (*Uromyces vicia -faba*) and suggested that a one time rating of final disease severity could be used to assess slow rusting rather than AUDPC values which require several ratings over time. In the assessment of quantitative resistance to *C. gloeosporioides* in *Stylosanthes hamata*, the final disease severity rating was also found to be highly correlated to AUDPC (Imasupasit et al. 1991). It was suggested that a single end-of-season assessment of disease was sufficient to differentiate resistance levels in several accessions. In the present study, correlation analysis was used to relate components of incomplete resistance among themselves and to the levels of incomplete resistance in different lentil lines/cultivars. Multi-variate analysis procedures such as principle component analysis and stepwise regression could be used in the future to help determine combinations of the important components of incomplete resistance in lentil. Results of the present study indicate that incomplete resistance in lentil can be selected readily by selecting one or a few components involved in this type of resistance. This would simplify the task of the plant breeder and enhance selection efficiency, reduce the time required to develop resistant cultivars and reduce costs. Breeding for incomplete resistance when it is controlled by minor genes may be considered complex and more difficult than when this type of resistance is controlled by major genes. The transfer of incomplete resistance to adapted cultivars in lentil could be achieved easily because incomplete resistance in a number of lentil lines is controlled by single dominant genes (L. Buchwaldt and C.C. Bernier, unpublished). To ensure durability of incomplete resistance a number of these genes could be transferred into one cultivar.

The methods of assessment and selection for incomplete resistance usually involve the use of mixtures of races/isolates or natural inoculum of the pathogen in the field. The assumption here is that the incomplete resistance present is race-non-specific in nature i.e. will show no specificity for individual isolates/races. This may not always be true as sometimes major genes may be involved in this type of resistance. The use of mixtures of races/isolates when major genes are involved does not provide a clear indication as to what races the host lines are reacting to in the mixture and therefore

confound the results (Parlevliet 1989). The use of natural inoculum may also not ensure the development of an effective epidemic during the evaluation and it is also difficult to determine the composition of races or isolates in the inoculum. An almost exclusive reliability on selection for incomplete resistance based on AUDPC values, also prolongs the selection time as several disease ratings have to be obtained during the epidemic for calculating AUDPC values. The best strategy for evaluation of this type of incomplete resistance should be done with a single isolate artificially inoculated to ensure effective disease development under which to assess the resistance. If required, other isolates can be tested later, one at a time.

Differences in virulence, i.e. relative ability to cause disease, among *C. truncatum* isolates on lentil have been observed recently (B.D. McCallum and C.C. Bernier, unpublished). The extent of pathogen variability however, is not fully known. This suggests the need for continued testing of lines with incomplete resistance to different pathogen isolates to determine if *C. truncatum* might be specific to certain host lines and whether the effectiveness of a given resistant line might eventually be reduced or lost over time. Developing differential lines for testing isolates, similar to what has been done in other host-pathogen systems will help in determining the extent of pathogen variability and further help to improve evaluation and selection methods for incomplete resistance. Hopefully this will ensure that levels of resistance in the future will be effective and durable.

In conclusion, the type of incomplete resistance to anthracnose in lentil in the present study produced smaller lesions on the incompletely resistant lines than those on the susceptible cultivar whereas the concept of partial resistance described by Parlevliet (1985), lesions in incompletely resistance lines/cultivars are as large as in the susceptible. In addition the incomplete resistance that is described as partial resistance is generally considered to be polygenic in nature which seems not to be the case at least in a few lentil lines/cultivars such as PI 320937, PI 345629 and Indianhead where

simple inheritance patterns for incomplete resistance have been observed (L. Buchwaldt and C.C. Bernier, unpublished).

The effectiveness of incomplete resistance in one line of lentil as a means for controlling anthracnose and increasing yields was demonstrated in fields with high levels of anthracnose. Fungicidal control of anthracnose in lentil was confirmed, and combining incomplete resistance with a fungicide also lead to an enhanced control of anthracnose and reductions in the amounts of fungicide used in the field when disease pressure is high. Information has been generated on the responses of incomplete resistance to *C. truncatum* under various environmental and pathogen factors which can serve as standards or guidelines in future studies. The incomplete resistance to anthracnose in lentil is based on longer IP and LP, smaller and fewer lesions and reduced sporulation.

