Role of Ethylene in the Response of Tomato Plants Susceptible and Resistant to *Meloidogyne incognita*

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ABSTRACT

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Ethylene concentrations were measured in gases from roots of tomato (*Lycopersicon esculentum*) plants of cultivars Vendor and Anahu which are susceptible (S) and resistant (R), respectively, to *Meloidogyne incognita*. No significant difference was found in root ethylene concentrations between nematode-treated and control R plants during a 30-day postinoculation period. Postinoculation ethylene concentrations in roots of infected S plants decreased significantly relative to those of the uninfected controls. Decreasing ethylene concentrations in infected S plants correlated inversely with root weight which increased significantly over the same period due to gall formation. Thus, galled roots contained less ethylene per gram than did healthy roots. These data do not support the hypothesis that increased ethylene production accompanies gall formation. Ethane concentrations varied directly with ethylene concentrations, but were invariably greater. These results are discussed with reference to recent literature on possible relationships between ethane and ethylene biosynthesis.

Ethylene is a plant hormone responsible for the regulation of a large number of plant growth responses (1). The synthetic compound Ethrel (2-chloroethylphosphonic acid) (Amchem Products Incorporated, Windsor, Ontario, Canada) is hydrolyzed by plants and ethylene is released. Orion and Minz (17) applied ethrel solutions to *Meloidogyne javanica* (Treub) Chitwood-infected tomato (cultivar Hossen Eilon) seedlings. Increasing ethrel concentrations resulted in linear increases in the weight of galls and the highest concentration applied (20.0 mg/plant) resulted in a 100% increase in gall weight. Histological examinations showed that increased gall weights were due to increased proliferation of parenchyma cells (15). A similar result was reported for tomato cultivar Fortos plants infected with *Meloidogyne incognita* (Kofoid and White) Chitwood (16) and, again, was suggested that ethylene was involved in the proliferation of parenchyma tissue during gall formation.

Resistance of plants to certain bacterial and fungal pathogens has been linked to elevated ethylene production (22). Potato root tissue normally susceptible to black rot fungus (*Ceratocystis fimbriata* Ell. and Halst.) became resistant when exposed to an atmosphere containing 8 ppm ethylene (24). Clare et al (7) also identified ethylene as a resistance-inducing agent in this same host-parasite combination. Ethylene released from ethrel depressed *Fusarium oxysporum* (f. sp. *lycopersici* race 1 IPO isolate 8) development on tomato plants (16). In addition the resistance of tomato cultivar Fortos plants to *F. oxysporum* was partially broken by root-knot nematode infection. Subsequent ethrel treatment of these plants restored full resistance against infection by the fungus (16).

The possible involvement of ethylene in the response of susceptible (S) and resistant (R) cultivars of tomato to *M. incognita* was investigated. Direct measurements of root ethylene concentrations were made over a 30-day period following exposure of S and R tomato plants to a large number of infectious second-stage larvae.

MATERIALS AND METHODS

Tomato seedlings of cultivars Vendor and Anahu, which are susceptible (S) and resistant (R), respectively, to *M. incognita* were transplanted into sterile sand in 10-cm square plastic pots when about 5 cm tall (cotyledon stage). Seedlings were grown in a greenhouse under fluorescent lights (GE reflector cool white CW-1500) and exposed to a 16-hr photoperiod. Plants were watered with a half-strength balanced nutrient hydroponics solution (20).

Stock supplies of *M. incognita* were maintained on tomato cultivars Tiny Tim and Glamour grown in greenhouses at Agriculture Canada Research Station, Vineland Station, Ontario. Severely infected stock roots were cleaned in water, chopped up thoroughly with scissors and placed in a 'Seinhorst' mistifier as described by Goodey (10). Hatching second-stage larvae were collected for 14 days, allowed to settle and were concentrated by decanting excess supernatant water. Nematodes were counted in 1-ml aliquots of this concentrate and the total number collected was calculated. Approximately 18,000 larvae suspended in 10 ml of water were applied to the sand around the roots of each plant with a Plastipak syringe and (18 gauge) 3.8-cm-long hypodermic needle. Control plants were treated with 10 ml of water in the same manner.

