FUTURE MANAGEMENT STRATEGIES IN DISEASE CONTROL

K.G. Pegg, Queensland Horticulture Institute

When creating a 'good vision' for plant disease management for the avocado industry in the future, the following need to be taken into account:-

- Due to its high yield potential and excellent eating quality, Hass will remain overwhelmingly the most important cultivar in Australasia.

- The trend towards larger production units will continue.

- Even more so than today, people of Australasia will want to live in a clean, pesticide-free and diverse environment, and want to eat 'cleaner' and 'greener' food. Thus all new developments in disease control must be environmentally acceptable.

- The movement which started with Rachael Carson's book *Silent Spring* in the 1960's towards safer and fewer pesticides will accelerate. However, pesticides will continue to play an important role in agriculture and horticulture but at a much reduced level. They will be used in a much more efficient and targeted way.

- The rapid changes occurring in science and horticulture.

- It is hoped that a major quarantine incursion will not occur inadvertently or through the level playing field policy which is based on economic and commercial agendas rather than the biology of plant pathogens.

What disease problems confronted the Australasian avocado industry 20 years ago?

The major stimulus that drove avocado disease research 20 years ago was Phytophthora root rot. In 1980 the industry was just recovering from the disastrous Phytophthora epidemics of the 1970's. With the development of cheap control of root rot by trunk injection with phosphonates (Darvas *et al.*, 1984; Pegg *et al.*, 1985; Guest *et al.*, 1995) most growers lost interest in research results of the previous decade which showed that mulching under avocado trees to stimulate soil biological activity in general reduced root rot (Broadbent and Baker 1974; Pegg 1977). At the present time, for economic reasons and the perceived injury inflicted on the water conducting tissue above and below injection sites, many growers are moving away from trunk injection to foliar spraying with phosphonates. Fortunately, the cost of the chemical remains low and it apparently causes little damage to the environment, natural predators and biological control agents.

Disease research also flourished during this era because of the enormous losses caused by pre- and postharvest anthracnose (*Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc.) in the popular 'green-skin' cultivars, such as Fuerte, which were preferred at the time. The current spray program for Hass is still based on the copper fungicide program developed for Fuerte in the late 1970's (Peterson and Inch, 1980). Also, during this period the fungicide prochloraz was found to be effective for the control of anthracnose when applied after harvest (Muirhead *et al.*, 1982). When the industry changed to Hass the popular opinion was that Hass was resistant to anthracnose and did not require field spraying. With the exception of 'pepper spot' (Willingham *et al.*, 2000), preharvest symptoms of anthracnose are rare in Hass but retail surveys in the 1990's (Ledger 1993) disproved the theory with respect to postharvest anthracnose. Controlled ripening and postharvest temperature management were shown to have a major influence on anthracnose development in ripened fruit. Fitzell and Muirhead (1983) found that temperatures exceeding 24°C increased anthracnose significantly in Fuerte and recommended that fruit be ripened at 17°C. Recent research has shown that the strobilurin – analogue fungicide azoxystrobin alone, or blocked with the standard copper spray program gives good control of anthracnose (L.M. Coates *et al.*, these proceedings). Also, rootstock studies have shown that rootstock has a significant impact on the incidence and severity of postharvest anthracnose in Hass. Differences in susceptibility have been related to differences in antifungal diene levels and fruit mineral nutrient concentrations (S.L. Willingham, these proceedings). The use of postharvest chemicals such as prochloraz has become a major issue and many countries will not accept prochloraz treated fruit. This alone provides a very strong incentive to look at new technologies for fruit disease control.

