CHARACTERIZATION OF DISEASES OF KENAF (*HIBISCUS CANNABINUS* L.) IN SOUTH AFRICA

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GENERAL INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) is a warm season, annual, herbaceous bast fibre crop that belongs to the family Malvaceae. Since cotton (*Gossypium hirsutum* L.) belongs to the same family it can be expected that kenaf will be susceptible to diseases of cotton. Although was originated in Africa it was not known as a fiber crop in South Africa until 1947, when it was considered as a suitable substitute for jute. It is currently being investigated in South Africa with a view to commercial production. Fibre obtained from kenaf can be used to produce pulp for paper, packing material, oil absorbents, fibreboards and animal bedding. In addition, its foliage can be used as animal fooder.

During the course of evaluation trials in South Africa over the past two years, certain pathogens of kenaf have been identified that cause disease on kenaf in other parts of the world. Powdery mildew caused by *Leveillula taurica* Lév attacks leaves and can cause significant losses in seed yield and reduce its quality. Grey mould caused by *Botrytis cinerea* Pers.:Fr. infects kenaf stems leading to lodging and results in severe losses of yield and decreased fibre quality. *Pythium* group G was identified for the first time as a pathogen of kenaf in South Africa. Diseased plants displayed large, black sunken lesions at the base of the stem and severe root rot. Rust caused by *Aecidium garkeanum* P. Hennings has also been observed on cultivated kenaf plants in South Africa.

This thesis is a compilation of four independent manuscripts based on research conducted over a period of two years. Each chapter is an independent entity and some redundancy between chapters has, therefore, been unavoidable.

The first chapter is a review of literature on diseases of kenaf and their control. It discusses the etiology and control of the major leaf and soilborne diseases of kenaf. An attempt was made to examine disease management practices on related crops and how they

can be adapted for kenaf cultivation. Thus integrated disease management, with specific reference to cotton, is discussed.

Kenaf is cultivated from seed and the uniform emergence of seedlings plays an important role in its production. It is in this context that the second chapter sets out to evaluate the factors that are related to the establishment of kenaf from seed. The chapter investigates the potential seed borne pathogens of kenaf and determines their pathogenicity to the crop. It also investigates the effect of fungicide and ComCat® seed treatment on seed germination, seedling emergence as well as the fresh and dry mass of roots and above ground parts of seedlings.

The characterization of *Botrytis cinerea*, the cause of grey mould of kenaf, is discussed in chapter 3. It investigates the pathogenicity of *B. cinerea* to kenaf, optimum temperature requirements for its mycelial growth, the effect of irrigation on grey mould incidence and *in vitro* sensitivity of *B. cinerea* to various fungicides.

In chapter 4, research on *Pythium* group G, a pathogen on kenaf, is reported. The chapter deals with pathogenicity trials in the field and glasshouse as well as growth studies *in vitro* with specific reference to the optimum growth temperature and fungicide sensitivity of the fungus.

The present study deals with important diseases of kenaf that are incited by various pathogens and particular emphasis is given to those reported in South Africa. It is the first time that such a study has been conducted on kenaf pathology in South Africa. It is my hope that the results of this study will contribute to a better understanding and knowledge of kenaf diseases and their management in South Africa.

CHAPTER 1

DISEASES OF KENAF WITH SPECIFIC REFERENCE TO THE POSSIBLE INTEGRATION OF MANAGEMENT PRACTICES RELEVANT TO THE CONTROL OF COTTON DISEASES

1.0 INTRODUCTION

Kenaf *(Hibiscus cannabinus* L.*)* is a warm season, annual, herbaceous plant belonging to the Malvaceae, a family known for both its economic and horticultural importance (Dempsey, 1975). Kenaf is a native of tropical Africa and the East Indies (Lawrence & McLean, 1991) and may have been domesticated as early as 4,000 B.C. in the Western Sudan (Dempsey, 1975). It can grow under various climatic conditions and probably has a wider range of adaptation to climate and soil than any other fibre plant grown for commercial use (Dempsey, 1975).

The economical potential of this crop is related to the gradual diminishing supply of hard and softwood in the world, and the increasing per capita consumption of paper and paperboard materials (Francois, Donovan & Maas, 1992). According to Francois *et al.* (1992), the entire kenaf plant can be utilized. The stem consists of two main fibres; the bast obtained from the outer bark and the core obtained from inside the stem. Both types of fibres may be used to produce packing material, oil absorbents, animal bedding, and pulp for paper (Colyer, Vernon & Caldwell, 1992; Baldwin *et al.*, 1996; Bagby, Princen & Rossi, 1996). The foliage can be used as a source of roughage and protein for cattle and sheep after it has been shredded (Francois *et al.*, 1992; Rojas, Dormond & Viquez, 1994). Oil extracted from kenaf has a similar nutritional value and palatability to sunflower oil and can thus be used as an alternative to sunflower oil (Valdes *et al.*, 1996).

Kenaf is as a source of cordage fibre, which can be used in the manufacture of rope, twine, carpet backing and burlap (Webber, 1993) as well as fiberboard and compressed board (Oliveros *et al.*, 1996). Kenaf also plays an important role in the removal of nitrogen and phosphorus from wastewater (Abe, Ozaki & Kihou, 1997; Mizuta, Abe & Ozaki, 1998). Russo, Webber & Myers (1997) reported the bio-catalytic effect of decomposed kenaf or extracts in enhancing the germination and post-germination

development of certain economic crops. Alternatively, non-decomposed kenaf and its extracts may be utilized to suppress weeds. The kenaf oil extracted from leaves is phytotoxic to certain weeds and also antifungal to *Colletotrichum* species (Kobaisy *et al.*, 2001).

Kenaf is closely related to cotton (*Gossypium hirsutum* L.) (Malvaceae) and cultural practices and growing conditions that favour cotton also favour the cultivation of kenaf (Baldwin *et al.*, 1996). As a result, pathogens that can cause disease in cotton can be expected to also attack kenaf. White *et al.* (1970)(as cited by Batson, Caceres & Carvajal, 2000) reported that soilborne diseases of cotton such as damping-off and root rot could become serious when kenaf is cultivated after cotton in the same field. Corato *et al.* (1999) reported that *Fusarium* isolates obtained from kenaf were similar in pathogenicity to those isolated from cotton.

Dempsey (1975) described the principal diseases of kenaf. This review will examine the most important leaf and stem as well as soilborne diseases of kenaf with specific reference to those that have been reported in South Africa or are expected to occur here. The review also deals with the integration of management practices relevant to the control of cotton diseases and how they can be adapted for kenaf cultivation.

2.0 LEAF AND STEM DISEASES OF KENAF.

2.1. Grey mould .

2.1.1 Etiology: *Botrytis cinerea* Pers.:Fr. is an ascomycete that causes pre- and postharvest diseases of many economically important crops (Have *et al.*, 2001). Fermaud & Le Menn (1989) described *B. cinerea* as a destructive pathogen that attacks a wide range of plants throughout the world. Grey mould caused by *B. cinerea* has been reported in numerous countries and on various crops. *Botrytis* spp. infects cotton lint after normal dehiscence resulting in lint discolouration (Hillocks, 1992c). It was recently reported on kenaf stems in South Africa for the first time (Swart, Tesfaendrias & Terblanche, 2001).

The pathogen attacks plants during periods of high humidity, and causes partial or total defoliation of kenaf (Dempsey, 1975). According to Swart *et al* (2001), infected plants display brown necrotic lesions that girdle the stem, resulting in wilting and lodging of the plants. De Cal & Melgarejo (1991) as well as Polverari, Tosi & Benincasa (1994) also observed the breaking of infected stems and wilting of foliage above the lesions. When the cortical tissues of the stem are affected considerable yield losses or a decrease in fiber quality can occur (Polverari *et al.*, 1994).

The presence of the fungus on infected stems is indicated by extensive mycelia, conidiophores and the characteristic grey conidia of the fungus (Polverari *et al.*, 1994; Swart *et al.*, 2001) which are liberated in a grey cloud in the slightest breeze (McPartland, Clarke & Watson, 2000). Disease is favoured by low temperatures and high humidity, and the incidence is highest in irrigated crops, especially those planted too densely (Polverari *et al.*, 1994). A number of mechanisms have been suggested for the primary infection of *B. cinerea* although the fungus is often regarded as an opportunistic pathogen that may penetrate through wounds or natural openings (Have *et al.*, 2001).

2.1.2 Control: McPartland *et al.* (2000) stated that the wounding of plants should be avoided during susceptible periods, except to remove injured or infected branches to protect plants from infection by *B. cinerea.* For the cultivation of grapes, Nair & Hill (1992) recommended the creation of a dry microclimate by means of adequate ventilation and management of plant nutrition to reduce the risk of *B. cinerea.* Since dead and moribund plant tissues are ideal for the maintenance and sporulation of *Botrytis* spp. Maude (1980) recommended removal and burial or burning of decaying infected plant tissues in order to reduce inoculum levels.

Morando, Morando & Morando (1998) reported that effective control of *B. cinerea* on grapes was achieved with the pyrimethanil and dicarboximide fungicides vincolozoline and procimiodine. Rosslenbroich *et al.* (1998) as well as Morando *et al.* (1998) reported protective action with the foliar fungicide, fenhexamide, a new chemical class of hydroxyanilides. Dempsey (1975) recommended dressing kenaf seed with *cis-N-*((trichloromethyl) thio)-4-cyclohexene-1, 2-dicarboximide (captan) to minimize damage by *B. cinerea.* According to Pappas (2000) the application of conventional compounds such as dichlorofluanid and chlorothalonil is recommended when disease pressure is low. However, under conditions very favourable to *Botrytis* infection, mixtures of conventional fungicides with reduced strength specific botrycides should preferably be applied to minimize losses (Nair & Hill, 1992; Pappas, 2000).

2.2 Powdery mildew.

2.2.1 Etiology: *Leveillula taurica* Lév can infect a large number of plant species (Correll, Gordon & Elliott, 1987). Mihail & Alcorn (1984) reported its association with more than 700 plant species worldwide. It principally attacks kenaf leaves during periods of high humidity and may cause partial or total defoliation (Dempsey, 1975).

Symptoms include extensive growth of white, superficial mycelial colonies on abaxial leaf surfaces, followed by partial or total leaf defoliation (Cook & Riggs, 1995; Swart & Terblanche, 2001) as well as the abortion of pre-bloom floral structures and early stage seedpods (Cook & Riggs, 1995). On older leaves, powdery mildew covers the abaxial leaf surface, while chlorotic and necrotic patches are visible on the adaxial surface (Swart & Terblanche, 2001). The disease can severely diminish seed yield and reduce seed quality in infected kenaf plants (Cook & Riggs, 1995).

2.2.2 Control: Due to the insignificant effect of the pathogen on cotton yield, control is not recommended for powdery mildew (Watkins, 1981; Hillocks, 1992b). However, numerous fungicides have been prescribed for the control of the disease caused by *L. taurica* in various crops. The superficial nature of the pathogen renders it susceptible to a foliar spray (McPartland *et al.*, 2000). A combined treatment of tetraconazole with neem oil was effective in controlling powdery mildew in sweet pepper caused by *L. taurica* (Fium, 1997). Penconazole followed by hexaconazole and propiconazole was reportedly effective in controlling powdery mildew in fenugreek (*Trigonella foenum-graecum*) (Dhruj *et al.*, 1996). Successful control of *L. taurica* on chickpea (*Cicer arietinum* L.) using tridemorph, bitertanol and carbendazim has been reported (Bidari, Dayanand, & Anahosur, 1998). A foliar spray consisting of a 1 % (w/v) solution of mono-potassium phosphate was effective in controlling powdery mildew caused by *L. taurica* on peppers (*Capsicum*) (Reuveni, Dor & Reuveni, 1998).

2.3 Zonate leaf spot.

2.3.1 Etiology: This disease can result in 50 –75% defoliation (Pollack & Waterworth, 1969; Holcomb, Viator & Brown, 1992) and can affect 98-100% of plants (Blake, Mueller & Lewis, 1994). In most cases, severe leaf spot, blight of foliage, and defoliation caused

by *Cristulariella moricola* (Hino) Redhead (syn. *C. pyramidalis* Waterman & Marshal) occur following a prolonged period of wet weather (Grand, 1978; Colyer *et al.*, 1992).

The pathogen has a wide host range and causes leaf spots and defoliation in numerous woody and annual species (Trolinger, Elliott & Young, 1978). The most frequently observed symptom of the disease involves target-like yellowish-grey to brown spots progressing from small, circular, tan lesions with dark brown margins to large lesions with concentric rings (Pollack & Waterworth, 1969; Trolinger *et al.*, 1978). The spots eventually cover the entire leaf, causing blight and eventual defoliation (Colyer *et al.*, 1992). Horst (1979) reported the appearance of yellow-grey spots with definite margins on infected *Hibiscus* plants. The necrotic lesions increase both in number and size from the lower to the upper part of the plant (Pollack & Waterworth, 1969).

2.3.2 Control: Resistance among kenaf cultivars to *C. moricola* has been observed and the cultivar, Gregg, is apparently resistant (Cook & Scott, 2000).

2.4 Rust.

2.4.1 Etiology: Rust has been reported on leaves of wild kenaf along the Rift Valley (Dempsey, 1975). A very high incidence of rust caused by *Aecidium garkeanum* P. Hennnings, with yellow pustules on the leaves, occurred during 2002 in kenaf trials near Makhatini, South Africa (Swart *et al.*, unpublished data).

2.4.2 Control: Cultivars resistant to the disease have been reported and Sarma (1972), as cited by Dempsey (1975), reported that the Guatemalan 4 variety was more resistant to rust than all other varieties of kenaf planted at Namalu, Uganda. Among cultivars tested in South Africa, Everglades 41, Dowling and Whitton were relatively resistant to the pathogen (Swart *et al.*, unpublished data). Tropical rust of cotton caused by *Phakospora gossypii* (Arth.) Hirat. f., can be controlled by burning or removing contaminated residues

(Watkins, 1981; Hillocks, 1992a), planting resistant varieties and avoiding seeds from diseases plants (Watkins, 1981).

3.0 SOILBORNE DISEASES OF KENAF

3.1 *Fusarium* spp.

3.1.1 Etiology: *Fusarium* spp. attack both young seedlings and older plants, causing black or brown stem lesions close to ground level that result in the lodging and death of plants (Dempsey, 1975). Fusarium wilt of cotton results when the pathogen penetrates through the roots and spreads upwards in the vascular tissue, thus depriving the plant of water (Munro, 1987). Wilting and necrosis caused by *F. oxysporum* f.sp. *vasinfectum* on kenaf which also attacks cotton (Corato *et al.*,1999) is favoured by hot and dry conditions followed by rain (Verma & Raj, 1992).

Extensive infection of cotton by *Fusarium* spp. is usually associated with the presence of nematodes, which provide wounds for easier infection and hence facilitate disease (Munro, 1987). Blake *et al.* (1994) reported the association of *Rhizoctonia* sp., *Pythium* sp. and *Fusarium* sp. with plants infected by root-knot nematodes. Potassium deficient plants are more prone to nematode damage and disease incidence caused by *Fusarium* spp. thus increases (Verma & Raj, 1992).

3.1.2 Control: Conditions at planting that favour rapid germination and seedling growth will help to reduce seedling diseases (Seney, 1984). Planting too deep is conducive to damping-off (Minton & Garber, 1983). This is because soil temperature decreases with depth and deep planting delays seedling emergence resulting in longer exposure of hypocotyl tissue to pathogen attack. It is therefore important to plant seeds at an optimal depth where adequate moisture for germination and seedling growth is available (Minton

& Garber, 1983). Fusarium wilt of cotton is transmitted in seeds; it thus advisable to avoid using seeds produced in infested fields (Bird, 1986).

Cook *et al.* (1996) recommended the use of newly developed cultivars of kenaf that are resistant to the root-knot nematode (*Meloidogyne incognita*) and fungus complex in order to reduce crop losses. Fusarium wilt can be controlled indirectly by destroying the nematodes in a wilt-nematode complex (Verma & Raj, 1992). However, the application of nematicide may not always be economical (Perry, 1962). Deep ploughing exposes inoculum under the soil surface to desiccation and radiation and ploughing before planting cotton reduces the incidence of the Fusarium wilt-nematode complex if it is practiced during the dry season (Maloy, 1993).

Zinc and manganese can reduce wilt by inhibiting the germination of conidia of *Fusarium* when added to soil (Verma & Raj, 1992). Treating cotton seeds with systemic fungicides such as chlorothalonil, thiabendazol, carbaxin and spraying the soil with zinc sulfate and systemic fungicides such as algin, was found to be effective against Fusarium diseases on cotton (Verma & Raj, 1992) and should therefore also be effective for kenaf.

3.2 Damping-off.

3.2.1 Etiology: Damping-off is probably the most common symptom caused by *Rhizoctonia solani* Kühn. Most plants are affected and the disease occurs primarily in cold and wet soils (Agrios, 1997). Damping-off caused by *R. solani* affects young kenaf seedlings at ground level, resulting in the lodging and the death of plants (Dempsey, 1975). The fungus is not only restricted to the seedling stage but also attacks older plants causing collar rot at the base of the stem if conditions remain favourable for disease development (Dempsey, 1975; Hillocks, 1992b). It attacks cotton seedlings during cold, moist weather or when the seedlings are weakened by insects such as thrips (Munro, 1987).

3.2.1 Control: Cultural practices such as crop rotation and measures which encourage rapid emergence and growth of seedlings, help to reduce disease incidence in cotton (Hillocks, 1992b). Sowing cotton on well drained soil along with balanced fertilization ensures quick germination and a vigorous start, thus reducing losses due to *R. solani* (Munro, 1987). Soil mulching with polyethylene film also greatly reduces the population of *R. solani* on cotton fields (Ahmed *et al.*, 2000). For maximum protection against soilborne pathogens of cotton, the treatment of seeds with the combination of two or more fungicides is recommended (Minton & Garber, 1983). Effective control of *R. solani* can be achieved by treating cotton seeds with a mixture of triadimenol, pencycuron and tolyfluanid (Goulart, 1999). Treating kenaf seeds with pentachlorobenzene (PCNB), (Batson *et al.* 2000) and spraying PCNB in seed furrows of cotton (Elad, Kalfon & Chet, 1982) is effective in reducing disease incidence in cotton fields.

3.3 *Pythium* diseases.

3.3.1 Etiology: *Pythium* infection occurs mainly on seedlings but may also occur during the early growing season (Watkins, 1981). Seed infection and infection of the radicle and seedling hypocotyls of cotton results in seed rot, pre-emergence damping-off or post-emergence damping-off (Hillocks, 1992). Basal stem rot of kenaf has been reported in South Africa where it results in large, black, sunken lesions at the base of the stem, and severe root rot due to infection by *Pythium* group G (Swart, Tesfaendrias & Botha, 2002). Damage related to *Pythium* infection is more severe when soil moisture is high and temperature low (Watkins, 1981; Hillocks, 1992; Agrios, 1997). Furthermore, Hillocks (1992b) noted that root-knot nematodes (*Meloidogyne* spp.) prolong the period of susceptibility of cotton seedlings to *Pythium* infection. Other factors such as the presence

of excessive nitrogen in the soil and planting the same crop in the same field for several consecutive years may also cause crop losses due to *Pythium* species (Agrios, 1997).

3.3.2 Control: Soil amended with urea is effective in reducing the number of viable propagules of *Pythium ultimum* Trow (Chun & Lockwood, 1985). The use of metalaxyl as a seed treatment when conditions favour the development of seedling diseases caused by *Pythium* has also shown positive results in kenaf (Batson *et al.*, 2000). The application of metalaxyl protects cotton seedlings from *Pythium* infection (Hillocks, 1992) while iprodione provides effective control of *Pythium* seedling diseases in cotton (Baldwin & Hogue, 1997). Kenaf seeds treated with triadimenol and carboxin-pentachloronitrobenzene (PCNB), alone or in combination, resulted in increased survival in plots where additional inoculum of *P. ultimum* had been applied (Batson *et al.*, 2000).

3.4 *Verticillium dahliae*

3.4.1 Etiology: *Verticillium dahliae* Klebahn is a soil inhabiting fungus with a wide host range that includes both cultivated crops and weed species in both temperate and tropical countries (Munro, 1987; Davis *et al.*, 2000). The disease is favoured by cool wet weather (Munro, 1987) and excessive moisture in the form of irrigation or rain as well as excessive nitrogen in the soil (Watkins, 1981). *V. dahliae* is now recognized as one of the most widely distributed and destructive pathogens with a wide host range in agricultural soils (El- Zik, 1985). Verticillium wilt of cotton caused by *V. dahliae* has been reported in various African countries including South Africa (Prentice, 1972). *V. dahliae* causes severe stunting and wilt of kenaf plants and the pathogen is most often isolated from the stem tissues of diseased kenaf, but not from seeds produced by diseased plants (Corato *et al.*, 1996; Polizzi, 1996). Diseased plants exhibit yellowing of leaves and discolouration of vascular stem tissue (Polizzi, 1996). In cotton, defoliation and terminal dieback occurs in

plants infected with *V. dahliae* (El-Zik, 1985), but regrowth of leaves from lower nodes often occurs after defoliation, if the plant is not killed. The pathogen persists in the soil for several years and enters the plant through its roots, resulting in wilt due to interference with water translocation (Prentice, 1972). Infection arises from microsclerotia, which overwinter in soil or in infected plant debris from previous crops (Lazarovits, Conn & Tenuta, 2000).