Root growth and root ethylene and ethane concentrations were determined between 1300 and 1600 hours immediately after inoculation and thereafter during the same time interval on alternate days for approximately 30-days. Each measurement time resulted in eight sets of data from four resistant and four susceptible plants (two control and two nematode-inoculated.) The experiment was replicated several times and typical results are reported.

Plants were cut at the base of the stem and the intact root system was quickly washed free of sand and placed in the gas extraction apparatus. The entire operation required less than 1 min. Gas extraction involved a vacuum technique (2) and continued until a partial pressure of 2.0–3.0 cm Hg was obtained. This generally required about 15 min, but varied slightly depending on available water main pressure. Aliquots of the extracted root gases at atmospheric pressure were withdrawn into a 1.0-ml gas-tight syringe (Hamilton) and injected into a gas chromatograph (Hewlett-Packard 5700A) equipped with a flame ionization detector. Gases were separated on a 183 × 0.64-cm teflon or glass column packed with Porapak-Q mesh range 80/100 (Waters Associates, Framingham, MA 01701) maintained at 55 C. Nitrogen (carrier gas) and hydrogen were supplied to the column and detector, respectively, at a rate of 60 ml/min. Areas under ethane and ethylene peaks were measured with a Fisher Recordall Series 5000 electronic recorder and integrator.

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Calibration curves which related gas peak size to gas concentration (microliters per liter) were obtained by analyzing authentic gas standards. Gas concentrations in microliters per liter were converted to nanograms of gas per gram fresh weight of root tissue, taking into consideration laboratory temperature and pressure conditions.

To minimize day-to-day variability due to changes in endogenous ethylene and ethane levels, statistical analyses were performed on the percentage differences in gas concentrations between control and inoculated plants. This procedure resulted in each day's data receiving equal weight. Student's *t*-tests were used to determine whether the mean percentage difference was statistically significant. Linear regression analysis determined whether percentage differences changed linearly with time.

RESULTS

Fresh root weights of infected S plants increased significantly over controls with time. Thirty days after inoculation the mean root weight of infected S plants was 16.9 g and that of controls 5.9 g (Fig. 1). This three-fold increase in root weight was due to gall formation, and is indicative of the heavy infections that were obtained. By comparison, galls were never detected on the resistant roots of Anahu tomatoes, and exposure to 18,000 *M. incognita* larvae had no significant effect on root weight (Fig. 1).

Ethylene concentrations from the roots of S and R plants are plotted against time after inoculation in Fig. 2. Ethylene concentrations were high in the first 2 days after inoculation in both R and S control and R and S inoculated plants (Fig. 2). This may have resulted from mechanical disturbance of plants when they were removed from the greenhouse for inoculation with nematodes. Ethylene concentrations remained relatively constant after day 2. Effects of *M. incognita* inoculation on root ethylene levels were determined by comparing ethylene concentrations in control and inoculated plants.

Ethylene concentrations in control and inoculated R plants were not significantly different; however, roots of control S tomatoes had greater ethylene concentrations than did roots of infected S tomatoes. Linear regression analysis determined that the percentage difference for S plants increased linearly with time ($P = 0.1$) which suggested a possible inverse correlation between decreasing root ethylene concentration and increasing weight of galled roots (compare Fig. 1 and 2). To further investigate this possibility, linear regressions of control and infected root ethylene concentrations paired with their corresponding root weights were computed. The best straight-line fit of this data for plants without galls (all R plants and control S plants) was not significantly different from the horizontal. Thus, as root weights increased in the course of normal growth, total ethylene levels increased proportionally, resulting in a constant ethylene concentration for the 30-day experimental period. However, a significant ($P = 0.01$) negative correlation was found between decreasing ethylene concentrations and increasing root weights of infected S plants (slope = -0.019 ng C_2H_4/g of root). Consequently as root weights of infected S plants increased, largely due to galling, ethylene concentrations decreased.

