Occurrence, prevalence and control methods of *Phaeoramularia* leaf and fruit spot disease of citrus in Ethiopia

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Occurrence, prevalence and control methods of *Phaeoramularia* leaf and fruit spot disease of citrus in Ethiopia.

Abstract — Introduction. In Ethiopia, sweet orange tree (*Citrus sinensis*) grows in most regions. In 1990, a new disease, which attacks fruits, young twigs and leaves of orange trees, was first observed in the south, at Bebeka State farm, and, within a very short period of time, it spread to the wet regions of south and south west parts of the country. A study was conducted to determine the occurrence, distribution and extent of damage, to identify the causative agent and to control the disease through fungicides. **Materials and methods**. Surveys were conducted at various locations to determine incidence and severity of the disease. Identification and pathogenecity tests were conducted in the laboratory. Fungicide screening trials were carried out at Bebeka and Metu. **Results and discussion**. Amongst the sites studied, those of Jima, Mizan, Bebeka, Metu and Limu were mostly affected. Overall mean incidence was estimated to be 88.19% while severity on leaves was 43% and on fruits 65%. The disease was identified as leaf and fruit spot of citrus caused by a fungus, *Phaeoramularia angolensis* (syn. *Cercospora angolensis*). During pathogenicity test, the fungus was reisolated from inoculated leaves, fruits and young twigs. Chlorothalonil controlled the disease better than prochloraz. **Conclusion**. The disease spreads very fast and efficient and economical control measures should be investigated. Moreover, epidemiology of the disease and survival mechanism of the fungus have to be thoroughly studied. (© Elsevier, Paris)

Ethiopia / *Citrus* / sweet orange / plant diseases / cercospora / disease occurrence / pathogens

Occurrence, répartition et méthodes de contrôle de la maladie des taches sur feuilles et fruits d'agrumes, causée par *Phaeoramularia*, en Éthiopie.

Résumé — Introduction. En Éthiopie, l'oranger (*Citrus sinensis*) pousse dans la plupart des régions. En 1990, une nouvelle maladie touchant les fruits, les jeunes rameaux et les feuilles d'agrumes a été observée d'abord dans la ferme d'État de Bebeka au sud du pays ; elle s'est propagée ensuite, très rapidement, dans toutes les régions humides du sud et du sud-ouest. Une étude a été entreprise pour déterminer l'occurrence, la répartition et l'extension des dégâts, pour identifier l'agent responsable de la maladie et en assurer le contrôle à l'aide de fongicides. **Matériel et méthodes**. Des enquêtes ont été effectuées sur différents sites pour déterminer l'incidence et la gravité de la maladie. Des tests d'identification et de virulence ont été menés en laboratoire. Des traitements fongicides ont été testés sur les sites Bebeka et Metu. **Résultats et discussion**. Parmi les sites étudiés, ceux de Jima, Mizan, Bebeka, Metu and Limu ont été les plus affectés. Sur l'ensemble de ces sites, la maladie a touché, en moyenne, 88,19 % des arbres, alors que 43 % des feuilles et 65 % des fruits d'un même arbre étaient atteints. Il s'agirait de la maladie des taches sur feuilles et fruits d'agrumes causée par un champignon, *Phaeoramularia angolensis* (syn. *Cercospora angolensis*). Les tests de virulence ont permis de réisoler le champignon à partir de feuilles, fruits et jeunes rameaux inoculés. Parmi les fongicides testés, le chlorothalonile a été plus efficace que le prochloraze. **Conclusion**. La maladie se propage très rapidement et des méthodes de contrôle efficaces et économiques devraient être mises au point. De plus, l'épidémiologie de la maladie et les mécanismes de survie du champignon doivent être minutieusement étudiés. (© Elsevier, Paris)

Éthiopie / *Citrus* / orange douce / maladie des plantes / cercospora / présence de maladie / agent pathogène

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1. introduction

Sweet orange tree (*Citrus sinensis*) grows best in warm sunny climates. In most months, the rainfall should be equal to or slightly higher than the potential evapotranspiration, although a few dry months are beneficial as they concentrate blooming. Where rainfall is much lower than potential evapotranspiration during more than 3 months, irrigation is required. Sweet orange tree does not tolerate much frost. In the tropics it can only be grown at an elevation of 1 000–2 000 m on most soil types provided that they are not saline, alkaline, water logged or acidic [1]. In Ethiopia, it grows in most parts of the country in gardens and back yards and, in some parts of the country, it is grown on small scale State farms. Citrus production in Ethiopia seem to have been little affected by external influences. In fact, most of the cultivation in the south and south-west region is based on a small number of local orange varieties. In most parts of the country, citrus is grown by small farmers with plantings of, often, less than 50 trees.

