INDEXING MANGO MOTHER TREES FOR THE PRESENCE OF *XANTHOMONAS CAMPESTRIS* PV *MANGIFERAEINDICAE* USING MONOCLONAL ANTIBODIES

ABSTRACT

Propagative material cut from symptomless mango *(Mangifera indica* L.) mother trees with latent infections of *Xanthomonas campestris* pv *mangiferaeindicae* is the main mechanism for bacterial blackspot dispersal. Therefore, it is important to test these mother trees for the industry to prevent further spread of the pathogen. Monoclonal antibodies raised against *X. campestris* pv *mangiferaeindicae* can now be used successfully to screen propagative material using the Enzyme-Linked Immunosorbent Assay (ELISA). Due to the latent nature of the infection, a selective enrichment step was developed to increase pathogen cell numbers present on the plant surface. Currently, mango mother trees can be screened commercially in South Africa for the presence of latent infections. This ensures prevention of further spread of the disease or its establishment in new plantings.

G Sanders and L Korsten
Department of Microbiology and Plant Pathology
Faculty of Agriculture Science
University of Pretoria
Pretoria 0002

Mangoes (*Mangifera indica* L.) are one of the most important subtropical fruits cultivated in South Africa, with the total value of production exceeding R5,7 million a year (Anon, 1983). Cultivars currently recommended in terms of external colour, percentage fibre in the flesh and disease resistance are Peach, Haden, Irwin, Zill, Fascell and Sensation (Anon, 1983).

Bacterial blackspot is the most important disease of mango in South Africa. This disease affects all cultivars with varying degrees of intensity (Viljoen & Kotzé, 1972) and has been observed in the major production areas of Letaba, Hoedspruit and districts of Barberton and Zoutpansberg (Kotzé, Viljoen & Steyn, 1976). Fibreless cultivars such as Kent and Haden are particularly susceptible and fruit losses of up to 50% and more have been recorded (Viljoen & Kotzé, 1972).

The etiological agent of mango blackspot disease was first described as *Bacillus, mangiferae* Doidge (Doidge, 1915), but was later changed to *Erwinia mangiferae* Doidge (Breed, Murray & Smith, 1957). Viljoen & Kotzé (1972) were unable to produce symptoms using *E. mangiferae,* while an organism isolated by them, identified as a *Pseudomonas* species caused typical disease symptoms. This isolate was positively identified as *P. mangiferaeindicae* and was implicated in the disease syndrome (Steyn, Viljoen & Kotzé, 1974). *P. mangiferaeindicae* was subsequently reclassified as *Xanthomonas mangiferaeindicae* (Robbs, Ribeiro & Kimura, 1974). Following the grouping of all phytopathogenic Xanthomonads as pathovars of *X. campestris,* the causal agent was finally designated *X. campestris* pv *mangiferaeindicae* (Dye, Bradbury, Hayward, Lelliot & Schroth, 1980).

Bacterial blackspot is a pre-harvest disease and new spots seldom appear on the fruit after picking (Viljoen & Kotzé, 1972). Symptom expression occurs on all above ground plant parts in various ways. The first signs of infection on the leaves are tiny water-soaked spots which form irregular black lesions surrounded by yellow haloes. However, once manifested, leaf and twig symptoms provide inoculum for fruit during the development stage. On twigs and petioles dark, longitudinal lesions, which later crack open are characteristic of blackspot infection. Recently, signs of infection have also been observed on flowers of heavily infested trees (J Lonsdale, pers. comm.). Fruit symptoms range from small, water soaked spots to larger star shaped cracks (Viljoen & Kotzé, 1972).

Warm and humid conditions with intermittent spells of rain was found to be conducive for disease development (Viljoen & Kotzé, 1972). Symptom development is also optimal under these conditions provided the relative humidity and temperature remain high enough. Under conditions unfavourable for disease development, the bacteria can survive on the plant as epiphytic populations (Leben, 1965; Hayward, 1974), thus providing inoculum in the absence of disease (Hirano & Upper, 1983). Propagation of these symptomless plants is the main means of disease dispersal. Propagative material obtained from these symptomless mother trees used for establishing new orchards would become symptomatic under favourable conditions. It is thus of paramount importance that all selected mother trees used in the mango industry should be screened for the presence of the pathogen.

Standard isolation techniques using non-selective medium, followed by identification using physiological and biochemical tests and final proof of pathogenicity with Koch's postulates have been used up to now to positively identify the pathogen. However, these tests are time consuming, expensive and not practical for large scale commercial screening. Bacterial infections on plant parts such as seeds (Claflin & Ramundo, 1987), and other plant tissue (Nomé, Raju, Goheen, Nyland & Docampo, 1980) are currently screened using rapid, accurate, inexpensive techniques such as selective media (Mulrean & Schroth, 1981; Schaad & Forster, 1985) or serological, techniques such as dot-immunobinding assay (DIA) (Claflin & Ramundo, 1987) immunofluorescence (IF) (Schaad, 1978) and ELISA (Civerolo & Fan, 1982). These techniques vary in accuracy, simplicity, degree of variability and cost. However, the ELISA has been used the most often on a commercial scale with great success (Dosba, Lansac, Pêcheur, Teyssier, Piquemal & Michel, 1986; De Boer, Wieczorek & Kummer, 1988).

Recently, a new approach has been developed for detecting mango bacterial blackspot infections (Sanders, Korsten & Kotzé, 1989) using selective media and serology. Due to the low cell concentrations associated with latent infections (Hayward, 1974), two new approaches have been followed. Firstly, the development of a selective medium specific for *X. c.* pv *mangiferaeindicae,* increasing cell numbers to a detectable level, since the ELISA requires a minimum number of bacterial cells (10^4 cells/mℓ) for a positive reaction (Smith, 1988). Secondly, using monoclonal antibodies being highly specific for the pathogen. Monoclonal antibodies are superior to conventional polyclonal antibodies in terms of specificity and stability. The concept of monoclonal antiserum was revolutionised by Köhler & Milstein (1975) who reported the successful fusion of mouse spleen cells with a murine myeloma cell line. These

hybrids or hybridomas could be cloned and cultured indefinitely, secreting homogenous or monoclonal antibodies identical in terms of specificity and isotype.

However, using the ELISA system on a commercial scale requires optimisation of the system in order to obtain maximum sensitivity. Several parameters should be taken into account when optimising the system, such as antigen fixation, duration of fixation, fixation temperature, blocking agents, buffer systems, antibody and conjugate concentrations and enzyme-substrate reaction time. Each parameter has been optimised using *X. c.* pv *mangiferaeindicae* as solid phase antigen (unpublished data). The sampling method is the most difficult to standardise and optimise due to the uncertainty as to the exact location of the organism on the plant. Furthermore, it is not known whether following infection, translocation of the organism within the plant is systemic. Systemic infections have been shown to occur with closely related organisms such as *Pseudomonas syringae* pv *syringae* on plum trees (Roos & Hattingh, 1987). Thus far, 7,8% of selected material collected after visual inspection for symptomless trees have tested positive (unpublished data). Thus although plant material appears healthy, this is not always the case.

In conclusion, the most difficult section of the indexing programme to control and optimise is the sampling method. It is not possible to test every twig, and for this reason, the pathogen may be missed. Therefore, results cannot be regarded as 100% accurate. Trees testing positive can be regarded as infected and should be removed from the mother block. However, trees testing negative should be retested at a later stage and monitored for symptom development, especially when conditions are favourable for symptom expression. In so doing, trees can be certified as pathogen-free thus preventing indiscriminate spread of the disease, ensuring establishment of healthy orchards.

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