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Fusarium wilt of banana in Australia

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Summary

Approaches to manage outbreaks of fusarium wilt of banana in Australia and current research towards long term disease management strategies are summarised in this paper.

Keywords: Fusarium wilt, banana, Australia.

Introduction

The first recorded description of the fungal disease of banana known as fusarium wilt or 'Panama Disease' was made in 1876 by Dr Joseph Bancroft from the 'Sugar' banana variety (Silk/Pisang Rastali, AAB) in Brisbane, Queensland, Australia (Bancroft 1876). Since this time, production of the popular banana varieties in Australia (Cavendish, Lady Finger, Ducasse and Sugar) has been constrained, at times severely, by various strains of *Fusarium* wilt. The Australian banana industry is primarily based on the Cavendish (AAA) group with smaller yet loyal domestic markets for the Lady Finger variety (Pacha Nadan, AAB from the Pome sub-group). Figures released in 1998 for the Australian banana industry showed 2400 growers with a total of 13 200 hectares under production in the states of Queensland, New South Wales and Western Australia and the Northern Territory (ABGC 1998). Annual production was in the order of 230 000 tonnes of fruit (17.6 million 15 kg cartons) with a gross value of AU\$3 200 million (ADGC 1998).

Early research on fusarium wilt of banana in Australia was largely carried out by the Queensland Department of Primary Industries (QDPI) and more recently also through the New South Wales Department of Agriculture, Queensland University of Technology, The University of Queensland, The University of Western Australia and other State and Territory government departments. Australian researchers have worked closely with banana growers and extension staff to focus research on industry priorities and ensure adoption of research outcomes. Over the past decade the human and funding resources dedicated to better understanding and managing this disease have been considerable. Currently in Australia, there are around fifteen researchers (including plant pathologists, molecular biologists, horticulturists, tissue culturists, physiologists, agronomists, and biotechnologists) assisted by some sixteen PhD students, post-doctoral and technical staff involved in various aspects of research on fusarium wilt of banana. This paper summarises some

of the current research and approaches to management of Fusarium wilt of banana in Australia.

Pathogen diversity and distribution

Techniques used to characterise other fungi (Cove 1976, Correll *et al.* 1987) have been adapted and used to divide the strains of FOC into meaningful subgroups in order to identify the different strains of this pathogen found in Australia (Brake *et al.* 1990, More *et al.* 1990, More *et al.* 1993, Pegg *et al.* 1995, Pegg *et al.* 1996, Bentley *et al.* 1998). Development and application of molecular characterisation techniques in the past decade has enabled Australian populations of FOC to be studied in depth and compared with those elsewhere in the world (Bentley *et al.* 1998, Bentley *et al.* in these proceedings). The diversity and distribution of populations of FOC in all banana-producing regions of Australia is now well characterised with several hundred isolates analysed by Vegetative Compatibility Group (VCG) analysis, volatile production, DNA amplification fingerprint (DAF) techniques and field observations of pathogenicity.

The race 1 FOC pathotypes belonging to VCGs 0124 and 0125 are non-volatile producing and are widespread throughout eastern Australia. Strains in the very closely related non-volatile producing VCGs 0128 and 01220 are narrowly distributed at a small number of sites in northern Queensland and New South Wales (NSW) and at Carnarvon in Western Australia, respectively (Moore *et al.* 1993, Pegg 1995, Pegg 1996). The Cavendish-competent, volatile producing race 4 pathotypes belong to VCGs 0120, 0129 and 01211 and are at present restricted to the banana growing regions of southern Queensland and northern NSW. They have not yet been recorded in the major Cavendish production areas of northern Queensland or the emerging production areas of Carnarvon and Kununurra in Western Australia or near Darwin in the Northern Territory. The strains of FOC known as 'tropical' race 4 have recently (1997, 1998 and 1999) been confirmed at three sites near Darwin in the Northern Territory. Isolates of this Cavendish competent strain of FOC produce volatile metabolites and belong to the VCGs 01213 or 01216 (see paper by Condé and Pitkethley in these proceedings). These are the only recorded cases of this strain of FOC in Australia although it is widespread in the islands of Indonesia and peninsular Malaysia to Australia's north.

