Citrus Greening Disease Survey in East and West African Countries South of Sahara

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ABSTRACT. A survey covering eight African countries south of the Sahara, was undertaken for Citrus Greening Disease (CGD) and its psylla vector *Trioza erytreae* (Del Guercio) on citrus orchards and indigenous plants belonging to the Rutaceae. Samples of columellas and leaf midveins were collected on presumably contaminated material for electron microscopic examination. Both the extent of the vector and the spread of the disease were then correlated with ambient conditions of temperature and relative humidity for assessing the outlines of the greening belt in these African countries.
Index words. African citrus psylla, greening organism, psylla parasitism.

Citrus Greening Disease (CGD) is known to exist in subsaharan Africa, where it is disseminated naturally by the African psylla vector *Trioza erytreae* (Del Guercio). In Africa, both the insect vector and the greening organism (G.O.) do not tolerate hot and dry climates (1, 2). The extent of the vector and the spread of the disease are therefore dependent on ambient conditions of temperature and relative humidity. CGD is in fact restricted to fresh and moist highlying areas, with different ranges of elevation according to local microclimates. The present study is an attempt to give the first description of the greening belt in different African regions south of the Sahara.

MATERIALS AND METHODS

The survey was conducted in three West African countries, and five East African countries and in the Comores Islands (fig. 1).

Citrus orchards and local rutaceous flora were checked following altitudinal transects, and examined for the presence of:

1) living psylla nymphs and/or adults on new flush (search of 10 minutes per tree)
2) traces of leaf galls and nymph mummies left by *T. erytreae* (search of 10 minutes per tree)
3) attendant primary and secondary parasites (hyperparasites), caught by placing psylla nymphs in hatching boxes (50 to 70 nymphs per box). Winged individuals chalcidoid insects obtained from the hatching boxes, were then dispatched dry for taxonomic identification. The exoskeleton of the nymph psylla mummies was also examined to check the characteristics of the exit hole as well as the meconium left by the attendant chalcidoid insects. The mummies of parasitized nymphs exhibit a conspicuous hole in the middle of the thorax when ectoparasites, e.g. *Tetrastichus dryi* Waterston, are dealt with. The emergence of the endoparasite, e.g. *Psyllaephagus pulvinatus* Waterston, usually takes place through a hole chewed in the abdomen, whereas lateral position of the exit hole is generally associated with hyperparasitism (fig. 2).
4) visual greening symptom characterized by sectorial dieback, zinc pattern deficiences, uneven coloration of the fruits, lobsided fruits with aborted seeds.

Samples of columellas and leaf midveins were collected on presumably contaminated material, then fixed immediately in a 4% buffered solution of glutaraldehyde, pH 7.4, and dispatched to the laboratory. The samples were subsequently postfixed in a 1% osmium tetroxide solution, dehyd-

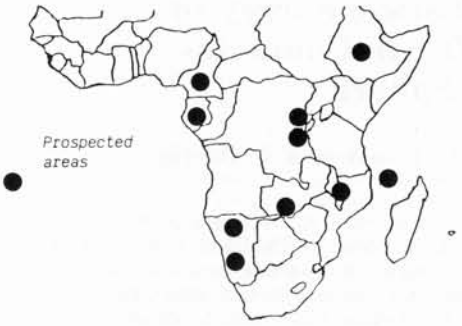

Fig. 1. Greening and *Trioza erytreae* survey in Africa south of the Sahara.

rated up to absolute alcohol and then embedded in Epon for ultrathin sections. Observations of phloem sieve tubes were made by transmission electron microscopy for the presence of endocellular bacteria asociated with CGD.

Meteorological records were collected from local national networks, when available at a given representative site. Attention was focused on maximum temperature and maximum vapor pressure saturation deficit.

1) *Temperature:* besides average monthly maximum temperature, the number of days per annum having a maximum temperature less than 25 C (TD/25) was computed. The latter index had been used previously for predicting symptom expression of CGD (6): it was found that severe symptoms occurred when TD/25 exceeds 60 days but usually mild symptoms or no symptoms appeared below a TD/25 of 30 days.
2) *Mean maximum air saturation deficit (SD):* SD is expressed in millibars and can be obtained by combining midday maximum temperature and lowest relative humidity. SD values above 30 mmb are considered as lethal for the African psylla vector, owing to the high rate of mortality of *T. erytreae* eggs and first instar nymphs (2).

