Citrus canker: a new disease of Mexican lime (*Citrus aurantifolia*) and sour orange (*C. aurantium)* in Ethiopia

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Citrus canker: a new disease of Mexican lime (*Citrus aurantifolia*) and sour orange (*C. aurantium)* in Ethiopia.

Abstract — Introduction. Citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. *citri,* is a serious disease of most commercial citrus cultivars and some citrus relatives. Surveys were conducted in small-scale farms, commercial plantations, backyard orchards and nurseries in order to quantitatively study the occurrence and distribution of the disease and determine the intensity and field host range. **Materials and methods**. Seventeen locations in four regional states of Ethiopia were surveyed. Incidence on leaves was determined by counting total number of leaves and expressed as a proportion of leaves with at least one lesion. Incidence on fruits was determined on attached fruits, recorded as presence or absence of the symptoms. Severity was also measured on leaves and fruits. The identity of the pathogen was confirmed by morphological, biochemical and physiological characterization and detached leaf tests of representative isolates. **Results**. The surveys indicated the occurrence of citrus canker in Ethiopia. Overall incidence on leaves was 71.4% of leaves with at least one lesion and severity was 26.8% of leaf area infected. Incidence on fruits was 30% of fruits infected and severity was 21.25% fruit area infected. Morphological, biochemical and physiological characterization and detached leaf tests of isolates indicated characteristics similar to *X. axonopodis* pv. *citri*. The host range for citrus canker in Ethiopia was limited to Mexican lime (*C. aurantifolia*) and sour orange (*C. aurantium*). Based on the field host range and detached leaf tests, it appears that the *X. axonopodis* pv. *citri* variant that occurs in Ethiopia has similar host ranges to that of atypical Asiatic (*Xac*-A*) form. Canker severity significantly correlated with temperature but not with rainfall, elevation or tree age. **Conclusion**. This is the first confirmed report of the disease in Ethiopia.

Ethiopia / *Citrus aurantium* / *Citrus aurantiifolia* / *Xanthomonas* / plant diseases / pathogens / identification / geographical distribution / host plants

Le chancre citrique : une nouvelle maladie de la lime mexicaine (*Citrus aurantifolia*) et de l'orange amère (*C. aurantium*) en Éthiopie.

Résumé — Introduction. Le chancre citrique, provoqué par la bactérie *Xanthomonas axonopodis* pv. *citri,* est une maladie sérieuse de la plupart des cultivars commerciaux d'agrumes et d'autres genres voisins. Des prospections ont été conduites en exploitations de petite taille, en plantations commerciales, en vergers de case et en pépinières afin d'étudier quantitativement l'occurrence et la distribution de la maladie et d'en déterminer l'intensité et les hôtes. **Matériel et méthodes**. Dix-sept lieux répartis dans quatre États d'Éthiopie ont été visités. L'incidence sur feuilles a été exprimée par la proportion de feuilles portant au moins une lésion parmi l'ensemble des feuilles échantillonnées. L'incidence sur fruits a été déterminée sur les fruits sur arbre, en notant la présence ou l'absence des symptômes. La sévérité a été également mesurée sur feuilles et fruits. L'identité du pathogène a été confirmée par caractérisation morphologique, biochimique, physiologique et par tests des isolats sur feuille détachée. **Résultats**. Les prospections ont indiqué la présence du chancre citrique en Éthiopie. Sur feuilles, l'incidence globale a été de 71,4 % de feuilles avec au moins une lésion et la sévérité a été de 26,8 % de surface foliaire infectée. Sur fruits, l'incidence a été de 30 % de fruits infectés et la sévérité a été de 21,25 % de surface de fruit infectée. Les tests morphologiques, biochimiques, physiologiques et d'isolats sur feuille détachée ont indiqué des caractéristiques semblables à *X. axonopodis* pv. *citri*. La gamme d'hôtes du chancre citrique en Éthiopie a été limitée à la lime mexicaine (*C. aurantifolia*) et à l'orange amère (*C. aurantium*). Sur la base de cette gamme d'hôtes et des tests sur feuille isolée, il s'avère que le variant de *X. axonopodis* pv. *citri* qui s'exprime en Éthiopie a les mêmes hôtes que ceux de la forme asiatique atypique (*Xac*-A*). La sévérité du chancre a été significativement corrélée à la température mais pas aux précipitations, à l'altitude ou à l'âge de l'arbre. **Conclusion**. C'est la première fois que la présence de cette maladie est confirmée en Éthiopie.