With the application of molecular methodologies, we have been able to confirm the stable nature of VCGs of FOC in Australia over time (see paper by Bentley *et al.* in these proceedings). From VCG and DNA fingerprint data from isolates from other countries, we have also been able to surmise that this pathogen did not originate in Australia but was rather introduced by Man and speculate on the likely origins and distribution of this pathogen. Also, the extensive DNA fingerprint library has been used to develop VCG specific primers in order to develop a DNA detection system for the common strains of FOC. In addition, strains of FOC have also been characterised by

restriction endonuclease analysis of the intergenic spacer (IGS) region of the ribosomal DNA (see paper by Bentley *et al.* in these proceedings).

Disease management strategies

Management of this disease in Australia is based around three key strategies: 1) early detection and containment of outbreaks of fusarium wilt and application of protocols to prevent introduction of FOC into disease-free production districts; 2) adoption of disease management strategies for farms with fusarium wilt to maintain production and; 3) conduct research to evaluate and develop long-term disease management strategies for the Australian banana industry.

1. Prevention, detection and containment

Local quarantine measures are in place in banana production areas of Australia to restrict the movement of banana planting material (particularly suckers and rhizome pieces). Australian banana growers require permits to move planting material to initiate new plantations and, if vegetative material is used (e.g. suckers or rhizome pieces) this must be from a certified source of 'clean' planting material. These protocols appear to have been successful in preventing the Cavendish competent race 4 strains of FOC (VCGs 0120 and 0129) from becoming established in the production districts of north Queensland where the majority of the Australian Cavendish industry is located. In Australia, banana growers and local department of agriculture officials are encouraged to send specimens from any plants suspected of Fusarium wilt to the DPI Indooroopilly laboratories for analysis. This enables new outbreaks to be mapped and the distribution of the different strains of FOC to be monitored, and any new or exotic strains of FOC to be detected (e.g. the recent outbreaks in Cavendish plantations in the Northern Territory that were caused by VCG 01213/16 see paper by Condé and Pitkethley in these proceedings).

2. On-farm disease management

Provided the strain of FOC involved in an outbreak of fusarium wilt is not of a quarantinable or exotic nature or the property concerned is not beyond a defined local quarantine zone, banana production is allowed to continue. Australian banana growers are advised to follow simple yet practical measures to minimise the spread of the disease and to extend the productive life of the plantation.

After removing possibilities for spreading the pathogen to other farms (e.g. no movement of planting material or infested soil and, if possible, containing or diverting contaminated drainage water), the primary concern is to minimise the spread of FOC within the affected farm. The common procedure in Queensland and New South Wales for isolated cases of fusarium wilt in an otherwise healthy block is to immediately inject the affected plants with

herbicide (e.g. Roundup®/glyphosate) and let them die *in situ* without further chopping or disturbance of the plants. Often a single or double ring of healthy plants around the affected ones may also be injected or otherwise treated with herbicide and allowed to die in place. This has the twofold effect of preventing mat to mat spread of the pathogen through living banana roots and also reducing the population of the fungus at that site by killing its preferred host plant. It is important that the affected plants and the soil around them be disturbed as little as possible in this process as any disturbance and movement of soil will increase the chances of moving the pathogen. Fencing or cordoning the area also signals to workers or visitors to keep out, reducing the risk of contaminated soil accidentally being moved on boots or machinery or in suckers. If appropriate, the area may also need to be fenced where animals are a problem (large animals may carry infested soil/mud throughout the plantation, particularly in wet weather).

If FOC has infected some plants at a site, it is very likely to also be present in some of the soil surrounding those plants. Any suckers that subsequently emerge from the herbicide-treated plants must also be treated in order to kill the mat completely. This requires follow-up inspections. If infected banana plants are allowed to persist, the pathogen population on that site will not decrease and will remain a source of inoculum for nearby banana plants. In the interests of least possible disturbance and movement of soil from the site, it is also useful to sow a persistent ground cover over the affected patch to control soil erosion after the banana plants die. Where practicable, other measures may include altering the location of roads, minimising access points to affected blocks, isolating the affected area from irrigation flows and changing the order in which the plantation blocks are worked so that infected patches are worked after disease free ones.

