Interrelationships between Ethylene Production, Gall Formation, and Root-knot Nematode Development in Tomato Plants Infected with Meloidogyne javanica[1]

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Abstract: Ethylene production was determined in excised tomato (*Lycopersicon esculentum*) root cultures of *Meloidogyne javanica* susceptible and resistant cultivars infected with *M. javanica*. Uninfected cultivars produced very low amounts of ethylene. Relatively high amounts of ethylene were produced by the infected susceptible cultivars. Peak production of 1.6 n moles • g root^{-1} • h^{1-} occurred between 9 and 16 days after inoculation (DAI). The period of high ethylene production coincided with that of rapid increase in gall weight. Low amounts of ethylene were also released by the infected resistant cultivar between 9 and 12 DAI, which follows the hypersensitivity reaction. Ethylene production in infected intact plants during the period of rapid gall growth was twice as much as in uninfected plants during the same time. Exposing excised root cultures to 0.5 or 10 ppm ethylene accelerated the rate of increase in gall weight of *M. javanica* infected roots. In contrast, overall root growth was inhibited by these treatments, compared to infected roots which were not exposed to ethylene. *Key words:* physiology, growth, inhibition, interactions, host-parasite relations. Journal of Nematology 15(4):539-544. 1983.

The role of plant growth substances in the development of galls induced by root-knot nematodes has been reviewed (6,8), and it is apparent that little work has been published on the involvement of ethylene in gall formation. Ethephon, an ethylene-releasing agent, applied to tomato plants infected by root-knot nematodes causes an increase in gall weight due to the proliferation of the parenchymatous cells (11,12). Orion and Netzer (13) demonstrated that ethephon mimicked the effect of high population of *Meloidogyne javanica* suppressing fusarium wilt. However, conflicting reports have been published (2,10) on the relative rates of ethylene production in nematode-infected and uninfected roots.

In view of the observation that microbial or insect infection (14) and wounding (1) induce increased ethylene production, we have undertaken a study to clarify the interrelationships between ethylene production, gall formation, and nematode development in tomato roots infected with *M. javanica*.

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MATERIALS AND METHODS

Ethylene evaluation by root cultures: Seeds of tomato *Lycopersicon esculentum* cv. Hosen-Eilon (susceptible to *M. javanica*) and cv. line 199-2 (resistant to *M. javanica*) were surface sterilized with 1% sodium hypochlorite, rinsed three times with sterile distilled water, and placed in petri dishes containing 1% water agar. After the seeds germinated, 1-cm-long primary root tips were exised and transferred into a chemically defined medium (15) in cotton plugged 25-ml conical flasks or petri dishes. The roots were inoculated with egg masses of *M. javanica*, obtained from a monoxenic culture of the nematode, by placing the eggs near newly emerged lateral roots. Controls consisted of similarly treated roots not inoculated with nematode. The cultures were incubated in the dark at 25 C. Each treatment was replicated eight times.

The ethylene content in each flask was measured at 3–4 day intervals for 35 days after inoculation (DAI). Twenty-four hours before each determination, the cotton plugs of the flasks were replaced by rubber caps to avoid gas exchange. A 1-ml air sample withdrawn from the conical flasks with an air-tight hypodermic syringe was injected into a gas chromatograph (GC) equipped with a flame ionization detector. Ethylene was separated on a 3-foot glass column packed with 80–100 mesh activated

alumina; minimum of detection was 50 ppb.

Nematode and gall development: At 5-day intervals for 35 days, the roots were harvested from each of five excised root cultivars and stained with acid fuchsin. Twenty-five stained galls were removed from each sample and weighed. The nematodes in the stained galls were manually extracted and the stages of development determined for 50 nematodes from each sample.

Ethylene evaluation by the intact plant: Susceptible tomato seedlings were transplanted in sterile quartz sand in 12 500-ml plastic pots. The plants were grown under greenhouse conditions and were fertilized with mineral nutrient solution once a week. When the plants were at the four-leaf stage, 1,000–1,200 eggs were added to each pot (7); controls consisted of uninfected plants. The control and treatment were replicated six times. Ethylene evaluation was determined by GC once a week for a 28-day period by enclosing the intact plant in a 6-liter sealed glass container for 24 hours. Gas sample collection and analysis were as described above.

Exposure of root culture to ethylene: Excised susceptible tomato root cultures infected with *M. javanica* were placed in 6-liter sealed containers (28 culture dishes in each) containing 0, 0.5, or 10 ppm ethylene. The containers were placed in the dark at 25 C. One, two, three, and four weeks after inoculation, seven replicates from each treatment were harvested and the root and gall weights determined.

RESULTS

The infected susceptible excised tomato root cultures (treatment No. 1) produced greater amounts of ethylene than did the uninfected cultures (Fig. 1). The highest rate of ethylene production was recorded between 9 and 16 DAI, reaching a peak value of 1.6 n mole • g root^{-1} • h^{-1}. Uninoculated excised root cultures of both cultivars produced barely detectable amounts of ethylene. Low amounts of ethylene were released by the infected resistant cultivar between 9 and 12 DAI (Fig. 1) even though no galls were formed.

Nematode and gall development: Gall weights increased rapidly between 7 and 16 DAI (Fig. 2); this coincided with the period of highest rate of ethylene production. During that period all of the nematodes were in the third and fourth larval stages (Fig. 2B).

