Involvement of Ethylene in Herbicide-Induced Resistance to *Fusarium oxysporum* f. sp. *melonis*

R. Cohen, J. Riov, N. Lisker, and J. Katan

Department of Plant Pathology and Microbiology (first and fourth authors), and Department of Horticulture (second author), The Hebrew University of Jerusalem, Faculty of Agriculture, Rehovot 76100; and Department of Stored Products, Agricultural Research Organization, The Volcani Center, P.O.B. 6 Bet Dagan 50250, Israel

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ABSTRACT

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Pretreatment of melon seedlings with the herbicide dinitramine induced resistance to subsequent inoculation with *Fusarium oxysporum* f. sp. *melonis*. Ethylene production in leaves and stems of the herbicide-treated plants or in genetically resistant ones was much suppressed compared with nontreated diseased seedlings. Ethylene production in diseased seedlings reached its peak shortly after the appearance of symptoms. Similarly, dinitramine increased resistance to Fusarium wilt and suppressed ethylene production in tomato seedlings. Suppression of ethylene production was positively correlated with levels of induced resistance in melon seedlings treated with various herbicides or with various concentrations of dinitramine. Aminoethoxyvinylglycine and aminooxyacetic acid, which inhibit ethylene biosynthesis, induced resistance to Fusarium wilt and suppressed ethylene production. Silver thiosulfate, an inhibitor of ethylene action, also increased resistance to Fusarium wilt.

Additional key words: AOA, AVG, benfluralin, ethalfluralin, isopropalin, STS, trifluralin.

Interactions between herbicides and plant pathogens are well-known phenomena (8–10). They stem from the fact that the biological activity of pesticides may extend beyond their effect on target organisms. Thus, herbicides may affect plant disease through their effect on the pathogen, plant resistance, or on the surrounding soil microorganisms.

Substituted dinitroanilines are selective, wide-spectrum herbicides, which are used extensively in vegetable and field crops. These herbicides affect many biochemical processes in the treated plants (2). An unusual side effect in various members of this group is the remarkable increase in resistance of pretreated plants to *Fusarium* and other soilborne pathogens at very low concentrations of 1 $\mu g \cdot g^{-1}$ of soil or less (8,9). This could not be attributed to a direct fungitoxic effect on the pathogen (6,8,9). In tomatoes and cotton plants pretreated with trifluralin, the induced resistance to the respective *Fusarium* pathogens was associated with the production of fungitoxic compounds (9). These toxicants accumulate only after inoculation and were regarded as phytoalexins.

Increase in the rate of ethylene production in infected plants and its involvement in pathogenesis in certain vascular wilt diseases are well established (1,4,12). Cronshow and Pegg (4) have suggested a more complex role for ethylene in which it may act synergistically, predisposing tissues to the damaging action of toxic substances and pectolytic enzymes produced by the pathogen. As with other compounds involved in disease syndromes, there are also studies describing involvement of ethylene in disease resistance (5,11), which might be attributed either to the stimulatory effect of ethylene on certain enzyme systems associated with resistance reactions, or to its effect on the synthesis of antifungal compounds. Various compounds such as aminoethoxyvinylglycine (AVG) and aminooxyacetic acid (AOA) suppress ethylene biosynthesis (14). Another compound, silver thiosulfate (STS), does not affect ethylene production but inhibits its action (13). These compounds are used as tools for studying the role of ethylene in physiological processes in plants and in host-pathogen interactions. (7,13).

The purpose of this work was to study the possible involvement of ethylene in the phenomena of plant resistance either induced by dinitroaniline herbicides or that existing in a genetically resistant cultivar. A brief report of some of the results reported here has been published (3).

MATERIALS AND METHODS

Pathogens. Pathogenic isolates of *Fusarium oxysporum* Schlecht. f. sp. *melonis* Snyder and Hansen race 0 and *F. o.* Schlecht. f. sp. *lycopersici* (Sacc.) Snyder and Hansen, (races 1 and 2) were used.