3.4.2 Control: Any cultural practice that increases soil temperature tends to reduce the amount of wilt caused by *Verticillium* spp. (Munro, 1987). Soil mulching with polyethylene provides the potential for long-term control of *V. dahliae* in pistachio groves due to high temperatures created by the plastic (Ashworth & Gaona, 1982). High plant density in cotton reportedly reduces the incidence and severity of Verticillium wilt (Watkins, 1981). Plants grown under high plant density have a relatively small root volume limiting the chance that roots will encounter inoculum of *Verticillium* in the soil (Seney, 1984). Soil solarization and flooding can also reduce the inoculum density of *V. dahliae* in soil and prevent the increase and spread of the pathogen (Pullman & De Vay, 1982; Lopez-Escudero & Blanco-Lopez, 2000; Termorshuizen, Blok, & Lamers, 2000). The success of these practices however depends on environmental conditions. Soil solarization is usually practiced to areas with a hot climate while flooding is best applied to soils with a low hydraulic conductivity (Termorshuizen *et al.*, 2000).

Nutrition determines the resistance or susceptibility of plants to disease, by influencing the ability of tissues to hasten or slow down pathogenesis, as well as the virulence and ability of pathogens to survive (Huber, 1980). The amendment of soil with nutrients can thus play a vital role in managing both foliar and soilborne diseases. For example, excessive application of nitrogen promotes disease, while high levels of potassium tends to reduce disease (Watkins, 1981;Verma & Raj, 1992). It is thus

advisable to apply fertilizers at the recommended rate if disease incidence and severity are to be controlled.

The application of nitrogen fertilizer in the form of ammonia and ammonium salt can reduce populations of *Pythium ultimum, Fusarium* spp., *Phymatotrichum omnivorum* Duggan and *Sclerotium rolfsii* Sacc. (Hodges, 1992). However, Presley & Bird (1968), Huber (1981) as well as Bell (1989) as cited by Hodges, (1992) reported that nitrogen application could increase the severity of both Fusarium and Verticillium wilt, especially when potassium is deficient. The use of properly balanced N, P and K fertilizers can therefore limit Verticillium wilt while maximizing yields (Hodges, 1992). Yields and profits are usually increased in proportion to applied N and hence one has to apply the recommended balance of fertilizers in order to combat pathogens and gain the expected yield. The application of potassium and micronutrients such as zinc and manganese can also reduce disease frequency caused by *V. dahliae* (El-Zik, 1985).

Fumigation with broad-spectrum fungicides such as methane sodium and methyl bromide can effectively eradicate *V. dahliae* in soils and thus reduce disease incidence (Fravel & Larkin, 2000). These broad-spectrum fungicides, however, also eliminate populations of beneficial, non-target soil microorganisms. A decline in microbial activity of soil can lead to increased pathogen populations due to reduced competition and antagonism (Lazarovits *et al.*, 2000).

3.5 Phymatotrichum root rot.

3.5.1 Etiology: Phymatotrichum root rot of kenaf is caused by *Phymatotrichum omnivorum* Duggan, a soil-borne fungal pathogen (Cook & Riggs 1993; Cook *et al.*, 1995). The pathogen causes severe reductions in plant height and stalk yield (Cook *et al.*, 1995) and thrives, causing considerably more damage, in alkaline, black, heavy clay soils that are

poorly aerated (Agrios, 1997). Sclerotia are the primary source of inoculum for initiating infection allowing the fungus to persist in soil for up to 12 years. (Kenerley & Jeger, 1992). Although conidia can sometimes be found on diseased plants, they have not been shown to function as infective propagules (Kenerley & Jeger, 1992). Cook & Riggs (1993) observed increased disease severity and intensity due to infection by *P. omnivorum* as the growing season progressed. Leaves of infected plants initially show yellowing and bronzing, which is followed by wilting and death of the plant (Watkins, 1981; Cook & Riggs, 1993; Agrios, 1997). When the bark of diseased plants is stripped, a reddish lesion is revealed above the crown (Cook & Riggs, 1993).

3.5.2 Control: Continuous cultivation of kenaf on soils infested with *P. omnivorum* causes a significant reduction in plant height and stalk yield with each successive year. Continuous kenaf cultivation should thus be avoided on soils with a high infestation of *P. omnivorum* (Cook *et al.*, 1995). Since grass crops are not susceptible to root rot caused by *P. omnivorum* (Seney, 1984), long rotations with grain crops in infested soils may reduce crop losses due to this pathogen (Seney, 1984; Agrios, 1997). Weed eradication, and frequent ploughing to keep soil well aerated are effective cultural practices in the control of *P. omnivorum* (Agrios, 1997). The application of green manure, using thickly planted maize, sorghum, or legumes, favours the build-up of large populations of microorganisms that are antagonistic to *P. omnivorum* (Agrios, 1997).

3.6 Collar rot.

3.6.1 Etiology: Collar rot caused by *Sclerotium rolfsii* Sacc. is a major disease of kenaf. It usually attacks plants that are 0.5-1.0 m in height, causing the formation of deep-seated lesions on the stem at the ground level (Dempsey, 1975). The mycelium of the pathogen affixes itself to the surface of plant parts in contact with the soil at or near the air-soil

interface when there is sufficient soil moisture (Watkins, 1981). *S. rolfsii* produces a canker or decayed zone that usually girdles the stem or root by liberating considerable amounts of oxalic acid and cell wall degrading enzymes (Watkins, 1981). Plants that are less than 1 m in height wilt quickly while older plants that have developed woody tissue develop lesions high up on the stem and leaves are also attacked (Dempsey, 1975; Agrios, 1997). The lesions have a light brown border and are shrunken to the extent that the bast fibres separate from the wood (Dempsey, 1975). The fungus grows in the cortex and girdles the stem causing the plant to eventually wilt and die (Watkins, 1981; Agrios, 1997).

3.6.2 Control: In general, control of *S. rolfsii* is difficult, but fertilizing the fields with ammonium-type fertilizers, applying calcium compounds, and fungicides such as PCNB during planting and preplanting, can provide partial control (Agrios, 1997). After screening kenaf cultivars, Majaumdar *et al* (1998) identified five kenaf genotypes, namely LC21846, Everglades 71, Everglades 41, C2032 (Type 1056C), and PI326024(A), that were resistant to collar and stem rot caused by *S. rolfsii*. According to Dempsey (1975) the isolation of infected sites, deep ploughing to inactivate the sclerotia, and crop rotation are important cultural practices to control collar rot.

4.0 INTEGRATED DISEASE MANAGEMENT OF COTTON: POSSIBLE APPLICATION FOR KENAF PRODUCTION

Integrated disease management combines all possible control measures and should be economical and environmentally friendly (Chahal *et al.*, 1996). An integrated disease management system should involve the identification and monitoring of diseases, environmental monitoring, deciding on proper intervention, implementing the intervention and post-intervention reassessment (McPartland *et al.*, 2000). Based on the analysis of potential disease problems, preventative measures such as modified cultural practices that

affect the potential pathogen adversely and aid the natural enemy of the pests should be taken (Jacobsen, 1997). Suppressive measures should only be taken when the threshold level at which significant crop damage occurs, due to pathogen attack, is likely to be exceeded (Jacobsen, 1997)

The integration of disease control strategies such as sanitation, crop rotation, cultural practices, sowing date, plant spacing, use of resistant cultivars, disease forecasting, biological control, as well as chemical control is often recommended for cotton (Minton & Garber, 1983) and should therefore be suitable for kenaf. The combined use of PCNB, bezimidazole fungicides, including benomyl, resistant cultivars and crop rotation significantly decreases *V. dahliae* infection of cotton (Verma & Raj, 1992). El-Zik (1985) recommended paying heed to the role crop and pest phenology, rhizoplane interactions, cultural practices and environmental factors play in formulating a disease management program for cotton.

The control of cotton seedling diseases starts with an overall disease management system that takes field preparation, planting dates, cultivar selection, seed treatment, field drainage and the use of in-furrow fungicides at planting into account (Basil *et al.*, 1998). The integration of resistant cotton cultivars, crop rotation, burial and destuction of crop residues, sanitation, proper fertilizer and water mangement can prevent serious yield losses due to disease (Bird, 1986). Allen *et al.* (2000) identified summer flooding of fields, crop rotation, seed treatment and farm hygiene as potential components of an integrated disease management strategy to provide effective control of Fusarium wilt of cotton. Significant progress has been made in improving both stalk fibre yield potential and tolerance to nematode/soil-borne fungi complex by introducing newly developed kenaf cultivars in conjunction with effective cultural practices such as crop rotation (Cook *et al.*, 1996). An integrated disease management program if practiced along with other improved agronomic

practices, would thus substantially reduce disease incidence and severity in kenaf fields and increase production in disease-prone areas.

5.0 CONCLUSIONS

Kenaf and cotton belong to the plant family Malvaceae and cultural practices that favour cotton production will presumably also favour kenaf production. By the same token, disease control tactics that are used for cotton diseases could be applicable for kenaf diseases. Integrated disease control measures have proven particularly effective in controlling many cotton pests and diseases. It is thus important to set an integrated disease management program for kenaf based on all relevant factors involved in disease incidence and severity. Preventative control measures such as the planting of disease resistant cultivars, sanitation, crop rotation and other cultural practices can effectively prevent or reduce crop losses. The use of chemicals, although an important option to manage plant diseases, should only be considered as a last option. Nevertheless, the judicious use of fungicides, including seed treatment and timely application of recommended fungicides in suitable concentrations are important control options. Selected chemicals suitable for the possible control of diseases of kenaf grown under South African condition must be evaluated and registered according to Act 36 of 1947.

Diseases encountered thus far in kenaf plantings in South Africa could become limiting factors if not properly managed. The impact of these diseases on kenaf production in South Africa should therefore be considered seriously. A holistic approach to disease management can help to substantially reduce disease incidence and severity in kenaf.

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CHAPTER 2

FACTORS RELATED TO THE ESTABLISHMENT OF KENAF FROM SEED

ABSTRACT

A total of nine fungal genera were isolated from surface disinfested seeds from ten kenaf cultivars. *Alternaria* spp. were isolated at the highest frequency followed by *Chaetomium* spp. from all cultivars. *Fusarium subglutinans* was pathogenic to kenaf and differences among cultivars in tolerance to infection by *F. subglutinans* were observed in glasshouse experiments. The effect of ComCat®, a commercial biostimulant, and a fungicide (thiram) on germination and early seedling growth of kenaf seeds was determined under laboratory and glasshouse conditions. Significant variation in the germination of kenaf seeds as well as emergence of seedlings was observed between cultivars. Neither ComCat® or thiram treatments, singly or combined, had any influence on the germination of kenaf seeds. Fungicide treatment did however significantly affect the emergence of kenaf seedlings. When seedlings were treated with ComCat® no significant effect on both the fresh and dry mass of roots as well as on the fresh mass of foliage was evident. However, when seeds were treated with ComCat®, the dry weight of the above ground parts of the seedlings was affected significantly.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.) (Malvaceae) is a promising source of high quality cellulose fibers and its cultivation in South Africa is being investigated with a view to producing it as an alternative fibre crop on a commercial scale. Its cultivation depends on seed and the uniform emergence of seedlings plays an important role in competitiveness, yield and harvestability of kenaf (White *et al.*, 1971). It is therefore important to firstly understand the conditions that will allow for optimal seed germination and secondly, any treatment that can stimulate germination.

Many serious plant diseases arise from seed borne pathogens and the establishment of crops can be significantly affected by the death of seedlings (Maude, 1973). The identification of seedborne fungi and evaluation of their pathogenicity to kenaf seedlings is thus crucial to the establishment of this crop in South Africa. Various techniques have been developed for the detection of seedborne inoculum amongst which agar testing gives the best indication of viable inoculum present inside a particular seed (Maude, 1996).

The elimination of seedborne pathogens by means of fungicides is standard practice for certain crops (McGee, 1981; Davis, Nunez & Subbarao, 1997). Treating seeds with fungicides can also protect emerging plants from damping-off caused by soilborne pathogens (Pennypacker & Stevenson, 1982). White *et al.* (1971) observed a significantly greater number of kenaf seedlings established from fungicide treated seeds than non-treated seeds. Batson, Caceres & Carvajal (2000) recommended treating kenaf seeds with a combination of fungicides in order to improve stand establishment.

ComCat® is a natural biostimulant of plant origin that contains a combination of natural biological substances involved in plant growth, the induction of plant defence mechanisms, root development and physiological efficiency (Hüster, 2001). ComCat® can enter the plant system by absorption through the leaves or the roots and, hence, can be

applied as a foliar spray, a soil drench, a soil additive or as a seed treatment. Cotton seeds treated with ComCat® showed a significant improvement in plant emergence and fibre strength (Hüster, 2001).

The objectives of this research were fourfold: (i) to identify potentially important fungal pathogens associated with kenaf seeds; (ii) to determine their pathogenicity to kenaf seedlings; (iii) to test thiram as a possible chemical seed treatment for kenaf and finally, (iv) to investigate the response of kenaf seeds to an industrial biostimulant, ComCat®.

MATERIALS AND METHODS

Isolation of fungi associated with kenaf seeds

Seeds (n = 100) from each of 10 kenaf cultivars namely, Cuba 108, Dowling, El Salvador, Endora, Everglades 41, Everglades 71, Gregg, SF 459, Tainung 2 and Whitten, were surface disinfested for 1 min in 3.5% (m/v) sodium hypochlorite. This was followed by three rinses in sterile distilled water (Baxter & van der Linde, 1999) and then blotting on sterilized filter paper. Seeds were then placed aseptically on potato dextrose agar (PDA) (Difco®) amended with streptomycin sulfate (0.3 ml/l) and incubated at 22 ^{0}C for 7 days. All fungal colonies growing from the seeds were transferred from colony margins to 1.5% water agar (WA) (Oxoid®) and the resulting cultures were identified using light microscopy.

Pathogenicity trial

Inoculum preparation: An isolate of *Fusarium subglutinans* (Wallenweb. & Reinking) Nelson, Toussoun, & Marasas, was selected as the only potential pathogen obtained from kenaf seeds. Inoculum of the pathogen was prepared by soaking wheat and barley grains (ratio 1:1) in 250 ml water in a 500 ml Erlenmeyer flask for 12 hr. Water was decanted,

and the grain mixture was autoclaved twice for 20 min at 121^{0}C. Agar colonized by *F. subglutinans* was homogenized, added to the grain mixture and incubated for 21 days at 25 ^{0}C to allow grain kernels to become colonized. The mixture was then air-dried at room temperature for two days, before being ground in a laboratory mill. The resulting powder was subsequently used for the artificial inoculation of kenaf seedlings.

Artificial inoculation: The reaction of the ten kenaf cultivars mentioned previously to artificial inoculation with *F. subglutinans* was tested in the glasshouse. Seedlings were sown in pots (400 cm^3) containing a 1:1 v/v steam sterilized soil/peatmoss mixture (200 g) after seeds of each kenaf cultivar had been pregerminated on (1.5%; m/v) water agar (WA) to ensure germination and the absence of seedborne *Fusarium* spp. (Van Wyk *et al.*, 1988). Seeds had previously been divided equally into two groups; one half was treated with thiram and the other was surface disinfested in sodium hypochlorite (3.5 %; m/v) for 60 sec, washed three times with sterile distilled water and blotted dry on sterile filter paper. Germinated seeds were placed on the soil surface in each pot and covered with 100 grams of the same soil. Inoculum powder of *F. subglutinans* (0.28 g) was sprinkled on the surface of the soil and covered by an additional 100 g soil. Twenty seeds from each cultivar with four replicates (5 seeds/pot) were used. For each main treatment, "fungicide treated" and "non-treated", a sterile wheat:barley powder served as the control treatment. The pots were watered as regularly as required. Plants were observed periodically for symptom development and the percentage mortality for each treatment was recorded after 3 weeks and the presence of the pathogen was confirmed.

The effect of ComCat® and fungicide

The germination and seedling emergence of five kenaf cultivars, namely Cuba 108, El Salvador, Everglades 41, SF 459 and Tainung 2 were examined in response to ComCat® and the fungicide thiram.

Seed germination: Germination trials were conducted in the laboratory according to the method of Curtis and Läuchli (1985). The treatments were seeds treated with ComCat®, a combination of ComCat® and thiram, thiram alone and non-treated control seeds. Each treatment was replicated three times for each cultivar. Twenty seeds per replicate were placed between two saturated germination papers. In the case where seeds were treated with ComCat®, the germination paper was saturated with the ComCat® solution at a concentration of 0.5 mg/l. Seeds that were not treated with ComCat® were saturated with distilled water.

The double layer germination paper was rolled up longitudinally and placed in Erlenmeyer flasks containing the same relevant test solution. Experiments were conducted in an incubator at 22°C. Germination percentage was recorded every 24 hrs and the final germination percentage was calculated after 96 hrs of incubation. Germination was scored as being positive when radicles had emerged.

Vegetative growth: Seedling emergence for each treatment was tested by planting 30 seeds per cultivar in three pots (10/pot) containing sandy loam soil. The pots were arranged in a complete randomised design in a glasshouse at 22°C and watered regularly. The treatments were seeds treated with ComCat®, a combination of ComCat® and thiram, thiram alone and non-treated control seeds. Each treatment was replicated three times for each cultivar. ComCat® was applied by dressing the seeds at a concentration of 0.5 mg/l before planting. Seedling emergence was monitored regularly and the final percentage was calculated 10 days after the first seedling had emerged. Seedlings were subsequently

removed from the pots and the soil removed from the root system with running water. The fresh mass of roots and the above ground parts (stems + leaves) were recorded separately while the dry mass was determined after drying for three days at 70 ^{0}C in a drying oven.

Data analyses

All statistical analyses were performed using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA) and Duncan's multiple range test was used to compare treatment means.

RESULTS

Isolation of fungi associated with kenaf seeds

In this study, a total of nine genera of fungi were identified from surface-disinfested seeds of the ten kenaf cultivars (Table 2.1). In all cases *Alternaria* spp was most common followed by *Chaetomium* spp. Whitten had the highest incidence with 60% of seeds contaminated by fungi. Of the fungal species in the nine genera, which were isolated from kenf seeds, *F. subglutinans* was considered to be the most potentially pathogenic to kenaf and pathogenicity studies on kenaf seedlings were subsequently conducted with an isolate of this fungus.

Pathogenicity trial

F. subglutinans produced disease symptoms on seedlings of all kenaf cultivars. Symptoms started to develop soon after seedlings started to emerge. Dark brown lesions that developed on the stem just above ground level girdled the stem resulting in wilting. When the stem was cut lengthwise, browning was clearly visisble in vascular bundles. The pathogen also caused necrosis and decay of the taproot. *F. subglutinans* was isolated from

symptomatic kenaf seedlings. No damping-off was evident in control treatments for any of the cultivars tested.

Cultivars varied significantly ($P \leq 0.05$) in their reaction to *F. subglutinans* in glasshouse trial (Fig. 2.1). Although damping-off occurred in all cultivars, El Salvador had the lowest degree of damping-off (5%) followed by Tainung 2 (15%), Cuba 108 (20%) and Endora (25%) on seeds that had been treated with thiram. Cultivars SF 459 and Whitten displayed 50% damping-off while 40% damping-off was observed for Dowling.

The effect of ComCat® and fungicide

Seed germination: Significant ($P \leq 0.05$) variation in % seed germination was observed among kenaf cultivars (Fig. 2.2). The mean germination percentage of Tainung 2 (98.33%), Everglades 41 (98.33%) and El Salvador (94.17%) was significantly ($P \leq 0.05$) higher than that of Cuba 108 (77.92%) and SF 459 (74.58%). Neither ComCat® nor fungicide treatment, alone or in combination, influenced the germination of the five kenaf cultivars.

Vegetative growth: In most cases seedling emergence from seeds treated with either the fungicide alone or combined with ComCat® was superior to both the untreated control and ComCat® treatment alone (Fig. 2.3). Variation between cultivars was significant ($P \leq 0.05$). El Salvador showed the highest emergence for all treatments with a mean percentage of 73.33%. El Salvador had the highest % seedling emergence for control treatments. El Salvador also had the highest significant seedling emergence followed by Everglades 41 and Tainung 2 when seeds were treated with ComCat®. Results confirmed the beneficial effect of treating kenaf seed with fungicide.

There was a significant difference ($P \leq 0.05$) between cultivars in terms of fresh weight of above ground parts. El Salvador (0.5908 g), SF 459 (0.5713 g) and Cuba 108 (0.5229 g) had a significantly greater fresh weight than Tainung 2 (0.4892 g) and Everglades 41 (0.4816 g) (Fig. 2.4). There was however no significant ($P \leq 0.05$) effect of seed treatment on fresh weight of above ground parts.

