COLLETOTRICHUM SPP. ON DRY BEANS AND LUPINS IN SOUTH AFRICA

by

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ABSTRACT

Three anthracnose diseases, caused by three different *Colletotrichum* spp., of importance in South Africa were studied. Anthracnose of *Lupinus albus* was recorded for the first time and the causal organism described as *C. tortuosum* Koch & Baxter. *Chamaecyticus palmensis* was added to the host range of *C. trifolii.* Differential *Phaseolus vulgaris* cultivars were used to distinquish seven races of *C. lindemuthianum*. None of 30 local bean cultivars showed resistance to the races of the fungus present in South Africa. Different methods for the detection of *C. lindemuthianum* on bean seed were evaluated. The paper doll method was found the most effective. Various fungicides were evaluated for their effectiveness to control seedborne *C. lindemuthianum*. Benomyl was found to be the most effective.

Keywords: control, *Chamaecytisus palmensis*, *C. lindemuthianum*, *C. tortuosum*, *C. trifolii*, *Lupinus albus*, *Phaseolus vulgaris*, races, seed-borne, taxonomy

CONTENTS

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CHAPTER 1

GENERAL INTRODUCTION

Several members of the family Fabaceae (legumes) are grown world-wide as a source of protein for man and animals. One of the major constraints in the production of these legumes is the occurrence of a wide variety of diseases. Of particular concern is a disease commonly referred to as anthracnose which is caused by several species of the fungal genus *Colletotrichum* Corda.

Anthracnose is of particular interest regarding several crops in South Africa. Of these, anthracnose of dry beans (*Phaseolus* spp.), lupins (*Lupinus* sp.), lucerne (*Medicago sativa*) and medics (annual *Medicago* spp.) are the most devastating. Successful production of these valuable sources of protein will in future rely strongly on the development and implementation of integrated disease control strategies.

An integrated control system is normally structured to include several components which control diseases. These components, in turn, are identified from the accumulated knowledge of the specific pathogenic relationships. The available information with respect to some of these relationships (Review of Literature, Chapter 2) highlighted the lack of information concerning several of these aspects. The aim of this study is to provide more information, with direct implication for the industry, on some economically important anthracnose diseases in South Africa.

Anthracnose of lupins (*Lupinus albus* L.) was only recently recorded in South Africa. Research into the identity of the causal fungus is of very low priority to producers. Taxonomy is the corner-stone of plant pathogenic relationships. The wrong identification of a pathogen could be misleading and subsequent epidemiological studies and control strategies worthless. As explained in Chapter 3, the aim was to apply classical taxonomical methods for the identification and separation of the species. The identity and taxonomic position of the causal organism of the lupin anthracnose in South Africa is unresolved and is addressed in Chapter 3.

Distinction between *C. trifolii* and *C. lindemuthianum* on medics is discussed in Chapter 2 and an additional host for *C. trifolii*, namely *Chameacyticus palmensis*, is recorded in Chapter 4. In this case pathogenicity was employed as a further tool in the identification of the casual organism.

Colletotrichum lindemuthianum on dry beans is of particular concerne to the local dry bean industry and therefor three aspects concerning anthracnose of dry beans were studied.

To control certain diseases it is sometimes necessary to identify pathological races of the fungi involved. A set of internationally recognized differentiating cultivars with distinct resistant genes is a prerequisite for such studies. Pathological races of *C. lindemuthianum* on *Phaseolus vulgaris* in South Africa have not previously been identified and is reported on in Chapter 5.

In an effective control programme it is of utmost importance to determine the presence of the pathogen on host material. Reliable detection methods are therefore essential. The causal organism of anthracnose of beans, *C. lindemuthianum,* is seedborne and detection of the fungus on seed is necessary to prevent, or at least curb, spreading of the disease and for the production of disease-free seed. In Chapter 6 different detection methods are evaluated according to their effectiveness, cost, efficiency in terms of time and space requirements for maintaining the samples in controlled environments.

As control of a plant disease is based on different aspects that reduce the inoculum potential, it is sometimes necessary to apply chemicals as control measure. These chemicals have to comply with certain standards and requirements. It must be effective against the pathogen involved, but must not harm the host plant in any way. Furthermore, it must be environmentally friendly, easy to apply and cost effective. In South Africa there is at present no registered seed-treatment to control spread of dry bean anthracnose through seed. As a supplementary control measure to the use of disease-free seed, fungicidal seed treatments were evaluated for their effectiveness in controlling seedborne anthracnose (Chapter 7).

CHAPTER 2*

A REVIEW OF LITERATURE

The world's crop plants form only a very small portion of the plant kingdom. Legumes are a large group that are second only to the cereals as a source of human and animal food. They belong to the family Fabaceae, formerly Leguminosae, characterized by the ability to form a symbiotic relationship with a group of bacteria (*Rhizobium* and *Bradyrhizobium* spp.) which can utilize atmospheric nitrogen. Legumes have a worldwide distribution. Their success may be due to their effective relationship with the nitrogen-fixing bacteria. The family comprises about 18 000 species, characterized by their fruits, which are pods, and by their (usually) alternate, compound, pinnate or trifoliate leaves (Cobley & Steele, 1976). Within the Fabaceae the growth form is diverse, ranging from hardwood trees in tropical rain forests to the herbs in temperate pastures.

The Fabaceae has three sub-families (Polhill & Raven, 1981), the Caesalpinioideae, the Mimosoideae, and the Papilionoideae; the first two consist mainly of tropical trees and shrubs with few economically important species. It is the Papilionoideae that is of agricultural importance. Their value lies in the nitrogen-rich plant material consumed by man and animals and the nitrogen-rich residues they leave in the soil, thus enhancing the productivity of other crops grown in association with them. Three kinds of human food are supplied, namely leaves, green pods and unripe seed (vegetable legumes), ripe dry seeds (grain legumes, pulses) and edible tubers. Together they form food legumes in contrast to forage or fodder consumed by animals.

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The Papilionoideae (or Faboideae) includes some 440 genera which consists of 1200 species. These are further classified in 32 botanical tribes (Polhill & Raven, 1981), six of which include the major vegetable and grain legumes. The Vicieae (Adans.) DC. which includes the genera *Vicia* L., *Pisum* L.and *Lens* Mill.; the Cicereae Alefeld which includes *Cicer*; the Phaseoleae DC. which includes *Phaseolus* L., *Vigna* Savi, *Glycine* Willd and *Cajanus* DC.; the Aeschynomeneae (Benth.) Hutch which contains *Arachis* L. and the Genisteae (Adans.) Benth. which includes *Lupinus* L. and *Chamaecytisus* Link.

In the summer rainfall area of South Africa *Arachis hypogaea* L., *Glycine max* (L.) Mer., *Phaseolus vulgaris* L. and *Vigna ungiculata* (L.) Walp. are extensively grown. In the winter rainfall area *Medicago sativa* L., *Trifolium* spp., medics (annual *Medicago* spp.) and *Lupinus* spp. are grown as fodder crops. *Chamaecytisus palmensis* Webb. is at present grown as an experimental fodder crop.

Many members of the genus *Colletotrichum* Corda of the Melanconiales (Coelomycetes) are the cause of anthracnose ("like coal") disease of many members of the family Fabaceae. Lenné (1992) reviewed colletotrichum diseases of legumes, providing a list of accessions of the International Mycological Institute Herbarium (IMI, International Mycological Institute, Egham Surrey, United Kingdom) and additional records of *Colletotrichum* species on legumes. The *Colletotrichum* spp. involved are: *C. acaciae* Gunter, *C. capsici* (H. Sydow) E. Butler and Bisby, *C. coccodes* (Wallr.) Hughes, *C. crassipes* (Speg.) Arx, *C. crotalariae* Petch, *C. dematium* (Pers.) Grove, *C. destructivum* O'Gara, *C. gloeosporioides* (Penz.) Penz. & Sacc., *C. lebbek* (H. Sydow) Petrak, *C.*

lindemuthianum (Sacc. and Magn.) Br. & Cav., *C. medicaginis* Pavgi and U. P. Singh, *C. pisi*, *C. rhynchosiae* Pavgi and U. P. Singh, *C. sesbaniae* Pavgi and U. P. Singh, *C. teramnicola* Pavgi and U. P. Singh, *C. trifolii* Bain and Essary and *C. truncatum* (Schw.) Andrus and Moore. Of these 17 species only *C. capsici*, *C. coccodes*, *C. crassipes*, *C. dematium*, *C. destructivum*, *C. gloeosporioides*, *C. lindemuthianum*, *C. trifolii* and *C. truncatum* are currently recognized as taxa (Sutton, 1980; 1992; Baxter, Van der Westhuizen & Eicker, 1983).

Colletotrichum spp. are not easily identifiable and no protocols for the identification of species exist (Sutton, 1980, 1992; Baxter *et al.*, 1983; Koch, Baxter & Knox-Davies, 1989; Walker, Nikandrow & Millar, 1991). Although morphology will always form the basis of separating species, or groups of species, other non-morphological methods might contribute to the range of characteristics used in identifications. This is of particular value between and below species level. Various attempts to categorise *Colletotrichum* have been published (Park *et al.*, 1987; Dale, Manners & Irwin, 1988; Ali *et al.*, 1989; Braithwaite and Manners, 1989; Braithwaite, Irwin & Manners, 1990; Sreenivasaprasad, Brown & Mills, 1992; Freeman, Pham & Rodrigues, 1993; Sherriff *et al.*, 1994). To date there is no review available of the biochemical and molecular methods which have been or may be used for this genus. Waller *et al.* (1993) successfully used a biochemical character (utilization of tartrate and/or citrate) in the separation of *C. kahawae* J.M. Waller & P.D. Bridge from *C. gloeosporioides*.

Sutton (1992) proposed that systems documented for other groups (Mugnai, Bridge &

Evans, 1989; Monte, Bridge & Sutton, 1991) be applied to the genus *Colletotrichum*. This includes items like standardization of media and conditions for growth, physiology and primary metabolism (including temperature relationships, pH, growth in the presence of various compounds, formation of inhibition zones with various compounds, primary biochemistry such as hydrolysis, reduction, liquefaction, C and N sources), API ZYM tests, secondary metabolites and Biolog™. The only attempt along these lines in *Colletotrichum* was by Ferraz (1977) and Ferraz & Lima (1982). Their work was unsuccessful due to the assumption that *Colletotrichum* differs from *Gloeosporium*, which proved to be incorrect (Sutton, 1992).

Lenné (1992) mentioned *C. gloeosporioides* on lupins, but in her list only *C. trifolii* on *Lupinus luteus* ex Chile and an unidentified *Colletotrichum* sp. from a *Lupinus* sp. are listed. Some confusion exists as to the identity of the causal organism of lupin anthracnose. In France, Chile, Canada and Australia the cause of anthracnose of *Lupinus albus* L. was reported to be *C. gloeosporioides* by Gondran (1994), Peredo & Valezuela (1988), Sweetingham *et al*. (1995) and Paulitz, Atlin & Gray (1995). Sherriff *et al*. (1994) found an isolate from lupins closely related to *C. musae* (Berk. and M.A. Curtis) von Arx with rDNA sequencing. Based on molecular identification by Sreenivasaprasad, Mills & Brown (1994), Reed, Dickens & O'Niel (1996) identified the cause of anthracnose of ornamental lupins (*L. polyphyllus* Lindl.) in the United Kingdom as *C. acutatum* J.H. Simmonds ex J.H. Simmonds. The *Colletotrichum* sp. associated with *Lupinus albus* in South Africa and France is believed to be a different, and unrelated to the above taxa.

Doidge (1915), Von Arx (1957, 1970) and Baxter *et al.* (1983) found *C. trifolii* and *C. lindemuthianum* morphologically closely related. Sutton (1980), however, did not include *C. trifolii* in his list of species, or even listed it as a synonym, but extended the host range of *C. lindemuthianum* to include *G. max*, *M. sativa* and various other members of the Fabaceae. Sherriff *et al.* (1994) found in an analysis of an 886-bp region of their 28S rDNA sequences, IST 2 regions and other domains that *C. lindemuthianum*, *C. orbiculare*, *C. malvarum* and *C. trifolii* are closely related and that they are distinct from *C. gloeosporioides*. They proposed that these must now be regarded as biological variants of *C. orbiculare* (Berk. and Mont) von Arx. and the host origin should, when necessary, be indicated by use of *var.* or *forma specialis*. Pending a comparative study which includes morphology, cultural, pathogenicity, molecular and monoclonal antibody characteristics, of these species, Koch *et al.* (1989) considered *C. trifolii* to be a distinct species. Koch *et al.* (1989) tabulated the recorded host ranges and confirmed the pathogenicity of five *Colletotrichum* species (*C. coccodes*, *C. dematium*, *C. destructivum*, *C. trifolii* and *C. truncatum*) isolated from *Medicago sativa* in South Africa. *C. trifolii* was recorded from *M. sativa* (Doidge, 1915; Koch, Knox-Davies & Lamprecht, 1988), *Glycine max* (Baxter *et al.*, 1983), *M. aculeata* Willd., *M. orbiculars* Bart., *M. tornata* Mill. and *M. truncatula* Gaertn. (Lamprecht & Knox-Davies, 1984). In Chapter 4 *Chamaecytisus palmensis* is added to the host range of *C. trifolii*.

Anthracnose of common beans (*Phaseolus vulgaris*), caused by *C. lindemuthianum,* is a world-wide problem in bean producing areas. The disease has been extensively studied in North America (Tu, 1988; 1992a,b; Kelly, Afanador & Cameron, 1994), Europe

(Drijfhout & Davis, 1989) and South America (Pastor-Corrales & Tu, 1989; Pastor-Corrales *et al.*, 1995). Although it is still regarded as one of the most serious bean diseases, its economic importance has declined in developed countries through the effective use of clean seed and widespread use of resistant varieties (Fouilloux, 1979; Allen, 1983; Pastor-Corrales & Tu, 1989). However, anthracnose outbreaks, either due to the development of new races or to the introduction of known races to new areas, still occur. Some typical examples are the introduction of the delta and lambda races into Canada in 1977 (Tu, 1988) and races 7 and 73 into North America in 1994 (Kelly, Afanador & Cameron, 1994). Two different nomenclature systems exits. The original one in which Greek alphabetical names was assigned, is relatively difficult to compare with the new binomial one in which values are assigned. Comparison is difficult because the differential cultivars in the two sets differ. Strict implementation of seed treatment, pedigree seed inspection, crop rotation and the development of resistant varieties have achieved effective control in developed countries (Tu, 1988). In developing countries, however, anthracnose remains a serious disease and is regarded as one of the principal diseases of beans throughout tropical regions including Latin America and East Africa (Allen, 1983; Pastor-Corrales & Tu, 1989).

Although pathogenic specialization in *C. lindemuthianum* has been known for more than 80 years (Barrus, 1911), little is known about it in Africa, including South Africa. Mainly due to the lack of an internationally acceptable set of differential cultivars little research was done, and those done were not comparable. At the time that Drijfhout & Davis (1989) made their set of homogenously reacting differential cultivars available, Pastor-Corrales and his Latin American co-workers (CIAT, 1988) made a set of differentials available. This was based on the nomenclature system of race designation proposed by Johnson *et al*. (1972).

