Angular leaf spot of Citrus - *Pseudocercospora angolensis*

*Pseudocercospora angolensis* is a dematiaceous hyphomycete occurring in sub-Saharan Africa and Yemen. This fungus requires moisture for infection and the production of wind-borne conidia and causes a devastating fruit and leaf spot disease of cultivated species of *Citrus*. Losses of 50-100% of yield can occur and growers may cease production where disease is endemic. Although species and cultivars of *Citrus* vary in susceptibility, no source of resistance is known (Kaute, 1998). An A1 quarantine pest for Europe and the Mediterranean region (EPPO, 2009), the fungus is also of concern for other warm humid regions where *Citrus* is grown, such as Florida. Other than by wind, conidia can be transported on infected fruit or propagative material.

*Pseudocercospora angolensis* (T. Carvalho & O. Mendes) Crous & U. Braun 2003

Conidiophores solitary, fasciculate, or forming loose synnemata 12-45 µm wide, unbranched, septate, smooth, pale-brown to brown, (60-)120-240 x 4.5-7 µm, usually arising from a dark stroma 30-60 µm diam.

Conidiogenous cells terminal, slightly geniculate, scars conspicuous but unthickened and only slightly pigmented.

Conidia solitary or in simple or branched chains of 2-4, cylindrical to narrowly obclavate, straight or slightly flexuous to more or less curved, smooth, hyaline to very pale brown, (1-)3-4(-6)-septate, 24-79 x 4-5 (-6.5) µm, apex rounded, base truncate. Basal and apical scars slightly thickened and pigmented, 2-3 µm.

**Symptoms:** On leaves the fungus produces circular, mostly solitary spots, which often coalesce, up to 10 mm in diameter, with a light brown or greyish centre when dormant and non-sporulating during the dry season, but becoming black with sporulation after the onset of the rainy season (Sief and Hillocks, 1993). The lesions are usually surrounded by a dark brown margin and a prominent yellow halo; occasionally the centre of the lesion falls out, creating a shot-hole effect. At first glance the young lesions appear similar to those of canker (caused by *Xanthomonas campestris pv. citri*), but differ in being flat or shrunken. Leaf spots, especially on younger leaves, often coalesce and together cause generalized chlorosis, followed by premature abscission and defoliation of the affected tree. Young leaves and fruit appear to be more susceptible than older mature leaves (Sief and Hillocks, 1999), but whether the leaves or fruit are more affected varies with the host species and variety (Manga et al., 1999) and location (Derso, 1999).

On fruit the spots are circular to irregular, discrete or coalescent, and mostly up to 10 mm in diameter. On young fruits, infection often results in hyperplasia, producing raised tumor-like growths surrounded by a yellow halo; these develop central necrosis and collapse (Kuate, 1998). Lesions on mature fruit are normally flat but sometimes have a slightly sunken brown centre. Diseased fruits ripen prematurely and drop or dry up and remain on the tree (Kuate, 1998). Infection by the fungus seems to predispose the fruit to secondary infection by *Colletotrichum gloeosporioides* (De Carvalho and Mendes, 1952; Seif and Kungu, 1990); it is common to find a dark-brown to black sunken margin of anthracnose around the fruit spots.

Stem lesions are not frequent and mostly occur as an extension of lesions on the petiole. Occurrence of several such lesions at the stem tip results in dieback; those on other parts of the stem coalesce, become corky, and crack. At the base of the dead stem there is usually a profuse growth of secondary shoots (Menyonga, 1971).

For additional details, see Kirk, 1986.

**Host range:** *Citrus* spp. (Rutaceae)

**Geographic distribution:** Africa, Asia (Yemen)

**NOTES ON TAXONOMY AND NOMENCLATURE**

This species was first described as *Cercospora angolensis* by de Carvalho and Mendes (1953), causing a leaf spot on *Citrus sinensis* in Angola. It was subsequently reported by Emechebe (1981) as *Phaeoisariopsis* sp. from citrus in Nigeria and from other citrus-growing areas in Africa (see Seif and Hillocks, 1993). Kirk (1986) transferred it to the genus *Phaeoramularia* because the pale brown conidia are produced in chains and the scars at the conidiogenous loci are conspicuous and slightly pigmented (Pretorius et al., 2003). Braun (1999) assigned it to a new genus *Pseudo phaeoramularia* because the scars on the conidiogenous cells are unthickened, i.e., the conidiogenous loci do not fit with those of the former genus *Phaeoramularia* (now *Passalora* emend., see Crous and Braun, 2003). Crous and Braun (in
Pretorius et al., 2003) carried out molecular analyses and reassessments of conidiogenesis and the structure of the conidiogenous loci. They determined that the conidiophore morphology is not distinct from that of the genus *Pseudocercospora*. Other species of *Pseudocercospora* also produce short conidial chains. Furthermore, *P. angolensis* clustered in molecular sequence analyses with other species of *Pseudocercospora*. Consequently, *Cercospora angolensis* is placed in *Pseudocercospora* (Pretorius et al., 2003).