It was also during this period that sunblotch was shown to be caused by a viroid (Allen *et al.*, 1981). This viroid has a single stranded RNA molecule comprising 247 nucleotides (Symons, 1981). Recently, variants of avocado sunblotch viroid of between 246 and 251 nucleotides have been detected in trees showing no symptoms of disease. Initially the viroid was detected using an RNA probe based procedure and recently using the more sensitive procedure of Reverse Transcription – Polymerase Chain Reaction (RT-PCR). Molecular techniques have had, and will continue to have, a major impact on improved disease diagnostics. Diagnostic science in plant pathology is being given more support and will eventually be as efficient as medical and veterinary diagnostics in the medical and veterinary pathology field.

The future direction

Taking into account the changes in science and in horticulture, the following issues will be addressed:-

- the future of phosphonates
- improving fruit disease control
    - plant activators
    - prediction systems
- the role of biotechnology
    - molecular makers
    - molecular diagnostics
    - transgenic plants

- biological control

    — enhancement of natural suppression and innundative biocontrol

- rootstocks

The future of phosphonates

Phytophthora root rot has been studied intensively in both basic and applied research programs, yet phosphonate still remains the only really reliable and cost effective tool to manage this disease. Mulching to reduce the impact of Phytophthora and improve root health has declined due to the cost and availability of suitable materials despite its importance in integrated management programs.

Foliar applications

Trunk injections give long-lasting protection to vulnerable trees with minimal environmental impact. This was achieved for avocado through a thorough understanding of tree architecture, phenology and physiology so that phosphonate is targeted to the vulnerable part of the tree (feeder roots) while minimising residues in fruit (Whiley *et al.*, 1995). High concentrations of phosphonates (0.5%) are now being applied up to eight times per growing season to trees as foliar sprays to run-off. I believe that insufficient is known about the relationship between crop phenology and the partitioning and persistence of foliar-applied phosphonate. In avocado phosphonate in fruit is simply a by-product of the treatment for root rot and serves no purpose. Also, residues that reach greater than 100 ppm are not permitted by health authorities. However, developing fruit because of their sink strength will accumulate phosphonate rapidly and possibly with priority over vegetative growth flushes and feeder roots.

There is the opportunity to reduce fruit levels of phosphonate by exploiting compounds that enhance the natural disease resistance mechanisms of plants. Such compounds may not always be sufficiently active when used alone but may have an additive effect when combined with phosphonates. Ali *et al.*, (2000) using horticulturally valuable Australian native plants, which were extremely sensitive to phosphorus and where phosphorous acid is phytotoxic at levels required for disease control, found that low doses of phosphonate that normally give marginal levels of control were as effective as higher doses when combined with the plant activator acibenzolar (Bion), which is also phloem translocated.

Combining acibenzolar with phosphonate may also reduce selection pressure on *P. cinnamomi* in the roots and soil, thus preventing a shift in sensitivity with the populations becoming dominated by less sensitive isolates (M.P. Weinert, 1997; Duvenhage, 1996). There is a range of variation in the ability of *P. cinnamomi* isolates to colonise phosphonate treated plants, even when the isolates have never been exposed to phosphonate (Hardy *et al.*, 2001). The selection for less tolerant isolates of *P. cinnamomi* needs to be monitored but it is important to remember that the *in vitro* sensitivity of an isolate to phosphite will only be of value if it correlates with that *in planta*. This is because phosphonates have a complex mode of action, acting directly on the pathogen and indirectly in inducing a strong and rapid defence response in the challenged plant (Guest and Grant, 1991).

Aerial spraying

As orchards become larger, there is also the possibility of applying phosphonate from the air. If applied from the air, it will be necessary to add an adjuvant (perhaps one based on canola oil, or a mineral oil surfactant) to increase spray coverage by droplet spreading, to promote spray retention, and to reduce spray drift, evaporation and wash-off. This technology is well developed for the application of phosphonates to natural ecosystems in Western Australia. The use of adjuvants should also be examined for the application of conventional foliar sprays to run-off from the ground. They may allow the concentration of phosphonates to be reduced, thus minimising residues in fruit.

