Distribution and management of *Phaeoramularia* leaf and fruit spot disease of citrus in Ethiopia

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Abstract — Introduction. Citrus is the most important fruit crop widely grown by small-scale and commercial farms in Ethiopia. However, productivity of citrus in many parts of the country is threatened by *Phaeoramularia* leaf and fruit spot disease caused by *Phaeoramularia angolensis*. Surveys and field experiments were conducted to map the geographical distribution, and to study the efficacy of different fungicides for the control of this disease. **Materials and methods**. Surveys were conducted in the major citrus-growing areas (south, south-west and north-west) of Ethiopia. Random sampling techniques were used for sample collection. The efficacy of different fungicides against *P. angolensis* was studied under a hot-spot area, based on disease incidence and severity, and marketable fruit yield. **Results and discussion**. *Phaeoramularia* leaf and fruit spot disease of citrus has been widespread throughout the south, south-west, and north-west of Ethiopia, causing complete crop failure. This disease was more severe in smallholder farmers' fields as compared with the commercial orchards. The severity of *P. angolensis* was varied between different citrus species and areas with different agro-ecologies. The south and south-west part of the country, characterized by high and frequent rainfall with high humidity, were severely affected by the disease. Field application of Benlate, Score and Cuproxat significantly reduced the incidence and severity of the disease and, thereby, increased marketable fruit yield. **Conclusion**. *Phaeoramularia* leaf and fruit spot disease of citrus has become widespread in many parts of Ethiopia within a short period. Further investigation on *P. angolensis* biology and detailed epidemiological studies are mandatory to develop a model, and, thereby, to facilitate forecasting systems for effective and sustainable management of leaf and fruit spot disease of citrus in Ethiopia.

Ethiopia / *Citrus* / plant diseases / blotches / *Phaeoramularia angolensis* / geographical distribution / disease control

Distribution et contrôle de la cercosporiose des agrumes en Éthiopie.

Résumé — Introduction. En Éthiopie, la culture des agrumes est la production fruitière la plus développée en petites exploitations comme en vergers commerciaux. Cependant, dans la majeure partie du pays la productivité des arbres est menacée par la cercosporiose, maladie affectant les feuilles et fruits des agrumes, et provoquée par *Phaeoramularia angolensis*. Des prospections et une expérimentation en champ ont été menées afin de définir la répartition géographique de la maladie, et d'étudier l'efficacité de divers fongicides utilisés pour son contrôle. **Matériel et méthodes**. Des prospections ont été conduites dans les principales régions productrices (sud, sud-ouest et nord-ouest) d'agrumes en Éthiopie. Des techniques d'échantillonnage randomisé ont été employées pour la collecte d'échantillons. L'efficacité de différents fongicides utilisés contre *P. angolensis* a été étudiée dans un secteur de forte infestation, en se basant sur l'incidence et la sévérité de la maladie, ainsi que sur le rendement en fruits commercialisables. **Résultats et discussion**. La cercosporiose des agrumes s'est révélée répandue dans tout le sud, le sud-ouest et le nord-ouest de l'Éthiopie y causant la perte décisive des récoltes. Cette maladie est apparue plus grave dans les petites exploitations que dans les vergers commerciaux. La sévérité des attaques dues à *P. angolensis* a varié selon les différentes espèces d'agrumes étudiées et l'agroécologie des régions de production. Les régions du sud et du sud-ouest du pays, caractérisées par de fortes et fréquentes précipitations et un taux d'humidité élevé, ont été sévèrement affectées par la maladie. L'application en champ de Benlate, Score et Cuproxat a sensiblement réduit l'incidence et la sévérité de la maladie et a accru le rendement en fruits commercialisables. **Conclusion**. La cercosporiose des agrumes s'est répandue dans de nombreuses régions d'Éthiopie en peu de temps. Davantage de recherche sur la biologie de *P. angolensis* et des études épidémiologiques détaillées seront nécessaires pour développer un modèle, et, de ce fait, faciliter un système d'avertissement pour une gestion efficace et durable de la cercosporiose des agrumes en Éthiopie.

