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The Symptoms and Cause of Guava Wilt in South Africa

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With 3 figures

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Abstract

Wilt of guava (*Psidium guajava*), a serious disease occurring in most guava producing areas of the Northern and Mpumalanga provinces of South Africa, was shown to be induced by a fungus tentatively identified as *Penicillium vermoesenii*. This fungus is the cause of a blight of ornamental palms in the United States and Belgium and its occurrence on guava is enigmatic. Pathogenicity tests were conducted in the glasshouse and under field conditions. Symptoms on trees include wilting, chlorosis and defoliation.

Zusammenfassung

Symptome und Ursache der Guaven-Welke in Südafrika

Es wurde gezeigt, daß die Welke der Guave (*Psidium guajava*)-eine schwere, in den meisten südafrikanischen Guavenanbaugebieten der Northern Province und der Mpumalanga Province auftretende Krankheit-durch einen Pilz hervorgerufen wird, der vorläufig als *Penicillium vermoesenii* bestimmt wurde. Dieser Pilz ruft bei Zier-Palmen in den USA und Belgien eine Fäule hervor, sein Vorkommen auf der Gauve gibt Rätsel auf. Im Gewächshaus und im Freiland wurden Pathogenitätstests durchgeführt. Zu den Befallssymptomen der Bäume gehören Welke, Chlorose und Blattverlust.

Introduction

Guava (*Psidium guajava* L.), a tree native to South and Central America, produces fruit of commercial importance in many tropical and sub-tropical areas of the world (Shigeura and Bullock, 1983). The guava industry in South Africa, was established in the Western Cape Province in the early 1900s and in the Northern and Mpumalanga provinces during 1938 (Bolt, 1984); it is now one of the world's three largest producers with production of c. 40 000 tonnes per annum (Anonymous, 1990). In South Africa the cultivar Fan Relief is most commonly grown (Fig. 1a); (Grech, 1987a).

A serious wilting disease of guava trees known as Guava Wilt Disease (GWD), was first reported and described from the Malelane area (Mpumalanga) in 1981 (Grech, 1985). By 1985, it was present in 85% of the guava producing areas of the Transvaal province and affected 12% of these areas (Grech, 1985). GWD continued to spread and now also occurs in the Northern Transvaal province (Grech, 1990). Its occurrence has halved the area of guavas grown in the Southern Lowveld area of Mpumalanga during the last 10 years. Quarantine measures in force since 1985 have undoubtedly prevented the disease from spreading to the Western Cape Province where guavas are also an important crop (Grech, 1987b).

There has been considerable confusion concerning the identity of the cause of GWD in South Africa. A similar disease, known since 1923 in Taiwan, is reported to be caused by *Myxosporium psidii* Sawada & Kurosawa (Leu et al., 1979). Until recently, it was suspected that this pathogen had been accidentally introduced into South Africa from Taiwan (Grech, 1988). More recent studies have suggested that the causal agent might belong in the genus *Acremonium* Link and perhaps be related to the persimmon wilt pathogen *Acremonium diospyri* (Crandall and Baker, 1950; Benade et al., 1991). A wilting disease of guava has also been reported from India, and has been reported to be caused by *Fusarium oxysporum* Schl. Emend. Snyder and Hanson *f.* sp. *psidii* Prasad, Mehta & Lal. (Pandey and Dwivedi, 1985) or *Macrophomina phaseolina* (Tassi) Goid. (Dwivedi, 1990).

The aim of this study was to describe GWD and the pathogenicity and taxonomy of the fungus consistently associated with the disease.

