Ethylene Production by Botrytis cinerea (Casual Organism of Gray Mold Disease) & Influence of the Exogenously Applied Growth Regulators & thier Inhibitor on Disease Development

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Ethylene Production by Botrytis cinerea (Causal Organism of Gray Mold Disease) and Influence of the Exogenously Applied Growth Regulators and their Inhibitor on Disease Development.

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Abstract
The fungus Botrytis cinerea grown on shaking potato dextrose broth (PDB) medium amended with 10 mM methionine produced ethylene. Ethylene production reached a peak after 4 days of incubation; however, there was a high variability in production between the isolates. The range of ethylene produced by twenty four isolates of the fungus was from 383 to 6789 μl/g/h with average production of 2091 μl/g/h. The results showed considerable intraspecific variation in ethylene production of B. cinerea.

The role of exogenously applied ethylene (Ethephon®) in the interaction between B. cinerea and tomato (Lycopersicon esculentum) and bean (Phaseolus vulgaris) plants was studied in vitro and in vivo. Ethephon® has not significantly affected the fungus mycelium growth rate on potato dextrose agar (PDA), while it significantly increased disease severity by 50.4% - 55.8% on tomato plants and by 27.6% - 33.8% on bean plants at concentration 200-600 μg/ml. Aminoethoxyvinylglycine (AVG) significantly decreased mycelium growth in vitro at 200 μg/ml by 47%, and it decreased disease severity by 45% - 49% at concentrations 200 - 300 μg/ml on tomato plants and 55% - 75% at 100-300 μg/ml on bean plants.

The results indicate that the fungus mycelium growth rate on potato dextrose agar (PDA), while it significantly increased disease severity by 50.4% - 55.8% on tomato plants and by 27.6% - 33.8% on bean plants at concentration 200-600 μg/ml. Aminoethoxyvinylglycine (AVG) significantly decreased mycelium growth in vitro at 200 μg/ml by 47%, and it decreased disease severity by 45% - 49% at concentrations 200 - 300 μg/ml on tomato plants and 55% - 75% at 100-300 μg/ml on bean plants.

Keywords: Ethylene, Ethephon®, Aminoethoxyvinylglycine, Botrytis cinerea, and Gray Mold

Introduction
Ethylene is a gaseous plant hormone produced by a number of plant pathogenic bacteria and fungi (Hay and Curtis, 1968). Many investigators showed that ethylene was produced by Botrytis cinerea when grown on PDA medium (Fukuda et al. 1993, Qadir et al. 1997, Chague et al. 2002). Ethylene production may be an inherent character of the species rather than a feature of specific isolates (Qadir, et al. 1997). The amount of ethylene production varied from case to another. This could be attributed to many factors, including temperature, pH of medium, culture age, incubation period, fungal species, and precursor materials (Strzelczyk et al., 1994).

Ethephon® (2-chloroethylphosphonic acid) as an ethylene-releasing product (Dennis et al., 1970), was exogenously applied in many...
agricultural practices. Ethylene is known to hasten multi-biological and physiological alterations such as proteolysis and other hydrolytic activities, stimulation of oxidative enzymes, loss of chlorophyll, and decline in photosynthetic rate. These alterations lead to early maturation and accelerated senescence (Abeles, 1973; Aharoni & Lieberman., 1979; Gepstein & Thimaan., 1981; Choe & Whang, 1986).