Éthiopie / *Citrus aurantium* / *Citrus aurantiifolia* / *Xanthomonas* / maladie des plantes / agent pathogène / identification / distribution géographique / plante hôte

1. Introduction

Citrus is attacked by various diseases including canker, caused by the bacterium *Xanthomonas axonopodis* pv. *citri*. It is a serious disease of most commercial citrus cultivars and some citrus relatives [1]. Currently, it is the most important and a very destructive disease that has received considerable press attention and legal challenges [2]. The economic importance of canker stems from the fall of the heavily infected fruits; another aspect lies in the fact that citrus fruits produced in canker-infected regions are placed under strict international regulation of plant quarantine [3], which restricts the region's market.

Several forms of the disease are recognized. It is believed that four forms of citrus canker; Asiatic (A), atypical Asiatic (A*), South American (B) and Mexican lime (C), occur around the world and are induced by variants of two causal agents, *X. axonopodis* pv. *citri* and *X. axonopodis* pv. *aurantifolii*. These variants are primarily distinguished by their geographical origin and their host range [4].

Asiatic canker, A type, is the most common and damaging of the canker strains [1] and is pathogenic to virtually all cultivated members of the family Rutaceae [5]. Vernière *et al.* [6] designated some isolates of the Asian strain with a host range restricted naturally to Key/Mexican lime (*C. aurantifolia*) as the A* strain. This strain was discovered in Oman, Saudi Arabia, Iran and India. An additional A strain variant, Aw (Wellington strain), is capable of inducing canker symptoms on a restricted range of citrus hosts, including Mexican lime and alemow (*C. macrophylla*), and is closely related to A* [7].

The B strain infects sour orange, lime and, rarely, sweet orange [8]. The C strain infects only Key/Mexican lime. The B and C strains are currently classified as *X. axonopodis* pv. *aurantifolii* [9].

Because symptoms are generally similar, separation of these types from each other is based on host range, and cultural and physiological characteristics [10].

X. axonopodis pv. *citri* does not require a specific vector but can be transmitted from plant to plant via wind, rain and human-derived materials such as contaminated cutting tools and infected plant clippings [11]. Long-distance dissemination of the pathogen occurs primarily via the movement of infected planting and propagating material, such as bud-wood and rootstock seedlings or budded trees from nurseries [10]. Citrus canker was reported, in the early 1980s, from Africa: Comoros, Côte d'Ivoire, Gabon, Madagascar, Mauritius, Mozambique (reported to be eradicated), Réunion, Seychelles and Zaire [12]. However, there is no recent report of the occurrence of the disease in the continent.

The disease has not been previously studied and documented in Ethiopia. Therefore, a study on the occurrence, distribution and intensity of the disease in the country would be of much benefit to avail data to be used in the planning of disease management practices, for policy-makers to formulate strategic plans, guide researchers in prioritization of research programs and for making growers aware. Our study was undertaken to quantitatively study the occurrence and distribution of citrus canker in Ethiopia, determine the incidence, severity and field host range of the disease, confirm the identity of the pathogen using morphological, biochemical and physiological characterization and detached leaf tests, and relate citrus canker severity to temperature, rainfall, elevation and tree age.

2. Materials and methods

2.1. Survey areas

Seventeen locations in four regional states were surveyed for citrus canker occurrence and intensity on commercial plantations, small-scale farms, research centers, and dooryard and backyard orchards between August and November 2004.

The geographic positions of the survey areas were recorded using the Geographic Positioning System (GPS). The areas covered in the survey lie between long. 36° to 40° 68' E and lat. 7° to 11° N. The surveys were conducted in four citrus-growing regional states; namely, the Afar, Amhara and Oromia regional states, and Southern

Nations and Nationalities and People's Regional State (SNNPRS). The surveys included sites in the regional states that were believed to be truly representative of the regions and were of sufficient size to obtain the required accuracy [13].

