# CHAPTER 10

# PHYTOHORMONES IN *BOTRYTIS*-PLANT INTERACTIONS

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**Abstract**. Several lines of evidence suggest that plant hormones are involved in mediating *Botrytis* interaction with plants. External treatments with some plant hormones such as auxins and gibberellins can suppress disease development, while ethylene and abscisic acid seem to enhance the disease. Increased ethylene levels by *Botrytis* infection are well documented. Not only the plant, but also the fungus is capable of producing different hormones and fungal development may be influenced by these hormones. Little direct evidence is available on the involvement of plant hormones in vegetative and pathogenic *Botrytis* development. Most of the data come from studies on the production of ethylene in infected plants, on its possible effect on the disease and on ethylene production by *Botrytis*. Production of other plant hormones by *Botrytis* and their possible role in disease and fungal development have hardly been studied. The production of various plant hormones in *Botrytis,* and the effect that they may have on disease and fungal development are reported.

## 1. Introduction

Plant hormones (phytohormones) are naturally occurring substances that at low concentration control various stages of plant growth and development. The important plant hormones are auxins, gibberellins, cytokinins, ethylene and abscisic acid. All classes of plant hormone have also been found in microorganisms (Tudzynski, 1997; Tudzynski and Sharon, 2002). The physiological condition of plant tissue affects susceptibility to infection and disease development. Plant hormones are involved in mediating a plant's susceptibility to pathogens. Hormone biosynthesis, transport, metabolism and action, as well as host tissue sensitivity to hormones, all contribute to the hormonal homeostatic balance of the tissue. This balance affects plant susceptibility to pathogen development and infection, and may change hormonal levels in the host tissue. Furthermore, pathogen susceptibility to plant hormones may affect its behaviour before and after infection of the plant (Elad



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and Evensen, 1995; Elad, 1997). This chapter will describe the involvement of plant hormones in the interaction of *Botrytis cinerea* with plants. Ethylene is described in detail compared with the other hormones because of the much larger extent of published data that is available on its biosynthesis, the effect on pathogen development, and association with *Botrytis*-incited diseases. We will summarize the current knowledge on the biosynthesis and influence of the three major plant hormones: the auxin indole-3-acetic acid (IAA), gibberellic acid ($GA_3$), abscisic acid (ABA) and cytokinins in *Botrytis*.

## 2. Biosynthesis of plant hormones by *B. cinerea*

### 2.1. Ethylene

Qadir et al. (1997) have shown that *B. cinerea* produces ethylene in shake cultures. They also found that ethylene production was methionine-dependent, but were unable to determine which enzymes were involved in ethylene biosynthesis. In higher plants ethylene is produced from methionine through the intermediate S-adenosyl methionine (AdoMet or SAM) and 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang, 1981; Johnson and Ecker, 1998). This pathway is uncommon in microorganisms and has been described in only a few fungal species (Amagai and Maeda, 1992; Jia et al., 1999). Two other ethylene biosynthetic pathways are known in bacteria and fungi. In the first pathway, 2-oxoglutarate is converted to ethylene by a multi-function enzyme called ethylene-forming enzyme (EFE). In the other pathway methionine is deaminated to produce α-keto γ-methylthiobutyric acid (KMBA) and ethylene is produced by spontaneous or enzymatic oxidation of KMBA (Yang, 1969). Chagué et al. (2002) and Cristescu et al. (2002) showed that in *B. cinerea* ethylene was produced from methionine through KMBA, but not through the ACC pathway. Ethylene production was light dependent: when the fungus was grown in the dark no ethylene was produced, but when the dark-grown cultures or culture filtrates were exposed to light they released large amounts of ethylene (Chagué et al., 2002). These and other results showed in *B. cinerea* that ethylene is released by photo-oxidation of KMBA produced from methionine and then secreted to the medium.

### 2.2. Auxins

Auxins are probably the most important group of plant growth regulators. Biosynthesis and function of the major auxin in plants, IAA, has been intensively studied for almost a century. In most plant species IAA is produced from tryptophan through the intermediate indole-3-pyruvic acid (IPA) but IAA can also be synthesized through additional pathways including tryptophan-independent pathways. Despite intensive efforts, none of the suggested pathways of IAA biosynthesis has been unequivocally established in plants. One reason for that is the complexity by which IAA levels are regulated in plants, which involves multiple pathways of IAA biosynthesis, conjugation of IAA to larger molecules, hydrolysis

of IAA conjugates by a variety of specific enzymes, and IAA oxidation (Bartel, 1997; Normanly and Bartel, 1999).

Four IAA biosynthesis pathways have been characterized in bacteria (Patten and Glick, 1996). Activities of the enzymes that compose these pathways were confirmed and the corresponding genes cloned from several bacterial species. The two primary IAA pathways identified in bacteria are the indole-3-pyruvic acid (IPA) pathway and the indole-3-acetamide (IAM) pathway, which is rare in plants. Most fungal species have the capacity to produce IAA, but relatively little information is available on the metabolic pathways of IAA biosynthesis in fungi. The IPA pathway has been identified in a few species, but only two IAA biosynthesis genes from *Ustilago maydis* have been analysed so far (Basse et al., 1996). Furukawa et al. (1996) found that fungi belonging to the genus *Rhizoctonia* converted tryptophan to IAA. Feeding experiments using indole precursors confirmed the activity of the IPA pathway in *Rhizoctonia*. No other IAA biosynthesis pathways were identified in this fungus. The bacterial pathway (through IAM) has been reported so far only in *Colletotrichum* (Robinson et al., 1998).

Tapani et al. (1993) reported that the mycelium of *B. cinerea* contained 128 ng/g IAA while less than 1 ng/ml was detected in the medium. However, we analysed IAA production by 30 *B. cinerea* isolates and found that most of the IAA was secreted to the medium (S. Haskin and A. Sharon, unpubl.). As was found in other species, IAA biosynthesis in *B. cinerea* requires tryptophan as a precursor. It has been suggested that IAA is produced via the IPA pathway, but the precise biosynthetic pathway is still unclear.