Éthiopie / *Citrus* / maladie des plantes / cercosporiose / *Phaeoramularia angolensis* / distribution géographique / contrôle de maladies

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***RESUMEN ESPAÑOL**, p. 106*

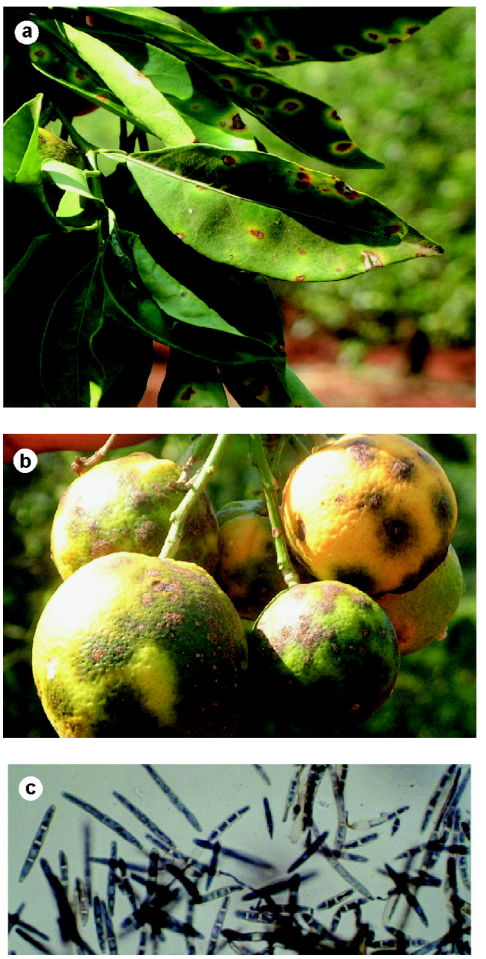

Figure 1.
Characteristic symptoms of *Phaeoramularia* leaf and fruit spot disease on sweet orange (*Citrus sinensis*): (a) infected leaves, (b) infected fruits, (c) conidia of *Phaeoramularia angolensis*.

1. Introduction

With a coverage estimated to be more than 5300 ha [1, 2], citrus is the most important fruit crop widely grown in Ethiopia. However, in many parts of the country, citrus productivity is threatened by a disease called *Phaeoramularia* leaf and fruit spot (*figure 1*). This disease, caused by a fungus, *Phaeoramularia angolensis* (Covalho & Mendes) Kirk, was first reported in Angola and Mozambique in 1952. Within a short period, the disease spread northwards to the south of the Sahara [3–5]. Later it spread to the eastern part of Africa: Uganda [6], Kenya [5] and Ethiopia [7]. The disease has also been reported in Yemen [6]. Leaf and fruit spot disease of citrus is transmitted by airborne conidia or infected planting materials [8, 9]. Currently, the disease is widespread and is becoming a major threat to citrus plantation in 20 African countries including Ethiopia [6, 10]. Leaf and fruit spot disease of citrus has not yet been reported in the rest of the world, except Yemen.

In Ethiopia, the characteristic symptoms of leaf and fruit spot disease of citrus were first observed in 1988 around the Aletawondo and Dale areas, near the border with Kenya [11]. Later, in 1990, similar disease symptoms were also apparent around Bebeka and the south-west part of Ethiopia [12]. The causal agent of the disease was positively identified for the first time in Ethiopia to be *Phaeoramularia angolensis* [7]. The disease can cause a yield loss of (50 to 100)% in areas with high rainfall and humidity during the active growth stages of the crop [13–15]. Currently, citrus plantations in the south and south-west of Ethiopia are seriously affected by this disease. Consequently, the supply of citrus fruits in these areas has been insufficient for the last ten years. Several small-scale citrus growers have abandoned their trees due to severe infection of citrus by the disease. The policy of the Ethiopian government is presently to promote fruit production, particularly citrus, mainly to improve the nutritional standard of the people and to obtain a source of income for resource-poor farmers. However, leaf and fruit spot disease is becoming the major limiting factor of the citrus industry. The national distribution, biology and factors of disease epidemics of this pathogen have not yet been exhaustively investigated. Despite the devastating nature of the disease, research efforts on disease distribution and management options to tackle this disease are negligible.

Therefore, our research was initiated to study the geographical distribution of the disease and efficacy of fungicides for its management in Ethiopia.