Materials and Methods

Isolation and identification of the pathogen

Ten diseased trees were collected randomly from three different orchards located in the Mpumalanga province. Thirty small pieces (5 mm^2) were cut randomly from the xylem of cross-sectional disks from each tree. Xylem

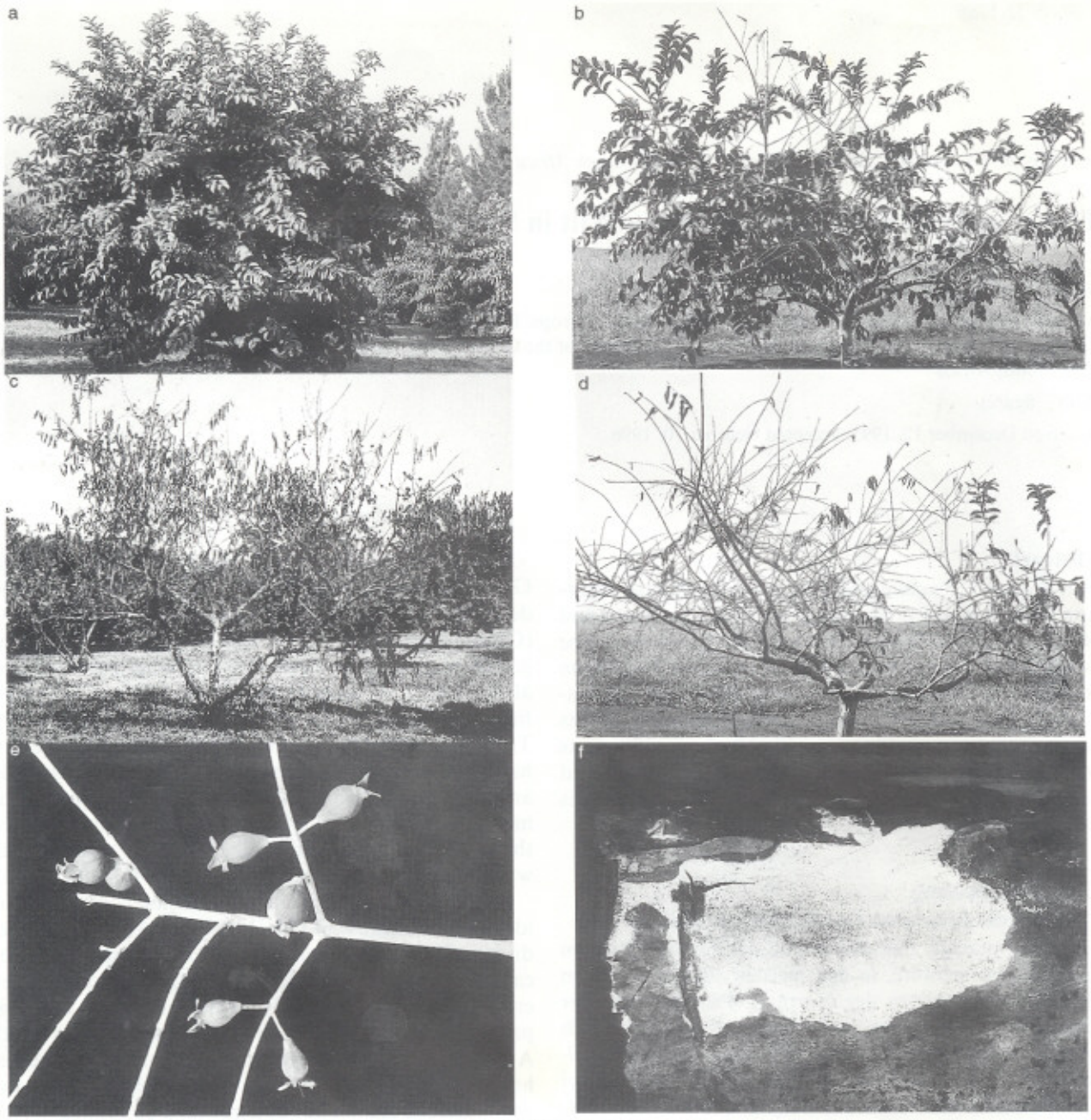

Fig. 1 A healthy guava tree and symptoms of wilt disease. (a) healthy; (b) wilting in the upper branches; (c) fire-scorched appearance; (d) defoliation almost complete, tree moribund; (e) mummified fruit on an infected tree; (f) masses of spores produced on infected tree

