EPIDEMIOLOGY AND CONTROL OF *PSEUDOCERCOSPORA ANGOLENSIS* FRUIT AND LEAF SPOT DISEASE ON CITRUS IN ZIMBABWE

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DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any University for a degree.

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M.C. Pretorius Date

SUMMARY

EPIDEMIOLOGY AND CONTROL OF *PSEUDOCERCOSPORA ANGOLENSIS* FRUIT AND LEAF SPOT DISEASE ON CITRUS IN ZIMBABWE

Fruit and Leaf Spot Disease (FLSD) of citrus, caused by *Phaeoramularia angolensis*, is found only in 18 countries in Africa, the Comores Islands in the Indian Ocean and Yemen in the Arabian peninsula. The major citrus export countries in Africa are Morocco, South Africa, Swaziland, and Zimbabwe. Zimbabwe is the only country affected by FLSD. FLSD is a disease of major phytosanitary and economic importance and its devastating effect on citrus is highlighted by the fact that the damage is cosmetic, which renders the fruit unmarketable. Total crop losses are not uncommon in Kenya. The aims of the present study, therefore, was was to determine the occurrence of *P. angolensis* in Zimbabwe and neighbouring Mozambique, to compare these isolates with the *Cercospora* Fresen. isolates from Swaziland and South Africa, to determine the epidemiology of the pathogen and to implement an effective control strategy to prevent the spread of FLSD.

Leaf samples with citrus canker-like lesions collected in the early 1990's in Zimbabwe were found to be infected by the fungus, *Phaeoramularia angolensis.* Surveys were undertaken to determine the spread and intensity of FLSD in Zimbabwe and Mozambique. In Zimbabwe, *P. angolensis* was limited to an area above the 19° south latitude, predominantly the moist areas and not the low-lying drier parts of the country. In Mozambique, no *P. angolensis* symptoms were found. Observations during the survey indicated that no proper management systems were implemented by Zimbabwean growers.

A cercosporoid fungus causing a new Fruit and Leaf Spot Disease on *Citrus* in South Africa was identified. From morphological and rDNA sequence data (ITS 1, 5.8S and ITS 2), it was concluded that the new disease was caused by *Cercospora penzigii*, belonging to the *Cercospora apii* species complex. The genera *Pseudophaeoramularia* and *Phaeoramularia* are regarded as synonyms of *Pseudocercospora*, and subsequently a new combination was proposed in *Pseudocercospora* as *P. angolensis*. *Cercospora gigantea* was shown to not represent a species of *Cercospora*, while *Mycosphaerella citri* was found to be morphologically variable, suggesting that it could represent more than one taxon.

A control strategy for the control of FLSD was evaluated in the study. The data showed that *P. angolensis* in Zimbabwe can be managed successfully by the removal of all old and

neglected orchards, and on timely fungicide applications. Trifloxystrobin + mancozeb + mineral spray oil (20 g + 200 g + 500 mℓ/100 ℓ water) applied in November, January and March was the most effective treatment. Three applications of benomyl + mancozeb + mineral spray oil (25 g + 200 g + 500 mℓ/100 ℓ water) applied during the same period, was the second most effective treatment, and two applications (November and January) of trifloxystrobin + mineral spray oil (20g + 500 mℓ/100 ℓ water) and difenoconazole (40 g) per 100 ℓ/water applied twice in November and January, the third most effective treatment.

The spore trap and weather data showed that *P. angolensis* needs high moisture and temperatures in excess of 25°C for disease development. It is concluded that *P. angolensis* in Zimbabwe can be managed successfully by implementing a holistic approach, which should be supported by the authorities, organised agriculture and all technical personnel involved in citrus production.

OPSOMMING

EPIDEMIOLOGIE EN BEHEER VAN *PSEUDOCERCOSPORA ANGOLENSIS* BLAAR EN VRUGVLEKSIEKTE OP SITRUS IN ZIMBABWE

Blaar- en vrugvleksiekte (BVVS) op sitrus, veroorsaak deur *Phaeoramularia angolensis,* kom in 18 lande in Afrika voor asook die Comores Eilande in die Indiese Oseaan en Yemen op die Arabiese skiereiland. Marokko, Suid Afrika, Swaziland en Zimbabwe is die belangrikste uitvoerders van sitrus in Afrika. Van dié lande het slegs Zimbabwe blaar en vrugvleksiekte op sitrus. Hierdie siekte is van fitosanitêre en ekonomiese waarde en die nadelige effek van die siekte, wat slegs kosmetiese van aard is, is venietigend aangesien vrugte onbemarkbaar is. Totale opbrengsverliese is nie ongewoon in lande soos Kenya nie. Die doelwitte van die studie was dus om die voorkoms van *P. angolensis* in Zimbabwe te bepaal, om die *Cercospora* Fresen. isolate vanaf Swaziland en Suid-Afrika met mekaar te vergelyk, om die epidemiologie van die siekte vas te stel en om ‘n effektiewe beheermaatreël teen die siekte te ondersoek.

Blaarmonsters met kankeragtige letsels wat in die vroeë 1990’s in Zimbabwe gevind is, het getoon dat die blare geinfekteer is met die swam, *Phaeoramularia angolensis.* Ondersoeke is geloots om die verspreiding en intensiteit van BVVS in Zimbabwe en Mosambiek te bepaal. In Zimbabwe was gevind dat *P. angolensis* beperk was tot gebiede bo die 19° Suid breedtegraad, wat die hoër vogtiger gebiede insluit eerder as die droeër, laagliggende gebiede. Geen *P. angolensis* simptome kon in Mosambiek gevind word nie. Tydens die opnames was dit duidelik dat geen geskikte beheerstrategieë toegepas word deur Zimbabwe se produsente nie.

‘n Nuwe cercosporoid swam, wat blaar en vrugvleksiekte op sitrus is in Suid Afrika veroorsaak is geidentifiseer. Morfologiese en rDNA volgorde (ITS 1, 5.8S en ITS 2) data het getoon dat die siekte veroorsaak word deur *Cercospora penzigii* wat tot die *Cercospora apii* spesie kompleks behoort. Die genus *Pseudophaeoramularia* kan as sinoniem van *Pseudocercospora* beskou word en ‘n nuwe kombinasie word voorgestel in *Pseudocercospora* as *P. angolensis. Cercospora gigantea* het getoon dat dit nie ‘n spesie

van *Cercospora* kon verteenwoordig nie terwyl *Mycosphaerella citri* varieërend voorkom en meer as een takson kan verteenwoordig.

‘n Beheerstrategie vir die beheer van BVVS is ondersoek. Die data wys dat *P. angolensis* in Zimbabwe doeltreffend beheer kan word deur die uitroeiing van ou en verwaarloosde bome, en deur goed beplande fungisied bespuiting. Trifloxystrobin + mancozeb + minerale spuitolie (20 g + 200 g + 500 mℓ/100 ℓ water), wat in November, Januarie en Maart toegedien is, was die mees effektiefste behandeling. Drie bespuitings van benomyl + mancozeb + minerale spuitolie (25 g + 200 g + 500 mℓ/100 ℓ water) wat oor dieselfde tydperk toegedien is, was die naas beste behandeling. Trifloxystrobin (20 g) + minerale spuitolie (500 mℓ) per 100 ℓ/water en difenoconazole (40 g) per 100 ℓ/water, beide as twee bespuitings toegedien in November en Januarie, het die derde beste resultaat opgelewer.

Die spoorlokval en klimatologiese data het getoon dat *P. angolensis* vogtige toestande en temperature hoër as 25°C benodig vir siekteontwikkeling. Die afleiding uit die studie is dat *P. angolensis* suksesvol beheer kan word indien ‘n holistiese benadering gevolg word en alle rolspelers naamlik die owerheid, georganiseerde landbou en tegniese personeel die proses ondersteun.

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CONTENTS

1. FRUIT AND LEAF SPOT DISEASE OF CITRUS CAUSED BY *PHAEORAMULARIA ANGOLENSIS* – AN OVERVIEW

INTRODUCTION

Citrus is primarily grown in the subtropics. In Africa, the major citrus producing countries are Algeria, Egypt, Morocco, South Africa, Swaziland, Tunisia and Zimbabwe. The average per capita consumption of citrus fruit in Africa is 3 kg compared to the world average of approximately 12 kg (Fortucci-Marongiu, 1988). In tropical Africa, citrus is planted by small scale resource-poor farmers for local consumption.

Fruit and leaf spot disease (FLSD) of citrus, caused by *Phaeoramularia angolensis* (De Cavalho & Mendes) Kirk [=*Phaeosoriopsis angolensis* = *Cercospora angolensis*] (Kirk, 1986), is found predominantly in Africa. The disease has been observed on all citrus species (Timmer *et al.*, 2000). It is a disease of major phytosanitary and economic importance for citrus producing countries in Africa. The disease was first reported in 1952 in Angola and Mozambique (De Cavalho & Mendes, 1953). Yemen in the Arabian peninsula and the Comores Islands are the only countries outside of continental Africa in which the disease is found.

ECONOMIC IMPORTANCE

The economic importance of FLSD and its devastating effect on citrus was highlighted by Seif (1995). The damage is cosmetic with the development of fruit spots that renders the fruit unmarketable. Total crop losses are not uncommon in Kenya. After the severe disease outbreak during the late 1980's, most growers in Trans-Nzoia, Kenya, replaced their citrus with cereals and vegetables (Seif, 1995).

In Zimbabwe, a totally different situation is evident regarding citrus production compared to most other African countries such as Kenya. In recent years, there has been an increase in the Zimbabwean citrus production with most of the crop destined for export mainly to European markets. It was projected that Zimbabwean exports would increase to 7 million cartons by the year 2000 (S. Hery; HPC – Horticultural Promotion Council, Harare, personal communication, 1999). F. du Pont (Interspan, Harare, Zimbabwe; personal communication 2003) considers citrus as one of the most important export commodities of Zimbabwe earning valuable foreign currency both for the citrus producers and the country as a whole. The quantity exported on average for 2000 (39 468 tons at US$0.34/kg = US$13 419 120), 2001 (45800 tons at

US$0.34/kg = US$15 572 000) and 2002 (43 000 tons at 0.34/kg = US$ 14 620 000) indicated the value of this crop to the country. As a result of the presence of *P. angolensis*, the Directorate of Plant and Quality Control in South Africa decided that fruit harvested from infested areas in Zimbabwe could not be sold in South Africa, and any fruit rejected in South African ports, for whatever reason, had to be destroyed. The presence of the disease is also of quarantine concern to the European Union and this could have major implications for the Zimbabwean citrus industry (H. le Roux, Citrus Research International, Nelspruit, personal communication, 1999).

Since the first report of this disease in Angola and Mozambique (De Calvalho & Mendes, 1952), further information on FLSD can be found in the publications of De Cavalho and Mendes (1953), Doutel (1963) and Querra (1963). Within a period of 38 years the disease has rapidly spread northwards to 14 countries, south of the Sahara (Menyonga, 1971; Brun, 1972; Emechebe, 1981; Seif & Whittle, 1984; Aubert, 1986; Kirk, 1986) and also (eastwards) to the Comores Islands (Aubert, 1986) in the Indian Ocean and Yemen, north of Africa (Kirk, 1986) in the Arabian peninsula (Table 1). Seif and Hillocks (1993) indicated the chronology of spread of FLSC (Table 1) and its distribution (Fig. 1).

SYMPTOMS

Economically important grapefruit (*Citrus grandis* [L.] Osb), orange (*Citrus sinensis* [L.] Osb), mandarin (*Citrus reticulata* Blanco), pomelo (*Citrus paradisi* Macf.), lemon (*Citrus lemon* [L.] Burn. F.), and lime (*Citrus aurantifolia* [Christm] Sw) harbour the disease. Grapefruit, sweet orange and tangerine are highly susceptible, lemon is less susceptible and lime the least (Seif, 2000).

On leaves, the fungus produces a circular spot approximately 10 mm in diameter with a light brown or greyish center. The spots generally occur singly and are usually surrounded by a prominent yellow halo (Fig. 2). However, during the rains, spots on young leaves may coalesce, and this may culminate in general chlorosis. Premature defoliation takes place when leaf petioles are infected. The paper-thin necrotic tissue in the center of old lesions occasionally falls out, creating a shot-hole effect.

On fruit, the infected areas or spots are circular to irregular, discrete or coalescent, and surrounded by yellow halos. Most spots measure up to 8 mm in diameter. On young fruit, symptoms often commence with nipple-like swellings without a yellow halo (Fig. 3). Severely infected fruitlets become mummified. Spots on mature fruit are normally flat and often a dark brown to black sunken margin of anthracnose around the spots is observed (Kirk, 1986).

CAUSAL ORGANISM

According to Seif and Hillocks (1993), the causal organism of FLSD of citrus was identified by De Cavalho and Mendes (1952) as *Cercospora angolensis* (De Cavalho & Mendes). The species was later transferred from *Cercospora* to *Phaeoramularia* (De Cavalho & Mendes) Kirk, comb. nov. (Kirk, 1986). The fungus forms dense tufts (synnemata) of light chestnut-coloured, multiseptate conidiophores (27-240 µm by 3-7 µm), which arises from a stroma and emerge through stomata on the lower leaf surfaces, bearing conidia singly or in chains of two to four (Fig. 4). The conidia are hyaline, cylindrical to slightly flexuous, 1-6 (usually 3 or 4) septate and 23-87 by 3-7 µm.

Isolates of *P. angolensis* were found to grow slowly on potato carrot agar (PCA), malt agar (MA) and carrot juice PDA (CJPDA) incubated at 25°C under continuous light. The surfaces of colonies are greyish in appearance, often velvety and raised at the central point, forming a gnarled mat. The colour of the underside of the colony is dark green. No sporulation was observed on these cultures (Seif & Hillocks, 1993). Ndzoumba (1985), however, obtained abundant sporulation of the fungus on V8A, PDA and mycophyl agar at 25°C irrespective of light regime (continuous light or alternating light and darkness).

EPIDEMIOLOGY

Little is known about the epidemiology of FLSD on citrus. The disease is restricted to the humid tropics in Africa between altitudes 80 and 1500 m (Brun, 1972; Seif & Kungu, 1989). Prolonged wet weather conditions, followed by dry spells coupled with moderate temperatures of 22-26°C favour the disease (Emechebe, 1981; Kungu *et al.*, 1989).

At the onset of rains, new disease-free flushes of leaves are formed, while older leaves may contain a varying number of non-sporulating lesions. These lesions sporulate 3-5 weeks after the start of the rainy season and symptoms on young leaves appear 2-3 weeks later. This suggests that conidia from lesions that were produced during the previous season infect the new flushes and thereby continue the disease cycle (Emechebe, 1981). Seif *et al.* (1993) reported that young fruit up to golf ball size are very susceptible to infection. The inoculum in citrus orchards is derived from infected fruit and foliage, but the contribution of external sources such as neglected orchards that are found in most citrus producing areas in Africa cannot be ignored. Long distance dispersal of the fungus is by windborne conidia (De Cavalho & Mendes, 1952), while spread within the orchards is primarily by means of rain drops laden with spores and rain splash. Humans may be responsible for the inadvertent movement of infected planting material

and fruit between areas. Lesions on leaves produce more conidia than those on fruit and therefore constitute the main source of inoculum for primary and secondary infections in endemic areas (Seif *et al.*, 1993).

DISEASE MANAGEMENT

When Maramba (1982) reported the FLSD problem on citrus in Zimbabwe, no control methods were known at that time. Work done by Rey *et al.* (1988) in West-Africa showed that Perenox (cuprous oxide) and Benlate (benomyl) were the most effective of the fungicides tested.

Seif and Hillocks (1996) reported that timing of chemical sprays is of far greater importance than the fungicides used. The treatments should be applied on the developing citrus crop throughout the rainy season when conditions are such that frequent infection will occur. However, the general use of fungicides is limited in Kenya because it is not cost effective for the small-scale farmers to implement.

CONCLUSION

Knowledge, information and understanding of the biology and epidemiology of *P. angolensis* is essential for the effective control of FLSD on citrus in Zimbabwe. Although the problem is restricted to the African continent, excluding Yemen in the Arabian peninsula and the Comores in the Indian Ocean, limited information on the epidemiology and control of *P. angolensis* on citrus is available. In contrast with tropical African countries where *P. angolensis* is always present, the Zimbabwean citrus industry consists mainly of commercial units and not small-scale, resource-poor farmers (Seif, 1995). In Zimbabwe, the disease only occurs on out-of-season citrus fruit but is visible on leaves (P. Caminada, Chairman of Consultant Association of Zimbabwe, Harare, personal communication, 1999). If this disease is not controlled effectively, it could have huge phytosanitary and economic consequences to both the Zimbabwean and South African citrus industries. The importance of FLSD for both citrus industries prompted these industries to support this study.

During surveys of FLSD on citrus conducted in Zimbabwe and Mozambique, previously unknown leaf and fruit spot disease symptoms were found to be associated with species of *Citrus* cultivated in Swaziland, and the Northern and Mpumalanga Provinces of South Africa. Although symptoms were not as severe as for *Phaeoramularia* fruit and leaf spot, the new cercosporoid disease was still regarded as a potential threat for *Citrus* cultivation. The aims of the present study, was therefore, to determine the spread of *P. angolensis* in Zimbabwe, to compare the *Cercospora* Fresen. isolates from Swaziland and South Africa, to determine the

epidemiology of the pathogen and to implement an effective control strategy to prevent the spread of FLSD.