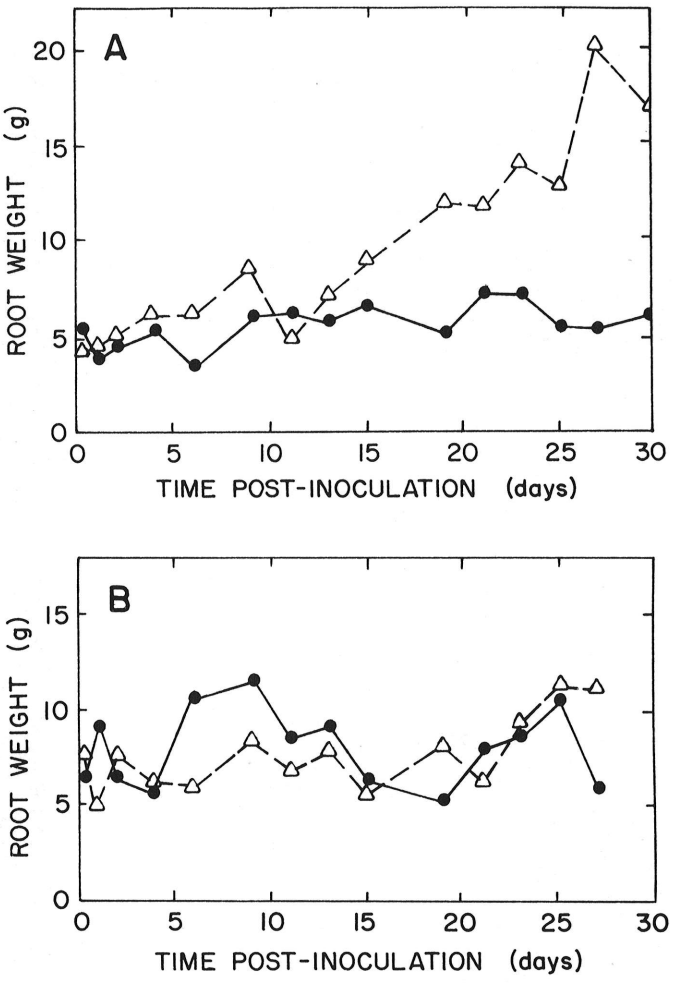

Fig. 1. Root weights of **A,** susceptible and **B,** resistant tomato plants for the 30-day period after inoculation with *Meloidogyne incognita*. Each point is the mean of two determinations ●——● = control; △---△ = inoculated.

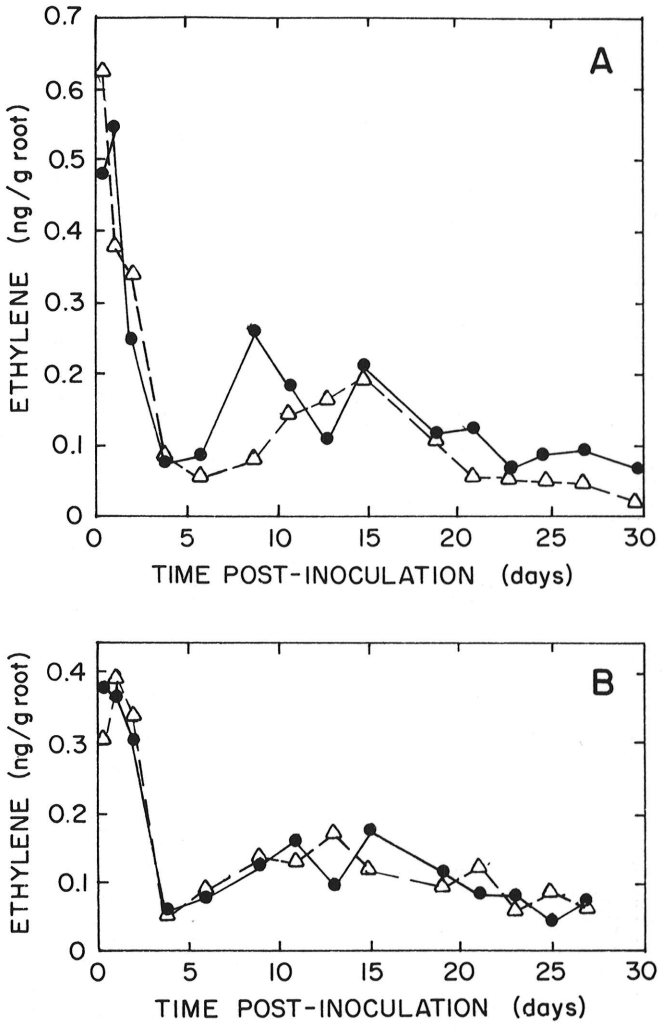

Fig. 2. Ethylene concentrations obtained from roots of **A,** susceptible and **B,** resistant tomato plants plotted against time after inoculation with *Meloidogyne incognita*. Each point is the mean of two determinations. ●——● = control; △---△ = inoculated.

