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Novel Elicitors Induce Defense Responses in Cut Flowers

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1. Introduction

Cut flower production and trade in the E.U. and the rest of the world holds the main share within the ornamental horticulture industry. Despite the global economic crisis started in 2008, changes in cut flower trade, such as the merge of the 2 major auctions in Holland (i.e. VBA and FloraHolland), resulted in stabilization or even small increases in stem number sales for the years 2008-2010 (Evans & Van der Ploeg, 2008; Anonymous, 2011). In other words, the importance of cut flower industry in global economy is undisputed, but also reflects the human need for ornamental plant consumption as part of a better life.

Product quality of horticultural crops has been the main area of research the past decades. Growers and sellers have been seeking for best possible product quality and highest possible profits. However, problems in quality after pathogen infections at some point of production, or during storage or transportation eventually result in economic losses (van Meeteren, 2009). *Botrytis cinerea* is the single-most important pathogen infecting ornamental plants and cut flowers postharvest and substantially reduce growers' and sellers' income by increasing product rejections.

B. cinerea Pers. is a common fungal pathogen that infects glasshouse-grown ornamental crops under cool and humid conditions with latent symptoms, which develop during storage or transportation (Elad, 1988). Growers and sellers around the world are deeply concerned by such infection problems. In Europe, for instance, large quantities of *B. cinerea*-infected cut freesias from The Netherlands are rejected in the UK by wholesalers and retailers at certain times of the year (Darras et al., 2004). These rejections result in immediate economic losses and make cooperation between growers and importers problematical. The problem is equally substantial for roses (Elad, 1988; Elad et al., 1993), gerberas (Salinas & Verhoeff, 1995) and Geraldton waxflowers (Joyce, 1993), although species such as chrysanthemum (Dirkse, 1982), narcissus (O'Neill et al., 2004), lisianthus (Wegulo & Vilchez, 2007), dianthus, ranunculus and cyclamen (Seglie et al., 2009) eventually suffer infections by *B. cinerea*, but to a lesser extend.

Infections by *B. cinerea* are usually managed by conventional fungicides applied protectively at certain times of the year and especially during autumn and spring when most infections occur. However, extensive use of fungicides such as dicarboximides, has led to the

appearance of resistant *B. cinerea* strains (Pappas, 1997). Alternative methods to control *B. cinerea* disease (i.e. grey mold) within the concept of integrated disease management (IDM) programs are sought by growers and help overcome resistance by the pathogen.

Elicitor-based disease management constitutes an attractive socio-environmentally sound strategy (Joyce & Johnson, 1999). Known activators of plant defence reactions, such as 2,6-dichloroisoniciotinic acid (INA), salicylic acid (SA), 3-aminobutyric acid (BABA), Acibenzolar-S-methyl [ASM; benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester; benzothiadiazole or BTH; CGA 245704] and methyl jasmonate (MeJA), have been shown to enhance natural defence mechanisms or induce systemic defence responses such as SAR or ISR in plants, thereby providing prospects for IDM (Terry & Joyce, 2004a).

1.1 *Botrytis cinerea* infecting cut flowers and ornamental pot plants

Botrytis cinerea Pers. belongs to the Class Deuteromycetes and the Phylum Ascomycota. The disease caused by *B. cinerea* is called grey mold. The fungus is pathogenic to most of the cultivated ornamental pot plants and cut flowers. For example, infection of gerbera (*Gerbera jamesonii*) flowers occurs inside the glasshouse during crop cultivation, but symptoms develop after a latent period at storage or transportation following fluctuations in temperature (Salinas & Verhoeff, 1995). Favourable temperature and relative humidity (RH) for the pathogen after harvest results in rapid disease development (Salinas et al., 1989). Grey mold on gerbera and freesia flowers is observed as small necrotic, dark-brown fleck lesions 'spots'. Similar symptoms developed in the laboratory under controlled conditions following artificial inoculation of gerbera or freesia inflorescences at temperatures ranging from 4 to 25°C (Salinas & Verhoeff, 1995; Darras et al., 2006a). Infection of freesia (*Freesia hybrida*) inflorescences after artificial inoculation occurred in less than 24 h at 12°C and 80-90% RH. Even at the low temperature of 5°C, disease symptoms were evident in a saturated atmosphere (ca. 100% RH) within the first 24 h of incubation.

B. cinerea is also pathogenic to Geraldton waxflower (*Chamelaucium uncinatum*), the Australian native plant which holds a high ornamental and commercial value (Joyce, 1993; Tomas et al., 1995). Geraldton waxflower sprigs artificially inoculated with *B. cinerea* showed increased abscission of flowers from their pedicels.

B. cinerea infects rose (*Rosa hybrida*) flowers and produces necrotic spots or blister-like patches on petal surfaces (Pie & De Leeuw, 1991; Williamson et al., 1995). Infection has been described by Elad (1988) as restricted, brown, volcano-like shaped lesions. *B. cinerea* damages phylloclades of ruscus (*Ruscus aculeatus*) by causing small, dark water soaked necrotic lesions encircled by a faint halo. These lesions later become brown without growing in size (Elad et al., 1993).

Infection of lisianthus (*Eustoma russellianum*) flowers has been recently reported by Wegulo & Vilchez (2007). Significant ($P \leq 0.03$) positive correlations between stem lesion length of naturally infected plants in the glasshouse ($R = 0.74$) and stem lesion length of artificially inoculated ones ($R = 0.62$) with the disease incidence score, and with the percent of necrosis ($R = 0.71$) of detached leaves were reported (Wegulo & Vilchez, 2007). From all the 12 lisianthus cultivars tested, 'Magic Champagne' was suggested as the most resistant and proposed as ideal for commercial cultivation.

In regards to pot plants, *B. cinerea* disease symptoms on geranium (*Pelargonium zonale*) flowers has been described by Strider (1985) as flower blight, leaf blight and stem rot. Martinez et al. (2008) published a detailed report on infection of *Pelargonium x hortorum, Euphorbia pulcherrima, Lantana camara, Lonicera japonica, Hydrangea macrophylla, and Cyclamen persicum* by *B. cinerea.* They reported that growth of *B. cinerea* isolates in-vitro from the above mentioned ornamentals varied significantly. *B. cinerea* showed a high degree of phenotypical variability among the isolates, not only as regards to visual aspects of the colonies but also to some morphological structures such as conidium length, conidiophores, sclerotia production, and hyphae (Martinez et al., 2008). Increased susceptibility to grey mold from 10% to 80% in stems and from 3% to 14% in leaves was observed after using elevated levels of N supply (i.e. from 7.15 to 57.1 mM) for begonia plant (*Begonia x tuberhybrida* Voss) cultivation (Pitchay et al., 2007).

1.2 Review on host-pathogen interactions and on defence responses

Host-pathogen specificity involves factors that determine the virulence of the pathogen and also factors that confer resistance on the host (Lucas, 1997). Many theories have been proposed concerning mechanisms by which pathogens either achieve or fail to infect host tissue. A model concerning specific gene-for-gene interactions determining the host range of pathogens in wild plant species was first proposed by Flor (1971). In a gene-for-gene system, recognition of the pathogen by the host occurs when a resistance (R) gene of the host interacts with an avirulence (avr) gene of the pathogen (Table 1).

Virulent or avirulent Pathogen genes	**Resistant or susceptible genes in the plant**	
	R (resistant) dominant	r (susceptible) recessive
A (avirulent) dominant	AR (-)[a]	Ar (+)
a (virulent) recessive	aR (+)	ar (+)

[a] (-) indicate incompatible interaction and, therefore, no infection. (+) indicate compatible interaction and, therefore, infection.

Table 1. Quadratic check of gene combinations and disease reaction types in host-pathogen systems in which the gene-for-gene concept for one gene operates (Lucas, 1997).

According to this model, avr gene products secreted by hyphae or located on the surface of the pathogen bind to a receptor located on the cell membranes of host's epidermal cells. Binding triggers a cascade of defence responses by the host. Every other possible match in the system could lead to infection (Table 1). Thus, a combination of a resistant host gene and a virulent pathogen leads to a compatible host-pathogen interaction. In both cases, when an avr race of the pathogen matches with a susceptible host and a virulent pathogen matches with a susceptible host, the host fails to recognize the pathogen and infection occurs (Flor, 1971).

