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Status of Citrus Canker Caused by *Xanthomonas axonopodis* PV. *CITRI* in Peninsular Malaysia

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ABSTRACT

Study on citrus canker caused by *Xanthomonas axonopodis* pv. *citri* was undertaken in Peninsular Malaysia. The disease occurred in eight states, where incidence of 36.5% and severity of 15.2% was observed on leaves, while incidence of 18.7% and severity of 7.5% was observed on fruits. Field host range included Mexican lime (*C. aurantifolia*), pomelo (*C. grandis*) and kaffier lime (*C. hysterix*). Canker severity significantly correlated ($r = 0.7041$, $p = 0.011$) with temperature but not with rainfall altitude and tree age.

Key Words: Citrus canker; *Xanthomonas axonopodis* pv. *citri*; West Malysia; Disease intensity; Citrus

INTRODUCTION

In Malaysia citrus is grown in small scale or commercial plantations and also in backyard orchards and small holdings in various parts of the country. Citrus is attacked by various diseases among, which canker is one. In Malaysia, information regarding the disease is rare even though its occurrence is known. Therefore a study on the occurrence, distribution and intensity of citrus canker 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 awareness to growers. This study was undertaken mainly to quantitatively study the occurrence and distribution of citrus canker caused by *Xanthomonas axonopodis* pv. *citri* in West Malaysia, to determine the incidence, severity and field host range of the disease and to relate citrus canker severity to temperature, rainfall, elevation and tree age.

MATERIALS AND METHODS

Survey areas. The survey was conducted in eight states and 16 locations between January to April 2004 (Table I). It was conducted mostly on conservation and experimental plots at Malaysian Agricultural Research and Development Institute (MARDI) stations, in commercial plantations, at University Malaya (UM) botanical garden and in backyard orchards in various states.

All surveyed sites were believed to be representative of their respective locations. The survey was conducted in high, mid and low altitudes, where citrus is grown. Geographic positions of the survey areas were recorded using the Geographic Positioning System (GPS). The areas covered in the surveys were situated between 100^0 to 103^0 east longitude and 1^0 to 6^0 latitude and classified based on altitudinal groups: (a) high altitude areas (> 2000 m) (b) medium altitude areas (1300 – 2000 m) and (c) low land areas below 1300 m.

Varieties encountered during the survey were mostly pomelo (*Citrus grandis*), Mexican lime (*C. aurantifolia*), kaffier lime (C. *hysterix*) and in rare cases rangpur lime (C. *limonia*) and honey mandarin (*C. reticulata*).

Sampling and data collection. The quantity of citrus canker present was assessed on five un-sprayed citrus trees from each cultivar at a site in the field by means of disease intensity, which was measured using severity and incidence. Forty attached leaves and 50 intact fruit samples were considered from each tree and were related to the spatial distribution of the disease and collected from upper and lower half canopies. Leaf and fruit samples with citrus canker symptoms were collected, from citrus trees, where data were taken and brought to the laboratory to confirm identity of the disease through microscopic examination of the pathogen (Holderness, 2002).

In order to have better coverage of the field, systematic sampling method with a "W" shaped sampling path was used to determine incidence in the field. To eliminate personal selection of the units, 20, 40 and 60 paces, depending on field size, were made between samples (IMI, 1995). Destructive sampling method was used for disease assessment on leaves and measurements were taken on intact fruits. Sample sizes were determined from similar studies conducted on Phaeoramularia leaf and fruit spot of

citrus (Seif & Hillocks, 1999). Incidence of disease on leaves was determined on eight random terminal shoots from lower and upper halves of a tree, total number of leaves counted and expressed as a proportion of leaves with at least one lesion (Teng & James, 2002).

Canker incidence on fruits was determined on the same five trees by taking randomly 50 intact fruits per tree (10 from four directions of the tree, five from lower & five from upper canopy), recorded as + or – diseased and expressed as a proportion of the total number of fruits (Seif & Hillocks, 1999).

Severity on leaves, were determined using Rayner's method (1969) of graphic representation, where sample leaves were put under graphic transparent paper with 2 cm squares. The numbers of squares covered by the whole leaf area and numbers of squares with citrus canker were recorded. Severity was determined as a percentage of squares covered by the whole leaf to those covered with spots (Teng & James, 2002) and was conducted on the same samples used for disease incidence.

