

Induced Resistance: Potential for Control of Postharvest Diseases of Horticultural Crops

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INTRODUCTION

Fortunately, plants have developed highly effective mechanisms with which to defend themselves when attacked by potentially disease-causing microorganisms. If not, then they would succumb to the many pathogenic fungi, bacteria, viruses, nematodes and insect pests, and disease would prevail. These natural defence systems of plants can be deliberately activated to provide some protection against the major pathogens responsible for causing severe yield losses in agricultural and horticultural crops. This is the basis of what is known as 'induced' or 'acquired' disease resistance in plants.

Although the phenomenon of induced resistance has been known amongst plant pathologists for over 100 years, its inclusion into pest and disease management programmes has been a relatively recent development, ie. within the last 5 years. This review will discuss very briefly some of the characteristics of the induced resistance phenomenon, outline some of the advantages and limitations to its implementation and provide some examples within a postharvest pathology context. Finally some approaches being investigated by the fruit pathology team at DPI Indooroopilly and collaborators will be outlined.

CHARACTERISTICS OF INDUCED RESISTANCE

One of the prime characteristics of this type of plant disease resistance is its **non-specificity** with respect to a) the many and varied types of treatments or 'activators' of plant defences, b) the broad range of plant species in which resistance can be induced and c) the broad spectrum of fungal, bacterial and viral pathogens and also nematode and arthropod pests against which the induced resistance may be effective. The volume of research and resulting literature on induced resistance has expanded enormously in the last 10 years, and several review papers have been published (e.g. Metraux et al., 2002). Therefore, only a few examples of resistance activators and plant/pathogen systems studied will be discussed here.

Induced resistance relies on the activation or elicitation of many different defence mechanisms within the plant, under the control of many genes. Very briefly, these **inducible defences** include the early formation of physical or **structural barriers** to pathogen ingress and/or the activation of **biochemical defences**. Structural barriers may include strengthening of the plant cell wall via phenolic deposition and lignification to prevent the pathogen progressing beyond the first few penetrated cells. Biochemical defences include the activation of **pathogenesis-related (PR) proteins**, some of which are enzymes (e.g. chitinase and β -1,3-glucanase) known to hydrolyse components of fungal and bacterial cell walls, and the *de novo* synthesis by the plant of antimicrobial secondary metabolites, known as **phytoalexins**. Levels of preformed or **constitutive** antimicrobial secondary metabolites may also be increased by some treatments.

The resistance may be activated within the plant locally, ie. at the site of the activating treatment, or **systemically** throughout the plant, termed systemic induced or acquired resistance. The systemic process thus requires the production and translocation of a **signal molecule(s)**. While many of the defence mechanisms operable in induced resistance have been identified and are well known, the identity of the potential signal molecule(s) is debated amongst researchers. Extensive research has

demonstrated that salicylic acid, jasmonates and ethylene are all important intermediates in the complex pathways culminating in resistance of plants to pathogens and pests. The processes of signalling and priming of defences in systemic tissues requires some time, thus a **lag period** is necessary between the inducing treatment and the expression of resistance. The lag period varies among plant/pathogen systems studied, but typically ranges from a few hours to a few days for maximum effect.

TYPES OF RESISTANCE ACTIVATORS, AND SOME EXAMPLES IN POSTHARVEST PATHOLOGY

There is an ever-increasing list of plant defence activators. These include:

- *Micro-organisms*, which may be pathogenic, weakly pathogenic or not pathogenic. For example, infection of banana with a fungus, *Phyllosticta musarum*, causes minute blackish brown spots on the skin, or 'freckle', but doesn't affect the pulp. Severity of postharvest anthracnose disease, caused by *Colletotrichum musae*, is significantly reduced in these freckle-infected fruit, and it is likely that the initial infection by the *Phyllosticta* sp. fungus activates defence mechanisms in the peel (Abayasekara et al., 1998).
- *Microbial products* or by-products that are non-infective. For example, researchers at Cornell University discovered that treatment of plants with harpin, the protein encoded by a gene of the bacterium *Erwinia amylovora*, could induce multiple defence responses and resistance to pathogens (e.g. Wei and Beer, 1996). EDEN Bioscience, U.S.A. (www.edenbioscience.com), have formulated the protein into a product called Messenger®. The *E. amylovora* bacterium actually causes fireblight disease of apples, but neither the harpin protein nor Messenger causes disease. Extensive field trials have demonstrated its effectiveness in activating resistance to pests and diseases in a wide range of crops (see website). Postharvest treatment reduces the severity of diseases caused by *Penicillium digitatum* and *Botrytis cinerea* in citrus, tomato, grape, apples and cherries. Messenger has been registered for agricultural and home garden use in the U.S.A. and elsewhere including Mexico, China, Central America and Germany.
- *Plant extracts* such as Milsana® (KHH BioSci Inc., USA), which is an extract from giant knotweed, *Reynoutria sachalinensis*. Milsana treatment induced resistance in cucumber to the powdery mildew fungus, *Sphaerotheca fuliginea*. The resistance correlated with the accumulation of flavonoid defence compounds, and the activation of two genes involved in their synthesis (Fofana et al., 2002). Milsana was registered in the USA in 2000 as a bioprotectant and approved for use in glasshouse ornamentals.
- *Synthetic chemicals* that have no direct pesticidal activity. This group of activators perhaps offers the greatest potential for implementation of induced resistance on a commercial basis. Acibenzolar-S-methyl (ASM) is a synthetic analogue of salicylic acid, that is, studies have shown that it acts similarly to salicylic acid in the defence pathway. ASM was developed by the Ciba-Geigy, Novartis, Syngenta succession of companies, and has been registered (trade names include Bion®, Boost® and Actigard®) and made available in many countries to help control specific disease problems in target crops.

Although ASM has not been registered and made available to Australian horticulturalists, it has been tested in many crops against a range of pathogens. For example, pre-flowering treatment of field-grown melons with ASM significantly reduced the severity of postharvest fungal rots (Huang et al., 2000; McConchie pers. comm., 2003). And in a glasshouse trial, severity of dry rot in potato tubers inoculated with *Fusarium semitectum* was significantly less severe if plants had received earlier foliar sprays of ASM (Bokshi et al., 2003). Both studies reported increased activities of PR proteins in ASM treated plants. Postharvest disease of strawberry fruit caused by *Botrytis cinerea* was suppressed and shelf life extended by ASM treatments applied through the vegetative and reproductive growth stages (Terry and Joyce, 2000).

Other promising treatments include the incorporation of various silicon-based amendments or fertilisers. Products such as potassium and sodium silicate, diatomite, rice hull wastes, Silica Plus™ (Grose'n'Grows P/L, QLD), Photo-Finish™ (Nutri-tech Solutions P/L, QLD, and Kasil 2040 (PQ Australia P/L, Vic.) are already available and widely used. There is much evidence to suggest that monosilicic acid, the form of silicon which is available to the plant, can increase resistance to disease and activate defence responses such as PR proteins (e.g. Dann and Muir, 2002) although there are limited reports of effects on postharvest diseases. It is likely that over the next few years many more resistance activators will be developed and made available to the agricultural and horticultural industries.

Other physiological treatments. For example, exposure of harvested fruits and vegetables to low doses of ultraviolet-C irradiation can reduce the development of postharvest rots and significantly extend shelf life (see review by Wilson et al., 1995). The activation by UV-C of defence enzymes in peaches (e.g. El Ghaouth et al., 2003) and phytoalexins in carrots (Mercier et al., 1993) has been reported, suggesting that the effect may at least partly be due to activation of defences.

CONSIDERATIONS FOR THE IMPLEMENTATION OF INDUCED RESISTANCE IN POSTHARVEST HORTICULTURE

Some of the above characteristics make induced resistance well suited for implementation into crop protection strategies for a range of agricultural and horticultural crops. Induced resistance is likely to be highly durable, as many genes are activated in the induced defence response. This is in contrast to the use of resistant cultivars, where one or a few single genes determine resistance, or synthetic pesticides, which often have specific sites of action within the pathogen. These types of control measures are less durable, as pathogens can evolve to overcome their very specific modes of action.

Conceivably, many of the treatments described above would be easily integrated into current pest protection programmes, utilising existing agronomic machinery and practices. For example, the treatments could be used in combination with reduced applications of synthetic pesticides. This may lessen dependency on pesticides and moderate some concerns associated with their use, such as environmental and residue issues, and reduced efficacy. The systemic nature of induced resistance means that the whole plant will often be protected from disease after an initial activator treatment to one part of the plant, e.g. roots, or lower leaves, so that the protection may be carried through to new growth or developing fruits. Another benefit is that a wide range of plants may be protected against a vast array of pathogens and even root-infecting nematodes and arthropod pests in some cases.