IAA biosynthesis in *B. cinerea* was studied by feeding with various IAA intermediates *in vitro* (S. Haskin and A. Sharon, unpubl.). To test the activity of the IPA pathway enzymes we used tryptophan, IPA, or indole-acetaldehyde (IAL) as precursors and then measured IAA production. Only low levels of IAA were produced in tryptophan-amended medium. Addition of IPA to the medium resulted in high levels of IAA, but similar levels also accumulated in the control treatment that included only medium without the fungus. Further analyses showed that IPA is spontaneously converted to IAA in the growth medium. Similar results were obtained by *in vitro* enzymatic assays using *B. cinerea* protein extracts under conditions specific for the activity of the enzyme indole pyruvic acid decarboxylase. These results indicated that IPA was not used as a precursor for IAA synthesis under these experimental conditions. The addition of indole-3-acetaldehyde (IAD), on the other hand, resulted in production of high levels of IAA an order of magnitude higher than the amounts of IAA that were produced with tryptophan. No IAA was detected in the control flasks that contained only culture medium without the fungus. These results suggested that the IPA pathway might not be active in *B. cinerea* under the experimental conditions, or that it is not a main pathway of IAA biosynthesis in the fungus. It also showed that *B. cinerea* was capable of producing high levels of IAA, but under the experimental conditions it could not use tryptophan efficiently and required an intermediate precursor such as IAD. These results suggested that pathways other than the IPA that lead to IAD and then to IAA production might be used for IAA synthesis.To test this possibility we added to the medium tryptamine,an intermediateof another microbial pathwayin which tryptophan is

first decarboxylated to tryptamine, which is then deaminated to produce IAD. The addition of tryptamine to the medium resulted in high levels of IAA that was completely dependent on the presence of the fungus. These results showed that in *B. cinerea* the major pathway of IAA biosynthesis is probably the tryptamine pathway, and not the IPA as was previously suggested. Once the intermediate precursors tryptamine or IAD are provided to the fungus, it can produce high amounts of IAA, but it is still unknown why only low levels are produced from tryptophan.

### 2.3. Gibberellic acid

The best investigated fungal phytohormone system, and the only one in which all the biosynthetic genes involved have been identified, is the production of GA by *Gibberella fujikuroi*: members of the mating population C (causing the so-called Bakanae disease in rice, characterized by an enormous stem elongation) produce high titres of $GA_3$; high producing strains are used for efficient biotechnological production of $GA_3$ (Tudzynski, 1999; Tudzynski and Sharon, 2002). It has been shown recently that the genes involved in GA biosynthesis in *G. fujikuroi* are arranged in a cluster (Tudzynski and Hölter, 1998), and that the biosynthesis in major aspects is different from the higher plant pathway (Hedden et al., 2002).

So far GA production in *Botrytis* has not been proved unequivocally. In extracts of a model strain (B05.10) not even kaurenes could be detected (V. Siewers and P. Tudzynski, unpubl.). A gene (*bccps*/*ks1*) showing significant homology to the first gene of the GA pathway in *G. fujikuroi* (encoding the bifunctional enzyme ent-copalyl diphosphate synthase/ent-kaurene synthase) was identified in *B. cinerea*; a knock-out of this gene had no effect on growth or morphology of the mutant in axenic culture nor on virulence. Since expression of the gene could not be detected under any growth conditions (by RT-PCR) and a neighbourhood sequence analysis showed absence of any possible GA cluster genes like in *G. fujikuroi*, the analyses were not carried on (V. Siewers, S. Giesbert and P. Tudzynski, unpubl.).

### 2.4. Abscisic acid

Strains of *B. cinerea* have been shown to synthesize ABA (Marumo et al., 1982; Hirai et al., 1986); overproducing strains are used for biotechnological production of ABA (Toray Industries, Japan). Biosynthesis of ABA in fungi also seems to be distinct from the pathway used by higher plants. Biochemical analyses in various *Cercospora* species have presented evidence that these fungi synthesize ABA directly from farnesylpyrophosphate (FPP) via different oxidative steps, with either 1',4'-dihydroxy-γ-ionylidone acetate, 1'-desoxy-ABA, or 1',4'-*trans*-diol-ABA as intermediates, and not via the carotenoid pathway as in higher plants (Assante et al., 1977; Neill et al., 1987; Okamoto et al., 1988a, b). In *B. cinerea*, biosynthesis of ABA seems to follow the same pathway as *Cercospora densiflorae*, i.e. via 1',4'-*trans*-diol-ABA (Hirai et al., 1986). The fact that ABA is produced mainly by pathogenic fungi, and given the effects of ABA on higher plants (induction of senescence, etc.), it has been speculated that ABA might be involved in pathogenesis, i.e. represents a virulence factor (Kettner and Dorffling, 1995). This idea was supported by Shaul et al. (1996) who showed that external application of

ABA enhanced disease development caused by *B. cinerea*. The unequivocal proof for a role of fungal ABA in the host-pathogen interaction would require defined mutants which are absolutely unable to produce ABA *in vivo*. (Strains not producing detectable amounts of ABA in axenic culture have been described, but it is open to question whether they still have the capability to produce ABA *in planta*.) Identification of genes involved in the ABA biosynthesis pathway in *B. cinerea* would be interesting for several reasons: they could be used for evolutionary research for comparison of the pathways in higher plants and fungi, for biotechnological purposes for the generation of overproducing strains, and for phytopathological analyses as outlined above.

The approach to clone ABA pathway genes (Siewers et al., 2004) is based on the first "genomic" tools available for *B. cinerea* (Chapter 4). The proposed direct biosynthetic pathway from FPP suggested the involvement of P450 monooxygenases, since several oxidation/hydroxylation steps would be involved. For a first step a P450-oxidoreductase gene (*bccpr1*) was cloned using a PCR approach; deletion of this gene resulted in a drastic reduction of ABA biosynthesis in an ABA overproducing strain (ATCC 58025), strongly supporting the concept that P450 monooxygenases are involved. Therefore, all P450 monooxygenase genes contained in the available EST libraries (28) were checked for induction under ABA biosynthesis conditions. Two genes up-regulated under ABA biosynthesis conditions were deleted by a gene-replacement approach. A mutant of one of the genes (*bcp450-16*) did not produce any ABA, but an ABA intermediate, proving that this gene encodes the first identified enzyme of the ABA biosynthetic pathway. Deletion of the homologous gene in strain B05.10 (strain ATCC 58025 is almost non-pathogenic) is under way; this will allow the first unequivocal test for the role of fungal ABA biosynthesis in pathogenicity of *Botrytis*.

The above mentioned examples suggest that the phytohormone biosynthesis pathways in *Botrytis* are unrelated to plant pathways and are in contrast to the hypothesis that phytohormones represent a good example for an horizontal gene transfer between plants and fungi. Ethylene is synthesized via KMBA (Chagué et al., 2002), as in prokaryotic systems, and not via the ACC pathway used in higher plants, and ABA is synthesized directly from FPP and not through the plant carotenoid pathways (Siewers et al., 2004). The details of IAA biosynthesis are not yet fully uncovered, but they include at least one non-plant pathway in which tryptophan is first decarboxylated to tryptamine which is then deaminated to form indole-3-acetaldehyde. These examples suggest that in *B. cinerea* phytohormone biosynthesis pathways are more similar to prokaryotes than to plants.