RESULTS

Results of the survey are summarized in table 1 for East and West Africa.

EAST AFRICA

Ethiopia. In Ethiopia, greening symptoms and *T. erytreae* are usually noticed above 1,500 m of elevation (5, 8). Most of the recent citrus plantations have been established in the middle Awash River valley between 1,100 and 1,200 m (especially in Nura Era and Merti Jeju). But citrus orchards were also traditionally planted in the lower Awash River (e.g. Melka Werer 730 m) or in the upper Awash River (e.g. Awasa-Koka 1,600 m). Furthermore, backyard citrus trees are commonly found at higher elevation, for instance at Debre Zeit (1,850 m) and near Addis Ababa (2,400 m). The present survey was carried out on orchards located along the altitudinal gradient which prevails between Melka Werer and Addis Ababa.

Table 1 shows that the T/25 index increases from zero to 100 days between 1,200 and 1,600 m. There is a concomittant appearance of severe CGD symptoms on the trees at the latter altitude. This field diagnosis was subsequently confirmed by the examination of the phloem ultrastruc-

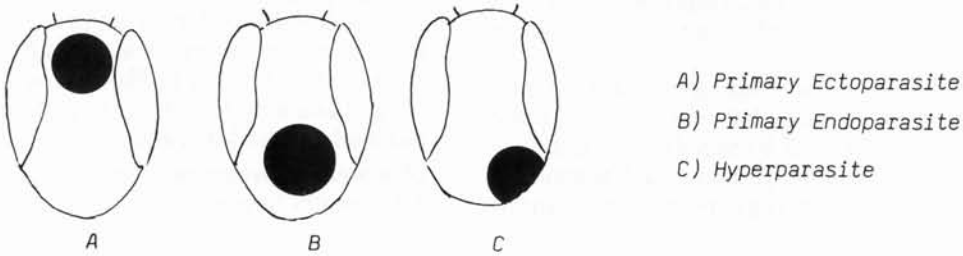

Fig. 2. Examples of leaf psylla mummies exhibiting different types of chalcidoid exit holes.

TABLE 1
CITRUS GREENING DISEASE SURVEY IN EAST AND WEST AFRICA

Country	Location	Altitude (a)	Climatic conditions: T° max[z]	TD/25[y]	SD (mmb)[x]: Mean	Max	Min	Greening: Visual Symptom	GO[v]	*Trioza erytreae*[w]	Primary parasite: Endo[w]	Ecto[w]	Secondary parasite[w]
Ethiopia	Melka Werer	730	34.3	0	32			—		—			
Lat. 4°-18°N	Awash	916	33.4	0	30	39	24	—		—			
	Metahara	950	32.3	0	25	31	19	—		—			
	Nura Era	1,200	30.8	0	25	30	12	—		—			
	Tibila	1,400	—	—	—	—	—	+		+			
	Awasa/Koka	1,600	24.3	100	16	24	8	+	+	+	+	—	+
	Debre Zeit	1,850	26.1	120	13	16	6	+	+	+	+	—	+
	Addis Abeba	2,400	22.8	337	14	13	7	+		+			
Rwanda-Burundi	Muronge							+	+	+			
Lat. 4°-7°S	Karama	1,403	27.9	25				+		+	+	+	+
	Butare	2,312	20.1	360				+	+	+	+	+	+
Malawi	Chileka	767	27.5	70	16	26	10	+	+	+			
Lat. 15°S	Chiteze	1,149	26.7	120	19	29	11	+	+	+		+	+
	Bvumbe	1,400	23.8	190	11	18	8	+	+	+		+	+
Cameroon	Garoua	183	34.4	0	37	61	18	—	—	—	—	—	—
Lat. 2-9°N	Sangmelima	730	28.8	30	17	28	10	+	+	+	—	—	
	Nkoundja	1,220	28.0	59	21	39	9	+		+	+	+	+
Gabon	Okoloville	380	28.0	30	22	12	40	—	—	+	—	—	—
Lat. 1°S													
Namibia	Swakopmund	5	29.3	32	29	40	17	—		—	—	—	—
Lat. 19-22°S	Grootfontein	1,400	27.2	45	28	41	17	—	—	—	—	—	—
Comores	Grande Comore	50	29.9					—	—	Diapho-	+	—	—
Lat. 12°S	Anjouan	350	25.7					—	—	rina sp.	+	—	—

