POSTHARVEST DISEASE CONTROL OF MELONS USING SYSTEMIC ACQUIRED RESISTANCE AND OTHER SAFE METHODS

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A thesis presented in fulfillment of the requirements for the degree of Doctor of Philosophy

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February 2008

ABSTRACT

The goal of this research was to test commercially viable alternative methods to fungicides for controlling storage diseases of melons that are safe for human health and the environment. Initially, experiments were conducted on melons to develop a protocol for optimum conditions of disease development during storage for different pathogens and for different stages of fruit maturity. For all pathogens tested, the study found that humidity greater than 90% and temperatures above 20° C support infection and rapid growth of disease.

Differences in the rate of infection and extent of disease development after the inoculation of different storage pathogens was observed between rockmelon and honeydew melons, indicating differences in host pathogen interactions. Among the tested pathogens, *Alternaria* spp. was the least aggressive in infection and disease severity, growing considerably slower than *Fusarium acuminatum* which was moderately aggressive and *Rhizopus* spp. which was very aggressive, in comparison. Green half-slip melons showed greater resistance to pathogen attack than green full-slip fruit, while yellow full-slip melons were highly susceptible to pathogen attack. Therefore, the laboratory experiments for postharvest treatments of rockmelons were performed using green full-slip fruit challenged with *F. acuminatum.*

Evaluation of physical and safe chemical methods of postharvest treatment to control postharvest diseases of melons showed that none of the treatments alone was as effective as the commercially available fungicide. Hot water solutions of safe compounds considerably increase their efficacy against postharvest rots, however, symptoms of phytotoxicity on the

rind after dipping made their use unacceptable. Iodine was the only safe chemical tested which did not cause any phytotoxicity on melons. When combined with hot water, iodine showed the best control of storage rots and was as good as the fungicides carbendazim or guazatine. Hot water iodine dipping of fruit also delayed ripening and fruit were firmer during storage for a longer period of time.

Systemic acquired resistance (SAR) was evaluated as a method of controlling powdery mildew in glasshouse grown rockmelon seedlings by treating with the activators 2,6-dichloroisonicotinic acid (INA) or benzothiadiazole (BTH) or water. Increased resistance due to application of INA or BTH, was observed by the reduction of powdery mildew on pre-inoculated detached leaves and also on intact leaves from natural infections. Heightened resistance due to spraying with elicitors of SAR, was further evident by the increased activities of the pathogenesis related proteins (PR proteins), peroxidase and accumulation of phenolics or antifungal compounds during and after challenge inoculation.

Field grown rockmelons were treated with INA or BTH or BABA (β-aminobutyric acid) or water at various stages of plant growth and evaluated for increased resistance against pre and postharvest diseases. Both powdery mildew and downy mildew were significantly less on the SAR elicitor treated plants. Preharvest treatment with SAR elicitors also reduced storage diseases of the harvested rockmelon fruit. The reduction in postharvest disease was similar whether plants were treated once, three weeks before harvest, or given four sprays during the growing season beginning at anthesis. A further postharvest dip with 500 ppm of guazatine gave substantial reduction of storage rots of melons. Enhanced activities of chitinase and peroxidase, two major PR-proteins, compared to the control, indicated induction of defence had occurred in the foliage and fruit as a result of SAR. Over the course of four field and one glasshouse experiments slight phytotoxicity was observed in

plants frequently sprayed with INA or BTH, but no phytotoxicity was seen after a single spray during the late stages of fruit development.

The combination of SAR elicitor treatment and use of a safe postharvest dip provided substantial control of storage rots of rockmelons. The best treatment for control of storage rots involved application of BTH (50 ppm) two weeks before harvest, combined with a hot iodine dip (55° C) of fruit, achieving equivalent or better disease control than use of guazatine fungicide dip.

DECLARATION OF ORIGINALITY

The contents and subject matter of this thesis are the original work conducted by the author, except where otherwise acknowledged. None of the work has been previously submitted either in whole or in part, for a higher degree at this or any other institute.

Anowarul I Bokshi

ACKNOWLEDGEMENTS

There have been many without whom I could not have finished my project. However, I wish to acknowledge a few of them for their outstanding support and assistance during my study.

It is my great pleasure to thank Dr. Robyn McConchie for her enormous support with continuous advice and guidance, right from the initiation of the project and on the preparation of this thesis. I would like to specially thank to Dr Jenny Jobling for her cheerful support and encouragement during my research. My grateful thanks to Dr. Kerrie McDonald for her initiatives and effort to enrich the molecular biology laboratory with sophisticated instruments and helping enthusiastically with the biochemical assays. Also thanks to Prof. Brian Deverall for his enthusiastic advice on a key portion of this project regarding systemic induced resistance and disease reactions. I would like specially thank to Prof. Burgess Lester and his student Tran Nguyen Ha for their generous advice and diagnosis of the plant pathogens involved in the research.

I express my deep gratitude to Dr Stephen Morris for his encouragement, scholastic guidance with constructive analysis and valuable suggestions given with great enthusiasm throughout the whole period of the project. To Michael Forbes Smith and Penelope Symes for their support for a comfortable working environment at Sydney Postharvest Laboratory. I would like to thank friends and colleagues at the Sydney Postharvest Laboratory and Food Science Australia, CSIRO North Ryde for their continuous support over the last four years.

Also grateful thanks to Glen Foxwell for his help and assistance in conducting field experiments at Camden, Ivan Desailly and Jarka Geisler for their help in the laboratory. My thanks also to all staff and students in the Faculty of Agriculture, Food and Natural Resources and to all my friends for their help and encouragement during my study.

At Griffith, Gary Amaro and his family and workers allowed me unlimited access to their farm and postharvest processing facilities, as well as helping with supplying and transporting melons to Sydney for my research. Their enthusiastic support during the field experiments definitely helped make life easier in a remote country area.

Finally, I would like to gratefully acknowledge my wife, Anjuara Begum for her continuous inspiration and also taking care of me and all the family during my study, giving me minimum burden in family management. Also grateful thanks to all of my family and friends who shared my difficulties and offered sympathy and compassion during my research.

Last but not least, my grateful acknowledgement of the F.H. Loxton foundation, which provided the scholarship for my degree and the Australian Centre for International Agricultural Research (ACIAR) for funding my research project.

CONFERENCES ATTENDED AND PUBLICATIONS FROM THIS THESIS

1. Conferences attended and presented during PhD:

28-30 September 2005:	Australasian Postharvest Conference, Rotorua, New Zealand.
1-3 September 2004:	International Symposium on Harnessing the Potential of Horticulture in the Asia Pacific Region, Coolum, Australia.
1-3 October 2003:	Australasian Postharvest Conference, Brisbane, Australia.
29 September – 2 October 2002:	Australian Society of Horticultural Science Conference, Sydney Australia.

2. Publications; independent but related

Bokshi, A.I., Morris, S.C., McDonald, K., and McConchie, R.M. 2007. Environmentally Safe Control of Postharvest Diseases of Melons by Integrating Heat Treatment, Safe Chemicals and Systemic Acquired Resistance. *New Zealand Journal of Crop and Horticultural Science*, 35: 179–186.

Bokshi, A.I., Morris, S.C., McConchie, R. and Deverall, B.J. 2006. Pre-harvest application of INA, BABA or BTH to control post-harvest storage diseases of melons by inducing systemic acquired resistance (SAR). *Journal of Horticultural Science and Biotechnology*, 81: 700-706.

Bokshi, A.I., Morris, S.C., McDonald, K., and McConchie, R.M. 2005. Application of INA and BABA control pre and postharvest diseases of melons through induction of systemic acquired resistance. *Acta Horticulturae*, 694: 416-419. Proceedings of the International Symposium on Harnessing the Potential of Horticulture in the Asia-Pacific Region.

Bokshi, A.I., Morris, S.C., An Li, Feng, Z., McDonald, K. and McConchie, R. 2005. Evaluation of conventional fungicides, heat treatment and safe compounds in hot solutions for the control of postharvest diseases of melons. *Acta Horticulturae*, 694: 411-415. Proceedings of the International Symposium on Harnessing the Potential of Horticulture in the Asia-Pacific Region.

McDonald, K.L., McConchie, R.M., **Bokshi**, **A.I.** and Morris, S.C. 2004. Heat treatment: A natural way to inhibit postharvest diseases in rockmelon. *Acta Horticulturae*, 682: 2029-2033. Proceedings of the V International Postharvest Symposium.

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ABBREVIATIONS

ABS	Australian Bureau of Statistics
ANOVA	analysis of variance
ASA	acetylsalicylic acid
BABA	β-aminobutyric acid
BTH	benzothiadiazole
cfu	colony forming unit
CH_3COONa	sodium acetate
CRD	completely randomized design
EDTA	ethelenediaminetetra acetic acid
ET	ethylene
GLM	generalized linear model
GRAS	generally regarded as safe
HCl	hydrochloric acid
H_2O_2	hydrogen peroxide
HR	hypersensitive response
INA	2,6-dichloroisonicotinic acid
JA	jasmonic acid
LSD	least significant difference
mRNA	messenger ribonucleic acid
$NaHCO_3$	sodium bicarbonate
Na_2MoO_4	sodium molybdate
NaOCl	sodium hypochlorite
$(NH_4)_2MoO_4$	ammonium molybdate
NS	not significant
PDA	potato dextrose agar
PM	powdery mildew
PR-proteins	pathogenesis related proteins
RCBD	randomized complete block design
RH	relative humidity
SA	salicylic acid
SAR	systemic acquired resistance
SE	standard error
SIR	systemic induced resistance
SPL	Sydney Postharvest Laboratory
TMV	tobacco mosaic virus
Tween 20	polyoxyethylene sorbitan monolaurate

CHAPTER I

INTRODUCTION

Melon production is an important horticultural industry in Australia, with a wide variety of melons grown throughout the country (Possingham, 1998). Melons are grown year round, across the country during the warmer seasons of the different regions, mostly for the domestic market (Morris *et al*., 2001). With increased production, export markets have begun to develop. However, overproduction and oversupply of melons in the local market has caused price fluctuations which has discouraged growers in recent years (ABS, 2004). A lack of economically feasible transport, unreliable postharvest storage technology and the relatively short storage life of melons are hindering the development of a solid export market (Sykes, 1990). Exporting melons to distant markets where it takes more than 3 weeks to transport them is of particular concern (Mayberry and Hatz, 1992).

Australian melons are popular in foreign markets and can attract high prices because of the superior fruit quality (Edwards and Blennerhassett, 1990). However, postharvest deterioration of melons caused by storage rots is a major concern especially during long distance transport to export markets (Wilson and Pusey, 1985). In Australia, postharvest decay of melons mainly occurs from infection by *Fusarium* spp., *Geotrichum*, *Rhizopus* spp., *Cladosporium* spp., *Alternaria* spp. and *Pseudomonas* spp. (Morris and Wade, 1983). Disorders in storage may also occur such as desiccation, chilling injury, over ripening, and loss of firmness, which all result in loss of marketability of melons.

Postharvest research into the development of technologies that enable produce to reach export markets without deterioration in quality, can be profitable to the industry, as well as

benefit consumers (Johnson *et al*., 2001). The current practice of control of storage rots of melons is through dipping harvested fruit in fungicides. Continuous use of fungicides has faced two major obstacles; increasing public concern regarding contamination of perishables with fungicidal residues, and proliferation of resistance in the pathogen populations (Tripathi and Dubey, 2004). Moreover, many countries do not allow importation of produce that has been treated with fungicides (Droby *et al*., 1998). These issues suggest there is an urgent need to develop safe, effective, non-pesticide treatments for disease control in fresh horticultural produce (Fallik, 2004).

With the present change in emphasis on the use of fungicides, hot water dipping of postharvest produce has been explored as one of the possibilities for controlling storage rot pathogens (Klein and Lurie, 1991). Hot water treatment is relatively easy to implement because of the short duration of its treatment. The hot water can be recycled effectively as most of the pathogens cannot survive at a temperature range above 50°C (Barkai-Golan and Phillips, 1991; Lurie, 1998). Hot water treatments are thought to be partly dependent on the elimination of incipient infections by removing spores from the wounds of fresh produce, as well as reducing their viability (Couey, 1989). The reduction of decay incidence from hot water treatment is also due to the induction of biochemical molecules in the host tissues which are responsible for inhibition of fungal growth (Ben-Yehoshua, 2003; Schirra *et al*., 2000). Hot water treatment keeps the fruit firmer, causes less weight loss and maintains fruit quality under storage (Fallik *et al*., 1999; Paull and Chen, 2000; Lingle *et al*., 1987).

Many chemicals which are used as food additives or for food processing have been reported to control the postharvest storage rots of fruit and vegetables (Palou *et al*, 2002). Some of the promising chemicals are bicarbonate salts (Smilanick *et al*, 1999), acetates and

molybdate salts (Palou *et al*, 2002) and iodine (Morris and Bokshi, 2002). These chemicals have shown broad-spectrum antimicrobial properties and are Generally Regarded As Safe (GRAS) compounds that do not require expensive testing and validation by regulatory agencies (Aharoni *et al*, 1997). However, none of the alternative physical or non-pesticide chemical treatments can, by themselves, provide equivalent control to that of synthetic fungicides (Palou *et al*., 2002). A combination of various alternatives could be a suitable technology that could equal the effectiveness of synthetic chemicals (Conway et al., 2004).

As a fungicide alternative, biological control of postharvest diseases using antagonists has been extensively studied (Nunes *et al*., 2001; Wei *et al*., 1999). However, the possible adverse effect of the microorganisms on the physiology of the plants and the expense of development has limited their use (Nunes *et al*., 2002). Another alternative is the development of disease resistant varieties either by conventional breeding or genetic engineering (transgenic plants). Both of these breeding processes take a long time and need a thorough study of target genes, the pathogens and host plant (Mount and Berman, 1994). Furthermore, there are still questions about the commercialisation of transgenic varieties due to public concern in many countries.

In recent years much attention has been given to the control of plant disease through induction of resistance by means of physical, biological or chemical elicitors. After treatment by an elicitor, a plant is stimulated to activate its defence mechanisms, such as formation of physical and chemical barriers, and so minimise disease incidence. (Kombrink and Schmelzer, 2001). Natural resistance mechanisms of the plant are mostly activated in response to pathogen attack; however, they also can be induced or accelerated by physical treatment or application of a biological agent or a chemical activator (Kuc, 1982; Ryals *et*

al., 1994). The mechanism of inducing systemic resistance in plants against pathogens works on the same principle both for biological and chemical inducers (Lucas, 1999).