Cultivars did not differ significantly ($P \leq 0.05$) in their above ground dry mass. The treatments however differ significantly ($P \leq 0.05$) in their effect on the mean above ground dry weight of seedlings (Fig. 2.5). Seedlings treated with ComCat® had a significantly higher above ground dry weight (0.0638 g) than seedlings treated with both ComCat® and fungicide (0.6028 g). Fungicide treated and untreated seeds had a dry mass of 0.0535 g and 0.0543 g respectively.

There was no significant difference between cultivars or between fresh and dry root mass. There was however a significant ($P \leq 0.05$) interaction between treatments and cultivars in the mean fresh and dry root mass (Fig. 2.6). For example, the mean fresh weight of kenaf cultivar SF 459 of the control treatment was higher than the rest, but was among the lowest on seeds treated with ComCat®.

DISCUSSION

In the present study, *F. subglutinans* was shown to be pathogenic to certain kenaf cultivars and can thus be expected to influence its establishment in South Africa. Dempsey (1964) as cited by Dempsey (1975) reported that Everglades 71 and Cuba 108 were only slightly attacked by *Fusarium* sp. when planted on *Fusarium*-infested soil in Iran while the susceptible local kenaf variety was wiped out by the disease. Our results are consistent with this report in that Everglades 71 had the highest survival rate when grown from untreated seed. Although 50% of Cuba 108 grown from untreated seeds was attacked by

F. subglutinans, this cultivar was among the most resistant when established from fungicide treated seeds. It is important to consider the variation between kenaf cultivars in their resistance to *F. subglutinans* alongside other differences in terms of plant height, percent core material and bark production.

Cook & Mullin (1994) reported an association between kenaf root nematodes and soilborne fungi whereby wounds created by nematodes provide portals of infection for fungal pathogens. The presence of nematodes in soil also increases Fusarium wilt of cotton by providing entry for the fungus (Verma & Raj, 1992). Root damage caused by nematodes has been suggested to be the main factor in nematode-fungi complex in cotton seedling diseases (Bridge, 1992). The effects of nematodes on Fusarium wilt of cotton is more pronounced when infection of the roots by the nematodes precedes infection by *Fusarium* by 3-4 weeks (Starr, *et al.*, 1989). Effective nematode control would thus be important to successful disease management of kenaf.

The treatment of kenaf seeds with ComCat® and fungicide singly or in a combination did not improve seed germination. White *et al.* (1971) and Cook *et al.* (1992) noted that there was no effect of a fungicide seed treatment on the germination of kenaf seeds. Fungicide however improved seedling emergence and all five kenaf cultivars tested showed similar responses in seedling emergence following treatment. Fungicide treatment can result in better seedling emergence and stand establishment of kenaf (White *et al.*, 1971; Cook *et al.*, 1992). Although disease control should not be totally dependent on chemical treatment, it would be advisable to integrate the use of fungicides with other cultural practices such as crop rotation and proper crop management as well as avoiding predisposing conditions that enhance infection in order to prevent stress to newly established plants.

Our results indicated that thiram on its own did not control damping-off. Thiram was registered in South Africa for cottonseed treatment to be applied in a mixture with carboxin to control seedling diseases caused by *Fusarium* spp. (Nel *et al.*, 1999). Watkins (1981) also recommended dressing of cottonseeds with two or more fungicides to improve their impact. Seed treatment with a combination of metalaxyl, carboxin and captan can result in improved establishment of kenaf (Cook *et al.*, 1992). It would thus be advisable to treat kenaf seeds with a combination of fungicides.

ComCat® is environmentally friendly product and no impact has been reported on non-target organisms (Hüster, 2001). Hüster (2001) reported that cottonseeds treated with ComCat® improved seedling emergence. In our experiment, however, ComCat® did not improve seedling emergence. Although ComCat® had no effect on seed germination, seedling emergence, fresh and dry root weights as well as above ground fresh weight, our results suggest that ComCat® was involved in an increase of dry mass of the above ground parts of seedlings. ComCat® could thus help to improve dry matter production and fibre yield of kenaf and further studies on the use of ComCat® in kenaf production should be conducted.

In the present study, variation between cultivars in terms of the frequency of fungi isolated from seeds, reaction of seedlings towards infection by *F. subglutinans*, germination, seedling emergence and fresh above ground weight of seedlings was observed. Cook & Smart (1994) also noted that kenaf cultivars can differ in seed quality, stand establishment and susceptibility towards seedling disease. The use of good quality seed combined with fungicide and ComCat® could therefore result in more uniform stand establishment, seedling vigour and resistance of kenaf to soilborne pathogens such as *F. subglutinans*.

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Table 2.1 Composition (percentage) of fungi in surface- disinfested kenaf seed.

	Fungal genus									
Cultivar	*Alternaria* spp.	*Aspergillus* spp.	*Bipolaris* spp.	*Botrytis spp.*	*Chaetomium* spp.	*Curvularia* spp.	*Fusarium* spp.	*Penicillium* spp.	*Phoma* spp.	Total %
Cuba 108	13				12	3	3			31
Dowling	9	2					4	2		17
El Salvador	10				14			3	2	29
Endora	5				3		5	2		15
Everglades 41	23		4		3					30
Everglades 71	6			4	2					12
Gregg	8				4		3			15
SF 459	15								4	19
Tainung 2	18				5		2	4		29
Whitten	13	31						16		60

Figure 2.1 The occurrence of damping-off in kenaf seedlings grown from fungicide treated and non-treated seeds inoculated with *F. subglutinans* previously isolated from kenaf seeds. Bars with the same letter are not significantly different at the P=0.05 level according to Duncan's multiple range test.

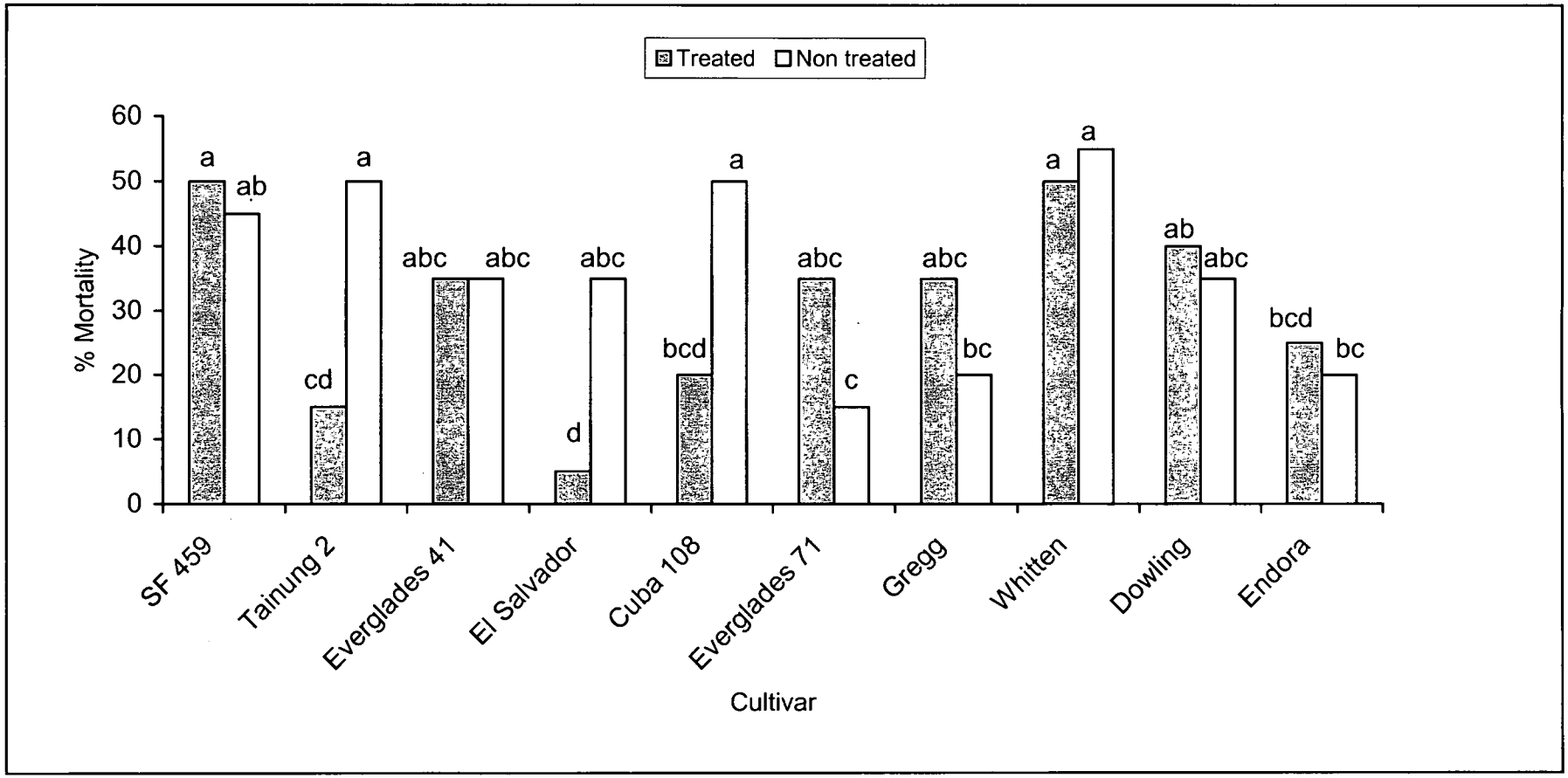

Treated
Non treated
% Mortality
60
50
40
30
20
10
0
a
ab
cd
a
abc
abc
d
abc
bcd
a
abc
c
abc
bc
a
a
ab
abc
bcd
bc
SF 459
Tainung 2
Everglades 41
El Salvador
Cuba 108
Everglades 71
Gregg
Whitten
Dowling
Endora
Cultivar

Figure 2.2 Percent germination of kenaf seeds. Values are the means of combined treatments (ComCat® treatment; fungicide and ComCat® combination treatment, fungicide treatment and control). Bars denoted by the same letter are not significantly different at the P = 0.05 level according to Duncan's multiple range test.

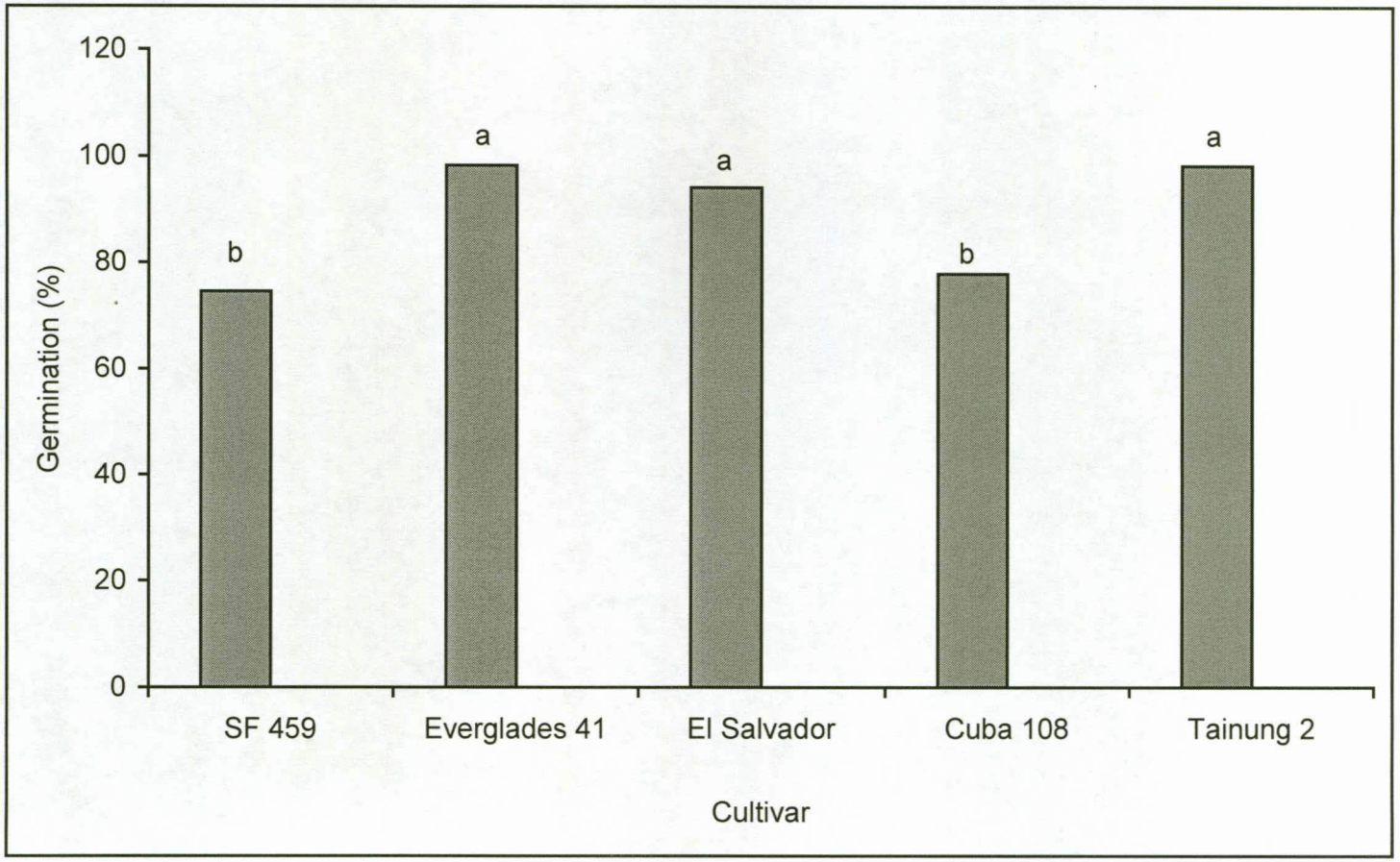

120
100
80
60
40
20
0
Germination (%)
b
a
a
b
a
SF 459
Everglades 41
El Salvador
Cuba 108
Tainung 2
Cultivar

Figure 2.3 Percentage seedling emergence of five kenaf cultivars from seeds treated with Com Cat® and fungicide (thiram) either separately or in combination. Bars with the same letter with in the same cultivar are not significantly different at the P = 0.05 level according to Duncan's multiple range test.

CC = ComCat® treatment; F + CC = Fungicide and ComCat® combination treatment; F= Fungicide treatment.

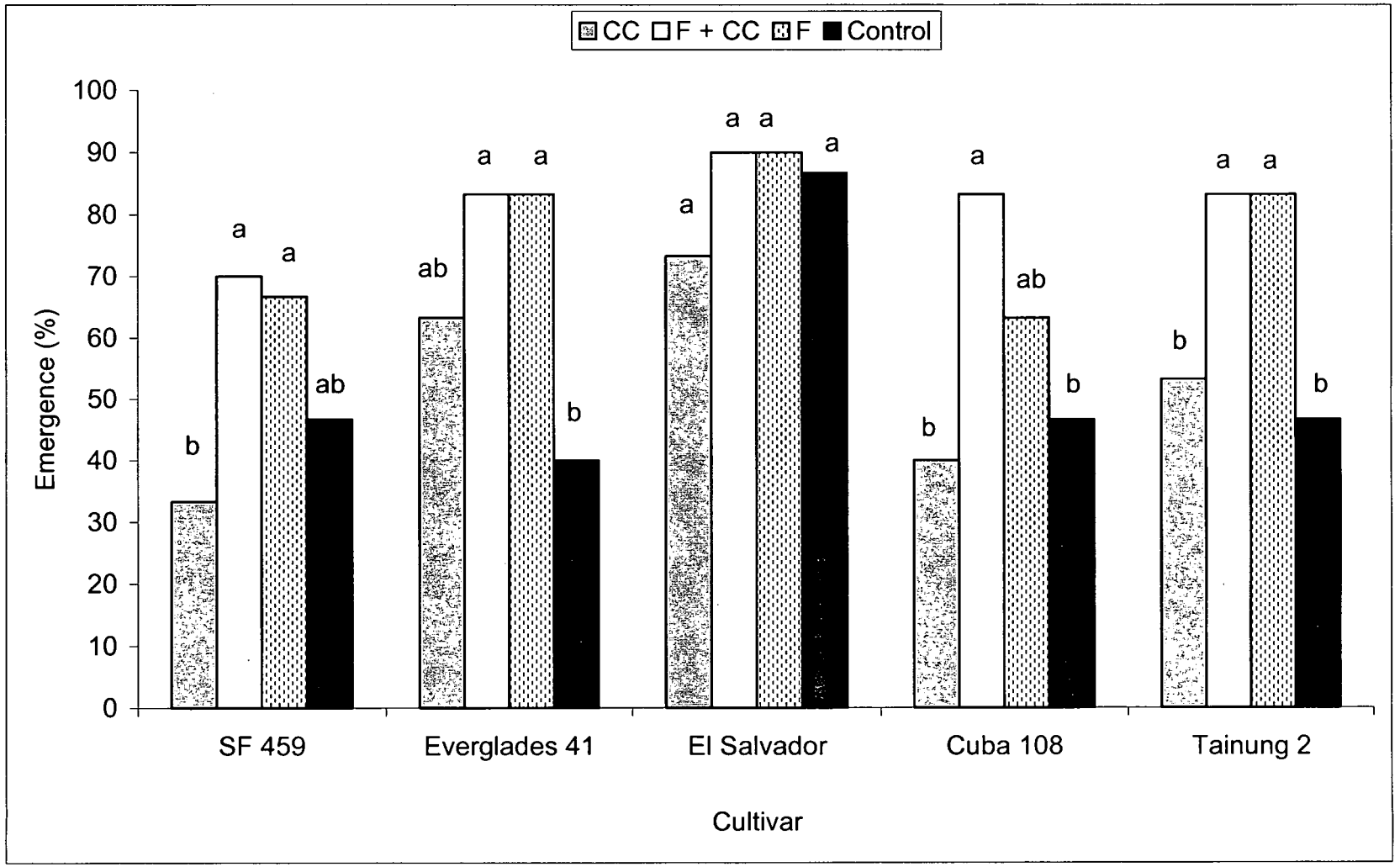

CC
F + CC
F
Control
Emergence (%)
100
90
80
70
60
50
40
30
20
10
0
b
a
a
ab
ab
a
a
b
a
a
a
b
a
ab
b
b
a
a
b
SF 459
Everglades 41
El Salvador
Cuba 108
Tainung 2
Cultivar

Figure 2.4 Differences between cultivars in terms of mean fresh weight of above ground parts of kenaf seedlings ten days post emergence. Values are the means of all treatments (ComCat® treatment; fungicide and ComCat® combination treatment. fungicide treatment and control). Bars denoted by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test.

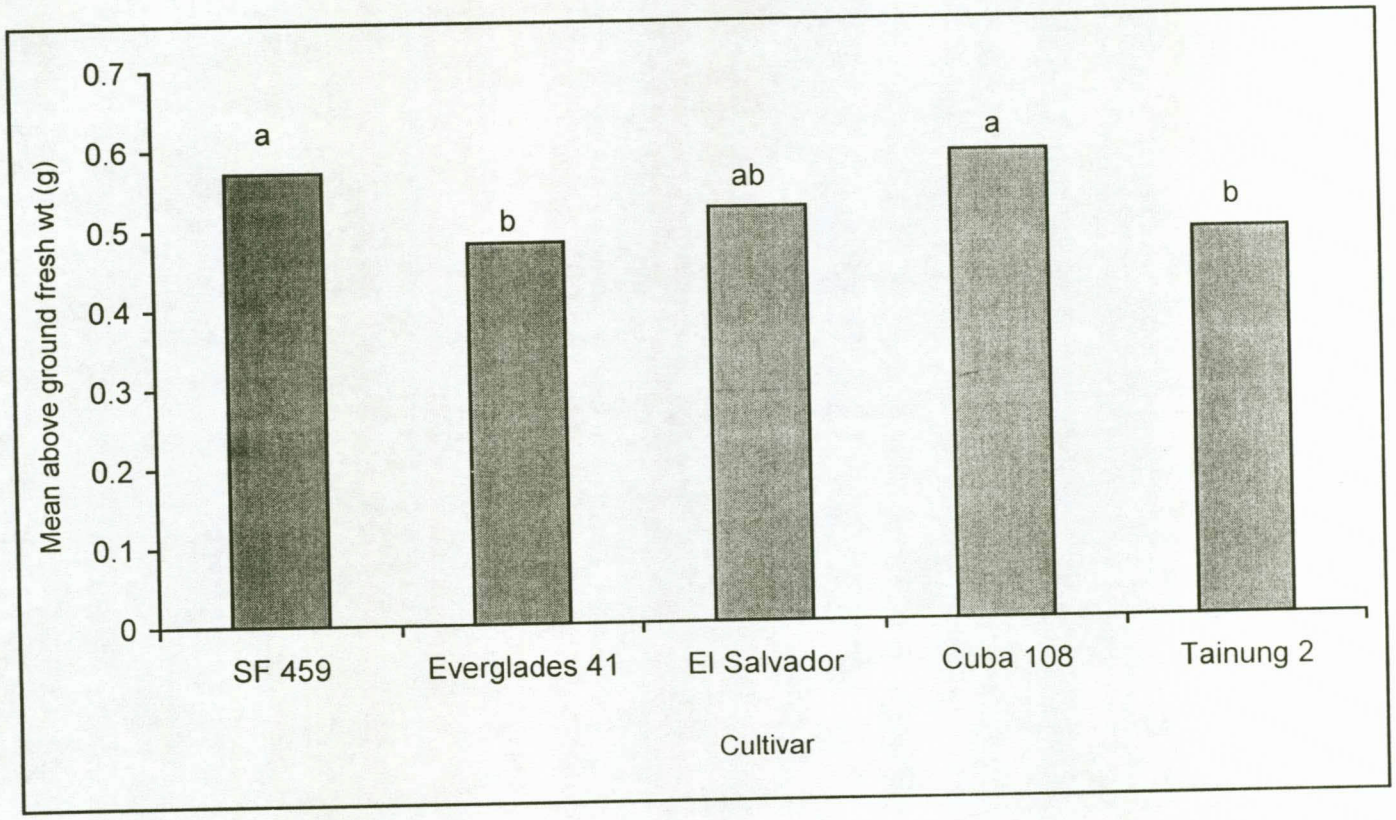

Mean above ground fresh wt (g)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
b
ab
a
b
SF 459
Everglades 41
El Salvador
Cuba 108
Tainung 2
Cultivar

Figure 2.5 Effect of ComCat® and fungicide (thiram) on the mean dry weight of above ground parts of kenaf seedlings. Values are the means of five kenaf cultivars. Bars denoted by the same letter are not significantly different at P = 0.05 according to Duncan's multiple range test.