The role of ethylene in disease resistance is difficult to interpret, because ethylene may have a stimulation, inhibition, or no effect on disease development (Archers et al., 1975). Studies showed both increased and decreased disease developments after ethephon treatments of various plants. In particular, it was reported that ethephon increased the resistance of cucumber to Erysiphe cichoracearum (Dehne et al., 1982). In addition, it inhibited Rhizoctonia solani development on mug bean, and Verticillium albo-atrum on tomato (Biles et al., 1990). In another study, ethylene promoted development of cucumber (Cucumis sativus) anthracnose caused by Colletotrichum lagenarium, B. cinerea on strawberry, and Helminthosporium sativum on barley (Biles et al., 1990). Exogenously applied ethephon at 7×10⁻⁵, 7×10⁻⁶ and 7×10⁻⁵ M slightly stimulated and at 7×10⁻⁴ M inhibited the hyphal growth of Botrytis cinerea in vitro and the disease on apples (Kepezyńska, 1993). However, Elad (1992) reported that ethephon increased the severity of gray mold caused by B. cinerea on leaves of Senecio sp. In addition the ethephon increased the gray mold disease severity by 23.5%-41.2% on tomato plants pretreated with 100-400 µg/ml respectively (Al-Masri et al. 2002, Barakat & Al-Masri, 2004).

Studies on the effect of ethylene in vitro and in vivo on growth of certain post-harvest fruit-infecting fungi indicated that the ethylene up to 10⁻⁵ µg/ml had no significant effect on percent spore germination of Alternaria alternata, Colletotrichum gloeosporioides, Penicillium expansum and Rhizopus stolonifer (El Kazaa et al., 1983). Kępezyńska, (1993) found that, when Ethylene was applied as ethephon at 7×10⁻⁶ and 7×10⁻⁵ M, the growth of hyphae of B. cinerea was slightly increased in vitro and on apple fruits. However, when applied at 7×10⁻⁴, hyphal growth was inhibited in vivo and in vitro.

Materials and methods

Fungal Isolates

Isolates of Botrytis cinerea used in the present study were recovered from infected tomato, Lycopersicon esculentum and cucumber, Cucumis sativus plants collected from various greenhouses and fields in the Palestinian agricultural areas. Twenty-four isolates had been purified by sub-culturing on potato dextrose agar (PDA) amended with 250 mg/l chloramphenicol. Mycella and spores of the isolates were lyophilized, suspended in skim milk and preserved in deep freezer at -80°C. In the ethylene production experiments, the isolates used were Bc.1, Bc.2, Bc.3, Bc.4, Bc.5, Bc.6, Bc.8, Bc.9, Bc.10, Bc.11, Bc.13, Bc.14, Bc.15, Bc.16, Bc.17, Bc.21, Bc.22, Bc.27, Bc.28, Bc.33, Bc.34, and Bc.35. In the exogenously applied ethephon and AVG experiments against gray mold disease, the isolates Bc.16, Bc.P.1, and Bc.3 were used. These isolates were recovered from flower of cucumber, stem of tomato, and fruit of eggplant, respectively.
Determination of ethylene production by *B. cinerea*

To determine ethylene production *in vitro*, ten-day-old single spore cultures of the 24 isolates of *B. cinerea* mentioned earlier were used. The isolates were grown on PDA medium amended with 250 mg/l chloramphenicol in 50 mm diameter plates under light at 20 ± 1°C and were used to prepare spore suspensions. Cultures (12-16 day old) were flooded with deionized sterilized water, and the conidia were scraped from the surface and transferred to 500 ml flask. The spore suspension was then filtered through 2-3 thin layers of cheese cloth. Number of spores in the suspension was adjusted to give a spore concentration of 10^6 /ml by using a haemacytometer. The tubes were then vortexed at 2000 rpm for 2 minutes, and spore counts were diluted to (10^7 spores /ml).

Autoclaved aluminum foil covered flasks containing 10 ml of potato dextrose broth (PDB) supplemented with 0.1 g /l chloramphenicol and 10 mM methionine (Sigma M-6039) were inoculated with 0.5 ml of the spore suspension. The flasks were fitted on a rotavator shaker at 150 rpm under light and at 20 ±1°C. On the fourth day the aluminum foil was replaced with a rubber stopper seal, and fitted on the shaker for 3 hours. Five ml of the gas was removed from each flask by using a gas-tight syringe and injected into a Gas Chromatograph (Varian 3400) fitted with apre-column for ethylene and calibrated by standard gas (1ppm). The mycelium in the flasks was harvested by filtration through filter paper, and was transferred to a tray for drying at room temperature. Ethylene production rates were calculated as micro liter ethylene produced in one hour per gram of fresh weight of mycelium by using the following equation: Ethylene production (µl/g/hr) = (R×V)/(W×D), where R is gas chromatography reading (ppm); V- volume of air in the flask (ml); W- mycelium fresh weight (g); and D - duration (hours).