Induction of systemic resistance, by the use of a pathogenic or a non-pathogenic microorganism, has been reported for many crops (Jenns and Kuc, 1980; Kroon *et al.*, 1991; Kuc, 1982). Although induction of systemic resistance in plants using biological elicitors may not yet have been useful for commercial purposes of disease control in the field, it has provided researchers with a basis for investigation and a platform for the development and selection of chemical agents that induce resistance (Owen, 1995).

Induction of natural disease resistance in harvested horticultural crops using chemical elicitors has received increasing attention over recent years (Terry and Joyce, 2004). There are a number of reports in recent times on induction of systemic resistance by spraying chemical activators on horticultural crops for the control of postharvest diseases (Bokshi *et al*., 2003; Huang *et al*., 2000; Terry and Joyce, 2000; Willingham *et al*., 2002). The induction of systemic acquired resistance (SAR) has mainly focused on the treatment of intact plants for the control of pre and postharvest diseases. However, in a recent report it is suggested that application of benzothiadiazole (BTH), an SAR inducer, to freshly harvested apples can lead to resistance against storage diseases (Liu *et al*., 2005).

A number of chemicals having no direct antifungal action have been reported to induce systemic resistance to pathogens when applied to plants (Kessmann *et al.,* 1994). Recently, a number of chemical activators have been identified that confer broad spectrum efficacy against pathogens on a wide variety of crops including cucurbits (Tally *et al*., 2000). The functional analogues of salicylic acid such as 2,6-dichloroisonicotinic acid (INA) have been

reported to induce resistance by mimicking some aspects of pathogen attack, possibly accelerating the normal responses to further infection (Uknes *et al*., 1992). β-aminobutyric acid (BABA) is another compound rarely found naturally in plants but also a potent inducer of systemic resistance (Jakab *et al.*, 2001). The resistance induced in INA or BTH or BABA-treated tissues correlated well with the accumulation of a number of enzymes such as chitinase and peroxidase; the antifungal potential of these enzymes has been demonstrated before (Ippolito *et al.*, 2000; Mauch *et al*., 1988).

Research conducted on chemical or biological elicitors shows potential for controlling field as well as postharvest diseases through induction of resistance in the host plant. After many years since the first report on SAR using the treatment of inducers, and many more publications around the world, the stage has been reached to utilise the technology for disease control on a commercial scale (Kuc, 2001). However, for their commercial use in a wider varieties of crops, a range of issues still needs to be addressed. Attention should be given to individual plant-pathogen interactions, to determine the effective inducers, their optimum concentration, as well as the putative defence compounds induced and the timing of their appearance (Kuc, 2001). In addition, more applied and basic research is required to fully understand the role systemic resistance can play in controlling postharvest diseases commercially (Terry and Joyce, 2004).

This study aims to assist in the expansion of local and export markets of Australian melons for the benefit of the growers as well as to increase customer satisfaction. The study investigates ways in which the use of conventional fungicides can be reduced as a postharvest treatment of melons, and can be replaced with safe compounds of low or no residual effect. In this study we investigated use of GRAS chemicals as well as different

temperature hot water treatments and compared them with current commercial application procedures.

Systemic induced resistance as a means of disease control strategy against field and storage diseases of melons was also investigated. We selected chemicals which had been reported to have no residual or fungicidal effect, to induce natural resistance and assess their efficacy in protecting against field and postharvest disease in melons. Studies sought to find the appropriate stages of plant growth for the development of systemic resistance in plants without compromising crop yield and quality. The concentration of the SAR elicitors were also investigated to enable maximum expression of induced resistance at the time of harvest for disease resistance of fruit in storage.

CHAPTER II

LITERATURE REVIEW

2.1 THE MELONS

Melons are well-known members of the Cucurbitaceae family, especially watermelons, rockmelons and honeydew melons. In addition to particular plant, fruit and seed characteristics and physiology, melons with excessive moisture are called watermelon and those with a pleasant flavour are called muskmelon (Mallick and Masui, 1986). Watermelons are grouped under the genus *Citrullus* and muskmelons under the genus *Cucumis*. The muskmelon (*Cucumis melo*) is a polymorphic species that historically has been divided into a number of botanical subspecies (Sykes, 1990). However, there are a number of other varieties, the main ones being Galia melons, Musk melons and Hami melons (Agri. Notes, 1998). Melons generally occupy a prestigious position in the vegetable market because of their attractive appearance and pleasant flavour (Mallick and Masui, 1986). 'Rockmelon' (also known as Cantaloupe) and 'honeydew' are the two main types of melons in Australia and are available year round (ABS, 2004).

2.2 AUSTRALIAN MELON INDUSTRY

With the rapid increase in melon production since the early '70s, Australia now has an important melon industry, providing produce for domestic and for export markets (Possingham, 1998). In the year 2005, there were about 300 commercial growers in the industry producing about 217,000 tonNes from 8,500 hectares of land (Aust. Melon Ass., 2005), 30% of which was rockmelons (Table 2.2.1). The production areas for cantaloupe and honeydew melons move across the country following the warmer seasons. Melons are therefore available all year round for the domestic market (Morris *et al*., 2001). The main

producing areas are the Burdekin Valley in Queensland, the Murrumbidgee Irrigation area in NSW, the Murray Valley region in NSW and Victoria and the Ord River area in Western Australia. The industry is estimated to be worth in the vicinity of about $100 million during the production year 2001/02 (ABS, 2004).

Table 2.2.1 Production of rockmelon (tonnes) by year for each state

Year	NSW	VIC	QLD	WA	SA	NT	**Total**
1998	16,500	6,232	42,427	14,523	4,016	1,418	**85,115**
1999	21,470	8,413	54,581	12,900	2,792	889	**101,045**
2000	16,381	5,429	49,012	12,470	2,726	1,046	**87,064**
2001	34,983	7,304	31,129	12,972	5,212	352	**91,952**
2002	21,480	5,159	36,163	9,656	773	870	**74,101**
2003	16,798	5,742	30,242	10,868	455	45	**64,150**
2004	14,801	5,028	43,154	11,850	375	1,460	**76,667**
2005	10,695	5,413	39,407	10,645	201	1,705	**68,066**

Source: ABS, 2007

2.3 PROSPECT OF EXPORTING AUSTRALIAN MELONS

The Australian melon industry is predominantly focused on local markets, however, in recent years the export market has grown considerably (Aust. Melon Ass., 2005). Australia has the potential to expand melon exports to countries of the northern hemisphere especially Asian markets and also to Europe. However, the distance from these potential export markets makes it difficult to maintain quality during shipment, because of their susceptibility to disease and the relatively short postharvest life. Despite these constraints Australia is developing a strong export market for melons in Hong Kong, Singapore and

New Zealand (Aust. Melon Ass., 2005). Expansion of the industry depends on minimising postharvest losses and quality deterioration during storage and long-distance transport (Morris *et al.*, 2001).

The current situation within the melon industry of Australia is one of overproduction and over supply (ABS, 2004). During the last several years, production has been increasing more than 40% each year, but there has not been a corresponding rise in exports (ABS, 2004). In fact, the last few years' exports have decreased by about 5% in tonnage and 14% in currency (Table 2.3.1). This is largely because of the lack of an economically feasible transportation system for this perishable product which needs precise storage conditions to control losses (Sykes, 1990).

Table 2.3.1 Australia's major rockmelon export markets

Country of destination	2001/02		2002/03		%variance	
	tonnes	$000	tonnes	$000	tonnes	$000
Hong Kong	4,694	7,396	3,142	4,584	-33	-38
Singapore	3,842	5,637	3,642	4,927	-5	-13
New Zealand	3,605	3,038	4,510	4,089	25	35
UAEM	1,271	2,052	1,543	1,968	21	-4
Malaysia	406	558	224	312	-45	-44
Brunei	85	246	92	272	8	11
Mauritius	45	43	49	60	9	40
Bahrain	119	181	80	146	-33	-19
Maldives	87	101	57	83	-34	-18
India	57	74	64	96	12	30
Others	140	208	221	250	58	20
Total	**14,351**	**19,534**	**13,624**	**16,787**	**-5**	**-14**

Source: ABS, 2004

Australian melons are very popular in foreign markets and can attract high prices providing the melons are of premium quality and are free of spoilage or any rind disorder (Edwards and Blennerhassett, 1990). A successful export market is dependent on development of technologies that improve quality, packaging and storage conditions.

2.4 PROBLEMS ASSOCIATED WITH EXPORTING AUSLRALIAN MELONS

Postharvest losses can be greater in export rather than domestic markets and often deter industries engaging in the export of fresh commodities (Wilson and Pusey, 1985). Postharvest disease management, which reduces shelf life and quality, is the main constraint on the Australian melon industry and is the major issue limiting the progress of export markets for Australian melons (Morris *et al*., 2001).

Shipment of honeydew melons to Europe and South East Asia is serviced by air freight which is costly and often difficult due to space limitations (Edwards and Blennerhassett, 1994). Sea freight is less costly and would allow larger consignments of melons enabling greater financial returns to growers. However, the relatively short storage life of melons (Mayberry and Hatz, 1992) limits the possibility of exporting to long distance markets via sea, since transport to those destinations takes more than three weeks. The lack of a well accepted and established postharvest handling method for shipment by sea, coupled with the poor understanding of cultural and varietal impacts on storage quality, often results in poor product outturn (Mayberry and Hartz, 1992).

2.5 POSTHARVEST LOSSES OF MELONS

2.5.1 *Losses from diseases and storage conditions*

Rockmelon fruit has a relatively shorter storage life than honeydew fruit. At temperatures between 2 and 5° C with a humidity level around 90%, rockmelon can be stored for two to three weeks, whereas honeydew melons for up to four to five weeks at a temperature of 8° C (Carr. Corp., 1995). In horticultural produce, postharvest disease caused by fungi usually begins as either latent infections established in the field or from infection through wounds during postharvest handling (Terry and Joyce, 2004).

A substantial loss of marketability may occur within a few weeks of storage if timely postharvest treatments have not been applied. Development of fungal rots is a key factor in postharvest deterioration of melons. In Australia, melon postharvest decay mainly occurs from development of the fungal pathogens *Fusarium* spp., *Geotrichum* spp., *Rhizopus* spp., *Cladosporium* spp., *Alternaria* spp. and the bacterial pathogen *Pseudomonas* spp. (Morris and Wade, 1983). It has also been estimated that the average disease loss after transport to distant markets on the east coast is 30-50% and has even reached 80% in extreme cases (Morris, 1977). Heavy postharvest losses may occur due to cultivar susceptibility to disease, rough handling after harvest, inadequate packaging and temperature management and long transport times (Mayberry and Hartz, 1992).

In addition to fungal rots, desiccation is another major limiting factor of melons in storage life (Lester and Bruton, 1986). Postharvest water loss in rockmelon is relatively rapid under low humidity conditions. For example, rockmelon stored at 4° C in 85-95% relative humidity, which are typical commercial storage conditions, may lose up to 5.7% of fresh

weight in 20 days of storage. The extent of this water loss causes a decrease in firmness and loss of membrane integrity which results in loss of visual quality and marketability.

2.5.2 *Losses from field diseases*

In cucurbits, powdery mildew (*Podosphaera xanthii*) is a major disease, attacking both field and glasshouse grown plants (Reuveni *et al*., 1997). Downy mildew (*Pseudoperonospora cubensis*) diseases have also been reported on several economically important crops, including cucurbits, causing severe economic losses in some regions and seasons (Thakur and Mathur, 2002). These diseases are prevalent in production areas during periods of high humidity often accompanied by frequent rainfall (Thomas and Caniglia, 1997), and are normally controlled by using protective fungicides. Disease outbreak in the field may cause defoliation of vines and it is suspected to cause premature ripening of fruits. The fruit is thus less sweet than fruit harvested from healthy vines (Vawdry, 1994).

Disease outbreak is difficult to control without a standard fungicide, especially powdery mildew which has demonstrated a high potential for developing resistance against common fungicides (DPI Notes, 2005). However, good control of powdery mildew is possible by using low toxic biocompatible controls that have low animal toxicity and less potential risk to the environment such as potassium salts early in the disease development stage (McGrath and Shishkoff, 1999). Cultivation of a resistant variety or use of protectant fungicides has been recommended for resistance management. However, the difficulty of adequate coverage of protectant fungicides on the plant limits the control of the fungus. Therefore, use of a resistant variety or inducted plant materials could be a practical strategy for the control of the disease.

2.6 POSTHARVEST STORAGE DISEASES: FACT AND FACTORS

The presence or penetration of pathogens on harvested fruits does not automatically ensure disease development. Rather, certain conditions are needed to establish the infection process and to aid further development of disease in stored produce. These conditions include appropriate temperature and humidity, available nutrients for the pathogens and other environmental conditions (Barkai-Golan, 2001). Temperature and humidity are the two basic or even limiting factors for infection development on postharvest produce. However, the growing conditions of various storage fungi can differ even among the fungus of different species isolates.

In order to choose the appropriate strategy for disease control, we require an understanding of the mode of infection of the pathogen, the biology of the host and the environmental factors that affect disease development. Temperature is usually the most important factor limiting the postharvest life of fruit and vegetables; fruit held at lower temperature have better appearance and maintain qualities such as flesh colour and firmness, than those held at higher temperatures (Yang *et al*., 2003). Lower temperatures significantly increase the storage life of rockmelons by reducing the breakdown of fruit caused by disease, dehydration and fruit ageing (Salvestrin, 1988).

2.6.1 *Effect of temperature on disease development*

Some pathogenic fungi can grow on the host tissue at low temperatures. It has been reported that *Alternaria alternata,* a pathogen of apples, can develop disease in storage at 0° C or below, and can be active at temperatures as low as -3° C (Sommer, 1985). On the other hand some pathogenic fungi cannot grow at all and do not survive at low storage temperatures. *Rhizopus stolonifer* for example, cannot generally develop spores at

temperatures below 5° C, although a certain percentage of the spores can germinate at temperatures as low as 2° C, but their germ tubes cannot continue growth (Dennis and Cohen, 1976). Therefore, these fungi are not active on the host tissue and cannot develop disease (Barkai-Golan, 2001). However, most of the storage pathogens can grow and develop rots over a range of 15 to 25° C, with a significant increase in rot severity at higher temperatures (Stephens *et al*., 1997). Temperature levels of 20-30° C were found to support many storage pathogens leading to development of postharvest diseases (Pardo *et al*., 2004, 2005).