Ethane concentrations from the roots of S and R plants are plotted against time after inoculation in Fig. 3. Concentrations in roots of R plants ranged between 0.28–1.22 ng C_2H_6/g root as compared to the range of 0.25–2.28 ng C_2H_6/g root found in roots of S plants. These concentrations are substantially higher than those found for ethylene, but corresponding to the ethylene levels did exhibit daily fluctuation. No significant difference was found in ethane concentration between control and nematode-treated resistant plants, a result similar to that for ethylene concentration from these plants. Ethane concentrations were significantly lower in infected galled S plants than in control plants. Thus, ethane and ethylene concentrations in roots of S plants were greater in the absence of infection.

DISCUSSION

M. incognita larvae penetrate the roots of resistant tomato plants as easily as susceptible ones (21). Resistance is controlled by a single incompletely dominant gene *Mi* (26) which is expressed in a hypersensitive host response (HR). The HR characteristically results in a walling off of the nematode by necrotic cells, and it has been suggested that necrosis occurs only in cells on which the parasite has fed (18,25). In the susceptible response, cells on which the nematode feeds normally develop into syncytia (3,4,9). *M. incognita* larvae may have penetrated roots of R plants without inducing gall formation.

In the present study a statistically significant increase in mean ethylene concentration of inoculated R plants over control plants was not found. The largest single increase in ethylene concentration of nematode treated R plants was 1.75 times that of controls at day 25 (Fig. 2B). In addition, no evidence was found of increased ethylene concentrations in the 10 days following inoculation. A similar study concerning the role of ethylene in resistance was conducted by Shain and Hillis (23). Arthrospores of the decay fungus *Amylostereum areolatum* (Fr.) Boldin are deposited with eggs by the wood wasp *Sirex noctilio* F. at oviposition. A 17-fold increase in ethylene production was observed in tissue surrounding oviposition wounds in *Pinus radiata* D. Don trees resistant to this fungus. This response is far greater than any detectable increase in ethylene concentration of inoculated R plants in our study.

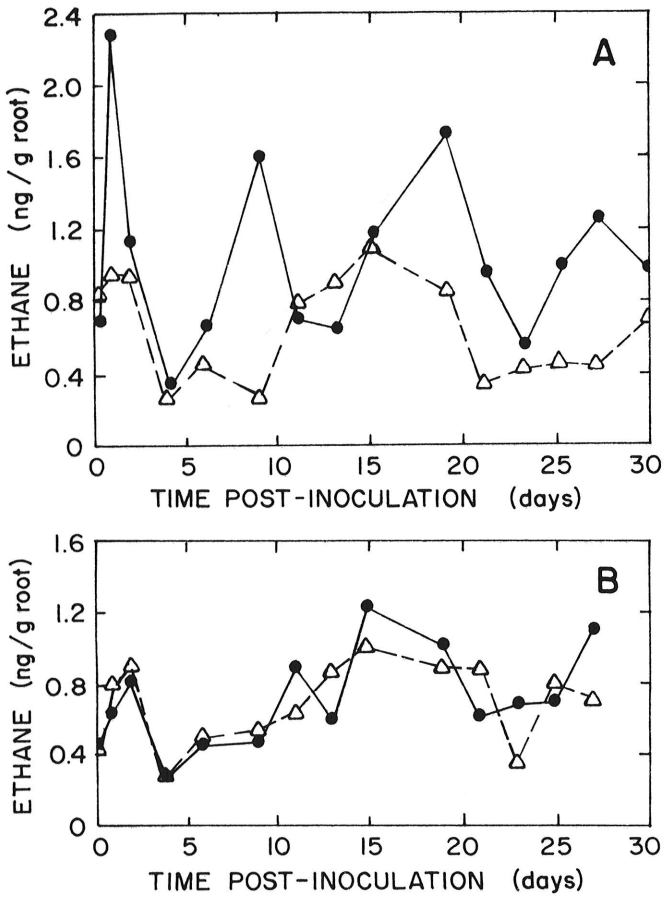

Fig. 3. Ethane concentrations obtained from roots of **A,** susceptible and **B,** resistant tomato plants plotted against time after inoculation with *Meloidogyne incognita.* Each point is the mean of two determinations. ●——● = control; △---△ = inoculated.