## 3. Effect of plant hormones on *B. cinerea* and on disease development

### 3.1. Ethylene

#### 3.1.1. Ethylene and fungal development

Stimulation by ethylene of conidial germination, germ tube elongation and appressorium formation were reported in several fungi (Kepczynski and Kepczynska, 1977; Kepczynska, 1989, 1994; Kolattukudy et al., 1995). These effects, however, are not general and may be significant in some, but not all, fungi. A considerable amount of work was dedicated to study the effects of ethylene in *B. cinerea*. Brown (1922) reported high rates of *B. cinerea* conidial germination in the presence of ripe apples, and suggested that the atmosphere of ripening fruits may have a stimulatory effect on conidial germination, and later studies supported these early observations. Kepczynski and Kepczynska (1977) reported that ethylene enhanced conidial germination of *B. cinerea*, and Kepczynska (1989) showed that 2,5-norbornadiene (NBD) (a specific inhibitor of ethylene action in plants) reversed this effect. Germination was effectively inhibited by NBD and was relieved by transfer of the conidia to fresh air suggesting an indispensable role for ethylene in the germination process. Ethylene was also reported to stimulate mycelial growth (Kepczynska, 1993, 1994). Treatment with ethylene up to $10^3$ µl/l air increased the total dry weight of *B. cinerea* grown both *in vitro* and *in vivo* on strawberries as determined by glucosamine content (El Kazzaz, 1983). It should be noted that ethylene inhibitors such as amino-ethoxy-vinyl-glycine (AVG) and NBD, and the ethylene donor ethephon (ethylene releasing agent) were used in these studies. While these results strongly suggest that ethylene affects *B. cinerea* development by enhancing conidial germination and hyphal growth, they do not present unequivocal proof for the direct effect of ethylene. Both AVG and ethephon have effects other than that of inhibition of the ACC pathway and release of gaseous ethylene, respectively. AVG was found to reduce mycelium growth and sporulation of the fungus (V. Chagué and A. Sharon, unpubl.), while ethephon is known to release phosphonic acid and is pH sensitive. To better assess the direct effect of ethylene on *B. cinerea* we conducted similar experiments to test the effect of pure ethylene on fungal development and found that it inhibited mycelium growth in culture (V. Chagué and A. Sharon, unpubl.). The rate of conidial germination and germ tube elongation on glass, or on tomato and bean leaf surfaces, were enhanced (Elad, 2002; Elad et al., 2002). Thus, ethylene may have different effects on the fungus at different developmental stages and in different systems.

Two lines of evidence appear to be significant in evaluating the possible role of ethylene in *B. cinerea*: 1) all strains tested so far produced significant amounts of ethylene (e.g. Qadir et al., 1997; Chagué et al., 2002) suggesting that this ability may be an inherent characteristic of the species; and 2) the accumulated data strongly support an effect of ethylene on fungal development, including conidial germination and mycelial growth. Taken together, these data suggest that changes in ethylene levels are sensed by the fungus and might affect its growth and development.

The mechanisms of ethylene perception and action in plants have been elucidated in a great detail. It has been shown that ethylene specifically binds to a number of "ethylene receptors" which are proteins with homology to two-component histidine kinase regulators (Kieber, 1997; Theologis, 1998). The binding of ethylene to these receptors triggers a kinase cascade resulting in transcriptional activation of nuclear genes (Kende and Zeevaart, 1997). It is rather plausible to assume that a similar cascade might mediate the effect of ethylene in fungi, including *B. cinerea.* However, in contrast to the wealth of physiological studies describing the effect of ethylene on fungal development, there are no molecular data in support of this hypothesis. Molecular studies are therefore needed to verify whether ethylene indeed affects development through transcriptional gene activation. Using differential expression techniques we were able to show differences in the transcription level of an array of genes caused by ethylene treatment (V. Chagué and A. Sharon, unpubl.). These preliminary results support the hypothesis that ethylene directly affects *B. cinerea.* More extensive research is required to determine how this effect is obtained.

### 3.1.2. Ethylene and disease

Enhanced ethylene production has been considered to be an early response of plants to pathogen attack. Although increase in ethylene levels has been associated both with resistance and susceptibility to disease, the working model has been that enhanced ethylene production is an early, active response of plants to pathogen attack.

Plants infected by *B. cinerea* certainly produce high levels of ethylene. Williamson (1950) noted that infection of chrysanthemum tissue by *B. cinerea* resulted in the release of ethylene. Smith et al. (1964) found that carnation infected by *B. cinerea* also produced more ethylene than non-infected plants; the ethylene predisposed the flowers to further attack by the pathogen. Leaves of pelargonium and ruscus, flowers of carnation and leaves and flowers of rose infected by *B. cinerea* produced much higher levels of ethylene (up to 12 nl/g/h) compared with wounded or healthy tissues, and ethylene production was correlated with the severity of grey mould. However, when the host became completely macerated, ethylene production diminished. Methionine sprays, incubation with exogenous ethylene, or pre-cooling of flowers at 4°C increased disease incidence considerably. On the other hand, sprays of the ethylene activity inhibitor silver thiosulphate (STS) and the ethylene biosynthesis inhibitors aminooxyacetic acid (AOA) and AVG decreased disease severity. The latter two compounds inhibited ethylene production in infected plants (Elad, 1988; Elad and Volpin, 1988). Leaves of tomato, sweet pepper, yellow bean, and cucumber behaved similarly. In addition, when *B. cinerea* was grown on autoclaved leaves supplemented with methionine it produced 0.14 nl/g/h ethylene (Elad, 1990).

Calcium can be added to cut flowers in the vase and in the fertilizing solution in the greenhouse. Ethylene production in flowers with high calcium content was decreased by 50-95% (Volpin and Elad, 1991). Calcium ions inhibited disease

development, whereas the ion chelator ethylene glycol-bis-(2-aminoethyl)-N,N,N', N'-tetraacetic acid (EGTA) enhanced it. Disease suppression by an excess of $Ca^{2+}$ was correlated with repression of ethylene production by the flowers (Elad and Volpin, 1988). It should be noted that calcium can also affect the susceptibility of plants to *B. cinerea* by affecting pectin resistance to *B. cinerea* enzymes or by directly inhibiting pectolytic enzymes (Chapter 7).

There are additional reports on production of ethylene by *B. cinerea*-infected plant tissues. A 3.3-fold increase in ethylene production by cell suspension cultures of *Papaver somniferum* was observed 7 h after elicitation with a *Botrytis* fungal homogenate (Songstad et al., 1989). Kiwifruit stored at 0-10°C produced significant amounts of ethylene 20-30 days after inoculation with *B. cinerea,* while only trace amounts were detected in healthy controls (Niklis et al., 1992). Symptomless, newly abscised blackcurrant flowers of many genotypes were found by fluorescence microscopy to contain infected ovules. Inoculated flowers produced higher ethylene levels than un-inoculated controls (McNicol et al., 1989). The use of NBD then confirmed that ethylene produced in response to infection was a major factor in premature flower abscission, and the sensitivity of blackcurrant genotypes to ethylene corresponded with their known susceptibility to fruit drop.

