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Aetiology and causal agents of mango sudden decline disease in the Sultanate of Oman

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Abstract

Mango sudden decline is a recently introduced, economically serious disease in Oman. Affected mango trees have wilting symptoms that usually begin on one side and later spread to involve the entire tree. Trees exude amber-coloured gum from the bark of their trunks or branches and vascular tissues are discoloured. Having entered Oman in the recent past, survey data is presented that shows the disease to have spread throughout the northern part of the country. Evidence is presented that the vascular wilt pathogen *Ceratocystis fimbriata* causes mango sudden decline disease in Oman, possibly in concert with *Lasiodiplodia theobromae* and the recently described *Ceratocystis omanensis*. Isolates of these fungi from affected trees, cause infection and can be recovered from inoculated seedlings. Bark beetles (*Hypocryphalus mangiferae*) are shown to carry *C. fimbriata* and *L. theobromae* and are presumably responsible for transmitting both pathogens to healthy mango trees. Acting as a wounding agent and vector, the bark beetle is likely to have assisted the rapid spread of the disease across Oman.

Introduction

Mango, date, lime and banana are the most important perennial fruit crops in the Sultanate of Oman. The area of mango production in 2004 was 2500 ha with production of over 8600 t, concentrated in the Al Batinah region along the northern coast of the country. Cultivation is based on local Omani varieties and exotic scions grafted onto Omani rootstocks.

During 1998, many mango trees began dying in the southern part of the Al Batinah region. The disease was locally called sudden decline in recognition of the rapid death of affected trees. It was first reported in the Barka area in the south of the Al Batinah region. The disease spread northwards and was subsequently reported in Masanah, Suwaiq, Khabora, Saham, Sohar, Liwa, and Shinas (Figure 1) (Al Adawi, 2002). Subsequently, sudden decline has increased in severity, threatening mango cultivation in the country (Al Adawi et al., 2003). To limit the spread of the disease, in 2001 the Ministry of Agriculture and Fisheries (MAF) embarked on an eradication programme to

remove infected trees. More than 13% of the trees were removed from some districts of the Al Batinah region (Table 1).

In 2000, during preliminary studies on the disease, *Lasiodiplodia theobromae* (CAB International reference W6341) was frequently isolated from mango trees affected by sudden decline. *Ceratocystis fimbriata* (culture collection CMW, Forestry and Agricultural Biotechnology Institute, University of Pretoria, South Africa; see van Wyk et al., 2005) was recovered more frequently, as was *C. omanensis* (culture collection CMW; see Al-Subhi et al., 2006). In addition, the bark beetle *Hypocryphalus mangiferae* (Coleoptera: Scolytidae) (Florida Department of Agriculture, identification reference E2005-1780-701) appeared to be consistently associated with the disease, possibly acting as a wounding agent and as a vector of spores.

Because of the importance of mango sudden decline in Oman and the consequent threat to local and regional mango production, research was conducted to establish by survey the distribution of the disease in northern Oman, to investigate the role of *C. fimbriata*, *C. omanensis* and *L. theobromae* in the aetiology of sudden decline disease, and to consider the role of the bark beetle in disease development.

Materials and methods

Disease distribution and symptom development

The field distribution of mango sudden decline was determined in 2000. The survey covered eight districts of the Al Batinah region where mango production is concentrated (Figure 1). Detailed examinations were made of trees at all stages of disease development in each of the areas visited.

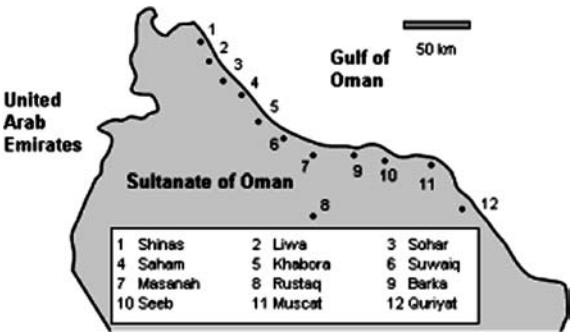

Figure 1. Map of northern Oman showing locations for districts covered by the survey quantifying the incidence of mango sudden decline disease.