It has been suggested that internal gas concentrations vary directly with the rate of production of that gas (1), and conversion constants quantifying the relationship between internal gas concentrations and their rate of production have been determined for a variety of plant tissues (5,6,13). Therefore comparison of results obtained on rates of gas production and internal gas concentrations can be made, although Jerie et al (11) recently suggested that vacuum-extracted ethylene levels may not be related to rates of production.

The mean ethylene concentration from roots of S plants for the 30-day postinoculation period was significantly greater in control than infected roots. This result does not support the hypothesis of Orion and Minz (17) that increased ethylene levels accompany nematode-stimulated gall formation. The results of other studies have shown great increases in ethylene production in susceptible plants after infection by pathogens. Ethylene production increased 44 times and 12 times over the uninfected control rates in leaves of tomatoes 12 days after infection with *Verticillium albo-atrum* Reinke et Berth (19). Internal ethylene concentrations of lemons and limes increased 110-fold after infection with stubborn virus (14). In the present study, the single greatest increase in ethylene concentration in infected S plants was 1.5 times that of controls 13 days after nematode inoculation. Conversely, 9 days after inoculation the ethylene concentration in control roots was 3.1 times greater than in infected roots and the mean ethylene concentration from control S plants was significantly greater than infected S plants (Fig. 2). This difference which increased with time was significantly correlated ($P = 0.01$) with increasing root weights resulting from the formation of galls. These results strongly suggest that gall tissue contains less ethylene per gram than normal root tissue. It may be that Orion and Minz (17) in exposing plants to high doses of ethrel caused nonphysiological levels of ethylene to be released which resulted in increased gall size in some hosts but not in others (16,17). In our study we found no evidence that gall formation was associated with an increase in root ethylene concentration in four separate experiments which involved 64 separate comparisons of ethylene levels in infected and control roots. Nevertheless the possibility that gall development involves small transient increases in ethylene concentration which are not detectable by the present procedures should not be overlooked. In addition, compartmentation of ethylene (11) between gall and normal root tissue could conceivably result in increased local concentrations of ethylene in gall tissue. However this suggestion is not consistent with the present data unless there is a decrease in normal root ethylene such that the average ethylene concentration in infected root systems is lowered.

Linolenic acid has been identified as the probable precursor of ethane biosynthesis in bean plants (12). Application of linolenic acid to oat root homogenates increased both ethane and ethylene production (12). These results suggest that some common factor may be involved in the biosynthesis of these gases. Elstner and Konz (8) however concluded, after investigating the rate of production of both ethane and ethylene from sugar beet leaf discs, that biosynthesis of these gases occurred via two independent pathways. In the present study neither ethane nor ethylene concentrations in roots of R plants were significantly altered by nematode attack. Further, both ethane and ethylene concentrations were significantly lower in S galled roots than in uninoculated control roots. These results are consistent with the findings of John and Curtis (12) that the biosyntheses of ethane and ethylene share a common factor.

