INTRODUCTION
Agricultural crops are under constant attack by pathogenic organisms. When varietal disease resistance, cultural practices and environmental conditions do not provide adequate protection against infection, the use of chemicals to control pathogen infections becomes essential.

When using chemicals to control diseases, difficulties may be experienced when the pathogen becomes resistant to the pesticide or fungicide. Pathogens adapt to changing environmental conditions over time through selection for strains that are able to survive under new, sometimes adverse, circumstances. The application of a fungicide constitutes an adverse change in environment for an organism that is sensitive to the compound, and this may lead to the organism developing resistance to the compound.

Green mould, Penicillium digitatum (Pers.Fr.) Saccardo and blue mould, P. italicum Wehmer are the most important post-harvest diseases of citrus fruit in South Africa, responsible for 90% of all losses caused by post-harvest pathogens (1). Pathogen resistance to post-harvest fungicides has always been a threat to the citrus industry.

In South African citrus packhouses, citrus fruits are treated with the post-harvest fungicides, imazalil, thiabendazole (TBZ) and guazatine (certain markets) to control Penicillium decay. Two other post-harvest fungicides, sodium ortho-phenylphenate (SOPP) and prochloraz, are also registered for the control of the Penicillium moulds, but have not been used in citrus packhouses for the last two decades.

TBZ and benomyl belong to the benzimidazole group of fungicides which all have the same mode of action. Intensive preharvest spraying of benomyl for black spot control over many years, exerted selection pressure on the naturally-occurring benzimidazole-resistant green and blue mould spore populations. The harvested fruit, contaminated with resistant spores, was treated with TBZ in the packhouse, continuing the selection process until a high proportion of the spore population had become resistant to TBZ. Allowing culled fruit and fruit for processing to decay within or near to the packhouse greatly increases the intensity of resistance selection pressure.

Green and blue mould resistance to TBZ has been in existence in South Africa for the last three decades. The same scenario exists in California for both SOPP and TBZ (3, 4, 6). This came about in California as a result of the long-term storage of fungicide-treated fruit that had been allowed to decay in the vicinity of packed fruit treated with the same fungicide (6).

Imazalil was introduced into the Californian and South African citrus industries in the early 1980s as a successful treatment for the TBZ-resistant biotypes. However, within 5 years of commercial imazalil usage in California packhouses, imazalil-resistant biotypes were detected and have subsequently been widely reported within the Californian industry (6). Imazalil-resistant biotypes have also been reported in Argentina (5) and Uruguay (8), but not in Spain, Japan, Australia (2, 7, 9) or Florida (USA) (10).

Random in vitro screening of 160-200 Penicillium spore samples from 2001-2005 in the South African citrus industry revealed 20 samples with imazalil resistance. The discovery of imazalil-resistant Penicillium biotypes in South Africa has prompted the introduction of a major screening strategy. Blue and green mould samples from all citrus production areas will be screened to determine the incidence and distribution of imazalil resistance and to determine the source of the inoculum (orchard or packhouse). In vitro testing will be conducted to quantify the proportion of samples found to be resistant and these resistant samples will then be exposed to further in vitro testing so that treatment strategies can be provided to packhouses to overcome the resistance.

STRATEGIES FOR PREVENTING RESISTANCE OF FUNGAL PATHOGENS TO POST-HARVEST FUNGICIDES
In any fungal spore population a small percentage (< 1%) of spores are resistant to one or more of the standard post-harvest fungicides. The proportion of the spore population that is resistant may increase or decrease, depending on the way in which the post-harvest fungicides are used and the handling and storage of fungicide-treated fruit.

How does fungicide resistance develop? Selection pressure is the main mechanism by which fungicide resistance develops. The intensive use of a fungicide or fungicides with the same mode of action, selectively inhibits the sensitive spores in a fungal spore population and consequently the resistant spores that survive make up a larger proportion of the spore population.

The following strategies are all part of the “basic practices” for decay control and are valuable in preventing the development of resistance to the post-harvest fungicides.

- **Pre-harvest disease control** It is undesirable to use fungicides with the same mode of action for pre- and post-harvest disease control. However, it is important to effectively implement alternative pre-harvest control practices to reduce the inoculum of pathogens that cause post-harvest decay.

- **Orchard sanitation** A certain threshold concentration of fungal spores in a wound is necessary before a fruit will rot. Therefore it is essential that the total spore load on the fruit be kept as low as possible. Fallen fruit should be removed from the orchard twice a week, before any mould spores are formed on the fruit surface.

- **Insect control, harvesting and handling of fruit** Insects that...
damage fruit must be controlled to reduce the incidence of infection sites on the fruit for blue and green mould and the sour rot fungi. Care should be exercised while harvesting and handling to avoid injuries to the fruit.

- **Disinfecting dump tanks and the fruit washing process** To further reduce the spore load on fruit surfaces, the dump tank water should be thoroughly sanitised. Where fruit is dumped dry, the first set of brushes must not be allowed to become a source of infection by concentrating spores that are brushed from the fruit surface. Fruit should be washed with a detergent containing a fungicide (e.g. Decosol) or other recommended sanitiser, or rinsed with chlorinated water. Water in dump tanks and fungicide baths should be changed regularly to prevent a build-up of spores. Avoid allowing rotten fruit to settle in these baths.