Such measures are simple but if they are carried out immediately and with a high degree of vigilance, growers have an excellent chance of minimising the pathogen's reproduction and movement within and to other plantations.

Banana growers must not be tempted to use planting material from known infected plantations, even if some rhizomes appear 'clean'. If FOC has been identified at a plantation, any movement of soil or plants from the affected block must be considered as an unacceptable risk.

When a plantation block has many wilted plants dispersed throughout, it is much harder to minimise the spread as the pathogen is obviously already well distributed throughout the patch. Early detection is a big advantage for long-term control. Even if disease resistant varieties are planted into a wilt-affected plantation, the soil and run-off water from that plantation will remain as a source of FOC for some time after, possibly as long as several decades.

If disease-free areas are available for banana production it is vitally important not to contaminate them through lack of basic farm hygiene. The key to

Keeping disease free areas free from FOC is to minimise, if not remove, all opportunities for infested soil, plant material or irrigation water (that may be carrying spores of the fungus attached to soil particles) from entering the area. Risk minimisation can be achieved in several ways:

- using only certified disease-free vegetative planting material or tissue-cultured plants to initiate new plantations. The location of the nursery and possible contamination from any nearby affected blocks must be carefully considered (i.e. possible contamination of the soil or water used for raising plants in the nursery).
- cleaning equipment, footwear and vehicles between plantations or blocks within a plantation to avoid moving infested soil or plant matter from an affected block to disease-free blocks.
- where practicable, isolating irrigation water that drains from diseased parts of a plantation. This should not be used to irrigate new or disease-free areas if possible. At this time no commercial treatments are known for controlling FOC in irrigation water or water storages.
- educating farm workers about how the fungus is spread and providing posters or other visual material to enable symptoms of fusarium wilt to be recognised to aid in early detection of new outbreaks.

'Farmcleanse' is a detergent-based degreaser with a quaternary ammonium additive produced by the Castrol company in Australia and it has been shown to have very high efficacy against spores of *F. oxysporum* f. sp. *vasinfectum* (Atk.) Snyd. & Hans. (FOV), the wilt pathogen of cotton (*Gossypium hirsutum* L.) (O'Neill 1999). This agent was shown to have much higher efficacy against spores of FOV in soil compared with other agents tested including a fungicide and a quaternary ammonium compound. No surviving spores were recovered in the tests using Farmcleanse compared with only a 40% reduction in viable spore numbers in treatments where the fungicide or quaternary ammonium solutions were used. This does not mean that *all* spores were killed by the Farmcleanse treatment but it was by far the most effective agent tested. The cotton industry in Australia has moved quickly to develop industry best practice protocols to ensure that contract machinery moving between farms and between districts is cleaned to remove as much attached soil and plant matter as possible (Moore & O'Neill 2000). Farmcleanse is then applied to vehicles (particularly tyres and inside wheel arches) as a foaming spray which is then allowed to soak into any soil that may still be attached and is then rinsed off. The fungus that causes Fusarium wilt of cotton (FOV) has a very similar life cycle and mode of spread to the form specialis *Fusarium oxysporum* that causes wilt in banana (FOC). Farmcleanse has subsequently been adopted for use in footbaths and for cleaning farm equipment in the banana and several vegetable industries in Australia.

Previously in Australia, chlorine bleach, methylated spirit or copper oxychloride solutions have been used in footbaths and for cleaning equipment and vehicles. However, these products have safety issues for workers and

the environment associated with their use and can cause corrosion of equipment and vehicles if used over long periods. Farmcleanse offers a far safer alternative and does not require registration for use in Australia. It is also biodegradable and does not cause corrosion. No agents should be seen as a substitute for the physical removal of contaminated soil or infected plant matter from boots, vehicles or equipment, but rather as giving optimum results or 'extra insurance' when used in conjunction with cleaning or washing procedures. Where Farmcleanse is not available, efficacy trials will need to be conducted with local equivalent agents to determine effective substitutes. Adopting more hygienic farm practices not only retards the spread of *Fusarium*, but other soil-borne diseases and weeds as well.