The surveyed areas were classified based on altitudinal groups, (a): high-altitude areas (> 2000 m), (b): medium-altitude areas [(1300–2000) m], and (c): lowland areas below 1300 m. These areas are categorized under semi-arid lowlands (SA1), sub-moist lowlands (SM1), moist cool (M2) and sub-humid (SH3) agroecological zones. Accordingly, representing the highlands and mid-altitudes, the survey started in Amhara region in Shoa Robit (1450 m) and continued further north to Habru, Bati, Haik, Mersa and Woldia. In Oromia region, the survey was conducted in backyard orchards, research centers and commercial plantations.

In Afar regional state, the survey was conducted in Awara Melka on a commercial citrus plantation and on experimental plots belonging to Melka Werer Agricultural Research Center. In the SNNPRS, the survey was conducted in the Gurage zone, near the town of Wolkite, in farmers' orchards.

2.2. Sampling and data collection

The quantity of citrus canker present in the field was assessed on five unsprayed trees from each cultivar by means of disease intensity that was measured using severity and incidence. A systematic sampling method with a "W"-shaped sampling path was used to select five trees in the field. To eliminate personal bias in selection of the units, 20, 40 and 60 paces, depending on field size, were taken between samples [14]. A destructive sampling method was used for disease assessment on leaves, and measurements were taken on attached fruits. Sample sizes were determined from similar studies conducted on *Phaeoramularia* leaf and fruit spot of citrus [15].

Disease incidence on leaves was determined on eight random terminal shoots where the total number of leaves was counted and expressed as a proportion of leaves with at least one lesion [16].

Canker incidence on fruits was determined on the same five trees by randomly taking 50 attached fruits per tree (10 from each of the four directions of the tree and an additional five fruits from the lower, and five from the upper canopy), recorded as disease present or absent, and expressed as a proportion of the total number of fruits [15].

To determine severity, forty leaves were randomly detached from the selected branches for disease incidence, and calculated using Rayner's method [17] of graphic representation as: disease severity = [(Number of squares covered with spots × 40) / (total number of squares covered for whole leaf × 40)] × 100, number of squares corresponding to leaf areas.

Percentage of severity on fruits was initially determined using a descriptive-type assessment key with a 0–4 score scale where 0: healthy, 1: 1–10%, 2: 11–25%, 3: 26–50% and 4: > 50% of fruit area infected [15]. The score scales were then converted into a disease severity index for non-parametric measurements [18] and expressed as percentages.

Incidence and severity at a location were determined by taking means of the disease at one to three sites depending on the availability of trees.

To confirm the identity of the causal organism, leaf samples with citrus canker symptoms from citrus trees where data were taken were collected and brought to the laboratory for isolation and characterization.

Secondary data on temperature and rainfall for the surveyed areas were obtained from the Ethiopian Meteorological Service. Tree age data were obtained from the respective plantations and private farms. Elevation data were recorded using an altimeter.

2.3. Data analysis

To stabilize variance, disease incidence and severity data on leaves and fruits were subjected to arcsine transformation prior to analysis using the Excel statistical software program [14]. The precision of the assessment methods was determined using the coefficient of variation and standard error [19] and data were analyzed using the statistical software package SAS V8 (SAS Inst., Carey, North Carolina, USA). Correlations between canker severity, temperature, rainfall, tree age and elevation were also determined using the same program.

2.4. Identity confirmation of isolates

Eight representative isolates, two from each site in Awara Melka, Melka Werer, Merti Jeju and Nura Era, were obtained from leaves showing canker symptoms on Mexican lime (*C. aurantifolia*). Ten different leaflets from each site were collected and washed for 10 min in running tap water, surface-sterilized by dipping in 70% ethanol for 1 min, and rinsed twice with sterile distilled water. Individual lesions were punched with a cork borer and separated from the leaves, finely chopped and placed on sterile Petri plates. The leaves were then put into McCartney bottles containing 5 mL sterilized distilled water and left for 15 min. From each sample, 0.1-mL aliquots of the wash suspension were subjected to tenfold serial dilutions, taken in a pipettor and streaked out on Petri plates containing the selective medium, yeast dextrose chalk agar (YDCA). The Petri plates were incubated at 30 °C for 48 h [20], and cellular and cultural characteristics of the isolates were observed. A simple staining procedure was used for morphological characterization that was carried out through examining the stained slides with smears by placing them under the oil-immersion objective of the light microscope (total magnification about ×100) [21].