Improving fruit disease control

The future of plant activators

Anthracnose and stem-end rot (*Dothiorella* spp.) are the most destructive postharvest pathogens of avocado. Control of these pathogens currently relies on fungicide use but recently S.L. Willingham (these proceedings) reported that avocado rootstocks, and mineral nutrient concentrations, influence the susceptibility of fruit to postharvest diseases. To reduce even further the reliance on fungicides, it may be possible to regulate the levels of preformed and inducible antifungal compounds in fruit that decrease during ripening. Certain treatments (both biotic and abiotic) have been found to induce antifungal production and protect fruit from postharvest decay. The abiotic inducers are the plant activators such as acibenzolar (Bion) previously referred to in the Phytophthora control section. These chemicals are not fungicides in their own right, but stimulate antifungal responses in the host plant. The response is known as Systemic Acquired Resistance and differs from genetic engineering in that it uses the genes already present in the plant. It is based on the fact that plants are resistant to most pathogens; that the genes necessary for defence are present in all plants, and resistance may depend on how quickly these genes for defence are activated. Disease resistance in plants involves three phases:- 1. A recognition phase, where an incompatible pathogen is perceived, most probably by host receptors on the cell surface and elsewhere, 2. A signal transduction phase, where various pathways of molecular signalling are activated to distribute the cellular message that the cell is under microbial challenge and 3. A response phase where plant defence mechanisms (e.g. production of antimicrobial compounds) are activated. Systemic Acquired Resistance needs a lag time to develop after the inducing treatment is applied. We have already shown that systemic acquired resistance can be used to improve the control of some mango and passionfruit diseases by adding a plant activator to multi-site standard industry protectant fungicides.

So far in our studies, the plant activator Bion has not boosted the level of fruit disease control in avocado. This may be because applications have been concentrated on the late stages of fruit growth rather than the early stages when cell division in the fruit is most rapid.

Develop prediction systems

By the year 2020, it will be important that fungicide only be applied when needed to counter infection. Computer-based equipment to monitor environmental variables (rainfall, leaf wetness, temperature) needs to be developed. However, such a prediction system will require the availability of a very effective systemic fungicide with the ability to penetrate and inactivate existing latent infections. Prochloraz does not have sufficient systemic activity for this purpose but some of the current strobilurin fungicides may be powerful enough. Other new systemic fungicides need to be evaluated for their ability to penetrate and inactivate existing latent infections.

The role of biotechnology

Molecular marker technology and molecular diagnostics

Biotechnology offers two activities which are important in conventional plant breeding and plant disease management. These are:-

- molecular marker technology — genetic markers for tracking the progress of populations under selection

- molecular diagnostics — have had a major impact on improved disease diagnostics and, given time, will be as effective as medical or veterinary diagnostics. A new molecular diversity and diagnostic research facility has recently been established at the DPI Indooroopilly Research Centre (IRC) to serve tropical and sub-tropical agriculture and horticulture. This laboratory is a joint venture between the Cooperative Research Centre for Tropical Plant Protection and DPI. Dr Andre Drenth of this laboratory has designed a PCR-based method for the rapid detection and identification of *Phytophthora* species. He has developed primers that are highly specific to the genus Phytophthora. The resulting PCR product from this genus specific assay then provides a DNA fragment pattern that is species specific. This assay combines highly sensitive detection using PCR with the ability to detect all species within the genus in a quick and cost effective way. This technology will be invaluable to the avocado nursery industry. The highly sensitive RT-PCR assay for the detection of avocado sunblotch viroid will also be necessary for preventing the distribution of non-symptomatic ASBV-infected germplasm.

- transgenic plants

There have been several predictions made that most major plant disease problems will be overcome using recombinant DNA technology. So far molecular biology has given us Roundup tolerance; corn, cotton, canola and soybean protected by the Bt gene of *Bacillus thuringiensis*; and PLRV-resistant potato and other virus resistant plants by the introduction of virus coat protein into the crop genome. To my knowledge no major fungal disease of any crop has yet fallen to molecular biology.