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Table 1. Chronology of spread of FLSD (*Phaeoramularia angolensis*) on citrus

Country	Year	References
Angola	1952	De Cavalho and Mendes (1952)
Mozambique	1952	De Cavalho and Mendes (1952)
Zaire (DRC)	1966	Brun (1972)
Central African Republic	1968	Brun (1972)
Cameroon	1969	Menyonga (1971)
Congo	1971	Brun (1972)
Togo	1972	Brun (1972)
Zambia	1973	Kirk (1986)
Nigeria	1978	Emechebe (1981)
Burundi	1980	IAPSO (1985)
Zimbabwe	1982	Maramba (1982)
Uganda	1983	Kirk (1986)
Kenya	1984	Seif and Whittle (1984)
Comores	1985	Aubert (1986)
Yemen	1986	Kirk (1986)
Tanzania	1990	National Agricultural Research Laboratories, Kenya

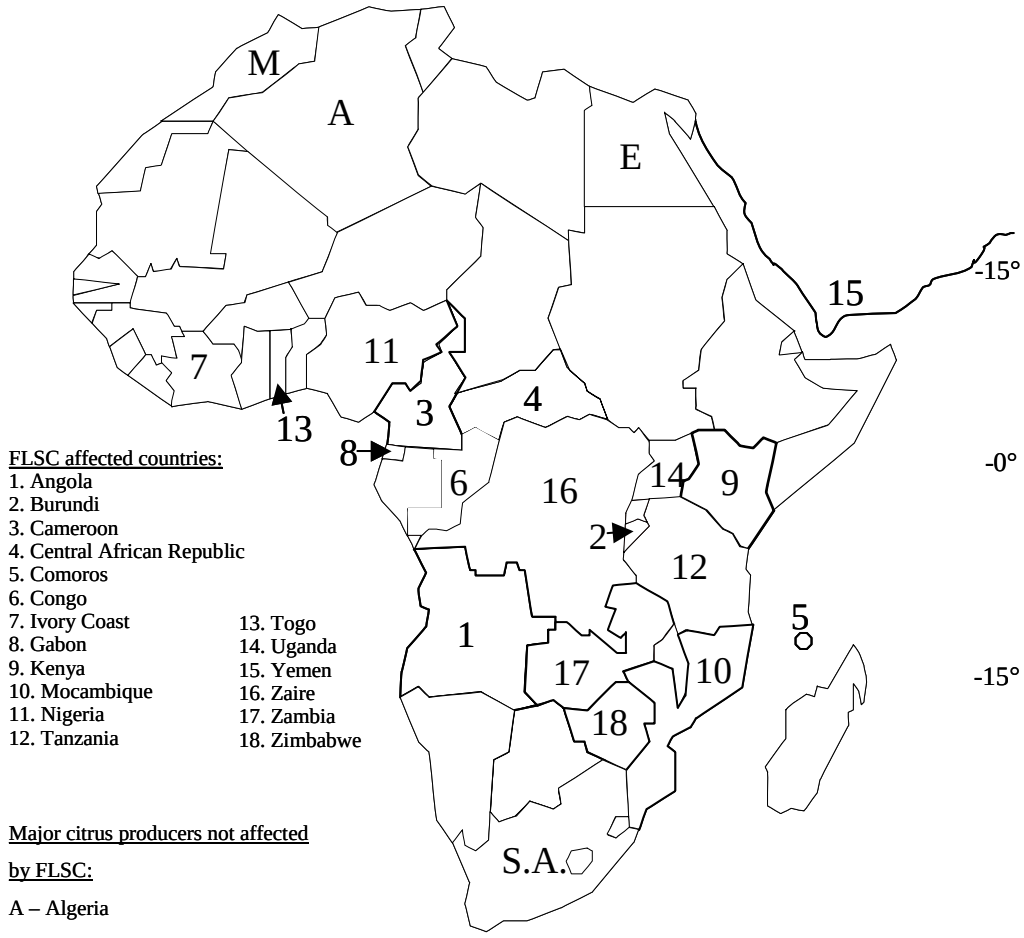

Fig. 1. Distribution of FLSD (*Phaeoramularia angolensis*) in Africa and the neighbouring countries.

Fig. 2. *Phaeoramularia angolensis* lesions surrounded by yellow halos.

Fig. 3. Young fruit infected with *Phaeoramularia angolensis* showing nipple-like symptoms.

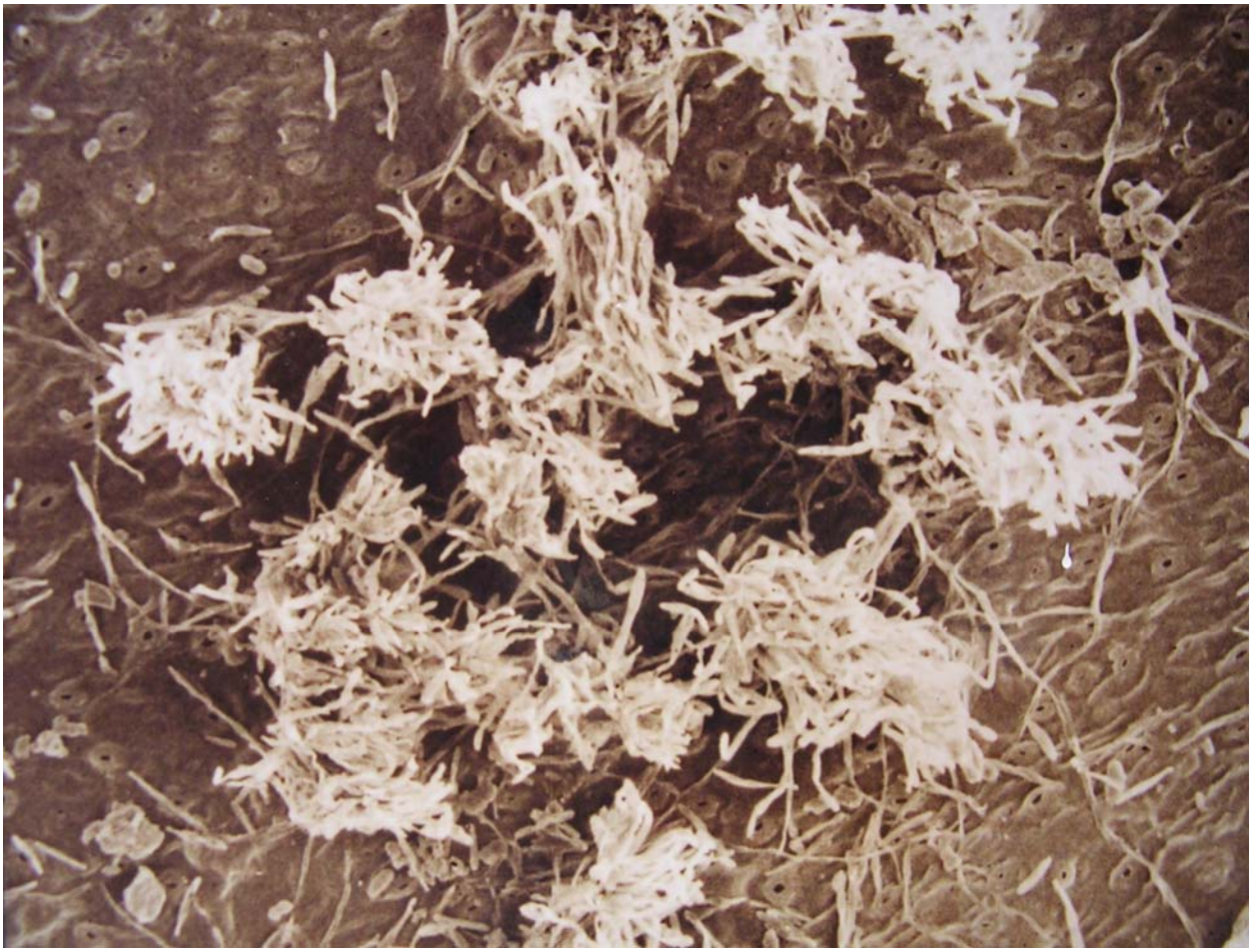

Fig. 4. An electron microscope image of multiseptate conidiophores (27-240 µm by 3-7 µm), which arises from a stroma and emerge through stomata on the lower leaf surfaces, bearing conidia singly or in chains of two to four.

2. OCCURRENCE OF FRUIT AND LEAF SPOT DISEASE (*PHAEORAMULARIA ANGOLENSIS*) ON CITRUS IN ZIMBABWE AND MOZAMBIQUE

ABSTRACT

Leaf samples with citrus canker-like lesions collected in the early 1990's in Zimbabwe were found to be infected by the fungus, *Phaeoramularia angolensis,* causative organism of Fruit and Leaf Spot Disease (FLSD) on citrus. This finding has phytosanitary implications for the region and the extent of infestation in this country needed to be examined. Three surveys were therefore undertaken in Zimbabwe (four geographical areas) and Mozambique (from the southern border to the Beira corridor). In Zimbabwe, *P. angolensis* was limited to an area above the 19° south latitude, predominantly the moist areas and not the low-lying drier parts of the country. In Mozambique, no *P. angolensis* symptoms were noted between the Swaziland border (27^{o} South) and as far north as Mocuba (17^{o} South). Observations during the survey indicated that no proper control management systems were undertaken by the Zimbabwean growers. A preventative control programme needs to be implemented to effectively control this phytosanitary threat in this country.

INTRODUCTION

The southern African citrus industry has been threatened by some serious citrus diseases over the past century. One of these threats was the introduction of citrus canker caused by *Xanthomonas axonopodis* pv. *citri* (Hasse) Dye, into South Africa early in the twentieth century. The successful eradication thereof is an achievement still mentioned in the international media (Schubert *et al.*, 2001). A second threat was citrus greening disease, Huanglongbing, caused by a non-culturable phloem-restricted α -proteobacterium, "*Candidatus* Liberibacter africanus" (Texeira *et al.*, 2005). During the 1970's four of the eleven million citrus trees in South Africa were destroyed by this disease. The citrus research infrastructure and authorities within South Africa had the capacity to address the problem and to develop strategies to cope with this disease (S.P. van Vuuren, Citrus Research International, Nelspruit, personal communication, 2005).

In 1990, citrus leaf samples from Zimbabwe were sent to the South African Co-operative Citrus Exchange's Diagnostic Centre at the Outspan Citrus Centre in Nelspruit, to confirm whether the visible lesions were caused by *Cercospora angolensis.* Although workers at the

Universities of Pretoria and Zimbabwe confirmed the presence of *Xanthomonas,* a citrus cancer specialist from Argentina maintained that citrus cancer is not implicated in the leaf spot lesions observed but confirmed the presence of *Cercospora angolensis* (Le Roux & Pretorius, 1991).

Cercospora angolensis (De Cavalho & Mendes) causes Fruit and Leaf Spot Disease (FLSD) on citrus (De Cavalho & Mendes, 1952). The species was later transferred from *Cercospora* to *Phaeoramularia* (De Cavalho & Mendes) Kirk, comb. nov. (Kirk, 1986). FLSD was reported for the first time in Angola and Mozambique in 1952 (De Calvalho & Mendes, 1952). In 1982, Maramba reported the presence of FLSD in Zimbabwe. Currently, the disease is prevalent in 18 African countries and the Comoros Islands in the Indian ocean, and Yemen on the Arabian Peninsula. All citrus varieties are susceptible to varying degrees with Marsh grapefruit and navel orange cultivars being highly susceptible, and lemons and limes the least (Seif, 1995).

The Zimbabwean citrus industry consists of commercial producers who produce high quality fruit for the export market. Maramba (1982) reported that the disease was present on three farms, all situated north of Harare. The incidence of FLSD on one of the farms was severe and the producer had to destroy his Washington navel orange cultivar orchard. The disease then spread to his 20 year old, highly productive Valencia and grapefruit orchards, which also had to be destroyed. Maramba (1982) hypothesised that the disease was introduced through airborne conidia into Zimbabwe from neighbouring countries north of Zimbabwe.

According to the Horticultural Promotion Council (HPC) an increase of up to 7 million cartons by the year 2000/2001 was anticipated as a result of an increased interest in citrus production by the Zimbabwean growers. Navels and mandarin types were specifically planted for the export market. *Phaeoramularia angolensis* has not been found on commercial fruit in Zimbabwe but can be found on out-of-season fruit in neglected orchards (S. Hery, HPC, Harare, personal communication, 1998). *P. angolensis* that is found on fruit in Kenya, causes yield losses of between 50-100% (Seif & Hillocks, 1997).

The Directorate of Plant and Quality Control of South Africa was concerned about samples taken and inspected in South African harbours from fruit originating from Zimbabwe. *P. angolensis* is regarded as a quarantine organism by South African authorities and imports from countries where the organism is present is not permitted. Consequently the Zimbabwean authorities were informed by South Africa that no fruit would be allowed into South Africa during the 1997 citrus season. It was suggested that a survey be conducted to identify the areas where the fungus occurs and citrus fruit from areas where the disease is present will only be

allowed to be transported in bond through the RSA. Only fruit originating from disease-free areas would be inspected. This is in accordance with the International Standards for Phytosanitary Measures regarding the establishment of pest-free areas. The European Union is also concerned about the presence of *P. angolensis* and this could have major repercussions on the Zimbabwean citrus industry. Therefore, the Ministry of Lands, Agriculture and Water Development was requested by the Zimbabwean citrus growers to co-ordinate a survey in Zimbabwe in order to establish which areas were infested and determine the severity of infestation.

During the late 1990s, visitors to the Inhambane area, in Mozambique, reported lesions similar to those caused by *P. angolensis* on mandarin trees. It was later shown that these symptoms were caused by *Alternaria*. There was, however, the perception that *P. angolensis* could move from the northern areas of Mozambique southwards. This could then pose a threat to the Mpumalanga Lowveld and Swaziland citrus industries. In the past, Outspan International was active in both the south of Mozambique and the Beira corridor. This involvement ceased a few years ago and monitoring on the citrus pest and disease status in Mozambique stopped. Although there is contact between the South African authorities and their Mozambiquan counterparts, the South African Citrus Growers Association was concerned about the threat posed by citrus in Mozambique to the rest of South Africa (H. le Roux, Citrus Research International, Nelspruit, personal communication, 2003).

During 2000, some of the citrus farms in Zimbabwe were taken over by "war veterans" and the trees on these farms were no longer subjected to chemical programmes designed to control diseases and pests, amongst these being *P. angolensis*. Furthermore, there were rumours that the disease had reoccured in the Chegutu area in Zimbabwe. The presence of the disease in this area and in areas where the disease was reduced to undetectable levels late in the 1990s early 2000, needed to be verified (C. Maggs, private consultant, Harare, personal communication, 2003). The aim of the present study, therefore, was to determine the occurrence of *P. angolensis* in Zimbabwe and Mozambique.

MATERIALS AND METHODS

Surveys to detect the presence of FLSD on citrus were conducted in Zimbabwe during August-September 1996 (survey 1), May 1999 (survey 2) and during June/July 2003 (survey 3). The objectives of the first survey were to identify the citrus growing areas of Zimbabwe where *P. angolensis* was present; to develop strategies to manage the disease and to restrict the spread of the disease to non-infected areas. This information would assist the South African

Directorate of Plant and Quality Control's decisions regarding import requirements for Zimbabwean citrus to be sold, inspected and transported through South Africa. The survey were undertaken by one research officer and a senior research technician from the Zimbabwean Directorate of Plant Protection Research Institute, one officer of the South African Directorate of Plant and Quality Control, researchers from Outspan International (Pty) Ltd., and the chairman of the Zimbabwean consultant organisation. Neither the Zimbabwean nor the South African authorities were familiar with the disease other than from photographs. In order to familiarise themselves with the symptoms, they visited a neglected orchard owned by Mr. T. Galante, on the Shamva road, north of Harare, three kilometres from Enterprise, before the survey commenced. Leaf lesions and infected fruit were observed on both navel oranges and grapefruit.

The Zimbabwean authorities selected farms that were representative of the major citrus producing areas throughout Zimbabwe. The production areas were divided into four geographical areas (Fig. 1). These were: Area 1 = north of 18° south latitude and east of the Umvukwe mountain range; Area 2 = north of 18° south latitude and west of the Umvukwe mountain range; Area 3 = between 18° south and 19° south latitude; and Area 4 = south of the 19° south latitude. Farms visited were chosen at random and also included orchards known to be infected by *P. angolensis*. At least 10% of the farms in each of the four production areas were visited and, where possible, all the orchards on each farm were inspected. When orchards exceeded a total of 300 ha, at least 300 ha were inspected. Each orchard was inspected in a criss-cross pattern. Several hundred trees were inspected on each of the farms visited.

After each inspection, the Zimbabwean and South African delegations independently verified the presence of the disease from a specific orchard. Samples were taken from those orchards with trees showing disease symptoms and from orchards where the presence of the disease was uncertain. Leaf samples were taken (no fruit was available during the first survey), placed in labelled brown paper bags and kept in cool box containers until they could be refrigerated.

In the laboratory, leaf samples were incubated at 25°C under high humidity (± 85% RH) to stimulate development or spore release production of *P. angolensis.* Some leaf samples were also placed on potato dextrose agar and incubated at 25°C for 3-14 days before being examined for the development of *P. angolensis.*

In the second survey, personnel of both countries, were again involved and the sampling methods were the same. Areas and orchards where FLSD was observed/identified during survey

1, were re-visited. This was done to confirm the disease status in those areas. The absence of FLSD was also confirmed through checks in orchards where it was absent in survey 1 as well as in Area 4.

Researchers from Citrus Research International and South Africa citrus growers undertook the third survey (Fig. 2). Distribution of the disease was determined in Jun/July 2003, from the most southern parts of Mozambique to the Beira corridor and all the previous inspected areas (Area 1- 4) in Zimbabwe. Only visual evaluations were done.

RESULTS

Data from the Survey, conducted during August-September 1996, are reported in Table 1 and are summarised below. *P. angolensis* was limited to areas north of the 19° south latitude (Areas 1, 2 and 3). Lesions on leaves were old and no longer active, which indicated that further surveys should be conducted earlier in the season. In Area 1, which consisted of 16 farms and covering a total of 1,121 ha, *P. angolensis* was positively identified on six farms. The pathogen was positively identified on three farms in Area 2, which consisted of 6 farms and covering a total of 362,6 ha. In Area 3 *P. angolensis* was identified on only one out of 10 farms, covering a total of 228 ha. The pathogen was not found on leaves from any of the 8 farms in Area 4, which covered a total of 858,5 ha.