2. Materials and methods

2.1. Disease survey and identification

Surveys were conducted in the major citrus-growing areas of Ethiopia during the 2001

and 2002 crop seasons. Samples of infected leaves and fruits were collected randomly from all citrus species (sweet orange, mandarin, lemon, lime and grapefruit) present in the 24 citrus orchards surveyed. From each of these citrus orchards, 10 trees were randomly selected towards the diagonal of the field. Leaf samples were taken from different parts of the canopy (lower, middle and upper) and, to assess the incidence and severity of *P. angolensis,* 25 fruits were sampled from each tree. Samples were kept at 5 °C in a refrigerator. To avoid secondary invaders, both leaf and fruit samples were surface-sterilized using 1% sodium hypochlorite (NaOCl). Isolation was done by placing pieces of tissue on Petri dishes with a freshly-prepared potato dextrose agar medium (PDA). The Petri dishes were then kept for 7 d in an incubator under a temperature of (22 ± 1) °C, and then fungal growth was examined under a binocular and compound microscope. Single spore isolation was carried out to obtain pure culture of *P. angolensis*.

2.2. Susceptibility of citrus species to *P. angolensis*

Evaluation of different *Citrus* species (*C. aurantifolia*, *C. limon*, *C. paradisi*, *C. reticulata* and *C. sinensis*) was done for their relative susceptibility to leaf and fruit spot disease of citrus in a hot-spot area. Disease assessment was superimposed on the already established citrus trees. From each species, five trees and fifty fruits per tree were randomly selected. Leaves were sampled from different canopy parts (lower, middle and upper) and directions. Assessment of percent severity (percent of leaf and fruit area covered by lesions) and number of lesions on leaves and fruits was recorded at different growth stages.

2.3. Fungicide screening

Various fungicides of different groups [Benlate® (benomyl, Du Pont de Nemours, France, S.A.S.), different formulations of Kocide® (copper oxychlorid, Du Pont de Nemours, France, S.A.S.), Score® (difenoconazole, Syngenta Agro S.A.), Cuproxat® (complex composition, Agrolinz, France), and Cuprofix® (sulfate copper, Cerexagri] were tested at Ghibe citrus orchard which is a hot-spot area for leaf and fruit spot disease of citrus in Ethiopia. The experiment was conducted for three consecutive years (2000, 2001 and 2002), during the main crop season, on sweet orange (*Citrus sinensis* cv. Valencia) using a randomized complete block design with four replications. Fungicides were applied, based on manufacturers' recommendations, every 2 weeks, using a motorized knapsack sprayer. Each treatment was replicated four times by considering a single tree as a replicate.

Disease incidence on the foliage was estimated by counting total and infected leaves of eight shoots randomly selected from four directions of a tree. Disease incidence on mature fruits was assessed using 50 randomly selected fruits from each tree after harvest. Severity on leaves and fruits was estimated based on percent area covered by lesions of the disease.

2.4. Data analysis

Data were analyzed using the analysis of variance (ANOVA) procedure. Mean separation tests were performed using Student's Newman Keuls test using SAS version 8.0.

3. Results and discussion

3.1. Disease surveys

The survey data indicated that *Phaeoramularia* leaf and fruit spot disease of citrus was widely distributed throughout citrus-growing areas of the south, south-west and north-west parts of Ethiopia (*figure 2*). Eshetu [7] reported in 1999 that this disease was distributed in the south and south-west of the country. However, we noted that it has also been widely distributed to the major citrus-growing areas of the north-west part of Ethiopia. The incidence and severity of the disease varied across the agro-ecology of the areas. *Phaeoramularia* leaf and fruit spot disease of citrus was found to cause complete crop loss, especially on small-scale peasant holdings. The south and south-west

Figure 2.
Geographical distribution of *Phaeoramularia* leaf and fruit spot disease of citrus in Ethiopia.

parts of Ethiopia (Ghibe, Jimma, Metu, Wolisso and Wolkite) were found to be the most affected as compared with the northwest regions (*table I*). Apart from *P. angolensis*, anthracnose caused by *Colletotrichum gloeosporioides*, root rot (*Phythophtora* sp.), leaf spot (*Alternaria* sp.) and fruit rot (*Penicillium* sp.) were found to be important pathogens associated with citrus in the surveyed areas, causing both preharvest and postharvest infection.