According to the ministry of State farms [2], a total of 7 290 ha of citrus planting was projected and this was expected to give a total output of 240,000 metric tons. Sweet orange tree is affected by a number of diseases caused by biotic and abiotic factors which induce considerable crop losses to citrus growers.

In 1990, a new disease, which indiscriminately attacks fruits, young twigs and leaves of orange trees, was observed in the south of Ethiopia, at Bebeka State farm, and, within a very short period of time, it spread to the wet regions of the south and south west parts of the country (*figure 1*). Currently the disease has reached epidemic level. The economic losses of the outbreak have resulted from direct fruit damage and premature leaf fall. The disease has become so drastic that, in some areas, farmers were compelled to up-root citrus trees and replace them with other crops.

The disease is known to occur in Mozambique [3], Angola [4], Nigeria [5], Cameroon [6], Kenya [7], Gabon, Uganda, Zambia and Zimbabwe [8]. However, in Ethiopia, it has not been reported earlier. This study was conducted to determine the occurrence, distribution and extent of damage induced by the disease, to identify the causative agent, and to control the disease through fungicides.

Figure 1.
Distribution of *Phaeromularia* leaf and fruit spot disease of citrus in Ethiopia.

2. materials and methods

Surveys were carried out in 1991–1993 in Jima, Seka, Bonga, Mizan, Bebeka, Tepi, Metu, Agaro and Limu (south west of Ethiopia) to determine the incidence and severity of the disease. This was done by taking randomly 10 trees at each site; incidence and severity from each orange tree was considered and the mean for the site was calculated. Incidence was taken as the number of orange trees affected by the disease while severity was taken as the num-

ber of diseased fruits and leaves on a single tree expressed as a percentage of total number of fruits and leaves on the tree. This was conducted using visual assessment technique which helps to evaluate many plantations in a relatively short period of time. The results were angularly transformed, which is appropriate when the percentage represents less than 100 observations [9]. Besides, data should be transformed if the range of percentage is greater than 40 [10].

Diseased leaves, young twigs and fruits from all surveyed areas were collected and brought to the laboratory for identification of the pathogen. Samples were surface sterilized with sodium hypochlorite solution and rinsed repeatedly with sterile water. Pieces of sterilized leaves, fruits and young twigs were cut and placed on petri dishes containing PDA (potato, dextrose, agar), NA (nutrient, agar) and MEA (malt, extract, agar) and were incubated at 26 °C. These media were chosen to encourage growth of possible primary pathogens, fungi or bacteria. After 3 d of incubation, isolates were taken from each petri dish and were characterized and identified. To induce sporulation, isolates were taken and grown on petri dishes containing nutritionaly weak media, PCA (potato, carrot, agar), and cultures were maintained for further uses.

Pathogenicity tests were conducted on apparently healthy leaves, fruits and young twigs. They were surface sterilized with sodium hypochlorite solution and were arranged in plastic boxes. Inoculation was carried out by placing a drop of conidial suspension, with concentration of 2×10^6 conidia·mL^{-1}, produced from diseased plant parts and from culture. The plastic boxes were closed for 24 h to maintain relative humidity and were kept in the laboratory. Control leaves, fruits and young twigs were inoculated with sterilized distilled water.

For fungicide screening trial, two sites were selected at Bebeka and Metu; on both sites, three plots, each having four trees, were selected. The design was randomized complete block design (RCBD) with three replications. Fungicides which are currently in use on coffee – chlorothalonil 75% wettable powder (w.p.) and prochloraz 50% w.p, both of which were known to have broad spectrum effects – were used in the experiment. The rates were 130 g active ingredient per 100 L of water for chlorothalonil and 50 g active ingredient per 100 L of water for prochloraz. First application was done at early stages of fruit formation (1 week after fruit setting); second application was done after 4 weeks. Disease severity on each experimental plots was recorded three times throughout the crop season. Fruits were collected from each tree, counted, weighed, and classified as marketable and unmarketable.