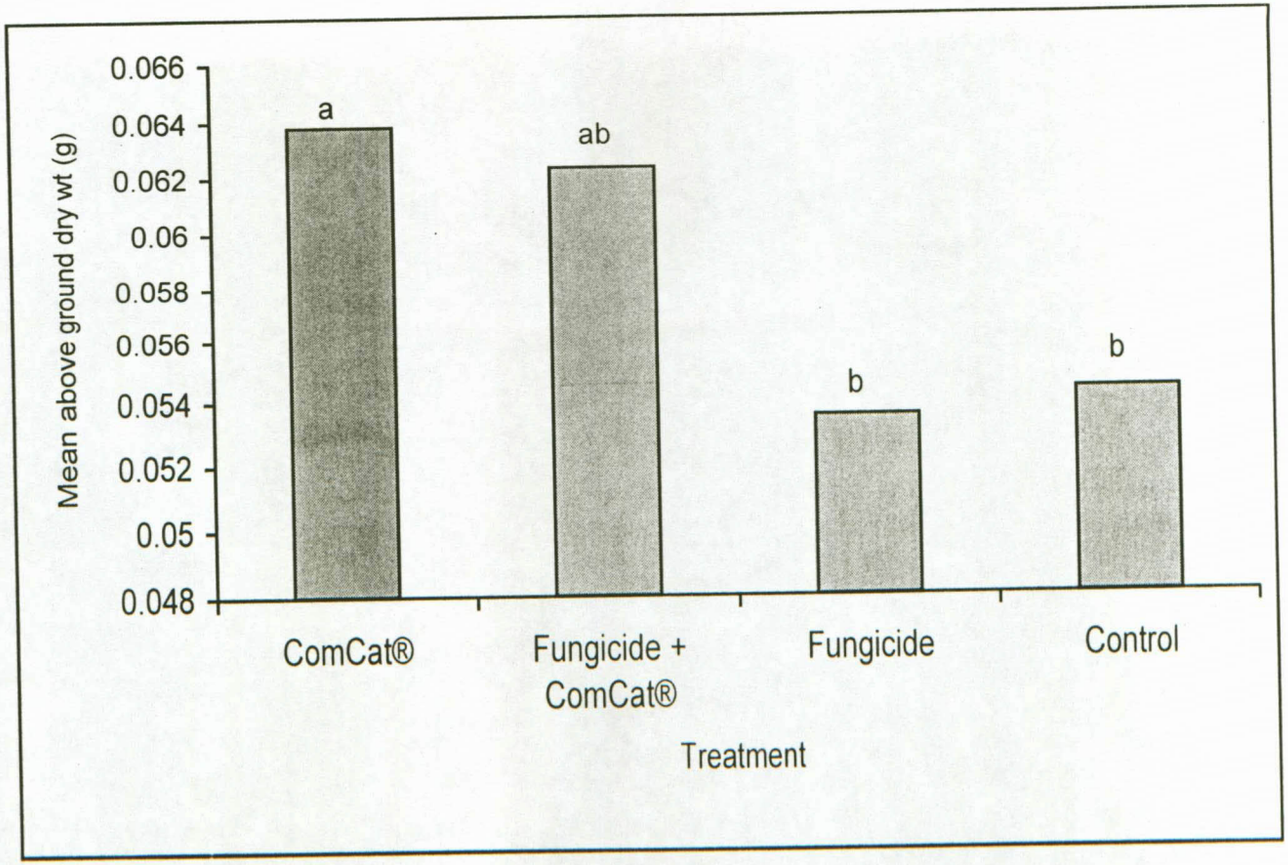
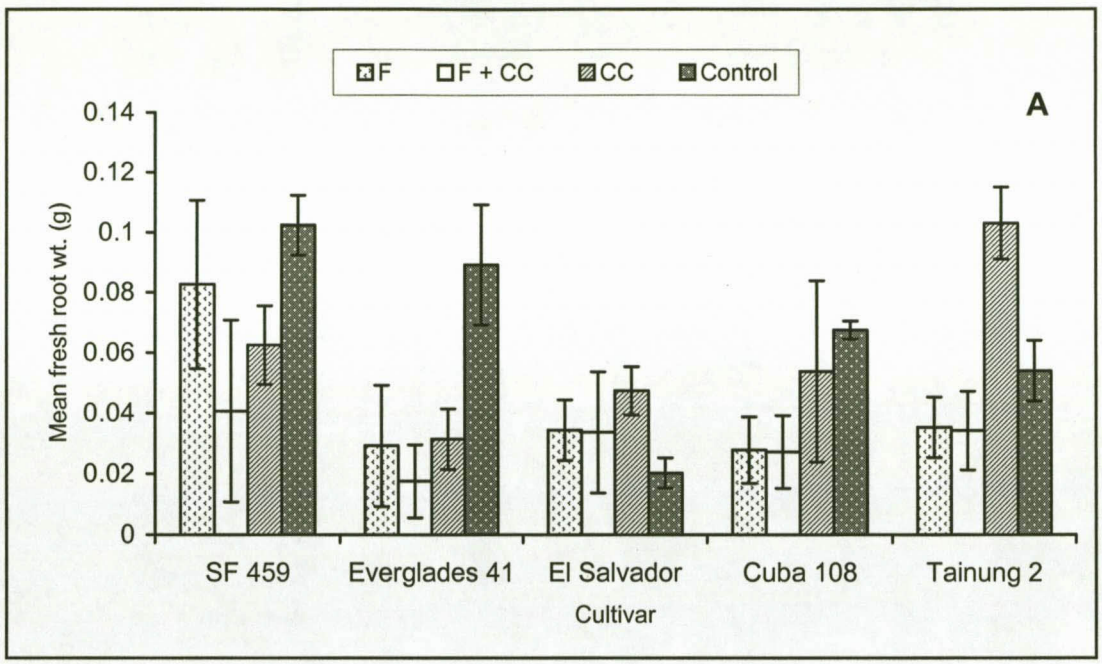
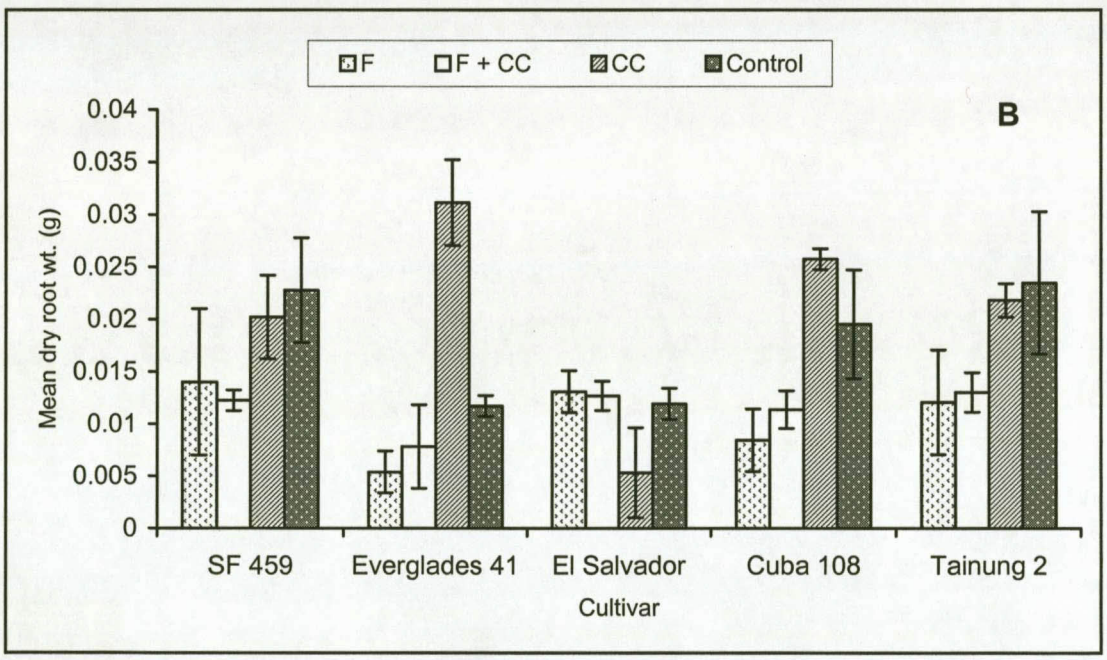

Mean above ground dry wt (g)
0.066
0.064
0.062
0.06
0.058
0.056
0.054
0.052
0.05
0.048
a
ab
b
b
ComCat®
Fungicide + ComCat®
Fungicide
Control
Treatment

Figure 2.6 The effect of treatments on the mean fresh (A) and dry (B) root weights of kenaf seedlings ten days after emergence. Bars represent standard deviation.

CC = ComCat® treatment; F + CC = Fungicide and ComCat® combination treatment; F= Fungicide treatment.


F
F + CC
CC
Control
A
Mean fresh root wt. (g)
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
SF 459
Everglades 41
El Salvador
Cuba 108
Tainung 2
Cultivar


F
F + CC
CC
Control
B
Mean dry root wt. (g)
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
SF 459
Everglades 41
El Salvador
Cuba 108
Tainung 2
Cultivar

CHAPTER 3

CHARACTERIZATION OF *BOTRYTIS CINEREA* ON KENAF IN SOUTH AFRICA

ABSTRACT

Diseased samples were obtained from 5-month-old kenaf plants from experimental plots near Rustenburg, Northwest Province, South Africa. The disease was subsequently identified as grey mould caused by *Botrytis cinerea*. Field trials were conducted by artificially inoculating the stems of 120-day-old plants from each of ten kenaf cultivars. Inoculation was performed by applying toothpicks that had previously been colonized by *B. cinerea*. The development of lesions on inoculated stems and the isolation of *B. cinerea* from cambial lesions confirmed that *B. cinerea* was the causal pathogen. Variation in susceptibility between cultivars was observed with Everglades 41 and SF 459 having the largest and smallest lesion lengths, respectively. Optimum temperature for mycelial growth of *B. cinerea* was determined by incubating the fungus at a range of temperatures from 5 to 30 °C. Maximum radial growth was recorded at 20°C and optimum temperature for its growth was between 15 and 20 °C. The effect of irrigation on the incidence of grey mould was determined by planting kenaf under three moisture regimes: dry land (rain-fed), medium and high moisture. There were no significant differences in grey mould incidence between the moisture regimes although cultivars varied significantly in susceptibility to *B. cinerea*. There was a significant ($P \leq 0.05$) interaction between moisture regimes and cultivars. The screening of seven fungicides was conducted on one isolate of *B. cinerea in vitro*. Benomyl was shown to be the most effective fungicide and variation between nine single conidial isolates of *B. cinerea* was also observed.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.), is an annual, herbaceous plant belonging to the family Malvaceae, grows in a wide range of climatic conditions. It is a source of good quality bast fibre (Dempsey, 1975; Lawrence & McLean, 1991), its fibers being used to produce packing material, oil absorbent, animal bedding and pulp for paper (Colyer, Vernon & Caldwell, 1992; Bagby, Princen & Rossi, 1996; Baldwin, *et al.*, 1996). An increased demand for kenaf is related to the gradual diminishing supply of wood in the world (Francois, Donovan & Mass, 1992).

During the evaluation of kenaf as a potential fibre crop for South Africa, grey mould caused by *Botrytis cinerea* Pers.:Fr. was found in experimental plots near Rustenburg, South Africa. Approximately 20 to 30% of 5-month-old kenaf plants displayed brown necrotic lesions which girdled the stem, resulting in wilting and lodging of the plants (Swart, Tesfaendrias & Terblanche, 2001). This was the first observation of *B. cinerea* on kenaf in South Africa. Grey mould caused by *B. cinerea* had previously been reported on roselle (*Hibiscus sabdariffa* L.) in South Africa (Swart & Langenhoven, 2000) and *Botrytis* spp. have also been known to contaminate cotton lint after natural dehiscence leading to lint discolouration (Hillocks, 1992).

The mean relative humidity of major kenaf growing areas ranges from 68 to 82% and a mean temperature range of 22.6 to 30.3°C during the growing season (Dempsey, 1975). Kenaf requires an average of 100 to 125 mm of rain (Dempsey, 1975) and in regions of low rainfall, supplementary irrigation is necessary for optimal kenaf cultivation (Rego, 1998). *B. cinerea* generally requires temperatures of 18 to 23°C for growth, sporulation, germination and the establishment of infection (Agrios, 1997). Grey mould caused by *B. cinerea* is favoured by low temperatures and high humidity, and disease incidence is high in irrigated crops and dense stands (Polverari, Tosi & Benincasa, 1994).

Swart & Langenhoven (2000) also reported that cool and humid conditions initiated rapid expansion of lesions on roselle stems infected with *B. cinerea*. The similar environmental requirements of kenaf and *B. cinerea* can thus be expected to play a role in the incidence of the disease.

Since kenaf is being investigated as a new crop, no fungicides are currently registered for the control of its diseases in South Africa. Various fungicides effective against *B. cinerea* have been shown to be successful on a variety of crops. Maude (1980) generally recommends the use of systemic fungicides such as benomyl and carbendazim to control *B. cinerea*. Disinfecting kenaf seeds with captan has been shown to minimize grey mould (Dempsey, 1975).

The objective of the current investigation was to characterize *B. cinerea* on kenaf in South Africa in terms of (i) it's relative pathogenicity to kenaf cultivars, (ii) optimum temperature requirements, (iii) optimum irrigation requirements and (iv) sensitivity, *in vitro*, to selected fungicides.

MATERIALS AND METHODS

Artificial inoculation

Isolation: The isolate used in this study was retrieved in April 2001 from a 5-month-old infected kenaf plant growing in experimental plots near Rustenburg, Northwest Province, South Africa. Sections of plant stem tissue with visible lesions were surface disinfested using a series of 1minute in 96% ethanol, 2 minutes in a 1.75% NaOCl solution and then rinsed three times in sterile distilled water. Tissue pieces (5 mm x 5 mm) were placed on corn meal agar (CMA, Hampshire, Basingstoke, England) containing streptomycin sulfate (0.3 ml Novo-strep/l; at 1 g per 3 ml a.i.). After 7 days, mycelium growing from the tissue was transferred to potato dextrose agar (Difco®) amended with streptomycin (PDAS).

Inoculum preparation: The pathogen was identified based on colony morphology and sporangial characteristics using light microscopy. Inoculum was prepared by culturing a single spore isolate of *B. cinerea* on PDAS. Toothpick tips (30 mm long), which had been autoclaved 3 times for 20 min at 121° C, were placed on the agar in petri plates and the medium was incubated at 25 ^{0}C for 7 days.

Field inoculation: Sterilized toothpick tips that had been colonized by *B. cinerea* on PDAS were applied to the stems of 120-day-old kenaf plants at approximately 25 cm above soil level. Ten plants of each of ten cultivars, Cuba 108, Tainung 2, Everglades 41, El Salvador, SF 459, Gregg, Dowling, Endora, Whitten and Everglades 71 were artificially inoculated. Stems were wounded using a sharp sterile dissecting needle at the point of inoculation (Sharabani *et al.*, 1999) before the toothpick was inserted to a depth not exceeding 10 mm. Control treatments comprised sterile toothpick tips applied to each of ten plants per cultivar. Inoculation points were wrapped in Parafilm®. Developing lesions were measured 7 days after inoculation. The fungus was reisolated on PDAS from all artificially inoculated plants.

The effect of temperature on mycelial growth of *B. cinerea*

A 5-mm-diameter mycelial plug, obtained from a stock culture of *B. cinerea* on PDA, was placed aseptically in an inverted position at the centre of Petri plates (90 mm) containing PDA. The plates were incubated at temperature ranges of 5, 10, 15, 20, 25 and 30 °C. Each treatment was replicated three times. Radial colony growth was determined by calculating the mean of two perpendicular measurements of colony diameter. Growth was recorded for 7 days at an interval of 24 hrs. starting at 24 hours. The optimum temperature for mycelial growth was determined seven days after seeding plates. The

experiment was conducted twice and means were pooled after conducting Bartlett's test for homogeneity of variances.

Effect of irrigation on incidence of grey mould

Natural infection of kenaf plants by *B. cinerea* during the 2000/2001 growing season was determined by monitoring the incidence grey mould under different moisture regimes in field trials at Rustenburg. Five kenaf cultivars namely, Cuba 108, Everglades 41, El Salvador, SF 459 and Tainung 2 were evaluated. A complete randomised block design with four replications per treatment was used. A between-row spacing of 35 cm and within-row spacing of 4.08 cm was used in 10 x 2.1 m plots. The soil was a vertisol (Arcadia form) with more than 30% clay content. The three treatments were (i) dry land or rain-fed regime, in which plants received water only via rainfall; (ii) medium soil moisture regime where plots were irrigated weekly to field water capacity (FWC); and (iii) high soil moisture regime, in which plots were irrigated every 2nd or 3rd day to FWC during the growing season.

In vitro* inhibition of fungicides on isolates of *B. cinerea

An isolate of *B. cinerea* isolated from symptomatic kenaf plants and previously determined to be highly virulent to kenaf was used in a trial to screen selected fungicides For the control of the pathogen. Seven fungicides, namely carbendazim/tebuconazole (Libero®-133/167 g/l SC), benomyl (Benomyl®-500 g/kg WP), vinclosolin (Ronilan®-500g/l SC), chlorothalonil (Bravo®-500 g/l SC), captan (Kaptan®-500 g/kg WP), thiram (Thiram®-750 g/kg WP) and iprodione (Rovral Flo®-255 g/l SC) were assayed for their effect on the radial growth of *B. cinerea*. Autoclaved potato dextrose agar (PDA) (Difco®) cooled to 45 ^{0}C was used as a basal medium. The medium was amended with an aqueous

stock solution of each fungicide at concentrations of 0.5, 1. 5, 10. 25 and 50 µg a. i. /ml. In a separate experiment, benomyl, libero and ronilan were also tested at lower concentrations of 0.01, 0.05, 0.1, 0.5, 1 and 5 µg a. i. /ml. Control treatments consisted of the unamended basal medium. Three replications per concentration were used and the experiment was conducted twice.

After evaluating the effect of all seven fungicides on the chosen isolate, benomyl was tested separately on nine monoconidial isolates of *B. cinerea*. Fungal isolates were grown for a week at 22 ^{0}C before being transferred to the amended basal medium. Agar discs (5 mm diameter) were removed from the margins of actively growing colonies and transferred to the middle of 90 mm Petri plates containing 25 ml fungicide amended PDA. Plates were incubated at 22 °C and radial colony growth was determined by calculating the mean of two perpendicular measurements of colony diameter on each plate 72 hours after incubation. The inhibition of colony diameter compared to the unamended control treatment was determined. The effective concentration (EC_{50}), the point on the regression line at which 50% of the isolate is inhibited, was determined by plotting the radial growth inhibition values of the isolate transformed to probits against the $\log_{10}$ concentration of fungicides (Fisher & Yates, 1963).

Data from all trials were subjected to an analysis of variance (ANOVA), and Duncan's multiple range test was used to separate treatment means. All data analyses were performed using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA).