**DISTRIBUTION**

*Pseudocercospora angolensis* appears to be restricted primarily to the humid tropics in Africa between altitudes of 80 and 1500 m (Brun, 1972; Seif et al., 1989). It has also been reported from Yemen (Kirk, 1986). Derso (1999) and Yusef (2007) describe the spread and impact of the fungus in Ethiopia. As of 2008, it was no closer to the citrus-producing regions of South Africa than the moister northern part of Zimbabwe (Pretorius and Holtz, 2008).

**HISTORY OF INTRODUCTION/SPREAD**

Since its identification in Angola and Mozambique in 1952 (de Carvalho and Mendes, 1952), *Pseudocercospora angolensis* has appeared to spread north to other parts of Africa (Seif and Hillocks, 1993). It was found in central African countries in the late 1960s, in West Africa in the next decade, and then in eastern Africa in the 1980s (Seif and Hillocks, 1993). Derso (1999) described the appearance of the disease in southern Ethiopia in 1990 and later in the highlands of Guinea in 1993 (Diallo, 2001). Because wild hosts may harbor this pathogen, it is not clear to what extent increased development of the cultivation of citrus has resulted in the fungus spreading from local sources, compared to the role of increased commercial and private transport of infected plant materials in carrying the pathogen across distances.

**RISK OF INTRODUCTION**

The fungus can be transported in or on infected fruit or propagative material. If conidia are not present, substantial moisture appears to be required for their production and for new infections (Emechebe 1981). Wind is the known means of local dissemination of conidia. Spread of the fungus is probable in warm regions where there is enough moisture, but it is not clear if the Mediterranean climate is suitable (Vicent and García-Jiménez, 2008). The warm and humid conditions of the important citrus-growing regions of Florida are likely to be favorable for this pathogen (Chung and Timmer, 2009).

**SIMILARITIES TO OTHER SPECIES/CONDITIONS**

At an early stage, the lesions caused by *Pseudocercospora angolensis* on leaves appear similar to those of citrus canker caused by the bacterium *Xanthomonas campestris* pv. *citri* (Hasse) Dye. They differ in being flat or shrunken, rather than raised (Seif and Hillocks, 1993). Canker lesions on leaves also have a yellow halo, but are distinguished by a water-soaked margin around the spot (Brlansky, 1988), as are the flat lesions caused by other bacterial pathogens of citrus (Duan et al., 2009).

The fungus *Guignardia citricarpa* Kiely also causes spots on leaves and/or fruits of Citrus in Africa, Asia, Australia, and South America. The lesions of the disease called ‘black spot’ may resemble those produced by *P. angolensis*, particularly on lemon leaves or take the form of ‘freckle spots’ or red-brown-bordered irregular sunken necrotic spots. Small, globose, black fungal pycnidia containing single-celled colorless spores often are produced in these spots (Kotze, 1988).

**DETECTION AND INSPECTION METHODS**

The lower sides of leaves should be examined for the dark sporulation of the fungus in grey to brown sunken lesions with yellow halos; the lesions are also visible from the upper surface (Kuate, 1998). Mature fruits also bear sunken brown lesions with a yellow halo, with sporulation occurring under wet or humid conditions.

**DIAGNOSTIC METHOD**

Sequences of the ITS1, ITS2 and 5.8s regions of ribosomal DNA are available in GenBank for comparison (NCBI, 2009).

**NOTES ON HABITAT**

Within the humid African range of the distribution of the fungus, elevation appears to play a role in the epidemiology of the disease. Kuate (1998) described the fungus as most common above 200 m, and Diallo (2001) reported that disease was serious on trees of the highlands of Guinea while the lowland areas appeared to be disease free. In Kenya, disease is serious at altitudes above 600 m (Seif and Hillocks, 1993). To the south in Angola, trees at lower altitudes were more at risk (Ragazzi, 1997).