The results (Table 2) of the second survey conducted during May 1999 confirmed that *P. angolensis* was present in two areas north of Harare (Bindura and Matepatepa), Area 1. No lesions caused by *P. angolensis* were found in the Karoi area (Area 2) where the disease was identified during the first survey. Areas 3 and 4 were confirmed as disease-free.

The following data were recorded during the Survey 3, which was conducted during June/July 2003 in Zimbabwe and neighbouring Mozambique.

Mozambique:

Area between Maputo and Swaziland – Area I (Fig. 2). Although citrus black spot (CBS) was commonly found on the Valencias, there were no lesions caused by *P. angolensis*.

Area between Maputo and Morrumbene – Area II (Fig. 2). No commercial citrus was left in this region but there were several neglected old orchards, mainly mandarins. No CBS was present and *P. angolensis* was not found on the leaves. Neither was the disease found on the fruit sold by roadside traders.

Area between Morrungulu and Inchope (Beira corridor) – Area III (Fig. 2). A few trees were found at Vilanculos and a few backyard trees along the road. No *P. angolensis* was found.

Area between Mutare (Machipanga) and Inchope (Beira corridor) – Area IV (Fig. 2). All the commercial citrus plantings from Machipanga on the Zimbabwean border at Mutare to Inchope, with the exception of less than ten hectares, had been neglected. In some cases trees still existed but the fruit were small because of a lack of irrigation and showed symptoms of CBS. No *P. angolensis* was found.

Area between the Beira corridor over the Zambezi river up to Nicaudala – Area V (Fig. 2). There were no commercial citrus orchards between Inchope to Nicaudala. No *P. angolensis* was found on leaves of trees or fruit sold by roadside traders.

Area between Quelimane, Nicaudala and Mocuba – Area VI (Fig. 2). Backyard Mandarin trees were abundant. At Nicaudala, Bahianina navels were grown on a government farm. CBS was common but no FLSD symptoms were found.

Zimbabwe – Area VII (Fig. 2*).* The Bindura (Area 1), Mvurwi (Area 2) and Chegutu (Area 3) areas were visited (Fig. 1) CBS was found in both areas. *P. angolensis* was again found in the Bindura area (Area 1). Symptoms had reappeared in all three of the orchards visited in the Mvurwi area (Area 2). No *P. angolensis* symptoms could be found on farms in the Chegutu area (Area 3).

DISCUSSION

The observation that *P. angolensis* was limited to areas above the 19° south latitude (Areas 1, 2 and 3) confirmed Maramba's (1982) findings. The results also indicated the southern citrus producing areas to be disease-free.

Based on the data of the survey, the South African authorities had notified the Zimbabweans that no fruit that was produced in disease-positive areas above the 19° south latitude (which include areas 1, 2 and 3) will be inspected or sold in South Africa. Citrus fruit from these areas may, however, be transported in bond through South Africa. Area 4 was excluded from this regulation but the Zimbabwean Government must undertake that fruit entering South Africa was indeed produced in this area and that these areas remain disease free. It was recommended that all the neglected orchards identified during the surveys be removed and burned in order to prevent the spread of the disease.

The second survey demonstrated the importance of conducting a survey earlier in the season. Much needed information regarding the status of the disease was gathered that assisted researchers and the authorities to formulate a control strategy to reduce or eradicate the disease from the infected areas. This information could be used in restricting the movement of plant material or fruit from infested areas to disease-free areas. It was also clear that producers were more aware of the disease as neglected orchards were being removed and burned since the first survey.

From the third survey, the following conclusions were made. In Mozambique, with the exception of Citrum, there was no commercial citrus export industry left. It was unlikely that any citrus industry would be re-established in the near future because of a lack of expertise and infrastructure. This was in spite of the fact that excellent grapefruit and Valencias could be produced in the southern parts of the country. No *P. angolensis* symptoms were seen on the leaves or fruit inspected on trees nor on any of the thousands of fruit sold by roadside traders between the Swaziland border (27^{o} south) and as far north as Mocuba (17^{o} south). At that stage, most of the citrus produced by subsistence farmers were Empress mandarins, old clone Valencias and Bahianina navels. The natural movement of citrus diseases from the Beira corridor to the south of Mozambique was unlikely. This was due to a 500 km citrus free zone south of the Beira corridor. There was still a danger that the disease could be spread through the movement of plant material To the north of the Beira corridor there was also an area of almost 400 km which is free of citrus, allowing a further buffer to the north. No citrus diseases, other than those already occurring in South Africa and Swaziland, could be found.

The citrus industry in Zimbabwe was still found to be in a surprisingly good condition after the third survey in July 2003, despite the fact that 40% of the farms had been taken over by war veterans. The exports were only expected to drop from 3 million to 2,5 million cartons that year (C. Maggs, private consultant, Harare, personal communication, 2003). This was because the farms occupied were in a good condition when they were taken over. Some farms obtained packouts as high as 60%. However, this situation would not continue unless they adhered to the standard spray, irrigation and fertilisation programmes that would ensure export quality yields in the coming seasons.

P. angolensis was again found at Bindura. There was also a low incidence of the disease on some of the farms occupied by the war veterans in the Mvurwi area, an area where *P. angolensis* had been once reduced to undetectable levels. Farms inspected in the Chegutu area were still free of the disease. It was believed that either leafhopper damage or severe grey mite

(Fig. 3), infestations and sunburn lesions on the concentric ring blotches may have been mistaken for *P. angolensis* (Fig. 4) in this region.

It is recommended that Zimbabwean authorities conduct surveys on a regular basis, at least every three years, to ensure that *P. angolensis* does not penetrate the disease-free areas and to determine the status of the disease in areas known to harbour the disease. Zimbabwe should implement control measures to prevent the movement of any plant material from the infected areas and to reduce or eradicate the disease by means of effective chemical control measures. An eradication programme in the infected areas should remain in place as a preventative programme to prevent orchards becoming the source of inoculum in these areas. All cultural practices, as recommended in the Citrus Production Guidelines (2003) should be followed. Irrigation should be scheduled so as to synchronise the blossom, and prevent an out-of-season blossom which would enhance the chances of out-of-season fruit being infected with *P. angolensis*.

Visual inspections in Zimbabwe indicated that *P. angolensis* only occurs in the moist areas rather than the low-lying drier parts of the country. This observation correlates with Seif & Hillocks (1995) observations in Kenya.

The surveys in Mozambique and Zimbabwe indicated that there was no immediate plant pathological invasion danger with specific reference to *P. angolensis* to the South African citrus industry from these geographical regions.

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Table 1. Area and citrus orchards inspected during August-September 1996 in Zimbabwe, and designated FLSD (*Phaeoramularia angolensis*) positive or negative

Magisterial District	Grower	Farm name	Cultivar	Result	Farm size (ha)	Tree age (years)
			AREA 1			
Bindura	W Reed	Chinyudze	Valencias Navels	Negative	100	12
	P Metcalf		Valencias	Negative	20	3
	C Taylor		Valencias	Negative	20	4-6
	E Fynes-Clinton	Satawa	Navels, Valencias	Positive	55	22
Matepatepa	B McKersie	Duna Verty	Lemons	Negative	0.5	13
	H Bosman	Argyle	Lemons	Negative	15	1-2
	R Tate	Frinton	Navels, Valencias Lemons	Positive	24	8-10
	A Harvey	LagLagnaha	Navels, Lemons	Positive	1	20-30
Shamva	L Steyn	Riverend	Valencias, grapefruit, Minneolas	Positive	80	2-5
Concession	R Tarr	Mountain Home	Valencias, Clementines	Negative	8	5
Glendale	D Sole	Bauhinia	Navels, Valencias	Positive	10	10-12
	Arrowsmith	Kwayedze	Navels	Positive	2	15
Mazowe	Mazowe citrus	Mazowe Citrus	Navels, Valencias, Lemons, grapefruit, Clementines	Negative	500	4-40
Mvurwi	J Perrot	Macheri	Navels	Negative	52	5
	C Maggs	Highveld Hort.	Lemons, navels, Valencias, grapefruit	Negative	210	1-2
	J Taylor	Vita fruits	Navels, Clementines, Valencias	Negative	24	2
TOTAL	**16 Farms**			**Neg 10 Pos 6**	**1121.5**	
			AREA 2			
Guruwe	C Deall		Valencias, navels	Negative	45	5
Banket	G Watson	Hillpass Ests	Navels	Negative	62	4
Chinhoyi	D Wilken	Gamanya	Valencias	Positive	210	1
Mhangura	M Hall	Chipiri	Novas	Positive	7	5
Karoi	S Botha	Childerly	Valencias, Clementines	Negative	3,6	8
	F Mitchell	Kevlyn	Navels, Valencias	Positive	35	7
TOTAL	**6 Farms**			**Neg 3 Pos 3**	**362.6**	
			AREA 3			
Bromley	M Cullingham	Sky Farms	Navels, Valencias, Clementines	Negative	10	6
Headlands	B Masson	Precincts	Clementines	Negative	10	5
Rusape	E Mordt	Rockingstone	Navels, lemons Clementines, Valencias	Positive	25	3
Nyazura	C van Vuuren	Christobello	Navels, Valencias	Negative	9	4
Odzi	F Holman	Amberwell	Navels, Clementines, Valencias	Negative	23	2

Table 1. Continued

Magisterial district	Grower	Farm name	Cultivar	Result	Farm size (ha)	Tree age (years)
			AREA 3			
Chegutu	T Beattie	Lions Vlei	Valencias, navels, Clementines	Negative	90	5
	M Campbell	Mount Carmel	Clementines, navels, Valencias	Negative	18	12
Kodoma	A Kirkman	Msweswe	Minneolas	Negative	15	14
Chakari	M Kemple	Blackmorevale	Navels, Valencias	Negative	15	10
Kwekwe	N Newbold	Umlala Park	Navels, Valencias	Negative	13	14
TOTAL	**10 Farms**			**Neg 9 Pos 1**	**228**	
			AREA 4			
Mid Savie	ADA, Mid Savie	ADA	Valencias, grapefruit	Negative	12,5	1
	J Souchen	Mauricia	Valencias, grapefruit	Negative	30	4
Chiredzi	Hippo Valley	Hippo Valley	Valencias, grapefruit	Negative	20	4-30
	J Baldwin	Mopani Vale	Lemons	Negative	11	1-4
	E Harrison	Maioio	Valencias	Negative	42	1-5
Beitbridge	R Park	Bishopstone	Navels, Valencias, grapefruit	Negative	350	5-20
	D Bristow	-	Valencias	Negative	28	2
	K Knott	Knottingham	Navels, Valencias, grapefruit	Negative	365	1-40
TOTAL	**8 Farms**			**Neg 8 Pos 0**	**858.5**	
GRAND TOTAL	**40 Farms**			**Neg 30 Pos 10**	**2570.6**	

Table 2. Area and citrus orchards inspected during May 1999 in Zimbabwe, and designated FLSD (*Phaeoramularia angolensis*) positive or negative

Magisterial district	Grower	Farm name	Cultivars	Result	Farm size (ha)	Tree age (years)
			AREA 1			
Bindura	W Reed	Chinyudze	Valencias Navels	Negative	100	15
	C Taylor		Valencias	Negative	20	9
	E Fynes-Clinton	Satawa	Navels, Valencias	Positive	55	25
Matepatepa	B McKersie	Duna Verty	Lemons	Negative	1	16
	R Tate	Frinton	Navels, Valencias Lemons	Positive	24	13
	A Harvey	LagLagnaha	Navels, Lemons	Negative	1	23-33
Shamva	L Steyn	Riverend	Valencias, grapefruit, Minneolas	Negative	80	8
Concession	R Tarr	Mountain Home	Valencias, Clementines	Negative	8	8
Glendale	D Sole	Bauhinia	Navels, Valencias	Negative	10	14
	Arrowsmith	Kwayedze	Navels	Negative	2	18
Mazowe	Mazowe citrus	Mazowe Citrus	Navels, Valencias, Lemons, grapefruit, Clementines	Negative	100	7-35+
Mvurwi	J Perrot	Macheri	Navels	Negative	52	8
	C Maggs	Highveld Hort.	Lemons, navels, Valencias, grapefruit	Negative	120	5
TOTAL	**13 Farms**			**Neg 11 Pos 2**	**573**	
			AREA 2			
Banket	G Watson	Hillpass Ests	Navels	Negative	62	7
Chinhoyi	D Wilken	Gamanya	Valencias	Negative	210	4
Mhangura	M Hall	Chipiri	Novas	Negative	7	8
Karoi	F Mitchell	Kevlyn	Navels, Valencias	Negative	35	10
TOTAL	**4 Farms**			**Neg 4 Pos 0**	**312**	
			AREA 3			
Headlands	B Masson	Precincts	Clementines	Negative	10	8
Rusape	E Mordt	Rockingstone	Navels, lemons Clementines, Valencias	Negative	25	8
Odzi	F Holman	Amberwell	Navels, Clementines, Valencias	Negative	20	5
Chegutu	T Beattie	Lions Vlei	Valencias, navels, Clementines	Negative	80	8
Kwekwe	N Newbold	Umlala Park	Navels, Valencias	Negative	10	17
TOTAL	**5 Farms**			**Neg 5 Pos 0**	**125**	
			AREA 4			
Mid Savie	J Souchen	Mauricia	Valencias, grapefruit	Negative	30	7
Chiredzi	Hippo Valley	HIppo Valley	Valencias, grapefruit	Negative	20	7-30+
Beitbridge	R Park	Bishopstone	Navels, Valencias, grapefruit	Negative	200	8-20+
TOTAL	**3 Farms**			**Neg 3 Pos 0**	**250**	
GRAND TOTAL	**25 Farms visited**			**Neg 23 Pos 2**	**1260**	

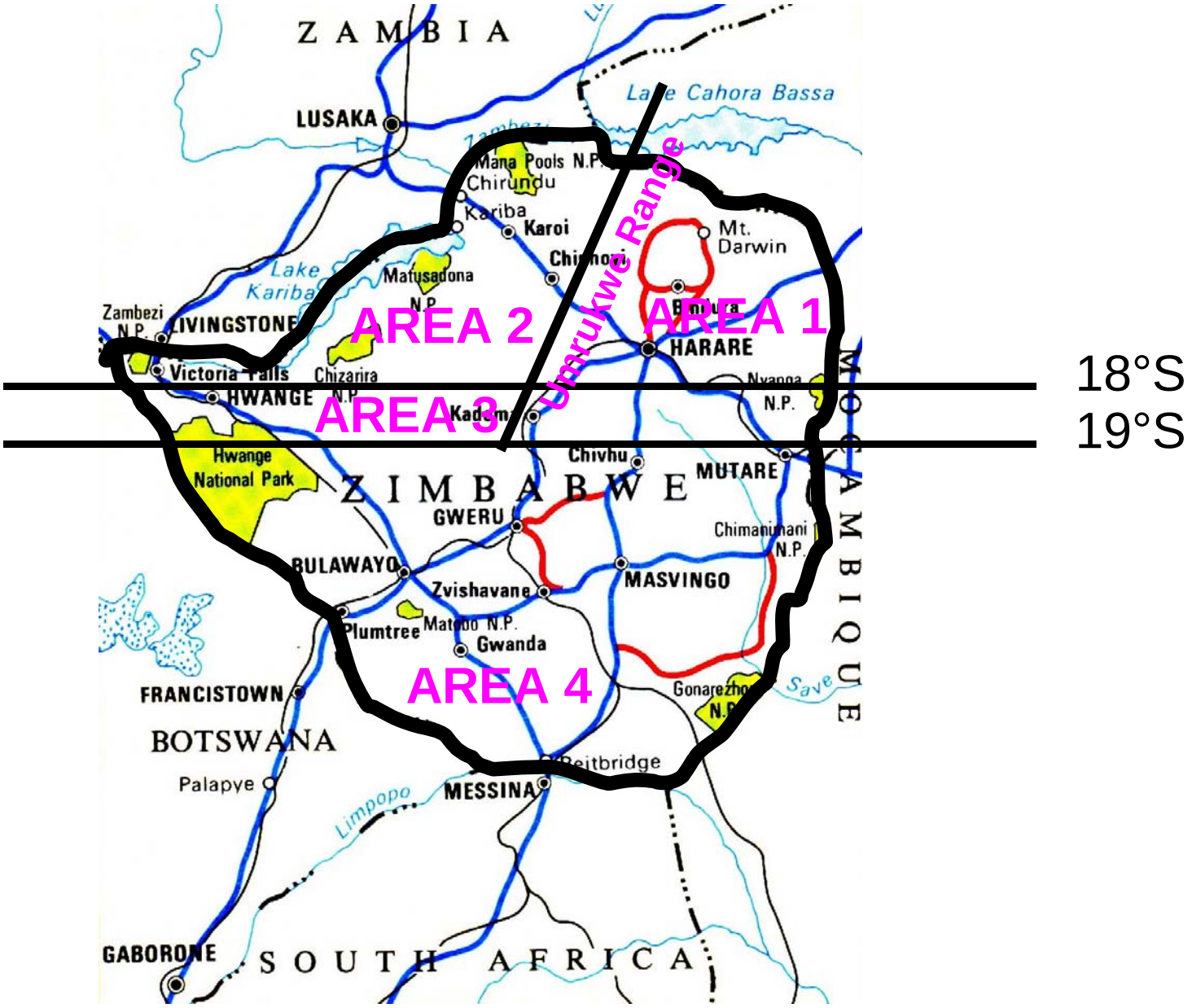

Fig. 1. The geographical citrus producing areas in Zimbabwe from where the surveys for FLSD was conducted during the years 1996, 1999 and 2003.

