Ethylene Production by *Botrytis cinerea* (Causal Organism of Gray Mold Disease) and Influence of the Exogenously Applied Growth Regulators and thier 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 intraspesific 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.

الملخص

تم دراسةقدرة فطر البوتريتيس المسبب لمرض العفن الرمادي على انتاجمنظم النمو النباتي الايثلين، ومقدرته ومنظم النمو النباتي المثبط على تطور المرض على نباتات البندورة والفاصوليا. اظهرت النتائج ان للفطر مقدرة عاليةعلى إنتاج غاز الايثلين عند تنميته على

الوسط المغذي (PDB) الممزوج مع الحمض الأميني الميثونين، حيث تم انتاج الهرمون بمعدل (µl/g/h) 2091 في يوم التحضين الرابع ، كما وجد تفاوت في كمية الايثلين المنتجة حيث تراوحت بين (µl/g/h) 383- 6789 ويرجع ذلك إلى التفاوت الوراثي بين عزلات الفطر.

عند دراسة اثرمنظم النمو النباتيالايثلين على تطور المرض تبين ان ه يلعب دورا مهما في تطور المرض على نباتات البندورة والفاصوليا حيث زاد المرض بنسبة % 50.4 - 55.8 % على نباتات البندورة وبنسبة 27.6% - 33.8% على نباتات الفاصوليا عند استخدامة بتركيز 200- 600 مليجرام/لتر بينما لم يؤثر على تطور نمو الفطر عند تنميتة على الوسط المغذي الممزوج مع منظم النمو النباتي . عند استخدام منظم النمو AVG والمستخدم لتثبيط انتاج الايثلين ، اظهرت النتائج ان لمنظم النموقدرة على تقليل اثر الايثلين على تطور المرض وعمل على تقليل المرض بنسبة 45% - 49% على نباتات البندورة وبنسبة 55% - 75% على نباتات الفاصوليا عند استخدامة بتركيز 100- 300 مليجرام / ملليتر.

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-chloroethylphosphanic acid), as an ethylene-releasing product (Dennis *et al*., 1970), was exogenously applied in many

agricultural practices. Ethylene is known to hasten multi-biochemical 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 *Rhizictonia 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 $7x10^{-7}$, $7x10^{-6}$ and $7x10^{-5}$ M slightly stimulated and at $7x10^{-4}$ M inhibited the hyphal growth of *Botrytis cinerea in vitro* and the disease on apples (Kepezynska, 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 10^3 µg/ml had no significant effect on percent spore germination of *Alternaria alternata, Colletotrichum gloesporioides, Penicillium expansum* and *Rhizopus stolonifer* (El Kazzaz *et al*., 1983). Kepczynska, (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*.

Aminoethoxyvinylglycine (AVG) inhibits the enzyme Aminocyclopropane carboxylic acid (ACC) synthase, thereby inhibiting ethylene formation in plants (Bae. *et al.* 1996; Shellie 1999, Amir 2004). Spraying of AVG as ethylene inhibitor agent on rose petals and leaves inoculated with mycelial plugs or conidia of *B. cinerea* was found to decrease the disease severity (Elad, 1988). The application at a concentration of 5×10^{-3} M, prevented the disease completely in bean and tomato leaves eight days after inoculation (Elad, 1990). AVG prevents ethylene production and has been used to control gray mold on different crops (Elad, 1988, and 1990). Also, AVG reduced the gray mold disease severity by 31.4%-80.3%on tomato plants treated at concentration 50-200 µg/ml respectively (Barakat & Al-Masri, 2004).

The objective of the present study was to check if the fungus *B. cinerea* has a potential to produce ethylene *in vitro,* and to examine the role of ethephon® and aminoethoxyvinylglycine in the interaction between *B. cinerea* and the host plants, tomato (*Lycopersicon esculentum*) and bean (*Phaseolus vulgaris*).

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. Mycelia 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 Bcp.1, Bc.1, Bc.2, Bc.3, Bc.4, Bc.5, Bc.6, Bc.8, Bc.9, Bc.10, Bo5.10, Bc.13, Bc.14, Bc.15, Bc.16, Bc.17, Bc.20, 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, BcP.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 10^5 /ml by using a haemacytometer. The tubes were then vortexes at 2000 rpm for 2 minutes, and spore counts were diluted to (10^5 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 rotovator 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 litter 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 ×34 cm wide × 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 ±1ºC. Colony diameter was measured after 24, 48, and 96 hours of incubation. The ninety six-hour-mycelium growth rate (cm^2/day) was presented.