3. results and discussion

Among the surveyed areas, Jima, Mizan, Bebeka, Metu and Limu were most seriously affected sites. In Metu at Dizi, on the way to Supe, complete damage of fruits was observed on a farmer's orchards. In Jima, the disease was very severe at Seka and particularly around Jima Research Center, at Melko and Doyo. Orange leaves were attacked less severly than the fruits. Disease severity at Mizan was high on leaves and fruits. In Bebeka, the disease was very serious at Abiye Arat where almost all of the orange trees were affected, and it was more pronounced on the fruits than on the leaves. Not a single tree was observed in which only fruits or leaves alone were affected. The same situation was observed in Limu. The disease was also observed at Gibe, Wolkite and in most parts of Sidamo. In the surveyed areas, randomly selected orange trees were all affected by the disease (*figure 2*). However, severity on leaves and fruits varied from site to site. Accordingly, transformed mean incidence value was 88.19% while severity on the leaves was 43% and on fruits 65% (*table I*).

At Bebeka and other areas, differences in susceptibility between individual trees were visually observed on local varieties. The disease was observed in all development stages of orange tree but maximum damage was inflicted on young leaves (*figure 3*) and fruit (*figure 4*).

Figure 2.
Citrus tree heavily infected with *Phaeromularia* leaf and fruit spot disease.

Critical examination of diseased material in the laboratory showed that leaf spots grew on both sides of the leaf and were pale brown to blackish brown. When sporulation is dense, surrounded by a dark brown margin and a yellow halo, the center often becomes detached resulting in shot-hole spot [8]. On the fruits, spots which develop are dark at first. However, at a later stage, they turn dark brown to blackish brown in colour, and their texture becomes rough and the whole fruit hardens. Sometimes spots are arranged on the fruits in curves or circles. Most infected fruits finally fall to the ground or become mummified and remain on the tree.

Isolates grown on media from leaves, fruits, and twigs were identical. The mycelium was creamy white in culture and compact. Better growth was observed after 3 d on potato dextrose agar media than on other media. Conidia borne in branched chains 2–4 conidia hyaline, cylindrical. It was observed that the optimum temperature for growth of the pathogen in the laboratory seems to range between 26–30 °C.

Using Koch's method [11], pathogenicity tests were conducted on detached apparently healthy fruits, leaves and young twigs. The same symptoms were observed on artificially inoculated leaves after 15 d. However, symptom development on inoculated fruit was slow. The same fungus having the same characteristics and morphology was re-isolated from inoculated leaves fruits and young twigs. The disease was identified as "leaf and fruit spot of citrus" and the causative agent was a fungus known as *Phaeoramularia angolensis* (syn. *Cercospora angolensis*) (Carvalho and Mendes [3, 4]). This species was fully described by P.M. Kirk [8]. The identification was confirmed by the Center for Agriculture and Bioscience International (CABI) Bioscience in the UK (IMI No.361170).

Table I.
Severity and incidence of leaf and fruit spot of orange at various sites of Ethiopia (data angularly transformed).

Location	Altitude (m asl)	Severity (%)		Incidence (%)	
		Leaf	Fruit	Leaf	Fruit
Bebeka	1 050	33.39 (31.0)	58.53 (71)	88.19 (100)	88.19 (100)
Mizan	1 260	62.97 (78.0)	70.68 (88)	88.19 (100)	88.19 (100)
Metu	1 750	41.40 (44.0)	88.19 (100)	88.19 (100)	88.19 (100)
Limu	1 690	53.13 (63.0)	56.37 (68)	88.19 (100)	88.19 (100)
Jima	1 760	24.79 (18.5)	52.98 (63)	88.19 (100)	88.19 (100)
Mean	–	43.14 (46.9)	65.35 (78.0)	88.19 (100)	88.19 (100)
S.E.	–	15.23 (21.4)	14.41 (13.8)	–	–
C.V. (%)	–	0.35 (45.6)	0.22 (17.7)	–	–

Figures in parenthesis show actual values.

According to the results of the chimical spray trial conducted at Bebeka (*table II*), even though there was no significant difference between treatments, relatively better disease control was observed on plots treated with chlorothalonil 75% w.p. Control of the disease with prochloraz 50% w.p. was somewhat lower than obtained with chlorothalonil. In all treatments, marketable yield was very low: 65.5 $q \cdot ha^{-1}$ for chlorothalonil and 38.7 $q \cdot ha^{-1}$ for prochloraz. Percentage of unmarketable fruits was high, 64% for chlorothalonil, 67.6% for prochloraz and 83.3% for the untreated control. The trial showed positive correlation between disease control and yield. However, the efficacy of both fungicides was not as expected, which might be attributed to the late time of fungicide application.