pieces were surface disinfected in 0.35% commercial household bleach (NaOCl) for 3 min and then rinsed in sterile distilled water. Ten xylem pieces were then randomly selected from the sets of 30 and plated on malt extract agar (MEA) in Petri dishes with 10 pieces per dish. Dishes were incubated at 28°C for 3–5 days and resulting isolates transferred to MEA. Pure cultures of the fungus most commonly isolated were examined and characterized using bright field microscopy. Two GWD isolates (PREM 51879 and PREM 51880; National Collection of Fungi, Pretoria, RSA) were used in characterisation of the fungus as well as in pathogenicity tests.

Pathogenicity tests

Pathogenicity studies were conducted both in the glasshouse and in the field. In glasshouse tests, wounds were made in the stems of c. 8-month-old cv. Fan Retief guava trees, with a 4 mm diameter cork borer. Inocula of the two isolates (PREM 51879 and PREM 51880) were grown on potato dextrose agar (PDA) at 25°C for 2 weeks. Plugs of agar bearing actively-growing mycelium were placed in the wounds on each of 20 trees for each isolate. The same number of trees inoculated with sterile PDA discs served as controls. Lesion lengths were measured after 37 days and compared according to Fischer's procedure for comparison of means.

For pathogenicity tests in the field, only one isolate (PREM 51879) grown for 10 days on MEA at 28°C was used. Structural roots of c. 90 mm diameter from nine trees (17 years old) were respectively used for inoculations. Roots were wounded by making a 30° angle cut with a machete, into the xylem, c. 10 mm deep. The inoculum (50.29 $cm^2 \times 5$ mm thick agar disc) was then placed onto the exposed xylem. The flap of the wound was pressed back in position and the exposed root covered with soil. Control trees were treated similarly but inoculated with sterile MEA. After 7 weeks the trees were observed for symptom development. The inoculated fungus was re-isolated from diseased tissue on all roots at the conclusion of the study.

Results

Disease symptoms

Symptoms of GWD include an abrupt wilting of the foliage that begins in the upper branches (Fig. 1b) and spreads to the whole tree; leaves become chlorotic and eventually most absciss. When decline is rapid, leaves tend to shrivel and die on the trees, which assume a fire-scorched appearance (Fig. 1c). When decline occurs more slowly, leaves drop gradually, resulting in complete defoliation (Fig. 1d). The development of fruit present on affected trees ceases, and the fruit eventually becomes mummified (Fig. 1e). In advanced stages of GWD, small red blisters develop on the branches or trunk and these contain masses of white to salmon pink spores (Fig. 1f).

Pathogen isolation and identification

One fungus was consistently isolated from all diseased trees sampled and this occurred in 93% of the total of 90 disks investigated (total disks from 10 trees). This fungus has tentatively been identified as being very similar, if not identical, to *Penicillium vermoesenii* (Biourge) Thom. by Dr Walter Gams, Centraalbureau voor Schimmelcultures, Baarn, The Netherlands. Typical isolates have also been assigned the collection numbers CBS 590.96 and CBS 591.96. Other fungi, such as *Fusarium* spp. and *Pestalotiopsis* spp. were occasionally present but were ignored due to their inconsistent occurrence.

The fungus grows rapidly in culture (MEA), forming radially streaked white to orange-pink mycelium which develops as fluffy aerial mycelium. Two types of conidiophores are present. The first type are simple solitary conidiophores with phialidic conidiogenous cells that give rise to large ellipsoidal to cylindrical conidia (6.1–6.6×1.5–1.9 μm) (Fig. 3a, b), carried in slimy masses. These conidia are formed abundantly in young cultures. The second type of conidiophores are penicillately branched (2.1–2.2×1.8–2.1) μm) (Fig. 3c) having lageniform conidiogenous cells with ovate conidia (Fig. 3c, d). These small, dry, ovate conidia are usually more abundant in older cultures.

