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Effect of Plant Growth Regulators on White Mould (*Sclerotinia sclerotiorum*) on Bean and Cucumber

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With 3 figures

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Abstract

The effect of plant growth regulators on the pathogenic fungus *Sclerotinia sclerotiorum* (white mould) was investigated under *in vitro* and *in vivo* conditions. Naphthalene acetic acid inhibits the fungus *in vitro* and *in vivo*. It reduced white mould disease severity on bean and cucumber plants at concentrations of 200–400 μg/ml. Gibberellic acid (GA_3) promoted both mycelium and white mould disease severity on plants at concentrations of 50–250 μg/ml. Methyl jasmonate (MeJa), and abscisic acid (ABA) decreased mycelium growth of *S. sclerotiorum in vitro*. MeJa decreased bean and cucumber white mould disease at concentrations of 75–250 μg/ml. ABA increased disease development on bean and cucumber plants at concentrations of 100–300 μg/ml.

Introduction

The role of plant growth regulators (PGRs) in plant diseases is not clearly identified. There is increasing evidence that both the pathogen and the host have the capacity to synthesize various PGRs. Alterations in the levels of PGRs and in disease susceptibility or resistance reaction are associated with plant pathogen interaction (Singh et al.,1997). Some investigations indicated that naphthalene acetic acid (NAA) is a potential antifungal agent (Nakamura et al., 1978; Tomita et al., 1984; Michniewicz and Rozej, 1988). Auxin strongly inhibited mycelial growth, sporulation, and spore germination of *Fusarium culmorum in vitro* (Michniewicz and Rozej, 1987). NAA, indole acetic acid (IAA), 2,4-diphenol acetic acid (2,4,D) and abscisic acid (ABA) were exogenously applied to control *Alternaria solani* caused early blight of potato. Auxins such as IAA, naphthalene acetic acid ethyl ester and *N*-metatotyl phthalamic acid reduced Botrytis blight of cut rose flowers (Elad, 1995).

Gibberellic acid (GA_3) does not act as a growth factor in fungi as a result of experiments in which growth retardants were used in cultures of *F. moniliforme* (Evans, 1984). GA inhibited sporulation in *Claviceps purpurea*, the causal agent of wheat Ergot in saprophytic and parasitic cultures (Ostrovsky et al., 1961). Nakamura et al. (1978) and Tomita et al. (1984) reported that GA_3 stimulated spore germination and elongation of young hyphae of *Neurospora crassa*. GA_3 at 10^{-9}–10^{-5} M slightly stimulated *F. culmorum* mycelium growth, and significantly stimulated sporulation and spore germination at 10^{-7} and 10^{-5} M *in vitro* under optimal condition (Michniewicz and Rozej, 1987, 1988). Botrytis blight of cut rose flowers has been controlled by GA_3 applications but the *in vitro* germination, growth and development of *Botrytis cinerea* were not affected by the hormone at the tested concentration; its effect resulted from GA_3-imposed inhibition of senescence processes in rose petals (Shaul et al., 1995).

Jasmonates, produced by some fungi, may be involved as antifungal agents by increasing plant defence (Paul, 1995). Methyl jasmonate (MeJa) inhibited the growth of mycorrhizal fungi (Gogal, 1991). Jasmonic acid and MeJa, applied to potato and tomato plants in the glasshouse, induced local protection against *Phytophthora infestans* (Cohen et al., 1993). In another study, jasmonates sprayed on barley plants in growth chamber gave protection against powdery mildew (Schweizer et al., 1993).

ABA is produced by phytopathogenic fungi of the genera *Botrytis*, *Ceratocystis*, *Fusarium*, *Rhizoctonia* and *Cercospora rosicola* (Asante and Nasini, 1977; Dorffling and Peterson, 1984). ABA was a potent inhibitor of growth, sporidiogenesis and teliospore germination of *Neovossia indica* under culture condition (Singh et al., 1997) and promotes *B. cinerea* infection of various plants (Elad, 1997; Shaul et al., 1996).