The eight representative isolates were also characterized on the basis of their biochemical, physiological and metabolic properties, following standard determinative tests: Gram reaction using 3% KOH, Voges-Proskauer and methyl red test, Levan production, nitrate reduction, hydrogen sulfide production, milk proteolysis casein hydrolysis, starch hydrolysis, sodium chloride tolerance, oxidase reaction oxidative/fermentative growth test, gelatin hydrolysis, esculin (aesculin) hydrolysis and esterase activity (Tween hydrolysis).

In order to reproduce the symptoms from pure culture, two-thirds to fully expanded immature leaves from seedlings of sour orange (*C. aurantium*), Mexican lime (*C. aurantifolia*) and sweet orange (*C. sinensis*) were detached and separated. Leaflets were washed for 10 min in running tap water, surface-sterilized by dipping in 70% ethanol for 1 min, rinsed twice with sterile distilled water, and placed with their abaxial surface exposed in 100 mm-diameter plastic Petri plates, each containing 20 mL of 1.5% water agar amended with 100 $mg \cdot L^{-1}$ benzimidazole. The design was a completely randomized design with three replications. Leaflets were punctured with a 26-gauge syringe needle [22] on five spots. Ten µL of a 24-h suspension of isolate, obtained from canker-infected Mexican lime leaves in Awara Melka, containing 10^8 $cfu \cdot mL^{-1}$ (determined spectrophotometrically), were placed on each wound site [6]. Non-inoculated controls were treated with an equal volume of sterile distilled water. Petri plates with inoculated detached leaves were placed in a growth chamber at 30 °C and incubated under fluorescent lights for 14-h photoperiods for nine days to induce optimal symptom expression [23].

3. Results

3.1. Surveyed areas

The disease was not found in the highlands or mid-altitudes of the country. It occurred in the lowlands of the Afar and Oromia regions, mainly in the Awash Agro-Industry plantations which are situated in the Rift valley. The areas are agroecologically classified as SA1, which are characterized as dry-warm semi-arid lowland plains.

3.2. Disease intensity in Ethiopia

Citrus canker was observed only on Mexican lime (*C. aurantifolia*) and sour orange (*C. aurantium*) in 4 of the 17 locations surveyed, and it was present only in plantations and research centers in the Afar and Oromia regions. However, in the rest of the 13 locations, that constitute 76.5% of the surveyed areas, citrus orchards belonging to small-scale farmers had no trees with visible symptoms of canker.

Overall disease incidence for the citrus canker affected locations in the Afar and Oromia regions; incidence on leaves was 71.4% of leaves with at least one lesion and severity was 26.78% (*table I*); incidence on fruits was 30% and severity was 21.25% (*table I*). Leaf spots were observed on both

Table I.
Intensity of canker assessed on citrus leaves in Ethiopia (2004).

Location	Variety	Crop age (year)	Incidence (%)[1]	Mean range[2]	Severity (%)	Mean range[2]
Awara Melka	Mexican lime	15	84 a	70–90	35.9 a	26–44.2
Melka Werer	Mexican lime	3	65 b	60–70	25.6 c	11.9–34.4
Merti Jeju	Mexican lime	10	71 ab	35–90	30.3 b	25–38.65
Merti Jeju	Sour orange	10	63 b	55–65	17.3 d	12.7–23
Nura Era	Mexican lime	1	74 ab	60–80	24.8 bc	16.7–34.9
Mean		–	71.4	–	26.78	–
Coefficient of variation (%)		–	11.65	–	25.76	–
Standard error of the mean		–	3.72	–	3..08	–

Means within a column followed by the same letters are not statistically different at $P \leq 0.05$ (Tukey test).
[1] % of leaves with at least one lesion, [2] Mean of five trees.

the upper and lower leaf canopies at all sites surveyed.