Anecdotal evidence from Australian plantations suggests that fertilizer regimes that are used to 'push' plants (e.g. excessive applications of nitrogen) also increase the incidence of fusarium wilt in affected plantations. In most plant diseases, disease expression exists in a shifting three-way balance between the host plant, the pathogen and the prevailing environmental and growing conditions. Modern agriculture practices often interfere with that balance and inadvertently hasten the spread and intensity of diseases such as fusarium wilt. If, however, the balance favours the natural vigour of the plant, its own defence mechanisms will keep the pathogen at as low a level as possible. Where growers wish to maintain production of susceptible varieties in the presence of FOC, they often reduce applications of nitrogenous fertilizers, but this in turn can lead to sacrifices in bunch size, fruit quality and financial returns.

As for many diseases, resistant or tolerant banana varieties offer the only long-term and environmentally sustainable solution for control of this disease. Where such varieties have not been available, plantation owners have managed the disease in susceptible varieties for many years through a combination of hygiene and disease management practices along with early detection and containment of new outbreaks. Conversely, if incorrect practices are followed (or no measures are taken at all), the disease will continue to spread around the farm and eventually to neighbouring farms.

3. Research towards long-term disease management strategies

3.1 Host plant resistance. Extensive field evaluations have been conducted in Australia to identify sources of resistance to 'race' 1 (VCG 0124/5) and 'race' 4 (VCG 0120) of FOC. Many varieties, breeding diploid lines and advanced hybrids (including tetraploid dessert bananas and triploid Silk/Pome dessert bananas) have been imported through the Australian Quarantine and Inspection Service (AQIS) and established at field sites in northern NSW and southern Queensland. The sites chosen for field evaluation were commercially productive plantations that developed high levels of fusarium wilt of different strains (VCG 0124/5 at Cudgen and Durambah and VCG 0120 at Wamuran).

Once they have cleared the post-entry quarantine process, tissue culture derived plants are raised in pasteurised potting mix in glasshouses until the 6-8 leaf stage (or approximately 30-50 cm in height) when they are taken to the field site for planting during the October to November period as per commercial practice. Plants are grown under normal commercial production regimes and inspected monthly for the development of external symptoms of fusarium wilt until at least bunching of the first ratoon is completed (fortnightly inspections are made during the warmer summer months of December to February). Trial designs and reference cultivars are available in the INIBAP guideline (Orjeda 1998).

Specimens of internal symptomatic vascular tissue are taken from affected plants for isolation and VCG analysis. This process confirms the disease but also enables monitoring to ensure that no other strains (VCGs) of the pathogen have been introduced to the site. Over many years of testing diverse *Musa* germplasm at the field sites, we have not recovered any strains of FOC from either of the sites other than those that were originally present. After harvest of the first ratoon bunches, surviving plants are levered out of the ground and cut open to inspect the pseudostem and rhizome tissue for evidence of vascular discoloration due to FOC. The INIBAP rating scheme for internal discoloration is used (Orjeda 1998). In collaboration with the NSW Department of Agriculture research station at Gosford, plant crop and first ratoon bunches from varieties growing in the FOC trial sites are sent for post harvest fruit quality evaluations.

Four tetraploid hybrids have shown good levels of resistance to FOC at both the race 1 and race 4 field evaluation sites in Australia. These are FHIA 01 (commercially marketed as Gold Finger in Australia), FHIA 18 and SH 3640-10 (known as 'High Noon' in South Africa) from the FHIA breeding programme in Honduras, and the 'Laknau' derived plantain variety TMBx 5295-1 which was bred by the IITA breeding programme in Nigeria. TMBx 5295-1 appeared to be more compromised by the cold winter conditions in sub-tropical production areas than these particular FHIA varieties. While SH 3640-10 showed few external symptoms consistent with FOC infection at the race 1 site and produced good bunches, when the plants were internally examined many were found to be infected with FOC with discoloured vascular tissues apparent in the rhizomes and pseudostems. In contrast very few plants of SH 3640-10 at the race 4 site showed internal symptoms after destructive sampling.