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The fungus *Sclerotinia sclerotiorum* (Lib.) de Bary belongs to the class Ascomycetes, and the family Sclerotiniaceae (Purdy, 1979). It is an important plant pathogen causing white mould, a serious yield-limiting disease of many crops in many countries (Steadman, 1983). The role of PGRs in the interaction of white mould and host plants has, to the best of our knowledge, not been identified. The aim of the present work was to investigate the effect of PGRs on the interaction between *S. sclerotiorum* and its host plants bean (*Phaseolus vulgaris*) and cucumber (*Cucumis sativus*).

Materials and Methods

Plant growth regulators

NAA (Fine Agrochemical, Worcester, UK), GA_3 (CTC, Petakh Tiqua, Israel), MeJa (Asia Riezel, Petakh Tiqua, Israel) and ABA (St. Louis, MO, USA) were used.

Fungal material

Collected isolates of *S. sclerotiorum* were recovered from infected bean and cucumber plants. Isolates had been cleaned up and kept lyophilized in skimmed milk at 30°C. Three isolates (SS.P.10, SS.I.18 and SS.P.26) had been used in all experiments.

Effect of plant growth regulators *in vitro*

Growth regulators were incorporated in potato-dextrose agar (PDA) (Oxoid) medium at 40°C to give final concentrations of active ingredient (μg/ml) of NAA 0, 1, 10, 50, 100, 150, and 200; GA_3 0, 10, 50, 100, 150, and 200; MeJa 0, 10, 50, 75, 100, and 150; and ABA 0, 25, 50, 75, 100, and 150. Fourteen millilitres of PGR-amended PDA were dispensed into each 90-mm diameter Petri plate. Mycelium plugs (5-mm diameter) taken from the edges of 6-day-old cultures of each *S. sclerotiorum* isolate were used to inoculate six replicate plates for each concentration. Plates were then incubated in the dark at 22°C. Colony diameter was measured at 44, 68, and 74 h. The 74-h mycelium growth rate (cm^2/day) is presented.

Effect of plant growth regulators on detached leaves

Two-week-old bean (cv. Hilda) seedlings and three-week-old cucumber seedlings (cv. Delila) were planted in 15 × 30 cm (breadth × height) pots in glasshouse. The planting medium comprised of a mixture of peat-moss and perlite (2 : 1 v/v). Plants were irrigated and a 20 : 20 : 20 NPK fertilizer was added twice a week.

At flowering phase (40 days after planting), fully expended young leaves were detached and placed in plastic boxes (40 × 25 × 15 cm), on a plastic mesh platform placed on sterilized wet towel paper to preserve high relative humidity in the polyethylene-covered box. Each box had six bean or three cucumber leaves.

The leaves were sprayed with the PGR solutions using a microsprayer. The concentrations (μg a.i./ml) of PGRs were as follows: NAA 0, 200, 300, 400, 500, and 600; GA_3 0, 50, 100, 150, 200 and 300; MeJa 0, 50, 75, 100, and 150; and ABA 0, 50, 100, 150, 200, and 250. After the PGR solutions were absorbed to the leaf, the detached leaves were inoculated on their lower surface with 5-mm diameter agar block, taken from the margins of 6-day-old PDA cultures of each of the three isolates of *S. sclerotiorum*. The boxes were moistened, covered by transparent plastic film, and incubated at 22°C with 12 h photoperiod. The development of *S. sclerotiorum* infection rot was evaluated by measuring disease lesions growth rate 93 h after inoculation. The experimental design used was completely randomized design, in six replicates where each of the six bean leaflets or three cucumber leaves was considered as a replicate.