Aminoethoxyvinylglycine (AVG) produced by Sigma was incorporated in PDA (Oxoid) medium at 40ºC 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 ±1ºC. Colony diameter was measured after 3 days. The experimental design used was completely randomized design (CRD) with six replicate plates employed 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 ×17 cm depth) with a peat - vermiculite - perlite mixture (2: 1: 1 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 × 45 ×15 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 (KH_2PO_4) 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 diameter×17 cm deep) with a peat - vermiculite - perlite mixture (2: 1: 1 v/v) in the

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 (10^5 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 Sigmastat® program, according to Tukey multiple comparison test ($P \leq 0.05$).

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, Bo5-10, 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)

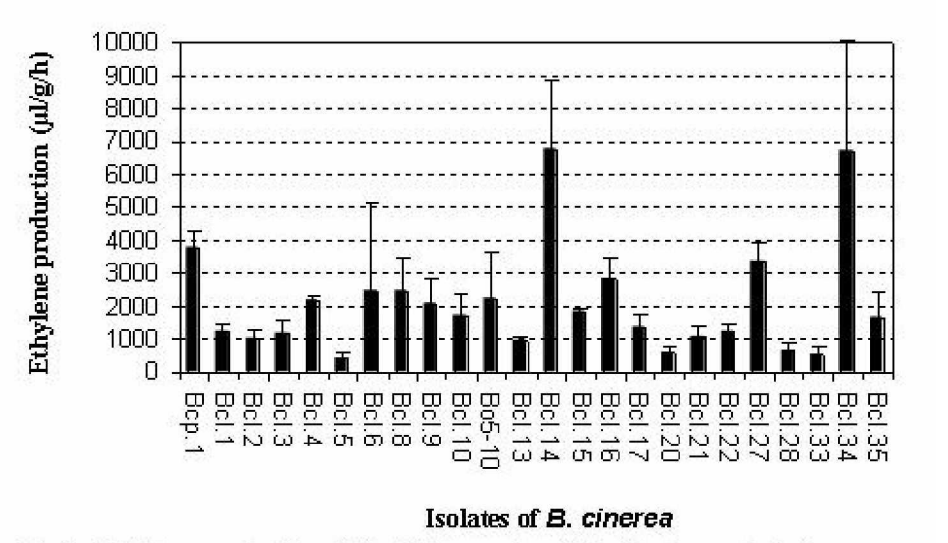

Fig.1 Ethylene production (µl/g/h) by various *Botrytis cinerea* isolates grown in potatoes dextrose broth amended with 10 mM methionine after four days of incubation at 20 °C.

Effect of exogenous application of plant growth regulators

In vitro

Ethephon® at low concentrations (10 and 50 µg/ml) hase 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 ($P \leq 0.05$) decreased mycelium growth rate of the three *B. cinerea* isolates (Bc.16, BcP.1, and BcI.3) at concentrations of 10, 50, 100, 150, and 200 µg/ml. The inhibition at 200 µg/ml was 47 %.