Culture filtrates or extracts from microbial cells can act as potent inducers of plant defence responses (Chappell & Hahlbrock, 1984; Kombrink & Hahlbrock, 1986; Fritzemeier et al.,

1987; Keller et al., 1999). For instance, extracts from fungal cell walls when applied to plant tissue induced the synthesis and accumulation of phytoalexins (Yoshikawa et al., 1993). Active components in such chemical, biological and physical extracts are referred to as elicitors. This term is now generally used to denote agents, which induce plant defence responses, including accumulation of PR-proteins, cell wall structural (strengthening) changes, and hypersensitive cell death (Kombrink & Hahlbrock, 1986).

1.2.1 Rapid defence responses

The first step in the rapid defence responses by plants is recognition of the infection attempt by the pathogen. Pathogen recognition results in a signalling cascade to neighbouring cells and in initial molecular defence responses (Kombrink & Somssich, 1995). Examples of elicitor-active components produced by pathogenic fungi include the β-glucan elicitor and the 42 kDa glycoprotein derived from the fungus *Phytophthora megasperma*, the oligo-1,4-α-galacturonides from *Cladosporium fulvum* and *Rhynchosporium secalis*, and the harpin protein from *Erwinia amylovora*. These compounds activate defence responses when they bind to host receptors during incompatible host-pathogen interactions (Ebel & Cosio, 1994; Kombrink & Somssich, 1995).

In parsley cells, the existence of a receptor was proposed by Ebel & Cosio (1994). The intracellular changes were subsequent signals activated by the receptor and transported to host plasma membrane. Changes in H^+, K^+, Cl^- and Ca^{2+} fluxes across the plasma membrane and H_2O_2 increase within 2-5 min can occur (Nurnberger et al., 1994; Nurnberger & Scheel, 2001).

The activity of active oxygen species (e.g. O^-, H_2O_2) and the rapidity of their production after invasion characterize the rapid defence response of the host (Dixon et al., 1994; Ebel & Cosio, 1994; Bolwell, 1999). These toxic active oxygen species cause host cell death at the infection site (Kombrink & Somssich, 1995). It has been suggested that reactive oxygen species (ROS) could have a dual function in disease resistance (Kombrink & Schmelzer, 2001). Firstly, ROS participate directly in cell death during HR and, thereby, results in direct pathogen inhibition. Secondly, ROS have a role in signal diffusion for cellular protectant induction and associated defence responses in neighbouring cells (Kombrink & Schmelzer, 2001).

The HR is part of the initial plant defence responses and involves localized cell death at the infection site (Kombrink & Schmelzer, 2001). Thus, the HR is a result of host recognition of infection attempts made by a pathogenic bacterium or fungus. Specific elicitor-molecules comprise signals, which induce these initial defence responses. When pathogenic bacteria are injected inside a non-host plant under artificial conditions they are killed by the HR as a result of being surrounded by dead cells. The HR may occur when either virulent strains of bacteria are injected inside a resistant host or avirulent strains of bacteria are injected inside a susceptible host (Agrios, 1997). HR associated isolation of the pathogen inside necrotic cells causes the pathogen loses its ability to take-up nutrients and grow into adjacent healthy cells (Kombrink & Schmelzer, 2001).

Elicitors which do not cause an HR can also activate defence-related compounds (Schroder et al., 1992; Atkinson, 1993; Kuć 1995; Kombrink & Schmelzer, 2001). Activation of these compounds can be similar for both compatible and incompatible host-pathogen interactions (Schroder et al., 1992; Kombrink and Schmelzer, 2001). However, only with compatible

interactions does the pathogen infect and colonize the host. Accumulation of phytoalexins can occur as part of the HR (Dixon et al., 1994). However, it is not clear whether the HR triggers the production of phytoalexins and other antimicrobial compounds or if their accumulation is a direct result of elicitation (Kombrink & Somssich, 1995).

1.2.2 Local acquired resistance

Phytoalexins are low molecular weight antimicrobial compounds produced de-novo by some plants. They accumulate during infection by pathogens or after injury or stress (Ebel, 1986; Isaac, 1992; Kuć, 1995). Accumulation of phytoalexins is mainly observed when fungi, rather than bacteria, viruses or nematodes, try to infect the plant. Accumulation is a result of specific elicitors released either by the fungal cell walls or by the plant cell walls (Ebel, 1986). Elicitors of phytoalexins include a large number of compounds including inorganic salts (Perrin & Cruickshank, 1965), oligoglucans (Sharp et al., 1984), ethylene (Chalutz & Stahmann, 1969), fatty acids (Bostock et al., 1981), and chitosan oligomers (Kendra & Hadwiger, 1984). Over 200 compounds, microorganisms and physiological stresses have been reported to elicit pisatin in pea, phaseollin and kievitone in green bean and glyceollin in soybean (Kuć, 1991).

Most phytoalexins have been isolated from dicot plants, but they are also present in monocots including rice, corn, sorghum, wheat, barley and onions (Kuć, 1995). There is no published work on phytoalexins in cut flower species. Phytoalexins have been found in almost every part of the plant including roots, stems, leaves and fruits (Kuć, 1995). Such plant species produces a characteristic set of phytoalexins derived from secondary metabolism, in most cases via the phenylpropanoid pathway (Ebel, 1986; Kombrink & Somssich, 1995; Kuć, 1995). Phytoalexins belong to a number of key chemical groups including phenolics (e.g. flavonoids and coumarins), polyacetylenes, isoprenes, terpenoids and steroids (Ebel, 1986). They are produced by both resistant and susceptible tissues and resistance appears to be related with the total phytoalexin concentration (Kuć, 1995). Phytoalexins can affect fungal growth by inhibiting germ tube elongation and colony growth (Elad, 1997). The main effect of phytoalexins on fungi is via their cell membranes. Direct contact of phytoalexins with fungal cell walls resulted in fungal plasma membrane disruption and loss of the ultrastructural integrity (Elad, 1997). In compatible interactions, the pathogen apparently tolerates accumulated phytoalexins, detoxifies them, suppresses phytoalexin accumulation and/or avoids eliciting phytoalexin production (Kuć, 1995). Overcoming phytoalexin accumulation is attributed to either suppressor molecules released by the pathogen (i.e. low molecular weight polysaccharides or glycopeptides) or suppression of the intensity and timing of signal genes that could trigger phytoalexin accumulation (Kuć, 1995).

Pathogenesis related proteins (PR-proteins) accumulate either in extracellular space or the vacuole after various types of plant stress, including pathogen infection (Stermer, 1995; Sticher et al., 1997). PR-proteins accumulate at the site of infection as well as in uninfected tissues (Van Loon & Gerritsen, 1989; Ryals et al., 1996). Although healthy plants may contain traces of PR-proteins, the transcription of genes encoding PR-proteins is up-regulated following pathogen attack, elicitor treatment, wounding or stress (Stermer, 1995; Sticher et al., 1997; Van Loon, 1997). Signal compounds responsible for initiating PR-protein production include salicylic acid, ethylene, the enzyme xylanase, the polypeptide systemin

and jasmonic acid (Agrios, 1997). The importance of PR-proteins lies in their range of defence activities (Van Loon et al., 1994). A number of PR-proteins release molecules that may act as elicitors (Keen & Yoshikawa, 1983). PR-proteins accumulation has been observed in monocots as well as in dicots (Redolfi, 1983). However, there is no published work on PR-proteins induced in flower species. Eleven families of PR-proteins have been recognized so far (Van Loon et al., 1994). Some inhibit pathogen development during microbial infection by inhibiting fungal spore production and germination. Others are associated with strengthening of the host cell wall via its outgrowths and papillae (Agrios, 1997). Both β-1,3-glucanases and chitinases, PR-2 and PR-3, respectively, are known to have direct antifungal activity (Mauch et al., 1988; Van Loon, 1997). However, many pathogens have evolved mechanisms to reduce the antifungal impact of PR-proteins (Van Loon, 1997). For example, many chitin-containing fungi are not inhibited by host-produced chitinases.