Pathogenicity tests

Glasshouse-grown guava plants were highly susceptible to the GWD isolates which caused dark brown discoloration of the cambium and associated xylem tissue (Fig. 2a). The average lesion length of 20 guava trees for the one isolate (PREM 51879) was slightly greater (84.9 mm) than that for the second isolate (PREM 51880) (70.3 mm). Average lesion lengths for both isolates, differed significantly ($P \leqslant 0.05$) from the controls (4.9 mm) according to Fischer's procedure for comparison of means.

Symptoms on inoculated established trees were first observed 7 weeks after inoculation and all trees died 2 weeks later. Wilting first occurred above the point of inoculation (Fig. 2b). Symptoms were similar to, and as severe as, those observed on naturally-infected trees. Blisters containing masses of white to salmon-pink conidia were subsequently observed on the trunks of dead trees and the GWD pathogen was re-isolated from all the dead trees. No symptoms developed on control trees.

Discussion

Isolations from diseased guava trees and pathogenicity tests in this study showed that the fungus identified as *P.*

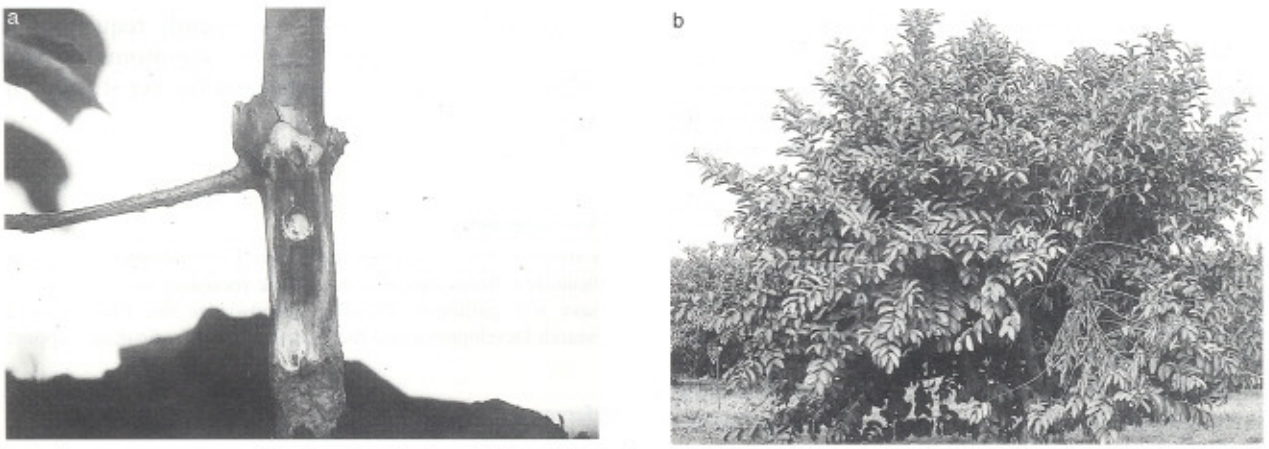

Fig. 2 Symptoms on guava following inoculation with the GWD isolate (a) dark brown disocolouration of the cambium and nearby xylem tissue on shoots inoculated in the glasshouse; (b) wilting, above the point of inoculation in established trees in the field