The tetraploid banana varieties FHIA 17 and SH 3641, also from the FHIA programme, showed resistance to race 1 of FOC but more than one third of the plants of both these varieties became diseased at the race 4 evaluation site. Conversely, the FHIA variety SH 3656 was susceptible to race 1 of FOC with more than half the plants tested developing symptoms within the first 12 months, but this variety showed good resistance to race 4 of FOC in field evaluations. The dessert varieties PA 03-22, PC 12-05 and PV 03-44 developed by the EMBRAPA-CNPMPF

breeding programme in Brazil showed good levels of resistance to race 1 of FOC in our trials.

The field evaluation of three triploid dessert varieties from the CIRAD-FLHOR breeding programme in Guadeloupe (IRFA 909, 910 and 914) could not be completed since these varieties all developed pronounced symptoms of banana streak virus (BSV) at both field sites during 1999. In line with Australian banana industry policies, all plants were destroyed and will not be able to be retested until BSV-free lines become available. This is most unfortunate since two of the varieties (IRFA 909 and IRFA 914) were showing little evidence of fusarium wilt symptoms at the time of their destruction. The IRFA 910 plants, however, developed disease symptoms at both evaluation sites.

Varieties that have completed evaluation but have shown little resistance to either races 1 or 4 of FOC in our trials include FHIA 02, FHIA 03 and Pisang Ceylan.

The cooking banana FHIA 25 which has proven to be highly resistant to black sigatoka (*Mycosphaerella fijiensis* Morelet) (P. Rowe, pers. comm.) is currently being evaluated in Australia against race 1 of FOC with Lady Finger, Gold Finger, FHIA 18 and SH 3640-10 for comparison. It will be assessed at the race 4 site in future trials.

Although the trial design for assessing FOC reaction is different to that for leaf spot resistance evaluations (Orjeda 1998), both Gold Finger, FHIA 18 and TMBx 5295-1 developed very little yellow sigatoka leaf spot (*Mycosphaerella musicola* Leach) in the FOC field evaluation trails which are not sprayed for leaf diseases. Significantly more leaf spot was apparent on SH 3640-10 at both field sites.

- 3.2 *Biocontrol research.* We are still a long way from understanding and applying robust, long-term biological control mechanisms for FOC. In current research projects in Australia, biological agents are being investigated for their potential to improve the vigour of tissue culture-derived banana plants and their resistance to FOC and nematodes. Micropropagated bananas are increasingly being used by the Australian banana industry as a source of disease and pest-free planting material. Recent work has shown that micropropagated bananas are more susceptible to fusarium wilt (Smith et al. 1990) and to burrowing (*Radopholus similis* [Cobb] Thorne.) and spiral (*Helicotylenchus multicinctus* [Cobb] Golden.) nematodes (J. Stanton, pers. comm.) than conventional planting material.

Projects currently underway aim to isolate beneficial microorganisms from plantations growing in wilt and nematode suppressive soils with the view to introducing them to micropropagated plants at the nursery stage before planting in infested fields, in order to obtain more vigorous

plants that are better able to utilize nutrients and with greater protection against disease.

Plantations of the Lady Finger variety in southern Queensland and northern NSW where race 1 (VCG 0124/5) is known to be present but where disease does not develop or has developed only slowly over time are described as having 'wilt-suppressive' soils. Conventional vegetative planting material (i.e. suckers and rhizome pieces) from 'wilt suppressive' soils may be protected by the presence of beneficial microorganisms such as fungi and bacteria that are not available to micropropagated plants that are raised in an aseptic environment.