RESULTS AND DISCUSSION

Artificial inoculation

Field inoculation trial: Inoculated plants displayed brown necrotic lesions, which girdled the stem after 7 days resulting in lodging of stems and wilting of foliage above the lesion. Artificially inoculated plants produced symptoms identical to those observed in the field (Fig. 3.1) (Swart, *et al.*, 2001). All diseased tissue from inoculated plants produced cultures of *B. cinerea* upon reisolation. Microscopic examination revealed hyaline, one-celled conidia and conidiophores conforming to the description of *B. cinerea* (Ellis & Ellis, 1997). No lesions developed on the control treatments and no *B. cinerea* was isolated from control treatments. Re-isolation from lesions of inoculated stems confirmed that *B. cinerea* was the responsible pathogen.

There were significant differences ($P \leq 0.05$) in susceptibility of kenaf cultivars to *B. cinerea* (Fig. 3.2). The most resistant cultivar was SF 459 followed by Gregg, Cuba 108, El Salvador and Whitten with mean lesion lengths of 24.62, 51.23, 52.55, 54.72 and 55.78 mm respectively. Of the remaining cultivars, Everglades 41 had the longest (79.45 mm) mean lesion length followed by Endora and Dowling with lesions of 67.1 and 66.01 mm respectively. Our results are consistent with those of Polverari *et al.* (1994), who reported Everglades 41 as being one of the most susceptible kenaf cultivars to *B. cinerea*. Grey mould results in the stem of infected plants breaking and foliage above the lesion wilting (De Cal & Melgarejo 1991; Polverari, *et al.*, 1994). The infection of cortical tissue results in yield losses and a decrease in fibre quality (Polverari, *et al.*, 1994). *B. cinerea*, therefore, has the potential to become one of the most serious pathogens of kenaf in South Africa.

B. cinerea can survive as a saprophyte on dead plant tissue (Maude, 1980; Trench, Wilkinson & Esterhuysen, 1992) and conidia that are formed on dying or necrotic tissue is

the source of primary inoculum (Maude, 1980). It is therefore advisable to destroy diseased plant material. Care should also be taken during the retting process to avoid possible sources of inoculum that may result in grey mould.

The effect of temperature on mycelial growth of *B. cinerea*

Mycelial growth started 24 hours after incubation at 15-30°C but at 10 and 5°C, mycelial growth only became visible 24 – 48 hours after incubation. Radial colony growth was significantly ($P < 0.05$) greater at 20°C (Fig 3.3).

The present study showed that optimum growth of *B. cinerea in vitro* occurred between 15 and 20°C. *B. cinerea* requires temperatures of 18-23°C for optimal growth, sporulation, spore release and germination, as well as establishment of infection (Agrios, 1997). Maximum condial germination of *B. cinerea* takes place when it is incubated between 18 and 25°C (Salinas, *et al.*, 1989). Conidia start to germinate two days after incubation at 4°C, while at room temperature one day is sufficient for the phenomena (Salinas, *et al.*, 1989). According to Sosa-Alvarez. Madden & Ellis (1995) optimum temperature for conidial production ranges between 15 to 22°C but decreases with an increase in temperature. Results of this study are therefore consistent with the findings of the above authors.

Cool and humid conditions initiate rapid expansion of lesions on roselle stems infected with *B. cinerea* (Swart & Langenhoven, 2000). The mean temperature range during April 2001 recorded at Rustenburg weather station, near kenaf plots, was between 12.3 and 25.1°C. It was during the same season that Swart *et al.* (2001) reported the incidence of grey mould on 5-month-old kenaf plants at Rustenburg. Kenaf requires a mean temperature ranges from 22.6 to 30.3°C during the growing season (Dempsey, 1975), which coincides with the optimum temperature requirement for *B. cinerea*. A sound

knowledge of the environmental conditions required for the development of primary inoculum of *B. cinerea* is therefore essential in identifying periods when the risk of infection is highest (Sosa-Alvarez, *et al.*, 1995). Knowledge of climatic conditions during which *Botrytis* infection occurs is also essential for the proper timing of fungicide applications (Coley-Smith, 1980).

Effect of irrigation on incidence of grey mould

Grey mould which developed in experimental plots in Rustenburg, during 2000/2001 growing season showed significant ($P \leq 0.05$) variation in severity among cultivars (Fig. 3.4). Although there was no significant ($P \leq 0.05$) variation between treatments, variation in disease incidence was affected by treatment as indicated by the significant ($P \leq 0.05$) interaction between cultivars and treatments. For example, grey mould incidence in SF 459 was significantly lower under dry land conditions but was higher under medium and high moisture regimes. Across all treatments, the mean grey mould incidence ranged from 2.5% to 35.5%. Under dry conditions, disease incidence was significantly higher on El Salvador (34%) compared to other cultivars in the same moisture regime. Disease incidence on SF 459 (25.25%) and El Salvador (17.5%) was significantly higher than the remaining cultivars under the high soil moisture regime. SF 459 (35.5%) and El Salvador (29.5%) followed by Cuba 108 (17.5%) had a significantly higher disease incidence than Tainung 2 and Everglades 41 under the medium soil moisture regime.

Results of the artificial inoculation trial and those results obtained from the effect of irrigation on natural infection were contradictory. In inoculation trials Everglades 41 was the most susceptible and SF 459 the most resistant cultivar. Results from natural infection (irrigation trial) were reversed. This phenomenon could be due to genetic variation of *B. cinerea* isolates that caused natural infection compared to infection

following artificial inoculation. This may also suggest that isolates of *B. cinerea* vary in their ability to infect different kenaf cultivars.

Surface wetness and temperature are important environmental factors in epidemics caused by *B. cinerea* (Jarvis, 1980) which is known to attack during periods of high humidity, and causes partial or total defoliation of kenaf plants (Dempsey, 1975). Grey mould is favoured by low temperatures and high humidity, and its incidence is usually more pronounced in irrigated crops (Polverari, *et al.*, 1994). Data from the 2000/01 season however, did not show a significant increase in disease incidence under medium and high moisture regimes. A relative humidity of 80% or more is very conducive to *Botrytis* infection (Nair & Hill, 1992). The weather data recorded at Rustenburg reflected a mean relative humidity of 92.5% during the growing season which is more than sufficient for infection to occur even under dry land conditions. Fields should thus be monitored regularly for the occurrence of grey mould during periods of high humidity (greater than 60%) (McPartland, *et al.*, 2000).

Severe infection of grape vines by *B. cinerea* is associated with late-season rain or overhead sprinkling, but substantial infection may also occur in the absence of rain or irrigation (Savage & Sall, 1983). The development of bunch rot caused by *B. cinerea* under dry conditions is most pronounced in cultivars that develop a dense canopy and compacted fruit clusters (Savage & Sall, 1983). Kenaf is planted very close together (Dempsey, 1975; Campbell & White, 1982; Bhangoo, Tehrani & Henderson, 1986; Francois *et al.*, 1992) which creates a cooler and more humid microclimate that is conducive to grey mould even under dry land conditions.

Intensive irrigation of certain crops may create microenvironments favourable to pathogens in locations where they were previously not favoured (Fry, 1982). In addition to dispersing fungal spores, sprinkler irrigation may extend periods of leaf wetness which

leads to easier infection by fungal pathogens and increases relative humidity which enhances sporulation (Fry, 1982). Overhead irrigation also promotes favourable conditions for disease development (Rotem, 1982; Hatfield, 1982). Thus, proper management of irrigation for kenaf should be implemented to minimize the effect of irrigation on disease development, especially when newly introduced into an area.

In vitro* inhibition of fungicides on isolates of *B. cinerea

Fungicides varied significantly ($P \leq 0.05$) in their ability to inhibit the radial mycelial growth of *B. cinerea in vitro*. Benomyl followed by vinclosolin, carbendazim/tebuconazole and iprodione significantly inhibited the radial growth of *B. cinerea* on PDA, as indicated by their low EC_{50} values of 0.2203, 0.4111, 0.4738 and 0.9084 µg a. i. /ml respectively (Fig 3.5). Lee, Choi & Cho (1998) also reported that iprodione and vinclosolin effectively inhibited mycelial growth of *B. cinerea*. Thiram (EC_{50} 419.3 µg a. i. /ml) was least effective in inhibiting the radial growth of *B. cinerea*. There was no significant difference between chlorothalonil and captan towards *B. cinerea* which had EC_{50} values of 31.12 and 26.49 µg/ml respectively.

The application of systemic fungicides such as benomyl and carbendazim can effectively control *Botrytis* diseases (Maude, 1980). When applied topically they can penetrate plant tissues sufficiently to eliminate established infections and when added to the soil they are taken up in by the roots to protect stem tissues from infection (Maude, 1980). Our results indicated that benomyl and carbendazim/tebuconazole were among the most effective fungicides that inhibited *in vitro* mycelial growth of *B. cinerea*. Treating kenaf with these fungicides may thus greatly reduce the incidence of grey mould. Since kenaf is being investigated as a new crop, no fungicides are presently registered in south Africa for the control of diseases to which it may be susceptible in South Africa. The

results reported here could thus be used as a guide for the selection of fungicides to control grey mould of kenaf in the field.

Various cultural and chemical control measures for diseases caused by *B. cinerea* have been tried and shown to be successful. Cultural practices such as sanitation that include removal and destruction of infected plant material (Maude, 1980; Hausbeck & Pennypacker, 1991; Mertely *et al.*, 2000), adequate ventilation as well as good management of plant nutrition (Nair & Hill, 1992) can be implemented to protect crops against infection by *B. cinerea*. Dempsey (1975) recommended dressing kenaf seeds with captan to minimize grey mould caused by *B. cinerea*. Our results indicated that captan showed only moderate inhibition of the fungus. Thiram was the least effective fungicide in inhibition of radial growth of *B. cinerea* even at higher concentrations. All the seed used in the experimental plots were treated with thiram, and in the light of the above results, could not control the grey mould in the filed.

Isolates of *B. cinerea* differed significantly ($P \leq 0.05$) in their sensitivity towards benomyl (Fig 3.6). *B. cinerea* is known for its rapid development of resistance to various classes of fungicides with different modes of action (Pepin & MacPherson, 1982; Schoonbeek, Del Sorbo & De Waard, 1999; Yourman & Jeffers, 1999; Yourman, Jeffers & Dean, 2000). Precautionary measures should therefore be taken to prevent the development of fungicide resistant strains of *B. cinerea* by applying fungicides in combinations of two or three (Agrios, 1997). Under conditions favourable to *Botrytis* infection on tomato, Pappas (2000) recommended the application of a mixture of conventional compounds with specific botrycides to prevent the development of fungicide resistance. Nair & Hill (1992) also recommended mixing fungicides such as thiram with promising new fungicides such as vinclosolin to control bunch rot of grapes caused by *B.*

cinerea. In the long term, however, fungicide application may not solve the problem posed by grey mould in kenaf production.

The results of this study suggested that *B. cinerea* is virulent to kenaf and could hamper the establishment of the crop in South Africa. The pathogen can also significantly reduce fibre yield and quality. Nevertheless, indications are that satisfactory control of grey mould could be achieved by the integration of chemical control and cultural practices such as adjustment of plant density, removal of infected plant material and proper irrigation management. Other measures such as biological control could be investigated.

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Figure 3.1 Early (A), late (B) symptoms and (C) breakage and lodging of kenaf plants infected by *B. cinerea*.

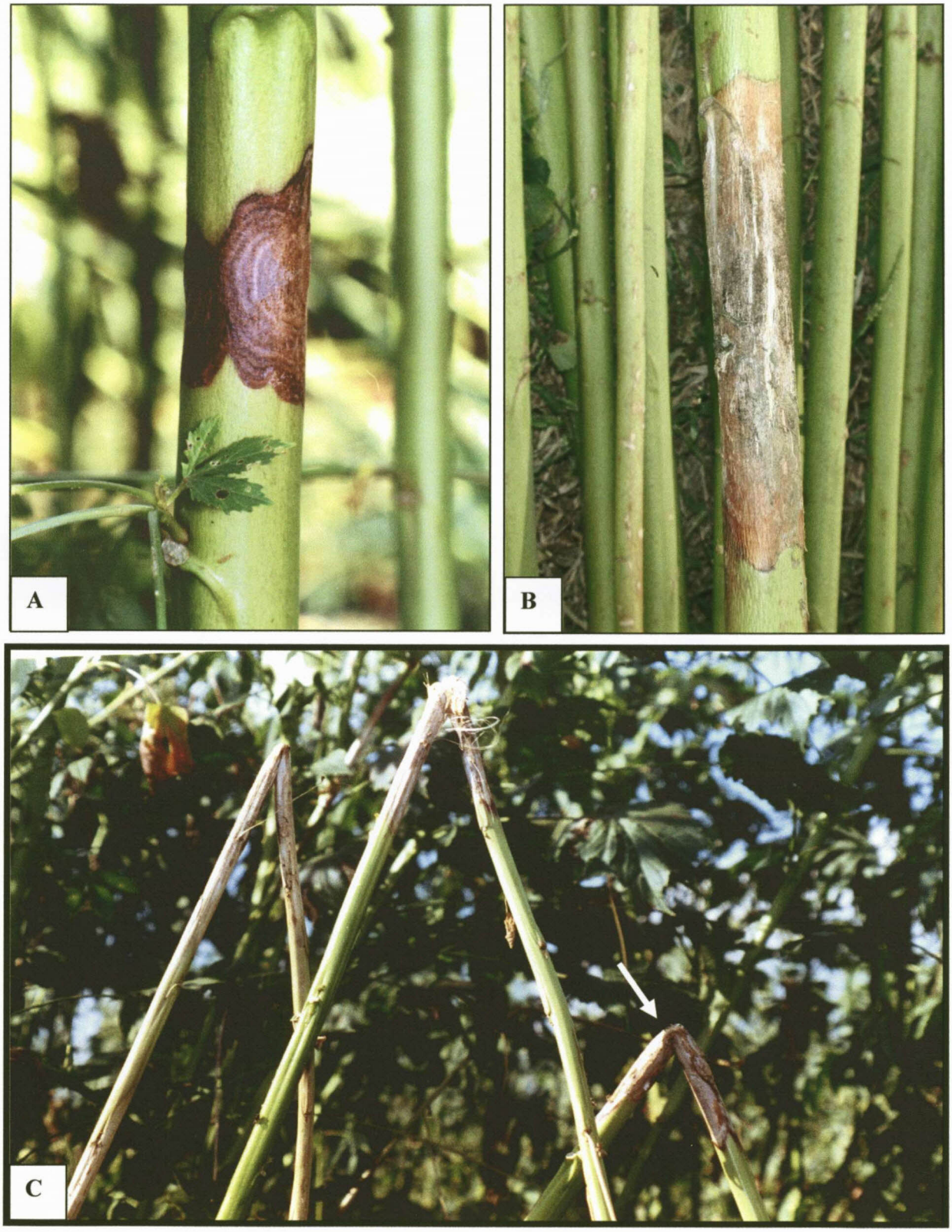

A
B
C

Figure 3.2 Mean lesion length on stems of mature kenaf plants artificially inoculated with *B. cinerea*. Bars denoted by different letters are significantly (P = 0.05) different according to Duncan's multiple range test.

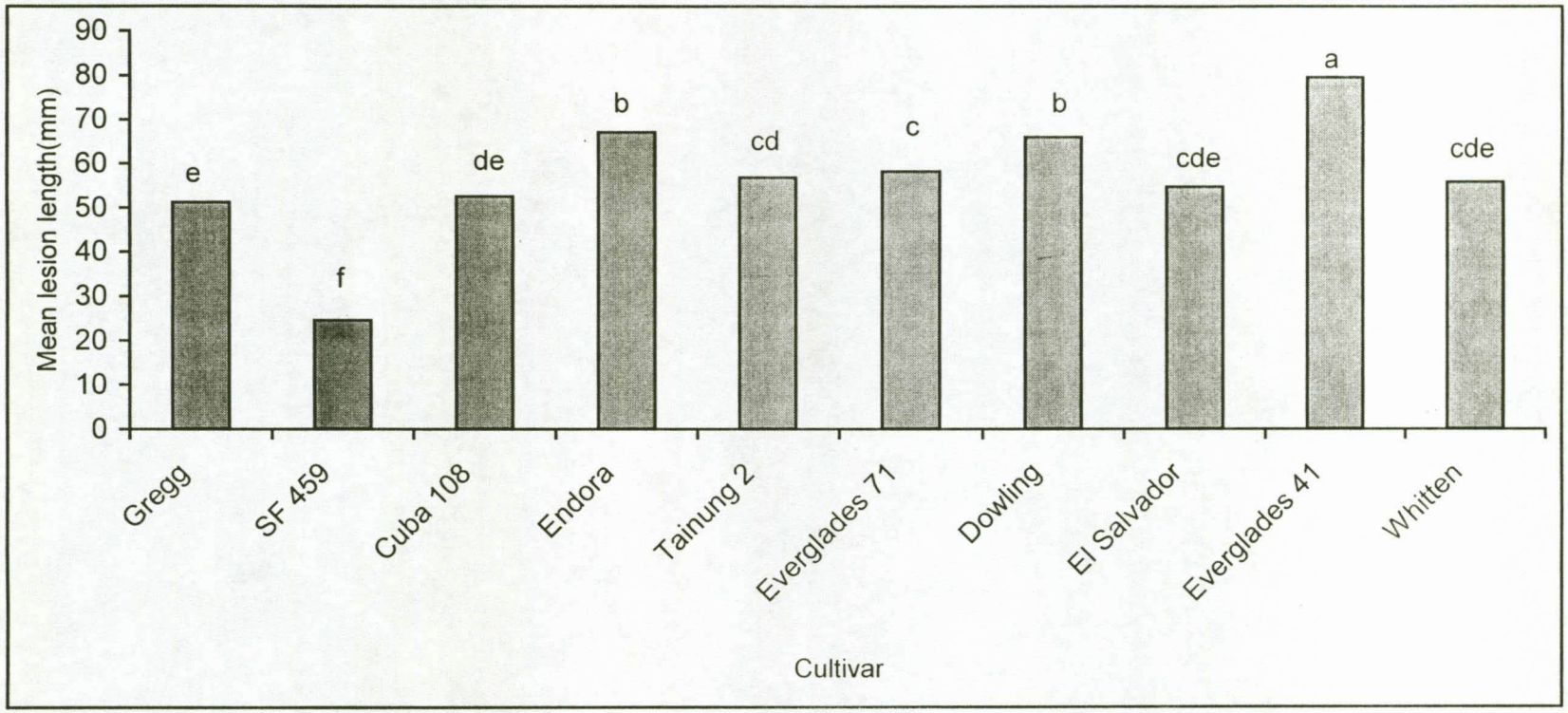

Mean lesion length(mm)
90
80
70
60
50
40
30
20
10
0
e
f
de
b
cd
c
b
cde
a
cde
Gregg
SF 459
Cuba 108
Endora
Tainung 2
Everglades 71
Dowling
El Salvador
Everglades 41
Whitten
Cultivar

Figure 3.3 Mycelial growth of *B. cinerea* under various temperature ranges seven days after incubation.

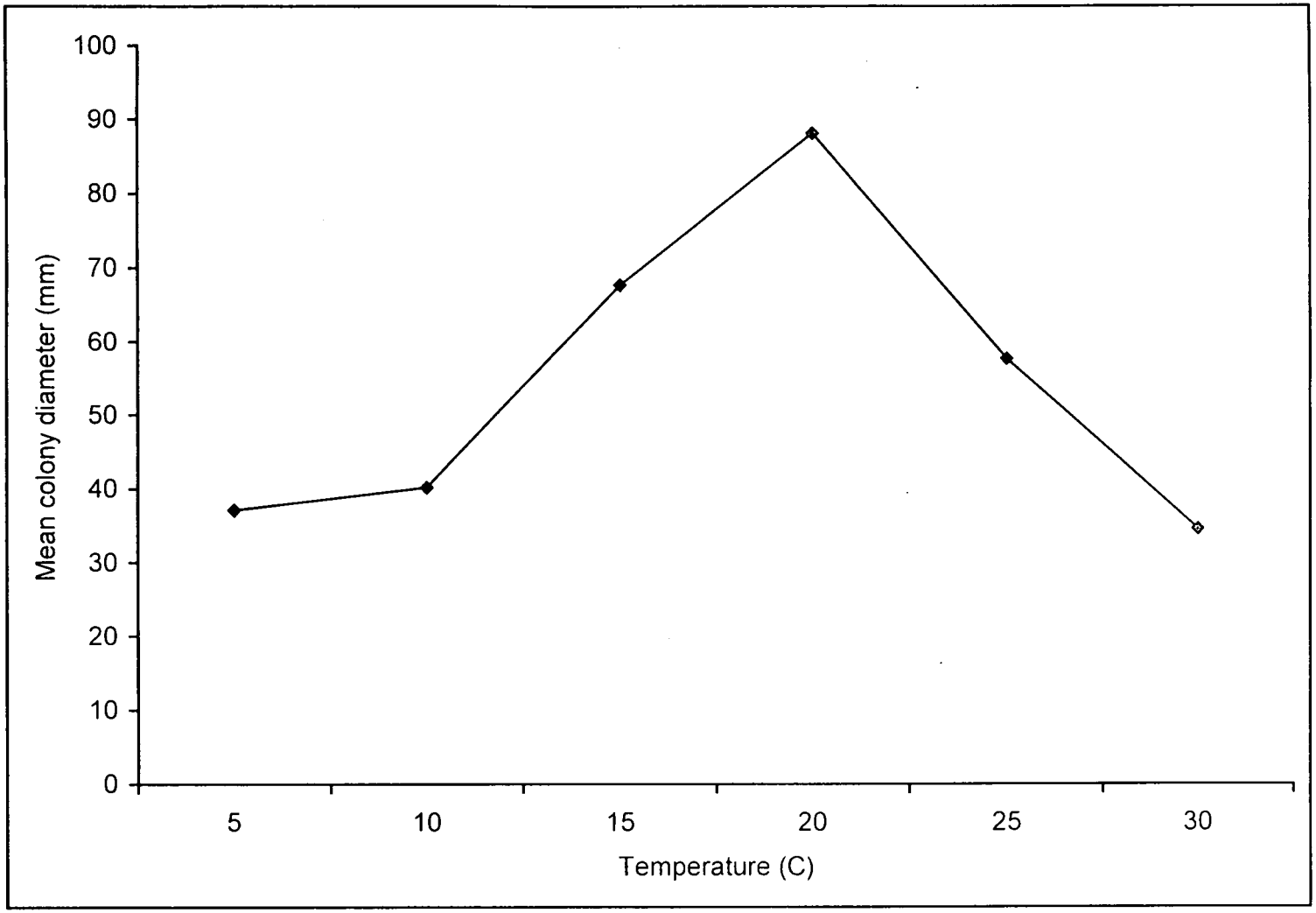

100
90
80
70
60
50
40
30
20
10
0
Mean colony diameter (mm)
5
10
15
20
25
30
Temperature (C)

Figure 3.4 Incidence (%) of grey mould caused by *B. cinerea* on kenaf under different moisture regimes during the 2000/2001 season. Standard error for treatment = 2.77 and for cultivar = 3.58. Bars designated with the same letter with in treatment are not significantly different at P = 0.05 according to Duncan's multiple range test.