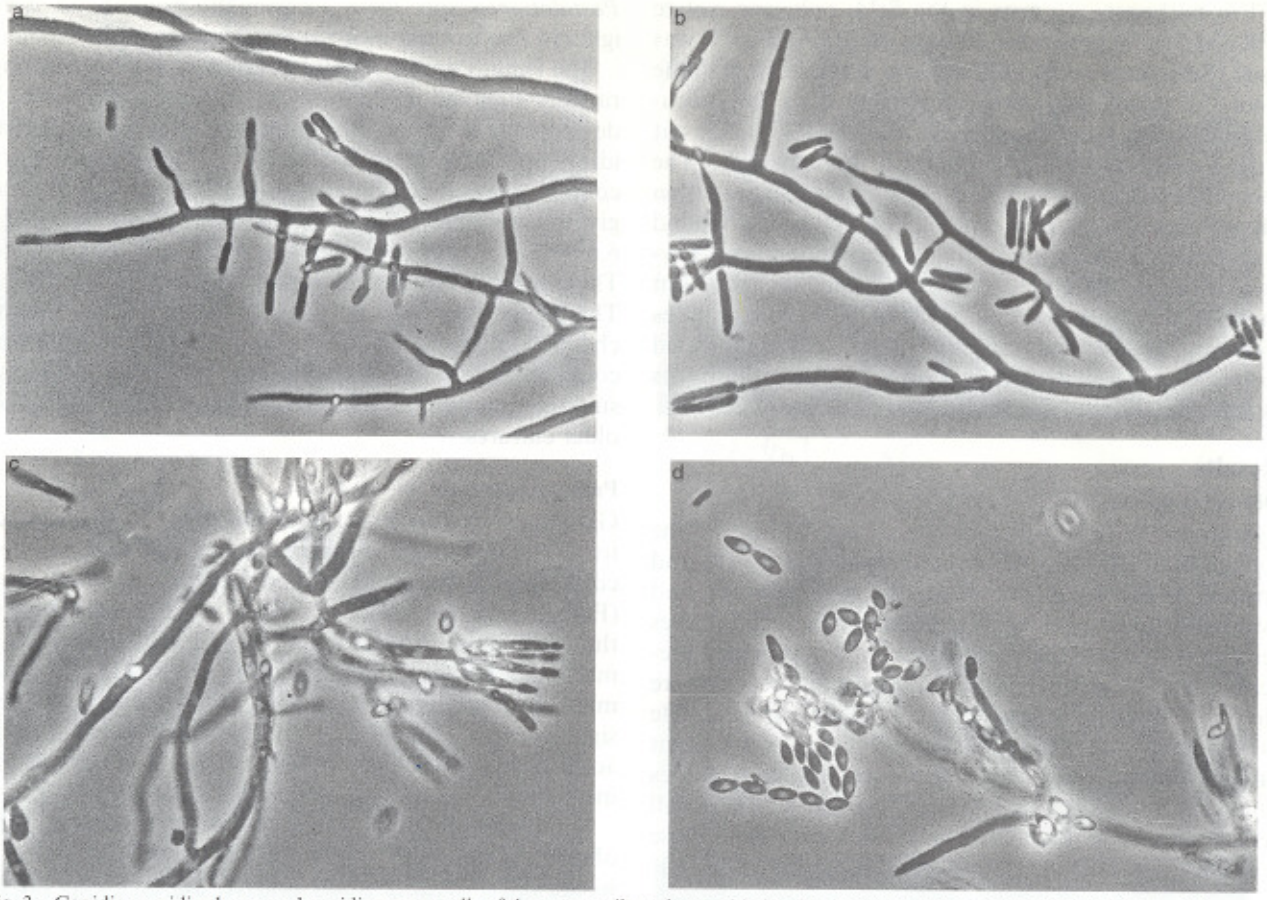

Fig. 3 Conidia, conidiophores and conidiogenous cells of the guava wilt pathogen (a) simple solitary conidiophores with phialidic conidiogenous cells producing large ellipsoidal conidia; (b) ellipsoidal conidia carried in a slimy mass; (c) pennicilately branched conidiophores with ovoid conidia; (d) ovoid conidia arranged in false chains

vermoesenii is the causal agent of GWD in South Africa. Although GWD has occurred in South Africa for more than a decade, this is the first attempt to describe the disease and identify the pathogen.

P. vermoesenii is the causal agent of a blight of ornamental palms in the United States and Belgium. The disease was first reported in 1932 on Areca palm (*Chrysalidocarpus lutescens* H. Wendl.) in Belgium and later in 1964 also on *Chamaedorea seifrizii* Burret. in Florida (Reynolds, 1964). Since then, many genera and species of palms have been listed as susceptible to the disease (Keim and Maire, 1975; Chase, 1987). However, this is the first record of a fungus resembling *P. vermoesenii* outside the United States and Belgium and on a host other than palms. In this sense, it is enigmatic and deserves further taxonomic consideration.