In an attempt to better understand how the mechanisms operate in these soils, research is underway into the microorganisms present in these soils. In particular the studies being conducted through the DPI and CRCTPP are investigating mycorrhizal fungi, non-pathogenic strains of *F. oxysporum* and rhizobacteria that are present as endophytes in the roots of banana plants in wilt suppressive plantations. Several hundred isolations have been made from the root stele of such plants. Twenty endophytic strains of *F. oxysporum* have been retained for testing. Of these, four isolates have provided additional levels of protection for mature Cavendish banana plants inoculated with race 4 of FOC in glasshouse trials. None of the isolates afforded the plant immunity from disease and none of the isolates tested to date have afforded Lady Finger plants significant levels of protection against race 1 of FOC. The size of plants required for these studies has precluded rapid evaluation of the endophytic isolates. New protocols for a reliable small plant test would expedite these studies. Ultimately, the isolates which give the greatest levels of protection to plants in the glasshouse, will be introduced to tissue cultured plants and then assessed in diseased field conditions.

One hundred and thirteen bacterial isolates have been recovered from the banana root steele and rhizosphere of plants growing at FOC suppressive sites. Thirty isolates have been selected for initial evaluation based on results from four tests that included inhibition of FOC *in vitro*, chitinolytic activity, siderophore production and ability to produce spores. Protocols for assessing the potential of these bacteria to protect tissue culture derived Lady Finger plants against infection by race 1 of FOC are being developed. So far 11 isolates have been tested and of these, two isolates appear to reduce the amount of disease development in Lady Finger plants in glasshouse trials. These studies are continuing to test the repeatability of the results. Also, mycorrhizal cultures are presently being collected from banana roots and maintained for incorporation into future studies.

In associated projects, Dr Julie Stanton and Mr Tony Pattison in cooperation with Dr Mike Smith and Mrs Linda Smith of the DPI, have isolated 30 non-pathogenic isolates of *F. oxysporum* from banana roots

growing in soil which is suppressive to *R. similis*. These isolates are currently being tested for their ability to inhibit reproduction of *R. similis* on banana plants in pots. Thus far, at least one isolate has inhibited nematode reproduction but is affected by plant age and relative inoculation times of the fungus and the nematode. Rhizosphere bacteria isolated from suppressive soils will also be assessed for their ability to reduce nematode reproduction.

Biofumigant crops such as Vetch (*Vicia villosa* L.) or fodder rapes and mustards (*Brassica* L. spp.) have been shown to have a deleterious effect on some soil-borne fungal pathogens in Australia (Allen & Nehl 1999, Kirkegaard & Matthiessen 1999) where they are used as a green manure crop in crop rotation strategies. However, their potential to reduce the populations of FOC in soil and the long term effect on populations of FOC in soil after incorporation of such crops has not yet been investigated. While such crops may not be applicable in perennial production systems, they may have potential to treat affected areas before replanting with different cultivars or in allowing the production of susceptible yet highly favoured clones to continue in areas where FOC occurs and alternative varieties are not acceptable.

Biological options for disease control would be particularly appropriate for sustainable agricultural practices, particularly where they offer alternatives to chemicals such as nematicides and fungicides. To undertake studies into biological agents for disease control in banana will require a long-term commitment of funds and research effort and collaboration with experts in other disciplines such as soil microbiology, biochemistry and plant physiology.

- 3.3 *Systemically Acquired Resistance.* Research efforts are currently underway through the DPI in Australia and with collaborators in South Africa to investigate plant resistance activators (such as BION®) for their efficacy in protecting immature, tissue culture-derived banana plants from infection by FOC through their ability to induce Systemic Acquired Resistance (SAR). Full evaluation of these agents against FOC in banana is yet to be completed. However, early results with tissue culture-derived plantlets in controlled environment cabinets have shown that foliar applications of BION® prior to inoculation provided significant levels of protection against race 4 of FOC for at least six weeks after inoculation. Field trials will commence next season to investigate whether the induced resistance can be maintained by 'booster' treatments of BION® throughout the season.

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