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CERATOCYSTIS WILT OF MANGO IN OMAN

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Abstract

Ceratocystis wilt of mango is arguably the most serious current threat to fruit production in Oman and the region. As proved by inoculation tests, the disease is caused by *Ceratocystis fimbriata sensu late* and was introduced into Oman in the late 1990s. Prior to the appearance in Oman the disease had only previously been reported from Brazil where mango production had been severely affected in the 20th century. The disease has devastated production in the Sultanate, killing more than 50% of trees in some regions of the country. During a four year period the disease has spread out from a focus close to Muscat to affect the whole of northern Oman. The rapid spread has been facilitated by a bark beetle (*Hypocryphalus mangiferae*) which acts as a wounding agent and vector for *C. fimbriata*. A second species, *Ceratocystis omanensis* is also involved in disease aetiology but is thought to be a relatively weak pathogen. Diseased trees show wilting of leaves, gummosis from stem and trunk lesions, vascular discoloration and eventually, tree death, usually within 6 months of symptom appearance. Local rootstocks, derived from seedling selections are especially susceptible and infection can lead to accelerated plant death. Scion varieties vary in susceptibility. Current research in Oman is examining sources of resistance to the disease with a view to introducing rootstocks derived from polyembryonic varieties.

Key words: Mango, *Ceratocystis fimbriata*, disease aetiology

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. 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. Subsequently, the disease 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. In 2000, during preliminary studies on the disease, *Lasiodiplodia theobromae* (Al Adawi et al., 2003), *Ceratocystis fimbriata* (van Wyk et al., 2005) and *C. omanensis* (Al-Subhi et al., 2006) were isolated from infected trees. In addition, the bark beetle *Hypocryphalus mangiferae* (Coleoptera: Scolytidae) appeared to be consistently associated with the disease, possibly acting as a wounding agent and as a vector of spores. 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 the disease, and to consider the role of the bark beetle in disease development.

MATERIALS AND METHOD

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. Detailed examinations were made of trees at all stages of disease development in each of the areas visited. Samples were collected from several trees at each farm surveyed. Most isolation was made from stem tissue. 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 placed between carrot discs or transferred to Petri dishes containing malt extract agar. Plates were incubated at room temperature and after 24 h subcultured onto fresh MEA plates.

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. Pathogenicity tests were conducted on stems of young mango plants (24 months old) growing in 13 cm diam pots. 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. 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. 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.

Over 700 *H. mangiferae* bark beetles, adults and larvae, were caught using aspirator traps from different locations. 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. 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. *C. fimbriata* was identified based on culture morphology, distinctive perithecia and culture aroma.

RESULTS

The distribution of the disease indicated highest disease levels in the east of the region, decreasing closer towards the United Arab Emirates. Initial disease symptoms were gummosis from the bark and branch death on affected trees; these affected trees usually displayed vascular discolouration beneath the gummosis. 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*. 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. 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.

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 were recovered from plant tissue. Less successful isolation were made from beetles. *Lasiodiplodia theobromae* represented 46.6% of the isolates, while *C. fimbriata* represented 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.

Mango plants inoculated with *C. fimbriata* developed gummosis and extensive lesions on treated seedlings. Wilting progressed 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.

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.

DISCUSSION

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.

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. This supports the hypothesis that *C. fimbriata* is the primary factor associated with sudden decline of mango in Oman. *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. *L. theobromae* has been present in Oman for many years, and has been recorded on mango causing dieback disease. The relationship between *C. fimbriata* and *L. theobromae* in mango decline aetiology needs further investigation.

This study provides clear evidence for the role of the bark beetle *H. mangiferae* in sudden decline of mango in Oman. In Brazil, *H. mangiferae* is reported as the primary species responsible for disseminating *C. fimbriata* (Ribiero, 1980). *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 tunneling 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 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.

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