The experimental design was completely randomized design (CRD) which considers the flask as replicate and for each isolate three flasks were used.

**In vitro Studies**

The effect of ethylene on *B. cinerea* mycelium growth rate was evaluated by adding ethephon ®, (an ethylene releaser produced by Chemical Manufactures LTD., AGAN, as Soluble suspension) at concentration of (0, 10, 50, 100, 150, and 200 µg /ml of air volume of sealed boxes,26 cm long x34 cm wide x 14 cm deep) in sodium hydroxide solution (0.001N, PH>8) and placed near the *Botrytis* plates before sealing the boxes with transparent plastic. Each 90-mm diameter Petri-dish contained 14 ml PDA. Five-mm diameter mycelium plugs from the edges of six-day old cultures of each *B. cinerea* isolate were used to inoculate 5 replicate plates for each concentration. Plates were incubated in the light at 20 ±1°C. Colony diameter was measured after 24, 48, and 96 hours of incubation. The ninety-six-hour-mycelium growth rate (cm²/day) was presented.

Aminoethoxyvinylglycine (AVG) produced by Sigma was incorporated in PDA (Oxoid) medium at 40°C to final concentrations of active ingredient 0, 10, 50, 100, 150, 200 µg /ml. Each 90-mm diameter Petri dish contained 14 ml PDA medium. Five-mm diameter mycelia plugs from the edges of six-day old cultures of each isolate of *B. cinerea* were used to inoculate 6 replicate plates for each concentration. Plates were incubated in the light at 20 ±1°C. Colony diameter was measured after 3 days. The experimental design used was completely randomized design (CRD) with six replicate plates for each concentration.

**In vivo Studies**

**Detached leaves**

Tomato and bean seedlings (*Lycopersicon esculentum* Mill, cv. Faculta 144; *Phaseolus vulgaris*. cv. Lolita) were grown in pots (15 cm diameter x17 cm depth) with a peat - vermiculite - perlite mixture (2: 1: 1 v/v v ) under greenhouse conditions (17 - 27 °C). Plants were irrigated daily and a 20:20:20 NPK fertilizer was added twice a week (5g /L). Tomato and bean detached leaves were taken from 7 and 5-week - old plants, respectively. Detached leaves were placed in plastic containers (30 x 45 x15 cm) with a grid on the bottom. A wet filter paper was placed beneath the grid to maintain high humidity. Leaves were placed upside down on the plastic grid in the bottom of the plastic containers. Six tomato and bean leaflets were sprayed with different concentrations of Ethephon® 0-400; and AVG 0-300 µg /ml. After two hours, the leaves had absorbed
the plant growth regulators (PGRs). Detached Tomato leaves were inoculated with 5-mm - diameter mycelial agar discs of B. cinerea taken from 6-day - old cultures on PDA with the disc placed in the middle of each leaflet (Elad, 1990). Three leaves of tomato plant (each one with 6 leaflets) were used for each box. Conidial inoculums were collected from sporulating cultures of B. cinerea obtained after growing the fungus on 50-mm diameter PDA plates and incubated in the light at 20 °C. Cultures (12 - 16 day - old) were flooded with deionized sterilized water, and the conidia were scraped from the surface and transferred to 500 ml flask. The spore suspension was then filtered through 2-3 thin layers of cheese cloth. A number of spores in the suspension was adjusted to give a spore concentration of 10^5 /ml by using a haemacytometer. Phosphate (KH2PO4) and glucose (D-glucose-monohydrate) were added to the suspension as nutrients (1 g/l). Twenty µl of spore suspension (10^5 /ml) were placed on the lower surface of the leaflet. The leaflets were left at room temperature for 30 minutes to allow the spores to settle down; the boxes were then closed with a plastic transparent cover in order to maintain air humidity above 95 % and incubated at 20 °C with 12 h photoperiod in the growth room. Six replicate leaflets were used for each treatment. Disease evaluation was carried out using a modified version of Zimand et al (1996) method, by measuring the radius of the growth zone of the fungus from the droplet according to a 0 - 8 scale where 0 = no infection (symptomless leaf tissue), 1 = 0.5% infection (weak, light brown discoloration), 2 = 2% (moderate browning), 3 = 5 % (dark brown local lesions), 4 = 10 % (black lesions), 5 = 20 % (black spreading lesions), and 6 = 40 % (expansion zone < 2 mm), 7 = 75 % (expansion zone 2 - 6 mm), and 8 = 100 % expansion zone > 6 mm. The experimental design used was completely randomized design (CRD). Colony diameters were measured during three days and percentage growth rate was calculated by considering radial growth values of each isolate.