Effective and simple methods which can be used for evaluation of new sources of incomplete resistance, selection for incomplete resistance in a breeding program and for retesting of lentil lines to new isolates under controlled and field conditions have also been demonstrated. However, a recent finding of differences in virulence amongst isolates of *C. truncatum*, suggest that there is a need to develop differential host lines and to continue to test more isolates to establish the extent of pathogen variability and to monitor any changes that might be taking place in the pathogen population. It would be important to investigate the role of interrupted leaf wetness period on germination of conidia and host infection and also the effect of the interactions of environmental factors on anthracnose development. The effect of chemical dessication on sporulation and formation of sclerotia should also be investigated as it seems that this practice may be responsible for the rapid increase in inoculum levels which has occurred in lentil fields in southern Manitoba.

7.0. REFERENCES

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8.0. APPENDICES

Appendix 2.0. Characteristics of a susceptible and the incompletely resistant lentil lines/cultivars used in the studies.

Cultivar/line	Year registered	Origin	Attributes	Disease reaction [‡]
Eston [†]	1980	Reselection of USDA PI 179307 (Turkey) (CDC)	High yielding cultivar, small seed, greenish seed coat, yellow cotyledon	S
Indianhead [†]	1986	Reselection of USDA PI 320952 (Czechoslovakia) (CDC)	Green manure cultivar, strongly intermediate growth habit, small black seed coat, yellow cotyledon	MR
458-57	-	Indianhead x Eston (CDC)	Small seed, tan-brown seed coat, yellow cotyledon	MR
PI 299331	-	Chile	Large seed, greenish seed coat, yellow cotyledon	MR-MS
PI 320937	-	Germany	Small seed, black seed coat, yellow cotyledon	MR
PI 345629	-	USSR	Small seed, tan-brown seed coat, yellow cotyledon	MR

USDA, United States Department of Agriculture; CDC, Crop Development Centre, Saskatoon, Sask.; USSR, Union of Soviet Socialist Republic.

[‡]Reaction to anthracnose (*Colletotrichum truncatum*) (Bernier et al. 1992).

[†]Adapted from Slinkard and Vandenberg 1995.

Appendix 3.0. Analysis of co-variance for the effect of incomplete resistance and fungicide application on anthracnose severity (%) and seed yield (kg/ha) in lentil at three anthracnose levels each combined over two locations and two years.

Source	DF	Mean Squares					
		Low		Moderate		High	
		Disease	Yield [§]	Disease	Yield [§]	Disease	Yield [§]
Year (Y)	1	20	13349***	10050***	148	129	5438***
Site (S)	1	749***	2673**	3751**	452**	908**	3119***
Reps/ (YL)	9	75	85	155	31	106	36
line/cv.(L/C)	1	613**	16	4318***	65	6629**	375**
Y x L/C	1	25	49	127	81	98	96
L x L/C	1	11	32	14	69	118	240
Error A	9	50	638	74	200	171	81
Fungicide (F)	3	589***	556****	905***	488***	1994***	357***
Y x F	3	114	259**	22	226**	111	50
S x F	3	5	43	144*	67	124*	33
L/C x F	3	77	41	3	20	99	286***
Disease	1		38		82		1
Error B	593	43	38	36	28	45	31

[§] Mean squares were divided by 1000.

*, **, *** Significant at p=0.05, 0.01 and 0.001, respectively.

Appendix 4.0. Analysis of variance for components of incomplete resistance and disease severity in three lentil lines/cultivars inoculated with *Colletotrichum truncatum* at four different growth stages.

Source	DF	Mean squares			
		IP	LP	LN	DS
Experiment (E)	1	16	9	0	397*
Reps/(E)	6	12	7	25*	95
Line/cultivar (L/C)	2	1227***	1925***	1884***	16460***
Stage (S)	3	215***	242***	502***	8803***
L/C x S	6	32***	38***	205***	388***
Error	173	6	8	8	76
C.V. (%)		22	16	53	24

IP=incubation period, LP=latent period, LN=lesion number/stem, DS=disease severity.