Subsequently, the highest disease incidence on leaves recorded in Awara Melka was significantly higher than in Merti Jeju on sour orange and in Melka Werer, while the incidence observed in Nura Era and Merti Jeju on Mexican lime was intermediary (*table I*). Canker severity on limes recorded in Awara Melka (35.9%) was significantly higher than for Merti Jeju, 30.3%, Melka Werer, 25.6%, or Nura Era, 24.8%, and, additionally, citrus canker severity on sour orange in Merti Jeju was significantly lower than on Mexican lime (*table I*). On Mexican lime, the disease induced erupted corky lesions that were rough to the touch, but produced only flat lesions on sour orange (*figure 1*).

On fruits, canker incidence on Mexican lime recorded in Awara Melka was again significantly higher than for other locations (*table II*). However, non-significant differences were observed between Merti Jeju (on Mexican limes and on sour oranges) and Melka Werer. Canker severity on Mexican lime fruits in Awara Melka, Merti Jeju and Melka Werer were not significantly different but it was significantly higher than on sour oranges in Merti Jeju (*table II*).

The disease was not observed in Ambo and Melkassa, where the lowest mean temperatures were recorded and the most significant annual rainfalls were registered (*table III*).

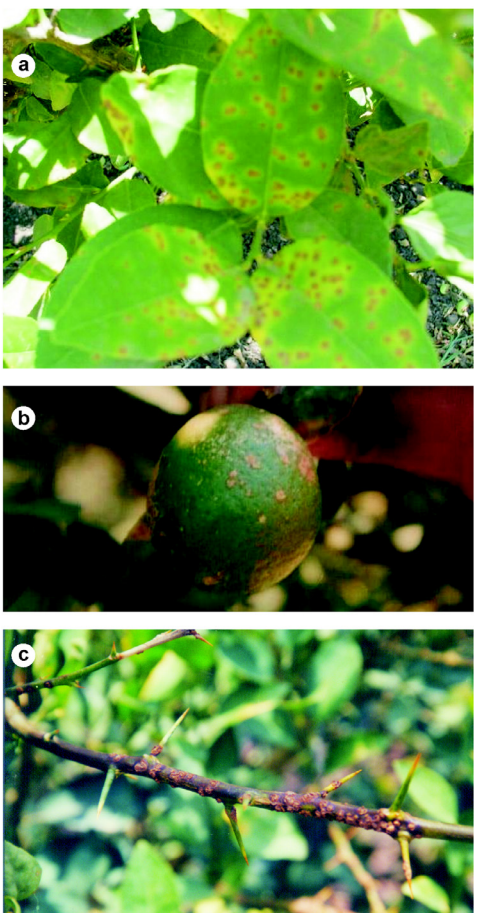

Figure 1.
Citrus canker on Mexican lime leaves (a), fruits (b) and twigs (c) in Ethiopia (2004). (A color version of this figure is available at www.edpsciences.org/fruits.)

In the survey year, the mean monthly temperature distribution in Awara Melka (28.86 °C) was significantly higher than that

Table II.
Intensity of canker assessed on citrus fruits in Ethiopia (2004).

Location	Variety	Crop age (year)	Incidence (%)[1]	Mean range[2]	Severity	Mean range[3]
Awara Melka	Mexican lime	15	60 a	40–80	30 a	0–3
Merti Jeju	Mexican lime	10	25 bc	15–35	20 ab	0–3
Merti Jeju	Sour orange	10	15 c	5–25	15 c	0–2
Nura Era	Mexican lime	1	Non-fruiting	–	Non-fruiting	–
Melka Werer	Mexican lime	3	20 bc	5–35	20 ab	0–2
Mean		–	30.00	–	21.25	–
Coefficient of variation (%)		–	68.03	–	29.6	–
Standard error of the mean		–	10.21	–	3.14	–

Means within a column followed by the same letters are not statistically different at $P \leq 0.05$ (Tukey test).

[1] % of fruits with disease presence.

[2] Mean of five trees; [3] 0–4 score scale, where 0 = 0%, 1 = (1 to 10)%, 2 = (11 to 25)%, 3 = (26 to 50)%, 4 = > 50% [15].

at other locations, followed by Melka Werer (26.65 °C), then Nura Era and Merti Jeju, both with 24.3 °C, but no difference in mean annual rainfall during the survey year was noticed for these locations (*table III*).

The relationship between canker severity and temperature in the above locations was relatively stronger (R^2 = 83%) than with rainfall (R^2 = 61%) (*figure 2*).