Tu (1992b) reviewed inoculation methods and disease ratings used by various researchers. Differences between the methods also contribute to variations in the results that are obtained. Temperature is a very important factor in race determinations. The dip method of Drijfhout & Davies (1989), adapted from Hubbeling (1961), is labour intensive. Furthermore, germinated seed is easily damaged when the seed coats are removed prior to dipping in the inoculum and planting. The method used by CIAT (International Center for Tropical Agriculture) is less labour intensive (M.A. Pastor-Corrales, CIAT, Cali, Columbia, personal communication), but seed germination is unreliable. All the seed for use in race determinations has to be produced in a glasshouse to keep them genetically pure and free from disease.

In Chapter 5 the pathological potential of South African races of the fungus by means of differential bean cultivars are evaluated. Cultivar resistance is still the most important control measure. Therefore, the local bean cultivars should be tested for resistance to the most important local races of *C. lindemuthianum*. Seven resistance genes to various races of *C. lindemuthianum* have been identified and is listed with their new names by Kelly & Young (1996).

In North America and Europe effective control of anthracnose was achieved by the production of disease free seed and chemical seed treatment (Tu, 1988). For the production of disease free seed, the backing of a reliable laboratory testing method is necessary. These laboratory tests must also conform to the requirements of the Rules of the International Seed Testing Association (ISTA, 1993).

Various chemical control methods, foliar sprays and seed treatments have been prescribed for anthracnose of beans (Sindhan & Bose, 1981; Tu, 1996). Little published information is available. Seed treatment is a cost-effective control measure. It eliminates or reduces the inoculum potential of a disease in the early stages of plant growth. No seed treatment for the control of seed-borne *C. lindemuthianum* is at present registered in South Africa in terms of Act 36 of 1947.

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CHAPTER 3*

MORPHOLOGICAL AND CULTURAL CHARACTERIZATION OF THE CAUSAL ORGANISM OF LUPIN ANTHRACNOSE, *COLLETOTRICHUM TORTUOSUM* SP. NOV.

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SUMMARY

Colletotrichum isolates associated with anthracnose of *Lupinus* spp. from South Africa and France were morphologically and culturally characterized on three different media. Based on average conidial size and shape as well as appressorial outline and conformation, and colony appearance and texture these cultures were distinguished from *C. acutatum*, *C. gloeosporioides* and *C. musae*. On the grounds of differences in these features the new name, *C. tortuosum,* is introduced for the organism causing lupin anthracnose. Disease symptoms are described and illustrated. This is the first report of lupin anthracnose in South Africa.

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Lupinus species are grown as a fodder crop in many areas of the world. In South Africa lupins were initially produced in the Western Cape Province as a winter crop. Recently they were also introduced to the cooler parts of the Free State and Mpumalanga Provinces as a summer crop.

Anthracnose of white lupins (*Lupinus albus* L.) is a major constraint to lupin production in many countries. The disease is well established in Brazil, Chile, southern Germany and France (Gondran, 1994; J.A.M. van der Mey, Oil and Protein Seed Centre, Grain Crops Institute, Potchefstroom, personal communication). In Austria lupin production started badly with the introduction of a large amount of contaminated seed from Chile. The effect on cultivar Amiga was so devastating that further development of the crop was discontinued (Ronell Keeve, Oil and Protein Seed Centre, Grain Crops Institute, Potchefstroom, personal communication). Anthracnose of lupins was also recently introduced into Western Australia (Sweetingham *et al*., 1995), the source of infection being traced to white lupin lines imported from Germany. Its introduction into New Zealand has led to the eradication of *Lupinus arboreus* Sims (Dick, 1994; J.A.M. van der Mey, Oil and Protein Seed Centre, Grain Crops Institute, Potchefstroom, personal communication). The disease has also been reported on white lupins in Canada (Paulitz, Atlin & Gray, 1995).

Anthracnose of lupins was first recorded on *L. albus* in South Africa during the summer of 1993/94 in the Free State Province. During the winters of 1995 and 1996 cultivar trials at Elsenburg, Department of Agriculture and Development, Western Cape Province, also became heavily infected.

There is some confusion regarding the identity of the pathogen. In France, Chile, Canada and Australia the cause of anthracnose of *L. albus*. was reported to be *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. by Gondran (1994), Peredo & Valenzuela (1988), Sweetingham *et al*. (1995) and Paulitz *et al*. (1995). Using rDNA sequencing Sherriff *et al*. (1994) found that an isolate from lupins was closely related to *C. musae* (Berk. & M.A. Curtis) von Arx. Also based on molecular characterization Reed, Dickens & O'Niel (1996) state the cause of anthracnose of ornamental lupins (*L. polyphyllus* Lindl.) in the United Kingdom to be *C. acutatum* J.H. Simmonds ex J.H. Simmonds based on the work of Sreenivasaprasad, Mills & Brown (1994).

This paper is the first report of anthracnose of *L. albus* in South Africa and the causal organism, *C. tortuosum* sp. nov., is formally named.

MATERIALS AND METHODS

Disease Characteristics

Diseased *L. albus* plants were collected from Bethlehem in the Free State Province (February 1994) and Elsenburg in the Western Cape Province (September 1995 and 1996). Visual disease symptoms were recorded. Pathogenicity studies were performed on six week old lupin seedlings.

Isolation

Diseased material was surface disinfected for 2 min. in 1% NaOCl solution and rinsed with sterile distilled H_2O. Small (ca. 5 mm) squares of diseased material were incubated in Petri dishes on wet filter paper. Conidial masses were transfered to potato dextrose agar (39 g Oxoid Potato Dextrose Agar/1000 ml H_2O supplemented with Streptomycin (0,05g/1000 ml and Chloramphenicol (0,05g/1000 ml) (PDA). Single spores were isolated and cultures were maintained on potato carrot agar (20 g potato, 20 g carrot, 15 g agar/1000 ml H_2O) (PCA) slants at 5 °C in the dark.

Isolates examined

PREM 54898, *L. albus* pods, Elsenburg near Stellenbosch, RSA, November 1995, S.H. Koch

PPRI 6128, *L. albus*, Bethlehem, RSA, 1994, A.B. van Jaarsveld [SHK 788]

PPRI 6129, *L. albus* cv. C5, Elsenburg near Stellenbosch, RSA, September 1995, S.H. Koch [SHK 1031]

PPRI 6130, *L. albus* cv. C38, Elsenburg near Stellenbosch, RSA, September 1995, S.H. Koch [SHK 1032]

PPRI 6131, *L. albus* cv. C25, Elsenburg near Stellenbosch, RSA, September 1995, S.H. Koch [SHK 1033]

PPRI 6132, *L. albus* cv. SAL36, Elsenburg near Stellenbosch, RSA, September 1995, S.H. Koch [SHK 1034]

PPRI 6133, *L. albus* cv. SAL14, Elsenburg near Stellenbosch, RSA, September 1995, S.H. Koch [SHK 1035]

PPRI 6134, *L. albus* cv. SAL12, Elsenburg near Stellenbosch, RSA, September 1995, S.H. Koch [SHK 1036]

PPRI 6135, *L. albus* cv. E25, Elsenburg near Stellenbosch, RSA, September 1995, S.H. Koch [SHK 1037]

PPRI 6136, *L. albus* cv. Tina, Elsenburg near Stellenbosch, RSA, September 1995, S.H. Koch [SHK 1038]

PPRI 6137, *L. mutabilis*, Dijon, France, 6 August 1982, J. Gondran [G1, SHK 1048]

PPRI 6138, *L. albus*, Jouy near Paris, France, 24 August 1984, J. Gondran [G4(a), SHK 1049]

PPRI 6139, *L. albus*, Jouy near Paris, France, 24 August 1984, J. Gondran [G4(b),SHK 1050]

PPRI 6140, *L. polyphyllus* var. Russel, France, 2 July 1987, J. Gondran [C87-139, SHK 1051]

PPRI 6141, *L. mutabilis*, Lusignan, France, 3 August 1994, J. Gondran [G47(a), SHK 1052]

PPRI 6142, *L. mutabilis*, Lusignan, France, 3 August 1994, J. Gondran [G47(b), SHK 1053]

PPRI 6143, *L. albus* cv. Lucky, Lusignan, France, 3 August 1994, J. Gondran [G50(a), SHK 1054]

PPRI 6144, *L. albus* cv. Lucky, Lusignan, France, 3 August 1994, J. Gondran [G50(b), SHK 1055]

Dried reference material (pods) (PREM) and cultures (PPRI) were deposited in the

National Collection of Fungi of the Plant Protection Research Institute, Pretoria. The French isolates were supplied by J. Gondran, Institut National de la Recherche Agronomigue (INRA), Ministère De L'Agriculture, 86600 Lusignan, France.

Identification

Cultures were grown on PDA, PCA (150 g potato, 30 g carrot, 15 g Merck Agar Agar/ 1000 ml) (Koch, Baxter & Knox-Davies, 1989) and malt salts agar (1 g ammonium nitrate, 1 g magnesium chloride, 1 g potassium phosphate, 5 g malt extract, 15 g Merck Agar Agar/1000 ml) (MSA) (Baxter, Van der Westhuizen & Eicker, 1983) for 10 days under 12 h fluctuating light/darkness regimes at 23 °C. Slide cultures, for appressoria formation, were prepared using PCA (Sutton, 1962).

Using standard terminology, colony characteristics such as colour (Rayner, 1970), texture (Long & Harsch, 1918), zonation, sectoring, edge form (Hawksworth, Sutton & Ainsworth, 1983) and appearance of conidial masses were determined on all three media. Morphology in culture was recorded only on PCA. Optical outline, conformation, relative abundance and size of mycelial appressoria were also recorded.

Slide preparations from 10-d-old cultures were made using the polyvinyl alcohol mounting medium (8·33 g polyvinyl alcohol, 50 ml lactic acid, 5 ml glycerine and 50 ml distilled water) of Koske & Tessier (1983). Appressoria were observed in slide cultures prepared according to Sutton (1962), using PCA. Observations were made with bright field and differential interference contrast illumination. Length and width of conidia and appressoria (30 each/isolate) were measured. Colony diameters after 7 days in culture on PDA, PCA and MSA at 25 °C were recorded.

RESULTS AND DISCUSSION

Disease characteristics

The first symptom of the disease is the wilting of growth tips. Severely affected plants show undetermined curling of the branches in different directions (Fig. 1). Large diamond shaped lesions with brown borders and orange centres are found on the branches (Fig. 2). As the disease progresses, flowers and pods are also affected. On pods the lesions are circular (Fig. 3), but still characterized by a slight brown border and orange centre, the latter due to the presence of conidial masses. The disease is seed-borne. Seed symptoms are difficult to characterize, varying from brown to black non-determined lesions (Fig. 4).

Identification of the pathogen

A *Colletotrichum* sp. has consistently been isolated from anthracnose lesions on lupin and its association with the disease confirmed. The fungus caused the same symptoms under glasshouse conditions and was successfully re-isolated.

Many *Colletotrichum* Corda sp. (Von Arx, 1957; Sutton, 1980; 1992) cause diseases known as anthracnose on plants in a wide variety of genera. The family Fabaceae, to which the genus *Lupinus* belongs, includes several examples of anthracnose diseases

(Tiffany & Gilman, 1954; Lenné, 1992). Currently there are no protocols regarding the use of cultural media and conditions for the evaluation and description of *Colletotrichum* isolates (Sutton, 1992). In recent studies various media have been used by different authors [Sutton (1980) = PDA; Baxter *et al.* (1983) = Czapek-Dox yeast extract and MSA; Gunnell and Gubler (1992) = strawberry leaf agar; Walker, Nikandrow & Millar (1991) = PDA and potato vegimite agar or carnation leaf agar; Koch *et al.* (1989) = PCA; Waller *et al.* (1993) = malt extract agar]. Most of these authors state that cultural variation is detected on the different media. In this study PDA was used for cultural characteristics, because of its universal availability. PCA (both ingredients available world-wide) was used as a sporulation medium and for characteristics such as sclerotia production and formation (Koch *et al.*, 1988). MSA was used for comparison with the work of Baxter *et al.* (1983). We would therefore propose that PDA is used, as in the case of *Fusarium* spp. (Nelson, Toussoun & Marasas, 1983), for colony morphology and PCA for conidial and sclerotial production and formation as standard media for identification of *Colletotrichum* spp.

Weimer (1943, 1951) and Weimer & Dunegan (1949) indicated *C. gloeosporioides* as the cause of anthracnose of *L. angustifolius* L. in the USA, although neither Von Arx (1957) nor Sutton (1980, 1992) referred to any *Colletotrichum* species on lupin. Lenné (1992) noted *C. gloeosporioides* on lupin, while mentioning two listed species on lupin, namely *C. trifolii* Bain & Essary and an unknown *Colletotrichum* spp. in IMI records. Tiffany & Gilman (1954) mentioned *Glomerella cingulata* (Stoneman) Spauld. & H. Schrenk on *L. angustifolius*.

Walker *et al.* (1991) stressed the importance of the correct identification of species to assist plant pathologists in their decision making. Strict quarantine measures are necessary to prevent further spread of this devastating disease. In Europe, South Africa and Australia lupin anthracnose is a major problem and at this stage control in South Africa and Australia is based on eradication (all diseased plant material must be destroyed by burning). No seed must be produced from infected plants. It is therefore of utmost importance that this fungus is recognised as a separate taxon. Along with Walker *et al.* (1991) we plead for clarification of the species concept in *Colletotrichum* as it is a matter of considerable practical importance.

This is the first report of anthracnose of *L. albus* in South Africa. Neither the keys of Sutton (1980) nor Baxter *et al.* (1983) enabled us to identify the fungus. The mycelium of the pathogen appear predominantly closely appressed on all three media used. Conidia are mostly borne on single hyphae over the whole colony although apparently constricted in the centre and in concentric zones. Although conidial length varies greatly, the average length of 12-13 µm is much less than that of *C. gloeosporioides* or *C. musae* and more than that of *C. acutatum* (Table 1). The conidia are oblong to cylindrical but mostly narrowly obovoid, and tapered towards a truncate base, whereas those of *C. acutatum* are fusiform and abruptly tapered at each end (Table 1). There are also differences in conidia produced from free hyphae in slide culture and conidia produced in pure culture (Cox & Irwin, 1988). This was also observed in this study where conidia from the host material had in length a narrower range and a higher mean.

Based on the traditional Saccardoan characteristics such as conidial size and shape, as well as the morphology of appressoria, the lupin anthracnose fungus (LAF) is distinguished from *C. gloeosporioides* by the shorter, wider conidia of the former, which taper to a truncate base. Appressoria of the LAF are generally smaller and colonies are brownish in colour apposed to grey and mycelium appressed opposed to even and felty or in tufts. *C. fragariae* Brooks, although having conidia of similar shape and size to those of the LAF, is separated by the presence of setae in culture. *C. musae* can be separated from the LAF by its higher growth rate and narrower conidia (Table 1). *C. acutatum* is separated from *C. musae* by the presence of fusiform conidia in the former and the pink to carmine pigment produced in culture. In terms of growth rate the LAF may compare with *C. kahawae* J.M. Waller & P.D. Bridge. The conidial morphology and the pathogenic specialization of the latter (Waller *et al*., 1993) clearly separate these two fungi. This species delimitation is complicated by the involvement of *C. acutatum* as the causal organism of anthracnose of ornamental lupins in the United Kingdom (Reed *et al*., 1996). These authors based their identification of *C. acutatum* on nucleotide sequence of the rDNA spacer 1 of some "atypical" isolates of *Colletotrichum* (Sreenivasaprasad *et al*., 1994). Earlier Sreenivasaprasad, Brown & Mills (1992) suggested that isolates of *C. fragariae* would fit in the group species concept of *C. gloeosporioides*. This suggestion was made following confirmation of a close molecular relationship of the supposedly morphologically indistinguishable *C. gloeosporioides* and *C. fragariae*. However, Gunnell and Gubler (1992) clearly distinguished *C. gloeosporioides* from *C. fragariae* on the grounds of morphological and colony characteristics. For the time being there are two opposing approaches: ignorance of morphological differences ("atypical" and morphologically indistinguishable isolates) where molecular data serves as the taxonomical tool; and characterization by means of morphological and cultural characteristics. The latter approach has been adopted in the case of our description of LAF as a new species. The *Colletotrichum* strains associated with anthracnose of *Lupinus* spp. are believed to be distinct from *C. acutatum, C. gloeosporioides* and *C. musae*. Host range and host specificity, as used by Von Arx (1957), Sutton (1980), Baxter *et al*. (1983) and Waller *et al*. (1993) were not considered. We propose the new name *Colletotrichum tortuosum* for the LAF as follows. A comparison of the four species in question is given in Table 1.