Treatment 1= dry land regime (rain-fed).

Treatment 2= Medium soil moisture regime (irrigated weekly at FWC).

Treatment 3= High soil moisture regime (irrigated every second or third day).

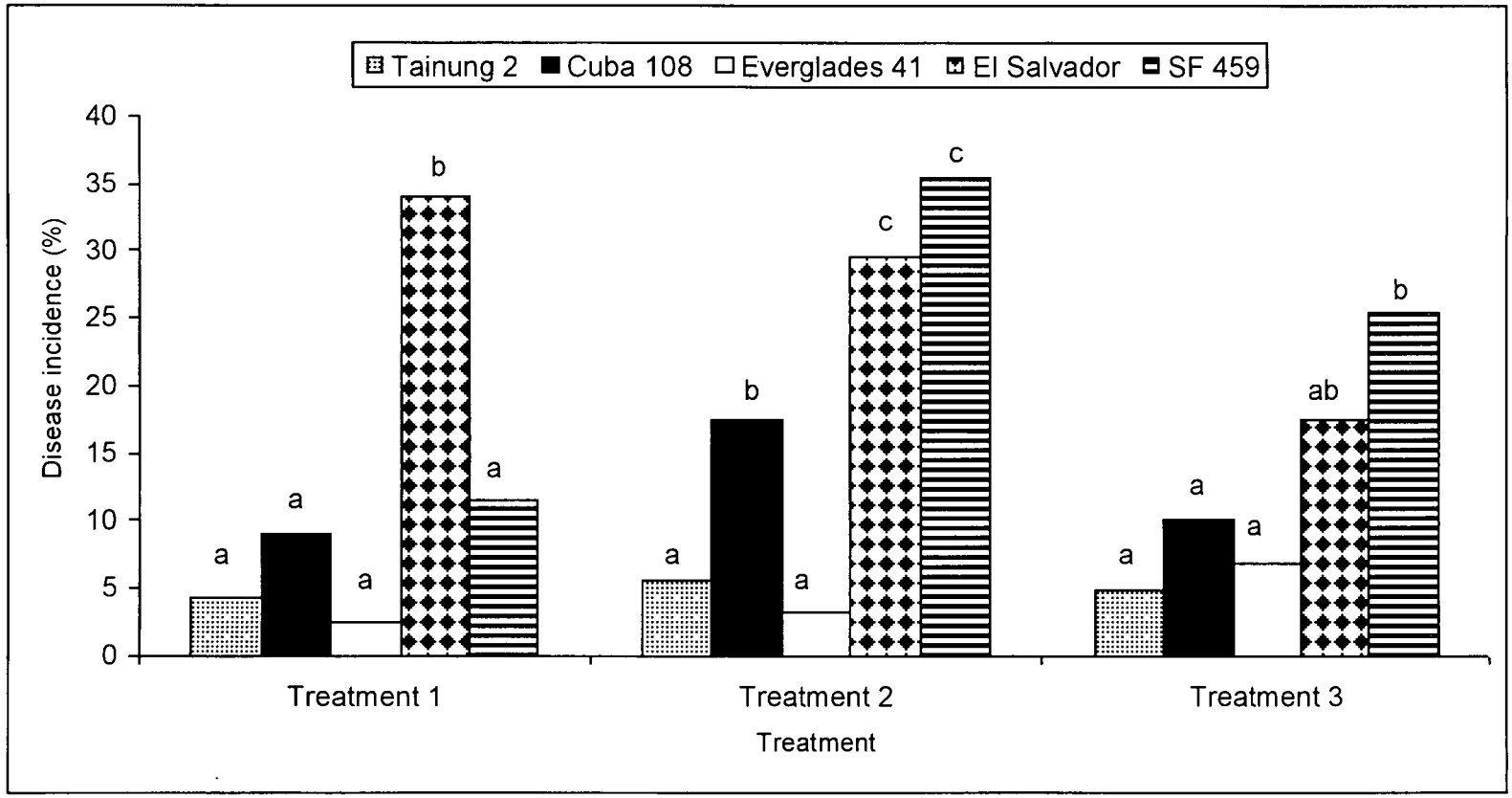
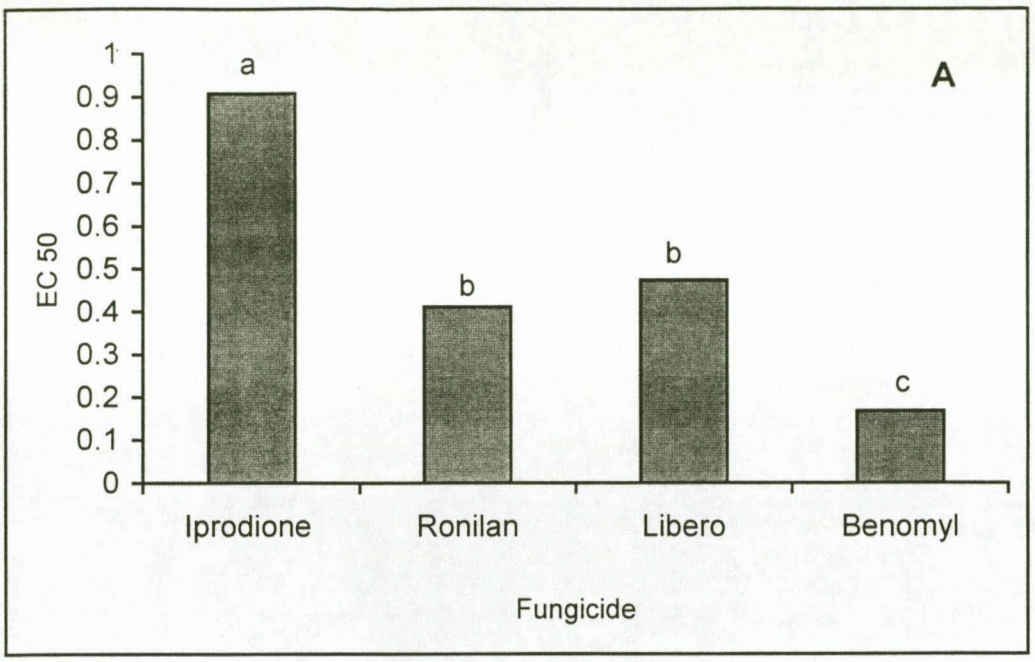

Tainung 2
Cuba 108
Everglades 41
El Salvador
SF 459
Disease incidence (%)
40
35
30
25
20
15
10
5
0
a
a
a
b
a
a
b
a
c
c
a
a
a
ab
b
Treatment 1
Treatment 2
Treatment 3
Treatment

Figure 3.5 Mean sensitivity (µg a.i./ml medium resulting in 50% inhibition of radial growth (EC_{50})) of *B. cinerea* isolate towards (A) the four most effective fungicides and (B) the three least effective fungicides. Bars denoted by different letters are significantly (P = 0.05) different according to Duncan's multiple range test.


A
EC 50
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
a
b
b
c
Iprodione
Ronilan
Libero
Benomyl
Fungicide

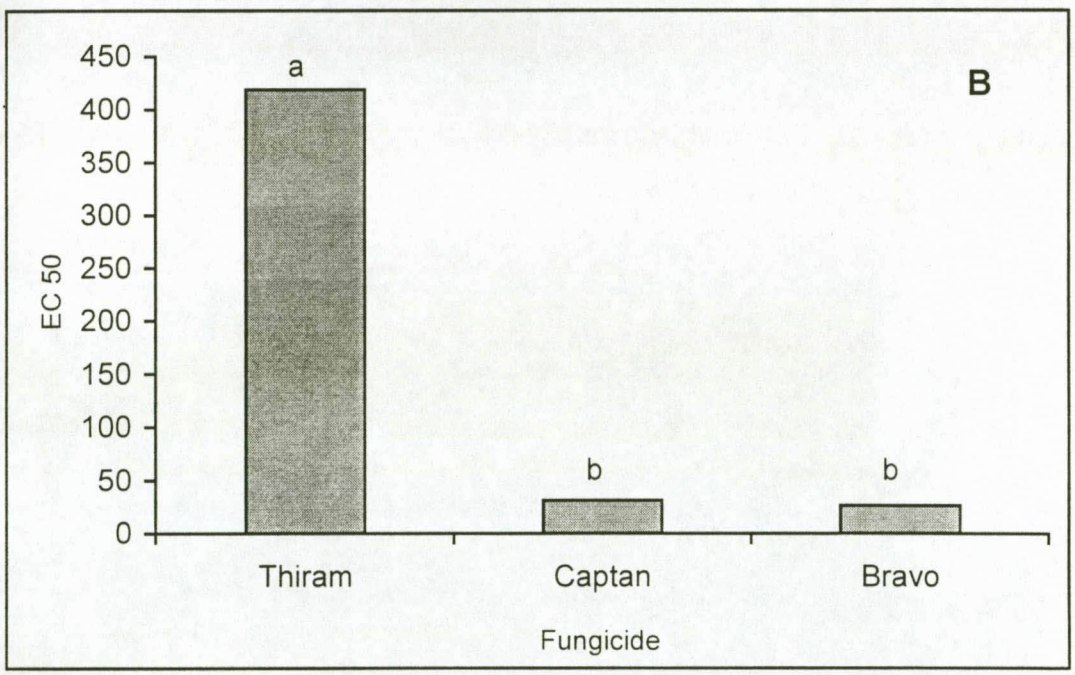
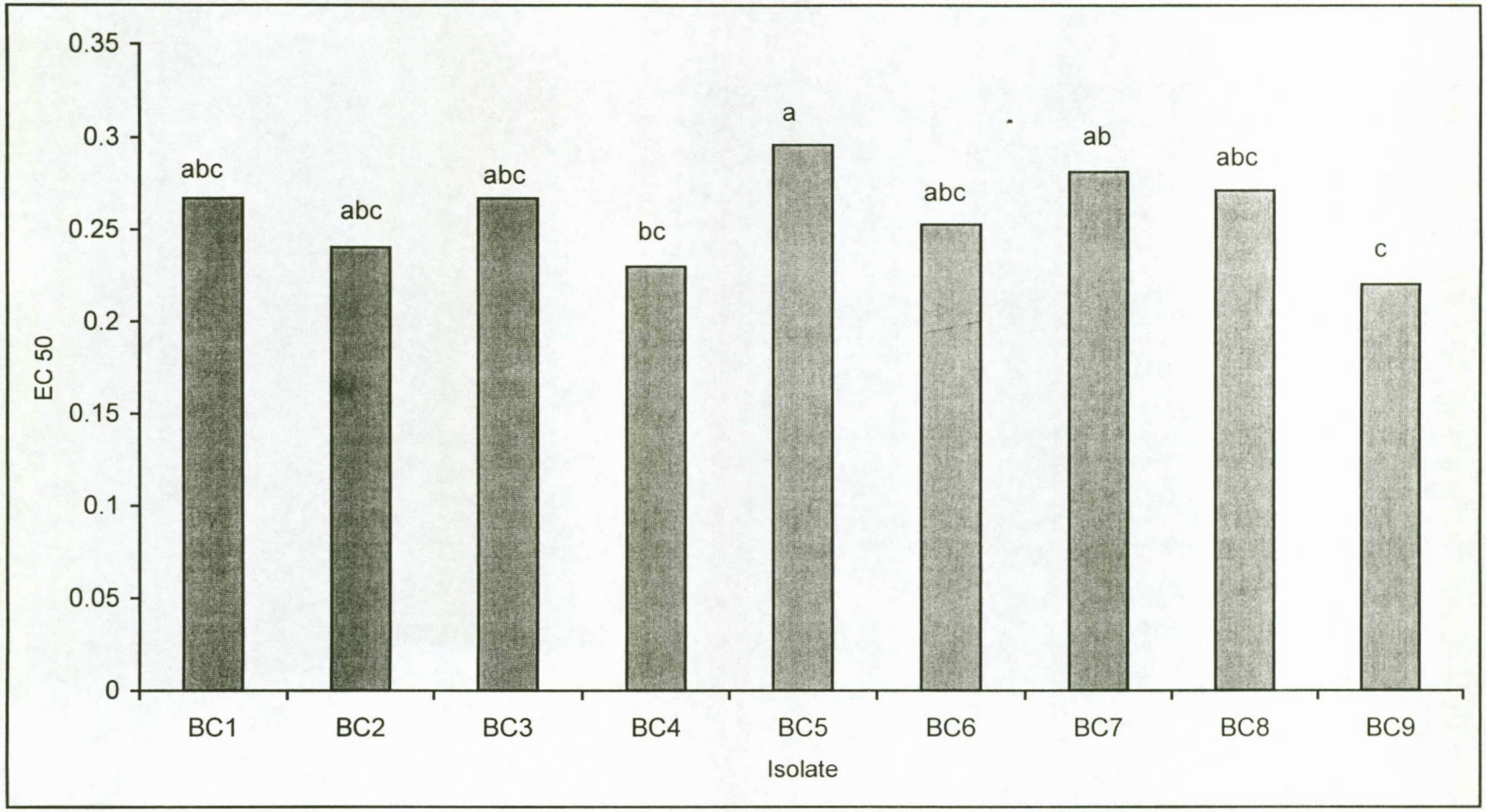

B
EC 50
450
400
350
300
250
200
150
100
50
0
a
b
b
Thiram
Captan
Bravo
Fungicide

Figure 3.6 Mean EC_{50} values (µg a.i./ml medium resulting in 50% inhibition of radial growth (EC_{50}) of benomyl tested against *B. cinerea* isolates. Bars denoted by different letters are significantly (P = 0.05) different according to Duncan's multiple range test.


0.35
0.3
0.25
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0.15
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EC 50
abc
abc
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bc
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ab
abc
c
BC1
BC2
BC3
BC4
BC5
BC6
BC7
BC8
BC9
Isolate

CHAPTER 4

THE CHARACTERIZATION OF *PYTHIUM* GROUP G OCCURRING ON KENAF IN SOUTH AFRICA

ABSTRACT

Kenaf (*Hibiscus cannabinus* L.)(Malvaceae) presents a source of high quality cellulose fibers and is being investigated in South Africa with a view to commercial production. In March 2001, kenaf plants grown from seed in experimental plots near Bloemfontein, South Africa, displayed large, black sunken lesions (10–20 cm long) at the base of the stem, and severe root rot. The causal agent was subsequently identified as *Pythium* group G and various studies were consequently undertaken to characterize the pathogen and the disease it causes on kenaf. The relative susceptibilities of kenaf cultivars (Cuba 108, Tainung 2, Everglades 41, El Salvador, SF 459, Gregg, Dowling, Endora, Whitten and Everglades 71) being considered for commercial cultivation were determined by means of artificial inoculations in the field and glasshouse. Field inoculation of 5-month-old kenaf plants revealed that the fungus was able to colonize cambial tissue resulting in cambial lesions ranging from 44.34 to 164.34 mm with an overall mean of 123.84 mm, seven days after inoculation. In glasshouse inoculation of potted plants, mean lesion lengths ranging from 43.38 mm to 58.27 mm with an overall mean of 52.72 mm, were measured seven days after inoculation. There were significant differences ($P < 0.05$) between the mean lesion lengths of the ten inoculated cultivars. Everglades 41 had the longest mean lesion length (58.27mm) while Everglades 71 had the shortest (43.06 mm). The optimum temperature for mycelial growth of *Pythium* group G was determined in the laboratory by incubating the fungus at a range of temperatures from 5 to 30°C. The optimum temperature range for growth was 20-30°C. Screening of six fungicides was conducted *in vitro* to determine their inhibitory effect on the radial growth of an isolate of *Pythium* group G. Dichlorophen and mancozeb + metalaxyl were the most effective fungicides. This study has important implications for the cultivation of kenaf in South Africa.

INTRODUCTION

Kenaf (*Hibiscus cannabinus* L.)(Malvaceae) is a warm season, annual, herbaceous plant which presents a source of high quality cellulose fibers. Its origin is in Africa and it was first domesticated and used in North Africa (Dempsey, 1975). It is currently being investigated in South Africa with a view to commercial production in the eastern part of the country. A sustainable pest and disease management strategy for the cultivation of kenaf in South Africa requires a sound understanding of biotic and abiotic factors that can affect the health of this crop. Trial plantings of kenaf varieties in various localities in South Africa have therefore been closely monitored for diseases and insect pests during the past three years.

During March 2001, a basal stem rot (Fig. 4.1) was observed on 12 five-month-old kenaf plants grown from seed in experimental plots near Bloemfontein in the Free State province. Diseased plants displayed large, black sunken lesions (10–20 cm long) at the base of the stem, and severe root rot. Isolations from diseased tissue onto malt-extract agar (MEA) consistently yielded a fungus subsequently identified as *Pythium* group G, a form of *Pythium ultimum* Trow (Swart, Tesfaendrias & Botha. 2002). This is the first published report of *Pythium* group G causing stem or root rot of kenaf.

Kenaf is a summer crop with climatic requirements similar to those of cotton (*Gossypium hirsutum* L.), a crop grown widely in South Africa. Kenaf and cotton both belong to the Malvaceae and pathogens that cause disease in cotton can therefore also be expected to attack kenaf. Several species of *Pythium* have been reported as seedling diseases of cotton especially if the soil has remained saturated for several days or is poorly drained (Koenning, 2000). Root rot of kenaf caused by *P. deliense* Meurs has been reported in Australia (Vawdrey and Peterson, 1990) but there are few reports of *Pythium* associated with diseases of *Hibiscus* species in general. In Florida, USA, root rot of *H.*

rosa-sinensis L. and in Hawaii, of *H. tilaceus* L. was attributed to *Pythium* sp. (Farr *et al.*, 1989). In South Carolina, USA *Pythium* and *Fusarium* spp. were associated with plants affected by the root-knot nematode (Blake, Mueller and Lewis, 1994).

P. ultimum is one of the most prevalent and pathogenic fungi associated with damping-off of cotton (Watkins, 1981; Minton & Garber, 1983). It is a very widely distributed species with a wide host range affecting roots and if the plant escapes death, the pathogen leads to poor root development, stunting and reduced yield (Hendrix & Campbell, 1973). Seedling disease incidence is greater when germination and seedling growth are slowed by cool, wet conditions and plants that survive infection often have a poor root system and are prone to water stress resulting in lower yield (Seney, 1984).

The association between *Pythium* group G and kenaf and the fact that cotton and kenaf both belong to the Malvaceae has important implications for the cultivation of kenaf in South Africa. Control measures prescribed for cotton diseases, especially those caused by *P. ultimum* (Bird, 1986) should therefore be examined with a view to applying them to the kenaf disease caused by *Pythium* group G. The objectives of this study were therefore to characterize *Pythium* group G on kenaf in South Africa in terms of (i) it's relative pathogenicity to kenaf cultivars, (ii) optimum temperature requirements and (iii) it's *in vitro* sensitivity to selected fungicides.

MATERIALS AND METHODS

Isolation and identification.