**Whole plants**

Tomato and bean seedlings were grown in pots (15 cm diameterx17 cm deep) with a peat - vermiculite - perlite mixture (2: 1: 1 v/v) in the

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**Ethylene Production by Botrytis cinerea…..**

Greenhouse at 17 - 27 °C. Plants were irrigated daily and a 20:20:20 NPK fertilizer was added twice a week. Nine - and seven - week - old tomato and bean whole plants (fruiting stage) were employed in this experiment. Tomato and bean plants were sprayed with different concentrations of PGRs. The concentrations (µg /ml) of active ingredient used were Ethephon 0 - 600 µg/ml; and AVG 0- 500 µg/ml. A hand sprayer was used for applying spray solution until run off from the edge of the leaves. The plants were sprayed with Ethephon or AVG. After two hours the treated plants were sprayed with conidial suspension (10^8 spores /ml) until the suspension drops started to run off; the plants were then covered with a transparent plastic bag to maintain air humidity above 95 % and incubated at 20 °C and 12 hour photoperiod-fluorescent light. Disease severity was evaluated after 6 days as percentage of rot covering the plant. Four replicate plants were used for each treatment and the experimental design used was CRD.

**Statistical analysis**

The data of ethylene production, mycelium growth rate, disease severity on detached leaves, and disease severity on tomato and bean plants were analyzed by using Sigmapstat® program, according to Tukey multiple comparison test (P≤0.05).

**Results**

**Ethylene production by strains of B. cinerea**

Twenty four strains of B. cinerea produced ethylene in vitro (Fig.1). The range of ethylene production was 383- 6789 µl/g/h with average 2091 µl/g/h. The results showed considerable intraspecific variation in ethylene production of B. cinerea after four days of inoculation. Fourteen isolates (Bc.5, Bc.33, Bc.20, Bc.28, Bc.13, Bc.2, Bc.21, Bc.3, Bc.1, Bc.22, Bc.17, Bc.35, Bc.10, and Bc.15 ) produced ethylene in the range of 383-1794 µl/g/h with an average of 1069 µl/g/h; the isolates (Bc.9, Bc.4, Bc.5-10, Bc.6, Bc.8, Bc.16, Bc.27, and Bcp.1) produced ethylene in the range of 2069-3759 µl/g/h with an average of 2671 µl/g/h with the isolates Bc.34 and Bc.14 producing 6698 and 6788 µl/g/h ethylene, respectively.(Fig.1)

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Khaled Hardan, Mohammad I. Al-Masri & Radwan Barakat

Effect of exogenous application of plant growth regulators

In vitro

Ethephon® at low concentrations (10 and 50 μg/ml) has not significantly affected the mycelium growth rate of *B. cinerea* isolates in vitro (Fig. 2). However, a minor reduction in growth was observed at high concentration (200 μg/ml). Variation between the three isolates in their effect on mycelial growth rate was not significant. AVG significantly (P ≤ 0.05) decreased mycelium growth rate of the three *B. cinerea* isolates (Bc.16, Bc.P.1, and Bc.L3) at concentrations of 10, 50, 100, 150, and 200 μg/ml. The inhibition at 200 μg/ml was 47%.