Pathogen isolation and identification

Samples were collected from several trees at each farm surveyed. Most isolations were made from stem tissue. Wood samples (0.5–1.5 × 2.00–3.00 cm) were chiselled from the margins of infected tissue after bark removal. Plant tissue was cut from the leading edges of lesions and washed with tap water, surface-sterilized in 1% NaOCl for 1 min., rinsed in sterile distilled water (SDW), blotted on sterile filter paper, and aseptically placed between carrot discs (Moller and DeVay, 1968) or transferred to Petri dishes containing malt extract agar (MEA). Plates were incubated at room temperature (22–25 °C) and after 24 h subcultured onto fresh MEA plates.

Table 1. Number of mango trees in the Al Batinah region eradicated by the MAF, in 2001 and incidence of mango sudden decline recorded during a survey in June 2000

District[a]	Number of trees prior to epidemic	Number of trees removed	Incidence (%) recorded during survey
Barka (9)	74,794	10,154 (13.6%)	76.0
Masanah (7)	24,012	591 (2.5%)	89.4
Suwaiq (6)	55,282	7,465 (13.5%)	60.2
Khabora (5)	35,969	1,212 (3.4%)	54.5
Saham (4)	95,918	4,182 (4.4%)	76.1
Sohar (3)	n/a[b]	n/a[b]	41.9
Liwa (2)	36,246	321 (0.9%)	19.9
Shinas (1)	65,670	189 (0.3%)	12.5

[a]District order reflects increasing distance from Barka, numbers in brackets refer to locations in Figure 1.
[b]Data not available.
Source: MAF (2002).

Carrot discs were incubated for 5–7 days at room temperature under high humidity. After ascomata developed, ascospore masses were transferred to MEA supplemented with streptomycin (500 mg l^{-1}). Isolations made directly onto MEA were purified by transferring hyphal tips onto new MEA plates. Isolated cultures are maintained at the Ghadafan Agriculture Research Station (GCC), MAF, Oman.

Pathogenicity tests

Using a local variety, pathogenicity tests were conducted on stems of young mango plants (24 months old) growing in 13 cm diam pots, containing a mixture of peat moss and loamy soil. The trees were inoculated with four isolates each of *C. fimbriata*, *C. omanensis* and *L. theobromae*. Five seedlings per treatment were inoculated by inserting an agar disc (3 mm diam) bearing mycelium taken from the leading edge of actively growing colonies on MEA, under the bark that had been lifted away from an I-shaped incision (10 mm long) made with a sterile scalpel (Mullen et al., 1991). Seedlings inoculated with uncolonized MEA served as controls. Moistened, sterile cotton pads were placed over wounds that were then wrapped loosely with Parafilm to maintain a humid environment. Parafilm wraps were removed one week after inoculation.

Inoculated seedlings were assessed weekly to record symptom development. As lesions developed, pieces of stem were plated on MEA to verify the presence of inoculated pathogens; fungi were recovered and re-cultured to confirm identity. Analysis of variance of lesion length values taken 42 days after inoculation was done using the GLM procedure in SAS (v8.2, SAS Institute, Cary, NC, USA). Means were compared with Tukey's Studentized Range (HSD) at $P \leq 0.05$.

Isolations from bark beetles

Over 700 *H. mangiferae* bark beetles, adults and larvae, were caught using aspirator traps from different locations in the Al Batinah region. Bark beetles were also taken from three mango log traps taken from healthy mango trees and placed, for 30 days, within an infested orchard in Barka. As a control, three similar logs were placed under polyester fleece tents within the same orchard for the same duration.

Insects were immersed in 1% NaOCl for 1 min., rinsed with SDW, blotted dry on sterile filter paper, and aseptically placed onto potato dextrose agar (PDA). Plates were incubated at room temperature for 3 days, after which colonies emerging from beetles were sub-cultured and identified. Bark beetles were also placed in a cavity made on the inner surface of a pair of carrot discs and incubated at room temperature for 4 days. For isolation from plant tissue and insects, the carrot disc technique was later modified to enhance *C. fimbriata* recovery by soaking carrot discs in streptomycin (100 mg l^{-1}) before incubation. In all cases, *C. fimbriata* was identified based on culture morphology, distinctive perithecia and culture aroma (Upadhyay, 1981).