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

Canker severity on fruits were initially determined using descriptive type assessment key with a 0 - 4 score scale, where 0 = 0%, 1 = 1 - 10%, 2 = 11 - 25, 3 = 26 - 50%, 4 = >50% (Seif & Hillocks, 1999). The score scales were then converted to disease severity index for non-parametric measurements (Kim *et al*., 2000) and expressed as percentages.

Secondary data on temperature and rainfall for the surveyed areas were obtained from Malaysian Meteorological Service. Tree age data were obtained from the respective plantations and MARDI stations. Elevation data were taken using an altimeter.

Data analysis. To stabilize variance, disease incidence and severity data on leaves and fruits in both countries were arcsine transformed before analysis using descriptive statistics (IMI, 1995). Pearson's correlation analysis were run using the statistical software package SAS V8 (SAS Institute, Carey, North Carolina, USA) program. The correlations between canker severity, temperature, rainfall, tree age and altitude were also determined using the same program.

RESULTS

Surveyed areas. In west Malaysia, the survey was conducted in eight states. The elevation in all surveyed areas, except Cameron Highlands, which is 1425 m, was less than 100 m.

Disease intensity. Citrus canker disease was observed in five of the eight states surveyed and was found in the lowlands, on Mexican lime (*C. aurantifolia*), pomelo (*C. grandis*) and kaffier lime (*C. hysterix*) and was not observed in the highlands.

Table I. Number of locations covered during the citrus canker surveys in West Malaysia states

State	Location
Perak	1
Pahang	2
Petaling Jaya	1
Kedah	3
Selangor	3
Terengganu	2
Kelantan	1
Johor	3
Total	16

Overall canker incidence in the country, on infected leaves was 36.5% and severity 15.1% and incidence on fruits was 18.7% and severity 7.5%. Moderate canker incidence was recorded on leaves of pomelo, Mexican lime and kaffier lime. Severity was relatively low when compared with incidence. The lowest incidence and severity was recorded on Mexican lime in Kluang at Bandar Tenggara on a commercial citrus plantation, where Mexican lime was widely grown. On the basis of specific location, the highest citrus canker severity of 22.21% on kaffier lime leaves was recorded at Serdang, on cultivars originated from Malaysia followed by 18.9% at Jerenggau, Terregganu, while the lowest, 3.15% was recorded at Kampung Krangi Hosba, Kedah. Kaffier lime cultivars originated from Thailand were less susceptible, 11.5%, than those originated from Malaysia. Similarly, the highest disease severity, 22.37%, on Mexican lime was recorded at Kampung Krangi Hosba in Kedah, while the lowest, 1.5% was observed in Kluang, Johor. On the other hand, both cultivars did not show any canker symptom at University Malaya botanical garden in Petaling Jaya. Disease severity on pomelo was relatively high, 17.75%, at Kuala Kangsar in Perak. However, no canker symptom on pomelo was observed at Kedah, Petaling Jaya and Jeranggau (Table II).

On the basis of disease intensity on cultivars the highest overall mean disease severity was recorded on kaffier lime (*C. hysterix*) followed by Mexican lime (*C. aurantifolia*) and pomelo (*C. grandis*) (Table III). On the other hand, canker was not observed at Tanah Rata and Ringlet on rangpur lime (C. *limonia*) and on honey mandarin (*C. reticulata*). The same situation was observed at Kesedar project in Kelantan.

Relatively high disease severity was observed in Kuala Kangsar followed by Serdang, Jerengau and Alor Setar. Very low severity was observed in Kluang, while no disease was recorded in Cameron Highlands. Minimum annual rainfall (2212.1 mm) was recorded at Kluang. The highest mean annual rainfall, 2760.8 mm, was recorded at Cameroon Highlands, while the lowest, 2212.1 mm was at Kluang (Table IV).

Ten years mean monthly rainfall in West Malaysia ranged from 170.2 in Kluang to 212.4 in Cameron Highlands. High rainfall intensities were observed from October through December. Un-usually high rainfall

Table II. Distribution and intensity of canker (*X. axanopodis.* Pv.*citri*) on citrus cultivars in West Malaysia