Extremely low temperature in storage does not always guarantee prevention of fungal development. There is also a danger in exposing fresh produce to conditions that may cause tissue damage to the fresh produce, yet do not inhibit infection or disease development, and possibly even enhance the infection and the disease development on the host tissues (Segall, 1967). For instance, an extremely low temperature, below 2.5° C for longer than two weeks, is believed to damage the skin tissues of melons, causing surface decay and the development of pitting like symptoms (Evensen, 1983). Increased incidence of decay also occurs with increase in storage temperature, indicating a need to critically manage the storage temperature. The industry standard temperature for melon storage in Australia has been recommended at 5-8° C (Agric. Notes, 1998). However, different types of melons have different recommended safe temperatures; for rockmelons it is 2-5° C and for honeydew it is 8° C (Morris, 1992).

2.6.2 *Effect of humidity on disease development*

A pathogen is much more damaging to plant tissue in conditions of high humidity. Plant tissues, even those considered to be resistant to pathogens, may become completely

susceptible with excessive water around the tissues during inoculation (Johnson, 1947). High humidity at ambient temperatures around harvested fruit, even for a short period of time, helps to establish infection by the pathogens (Bonnardeaux and Robinson, 1994). However, every fungal spore or bacterial cell that reaches the harvested product can not develop to cause decay, even when conditions are favourable. Only a small group of pathogens can develop rots on harvested fruit and vegetable and each fruit and vegetable has typical fungal or bacterial strains that can grow on the product. The two abiotic factors and their interaction (humidity x temperature) significantly affect the infection and growth of rots in fresh produce (Pardo *et al*., 2005).

Maintenance of a high humidity micro-atmosphere is also needed to prevent changes related to senescence, such as deterioration of membrane integrity and softening (Ben-Yehoshua, 1985). Good appearance and marketability of melon fruit requires a saturated atmosphere in cold storage to keep the weight loss less than 1% (Lester and Bruton, 1986). Weight loss of more than 5% reduces marketability of most fruit because of shrinkage. It is reported that a low level of humidity contributes to the relatively short storage life of netted melons due to high transpiration rate, with fruit becoming soft and shrivelled even stored under cool conditions (Ryall *et al*., 1979). On the other hand a high relative humidity in storage encourages most of the postharvest diseases of melons (Wadia *et al*., 1986). Hence, proper surface disinfection, performed prior to storage, becomes a significant factor in limiting storage losses arising from infections (Halloran *et al*., 1999) especially when the produce has to be stored at high humidity.

2.6.3 *Effect of harvest maturity on disease development*

A mature melon is defined as one that has reached the stage of physio-biochemical maturity which will ensure proper completion of the normal ripening process (Forbus *et al.*, 1991). Melons should be harvested as soon as they reach maturity to enable produce to reach the consumer in a good quality. On the other hand storage quality of melons is significantly affected by maturity at harvest and it is recommended that for a superior quality and longer storage life, rockmelons should be harvested at green full-slip (Evensen, 1983). Harvesting melons at green half-slip maintains superior appearance and quality in storage, but results in lack of flavour. In contrast, yellow full-slip melons are the most affected by changes in the measurement of quality during storage and marketing. Therefore, green full-slip melons have superior flavour, sugar and acid content and attractive appearance compared to other stages of maturity (Evensen, 1983).

Harvest maturity is also a determining factor in the susceptibility of fruit and vegetable to storage diseases (Eckert, 1975). Most fruit are more susceptible to pathogen attack as ripening progresses and when host tissue characteristics change during senescence. With ripening, acidity level, turgor state of the tissues and nutrient availability are the main factors that might separately, or in combination, enhance susceptibility to disease (Barkai-Golan, 2001). As the fruit ripens, tissues soften due to the solubility of cell wall compounds such as pectin or hemicellulose (Paull *et al.*, 1999; Eckert, 1978). This softening of tissues adds to the breakdown of the defence mechanism that makes fruit more susceptible to the invasion of pathogens.

During ripening the ability of tissues to produce antimicrobial compounds which inhibit the pathogenic infections and their growth diminishes (Verhoeff and Liem, 1975). Conditions

that reduce the concentration of antimicrobial compounds in the host tissues, result in increased infection and disease development. The increased availability of nutrients, softening of the tissues and reduced activity of antifungal compounds in the ripe fruit, make them prone to attack by the pathogen. Therefore, harvesting fruit at its optimum stage of maturity, before softening has occurred and when levels of antifungal compounds have not totally declined, helps to inhibit development of storage rots and provides longer storage of the fruit. Furthermore, treatments and conditions that lead to delayed ripening and senescence can thereby indirectly suppress postharvest disease development. Therefore, knowledge of biological and environmental factors for the development of storage rots in melon would help optimisation of postharvest techniques for storage.

2.7 CURRENT PRACTICES OF POSTHARVEST TREATMENT OF MELONS

After harvest and throughout the handling chain, fruit are exposed to a huge number of fungal and bacterial pathogens. To ensure a healthy storage life, postharvest washing of melons must be done as soon as possible after harvest (Bonnardeaux and Robinson, 1994). The current practice to control storage rots of melons is dipping harvested fruit in fungicides. There are a number of recommended synthetic fungicides such as benomyl and guazatine, used by growers as postharvest treatments. Since no single chemical has a sufficiently wide spectrum of activity to control all postharvest diseases of rockmelons, a mixture of fungicides is recommended at particular concentrations for effective control of rots (Edwards and Blennerhassett, 1990). The current fungicide recommendation is a mixture of benomyl (Benlate) and guazatine (Panoctine) both at 500 ppm (Dimsey, 1995).

Washing fruit in chlorine solution is a relatively safe method that is commonly used for postharvest washing of melons to control pathogenic micro-organisms during storage,

transportation and marketing (Bonnardeaux and Robinson, 1994). However, the antimicrobial activity and stability of the chlorine solution depends on the pH, the temperature and organic matter in the solution. Fungicide dips are commonly used also and the concentration can be reduced if used in combination with hot water. One such practice is hot water drench with fungicides like imazalil which appears to be more effective than cold water application for postharvest treatments and is already being implemented in many packaging houses (Ben-Yehoshua, 2003).

2.8 PROBLEMS WITH CURRENT PRACTICES OF POSTHARVEST TREATMENTS

Many synthetic chemicals are employed today for postharvest treatment of fruit and vegetables throughout the world. Fungicide residues often represent a major threat to human health, with unpredictable consequences for the trade, the economy and the environment (Schirra *et al*., 2000). However, without their use the production and marketing of these fresh produce would not be possible (Ragsdale and Sisler, 1994). Fungicides used for the control of postharvest rots of fruit and vegetable are potentially more harmful to humans than on-farm fungicide application. Postharvest pesticides are detected at relatively high frequencies and high concentrations because they are applied later than pesticides applied on-farm, and their residues are usually not exposed to rain, wind, high temperature or sunlight which reduces the residue levels (Kuchler *et al*., 1997). Furthermore, they are applied directly to edible products, sometimes with wax to ensure that they remain in contact with fruit and vegetable surfaces.

Continuous use of fungicides has faced two major obstacles; firstly, increasing public concern regarding contamination of perishables with fungicidal residues, and secondly,

proliferation of resistance in the pathogen populations (Tripathi and Dubey, 2004). Problems have arisen in many countries with regards to the use of synthetic fungicides on melons when residual levels of the fungicides were found to be several times higher than the maximum permissible levels (Anon. 1991). The use of imazalil fungicide as part of a wax application on 'Galia' melon cultivar has been shown to prevent development of *Fusarium* spp. and *Alternaria alternata*, but the treatment resulted in a high level of residue measuring 4-5 ppm, and persisted after the storage period (Aharoni *et al*., 1993). This amount of residue exceeds the acceptable tolerance of some European countries that have a residue tolerance below 0.5 ppm (Aharoni *et al*., 1992).

Consequently, there is renewed interest in alternative postharvest disease management practices that can reduce consumer and environmental risks (Droby *et al*., 1998). Because of increasing public demand to reduce the use of synthetic fungicides there is an urgent need to develop effective, non-damaging physical or non-pesticide treatment for disease control in fresh horticultural produce (Fallik, 2004).

Efforts to reduce reliance on fungicides in postharvest treatments of fresh produce have included dipping in hot water for short periods. Coates and Johnson (1993) showed that the combination of hot water and a fungicide is more effective in preventing fungal development in mangoes during postharvest storage. Although hot imazalil at 250 ppm reduced decay to the same level as cold imazalil at 1000 ppm (Ben-Yehoshua, 2003), residues from hot water are five to eight times higher in citrus fruit than residues on fruit treated in cool temperatures (Schirra *et al*., 1996, 1998). For melons, postharvest dipping in hot imazalil is not always more effective than normal cool application (Mayberry and Hartz, 1992).

Countries importing agricultural commodities have great concerns about the health risk and the environment, in relation to pesticide operations in field crops as well as postharvest treatments (Kuchler *et al*., 1997). Australian melons which are mostly dependent on fungicide for postharvest disease control are facing strict guidelines from importing countries. For these reasons there is an urgent need to find an alternative for storage disease control of melons. Australia has favourable geographical and climatic conditions for growing melons and the product is renowned for its excellent fruit quality (Sykes, 1990). The demand for Australian product is therefore very high and can be further expanded by using a safe and effective alternative to fungicides for postharvest treatment.

2.9 ALETRNATIVES TO FUNGICIDE FOR POSTHARVEST TREATMENT

2.9.1 *Biological agents*

In last fifteen years biological control of postharvest diseases has been extensively studied by using yeast and bacteria as antagonists (Filnonow *et al*., 1996; Janisiewicz, and Marchi, 1992; Madrigal *et al*., 1994; Nunes *et al*., 2001; Vinas *et al*., 1998; Wei *et al*., 1999). However, microorganisms as biological agents have a relatively narrow spectrum of activity compared to synthetic fungicides (Janisiewicz *et al*., 1992), and use of an antagonist for biological control is less economical (Nunes *et al*., 2002). These organisms can have adverse effects on the physio-metabolic processes of the plant or plant parts and also might face legislative objections (Marquenie *et al*., 2002). Therefore, physical methods that do not leave any residue on the treated products are more appropriate as alternatives to the use of chemicals.

Commercialisation of biological agents for use as postharvest treatment of fresh produce involves costly and thorough taxonomical studies on the antagonists. Huang *et al*. (1992,

1993, 1995) conducted trials for biological control of storage diseases of oranges using different isolates in the laboratory as well as a commercial packaging line. They found that antagonists can provide significant control of the disease, often as good as fungicides. However, for commercialization, companies are reluctant to invest because these biocontrol agents are linked taxonomically to pathogenic strains and further extensive investigation should be necessary to develop the technology for commercial practice (Huang *et al*., 1993). Also, the introduction of exogenous microorganisms into the food chain for biocontrol would require a number of safety procedures which would still need to gain public acceptance (Wilson and Pusey, 1985).

There are reports on decreased efficacy and lack of consistency of biological agents over generations; hence, application as a stand-alone treatment limits their use in commercial conditions (Droby *et al*., 2001). These drawbacks in the use of biological antagonists have increased interest in developing alternative control methods, particularly those which are environmentally sound and biodegradable (Tripathi and Dubey, 2004). Thus replacement of synthetic fungicides by non-toxic treatments specific in action, is gaining considerable attention.

2.9.2 *Physical treatment (heat/ hot water treatment)*

With the present change in emphasis on the use of chemical treatments, interest in heat disinfection has been revived (Spotts and Chen, 1987). Among several physical and non-pesticide technologies that are being investigated to extend the storage life of fresh produce, postharvest heat treatment has been shown to be an effective physical method for the control of a wide range of pathogens for storage rots (Lurie, 1998; Palou *et al*., 2001; Schirra *et al*., 2000; Teitel *et al*., 1989). High temperatures inhibit fungal germination and

growth. This is the basis of hot water dips which have been developed as a method of disinfection (Klein and Lurie, 1991). In recent times there has been increasing interest in the use of heat treatments on postharvest fresh produce to control insect pests, prevent fungal rots and manipulate the ripening of the commodity. Interest is mostly promoted because of increased public demand for the decrease of postharvest use of fungicides against insects and diseases (Fallik, 2004).

2.9.2.1 *Postharvest disease control by treatment with hot water*

Hot water dipping of postharvest produce has been explored as one of the possibilities for controlling storage rot pathogens (Klein and Lurie, 1991). The advantages of hot water treatment are that it is an efficient medium for heat transfer and is relatively easy to use. The water used for heat treatment can be recycled effectively as most of the pathogens cannot survive at the temperature range recommended for variouscrops (Barkai-Golan and Phillips, 1991; Lurie, 1998). In addition to killing the pathogens, hot water treatment helps in maintaining fruit quality during prolonged storage and marketing (Fallik, 2004).

Treatments with hot water for the control of postharvest decay are often applied for only a few minutes because only the surface of the commodity requires heating (Lurie, 1998). However, many fruit and vegetables tolerate exposure to water temperatures of 50-60°C for up to 10 min. However, such a long duration of dipping is not required for the control of most postharvest pathogens (Barkai-Golan and Phillips, 1991). Melons treated with hot water for as little as 15 sec had significantly less disease than untreated fruit, after a prolonged period of storage and marketing (Fallik *et al*., 2000). The efficiency of hot water treatment is dependent on the temperature and time of exposure of the product to hot water.

Therefore, a higher water temperature (about 60°C) for the treatment of fruit can compensate for a short time of exposure (10 sec.) to heat (Ben-Yehoshua, 2003).

Postharvest treatment with hot water is thought to reduce decay incidence by limiting the sites of fungal penetration into the fruit and to keep fruit firmer due to less weight loss (Fallik *et al*., 1999). Reduced fungal infection after hot water application may be due to recrystallization or melting of the wax layer, which seals the micro-openings through which pathogens could get entry. Improvement of the physical barrier due to heat treatment through redistribution of the epicuticular wax layer resulting in a significant reduction in cuticular cracks, has also been reported by Schirra and D'Hallewin, (1997), Fallik *et al*. (2000) and Porat *et al*. (2000a).

2.9.2.2 *Hot water treatment enhances quality of fresh produce*

Heat treatment not only reduces the incidence of diseases but also improves the quality of fruit under prolonged and controlled storage conditions. A two min dip of citrus at 53°C has been found to markedly reduce the sensitivity of citrus and other fruit to chilling injury (Ben-Yehoshua, 2003). Heat treatment has been found to induce activities of catalase, ascorbate peroxidase and superoxide dismutase. Catalase is thought to be a major antioxidant operating in the heat-induced chilling tolerance of cold-stored mandarin (Sal and LaFuente, 1999). Postharvest dipping of some commodities, such as muskmelons in hot water improves the sugar content by preventing the loss of sucrose that occur in non-heated fruit during storage (Lingle *et al*., 1987).