Effect of plant growth regulators on white mould disease severity

Forty-day-old bean plants and 30-day-old cucumber plants for each concentration, were sprayed until run-off by the following PGRs (μg a.i./ml): NAA 0, 200, 400, and 600; GA_3 0, 50, 150, and 250; MeJa 0, 75, 150, and 250; and ABA 0, 100, 200, and 300 μg/ml. The PGR solutions had been adsorbed within 2 h. Treated plants were inoculated with homogenized mycelium of each of the *S. sclerotiorum* isolates suspended in deionized sterile water containing 2 g/l glucose, and 1 g/l KH_2PO_4 at volume of 20 ml/plant. Plants were covered with transparent plastic bags to preserve humidity, and incubated in a growth chamber at 22°C, with 12 h photoperiod. The severity of white mould was evaluated by estimating the percent of leaf coverage 11 days after inoculation. There were 12 plants for each replicate and four replicates per treatment arranged in complete randomized design.

Statistical analysis

Results were analysed statistically using one-way analysis of variance (ANOVA) to test significance, and the Tukey test was used for means separations by Sigma Stat Software Program (1997).

Results

In vitro effect of plant growth regulators on mycelial growth of *S. sclerotiorum*

Growth of the three isolates of *S. sclerotiorum* in the non-amended control plates was 12–18 cm^2/day (Fig. 1). NAA significantly ($P = 0.05$) decreased the mycelium growth rate of the three isolates of *S. sclerotiorum* on PDA at concentrations 1–200 μg/ml. The inhibitory effect of NAA increased significantly with increasing concentration. At 200 μg/ml, the mycelium growth rate of the three isolate was reduced to 2.9% of mycelium growth rate of the non-treated mycelium (Fig. 1). GA_3 did not significantly ($P = 0.05$) reduce the mycelium growth rates of *S. sclerotiorum* isolates at concentrations lower than 100 μg/ml (Fig. 1). The mean mycelium growth rate was reduced significantly at 200 μg/ml of GA_3 by 51% as compared with the control. MeJa significantly ($P = 0.05$) reduced mycelium growth rate of the three isolates of *S. sclerotiorum* at

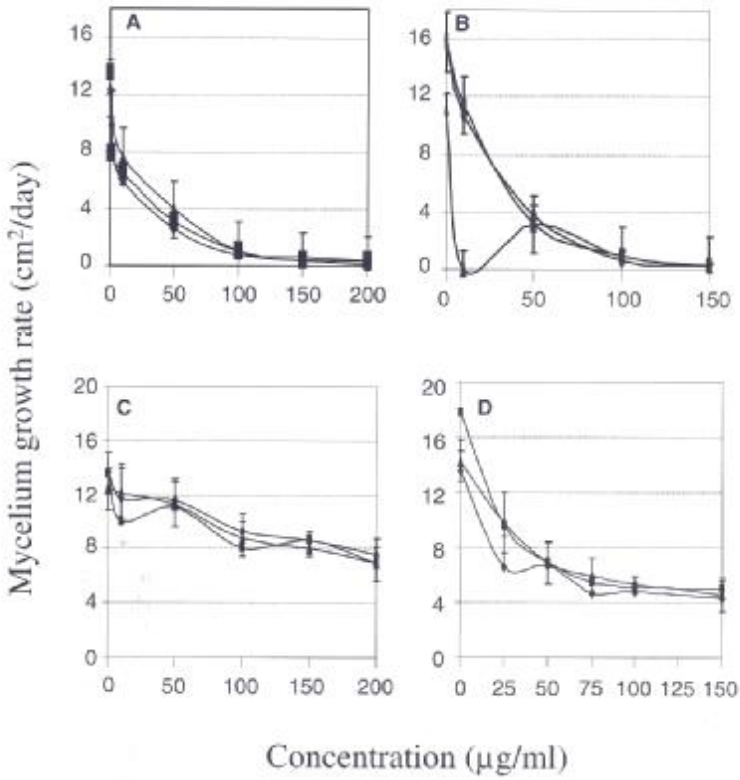

Fig. 1 Effect of naphthalene acetic acid (A); gibberellic acid (B); methyl jasmonate (C); and abscisic acid (D) on mycelium growth rate of isolates SS.P.10 (-◆-), SS.I.18 (-■-), SS.P.26 (-▲-) of *Sclerotinia sclerotiorum* on PDA-amended with different concentration of PGRs

10–150 µg/ml. Mycelium growth at 150 µg/ml was 2% of the control. ABA significantly ($P = 0.05$) reduced mycelium growth rate of the three isolates of *S. sclerotiorum* growing on PDA amended with 25–150 µg/ml, with no differences between the concentrations (Fig. 1).