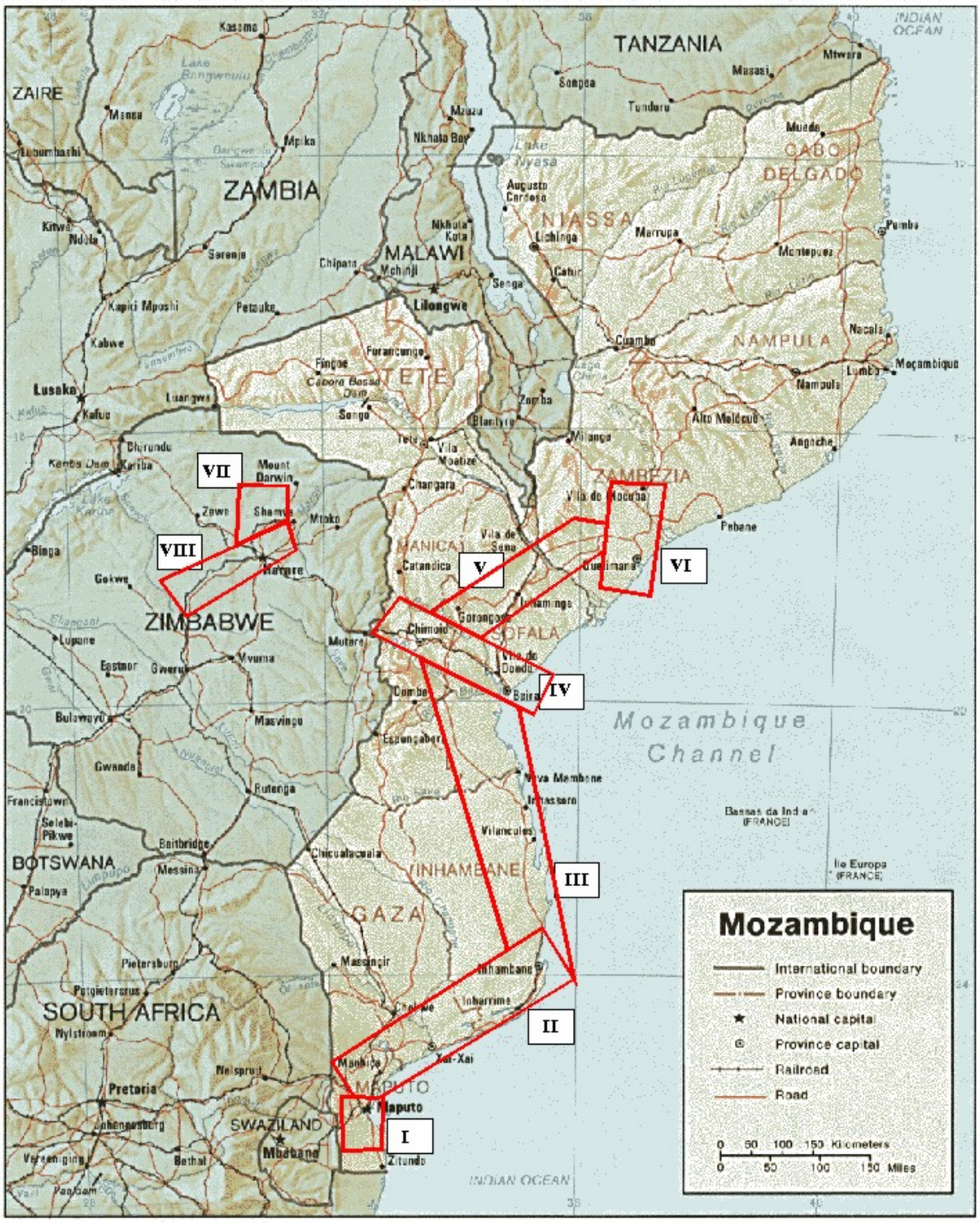

Fig. 2. Areas (I-VIII) inspected for FLSD (*Phaeoramularia angolensis*) during June/July 2003 in Mozambique and Zimbabwe.

Fig. 3. Concentric ring blotch on citrus leaves caused by citrus grey mite found in the Chegutu region in Zimbabwe.

Fig. 4. Typical *Phaeoramularia angolensis* lesions on fruit and leaves of citrus found in Zimbabwe.

3. PHYLOGENY OF SOME CERCOSPOROID FUNGI FROM *CITRUS IN SOUTHERN AFRICA*

ABSTRACT

This study examines several cercosporoid species that are known to cause foliar diseases of *Citrus*. A cercosporoid fungus causing a new fruit and leaf spot disease on *Citrus* in South Africa was identified. From morphological and rDNA sequence data (ITS 1, 5.8S and ITS 2), it was concluded that the new disease was caused by *Cercospora penzigii*, belonging to the *Cercospora apii* species complex. It was subsequently compared with a similar organism, *Pseudophaeoramularia angolensis*, which is of quarantine significance to the citrus industry. The genus *Pseudophaeoramularia* is regarded as synonym of *Pseudocercospora*, and subsequently a new combination is proposed in *Pseudocercospora* as *P. angolensis*. *Cercospora gigantea* was shown to not represent a species of *Cercospora*, while *Mycosphaerella citri* was found to be morphologically variable, suggesting that it could represent more than one taxon. A key is also provided to the cercosporoid species occurring on *Citrus*.

INTRODUCTION

A wide range of *Mycosphaerella* Johanson species with cercosporoid anamorphs are commonly associated with fruit and leaf spot diseases of species of *Citrus* L. Of these, two are regarded as being particularly serious. Greasy spot, caused by *Mycosphaerella citri* Whiteside (anamorph *Stenella citri-grisea* (F.E. Fisher) Sivan.) (Sivanesan, 1984), occurs in Florida and Texas (USA), the Caribbean, and Central and South America (Timmer & Gottwald, 2000). *Phaeoramularia* fruit and leaf spot, caused by *Phaeoramularia angolensis* (T. Carvalho & O. Mendes) U. Braun, is common in sub-Saharan Africa, the Comoro Islands, and has also been reported from Yemen on the Arabian Peninsula (Seif, 2000). The most devastating effect of *Phaeoramularia* fruit and leaf spot is the development of fruit spots, which render the crop unmarketable. A yield loss of 50–100% is common in highly effected areas (Seif, 1995). As *Phaeoramularia* fruit and leaf spot also occurs in Zimbabwe, which borders South Africa, it is of particular concern to the local citrus industry. Although the disease is presently restricted to two areas north of Harare in Zimbabwe, it has not yet spread to South Africa (Crous *et al*., 2000b), presumably due to unfavourable climatic conditions. However, this organism is still regarded as of extreme phytosanitary importance.

During the course of 2000, previously unknown leaf and fruit spot disease symptoms were found associated with species of *Citrus* cultivated in Swaziland, and the Northern and Mpumalanga Provinces of South Africa. Although symptoms were not as severe as for *Phaeoramularia* fruit and leaf spot, the new cercosporoid disease was still regarded as a potential threat for *Citrus* cultivation. The aim of the present study, was therefore, to identify the *Cercospora* Fresen. isolates from Swaziland and South Africa. These isolates were also phylogenetically compared with other cercosporoid fungi occurring on *Citrus* spp., and specifically to *C. apii* Fresen., to which they were morphologically similar.

MATERIALS AND METHODS

Morphology. Herbarium and type specimens were obtained from USDA U.S. National Fungus Collections, Beltsville (BPI), CABI Bioscience, Egham, England (IMI), and the Department of Plant Pathology at the University of Florida (F). Morphological observations were made from structures mounted in clear lactophenol, and descriptions were based on collections from host material. All measurements were derived from at least 30 observations of each respective structure. Cultures were obtained from freshly collected field material (*Cercospora* sp. and *P. angolensis*) by establishing colonies from single conidia on 2% malt extract agar (MEA) (Biolab, Midrand, Johannesburg). Isolates are maintained in the culture collection of the Department of Plant Pathology, University of Stellenbosch, South Africa (STE-U) and the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands.

PCR amplification and sequencing. The isolation protocol of Crous *et al*. (2000a) was used to isolate genomic DNA from fungal mycelia grown on MEA plates. The primers ITS1 and ITS4 were used to amplify part of the nuclear rRNA operon using the PCR conditions recommended by White *et al*., (1990). The amplified region included the 3' end of the 18S (small subunit) rRNA gene, the first internal transcribed spacer (ITS1), the 5.8S rRNA gene, the second ITS (ITS2) region and the 5'end of the 28S (large subunit) of the rRNA gene. PCR products were separated by electrophoresis at 75 V for 1 h in a 0.8% (w/v) agarose gel in 0.5 x TAE buffer (0.4 M Tris, 0.05 M NaAc, and 0.01 M EDTA, pH 7.85) and visualised under UV light using a GeneGenius Gel Documentation and Analysis System (Syngene, Cambridge, UK) following ethidium bromide staining.

PCR products were purified by using a NucleoSpin Extract 2 in 1 Purification Kit (Macherey-Nagel GmbH, Germany). The cycle sequencing reaction of purified PCR products was carried out with an ABI PRISM BigDye Terminator v3.0 Cycle Sequencing Ready Reaction Kit (PE

Biosystems, Foster City, CA, USA) following the instructions of the manufacturer. The resulting fragments were analysed on an ABI Prism 3100 DNA Sequencer (Perkin-Elmer, Norwalk, Connecticut). The isolates that were subjected to molecular analysis are listed in Table 1. The unidentified *Cercospora* isolates from *Citrus* were compared to other species of *Cercospora*, and to *C. apii*, from which they were morphologically indistinguishable.

Phylogenetic analysis. The nucleotide sequences generated in this study were added to a previously published data matrix (TreeBase M691, Stewart *et al*., 1999). *Mycocentrospora acerina* (R. Hartig) Deighton AY266155 served as outgroup. Sequences were assembled using the editor in PAUP (Phylogenetic Analysis Using Parsimony) version 4.0b8a (Swofford, 2000), and aligned using the CLUSTAL W software (Thompson *et al*., 1994). Adjustments for improvement were done manually where necessary. Phylogenetic analyses were undertaken using PAUP. Gaps were treated as a new state and all characters were unordered and weighted equally. Heuristic searches were conducted using stepwise simple addition and tree bisection and reconstruction (TBR). The robustness of the branches was evaluated by 1000 bootstrap replications (Hillis & Bull, 1993). A second parsimony analysis was also performed for which all missing and ambiguous characters were excluded. Tree length, consistency index, retention index and rescaled consistency index (CI, RI and RC, respectively) were also calculated. Resulting trees were printed with TreeView Version 1.6.5 (Page, 1996) and decay indices were calculated with AutoDecay Version 4.0.2 (Eriksson, 1998). Sequences were deposited at GenBank (Table 1), and the alignment in TreeBase (submission number SN1397).

Nucleotide differences between and within *Cercospora* species. The number and type of nucleotide differences between the 25 *Cercospora* sequences used in this study were tabulated using *C. apii* Fresen. AY266168 (TreeBase matrix M691; Stewart *et al*., 1999) as reference sequence. The differences within isolates of *C. penzigii* Sacc. were calculated separately. Separate counts for transversions, transitions, insertions and deletions in the ITS1, 5.8S and ITS2 regions, respectively, were made for all of the *Cercospora* sequences included in this paper.

RESULTS AND DISCUSSION

Morphology. Isolates causing the new disease on *Citrus* in Swaziland and South Africa were morphologically similar and were indistinguishable from *C. penzigii*, which is the common species of *Cercospora* occurring on this host (Chupp, 1954). They had long, fasciculate, septate, smooth, pigmented conidiophores with thickened, darkened and refractive loci. Fully developed

long conidia were acicular with truncate bases, whereas young, shorter conidia were obclavate to subcylindrical with obconically subtruncate bases and darkened, thickened, refractive hila. *Cercospora apii* is a species with a wide host range and geographical distribution (Pons & Sutton, 1988), with which *C. penzigii* appears to be synonymous.

Sequence alignment. All the *Cercospora* sequences used in the phylogenetic analysis, except for *C. oryzae* STE-U 4303 (one nucleotide shorter) and *C. asparagi* Sacc. AF297229 (one nucleotide longer), were exactly the same length (462 bp, including 5 bp of the 3' end of the 18S rDNA gene and 11 bp of the 5' end of the 28S rDNA gene) when alignment gaps were excluded. The alignment contained the complete sequences of the 5.8S rRNA gene, the second ITS (ITS2) region and the 5'end of the 28S (large subunit) of the rRNA gene. The complete ITS1 region was not included in the phylogenetic analysis of this study as the sequences of *P. angolensis* STE-U 4115, 4116 and 4118 included in the alignment did not contain the first eighteen nucleotides of the ITS1 region. However, for counting the nucleotide changes between the *Cercospora* species, the complete ITS1 was included. The manually adjusted alignments of the nucleotide sequences contained 520 sites for the data set (data not shown). Of the aligned nucleotide sites for the data set, 166 characters were parsimony-informative, 127 variable characters were parsimony-uninformative and 227 were constant.

Phylogenetic relationships. The aligned sequences of 37 isolates and an outgroup were subjected to maximum parsimony analysis, and only a single most parsimonious tree was obtained and evaluated with 1000 bootstrap replications. All 25 *Cercospora* sequences grouped in a strongly supported clade (99 % support) (Fig. 1) as did *Pseudocercospora* Speg. (99 %) and *Stenella* Syd. (100 %). In the main *Cercospora* clade, *C. oryzae* (= *Passalora janseana* (Racib.) U. Braun) STE-U 4303 and *C. canescens* AY266164 (TreeBase matrix M691; Stewart *et al*., 1999) (identifications could not be confirmed) were found outside a clade containing the majority of the *Cercospora* species (74 %). *Cercospora zebrina* Pass., a species with acicular to cylindrical–filiform conidia, formed a clade with a bootstrap support value of 63 % within the larger *Cercospora* clade. Exclusion of missing and ambiguous characters from the analysis did not change the topology of the tree.

Nucleotide differences between and within *Cercospora* species. The decrease in length for '*C. oryzae*' STE-U 4303 can be ascribed to a deletion of a G at character 357, and the increase in length for *C. asparagi* AF297229 can be accounted for by an extra C at character 101 of the alignment (Table 2). Eight isolates had sequences identical to *C. apii* CA1 (TreeBase matrix

M691; Stewart *et al*., 1999): *C. canescens* Ellis and G. Martin STE-U 1137 and 1138, *C. nicotianae* Ellis and Everh. AF297230, *C. sorghi* Ellis and Everh. *f. maydis* AF297232, *C. beticola* Sacc. AF222827, *C. penzigii* STE-U 4408 and 4409 and *C. hayi* Calp. CH6 (TreeBase matrix M691, Stewart *et al*., 1999). Of the remaining sixteen isolates, '*C. oryzae*' STE-U 4303 and '*C. canescens*' AY266164 (TreeBase matrix M691, Stewart *et al.,* 1999) differed most from *C. apii* AY266168 (TreeBase matrix M691, Stewart *et al*., 1999), with changes at nine and three positions respectively. Eighteen changes were observed for the eleven *Cercospora* species studied (Table 2), resulting in a difference of 1.64 (18 changes over 11 species) nucleotides between species. Goodwin *et al*. (2001) calculated an overall mean of 1.27 differences between taxa in their *Cercospora* cluster, which is slightly lower than what we found. This might be ascribed to the sampling of 18 isolates representing 11 species by Goodwin *et al*. (2001) whereas 25 isolates representing 11 species was sampled in the present study. Within *Cercospora*, twelve transitions, four transversions and a single duplication and deletion were observed. Goodwin *et al*. (2001) also observed more transitions than transversions for *Cercospora* and *Mycosphaerella* based on the ITS region.

Based on the ITS sequence, *C. penzigii* is distributed over three groups (Table 2): the first group contains two isolates (STE-U 4408 and 4409) identical to *C. apii* AY266168 (TreeBase matrix M691); the second group contains five isolates (STE-U 4410, 4411, 3946, 3947 and 3945) as well as *C. populicola* Tharp STE-U 1051, that differed from *C. apii* AY266168 (TreeBase matrix M691) at character 502; and the final group consisted of three isolates (STE-U 3948, 3949 and 3950) that contained the same two changes as *C. hayi* AY266163 (TreeBase matrix M691). The third group has the same change at character 502 as the second group, but also an additional change at character 500. The *C. penzigii* of the first group was isolated on citrus fruit, whereas the *C. penzigii* isolates in groups two and three were isolated from leaf spots.

There was no difference between the number of changes in the ITS1 and ITS2 regions of the *Cercospora* sequences (5 changes each between the eleven species). However, eight changes in the sequence of the 5.8S gene were observed between the eleven species. All eight changes occurred in *C. oryzae* STE-U 4303 and *C. canescens* AY266164 (TreeBase matrix M691), whereas no changes were observed for this region in the rest of the *Cercospora* isolates. Goodwin *et al*. (2001) also reported a very small difference in the number of changes between the ITS1 and ITS2 region, but found no changes in the 5.8S gene. As *C. oryzae* STE-U 4303 and *C. canescens* AY266164 (TreeBase matrix M691) clustered outside the main *Cercospora* clade,

it appears that they are not part of the *C. apii* complex, and that the *Cercospora* isolates in the main clade (74 % bootstrap support) represent *C. apii sensu lato*.

TREATMENT OF SPECIES

***Cercospora gigantea* F.E. Fisher, Phytopathology 51: 300. 1961 (Fig. 2).**

Hosts and distribution. *Citrus sinensis* Pers., *C. paradisi* Macfad. (Rutaceae), USA (FL).

Specimen examined. USA, Florida, Orange County, Winter Park, on grapefruit leaves, F. Fisher, 28 May 1957, F-46419 (holotype).

Cercospora gigantea was described as having straight, fasciculate conidiophores with broad, 3–12-septate, brown conidia with rounded apices and beveled bases, 80–180 x 6–8 µm (Fisher, 1961). Although the specimen is in a poor condition, a few conidia fitting this description were found. However, the conidia are distoseptate with darkened, thickened hila, and resemble those of *Corynespora citricola* M. B. Ellis (Ellis, 1971). The poor quality of the type specimen, however, made it impossible to resolve this issue.

***Cercospora penzigii* Sacc., Syll. Fung. 15: 84. 1901(Fig. 3).**

≡ *Cercospora fumosa* Penz., Michelia 2: 476. 1882, non *C. fumosa* Speg., 1880.
= *Cercospora aurantia* Heald and F.A. Wolf, Mycologia 3: 15. 1911.
= *Cercospora daidai* Hara, List of Japanese Fungi ed. 4: 400. 1954.