Plant secondary metabolites are divided into the three main categories of terpenes, phenolic compounds and nitrogen containing secondary metabolites (i.e. alkaloids) (Taiz & Zeiger, 1998). All secondary metabolites are produced through one of the major mevalonic, malonic or shikimic pathways (Taiz & Zeiger, 1998). Phenylalanine is a common amino acid produced via the shikimic pathway (Hahlbrock & Scheel, 1989). The most abundant classes of secondary phenolic compounds in plants are derived from phenylalanine via elimination of an ammonia molecule to form cinnamic acid. This reaction is catalyzed by phenylalanine ammonia lyase (PAL), the key enzyme of phenylpropanoid metabolism (Hahlbrock & Scheel, 1989). Derivatives of phenylpropanoid pathway include low-molecular-weight flower pigments, antibiotic phytoalexins, UV-protectants, insect repellents, and signal molecules in plant-microbe interactions (Hahlbrock & Scheel, 1989; Kombrink & Somssich, 1995).

The main phenylpropanoid pathway branches leading to formation of flavonoids, isoflavonoids, coumarins, soluble esters, wall bound phenolics, lignin and suberin. This diverse spectrum of compounds has a wide range of properties (Hahlbrock & Scheel, 1989). For example, the lignin pathway is an important phenylpropanoid pathway branch that produces precursors for lignin deposition (Grisebach, 1981). Various enzymes implicated in the biosynthesis of lignin appeared to be induced in plants in response to infection or elicitor treatment (Matern & Kneusel, 1988). However, not all studies show a role of lignin and cell lignification in disease inhibition (Garrod et al., 1982). Furanocoumarins derived from the furanocoumarin pathway in parsley are considered potent phytoalexins (Beier & Oertli, 1983). Flavonoid and furanocoumarin production as a response to UV light or fungal elicitor treatment respectively was associated with up-regulation of PAL, 4-coumarate: CoA-ligase (4CL) and chalcone synthase (CHS). Up-regulation was based on rapid changes in amounts and activities of the corresponding mRNAs (Chappell & Hahlbrock, 1984).

After pathogen recognition by the host, a cascade of early responses is induced including ion fluxes, phosphorylation events, and generation of active oxygen species (Kombrink & Somssich, 1995). SA acts as a secondary signal molecule and its levels increase during the defence induction process. Thus, SA is required for initiation of synthesis of various defence-related proteins such as the PR-proteins (Van Loon, 1997; Metraux, 2001). SA accumulation endogenously in tobacco and cucumber plants lead to the HR and the SAR responses. However, SA is not necessarily the translocated signal (elicitor) for the onset of SAR. Rather, SA exerts an effect locally (Vernooij et al., 1994; Ryals et al., 1996). Nonetheless,

SA is still required for SAR expression (Van Loon, 1997). The importance of SA in the onset of SAR was determined using transgenic tobacco and Arabidopsis plants engineered to over-express SA-hydroxylase. Transformed plants with the naphthalene hydroxylase G (NahG) gene produced low levels of SA and SAR expression was blocked.

SA is produced from phenylalanine via coumaric and benzoic acid (Mauch-Mani and Slusarenko, 1996; Ryals et al., 1996; Sticher et al., 1997). Biosynthesis of SA starts with the conversion of phenylalanine to trans-cinnamic acid (Sticher et al., 1997). From trans-cinnamic acid, either benzoic acid (BA) or ortho-coumaric acid are produced. Both compounds are SA precursors (Sticher et al., 1997). Pallas et al. (1996) showed that tobacco plants epigenetically suppressed in PAL expression produced a much lower concentration of SA and other phenylpropanoid derivatives when artificially inoculated with tobacco mosaic virus (TMV). This was seen, firstly, due to the lack of resistance to TMV upon secondary infection, and, secondly, to the absence of PR protein induction in systemic leaves (Pallas et al., 1996).

Jasmonic acid (JA) and its methyl ester (MeJA) are derived from linolenic acid. They are cyclopentanine-based compounds that occur naturally in many plant species (Sembdner & Parthier, 1993; Creelman & Mullet, 1997). Linolenic acid levels or its availability could determine JA biosynthetic rate (Farmer & Ryan, 1992; Conconi et al., 1996). The level of JA in plants varies as a function of tissue and cell type, developmental stage, and in response to various environmental stimuli (Creelman & Mullet, 1997). For example, in soybean seedlings, JA levels are higher in the hypocotyls hook (a zone of cell division) and young plumules as compared to the zone of cell elongation and more mature regions of the stem, older leaves and roots (Creelman & Mullet, 1997). High JA levels are also found in flowers and pericarp tissues of developing reproductive structures (Creelman & Mullet, 1997). Jasmonates are wide spread in Angiosperms, Gymnosperms and algae (Parthier, 1991). They can mediate gene expression in response to various environmental and developmental processes (Wasternack & Parthier, 1997). These processes include wounding (Schaller & Ryan, 1995), pathogen attack (Epple et al., 1997), fungal elicitation (Nojiri et al., 1996), touch (Sharkey, 1996), nitrogen storage (Staswick, 1990), and cell wall strengthening (Creelman et al., 1992). Wounding of tomato leaves produced an 18-amino acid polypeptide called systemin, the first polypeptide hormone discovered in plants so far (Pearce et al., 1991). Systemin was released from damaged cells into the apoplast and transported out of the wounded leaf via the phloem (Schaller & Ryan, 1995) (Fig. 1).

Upon herbivore wounding, a systemic signal is delivered from systemin and results in an ABA-dependent rise of linoleic acid. Systemin was believed to bind to the plasma membrane of target cells and thereby initiate JA biosynthesis (Schaller & Ryan, 1995). JA accumulation can also be induced by oligosaccharides derived from plant cell walls and by elicitors, such as chitosans derived from fungal cell walls (Gundlach et al., 1992; Doares et al., 1995; Nojiri et al., 1996). JA also activates gene expression encoding proteinase inhibitors (Creelman & Mullet, 1997). Proteinase inhibitors are known antidigestive proteins that block the action of herbivore proteolytic enzymes (Creelman & Mullet, 1997). Thereby, proteinase inhibitors help the host to avoid consumption by herbivores. Proteinase inhibitors were accumulated in tomato plants after wounding (O'Donnell et al., 1996) and after irradiation with UV-C (Conconi et al. 1996). In response to wounding, ethylene and JA act together to regulate gene expression of proteinase inhibitors (O'Donnell et al., 1996). Exposing tomato

leaves to increasing doses of 254 nm UV-C resulted in increased proteinase inhibitors gene expression. Expression of proteinase inhibitors in wounded (Doares et al., 1995; O'Donnell et al., 1996) or UV-C treated (Conconi et al., 1996) tomato leaves was markedly reduced upon treatment with SA. From linoleic acid, jasmonic acid is produced. Ethylene is required in the jasmonic-signalling cascade (O'Donnell et al., 1995).