Plants. The following plants were used: melon (*Cucumis melo* L.) 'Ananas Yokneam' susceptible to *F. o.* f. sp. *melonis* and 'Hemed', resistant to this pathogen, tomato (*Lycopersicon esculentum* Mill.) 'Marmande', susceptible to both races 1 and 2 of *F. o.* f. sp. *lycopersici* and 'Rehovot 13', resistant to race 1 and susceptible to race 2 of this pathogen.

Herbicides. The following herbicides were used as emulsifiable concentrates: *N*,*N*-diethyl-2,6 dinitro-4-trifluromethyl-m-phenylenediamine (dinitramine), α,α,α-trifluoro-2,6-dinitro-*N*,*N*-di-prop-yl-p-toluidine (trifluralin), *N*-ethyl-α,α,αtrifluoro-*N*-(2-methylallyl)-2,6-dinitro p-toluidine (ethalfluralin), *N*-butyl-*N*- ethyl-α,α,αtrifluoro-2,6-dinitro p-toluidine (benfluralin), and 4-

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isopropyl 2,6 dinitro-*N,N* dipropylaniline (isopropalin). Water emulsions of the herbicides were mixed with the soil to give the desired concentration of active ingredient. Water-treated soils were used as controls.

The effect of herbicides on host resistance. Experiments to test this effect were carried out as described (8). Seeds of the tested plants were sown in a pathogen-free natural sandy loam soil, which was mixed previously with herbicides or was left untreated. One day after emergence, the seedlings were removed and washed thoroughly to remove the adhering soil and traces of the herbicide. They were transplanted into herbicide-free soil after being inoculated by dipping their roots for 2 min in a $10^6 \cdot ml^{-1}$ conidial suspension of *F. oxysprorum* of the appropriate forma specialis and race. Plants were then grown under greenhouse conditions at 24–30 C and examined daily to determine the percentage of diseased seedlings. Greenhouse experiments were carried out in five replicates of 20 seedlings each, and were repeated at least twice. The same procedure was used to produce plants for ethylene production measurements.

Determination of ethylene. Plants were removed from soil and washed under running tap water. Roots, stems, and leaves (cotyledons plus true leaves) were cut from five plants and transferred to 60-ml test tubes for melon seedlings or to 14-ml test tubes for tomato seedlings. The tubes were sealed for 4 hr with rubber caps and incubated in the dark at 27 C. A 1-ml air sample was withdrawn from the test tubes with an air-tight hypodermic syringe, and ethylene was assayed by gas chromatography using an activated alumina column and a flame ionizing detector.

Foliar treatments. Aqueous solutions of AVG, AOA, and STS at various concentrations were applied to melon plants by a foliar spray. STS was prepared from $AgNO_3$ and $Na_2S_2O_3$ (1:8 mol/mol). Number of diseased plants and ethylene production level were recorded periodically.

RESULTS

Effect of dinitramine on resistance of melon seedlings to Fusarium wilt and on ethylene production. Dinitramine pretreatment of melon seedlings of the susceptible cultivar induced resistance to Fusarium wilt, thus confirming earlier results (9). Thirteen days after inoculation, percentages of disease in the nontreated plants and in those grown in soil treated with dinitramine at 0.5 $\mu g \cdot g^{-1}$ of soil were 90 and 2, respectively. This phenomenon of induced resistance was observed in all experiments, and disease reduction by dinitramine was usually above 90%. In both leaves and stems, ethylene production in the nontreated inoculated plants was detected about 2 days before the appearance of first wilt symptoms, usually 6–8 days after inoculation. Ethylene levels increased rapidly in these plants shortly before or at about the same time of symptom appearance (Figs. 1 and 2). The highest levels of ethylene were detected in the leaves of the nontreated inoculated plants. Both noninoculated plants, with or without herbicide pretreatment, and herbicide-treated inoculated plants showing no disease symptoms produced smaller or undetectable amounts of ethylene.

Disease progress and ethylene production were also followed in noninoculated and inoculated melon plants of a genetically resistant cultivar (Hemed), with or without herbicide treatment. No diseased plants were observed and no ethylene was detected in the stems of any of the four tested combinations. Relatively small amounts of ethylene were detected only in the leaves of plants without herbicide treatment (Fig. 2).