The observation that the severity of leaf and fruit spot disease of citrus was more pronounced in smallholder farmers' orchards as compared with the commercial orchards might be due to the fact that smallholder farmers do not have technical know-how and access to disease management practices. For instance, some commercial citrus farms practiced sanitation measures and were able to reduce pathogen buildup for the coming crop season.

3.2. Susceptibility of citrus species to *P. angolensis*

Evaluation of different citrus species in a hot-spot area for relative susceptibility of *P. angolensis* based on incidence and severity assessment indicated that grapefruit (*Citrus paradisi*) was the most susceptible citrus species, followed by sweet orange (*C. sinensis*), mandarin (*C. reticulata*) and lemon (*C. limon*), with lime (*C. aurantifolia*) being the least susceptible (*table II*). More lesions

Table I.
Incidence and severity of *Phaeoramularia* leaf and fruit spot disease of citrus assessed by surveys in different citrus-growing regions of Ethiopia.

Locations	Incidence[1] %		Severity[2] %	
	Leaves	Fruits	Leaves	Fruits
Aletawondo	89.6	88.4	78.4	82.4
Areka	85.5	83.8	76.2	78.5
Chagni	84.8	82.8	68.9	76.2
Fnoteselam	82.6	78.6	72.6	78.4
Ghibe	92.2	88.5	81.8	86.0
Gonder	64.5	78.6	52.4	65.6
Jimma	96.6	90.2	83.5	90.4
Metu	95.4	89.4	84.0	89.8
Wolkite	98.6	94.2	84.0	88.6
Wolliso	96.4	90.4	80.6	84.2

[1] Incidence % = percent of leaves and fruits affected from the total per tree.

[2] Severity % = leaf and fruit surface area covered by lesions (%).

were counted during the wet and humid seasons than during the dry seasons. The number of lesions on both leaves and fruits was higher around the lower tree canopy than in the middle and upper canopy of trees. This could be substantiated mainly due to high moisture and humidity around the lower tree canopy. Extended and high rainfall during November and December created favorable conditions for infection of leaves and fruits in the Ghibe orchard. At the early fruit-setting stage of the crop, the severity of *P. angolensis* was more intense on leaves than on young fruits. However, at the later crop stages, the severity of the disease increased on fruits (*figure 3*).

Table II.
Relative susceptibility of different *Citrus* species, based on incidence and severity assessment, to *Phaeoramularia* leaf and fruit spot disease in a Ghibe citrus orchard (Ethiopia).

Citrus species	Incidence[1] %	Severity[2] %
C. aurantifolia	40	28
C. limon	56	33
C. paradisi	90	81
C. sinensis	75	52
C. reticulata	68	42

[1] Incidence % = percent of leaves and fruits affected from the total per tree.

[2] Severity % = leaf and fruit surface area covered by lesions (%).

3.3. Fungicide screening

Lower disease incidence and severity was recorded from trees treated with Benlate, Score and Cuproxat. Higher marketable fruit yield was also obtained from trees treated with Benlate, followed by Score and Cuproxat (*tables III, IV*). Field application of chlorothalonil was reported as effective against leaf and fruit spot disease of citrus in Ethiopia [7]. Application of Benlate by alternating with copper compounds has also been found effective to control *P. angolensis* in Kenya [16].

4. Conclusion

Phaeoramularia leaf and fruit spot disease of citrus has been a recent introduction into Ethiopia, and it was a problem in the south and south-west part of the country [7]. However, in a very short time, this disease was widespread in many citrus-growing areas of the country including the south, south-west and north-west parts of Ethiopia. The disease causes heavy loss to the citrus industry in these areas. The most devastating effect of the disease on all citrus species surveyed is premature defoliation of young leaves and fruit drop, and sunken lesions on the fruit surface, which seriously affect their market value. Infected fruits became extremely hard, juiceless and unattractive (*figure 1*).