Results

Disease distribution and symptom development

The distribution of the disease across the different districts of Al Batinah region showed the same pattern as the distribution of numbers of affected trees subsequently removed by the MAF. These data (Table 1) indicate highest disease levels in the east of the region (76% in Barka, location 9 in Figure 1), decreasing closer to the border with the United Arab Emirates (12.5% in Shinas, location 1 in Figure 1).

Initial disease symptoms were gummosis from the bark and branch death (Figure 2a) on affected trees; these affected trees usually displayed other symptoms, including vascular discolouration beneath the gummosis (Figure 2b). Tree death usually occurred within 6 months of first symptom appearance. Diseased trees always showed signs of damage caused by the bark beetle *H. mangiferae* (Figure 2c, d). The majority of diseased trees had developed large, inconspicuous trunk cankers where the bark appeared darker than normal. Beneath the affected bark underlying tissues were discoloured brown to black. Rootstocks of grafted trees were frequently severely affected compared with the scion, which was commonly asymptomatic (Figure 2e). Cankers located near ground level often resulted in death of the entire tree, especially with grafted trees. However, local varieties appeared to be more severely affected than exotic scions on grafted trees.

Figure 2. Symptoms of mango sudden decline: (a) branch death; (b) gummosis on bark and vascular discolouration; (c) bark beetle damage; (d) adult bark beetle; (e) dead tree with severely affected rootstock but asymptomatic scion.

Pathogen isolation and identification

From April 2000 to May 2004, samples were collected from nine areas of the Al Batinah region. Of the 294 fungal isolates made from a random selection of plant tissue and beetles, the majority (193 = 65.6%) were recovered from plant tissue. Fewer successful isolations (101 = 34.4%) were made from beetles. *Lasiodiplodia theobromae* represented 137 (46.6%) of the isolates, while *C. fimbriata* represented 83 (28.2%) and *C. omanensis* represented 8.2%. Fifty isolates (17%) remained unidentified, although some were possibly *L. theobromae* that failed to produce pycnidia in culture. *Lasiodiplodia theobromae* and *C. fimbriata* were isolated in approximately equal frequency from wood; *C. omanensis* was isolated predominantly from wood. Some samples appeared to yield two or more of the pathogens and it is possible that excessive growth of one, especially *L. theobromae*, masked

the appearance of *C. fimbriata*, causing an underestimation of the frequency of isolation of this pathogen.

Pathogenicity tests

Mango plants inoculated with *C. fimbriata* developed gummosis and extensive lesions on all treated seedlings (Table 2). Wilting progressed over a few days into a permanent wilt, with leaves still attached. Longitudinal sections under the bark revealed dark brown discolouration extending above and below the inoculation site.

Lesions also developed on plants inoculated with *C. omanensis* and *L. theobromae*. However, mean lesion length was significantly longer on stems inoculated with *C. fimbriata* (29.4 cm) compared with *C. omanensis* (1.8 cm) and *L. theobromae* (1.8 cm). Control seedlings did not display lesions. In each case the fungus used as the inoculant was reisolated from infected seedlings.

Isolation from bark beetles

Both *C. fimbriata* and *L. theobromae* were isolated from adult beetles. Between 2000 and 2002, *C. fimbriata* was isolated at relatively low frequency (0–13.2%) from beetles, even when the carrot baiting method was used. When this method was modified by treating carrot slices with streptomycin to reduce bacterial contamination, the recovery percentage of *C. fimbriata* from adult beetles improved significantly (Table 3).

The frequency of *L. theobromae* isolation from beetles ranged from 1.3% to 72% for adults and 71% to 100% for larvae (Table 3). In 2000, *L. theobromae* was isolated from 72% of adult beetles collected from Barka, and from 100% of larvae collected in the same orchard. In 2001, beetles were collected from the same area and 80% of larvae and adults yielded isolates of *L. theobromae*. The fungus was isolated from 43% and 23% of bark beetles collected from Khabora and Sohar respectively during 2002. However, the rate of recovery of this fungus was lower for samples collected from Masanah (1.3%) and Seeb (3.9%).

Where isolations were made from adult beetles collected from three log traps, 3/15 (20%), 25/60 (42%) and 6/48 (13%) of the insects yielded *L. theobromae*. However, *L. theobromae* was isolated from 27/38 (71%) of larvae collected from these traps. Removal of the bark of the log traps showed that tissue directly beneath the beetle infestation holes was discoloured. There was no discolouration below the bark on logs without insect injury. Isolation of fungi from the margins of the discoloured tissue yielded *L. theobromae*. No insects were found on log traps placed under polyester fleece and no vascular discolouration was evident. *Ceratocystis fimbriata* was not isolated from log traps.