The same trend of increasing ethylene production in diseased plants and its suppression in herbicide-treated ones was observed in the roots. The amounts of ethylene produced in roots were higher than in leaves and stems, reaching 9.8 $nl \cdot g^{-1} \cdot h^{-1}$ in the roots of the nontreated diseased plants 9 days after inoculation. Dinitramine suppressed ethylene production by 54–100% during the experimental period.

Increased herbicide concentration in the soil was positively correlated with an increase in plant resistance (Fig. 3A). At all tested concentrations, disease was significantly reduced by dinitramine. At 1 $\mu g \cdot g^{-1}$ of soil, disease percentage was reduced to

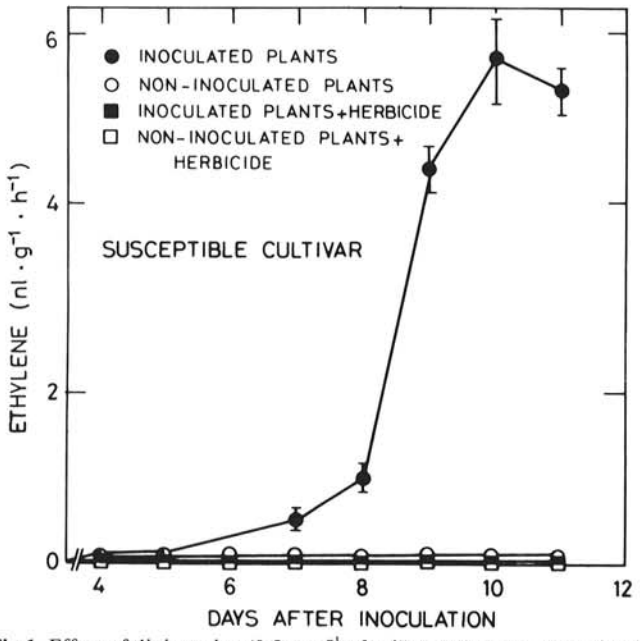

Fig. 1. Effect of dinitramine (0.5 $\mu g \cdot g^{-1}$ of soil) on ethylene production in stems of melon plants at various periods after inoculation with *Fusarium*. Vertical bars represent 1 SE.

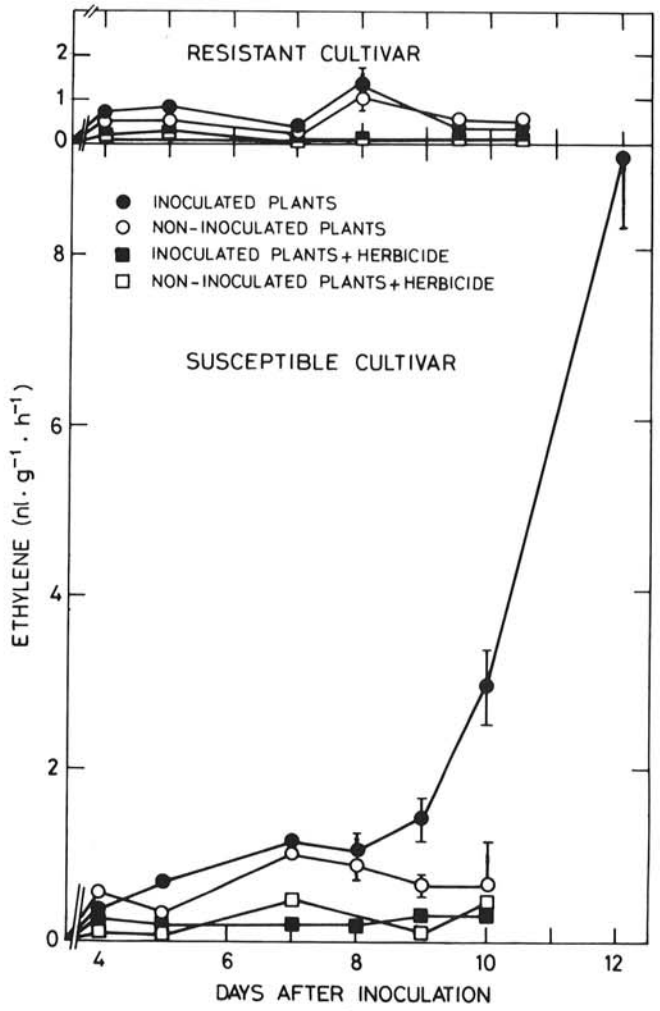

Fig. 2. Effect of dinitramine (0.5 $\mu g \cdot g^{-1}$ of soil) on ethylene production in cotyledons and true leaves of melon plants (two cultivars) at various periods after inoculation with *Fusarium*. Vertical bars represent 1 SE.