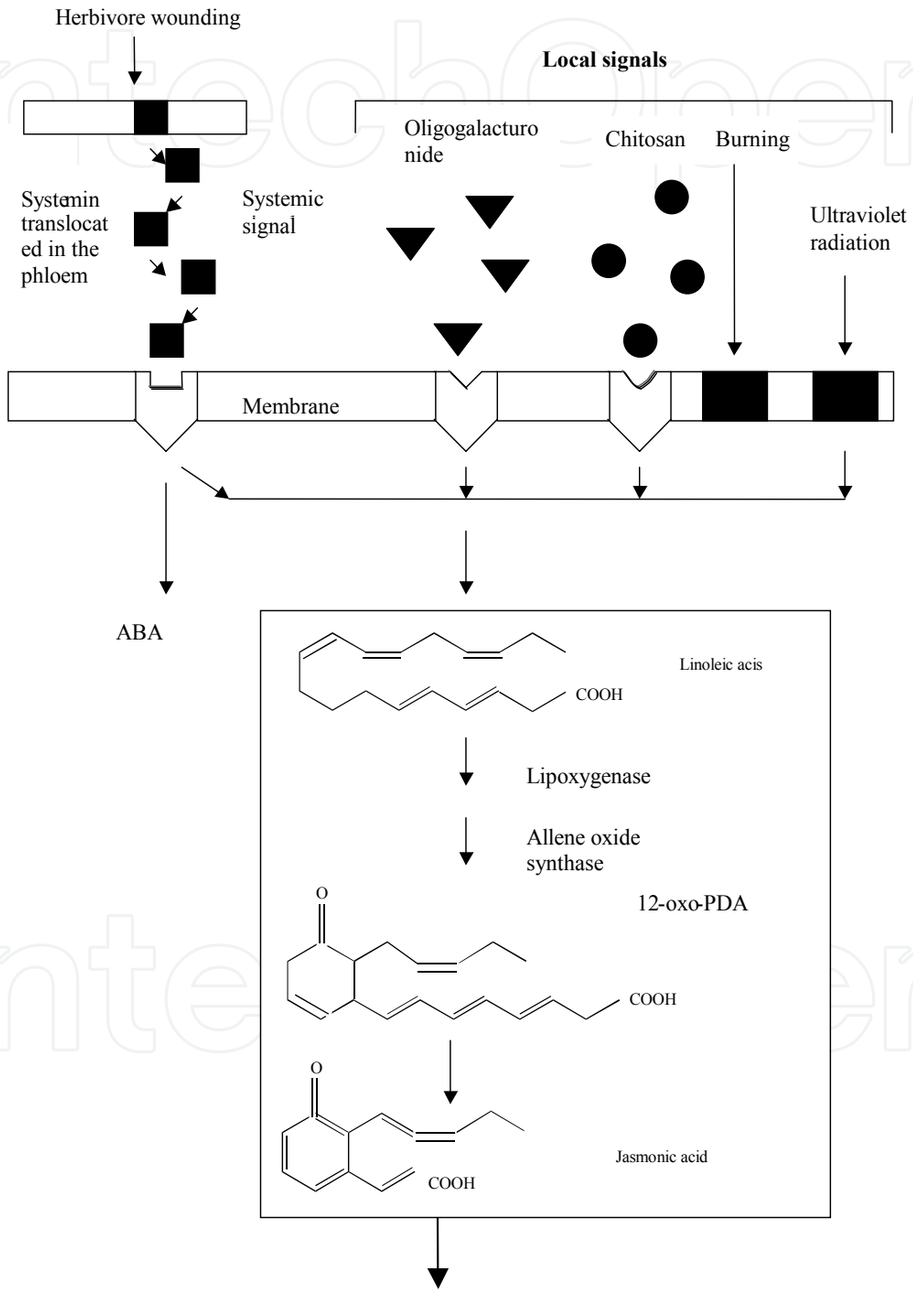

Fig. 1. The octadecanoid-signalling pathway for defence gene expression in tomato (Schaller and Ryan, 1995).

1.2.3 Systemic defence responses (i.e. SAR, ISR) and signalling pathways

SAR is activated following induction of local acquired resistance (LAR). SAR is potentially induced after the HR and after challenge with virulent strains of a pathogen or elicitor treatment. It develops systemically in distant parts of the infected plant (Lawton et al., 1996; Ryals et al., 1996; Metraux, 2001). SAR protects plants from a broad range of potential pathogens (Kessmann et al, 1994).

Specific genes induced in different plant species during SAR have been called SAR-genes (Kessmann et al., 1994; Stermer, 1995; Ryals et al., 1996; Sticher et al., 1997). Most of SAR-genes encode PR-proteins such as those accumulated after inoculation of tobacco with TMV (Ward et al., 1991). These include PR-1 (PR-1a, PR-1b, PR-1c), β-1,3-glucanases (PR-2a, PR-2b, PR-2c), chitinases (PR-3a, PR-3b), hevein-like proteins (PR-4a, PR-4b), thaumatin like proteins (PR-5a, PR-5b), acidic and basic isoforms of class III chitinase, an extracellular β-1,3-glucanase and the basic isoform of PR-1 (Ward et al., 1991). SAR and SAR-gene activation has been observed in various dicots (Kessmann et al., 1994; Ryals et al., 1996). SAR activation involves species specificity (Ryals et al., 1992). For example, acidic PR-1 is only weakly expressed in cucumber. In contrast, acidic PR-1 is the main protein accumulating in tobacco and Arabidopsis. A number of homologous SAR-genes have been identified in monocots. Homologs of the PR-1 family were found in maize and barley and other PR-proteins in maize (Nasser et al., 1988). Gorlach et al. (1996) isolated a group of wheat genes (WCI or wheat chemically induced) induced after chemical treatment with potent SAR inducers. WCI genes seemed to act in a similar manner to SAR-genes in dicots after chemical treatment with plant activators (Gorlach et al., 1996).

Recent research has revealed that JA and ethylene play key roles in signal transduction pathways associated with plant defence responses (Pieterse and van Loon, 1999; Thomma et al., 2000). Inoculation with a necrotizing pathogen resulted predominantly in activation of the SA-dependent SAR response. This response leads to the accumulation of salicylic acid inducible PR-proteins and the expression of SAR (Ryals et al., 1996; Pieterse & van Loon, 1999) (Fig. 2, pathway 2). JA and ethylene inducible defence responses are induced by non-necrotizing rhizobacteria and lead to the ISR phenomenon (Pieterse et al., 1996; Pieterse et al., 1998) (Fig. 2, pathway 1). Both pathways 1 and 2 are regulated in Arabidopsis plants carrying the NPR1 gene.

Depending on the invading pathogen, the composition of defence compounds produced after infection can vary between SA- and JA/ethylene-inducible pathways (Fig. 2, pathways 2 and 3) (Ryals et al., 1996; Epple et al., 1997; Dong, 1998).

Wounding can also result in JA and ethylene inducible defence response activation (Fig. 2, pathway 4) (O'Donnell et al., 1996; Wasternack & Parthier, 1997). However, resultant products of the wounding pathway differ from those induced upon pathogen infection (O'Donnell et al., 1996; Rojo et al., 1999). A second distinct wound-signalling pathway leading to wound responsive (WR) gene expression has been found in Arabidopsis plants (Titarenko et al., 1997; Rojo et al., 1998) (Fig. 2, pathway 6). Upon wounding, Arabidopsis plants carrying the coi1 (JA-insensitive) mutant gene expressed the wound responsive genes choline kinase (CK) and wound responsive (WR3) indicating that the induced pathway was totally independent of JA. UV irradiation of tomato leaves also resulted in induction of the same defensive genes normally activated through the octadecanoid pathway after wounding (Conconi et al., 1996). This response is blocked after SA treatment, confirming the

antagonistic regulation of the two distinct pathways (Pena-Cortes et al., 1993; Lawton et al., 1995; Xu et al., 1994; Doares et al., 1995; O'Donnell et al., 1996; Niki et al., 1998; Gupta et al., 2000; Rao et al., 2000).

In the rhizobacteria-mediated induced systemic resistance (ISR) pathway, components from the JA/ethylene response acted in sequence in activating a systemic resistance response that, like pathogen induced SAR, was dependent on the regulatory protein NPR1 (Pieterse & van Loon, 1999). The ISR pathway shares signalling events with pathways initiated upon pathogen infection, but is not associated with the activation of genes encoding plant defensins, thionins or PR-proteins (Pieterse & van Loon, 1999) (Fig. 2, pathway 3). This observation indicates that ISR inducing rhizobacteria, such as P. fluorescens strain WCS417r, trigger a novel signalling pathway leading to the production of so far unidentified defense compounds (Pieterse et al., 1996; Pietrese et al., 1998). Protection of NahG Arabidopsis plants by gaseous MeJA suggested that induction of a SA non-dependent systemic pathway was regulated by JA (Thomma et al., 2000) (Fig. 2, pathway 3). Protection was provided against two necrotrophic fungi, *A. brassicicola* and *B. cinerea*.