[z]Mean annual maximum temperature.
[y]Total number of day per annuum having a maximum temperature less than 25 C.
[x]Saturation deficit (SD) in millibars (mmb) at mid day; mean = annual average; Max = highest monthly saturation deficit; Min = lowest monthly saturation deficit at midday.
[w]— = not seen; + = considered organism or symptom present.
[v]— = apparently healthy phloem + = presence of endocellular bacteria in the phloem tissue.

ture attesting to the presence of endocellular GOs in the sieve tubes (fig. 3).

Mean SD maximum is only 16.7 mmb at 1,600 m with highest peaks of only 24 mmb, this means a low rate of air desiccation strongly favourable to psylla build-up. Between 1,000 and 1,200 m, SD values of 30 mmb bring about high mortality rate with low chance of psylla proliferation.

Ethiopian endemic Rutaceae flora include the following species *Clausena anisata* (Wild), *Vepris glomerata* (EH. Hoffm.) *Fagaropsis chalybea* (Engl) Verdoon, *Toddalia asiatica* (L.) Harm and *Zantoxylum chalyteum* Engl.. But so far only *C. anisata* was found harbouring *T. erytreae* in Ethiopia and a more complete survey is needed for the other species, mainly the *Fagariops*. *Trioza* species described recently on *Ficus sycamorus*, *Pygium africanum* and *Stephania abyssinica* (7) belong to different psyllid species, since *Trioza erytreae* is the only member of this group known to develop on the Rutaceae flora (3).

Natural parasites obtained from *T. erytreae* include *Psyllaephagus pulvinatus* Waterston, *Psyllaephagus* sp. and a secondary parasite: *Cheiloneurus cyanonotus* Waterston. To date, no *Tetrastichus* sp. has been obtained from *T. erytreae* in Ethiopia.

Rwanda, Burundi. *Trioza erytreae*, as well as symptoms of CGD, and endocellular phloem restricted GOs, were found in Rwanda and Burundi above 1,400 m of elevation. An example of GOs observed in the columella of a contaminated lemon tree at Butare is shown on fig. 4. Wild rutaceous plants of these countries include *Clausena anisata*, *Citropsis schweinfurtzi*, and Balsamocitrus sp. but more inspections are needed to check for the presence of *T. erytreae* reservoirs on this indigenous flora.