Disease severity was recorded only from infected leaves in the spray trial at Metu, since fruit development was inhibited due to unknown reason. Better control was given by chlorothalonil which reduced disease severity to only 28% when compared to 37% for prochloraz.

In 1991–1993, there was high rainfall at Bebeka and Metu from April to October. Average monthly rainfall recorded at Metu was 153.6, 154.5 and 166.8 mm in 1991, 1992 and 1993, respectively (*figure 5*), while, at Bebeka, it was 119.2, 125.8 and 122 mm in 1991, 1992 and 1993, respectively (*figure 6*).

During the 3 years, the south western region experienced high rainfall followed by prolonged periods of wetness throughout the year. This unusual condition might have led to the sporadic nature of the outbreak of the disease. Inoculum for the infection of blossoms might have originated from latent infections on the leaves, twigs, and from fallen diseased fruit.

4. conclusion

Phaeoramularia leaf and fruit spot of citrus has become a menace to citrus production in many parts of Ethiopia. Complete damage of fruits have been observed in some areas; therefore, the outbreak of the disease has to be halted and the problem has to get due attention.

The epidemiology of the disease and the survival mechanism of the fungus have to be thoroughly studied. At the same time, since differences in susceptibility were observed between cultivars, local and exotic resistant varieties should be developed and provided to farmers. Meanwhile,

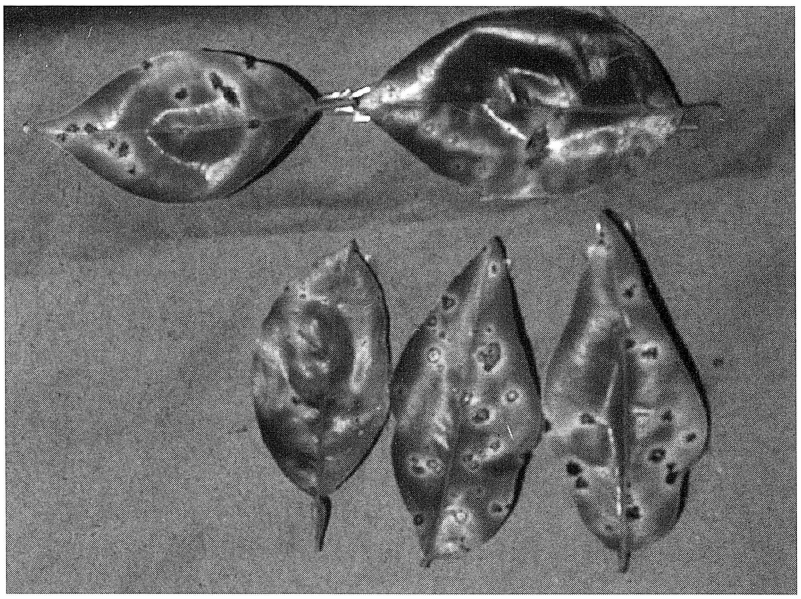

Figure 3.
Citrus leaves attacked by *Phaeromularia angolensis*.

Figure 4.
Typical symptoms of *Phaeromularia* leaf and fruit spot disease of citrus fruits.

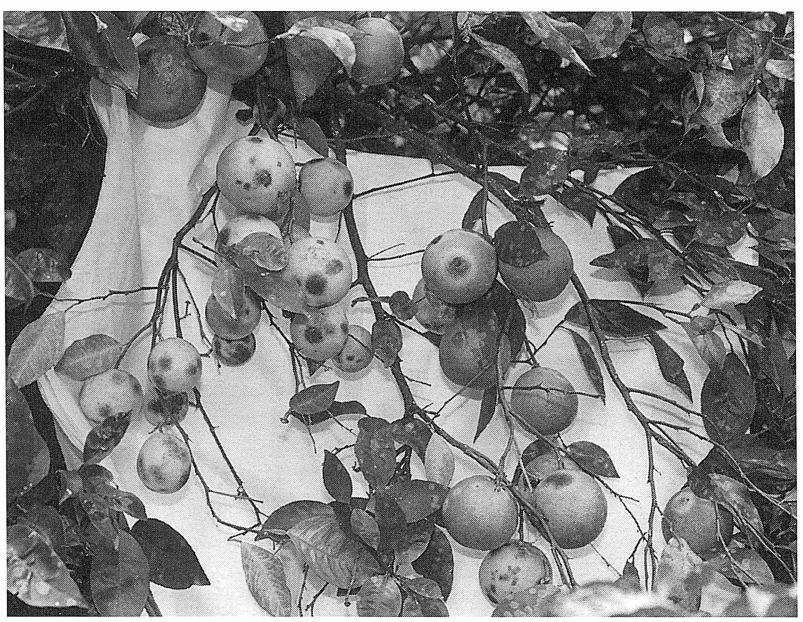

Table II.
Results of fungicide spray trials against *Phaeoramularia* leaf and fruit spot of orange at Bebeka (in south west of Ethiopia).