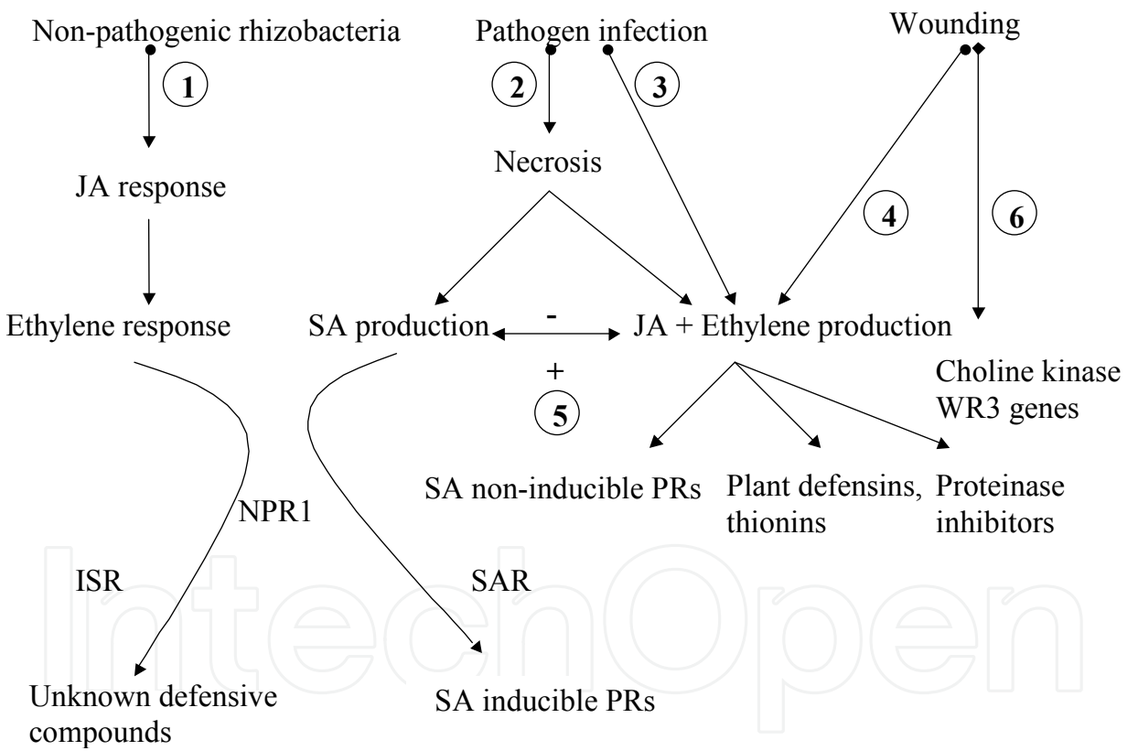

Fig. 2. Model showing systemic signalling pathways that can be induced in plants by non-pathogenic rhizobacteria, pathogen infection and wounding, such as caused by foraging insects. 1: ISR is induced in NPR1 Arabidopsis plants as a result of JA and ethylene responses. 2: SAR is induced in NPR1 Arabidopsis plants after necrosis by pathogenic fungi, bacteria or virus. 3: JA/ethylene pathway is up- regulated after fungal infection. JA/ethylene expression leads to genes encoding plant defensins, thionins, proteinase inhibitors and SA-independent PR-proteins. 4 and 6: A number of genes are regulated after mechanical wounding. JA and ethylene levels rise after mechanical wounding. 5: Cross-talk between SA- and JA- dependent pathways exist. Adopted from Pieterse & van Loon (1999).

1.3 Elicitation of defence responses with chemical activators

Disease management in the past has been achieved by various methods including resistant cultivars, biological control, crop rotation, tillage, and chemical pesticides (Kessmann et al., 1994). Recently, the use of abiotic and/or biotic agents, as well as, synthetic compounds that induce host immune systems have offered a new prospect for disease management.

A chemical is generally characterized as a plant activator when it induces natural and/or systemic defence responses, activate gene expression and provide protection on the same spectrum of diseases exerted by a wild type host (Kessmann et al., 1996; Ruess et al., 1996). Plant activators, normally, do not exert direct antimicrobial activity against pathogens when used for disease control, but rather work through their mutagenic elicitation effect and help eliminate the risk of the development of resistant strains by the pathogen.

1.3.1 Acibenzolar-S-methyl (ASM; benzo[1,2,3]thiadiazole-7-carbothioic acid S-methyl ester; CGA 245704; benzothiadiazole or BTH)

The efficacy of ASM has been tested in field, glasshouse and pot trials (Table 2).

Host	Pathogen	Induced response	ASM concentration	Reference
Apple seedlings cv. Golden Delicious	*Erwinia amylovora*	β-1,3-glucanases, peroxidases	0.1-0.2 g AI L^{-1}	Brisset et al., 2000
Cauliflower (*Brassica oleracea*)	*Peronospora parasitica*	ns	0.0015-0.075 g AI L^{-1}	Godard et al., 1999
Cereals, tobacco	*Erysiphe graminis*, *Septoria* spp., *Puccinia* spp., *Peronospora hyoscyami* f. sp. *tabacina*	ns	12-30 g AI ha^{-1}	Ruess et al., 1996
Cucumber (*Cucumis sativus* L.)	*Cladosporium cucumerinum*	Acidic peroxidase, class III chitinase and β-1,3-glucanase	32.4 g AI L^{-1}	Narusaka et al., 1999
Cucumber (*Cucumis sativus* L.) and Japanese pear (*Pyrus pyrifolia* Nakai var. culta Nakai)	*Many pathogens*	ns	0.05-0.1 g AI L^{-1}	Ishii et al., 1999
Cauliflower (*Brassica oleracea*)	*P. parasitica*	β-1,3-glucanase. PR-1 and PR-5	0.05 g AI L^{-1}	Ziadi et al., 2001
Grapevine cv Merlot	*B. cinerea*	na	0.3 mM	Iriti et al., 2004
Melons cv. Early Yellow Hami	*Fusarium* spp., *Alternaria* spp., *Rhizopus* spp. *Trichothecium* sp.	ns	0.025 or 0.05 g AI L^{-1}	Huang et al., 2000

Host	Pathogen	Induced response	ASM concentration	Reference
Melon fruit	*Fusarium pallidoroseum*	ascorbate peroxidase, guaiacol peroxidase, PAL, β-1,3-glucanase		Gondim et al., 2008
Parsley cells (*Petroselinum crispum* L.)	With or without elicitor (Pmg)	PAL, coumarins	0.32-6.48 g AI L^{-1}	Katz et al., 1998
Pepper (*Capsicum annuum* L.)	*Xanthomonas campestris* pv. *vesicatoria*	ns	1.25-5 g AI L^{-1}	Buonaurio et al., 2002
Soybean seedlings	*Sclerotinia sclerotiorum*	ns	0.035-0.375 g AI L^{-1}	Dann et al., 1998
Strawberry plants cvs. Elsanta and Andana	*B. cinerea*	ns	0.25-2 g AI L^{-1}	Terry and Joyce, 2000
Strawberry	Microbial populations	chitinase and β-1,3-glucanase	0.05-0.5 g.L^{-1}	Cao et al., 2010
Tobacco plants cv. Kutsaga Mammoth 10	*Pseudomonas syringae* pv *tabaci, Thanatephorus cucumeris, Cercospora nicotianae*	ns	0.05-30 g AI L^{-1}	Cole, 1999
Tomato plants (*Lycopersicon esculentum*)	*Fusarium oxysporum* f.sp. *radicis-lycopersici*	Callose enriched wall appositions phenolic compounds	97.2 g AI L^{-1}	Benhamou & Belanger, 1998
Tomato plants cv. Vollendung	*Cucumber mosaic virus (CMV)*	ns	0.1 mM	Anfoka, 2000
Wheat	*Erysiphe graminis* f.sp. *tritici*	WCI genes (1-5)	0.3 mM	Gorlach et al., 1996

Table 2. Effects of ASM on different host-pathogen interactions (ns: not shown, na: not applicable).

Although, most of ASM application were carried out preharvest, there is number of published research on ASM postharvest applications (i.e. Cao et al., 2010). Additionally, a considerable work on postharvest application of ASM on ornamentals has been published the recent years (i.e. Darras et al., 2007). ASM was introduced as a potent inducer of SAR and treated plants were resistant to the same spectrum of diseases as plants activated naturally (Kessmann et al., 1996; Friedrich et al., 1996). Although ASM and its metabolites exhibited no direct antimicrobial activity towards plant pathogens tested, they induced the same biochemical processes in the plant as those observed after natural activation of SAR (Friedrich et al., 1996; Lawton et al., 1996). The compound, which was inactive in plants that do not express the SAR-signaling pathway, required a lag time of approximately 30 days between application and protection (Lawton et al., 1996).