The effect of plant growth regulators on white mould infection of detached leaves

In most cases, the formation of lesions by the different isolates of *S. sclerotiorum* was similar but varied according to the experiment and plant host (Fig. 2). NAA at 200–600 µg/ml significantly ($P = 0.05$) reduced development of *S. sclerotiorum* lesions on bean and cucumber detached leaves (Fig. 2), with no significant differences found between the three isolates at 200–400 µg/ml, whereas at concentrations of 500 and 600 µg/ml, the differences between isolates were significant, isolate SS.P.26 being less susceptible. In addition, chlorosis was observed on bean leaves treated with 600 µg/ml of NAA.

GA_3 did not significantly ($P = 0.05$) affect *S. sclerotiorum* lesions development on bean detached leaves pretreated with concentrations of 150 µg/ml and below but it increased disease severity at concentrations of 200–250 µg/ml. It did not affect cucumber infection at concentrations of 50–300 µg/ml (Fig. 2).

MeJa significantly ($P = 0.05$) decreased *S. sclerotiorum* lesions development on bean and cucumber detached leaves pretreated at concentrations of 50–150 µg/ml. Variations between isolates were significant at 50 and 100 µg/ml MeJa on bean leaves, and at 50 and 75 µg/ml on cucumber leaves. A positive correlation was observed between MeJa concentration and its inhibitory effect on lesion development. At 150 µg/ml, the mean lesion growth rate was 14% ($r^2 = 0.84$) and 9% ($r^2 = 0.94$) on bean and cucumber detached leaves, respectively, compared with the growth rate of the untreated control.

ABA did not significantly ($P = 0.05$) affect lesion development on cucumber leaves pretreated at concentrations of 50–250 µg/ml. ABA slightly reduced lesions development on bean leaves at 50–200 µg/ml with no effect at 250 µg/ml (Fig. 2).

The effect of plant growth regulators on white mould severity on whole bean and cucumber plants

In general, no variation was detected between isolates in respect to the formation of lesions (Fig. 3). NAA significantly ($P = 0.05$) reduced bean white mould disease severity at 200–400 µg/ml. Differences in disease severity of the various isolates at the same concentrations were not significant. At 600 µg/ml NAA, white