Leaf spots amphigenous, circular to irregular, 2–30 mm diam., pale to dark brown, margin raised on lower surface, medium brown, surrounded by a chlorotic zone. – Caespituli chiefly hypophyllous, fascicles dense to loose and divergent; more compact with shorter conidiophores on epiphyllous surface. – Stromata medium to dark brown, erumpent, up to 70 µm diam.; fascicles grey (compared to brown tufts of *P. angolensis*). – Mycelium internal, pale brown, consisting of septate, branched, smooth hyphae, 3–4 µm. – Conidiophores in loose to dense fascicles, arising from stromata, straight to geniculate-sinuous, subcylindrical, unbranched, 20–300 x 4–6.5 µm, multi-septate, pale to medium brown, smooth. – Conidiogenous cells terminal, pale brown, smooth, tapering to an subobtuse or swollen apex, 20–60 x 3–5 µm; scars thickened, darkened and refractive. – Conidia solitary, long, fully developed conidia acicular, short conidia obclavate or subcylindrical, 50–300 x 2.5–5 µm, multi-septate, hyaline, apex obtuse to subacute to subobtuse, base truncate in acicular conidia or long obconically subtruncate

in obclavate-cylindrical conidia, hilum thickened, darkened and refractive; secondary conidia arising via microcyclic conidiation hyaline, subcylindrical to acicular or obclavate, 1–3-septate, 15–35 x 2–3.5 μm, with thickened, darkened and refractive hila.

Hosts and distribution. Araujia albens G. Don, Citrus aurantium L., C. junos Sieb. ex Tanaka, C. limon (L.) Burm.f, C. natsudaidai Hayata, C. nobilis Lour., C. paradisi hybrid, C. sinensis, Dictamnus dasycarpus Turcz., Poncirus trifoliatus Rafin. (Rutaceae), Algeria, Argentina, Azerbaijan, Bhutan, Caucasus, China, Cuba, Dominican Republic, India, Italy, Japan, Mexico, Papua New Guinea, Senegal, South Africa, Swaziland, USA (FL, MS, TX), Venezuela.

Specimens examined. ITALY, Padova, 'R. Horto Agrario', on leaves of *Citrus medica*, O. Penzig, 15 Jun. 1881, ex herb. Penzig [Staz. Patol. Veg. Roma], IMI 47696 (slide), type material of *C. penzigii*. BHUTAN, on leaves of *Citrus medica* v. *limon*, W.T.H. Peregrine SIB 385, 28 May 1985, IMI 295922. DOMINICAN REPUBLIC, intercepted at JFK airport, USA, on leaves of *Citrus* sp., K. Uchida, 6 Jun. 1975, BPI 439366; *Citrus* sp., R. Green, 29 Jun. 1983, BPI 439362. MEXICO, intercepted at Nogales, on living leaves of *Citrus* sp., W. Jackson, 15 Oct. 1965, BPI 439359. PAPUA NEW GUINEA, Laloki Q Stn., Port Moresby, on leaves of *Citrus* sp., A. Williams 6234b, 10 Oct. 1968, IMI 136538b. SOUTH AFRICA, Komatipoort, on leaves of *Citrus sinensis*, M.C. Pretorius, 6 Sep. 2000, herb CBS 6591, culture STE-U 3948-3950; Northern Province, Tsipise, on leaves and fruit of citrus pomelo, K. Serfontein, Sep. 2001, STE-U 4408, 4409; Northern Province, Messina, on leaves and fruit of *Citrus* sp., M. C. Pretorius, Sep. 2001, STE-U 4410, 4411. SWAZILAND, on leaves of *Citrus sinensis*, M. C. Pretorius, 6 Sep. 2000, herb CBS 6592, culture STE-U 4001; on leaves of *Citrus sinensis*, M. C. Pretorius, Oct. 2000, herb CBS 6593, culture STE-U 4002. USA, Texas, Falfurrias, on leaves of *Citrus aurantium*, F. D. Heald and F. A. Wolf, no. 2446, 14 Sept. 1909, BPI 433199 (holotype of *C. aurantia*), BPI 433198, photomicrographs of type.

Cercospora penzigii is morphologically similar to other cercosporoid species that are commonly referred to as part of the *Cercospora apii*-complex. This suggests that *C. penzigii* could have a wide host range (other than Rutaceae) and distribution. Morphologically this is a highly variable taxon with regards to conidiophore length, arrangement of scars on the conidiogenous cells (*in vitro* vs. *in vivo*), conidium length, shape, basal cell taper and fascicle morphology.

As shown in the present study, numerous *Cercospora* species are indistinguishable from the *C. apii* complex based on morphology and ITS sequence data (Fig. 1). It is tempting to reduce

them all to synonymy with *C. apii*, as inoculation studies have also shown many of these taxa to exhibit cross-pathogenicity between hosts (Johnston & Valleau, 1949; Berger & Hanson, 1963; Kaiser & Lukezic, 1965), but as we presently only have one molecular data set at our disposal, we will refrain from doing this step formally until a multi-locus DNA data set has been established for the *Cercospora* complex surrounding *C. apii*. ITS sequence data is a valuable tool for species identification, but insufficient as sole data set on which to base species synonymies. Additional data sets are therefore presently being generated to address host specificity in *Cercospora*.

***Mycosphaerella citri* Whiteside, Phytopathology 62: 263. 1972 (Fig. 4).**

Anamorph: *Stenella citri-grisea* (F.E. Fisher) Sivan., In Sivanesan, The bitunicate ascomycetes and their anamorphs: 226. 1984.

≡ *Cercospora citri-grisea* F.E. Fisher, Phytopathology 51: 300. 1961.

This species was treated in detail by Sivanesan (1984, pp. 226–228).

Hosts and distribution. Species of *Aeglopsis* Swingle, *Citrus*, *Fortunella* Swingle, *Murraya* L., *Poncirus* Rafin. (Rutaceae), Brazil, Costa Rica, Cuba, Dominican Republic, El-Salvador, Gabon, Haiti, Hong Kong, Japan, Puerto Rico, Surinam, Taiwan, Thailand, USA (FL, HI, TX), Venezuela, Virgin Islands.

Specimens examined. USA, Florida, Lake Alfred and Haines City, on leaves of *Citrus* sp., F. E. Fisher, May 1970, IMI 148810. VENEZUELA, on leaves of *Citrus sinensis*, R. Urtiaga 1560, 5 Jun. 1972, IMI 166609. DOMINICAN REPUBLIC, intercepted at JFK airport, USA, on leaves of *Citrus* sp., H. Shinsato, 19 Oct. 1967, BPI 439357; intercepted at JFK airport, USA, on leaves of *Citrus* sp., H. Shinsato, 23 Nov. 1969, BPI 439358; intercepted at JFK airport, USA, on leaves of *Citrus* sp., H. Shinsato, 21 Dec. 1968, BPI 439360; intercepted at JFK airport, USA, on leaves of *Citrus* sp., D. Walters, 19 Jun. 1970, BPI 439361; intercepted at JFK airport, USA, on leaves of *Citrus* sp., Heliczer, 23 Oct. 1969, BPI 439364; intercepted at JFK airport, USA, on leaves of *Citrus* sp., C. Locklear, 14 Nov. 1969, BPI 439365; intercepted at JFK airport, USA, on leaves of *Citrus* sp., R. Iwamoto, 3 Oct. 1967, BPI 439355; intercepted at JFK airport, USA, on leaves of *Citrus* sp., C. Smock, 26 Feb. 1968, BPI 439354; intercepted at JFK airport, USA, on leaves of *Citrus* sp., H. Wong, 4 Apr. 1968, BPI 439356. HAITI, on leaves of *Citrus* sp., N.R. Manalo, 14 Apr. 1985, BPI 439367. WEST INDIES, intercepted at JFK airport, USA, on leaves of *Citrus* sp., C. Locklear, 17 Aug. 1968, BPI 439363. PUERTO RICO, intercepted at San Juan airport, on leaves of *Citrus* sp., C. M. Looke, 24 Oct. 1950, BPI 439353. HONG KONG, on

leaves of *Citrus sinensis*, H. R. Mills, 13 Mar. 1970, BPI 431901. EL SALVADOR, on leaf of *Citrus* sp., J. Okamura, 1 Feb. 1983, BPI 420196; on leaf of *Citrus sinensis*, D. Bickell, 11 Aug. 1984, BPI 607682. HAWAII, Poamoho, on leaves of *Citrus sinensis*, J. Fine and L. M. Chilson, 20 Dec. 1954, BPI 602133.

A similar disease, also known as greasy spot, has been observed on *Citrus* in Australia (Timmer & Gottwald, 2000). Examination of a voucher specimen (Australia, Nambour, on leaves of *Citrus latifolia*, R. Thomas, BRIP 14527, 13 Jun. 1984, IMI 290702) found ascospores to be similar in size (10–12 x 2.5–3 μm) to those of *M. citri*, guttulate and fusiform, widest in the middle of the apical cell, and not constricted at the median septum. Symptoms vary, however, in being small, hypophyllous black specks surrounded by chlorotic halos (Timmer & Gottwald, 2000). It appears, therefore, that the Australian species represents yet another distinct species on *Citrus*. A further species of *Mycosphaerella* known to occur on *Citrus* in Japan is *M. horii* Hara (Timmer & Gottwald, 2000). After numerous attempts, however, we were unable to obtain any type material. Ascospores were reported to be 9–12.5 x 2.5–3 μm (Corlett, 1991).

Conidiophore fascicles of *S. citri-grisea* tend to be predominantly associated with spermatogonia or pseudothecia. Specimen BPI 439371 showed a lot of variation regarding ascospore size, with ascospores being up to 15 μm long and 4 μm wide. The anamorph is also highly variable, suggesting that this may, in fact, be a species complex. In some collections there is abundant superficial mycelium (BPI 420196), and short, narrow conidia, while in others conidia are borne on fascicles, and are long, wide and flexuous. Cultures and molecular studies would be required, however, to resolve the variation observed within *S. citri-grisea*.

Pseudocercospora angolensis **(T. Carvalho & O. Mendes) Crous and U. Braun, comb. Nov (Fig. 5).**

≡ *Cercospora angolensis* T. Carvalho and O. Mendes, Bol. Soc. Brot. 27: 201. 1953.

≡ *Phaeoramularia angolensis* (T. Carvalho & O. Mendes) P.M. Kirk, Mycopathologia 94: 177. 1986.

≡ *Pseudophaeoramularia angolensis* (T. Carvalho & O. Mendes) U. Braun, Crypt. Mycol. 20: 171. 1999.

This species was treated in detail by Kirk (1986).

Host range and distribution. *Citrus sinensis*, *Citrus* spp. (Rutaceae), Angola, Burundi, Cameroon, Central African Republic, Comoros, Congo, Ethiopia, Gabon, Gambia, Guinea, Ivory

Coast, Kenya, Mozambique, Nigeria, Tanzania, Togo, Uganda, West Africa, Yemen, Zaire, Zambia, Zimbabwe.

Specimens examined. ANGOLA, Mozambique Province, on leaves of *Citrus sinensis*, T. Carvalho and O. Mendes, Dec. 1951, BPI 432660, BPI 442839 (isosyntype), BPI 442837 (type). CAMAROON, Yaoundé, on leaves of *Citrus sinensis*, E. Milla, 17 Mar. 1978, IMI 252792. ETHIOPIA, on leaves of *Citrus* sp., ??, IMI 361170. KENYA, on leaves of *Citrus sinensis*, A. Seif W3753, 15 Nov. 1991, IMI 351626. UGANDA, on leaves of *Citrus sinensis*, W.T.H. Peregrine, 14 Jun. 1991, IMI 384297. WEST AFRICA, intercepted at San Pedro, California, USA, on leaves of *Citrus* sp., L. A. Hart, 2 Oct. 1953, BPI 432661, BPI 432659. ZAMBIA, on leaves of *Citrus* sp., R. H. Raemakers 7837, 18 Jun. 1973, IMI 176562; Chilanga, on leaves of *Citrus aurantium*, D. M. Naik, 28 Sep. 1983, IMI 280618; Chilanga, on leaves of *Citrus* sp., B. K. Patel, 18 Jul. 1975, IMI 196889; Lusaka, on leaves of *Citrus* sp., I. Javaid, 17 Jun. 1977, IMI 214501. ZIMBABWE, Bindura, on leaves of *Citrus* sp., A. Rothwell, 13 Aug. 1979, IMI 240682; on leaves of *Citrus* sp., M. C. Pretorius, Sep. 2000, STE-U 4111–4118.

Cercospora angolensis was originally described as having hyaline, subclavate conidia (De Carvalho & Mendes, 1953). For this reason it was seen as distinct from a *Phaeoisariopsis* Ferraris species causing a severe disease on *Citrus* in Nigeria (Emechebe, 1980). Kirk (1986) found this to be the same organism, and placed the fungus in *Phaeoramularia* Munt.-Cvetk. as *P. angolensis* based on its conspicuous, slightly pigmented scars, and pale brown catenulate conidia. Braun and Melnik (1997) established the genus *Pseudophaeoramularia* U. Braun for species with unthickened or only very slightly thickened, but somewhat darkened-refractive scars (intermediate between *Pseudocercospora* and *Phaeoramularia*) and hence proposed the combination *Pseudophaeoramularia angolensis*. In a later molecular study, however, Crous *et al*. (2001) reported that genera with such scars as in *Paracercospora* and *Pseudophaeoramularia* belong in *Pseudocercospora* (Fig. 1). Because this has again been confirmed in the present study, a new combination for *Cercospora angolensis* is herewith proposed in *Pseudocercospora*.

Key to cercosporoid species on *Citrus*[1]

1. Conidia hyaline, acicular or obclavate to subcylindrical, 50–300 x 2.5–5 µm, multiseptate,with thickened, darkened, refractive hila .*Cercospora penzigii* (= *C. apii s.lat.*)

1. Conidia pigmented…..…….…….....……...2.

2. Conidia and superficial mycelium verruculose; conidia pale olivaceous, subcylindrical to narrowly obclavate, catenulate, hila thickened, darkened, refractive, 6–50 x 2–4.5 µm, (0–)3–6(–9)-septate…….*Stenella citri-grisea* (*M. citri*)[2].

2. Conidia and superficial mycelium smooth……..…….3.

3. Conidial hila and scars inconspicuous, or minutely thickened.…….…....….4.

3. Conidial hila and scars prominently thickened, darkened and refractive…….5.

4. Conidia solitary, narrowly obclavate, base narrowly subtruncate, 3–4 µm wide, scars inconspicuous; occurring on leaves only…….*Pseudocercospora citri* Crous and U. Braun[3].

4. Conidia solitary or catenulate, cylindrical to obclavate, base truncate, 4–5(–6.5 µm) wide, scars inconspicuous or minutely thickened; occurring on leaves and fruit……...…….*Pseudocercospora angolensis.*

5. Conidia 1–6-septate, 28–60 x (1.5–)2(–2.5) µm…….. ……*Passalora citrigena* Crous and U. Braun (*Mycosphaerella citrigena* Crous and U. Braun)[1].

5. Conidia 0–1(–3)-septate, 18–35 x 4–5 µm……..……. ..*Passalora citricola*[1].

[1] For additional species on *Citrus* and Rutaceae see Crous and Braun (2003).

[2] Regarded as a species complex.

[3] Described in Braun and *et al*. (2003).

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Table 1. Isolates collected from citrus producing areas in Zimbabwe and South Africa during the year 2000 and other isolates of cercosporoid species were sequenced.

Anamorph	Teleomorph	Host	Origin	Collector	Date isolated	Accession no.	Gen Bank no.
Cercospora canescens	Unknown	*Vigna*	Free State,South Africa	P. S. Van Wyk	1995	STE-U 1137	AY260065
C. canescens	Unknown	*Vigna*	Free State,South Africa	P. S. Van Wyk	1995	STE-U 1138	AY260066
C. oryzae	'*Sphaerulina oryzina*'	*Oryza*	Arkansas, U.S.A.	E. C. Tullis	–	STE-U 4303, IMI 303642, CBS 145.37	AY260064
C. penzigii	Unknown	*Citrus*	Northern Province, South Africa	K. Serfontein	2000	STE-U 4408	AY260067
C. penzigii	Unknown	*Citrus*	Northern Province, South Africa	K. Serfontein	2000	STE-U 4409	AY260068
C. penzigii	Unknown	*Citrus*	Northern Province, South Africa	M. C. Pretorius	2000	STE-U 4410	AY260070
C. penzigii	Unknown	*Citrus*	Northern Province, South Africa	M. C. Pretorius	2000	STE-U 4411	AY260071
C. penzigii	Unknown	*Citrus*	Swaziland	M. C. Pretorius	2000	STE-U 3946	AY260072
C. penzigii	Unknown	*Citrus*	Swaziland	M. C. Pretorius	2000	STE-U 3947	AY260073
C. penzigii	Unknown	*Citrus*	Swaziland	M. C. Pretorius	2000	STE-U 3945	AY260074
C. penzigii	Unknown	*Citrus*	Mpumalanga, South Africa	M. C. Pretorius	2000	STE-U 3948	AY260075
C. penzigii	Unknown	*Citrus*	Northern Province, South Africa	M. C. Pretorius	2000	STE-U 3949	AY260076
C. penzigii	Unknown	*Citrus*	Northern Province, South Africa	M. C. Pretorius	2000	STE-U 3950	AY260077

Table 1. Continued

Anamorph	Teleomorph	Host	Origin	Collector	Date isolated	Accession no.	Gen Bank no.
C. populicola	Unknown	*Populus*	KwaZulu-Natal, South Africa	M. J. Wingfield	1995	STE-U 1051	AY260069
C. zebrine	Unknown	*Trifolium pratense*	Ottawa, Canada	K. A. Seifert	2000	STE-U 3955	AY260078
C. zebrine	Unknown	*Trifolium repens*	Ottawa, Canada	K. A. Seifert	2000	STE-U 3957	AY260079
C. zebrine	Unknown	*Trifolium repens*	Ottawa, Canada	K. A. Seifert	2000	STE-U 3958	AY260080
Pseudocercospora angolensis	Unknown	*Citrus*	Zimbabwe	M. C. Pretorius	2000	STE-U 4116	AY260061
P. angolensis	Unknown	*Citrus*	Zimbabwe	M. C. Pretorius	2000	STE-U 4115	AY260062
P. angolensis	Unknown	*Citrus*	Zimbabwe	M. C. Pretorius	2000	STE-U 4118	AY260063
Pseudocercospora sp.	*Mycosphaerella* sp.	*Acacia*	Venezuela	M. J. Wingfield	2000	STE-U 3837	AY260060

Table 2. Nucleotide differences observed for *Cercospora* species included in this study. Base positions include spaces caused by alignment gaps

Species	ITS1					5.8S rRNA gene					ITS2		
	base 69	base 101	base 147	base 148	base 149	base 280	base 293	base 334	base 357	base 360	base 464	base 500	base 502
C. apii CA1[5,6]	T	-	A	G	T	C	G	C	G	T	G	C	C
C. asparagi AF297229		C[3]											T[1]
C. canescens CCA19[6]						T[1]	A[1]	A[2]					
C. oryzae STE-U 4303	C[1]		C[2]	A[1]	C[1]	T[1]	A[1]	A[2]	-[4]	C[1]			
C. penzigii STE-U 4410													T[1]
C. penzigii STE-U 4411													T[1]
C. penzigii STE-U 3946													T[1]
C. penzigii STE-U 3947													T[1]
C. penzigii STE-U 3945													T[1]
C. populicola STE-U 1051													T[1]
C. penzigii STE-U 3948												T[1]	T[1]
C. penzigii STE-U 3949												T[1]	T[1]
C. penzigii STE-U 3950												T[1]	T[1]
C.haÿi CH5[6]												T[1]	T[1]
C. zebrina STE-U 3955											C[2]		
C. zebrina STE-U 3957											C[2]		
C. zebrina STE-U 3958											C[2]		

[1] Transition.
[2] Transversion.
[3] Insertion / duplication of leading nucleotide.
[4] Deletion.
[5] Sequences identical to *C. apii*: *C. canescens* STE-U 1137, *C. canescens* STE-U 1138, *C. nicotianae* AF297230, *C. sorghi* f. *maydis* AF297232, *C. beticola* AF222827, *C. penzigii* STE-U 4408, 4409, *C. hayi* CH6*.
[6] Sequences obtained from TreeBase matrix M691.