1.3.2 Jasmonates (plant hormones produced through the octadecanoid pathway)

The efficacy of jasmonates has been tested in field, glasshouse and pot trials (Table 3).

Host	Pathogen	Induced response	MeJA concentration	Reference
Arabidopsis (*Arabidopsis thaliana*)	*B. cinerea, A.brassicicola, Plectosphaerella cucumerina*	ns	0.5-50 µM and 0.001-1 µL L^{-1} air	Thomma et al., 2000
Arabidopsis (*Arabidopsis thaliana*)	*A. brassicicola*	PDF1.2	45 µM	Penninckx et al., 1996
Grapefruit (*Citrus paradisi*) var. 'Marsh Seedless	*Penicillium digitatum*	ns	1-50 µM	Droby et al., 1999
Large number of species	na	PPO	na	Constabel and Ryan, 1998
Loquat fruit	*Colletotrichum acutatum*	chitinase and β-1,3-glucanase	10 µmol L^{-1}	Cao et al., 2008
Potato plants (*Solanum tuberosum*)	*Phytophthora infestans*	phytoalexins	1-10 µM	Il'inskaya et al., 1996
Sweet cherry	*Monilinia fructicola*	PAL, β-1,3-glucanase	0.2 mM	Yao & Tian, 2005
Tobacco cell cultures	na	β-glucuronidase (GUS), osmotin protein	0.045-4550 µM	Xu et al., 1994
Tobacco cv. Xanthi-nc	*Phytophthora parasitica var. nicotianae, Cercospora nicotianae, TMV*	β-glucuronidase (GUS)	45 µM	Mitter et al., 1998
Tomato plants (*Lycopersicon esculentum*)	*Helicoverpa zea, Spodoptera exigua*	PPO, POD, LOX and PIs	0.1-10 mM	Thaler et al., 1996
Tomato plants (*Lycopersicon esculentum*)	*Spodoptera exigua, Pseudomonas syringae* pv. *tomato*	PPO	1 mM	Thaler et al., 1999

Table 3. Effects of MeJA on different host-pathogen interactions. (ns: not shown, na: not applicable).

Although, firstly tested preharvest, JA or MeJA has been extensively used postharvest at different hosts (i.e. fruits, vegetables, cut flowers), application modes (i.e. spray, pulse, gas) and incubation environments (i.e. storage or ambient temperatures). For example, JA and MeJA were tested on grapefruit for suppressing postharvest green mold decay [*Penicillium digitatum* (Pers.:Fr.) Sacc.] (Droby et al., 1999). Studies showed that 50 µM and 1 µM MeJA concentrations were effective against the disease and that the reduction in the decay was the same at incubation temperatures of 2 or 20°C. Moreover, as the in-vitro tests showed no direct antifungal activity of JA and MeJA, it was suggested that the disease suppression was achieved via natural resistance induction (Droby et al., 1999). Treatment of Arabidopsis plants with MeJA reduced *A. brassicicola, B. cinerea* and *Plectosphaerella cucumerina* disease development (Thomma et al., 2000). Application of gaseous MeJA to plants resulted in a greater disease reduction compared to that on plants sprayed with MeJA or treated with INA. Gaseous MeJA protected SA-degrading transformant NahG plants, suggesting that gaseous MeJA induced a non-SA dependent systemic response (Thomma et al., 2000). Combination of ASM and JA was tested against bacterial and insect attack on field grown tomato plants (Thaler et al., 1999). Two signaling pathways, one involving SA and another involving JA were proposed to provide resistance against pathogens and insect herbivores, respectively (Thaler et al., 1999).

1.4 Elicitation of defence responses in floriculture

The efficacy of ASM and MeJA on ornamental pot plants and on cut flowers has been tested pre- and postharvest, respectively (Table 4). Most of such tests were carried out in the very recent years and still increasing. For example, pre- and postharvest treatments with MeJA or ASM on cut flowers conferred a variable measure of protection against postharvest infections by *B. cinerea* (Dinh et al., 2007).

JA and MeJA provided systemic protection to various rose cultivars (e.g. Mercedes, Europa, Lambada, Frisco, Sacha and Eskimo) against *B. cinerea* (Meir et al., 1998). MeJA applied as postharvest pulse, significantly reduced *B. cinerea* lesion size on detached rose petals. In the same study, MeJA at concentrations of 100-400 µM showed in-vitro antifungal activity on *B. cinerea* spore germination and germ-tube elongation. Similarly, a postharvest pulse, spray, or vapour treatment with MeJA 200 µM, 600 µM or 1 µL L^{-1}, respectively, significantly reduced petal specking by *B. cinerea* on cut inflorescences of *Freesia hybrida* 'Cote d'Azur' (Darras et al., 2005; 2007). Moreover, 1-100 µL L^{-1} MeJA postharvest vapour treatment reduced *B. cinerea* development on cut Geraldton waxflower 'Purple Pride' and 'Mullering Brook' sprigs (Eyre et al., 2006). Application of gaseous MeJA to fresh cut peonies resulted in the lowest disease severity and in an improvement of vase life compared to the untreated controls (Gast, 2001).

MeJA and ASM, applied preharvest had variable responses against postharvest infection by *B. cinerea*. ASM was not as effective as MeJA in suppressing the development of postharvest *B. cinerea* disease for glasshouse grown freesias (Darras et al., 2006b). Dinh et al. (2007) reported that multiple sprays of ≤1000 µM MeJA to field grown plants significantly reduced *B. cinerea* on Geraldton waxflower 'My Sweet Sixteen' cut sprigs, that were un-inoculated or artificially inoculated with *B. cinerea* (Dinh et al., 2007).

Host	Elicitor	Target pathogen	Application method and timing	Reference
a. Cut flowers				
Rose (*Rosa hybrida*)	ASM	*Diplocarpon rosae*	Spray - preharvest	Suo & Leung, 2002
	MeJA	*B. cinerea*	Pulse, spray - postharvest	Meir et al., 1998; 2005
Gerbera (*Gerbera jamesonii*)	UV-C	*B. cinerea*	Postharvest	Darras et al., 2012
Freesia (*Freesia hybrida*)	MeJA & ASM	*B. cinerea*	Spray - preharvest	Darras et al., 2006b
	MeJA	*B. cinerea*	Pulse, spray, gas - postharvest	Darras et al., 2005; 2007
Sunflower plants (*Helianthus annuus*)	ASM, MeJA, SA, INA	*B. cinerea*	Spray - preharvest	Dimitriev et al., 2003
Sunflower plants (Helianthus annuus)	*ASM*	*Plasmopara helianthi*	*Spray - preharvest*	*Tosi et al., 1999*
Geraldton waxflower (*Chamelaucium uncinatum*)	SA	*Alternaria* sp., *Epicoccum* sp.	Spray - preharvest	Beasley, 2001
	MeJA	*B. cinerea*	Gas - postharvest	Eyre et al., 2006
	MeJA & ASM	*B. cinerea*	Spray – pre- and postharvest	Dinh et al., 2007; 2008
Peonies (*Paeonia lactiflora)*	MeJA	*B. cinerea*	Gas - postharvest	Gast, 2001
b. Pot plants				
Cyclamen (*Cyclamen persicum*)	ASM	*Fusarium oxysporum* f. sp. *cyclaminis*	Spray - preharvest	Elmer, 2006a
Petunia (*Petunia hybrida*)		*Phytophthora infestans*	Spray - preharvest	Becktell et al., 2005
c. Landscape architecture plants				
Date palm	ASM	*Fusarium oxysporum* f. sp. *albedinis*	Injection in the trunk	Jaiti et al., 2009
d. Propagation material				
Gladiolus corms (*Gladiolus x hortulanus*)	ASM	*Fusarium oxysporum* f. sp. *gladioli*	Dip	Elmer, 2006b

Table 4. Chemical and biological elicitors tested on cut flowers and ornamental pot plants against various pathogens infecting either pre- or postharvest.