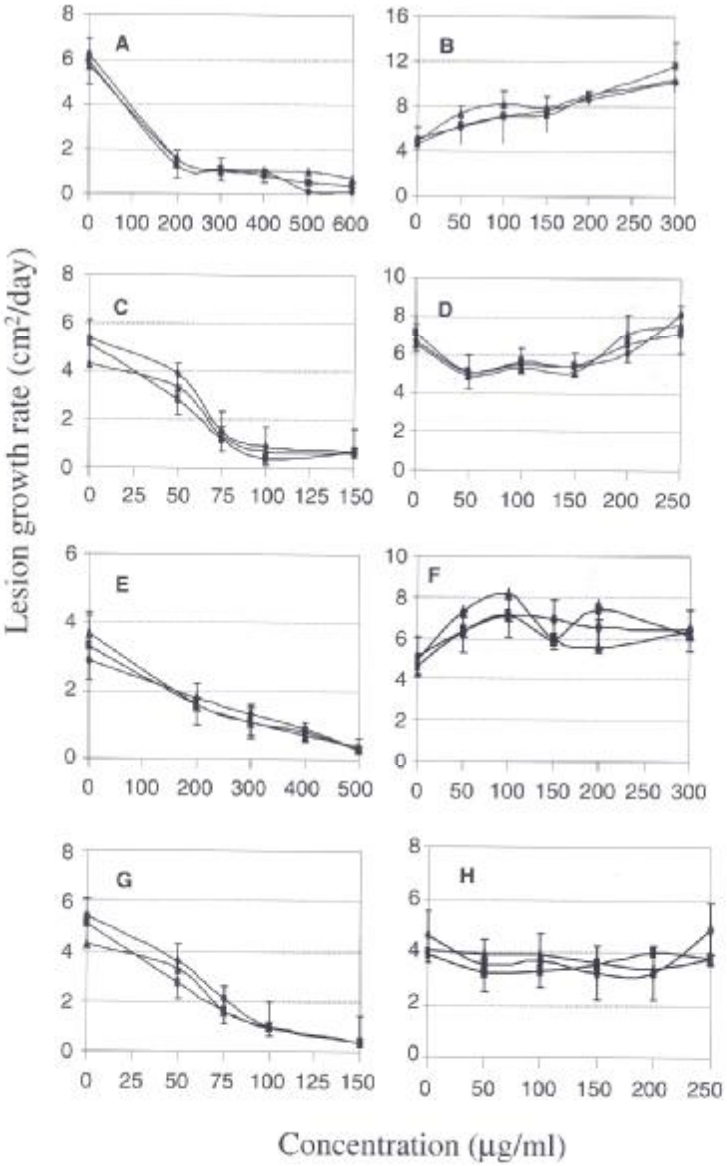

Fig. 2 Effect of naphthalene acetic acid (A, E); gibberellic acid (B, F); methyl jasmonate (C, G); and abscisic acid (D, H) on white mould lesion growth rate caused by isolates SS.P.10 (-◆-), SS.I.18 (-■-), SS.P.26 (-▲-) of *Sclerotinia sclerotiorum* on bean (A–D) and cucumber (E–H) detached leaves after 4-day incubation period

mould severity on bean and cucumber for three isolates except isolate SS.P.26 on cucumber plants were not affected. The latter concentration also caused leaf chlorosis. Mean disease severity in bean plants was significantly higher at 600 µg/ml compared with those at 200 and 400 µg/ml. Cucumber white mould disease severity was not significantly ($P = 0.05$) reduced at 200–600 µg/ml NAA compared with the control (Fig. 3).

GA_3 significantly ($P = 0.05$) increased disease severity on beans at 50–250 µg/ml (Fig. 3). The effect was more severe as concentration of GA_3 increased. However, disease severity was not affected on cucumber plants at 50 250 µg/ml of GA_3. Variation in disease severity between isolates was not significant at these concentrations. MeJa significantly ($P = 0.05$) reduced bean and cucumber white mould disease severity at 75–250 µg/ml (Fig. 3). A positive correlation ($r^2 = 0.99$ on bean, $r^2 = 0.75$ on cucumber) was found between concentrations of MeJa and its inhibitory effect on bean and cucumber plants, however, induced leaf chlorosis on plants. ABA significantly ($P = 0.05$) increased white mould severity on cucumber plants pretreated