LITERATURE CITED

1. ABELES, F. B. 1973. Ethylene in Plant Biology. Academic Press, New York. 302 pp.

2. BEYER, E. M., and P. W. MORGAN. 1970. A method for determining the concentration of ethylene in the gas phase of vegetative plant tissue. Plant Physiol. 46:352-354.
3. BIRD, A. F. 1962. The inducement of giant cells by *Meloidogyne javanica*. Nematologica 8:1-10.
4. BIRD, A. F. 1974. Plant response to root-knot nematode. Annu. Rev. Phytopathology 12:69-85.
5. BURG, S. P., and E. A. BURG. 1962. Role of ethylene in fruit ripening. Plant Physiol. 37:179-189.
6. CHADWICK, A. V., and S. P. BURG. 1967. An explanation of the inhibition of root growth caused by indole-3-acetic acid. Plant Physiol. 42:415-420.
7. CLARE, B., D. J. WEBER, and M. A. STAHMANN. 1966. Peroxidase and resistance to *Ceratocystis* in sweet potato increased by volatile materials. Science 153:62-63.
8. ELSTNER, E. F., and J. R. KONZ. 1976. Effect of point freezing on ethylene and ethane production by sugar beet leaf disks. Nature 263: 351-352.
9. ENDO, B. Y. 1975. Pathogenesis of nematode-infected plants. Annu. Rev. Phytopathol. 13:213-238.
10. GOODEY, T. 1951. Laboratory Methods for Work with Plant and Soil Nematodes. HMSO (Her Majesty's Stationery Office), London.
11. JERIE, P. H., M. ZERONI, and M. A. HALL. 1978. Movement and distribution of ethylene in plants in relation to the regulation of growth and development. Pestic. Sci. 9:162-168.
12. JOHN. W. W., and R. W. CURTIS. 1977. Isolation and identification of the precursor of ethane in *Phaseolus vulgaris* L. Plant Physiol. 59: 521-522.
13. KANG, B. G., and P. M. RAY. 1969. Ethylene and carbon dioxide as mediators in the response of the bean hypocotyl hook to light and auxins. Planta (Berlin) 87:206-216.
14. OLSON, E. O., B. ROGERS, and G. K. RASMUSSEN. 1970. Abscission of citrus fruit and ethylene production by Clementine tangerine fruit grafted onto seedlings infected with stubborn virus. Phytopathology 60:155-157.
15. ORION, D. 1973. Studies on plant root-knot nematode interrelationships. OEPP/EPPO (Organ. Eur. Plant Pathologists, Eur. Mediterr. Plant Prot. Organ.) Bull. 9:67-71.
16. ORION, D., and H. HOESTRA. 1974. The effect of root-knot nematodes and ethrel on fusarium wilt of tomatoes. Neth. J. Plant Pathol. 80:28-36.
17. ORION, D., and G. MINZ. 1969. The effect of ethrel (2-chloroethane phosphonic acid) on the pathogenicity of the root-knot nematode *Meloidogyne javanica*. Nematologica 15:608-614.
18. PAULSON, R. E., and J. M. WEBSTER. 1970. Giant cell formation in tomato roots caused by *Meloidogyne incognita* and *Meloidogyne hapla* (Nematoda) infection. A light and electron microscope study. Can. J. Bot. 48:271-276.
19. PEGG, G. F., and D. K. CRONSHAW. 1976. Ethylene production in tomato plants infected with *Verticillium albo-atrum*. Physiol. Plant Pathol. 8:279-295.
20. RIEKELS, J. W. 1973. Nutrient solutions for hydroponics. Ont. Minist. Agric. Food Factsheet 200-532.
21. RIGGS, R. D., and N. N. WINSTEAD. 1959. Studies on resistance in tomato to root-knot nematodes and on the occurrence of pathogenic biotypes. Phytopathology 49:716-724.
22. SEQUEIRA, L. 1973. Hormone metabolism in diseased plants. Annu. Rev. Plant Physiol. 24:353-380.
23. SHAIN, L., and W. E. HILLIS. 1972. Ethylene production in *Pinus radiata* in response to Sirex-Amylostereum attack. Phytopathology 62: 1407-1409.
24. STAHMANN, M. A., B. G. CLARE, and W. WOODBURY. 1966. Increased disease resistance and enzyme activity induced by ethylene and ethylene production by black rot infected sweet potato tissue. Plant Physiol. 41:1505-1512.
25. WEBSTER, J. M., and R. E. PAULSON. 1972. An interpretation of the ultrastructural response of tomato roots susceptible and resistant to *Meloidogyne incognita*. OEPP/EPPO (Organ. Eur. Plant Pathologists, Eur. Mediterr. Plant Prot. Organ.) Bull. 6:33-39.
26. WINSTEAD, N. N., and R. D. RIGGS. 1963. Stability of pathogenicity of B Biotypes of the root-knot nematode *Meloidogyne incognita* on tomato. Plant Dis. Rep. 47:870-871.