zero compared with 95% in the nontreated control. Concomitantly, ethylene production decreased in the herbicide-treated plants (Fig. 3B). This reduction was also positively correlated with the reduction in disease incidence.

Effect of various dinitroaniline herbicides on the resistance of melon seedlings to Fusarium wilt and on ethylene production. Dinitroaniline herbicides at 0.5 $\mu g \cdot g^{-1}$ of soil reduced disease incidence by various degrees (Fig. 4A). Isopropalin was the least effective, causing only 14% reduction at the end of the experiment, compared with 100% reduction by dinitramine and trifluralin. Benfluralin and ethalfluralin had an intermediate effect in reducing disease incidence. The course of ethylene production in the nontreated plants and its suppression by dinitramine in this experiment (Fig. 4B) were similar to those described in Figs. 1 and 3B. Generally, suppression of ethylene production in the various herbicide-plant combinations was positively correlated with disease reduction.

Ethyelene production in dinitramine-treated and genetically resistant tomato seedlings. Stems of tomato seedlings of the susceptible cultivar Marmande inoculated with race 1 of *F. o.* f. sp. *lycopersici* produced the highest amounts of ethylene (Table 1). As was observed with melon seedlings, dinitramine induced resistance in the inoculated tomato seedlings (93% disease reduction).

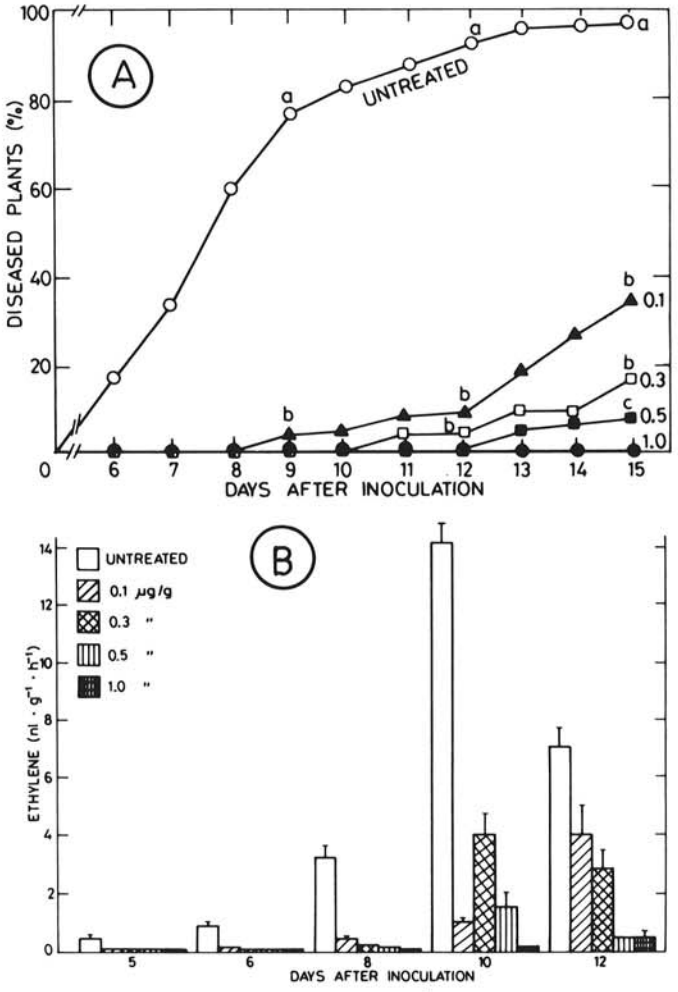

Fig.3. Effect of dinitramine at 0.1–1.0 $\mu g \cdot g^{-1}$ of soil on Fusarium wilt incidence **(A)** and on ethylene production in stems of melon plants **(B),** at various periods after inoculation. Data in A, at each period, with a common letter are not significantly different ($P = 0.05$). Vertical bars in B represent 1 SE.