Heat treatment has been reported to affect the ripening of climacteric fruits. Ripening of most climacteric fruit is characterised by softening of the flesh, an increase in the sugar/acid

ratio, enhanced colour development, increase in ethylene production and respiratory activity, and elevated rate of protein synthesis. Exposing fruit to high temperatures attenuates some of these processes, and enhances others (Klein and Lurie, 1991). Ripening of climacteric fruit is known to be inhibited or accelerated by heat treatment following an alteration of gene expression (Paull and Chen, 2000). However, they also reported that the extent of the alteration of fruit ripening is a function of the exposure temperature and duration, and how quickly the commodity is cooled following the heat treatment.

Ripening of green tomatoes is inhibited by heat treatment (Lurie, 1998), however, ripening of mangoes is accelerated (Prusky, 1996). Paull (1990) also reported delay in ripening and increase of storage life of fruit and vegetables treated with hot water. The delay in ripening of heat treated mature green tomatoes was because the degradation rate of peroxidase was delayed, which maintained resistance against decay pathogens (Lurie *et al*., 1997). Similarly, Sherf and Kolattukudy (1993) reported an association between increased susceptibility of tomatoes to decay and decrease or disappearance of peroxidase. Postharvest heat treatment actually delays other important ripening characteristics involved with maintaining fruit quality in storage by transiently inhibiting volatile production (Fallik *et al*., 1997). Hence, mature green fruit are more resistant to infection than ripe yellow fruit.

Hot water treatment also helps to maintain fruit firmness and freshness during storage. The sealing of cracks by melting waxes reduces weight loss, thus maintaining fruit firmness after prolonged storage (Fallik, 2004). One report shows that melons lost their firmness when treated in hot water at 55°C for 90 sec (Halloran *et al*., 1999). However, others did not show any loss of firmness in respect to hot water treatments (Barkai-Golan *et al*., 1994; Lester and Tyrley, 1992; Yahia and Rivera, 1994).

2.9.2.3 *Mode of action of hot water treatment*

2.9.2.3.1 *Effects on pathogen from hot water treatment*:

Hot water treatments are thought to be partly dependent on the elimination of incipient infections by removing spores from wounds of the fresh produce and acting directly on their viability (Couey, 1989). The mode of action of hot water dips on decay of fresh produce has been reported to be a direct interaction with the fungi, perhaps by killing ungerminated spores and slowing germ tube elongation, thus slowing disease development in storage (Fallik *et al*., 1996). Previously it has been reported that failure or delay in rot development is a result of slow growth of distorted hyphae germinated from the inoculated spores of heat treated fruit (Fallik *et al*., 1995). Similar observations have been made by Schirra *et al*. (2000) and Wijeratnam *et al*. (2005) who stated that heat treatments have a direct effect of slowing germ tube elongation or of inactivating or outright killing germinating spores.

Hot water treatment for postharvest dipping is not always a reliable disinfection method for the fresh produce. It has been reported that hot water dips at 50-53°C have proven to be ineffective in killing dormant spores but can reduce superficial pathogens effectively (Barkai-Golan and Phillips, 1991; Dettori *et al*., 1996). In contrast, germinating spores were found to be more sensitive to heat treatment than was mycelial growth (Fallik *et al*., 1993). An investigation by Schirra *et al*. (2000) found a negligible amount of fungal spores were eliminated or removed from wound inoculated fruit following a two min wash with hot water at 52°C. However, the reduction of rot development following hot water treatment was explained as a reduction of spore survival of various decay causing pathogens (Williams *et al*., 1994). The water is recycled, but because temperatures used are more than 50°C, organisms which are washed off the product into the water do not survive (Lurie, 1998).

2.9.2.3.2 *Induction of resistance in the host tissue from hot water treatment*:

The mode of action of the heat treatment has been attributed to inhibiting fungal penetration of the host tissue as well as to the host tissue developing resistance via the formation of physical and chemical barriers against fungal growth (Ben-Yehoshu, 2003; Schirra *et al*., 2000). The formation of chemical barriers includes inducing antifungal substances that inhibit fungal development in the fruit tissue, and enhancing wound healing. The heat shock response is manifested in most living organisms as induction or enhanced synthesis of heat shock proteins (Ferguson *et al*., 2000). Heat treatment can induce PR-proteins such as chitinase and β-1,3-glucanase, stabilise membranes, elicit antifungal compounds, or inhibit the cell wall degrading enzymes and delay the degradation rate of antifungal compounds that are present in freshly harvested fruit (Schirra *et al*., 2000).

It is also thought that heat treatment can delay the incidence of decay for a few days by arresting the growth of pathogens and enabling the fruit to build up its mechanism of resistance (Ben-Yehoshua, 2003). Whatever the mechanism involved, it is obvious that employing hot water treatments has significantly reduced decay development on several fresh harvested commodities (Ben-Yehoshua *et al*., 2000; Fallik *et al*., 2000; Porat *et al*., 2000a). However, the defence mechanisms against the pathogens are complex interactions with various types of response such as formation of physical barriers to pathogens and/or induction of antimicrobial compounds like PR-proteins (Bell, 1981; Couey, 1989). Evidence suggests that heat treatments are fungistatic but not fungicidal where pathogens are inhibited by both thermal inhibitions, and by the enhanced resistance of the fruit against the pathogen (Schirra *et al*., 2000).

Heat treatment may be effective either by directly inhibiting pathogen development, or by inducing natural resistance in the fruit (Klein and Lurie, 1991). Induction of resistance against decay due to hot water treatment of 'Star Ruby' grapefruit for two min at 62°C before inoculation has been reported by Porat *et al*. (2000b). The resistance was found to be most effective when inoculation was carried out one day after hot water treatment. Less effective resistance was observed when inoculation was carried out on the same day or seven days after heat treatment.

2.9.2.3.3 *Changes in physiology from hot water treatment*:

Hot water treatments are thought not only to retard pathogenic infection, but also to develop resistance in fruit due to changes of the physiological state of the peripheral cells (Schirra *et al*., 2000). Temperatures which inhibit the growth and penetration of fungal cells are also likely to disturb adjacent pericarp cells (Olesen *et al*., 2004). Moreover, Lurie (1998) suggested that hot water treatment inhibits pectic hydrolysis because of reduced level of cell wall degrading enzyme activity and ethylene production due to a reduction in the activity of ethylene-forming enzymes. Klein and Lurie (1991) suggested that treating fruit in hot water results in limited damage to the respiratory mechanism, which in turn delays ripening and ultimately extends the storage life of hot water treated produce. Hot water treatment of harvested fruit before storage reduces respiration and ethylene evolution and thus inhibits ripening and increases the shelf life of fruit (Fallik *et al*., 1999, 2000, 2001; Ilic *et al.*, 2001).

2.9.2.4 *Factors affecting the efficacy of hot water treatment*

Dipping melons in hot water not only reduces pathogens causing storage disease but can also significantly improve the storage life and marketability of fruit (Fallik *et al*., 2000).

The effectiveness of dipping fruit in hot water against storage pathogens depends on the temperature and/or duration of dip time of fresh produce in hot water. It was observed that a higher water temperature could compensate for a shorter dip time of fresh produce (Ben-Yehoshua, 2003). But a higher water temperature for dipping fresh produce even for a short period of time can damage the rind tissues. Teitel *et al*., (1991), showed that 'Galia' melons were damaged when dipped in hot water at 60° C for 30 sec only; however, this did not significantly compromise marketability.

On the other hand Mayberry and Hartz (1992) found that dipping muskmelon at 60° C for 3 mins controls surface mould development in storage without any heat injury to the rind. They also stated that a lower temperature and/or shorter exposure treatments were less effective. They suggested a hot water dip at 55° C for 1-2 min as optimum for a postharvest anti-fungal treatment for 'Galia' melons. However, 'Galia' melon fruit dipped in hot water above 55^{o} C and for more than two mins, damaged the skin of the fruit due to overheating and longer dip time (Mayberry and Hartz, 1992). Dipping fruit for less than half a minute under 55^{o} C has been shown to be least effective for the control of storage rots.

There are discrepancies regarding the effect of hot water dip of melons for the control of storage rots even within the same species, which may be largely because of variations in growing areas (Mayberry and Hartz, 1992). Carter (1981), in his study on postharvest decay of muskmelon, did not find significant differences in the incidence of Fusarium rot, stem scar and rind decay fungi, when dipped in water at 24° C and 57° C for 30 sec. However, Teitel *et al*. (1989) found that with a longer immersion time, a hot water dip may have provided effective protection for melon fruit against storage rots. They observed that a reduced temperature of 52° C and a longer dip time of two min controlled decay from

Alternaria spp., *Fusarium* spp., *Rhizopus* spp. and *Mucor* spp. without causing external heat injury. Furthermore, there was no evidence of heat effect on the quality and ripening variates. Similar observation made by Barkai-Golan *et al.* (1994), who reported that hot water treatment of 'Galia' melon at 52-55° C can effectively prevent storage losses caused by *Alternaria alternata, Fusarium* spp. and *Trichothecium roseum.*

2.9.2.5 *Prospects and problems of hot water treatment*

Hot water treatments of fresh produce remove not only soil and dust, but also fungal spores from the fruit surface more efficiently than washing at room temperature. The simple technique of hot water treatment of fresh produce should be explored on a broader range to reduce our current reliance on synthetic fungicides as it is environmentally friendly and involves no risk to health. Hot water treatment would reduce production costs and, in turn, would cost less for the consumers (Lurie, 1998). With the trend toward less reliance on chemical control, postharvest use of heat treatment warrants greater study and further development (Barkai-Golan and Phillips, 1991). A better understanding of the physiology, pathology, biochemistry and molecular biology of hot water-treated produce will enable the development of more precise and effective procedures in the near future (Fallik, 2004).

Postharvest heat treatment of fresh fruit and vegetable can provide good control of decay but does not provide the same protection of fruit quality as postharvest fungicides (Barkai-Golan and Phillips, 1991). One should not infer that hot water treatment alone would provide acceptable decay control in commercial packaging situations (Mayberry and Hartz, 1992). Carter (1981) suggested a longer dipping time (more than 30 sec.) and/or a mixture of fungicides and heated water for effective postharvest treatments of muskmelon. Similarly, the addition of nonpesticidal chemicals such as food additives to hot water may

increase the effectiveness of heat treatment; however, more effort is needed to find out the effective methods for the various commodities.

Although most reports have focused on the positive response of commodities to heat treatment, it is not without problems. There is always a danger of tissue damage (Lurie, 1998). This is one reason why there are a multitude of treatments and why there is a need to find a time-temperature regime which will produce the desired effect on decay control, whilst maintaining quality in storage. Tissue damage caused by heat may also result in increased decay development and may result in a poor quality commodity (Jacobi and Wong, 1992; Jacobi *et al*., 1993). Moreover, hot water at high temperatures may cause damage to the rind tissue that may affect the marketability of the fresh produce (Teitel *et al*., 1991).

The efficacy of hot water treatment on the pathogens is usually measured by reduced viability of the heated propagules (Schirra *et al*., 2000). The response of a pathogenic agent to heat can be influenced by the state of the pathogens such as maturity of the spores and amount of inoculum (Barkai-Golan and Phillips, 1991), but it does not always necessarily depend on these factors (Schirra *et al*., 2000). Although a linear relationship between the logarithms of reduction of fungal spores and time and temperature of heat treatment have been reported (Pullman *et al*., 1981; Roebroeck *et al*., 1991), the kill rate of the pathogen and the time and temperature treatment are not always proportional. Similarly fungal spores vary considerably in sensitivity to heat treatments (Rappel *et al*., 1991; Fallik *et al*., 2000).

Postharvest treatments of fresh produce with hot water only are reported to be about half as effective as a hot conventional fungicide like benomyl (Olesen *et al*., 2004; Johnson *et al*.,

2002). However, mixed results comparing the relative effectiveness of hot water and synthetic fungicides were observed by McGuire and Campbell (1993). Almost equivalent control of anthracnose on 'Tommy Atkins' mangoes was obtained from hot water dip for 3 min at 53°C and 2000 ppm of imazalil at room temperature, but the hot water treatment in similar conditions was not effective on 'Keitt' mangoes.

If a long period of dipping fresh produce is not required then a short hot water treatment by rinsing and brushing can be used to maintain fruit quality during prolonged storage (Fallik, 2004). In Australia, spray is the preferred option for washing fresh produce in the sheds and already hot water treatments by spray is practised in a number of horticultural industries such as mangoes and apples. Although dips and spray are likely to have different heat transfer characteristics, there is little difference in their effect. The concept is supported by Olesen *et al*. (2004), who compared hot water dip and hot water spray for the control of rots in lychee, and found that the hot water spray was equally effective as hot water dip.

2.9.3 *Postharvest disease control by the treatment with safe chemicals*

2.9.3.1 *Safe chemicals for postharvest dip*

In recent years, many chemicals which are used as food additives or for food processing have been evaluated for their efficacy to control the postharvest storage rots of fruit and vegetable (Palou *et al.*, 2002). Some promising chemicals include bicarbonate salts (Smilanick *et al.*, 1999), acetates and molybdate salts (Palou *et al.*, 2002) and iodine (Morris and Bokshi, SPL internal report). These have shown broad-spectrum antimicrobial properties and are generally regarded as safe (GRAS) compounds that do not require expensive testing and validation by regulatory agencies (Aharoni *et al.*, 1997).

Sodium bicarbonate is classified as GRAS by the United States Food and Drug Administration and is also proposed as exempt from residue tolerances on all agricultural commodities by the USA Environmental Protection Agency (Palou *et al*., 2001). Also sodium bicarbonate has been listed as an approved ingredient on products labelled 'organic', proposed by the United States Department of Agriculture. This salt can be a useful tool to manage postharvest decay as it is inexpensive, readily available, and can be used with minimal risk of injury to the fruit. Smilanik *et al*. (1997) stated that sodium bicarbonate solution, when used correctly, approached the effectiveness of common synthetic fungicides for the control of *Penicillium digitatum* on oranges. Although salts containing carbonate and bicarbonate anions reduced disease development compared to the water control, control was not at the level that would be considered commercially acceptable (Punja and Gaye, 1993).

A substantial reduction of blue mould incidence on oranges was reported by Palou *et al*. (2001) after a postharvest dip with 2-4% of sodium bicarbonate solution for 150 sec at room temperature, following a challenge inoculation of the pathogen. However, a high proportion of inoculum of *P. italicum* and *P. digitatum* conidia remained viable even after 5 min of exposure in a highly concentrated (10%) solution of sodium bicarbonate (Marloth, 1931). Although sodium bicarbonate more effectively controls naturally inoculated citrus fruit than artificially inoculated fruit (Smilanick *et al*., 1997), the effect is primarily fungistatic and not very persistent. Control may be due to the presence of salt residues in the wound infection courts occupied by the fungus (Palou *et al*., 2001) and therefore, the compound probably is not lethal.

