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

M. I. Al-Masri1, M. S. Al-Shtayer2, Y. Elad3, A. Sharon4, P. Tudzynski5 and R. Barakat1

Authors’ addresses: 1College of Agriculture, Hebron University, Hebron, Palestinian Authority; 2College of Science, An-Najah National University, Nablus, Palestinian Authority; 3Department of Plant Biology, ARO, The Volcani Center, Bet dagan 50250, Israel; 4Department of Plant Sciences, Tel Aviv University, Tel Aviv 69978, Israel; 5Institut für Botanik, Westf. Wilhelms Universität, D-48149 Münster, Germany (correspondence to R. Barakat. E-mail: rbarakat@netvision.net.il)

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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 (GA3) 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 under culture condition (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 (GA3) 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 GA3 stimulated spore germination and elongation of young hyphae of Neurospora crassa. GA3 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 GA3 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 GA3-imposed inhibition of senescence processes in rose petals (Shaful 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; Dorfling and Peterson, 1984). ABA was a potent inhibitor of growth, sporidogenesis and teliospore germination of Neovossia indica under culture condition (Singh et al., 1997) and promotes B. cinerea infection of various plants (Elad, 1997; Shaful et al., 1996).
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\textsubscript{3} (CTC, Petakh Tiqwa, Israel), MeJa (Asia Riezel, Petakh Tiqwa, 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\textsubscript{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 (cm²/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 x 30 cm (breadth x height) pots in glasshouse. The planting medium comprised of a mixture of peat-moss and perlite (2 : 1 v/v). Plants were irrigated and 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\textsubscript{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 leaves, 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.

#### 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 cm²/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\textsubscript{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\textsubscript{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
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.

GA₃ 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% (r² = 0.84) and 9% (r² = 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
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). 

GA₃ significantly (P = 0.05) increased disease severity on beans at 50–250 µg/ml (Fig. 3). The effect was more severe as concentration of GA₃ increased. However, disease severity was not affected on cucumber plants at 50-250 µg/ml of GA₃. 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. 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.](image-url)
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).

GA₃ significantly reduced mycelium growth rate *in vitro* at 150 and 200 μg/ml probably because GA₃ slightly reduced pH value of medium from 4.1 to 3.74 and 3.22, respectively (data not shown). However, GA₃ increased lesion 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 GA₃ 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 GA₃ acts as a regulator of nitrogen and carbon metabolism. Tomita et al. (1984) also came to the conclusion that GA₃ 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 GA₃ on the interaction of *S. sclerotiorum* with bean and cucumber plants is probably complex. The stimulating effect of GA₃ 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 activity, 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 GA₃ 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 mycelial 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 mycelial growth rate *in vitro* at 150 and 200 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 GA₃ and ABA stimulate disease development.

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**Literature**