Colletotrichum tortuosum S.H. Koch and A.P. Baxter, sp. nov.

Etym.: Latin *tortuosus* - winding, having an irregular, bending and turning direction, referring to the characteristic disease symptoms.

Coloniae in agaro dextroso cum *Solano tuberoso* (PDA) in 25 °C post 7 dies 27 mm diam., ubique arte appressae, brunneae: in margine bubalinae ad pallide melleae, in medio fulvae vel melleae vel luteo-electrinae vel cinnamomeae ad hinnuleae, in centro atrosepiaceae ad sepiaceae vel propter massam conidiorum sectores cinnamomeas efformantes, aut hinnuleae. Aspectus reversus similis. *Margo* aequatus, interdum sinuatus vel irregularis. *Conidiomata* parva, non stromatica. *Conidiophora* simplicia vel parce ramosa, plerumque in hyphis aeriis aut ex conidiomatibus oriunda. *Cellulae conidiogenae* enteroblasticae, monophialidicae, saepe ex hyphis simplicibus oriundae. *Conidia* hyalina, recta, subtiliter guttulata, non constricta, oblonga vel cylindrica sed plerumque amguste obovata, ad apicem obtusa, ad basim leniter attenuata, hilum truncatum saepe distincte protruberans, 8·0-(12·0-13·0)- 20·0 X 4·0-(4·1)-6·0 µm, in massis mucosis cinnamomea. *Appressoria mycelialia* abundantia, brunnea vel atrobrunnea, circularia vel clavata vel irregulariter lobata, interdum catenulata, foramen germinale saepe conspicuum exhibentia, 6·0-(8·9)-14·0 X 4·0-(6·9)-10·0 µm. *Setae* nullae.

In vivo Lupino albo; holotypus ex natura: in laesionibus in leguminibus, collectus in fundo Elsenburg, prope Stellenbosch, Prov. Capensis, Africa Meridionalis, S.H. Koch, Nov. 1995, PREM 54898.

Colonies on **PDA** at 25 °C after 7 d 27 mm diam., closely appressed throughout and brownish: margin Buff to pale or light Honey, middle zone Fulvous to Honey, Luteous Amber or Cinnamon to Fawn, centre dark Sepia to Sepia with Cinnamon sectors due to conidial masses, or Fawn. *Reverse* similar: middle zone sometimes pale Luteous and centre with Hazel sectors. *Edge* even, sometimes tending to sinuate or uneven. *Zonation* slight, concentric. *Sectoring* absent to slight. *Conidial masses* mostly Cinnamon, abundant, evenly distributed or sometimes more prominent in concentric bands or centre.

Colonies on **PCA** at 25 °C after 7 d 49 mm, closely appressed throughout or slightly downy: margin Buff, middle zone becoming Hazel, centre Isabelline. *Reverse*: margin Buff, middle zone Isabelline, centre Cinnamon-Isabelline. *Edge* even. *Zonation* slight, concentric. *Sectoring* absent. *Conidial masses* mostly Cinnamon, abundant, evenly distributed or sometimes more prominent in centre.

Colonies on **MSA** at 25 °C after 7 d 49 mm, closely appressed at margin and in middle zone, centre sometimes downy: margin Buff, light Rosy buff to light Isabelline, sometimes greyish Buff, middle zone concolorous, centre becoming Rosy Buff to Isabelline. *Reverse* similar: middle zone to centre light Buff, greyish Buff or Rosy Buff to Isabelline. *Edge* even. *Zonation* slight, concentric. *Sectoring* absent. *Conidial masses* pale Apricot to Apricot or sometimes Saffron to Salmon, abundant in discrete concentric bands and centre.

Conidiomata (Fig 9) small, not stromatic. *Conidiophores* simple to sparingly branched, mostly borne on aerial hyphae but also in conidiomata. *Conidiogenous cells* (Figs. 7-9) enteroblastic, monophialidic, often borne on simple hyphae. *Conidia* (Fig. 5) hyaline, straight, finely guttulate, not constricted, oblong to cylindrical but mostly narrowly obovate, apex obtuse, mostly tapered towards a truncate base, base often protuberant; 8·0-(12·0-13·0)-20·0 X 4·0-(4·1)-6·0 µm, Cinnamon in mucoid masses. *Mycelial appressoria* (Fig. 6) abundant, circular to clavate to irregularly lobed, sometimes forming short chains, with an often conspicuous germ pore, 6·0-(8·9)-14·0 X 4·0-(6·9)-10·0 µm. *Setae* absent.

Conidia on *Lupinus albus* mostly narrowly obovate, to cylindrical or somewhat oval in optical section, often unilaterally tapered towards a truncate base, base often protuberant, conidiogenous cells discrete, or integrated on conidiophores which are unbranched or branched only at the base, apex often with a distinct collarette and periclinal thickening. Setae present, common but not abundant, relatively small, generally shorter than conidial masses, 40·0 (60·0)-90·0 X 3·0-(4·0)-4·5 µm, septa 0-3 but mostly absent; mostly sinuate or irregular in optical section, often with deep constrictions which are mostly not associated with septa, mostly narrowing towards the base, basal cell often paler than the upper part but a few with a swollen, dark brown base or arising from a broader, rounded cell; apex mostly obtuse.

In living *Lupinus albus*; Holotype *ex natura*: in lesions on pods collected in Elsenburg near Stellenbosch, Western Cape Province, South Africa, S.H. Koch, Nov. 1995, PREM 54898.

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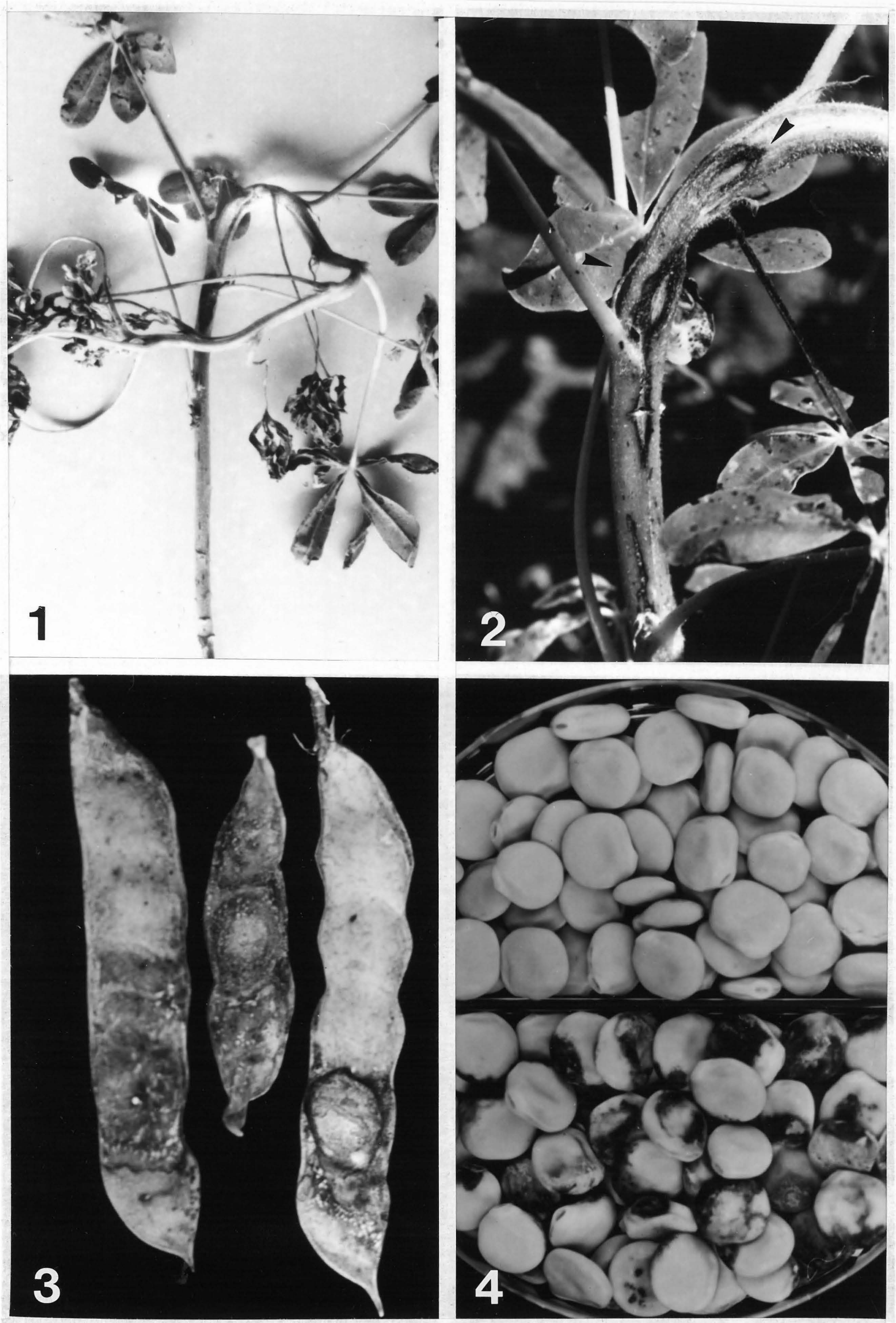
Table 1 Comparison of *C. tortuosum* sp. nov., *C. acutatum*, *C. gloeosporioides* and *C. musae*.

Cultural Characteristics	*Colletotrichum* spp.			
	C. tortuosum	*C. acutatum* [1]	*C. gloeosporioides* [1]	*C. musae* [1]
Conidial shape	Oblong to cylindrical, mostly narrowly obovate, tapered towards truncate base	Fusiform, abruptly tapered at each end, sometimes medianly constricted	Cylindrical	Cylindrical
Conidial length	8-(12-13)-20 µm	8·5-16·5 µm, (10-15 µm)[2] (12·5-22·5 µm)[3]	12-17 µm (10-23 µm)[2] (13-21 µm)[3]	12-17 µm (9-17 µm)[2]
Conidial width	4·0-(4·1)-6·0 µm	2·5-4·0 µm (3·0 -3·5 µm)[2] (3·0-4·5 µm)[3]	3·5-6·0 µm (3·0-5·0 µm)[2] (3·5-5·0 µm)[3]	4·5-5·5 µm (3-5 µm)[2]
Appressorial outline and conformation	Circular to clavate, to irregularly lobed, sometimes forming short chains	Clavate, ovate to obovate to slightly irregular with margins entire or slightly lobed	Clavate, ovate, obovate, sometimes lobed	Irregular in shape and often with large or deep lobes
Appressoria length	6·0-(8·9)-14·0 µm	8-10 µm	6-20 µm	9-13 µm
Appressoria width	4·0-(6.9)-10·0 µm	4·5-6·0 µm	4-12 µm	9·0-11.5 µm
Setae	Absent	Mostly absent	Present or absent	Absent
Colony	Brownish: Buff, pale to light honey, becoming luteus amber or cinnamon to fawn, dark sepia, sepia with cinnamon sectors in centre, aerial mycelium appressed	White, becoming grey to greyish brown, reverse pink to carmine, aerial mycelium dense	Variable, greyish white to dark grey, aerial mycelium even and felted or in tufts, reverse unevenly white to grey or darker with age	Abundant white aerial mycelium becoming grey with age, reverse light cinnamon drab to orange cinnamon, margin entire, thin, appressed
Growth Rate (Colony diameter on MSA after 7 d)	45-54 mm	45-50 mm[2]	35-65 mm[2]	70-80 mm[2]

[1] From Sutton, 1992
[2] From Baxter, Van der Westhuizen & Eicker, 1983
[3] From Gunnell & Gubler, 1992

1. *Lupinus albus* plant infected by *Colletotrichum tortuosum* and showing undetermined curling.
2. Elongated diamond shaped lesions caused by *Colletotrichum tortuosum* on lupin stem.
3. Anthracnose lesions on lupin pod.
4. Clean lupin seed compared with anthracnose seed below.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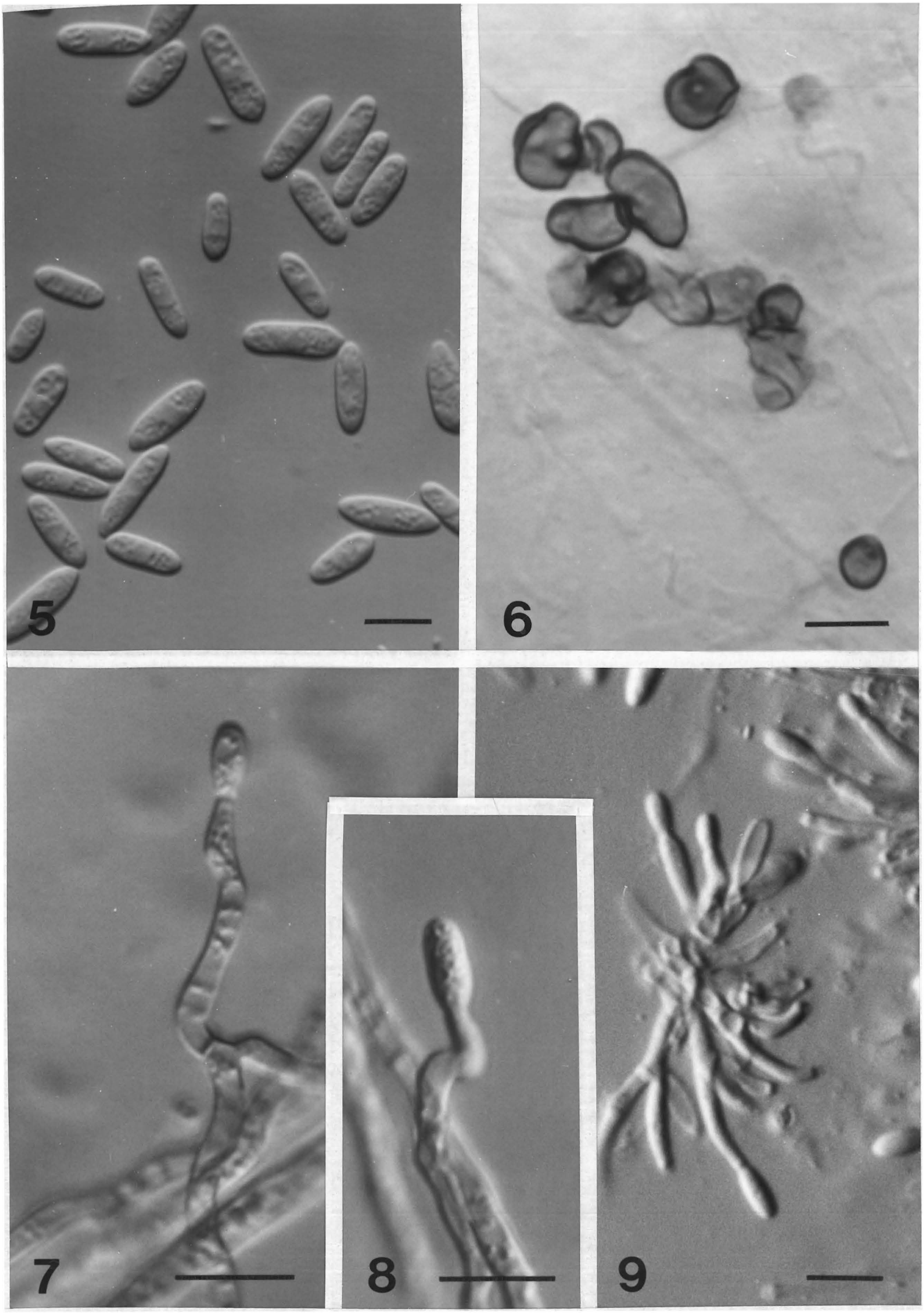
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5. Interference micrograph of conidia of *C. tortuosum* PREM 6131 (Bar = 10 µm).