Pearson's correlation analysis indicated highly significant positive correlation (r = 0.9321, $P \leq 0.001$) between disease severity and temperature and negative non-significant correlation (r = –0.8618, $P \leq 0.05$) between disease severity and rainfall, respectively. There was also negative non-significant correlation between canker severity and elevation (r = – 0.8977, $P \leq 0.05$). On the other hand, a strong but non-significant negative and linear association (r = –0.9256, $P \leq 0.05$) was observed between rainfall and temperature. Elevation and temperature also showed negative correlation (r = –0.8031, $P \leq 0.05$) (*table IV*).

3.3. Identity confirmation of isolates

The Gram stain allowed observation of cell size, shape and arrangement. The bacterial cells were Gram-negative, rod-shaped and single (*table V*).

Table III.
Citrus canker severity assessed in relation to mean monthly temperature, mean annual rainfall, elevation and citrus tree age at different locations in Ethiopia (2004).

Location	Canker severity[1] (%)	Temperature (°C)	Rainfall (mm)	Elevation (m)	Tree age (year)
Ambo	0 d	17.77 e	1079.58 a	2130	15
Awara Melka	35.9 a	28.86 a	588.00 ab	960	15
Melka Werer	25.6 bc	26.65 b	598.41 ab	800	3
Melkassa	0 d	21.03 d	711.47 ab	1622	16
Merti Jeju	30.3 b	24.30 c	515.40 b	1250	10
Nura Era	24.8 c	24.30 c	515.40 b	1140	1

Means within a column followed by the same letters are not statistically different at $P \leq 0.05$ (Tukey test).

[1] Canker severity on Mexican lime leaves. Mean of three sites.

In the cultural characterization, yellow, domed, mucoid colonies indicative of *Xanthomonas* were isolated after 48 h of incubation at 30 °C. The yellow pigment consists of a unique family of brominated aryl octanes, which have been called xanthomonadins, and is characteristic of the genus *Xanthomonas* [24].

In the biochemical and physiological characterization, all eight representative isolates showed similar reactions to the standard determinative tests; they were negative to the nitrate reduction, methyl red test, fermentative growth test and oxidase reaction, while they showed positive reactions to the rest of the tests (*table VI*).

In the detached leaf tests, less erumpent and non-spreading lesions were formed and *Xanthomonas* isolates were consistently recovered from inoculated detached leaves of Mexican lime and sour orange, and were not detected on inoculated sweet orange leaves or control leaves inoculated with sterile distilled water (*table VII*).

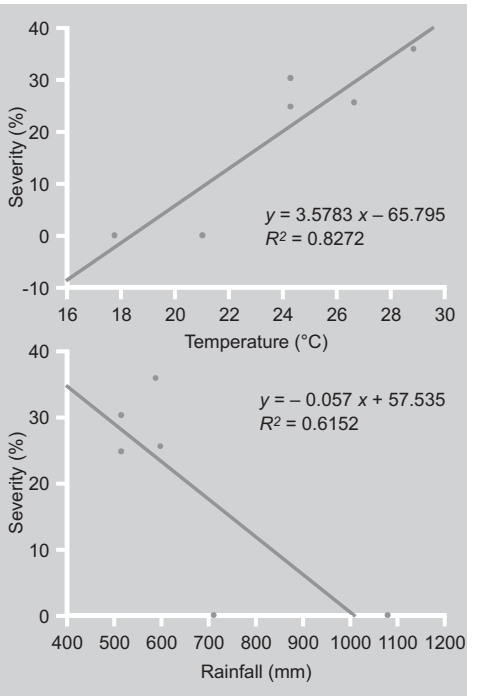

Figure 2.
Relationship between citrus canker severity, temperature and rainfall in Ethiopia (2004).

4. Discussion

Even though there were reports of the disease in the early 1980s, it appears that this is the most recent report on the occurrence of citrus canker in the African continent.

In Ethiopia, commercial plantations, backyard orchards and small farmers' holdings were examined. During the survey, citrus canker was not observed on citrus in the Amhara and SNNPR states. However, it was found to be widespread in areas situated in the Rift Valley, in the Afar and Oromia regional states, attacking solely Mexican lime at a high level and sour orange at a medium level. Other citrus varieties in the vicinity were not affected by the disease and that, hence, indicated the host specificity of the pathogen.