**NOTES ON CROPS/OTHER PLANTS AFFECTED**
All species of cultivated Citrus appear to be susceptible, although the lime (C. latifolia) and smooth lemon (C. limon) are often reported to be relatively resistant. Of the other members of the Rutaceae in Africa, Citropsis tanakae is known to be infected (Kuate, 1998). The susceptibilities of the many wild Citrus species in Asia (USDA-ARS, 2009) remain unknown.

BIOLGY AND ECOLOGY

Limited studies on field epidemiology have been carried out in western Africa (Emechebe, 1981; Kuante and Foure, 1988). The disease is favoured by prolonged wet weather conditions followed by dry spells (Emechebe, 1981; Kungu et al., 1989) coupled with moderately cool temperatures of 22-26°C (Kungu et al., 1989). Disease incidence varies with the amount of rainfall (Kuate et al., 1994).

At the onset of a rainy season, non-sporulating lesions may be present on older leaves when the new disease-free leaves are formed. Sporulation begins in the old lesions after a further 3-5 weeks and new symptoms appear on young leaves 2-3 weeks later (Emechebe, 1981). The old lesions appear to be the source of inoculum when conditions favor infection.

Long-distance dispersal of the fungus is by windborne conidia (De Carvalho and Mendes, 1952); locally dispersal it is primarily by rain-splash or raindrops (Seif et al., 1989). Undoubtedly humans mediate in the dissemination of the fungus through transport of infected plant material and/or fruits from infected areas. Because leaf lesions produce more conidia than similar lesions on fruit (Seif and Hillocks, 1993), it is most likely that they constitute the main source of infection during disease spread in infected areas.

Survival mechanisms are unknown; the fungus probably survives in dormant lesions on infected material until the onset of conditions conducive to sporulation.

No physiological specialization is known in this pathogen. In his study of vegetative compatibility groups among isolates from Angola, Ragazzi (1997) found their distribution in the population to be homogeneous. There was little variation in the disease caused by ten isolates used in inoculations in Cameroon (Kuate et al. 1997).

MOVEMENT AND DISPERSAL

Natural dispersal: Windborne conidia are the apparent sole means of natural dispersal. No sexual form is known. The possibility of wild hosts of the fungus should be examined (Kuate, 1998).

Vector transmission: Insect transmission may occur, but is not reported.

Accidental introduction: Human transport of infected fruit and propagating material has undoubtedly played some role in the spread of the fungus in Africa (Sief and Hillocks, 1993; Kuate, 1998).

ECONOMIC IMPACT

Pseudocercospora angolensis causes a significant disease of citrus, the most devastating effect being the premature abscission of young fruit and leaves. The development of even a few fruit lesions renders the fruit unmarketable. A yield loss of 50-100% is not uncommon in most disease-affected areas (Menyonga, 1971; Brun, 1972; Seif and Kungu, 1989). The loss of leaves and dessication of shoots can have a significant debilitating effect on the tree, which will affect subsequent fruit yields (Kuate, 1998). In some areas, farmers have abandoned citrus plantings or replaced them with other crops (Seif and Hillocks, 1993; Kuate, 1998; Kassahun et al., 2006).

PREVENTION

Prevention of the transport of infected trees and fruit from contaminated areas is an important measure for inhibiting the spread of the pathogen in and from Africa (Kuate, 1998). The most significant citrus production in Africa, near the Mediterranean in North Africa and in the Republic of South Africa, occurs in countries currently outside of the range of distribution of Pseudocercospora angolensis (Sief and Hillocks, 1993). Vicent and Garcia-Jimenez (2008) suggest that the relative aridity of the Mediterranean climate may make it unsuitable for wind-disseminated pathogens such as P. angolensis; the periods of interrupted leaf wetness provided by dew in Spain might be sufficient for infection. This fungus is an A1 level restricted organism for Europe (EPPO, 2009).

CULTURAL CONTROL AND SANITARY MEASURES

The following disease management methods have been recommended (Seif and Kungu, 1989):

- Collection and destruction by burying and/or burning of all fallen fruit and leaves in affected orchards. This may drastically reduce the inoculum pressure in the field.

- Planting of windbreaks around the citrus orchards to minimize the impact of wind, which is the primary dispersal agent for spores.
- Discouraging inter-planting in affected orchards composed of mature producing trees, fostering a microclimate of relatively cool temperatures and high relative humidity (RH) thus preventing disease development.