Treatments	Rate (g a.i.·100 L^{-1})	Disease severity on leaves (%)	Average fruit yield ($q \cdot ha^{-1}$)		Unmarketable fruits (%)
			Total	Marketable	
Unsprayed (control)	–	54.17	65	13	83.30
Chlorothalonil (75% w.p.)	130	24.64	182	65.52	64.07
Prochloraz (50% w.p.)	50	35.78	119.70	38.71	67.60

a.i.: active ingredient.

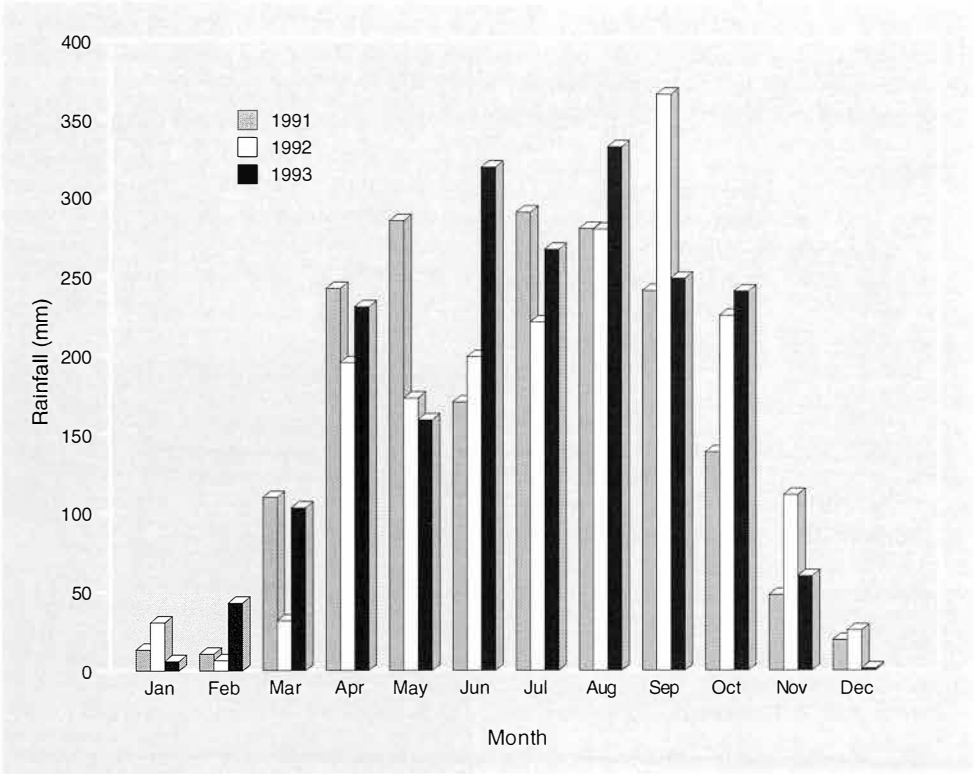

Figure 5.
Amount of rainfall registered at Metu, in south west of Ethiopia, for the years 1991–1993.

farmers should be advised to use sanitary measures. Removal of infected fruits and leaves should be practised, pruning operations should eliminate dead branches and twigs so as to reduce inoculum density.

acknowledgments

The author is highly indebted to the pathology section at Jima Research Center for collecting and summarizing data.