Application of Benlate, Score and Cuproxat every 2 weeks significantly reduced the incidence and severity of the citrus leaf and fruit spot disease at Ghibe. Benlate and copper fungicides have been reported to be effective against *P. angolensis* [16]. Field application of chlorothalonil, copper hydroxide, flusilazole and propinebe provided good control of the pathogen in East Africa [17]. Fungicide application should be done by alternating from different chemical groups, thereby avoiding development of resistance by the target pathogen population. Since Benlate is a systemic and expensive fungicide, it could be used by alternating with

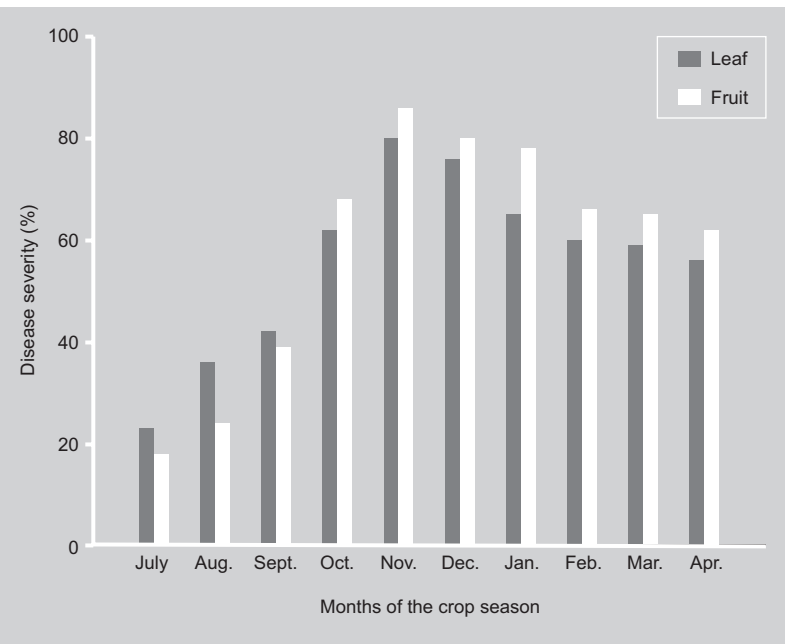

Figure 3.
Severity of *Phaeoramularia* leaf and fruit spot disease on sweet orange cv. Valencia based on area covered by lesions on leaves and fruits at different growing periods in Ghibe (Ethiopia).

Table III.
Effect of fungicide spray on the incidence of *Phaeoramularia* leaf and fruit spot disease of citrus and marketable fruit yield of sweet orange cv. Valencia in Ghibe (Ethiopia).

Fungicide	Active substances	2000/2001		2001/2002		
		Incidence on leaves (%)	Incidence on fruits (%)	Incidence on leaves (%)	Incidence on fruits (%)	Marketable fruit yield per tree (kg)
Control	–	58.7 a	67.3 a	58.0 a	69.3 a	75.6 c
Benlate 50% WP	Benomyl	31.0 f	38.0 d	31.3 f	40.7 de	130.7 a
–	Copper oxychloride 50% WP	44.3 bc	58.4 b	48.3 bc	58.0 b	79.3 bc
Cuprofix 78% WP	Copper sulfate + mancozeb	48.7 b	60.3 b	51.3 b	58.0 b	84.6 bc
Cuproxat 54% SC	Complex composition with copper sulfate	40.7 cd	48.7 c	39.0 d	45.7 cd	91.8 b
Kocide 101 77% WP	Cupric hydroxide	47.3 b	52.0 c	44.7 c	49.3 c	84.0 bc
Kocide 2000 53.8% WP	Copper hydroxide	36.3 de	48.7 c	37.3 de	48.3 c	82.3 bc
Score 25% EC	Diphenoconazol	32.0 ef	41.7 d	34.0 ef	39.3 e	124.7 a
Mean		42.4	51.9	43.0	51.1	94.1
Coefficient of variation (%)		12.6	10.8	14.5	11.4	9.6

Means followed by the same letter(s) in a column are not significantly different using Student's Newman Keuls test at (P = 0.05).

Table IV.
Effect of fungicide spray on the severity of *Phaeoramularia* leaf and fruit spot disease of citrus and marketable fruit yield of sweet orange cv. Valencia in Ghibe (Ethiopia).