Although bicarbonate salts are widely used in the food industry and have broad-spectrum antimicrobial activity, a higher concentration (>3%) may significantly reduce fruit quality (Fallik *et al*., 2004; Aharoni *et al*., 1997). Sodium carbonate and bicarbonate were equal and superior to the other carbonate and bicarbonate salts for the control of green mould on lemons and oranges (Smilanick *et al*., 1999); however, sodium bicarbonate was not recommended for use with hot water. The pH of sodium bicarbonate solutions rises rapidly at high temperatures because of carbon dioxide evolution in the air; therefore, only room temperature is advisable for use of this compound for the postharvest treatment of fruit (Smilanick *et al*., 1999).

The ability of molybdate salts such as sodium and ammonium molybdate to affect the metabolic process in several organisms and to control disease development has been reported (Grangeasse *et al*., 1998; Nunes *et al*., 2002; Wang *et al*., 1995). In laboratory conditions, the potential to control blue and grey moulds of pears by the antagonist *Candida sake,* was enhanced with the application of ammonium molybdate (Nunes *et al*., 2002). The same authors conducted a field trial where a preharvest application of ammonium molybdate followed by a postharvest treatment with the antagonist, showed a significant reduction of blue mould on pear in storage. However, preharvest applications of sodium molybdate only, without any further antagonist treatment, did not reduce blue mould on harvested pear.

The molybdate salts of sodium and ammonium gave satisfactory control of green and blue mould of lemon and oranges when treated in hot water at 48 or 50° C (Palou *et al*., 2002). The effectiveness of the salts was not increased with a further increase of temperature to 53° C. In a screening trial of low-toxic chemicals for the control of green and blue moulds, Palou *et al*. (2002) found that sodium molybdate at 24.2 mM and ammonium molybdate at

1.0 mM were effective. It was found that molybdate salts at higher concentrations were phytotoxic and stained the fruit. They also found that at non-phytotoxic concentrations, the effectiveness of these chemicals was more influenced by temperature than by concentration. The study stated that the inhibitory effects of these salts were not fungicidal but fungistatic and not very persistent. However, Nunes *et al*. (2001) found that 5 mM solutions of ammonium molybdate at room temperature were effective in controlling postharvest decay on apples caused by *Penicillium, Botrytis* and *Rhizopus* spp.

Organic acid salts such as sodium acetate were found to be as effective as sodium carbonate for the control of green and blue moulds of lemons and oranges (Palou *et al*., 2002). The salt has been reported to control postharvest diseases on lemons and oranges and have the added advantage of not being phytotoxic to the rind. However, the acid salts appear to show some selective control in that they were comparatively more effective in controlling green mould on lemons than on oranges (Palou *et al*., 2002). Like carbonate or bicarbonate salts, the inhibitory effects of the organic acid salts have been suggested to be dependent on the presence of residue within the wound infection court occupied by the fungus and on interactions between this residue and constituents of the rind.

Chlorine solutions, especially hypochlorites, are another sanitizer element being used by the growers for washing fruits and vegetables (Koponen *et al.,* 1993). Sodium hypochlorite as an active chlorine is currently recommended for the postharvest treatment of many fresh produce, but it appears to be ineffective against some of the decay pathogens even when used with common fungicides like imazalil or benomyl (Carter, 1981). The use of chlorine as a sanitizer requires extra care to keep it active because the concentration in the dip tank may drop from sequestration by organic matter present in the water (Punja and Gaye, 1993). The effectiveness of chlorine compounds usually declines because of the reduced stability

of the compound, which is mainly due to interaction with organic substances that modify the pH of the solution (Prusky *et al*., 2001).

Elemental iodine is another general sanitizer and a water purifier (Chang, 1958). The biocidally active form of iodine is widely used as a sanitizing compound in the food processing industry, especially with dairy. Iodine has been reported to be more active than chlorine against a number of organisms in water (Koponen *et al.*, 1993). The efficiency of iodine for the control of microorganisms in dirty water was found to increase at a higher temperature and pH values (Ellis *et al.*, 1993). Also the effectiveness of iodine on pathogenic organisms at low concentration makes it cost effective and less hazardous for the users (Oliver *et al.*, 1991).

2.9.4 *Combination of hot water and safe chemicals for postharvest dips*

Unfortunately, none of the alternative physical or non-pesticide controls such as food additives and low-toxicity chemical treatments that have been evaluated to date can, by themselves, provide equivalent control to those of synthetic fungicides (Palou *et al*., 2002). The need for finding suitable alternatives to fungicides to control postharvest decay has prompted research aimed at combining various alternatives into a control strategy that equals the effectiveness of synthetic chemicals (Conway *et al*., 2004). Therefore, it is important to integrate these alternative technologies to develop a treatment strategy able to reach the required levels without compromising the quality of the produce and cost to the consumer.

Over the years, combined heat-plus-chemical treatments have been developed in order to achieve decay control by using lower temperatures and shortened exposure time on the one hand and reduced fungicide concentration, on the other (Barkai-Golan, 2001). The

possibility of using hot water in combination with chemicals of low residual effect for the control of storage diseases has also been suggested (Marquenie *et al*., 2002). There are several reports on the effects of non-pesticide chemicals that suggest improvement in the efficacy by using heated solutions of the chemicals (Palou *et al*., 2002; Smilanick and Sorenson, 2001; Palou *et al*., 2001). Furthermore, other pre-storage treatments may act synergistically with heating (Ferguson *et al.*, 2000). It is speculated that a combination of the alternative methods may complement one another to overcome shortcomings of each (Conway *et al*., 2004).

We have taken the initiative to integrate alternative methods of postharvest disease control of melons to develop a treatment strategy for the quality of melons in long term storage. In our recent studies it was found that iodine as a sanitizing agent is very effective at room temperature. Moreover, use of iodine in hot water is even more effective for the control of postharvest diseases of fresh products such as melons, mangoes, avocados, oranges and many other vegetables (Morris and Bokshi, SPL internal report, 2002). As melon packaging uses dip and/or spray to wash the fruit, the addition of an extra hot water tank perhaps in lieu of extra machinery needed for the combination of safe chemical(s) would be easy to include with a minimum of re-tooling and outlay.

2.10 PLANT RESISTANCE MECHANISMS

2.10.1 *Inherent resistance mechanisms in the plant*

Plants present numerous barriers to inhibit pathogenic invasion. Physical barriers such as waxes and cuticles inhibit the penetration of pathogens (Hammerschmidt and Smith, 1997). In addition numerous chemical compounds widely present in plant species and toxic to

pathogens, are used in the defence mechanism to prevent fungal infection. Some of these compounds are already present in plants, while others are formed only in response to certain stress situations such as environmental or nutritional stresses or infection by pathogens (Kuc, 1987). The activation of chemical defence may occur systemically which spreads to other parts of the plant or remains localised and causes accumulation of the defence compound at the site of infection or stress (Ryan, 1984).

Plants have evolved a large variety of sophisticated defence mechanisms to resist colonisation by microbial pathogens and parasites (Kombrink and Schmelzer, 2001). Kombrink and Somssich (1995) described three major categories of plant defence mechanisms:

(i) immediate, early defence responses of directly invaded plant cells, starting with signal recognition and transduction and frequently leading to rapid cell death, the so-called hypersensitive response (HR);

(ii) local gene activation in the close vicinity of infection sites, resulting in the *de novo* synthesis of numerous secondary products, including phytoalexins, in the reinforcement of structural barriers, such as the cell wall, or in indirect inhibition of the pathogens;

(iii) systemic activation of genes encoding pathogenesis-related (PR) proteins, including chitinases and 1,3-β-glucanases, which are directly or indirectly inhibitory towards pathogens and have been associated with the phenomenon of systemic acquired resistance (SAR).

When plant-pathogen interactions result in disease establishment or successful host colonization, it is probably due to delayed plant defence expression, rather than to absence or inactivation of defence mechanisms (Benhamou *et al.*, 1994). Plants defend themselves

from pathogenic infection through developing resistance by a wide variety of mechanisms such as local or systemic, constitutive or inducible (Ryals *et al.*, 1994). However, the expression of resistance to a pathogen by a plant requires all mechanisms to be co-ordinately regulated (Ward *et al.*, 1991).

In response to pathogenic infection, plants rapidly sensitise multicomponent responses. Among the responses are rapid lignification in the cell wall and accumulation of antimicrobial substances around the site of infection (Kuc, 1987). Several reports suggest that lignification is a mechanism for resistance against pathogenic invasion (Massala *et al.*, 1980; Morris *et al.*, 1989; Pearce and Ride 1980; Ride, 1980; Vance *et al.*, 1980). Through lignification the infection process can be restricted in several ways. These occur mostly by i) increased mechanical resistance and ii) reduced degradation of host cell wall. The defence mechanism developed thus restricts the diffusion of antimicrobial substances and nutrients from wounds, and thus inhibits the growth of the pathogen in the host.

The mechanisms which require a plant's host metabolism to induce resistance during the course of pathogenic infection or in response to physical and chemical stresses are described as active mechanisms (Keen, 1992). In most plants including fruit and vegetables, the mechanisms limiting pathogen aggression are associated with i) pre-formed antimicrobial substances (phytoncides), ii) phytoalexins, enzymes and iii) physically resistant structures (Jarvis, 1994). Many of the efforts to understand the components forming the basis for disease resistance have revealed plant proteins and phenolic compounds accumulating to enhanced levels during the induction of resistance (Stermer, 1995). A positive correlation between plant resistance and synthesis of several proteins was found during pathogenic infection (Binder *et al.*, 1989, Christ and Mösinger, 1989).

2.10.2 *Induction of resistance in the plant*

The idea that plants might be able to develop a form of acquired immunity to infection following exposure to a pathogen has been current ever since discovery of the animal immune system in the late nineteenth century (Lucas, 1999). In the early part of the twentieth century, Chester (1933) reviewed the history of research on plant immunity and revealed that plants can protect against pathogenic infection by initiating local and systemic defence. Richmond *et al.* (1979) and Jenns and Kuc (1980) in their histological studies on induced resistance found that *Colletotrichum lagenarium* (a pathogen of cucumber) is inhibited at the point of penetration in a leaf of a plant previously inoculated with the pathogen on another leaf.

The phenomenon of induced resistance has been variously described as systemic acquired resistance (SAR) or induced systemic resistance (ISR) or systemic induced resistance (SIR). Although the terminology is not yet firmly established in this new branch of phytoimmunology, a compromise has been made to use the terms ISR and SAR synonymously (Hammerschmidt *et al*., 2001). However, in principle, all these terms denote the same phenomenon, that is, an activation of defence mechanisms in plants and several ways have been found for inducing resistance in a wide variety of plants (Cohen *et al.*, 1991; Ozeretskkovskaya, 1995; Stromberg and Brishammar, 1991). Therefore, SAR is frequently referred to as immunisation, sensitisation, vaccination, acquired immunity and sometimes cross-protection (Hammerschmidt *et al*., 2001; Lucas, 1999). The term SAR has now been established for a long-lasting response typically induced in plants in reaction to pathogen infection (Durrant and Dong, 2004).

Induction of SAR is taken to mean enhancement of resistance in a plant towards pathogens. It may result from previous treatment with a pathogen, an attenuated pathogen or a chemical that is not itself a pesticide (Deverall and Dann, 1995). Many recent studies provided evidence that immunization of plants with biotic or abiotic inducers could also effectively control disease in the field (Tuzun and Kloepper, 1995). Because of the apparent safety and broad-spectrum action of induced resistance, research is under way to identify and develop microbes, or nontoxic chemicals that can be used to induce resistance in plants and thus make this type of resistance directly applicable to disease control in the field (Kessman *et al.*, 1994; Kuc, 1995).

A simple model of SAR envisages that the initial inducer treatment (could be a physical or chemical or biological elicitor) generates a signal in the exposed tissues, which is then translocated to the remote parts of the plant, where cells are somehow primed to resist the fungal invasion (Lucas, 1999). Induction of systemic resistance now seems to be an encouraging technique for plant protection. This method is based not primarily on pathogen suppression as occurs in the application of pesticides, but on stimulating the natural defence mechanisms in plant tissues (Ozeretskovskaya, 1995). It is also suggested that a high level of resistance can be achieved even without any specific resistance genes. Therefore, SAR appears to be an essential component of the defensive repertoire that ensures plant health in nature (Uknes *et al*., 1996).

The mechanisms underlying the expression of plant defence genes indicate that artificial manipulation of plant defence could provide a biologically, environmentally and commercially viable alternative to existing pathogen control methods (Dixon and Lamb, 1990). The mechanisms of defence in plants exhibiting induced resistance are more or less similar to the mechanisms exhibiting resistance that is controlled by resistance genes or

non-host resistance (Hammerschmidt and Yang-Cashman, 1995). Many support the hypothesis that the genetic potential for resistance is present in all plants (Kuc, 1982) and that the presence of a strain-specific resistance gene is apparently not required for induced resistance to develop (Kessmann *et al.*, 1994). For instance, increased resistance to late blight from 30% to 70% in potato cultivar Bintji, which has no gene for resistance to *Phytophthora infestans*, was obtained by prior inoculation with an incompatible (cannot develop disease) race of *Phytophthora* (Stromberg and Brishammar, 1991).

Although the defence mechanism of a plant is mostly activated in response to pathogen attack, it also could be induced or accelerated by physical treatment or application of a biological agent or a chemical activator. Many reports have found that plants enhance resistance against pathogens after artificial inoculation or treatment with chemical activators (Kuc, 1982; Ryals *et al.*, 1994). Activation of defence responses in harvested crops has also been demonstrated in various host-pathogen interactions from physical, chemical and biological elicitors (Barkai-Golan, 2001). Inducible resistance in harvested tissues joins the general concept that resistance in plants can be enhanced by modulating their natural defence mechanisms (Kuc, 1995b).