Figure 2. Effect of Ethephon® (A) and Aminoethoxyvinylglycine (B) on mycelium growth rate of three isolates Bc.16 (●), Bc.P.1 (■) and Bc.3 (▲) growing on PDA amended medium and incubated at 20°C.

Detached leaves

Ethephon® has not affected significantly (P ≤ 0.05) the lesion growth rate of *B. cinerea* on tomato and bean detached leaves inoculated by mycelium disk at 400 μg/ml (Table 1). AVG reduced significantly (P ≤ 0.05) rot the disease development rate on tomato and bean detached leaves inoculated by mycelium disk at 200-300 μg/ml (Table 2).

Table 1. Effect of different concentrations of Ethephon® and Aminoethoxyvinylglycine (AVG) on gray mold lesions growth rate on tomato detached leaves (cm²/day) inoculated by mycelium disk after 4-days of incubation at 20°C.
### Table 2. Effect of different concentrations of Ethephon® and Aminoethoxyvinylglycine (AVG) on gray mold lesions growth rate on bean detached leaves (cm²/day) inoculated by mycelium disk after 4-days of incubation at 20°C.

<table>
<thead>
<tr>
<th>Plant Growth Regulators</th>
<th>Concentration (µg/ml)</th>
<th>Isolate Bc.16</th>
<th>Isolate Bcp.1</th>
<th>Isolate Bc.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethephon®</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1* efg</td>
<td>1.9 ab</td>
<td>1.7 ab</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.9 fg</td>
<td>1.8 abc</td>
<td>0.9 fg</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.8 g</td>
<td>1.6 abcde</td>
<td>1.2 cdefg</td>
<td></td>
</tr>
<tr>
<td>300</td>
<td>1.2 cdefg</td>
<td>1.5 bcdedef</td>
<td>1.1 defg</td>
<td></td>
</tr>
<tr>
<td>400</td>
<td>0.8 g</td>
<td>2.2 a</td>
<td>1.9 ab</td>
<td></td>
</tr>
<tr>
<td>AVG</td>
<td>0</td>
<td>1.9 a</td>
<td>1.7 abc</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.9 a</td>
<td>1.6 abc</td>
<td>1.6 abc</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.9 de</td>
<td>1.3 bcd</td>
<td>1.8 ab</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>0.7 e</td>
<td>1.3 bcd</td>
<td>0.9 de</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>0.5 e</td>
<td>1.3 bcd</td>
<td>1.3 bcd</td>
<td></td>
</tr>
</tbody>
</table>

*Means of six replicate leaflets; means followed by the same letters within the same column or row for the same plant growth regulator are not significantly different according to Tukey multiple comparison test (P≤0.05).

Variations between the three isolates tested were significant. In addition, ethephon was not effective in reducing gray mold disease severity of the three isolates on tomato and bean detached leaves inoculated by conidia suspension at 100-400 µg/ml (Table 3). Variations between the three isolates tested were significant with minor obvious trends. AVG however, completely suppressed gray mold disease severity on tomato plants at all concentrations (10-100 µg/ml) while reduced disease severity on bean plants by (93% - 94%) at 50-300 µg/ml (Fig. 3).

### Table 3. Effect of different concentrations of Ethephone® on gray mold disease severity (as radius growth zone of the lesion arising from conidial drop inoculation) in tomato and bean detached leaves after four days of incubation at 20°C.