As mentioned above, external ethylene (applied as ethephon) may enhance grey mould, whereas ethylene inhibitors may suppress the disease. *In vitro* tests showed that the plant growth regulator 4-chlorophenoxyacetic acid (4-CPA) at 10 µg/ml inhibited mycelial growth and conidial germination of three *B. cinerea* isolates. Ethephon had a slight effect on mycelium growth. Tomato plants sprayed with 4-CPA and ethephon showed 50 and 80% infection by *B. cinerea*, respectively, compared with 60% infection on an untreated control (Benliogulu and Yilmaz, 1992). On the contrary, ethylene exogenously applied as ethephon stimulated grey mould disease severity on both tomato and bean plants at 100-400 µg/ml (M.I. Al-Masri, Y. Elad, A. Sharon, and R. Barakat, unpubl.). Grapevines were sprayed with ethephon, harvested and the grapes were stored. *B. cinerea* infection and the percentage of soft berries were increased in these grapes (Hartmann, 1988).

Several compounds were tested for their ability to reduce development of *B. cinerea* on rose, tomato, sweet pepper, aubergine, French bean and *Senecio* sp. Use of potassium permanganate to absorb ethylene from the atmosphere surrounding rose flowers, or leaves of tomato and pepper, resulted in slower fungal development (Elad, 1993). Inhibition of ethylene activity by NBD controlled the disease on all crops other than tomato, and carbon dioxide controlled the fungus on roses. Inhibitors of ethylene biosynthesis such as AOA, $Co^{2+}$, the uncoupler 2,4-dinitrophenol and the radical scavenger salicyclic acid varied in effectiveness in controlling the disease on the various hosts. The cytokinin benzyl adenine, which reduces host responsiveness to ethylene, resulted in 39-99% disease reduction in rose flowers and in leaves of tomato and *Senecio* sp. but was not effective on aubergine or pepper (Elad, 1993). Most, if not all, of the compounds affect other processes in addition to ethylene and therefore the observed effects may not be ethylene-specific. Interestingly, the opposite was reported by Hoffman et al. (1988) who worked with a below ground plant organ. Carrot slices originally resistant

became susceptible to a normally non-invasive level of *B. cinerea* conidia after treatment with AVG; ACC partially reversed the susceptibility induced by AVG.

Antioxidants restrain grey mould on various plants (Elad, 1992; Chapter 8). Ethylene production was inhibited in tomato leaves treated with the antioxidants propyl gallate, ascorbic acid and benzoic acid, but not in pepper leaves. Ethephon or $H_2O_2$ increased the severity of grey mould on leaves of *Senecio* sp. This effect was controlled by the antioxidants butylated hydroxytoluene (BHT) and benzoic acid, or by BHT alone, respectively (Elad, 1992). Ethylene stimulated germ-tube elongation of *B. cinerea* conidia incubated within normal and non-ripening *nor* tomato fruits, but had little influence on the total percentage of germination. Exposure of the normal and the mutant fruits to ethylene immediately after inoculation increased sporulation. When tomato fruits were exposed to ethylene for 3 days before inoculation, rot was stimulated on the mature-green normal fruits, but not on the *nor* mutant fruits. It was suggested that exogenous ethylene might directly stimulate germ tube growth of *B. cinerea* in both normal and mutant fruit, but that it may affect subsequent fungal growth indirectly, via stimulation of the ripening process, only in pre-climacteric normal tomato fruit (Barkai Golan et al., 1989).

The effect of ethylene on *B. cinerea*-host interaction was further described using French beans, tomato and *Arabidopsis thaliana* plants (Elad, 2002; Elad et al., 2002; M.I. Al-Masri, Y. Elad, A. Sharon and R. Barakat, unpubl.). Infected resistant *Arabidopsis* plants produced less ethylene than sensitive plants. Interestingly, not only did ethylene enhance *B. cinerea* germination (Sect. 1.2), but it also increased the number of infection structures per germ tube and subsequent penetration of the host tissue (Elad, 2002; Y. Elad, unpubl.).

It is possible to study the impact of phytohormones produced by plants on the host-parasite interaction using plant mutants with altered hormone susceptibility or production (Korolev and Elad, 2004). Ethylene-related mutants include ethylene-insensitive, ethylene-overproducers and ethylene-reduced mutants. Ethylene-insensitive mutants *ein2-1, ein-6, etr1-1, etr1-3*, ethylene-overproducers *eto1-1, eto2* and ethylene-reduced production mutant *hls1-1* were more susceptible than the wild type (WT) *Arabidopsis*, whereas other mutants did not differ from their WT background. AVG significantly inhibited disease on both *ein2-1* and *hls1-1* mutants, whereas ethephon did not change the level of disease on *ein2-1* and slightly stimulated disease on *hls1-1* (Korolev and Elad, 2004). It is possible that the pathway that leads to susceptibility is independent of other ethylene signalling transduction pathways.

In summary, *Botrytis* infection induces increases in both active oxygen species (AOS) and ethylene production. Ethylene induces auto-catalytic production of that same hormone in exposed tissue. Ethylene promotes deterioration of the infected tissue and the AOS development and *vice versa*. Consequently, antioxidant levels are changed in the rot area or in asymptomatic tissues surrounding the rot. The reaction can be prevented by inhibition of ethylene or external application of antioxidants (Elad, 2002). Govrin and Levin (2000) suggested that the oxidative burst during plant infection enhances infection by *Botrytis.* They found an ethylene burst from the infected tissue that coincided with the peak of oxidative burst. *B. cinerea* perturbs redox processes involvement in pathogenesis (Chapters 8 and 9).

Cristescu et al. (2002) compared *in vitro* ethylene production by *Botrytis* with the ethylene produced during plant infection and found that the levels of emission during plant infection were 100-fold higher. The time of evolution of enhanced ethylene production by infected tomatoes and the cytological observations indicated that ethylene emission was not triggered by *B. cinerea*-produced ethylene, although it was strongly synchronized with the growth rate of the fungus inside the plant. Chagué et al. (2002) showed that peroxidase is capable of catalysing *in vitro* KMBA oxidation and release of ethylene. Taken together these results suggest that *Botrytis* has the potential to produce ethylene during plant infection. Further research is necessary to determine whether ethylene is indeed produced *in planta* by *B. cinerea* and to better assess the influence of ethylene on disease development by specifically determining the effect on both the plant and the fungus.