The mechanism of protection against pathogens by induction of systemic resistance in plants works on the same principle both for biological and chemical elicitors (Lucas, 1999). Significant advances have been made in understanding the genes involved in regulating the resistant state as well as the chemical signals modulating the responses, however, the actual mechanism(s) stopping pathogen development has not conclusively been revealed (Kombrink and Schmelzer, 2001). However, it is evident that the SAR elicitors are non-specific, some are more effective against some diseases than others (Kuc, 2001). It is also stated that the elicitors may affect different components of the resistance mechanism and

that all components are not equally effective against all pathogens. Some of the biological characteristics of systemic acquired resistance have been described by Lucas (1999) in the Table 2.10.2 below:

Table 2.10.2 Characteristics of systemic acquired resistance (SAR)

1.	Induced by agents or pathogens causing necrosis e.g. local lesions.
2.	Delay of several days between induction and full expression.
3.	Protection conferred on tissues not exposed to inducer inoculation.
4.	Expressed as reduction in lesion number, size, spore production, pathogen multiplication etc.
5.	Protection is long-lasting, often for weeks or even months.
6.	Protection is non-specific i.e. effective against pathogens unrelated to inducing agents.
7.	The signal for SAR is translocated and graft-transmissible.
8.	Protection not passed on to seed progeny; transmission to vegetatively propagated tissues has not been fully resolved.

There is a lag period required for the initiation of systemic resistance and for the plant to reach its heightened state of resistance following elicitor treatment. Platonova *et al.* (1982) suggested that induction of resistance in plants is the result of a rearrangement of cell ultrastructure that requires a certain time interval. They speculated that in potato a period of 72-96 hours is required for completion of this rearrangement and once these cellular changes have occurred they cannot be reversed. To develop complete resistance in potato plants against Alternaria leaf spot disease required about seven days after induction treatment (Bokshi *et al.*, 2003; Mauch-Mani and Slusarenko, 1994). They observed partial

resistance after 4 days of foliar infection but no systemic resistance was found after one day.

Although the initial response of pathogen-invaded or elicitor-treated plant cells occurs within a few minutes and is rapidly followed by local gene activation (Somssich and Hahlbrock, 1998) a minimum time period is required for full expression of systemic resistance (Kuc, 1983). Colson and Deverall (1996) suggested that the chemical activators required the same lag period as biological agents following application for the appearance of resistance in cotton plants. However, a change in the activity of the enzymes such as peroxidase and lypoxygenase due to application of inducers, could be detected in cucumber within 12 hours of treatment (Fritz *et al*., 1996). Martinez *et al*. (2001) found that the activities of defence related enzymes in melon seedlings began to increase 8 hours after elicitor treatment, reaching a maximum between 48 and 72 hours.

Once plants are induced the resistance symptoms last long beyond the period of pathogenic infection or elicitor treatment. Kuc and Richmond (1977) observed that induction of cucumber seedlings with a preliminary inoculation by *Colletotrichum lagenarium* can protect the plants against a wide range of pathogens for 4-6 weeks. A further induction after 2-3 weeks of primary infection led to persistence of resistance throughout the crop season. Guedes *et al.* (1980) reported that induction of systemic resistance is influenced by the age of the plants. They suggested that for effective protection plants should be treated for induction of resistance before flowering and fruiting. Hence, the conditioning of resistance is affected by the physio-chemical factors of the plants.

Although emphasis has been directed towards controlling diseases of growing plants by SAR, there are numerous reports in recent times on postharvest diseases (Bokshi *et al*.,

2003; Huang *et al*., 2000; Liu *et al*., 2005; Terry and Joyce, 2000; Willingham *et al*., 2002). Field application of elicitors on potato plants was found to develop resistance in the tuber that can reduce the infection of storage rots (Bokshi *et al*., 2003). They found that application of chemical elicitors during the formation of tuber is more effective for the development of systemic resistance than applying them at later stages of crop. Huang *et al*. (2000) also found application of chemical elicitors before flowering and a postharvest dip in fungicide reduced storage rots of melons. Similarly, field application of BTH weekly for nine successive sprays during flowering and fruit development was found to suppress grey mould of strawberry in storage (Terry and Joyce, 2000). A postharvest dip with BTH has been reported to induce resistance in peach against Penicillium rots (Liu *et al*., 2005).

Induction of natural resistance in harvested horticultural crops using physical, biological and chemical elicitors has received increasing attention over recent years. It is being considered a preferred strategy for disease management (Terry and Joyce, 2004). It is fresh produce which elicits most concern over the use of fungicides. More applied and basic research is required for understanding the role of SAR and the development of a strategy for the control of postharvest diseases that would benefit health and environment.

2.10.3 *Mechanisms involving SAR*

2.10.3.1 *Systemic signals for induction of systemic resistance*

Research provides strong evidence that a signal for SAR is produced at an induction site (Dean and Kuc, 1986a, 1986b) and is translocated throughout the plant, where it conditions resistance to disease (Jenns and Kuc, 1979; Tuzun and Kuc, 1985). These signals are generated and/or released during lesion development and are phloem transmitted moving both above and below the induction site and also into roots. More specific timing of host

cell death and generation of the signal were provided by Smith *et al*. (1991). They found that when a cucumber plant was inoculated with *Pseudomonas syringae* pv *syringae* a hypersensitive response resulted in a systemic expression of resistance within 24 hours.

Phenolic compounds such as salicylic acid (SA) that accumulate due to induction of systemic resistance in plants, are thought to play a role of primary signal (Enyedi *et al.*, 1992) with some different views seeing them only as inducers of resistance (Rasmussen *et al.*, 1991). The primary signal received by the vascular tissue may induce synthesis of SA and its transport throughout the plant. SA is a likely natural inducer of disease resistance in plants since it acts by stimulating local and systemic accumulation of defence-related proteins that are responsible for increased disease resistance of the plant (Raskin, 1998). In a classic model of systemic induced resistance, a signal produced by an infected leaf moves through the vascular tissue to uninfected leaves, where it induces PR-proteins and associated resistance against further pathogens (Smith-Becker *et al.*, 1998).

Correlation of endogenous SA with the onset of systemic induced resistance and accumulation of PR-proteins clearly indicates the role of SA in the pathway of systemic transduction of resistance (Malamy *et al.*, 1990; Métraux *et al.*, 1990). As a result of induction of systemic resistance, SA was detected in the phloem sap of cucumber and tobacco plants and appeared to act as a signal. Similar results by Yalpani *et al.* (1991) also suggested that SA functioned as the endogenous signal for accumulation of PR-protein involved in development of systemic induced resistance.

It has been reported that the signal for systemic resistance develops within 6 hours after inoculation and occurs before any visible sign of host cell necrosis (Smith *et al*., 1991). Immediately after perception of the signal, corresponding changes in metabolic activities,

including those leading to secondary product accumulation, provide defence against pathogens (Hahlbrock *et al.*, 1998). Madi and Katan (1998), suggested the induction of melon and cotton plants, by treatment with biological elicitors triggers the signal transduction cascade and activates different genes in the plants resulting in an increase in exogenous peroxidase and β-1,3-glucanase. Apparently, signals originating in the leaves reach the lower part of the stem and activate the defence mechanisms.

There is strong evidence that a signal for SAR is produced at the induction site (Dean and Kuc, 1986a, 1986b) and translocated throughout the plant where it conditions resistance to disease (Jenns and Kuc, 1979, Tuzun and Kuc, 1985). Hahlbrock *et al.* (1998) reported that the change in metabolic activities leading to secondary product accumulation that increases defence against pathogens is due to perception of a signal. A recent report demonstrated an SAR signal can also move to neighbouring plants through an airborne signalling mechanism (Lucas, 1999). Methyl salicylate which is a volatile metabolite of SA produced by pathogen inoculated parts of plants can activate resistance in adjacent plants to pathogenic infection and respond collectively to a perceived biological threat (Shulaev *et al*., 1997).

Reports by Shulaev *et al.* (1995) previously suggested the role of SA is as an endogenous signal for the development of systemic induced resistance in tobacco plants. In an experiment with radio-labelled oxygen, they found that a substantial part of SA accumulated in the upper leaves had been transported from the inoculated leaf. They also observed that the highest amount of SA accumulated in the youngest leaf directly above the induced leaf having a good vascular (phloem) communication. A little accumulation of SA occurred in the leaf that was located opposite to the inoculated leaf and which had a less direct vascular connection. However, when they detached the inoculated leaf after a definite period of induction it was found that the accumulation of SA in the upper leaves was

significantly reduced. Similar results shown by Rasmussen *et al.* (1991) raised doubts about the role of SA as a systemic signal for induction of systemic induced resistance. A recent development observed that SA-mediated plant immunity plays a central role in the plant defence response (Lee *et al*., 2007).

Two signalling pathways have been described by Thaler *et al*. (1999), one involving SA and another involving jasmonic acid (JA), which participates in the expression of plant resistance to pathogens and insect herbivores. SA is thought to be a key compound in the regulation of resistance to fungal, bacterial and viral pathogens and provides a signal for expression of PR-proteins and other potential protective compounds (Ryals *et al*., 1996). However, exogenous application of JA has been demonstrated to induce SAR in plants by stimulating many of the systemic metabolites, similar to that which occurs from challenge with pathogens or insects (Kessmann *et al*., 1994; Maleck and Dietrich, 1999).

The importance of the phytohormones SA and JA as critical signals in induced resistance response in plants is recognised (Bostock, 1999). As these chemicals can strongly influence other processes in plant growth and development, it would not be unexpected to see interactions between the pathways and with other phytohormones and signal molecules (Raskin, 1992; Staswick, 1992). There is evidence for a negative interaction between the SA and JA pathways in models of defence signalling, however, in some plants the two pathways have been reported to be complementary (Bostock, 1999).

The negative interaction between SA and JA signalling pathways has been demonstrated at the biochemical level, which may compromise the ability of the plants to coordinate defence against simultaneous challenge from pathogens and herbivores (Thaler *et al*., 1999). The best evidence for cross-talk between different induced defence responses is seen

in the SAR and wound response pathways (Maleck and Dietrich, 1999). Laboratory studies have revealed interaction between the salicylate and jasmonate pathways where SA appears to inhibit JA biosynthesis and the subsequent chemical responses (Doares *et al.*, 1995). Therefore, when plants are sprayed to stimulate both response pathways simultaneously, the corresponding biological effects on resistance to the pathogen and the insect are compromised (Thaler *et al.*, 1999).

Evidence is accumulating that the various induced defence responses might not be controlled by independent linear signalling cascades, but that components of one pathway can affect the signalling through other pathways (Maleck and Dietrich, 1999). The nature of the systemic signal has been a subject of controversy for many years (Durrant and Dong, 2004). In simple terms, it could be suggested that a systemic signal(s) is initiated in the induced part(s) of the plant and is translocated throughout the plant and conditions for resistance (Dean and Kuc, 1986a). It is also thought that the tissue receiving the primary signal does not generate more signals. Several signals may be involved as a result of systemic induced resistance (Kuc, 1995a). Only the translocatable signal(s) results directly or indirectly in the elicitation of defence compounds. Despite a long-standing notion of long-distance signals triggering systemic acquired resistance (SAR), the translocation pathway and the identity of the signals involved have not been determined with any degree of certainty (Van Bell and Gaupels, 2004).

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2.10.3.2 *Accumulation of antifungal compounds following SAR*

A number of known and unknown compounds are produced as a result of resistance at the infection site of the plant, ultimately determining the outcome of the host response (Pieterse *et al.*, 1992). In the past decade, considerable progress has been made in understanding the

proteins associated with induced resistance and the genes which encode them. The concentration of a particular elicitor influences the level of resistance induced by the accumulation of pathogen-resistance compounds on its application (Ozeretskovskaya, 1995). The plants respond through an increased accumulation of certain group of primary proteins such as to display an active defence mechanism that initiates the production of secondary metabolites such as phytoalexins and lignin (Lamb *et al.*, 1989).

The interaction of pathogens with plants leads to a disruption in cellular homeostasis, often leading to cell death, in both compatible and incompatible pathogens (Gilchrist, 1998). Cell death is most commonly followed by necrosis which results from exposure to highly toxic compounds that lead to immediate damage to membranes or cellular organelles (Cohen, 1993). The hypersensitive response that causes cell death is often associated with plant resistance to pathogen infection (Morel and Dangl, 1997) and onset of systemic acquired resistance (Kombrink and Schmelzer, 2001).

When plants are attacked by pathogens, they accumulate groups of proteins which are collectively known as pathogenesis-related proteins (PR-proteins) (Bol and Linthorst, 1990). PR-proteins are generally induced under a specific condition such as pathogenic infection, leading to a hypersensitive response (Van Loon, 1985). Although PR-proteins are induced in response to pathogenic infection, they can also be induced by the application of chemicals which mimic the effects of pathogenic infection (Buchel and Linthorst, 1999). Both biotic and abiotic inducers result in the accumulation of PR-proteins in leaves flowers and roots (Fluhr *et al.*, 1991). Also plants grown under unfavourable and artificial conditions such as in the glasshouse, stimulated PR-proteins to accumulate, providing increased resistance.

Plants are able to synthesis a wide diversity of PR-proteins (Monteiro et al., 2007). For example, seven different classes of chitinases can be synthesised by a higher plant which differs in protein structure, substrate specificity, mechanism of catalysis and sensitivity to inhibition (Kasprzewska, 2003). Many of these enzymes do not exhibit *in vitro* anti-fungal activity and this has led to the suggestion that plant chitinases are involved not only in defense-related processes or a general stress response, but also in numerous physiological events, including growth and development.

2.10.3.3 *Chitinase, antifungal activity and evidence for SAR*

When a plant is infected locally, it displays a marked increase in both exo- and endo-chitinase activities throughout the whole plant (Roby *et al.*, 1988). During the defence response, stimulation of chitinase and β-1,3-glucanase activity occurs as well as synthesis of phytoalexins (Matton *et al.*, 1990). The increase in chitinase, reported to begin immediately after inoculation, occurs sequentially in non-infected tissues as well. This suggests a correlation between increased chitinase activity and increase in resistance conferred. However, an elicitor derived from fungal mycelium was found to induce enzyme activities faster than the fungus itself when inoculated to a plant (Kombrink *et al.*, 1988). This is probably due to the rapid uptake and distribution of the elicitor throughout the plant leaf.

Other than pathogen attack, plants can induce PR-proteins in response to a number of factors like physical and chemical treatments. Matton and Brisson (1989) reported the accumulation of at least two clones of mRNAs at high levels in response to treatment with arachidonic acid and eicosapentaenoic acid of tuber disks. A similar response was observed in treatment with BTH on rose where enhancement of chitinase occurred due to expression

of the same isoforms as those in response to pathogen attack (Suo and Leung, 2001). In contrast, BTH treatment on cauliflower was found to induce β-1,3-glucanase activity but not chitinase and induction was more prominent after challenge inoculation (Ziadi *et al*., 2001). However, the increase in activity of particular enzymes is not correlated with induced resistance and varies with the inducer and host plant (Schneider and Ullrich, 1994).