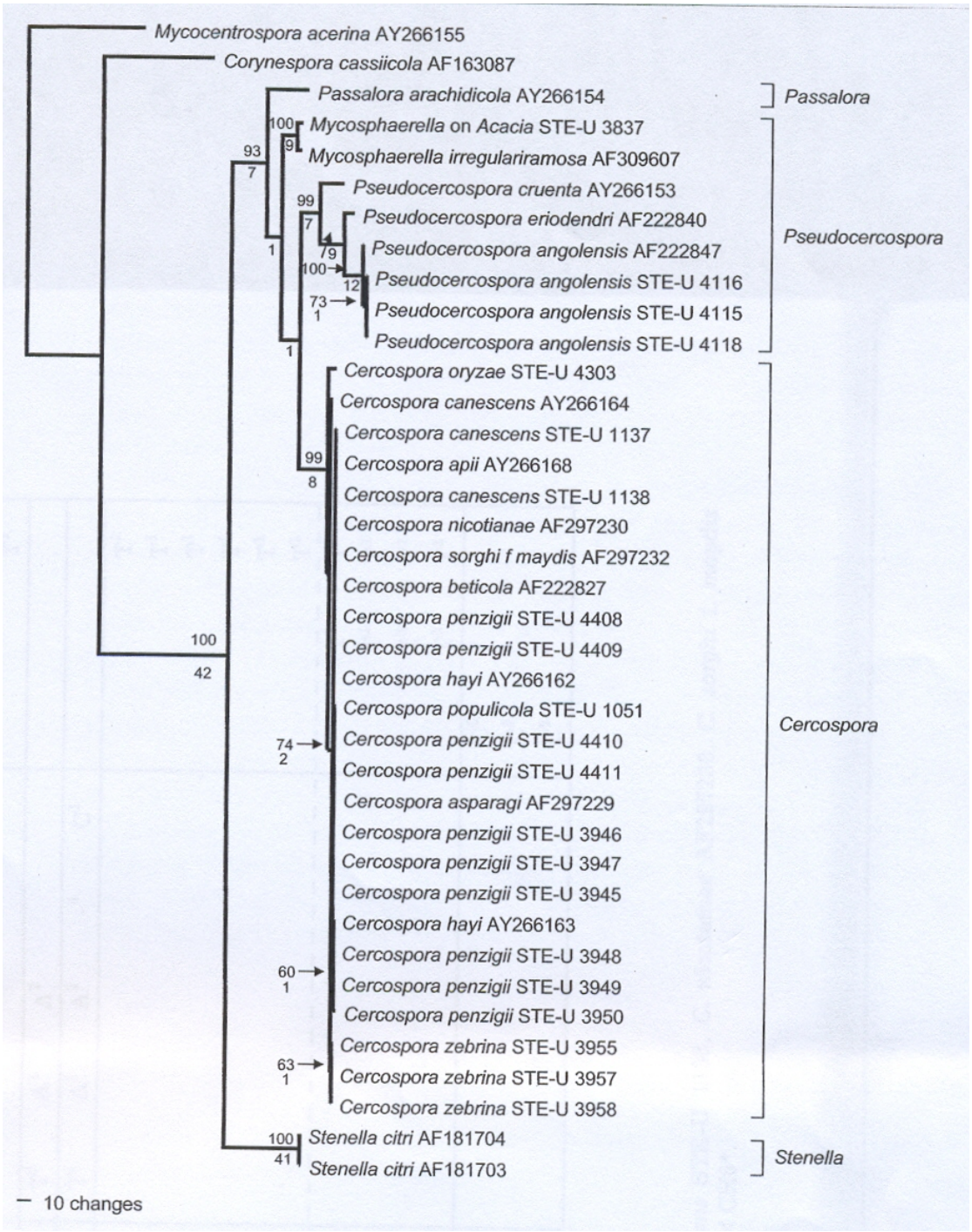

Fig. 1. Single most parsimonious tree obtained from a heuristic search using simple taxon additions (TL = 550 steps, CI = 0.836, RI = 0.852, RC = 0.712). Bootstrap and decay values are shown at the nodes, above and below the branches, respectively. *Mycocentrospora acerina* was included as outgroup.

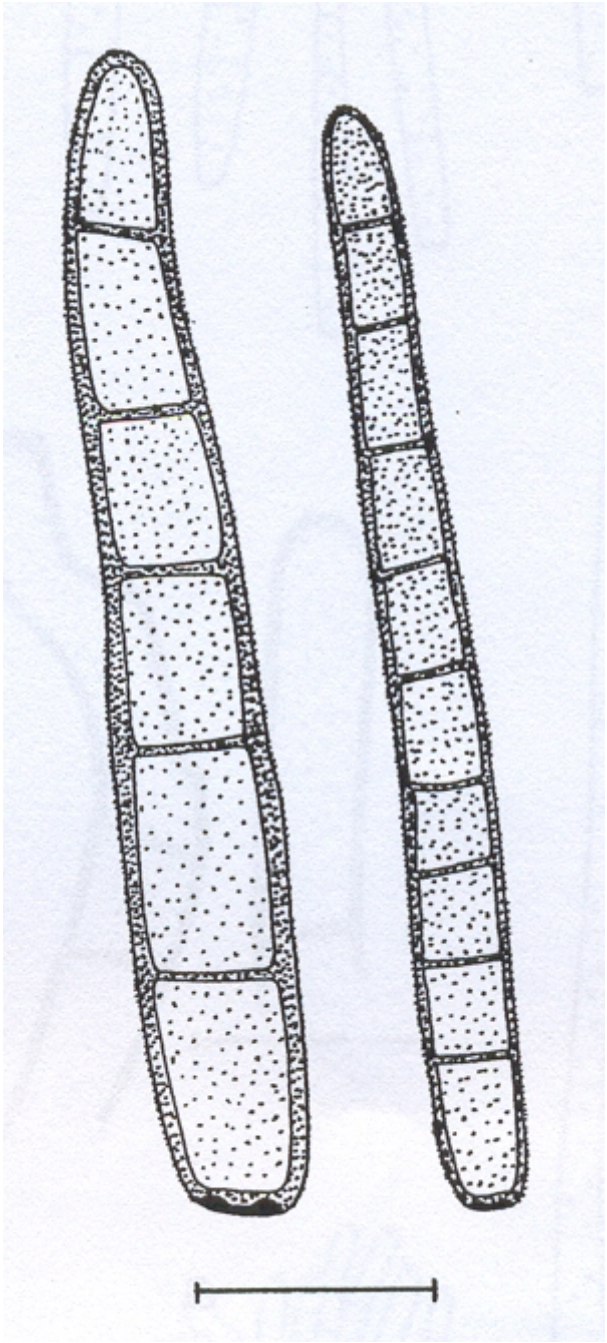

Fig. 2. Conidia of *Cercospora gigantea* (F 46419). Bar = 10 µm.

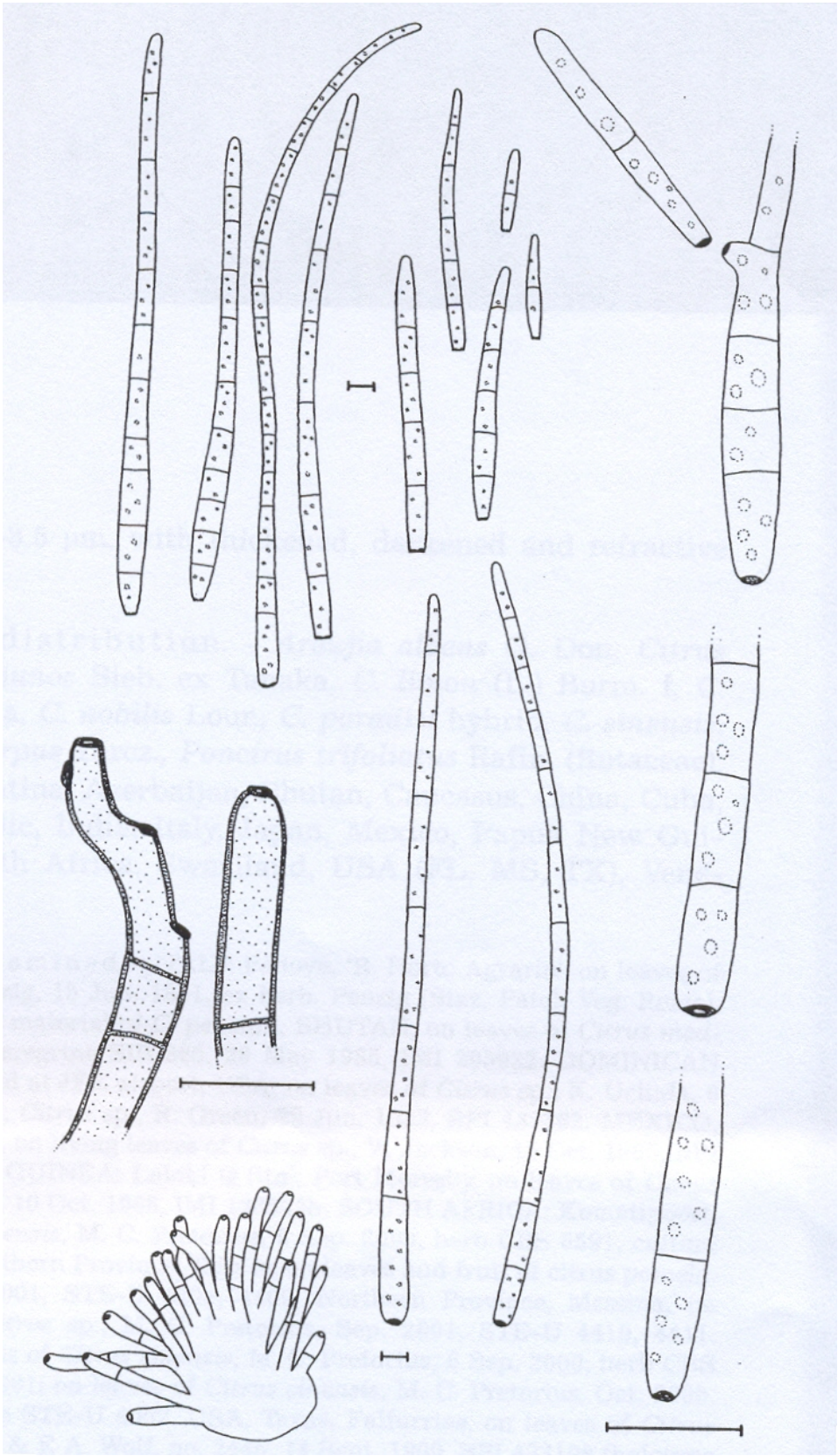

Fig. 3. Fascicles, conidiophores and conidia of *Cercospora penzigii* (herb CBS 6591). Bars = 10 μm.

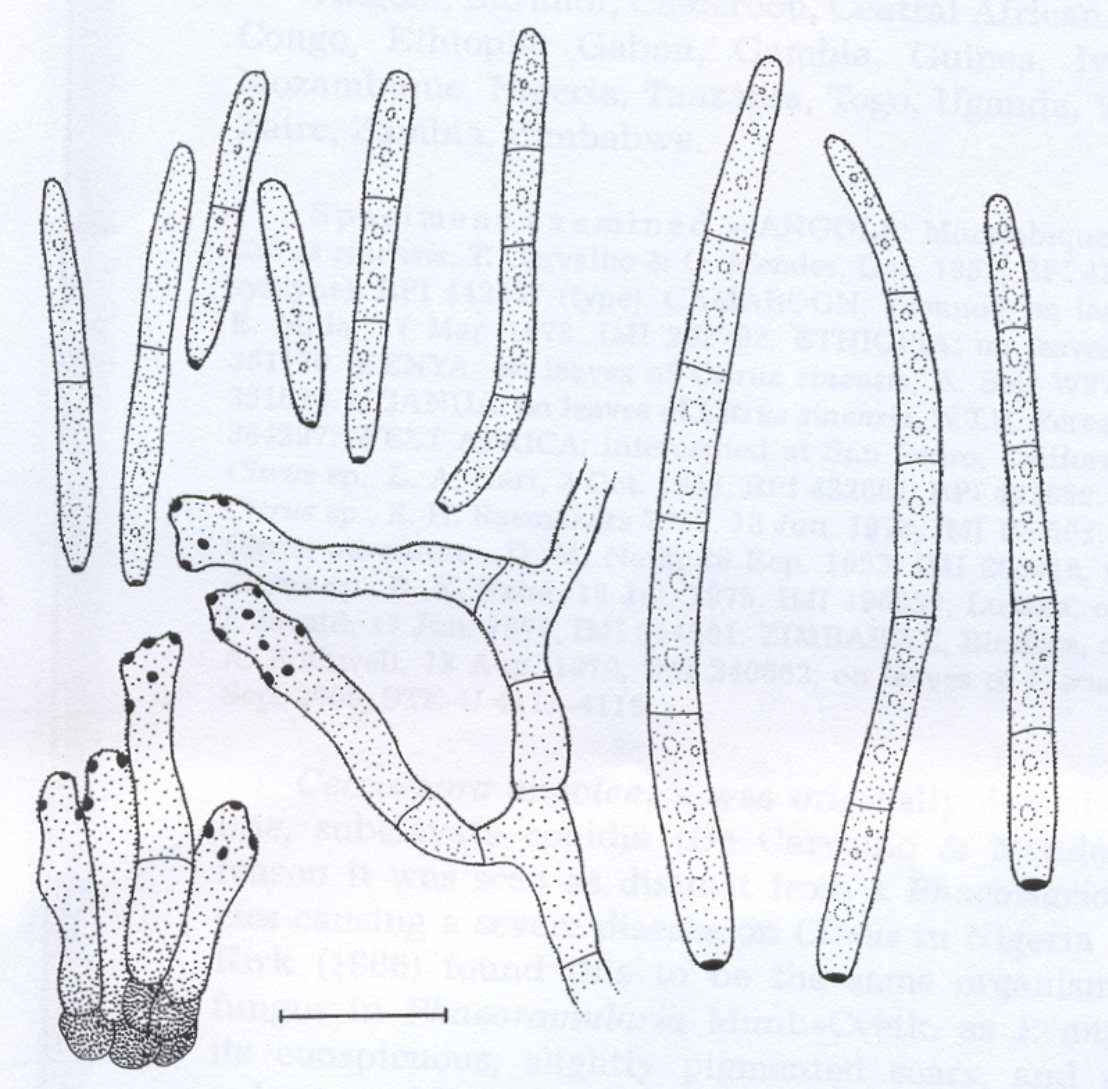

Fig. 4. Conidiophores and conidia of *Stenella citri-grisea*, the anamorph of *Mycosphaerella citri* (IMI 148810). Bar = 10 μm.