Chemical elicitors such as ASM have been applied in pot ornamentals such as petunia (Becktell et al., 2005), cyclamen (Elmer, 2006a) and in gladiolus corms (Elmer, 2006b), but effectiveness varied within the different experimental designs and conditions. In cyclamen, infection by *Fusarium oxysporum* f.sp. *cyclaminis* was reduced with increasing ASM doses (Elmer, 2006a). Additionally, the dry mass of ASM treated cyclamen plants increased with increasing ASM rates. However, as no further assays were carried out to assess possible induction of defence responses, it was not clear whether ASM reduced *F. oxysporum* f.sp. *cyclaminis* via induction of defence mechanisms or via a profound fungitoxic effect. It has been demonstrated in other research that ASM may exert direct toxic activity against *B. cinerea* (Darras et al., 2006b). In addition, ASM did not confer a significant level of protection on gladiolus corms against *F. oxysporum* f. sp. *gladioli*, and compared to conventional fungicides, although, the number of emerging flower spikes increased significantly compared to the ASM-untreated corms (Elmer, 2006b).

2. Elicitation of defence responses in cut *Freesia hybrida* flowers – A typical example

2.1 Background

Infection problems by *Botrytis cinerea* are typical to most geographical areas around the world and concern cut flower industry. Infection of cut flowers by the fungus results in visible lesions on flower petals (petal spotting or petal specking). According to Darras et al. (2004) freesia flower rejections at certain periods of the year (viz. April, May, October) lead in severe economic losses to growers, importers and sellers. Infection by *B. cinerea* of most cut flowers occurs in the glasshouse when a single conidium germinates and penetrates petal epidermal cells. A necrotic lesion appears postharvest after a brief incubation period under favourable environmental conditions (Darras et al., 2006a). Infection is difficult to control as it appears later in handling chain under various conditions during transport or storage.

In most cases, *B. cinerea* disease is controlled by conventional fungicides. However, extensive use of fungicides such as dicarboximides in the glasshouse has led to appearance of fungicide resistance (Pappas 1997). Alternative management methods within the concept of IDM can help overcome such problems.

For this reason, plant defence inducers (i.e. elicitors) such as ASM and MeJA have been tested with applications at various intervals, pre- or postharvest to activate systemic defence responses of the host (Kessmann et al., 1994; Meir et al., 1998; Thomma et al., 2000). For cut freesia flowers postharvest pulse, spray, or gaseous MeJA treatment at 200 µM, 600 µM, or 1 µL L^{-1}, respectively, significantly reduced petal specking by *B. cinerea* on cv. 'Cote d'Azur' inflorescences (Darras et al., 2005; 2007). An apparent induced defence response was recorded by both ASM and MeJA treatment. However, only MeJA conferred constant and significant disease reductions. MeJA vapour at 1 µL L^{-1} significantly reduced lesion numbers and diameters on freesia petals by up to 56% and 50%, respectively (Darras et al., 2005).

2.2 Overview of published research and further discussion

Freesia inflorescences cv 'Cote d'Azur' gassed with 0.1 µL L^{-1} MeJA showed significantly smaller lesions after artificial inoculation with *B. cinerea* (Fig. 3). Gaseous MeJA might have

induced a range of defence mechanisms to halt infection development. MeJA applied post-harvest as vapour at 1-100 µL L^{-1} significantly reduced the development of *B. cinerea* on cut Geraldton waxflower 'Purple Pride' and 'Mullering Brook' sprigs (Eyre et al., 2006). In a very recent study, Darras et al. (2011) demonstrated that gaseous MeJA at 0.1 µL L^{-1} significantly increased polythenol oxidase (PPO) activities 24 and 36 h post-treatment. This observation suggests that MeJA-induced defence mechanisms might be associated with the production of quinones (Constabel and Ryan, 1998), which probably helped in *B. cinerea* disease reduction. The effects of PPO in *B. cinerea* disease control have been confirmed for gerbera flowers (Darras et al., 2012). A low dose of UV-C irradiation increased PPO activity and was positively correlated with the reduction of *B. cinerea* disease symptoms on the florets (Darras et al., 2012). This indicates that PPO might play an important role in *B. cinerea* disease control on cut flowers.

Fig. 3. *B. cinerea* necrotic lesions on artificially inoculated freesia cv. 'Cote d'Azur' flowers treated with 0.1 µL L^{-1} gaseous MeJA (left) or left un-treated (control) (right) and incubated for 48 h at 20°C (Darras, 2003).

Lesion diameters on the detached freesia petals were significantly reduced with increasing MeJA spray, pulse or gaseous concentrations (Darras et al., 2007). The first published evidence of postharvest MeJA spray treatments enhancing protection of cut flowers against *B. cinerea* was the work by Meir et al. (2005) on cut roses. According to Meir et al. (2005), simultaneous MeJA pulsing and spraying under handling conditions resulted in suppression of gray mold in seven rose cultivars ('Eskimo', 'Profita', 'Tamara', 'Sun Beam', 'Pink Tango', 'Carmen', 'Golden Gate'). In an earlier study MeJA applied as a pulse variably reduced *B. cinerea* lesion numbers and diameters (Meir et al., 1998). Our findings are in agreement with those by Meir et al. (1998) that disease severity in both artificially inoculated and naturally infected rose flowers was reduced by a MeJA pulse at 0.2 mM at 20°C. On cut Geraldton waxflower 'Purple Pride' and 'Mullering Brook' sprigs, 1-100 µL L^{-1} MeJA postharvest vapour treatment significantly reduced the development of *B. cinerea* (Eyre et al., 2006). However, it also induced flower fall incidence, which was correlated with a systemic resistance-associated up-regulation of ethylene biosynthesis.

Irrespective to the concentration tested, ASM provided no protection to artificially inoculated freesia flowers (Darras et al., 2007). However, natural infection was significantly ($P < 0.05$) reduced after ASM treatment during storage at 5 and at 12°C. On the contrary, postharvest treatments of strawberry cv. Camarosa fruit with ASM failed to reduce natural infection by *B. cinerea* at 5°C (Terry & Joyce, 2004b). Generally, ASM tended to provide protection on freesia flowers at lower incubation temperatures (Darras et al., 2007). However, it was not clear whether such disease reductions were the result of the induction of host's defence responses or a direct fungitoxic activity measured in the same study. Likewise, Terry & Joyce (2000) showed that ASM reduced in-vitro *B. cinerea* mycelial growth on ASM-amended agar. It is possible that the limited disease control on freesia flowers at 5°C was due to direct toxic effect of ASM rather than via SAR induction.

Elicitation of defence responses in cut flowers is an interesting prospect for *B. cinerea* disease control especially as it may offer alternatives to fungicide application. In series of postharvest experiments with freesia inflorescences the potential to induce natural defence mechanisms or directly controlling *B. cinerea* disease by application of biological and chemical elicitors was investigated. Postharvest treatments with ASM, MeJA or UV-C irradiation markedly suppressed *B. cinerea* specking on freesia petals by reducing disease severity, lesion numbers and lesion diameters. However, attempts to further minimise disease damage caused by *B. cinerea* using combined treatments with different plant activators (i.e. both ASM and MeJA), were not successful (Darras et al., 2011).

In summary, ASM was the least effective in reducing *B. cinerea* specking on cut freesia flowers (Fig. 4). In addition, it remained unclear as to whether or not SAR was induced. In contrast, gaseous MeJA reduced disease severity most probably by inducing JA-dependant biochemical responses. These contrasting results tend to concur with observations by Pieterse & van Loon (1999) and Thomma et al. (2001) that SA- versus JA-dependent pathways are effective against different pathogens. The results of a most recent paper (i.e. Darras et al., 2011)) suggested that, SA-dependant pathway and consequently the SAR response was not effective in freesia flowers against *B. cinerea* infection. In contrast, the JA-dependant pathway was apparently induced and suppressive of *B. cinerea* infection (Darras et al., 2011).