The examination of 100 *T. erytreae* nymphs in Gisovu, Karama and Bu-

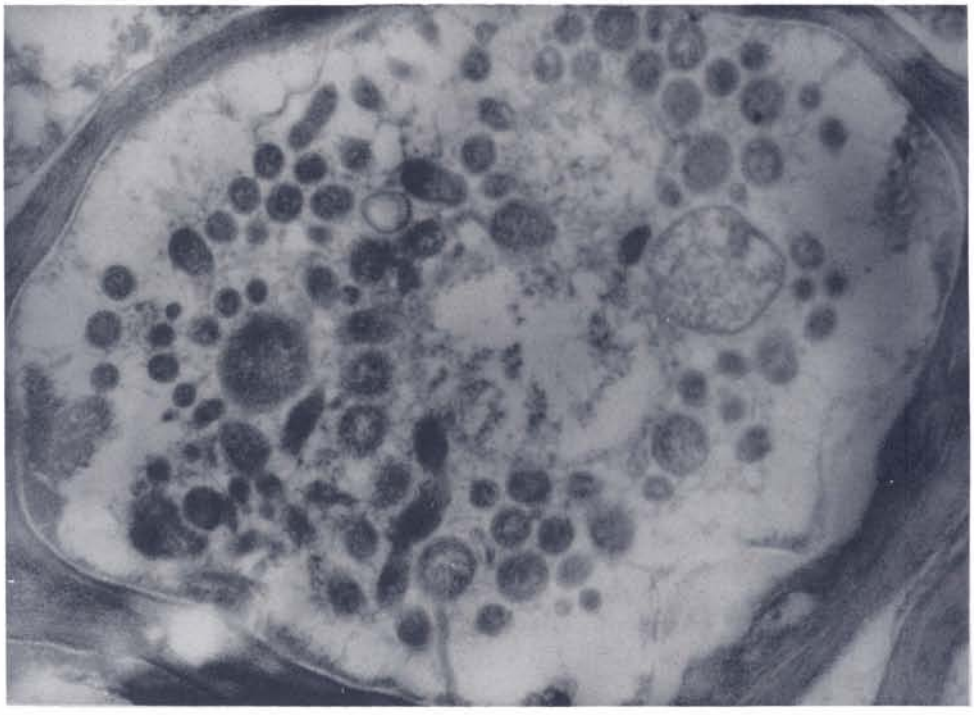

Fig. 3. Greening organism in the sieve tube of an orange tree, Koka, Ethiopa, 1,600 m. (X 25,000).

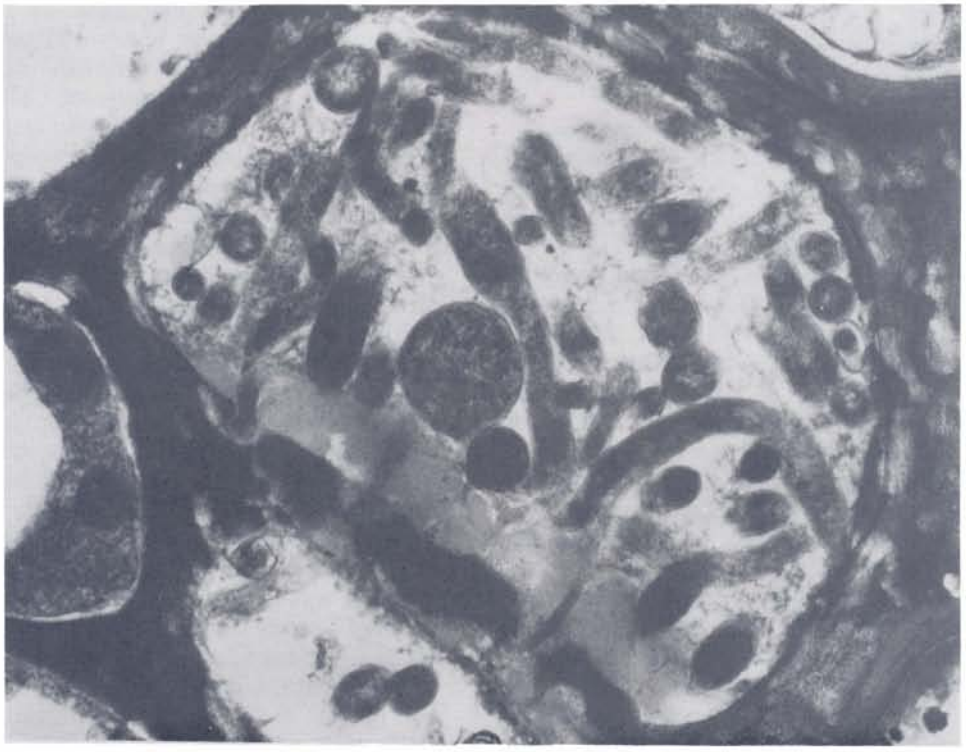

Fig. 4. Greening organism in the sieve tubes of a lemon tree, Butare, Rwanda, 2,312 m. (X 25,000).

tare has shown a high rate of parasitism (90 to 92%) in the first two places induced by what is probably *Tetrastichus dryi*. Endoparasites belonging to the *Psyllaephagus* group as well as unidentified secondary parasites, were obtained from the Butare samples.

Malawi. In Malawi, located at a latitude of 15° south, both CGD symptoms and the African psylla vector were observed at only 760 m of altitude. Phloem restricted GOs were detected from different samples of fruit columellas sampled on suspected trees. Since most of the territory of the Republic of Malawi is located above 1,000 m elevation, CGD is widespread all over the country and there is virtually no production of oranges or mandarins. Local rough lemon seedlings which are quite common in rural areas as backyard trees, exhibit several flushes per year which strongly attract *T. erytreae* adults. These plants are moderately tolerant to CGD, and have been found contaminated by the GOs. They constitute an important source of inoculum.