6. Appressoria of *C. tortuosum* PREM 6131 (Bar = 10 µm).

7 & 8. Conidiophore of *C. tortuosum* PREM 6128 (Bar = 10 µm).

9. Conidiomata of *C. tortuosum* PREM 6128 (Bar = 10 µm).

CHAPTER 4*

Chamaecytisus palmensis, a new host of *Colletotrichum trifolii*

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Anthracnose of two to three-month-old seedlings of *Chamaecytisus palmensis* (tagasaste) was observed during 1989 in an open-rooted nursery in the western Cape Province, South Africa. *Colletotrichum trifolii* was consistently isolated from the lesions. The disease was successfully reproduced on three-week-old tagasaste seedlings. Both tagasaste and *Medicago sativa* (lucerne) were susceptible to isolates of *C. trifolii* from either lucerne or tagasaste. Mature tagasaste plants were also susceptible to infection. This is the first report of *C. trifolii* on tagasaste, an important forage tree used in agro-afforestation.

Key words: *Chamaecytisus palmensis*, *Colletotrichum trifolii*, lucerne, tagasaste.

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Chamaecytisus palmensis Webb. (Syn. *Cytisus proliferus* L.f. (Link)) (tagasaste) a member of the Fabaceae and native to the island Las Palma in the Canary Islands, was introduced into South Africa as a fodder crop which also serves as shelter and shade, for honey production and low windbreaks (Esterhuyse, 1989). Tagasaste is drought resistant and well adapted to the Mediterranean climate of the Western and Eastern Cape Provinces of South Africa. It is also a nitrogen-fixing crop and is inter-cropped with lucerne (*Medicago sativa* L. and annual *Medicago* spp.

Material and methods

During May 1989 seedling die-back, wilted tillers and chlorotic diamond shaped lesions, with acervuli bearing setae and orange conidial masses, on the stems of two to three-months-old tagasaste seedlings were observed. The plants were grown in poor sandy soil in an open-rooted nursery near Atlantis, Western Cape Province, South Africa.

Diseased plants were washed under running tap water, surface disinfested with a 1% NaOCl-solution for 1 min and rinsed with three changes of sterile distilled water. Stem pieces (2 mm^3) cut from the edge of lesions were plated on malt-extract agar and potato-dextrose agar. Isolation plates were incubated at room temperature (20-25°C) for two to five days. One fungus was consistently isolated from the lesions. Cultures were grown from single spores and identified as *Colletotrichum trifolii* Bain & Essary according to the methods described by Koch et al. (1989).

Cultures were stored on potato-carrot agar (PCA1) (20 g potato, 20 g carrot, 20 g $agar.l^{-1}$) slants at 10°C in the dark. Reference cultures (PREM 47787 and PREM 47788) were deposited in the National Collection of Fungi (PREM) of the Plant Protection

Research Institute, Pretoria.

Seed of tagasaste and *Medicago sativa* L. (lucerne) cvs. Cuf 101 and SA Standard was sown in steam-sterilised soil/perlite (1:1) mixture in seedling trays and maintained in a greenhouse at 15 - 25°C. Two mature tagasaste plants, grown from seed in plastic planting bags containing 2 kg steam-sterilised soil were maintained under similar conditions.

Two isolates of *C. trifolii* from tagasaste (PREM 47787 & PREM 47788) and two isolates from lucerne (PREM 47769 & PREM 47770 (Koch et al., 1989)) were grown on PCA2 (150 g potato, 30 g carrot, 20 g agar.l^{-1}). Plates were incubated at 18°C in 12 hour/day light provided by mixed near-ultraviolet and daylight type fluorescent tubes positioned 30 cm above the plates. Conidial suspensions were prepared in sterile distilled water containing two drops.l^{-1} Tween 20 from 10-day-old cultures and the concentration adjusted to 10^6 conidia.ml^{-1}.

For each fungal isolate, 10 three-week-old seedlings of tagasaste and lucerne cvs. Cuf 101 and SA Standard respectively, were inoculated by spraying with the conidial suspensions until runoff. Ten control plants were sprayed with sterile distilled water supplemented with two drops.l^{-1} Tween 20. The two mature tagasaste plants were inoculated with isolates from tagasaste only. Plants were covered with black plastic bags for three days to maintain high relative humidity and to protect them from direct sunlight.

Disease symptoms and severity (scored on a 0-2 scale with 0 = no symptoms, 2 = plants dead) were recorded after eight days on the seedlings and after 14 days to two months on the mature tagasaste plants. Reisolations were made from diseased tissue on inoculated seedlings and mature plants.

Results

Within eight days all the three-weeks-old tagasaste and lucerne cv. Cuf 101 seedlings inoculated the with four *C. trifolii* isolates were dead, but lucerne cv. SA Standard was, affected less severely. Leaves of the inoculated mature tagasaste plants were shrivelled and severe leaf-drop occurred within 14 days after inoculation. Typical anthracnose lesions developed on the side branches and on the main stem. Within two months these lesions had enlarged, girdled the stems and the upper parts of the branches had wilted and died. *C. trifolii* was isolated from diseased tissue on seedlings and mature plants.

Discussion

This is the first report of *C. trifolii* in association with a disease of tagasaste. All the isolates tested were pathogenic to both tagasaste and lucerne. *C. trifolii* appears to be an important nursery pathogen affecting the establishment of tagasaste in open-rooted nursery beds. With the current emphasis on agroforestry and the establishment of pasture crops in South Africa, caution must be exercised when inter-cropping tagasaste with lucerne and annual *Medicago* spp. susceptible to anthracnose and crown rot caused by *C. trifolii*. Lucerne and medic cultivars resistant to *C. trifolii* are available (Lamprecht, 1986; Koch & Knox-Davies, 1989) and the present results confirm that lucerne cv. SA Standard is less susceptible than cv. Cuf 101.

Little is known about tagasaste diseases, although the crop has been cultivated for some time in Australia (Snook, 1986) and New Zealand (Webb, 1982). The diseases affecting this plant should therefore be further evaluated before it is extensively cultivated in South Africa.

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CHAPTER 5[§]

THE IDENTIFICATION OF RACES OF *COLLETOTRICHUM LINDEMUTHIANUM* IN SOUTH AFRICA

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Anthracnose of dry beans (*Phaseolus vulgaris*), caused by *Colletotrichum lindemuthianum*, is a world wide problem. In South Africa the occurrence of the disease is sporadic. Isolates of the fungus were collected from the major dry bean production areas. Fourteen-day-old seedlings from two sets of differential cultivars were inoculated by spraying with conidial suspensions of the fungus. Inoculated seedlings were incubated for seven days at 20 °C in plastic bags to maintain high humidity levels. Disease severity was rated 10 days after inoculation. By using the binomial system proposed by CIAT, races 3, 65, 80, 81, 83, 119 and 593 were found in South Africa. Races 65, 80, 81 and 593 resemble the race alpha-Brazil according to the Drijfhout and Davis system. None of the local bean cultivars showed resistance to five of the local races of the fungus under glasshouse conditions. These results suggest that anthracnose resistance in the local dry bean breeding programme should receive attention.

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INTRODUCTION

Anthracnose of beans (*Phaseolus vulgaris* L.), a fungal disease caused by *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cav., is a world wide problem under cool humid conditions (Schwartz, 1991). The first report of anthracnose of beans in South Africa was in 1906 (Evans, 1906). Doidge (1950) listed early references to the disease in southern Africa. In most parts of South Africa the occurrence of the disease is sporadic, but in Kwazulu-Natal it is more prevalent. All above ground parts of the bean plant are affected and the first signs of disease are a brick-red to purplish red discolouration of the veins on the underside of the leaves. The most characteristic symptom is rusty brown lesions with small brown spots on the pods.

Yield losses are more severe when bean plants are infected early. For example, yield losses of 95% and 38% were recorded when a susceptible bean cultivar was inoculated one and six weeks after plant emergence respectively (CIAT, 1976; Guzmán-Vargas and De la Rosa, 1975). Data on yield losses in South Africa are not available.

Dry beans are an important source of protein in Africa and are readily produced in sustainable agricultural systems. In South Africa, on the other hand, dry beans are mainly produced on a large scale by commercial farmers. Seed production also falls under a disease-free seed scheme which includes anthracnose as a limitation. Due to regular seed shortages, uncertified seed is often planted. This practice enhances spread of the disease.

Production of dry beans (commercial and sustainable) are on the increase in South Africa (J.C. van Wyk, Dry Bean Producer's Organization, Pretoria, Republic of South Africa, personal communication). Disease resistance is the most effective control measure. Differences in susceptibility to anthracnose were noted in South Africa (Dr A.J. Liebenberg, Oil and Protein Seed Centre, Grain Crops Institute, Agricultural Research Council, Private Bag X1251, Potchefstroom 2520, Republic of South Africa) and to assist the breeders, a study was launched to characterize and identify the races of the fungus involved and to evaluate the local cultivars for resistance to the different races.

Attempts have been made to identify races in Africa (Leaky & Simbwa-Bunnya, 1972; Edington, 1990; Kannaiyan & Haciwa, 1993; Allen, 1992), but most of the results were inconclusive. Comparisons of the results were difficult because an international set of differentials was not available. In this sudy we compare two set of differential cultivars in the identification of races of *C. lindemuthianum*.

MATERIALS AND METHODS

A limited number of anthracose outbreaks was recorded during the period 1992 to 1995 in the major dry bean production areas (Table 1). Pods and leaves from dry bean plants which showed typical anthracnose lesions were collected in paper bags and was air dried. The dried plant parts were rehydrated by dipping into 70% ethanol solution and then soaked for 30 min. in sterile distilled H_2O. The pieces were surface disinfected for 2 min in 1% NaOCl solution and rinsed three times with sterile distilled H_2O, dried on paper towelling for 30 min. and plased on moist filter paper in Petri dishes or 2% water agar. Conidia produced on the plant material were transferred to potato dextrose agar (39 g Oxoid Potato Dextrose Agar/1000 ml H_2O supplemented with Chloramphenicol (0,05g/1000ml) and Streptomycin (0,05g/1000ml) and single spore isolates were made. Cultures were identified according to Baxter *et al.* (1983). The isolates were stored at 5 °C on potato carrot agar (20 g potato, 20 g carrot, 15 g agar/1000 ml H_2O).

Inoculum, from representitive isolates, was produced on bean pod agar (250 g frozen young bean pods, autoclaved and strained, 15 g agar added to the supernatant and made up to 1000 ml). The conidia were scraped off and suspended in sterile distilled H_2O. The spore suspensions were then adjusted to between 5 x 10^5 and 1 x 10^6 conidia/ml. Two sets of differential cultivars, one supplied by Dr M.A. Pastor-Corrales, CIAT (International Center for Tropical Agriculture), Cali, Colombia and the other by Drs E. Drijfhout and J.H.C. Davis, Institute for Horticultural Plant Breeding, Wageningen, The Netherlands, were used (Tables 2 & 3). Seed of both sets of differentials were multiplied under glasshouse conditions in the absence of the anthracnose disease. The seeds were surface disinfected for 10 min in 1% NaOCl solution and then germinated between moist filter papers in Petri dishes for three days at room temperature (20-25 °C). The seedlings were planted in plastic trays which contained a mixture of 50% virgin sandy loam soil and 50% perlite. Plants were grown in a greenhouse with automatic mechanical cooling and heating units to maintain a temperature of 20 ±2 °C. Eight seedlings served as a treatment. Fourteen-day-old seedlings were inoculated by spraying with conidial suspensions of the fungus until runoff. Inoculated seedlings were incubated for seven days at 20 ±2 °C under plastic bags to maintain high humidity levels. Disease severity was rated 10 days after inoculation on a scale of 0-5 (0 = no symptoms, 1 = pinpoint lesions, 2 = small lesions, not sunken, 3 = larger, sunken lesions, 4 = large, deep lesions, up to stem centre and 5 = seedling killed by pathogen). The results were recorded and those with 0, 1 and 2 (either in one of these classes or more than one) were classified as resistant (designated as - or R). Those with 3, 4 and 5 (either in one of these classes or in more than one) were classified as susceptible (designated as + or S).

Dry bean cultivars currently being assessed for their agronomic value in South Africa were also evaluated for their susceptibility to five of the most prominent of the anthracnose races identified (Table 4). The seed was supplied by Dr. A.J. Liebenberg, Oil and Protein Seed Centre, Grain Crops Institute, Agricultural Research Council, Private Bag X1251, Potchefstroom 2520, Republic of South Africa. The same growth conditions, inoculation and evaluation methods as previously described were used.

RESULTS

The occurrence of the disease is limited, therefor only twenty-one isolates of *C. lindemuthianum* were collected throughout the bean production areas (Table 1). The *C. lindemuthianum* isolates used gave differential reactions on both sets of differential cultivars used and pathological races of the fungus could be determined. The results of tests using the Drijfhout & Davis differentials are presented in Table 2. The nomenclature of races of the fungus is presented in accordance with Drijfhout & Davis (1989) and Rawa *et al*. (1994). The CIAT differentials gave a more informative result which is presented in Table 3. The differential cultivar origin and gene dominations where available (Kelly & Young, 1996), are presented. By using the binominal system proposed by CIAT, races 3, 65, 80, 81, 83, 119 and 593 were found to be present in South Africa. Races 65, 80, 81 and 593 resemble the race alpha-Brazil of Drijfhout & Davis (1989) which falls in the alpha group. Race 3, 83 and 119 could not be named when the Drijfhot & Davis system was used. According to Rava et al. (1994) race 65 could also be epsilon and race 81 could be eta. Race 83 can be mex 11 and race 119 can be lambda. In both cases race 3 could not be named.

Differential resistance to five of the local races of the fungus was identified in the dry bean cultivars currently being evaluated in South Africa (Table 4). None of the local dry bean cultivars showed resistance to all local races of the fungus under glasshouse conditions.

DISCUSSION

Since Barrus (1911) described the presence of a pathological race in *C. lindemuthianum* by means of inoculating differential bean cultivars, numerous races have been reported (Burkholder, 1923; Andrus & Wade, 1942; Yerkes & Ortiz, 1956; Blondet, 1963; Hubbeling, 1961a,b, 1976, 1977; Oliari *et al.*, 1973; Hoffmann *et al.*, 1974; Fouilloux, 1976; Schwartz *et al.*, 1982).