P. vermoesenii has a taxonomically confused status. The fungus has been known as *Gliocladium vermoesenii* (Biourge) Thom. but is most likely neither a species of *Gliocladium* or of *Penicillium* (W. Gams, pers. comm.). The isolates of the guava wilt pathogen are slightly different in colour to those typical of *G. vermoesenii* and it is possible that they represent a different, but closely related species. Taxonomic studies at the molecular level are planned to resolve this question.

We have been unable to obtain isolates of the fungus associated with wilt of guava in Taiwan and comparisons have, therefore, been impossible. However, the fungus from Taiwan appears to be a Coelomycete with chains of conidia unlike those of *P. vermoesenii* (Tzean, pers. comm.), and is thus different from that causing wilt of guava in South Africa. The cause of guava wilt in India (Pandey and Dwivedi, 1985; Dwivedi, 1990) has apparently not been resolved. Whether it is similar to that in Taiwan or South Africa is unknown.

Strategies to manage GWD and to reduce its impact on the local guava industry are urgently required. This preliminary report, of the cause and symptoms of GWD, will hopefully stimulate interest and further study of the problem.

Acknowledgements

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References

Anonymous (1990): Research Liaison Committee on guava production. CSFRI, Nelspruit, Department of Agriculture.

Benade, E., G. H. J. Kemp, M. J. Wingfield, J. F. L. Kock (1991): Comparison of *Acremonium diospyri* with the guava wilt pathogen in South Africa. Phytophylactica **23,** 98.

Bolt, L. C. (1984): Geskiedenis van die Fan Retief koejawel in SA. CSFRI Info. Bull. **137,** 1–2.

Chase, A. R. (1987): Compendium of Ornamental Foliage Plant Diseases. APS Press, St. Paul, Minnesota.

Crandall, B. S., W. L. Baker (1950): The wilt disease of American persimmon, caused by *Cephalosporium diospyri*. Phytopathology **40,** 307–325.

Dwivedi, S. K. (1990): Guava wilt incited by *Macrophomina phaseolina*. National Acad. Sci. Lett. **13,** 301–303.

Grech, N. M. (1985): First report of guava rapid death syndrome caused by *Septofusidium* sp. In South Africa. Plant Dis. **69,** 726.

Grech, N. M. (1987a): Guava wilting disease: search for resistance. CSFRI Info. Bull. **178,** 1–2.

Grech, N. M. (1987b): Guava wilting disease: The Cape scenario. CSFRI Info. Bull. **179,** 1–2.

Grech, N. M. (1988): Aspects of guava cultivation in Taiwan. Citrus Subtrop. Fruit J. **642,** 9–13.

Grech, N. M. (1990): Guava wilting disease in Levubu. CSFRI Info. Bull. **218,** 8.

Keim, R., R. G. Maire (1985): Gliocladium disease of palm. California Plant Pathol. **27,** 1–2.

Leu, L. S., C. W. Kao, C. C. Wang, W. J. Liang, S. P. Y. Hsieh (1979): *Myxosporium* wilt of guava and its control. Pl. Disease Reptr. **63,** 1075–1080.

Pandey, R. R., R. S. Dwivedi (1985): *Fusarium oxysporum f.* sp. *psidii* as a pathogen causing wilt of guava in Varanasi District, India. Phytopath. Z. **114,** 243–248.

Reynolds, J. E. (1964): Gliocladium disease of palm in Dade County, Florida. Pl. Disease Reptr. **48,** 718–720.

Shigeura, G. T., R. M. Bullock (1983): Guava (*Psidium guajava* L.) in Hawaii history and production. Research Extension Series 35. Hawaii Institute of Tropical Agriculture and Human Resources, Univ. of Hawaii, Hawaii.