It is a popular belief that all that needs to be done to produce a disease resistant plant is to clone a resistant gene from one plant, and transfer it to another to make it resistant as well. Although resistance genes have been cloned, they seem to function in signalling pathways that produce different results when placed into different genetic backgrounds. Another concept, yet to be proven, is to insert genes encoding antimicrobial proteins into transgenic plants to confer wide-ranging resistance to pests and diseases.

Before biotechnology delivers, more needs to be known about the complexity of cells and organisms. It is sobering to realise that previous ‘wonder’ treatments (resistance genes, systemic fungicides etc.) have not lived up to expectations. This is because plant pathogens are comprised of variable and highly flexible populations of organisms that soon adapt to environmental changes. Molecular biology is a powerful tool but I very much doubt that we will see hundreds of hectares of Phytophthora and Colletotrichum resistant avocado trees in the field by the year 2020 using this technology. Even though biotechnology (genetic manipulation) is unlikely to solve all disease problems in the avocado industry in the near future, it should be looked upon as an important tool to value-add to, but not displace, traditional methods used in horticulture.

Biological control

The concept of using benign microorganisms as viable alternatives to use of fungicides is most appealing. Research in Australia has focussed on attempts at the recreation of natural biocontrol for Phytophthora root rot and innundative biocontrol and enhancement of natural suppression for anthracnose control.

Biological control of Phytophthora root rot

Naturally suppressive soils were first identified in Australia by Broadbent and Baker (1974) when they surveyed Australian avocado orchards. They found widespread occurrence of root rot caused by *P. cinnamomi* in northern New South Wales and southern Queensland, with many trees declining with the disease. However, certain orchards in rainforest soils near Tamborine Mountain in south-eastern Queensland were relatively free from Phytophthora root rot. Trees in these orchards were outwardly healthy despite the presence of the pathogen in the soil and prevailing favourable environmental conditions for disease development. In orchards where *P. cinnamomi* was apparently suppressed, a high level of organic matter was maintained around young trees by cover crops (legumes and grasses) which were regularly slashed and incorporated into the soil. Additional high fibre straw mulches (wheat, barley, sorghum) plus chicken manure and gypsum were added to the leaf litter under trees (Pegg, 1977). The above practices were aimed at simulating conditions found in the undisturbed soil of the adjacent rainforests where a large quantity of deposited organic matter is present. These soils are naturally suppressive to *P. cinnamomi* (Cook and Baker, 1983). Broadbent and Baker (1974) found that bacteria and antinomycetes were abundant in suppressive rainforest soils and suggested that pseudomonads, antinomycetes and *Bacillus* spp. were involved in the suppression of *P. cinnamomi*. Although individual antagonistic microorganisms have been isolated from these suppressive soils (Stirling *et al.*, 1992), research on the addition of a single antagonist to conducive soils did not continue.

Mulching under avocado trees, which stimulates soil biological activity in general, creates a suppressive environment for *P. cinnamomi*. Microorganisms interact with Phytophthora at all stages of the disease cycle. Turney and Menge (1994) have reviewed the mechanisms involved. These include increased populations of microbial flora that are antagonistic to pathogen activity (lysis of hyphae and production of many abortive sporangia); the production of inhibitory volatile compounds such as ammonia and nitrite and toxins such as saponins and organic acids; encystment of zoospores by organic matter; increased host resistance (phytoalexins); improved aeration and drainage in the mulch and soil; and improvement in root growth and reduced plant stress. Wolstenholme *et al.* (1996) emphasised the value of regulated mulches to reinforce the natural leaf and litter mulch under the tree for root rot control. They described suitable mulching materials based on their C:N ratios and their speed of decomposition. High fibre straw mulches (wheat, oats, barley) or composed chunky *Pinus radiata* D. Don bark were recommended.