Discussion

The disease was locally called sudden decline in recognition of the rapid death of affected trees. It

Table 2. Lesion development (mean of five replicates) on mango seedlings 42 days after inoculation with *C. fimbriata*, *C. omanensis* and *L. theobromae*

Isolate	Pathogen	Source	Location	Mean lesion length (cm)[a]
GCC55	*C. fimbriata*	Mango	Sohar	19.8 a
GCC58	*C. fimbriata*	Bark beetle	Sohar	24.5 a
GCC120	*C. fimbriata*	Mango	Shinas	27.1 a
GCC138	*C. fimbriata*	Mango	Shinas	46.1 a
GCC43	*C. omanensis*	Mango	Sohar	1.4 b
GCC48	*C. omanensis*	Mango	Sohar	3.8 b
GCC49	*C. omanensis*	Mango	Sohar	1.0 b
GCC52	*C. omanensis*	Mango	Sohar	1.0 b
GCC134	*L. theobromae*	Mango	Shinas	2.0 b
GCC100	*L. theobromae*	Mango	Sohar	1.7 b
GCC174	*L. theobromae*	Mango	Sohar	2.4 b
GCC356	*L. theobromae*	Bark beetle	Liwa	1.0 b

[a]Values not having a letter in common are significantly different at $P = 0.05$ according to Tukey's Studentized Range (HSD).

Table 3. Recovery of *C. fimbriata* and *L. theobromae* from adult bark beetles isolated using PDA and carrot baiting techniques

Location[a]	Isolation date	Isolation medium		
		PDA		Carrot
		C. fimbriata	*L. theobromae*	*C. fimbriata*
Barka (9)	October 2000	0/25 (0.0)[b]	18/25 (72.0)[b]	0/18 (0.0)[b]
Barka (9)	October 2000	0/10 (0.0)	10/10 (100.0)[c]	4/52 (7.7)
Barka (9)	January 2001	0/20 (0.0)	16/20 (80.0)	
Barka (9)	January 2001	0/10 (0.0)	8/10 (80.0)[c]	
Masanah (7)	March 2002	0/78 (0.0)	1/78 (1.3)	
Rustaq (8)	January 2004			2/3 (66.7)[d]
Khabora (5)	February 2002	0/100 (0.0)	43/100 (43.0)	
Saham (4)	April 2001			5/38 (13.2)
Sohar (3)	March 2002	0/100 (0.0)	23/100(23.0)	
Sohar (3)	November 2003			23/40 (57.5)[d]
Liwa (2)	January 2004			13/24 (54.2)[d]
Liwa (2)	January 2004			1/4 (25.0)[d]
Liwa (2)	May 2004			2/10 (20.0)[d]
Quriyat (12)	March 2004			5/6 (83.3)[d]
Seeb (10)	March 2002	1/76 (1.3)	3/76 (3.9)	0/26 (0.0)

[a]Numbers in brackets refer to locations in Figure 1.
[b]Numbers in parentheses represent percent recovery.
[c]Isolated from larval stage.
[d]Indicates use of streptomycin pretreatment of carrot slices.

was first reported in the Barka area in the south of the Al Batinah region in 1998. The disease spread northwards and was subsequently reported in Masanah, Suwaiq, Rustaq, Khabora, Saham, Sohar, Liwa and Shinas (Al Adawi, 2002; Al Adawi et al., 2003). Results of this study show that three fungi, *C. fimbriata*, *C. omanensis* and *L. theobromae* are closely associated with sudden decline disease of mango in Oman. This conclusion is based on the consistent isolation of the three species from stems of affected trees, the ability of these fungi to cause lesions in inoculated seedlings, and their recovery from the diseased tissue of inoculated plants. The results show a close association of the bark beetle, *H. mangiferae*, with the disease and its ability to transmit these fungi. Although *C. fimbriata* was not isolated from beetles collected in the logs placed in infected orchards, this was primarily because the optimized protocol for isolating *Ceratocystis* spp. had not been developed at the time.