### 3.2. Auxins

High auxin levels were found in infected plants, and symptoms resembling the effects of high auxin, such as epinasty and plant organ deformations, are associated with many fungal diseases (Tudzynski and Sharon, 2002). Auxin may affect both the fungus and the plant. Addition of IAA and gibberellic acid affected sporulation and cell elongation in yeast (Yanagishima, 1965; Kamisaka et al., 1967), and enhanced germination of *Neurospora crassa* conidia (Nakamura et al., 1978, 1982). Elevated IAA levels are associated with tumours and growth abnormalities caused by several pathogens. Wolf (1952) reported that only those species of *Ustilago maydis* that produced auxin in culture caused gall formation on their hosts. Naphthalene acetic acid (NAA) reduced the mycelial growth rate of *Sclerotinia sclerotiorum in vitro* and white mould disease severity on detached leaves and whole bean and cucumber plants at concentrations of 200-600 µg/ml (Al-Masri et al., 2002).

Various auxins such as IAA, naphthalene acetic acid ethyl ester (NAAEE) and N-*meta*-tolylphthalamic acid (NMT) reduced botrytis blight of cut rose flowers (Elad, 1995). Application of auxin to enhance fruit setting of aubergine reduced susceptibility to the disease (Elad et al., 1992). IAA and NAA reduced disease on tomato leaves at concentrations of $10^{-4}$-$10^{-3}$ M and also reduced *B. cinerea* germination *in vitro* or on leaves, with lower efficacy of the lower concentrations of NAA combined with $GA_3$ being inhibitory on tomato and bean leaves. The inhibitory effect on disease was sometimes additive (Y. Elad, unpubl.). The auxins, NAA and 2,3,5-triiodobenzoic acid (TIBA) reduced the mycelial growth rate of *B. cinerea in vitro*, and grey mould disease severity on tomato plants at various concentrations (R. Barakat, unpubl.). Delen and Özbek (1989) observed increased grey mould severity in tomato greenhouses treated with auxins. 2,4-dichlorophenoxy-acetic acid (2,4-D) increased *B. cinerea* growth and sporulation at concentrations of 0.01–10.0 and IAA increased the mycelial growth and sporulation at 0.01–1.0 µg/ml, but decreased it at 50.0 – 500.0 µg/ml. On tomato plants 2.4-D stimulated grey mould when applied at 0.5 – 1.0 µg/ml (Delen and Özbek, 1989).

Korolev and Elad (2004) infected auxin-resistant *Arabidopsis* plant mutants. Most auxin-resistant mutants developed rot similar to the WT background, whereas

the mutants *axr1-3* and *aux1*-7 were more susceptible than the WT; external application of NAA stimulated disease on *axr1-3*.

### 3.3. Gibberellic acid

There was less fungal decay due to *B. cinerea*, *Monilia* (*Monilinia*) *fructigena* and *Penicillium expansum* in treated nectarine fruits after gibberellic acid sprays were applied to trees in an orchard, which also delayed ripening and increased firmness (Lurie et al., 1998). $GA_3$ increased the mycelial growth and sporulation of *B. cinerea in vitro,* at concentrations of 1.0–50.0 µg/ml, respectively. On tomato plants it promoted disease at concentrations of 100–300 µg/ml (Delen and Özbek, 1989). When celery was treated with $GA_3$ 1 month prior to storage at 2°C, decay was decreased and the concentration of psoralens increased because $GA_3$ slowed down the conversion of (+)marmesin to psoralens, thereby increasing the resistance to *B. cinerea* and other pathogens (Afek et al., 1995). Gibberellin treatment reduces stalk rot in grapevine due to *B. cinerea* (Brechbuhler, 1982). $GA_3$ suppressed grey mould on tomato and bean plants (Elad, 1995, 1997). Botrytis blight of cut rose flowers has been controlled by $GA_3$ applications to detached petals or to whole cut flowers (Shaul et al., 1992). At the concentrations used in this work the germination, growth and development of the fungus were not affected, but in later work it was found that higher concentrations of $GA_3$ inhibited the fungus (Y. Elad, unpubl.). In the case of rose flowers the effect of blight suppression resulted from $GA_3$-imposed inhibition of senescence processes in the petals (Shaul et al., 1995a, b). $GA_3$ inhibits the senescence-related increase in the permeability of the cell membranes, reduced leakage of nutrients from the tissue and increased production of *Botrytis*-inhibiting phenolic compounds. The possibility that $GA_3$-stimulated formation of phenolic glycosides and other phenolic saccharides reduces the availability of nutrients to the pathogen and introduces inhibitory properties has been proposed (Zieslin et al., 1996). Other GA effects that may lead to reduced plant susceptibility to *Botrytis*, such as effects on pectin solubility, reduction of polygalacturonase activity and ethylene evolution, were reviewed by Elad (1997).

Korolev and Elad (2004) inoculated *Arabidopsis* mutants that affected GA metabolism and all were strongly affected by *B. cinerea*. GA-deficient mutants developed more severe rot than GA-resistant or GA-insensitive ones. External application of $GA_3$ or the inhibitor 2'-isopropyl-4'-(trimethylammonium chloride)-5'-methylphenyl piperidine-1-carboxylate (AMO-1618) did not change the level of disease on two GA-deficient mutants, *ga1-4* and *ga2-1*.

### 3.4. Abscisic acid

ABA is associated with negative effects on infected plants. Kettner and Dorffling (1995) studied tomato ABA mutants and concluded that at least four processes control the level of ABA in WT tomato leaves infected with *B. cinerea*: stimulation of fungal ABA biosynthesis by the host; release of ABA or its precursor by the fungus; stimulation of biosynthesis of plant ABA by the fungus; and inhibition of its metabolism by the fungus. Application of ABA together with fungal conidia to tomato leaves caused a faster development of necrotic leaf area than conidial

inoculation only (Kettner and Dorffling, 1995). ABA is formed during plant tissue aging and may antagonize the function of gibberellins in the plant tissue and increase the susceptibility to *Botrytis* (Elad, 1997). Indeed, botrytis blight of roses is promoted by ABA and ABA antagonizes $GA_3$-suppression of the disease on rose flowers (Shaul et al., 1996). Similarly, tomato and bean leaf infection is promoted by ABA. Additionally, ABA and ethylene act synergistically in promoting infection by *B. cinerea*. Mevalonic acid lactone, a precursor of ABA biosynthesis, promoted disease at 1 μM concentrations, similar to the ABA effect. It promoted germination of the conidia on glass and on leaves and even had an additive effect on germ tube elongation when combined with ABA (Y. Elad, unpubl.).