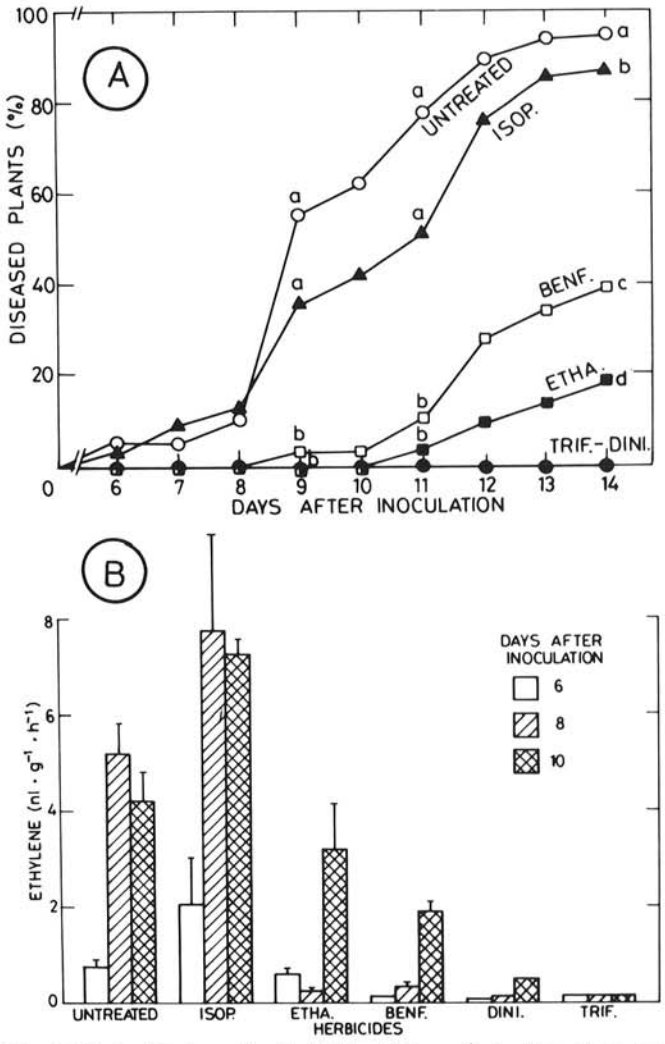

Fig. 4. Effect of dinitroaniline herbicides (0.5 $\mu g \cdot g^{-1}$ of soil) on Fusarium wilt incidence **(A)** and on ethylene production in stems of melon plants **(B),** at various periods after inoculation. ISOP = isopropalin, BENF = benfluralin, ETHA = ethalfluralin, TRIF = trifluralin, and DINI = dinitramine. Data in A, at each period, with a common letter are not significantly different ($P = 0.05$). Vertical bars in B represent 1 SE.

TABLE 1. Effect of dinitramine (0.5 μg/g of soil) on ethylene production in stems and leaves (cotyledons and true leaves) of tomato plants at various periods after inoculation with *Fusarium oxysporum* f. sp. *lycopersici*

Cultivar	Herbicide treatment	Fusarium race	Plant response	Ethylene, $nl \cdot g^{-1} \cdot h^{-1}$[a] Stems, days after inoculation 5	7	9	12	Leaves, days after inoculation 5	7	9	12
Marmande	···	1	Susceptible	0.97 ± 0.24	2.0 ± 0.74	9.9 ± 0.71	11.1 ± 3.9	2.5 ± 0.5	3.3 ± 0.82	6.0 ± 0.73	15 ± 4.2
Rehovot 13	···	2	Susceptible	0.45 ± 0.12	1.16 ± 0.14	3.1 ± 0.44	4.5 ± 0.19	2.5 ± 0.5	3.1 ± 0.15	2.3 ± 0.05	4.3 ± 0.54
Rehovot 13	···	1	Genetic resistance	0.83 ± 0.06	1.09 ± 0.32	0.96 ± 0.04	1.2 ± 0.23	2.68 ± 0.43	2.5 ± 0.15	2.7 ± 0.31	2.1 ± 0.11
Rehovot 13	+	2	Induced resistance	0.3 ± 0.04	0.56 ± 0.04	0.38 ± 0.1	1.2 ± 0.18	4.0 ± 0.68	4.3 ± 0.36	4.0 ± 1.1	3.15 ± 1.0
Marmande	+	1	Induced resistance	0.24 ± 0.014	0.33 ± 0.17	···	2.1 ± 0.17	3.5 ± 0.19	4.0 ± 0.61	···	2.52 ± 0.23