- Judicious pruning of shoots, particularly those that have died back, to allow light penetration into and free aeration within the tree canopy, thus making the environment in the phyllosphere less conducive to disease development i.e. shorter leaf wetness period, lower RH, moderate temperatures.


CHEMICAL CONTROL

The most effective fungicides tested on fruit and leaf spot of citrus in Cameroon were copper oxide and benomyl (Menyonga, 1971; Rey et al., 1988). Others found to be effective were mancozeb, triademorph, triadimenol, captan and propiconazole (Rey et al., 1988). Applications of mancozeb in the rainy season were not effective in Zimbabwe (Pretorius and Holtz, 2008).

Use of benomyl alone may lead to development of resistant strains of the fungus (Kaute, 1998). Treatments with benomyl, alternated with copper-based fungicides, may be applied at two-week intervals beginning a week after the onset of rains (Sief and Hillocks, 1993). A further three fortnightly applications with copper-based fungicides followed by one of benomyl can be made when the fruits are the size of golf balls (approximately 3 cm diam). Sief and Hillocks (1997) recommend spraying after rainfall, rather than on a fixed schedule, because rain stimulates spore production and favors infection (see Kuate et al., 1994).

Of the newer triazole fungicides, fluzilazole provided the best control of disease in the field, while tebuconazole was not as effective as a copper hydroxide formulation (Sief and Hillocks, 1997). Pretorius and Holtz (2008) reported that a trifloxystrobin + mancozeb + mineral spray oil combination applied in November, January and March provided the best control of the disease on foliage in Zimbabwe. A mixture of benomyl and chlorothalonil applied at 15-day intervals was most effective in controlling the disease on the leaves of sweet orange in Ethiopia, compared to a mixture of benomyl and copper hydroxide or any of the fungicides alone (Kassahun et al., 2006).

Some recent attention has focused on the possibility for use of natural oils, which should be relatively cheap, available, and safer in the environment, in place of synthetic chemicals. Oils extracted from the skin of fruits of more resistant species of Citrus, C. latifolia (lime) and C. limon (lemon) reduced growth of an isolate of the pathogen more than did the extracts of susceptible species (Jazet Dongmo et al., 2002). Furthermore, among the oil extracts of 22 varieties of cultivated Citrus, those of the more disease-tolerant varieties were the most effective in reducing radial growth of the fungus in culture, but those of the susceptible varieties were more effective in inhibiting sporulation (Kuate et al., 2006). Oil extracts from the leaves of two Eucalyptus species had minimum inhibitory concentrations (MICs) of 6000 and 6500 ppm (Jazet Dongmo et al., 2008a).

Essential oils of the bottlebrush plants Callistemon citrinus and C. rigidus had similar activity (Jazet et al., 2009). Lime leaf oil extract had an MIC of 1600 ppm, with both fungistatic and fungitoxic effects (Jazet Dongmo et al., 2008b), and oils of Citrus aurantifolia (Key lime) had MICs of 1400 to 1500 ppm (Jazet Dongmo et al., 2009). The antifungal activity of certain fractions of the lime oil appeared to be due to their high content of neral and geranial. Of the extracts reported so far, those of the grass Cymbopogon citratus were most effective at inhibiting fungal growth in the laboratory, with activity at 600 ppm comparable to that of a fungicide (Tchinda et al., 2009).

HOST RESISTANCE

Cultivated species and varieties vary in susceptibility to the fungus and effects of the disease (Manga et al., 1999; Sief and Hillocks, 1999). Use and development of resistant varieties would benefit growers with small orchards or a few trees, who cannot afford fungicide treatments (Yesuf, 2007). Nevertheless, progress in this effort is hindered by an absence of strong resistance, loss of apparent resistance in different ecological zones or in different seasons, and the need to evaluate the susceptibilities of leaves and fruits (Kuate, 1998).

GAPS IN KNOWLEDGE/RESEARCH NEEDS

Additional knowledge is needed of the conditions that favor infection and sporulation. Sources of resistance should be sought among the wild species of Citrus and related genera, while the role of wild hosts in persistence and spread of the pathogen in Africa should be explored.

References
Use this link to revisit SMML website
1. Sporulation in lesion on *Citrus sinensis* BPI 0432660

Conidia. 1000X. BPI 0432660