Fungicides	Active substances	2000 / 2001			2001 / 2002	
		Severity on leaves (%)	Severity on fruits (%)	Marketable fruit yield/tree (kg)	Severity on leaves (%)	Severity on fruits (%)
Control	–	21.4 a	31.7 a	68.4 c	21.7 a	29.9 a
Benlate 50% WP	Benomyl	9.1 c	13.3 d	126.8 a	11.8 de	10.1 d
–	Copper oxychloride 50% WP	16.3 b	25.1 b	75.0 bc	17.8 abc	19.0 ab
Cuprofix 78% WP	Copper sulfate + mancozeb	14.7 b	21.6 bc	83.8 bc	18.5 ab	16.3 abc
Cuproxat 54% SC	Complex composition with copper sulfate	12.4 b	19.6 c	88.4 b	14.4 cd	14.4 c
Kocide 101 77% WP	Cupric hydroxide	16.3 b	21.2 bc	76.2 bc	16.3 bc	16.6 abc
Kocide 2000 53.8% WP	Copper hydroxide	14.2 b	18.8 c	74.5 bc	14.3 cd	15.2 bc
Score 25% EC	Diphenoconazol	8.9 c	12.7 d	118.6 a	9.9 e	10.6 d
Mean		14.2	20.5	89.0	15.6	15.3
Coefficient of variation (%)		16.1	12.4	12.2	13.9	13.8

Means followed by the same letter(s) in a column are not significantly different using Student's Newman Keuls test (P = 0.05).

copper fungicides, thereby avoiding pathogen resistance and reducing fungicide application costs. Production of citrus should concentrate on the lower altitude and semi-arid areas of Ethiopia with low rainfall, which are not conducive to the development of leaf and fruit spot disease of citrus. Sources of resistance of citrus species against *P. angolensis* and detailed epidemiological studies should receive future research attention.

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Distribución y control de la cercosporosis de los cítricos en Etiopía.

Resumen — Introducción. En Etiopía, el cultivo de los cítricos es la producción de frutos más desarrollada en pequeñas explotaciones como en los huertos comerciales. No obstante, en la mayor parte del país, la productividad de los árboles está amenazada por la cercosporosis, enfermedad que afecta las hojas y los frutos de los cítricos y provocada por *Phaeoramularia angolensis*. Se llevaron a cabo prospecciones así como un experimento en campo, con el fin de definir el reparto geográfico de la enfermedad y de estudiar la eficacia de diversos fungicidas usados para su control. **Material y métodos**. Se llevaron a cabo prospecciones en las principales regiones productoras (sur, sudoeste y noroeste) de cítricos en Etiopía. Se emplearon técnicas de muestreo aleatorio para la colecta de muestras. Se estudió la eficacia de los diferentes fungicidas empleados contra *P. angolensis* en un sector con plagas pronunciadas, basándose en la incidencia y en la gravedad de la enfermedad, así como en el rendimiento en frutos comercializables. **Resultados y discusión**. La cercosporosis de los cítricos resultó extendida por todo el sur, suroeste y en el nordeste de Etiopía, causando de este modo la pérdida importante de las cosechas. Esta enfermedad se mostró más severa en las pequeñas explotaciones que en los huertos comerciales. La gravedad de los ataques causados por *P. angolensis* varió de acuerdo con las diferentes especies de cítricos estudiados y con la ecología agraria de las regiones de producción. Las regiones del sur y del suroeste del país, caracterizados por precipitaciones fuertes y frecuentes y por un alto índice de humedad, fueron gravemente afectadas por la enfermedad. La aplicación en campo de Benlate, Score y Cuproxat redujo sensiblemente la incidencia y la gravedad de la enfermedad y aumentó el rendimiento de frutos comercializables. **Conclusión**. La cercosporosis de los cítricos se extendió en numerosas regiones de Etiopía en poco tiempo. Serán necesarias más investigaciones de la biología de *P. angolensis*, así como estudios epidemiológicos detallados con el fin de desarrollar un modelo; y, de este modo, facilitar un sistema de advertencia para una gestión eficaz y duradera de la cercosporosis de los cítricos en Etiopía.

Etíopia / *Citrus* / enfermedades de las plantas / cercosporiose / *Phaeoramularia angolensis* / distribución geográfica / control de enfermedades