- **Prompt treatment of fruit after harvest** Fruit should be treated promptly after harvest to prevent sporulation of green and blue mould on fruit in lug-boxes or trailers. This will prevent a high spore load on fruit going into the packhouse.

**Sanitation in the packhouse, cold rooms and degreening rooms** It is of utmost importance to avoid the production of spores in the packhouse, cold rooms and degreening rooms. Store local market fruit in another building, see to it that factory-bound fruit is removed regularly, and destroy fungicide-treated culled fruit before spores are formed on them.

Do not repack fruit rejected for decay at the ports or at inland packhouses. If waste fungicide-treated fruit is repacked in an inland packhouse, the whole packhouse should immediately be thoroughly sanitised after completing the exercise, at least nightly. The same applies to Valencias stored for sale in late summer.

Degreening of citrus has become an integral part of citrus production in Southern Africa. Multiple exposure of citrus to fungicides in a pre-degreening drench and thereafter in packhouse treatments gives rise to heightened resistance selection pressure. If not carefully managed, this could lead to rapid development of resistance. The following practices should be specifically avoided:

- using imazalil in the pre-degreening drench as well as in the packhouse treatment;
- returning degreened fruit from the packhouse (after treatment) to the degreening process for a second time.

**TREATMENT STRATEGY IN A CITRUS PACKHOUSE TO REVERSE RESISTANCE AND AVOID DEVELOPMENT OF FURTHER RESISTANCE**

The first step is to identify the cause of the problem and eliminate it. For example, storage of processing fruit in close proximity to the packhouse would expose the packhouse to unnecessarily high spore inoculum. If the source of the resistance development is not removed, implementation of various strategies aimed at eliminating resistance may have the opposite effect and result in the selection of super-resistant strains.

The use of mixtures of fungicides The use of fungicide mixtures with different modes of action will greatly retard the onset of
Resistance to guazatine, which is effective in controlling green and blue mould, has not yet been detected in South Africa.

The standard packhouse treatment recommended in South African citrus packhouses is the mixture of imazalil and guazatine (only for markets that allow guazatine) in a hot water fungicide bath and thiabendazole (TBZ) in the wax. For markets where guazatine is not permitted, packhouses must use Sporekill in their fruit washing systems and imazalil alone in the hot water bath. If imazalil-resistant spores are detected at a packhouse/producer then one of the following two treatment options would be appropriate.

- Withdraw imazalil from the mixture in the bath and use guazatine for an entire season to reduce the imazalil resistant spores. Re-evaluate the status of the resistant spores. If the resistant spores have been reduced or nullified then reintroduce imazalil into the treatment programme. Advise the producer not to export fruit to markets where guazatine is not allowed. Use only Sporekill in the fruit washing systems.
- **Alternation of fungicides** It has been established that a fitness cost is attached to resistance by green and/or blue mould biotypes to imazalil or TBZ. In other words, the resistant biotypes cannot compete successfully with fungicide-sensitive biotypes in the absence of that fungicide, and the proportion of resistant biotypes will gradually decrease in the population as long as this fungicide is not in use.

After 30 years of Penicillium resistance to TBZ, imazalil was introduced into the industry and proved to be an effective replacement for TBZ. Imazalil should, after extensive use, inhibit the TBZ-resistant Penicillium spores and TBZ should once again become effective against green and blue mould. This can only be determined over a long time by screening all imazalil resistant spores against TBZ.

If imazalil resistance is detected, its further use should be curtailed and an alternative fungicide with a different mode of action (TBZ or guazatine) used until the number of resistant spores is very low or cannot be detected anymore, then imazalil can be reintroduced. This cycle is then repeated.

- **Increased concentration of the fungicide at risk** In the process of screening spore samples for resistance, spores are exposed to a higher than standard concentration (i.e. 2x) of the fungicide. If the higher concentration of the fungicide inhibits infection by the resistant spores, the packhouse can increase the concentration of fungicide for a production season. Upon completion of the season, fungal spore samples will have to be screened to determine whether the resistant spores have been reduced. If not, another treatment strategy is recommended.

- **Sporulation inhibition** Sporulation inhibition of green and blue mould is one of the primary properties of imazalil. For sporulation inhibition to be achieved, a residue of 1-2 ppm of imazalil needs to be retained on the fruit rind. Sporulation inhibition effectively reduces resistance selection pressure.

Residue analyses over recent years have indicated that the vast majority of fruit, treated in the standard hot water dip treatment, retain an imazalil residue level of below 1 ppm which does not inhibit sporulation. To overcome this problem and reduce the selection for resistant spores, a new standard treatment strategy will be recommended for the coming 2006 season.

**CONCLUSION**

It is important for citrus producers to realise that all the basic principles listed in this article for both the prevention of waste and the prevention of pathogen resistance development to our postharvest fungicides, are the same principles. These principles must be adhered to diligently.

The fact that the incidence of waste has been high this season, as well as last season, and that more imazalil-resistant spores have been detected, indicates that producers and packhouses are not diligently applying the above principles.

Due to the limited selection and efficacy of the available postharvest fungicides, the industry must prevent the widespread development of resistance to imazalil. If this is allowed to happen, the financial losses will be far higher than the losses experienced this 2005 season.

**REFERENCES**