<table>
<thead>
<tr>
<th>Plant Growth Regulators</th>
<th>Concentration (µg/ml)</th>
<th>Isolate Bc.16</th>
<th>Isolate Bcp.1</th>
<th>Isolate Bc.3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethephone</td>
<td>0</td>
<td>2.6ª ab</td>
<td>1.3 de</td>
<td>1.1 e</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>2 abcde</td>
<td>0.9 e</td>
<td>1.4 cde</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>2.3 abcd</td>
<td>1.2±0.3</td>
<td>1 e</td>
</tr>
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<td></td>
<td>300</td>
<td>3 a</td>
<td>1.2 de</td>
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<tr>
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<td>2.4 abc</td>
<td>1.3 cde</td>
<td>1.7 bcde</td>
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<tr>
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<td>0.6 bc</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>0.7 b</td>
<td>0.6 bc</td>
<td>0.7 b</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.6 bc</td>
<td>0.3 bc</td>
<td>0.2 cd</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>0.5 bc</td>
<td>0.2 cd</td>
<td>0.03 d</td>
</tr>
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ªMeans of six replicate leaflets; means followed by the same letters within the same column or row for the same plant growth regulator are not significantly different according to Tukey multiple comparison test (P≤0.05).

### Reference

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<table>
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<tr>
<th>Plant Growth Regulators</th>
<th>Concentration (μg/ml)</th>
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<th>Isolate Bc.1p</th>
<th>Isolate Bc.3</th>
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<td>57.5 def</td>
<td>80 abc</td>
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<tr>
<td></td>
<td>100</td>
<td>40 fgh</td>
<td>87.5 a</td>
<td>86.7 ab</td>
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<td>67.5 bcd</td>
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<td>41.7 efgh</td>
<td>60.8 cde</td>
<td>67.5 bcd</td>
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<tr>
<td>Bean</td>
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<td>12.5 bcd</td>
<td>43.3 a</td>
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<td></td>
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<td>11.7 bcd</td>
<td>14.5 bcd</td>
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</table>

* Means of six replicate leaflets; means followed by the same letters within the same column or row for the same plant growth regulator are not significantly different according to Tukey multiple comparison test (P≤0.05).

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**Figure 3.** Effect of Aminoethoxyvinylglycine on gray mold disease severity caused by three isolates Bc.16 (--●--), Bc.1p (■--) and Bc.3 (▲--) on tomato (A) and bean (B) detached leaves inoculated by spore suspension of B. cinerea and incubated at 20°C.

**Whole plant**

Ethephon significantly (P ≤ 0.05) increased gray mold disease severity caused by the three B. cinerea isolates on tomato and bean plants at 200-600 μg/ml. Disease severity was increased by (50.4%-55.8%) on tomato plants and by (27.6%-33.8%) on bean plants at the mentioned concentration (200-600 μg/ml), respectively. There was a high correlation between disease severity and the concentration of Ethephon applied on bean plants (r² = 0.89). However, AVG significantly (P ≤ 0.05) reduced disease severity (45 - 49%) at the concentrations 200 - 300 μg/ml on tomato plants and (55 - 75%) at 100-300 μg/ml on bean plants (Fig.4) there was relatively a high correlation between disease severity and the concentrations of AVG applied on tomato plants (r² = 0.77) and bean plants (r² = 0.66). The variations between isolates were significantly
observed when Ethephon® was used at the concentration of 400µg/ml and AVG at 100µg/ml on tomato plants.

**Discussion**

Ethylene was produced by the twenty four strains of *B. cinerea* used in the present study. Ethylene production may be an inherent character of the fungal species rather than a feature of specific isolates (Qadir, et al. 1997, Amir et al., 2004). However, a high variability in ethylene production between the isolates was also obtained in the present work. Similar results were observed by (Fukuda et al. 1993 and Qadir et al. 1997, Chague et al. 2002, Amir et al., 2004), who reported that ethylene is produced by *B. cinerea* when grown on PDA medium; this was attributed to many factors, including temperature, pH of medium, culture age, incubation period, fungal species, and precursor materials (Szinelczy et al., 1994). Ethylene production has also been observed in *Endothia gyrosa* and *Cytospora eucalypticola* (Wilkes et al., 1989). It is however difficult to be determined if this is related to variation in pathogenicity. In the present work, considerable variations in the pathogenicity of *B. cinerea* isolates tested on tomato and bean detached leaves by drop and plug inoculation methods were noted. Variability in ethylene production may be partly attributed to the genetic variation between *B. cinerea* isolates, but this needs further studies to be confirmed.