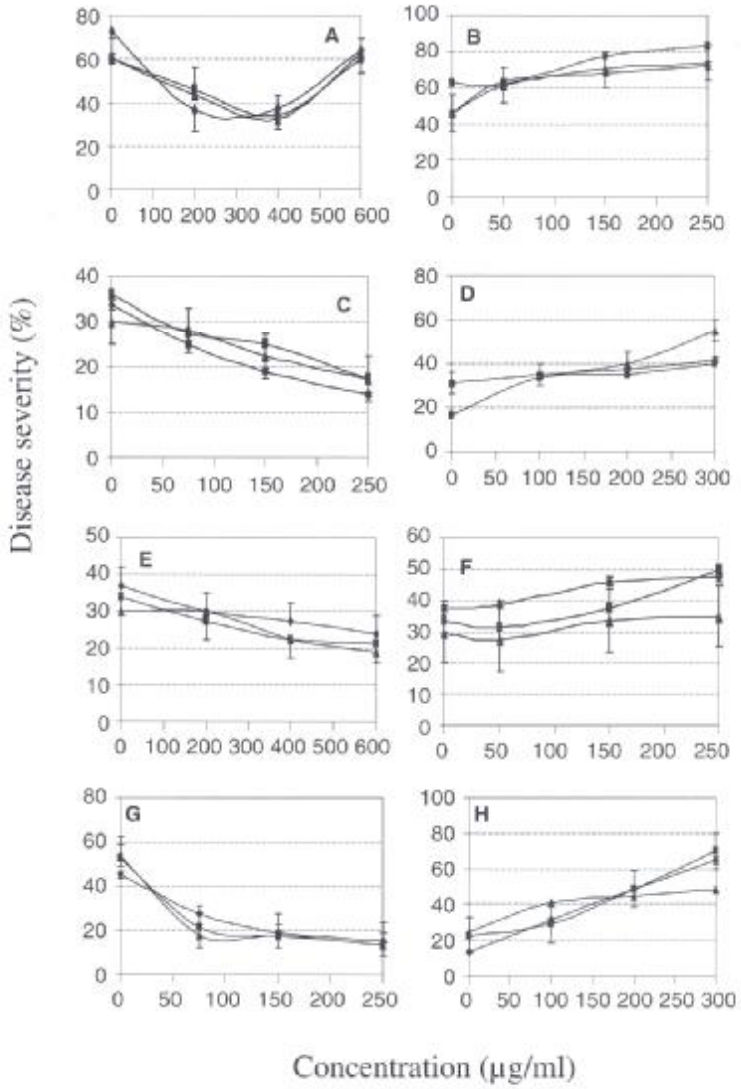

Fig. 3 Effect of naphthalene acetic acid (A, E); gibberellic acid (B, F); methyl jasmonate (C, G); and abscisic acid (D, H) on white mould disease severity (%) caused by isolates SS.P.10 (-◆-), SS.1.18 (-■-), SS.P.26 (-▲-) of *Sclerotinia sclerotiorum* on bean (A–D) and cucumber (E–H) plants pretreated with different concentrations of PGRs after 11-day incubation period

with 100–300 µg/ml, and on bean plants at 200 and 300 µg/ml (Fig. 3). Variable response to the three isolates in relation to disease development was observed at 300 µg/ml on bean and cucumber plants.

Discussion

NAA reduced mycelium growth rate of *S. sclerotiorum in vitro* and white mould disease severity on detached leaves and in whole bean and cucumber plants at concentrations of 200–600 µg/ml. Similarly, NAA strongly inhibited mycelium growth, sporulation, and spore germination of *F. culmorum in vitro*, whereas it increased spore production and germination at low concentrations (Michniewicz and Rozej, 1987). NAA increase the resistance of potato plants to early blight (Melinda and Stevenson, 1991). Michniewicz and Rozej (1987) and Melinda and Stevenson (1991) have pointed out that auxin acts as a fungal growth and development-controlling factor, while its role in growth and development processes may vary in different species. The velocity and flux of auxin transport varies depending on many factors, including nature of auxin, and nature and maturity of tissues. NAA moves slightly slower than other auxins and the transport rate of NAA in bean tissues is slow (Hertel and Flory, 1968). So, when NAA is exogenously applied on bean and cucumber plant tissues, it accumulates in the upper cell layers where it initiates a short-lived partial resistance against

the fungus. *S. sclerotiorum* penetration through tissues is associated with enzymes capable of degrading the middle lamella of host cell. Optimum pH-value range of these enzymes (e.g. pectinases and cellulases, Lumsden, 1976; and proteolytic enzymes, Khare and Bompeix, 1976) is 4.3–5.5. The pH value of NAA spray solutions used in this study was 6.63–7.69 depending on concentrations. This may have contributed to the reduction of enzyme activity and thus, the ability of *S. sclerotiorum* to degrade the host tissue. NAA increased disease severity on bean plants at 600 μg/ml possibly due to a deleterious effect on the host tissue rendering it more susceptible. For instance, when exogenously applied, it may stimulate endogenous ethylene production in plant tissue at high concentrations (Saniewski et al., 1990).