State	Locations	Cultivar	Disease Intensity			
			Incidence (%) Mean (Range)		Severity (%) Mean (Range	
			Leaves	Fruit	Leaves	Fruit
Pahang	Tanah Rata	Rangpur lime (*C.limonia*)	0	0	0	0
	Ringlet	Honey Mandarin (*C. reticulata)*	0	0	0	0
	MARDI	ditto	0	0	0	0
Perak	Kuala Kangsar	Pomelo (*C. grandis*)	45bc (40-50)	15ab (5-35)	17.75ab (9.6-26.14)	5c (0-2)
Selangor	UPM	Mexican lime (*C.aurantifolia*)	42.5cd (40-45)	25a (20-30)	10.53c (8.5-13.76)	10b (0-2)
	MARDI	Pomelo (*C. grandis*)	52.5a (50-55)	25a (20-30)	9.67c (7.83-11.67)	10b (0-2)
		Kaffier –M* (*C .hysterix*)	51.66ab (45-55)	28.33a (25-35)	22.21a (15.07-29.75)	15a (0-2)
		Kaffier –T** (*C. hysterix*)	37.5d (35-40)	18.33ab (15-20)	11.5c (8.13-16.43)	5c.(0-1)
Petaling Jaya	UM	Kaffier lime *(C. hysterix*)	0	0	0	0
		Pomelo (*C.grandis*)	0	0	0	0
		Mexican. lime (*C.aurantifolia)*	0	0	0	0
Kedah	Guar Batu Hitam	Pomelo (*C. grandis*)	0	0	0	0
	Kuala Lanjut	Honey Mandarin (*C. reticulata)*	0	0	0	0
	Gula Padang Terap	Honey Mandarin (*C. reticulata)*	0	0	0	0
	Kampung	Kaffier lime (*C. hysterix*)	21.66e (15-25)	15ab (0.94-7.11)	15b (10-20)	5c (0-1)
	Krangi Hosba	Mexican. lime (*C. aurantifolia*)	45bc (40-50)	37.5a (20-45)	37.5a (35-40)	15a (0-3)
Teregganu	Jerangau	Kaffier lime *(C. hysterix)*	45bc (40-50)	21.6a (10-40)	18.9ab (8.9-26.2)	10b (0-2)
		Mexican.lime (*C. aurantifolia*)	17.5 g (15-20)	0.8b (0.4-1.12)	7.5d (5-10)	Tr
		Pomelo (*C. grandis*)	0	0	0	0
	Durian Mentangau	Honey Mandarin (*C. reticulata*	0	0	0	0
Kelantan	Kesedar project	Honey mandarin (*C. reticulata)*	0	0	0	0
Johor	Bandar Tenggara	Mexican.lime (*C. aurantifolia*)	6.25f (0-10)	1.15b (0-3.4)	1.25e (0-5)	Tr
Mean			36.5	18.7	15.2	7..5
CV (%)			1.9	1.9	1.6	
SEM			0.06	0.06	0.05	0.02

Kaffier –M* = Malay type, Kaffier –T** = Thai type. Means within a column followed by the same letters are not Statistically different at $p \leq 0.05$. Tr < 0.01%

Table III. Mean* percentage of citrus canker severity on leaves on citrus cultivars in West Malaysia in 2004

Cultivar	Mean*% severity
Rangpur lime (*Citrus limonia)*	0c
Honey Mandarin(*C.casturi*)	0c
Mexican lime (*C.aurantifolia*)	14.2b
Pomelo (*C.grandis*)	13.71b
Kaffier lime (*C. hysterix*)	17.6a

Means within a column followed by the same letters are not statistically different at $p \leq 0.05$; *Mean of three sites.

Table IV. Canker severity, mean monthly temperature, mean annual rainfall, elevation and tree age at different locations in West Malaysia (2004)

Location	Canker severity (%)*	Temperature (°C)	Rainfall (mm)	Elevation (m)	Tree age (yr)
Kuala kangsar	17.75a	27.2b	2642ab	66	2
Serdang	13.5b	27.8a	2443ab	39.7	8
Alor Setar	12.76	27.8a	2258bc	4	5
Jerengau	13.2b	27c	2705a	7	10
Kluang	1.25d	26.6d	2212.1c	88.1	5
Cameron Highlands	0e	17.9e	2760.8a	1425	5

Means within a column followed by the same letters are not statistically different at $p \leq 0.05$

*Mean of three sites

occurred at Jerangau in November. The least rainfall record was in Kota Baru in May. Ten years mean monthly temperature in surveyed areas of West Malaysia was exceptionally uniform through out the year. It showed the highest mean monthly temperature at Alor Setar 29.0°C and was recorded in May, while the least temperature, 26.4°C, was recorded in October. At Jerangau the highest temperature was (28.3°C) and was recorded in May, while the lowest was 25.9°C and recorded in December. At Kuala Kangsar the highest temperature was observed in May (28.6°C) and the lowest in December (26.2°C). Highest temperature in Cameron Highlands was 18.7°C and was recorded in April and the lowest, 17.1°C, in October and December (Fig. 2). There was significant correlation between disease severity and temperature ($r = 0.7041$, $p = 0.011$) and non-significant linear association was observed between disease severity and rainfall ($r = 0.0935$) (Fig. 4 & 5). On the other hand, there was significant correlation between elevation and temperature ($r = -0.9951$, $p \leq 0.0001$) and non-significant linear association between disease severity and tree age ($r = 0.0442$), disease severity and elevation ($r = -0.6741$) (Table V).