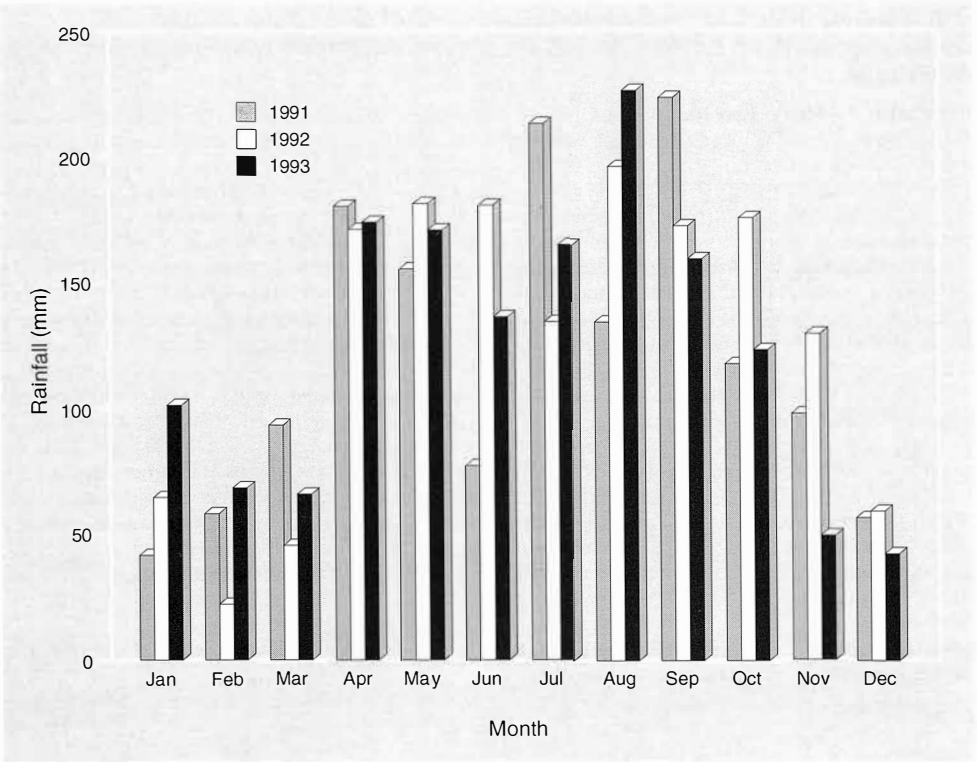

Figure 6.
Amount of rainfall registered at Bebeka, in south west of Ethiopia, for the years 1991–1993.

Thanks also go to Bebeka State farm for recording and collecting yield from experimental plots. Finally, I would also like to thank the Center for Agriculture and Bioscience International (CABI) Bioscience in the UK for confirming the identification.

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Ocurrencia, distribución y métodos de control de la enfermedad de las manchas en hojas y frutos de agrios, causadas por *Phaeoramularia*, en Étiopia.

Resumen — Introducción. En Etiopia, el naranjo (*Citrus sinensis*) crece en la mayoría de las regiones. En 1990, se observó una nueva enfermedad afectando los frutos, las jóvenes ramas y las hojas de agrios en primer lugar en la finca de estado de Bebeka al sur del país; se propagó luego, muy rápidamente, a todas las regiones húmedas del sur y del suroeste. Se emprendió un estudio para determinar la ocurrencia, la distribución y la extensión de los deterioros, para identificar el agente responsable de la enfermedad y asegurar su control mediante fungicidas. **Material y métodos**. Se efectuaron encuestas en distintos sitios para determinar la incidencia y la gravedad de la enfermedad. Se llevaron a cabo tests de identificación y de virulencia en laboratorio. Se sometieron a prueba tratamientos fungicidas en los sitios Bebeka y Metu. **Resultados y discusión**. Entre los sitios estudiados, los de Jima, Mizan, Bebeka, Metu y Limu fueron más afectados. En todos estos sitios, la enfermedad afectó, en término medio, 88,19 % de los árboles, mientras que el 43% de las hojas y el 65% de los frutos de un mismo árbol estaban afectados. Se trataría de la enfermedad de las manchas en hojas y frutos de agrios causada por un hongo, *Phaeoramularia angolensis* (syn.*Cercospora angolensis*). Los tests de virulencia permitieron aislar de nuevo el hongo a partir de hojas, frutos y jóvenes ramas inoculadas. Entre los fungicidas sometidos a prueba, el clorotalonil fue más eficaz que el procloraze. **Conclusión**. La enfermedad se propaga muy rápidamente y se deberían de poner a punto métodos de control eficaces y económicos. Además, deben estudiarse meticulosamente la epidemiologia de la enfermedad y los mecanismos de supervivencia del hongo. (© Elsevier, Paris)

Étiopia / *Citrus* / naranja dulce / enfermedades de las plantas / cercospora / presencia de enfermedad / organismos patogenos