According to Audenaert et al. (2002), ABA plays a major role in the susceptibility of tomato to *B. cinerea*. Tomato mutants with reduced ABA levels (sitiens plants) are more resistant to *B. cinerea* than WT plants. Exogenous application of ABA restored susceptibility to *B. cinerea* in sitiens plants and increased susceptibility in WT plants. ABA appeared to interact with a functional plant defence response against *B. cinerea*. Thus, ABA appears to negatively modulate the salicylic acid-dependent defence pathway in tomato, which may be one of the mechanisms by which ABA levels determine susceptibility to *B. cinerea* (Audenaert et al., 2002).

Korolev and Elad (2004) infected *Arabidopsis* mutants expressing both deficient and insensitive responses to ABA that were significantly more susceptible to *B. cinerea* than corresponding background lines. Application of ABA and mevalonic acid lactone external applications did not change the level of disease on the ABA-insensitive mutant *abi2-1*, but significantly reduced disease on the ABA-deficient mutant *aba1-3*.

When *B. cinerea* infects leaves of raspberry (*Rubus idaeus*) primocanes, it causes dwarfing of the axillary buds in the growing season; axillary buds at infected nodes on overwintered canes then usually fail to develop into lateral shoots in the following spring, thus causing yield loss (Williamson and Hargreaves, 1981). This suppression of axillary buds was postulated to be due to a fungal toxin, but in view of new research on the synthesis of ABA by *B. cinerea* it seems more likely that hormone inhibition is a cause of the retardation of bud development in the first season, followed by strong correlative inhibition from larger distal buds at healthy nodes above.

### 3.5. Cytokinins

There are only a few reports on the role of cytokinins in *B. cinerea* infection. Increased concentration of kinetin or 6-benzyladenine decreased mycelial growth of the onion pathogens *B. allii* and *Colletotrichum dematium*. Sporulation of *B. allii* increased by 60% as amendment concentration increased from $0\text{-}10^{-5}$ M and then decreased 25% at $10^{-3}$ M (Russo and Pappelis, 1993). Benzyladenine and kinetin reduced botrytis blight of rose flowers (Elad, 1995) and other plants (Elad et al., 1993).

## 4. Conclusions

*B. cinerea* is capable of producing several plant hormones in axenic cultures. All tested strains produce large quantities of ethylene and low levels of IAA, while ABA is produced only by some, but not all, strains in culture. None of the tested isolates produced gibberellins. External supply of a precursor is required for (ethylene and IAA) or significantly enhances (ABA) production of the phytohormones. No ethylene or IAA are produced in media without methionine or tryptophan respectively, and mevalonic acid is necessary for production of substantial levels of ABA. Since external supply of substrates is required for phytohormone production by the fungus, *B. cinerea* must utilize plant metabolites in order to produce phytohormones during plant colonization. Production of the phytohormones by *Botrytis in planta* has not been demonstrated, and it remains uncertain whether the fungus indeed utilizes plant substrates to produce plant hormones, and whether fungal-produced phytohormones affect disease development. *B. cinerea* employs biosynthetic pathways that are different from the plant pathways for synthesis of ethylene, IAA and ABA. This fact might be utilized to obtain information on phytohormone production *in planta* by the fungus through feeding experiments and measurement of pathway-specific intermediate compounds or by using pathway-specific inhibitors.

A *B. cinerea* biosynthetic gene was recently cloned for the ABA pathway. This will contribute to our ability to study the evolution and function of this phytohormone in fungi, e.g. by sequence and expression pattern comparisons with bacteria and plants, and by testing pathogenicity and development of null mutants. Isolation of ethylene biosynthesis genes might be more difficult since only one non-specific aminoacid transferase seems to be involved in ethylene production. Other methods including isolation of ethylene-regulated *Botrytis* genes may contribute to understanding ethylene function in *Botrytis.*

Phytohormones produced by the infected plant, or external supply of plant hormones, clearly affect disease development. External supply of ABA and ethylene seem to enhance disease, while IAA and $GA_3$ reduce it. As might be expected, the effect is highly sensitive to phytohormone concentrations and time of application. Nevertheless, the cumulative data suggest that when administered at the right time and concentrations, plant hormones might be very useful in preventing *Botrytis* diseases.

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## 6. References

Adams D and Yang S (1981) Ethylene the gaseous plant hormone: Mechanisms and regulation of biosynthesis. Trends in Biochemical Science 4: 161-164

Afek U, Aharoni N and Carmeli S (1995) Increasing celery resistance to pathogens during storage and reducing high-risk psoralen concentration by treatment with $GA_3$. Journal of the American Society for Horticultural Science 120: 562-565

Al-Masri MI, Ali-Shtayeh MS, Elad Y, Sharon A, Tudzynski P and Barakat R (2002) Effect of plant growth regulators on white mould (*Sclerotinia sclerotiorum*) on bean and cucumber. Journal of Phytopathology 150: 481-487

Amagai A and Maeda Y (1992) The ethylene action in the development of cellular slime molds: an analogy to higher plants. Protoplasma 167: 159-168

Assante G, Merlini L and Nasini G (1977) (+)-Abscisic acid, a metabolite of the fungus *Cercospora rosicola*. Experimentia 33: 1556-1557

Audenaert K, De Meyer GB and Höfte MM (2002) Abscisic acid determines basal susceptibility of tomato to *Botrytis cinerea* and suppresses salicylic acid-dependent signaling mechanisms. Plant Physiology 128: 491-501

Barkai-Golan R, Lavy Meir G and Kopeliovitch E (1989) Effects of ethylene on the susceptibility to *Botrytis cinerea* infection of different tomato genotypes. Annals of Applied Biology 114: 391-396

Bartel B (1997) Auxin biosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 48: 51-66

Basse CW, Lottspeich F, Steglich W and Kahmann R (1996) Two potential indole-3-acetaldehyde dehydrogenases in the phytopathogenic fungus *Ustilago maydis*. European Journal of Biochemistry 242: 648-656

Benliogulu S and Yilmaz D (1992) Influence of plant growth regulators on mycelial growth, germination of conidia and pathogenicity of *Botrytis cinerea*. In: Verhoeff K, Malathrakis NE and Williamson B (eds) Recent Advances in *Botrytis* Research. (pp. 119-122) Pudoc Scientific Publishers, Wageningen, The Netherlands

Brechbuhler C (1982) Relation between stalk necrosis and *Botrytis cinerea* on grapevine. EPPO Bulletin 12: 29-35

Brown W (1922) Studies in the physiology of parasitism. Annals of Botany 36: 285-300

Chagué V, Elad Y, Barakat R, Tudzynski P and Sharon A (2002) Ethylene biosynthesis in *Botrytis cinerea*. FEMS Microbial Ecology 40: 143-149

Cristescu SM, De Martinis D, Te Lintel Hekkert S, Parker DH and Harren FJM (2002) Ethylene production by *Botrytis cinerea in vitro* and in tomatoes. Applied and Environmental Microbiology 68: 5342-5350

Delen N and Özbek T (1989) Effects of certain plant growth regulators on the growth of *Botrytis cinerea.* Abstracts of the IXth *Botrytis* Symposium, Neustadt/Weintrasse, p. 16.