Survey locations in the Rift Valley, Awara Melka, Merti Jeju, Melka Werer and Nura Era, are classified under the SA1 agroecological zone. This zone is characterized by the presence of hot to warm arid valleys and escarpments. Altitude ranges from (500 to 1300) m, while mean annual rainfall is below 800 mm and mean annual temperature is between (21 and 28) °C. It has no growing period from rainfall unless irrigated [25]. High rainfall and low temperature, which could lead to the rapid decline of bacterial populations [23], and the absence of contaminated seedling materials could probably explain why citrus canker did not occur in the high and mid-altitudes of Ethiopia (Melkassa and Ambo).

All of the commercial plantations and the research center with canker in the Rift Valley areas use flood irrigation. In these farms, the

Table IV.
Pearson's correlation analysis between disease severity, temperature, rainfall, altitude and citrus tree age in the surveyed areas of Ethiopia (2004).

Parameter studied	Disease severity	Temperature	Rainfall	Altitude	Tree age
Disease severity	–	0.9321 **	– 0.8618 ns	– 0.8977 ns	– 0.6327 ns
Temperature	–	–	– 0.9256 ns	– 0.8031 ns	– 0.4945 ns
Rainfall	–	–	–	0.7927 ns	0.7005 ns
Elevation	–	–	–	–	0.5734 ns
Tree age	–	–	–	–	–

** Significant at $P \leq 0.001$ (Tukey test). ns = not significant.

Table V.
Cellular and cultural characteristics of *Xanthomonas axonopodis* pv. *citri* isolates from Ethiopia.

Parameter	Description
Shape	Rod
Size	0.48 µm × 1.58 µm
Arrangement	Single
Spore	Absent
Capsule	Absent
Staining	Gram-negative
Color	Yellow, mucoid

Table VI.
Biochemical and physiological characteristics of citrus canker causal agent isolates in Ethiopia.

Determinative test	Reaction
Gram stain using 3% KOH	+
Levan formation	+
Nitrate reduction	–
Voges-Proskauer and methyl red test	+ / –
Hydrogen sulfide production	+
Casein hydrolysis	+
Starch hydrolysis	+
Sodium chloride tolerance	+
Oxidative / fermentative growth test	+ / –
Gelatin hydrolysis	+
Aesculin hydrolysis	+
Tween hydrolysis	+
Oxidase reaction	–

Table VII.
Reaction of citrus cultivars to detached leaf tests with *Xanthomonas* isolates from Awara Melka (Rift Valley, Ethiopia).

Citrus cultivar	Lesion	Recovery of *Xanthomonas*
Mexican lime	Present	Present
Sour orange	Present	Present
Sweet orange	Absent	Absent
Control	Absent	Absent

temperature is relatively high and seems to be favorable for citrus canker development. However, it was not accompanied by high rainfall, which was low and erratic at all sites. On the other hand, in order for citrus canker disease to develop, high temperature should be accompanied by high rainfall [10]. This was not the case in the plantations. However, all farms in the Rift Valley benefited from the presence of the Awash river, which covers most parts of the plantations. Hence, the use of flood irrigation and contaminated seedling materials might have disseminated citrus canker within the Rift Valley region. Irrigation also might have made citrus trees to yield more susceptible flushes, which resulted in high disease incidence.

The fact that citrus canker in Ethiopia was not observed on small farmers' holdings and backyard orchards but on commercial plantations and experimental plots at research centers might indicate that the bacteria was probably introduced from abroad by researchers and technical staff in the state farms and research centers after the people returned from travels abroad. Due to the predominance and establishment of citrus canker, the potential for substantial economic damage to citrus trees in the Rift Valley regions of Ethiopia remains high. Subsequently, the regulatory authorities must maintain a constant vigil against such introductions in Ethiopia and should implement prohibitive measures to exclude the disease from spreading to the rest of country.