Diseased kenaf stems obtained from experimental plots near Bloemfontein were rinsed in tap water and then surface sterilized with 0.5% (v/v) NaOCl. The diseased bark was cut away with a sterile scalpel and small sections from the margins of discoloured tissue were plated on 1.5% malt extract agar (MEA) amended with streptomycin sulfate

(0.33g l^{-1} water) and incubated at 25°C for three days. Fungi that grew from the tissue into the agar were subsequently transferred to fresh plates containing MEA and incubated at 25 °C.

Fungal cultures that were tentatively identified as *Pythium* sp. were consistently isolated. Cultures were purified on 1% water agar with streptomycin sulphate (50 mg/l distilled water) and chloramphenicol (50 mg/l distilled water) using the single hyphal tip method. Single hyphal tips were transferred to 0.5% water agar with β-sitosterol (30 mg/l distilled water) and incubated at 25°C for 7 days. Five inoculum plugs (5 mm diameter) were placed in water cultures consisting of soil extract (15 g air-dried soil/1 distilled water) and two hemp seeds per Petri dish to induce sporulation. Cultures were incubated for 3 days at 25°C and morphological structures were observed with a light microscope. The taxonomic keys for *Pythium* (Dick, 1990) was used to identify the *Pythium* sporangial type and group.

Artificial inoculation

Field inoculations. Koch's postulates were satisfied by inoculating 4-month-old healthy kenaf plants (stem diameter 25-35 mm) growing in the same experimental plots that the disease was observed. Wooden toothpick tips (25 mm long) were autoclaved for 20 min in distilled water, removed from the water, blotted dry, autoclaved a second time in water to remove substances that might inhibit fungal growth, then autoclaved for a third time in potato dextrose broth (PDB). The toothpick tips were cooled in a sterile petri dish and transferred individually to the margins of colonies of the *Pythium* sp. on 2% MEA. After incubation at room temperature for 48 hours, the colonized toothpick tips were removed and used as an inoculum source.

Ten plants of each of five cultivars (Everglades 41, Cuba 108, El Salvador, SF459 and Tainung 2) were randomly selected for inoculation. Colonized tips were inserted approximately 25 cm above soil level into the stems of the selected plants. Corresponding control treatments consisted of 10 additional plants per cultivar were mock inoculated at the same height above soil level with sterile toothpick tips. Parafilm was wrapped around each inoculation site to prevent dessication. The length of the necrotic lesion extending either side of the inoculation wound was measured after 7 days. A completely randomized design was used.

Inoculation of potted plants. Inoculum was prepared by growing an isolate of *Pythium* group G on potato dextrose agar (PDA) (Difco®) for five days at 25°C. Agar in plates was overlaid with gauze strips (10 x 20 mm) that had been autoclaved twice for 20 min at 121°C. Five potted plants (3 months old) from each of 10 kenaf cultivars (Cuba 108, Tainung 2, Everglades 41, El Salvador, SF 459, Gregg, Dowling, Endora, Whitten and Everglades 71) were inoculated by puncturing the stems with a sterile dissecting needle, applying the colonized gauze strips to the wound and then wrapping the inoculation site with Parafilm to prevent desiccation. Control treatments consisted of sterile gauze strips applied to five plants of each cultivar. The developing lesion was measured 7 days after inoculation.

Damping-off studies. The ability of *Pythium* group G to cause damping-off on kenaf was tested by artificially inoculating the ten above mentioned cultivars. Inoculum of the fungus was established in a sterile growth medium consisting of 200 ml perlite, 60 g corn meal and 70 ml distilled water, mixed in 500 ml Erlenmeyer flasks. The medium was autoclaved twice for 20 min at 120°C. Agar plugs taken from the edge of a growing

colony, and aseptically transferred to Erlenmeyer flasks containing the growth medium. Flasks were incubated for three weeks at 25°C and shaken periodically to ensure uniform growth of inoculum.

Seeds of each kenaf cultivar were pre-germinated on 1.5% water agar (WA) to ensure germination and the absence of soilborne pathogens. Seeds had previously been equally divided into two groups; one half of which was treated with thiram. The other half was surface disinfested first with 96% ethanol for 30 seconds, then with NaOCl (1.75%) for 60 seconds and finally rinsed with sterile distilled water before being dried on sterile filter paper.

Seedlings were grown in pots (400 cm^3) containing a 1:1 steam sterilized soil/peatmoss mixture in the glasshouse. Inoculum prepared as above (0.4 g) was placed 1 cm below each germinating seed. Twenty seedlings for each cultivar with four replicates (5 seeds/pot) were used. For each main treatment, fungicide treated and non-treated, a sterile growth medium without inoculum served as control treatment. Plants were watered regularly as required. Plants were observed periodically for symptom development and the percentage mortality for each treatment was recorded three weeks after inoculation.

In vitro growth studies

Temperature growth studies. The vegetative growth of the *Pythium* group G isolate used for artificial inoculations was determined at temperatures ranging from 5–30°C at 5-degree intervals. The isolate was maintained on PDA for 5 days at 24°C. Mycelial plugs were removed from the edge of actively growing colonies with a sterile 5-mm-diameter cork borer and placed upside down in the centre of 90 mm Petri plates containing PDA. Plates were incubated at the six different temperatures and mycelial growth of the fungal colony in each of three replicate plates per treatment was measured after 24, 48 and 72 hr. Colony

size was determined by calculating the mean of two perpendicular measurements of colony diameter.

Fungicide sensitivity. Six fungicides namely, quintozene ((Pentacholoronitrobenzene (PCNB®)- 750 g/kg WP), mancozeb/metalaxyl (Expose MZ®- 600/100 g/kg), mancozeb (Mancozeb®- 800 g/kg WP), dicholorophen (Xanbac D®- 200g/l EC), thiram (Thiram®- 750 g/kg WP) and captan (Kaptan®- 500 g/kg WP) were evaluated *in vitro* for their effect on the radial growth of the *Pythium* isolate used in artificial inoculations. Autoclaved PDA cooled to 45°C was used as a basal medium. It was amended with an aqueous stock solution of each of the fungicide at the concentrations of 0.5, 1, 5, 10, 25 and 50 µg a.i./ml. Each treatment was replicated three times and the experiment was conducted twice.

The fungal isolate was grown for 5 days at 25°C before being transferred to the amended basal medium. A small agar disc (5 mm diameter) of the fungus culture was cut with a sterile cork-borer and transferred aseptically to the centre of 90 mm Petri plates containing the fungicide amended basal medium. Control treatments consisted of the unamended basal medium only. Plates were incubated at 25°C and colony size was determined by calculating the mean of two perpendicular measurements of colony diameter on each plate after 72 hours.

Inhibition of colony growth compared to the unamended control treatment was determined. The effective concentration (EC_{50}), the point on the regression line at which 50% of the isolate is inhibited, was determined by plotting the radial growth inhibition values of the isolate transformed to probits against the $\log_{10}$ concentration of fungicides (Fisher & Yates, 1963).

Statistical analyses.

All the above experiments were conducted twice and variances among the respective pairs of trials were tested for homogeneity using Bartlett's test before a two-way analysis of variance (ANOVA) was performed on the pooled data using the statistical program NCSS 2000 (BMDP Statistical Software Inc., Los Angeles, CA). Treatment means were separated using Duncan's multiple range test.

RESULTS

Isolation and identification

Dimensions of the morphological structures of the isolated fungus were as follows: Sporangia: 18-27 (av. 23) µm diam, terminal and intercalery, globose, non-proliferating; discharge-tubes: 2-4 µm wide and 7-11µm long; number of zoospores/sporangium: 5-10; hyphal swellings globose, terminal, 14-25 (av. 20) µm diam.; encysted zoospores: 9-16 µm diam. No oogonia or oospores were observed.

Artificial inoculation

Field inoculations. The artificially inoculated fungus colonized the cambial tissue of all five kenaf cultivars and cambial lesions ranging from 44.34 to 164.34 mm with an overall mean of 123.84 mm were measured seven days after inoculation. No lesions resulted from the control treatment on any of the five cultivars. Mean cambial lesion lengths for both experiments measured 116.7, 139.1, 119.5, 119.4 and 121.8 mm for Tainung-2, Everglades 41, SF-459, Cuba 108 and El Salvador, respectively. Although Everglades 41 had the longest lesions, there were no significant differences ($P < 0.05$) among cultivars. In all cases in which infection had been successful, discolouration of the bark surrounding the wound was clearly visible and girdling of the stem had resulted in wilting of foliage above

the point of inoculation. Removal of the bark revealed that the cambial tissue was severely discoloured and that discolouration had spread to the xylem in most cases. The pathogen was successfully re-isolated from all artificially inoculated tissue.

Inoculation of potted plants. Rapidly developing black lesions were observed in all artificially inoculated plants. A mean lesion length ranging from 43.38 mm to 58.27 mm with an overall mean of 52.72 mm was measured seven days after inoculation. There were significant differences ($P < 0.05$) between mean lesion lengths observed on the ten cultivars. Everglades 41 had the longest mean lesion length (58.27) while Everglades 71 had the shortest (43.06 mm) mean lesion length (Fig. 4.2).

Infection resulted in discolouration of the bark surrounding the point of inoculation and girdling of the stem. The cambial tissue was severely discoloured and discolouration had also spread to the xylem. The pathogen was successfully re-isolated from all artificially inoculated plants. No symptoms appeared on the control treatments.

Damping-off studies. Mortality of kenaf seedlings inoculated with the *Pythium* isolate occurred rapidly and were clearly discernible after seven days although seedlings continued to die during the subsequent 3 weeks. Mean damping-off of seedlings ranged between 35-60% (Fig. 4.3). Damaged seedlings had symptoms of partial or complete girdling of the emerged seedling stems and seedling rot. Damage to the roots was visible as soft, light brown lesions on tap and secondary roots. The pathogen was re-isolated from all artificially inoculated seedlings. No significant ($P < 0.05$) differences in susceptibility were observed between cultivars. Thiram treated seeds did not result in less damping-off than untreated seeds.

In vitro growth studies

Temperature growth studies. Mycelial growth was visible after 24 hours at temperatures of 15 - 30°C. No mycelial growth took place at 5 and 10°C during the entire evaluation period. Radial growth of the fungus was significantly higher ($P < 0.05$) (88.18 mm) at temperatures of 20, 25 and 30°C followed by 15°C which had mean radial growth of 78 mm 72 hrs after incubation (Fig. 4.4).

Fungicide sensitivity. Fungicides generally had a significant inhibitory effect on mycelial growth of the *Pythium* isolate. The fungicides tested differed significantly ($P < 0.05$) in their abilty to inhibit *in vitro* vegetative growth of *Pythium* group G. Dichlorophen and mancozeb+metalaxyl were the most effective fungicides. The concentration of these two fungicides required for 50% inhibition of colony diameter of the fungus was 4.71 and 4.75 µg a.i/ml respectively (Fig. 4.5). Captan was inferior to other fungicides with the highest EC_{50} value of 94.0414 µg a.i/ml.

DISCUSSION

The results of the present study confirm that the *Pythium* group G isolate isolated from diseased kenaf plants (Swart *et al.*, 2002) has the potential to cause serious losses in kenaf stands in South Africa. There can be little doubt that the isolate belongs to *Pythium* group G since isolates belonging to *Pythium* G-group do not produce the oogonia essential in classical taxonomy of Oomycetes. They produce only globose or subglobose spherical sporangia either in a terminal or intercalery position. Zoospores accumulate in a vesicle at the tip of a short discharge tube before the motile biflaggelate zoospores are released. No

oogonia with oospores were observed in the cultures we examined even after prolonged incubation of purified cultures.

Based on mtDNA profiles Huang *et al.* (Huang *et al.*, 1992), showed that an isolate of *Pythium* group G from safflower could be a variant form of *P. ultimum* Trow. These mtDNA patterns were similar to patterns of three isolates belonging to regional subpopulations of *P. ultimum* (Martin, 1990). Therefore, using mitochondrial and ribosomal genetic data, Klassen & Buchko (1990), indicated that their safflower isolate might be a variant form of *P. ultimum.* In a more recent study using amplified ribosomal DNA to develop an ITS I probe of *P. ultimum* (Levesque, Vrain and De Beer, 1994), no difference in hybridization could be detected among 13 isolates of *P. ultimum* (var. *ultimum* and var. *sporangiferum* from 8 countries) and 2 isolates of *Pythium* group G thus supporting the fact that it is merely a different form of *P. ultimum.* It is therefore highly probable that the isolate from kenaf may also be a heterothallic variant form of *P. ultimum.*

Many species of *Pythium* have been recognized as part of the cotton seedling disease complex (Farr *et al.*, 1989) and *P. ultimum* is the most prevalent and pathogenic of these species (Watkins, 1981). A study in Mississippi, USA showed that disease pressure on kenaf seedlings was greater and seedling survival lower in plots to which additional inoculum of *Rhizoctonia solani* Kühn and *P. ultimum* was applied than in plots with indigenous populations of these two fungi (Batson, Caceres & Carvajal, 2000). The study found that disease development incited by *P. ultimum* was favoured by cool damp soils.

To reduce severity of diseases caused by *Pythium* and optimise stand establishment, Batson *et al.* (2000) recommended dressing of kenaf seeds with selected combinations of fungicides. They recommended that metalaxyl be used as a seed treatment in combination with triadimenol and carboxin-pcnb for controlling *R. solani.* Other chemical control measures prescribed for kenaf diseases (White *et al.*, 1971; Cook *et al.*, 1992) as well as

those pertaining to the control of cotton seedling diseases caused by *P. ultimum* should therefore be examined with a view to applying them in controlling the disease of kenaf reported here.

Temperature and moisture are the most important factors that influence infection by *Pythium* (Hendrix & Campbell, 1973). Our results indicated that the optimum temperature for the radial growth of *Pythium* group G ranged from 20–30°C. Growth also took place as low as 15°C at lower rate than optimum temperature. According to Van der Plaats-Niterink (1981) optimum temperature for *P. ultimum* is 20-25°C. Lifshitz & Hancock (1983) reported that high *P. ultimum* population build up in soil occurred between 16-21°C. They also noted that maximum colonization of dry cotton leaves inoculated with *P. ultimum* occurred between 18-30°C. Arndt (1943) as cited by Roncadori & McCarter (1972) reported that root necrosis and stunting of cotton caused by *P. ultimum* occurred at 24 and 27°C. *P. ultimum* is the most virulent pathogen than other *Pythium* spp of soybean at 15-20°C (Thomson, Athow & Laviolette, 1971).

The optimum temperature for growth in culture and disease development would not always be similar (Hendrix & Campbell, 1973). When low soil temperature occurred in early season plantings, *P. ultimum* is considered the most important seedling pathogen of cotton (De Vay, Garber & Matheron, 1982). McCarter & Roncardori (1971) also reported that low temperature during germination reduces subsequent growth of cotton and increases the susceptibility of germinating seed to certain soilborne pathogens causing seed rot and damping-off. This may be due to slow germination of seeds at lower temperature that render the seed more subject to the attack by the species which grow at low temperatures (Thomson *et al.*, 1971). If soil temperature lowers due to various factors during the planting season of kenaf, there would be thus a possibility of diseases incidence incited by *Pythium* group G.

Soil temperature can be affected by the presence of a plant canopy, soil moisture and exposure to sunlight (Hatfield, 1982). Minton & Garber (1983) recommended the manipulation of these factors by planting cottonseeds when temperatures and moisture are favourable for cotton. If cotton is planted when soil moisture at planting depth has drained to field capacity and debris are removed or buried, seedling disease can be minimized (Minton & Garber, 1983). Thus, it would be suggested that attention be given to the factors that attribute to the fluctuation of soil temperature, such as irrigation, particularly during the vulnerable stage of kenaf seedlings. It would thus be important to take account of soil temperature when evaluating control strategies for *Pythium* infection.

The results of fungicide experiments indicated that dichlorophen and mancozeb+metalaxyl were the most effective fungicides in the inhibition of radial growth of *Pythium* group G. Mancozeb+metalaxyl is registered in South Africa for the control of late blight of potato and tomato caused by *Phytophthora infestans* (Mont.) de Bary (Nel *et al.*, 1999). However, metalaxyl both singly and/or in combination with other fungicides has been reported for the control of *Pythium* in cotton (Bassi *et al.*, 1998) and kenaf (Batson *et al.*, 2000). Minton & Garber (1983) reported that metalaxyl has shown effective control of *Pythium* in both greenhouse and field trials of cotton. Metalaxyl has also been reported in controlling *Pythium* in other crops. Brantner & Windels (1998) reported the *in vitro* sensitivity of *P. ultimum* isolated from sugar beet to metalaxyl. They also noted a significant reduction of disease when *Pythium* infested soil was planted with metalaxyl treated sugar beet seeds. Either alone or in combination with ziram, metalaxyl can effectively control tobacco seedling damping-off caused by *Pythium* (Patel, Patel & Patel, 1996). Taylor *et al.* (2002) also reported the sensitivity of *P. ultimum* isolates from rotted potato to metalaxyl. Dichlorophen is registered in South Africa for the control of wide range of pathogens. For cotton and groundnut, the fungicide is recommended for leaf spot

diseases; root rot and wilting of cucurbits and lettuce can be controlled by dichlorophen applied as a soil drench (Nel *et al.*, 1999). Although dichlorophen and metalaxyl are not yet registered in South Africa for *Pythium* in cotton and/or kenaf, evaluating the sensitivity of *Pythium* to both fungicides should be considered for integration into the overall disease management strategy for kenaf in South Africa.

A combination of fungicides is more effective in controlling soilborne pathogens than single fungicide treatments (Cook *et al.*, 1992). This is due to the fact that protecting seed and seedling against infection by one pathogen may increase infection by other pathogenic soilborne microorganisms (Davis, Nunez & Subbarao, 1997). Fungicide seed treatment is feasible due to low cost of seed treatment, unpredictability of weather and lack of information about the population of soilborne pathogens (Davis *et al.*, 1997) and therefore in areas where kenaf production is to be conducted, controlling seedling diseases of kenaf by treating seeds with fungicides in combination would be justifiable. Furthermore, it would be advisable to conduct continuous monitoring and surveying of all potential kenaf growing areas to assess the composition of soilborne pathogens in respective areas. Seedling disease of cotton is a complex involving more than one pathogen (Johnson *et al.*, 1978; Watkins, 1981), which also could be true for kenaf seedling diseases. To overcome the possible threat that would be encountered by the complex of soilborne pathogens, it would thus be advisable to consider all the pathogens involved before selecting fungicides for seed treatment.

Chemical disease control should not be seen as the only option to control kenaf diseases. It should be part of an integrated disease management program. Under favourable conditions, seedlings quickly outgrow the most vulnerable stage and infection is less likely and thus conditions at planting should favour rapid germination and seedling growth (Seney, 1984). Therefore, other factors that can affect disease incidence such as

soil fertility, soil moisture content, temperature, planting time and cultural practices should be properly managed to reduce the incidence and severity of *Pythium* group G as well as other soilborne pathogens of kenaf.

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Figure 4.1 Basal stem and root rot of kenaf resulting from natural infection by *Pythium* group G in the field.

Figure 4.2 Mean lesion length on stems of kenaf cultivars inoculated with *Pythium* group G in the field. Bars denoted by the same letter are not significantly different at ($P < 0.05$) according to Duncan's multiple range test.