DISCUSSION

Citrus canker occurrence and distribution in surveyed areas in west Malaysia varied from location to location depending mainly on the presence or absence of susceptible hosts. The survey indicated that in most cases, where ever kaffier lime was grown the disease was also observed. However, the same was not true for Mexican lime and pomelo. In some areas like Jeranggau no canker symptoms were observed on Mexican lime, where as kaffier lime was affected at the same location. In Jeranggau and Kedah one

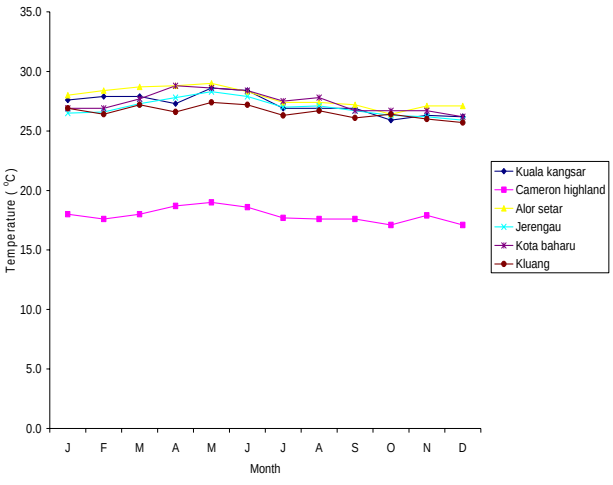
Table V. Correlation between canker severity, temperature, RF, elevation and tree age in surveyed areas in West Malaysia (2004)

DI	Temp. (°C)	RF (mm)	Elevation (m)	Tree age (yr)
DI	0.7041**	0.0935^{ns}	-0.6741 ^{ns}	0.0442 ^{ns}
Temp. (°C)		-0.5397 ^{ns}	-0.9951**	0.1528 ^{ns}
RF (mm)			0. 5479	0.1252 ^{ns}
Elevation (m)				-0.0178 ^{ns}
Tree age (yr).				

DI =Disease severity, RF = Rainfall, * Significant at $p \leq 0.05$

Fig. 1. Distribution map of citrus canker and locations covered during the survey in West Malaysia

Fig. 2. Ten year's mean monthly rainfall and mean monthly temperature through out the years in West Malaysia (1994-2004)



to two years old plantings of pomelo were not attacked by the disease. It was learnt that in those areas there was drought during the previous three months (Kairudin, pers. comm.). Subsequently, there was no favorable environment for the growth and development of the pathogen on pomelo, resulting in the absence of the disease. However, disease incidence in the vicinity was recorded on kaffier lime and Mexican lime. Field host range of citrus canker in West Malaysia appears to be wide.

During the survey low disease incidence has been observed in isolated fields at a commercial plantation in Bandar Tenggara/Kluang. Many citrus trees in this farm had been sprayed with foliar fungicide, which likely contributed to the lower canker levels in the season.

The reasons for the absence of the disease on known susceptible cultivars during the survey in some parts of Malaysia are not clear but, might be attributed to absence of favorable environmental factors especially rainfall and temperature, required for the rapid growth and development of *X. axonopodis* pv. *citri* (Kema *et al*., 1996). Another possible explanation for absence of the disease on some cultivars could be because such results, except at University Malaya botanical garden, usually occurred on young citrus plants that have less susceptible foliage (Ngugi *et al*., 2002).

The absence of disease on kaffier lime, Mexican lime and pomelo at University Malaya botanical garden in Petaling Jaya could be attributed to the application of disease management practices, like chemical control, in order to conserve the various plant genetic resources that were collected from different sources and maintained for further scientific studies.