Environmental stresses that cause rapid changes in plants stimulate the accumulation of enzymes for synthesis of antimicrobial substances. Pierpoint *et al.* (1990) detected higher activities of PR-proteins like chitinase and β-1,3-glucanase in potato leaves at early stages of plant growth grown in glasshouse conditions. However, Fraser (1981) reported the accumulation of increased amounts of PR-proteins in healthy plants during flowering and senescence. Interestingly, the same enzymes or PR-proteins are not produced as a result of pathogenesis or stresses which suggest that different kinds of inducing agents may have different effects on plant physiology (Tuzun, 2001). Depending on the particular interactions between elicitors and suppressors with their cognate plant targets, defence response cascades may or may not become activated during pathogenesis (Thomma *et al*., 2001).

The expression of chitinase is regulated by developmental factors of the plant like age of the organ and is also regulated in an organ specific manner (Beerhues and Kombrink, 1994). It has been suggested that in young leaves and stems, expression of chitinase and β-1,3-glucanase occurs differently. The activity of chitinase mRNA was abundant in young organs whereas β-1,3-glucanase mRNA was absent. Also different varieties of the same crop elicit various classes of enzymes; different species of pathogens of the same variety elicit various enzymes (Rahimi *et al.* 1996). Dann and Deverall (1995) stated that the site of

local infection is an important factor for induction. They speculated that differences between the epidermis and parenchyma near veins in leaves, and the epidermis and cortex of roots and stem bases affect the accumulation and distribution of induced resistance.

Efforts made by Beerhues and Kombrink (1994) for isolation and characterisation of chitinase and β-1,3-glucanase found that these enzymes are basically strongly and coordinately induced in response to infection or elicitor treatment. In a study for localization of chitinase and β-1,3-glucanase Keefe *et al.* (1990) suggested that the basic isoforms of these enzymes are intracellular whereas the acidic isoforms are secreted into the extracellular space. However, a report made by Boller and Metraux (1988) suggested that in cucumber leaves most of the chitinase activity is located in the extracellular space. In the case of pathogen attack on an induced plant the accumulated chitinase in the extracellular spaces is in a position to directly attack the incoming fungal hyphae. Thus the plants escape from infection and disease development by the pathogens.

In vitro trials with different fungi found that chitinase alone or in combination with β-1,3-glucanase can effectively restrict their growth (Mauch *et al.*, 1988; Schlumbaum *et al.*, 1986). In the situation of pathogenic attack the activities of both enzymes increased strongly and co-ordinately throughout an induction period in response to both compatible and incompatible pathogens (Schröder *et al.*, 1992). Meins *et al.* (1992) reported that the products of genes induced by systemic resistance have direct antimicrobial activity. These systemic products are closely related to the classes of antimicrobial proteins among which β-1,3-glucanase and chitinase are important. It is obvious that these two enzymes are not products of so-called resistance genes which determine the specificity of plant-pathogen-interactions; rather they are the defence-related gene products that are induced in response

to pathogens and may contribute to the inhibition of a potential pathogen when the plant expresses resistance (Mauch *et al*., 1988).

2.10.3.4 *Peroxidase, antifungal activity and evidence for SAR*

The role of peroxidase activity in plant resistance mechanisms during host-pathogen interaction has been described by many authors (Burdon and Marshall, 1983; Reuveni *et al*., 1992). Lebeda *et al*. (1999) indicated that there are differences in peroxidase activity in intact and infected cucurbits plants and suggests increased peroxidase activity is a biochemical marker for the prediction of resistance mechanism in the plants. A strong correlation has been revealed between peroxidase activity and level of disease control due to induction of resistance by physical, chemical or biological elicitors (Madi and Katan, 1998; Stermer and Hammerschmidt, 1984). Measurement of peroxidase activity may denote the state of resistance in the plants, although assessment of disease incidence is still needed for more accuracy (Reuveni *et al*., 1990).

Systemic induction of peroxidase by the treatment of chemical activators is thought to be the part of the action mechanisms restricting disease development (Reuveni *et al*., 1997). Antifungal activity of peroxidases is considered potentially important in host resistance mechanisms (Ippolito *et al*., 2000). The induction and accumulation of peroxidases is correlated with the onset of induced resistance, suggesting an active role for these enzymes in defence against pathogenic fungi, expected to retard fungal growth (Van Loon *et al*., 1998).

Peroxidase is involved in lignin formation (Conti *et al*., 1990; Hammerschmidt and Kuc, 1982; Kuc, 1990), while in combination with other enzymes activities and mechanisms they inhibit the growth of pathogenic fungi in the host tissue (Ippolito *et al*., 2000; Irving and

Kuc, 1990). Stimulation of extracellular peroxidase activities in plants during pathogen attack and their involvement in cell wall lignification and formation of hydrogen peroxide suggest it is the antimicrobial activity of the enzyme which limits the extent of pathogen spread (Tuzun, 2001).

Cools and Ishii (2002) demonstrated that benzothiadiazole (BTH) (a product of Syngenta) treatment, causes priming of the defence response of the plant, by systemically inducing the expression of an acidic peroxidase-encoding gene which, upon subsequent inoculation with the pathogen, enhances peroxidase activity. A positive correlation between peroxidase activity in non-infected leaves of melon cultivars and their resistance to powdery mildew also has been reported by Reuveni and Bothma (1985). Similarly, Smith and Hammerschmidt (1988) found that induced resistance in cucumber, muskmelon and watermelon is accompanied by a systemic increase in peroxidase activity. They speculated that the structural and regulatory similarity of peroxidase in cucumber, muskmelon and watermelon may reflect a similar role for the enzymes in the SAR response.

Increase in cell wall-bound peroxidase activity has also been reported to be a result of stress or tissue wounding from mechanical damage (Kawaoka *et al*., 1994) or in response to herbivory (Moore *et al*., 2003). The increase in peroxidase activity is reported to be linked with a reduction in leaf growth rate after herbivore grazing, due to a reduction in epidermal cell area. In response to wounds caused by mechanical stress increased peroxidase activity was found in harvested cucumber (Miller and Kelley, 1989). It is postulated that the biological role of peroxidase activity following mechanical stress may involve suberization and lignification during the wound healing process.

The change in peroxidase activity corresponds to fruit development and is associated with susceptibility to fruit rot. Peroxidase activities in muskmelon have been reported to decrease with the advancement of fruit maturity and are found at the highest levels in the outer skin at all times (Biles *et al*., 2000). A high level of peroxidase activity during the fruit development stage appears to correspond with the latent period of postharvest pathogens (Bruton *et al*., 1998). However, after harvest the fruit peroxidase decreases (Lacan and Baccou, 1998), the pathogen becomes active, thus fruits are more susceptible to rots. Hence, a less mature fruit is more resistant compared to fruit at an advanced stage of maturity (Zhang *et al*., 1999).

2.11 AGENTS FOR INDUCTION OF SAR

2.11.1 *Biological agents of SAR*

In recent years much attention has been given to the control of plant disease through induction of resistance by means of biological elicitors. Induction of systemic resistance by the use of a pathogenic or a non-pathogenic microorganism has been reported for many crops (Jenns and Kuc, 1980; Kroon *et al.*, 1991; Kuc, 1982). Resistance induction by prior inoculation with antagonists, mostly results in the formation of a necrotic lesion around the point of initial penetration, restricting further spread of the pathogen (Barkai-Golan, 2001). The resistance was evident by observing a delay in symptom development and a reduction in severity. It was thought that the reduction of disease severity was the result of the combined action of several mechanisms. For example an initial response may be increase of physical resistance of the cell wall by papillae deposition followed by assembly of antifungal metabolites, such as β-glucans in induced plants (Stromberg and Brishammar, 1993).

The enhancement of antimicrobial compounds in the host tissue by antagonistic microorganisms, has been commonly reported. Application of *Pichia guilliermondii*, an antagonist yeast, has been found to control a wide range of postharvest diseases of citrus, apples and peaches through induction of enhanced levels of phenylalanine ammonia lyase in citrus peel (Wisniewski and Wilson, 1992). Also, Ippolito *et al*. (2000) found that the antagonist yeast-like-fungus, *Aureobasidium pullulans*, reduces apple rot caused by *Botrytis cinerea* and *Penicillium expansum*, by inducing the activities of β-1,3-glucanase, chitinase and peroxidase. Pre-treatment with *Pseudomonas fluorescens* has been reported to significantly reduce soft rot caused by *Erwinia carotovora* subsp. *carotovora* of melon seedlings (El-Hendawy *et al*., 1998).

Application of a culture filtrate of fungal mycelium to plant can induce systemic resistance similar to that observed after pre-inoculation with conidia and may even protect the plant better from diseases (Madi and Katan, 1998). There was up to 100% reduction in the incidence of damping-off caused by *Rhizoctonia solani* on stems of melon and cotton plants, when treated with culture filtrate or conidia of *Penicillium janczewskii.* Induction of systemic resistance was evident by the hypersensitive-reaction-like responses and increase in extracellular peroxidase and β-1,3-glucanase.

There is a lag period between inoculation and initiation of the formation of antifungal compounds as a result of induction. It was found that the induction of increased enzyme activities initiates after 24 hours, and reaches its peak at 48 to 96 hours after inoculation (Ippolito *et al*., 2000). This suggests that the increased levels of the antifungal compounds develop structural and chemical barriers and out-compete the pathogens for nutrition and development in the host tissue. Similarly systemic resistance in plants was induced within one day by the pathogen, the resistance decreasing with increasing time (Murray and

Walters, 1991). Prior inoculation of the lower leaves of young broad bean plants against rust also induced resistance to rust in the upper leaves and was found to be effective for 9 days following inoculation (Murray and Walters, 1991).

The response to induced resistance by biological elicitors is not the same for all plants (Cohen and Kuc, 1981). It was found that treatment with *Peronospora tabacini* spores, injected into the internal cambium of tobacco stems, severely affects the plant growth, causing dwarfing, premature senescence and symptoms resembling nitrogen deficiency in plants. However, a subsequent injection into the external cambium results in increased plant growth; increase in weight and number of leaves in tobacco (Tuzun and Kuc, 1985). Similarly, infiltration of culture filtrates of antagonist to melon plants increases the level of peroxidase activity in leaf and stem but not in root. In contrast, infiltration on cotton results in increased activity of peroxidase in leaf and root but not in stem (Madi and Katan, 1998). This suggests that the differences in induction of different plants are because of their differences in physiological and biochemical response to the treatment.

A number of *Trichoderma* fungi are well known for their antagonism against several soil-phytopathogens, involving fungi, invertebrates, and bacteria (Verma *et al*. 2007). In addition to the ability of *Trichoderma* spp. to attack or inhibit the growth of plant pathogens, the organism can also induce systemic and localized resistance to a variety of plant pathogens and has substantial influence on plant growth and development (Harman et al., 2004). However, full-scale application of *Trichoderma* for biological control of plant pathogens has not been widespread because biocontrol agents generally do not perform well enough under field conditions to compete with chemical fungicides (Brunner *et al*., 2005).

2.11.2 *Chemical elicitors of SAR*

Chemically mediated disease control can be based on compounds that would induce the response for developing plant resistance (Kessmann *et al.*, 1994). A chemical is considered as an activator of systemic acquired resistance when it induces resistance to the same spectrum of pathogens and gives expression to the same biochemical markers as the biological model. Furthermore, the chemical should have no antimicrobial activity. Treatments with chemicals which have no direct antimicrobial activity are now a well established fact for the induction of systemic acquired resistance in plants. Chemicals that activate SAR in the field, as well as crop varieties with constitutive SAR gene expression, could provide solutions to disease problems for growers (Uknes *et al*., 1996).

Several chemicals having no direct antibiotic action, have been reported to induce resistance to pathogens when applied to plants (Kessmann *et al.*, 1994). Natural and synthetic molecules that have such a capacity are already known (Cohen *et al.*, 1991). Over the last 30 years, a number of compounds have been shown to increase resistance, or at least to decrease symptoms in some host-pathogen interactions (Hammerschmidt and Smith, 1997). These chemicals have been found to induce either systemic or local resistance in an otherwise susceptible host. In the last decades research has focused upon developing novel synthetic chemical activators with increased efficacy (Gorlach *et al*., 1996; Tally *et al*., 2000). Many chemical activators that confer broad-spectrum efficacy against pathogens on a number of crops, including cucurbits have been identified (Tally *et al*., 2000).

Although most of the literature has shown positive effects of chemical activators in inducing SAR, there are reports that obscure the fact that in some plant pathogen systems or environments SAR activators are relatively ineffectual (Terry and Joyce, 2004). There are suggestions that the timing of induction by chemical treatment and growth stage of the plant

may have important influence on the development of SAR (Bokshi *et al*., 2003; Huang *et al*., 2000). Multiple applications of SAR chemicals in both field and glasshouse conditions are recommended for the effective control of disease (Dann *et al*., 1998). Similar results have been found by Nielsen *et al.* (1994) where fewer than three applications of INA were ineffective in inducing resistance in glasshouse-grown sugar beets against *Cercospora beticola*. However, there are reports which demonstrate that one application of SAR chemicals is sufficient to induce significant disease resistance (Bokshi *et al*., 2003; Dann and Deverall, 1996; Metraux *et al*., 1991)

2.11.2.1 *SA and SA derivatives as chemical activators*

For a long time it has been thought that salicylic acid (SA) plays a role in signal transduction of systemic resistance after pathogenic infection as well as being an activator of systemic resistance and accumulation of PR-proteins (White, 1979). The application of SA to leaves of tobacco plants has been reported to induce SAR and to develop resistance against the same diseases as TMV (Ward *et al*., 1991). Unfortunately, there have been problems associated with exogenous application of SA for induction of resistance. Kessmann *et al.* (1994) stated that the use of SA as an inducer is probably not practical because of severe crop tolerance problems. The range between the efficacy of the compound and its toxicity to the plants is separated only by a narrow safety margin. However, failure of induction of systemic resistance by exogenous application of SA has also been reported by Siegrist *et al*. (1994).

A number of other SA derivatives studied by White (1979), Mills and Wood (1984) and Walters *et al.* (1993) were found to be effective in inducing resistance in tobacco, cucumber and barley. However, in tomato plants acetylsalicylic acid (ASA) was not effective in

inducing systemic resistance or accumulation of PR-proteins (Christ and Mösinger, 1989). The low rate of success (50%) for induction of systemic resistance with the application of SA has also been reported by Van Loon and Antoniw (1982). They could not find any obvious reason for this. According to Yalpani *et al.* (1993) the variation in sensitivity of SA to induce systemic resistance in tobacco might be due to changes in environmental and developmental factors of the plants. In contrast, Lopez-Lopez *et al.* (1995) observed an increase in resistance in potato tubers against *Erwinia carotovora* ssp. *carotovora* when dipped in ASA for 60 min but they did not report any enzymatic changes as evidence of systemic induction of resistance.