GA_3 significantly reduced mycelium growth rate *in vitro* at 150 and 200 μg/ml, probably because GA_3 slightly reduced pH value of medium from 4.1 to 3.74 and 3.22, respectively (data not shown). However, GA_3 increased lesions development on detached bean leaves at 200 and 300 μg/ml, and it stimulated white mould development on both bean and cucumber plants at concentrations higher than 150 μg/ml. Michniewicz and Rozej (1987, 1988) reported that GA_3 slightly stimulated mycelium growth of *F. culmorum in vitro* and significantly stimulated sporulation and spore germination (at 10^{-7} and 10^{-5} M). These authors speculate that GA_3 acts as a regulator of nitrogen and carbon metabolism. Tomita et al. (1984) also came to the conclusion that GA_3 is a growth and differentiation regulator in fungi just as it is in higher plants. Gibberellins perform a variety of activities in infected plants. Their most common effect on plants is promotion of plant cell elongation. Additional effects like induction of amylase and cellulase and stimulation or inhibition of ethylene production depend on the plant species (Pegg, 1981). The effect of exogenously applied GA_3 on the interaction of *S. sclerotiorum* with bean and cucumber plants is probably complex. The stimulating effect of GA_3 on white mould disease may be due to increase in sensitivity of the host plant tissue. In this process, elongation of plant cells and enhancement of α-amylase, β-amylase and cellulase activities stimulates the hydrolysis of starch and cellulose which may provide the white mould fungus with easily accessible nutrients like glucose (Lumsden, 1976, 1979). The pH value of GA_3 spray solutions in the present work was 3.46–4.92, which is – as mentioned above – in the optimum pH range for *S. sclerotiorum* enzymes; this could have an additional growth-promoting effect on the fungus.

MeJa significantly decreased myceliar growth rate of *S. sclerotiorum in vitro* and reduced white mould disease severity on both detached leaves and whole plants of bean and cucumber (Figs 2 and 3), in a concentration-dependent manner. This indicates that MeJa may be considered for control of white mould on bean and cucumber plants. Investigations with other plant pathogens support this view: Cohen et al. (1993) applied MeJa exogenously to control late blight on potato and tomato plants in greenhouse and achieved 92 and 100% control of the disease, respectively. When applied as a topical spray against barley powdery mildew, jasmonates gave 80% protection to plants inoculated 3 days after treatment (Schweizer et al., 1993). The level of protection was however, short-lived, and necrosis occurred on leaf tips at 10 μg/ml. Several investigators had attempted to explain low dose function of MeJa and how it induces partial resistance in diseased plants. The interaction between MeJa and systemin, ethylene, ABA, and electrical current in plant tissue initiates defence against pathogen (Xu et al., 1994; Shigemi et al., 1997). So it is possible that MeJa induced partial resistance against white mould on bean and cucumber plants. Chlorosis caused by exogenous application of MeJa at high concentrations (150 and 250 μg/ml) observed on bean and cucumber plants may be due to MeJa promotion of degradation of cell wall polysaccharides and chlorophyll (Parthier, 1990) and inhibition of photosynthetic activities (Popova et al., 1988). Similar observations were made by Vedo et al. (1996) and Miamoto et al. (1997)who reported that MeJa promoted the degradation of cell wall polysaccharides in petioles of *P. vulgaris* plants.

ABA slightly reduced myceliar growth rate on PDA amended with different concentrations up to 150 μg/ml, it stimulated white mould on bean and cucumber plants at 100–300 μg/ml concentrations. This may be due to ABA effects on plant cell membranes, stomata closure (Ya'acov et al., 1990), and turgidity of tissues due to its effect on membrane phospholipids. Thus externally applied ABA could reduce the resistance of plant tissues and enhance fungal penetration and colonization, promoting white mould disease.

Taken together, the results presented indicate that NAA and MeJa may have a potential to reduce white mould disease on bean and cucumber in whole plants, while GA_3 and ABA stimulate disease development.

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