The characteristic raised lesions on leaves, fruits and twigs of Mexican lime were easily identifiable, and permitted relatively quick and accurate diagnosis of the disease. Moreover, as the use of selective and differential media in the isolation of a bacterium provides information that helps to identify an organism [26], growth and production of yellow pigments on the use of the selective and differential medium, YDCA, clearly indicated that the eight test isolates were similar in characteristics to those of *Xanthomonas*. Hence, disease symptoms on infected leaves, fruits and twigs, the use of YDCA and the observed cellular and cultural characteristics were very useful tools in the identification of the eight representative Ethiopian isolates as *X. axonopodis* pv. *citri*. Based on the use of YDCA medium, bacterial species may be identified with fewer tests [27].

Further confirmation of the identity of the isolates was effected in subsequent studies on morphological, biochemical and

physiological characterization and detached leaf tests. The detached leaf test indicated that the test isolate was a pathogen of Mexican lime and sour orange. Moreover, the isolate was recovered again from inoculated leaves of these cultivars; the recovered isolates had similar cellular and cultural characteristics to the original isolate, hence proving Koch's rule of proof [28].

In conclusion, the current work indicated the occurrence of citrus canker in Ethiopia. To the authors' knowledge, this is the first confirmed report of the disease in Ethiopia.

The host range for citrus canker in Ethiopia was limited to Mexican lime (*C. aurantifolia*) and sour orange (*C. aurantium*). Based on the field host range and detached leaf tests, it appears that the *X. axonopodis* pv. *citri* variant that occurs in Ethiopia has similar host ranges to that of atypical Asiatic, *Xac*-A*, which has also been described in the Middle East region and India [9]. However, though the Rift Valley region in Ethiopia has similar climatic factors to the above regions, the common factor that induces citrus canker in these countries should further be studied. Molecular characterization, host range and losses incurred due to the disease in Ethiopia also require further investigation.

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La cancrosis de los cítricos: una nueva enfermedad de la lima mejicana (*Citrus aurantifolia*) y de la naranja amarga (*C. aurantium*) en Etiopía.

Resumen — Introducción. La cancrosis de los cítricos, provocada por la bacteria *Xanthomonas axonopodis* pv. *citri,* es una enfermedad grave de la mayoría de los cultivares comerciales de cítricos y de otras especies vecinas. Se llevaron a cabo prospecciones en explotaciones de talla pequeña, en plantaciones comerciales, en huertos de divisiones y en viveros, con el fin de estudiar cuantitativamente el caso y la distribución de la enfermedad y determinar su intensidad y sus huéspedes. **Material y métodos**. Se visitaron diecisiete lugares repartidos entre cuatro estados de Etiopía. Se determinó la incidencia en hoja mediante el número total de hojas y se efectuó en proporción de hojas con al menos una lesión. Se determinó la incidencia en frutos, en los frutos de árboles, con la anotación de presencia o de ausencia de síntomas. Asimismo se midió la gravedad en hojas y en frutos. La identidad del patógeno se confirmó mediante caracterización morfológica, bioquímica, fisiológica, así como mediante muestras del aislado en hoja desprendida. **Resultados**. Las prospecciones indicaron la presencia de la cancrosis de los cítricos en Etiopía. En las hojas, la incidencia global fue del 71,4 % de hojas con al menos una lesión; y, la gravedad fue del 26,8 % de superficie foliar infectada. En frutos, la incidencia fue del 30 % de los frutos infectados; y, la gravedad fue del 21,25 % de superficie del fruto infectada. Los testeos morfológicos, bioquímicos, fisiológicos y del aislado en hoja desprendida indicaron semejantes características *Xanthomonas axonopodis* pv. *citri*. La gama de huéspedes de la cancrosis de los cítricos en Etiopía se limitó a la lima mejicana (*C. aurantifolia*) y a la naranja amarga (*C. aurantium*). En base a esta gama de huéspedes y de testeos en hoja aislada, resulta que la variante de *X. axonopodis* pv. *citri,* la cual se refleja en Etiopía posee los mismo huéspedes que los de la forma asiática atípica (*Xac*-A*). La gravedad de la cancrosis se ligó significativamente a la temperatura, pero no a las precipitaciones, ni a la altitud, ni a la edad del árbol. **Conclusión**. Es la primera vez que se confirma la presencia de la enfermedad en Etiopía.

Etíopia / *Citrus aurantium* / *Citrus aurantiifolia* / *Xanthomonas* / enfermedades de las plantas / organismos patógenos / identificación / distribución geográfica / plantas huéspedes