[a]Data presented are means ±SE.

Amounts of ethylene produced in the susceptible combinations were higher than those in the resistant ones. These differences were especially pronounced in stems. Disease progress was slower and ethylene production was lower in Rehovot 13 seedlings inoculated with race 2 of the pathogen, compared with the Marmande-race 1 combination.

Effect of ethylene biosynthesis inhibitors and ethylene action inhibitor on wilt incidence and on ethylene production. AVG treatments. Spraying melon plants with AVG 3 days after inoculation resulted in a reduction in disease incidence (Fig. 5A). The first wilt symptoms were observed 9 days after inoculation. At that time, 20% of the untreated plants wilted, compared with 6–12% in the AVG-treated plants. The calculated slopes showed that the disease rate was significantly reduced ($P = 0.05$) in seedlings treated with the highest AVG concentration. AVG suppressed ethylene production 7 days after inoculation (Fig. 5B). On day 10, ethylene production in the AVG-treated plants was similar to or higher than the nontreated ones.

AOA treatments. Application of AOA to the plants reduced both disease incidence and ethylene production (Fig. 6A and B). Disease reduction was especially pronounced in the first 9 days after inoculation. Seven days after inoculation (3 days after AOA application), disease percentages in the nontreated and in the 0.3 mM and 1.0 mM AOA-treated plants were 27, 10, and 2, respectively. At the same period, ethylene production in the AOA treatments was significantly reduced by 99 and 78%, respectively. In the later stage, disease incidence remained low only at 0.3 mM concentration. Nine days after inoculation, ethylene production in the AOA-treated plants was higher than in the nontreated ones.

STS treatments. Application of STS (ethylene action inhibitor) to inoculated melon seedlings generally reduced disease incidence, and this was especially evident with 0.01 mM treatments (Table 2). In STS-treated plants, ethylene levels did not significantly differ from untreated plants. In some cases, STS even increased its production.