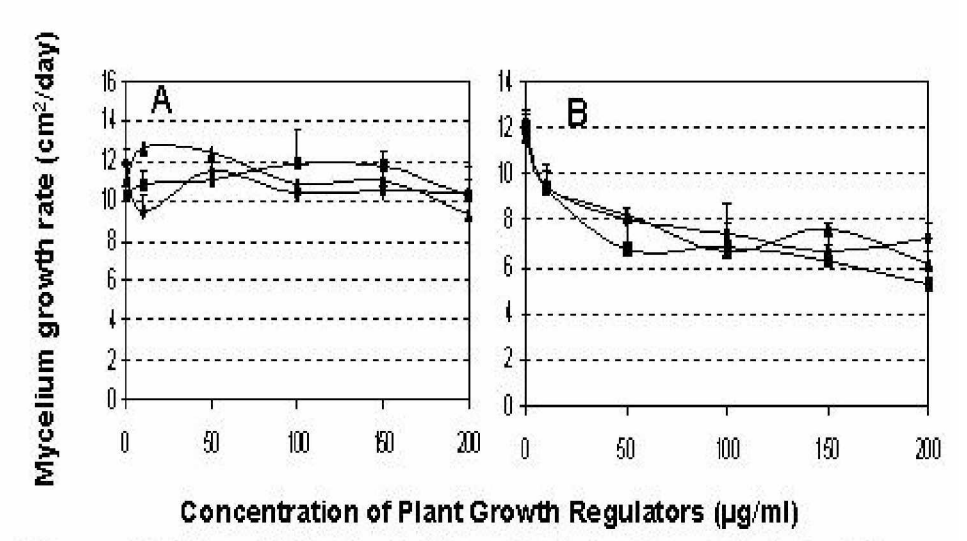

Figure. 2. Effect of Ethephon® (A) and Aminoethoxyvinylglycine (B) on mycelium growth rate of three isolates Bc.16 (-♦-), Bc.p.1 (-■-) and Bc.3 (-▲-) growing on PDA amended medium and incubated at 20° C.

Detached leaves

Ethephon® has not affected significantly ($P \leq 0.05$) 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 ($P \leq 0.05$) rot the disease development rate on tomato and bean detached leaves inoculated by mycelium disk at 200-300 µg/ml (Table 2).

Table1. Effect of different concentrations of Ethephon® and Aminoethoxyvinylglycine (AVG) on gray mold lesions growth rate on tomato detached leaves (cm^2/day) inoculated by mycelium disk after 4-days of incubation at 20°C.

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

* 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 ($P \leq 0.05$).

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

Plant Growth Regulators	Concentration (µg/ml)	Isolate Bc.16	Isolate Bcp.1	Isolate Bc.3
Ethephone	0	2.6* ab	1.3 de	1.1 e
	50	2 abcde	0.9 e	1.4 cde
	250	2.3 abcd	1.2±0.3	1 e
	300	3 a	1.2 de	1.6 bcde
	400	2.4 abc	1.3 ce	1.7 bcde
AVG	0	1.4 a	1.4 a	0.6 bc
	100	0.7 b	0.6 bc	0.7 b
	200	0.6 bc	0.3 bc	0.2 cd
	300	0.5 bc	0.2 cd	0.03 d

*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 ($P \leq 0.05$).

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.

Plant Growth Regulators	Concentration (µg/ml)	Isolate Bc.16	Isolate Bcp.1	Isolate Bc.3
Tomato	0	45.8* efg	81.7 ab	89.2 a
	50	16.2 i	57.5 def	80 abc
	100	40 fgh	87.5 a	86.7 ab
	200	36.7 gh	22.3 hi	67.5 bcd
	300	41.7 efgh	60.8 cde	67.5 bcd
Bean	0	14.2 bcd	12.5 bcd	43.3 a
	100	13.3 bcd	6 cd	16.7 bcd
	200	14.5 bcd	19.2 bc	57.5 a
	300	3.4 cd	26.7 b	43.3 a
	400	1.8 d	11.7 bcd	14.5 bcd

* 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 ($P \leq 0.05$).

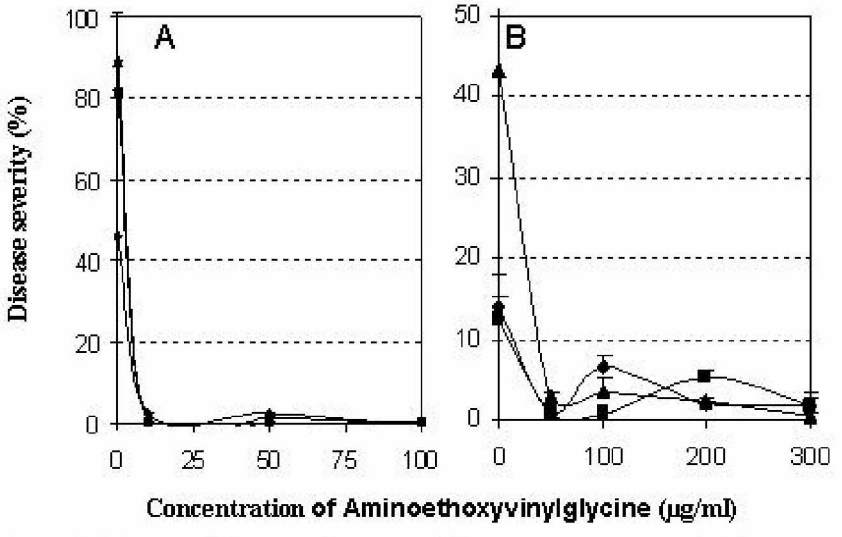

Figure 3. Effect of Aminoethoxyvinylglycine on gray mold disease severity caused by three isolates Bc.16 (-♦-), Bc.p.1 (-■-) and Bc.3 (-▲-) on tomato (A) and bean (B) detached leaves inoculated by spore suspension of *B. cinerea* and incubated at 20° C.