*, *** Significant at p=0.01 and 0.001, respectively.

Appendix 4.1. Analysis of variance and orthogonal polynomials and contrasts for lesion number per stem and disease severity in three lentil lines/cultivars exposed to four different temperatures after inoculation with isolate JPPTNL 882 of *Colletotrichum truncatum*.

Source	DF	Mean squares	
		Lesion number/stem	Disease severity
Experiment (E)	1	7	715
Reps/(E)	2	2	72
Temperature (T)	3	137***	3586***
T_{linear}	1	407***	9900***
$T_{\text{quadratic}}$	1	5*	57
T_{cubic}	1	0	801
Error A	9	3	146
Line/cultivar (L/C)	2	1557***	14139***
L/C x T	6	19*	126
Error B	72	3	128
C.V. (%)		26	27
Orthogonal contrasts			
T_{linear} x Eston	1	309***	-
T_{linear} x 458-57	1	109***	-
T_{linear} x Indianhead	1	47***	-
$T_{\text{quadratic}}$ x Eston	1	2	-
$T_{\text{quadratic}}$ x 458-57	1	12*	-
$T_{\text{quadratic}}$ x Indianhead	1	4	-
T_{cubic} x Eston	1	28**	-
T_{cubic} x 458-57	1	12*	-
T_{cubic} x Indianhead	1	1	-

***, ** Significant at $p=0.05$, 0.01 and 0.001 , respectively.

Appendix 5.0. Analysis of variance for components of incomplete resistance and disease severity in six lentil lines/cultivars tested with isolate JPPTNL 882 of *Colletotrichum truncatum* in the growth chamber.

Source	DF	Mean squares					
		IP	LP	LN	LS	SLS	DS
Expt (E)	1	27	5	8.0**	0.4	68	146**
Reps/(E)	6	7	8	0.1	0.3	37	5
Lines/cv. (L/C)	5	99***	155***	82***	15.4***	3749***	2862***
L/C x E	5	8	9	0.4	1.5**	110	18
Error	30	5	8	0.3	0.3	190	15
C.V. (%)		20	17	13	12	31	19

IP=incubation period, LP=latent period, LN=lesion number, LS=lesion size, SLS=lesions sporulating per stem, DS=disease severity.

, Signifcant at $p=0.01$ and 0.001 , respectively.