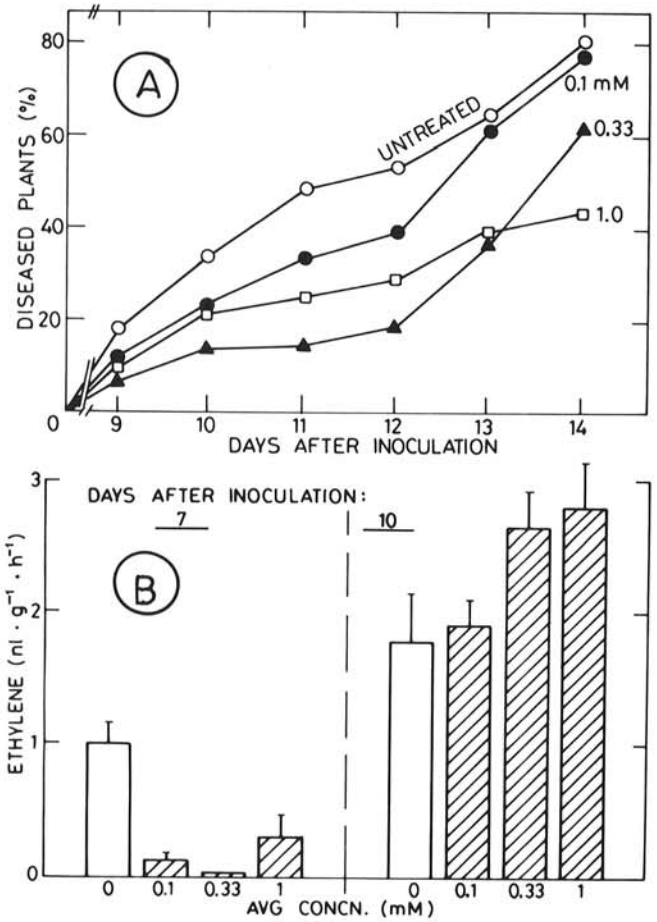

Fig. 5. Effect of aminoethoxyvinylglycine at three concentrations on Fusarium wilt incidence **(A)** and on ethylene production in stems of melon plants **(B)** at various periods after inoculation. Calculated slopes in A show that disease rate was significantly reduced ($P = 0.05$) with 1mM AVG. Vertical bars in B represent 1 SE.

DISCUSSION

As in other studies with wilt pathogens (1,12), *Fusarium*-inoculated plants in our experiments produced increased amounts of ethylene. Ethylene production started to increase about 2 days before visible symptoms were detected. Thus, it appears that this phenomenon can be used as a predictive indicator for disease appearance. Levels of induced resistance obtained by various herbicides or by ethylene synthesis inhibitors were well correlated with comparable degrees of ethylene suppression. At high levels of dinitramine or trifluralin, disease reduction was 100% and ethylene suppression was long-lasting (Figs. 3 and 4). At lower herbicide concentrations or with less effective substances, less disease reduction occurred and ethylene production was temporarily suppressed, followed by a rapid increase in ethylene production which in certain cases even exceeded that of nontreated inoculated

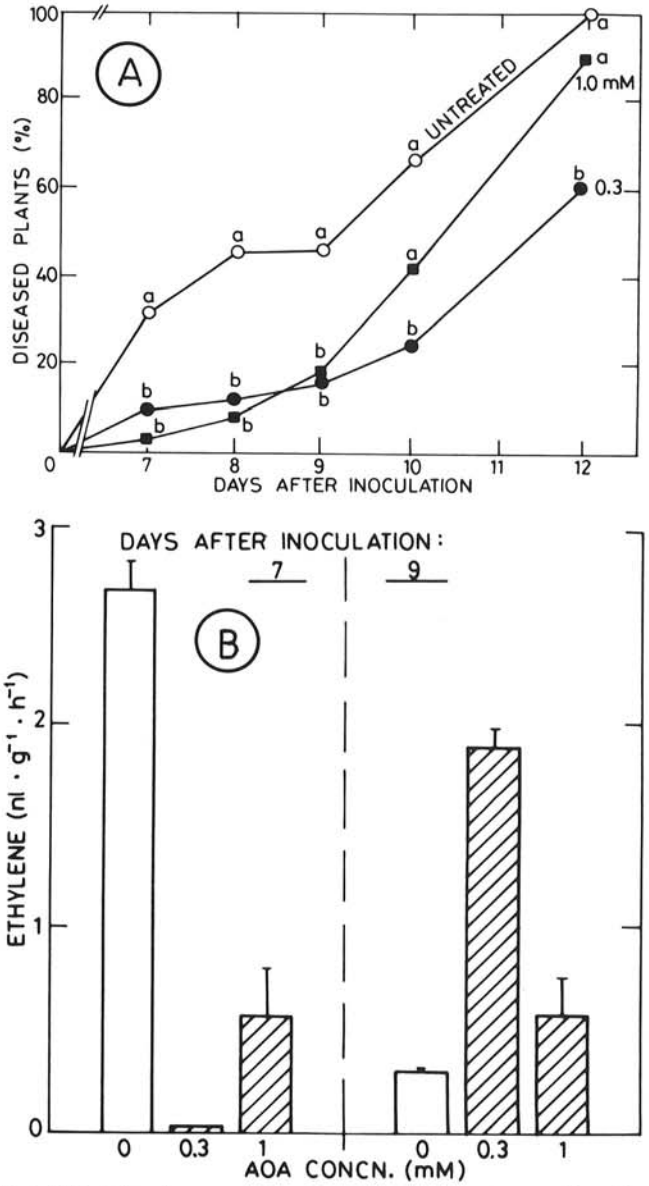

Fig.6. Effect of aminooxyacetic acid at two concentrations on Fusarium wilt incidence **(A)** and on ethylene production in stems of melon plants **(B)** at various periods after inoculation. Data in A, at each period, with a common letter are not significantly different ($P = 0.05$). Vertical bars in B represent 1 SE.