Survey of parasites has revealed the presence of a new species of *Tetrastichus* obtained from hatching boxes and presently under taxonomic investigation. This *Tetrastichus* sp. seems less effective than *Tetrastichus dryi* for controlling psylla buildup.

The examination of 379 nymphs collected in the citrus collection of Bvumbve has given a percentage of parasitism of only 27%. Among the parasitized nymphs 4% were attacked by the ectoparasite belonging to the group of *Tetrastichus*, 7% by an endoparasite of the group *Psyllaephagus*, whereas 16% exhibited lateral exit holes presumably induced by hyperparasites (fig. 2 c).

Zambia. In Zambia, the survey was carried out only in Lusaka area on orange or mandarin orchards. No trace of *Trioza erytreae* was detected and the survey did not show, either

Trioza erytreae or CGD symptoms. The observation of columella samples under electron microscope gave negative results.

WEST AFRICA

Cameroon. In the Cameroon, important outbreaks of *T. erytreae* have been noticed in the volcanic highlands (e.g. Nkoundja area 1,220 m), where the mean annual saturation deficit is only 21 mmb. Nonetheless extreme value of SD varies from 9 mmb in rainy season to 39 mmb during the hot dry season. The latter is too arid for the survival of eggs and first instar nymphs, hence there are strong fluctuations of psylla colonies throughout the year, with only two or three generations concentrated during the wet flush season.

In the hilly rainforest area of Sangmelima (700 m), permanent humid and fresh conditions support psylla outbreaks all year round with probably six or seven annual generations. Extremely high populations (exceeding 200 galls/m^2 of canopy area) were observed on citrus trees and *Clausena anisata*. Apart from *C. anisata*, the endemic rutaceous flora has not yet been surveyed.

One of the main characteristics of the Cameroon *T. erytreae* ecosystem, is the absence of effective predators and a very low percentage of natural parasitism. A new species of *Tetrastichus* apparently similar to that of Malawi has been found but its impact on regulation of the psyllid outbreak is too low. Of 245 psylla nymphs examined, only 25 were found parasitized (i.e. 10%).

Greening symptoms on citrus trees were seen in Nkoundja and Sangmelima. However, the Sangmelima samples were the only ones to reveal phloem restricted GOs. No GO was found in *C. anisata* mid-veins.

In the northern part of the country near Garoua, the climate is far too arid for allowing psylla buildup or symptom expression of the disease. For this reason, all the new disease-free germplasm recently has been established in this area.

Gabon. Typical leaf galls of *T. erytreae* were noticed in several private orchards of Okoloville (380 m), but no clear-cut symptoms of CGD were seen on orange or mandarin trees, and electron microscopic examinations of phloem columellas gave negative results.

Namibia. The survey of several citrus trees in Swakopmund (7 m) and Grootfontein (1,400 m) showed neither *T. erytreae* nor CGD symptoms, and electron microscopic examination of tissue samples failed to detect the GO. In the areas visited, the climate is apparently too arid for the psyllid borne CGD, even near 1,500 m of elevation.

Comores Islands. A new species of *Diaphorina*, close to *Diaphorina amoena* was discovered on the citrus trees of Grande Comore, Anjouan and Moheli. A taxonomic description of this new citrus psyllid was given recently (4). No typical symptoms of CGD were noticed in this archipelago and the results of electron microscopic examination of citrus phloem were negative. Whether the new Comorian *Diaphorina* is able to transmit CGD remains to be demonstrated.

DISCUSSION AND CONCLUSION

Although the distribution map of *Trioza erytreae* is presently known to cover 20 African countries or territories, less information was available on the citrus greening disease itself. During the present survey, the Greening Organism was detected on citrus trees in Malawi, Rwanda, Burundi, Ethiopia and Cameroon to augment electron microscopic evidence of CGD obtained previously from South Africa, Kenya, Reunion and Mauritius. With the exception of Gabon and certain areas of Cameroons we found that *T. erytreae* was associated with CGD.

In all the inspected areas, the observed psylla outbreaks were consistent with the expected populations deducted from climatic data. But the