El Kazzaz MK, Sommer NF and Kader AA (1983) Ethylene effects on *in vitro* and *in vivo* growth of certain postharvest fruit-infecting fungi. Phytopathology 73: 998-1001

Elad Y (1988) Involvement of ethylene in the disease caused by *Botrytis cinerea* on rose and carnation flowers and the possibility of control. Annals of Applied Biology 113: 589-598

Elad Y (1990) Production of ethylene by tissues of tomato, pepper, French bean and cucumber in response to infection by *Botrytis cinerea*. Physiological and Molecular Plant Pathology 36: 277-287

Elad Y (1992) The use of antioxidants (free radical scavengers) to control grey mould (*Botrytis cinerea*) and white mould (*Sclerotinia sclerotiorum*) in various crops. Plant Pathology 41: 417-426

Elad Y (1993) Regulators of ethylene biosynthesis or activity as a tool for reducing susceptibility of host plant tissues to infection by *Botrytis cinerea*. Netherlands Journal of Plant Pathology 99: 105-113

Elad Y (1995) Physiological factors involved in susceptibility of plants to pathogens and possibilities for disease control - The *Botrytis cinerea* example. In: Lyr D (ed.) Modern Fungicides and Antifungal Compounds. (pp. 217-233) Intercept Ltd, Andover, Hampshire, UK

Elad Y (1997) Responses of plants to infection by *Botrytis cinerea* and novel means involved in reducing their susceptibility to infection. Biological Reviews 72: 381-422

Elad Y (2002) Ethylene and reactive oxygen species in a plant–pathogen system. Phytoparasitica 30: 307

Elad Y and Evensen K (1995) Physiological aspects of resistance to *Botrytis cinerea*. Phytopathology 85: 637-643

Elad Y, Lapsker Z, Kolesnik I, Korolev N and Kirshner B (2002) Involvement of ethylene in plant *Botrytis cinerea* interaction. Abstracts of the 7th International Mycological Congress, Oslo, p. 28

Elad Y, Shtienberg D, Yunis H and Mahrer Y (1992) Epidemiology of grey mould, caused by *Botrytis cinerea* in vegetable greenhouses. In: Verhoeff K, Malathrakis NE and Williamson B (eds) Recent Advances in *Botrytis* Research. (pp. 147-158) Pudoc Scientific Publishers, Wageningen, The Netherlands

Elad Y and Volpin H (1988) The involvement of ethylene and calcium in gray mould of Pelargonium, Ruscus and rose plants. Phytoparasitica 16: 119-131

Elad Y, Yunis H and Volpin H (1993) Effect of nutrition on susceptibility of cucumber, eggplant and pepper crops to *Botrytis cinerea*. Canadian Journal of Botany 71: 602-608

Furukowa T, Koga J, Adachi T, Kishi K and Syono K (1996) Efficient conversion of L-tryptophan to indole-3-acetic acid and/or tryptophol by some species of *Rhizoctonia*. Plant and Cell Physiology 37: 899-905

Govrin EM and Levine A (2000) The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. Current Biology 10: 751-757

Hartmann PEO (1988) The effect of ethephon sprays on the quality of Barlinka grapes. Deciduous Fruit Grower 38: 186-188

Hedden P, Phillips AL, Rojas MC, Carrera E and Tudzynski B (2002) Gibberellin biosynthesis in plants and fungi: a case of convergent evolution? Journal of Plant Growth Regulation 20: 319-331

Hirai N, Okamoto M and Koshimizu K (1986) The 1',4'-*trans*-diol of abscisic acid, a possible precursor of abscisic acid in *Botrytis cinerea*. Phytochemistry 25: 1865-1868

Hoffman R, Roebroeck E and Heale JB (1988) Effects of ethylene biosynthesis in carrot root slices on 6-methoxymellein accumulation and resistance to *Botrytis cinerea.* Physiologia Plantarum 73: 71-76

Jia Y, Kakuta Y, Sugawara M, Igarashi T, Oki N, Kisaki M, Shoji T, Kanetuna Y, Horita T, Matsui H, and Honma M (1999) Synthesis and degradation of 1-aminocyclopropane-1-carboxylic acid by *Penicillium citrinum.* Bioscience Biotechnology Biochemistry 63: 542-549

Johnson P and Ecker J (1998) The ethylene gas signal transduction pathway: A molecular perspective. Annual Review of Genetics 32: 227-254

Kamisaka S, Yanagishima N and Masuda Y (1967) Effect of auxin and gibberellin on sporulation in yeast. Physiologia Plantarum 20: 90-97

Kende H and Zeevaart JAD (1997) The five "classical" plant hormones. Plant Cell 9: 1197-1210

Kepczynska E (1989) Ethylene requirement during germination of *Botrytis cinerea* spores. Physiologia Plantarum 77: 369-372

Kepczynska E (1993) Involvement of ethylene in the regulation of growth and development of the fungus *Botrytis cinerea* Pers. ex. Fr. Plant Growth Regulation 13: 65-69

Kepczynska E (1994) Involvement of ethylene in spore germination and mycelial growth of *Alternaria alternata*. Mycological Research 98: 118-120

Kepczynski J and Kepczynska E (1977) Effect of ethylene on germination of fungal spores causing fruit rot. Fruit Science Reports 4: 31-35

Kettner J and Dorffling K (1995) Biosynthesis and metabolism of abscisic acid in tomato leaves infected with *Botrytis cinerea*. Planta 196: 627-634

Kieber JJ (1997) The ethylene response pathway in *Arabidopsis*. Annual Review of Plant Physiology and Plant Molecular Biology 48: 277-296

Kolattukudy P, Li D, Huang C and Flaishman M (1995) Host signals in fungal gene expression involved in penetration into the host. Canadian Journal of Botany 73: 160-168

Korolev N and Elad Y (2004) The role of phytohormone balance in the plant-pathogen interaction (*Arabidopsis thaliana – Botrytis cinerea*). Phytoparasitica 32 186-187

Lurie S, Ben Arie R and Zilkah S (1998) The ripening and storage quality of nectarine fruits in response to preharvest application of gibberellic acid. Acta Horticulturae No. 463: 341-347

Marumo S, Katayama M, Komori E, Ozaki Y, Natsume M and Kondo S (1982) Microbial production of abscisic acid by *Botrytis cinerea*. Agricultural and Biological Chemistry 46: 1967-1968