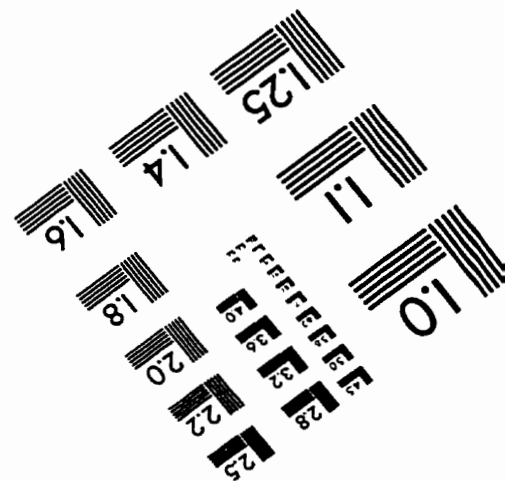
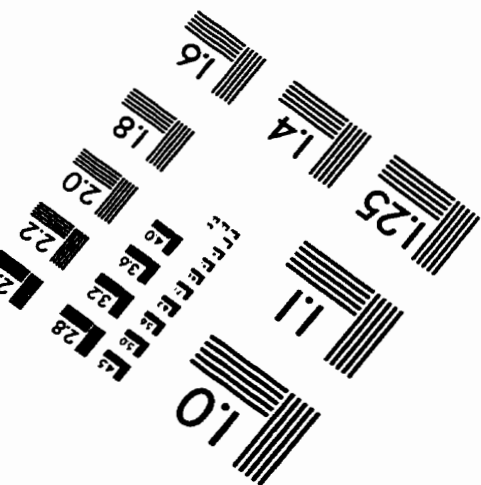
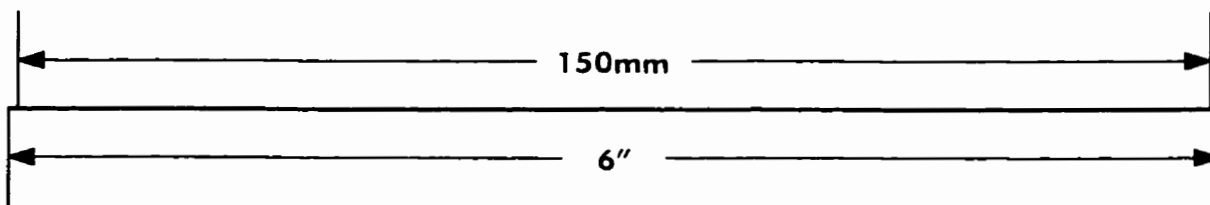
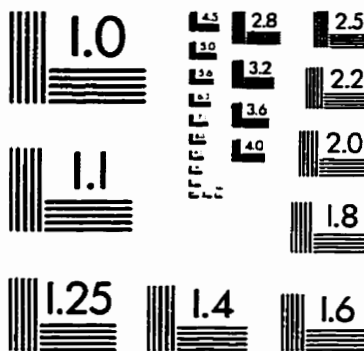
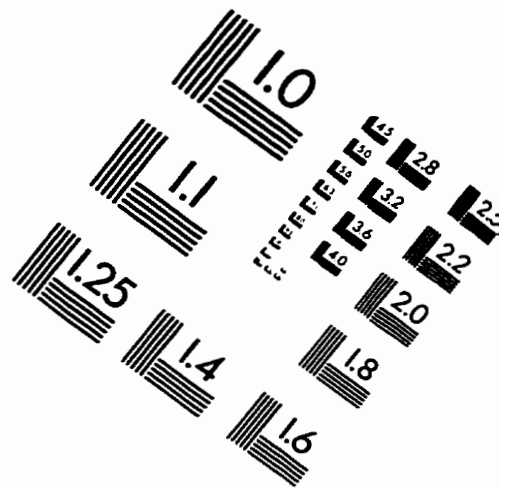
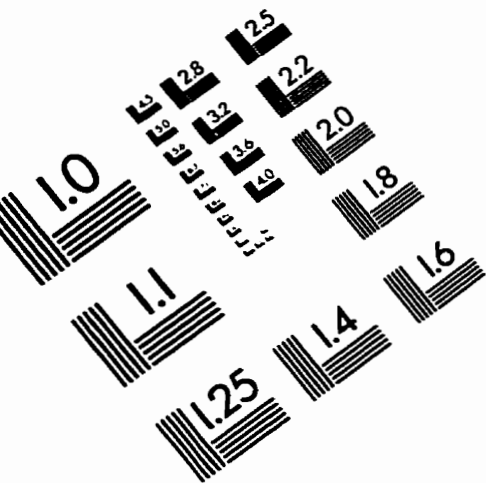
Appendix 5.1. Analysis of variance for components of incomplete resistance, disease severity and area under the disease progress curve values in six lentil lines/cultivars tested with isolate JPPTNL 882 of *Colletotrichum truncatum* in the field.

Source	DF	Mean squares						AUDPC
		IP	LP	LN	LS	SLS	DS	
Year (Y)	1	266***	1064***	3.0*	1.3	68	2	8911*
Reps/(Y)	6	3	3	0.5	0.3	216	7	549
Line/cv. (L/C)	5	126***	396***	53***	73***	4644***	1162***	72699**
L/C x Y	5	21**	33*	0.5	1.5*	126	10	1378
Error	30	2	10	0.5	0.5	140	16	640
C.V. (%)		10	13	26	22	32	35	65

IP=incubation period, LP=latent period, LN=lesion number, LS=lesion size, SLS=lesions sporulating per stem, DS=disease severity, AUDPC=area under the disease progress curve.

*,**,** Signifcant at $p=0.05$, $p=0.01$ and 0.001 , respectively.

IMAGE EVALUATION TEST TARGET (QA-3)



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