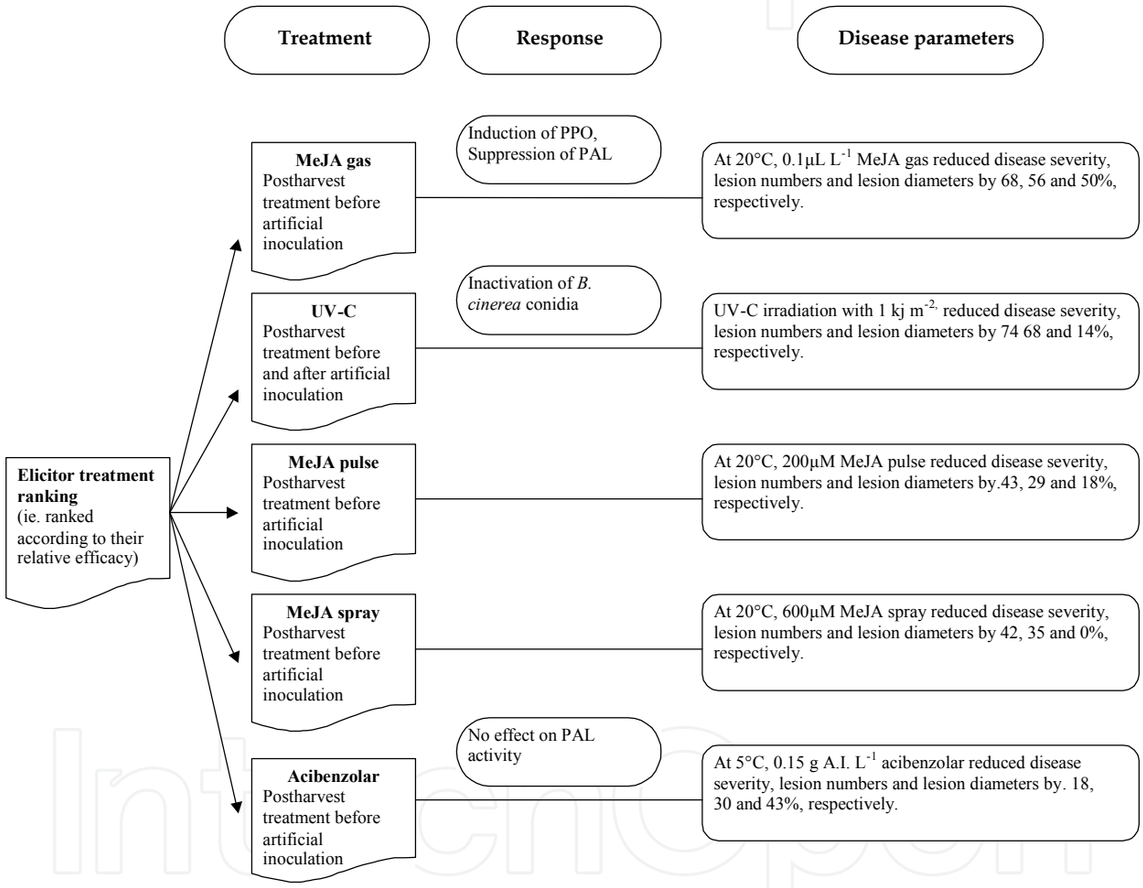

Fig. 4. Ranking, in terms of relative efficacy, of postharvest biological (i.e. UV-C) and chemical (i.e. ASM, MeJA) elicitors tested on cut freesia inflorescences to control *B. cinerea* infection starting with the most effective (Darras, 2003).

3. Conclusions and recommendations for future research

Management of postharvest infection of cut freesia flowers by *B. cinerea*, was, in most cases, successful. ASM was somewhat effective compared to untreated controls mostly when applied preharvest at 1.43 µM. In-vitro studies showed direct antifungal activity of ASM against *B. cinerea* colony growth and conidial germination. Inconsistency of ASM applied pre- or postharvest may be explained by: 1) variability of environmental conditions in the glasshouse, which may affected defence enhancement (Herms & Mattson, 1992; Terry, 2002); and, 2) infection by *B. cinerea* might not necessarily be sensitive to induced SAR responses, and thus ASM treatments may not correspond to *B. cinerea* disease suppression (Thomma et al., 1998; Govrin & Levine, 2002). Friedrich et al. (1996) reported that ASM failed to control *B. cinerea* in tobacco, but was effective against other pathogens. The apparent inability of ASM to control *B. cinerea* was seemingly supported by the observation that PAL activity in ASM treated freesia inflorescences was not higher compared to the untreated controls. Therefore, ASM did not induce biochemical defence processes, such as the production of antifungal secondary metabolites like phytoalexins through the phenylpropanoid pathway (Kombrink & Somssich, 1995; Kuć, 1995).

In contrast to inconsistent effects of ASM, MeJA was markedly effective in suppressing *B. cinerea* specking on cut freesia flowers when applied either pre- or postharvest. MeJA effectiveness was application method and concentration dependent. MeJA applied as gas was more effective compared to pulsing or spraying. It is possible that MeJA may function as an airborne signal which activated disease resistance and the expression of defence related genes in plant tissue (Shulaev et al., 1997). This finding agrees with earlier findings in Arabidopsis presented by Thomma et al. (2000). In Arabidopsis, this effect was mediated via the JA-dependent defence responses (Thomma et al., 2000). MeJA did not exert any direct antifungal activity in-vitro except at the concentration of 600 µM and therefore it is possible that MeJA reduced *B. cinerea* disease on freesia flowers by inducing responses correlated with the JA-dapendent pathway (Darras et al., 2005). PPO levels in freesia flowers after MeJA gaseous treatment increased by 47 and 57% compared to the untreated controls (Darras et al., 2011). However, PAL activity decreased markedly compared 12 h post MeJA application and maintained at minimum level (i.e. ≈ 0). These findings suggest that MeJA might suppress the action of PAL in the phenylpropanoid pathway and consequently reduce or block SA production. Antagonistic regulation of JA- and SA-dependent pathways has been documented in the past by Pena-Cortes et al. (1993), Conconi et al. (1996), Niki et al. (1998), Gupta et al. (2000), and Rao et al. (2000). The apparent suppression of PAL in freesia flowers by MeJA constitute additional evidence of a JA- and SA- antagonistic response.

MeJA applied to freesia plants 28 days before harvest suppressed postharvest flower specking caused by *B. cinerea* in both a temperature and variety dependent fashion. MeJA was highly effective when flowers were incubated at 20°C compared to incubation at 5 or 12°C. It is likely that low incubation temperatures slow down plant's metabolism and also the production of defence related compounds (Jarvis, 1980). Overall, MeJA provided a considerable level of protection against *B. cinerea* when applied preharvest and, thus, could be considered a promising tool in an IDM context. Further study at the molecular level is

warranted to help interpret the MeJA mode of action in cut flowers. Also, additional in-planta trials on extra freesia varieties and a wider range of MeJA concentrations may help in better understanding MeJA efficacy.

In view to the promising results using MeJA, it is likely that elicitor based strategies within IDM could be used for the control of Botrytis or other pathogens on freesias and ornamental pot plants, as well as on various cut flowers. In turn, IDM would minimise the risk of pathogens developing resistance to fungicides and also reduce public concerns over extensive fungicide use (Jacobsen & Backman, 1993).

More research could be undertaken into potential synergistic effects of combined pre- and postharvest treatments with plant activators and/or abiotic biological agents (i.e. UV-C irradiation). In due course, pre and/or postharvest use of plant activators could have commercial potential for postharvest disease suppression (Kessmann et al., 1994; Kessmann et al., 1996; Thaler et al., 1996; Meir et al., 1998; Huang et al., 2000; (Darras et al., 2011).

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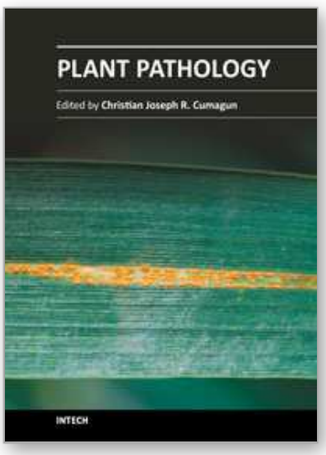

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