Drijfhout & Davies (1989) made available a set of homogenously reacting bean differentials to differentiate 10 races of *C. lindemuthianum*. After the first Latin American

Bean Anthracnose workshop held at CIAT in 1988, Pastor-Corrales and co-workers introduced a new system of race classification for anthracnose of beans (CIAT, 1988). This was due to the large number of races that exist in South and Central America. It is believed that the fungus and its host co-evolved in this area (Dr M.A. Pastor-Corrales, CIAT, Cali, Colombia, personal communication). The new system exists of a fixed number of differentials in which certain independent resistance genes are known, where as in the old system the number and combination of differentials always varied and for most the resistance gene were not identified. With the new system the diversity of races can be stressed, and is therefore applicable in South America and Eastern Africa where many races of the fungus are present. Although both methods were used in this study, the CIAT method was more satisfactory and will be adopted in future work.

Tu (1992) reviewed inoculation methods and disease ratings used by various researchers. Differences in methods also contribute to variations in the results that are obtained. Temperature is a very important factor in race determinations. In most parts of Africa it is very difficult to control temperatures of glasshouses between 18°C and 22°C for most of the year. In many cases glasshouses and other infrastructure necessary for inoculations and production of seed of differential cultivars, are not available. It is also necessary to have access to quarantine facilities. The dip method of Drijfhout & Davis (1989) adapted from the system suggested by Hubbeling (1961b), proved too labour intensive. Furthermore, germinated seed is easily damaged when the seed coats are removed prior to dipping in the inoculum and planting. The method used by CIAT is less labour intensive, but seed germination is unreliable. All seed used in race determinations have to be produced in a glasshouse to keep them genetically pure and free of disease.

Edington (1990) showed that the Kwazulu-Natal (Greytown and Cedara) races resemble the alpha-Brazil race. Our results correspond with those, except that we also distinguish between races 80, 81, 83, 119 and 593 in that area.

The dry bean industry in South Africa relies heavily on red speckled sugar beans. At this stage races 3 and 119 are our greatest concern, because most of the red speckled sugar beans are susceptible to these races (Table 4). Only one (cv. Pan 148) of our red speckled sugar beans showed possible resistance to race 3 and two (cvs. Monati and Pan 127) to race 119 in the glasshouse. It is believed that race 3 developed in a former seed producing area, namely Komatipoort, close to the Mozambican border. Seed of the cultivar Bonus, on which race 3 was found, was extensively produced in that area under irrigation during the winter months up to 1994.

At present our local races have not yet overcome the resistance that the *Are* (*Co-2*) gene in Cornell 49-242, Mex 2 (Co-4) in To, Co-6 in AB 136 and Co-7 in G2333 provides (Table 3). The *A* (*Co-1*) gene in Michigan Dark Red Kidney, the *Mex 1* (*Co-3*) gene in Mexico 222 and the *Mex 3* (*Co-5*) gene in Tu are no longer suitable on their own under South African conditions. Pastor-Corrales *et al.* (1994) found the germ plasm accession G2333 (Colorado de Teopisca) from Mexico resistant to 380 isolates of *C. lindemuthianum* from 11 Latin American countries. Because of its resistance to anthracnose, G2333 was released as a commercial cultivar in Rwanda, where it is known as Umubano. It is also effective against 15 isolates of the fungus in Tanzania (Allen, 1992). This cultivar is also widely grown in neighbouring areas: Burundi, the Kivu region of Zaire, and parts of southern Uganda. Other important characteristics are its shiny red seed coats (a preferred seed colour in Central-African markets), high tolerance of low soil fertility, and stable and exceptionally high yields in diverse environments (Allen, 1992). According to Pastor-Corrales *et al*. (1994), two independent dominant genes controlled resistance in the seedling and adult plant stages of G2333. A segregation ratio of 15 resistant to one susceptible in the F_2 was found. All were resistant in the backcross to G 2333, and three resistant to one susceptible in the backcross to ICA Pijao.

The random amplified polymorphic DNA (RAPD) technique is proposed by Vilarinhos, *et al*. (1995) as a fast and reliable alternative to distinguish races of *C. lindemuthianum*. Pathotype 585 was the most distinct one, showing a high dissimilarity in relation to the other races. Races alpha-Brazil, Zeta, kappa and Delta, on the other hand, were very closely related to one another. It is noteworthy that their results allowed the separation of the races into different groups from those defined by the use of common bean differentials. No correlation between the above mentioned work and genetic distances in differential cultivars was made. Undoubtedly, DNA-based molecular markers represent a valuable tool for the identification of races of *C. lindemuthianum*. The development of DNA-probes for the different races may also be of value to monitor and identify the development of new races of the fungus.

On the other hand, Vilarinhos *et al*. (1995) used the RAPD technique to fingerprint and determine the genetic distances among the twelve genotypes of *P. vulgaris* used internationally to differentiate races of *C. lindemuthianum*. The bidimensional graphic dispersions of the individuals demonstrate that the largest genetic distance was between cultivars PI 207-262 and AB 136 on the one hand, and the cultivars Perry Marrow and Dark Red Kidney on the other. The shortest distance was between Dark Red Kidney and Perry Marrow. Young and Kelly (1994) have identified a random amplified polymorphic DNA (RAPD) marker linked tightly (2.0 cM) to the *Are* gene. Some of the molecular markers to the other genes still have to be identified.

This paper represents the identification of seven races of *C. lindemuthianum* in South Africa. Races 3 and 119 are currently our major concern. To assist the breeding programme, molecular markers for the different resistance genes must be identified. It has to be determined whether the *Are* gene is present in our local cultivars and other resistance genes should be incorporated. For the time being the industry will have to rely on crop rotation, the production of healthy seed and effective seed treatments to control the disease.

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Table 1 Origin and distribution of 21 isolates of *Colletotrichum lindemuthianum* present in South Africa and classified according to the binary race distinction used by CIAT (Pastor-Corrales *et al*., 1995)

Origin	Number of isolates	Races of *Colletotrichum lindemuthianum*						
		3	65	80	81	83	119	593
Northern Province								
Pietersburg	1				+			
Mpumalanga								
Ermelo	4		+		+			+
Ogies/Delmas	4				+			+
Free State								
	2	+						
Bethlehem/Villiers								
Kwazulu-Natal								
Greytown	7			+		+	+	+
Cedara	3				+			+

+, race present

Table 2 Characterization of South African races of *Colletotrichum lindemuthianum* on the Drijfhout and Davis (1989) set of differential common bean (*Phaseolus vulgaris*) cultivars

Differential cultivars	South African races of *Colletotrichum lindemuthianum*						
	3	65	80	81	83	119	593
Michigan Dark Red Kidney	+				+	+	
Coco à la Crème	+					+	
PI 165-422	+	+	+	+	+	+	+
Kaboon						+	
Cornell 49-242							
PI 207-262							
Evolutie						+	
Mexico 222		+	+	+	+	+	+
Race designation							
Drijfhout & Davis (1989)	?	Alpha-Brazil	Alpha-Brazil	Alpha-Brazil	?	?	Alpha-Brazil
Rava *et al*. (1994)	?	Epsilon	Alpha-Brazil	Eta	Mex II	Lambda	?

+, Susceptible reaction, absence of + indicates resistant reaction

Table 3 Characterization of 21 South African isolates of *Colletotrichum lindemuthianum* to the CIAT set of differential common bean (*Phaseolus vulgaris*) cultivars, their origin, gene domination and their race allocation

Differential cultivars	Origin of cultivars	Gene domination[a]	Binary value	Number of South African isolates tested						
				2	1	1	10	1	1	5
Michelite	Meso-america		1	1[b]	1		1	1	1	1
Michigan Dark Red Kidney	Nueva Granada	*Co-1* (*A*)	2	2				2	2	
Perry Marrow	Chile		4						4	
Cornell 49-242	Meso-america	*Co-2* (*Are*)	8							
Widusa	Durango		16			16	16	16	16	16
Kaboon	Nueva Granada		32						32	
Mexico 222	Durango	*Co-3* (*Mex 1*)	64		64	64	64	64	64	64
PI 207-262	Meso-america	Undetermined	128							
To	Durango	*Co-4* (*Mex 2*)	256							
Tu	Durango	*Co-5* (*Mex 3*)	512							512
AB 136	Meso-america	*Co-6* (Undetermined)	1024							
G2333	Meso-america	*Co-7* (2 genes, not named)	2048							
Pathological races of South African *Colletotrichum lindemuthianum* isolates				3	65	80	81	83	119	593

[a]Kelly & Young, 1996

[b]Presence of value indicates susceptible reaction, absence of value indicates resistant reaction

Table 4 Reaction of local South African common bean (*Phaseolus vulgaris*) cultivars to five of the local races of *Colletotrichum lindemuthianum*

Local bean cultivars	South African races of *Colletotrichum lindemuthianum*				
	3	65	81	119	593
Small White					
Kamberg	R	S	-	S	-
Teebus	-	S	S	S	S
Kosi	R	S	S	S	S
Helderberg	R	S	S	R	S
Pan 122	-	S	-	-	-
Pan 143	-	R	R	R	R
Red Speckled Sugar					
Bonus	S	R	S	S	S
Stormberg	S	R	S	S	S
Leeukop	S	R	S	S	S
Kranskop	S	R	R	S	R
Jenny	S	R	R	S	R
Lisa	-	R	R	S	R
Monati	S	R	R	R	R
Pan 127	-	R	R	R	R
Pan 148	SR	R	S	S	R
SSB 10	S	S	S	S	S
Sani	R	S	S	S	S
Drakensberg	S	S	S	S	S
Sabie	S	R	R	S	R
Limpopo	SR	S	S	S	S

Local bean cultivars	South African races of *Colletotrichum lindemuthianum*				
	3	65	81	119	593
Yellow Harricot					
Katberg	S	S	S	S	S
Majuba	R	-	S	S	S
Brown Harricot					
Nuweveld	R	S	S	S	S
Donkerberg	S	S	S	S	S
Carioca					
Patrys	R	S	S	S	S
Nandi	R	S	S	R	S
Mkuzi	R	S	S	SR	S
Zambezi	R	S	S	S	S
Alubia					
Alubia Cerrillos	S	R	R	S	R
Cannelino	S	R	R	S	S

R, Resistant; S, Susceptible; -, Data not available

CHAPTER 6*

Detection of seed-borne *Colletotrichum lindemuthianum* on seed of *Phaseolus vulgaris*

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Summary

Colletotrichum lindemuthianum, the cause of anthracnose of beans (*Phaseolus vulgaris*), is a seed-borne fungal pathogen. The use of disease-free seed is an important disease control measure. In the case of anthracnose it also ensures that different races of the fungus are not spread to uninfested areas; this is particularly important if resistant lines are not available. Different laboratory detection methods, namely the blotter test proposed by ISTA, the use of 2 % water agar, filter paper in Petri dishes and the "paper doll" method were compared. All methods were successful. The "paper doll" method turned out to be the most reliable and inexpensive. This method is currently used by South African bean seed testing laboratories.

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Introduction

Colletotrichum lindemuthianum (Sacc. and Magnus) Briosi and Cav. is world-wide a problem wherever *Phaseolus vulgaris* L. is grown. It is also one of the most important seed-borne and seed transmitted diseases on *P. vulgaris*. The fungus causes disease of all the above-ground parts of the plant. When seed-borne, it infects the cotyledons and spreads from there to the hypocotyl. The sporulating lesions can then serve as a source of inoculum for the rest of the crop. Under suitable conditions (splashing rain and wind) the fungus can spread up to 4,6 m at a time (Tu, 1981). If diseased seed is planted, losses of up to 100% can be encountered when suitable conditions for disease development occur (Schwartz, 1991).

The disease, also known as anthracnose, is transported over long distances by means of seed. It is also believed that new physiological races of the fungus are introduced into areas where the disease did not previously occur by means of seed (Tu, 1983; Kelly, Afanador and Cameron, 1995). In developed countries the disease is controlled by the production of disease-free seed in areas where the disease is not likely to be present. Seed plantings are inspected during production and is followed by laboratory testing of the seed for the presence of disease causing organisms.

Seed health management integrates all activities in the chain of production of quality seed. It cannot be based on laboratory tests alone. On the other hand, field inspections requires the availability of a reliable test to refer to in cases of doubt or dispute

(Meijerink & Van Breukelen, 1995). Various detection methods for bean anthracnose have been described (Anselme and Champion, 1981; ISTA, 1993). This laboratory was requested to evaluate the reliability of the different methods and to compare them with the method prescribed by the International Seed Testing Association (ISTA). This paper reports on the effectiveness of different methods to detect *C. lindemuthianum* on bean seed in the laboratory.

Materials and Methods

A large sample of bean seed of the cultivar Zambezi (a Natal sugar bean or Carioca), infected with anthracnose, was collected during 1995 near Cedara in Kwazulu-Natal. The seed was sorted by hand and only the seed which showed no symptoms of the disease was used in the experiments. Each sample consisted of 400 seeds. The seed was not surface disinfected. All seed was tested directly by means of four incubation methods, namely the proposed ISTA (1993) method, the "paper doll" method which resembles the method described in the ISTA work sheet No. 45 (Anselme and Champion, 1981), water agar in Petri dishes and wetted filter paper in Petri dishes.

ISTA method (ISTA, 1993): Seed was spread in eight replicates of 50 seeds on double sheets of filter paper (Whatman No. 1), 350 X 450 mm, which were wetted with 60 ml sterile distilled water, and seeds covered with another sheet of the filter paper. The paper was then folded twice lengthways and covered with plastic bags to maintain high humidity during incubation.

Paper doll method: Seeds were spread in eight replicates of 50 seeds on absorbent cellulose wadding B.P.C. supplied by Multa Seed (Pty.) Ltd., 305 X 560 mm and backed with two layers of 305 X 560 mm germination paper (Anchor). The wadding was wetted with 60 ml sterile distilled water before seed was placed. After placement of the seed, the paper was rolled sideways and secured with elastic bands. The "paper doll" was then inserted into plastic bags and incubated in an upright position.

Water agar method: Seed, in replicates of five per plate, was incubated on 20 ml water agar (15 g Merck Agar Agar/1000 ml H_20) in 90 mm diameter Petri dishes. Ten plates were held in plastic bags to maintain high humidity levels.

Filter paper method: Seed, in replicates of five per plate, was incubated between two layers of 90 mm diameter Whatman No. 1 filter paper discs in 90 mm diameter Petri dishes. The filter paper was wetted with 5 ml sterile distilled water. Ten plates were held in plastic bags to maintain high humidity levels.

All the treatments were incubated at 20 °C in the dark. After ten days the seed coats were removed and the cotyledons examined for the presence of dark black, round lessions with a pinkish center, the typical symptoms of *C. lindemuthianum*. The presence of the fungus was further determined by means of a stereo-microscope at 60 X magnification or transmission microscope at 200 X magnification. Seed with symptoms to be verified were placed in 15 cm test tubes, 1 ml sterile water was added and the contents vortexed. A microscope slide was made of the water and checked for the presence of *C. lindemuthianum* conidia. The data were recorded and the percentage infected seeds determined.

These experiments were repeated three times over a period of six weeks and an analyses of variance was conducted to compare the obtained results. All seeds were stored at 5 °C between evaluations.

Cost of consumables and electricity used was calculated in South African Rand, the times spend in the initial preparation was recorded and calculated, and the space consumed by a sample measured in cm^3.