Concerning to the results, Ethephon® at low concentrations (10 and 50 µg/ml) has not affected significantly the mycelium growth rate of *B. cinerea* isolates *in vitro*. However, at high concentration (200 µg/ml), a minor inhibition was observed. These results are in consistence with those of Kepczynska (1993) who found that growth of hyphae of *B. cinerea* was slightly increased at low ethephon® concentrations (7×10⁻⁶, 7×10⁻⁵ M), while at high concentration (7×10⁻⁴ M) hyphal growth was inhibited *in vitro*. Tomato and bean gray mold disease severity caused by conidial inoculation on whole plants (Fig.4) was increased by (50.4 % and 33.8 %, respectively) when treated with ethephon at 600 µg/ml. This increase may be related to the fact that, ethephon® may stimulate ethylene production (Dennis et al. 1970). It is well known that ethylene encourages infection and senescence through many processes such as loss of chlorophyll and...
the decrease in the photosynthetic rate (Abeles, 1973; Aharoni & Lieberman, 1979; Gepslein & Thimaan, 1981; and Choe & Whang, 1986). The variation between tomato and bean plants in relation to disease severity may be related to the variability between host plant response to the phytohormones; similar results were observed by Elad (1992) who found that ethephon increased the sensativity of Senecio sp to grey mold. Also, it increased the grey mold disease severity by 23.5%-41.2% on tomato plants pretreated with 100-400 μg/ml respectively (Al-Masri et al., 2002, Barakat & Al-Masri, 2004).

Ethephon is considered an ethylene releasing product, and known to play an important role in the induction of plant disease. However, ethylene does not always promote disease development in many plant tissues; it induces the synthesis of proteins which functions in marshalling the host defense response (Lyon et al., 1995). Hence, variability in the results from host to host may be a normal phenomenon. Variability in the results between isolates at the same concentration may be related to the age of the inoculated tissues (Deverall & Wood, 1961).

Results showed that AVG at the concentration (200 μg/ml) significantly reduced the mycelium growth by 47% of B. cinerea isolates in vitro. Gray mold disease severity was also reduced at (300 μg/ml of AVG) on tomato by (49%) and on bean whole plant by (75%). In addition, AVG also reduced the percentage of rot development rate on tomato and bean detached leaves inoculated with mycelial agar disc by 32% in tomato and 78% in bean at 200 and 300 μg/ml, respectively. In addition, AVG seemed to stop rot development on leaves caused by conidial inoculum of B. cinerea isolates on tomato in the percentage of (100%) at 100 μg/ml and (94%) on bean at 300 μg/ml. These results are in consistence with those of Elad (1988) who reported that application of AVG to rose petals at concentration of 0.5 or 5 mM delayed the appearance of disease symptoms by 3-4 days. The two concentrations of AVG had also decreased disease incidence by 50-70% using mycelium plugs and by 26-32% by using conidial inoculation (Elad, 1988). Reduction in disease severity may be related to the fact that AVG suppresses ethylene biosynthesis (Yang & Hoffman, 1984). Adams & Abeles. (1979) found that AVG is a potent inhibitor of ethylene biosynthesis, through inhibiting the immediate precursor of ethylene production, 1-amino-cyclopropane-1-carboxylic acid (ACC) synthase production in the pathway of ethylene biosynthesis.

Reduction in disease severity may also be attributed to comparable ethylene suppression (Elad, 1988). Since AVG does not influence the growth and ability of the pathogen to germinate, it was concluded that ethylene is involved in rendering host tissue more susceptible without affecting the infectivity of the pathogen (Elad, 1988).

Over all results revealed that the phytopathogenic fungus B. cinerea produces ethylene in high variability due to intraspecific variation between isolates. The exogenously applied ethylene in general significantly increased gray mold disease severity, while the ethylene inhibitor aminoethoxyvinylglycine (AVG) significantly decreased gray mold disease severity on tomato and bean plants. However, further studies are needed to reveal the complex interaction that occurs in the host-parasite relationship in respect to phytohormones.

References