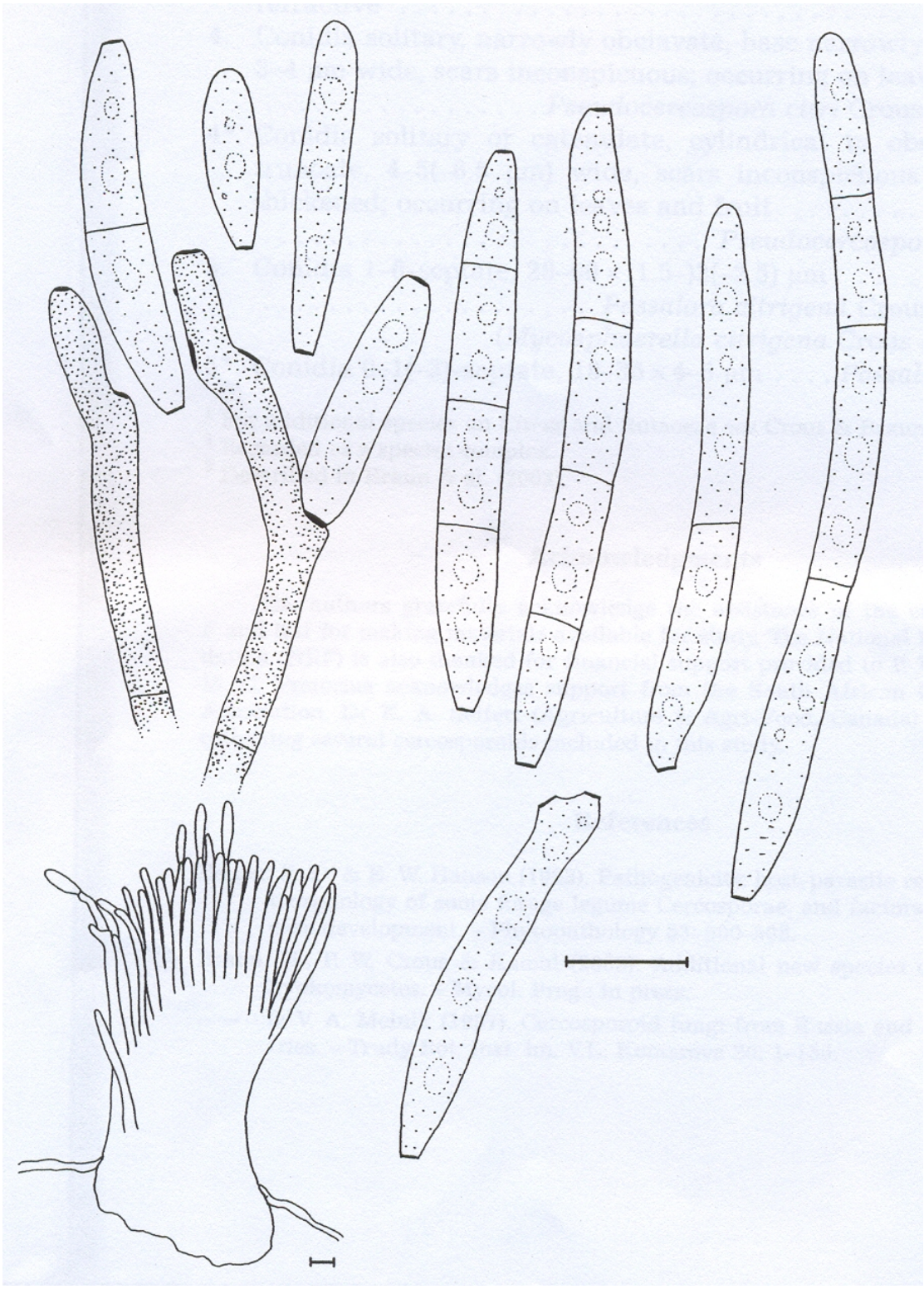

Fig. 5. Conidiophores, conidiogenous cells and conidia of *Pseudocercospora angolensis* (IMI 176562). Bars = 10 μm.

4. CONTROL OF *PSEUDOCERCOSPORA ANGOLENSIS* IN ZIMBABWE

ABSTRACT

Fruit and leaf spot disease (FLSD) caused by *Pseudocercospora angolensis* is a "cosmetic disease of quarantine importance" disease, and even a single lesion renders fruit unmarketable. Limited information is available on the epidemiology and control of FLSD on citrus. A control strategy was therefore investigated. The aims of the control strategy examined consisted of identifying and eradicating infected trees, the installation of a spore trap and weather station in an infected orchard to determine the epidemiology of the disease and to evaluate fungicide efficacy. The removal of old neglected orchards reduced the inoculum. Three applications of trifloxystrobin + mancozeb + mineral spray oil (20 g +200 g + 500 mℓ/100 ℓ water) sprayed during November, January and March reduced *P. angolensis* most effectively and resulted in the highest percentage flushes (72.8%) free from symptoms (index 0). The percentage flushes recorded in index 3 was significantly less than that for the control, and none of the flushes were catagorized in index 4. Three applications with benomyl + mancozeb + mineral spray oil (25 g + 200 g + 500 mℓ/100 ℓ water) also caused a significant reduction relative to the control in percentage flushes recorded in indices 0, 3 and 4. Two applications during November and January of trifloxystrobin + mancozeb + mineral spray oil (20 g + 500 mℓ /100 ℓ water), and difenoconazole (40 mℓ/100 ℓ water), resulted in 57.4% and 55.4% flushes catagorised in index 0, respectively. Poor results were obtained with Mancozeb at 200 g, applied three times during the season, the results indicated that chemical control with a contact fungicide allone was insufficient. The weather data and spore counts indicated that these factors were correlated, as *P. angolensis* needed moisture and temperatures in excess of 25°C for disease development. Recommendations for the chemical control of FSLD are made. *P. angolensis* in Zimbabwe can be managed successfully by implementing a holistic approach that should be supported by the authorities, organised agriculture and all technical personnel involved in citrus production.

INTRODUCTION

Fruit and leaf spot disease (FLSD) of citrus, caused by *Pseudocercospora angolensis* (Chapter 3) is restricted to countries in sub-Sahara Africa especially at altitudes above 600 m (Timmer & Gottwald, 2000). All *Citrus* species that were tested are susceptible. The order of susceptibility from the most to the least susceptible are, grapefruit (*C. paradisi* Macf.), oranges (*C. sinensis* [L.] Osb), mandarins (*C. reticulata* Blanco), lemons (*C. lemon* [L.] Burm) and lime (*C. aurantifolia* [Christm] Sw), although the degree of susceptibility varied amongst the different varieties (Kaute, 1993; Seif, 1996).

Infection occurs through splash-dispersed conidia that invades the undersides of leaves to produce irregular necrotic spots up to 1 cm in diameter that are visible on the upper surfaces, surrounded by a chlorotic halo (Kirk, 1986). Several spots may coalesce to affect much of the leaf area. Early infections of fruit lead to the abortion or mummification of young fruit. Later in fruit development, infection causes circular necrotic lesions that are 1-2 cm in diameter, surrounded by an area of chlorosis. Lesions appear sooty black in wet weather due to sporulation by the pathogen. FLSD is a "cosmetic" disease and a single lesion renders the fruit unmarketable. The economic loss to the producer is therefore much greater than would appear from yield figures expressed as fruit weight alone (Seif, 1997).

Information on the epidemiology and control of FLSD of citrus is limited (Seif, 1996). Reports are mostly based on the distribution of the disease in relation to altitude in West Africa (Oberti, 1971; Brun, 1972; Kuate & Foure, 1988) and on the incidence of fruit infection on different citrus cultivars in Cameroon (Rey *et al.*, 1988). Studies on chemical control of the disease in Cameroon (Menyonga, 1971; Rey *et al*., 1988) and Uganda (Emchebe, 1975) indicated that good control can be achieved by applying fortnightly sprays of benomyl or copper fungicides throughout the rainy season. A control programme, involving five or more sprays during the season, is beyond the means of most small-scale citrus growers in tropical Africa. This multiple spray programme scenario was not problematic in Zimbabwe prior to the land claim debacle (which had a negative impact on most of Zimbabwe's agricultural industry) because most of the citrus producers were commercial exporters of citrus. They were able to apply a high standard of sound agricultural practices that resulted in the sustainable production of high quality yields (S. Hery, Horticultural Promotion Council, Harare, personal communication, 2001).

Seif (1998) indicated that infection by *P. angolensis* takes place over a wide range of temperatures. The optimal temperature for lesion formation was 25°C. No lesions were produced at 35°C indicating that the disease is likely to be more of a problem in cooler high altitude citrus regions than in the hot low altitude regions. *P. angolensis* was found to be depended on high moisture for infection but not for lesion expansion (Seif, 1998). In Kenya, fungicides are usually applied at 14 day intervals throughout the rainy season and are discontinued a month prior to harvest (Seif & Hillocks, 1997). This approach to chemical control requires further validation in the field. If off-season rains occur, there may be a need for supplementary sprays with curative fungicides. Timing of applications with regards to weather condition and suseptible stage of fruit development should make chemical control of the disease more effective (Seif, 1998).

Because of the limited information available, the Zimbabwean and Southern African citrus growers associations recommended that a *P. angolensis* control strategy be investigated (S. Hery; Horticultural Promotion Council, Harare, personal communication, 1999). This strategy should restrict this “cosmetic” disease in commercial orchards and also eradicate old neglected orchards to reduce the threat of its spread to South Africa. The aims of the control strategy examined in this study therefore consisted of identifying and eradicating infected trees, the installation of a spore trap and weather station in an infected orchard to determine the epidemiology of the disease and to evaluate the efficacy of fungicide sprays.

MATERIALS AND METHODS

Eradication of infected trees. The first action which was taken with the assistance of the Horticultural Promotion Council (HPC) and Plant Protection Research Institute (PPRI), Zimbabwe, was an attempt to reduce the inoculum in infested regions. An orchard in the Enterprise region just north of Harare, that was believed to be the main source of infection in that region, and two orchards in the Bindura region were removed and burned (Fig. 1).

Field evaluation of fungicides. Four preliminary fungicide trials were conducted to screen the efficacy and timing of a restricted range of fungicides available in Zimbabwe. The unpublished trial data of these trials were utilised as a basis for planning of the final fungicide trial.

During March 1997 in the Bindura region a twelve-year-old navel orange orchard was selected for the first trial. The effect of a single application of various fungicides on *P. angolensis* was determined four months after application by inspecting the leaf flush. None of the products applied were successful in preventing disease development and it was concluded that a single fungicide application would not control *P. angolensis* effectively. The second trial was executed in a five-year-old navel orange orchard in the Karoi region, which showed leaf symptoms during the 1996 survey. Although no symptoms were found in 1998 when the trial commenced, the orchard was sprayed nevertheless. No lesions were observed in the orchard that season and therefore no conclusion could be drawn. The third trial consisted of two fungicide trials laid out in January 1999, in two different regions, one in the Bindura region and one in the Matepatepa region. Trees at the Bindura trial site were infected with *P. angolensis*. The trees were a mixture of 30 year old Valencia and navel orange trees. This was the only orchard to be found in this area that has not been on a standard CBS spray programme. A light infestation was visible on some of the trees in the orchard prior to the first application. The Matepatepa orchard was a 15-year-old navel orange orchard infected with *P. angolensis*. The trees in the Matepatepa trial were in good condition, whereas the trees at the Bindura trial site were older and not in such a good condition. No conclusion could be drawn from the Bindura trial site because enough lesions did not develop on most of the trees to make an evaluation. It is believed that this was due to the large numbers of Valencia orange trees, which is not as susceptible as the navel orange trees. The poor tree condition could also have an effect. The data (not shown) gathered from the Matepatepa trial site indicated that the benomyl and mineral oil group of treatments, the phosphonate treatment and the difenoconazole treatment gave the best result in preventing new lesion development on the leaf flushes. It was evident that the timing of the first sprays was important and had to be done even earlier as was anticipated i.e. before the January 1999 applications was done. The reason being that lesions were visible on the new leaf flushes two days after the first application was done during the January 1999 applications.

The information gathered from the four preliminary trials was used in planning the final trial, which was conducted at Matepatepa in a 15-year-old navel orchard next to the site that was utilised in the preliminary study. Each treatment was replicated five times in single tree plots arranged in a randomised block design. The fungicides used in the trial were benomyl (Benlate, 500 g WP; Du Pont), carbendazim/difenoconazole (Eria, 125/62.5 g/ℓ SC;

Syngenta), difenoconazole (Score 250 g/ℓ EC; Syngenta), bitertanol (Baycor, 300 g/ℓ EC; Bayer), azoxystrobin (Ortiva, 250 g/ℓ SC; Syngenta), trifloxystrobin (Flint, 500 g/kg WG; Bayer), pyraclostrobin (Cabrio, 500 g/kg WG; BASF), potassium phosphonate (Phytex, 200 g/ℓ SL; Horticura), mancozeb (Dithane M45, 750 g/kg WS, Dow AgroScience) and mineral spray oil (Sunspray 6E, Avima). Fungicides were applied with a trailer-mounted, high volume, high-pressure (2500 to 3000 kPa) sprayer with two hand-held spray guns and spray volumes cosequently varied according to the size and canopy density of each. Trees were sprayed to the point of runoff. The spray programme, combination of products and product concentrations are listed in Table 1.

Because lesions were not observed on any fruit, leaves were used to determine the fungicidal effect of the sprays. Thirty leaf flushes per tree according to a four-point *P. angolensis* lesion index were used: 0 = no lesions; 1 = 1-4 lesions per leaf flush; 2 = 5-14 lesions per leaf flush, and 3 = >15 lesions per leaf flush. Two months after the final fungicide application were made in May 2000, flushes were collected and indices determined. The data was analysed by analysis of variance (ANOVA) and Fisher's Least Significant Difference (LSD) test (P = 0.05).

Epidemiology. A battery operated Quest spore trap and a solar energy operated electronic weather station (Metos-Pessel instruments) were installed next to each other at Sangere farm, in the Bindura region, during October 1999 (Fig. 2). The Quest spore trap collected spores on an hourly basis for eight days on a poly-carbonate disc sprayed with a thin layer of aerosol Vaseline. The location of the trial site to where the research team was situated was more than 2500 km, the fact that a phytosanitary disease was investigated in another country, that no infected plant material could be investigated in South Africa and the economics regarding distances travelled by the research team, demanded exceptional input by people conducting the study. The farm owner was asked to maintain the apparatus and to change the spore trap discs on a weekly basis. The discs were transported to Kutsaga Research Station on a weekly basis. The weather data were downloaded from the weather station by researchers from Citrus Research International or by private consultants. As no infected material could be transferred into South Africa, the spore trap discs were microscopically examined in Zimbabwe by Dr. Desiree Cole, a plant pathologist (Fig. 3). The identification of the conidia was based on the morphology of conidia of *P. angolensis*. The data were expressed as a total

number of spores per month The weather station monitored the average day temperatures, percentage relative humidity (RH) as well as rainfall. Ninety six readings were taken daily (one every 15 minutes). These readings were then averaged to give a single daily reading that was used for further computations. The weather and spore count data were expressed on a monthly basis from October 1999 to October 2001. The trial was terminated in October 2001.

RESULTS

Eradication of infected trees. The result of removing the main source of infection in the Enterprise and Bindura regions was evident in the last survey conducted during July 2003 (Chapter 2) where it was reported that there was a low incidence of the disease compared with a high incidence before the eradication prosess. It was also reported by the HPC and private consultants that producers in other infested regions had identified neglected orchards and had destroyed them.

Field evaluation of fungicides. The percentage leaf flushes recorded in each of the four lesion indices are given in Table 1. Three applications of trifloxystrobin + mancozeb + mineral spray oil (20 g + 200 g + 500 mℓ/100 ℓ water) sprayed during November, January and March reduced *P. angolensis* most effectively and resulted in the highest percentage flushes (72.8%) free from symptoms (index 0). The percentage flushes recorded in index 3 was significantly less than that for the control, and none of the flushes were catagorised in index 4. Three application of benomyl + mancozeb + mineral spray oil (25 g + 200 g + 500 mℓ/100 ℓ water) during November, January and March also caused a significant reduction in percentage flushes recorded in indices 0, 3 and 4 relative to the control. Two applications (during November and January) of trifloxystrobin + mancozeb + mineral spray oil (20 g + 500 mℓ / 100 ℓ water), and also of difenoconazole (40 mℓ/100 ℓ water), resulted in 57.4% and 55.4% flushes catagorized in index 0, respectively.

Epidemiology. The number of conidia recorded on the spore trap was substantially higher during the rain season when higher average temperatures and higher relative humidity prevailed (Figures 4, 5 & 6). In the first season, conidial numbers peaked from March to May. In the second season, the number of conidia peaked during March and April. In both seasons low conidial numbers were recorded from June to October. The highest rainfall

occurred during February and March, with low to no rainfall from June to October. The average monthly temperature coincided with the change of the seasons and observed spore releases. The average winter and summer temperatures were 15 and 25°C respectively.

DISCUSSION

The data showed that *P. angolensis* in Zimbabwe can be managed successfully through the implementation of a holistic approach. This must, however, be supported by the authorities, organised agriculture and all technical personnel involved in citrus production. The recommended control strategy is based on the removal of all old and neglected orchards that could pose a threat to a region, and on timely fungicide applications. This strategy should reduce the inoculum in a region and consequently reduce the incidence and spread of the disease. The removal of old orchards resulted in only two regions (Area 1 & 2) being regarded as infested and the other areas (Area 3 &4) as disease free, as reported during the 1999 survey (Chapter 2), and it is believed that the removal of these orchards contributed to this scenario. Measures must be implemented to restrict the movement of plant material or fruit from infested areas to disease-free areas.

The implementation of an effective spray programme to protect the trees against *P. angolensis* infection during the rainy season further reduced the incidence of the disease and the accumulation of spores. This will have a negative effect on the following season's infection rate. Three applications with trifloxystrobin + mancozeb + mineral spray oil (20 g + 200 g + 500 mℓ /100 ℓ water) was found to be the most effective treatment with the highest percentage uninfected flushes. Three applications with benomyl + mancozeb + mineral spray oil (25 g + 200 g + 500 mℓ /100 ℓ water) was the second most effective treatment with 62% unifected leaf flushes. It is known that the inclusion of mancozeb with the benzimidazole and strobilurin group of fungicides improves their efficacy against citrus black spot disease (CBS). Two applications of trifloxystrobin (20 g) + mineral spray oil (500 mℓ) per 100 ℓ/water and two difenoconazole (40 g) applications were the third most effective treatments with 57.4% and 55.4% clean leaf flushes respectively.

Three applications with the contact fungicide mancozeb, at 200 g per 100 ℓ/water, gave poor results. Seif (1998) recommended a fortnightly application of a contact fungicide

commencing after the onset of the first rains. This data, however, clearly indicated that a contact fungicide, applied three times at two monthly intervals during the rainy season, was not effective and therefore cannot be recommended.

Emchebe (1975) recommended that benomyl should be applied at fortnightly intervals during the rainy season, but good results from this trial clearly indicated that spray intervals with benomyl and a contact fungicide such as mancozeb and mineral spray oil, could be as long as two months.