2.11.2.2 *INA as chemical activator*

Publications from several workers have demonstrated that SA and its functional analogues like 2,6-dichloroisonicotinic acid (INA) protect many crops against their pathogens. INA is weakly fungistatic *in vitro*, but effectively elicits SAR genes in tobacco prior to TMV challenge inoculation (Ward *et al*., 1991). Vernooij *et al.* (1995) showed that TMV, INA and SA induce the same nine genes in tobacco against five similar pathogens, however, INA acts independently of SA where INA itself operates downstream of SA. Nielsen *et al.* (1994) successfully induced resistance in sugar beet against *Cercospora beticola* by treating with INA but they did not find any evidence of PR-protein induction.

INA has successfully induced systemic resistance in a tobacco plant of a transgenic variety which previously was shown unable to be induced following pathogenic infection or with application of SA (Vernooij *et al.,* 1995). It was deduced that INA induced resistance through the SAR signal transduction pathway at the same step as SA by acting downstream of SA accumulation. Similarly, Métraux *et al.* (1991) have shown that induction of

resistance by INA does not require SA to accumulate. They showed that INA can move systemically in the plant when injected and then induce pathogen resistance without accumulation of SA. It was thought that INA can only compensate in SAR signal transduction rather than in the hypersensitive response. Schweizer *et al.* (1997) suggested that INA, aside from activating a pathogen-induced signalling pathway, also induces events that are not related to pathogenesis. The roles of SA and INA in disease resistance still require further research.

INA has been reported to induce resistance by mimicking some aspects of pathogen attack, possibly accelerating normal responses to further infection (Uknes *et al.*, 1992). It has been found that the INA-mediated acquired resistance in Arabidopsis was not specific to a given pathogen, as manifested by its effectiveness with *Pseudomonas syringae* tomato infection. The reduced pathogenesis observed in INA-treated tissues correlated well with the accumulation of a number of PR-proteins such as β-1,3-glucanase and chitinase; the increased antifungal potential of these enzymes in combination has been demonstrated before (Mauch *et al*., 1988). Following INA treatment on cotton cotyledons, the activities of β-1,3-glucanase were elevated in the true leaves and had a marked effect in decreasing susceptibility to *Alternaria macrospore*.

Whatever mechanisms are involved, it was found that INA can induce resistance in plants through induction of a full complement of genes that are associated with biological induction (Kessmann *et al.*, 1994). It has also been reported that INA induced resistance in bean against rust, anthracnose and halo blight (Dann and Deverall, 1995). In cucumber it induces resistance against anthracnose, and the nature of the induction was found to be identical to that of biological induction of resistance (Hammerschmidt and Kuc, 1982). Like most other biological activators, INA increased the expression of chitinase or glucanase

genes of tobacco but not through an accumulation of SA (Vernooij *et al.*, 1995). Dann *et al*. (1998) reported a significant reduction of white mould disease in field-grown soybeans by spraying INA, but the molecular basis of the resistance was not known.

However, there are contradictory reports on the effectiveness of INA and SA and their derivatives to induce systemic resistance in plants. In a comparative study on the effect of INA and SA and their derivatives, Kauss *et al*. (1992) found SA and its derivatives to be more potent than INA for induction of systemic resistance. A reverse result has been observed by Neilsen *et al.* (1994) who found that in sugar beet INA application induced systemic resistance, but not SA. Owen (1995) in a series of experiments with INA application on wheat found a slight reduction of rust disease over the control plants. Application of SA did not induce resistance in any of the experiments whereas its derivatives in most of the experiments significantly reduced the rust infection on wheat. This study also indicated that the methods of application of SA may be a considerable factor. The distribution of induced resistance from the treatment of INA has been reported specific to plant parts; relatively less in the stem and roots than the younger leaves and the growing points of the plants (Métraux *et al*., 1991).

2.11.2.3 *BTH (benzothiadiazole) as chemical activator*

Benzothiadiazole (BTH), a product of Syngenta, promoted as a safe, reliable and non-phytotoxic plant protection agent, was identified as a novel class of compound for induction of systemic resistance (Görlach *et al.*, 1996). BTH has been introduced on the market by Novartis as the first commercial chemical triggering induced resistance in plants under the tradenames Actigard® in USA and BION® in Europe (Hammerschmidt *et al*., 2001). Foliar spray of BTH on tomato plants has been reported to induce resistance against root diseases

on challenge inoculation with a soil-borne pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Benhamou and Belanger, 1998). From the evidence of histological and biochemical studies of BTH treated tomato roots, they suggested that BTH treatment confers increased protection of tomato plants against pathogenic infection by stimulating a number of defence reactions.

BTH was developed as a plant activator for commercial use in a wide range of crops by acting as a functional analogue of SA in the pathway leading to systemic acquired resistance (Kessmann *et al*., 1996). BTH treatment to tobacco plants was characterised by Friedrich *et al*. (1996) and the disease control was found to act through systemic acquired resistance. Like INA, BTH treatment does not cause systemic accumulation of SA but it appears to activate the systemic resistance signal transduction pathway at the site of, or downstream of, SA accumulation. However, both INA and BTH induced similar patterns of gene expression, suggesting that these compounds are functional analogues (Friedrich *et al*., 1996).

BTH has the potential of synergistic action with other treatments of disease and pest control. Melons induced for resistance from field sprays with BTH, were treated with biological control agents after harvest, and found to effectively control postharvest diseases and extend the shelf life for two to three weeks (Wei *et al*., 1999). In combination with insecticides, BTH was also used successfully on tomato crops against *Bemisa tabaci*, the vector of the tomato leaf curl virus, resulting in better yields and less disease incidence (Hammerschmidt *et al*., 2001).

BTH can protect a diverse group of both monocot and dicot plants including tobacco, *Arabidopsis* spp., wheat, barley and cotton (Colson-Hanks, 1998; Görlach *et al.*, 1996;

Lawton *et al.*, 1996). It is a broad spectrum plant protection compound with respect to both plants and pathogen species. The compound induces resistance systemically against pathogens by affecting multiple steps in the infection process. Ishii *et al.* (1999) found BTH effective against anthracnose and scab of cucumber and rust of Japanese pear. However, the compound did not control all diseases of the above hosts. Ziadi *et al*. (2001) confirmed that BTH induces resistance in cauliflower against downy mildew, and that the induced resistance is dose-dependent.

Studies with BTH for the induction of systemic resistance were mainly focused on the treatment of intact plants for the control of pre and postharvest diseases (Bokshi *et al*., 2003; Huang *et al*., 2000; Terry and Joyce, 2004). A recent report by Liu *et al*. (2005) found that BTH treatment at a concentration of 200 mg/L on freshly harvested peach fruit can significantly enhance resistance against *Penicillium expansum*. In contrast, a series of treatments of BTH on harvested whole potato tubers was not effective for the control of *Fusarium semitectum* even with much higher concentration of up to 500 mg/L and prolonged dip time of up to 1 hour (Bokshi, 2000). However, treatment of potato tuber disc with a low concentration of 25 or 75 mg/L of BTH was found to significantly control the same fungus. Root treatment with BTH was found to activate plant resistance more rapidly than a foliar application suggesting a quicker upward translocation of the activator than downward (Rohilla *et al*., 2001).

2.11.2.4 *BABA (β-aminobutyric acid) as chemical activator*

Although β-aminobutyric acid (BABA) is only rarely found naturally in plants, like BTH it has proved to be a potent inducer of acquired resistance and has a broad spectrum of activity against many disease-causing organisms (Cohen, 2002; Jakab *et al*., 2001),

including nematodes (Oka *et al*., 1999). The protective effect of BABA has been described as triggering the potential of natural defence mechanisms against biotic and abiotic stresses. BABA has been reported to induce local and systemic resistance against a variety of fungal plant diseases in crop plants by strongly and rapidly activating the accumulation of PR-proteins (Cohen, 1996).

The possibility of a phytotoxic effect of BABA at medium or low concentration has been ruled out since no toxic effects have ever been observed (Cohen, 1994; Cohen *et al*., 1994;; 1999; 1996; Hong *et al*., 1999; Tosi *et al*., 1999). However, a higher dose (10 mM compared to 1 mM as previously recommended) was found to cause rapid cell death in tobacco leaf tissue after foliar application resulting in the development of small necrotic lesions (Siegrist *et al*., 2000). However, β-aminobutyric acid (BABA) and its derivatives have been reported to activate disease resistance in various plants when used at relatively high rates (Oostendorp *et al*., 2001). BABA mediated induced resistance does not always involve PR-proteins, but rather primes the defence system to provide required resistance under biotic or abiotic stress (Ton *et al*., 2005).

The mechanisms governing resistance induced by BABA are not yet clear (Silue *et al*., 2002). BABA-induced resistance in plants has been suggested to operate through a variety of defence mechanisms, including formation of physical barriers and biochemical changes leading to resistance (Cohen, 2002). Application of BABA on tomato plants protected against late blight development and a positive correlation was found with the accumulation of high levels of PR-proteins like P14a, β-1,3-glucanase and chitinase (Cohen *et al*., 1994). Siegrist *et al*. (2000) suggested an increased level of SA as a consequence of cell death following BABA treatment on tobacco plants causes expression of PR-1a, a molecular

marker of SAR. The evidence suggests that foliar application of BABA can trigger a process which resembles resistance development or a hypersensitive response during microbial attack that results in SAR activation. However, the general mechanism of BABA-mediated priming and the effector genes which confer BABA-induced resistance towards pathogens are not known (Si-Ammour *et al*., 2003).

Experiments performed with radio-labelled BABA helped to show that it is taken up and transported through the plants. Cohen and Gisi (1994) tested different methods to determine the transportation in tomato plants and found that BABA penetrated through leaves and was transported mainly acropetally. They reported that transportation was not totally unidirectional since some label was recovered in the roots. When BABA is applied as a soil drench, it can be taken up by the roots and translocated through the tomato plantlets. The activation of resistance in tobacco by the treatment of BABA has been reported to occur in the SA accumulation pathway (Siegrist *at al*., 2000). However, BABA treatment on Arabidopsis did not respond through the SA, jasmonic acid (JA) or ethylene (ET) pathway (Mauch-Mani, 1999).

Cohen *et al*. (1994) and Tosi *et al*. (1998) have also reported the curative effect of BABA but the mode of resistance has not been well described. Inoculation of cauliflower seedlings with *Peronospora parasitica*, downy mildew, three days or one day before BABA treatment, significantly controlled disease development (Silue *et al*., 2002). In contrast Zimmerli *et al*. (2000) suggested that inoculation of *Peronospora parasitica* in *Arabidopsis thaliana* six days before BABA treatment had no curative effect. They mentioned that such a late application of treatment is ineffective even with a systemic fungicide. In general, a curative effect is not claimed for SAR or SAR-inducing compounds (Ryals *et al*., 1996). However, the curative effect of fungicide mancozeb was found in BABA-induced plants for

the control of late blight or downy mildew under field conditions, suggesting BABA can synergistically increase the efficacy of the fungicides (Baider and Cohen, 2003).

Induction of PR1 has been reported (Cohen *et al*., 1994) to occur within 1 day of treatment of BABA at high concentration (2000 ppm) which reached peak levels 3 days afterwards. Further, the resistance was found to persist longer than 11 days after BABA treatment. It has been observed that relatively high concentrations of BABA are needed for plant activation to trigger resistance in almost all systems tested so far (Siegrist *et al*., 2000). BABA has been found much better tolerated when applied to roots, without deleterious effects in the concentration range used to induce resistance (Jakab *et al*., 2001). However, through root application only one-third of the applied chemical is taken up by the plants whereas through foliar treatments plants can uptake almost the entire chemical applied (Cohen and Gisi, 1994).

2.11.3 *Potentials and problems of SAR by chemicals*

Although SAR by chemical or biological elicitors has demonstrated potential for controlling field as well as postharvest diseases a lot of questions have arisen regarding its wide commercialization. The potential cost of induced resistance with BTH in wheat has been reported in that it has been shown to produce fewer numbers of lateral shoots and a reduced grain yield (Heil *et al*., 2000). Early senescence of potato plants observed in field and glasshouse conditions with the application of BTH at 100 ppm resulted in stunting of plant growth and insignificantly lower yield compared to control plants (Bokshi, 2000). However, Kuc (2001) described both favourable and unfavourable factors for the development and use of SAR for commercial practices. These are listed in Table 2.11.3 below.

Table 2.11.3 Favourable and unfavourable factors for the development and use of SAR

Favourable factors include:

1. Problems with the resistance of pathogens to classical pesticides
2. The necessity to remove some pesticides from the market, the increased testing and cost of testing to meet requirements of regulatory agencies and the lack of substitutes for removed compounds.
3. Health and environmental problems, real and perceived, associated with pesticides and the increased popularity of 'organic crops' and 'sustainable agriculture'.
4. The inability of pesticides to effectively control some pathogens, e.g., virus and soilborne pathogens.
5. Classical pesticides may not be economically feasible for farmers in developing countries. In these countries the level of awareness for the safe and effective application of classical pesticides is low, thus creating dangers to human health and the environment.
6. Resistance of the public to genetically modified plants. In SAR, foreign genes are not introduced. The 'traditional' genes for resistance in the plant are those that are expressed.
7. SAR has a broad spectrum and is effective for a long time.
8. Since many defences are activated, SAR is less likely to develop resistance in pathogens.

Unfavourable factors include:

1. Some plant pathologists still ridicule the applicability of SAR.
2. Only high profit, patented and complex inducers make the major markets. Who champions the simple non-patented compounds?

3. Lack of sufficient information exchange and financial support for non mega-agribusiness-oriented scientists and a lack of adequate information flow to farmers and the public.
4. Unlike classical pesticides which directly kill or inhibit development of a pathogen, SAR depends upon the expression of genes for resistance in the plant. Therefore, SAR is more subject to physiological and environmental influences for effectiveness.
5. Public and farmer apprehension of new technologies.

Factors delaying the commercial use of SAR for postharvest storage diseases need to be overcome by increasing research on different commodities under a range of crop management practices. Already it has been reported that negative responses of crop growth and yield are related to the developmental stage of the plants when treated, as well as to the possible available nutrient supply (Hammerschmidt *et al*., 2001). More attention should be given to individual plant-pathogen interactions, to determine the doses of the inducer, as well as the putative defence compounds responsible for the SAR with the timing of their appearance. These are important for the development of SAR compounds for use commercially (Kuc, 2001). Hence, considerably more applied and basic research is required to fully understand the role systemic resistance can play in controlling postharvest diseases commercially (Terry and Joyce, 2004).