In most surveyed areas in west Malaysia high rainfall was accompanied with high temperature, with the exception of Cameron Highlands, where relatively high annual rainfall (2760.8 mm) and relatively low mean monthly temperature of 17.9°C were observed. Since high rainfall and temperature appear to be the main factors distinguishing west Malaysia, the probability of citrus canker to occur in any part of the country, where susceptible citrus cultivars are grown seems to be very high. Major out-breaks of citrus canker can occur primarily in regions, where rainfall increases and temperatures rise simultaneously and when new shoots are emerging or when fruits are in the early stages of development. Frequent rainfall in warm weather, especially during storms, contributes to disease development (Gottwald *et al*., 2002).

During the entire survey in West Malaysia kaffier lime (*Citrus hystrix*) was consistently found infected with canker. Kaffir lime is from Southeast Asia and popular in Thailand, Cambodia, Malaysia and Indonesia. It is a small, slow growing tree with dark green leaves and thorns. The fruit juice is used as anti-scurvy, expectorant; external use as antidandruff shampoo, fruit peel is also used for relief of stomach pain carminative and as traditional medicine in Indonesia (Anonymous, 2002). Though kaffier lime is a very valuable plant (Anonymous, 2004) it does not seem to be properly exploited in Malaysia and in most areas it was neglected. However, because of its high susceptibility to

Fig. 3. Relationship between citrus canker severity, temperature and rainfall in West Malaysia

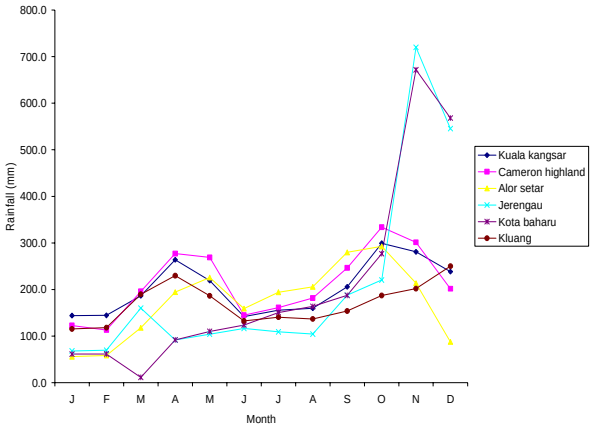


Fig. 4. Relationship between citrus canker severity and temperature in West Malaysia

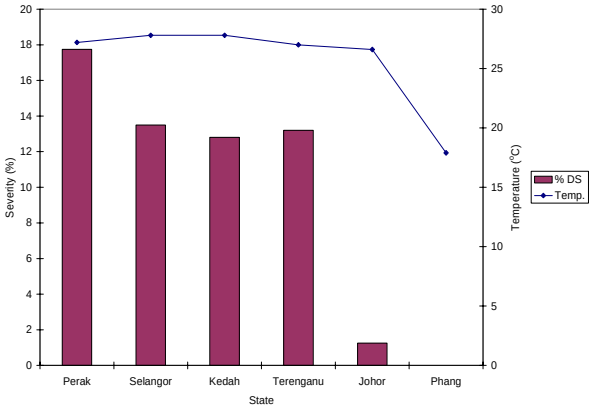
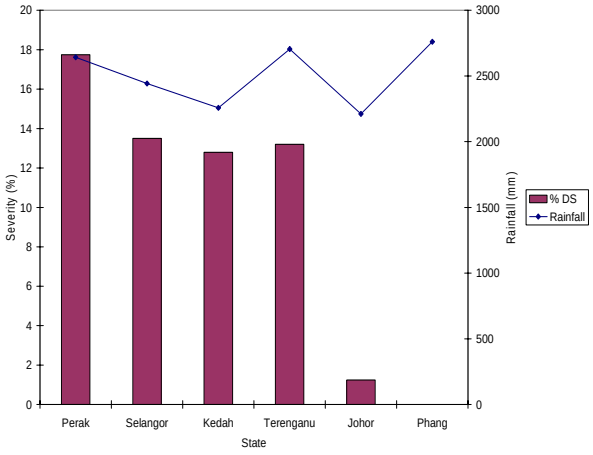


Fig. 5. Relationship between citrus canker severity and rainfall in West Malaysia



citrus canker it appears that the cultivar is an infective canker inoculum reservoir in the country.

In conclusion, based on the results, the current surveys indicated the occurrence of citrus canker in Malaysia. Citrus canker was severe at Kuala Kangsar in West Malaysia. Field host range for citrus canker in West Malaysia included Mexican lime (*C. aurantifolia*), pomelo (*C. grandis*) and kaffier lime (*C. hysterix*). Canker severity significantly correlated with temperature but not with rainfall elevation and tree age.

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