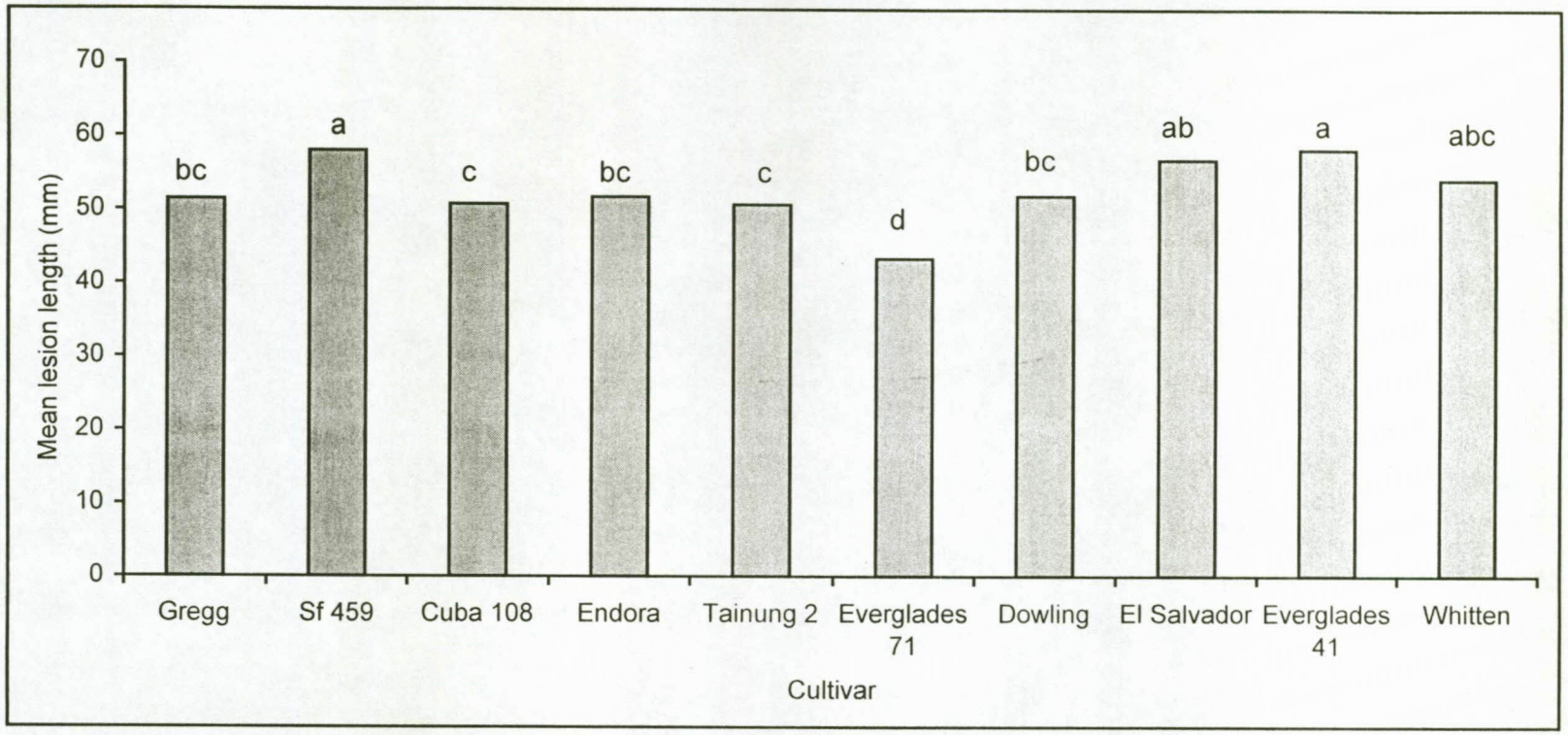

Mean lesion length (mm)
70
60
50
40
30
20
10
0
bc
a
c
bc
c
d
bc
ab
a
abc
Gregg
Sf 459
Cuba 108
Endora
Tainung 2
Everglades 71
Dowling
El Salvador
Everglades 41
Whitten
Cultivar

Figure 4.3 Damping-off of kenaf seedlings after inoculation of germinated kenaf seeds with *Pythium* group G.

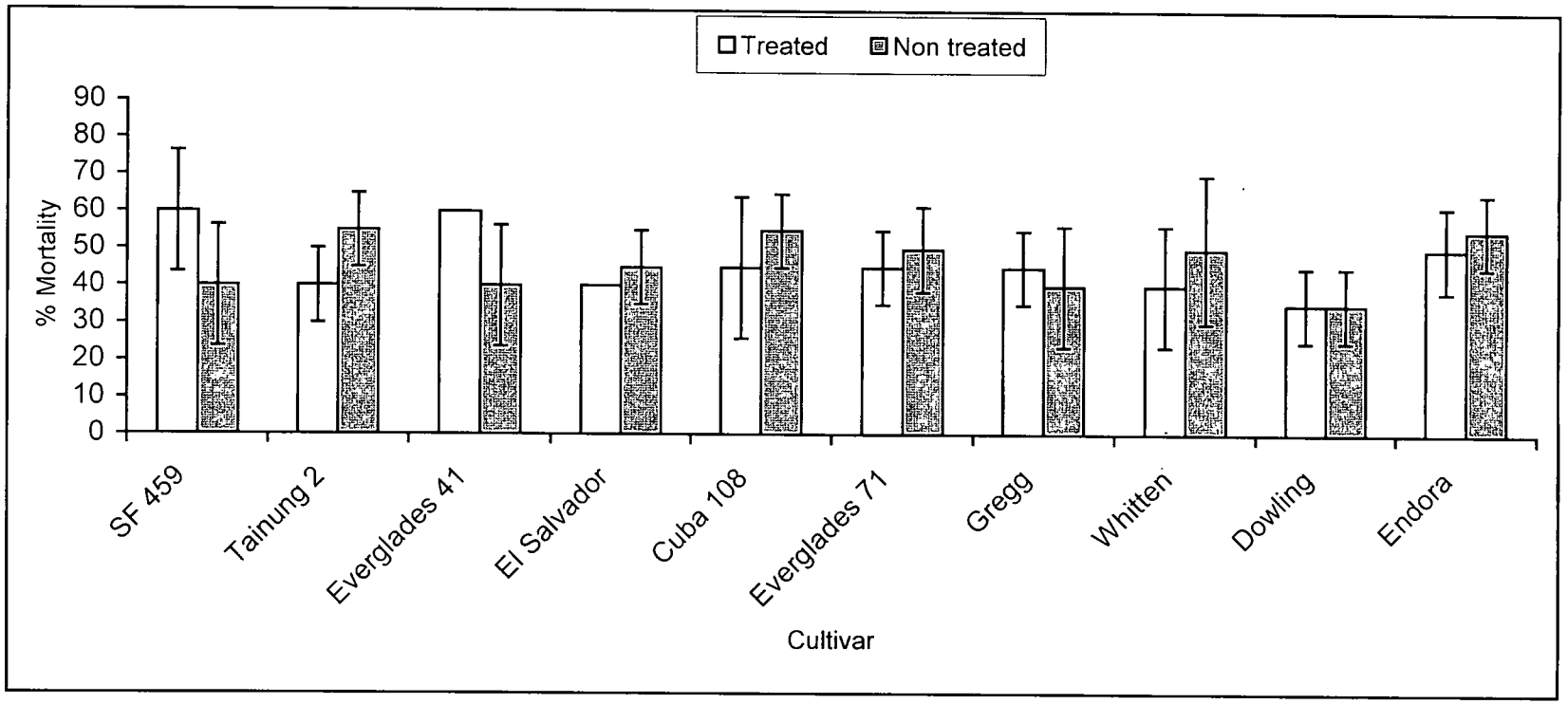

Treated
Non treated
% Mortality
90
80
70
60
50
40
30
20
10
0
SF 459
Tainung 2
Everglades 41
El Salvador
Cuba 108
Everglades 71
Gregg
Whitten
Dowling
Endora
Cultivar

Figure 4.4 Effect of temperature on mycelial growth of *Pythium* group G on potato dextrose agar after 72 hr.

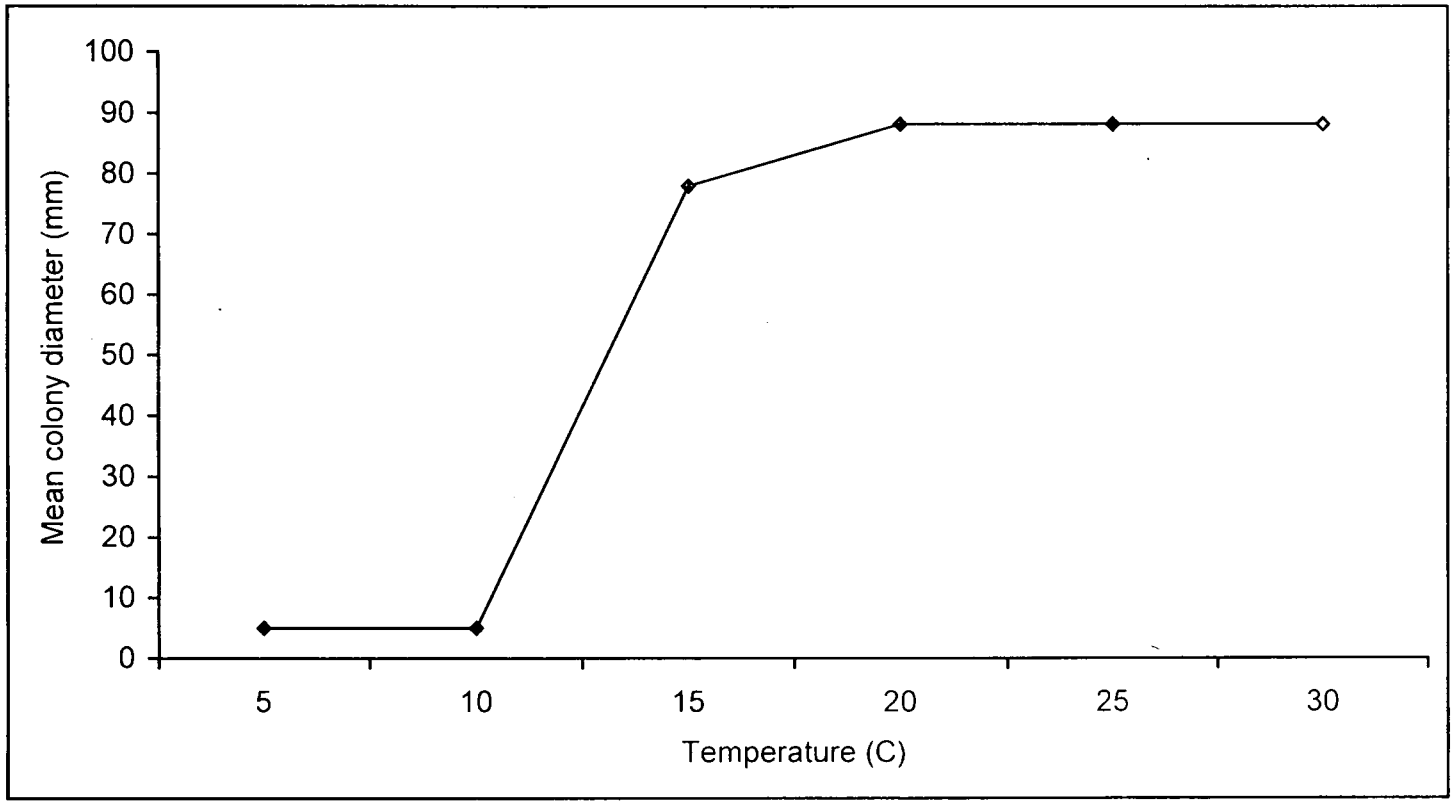
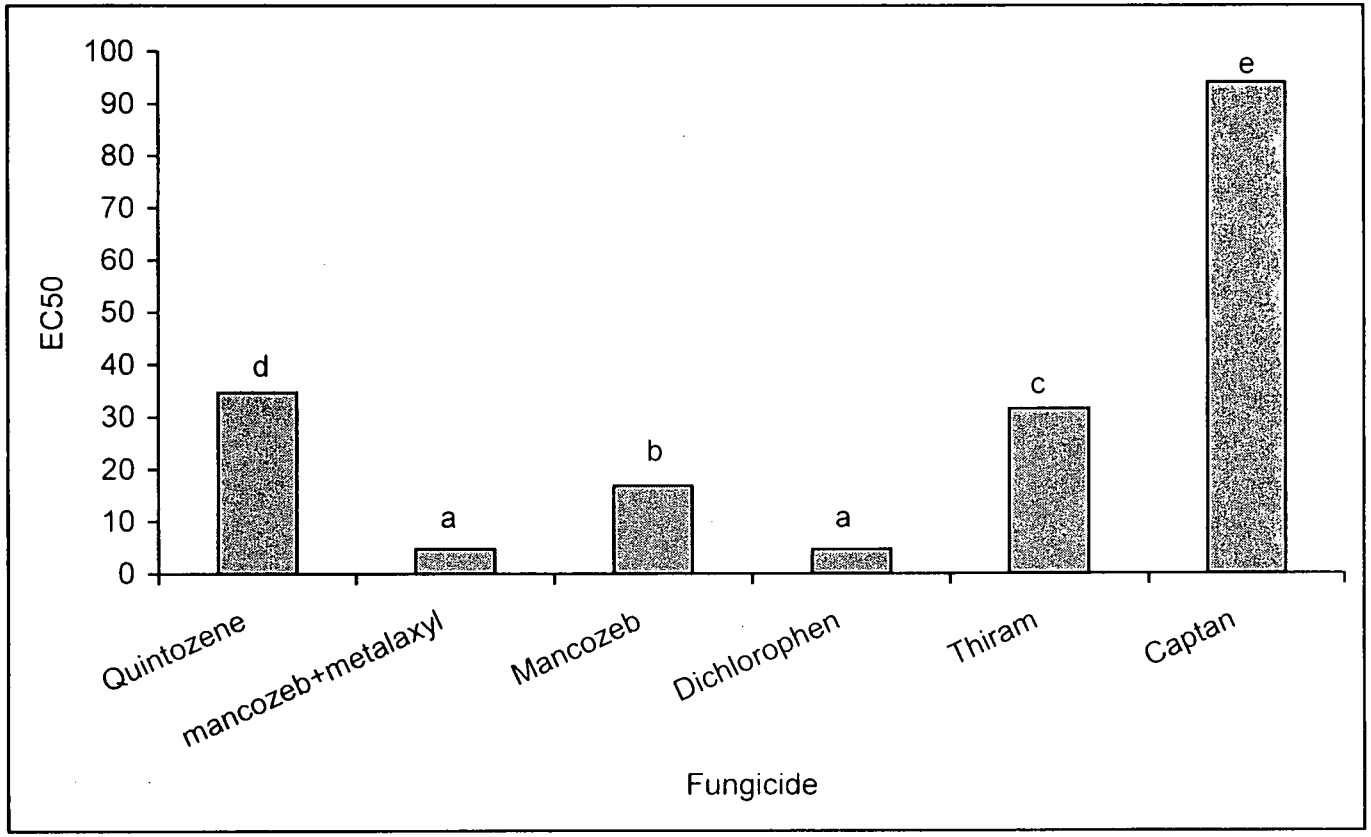

100
90
80
70
60
50
40
30
20
10
0
Mean colony diameter (mm)
5
10
15
20
25
30
Temperature (C)

Figure 4.5 Mean EC_{50} values (μg a.i./ml medium resulting in 50% inhibition of radial growth) of six fungicides tested against *Pythium* group G. Bars denoted by the same letter are not significantly different at $P < 0.05$ according to Duncan's multiple range test.


100
90
80
70
60
50
40
30
20
10
0
EC50
d
a
b
a
c
e
Quintozene
mancozeb+metalaxyl
Mancozeb
Dichlorophen
Thiram
Captan
Fungicide

SUMMARY

Kenaf is propagated by seed and thus its cultivation depends on good quality seed and uniform emergence of seedlings. The aim of chapter two of this study was to investigate factors that are related to the establishment of kenaf from seed. Consequently, a total of nine fungal genera were identified from surface disinfested kenaf seeds of ten cultivars. In all cases *Alternaria* spp. was the most common followed by *Chaetomium* spp. The cultivar, Whitten had the highest incidence with 60% of seeds contaminated. Of the fungal species isolated from nine genera, *Fusarium subglutinans* was considered to be the most potentially pathogenic. In the glasshouse trials, kenaf cultivars artificially inoculated with *F. subglutinans* produced disease symptoms which resulted in damping-off of seedlings. This suggests that the pathogen can be expected to influence kenaf production in South Africa.

Kenaf seed treatment with ComCat® and thiram did not improve seed germination. A stimulatory effect of ComCat® on seedling emergence, fresh and dry root weight as well as fresh foliage of kenaf seedlings was not observed. ComCat® was nevertheless involved in the increase of dry weight of the above ground parts. The latter suggests that it has a potential role in improving dry matter production of kenaf.

In the field trials, kenaf plants inoculated with *Botrytis cinerea* displayed brown necrotic lesions and girdling of the stem which resulted in loding. There was variation in susceptibility between cultivars. Everglades 41 and SF 459 had the largest and smallest lesion lengths respectively. Surface wetness and temperature are important factors in epidemics of *B. cinerea*. It was found that optimum temperature for mycelial growth of *B. cinerea* isolated from kenaf plants occurred between 15 and 20°C. Studies on the effect of irrigation on the incidence of grey mould, found no significant difference in grey mould incidence between three moisture regimes. Trials were conducted *in vitro* to determine possible fungicides that

would be employed for the control of *B. cinerea*. Benomyl displayed the highest inhibition. Variation in sensitivity to benomyl was observed between *B. cinerea* isolates.

The objectives of the fourth chapter were to characterize *Pyhtium* group G on kenaf in terms of its pathogenicity to kenaf cultivars, optimum temperature requirements and its sensitivity to selected fungicides. In pathogenicity trials, the artificially inoculated fungus colonized the cambial tissue of all ten kenaf cultivars. Reisolation of *Pythium* group G from artificially inoculated tissue confirmed its pathogenicity to kenaf plants. Seedling damping-off studies were conducted by artificially inoculating kenaf seedlings grown in pots in the glasshouse. Mortality of kenaf seedlings occurred rapidly but no significant difference in susceptibility was observed between cultivars. Growth studies conducted *in vitro* found that the optimum temperature for mycelial growth for *Pythium* group G ranged between 20-30°C. Screening of six fungicides was conducted *in vitro* to determine their inhibitory effect on radial colony growth of *Pythium* group G isolate. Dichlorophen and mancozeb/metalaxyl were found to be the most effective fungicides.

Results of this study established that *B. cinerea* and *Pythium* group G are virulent to kenaf and could hamper its establishment as a new crop in South Africa. Satisfactory control of the pathogens could be achieved by the integration of cultural practices and chemical control. Effective programs to monitor the distribution of the pathogens and control strategies should be implemented to prevent serious losses to kenaf.

OPSOMMING

Kenaf word deur middel van saad gepropageer en die verbouing daarvan is dus van hoë kwalitiet saad en die gelymatige opkoms van saailinge afhanklik. Die doel van hoofstuk twee van die huidige studie was om faktore wat verband hou met die vestiging van kenaf vanaf saad te ondersoek. Nege swam-genera is vanaf oppervlakkig-gedisinfesteerde saad van tien kenaf kultivars geïdentifiseer. In alle gevalle was *Alternaria* spp. die mees algemene swamspesie gevolg deur *Chaetomium* spp. Die kultivar, Whitten het die hoogste voorkoms van swamme getoon, met 60% van saad wat besmet was. Van al die swamspesies wat geïsoleer is, was *F. subglutinans* potensieel, die mees patogeniese spesie. In glashuisproewe, het kenaf kultivars wat kunsmatig met *F. subglutinans* geïnokuleer is, siektesimptome getoon wat tot omval-siekte aanleiding gegee het. Dit impliseer dat hierdie patogeen 'n negatiewe impak op kenaf verbouing in Suid-Afrika kan meebring.

Die behandeling van kenaf saad met ComCat® en thiram het nie saadontkieming verbeter nie. 'n Stimulerende effek van ComCat® op saadopkoms, vars- en droëgewig van wortels asook die varsgewig van blare was nie te bespeur nie. ComCat® het desnieteenstaande 'n toename in die bogrondse droëgewig van saailinge veroorsaak wat impliseer dat die produk 'n potensieele rol in die verbouing van kenaf het.

In veldproewe, het kenaf plante wat met *Botrytis cinerea* geïnokuleer is, bruin nekrotiese letsels op die stam getoon wat tot knak van die stam gelei het. Variasie in vatbaarheid tussen kenaf kultivars was duidelik waarneembaar. Everglades 41 en SF 459 het onderskeidelik die grootste en kleinste stamletsels getoon. Vog en temperatuur is belangrike faktore in die infeksie van *B. cinerea*. Daar was gevind dat die optimum

temperatuur vir die groei van *B. cinerea* miselium tussen 15 en 20°C plaasgevind het. Studies op die effek van besproeing op die voorkoms van *B. cinerea* het geen noemenswaardige verskil tussen drie verskillende vogvlakke getoon nie. Proewe was *in vitro* uitgevoer om moontlike fungisiedes vir die beheer van *B. cinerea* te toets. Benomyl het die meeste inhibisie van miseliumgroei getoon. Variasie tussen isolate in terme van hul sensitiwiteit teenoor benomyl was ook waargeneem.

The doelwitte van die vierde hoofstuk was om *Pythium* groep G op kenaf te karaktiseer in terme van patogenisiteit teenoor kultivars, optimum temperatuur en sensitiwiteit teenoor geselekteerde fungisiedes. In patogenisiteitsproewe, het die inokulasie van volwasse plante met die swam duidelike simptome in die cambium veroorsaak. Vooropkomsafsterwing van saailinge was ook ondersoek deur om plante in potte in die glashuis te inokuleer. Afsterwing van saailinge het vinnig plaasgevind maar geen beduidende verskil in vatbaarheid tussen kultivars was waargeneem nie. Studies wat *in vitro* uitgevoer is het gevind dat die optimum groei-temperatuur vir *Pythium* groep G tussen 20 en 30° C is. Proewe was *in vitro* uitgevoer om ses moontlike fungisiedes vir die beheer van 'n *Pythium* groep G isolaat te toets. Dichlorophen en mancozeb/metalaxyl was die mees inhiberende fungisiedes.

Resultate van die huidige studie bewys dat *B. cinerea* en *Pythium* groep G potensieel die verbouing van kenaf as 'n nuwe gewas in Suid-Afrika grootliks kan belemmer. 'n Redelike mate van beheer van hierdie twee patogene kan wel deur middel van geïntegreerde siektebeheermaatreëls bewerkstellig word. Effektiewe programme om die verspreiding van die patogene te monitor asook beheer strategieë behoort dus geïmplementeer te word om ernstige oesverliese te vermy.