Results

All the methods enabled the detection of *C. lindemuthianum* on initially symptomless bean seed. The percentage infection by the fungus is presented in Table 1. No significant difference in the ability to detect the fungus using the different methods was recorded. The compared cost effectiveness, space usage and time required is presented in Table 2. The paper doll method was found to be the best, because the disease symptoms were easily detected on lesion less seed. The fungus and symptoms were seldomly masked by the presence of other fungi. It is cheap by comparison with the other methods and less space was used.

Discussion

The method in the appendix of the ISTA rules (1993) was found uninformative. It also differs from the one described by Anselme and Champion (1981). When seedlots in the ISTA method were placed on top of each other, germination of the seed was influenced and therefore the detection of the fungus complicated. When seed is planted in the field it is not practice to disinfect seed and therefor seed disinfection was left out. Disinfection might influence the results obtained in the test. The aim is to detect contamination with the fungus as well.

The use of all these methods relies heavily on the development of disease symptoms. The formation of sporulating structures are often masked by the presence of other fungi. Setae of *C. lindemuthianum* have very rarely been observed. Conidial masses of the fungus were more prominent. They varied from a dried to a slimy mass of orange spores.

Macrophomina phaseolina (Tassi) Goid. was also easily detected on the paper dolls. The fungus spreads rapidly and forms masses of microsclerotia. *Rhizoctonia solani* Kühn and *Fusarium* spp. infections were less obvious. *Rhizoctonia* sp. infections were associated with undeveloped primary roots which had a water soaked appearance. Isolation onto media is necessary for this identification. A *Botrytis* sp. which is associated with rot and discolouration of the primary root was also detected.

The paper doll method corresponds with the method described by Anselme and Champion (1981) in the ISTA Handbook on Seed Health Testing and is currently being used by local bean seed testing laboratories.

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Table 1. Percentages of bean seedlings with *Colletotrichum lindemuthianum* symptoms detected by means of different detection methods.

Detection method	*C. lindemuthianum* (%)			
	Replicates			Mean*
	1	2	3	
ISTA	10	10	8	9
Paper doll	12	8	16	12
Water agar	10	12	6	9
Filter paper	8	14	8	10

* Means do not differ significantly

Table 2. Visual detection of symptoms of anthracnose on seed, cost of consumables and electricity, space usage and time required to detect *C. lindemuthianum* by means of different methods.

Methods	Detection	Cost	Space (cm^3)	Time
ISTA	variable	expensive	9 000	fast
Paper doll	good	cheap	9 000	fast
Water agar	good	average	12 000	slow
Filter paper	good	average	12 000	slower

CHAPTER 7[§]

The effect of different seed treatments in the control of seedborne *Colletotrichum lindemuthianum* on dry beans (*Phaseolus vulgaris*)

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Control of seedborne *Colletotrichum lindemuthianum*, the cause of anthracnose on dry beans (*Phaseolus vulgaris*), is lacking in South Africa. Different fungicides were tested *in vitro* and *in vivo* for their effect on the fungus and seedling protection following seed treatment. Benomyl was found the most effective. As a single broad spectrum seed treatment for the control of seedborne and soilborne diseases is needed, it is recommended that benomyl is used in combination with metalaxyl. Sodium molybdate in carboxymethyl-cellulose diluted with water was used as a sticker and wetter. This treatment was found to be effective for the control of seedborne *C. lindemuthianum* means of the "paper doll" method and glasshouse tests. It will also control seedborne *Sclerotinia sclerotiorum, Macrophomina phaseolina* and pythium damping-off.

Keywords: *Phaseolus vulgaris*, *Colletotrichum lindemuthianum*, seedborne, control, seed treatment

[§]*In format of* Crop Protection.

Dry beans (*Phaseolus vulgaris* L.) production is affected by a wide range of fungi (Schwartz, 1991), many of which are seedborne or soilborne. Some of the major constraints are pythium damping-off, rhizoctonia root rot, anthracnose and sclerotinia mould. Of these, anthracnose, sclerotinia and rhizoctonia can be seedborne (Richardson, 1979). In South African dry bean production it is a stipulation of the plant improvement act, 1976 (Act 53 of 1976) that seeds must be free of seedborne *Colletotrichum lindemuthianum* (Sacc. & Magnus) Briosi & Cav., the cause of anthracnose, and *Sclerotinia sclerotiorum* (Lib.) de Bary, the cause of sclerotinia mold. Seed is also laboratory tested for the presence of these two fungi by assigned laboratories.

Protecting seedlings against soilborne fungi implies that the fungicide is to be systemic in the plants. Currently only metalaxyl for the control of pythium damping-off and tolclofos-methyl for the control of rhizoctonia root rot, is registered in South Africa (Krause, Nel and van Zyl, 1996). A.J.L. Phillips (unpublished data) proposed the use of benomyl to control seedborne sclerotinia. Producers are therefore left with a choice of these compounds. Normally they cannot predict which of the soilborne fungi are likely to affect the crop in the following season. Since the need for a single multispectrum seed treatment is long overdue, the objective of this project was to formulate a seed treatment effective against seedborne *C. lindemuthianum* and the more common damping-off fungi. In this paper we investigated the effect of different fungicides on *C. lindemuthianum* and whether some of these fungicides can be mixed for the control of seedborne *C. lindemuthianum*.

Materials and methods

Test Organism

The test fungus was *C. lindemuthianum* (SHK 785) isolated from *P. vulgaris*. The fungus was maintained on potato-carrot agar slants in the dark at 4 °C. For the preparation of inoculum the cultures were grown on 90 mm diameter Petri dishes containing 20 ml bean pod agar (BPA; 250 g frozen young bean pods, 15 g agar, 1000 ml H_2O). After 10 days conidia were collected by rinsing colonies with 10 ml sterile distilled water. Spore suspensions were standardized with a haemacytometer to contain 1 X 10^6 condia/ml and used in all experiments except where stated otherwise.

Petri dish disc tests

Petri dishes containing 20 ml BPA, were inoculated with a 5 ml conidial suspension (half concentration: 1 X 10^3 condia/ml) of *C. lindemuthianum*. The excess conidial suspension was decanted. In the screening tests 14 fungicides (Table 1) were suspended in water at the rate of 2 g or 2 ml of the formulated compounds into 10 ml of water or as indicated in Table 1. Formulations were further diluted to obtain a serial dilution of 1 X 10^{-1} to 1 X 10^{-4}. One ml of each dilution was adsorbed on sterile 12,5 mm diameter antibiotic test discs. Discs were arranged in the centre of each seeded plate and each treatment was replicated three times. The radii of the inhibition zone were measured and the percentage inhibition area determined, where (πr^2 inhibition zone/πr^2 Petri dish) X 100 = % inhibition area.

Test for seedling protection following seed treatment

Disease free dry bean seed of the cultivar Nuweveld was treated with 14 fungicides in Petri dish disc tests (Table 1). Treated seeds were planted in seedling trays filled with 50% soil/perlite mixture in a glasshouse at 20°C. Four replicates of eight seeds were used. Germination was recorded after 14 days and the seedlings were then inoculated with a spore suspension (1 X 10^6 condia/ml) of *C. lindemuthianum*. After inoculation seedling trays were placed in plastic bags to maintain high humidity for a further 7 days. After 10 days the disease severity was scored on a 0-5 scale where 0 represents healthy and 5 dead seedlings. The data was anlysed by the non-parametric Kruskal-Wallis method. The mean rank scores for the different treatments were compared by the Dunn pairwise comparison test (Siegel, S. 1956).

Effect of fungicides on seedborne *C. lindemuthianum*

Seed of the dry bean cultivar Zambezi, naturally infected by *C. lindemuthianum,* was separated into three categories according to lesion size (no visual lesions, trace to 2 mm diameter lesions and 2 - 4 mm diameter lesions). Seed was wetted with 4 ml Mollyflo^{200} (59,7 g sodium molybdate /1000 ml 1,25% carboxymethyl-cellulose)/kg seed and 10 ml H_2O. Benomyl was added at a rate of 1,0; 1,5 and 2 g a.i./kg seed and mixed with metalaxyl at a constant rate of 0,1 g for each of the benomyl rates. The benomyl and metalaxyl were mixed prior to application to wetted seed. Untreated and unwetted seed served as control. Four replications of 50 seeds per paper doll (Koch, Van Wyk & Eicker, submitted a) were seeded for each of the 12 treatments and incubated for 10 days at ± 20 °C. Paper dolls were opened and the number of seedlings with anthracnose lesions recorded. Seedlings with anthracnose lesions were transferred to 500 ml Erlenmeyer flasks. Sterile distilled H_2O (100 ml) was added and the conidia formed on lesions on infected seedlings suspended. The conidal numbers were determined with a haemacytometer.

The experiment was repeated in soil in 27 X 31 X 11 cm trays in a glasshouse at ± 20 °C. Fourteen days after planting, trays containing seedlings were placed in large plastic bags to maintain high humidity. After a further 7 days in the glasshouse the plastic bags were removed and the percentage germination and percentage infected seedlings recorded. Seedlings were rated for disease severity on a scale of 0 - 5 (0 = no disease symptoms; 5 = seedlings dead).

A logistic regression model was fitted to the data of the paper doll and glasshouse results (McCullagh & Nelder, 1984). Differences between treatments were examined by comparing the parameters of the treatments estimated by the model with a *t*-test. Data were analysed by the PROC GENMOD procedure of the SAS System statistical package (SAS Institute Inc., 1993)

Determination of phytotoxicity of the benomyl/metalaxyl mixture

Seed of the dry bean cultivar Zambezi was wetted with 4 ml Mollyflo[200] and 10-ml H_2O/kg seed and thoroughly mixed with nine different concentrations of benomyl and metalaxyl (Table 8). Untreated seed served as controls Fifty seeds in each of four replicates were planted in soil in 27 X 31 X 11 cm plastic trays. The trays were kept in a glasshouse at ±

20 °C day and night. Percentage germination and total plant dry mass per treatment were recorded after 21 days. An analysis of variance was done. Treatment means were compared by the Bonferroni multiple comparison method. Data were analysed by the PROC GLM procedure of the SAS Stystem statistical package (SAS Institute Inc., 1988).

Effect of fungicidal treatment over time

Seed of the dry bean cultivar Zambezi was wetted with 4 ml Mollyflo[200] and 10 ml H_2O/kg seed and then treated with 1g benomyl and 0,1 g metalaxyl/kg seed. Untreated and unwetted seed served as a control. To measure the effect of storage after treatment, seed lots were planted immediately after treatment and 1, 5 and 8 weeks later. Fifty seeds were planted in 27 X 31 X 11 cm plastic trays and kept in a glasshouse at ± 20 °C day and night. Eight replicates were included per treatment. The percentage germination and total dry mass of seedlings per treatment were recorded 21 days after planting.

An analysis of covariance (ANCOVA) with time as covariate was used for analysing the germination data and time and time squared as covariates was used for analysing the dry mass data (Huitema, 1980). This was done to determine whether time had an effect on the difference between the two treatments (treatment and control).

Petri dish incorporation tests

Various concentrations (0,0; 0,001; 0,01; 0,1 and 1,0 mg a.i./ml agar) of benomyl (Benlate), captab (Captab), metalaxyl (Apron) and tolclofos-methyl (Rhizolex) were incorporated into sterilized potato dextrose agar (PDA) prior to pouring the agar into plates. Five plates for each treatment were included. Starter colonies of *C. lindemuthianum*, *C. coccodes* (Wallr.) S. Hughes, *Rhizoctonia solani* Kühn, *S. sclerotiorum*, *Macrophomina phaseolina* (Tassi) Goid, *Fusarium oxysporum* Schlecht. emend. Snyd. & Hans, *F. solani* (Mart.) Appel & Wollenw. emend. Snyd. & Hans. and *Pythium aphanidermatum* (Edson) Fitzp. were grown on PDA. Discs of actively growing mycelium were cut with a sterilized no. 3 cork borer and transferred to the test plates. Plates were incubated at 22°C in the dark and inspected regularly. When the fungi on control plates reached the perimeters, or after 7 days, the radial growth of the different test organisms for each concentration of fungicide was measured. The percentage growth was determined by comparing the treatments with their controls.

Results

Petri dish disc tests

The results are presented in Table 2. Of the 14 fungicides tested, captab, both formulations of iprodione, iprodione/imazalil, procymidone, tebuconazole, thiophanate-methyl, triticonazole, vinclozolin and the water control gave less than 50% growth inhibition at 10^{-2} dilution. Benomyl, carbendazim and carbendazim/tebuconazole gave more than 50% reduction in growth at 10^{-4} dilution.

Seedling protection following seed treatment

Results of the effect of seed treatment on germination and disease severity are presented in Table 3. Seed germination ranged from 84 to 100%. Of the 14 fungicides tested, the disease severity ratings for benomyl and carbendazim/tebuconazole and thiophanate-methyl were significantly lower than the rest. Carbendazim/tebuconazole treatment completely reduced growth of the seedlings and the seedlings did not develop beyond the primary leaf stage. No phytotoxic effect was recorded for benomyl and thiophanate-methyl.

Effect of fungicides on seedborne *C. lindemuthianum*

The effect of the benomyl/metalaxyl mixtures on the development of anthracnose lesions in the paper doll test is presented in Table 4. All the treatments significantly reduced the development of anthracnose lesions from the control. The development of anthracnose lesions decreased with a higher application of benomyl. The deep-seated lesions associated with the 2-4 mm lesion category were not reduced by higher applications of the benomyl mixture.

There was a significant difference in the amount of conidia produced between the lesion less control, and the trace - 2 mm and 2 - 4 mm categories of seed (Table 5). Conidial production in all the treated seed was reduced to less than 1.1 X 10^3 conidia/ml.

A significant difference in germination between the untreated control seed categories was recorded. Germination decreased with increasing lesion size (Table 6). The germination of the trace - 2 mm and 2 - 4 mm categories treated with 1 g benomyl and 0,1 g metalaxyl improved significantly. The higher rates of benomyl + metalaxyl treatments did not further enhance germination.

There was a significant difference in the incidence of anthracnose lesions between lesion categories (Table 7). In the trace - 2 mm and 2 - 4 mm categories the 1 g benomyl + 0,1 g metalaxyl decreased anthracnose development significantly. Increasing rates of benomyl did not inhibit disease development further. Although some lesions occurred on treated seedlings, the disease did not develop further.

Phytotoxicity of benomyl/metalaxyl mixture

No significant effect on germination or seedling growth was recorded at concentrations of benomyl/metalaxyl mixtures as high as 5 g benomyl + 0,5 g metalaxyl/kg seeds (Table 8). Only slight differences dry mass of seedlings could be detected when more than 10g benomyl + 1 g metalaxyl was used.

Effect of fungicidel treatment over time

Germination rates and the total dry mass of germinated seedlings differed significantly when seeds were planted immediately after treatment (Fig 1a & b). The positive effect of the treatment was reduced when the seeds were stored following treatment. Between week one and five the differences were eliminated and treatment negatively effected germination and total dry mass.

Petri dish incorporation tests

Benomyl at a concentration of 0,001 mg a.i./ml PDA completely reduced growth of *C.*

lindemuthianum, *S. sclerotiorum* and *M. phaseolina* (Fig 2). Concentrations of 0,01mg a.i./ml also reduced the growth of *R. solani*, *F. oxysporum* and *F. solani* by more than 50%. At these concentrations there were no effect against *P. aphanidermatum*. Metalaxyl at 0,001 mg a.i./ml gave more than 50% reduction in growth of *P. aphanidermatum* and at 0,01 mg a.i./ml a 100% reduction in growth. At these concentrations it was ineffective against the other test fungi.