Whole plant

Ethephon® significantly ($P \leq 0.05$) 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 ($r^2= 0.89$). However, AVG significantly ($P \leq 0.05$) 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 ($r^2= 0.77$) and bean plants ($r^2=0.66$). 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.

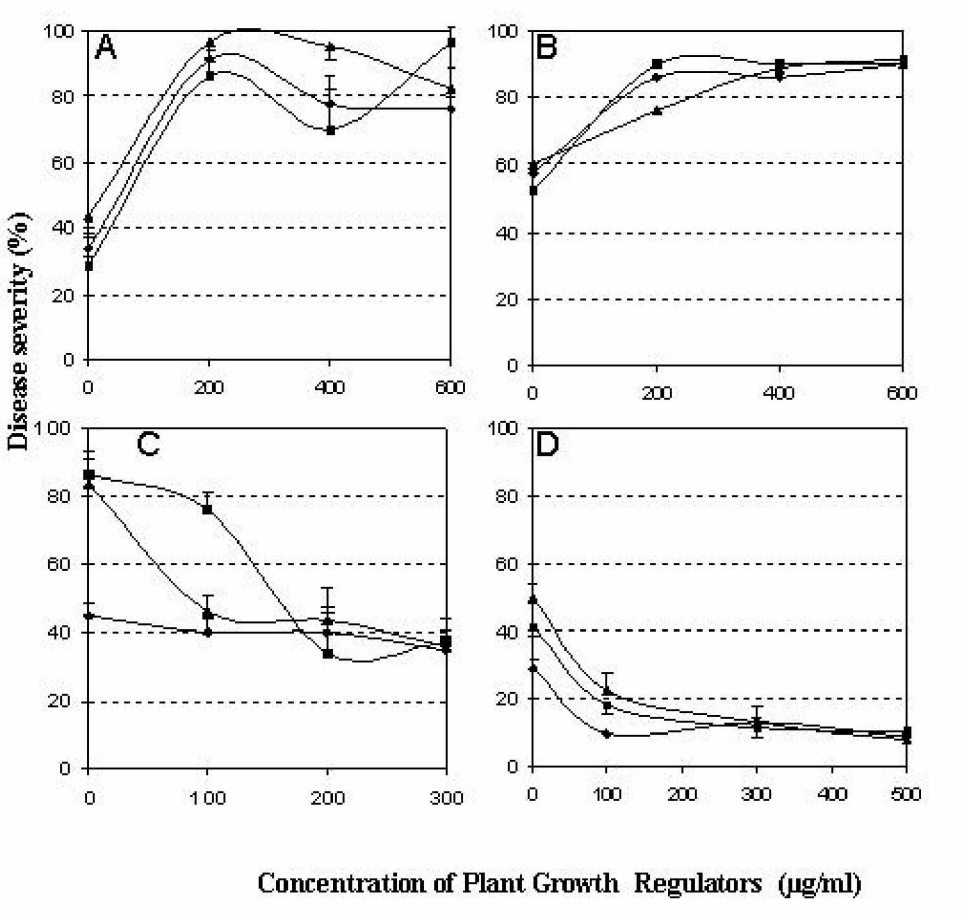

Fig. 4 Effect of different concentrations of Ethephon® (A,B) and aminoethoxyvinylglycine (C, D) on gray mold disease severity caused by three isolates Bc.16 (-♦-), Bc.p.1 (-■-) and Bc.3 (-▲-) on tomato (A, C) and bean (B,D) plants incubated in growth chamber at 20° C.

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 (Strzelczyk *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; Gepstein & 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 responses to the phytohormones; similar results were observed by Elad (1992) who found that ethephon® increased the sensetivity of *Senecio* sp to gray mold. Also, it 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).
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 %). Inaddition, 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-aminocyclopropane-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 intraspesific 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.

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