Biological control of anthracnose
In Australia, several bacteria and yeasts have been selected in laboratory studies for their ability to suppress anthracnose development in detached avocado fruit (Stirling *et al.,* 1995). Some success has also been achieved in the field when antagonistic organisms have been applied in an innundative manner (Coates *et al.,* 1995) or when natural suppression shown to exist in some orchards was augmented using specific nutrients (urea, yeast extract or molasses) (Stirling, 1996).

The full potential of innundative biocontrol has not been realised for two major reasons:-

- the cost of commercialisation and

- inconsistent disease control in the field

For managing root diseases, there are now some 30 biocontrol products available worldwide with those based on *Trichoderma* and *Bacillus,* the most popular. Some products are highly specific towards a certain pathogen (e.g. *Agrobacterium radiobacter* to control crown gall) or have been developed for niche markets (e.g. the nursery industry). To control a root disease, these agents must overcome the phenomenal buffering capacity of the resident soil organisms. If we apply a *Trichoderma,* it may work initially because of the very high inoculum levels applied but in time it will be restricted in its activity. Besides coping with the resident organisms, it must also cope with changes in the abiotic environment (temperature, mixture, pH, nutrients, organic matter etc.). We cannot expect a biocontrol agent to behave any differently to a soil-borne plant pathogen. For example, with *Phytophthora cinnamomi* we can have similar inoculum levels in different soils yet sites are classified as high, medium or low disease hazard soils. *P. cinnamomi* does not cause disease in a dry month yet massive root loss occurs in a wet month. Why should a biocontrol agent be any different? No large agrochemical or biotechnology company is likely to spend money and commercialise a product specifically for the avocado industry — the returns are just not there. Even though biocontrol is an exciting area of research, the industry has already demonstrated that it prefers to direct resources towards other fields of endeavour because of the high developmental costs.

Rootstocks
The ultimate solution to the Phytophthora root rot problem will be to select or breed a resistant rootstock. As Australasia does not have a breeding program, avocado germplasm exhibiting resistance to *P. cinnamomi* may need to be imported from California, South Africa or Israel.

Also, rootstocks need to be selected which give high levels of antifungal compounds and increase the allocation of Ca to fruit, which when provided with managed nitrogen nutrition and irrigation, particularly in the critical early period of fruit development, will significantly reduce anthracnose.

Summary and Conclusions

There are several exciting areas of research and development required for addressing some of industry's biggest issues for the future. These are:-

- Combining plant activators with phosphonate to minimise fruit residues and reduce selection pressure on *P. cinnamomi* in the roots and soil.

- Aerial application of phosphonate for larger production units using technology developed for the aerial applications to natural ecosystems in Western Australia.

- The use of plant activators to switch on the plant's defences and increase the concentration of antifungal compounds to reduce anthracnose and stem-end rot.

- Develop a computer-based prediction system so that fungicides only be applied when needed. Evaluate new systemic fungicides for their ability to penetrate and inactivate existing latent infections and incorporate into integrated management practices.

- Use molecular technology to assist in producing nursery trees free of Phytophthora and avocado sunblotch viroid; for detecting major endemic and exotic pathogens of the avocado; and for understanding pathogen variability.

- Develop biocontrol so that it forms an integral component of disease management. This will involve mulching, which alone offers a practical and environmentally friendly solution for reducing root rot and overall tree stress, as well as innundative biocontrol for Phytophthora and fruit diseases. Some of these biocontrol agents, besides having antibiosis capabilities against pathogens, also have the ability to induce plant resistance and increase plant growth.

- Select or import avocado rootstocks which exhibit resistance to *P. cinnamomi,* and/or reduce anthracnose susceptibility through influencing the levels of antifungal compounds and mineral nutrient concentrations of the fruit.

It will be a challenge to be able to direct resources towards the most fruitful and targeted areas of research. Because of the rapid advances in technology, a fairly liberal attitude will need to be taken towards more strategic research (discovery/ science activities) when considering resource allocation.

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