Discussion

From the Petri dish disc test, the seedling protection following seed treatment test and the Petri dish incorporation test benomyl was found the most effective fungicide against *C. lindemuthianum*. In the Petri dish incorporation test benomyl also controlled *S. sclerotiorum* and *M. phaseolina* and to a lesser extent *R. solani*, *F. oxysporum* and *F. solani,* but was ineffective against *P. aphanidermatum*. Therefore, it was decided to test benomyl in combination with metalaxyl on seed-borne *C. lindemuthianum*. Sodium molybdate, an essential micro-element in biological nitrogen-fixation, was used in combination with water, as a sticker and wetter for the fungicidal mixture. The mixture of benomyl and metalaxyl effectively reduced secondary inoculum from seedborne infections of *C. lindemuthianum*. Edgington and French (1981) and Tu (1996) found that lesions already present on seed before treatment were still present after germination. Sporulation was strongly inhibited and lesions did not develop any further. No phytotoxicity, at the recommended dosage, was found in the glasshouse experiments. The use of benomyl was also proposed by Egdington and MacNiell (1978), Sindhan and Bose (1981) and Tu (1996) for control of seedborne *C. lindemuthianum*. Although Tu and Jarvis

(1979a, b) and Tu and MacNaughton (1980) showed that the fungus is able to build up resistance towards benomyl under laboratory conditions, this has not yet been reported under field conditions.

Cultivar resistance is the best control measure against anthracnose on beans. In South Africa race non-specific resistance is not available (Koch, Van Wyk and Eicker, submitted b), therefore seed treatment as a control measure is of utmost importance. It will also help prevent spread of the different races of the fungus to new localities.

From the results presented in this paper we propose the use of 1 g benomyl plus 0,1 g metalaxyl/kg bean seed in combination with 4 ml Mollyflo^{200} and 10 ml water as a seed-treatment for the control of seed-borne *C. lindemuthianum*. Storage time had a significant effect on germination and dry mass of seedlings, therefore it is recommended that seed is treated immediately before planting. This seed treatment could be applied to seed that tested positively for anthracnose in the laboratory and of which the general fitness is good. When deep seated infections are avoided, treated seed can be presented as seed free from anthracnose. The treatment will also control seedborne *S. sclerotiorum* (A.J.L. Phillips, unpublished data), *M. phaseolina* (Abawi and Pastor-Corrales, 1990) and pythium damping-off (Krause *et al*., 1996).

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Table 1. List of fungicides, type of formulation, active ingredient, trade name, and application rate/kg seed used in the Petri dish disc test and the seed treatment test

Active ingredient(s)	Type of formulation*	Active ingredient(s)/ content (pure)	Trade name	Application rate/kg seed
benomyl	WP	500 g/kg	Benlate	2 g
cabtab	SC	500 g/l	Kaptan Flo	2 ml
carbendazim	SC	500 g/l	Bavistin	2 ml
carbendazim/tebuconazole	SC	133/167 g/l	Libero	2 ml
chlorothalonil	SC	500 g/l	Bravo	2 ml
iprodione	WP	500 g/kg	Rovral	2 g
iprodione	SC	250 g/l	Rovral Flo	6 ml
iprodione/carbendazim	SC	175/87,5 g/l	Calidan	2 g
iprodione/imazalil	FS	350/50 g/l	Exp 022 65E	2 ml
procymidone	SC	500 g/kg	Sumislex	2 g
tebuconazole	ES	15 g/l	Raxil	3 ml
thiophanate-methyl	SC	480 g/l	Topsin Flo	2 ml
triticonazole	FS	25 g/l	Exp 804 72A	2 ml
vinclozolin	SC	500 g/l	Ronilan	2 ml

*ES = emulsion for seed treatment; FS = flowable concentrate for seed treatment; WP = wettable powder; SC = suspension concentrate

Table 2. Percentage inhibition* of growth of *Colletotrichum lindemuthianum* on a seeded plate in the presence of filter paper discs impregnated with different fungicides in a dilution series

Fungicides	Initial quantity	Dilutions				
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
benomyl	2 g	100	100	85	63	0
cabtab	2 ml	95	40	34	1	0
carbendazim	2 ml	100	100	95	90	31
carbendazim/tebuconazole	2 ml	100	95	100	76	0
chlorothanonil	2 ml	95	80	34	28	6
iprodione	2 g	11	0	0	0	0
iprodione	6 ml	31	8	1	0	0
iprodione/carbendazim	2 ml	100	100	90	44	0
iprodione/imazalil	2 ml	100	25	2	0	0
procymidone	2 g	11	0	0	0	0
tebuconazole	3 ml	100	23	0	0	0
thiophanate methyl	2 ml	80	5	0	0	0
triticonazole	2 ml	8	1	0	0	0
vinclozolin	2 ml	0	0	0	0	0
water		0	0	0	0	0

* = (πr^2 inhibition area / πr^2 agar plate) X 100

Table 3. The effect of different fungicides as seed treatments on the germination after 14 days and development of anthracnose on dry bean seedlings following (seven days) post germination inoculation with *Colletotrichum lindemuthianum*

Fungicides	Germination (%)	Disease severity index*	
		Uninoculated	Inoculated
benomyl	88	0	0.7a
cabtab	97	0	4.8c
carbendazim	88	0	4.0c
carbendazim/tebuconazole	88	0	0.0a
chlorothalonil	97	0	4.0c
iprodione	94	0	4.9c
iprodione	94	0	3.5bc
iprodione/carbendazim	84	0	1.8ab
iprodione/imazalil	97	0	4.7c
procimidone	94	0	5.0c
tebuconazole	97	0	4.3c
thiophanate-methyl	100	0	1.4a
triticonazole	94	0	4.0c
vinclozolin	97	0	5.0c

*0 = no symptoms; 5 = plants dead
Means followed by the same letter are not significantly different as $P < 0.05$, mean rank scores compared by Dunn pairwise comparison.

Table 4. Predicted percentage seedlings that developed anthracnose lesions in the paper doll test 10 days after four different fungicide concentrations were applied to bean seed in three lesion size categories

Lesion size	Fungicide concentration (g a.i. /kg seed)			
	0 g benomyl + 0 g metalaxyl	1,0 g benomyl + 0,1 g metalaxyl	1,5 g benomyl + 0,1 g metalaxyl	2,0 g benomyl + 0,1 g metalaxyl
0	10 b	1 a	3 a	0 a
trace - 2 mm	52 e	36 d	22 c	24 c
2 - 4 mm	55 e	34 d	39 d	30 cd

Means followed by the same letter are not significantly different at $P < 0.05$, calculated after fit of a logistic regression model.

Table 5. Predicted production of conidia (conidia/ml) from the anthracnose lesions produced in the paper doll test 10 days after four different fungicide concentrations were applied to bean seed in three lesion size categories

Lesion size	Fungicide concentration (g a.i. /kg seed)			
	0 g benomyl + 0 g metalaxyl	1,0 g benomyl + 0,1 g metalaxyl	1,5 g benomyl + 0,1 g metalaxyl	2,0 g benomyl + 0,1 g metalaxyl
0	9,1 X 10^4 b	<1.1 X 10^3	<1.1 X 10^3	<1.1 X 10^3
trace - 2 mm	4,4 X 10^6 c	<1.1 X 10^3	<1.1 X 10^3	<1.1 X 10^3
2 - 4 mm	1,8 X 10^6 c	<1.1 X 10^3	<1.1 X 10^3	<1.1 X 10^3

Means followed by the same letter are not significantly different at $P < 0.05$, calculated after fit of logistic regression model.

Table 6. Predicted percentage germination of seed in a glasshouse trial 21 days after four different fungicide concentrations were applied to bean seed in three lesion size categories

Lesion size	Fungicide concentration (g a.i. /kg seed)			
	0 g benomyl + 0 g metalaxyl	1 g benomyl + 0,1 g metalaxyl	1,5 g benomyl + 0,1 g metalaxyl	2 g benomyl + 0,1 g metalaxyl
0	96 d	96 d	98 d	96 d
trace - 2 mm	76 b	94 cd	90 c	86 bc
2 - 4 mm	64 a	88 c	76 b	78 b

Means followed by the same letter are not significantly different at $P < 0.05$, calculated after fit of logistic regression model.

Table 7. Predicted percentage seedlings that developed anthracnose lesions and the general disease severity ( ) displayed in the glasshouse test 21 days after four different fungicide concentrations were applied to bean seed in three lesion size categories

Lesion size	Fungicide concentration (g a.i. /kg seed)			
	0 g benomyl + 0 g metalaxyl	1 g benomyl + 0,1 g metalaxyl	1,5 g benomyl + 0,1 g metalaxyl	2 g benomyl + 0,1 g metalaxyl
0	13 ab (3)*	10 a (1)	9 a (1)	14 ab (1)
trace - 2 mm	59 d (4)	21 b (1)	26 bc (1)	23 bc (1)
2 - 4 mm	56 d (4)	31 c (1)	31 c (1)	31 c (1)

Means followed by the same letter are not significantly different at $P < 0.05$, calculated after fit of logistic regression model.
* 1 = no symptoms, 5 = plant dead.

Table 8. Effect of different concentrations of benomyl and metalaxyl as seed treatments on the germination and dry mass of 50 *Phaseolus vulgaris* cv Zambezi seedlings 21 days after treatment

Benomyl+metalaxyl g/kg seed	Predicted germination/50 plants	Total dry mass (g/50 plants)
0 + 0	39 a	8,3 ab
0,5 + 0,05	37 a	8,1 ab
1,0 + 0,1	36 ab	8,3 ab
1,5 + 0,15	39 a	9,9 a
2,0 + 0,2	40 a	8,2 ab
5,0 + 0,5	37 ab	8,8 ab
10,0 + 1,0	31 ab	7,2 abc
15,0 + 1,5	31 ab	7,0 bc
20,0 + 2,0	25 b	4,8 c

Means followed by the same letter are not significantly different at $P < 0,05$.

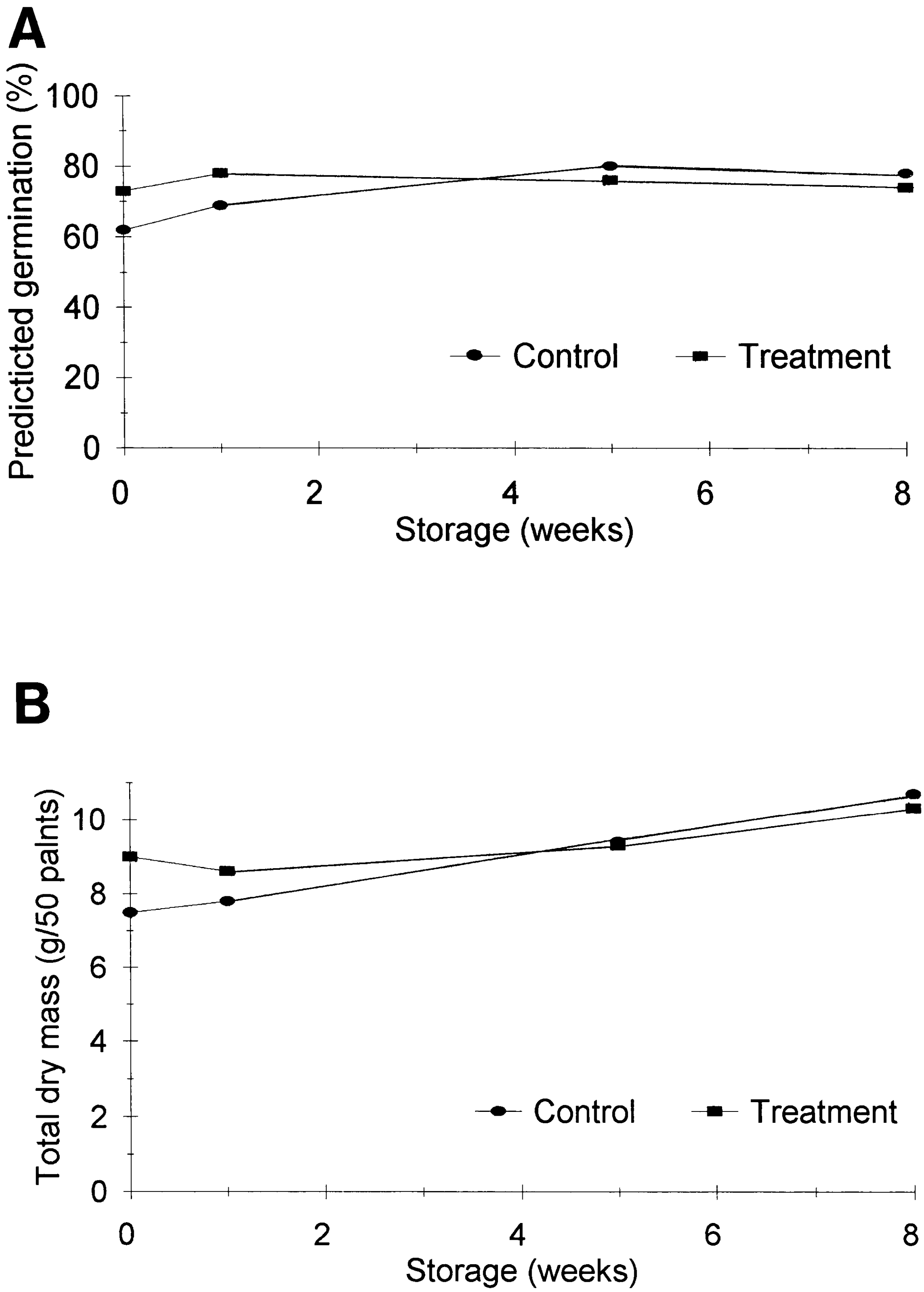

Figure 1. Effect of fungicidal seed treatment (1 g benomyl + 0,1 g metalaxyl/kg seed) on (A) germination and (B) total dry mass of *Phaseolus vulgaris* cv. Zambezi when seed was stored after treatment