TABLE 2. Effect of silver thiosulfate (STS) treatments on Fusarium disease in melon seedlings

STS concn (mM)	Applications (days after inoculation)	Diseased plants (%) days after inoculation			Disease rate (slope)[a]
		6	10	13	
Untreated	...	1.4	49.2	74.6	10.70
0.01	4	2.4	31.3	56.5	7.68[b]
0.10	4	0	31.3	68.6	9.85
1.00	4	0	44.8	80.7	12.10
0.10	4+5[c]	0	29.6	69.1	9.58
0.01	4+5[c]	3.7	26.2	61.2	7.92
0.01	4+5+6[c]	0	19.1	57.5	8.00[b]

[a] Slopes determined by linear regression analysis of cumulative number of diseased plants in time throughout the experiment.

[b] Slope values are significantly different from untreated plants according to Student's *t*-test analysis. All correlation coefficients (*r*) were significant at a 95% confidence level.

[c] STS was applied in the 4th and the 5th day or in the 4th, 5th, and 6th day after inoculation, as indicated.

plants (Figs. 3–6). In genetically resistant plants, reduction of both disease and ethylene was complete and long-lasting. Low levels of ethylene production in resistant cultivars were also reported in other studies (1,12). Though the trend of the concomitant reduction in disease incidence and in ethylene production was similar in both induced and genetic resistance, it still remains to be determined whether the mechanisms responsible for these two phenomena are similar in both systems of resistance. In *Fusarium*-inoculated tomato plants, the pattern of production of compounds inhibitory to the pathogen was similar in both herbicide-treated and in genetically resistant plants (9).

The involvement of ethylene in vascular wilt diseases is well established (1,12). Neither the increased production of ethylene in the diseased plants nor the reduced levels of ethylene in resistant ones necessarily indicate that ethylene plays an active role in the disease syndrome, because its formation might be the result rather than the cause of the pathological changes involved in the disease syndrome. However, accumulating evidence supports the idea that ethylene plays a role in inducing at least certain disease symptoms, either as a toxin or as a synergist predisposing tissues to the damaging action of other substances (4). In the present study, disease incidence was reduced not only by various dinitroaniline herbicides but also by AOA and AVG, which are inhibitors of 1-aminocyclopropane-1-carboxylic acid synthase, a key enzyme in ethylene biosynthesis (14). Moreover, STS, an inhibitor of ethylene action (13), also reduced disease incidence. Thus, in this study the idea that ethylene is actively involved in disease symptom formation is supported by different lines of evidence, using compounds differing in their mode of action and in their effect on plant metabolism. In an additional system, genetic or induced resistance in tomato plants inoculated with *Fusarium* was also correlated with reduced production of ethylene.

The involvement of ethylene in certain resistance reactions (5,11) does not necessarily preclude its involvement in pathogenesis because this might be related to the timing of the ethylene response in relation to the degree of establishment of the pathogen (11). The mechanism of ethylene suppression by dinitramine is still under investigation. Preliminary results show that dinitramine does not affect ethylene production directly but by interfering with IAA metabolism.

The present study shows that herbicides that suppress ethylene formation induce resistance in *Fusarium*-inoculated plants. This does not exclude the possibility of the existence of additional mechanisms of resistance in the herbicide-treated plants such as formation of antifungal compounds as shown with trifluralin-treated tomato and cotton plants (9), an effect on the toxins of the pathogen, or resistance through vascular occlusion.

Compounds that induce disease resistance have a great potential for disease control, perhaps even in the cure of previously infected plants. A better understanding of the mechanisms of induced resistance by these herbicides (which in the present study were used at very low concentrations) may provide relatively inexpensive tools for manipulating vascular diseases that are difficult to control by conventional fungicides.

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