Multiple applications of selective fungicides enhance selection for resistance in the pathogen population to these products, as was found with the benzimidazole group of fungicides. In some citrus producing regions in South Africa and Zimbabwe, where producers have been controlling CBS for many years, resistance developed in the pathogen population to benomyl (De Wet, 1987). **T**hese products should therefore be alternated with nonselective fungicides or fungicides from different chemical classes. The following control programme was recommended and should coincide with an existing CBS control programme and also adresses anti-resistance strategies:

a) First application: Mid-October – contact fungicide such as mancozeb at 200 g + mineral spray oil 500 mℓ100 ℓ / 100 ℓ of water. This application will protect the young fruit and leaves from any spores being released earlier than anticipated or if an early downpour should occur.

b) Second application: Mid-November – a systemic fungicide spray: benomyl 25 g + mancozeb 200 g + mineral spray oil 500 mℓ/100 ℓ of water; or trifloxystrobin 20 g + mancozeb 200 g + mineral spray oil 500 mℓ / 100 ℓ of water.

c) Third application: Mid –January - a second systemic fungicide spray, similar to, or alternating mixture used for the second application.

d) Fourth application: Mid-March - A contact fungicide such as mancozeb 200 g + mineral spray oil 500 mℓ / 100 ℓ water.

This programme should reduce sporulation during the latter part of the season, during or after heavy rainfall. Certain systemic compounds used at that time of the season, may result in high residue levels. Therefore a contact fungicide applied shortly before harvest would be

more appropriate. A difenoconazole application would be an option, but because of the residues the product can not be applied later than January.

The spore trap data showed that *P. angolensis* needed moisture for spore dispersal. The disease cycle coincided with high rainfall during the warmer summer months and therefore a spray programme consisting of systemic compounds is recommended. This approach should be more effective than a contact compound. As rainfall, temperature and humidity increases, more spores are released. At the beginning of the winter season, the spores decrease as rainfall, humidity and temperatures decrease. This data supported Seif's (1998) observation that the optimal temperature for lesion formation is 25°C. The data also explains the reason why lesions were only visible on the leaves and not on the fruit in Zimbabwe. In spring, during blossoming and fruit formation no moisture was present to facilitate infection. The temperature is still below 20°C, and therefore the disease remains inactive. When out of season fruit appears in November /December months, lesions were present on these fruit. Seif (1993) reported that young fruit up to golf ball size are very susceptible to infection. The results emphasised the fact that the first application must be applied before the 15th of November, before most of the spores are released. The *P. angolensis* disease cycle coincide with the CBS disease cycle and spray programmes could be designed to control both diseases.

In conclusion, *P. angolensis* cause a cosmetic disease and needs moisture and temperatures in excess of 25°C but below 35°C to pose a threat to citrus producing regions. When infected old and neglected orchards are removed and a well-managed, and a timely spray programme is implemented, the spread and intensity of the disease can be reduced in Zimbabwe, which will enable producers to produce lesion free fruit.

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Table 1. Percentage leaf flushes collected from navel oranges at Frinton farm, Matepatepa, Zimbabwe, categorized in four *Pseudocercospora angolensis* lesion indices two months after the applications of fungicide sprays during 1999 to 2000

Treatment	**Concentration (rate/100ℓ water)**	**Time of application**			**Lesions per flush (%)** [x]			
		Nov 1999	**Jan 2000**	**Mar 2000**	**0**	**1-5**	**6-14**	**>15**
Untreated control	-	-	-	-	15.8 f	17.4 c	27.4 ab	39.2 ab
Mancozeb + mineral spray oil	200 g + 500 mℓ	X	X	X	14.2 f	22.0 bc	16.6 bcde	47.0 a
Benomyl + mineral spray oil	25 g + 500 mℓ	X	X		48.0 bcde	30.6 bc	15.2 cde	6.0 efgh
Benomyl + mineral spray oil	50 g + 500 mℓ	X		X	40.6 cde	24.0 bc	21.6 abc	16.8 cd
Benomyl + mineral spray oil	50 g + 500 mℓ	X	X	X	49.4 bcde	26.8 abc	15.4 cde	8.8 defgh
Benomyl + mancozeb + mineral spray oil	25 g + 200 g + 500mℓ	X	X	X	62.0 ab	20.8 bc	12.0 cde	5.2 fgh
Pyraclostrobin + mineral spray oil	10 mℓ + 500 mℓ	X	X		34.0 de	27.4 abc	18.8 bcde	19.8 c
Pyraclostrobin + mineral spray oil	20 mℓ + 500 mℓ	X	X		54.0 bc	24.6 bc	8.6 de	12.6 cdefg
Pyraclostrobin + mineral spray oil	30 mℓ + 500 mℓ	X	X		43.2 cde	29.2 ab	18.8 bcde	8.6 defgh
Pyraclostrobin + mineral spray oil	10 mℓ + 500 mℓ	X	X	X	54.0 bc	23.4 bc	15.2 cde	7.4 defgh
Azoxystrobin + mineral spray oil	10 mℓ + 500 mℓ	X	X		33.4 e	30.0 ab	24.2 abc	15.2 cdef
Azoxystrobin + mineral spray oil	20 mℓ + 500 mℓ	X	X		51.2 bcd	30.6 ab	13.8 cde	3.8 gh
Azoxystrobin + mineral oil	30 mℓ + 500 mℓ	X	X		41.1 cde	38.0 a	11.8 cde	8.8 defgh

Table 1. Continued

Treatment	Concentration (rate/100ℓ water)	Time of application			Lesions per flush (%)[x]			
		Nov 1999	Jan 2000	Mar 2000	0	1-5	6-14	>15
Azoxystrobin + mancozeb + mineral spray oil	20 mℓ + 200 g + 500 mℓ	X	X	X	50.0 bcde	19.8 bc	18.6 bcde	11.4 cdefg
Trifloxystrobin + mineral spray oil	10 g + 500 mℓ	X	X		46.6 bcde	22.6 bc	20.6 abc	10.2 cdefgh
Trifloxystrobin + mineral oil spray	20 g + 500 mℓ	X	X		57.4 abc	24.8 bc	14.8 cde	3.2 gh
Trifloxystrobin + mineral spray oil	30 g + 500 mℓ	X	X		50.0 bcde	23.4 bc	19.4 bcd	7.4 defgh
Trifloxystrobin + mancozeb + mineral spray oil	20 g + 200 g + 500 mℓ	X	X	X	72.8 a	20.0 bc	7.4 e	0 h
Bitertanol	100 mℓ	X	X		40.6 cde	24.6 ab	18.8 bcde	15.8 cde
Bitertanol	200 mℓ	X	X		46.2 bcde	29.4 bc	16.6 bcde	8.0 defgh
Carbendazim / difenoconazole	50 mℓ	X	X		54.0 bc	22.0 bc	12.6 cde	11.2 cdefg
Carbendazim / difenoconazole	100 mℓ	X	X		54.2 bc	19.4 bc	16.0 bcde	10.6 cdefg
Difenoconazole	40 mℓ	X	X		55.4 abc	20.0 bc	14.6 cde	10.0 cdefgh
Difenoconazole	80 mℓ	X	X		48.8 bcde	27.4 abc	17.6 bcde	7.0 defgh
Potassium phosphonate	1 ℓ	X	X	X	11.0 f	21.8 bc	31.2 a	36.0 b

[x] Means in a column followed by the same letter are not significantly different (P>0.05) according to Fisher's Least Significant Different test.

Fig. 1. Removal and burning of **c**itrus orchards that were heavily infected with *Pseudocercospora angolensis* in Zimbabwe.

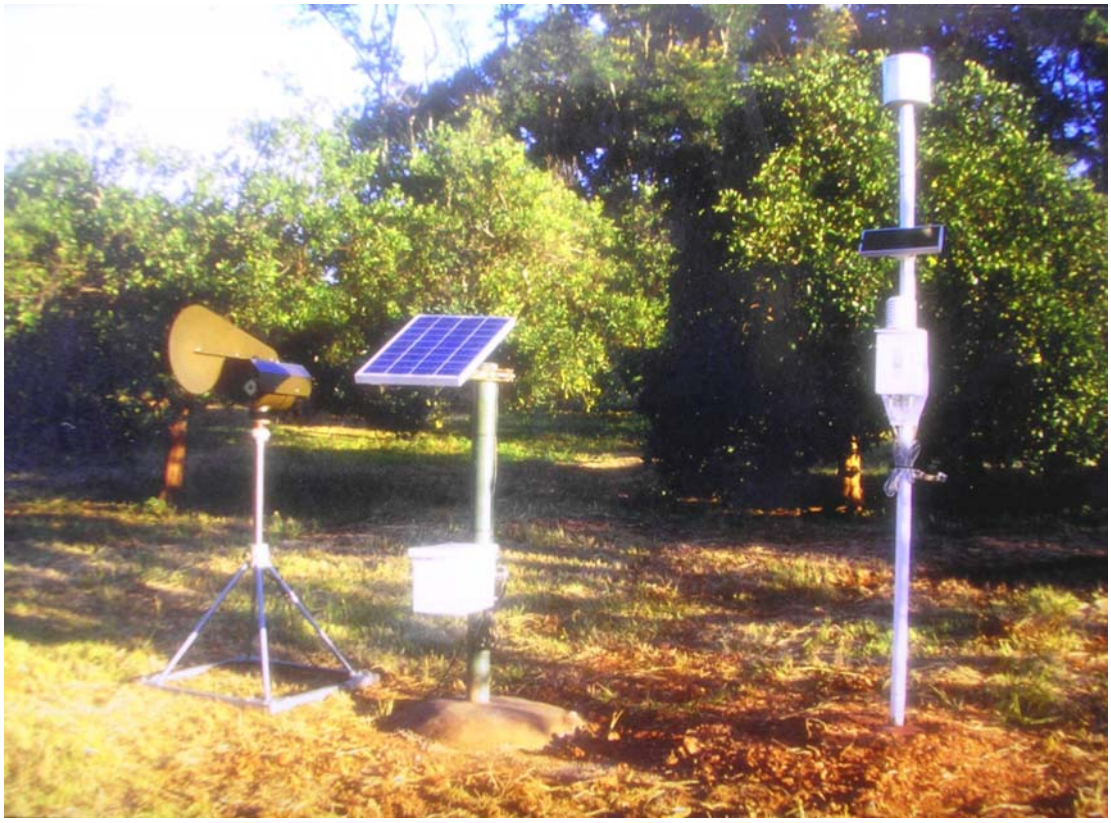

Fig. 2. A spore trap and electronic weather station installed at Sangere Farm in the Bindura region of Zimbabwe used for monitoring of conidia of *Pseudocercospora angolensis* and the gathering of weather data .

Fig. 3. Poly-carbonate disc of a Quest spore trap used to monitor conidia of *Pseudocercospora angolensis* on a weekly basis.

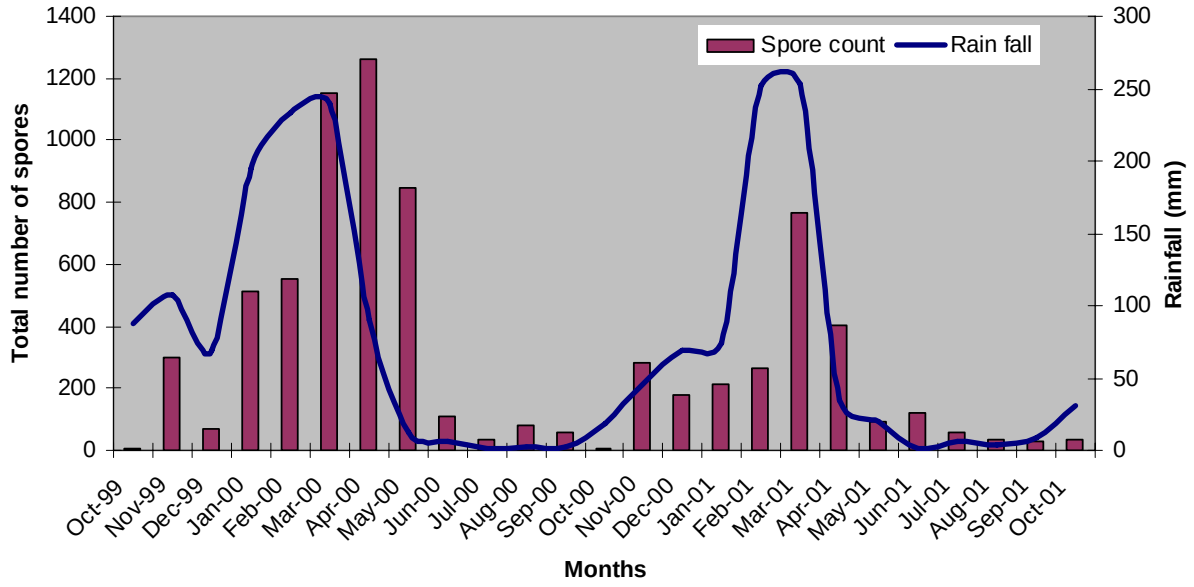

Fig 4. Number of *P. angolensis* spores recorded on a spore trap and average rainfall recorded on a data logger from October 1999 to October 2001 in a citrus orchard located at Sangere farm in the Bindura region, Zimbabwe.

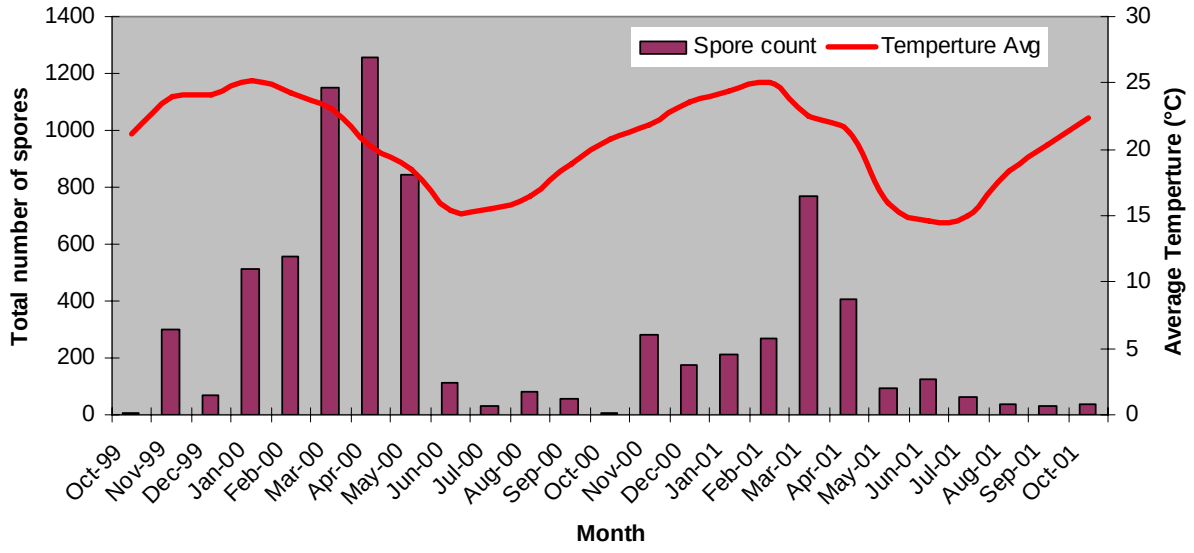

Fig 5. Number of *P. angolensis* spores recorded on a spore trap and average temperatures recorded on a data logger from October 1999 to October 2001 in a citrus orchard located at Sangere farm in the Bindura region, Zimbabwe.

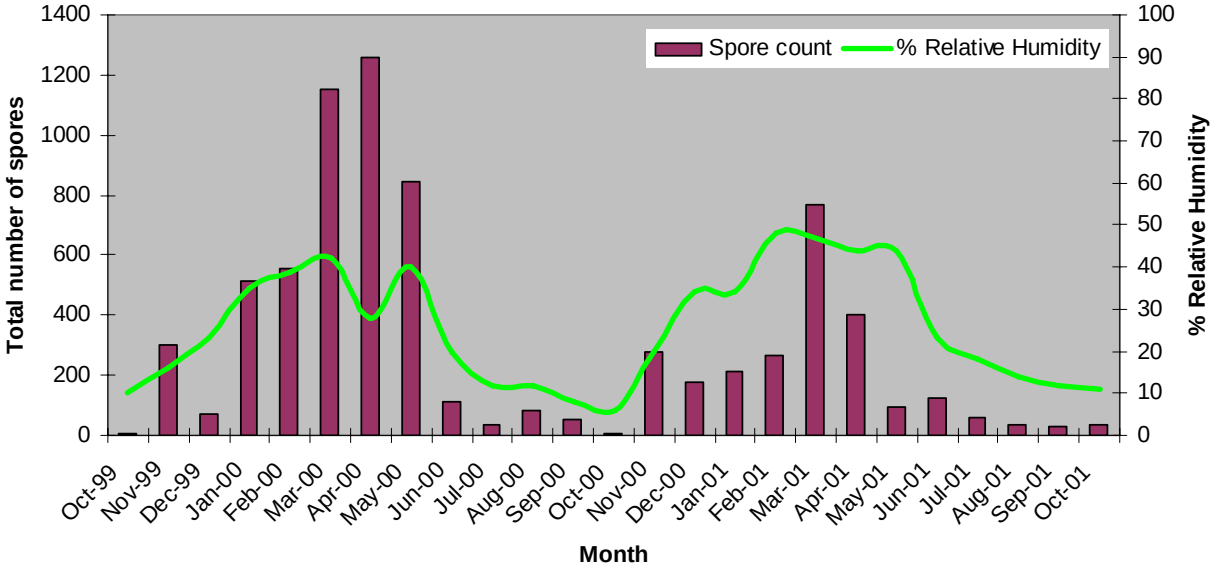

Fig 6. Number of *P. angolensis* spores recorded on a spore trap and percentage relative humidity recorded on a data logger from October 1999 to October 2001 in a citrus orchard located at Sangere farm in the Bindura region, Zimbabwe.