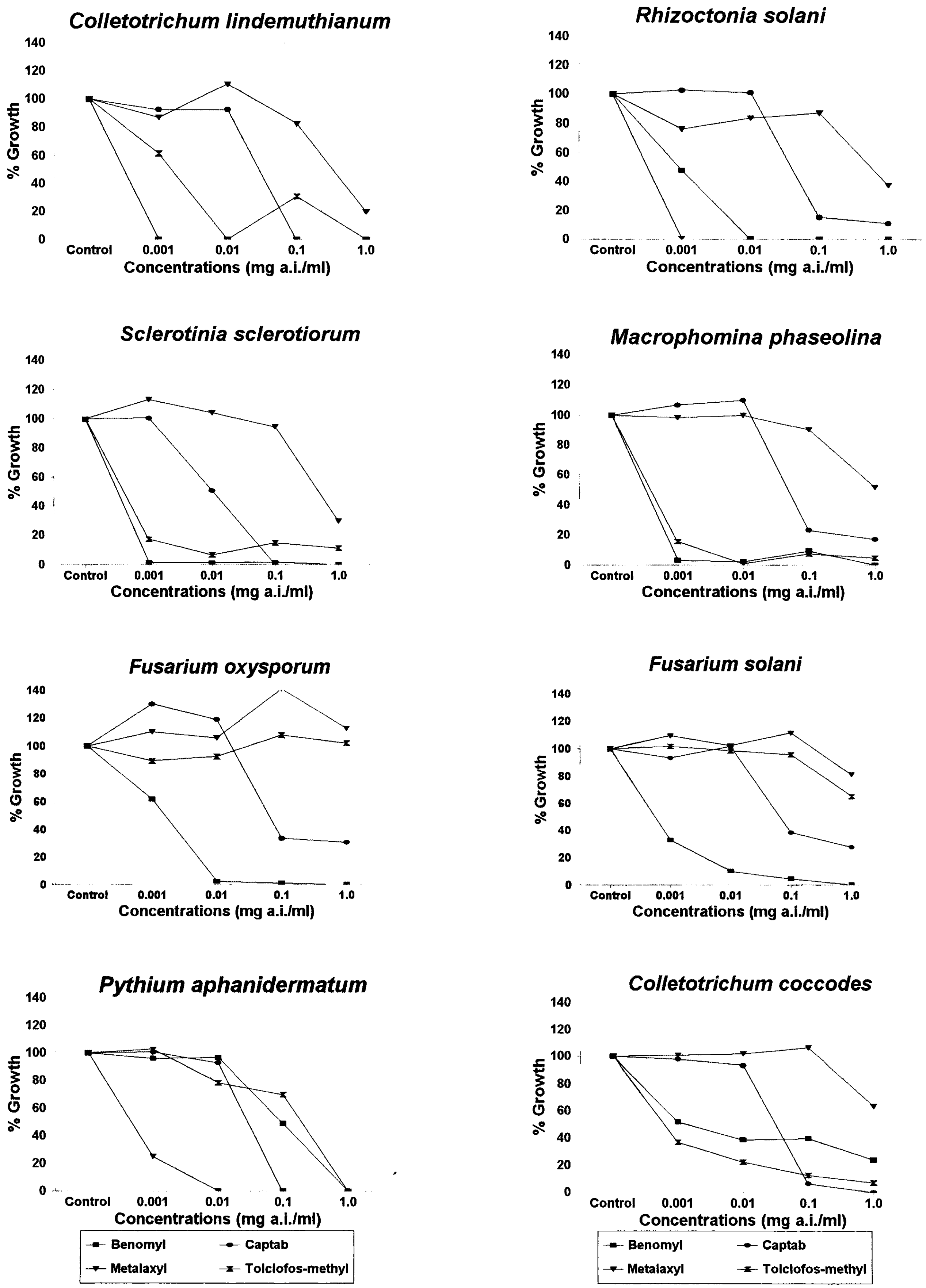

Figure 2. Percentage growth of different fungi on different fungicide concentrations in potato dextrose agar

CHAPTER 8*

GENERAL DISCUSSION

In this study three anthracnose diseases of importance in South Africa were studied. The emphasis was on *Colletotrichum lindemuthianum* (Sacc. and Magnus) Briosi and Cav., the cause of anthracnose of bean, *Phaseolus vulgaris* L.

To solve the taxonomic and pathological problems within the genus *Colletotrichum* Corda a considerable amount of basic research at the molecular level is needed. The results must be compared to those obtained with traditional morphological methods. There is a dire need for standardized and user friendly protocols and keys to identify the fungi to species level. Indeed this needs to be extended to sub-specific and even race levels as resistance seems to be a characteristic of races.

Although efforts were made to integrate the research into a holistic approach to anthracnose diseases of the plant family Fabaceae, the lack of certain basic information rendered meaningful linkages extremely difficult and tenuous.

An appreciable amount of fragmented information is available and efforts to consolidate this information was made during December 1990 at the President's Meeting of the British Society for Plant Pathology, held at the University of Bath. The results of this meeting were published by Bailey & Jeger (1992) and is a summary of the problems with

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Colletotrichum diseases rather than a clarification of the *Colletotrichum* species and the plant diseases associated with them. The lack of information in certain areas such as sub-specific variation, host ranges, genetics and quantitative epidemiology was identified and the necessity for certain basic research needed was emphasized.

To many researchers in other fields the identity of a causal organism is often of minor importance. In quarantine lists the causal organism of lupin anthracnose is listed as *C. gloeosporioides*, a cosmopolitan fungus present on a wide range of hosts in virtually every country. It is generally not considered to be an important pathogen. On the other hand, when a pathogen was previously recorded in a country, regardless its host range, it is not considered a quarantine problem any more. This misconception has led to the relaxing of import restrictions. This is probably the main reason for the lupin anthracnose problem currently experienced in South Africa. No detection methods and no control measures are available. These all need to be established, especially the identification of possible cultivar resistance.

The delimitation of *Colletotrichum* species is complex and, due to the lack of protocols regarding species identifications, very difficult (Sutton, 1992). Walker, Nikandrow & Millar (1991) stressed the importance of this aspect in making plant pathological decisions. In the case of the causal organism of lupin anthracnose such a situation was evident. In my opinion, and after considering the available published data, the *Colletotrichum* sp. involved did not fit the description of any accepted *Colletotrichum* spp. as listed by Sutton (1980; 1992) and Baxter, Van der Westhuizen & Eicker (1983). A study was therefore undertaken (Chapter 3) to describe this organism as a distinct species. It must be kept in mind that Sreenivasaprasad, Brown & Mills (1992), Sreenivasaprasad, Mills & Brown (1994) and Brown, Sreenivasaprasad & Timmer (1996) disregard conidial form and cultural characteristics in their molecular studies of *C. acutatum*. In their species concept they allow for more than 30% variation in a pairwise comparison of ribosomal and mitochondrial DNA restriction fragment bonding patterns. They concluded that the causal fungus of strawberry and lupin anthracnose is the same. However, they did not substantiate this claim by cross inoculation experiments. They also included the slow-growing orange and key lime anthracnose strains from *Citrus* in their species concept. In this study it was clearly shown on the basis of conidial morphology and cultural characteristics that the causal organism of lupin anthracnose is distinct from *C. acutatum*, *C. gloeosporioides* and *C. musae*. The species was therefore described and named *C. tortuosum* Koch & Baxter. The importance of this disease must be emphasized as lupin production all over the world is threatened by the disease and appropriate quarantine measures should therefore be invoked.

Molecular techniques can play a major role in the identification of a pathogen. Consensus on the most applicable technique in species delimitation must be reached. Basic research on the molecular level needs to be done on a global basis and a comparative approach with traditional morphological identification methods must be undertaken.

The Biolog System (Biolog, Inc., Hayward, CA), which is widely applied for bacteria and yeast identification as an additional aid in the identification of the lupin anthracnose causing organism was also investigated. The SF-N & SF-P Microplate™ were used. In preliminary runs the lupin anthracnose isolates grouped together. Unfortunately not enough representative isolates of other species for comparison were available and this approach could not be pursued. However, the technique has obvious merits and deserves attention.

Sheriff *et al*. (1994) proposed that *C. trifolii* and *C. lindemuthianum* must be included in *C. orbiculare* as pathovars. In this study the distinction between *C. trifolii*, *C. lindemuthianum* and *C. orbiculare* was upheld. In the fourth chapter *Chameacytycis palmensis* was added to the host range of *C. trifolii*. This is also a first report of anthracnose on this host.

A diversity of pathological races of the fungus *C. lindemuthianum,* the cause of anthracnose of beans, is known to occur in Africa (Allen, 1992) and South and Central America (Pastor-Corrales & Tu, 1989; Pastor-Corrales *et al*., 1995). Until the late 1980's, an internationally available set of differential cultivars, to distinguish between the races of the fungus, was not available. For the first time races of the fungus *C. lindemuthianum* were successfully determined in South Africa with seven races of the fungus evident (Chapter 5). They were also distinct and less diverse than those that occurs in Central and East Africa. No geographical regions of race predomination in South Africa could be established. This is probably due to unrestricted movement of diseased seed within the country. In South America they have applied random amplified DNA polymorphisms (RAPD's) (Vilarinhos *et al*., 1995; Otoya, Restrepo & Pastor-Corrales, 1995) in the characterization of races of *C. lindemuthianum*. They had no success in identifying races of the fungus and could only group the fungus according to geographical regions. It was also shown that no local cultivar had resistance against all the races of the fungus recorded in South Africa. It is therefore necessary to adapt the local dry bean breeding programme to include resistance to anthracnose. Caution should be exercised against the importation other races and the possible development of new races of the fungus must be monitored.

From the results obtained in Chapter 5 it was shown that the genes Co-1, Co-3 and Co-5 can not be used on their own any more. Co-2 in Cornell 49-242 and the genes in cultivar G2333 is still effective and can be used in further breeding programmes to enhance resistance to anthracnose.

When resistance to *C. lindemuthianum* is not available, as is the case in South Africa, dry bean production must rely on other control measures such as the use of disease-free seed and the chemical sanitation of diseased seed. This also implies that effective tests for the detection of infected seed must be available.

To ensure that producers receive disease-free seed, seed production fields are regularly inspected for the presence of anthracnose by inspectors of the South African National Seed Organisation (SANSOR) as required by the Plant Improvement Act, 1976 (Act 53 of 1976). Field inspections alone cannot ensure that seedlots are free of anthracnose and it must be followed by a reliable laboratory test. The so called "paper doll" method was found the most effective, user friendly and reliable in the detection of *C. lindemuthianum* on bean seed (Chapter 6). It is cost and time effective, and minimal space is required.

This method resembles the method described in the ISTA handbook (Anselme & Champion, 1981), but differs from that prescribed in the ISTA rules (ISTA, 1993). This technique has been successfully employed by South African seed testing laboratories of the Dry Bean Producers Organisation, but nevertheless suffers from significant drawbacks. Results are only available after 10 to 14 days and the method is labour intensive. Trained technicians are needed. The use of the PCR (polymerised chain reaction) and monoclonal antibodies in an adapted ELISA (enzyme linked immuno adsorbent assays) must be investigated.

Benomyl appears to be the most effective fungicide for the control of seedborne infections of *C. lindemuthianum*, both *in vitro* and *in vivo* (Chapter 7). Although it does not control deep-seated seed infections, infection rates were significantly reduced. This method could be used to treat seed with low percentages of infection. Treated seed can then be presented as anthracnose free. Resistance to benomyl is known to occur in fungi (Frahm, 1973) and it was experimentally proved that *C. lindemuthianum* acquired resistance to benomyl (Tu & Jarvis, 1979a,b; Tu & McNaughton, 1980), strains of the fungus resistant to benomyl have not yet been recorded under field conditions. Due to the above mentioned shortcomings of benomyl, new fungicides must be developed for the control of seedborne anthracnose of beans. The benomyl seed treatment should be regarded as an additional control method and must not be used excessively. This is to prevent the development of resistance to the fungicide.

Although the genus *Colletotrichum* has been extensively studied, many unanswered questions remain. This study revealed some answers with practical implications, but these economically important diseases warrant further in depth study. Protocols for identification of the causal organisms must be set and the host ranges of the species identified. Where races of certain species exists, they should be linked or associated to geographical regions. There is a need to identify and isolate resistant genes and to incorporate them into plants. Short term relief can be achieved by ensuring that plant material is disease-free. Reliable laboratory tests will play a major role in establishing whether the fungus is present. Another alternative is chemical control, but not many safe and economically justifiable fungicides effective against the genus *Colletotrichum* are available. The possibility of fungicide resistance cannot be ignored.

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CHAPTER 9

SUMMARY

Three different anthracnose diseases of importance in South Africa were studied. The main emphasis was on anthracnose of dry beans (*Phaseolus vulgaris*), caused by *Colletotrichum lindemuthianum*. The aim of the study was to provide more information, with direct implication for the industry, on these economically important anthracnose diseases.

Anthracnose of *Lupinus* spp. was for the first time recorded in South Africa. Based on average conidial size and shape as well as appressorial outline and conformation, and colony appearance and texture the causal organism was distinguished from all other *Colletotrichum* spp. On these grounds the new name, *C. tortuosum*, was introduced for the organism causing lupin anthracnose. Anthracnose of *Chamaecytisus palmensis* (tagasaste), caused by *C. trifolii* was also recorded for the first time.

By using the binomial system proposed by CIAT (Centro Internacional de Agricultura Tropical), races 3, 65, 80, 83, 119 and 593 of *C. lindemuthianum*, were found to be present in South Africa. Differential resistance to the local races of the fungus was identified in the bean cultivars currently being evaluated in South Africa. None of the local bean cultivars showed resistance to five of the local races of the fungus under glasshouse conditions. The use of disease-free seed is important in the control of anthracnose of beans. Different methods for the detection of *C. lindemuthianum* on bean seed were evaluated. The so called paper-doll method was found the most effective and is currently used by South African bean seed testing laboratories. Various fungicides were evaluated for their

effectiveness to control seedborne *C. lindemuthianum*. Benomyl was found the most effective. It is recommended that benomyl is used in combination with metalaxyl and that sodium molybdate in carboxymethyl-cellulose diluted with water, is used as sticker and wetter.

This study revealed some answers with practical implications, but also showed that these economically important diseases warrant further study in depth.

OPSOMMING

Drie verskillende antraknose siektes, van belang in Suid-Afrika, is bestudeer. Die klem was hoofsaaklik op antraknose van droë bone *(Phaseolus vulgaris*), wat deur *Colletotrichum lindemuthianum* veroorsaak word. Die doel van die studie was om meer inligting, met direkte implikasies op die bedryf, oor hierdie ekonomies belangrike antraknose siektes daar te stel.

Antraknose van *Lupinus* spp. is vir die eerste keer in Suid-Afrika aangeteken. Gebaseer op die algemene konidia grootte en vorm sowel as die appressoria buitelyn en konformasie, en kolonie voorkoms en tekstuur is die veroorsakende organisme van alle ander *Colletotrichum* spp. onderskei. Op grond hiervan is die nuwe naam, *C. tortuosum*, aan die organisme wat antraknose van lupiene veroorsaak, gegee. Antraknose van *Chamaecytisus palmensis* (tagasaste), veroorsaak deur *C. trifolii* is ook vir die eerste keer aangeteken.

Deur gebruik te gemaak het van die binominale sisteem voorgestel deur CIAT (Centro Internacional de Agricultura Tropical), is rasse 3, 65, 80, 81, 83, 119 en 593 van *C. lindemuthianum* in Suid-Afrika teenwoordig gevind. Weerstandsverskille ten opsigte van die plaaslike rasse van die swam is, in die boontjie kultivars wat tans in Suid-Afrika geevalueer word, geïdentifiseer. Geen van die plaaslike kultivars het weerstand teen vyf van die rasse onder glashuis toestande getoon nie. Die gebruik van siektevrye saad is belangrik in die beheer van antraknose van bone. Verskillende tegnieke vir die opsporing van *C. lindemuthianum* op boontjiesaad is geëvalueer. Die sogenaamde papierpop-metode is as die mees effektieve gevind en word tans deur Suid-Afrikaanse boontjiesaad

toetslaboratoriums gebruik. Verskillende fungisiede is vir hul doeltreffendheid in die beheer van saadgedraagde *C. lindemuthianum* geëvalueer. Benomyl is as die mees doeltreffendste gevind. Daar word aanbeveel dat benomyl, in kombinasie met metalaxyl en natriummolibdaat in karboksiemetielsellulose verdun met water, as bindmiddel en benatter, gebruik word.

Hierdie studie het sekere antwoorde met praktiese implikasies aan die dag gebring, maar het ook getoon dat hierdie ekonomies belangrike groep siektes in diepte verder bestudeer moet word.