The roles of the three fungi associated with mango sudden decline is uncertain. All three fungi were able to cause lesions on inoculated seedlings and they might all contribute to symptom development. However, since *C. fimbriata* was the most pathogenic fungus in inoculation tests, it may be the most important component in disease development. This fungus has caused a similar devastating disease of mango in Brazil known as *Seca*, since the late 1930s (Ribiero, 1980). Symptoms of that disease are similar to symptoms in Oman, including wilting, vascular discolouration, gummosis, blighting and tree death (Ploetz and Prakash, 1997). This supports the hypothesis that *C. fimbriata* is the primary factor associated with sudden decline of mango in Oman. The fungus is a well known vascular wilt pathogen of many tree species (Kile, 1993). On mango, before the present study, *C. fimbriata* had only been recorded in Brazil.

The most extensive lesion development in this study occurred following inoculation with *C. fimbriata*. Thus, *L. theobromae* may act as a secondary pathogen, colonizing lesions produced by *C. fimbriata*. The low frequency of isolation of *C. fimbriata* when specialized techniques are not used, and the relative ease with which *L. theobromae* is isolated, could have led researchers to conclude that *L. theobromae* was the causal agent of the disease in initial studies on mango sudden decline.

Lasiodiplodia theobromae is nonetheless closely associated with sudden decline of mango in Oman. This fungus was consistently isolated from symp-

tomatic tissue on dying trees and it also gave rise to distinctive lesions in inoculation tests. *Lasiodiplodia theobromae* is a well known opportunistic tree pathogen in the tropics and sub-tropics (Punithalingam, 1980). The fungus is the asexual state of *Botryosphaeria rhodina* (Sutton, 1980) and like many other species of *Botryosphaeria*, exists as an endophyte in asymptomatic tree tissue (Johnson, 1992, 1994). It is a well documented pathogen of mango (Johnson, 1992; 1994; Ploetz and Prakash, 1997; Ploetz, 2003). *Lasiodiplodia theobromae* has been present in Oman for many years, and has been recorded on mango causing dieback disease (Moghal et al., 1993). *Lasiodiplodia theobromae* may contribute to tree death in Oman after insect damage and infection by *C. fimbriata.*

The relationship between *C. fimbriata* and *L. theobromae* in mango decline aetiology needs further investigation. In Brazil, symptoms associated with *C. fimbriata*, causing *Seca* disease are identical to those associated with *Diplodia recifensis* Batista (morphologically similar to *L. theobromae*) causing Recife sickness; both diseases are found in the same area (Ploetz and Prakash, 1997). It is possible that Recife sickness and *Seca* may be the same disease, but the literature on these diseases is confused and difficult to interpret (R.C. Ploetz, personal communication).

This study provides clear evidence for the role of the bark beetle *H. mangiferae* in sudden decline of mango in Oman. Scolytid beetles are also thought to play key roles in the development of Recife sickness and *Seca* diseases of mango in Brazil (Ribiero, 1980). In Brazil, *H. mangiferae* is reported as the primary species responsible for disseminating *C. fimbriata* (Ribiero, 1980). *Ceratocystis* spp. are typically vectored by bark beetles and other insects (Graham, 1967). More specifically, *C. fimbriata* produces a fruity aroma that is attractive to insects and sticky spores, produced at the apices of long and exposed perithecial necks. The pathogen is ideally suited to transport by Scolytid beetles (Hansen, 1993; Christen et al., 1997). The tunnelling of these beetles into the stems of mango trees provides rapid access to host tissue.

The random distribution and rapid progress of mango sudden decline disease across northern Oman suggests the involvement of an insect vector. In Oman, the disease was first observed in Barka in 1998. By 2001 it had spread to Shinas (Table 1), approximately 200 km distant; an apparent spread rate of 60 km per year.

The involvement of *C. omanensis* in mango sudden decline requires further investigation. The results of this study suggest that it is a weaker pathogen than *C. fimbriata*, but is nonetheless capable of causing lesions. The pathogenicity of a larger number of isolates of all three fungi associated with sudden decline, an analysis of differences in susceptibility between local and exotic varieties and investigations into the role of the bark beetle in disease development are being considered. Furthermore, isolation of *C. fimbriata* from mango for the first time outside South America suggests a serious lapse in local quarantine procedures that requires investigation.

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