While discussing the positive attributes of induced resistance and its potential in horticultural systems, it is important to also mention the likely limitations, and gaps in knowledge. It is essential to bear in mind that this type of plant protection acts indirectly on the pathogen via boosting plants' natural defence mechanisms. It does not target the pathogen directly, as fungicide treatments do, and thus may require a fundamental shift in thinking if it is to be implemented effectively. In most cases, the resistance-activating treatments need to be applied prior to infection occurring, that is, when there are no obvious symptoms, so that the plant has time to boost its defences before the pathogens infect.

Induced resistance may not be effective against all pathogens of economic importance for a particular crop, and the protection is rarely complete, ie. some disease symptoms will probably still occur, although at lower incidence and severity. The environmental conditions may influence the plant's ability to stimulate its defence mechanisms, so that the resistance can be inconsistent or variable from season to season, and growers will not necessarily benefit from yield increases. Also, crops, particularly dicotyledons, may require multiple or 'booster' applications of a resistance activating treatment.

There is still much to learn about the various and complex defence mechanisms involved, and there are likely to be subtle differences among the different plant species and fruit and vegetable types. There needs to be assurance that the boosted natural defence compounds effective against pathogens are not present in consumed tissues at levels toxic to mammals. Also, most research and commercial applications so far have been to minimise losses due to foliar pathogens, and there is comparatively little information on the occurrence and/or effectiveness of induced resistance to root or storage diseases. In fact, the examples given above represent the majority of published research on induced resistance to postharvest diseases in horticultural crops.

WORK IN PROGRESS AT DPI AND UQ: INVESTIGATION OF INDUCED RESISTANCE TO POSTHARVEST DISEASES AND ACTIVATION OF DEFENCES IN MANGO

One of the many projects currently underway within the fruit pathology team at DPI Indooroopilly, under the leadership of Dr Lindy Coates, is investigating the management of postharvest diseases of mango, avocado and banana using their natural resistance mechanisms. The project is funded by ACIAR and involves research collaborators in Sri Lanka and at the University of Queensland, Gatton and St Lucia. One of the objectives is to explore the induced resistance response in Australian-grown mango, as there is a complete gap in information in this area of mango research.

Preliminary field trials in the 2002/2003 season were conducted at commercial mango orchards at Brookfield and Gatton. Results from the Gatton trial show that postharvest anthracnose disease severity after inoculation with *Colletotrichum gloeosporioides* was significantly lower in fruits which had been treated 7 days before harvest with ASM or *C. gloeosporioides*, compared with water-treated controls (Zainuri et al., these conference proceedings). At both sites, ASM treatments tended to delay anthracnose disease progression caused by natural field infections. These trials will be repeated and expanded to include earlier applications of ASM to trees in the orchard. Infection by the fungi causing anthracnose disease of mango actually occurs very early in the season when fruit are immature. After infection the fungus remains dormant or 'latent' and as fruits ripen after harvest the latency breaks and the fungus ramifies through the tissue causing the severe black lesions on the skin and rotting of the pulp. It is possible that earlier enhancement of defence mechanisms may reduce the incidence of the initial fruit infection. Also, early season applications of ASM may induce resistance to foliar pathogens within the canopy, thus reducing the inoculum loads of pathogens that may infect foliage or fruit later in the season.

There is strong evidence that antifungal (or fungistatic) compounds in mango and avocado contribute to the latency of fungi in immature fruit (see review by Prusky and Keen, 1993). Peel of unripe mature mango fruit naturally contains high levels of the 5-alkyl resorcinol secondary metabolites, which have demonstrated antifungal activity in bioassays with test fungi. As fruit ripen after harvest the concentrations of these compounds decline, which coincides with the breaking of pathogen latency and the development of disease symptoms. The laboratory component of the DPI/UQ work will firstly identify known constitutive and inducible defensive compounds in Australian mango cultivars and characterise any new antifungal metabolites. Further work will determine whether resistance-activating treatments can maintain high levels of these compounds through ripening, so that the onset of disease is delayed and shelf life extended. Another lab/glasshouse component will measure changes in activities of pathogenesis-related proteins and other defence enzymes following treatments.

CONCLUSION

The implementation of induced resistance in pest and disease management strategies of horticultural crops should be approached with cautious optimism. The enormous potential for minimising postharvest diseases of fruits and vegetables via their natural disease resistance mechanisms has been demonstrated. However, more information is required on a crop-by-crop basis to ensure that this type of resistance offers a safe, effective and reliable complement to the existing methods.

ACKNOWLEDGEMENTS

Funding from The Australian Centre for International Agricultural Research (ACIAR) is acknowledged. The effort of Dr Greg Johnson (ACIAR) in project development and operation is appreciated. All members of the fruit pathology team at DPI Indooroopilly are thanked for their support.

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