McNicol RJ, Williamson B and Young K (1989) Ethylene production by black currant flowers infected by *Botrytis cinerea*. Acta Horticulturae No. 262: 209-215

Nakamura T, Kawanabe Y, Takiyama E, Takahashi N and Murayama T (1978) Effects of auxin and gibberellin on conidial germination in *Neurospora crassa*. Plant Cell Physiology 19: 705-709

Nakamura T, Tomita K, Kawanabe Y and Murayama T (1982) Effect of auxin and gibberellin on conidial germination in *Neurospora crassa* II. "Conidial density effect" and auxin. Plant Cell Physiology 23: 1363-1369

Neill SJ, Horgan R, Walton DC and Mercer CAM (1987) The metabolism of α-ionylidene compounds by *Cercospora rosicola*. Phytochemistry 26: 2515-2519

Niklis ND, Thanassoulopoulos CC and Sfakiotakis EM (1992) Ethylene production and growth of *Botrytis cinerea* in kiwifruit as influenced by temperature and low oxygen storage. In: Verhoeff K, Malathrakis NE and Williamson B (eds) Recent Advances in *Botrytis* Research. (pp. 113-118) Pudoc Scientific Publishers, Wageningen, The Netherlands

Normanly J and Bartel B (1999) Redundancy as a way of life - IAA metabolism. Current Opinions in Plant Biology 2: 207-213

Okamoto M, Hirai N and Koshimizu K (1988a) Biosynthesis of abscisic acid. Memoirs of the College of Agriculture, Kyoto University 132: 79-115

Okamoto M, Hirai N and Koshimizu K (1988b) Biosynthesis of abscisic acid from α-ionylideneethanol in *Cercospora pini-densiflorae*. Phytochemistry 27: 3465-3469

Qadir A, Hewett E and Long P (1997) Ethylene production by *Botrytis cinerea*. Postharvest Biology and Technology 11: 85-91

Patten CL and Glick M (1995) Bacterial biosynthesis of indole-3-acetic acid. Canadian Journal of Microbiology 42: 207-220

Robinson M, Riov J and Sharon A (1998) Indole-3-acetic acid biosynthesis in *Colletotrichum gloeosporioides* f. sp. *aeschynomene*. Applied and Environmental Microbiology 64: 5030-5032

Russo VM and Pappelis AJ (1993) Mycelial elongation and sporulation of two fungi on amended media in light or dark. Antonie van Leeuwenhoek 63: 23-27

Shaul O, Elad Y and Zieslin N (1995a) Suppression of Botrytis blight disease of rose flowers with gibberellic acid: effect of concentration and mode of application. Postharvest Biology and Technology 6: 321-330

Shaul O, Elad Y and Zieslin N (1995b) Suppression of Botrytis blight disease of rose flowers with gibberellic acid: effects of postharvest timing of the gibberellin treatment, conidial inoculation and cold storage period. Postharvest Biology and Technology 6: 331-339

Shaul O, Elad Y and Zieslin N (1996) Suppression of Botrytis blight disease of rose flowers with gibberellic acid: effect of abscisic acid and paclobutrazol. Postharvest Biology and Technology 7: 145-150

Shaul O, Elad Y, Kirshner B, Volpin H, Zieslin N, Elad Y, Shtienberg D, Yunis H and Mahrer Y (1992) Control of *Botrytis cinerea* in cut rose flowers by gibberellic acid, ethylene inhibitors and calcium. In: Verhoeff K, Malathrakis NE and Williamson B (eds) Recent Advances in *Botrytis* Research. (pp. 257-261) Pudoc Scientific Publishers, Wageningen, The Netherlands

Siewers V, Smedsgaard J and Tudzynski P (2004) The P450 monooxygenase BcABA is involved in abscisic acid biosynthesis in *Botrytis cinerea*. Journal of Applied and Environmental Microbiology 70: 3868-3876

Smith WH, Meigh DF and Parker JC (1964) Effect of damage and fungal infection on the production of ethylene by carnation. Nature 204: 92-93

Songstad DD, Giles KL, Park J, Novakovski D, Epp D, Friesen L and Roewer I (1989) Effect of ethylene on sanguinarine production from *Papaver somniferum* cell cultures. Plant Cell Reports 8: 463-466

Tapani T, Livesoksa J, Laasko S and Rosenqvist H (1993) Interaction of abscisic acid and indole-3-acetic acid-producing fungi with *Salix* leaves. Journal of Plant Growth Regulators 12: 149-156

Theologis A (1998) Ethylene signaling: redundant receptors all have their say. Current Biology 8: R875-R878

Tudzynski B (1997) Fungal phytohormones in pathogenic and mutualistic associations. In: Carroll GC and Tudzynski P (eds), The Mycota V, Part A, Plant Relationships. (pp. 167-184) Springer-Verlag, Berlin, Heidelberg, Germany

Tudzynski B (1999) Biosynthesis of gibberellins in *Gibberella fujikuroi*: Biomolecular aspects. Applied Microbiology and Biotechnology 52: 298-310

Tudzynski B and Hölter K (1998) The gibberellin biosynthetic pathway in *Gibberella fujikuroi*: evidence for a gene cluster. Fungal Genetics and Biology 25: 157-170

Tudzynski B and Sharon A (2002) Biosynthesis, biological role and application of fungal hormones. In: Osiewacz HD (ed.), The Mycota X: Industrial Applications. (pp. 183-211) Springer-Verlag, Berlin, Heidelberg, Germany

Volpin H and Elad Y (1991) Influence of calcium nutrition on susceptibility of rose flowers to Botrytis blight. Phytopathology 81: 1390-1394

Williamson CE (1950) Ethylene, a metabolic product of diseased or injured plants. Phytopathology 40: 205-208

Williamson B and Hargreaves AJ (1981) Effects of *Didymella applanata* and *Botrytis cinerea* on axillary buds, lateral shoots and yield of red raspberry. Annals of Applied Biology 97: 55-64

Wolf FT (1952) The production of indoleacetic acid by *Ustilago zeae* and its possible significance in tumor formation. Proceedings of the National Academy of Sciences of the USA 38: 106-111

Yanagishima N (1965) Role of gibberellic acid in the growth response of yeast to auxin. Physiologia Plantarum 18: 306-312

Yang S (1969) Further studies on ethylene formation from 2-keto-4-methylthiobutyric acid or 3-methylthiopropionaldehyde by peroxidase in the presence of sulfite or oxygen. Journal of Biological Chemistry 244: 4360-4365

Zieslin N, Shaul O and Elad Y (1996) Suppression of Botrytis blight in rose flowers with gibberellic acid. Formation of endogenous inhibitory compounds. Journal of Plant Physiology 149: 580-584