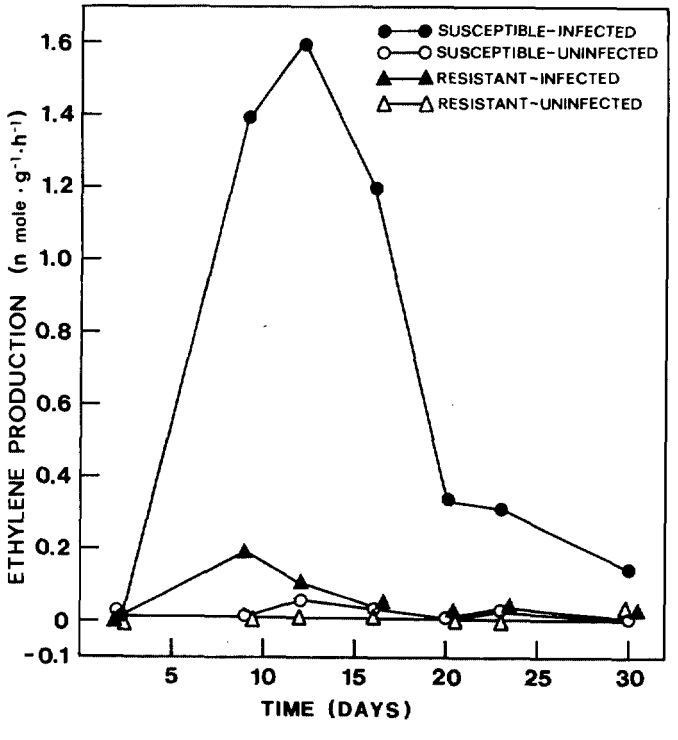

Fig. 1. Ethylene evaluation in *Meloidogyne javanica*-infected and uninfected susceptible excised tomato roots.

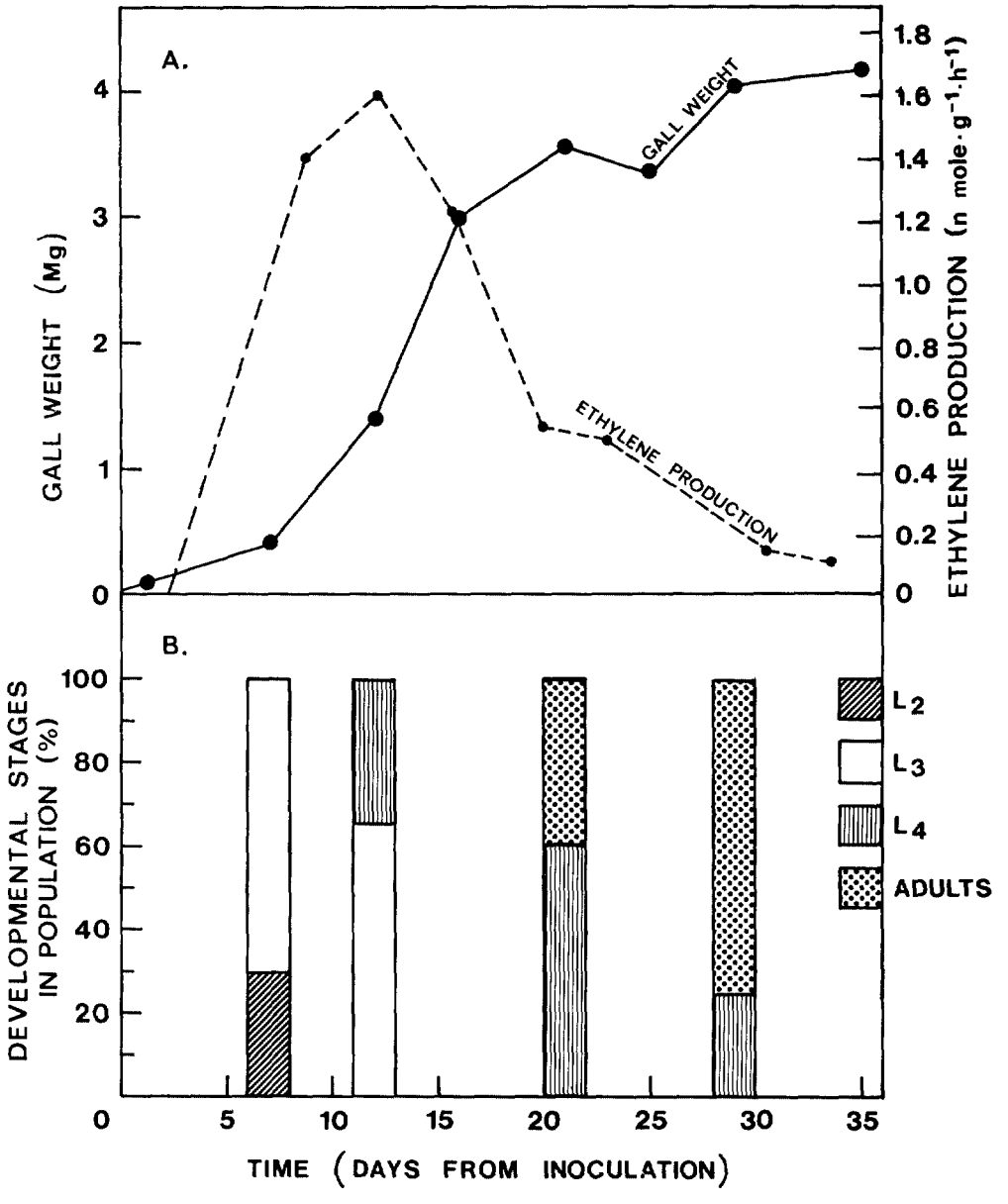

Fig. 2. Relationship between ethylene evolution and the weight of *Meloidogyne javanica*-induced gall in excised root culture of susceptible tomato and the development of the nematode. A) Ethylene evaluation and gall weight. B) Stages of nematode development.

Ethylene evaluation by the intact plant: Between 7 and 14 DAI, the infected plants released twice as much ethylene as did the uninfected plants (Fig. 3). However, no differences were recorded after this period of time.

Exposure of root cultures to ethylene: Although exposure to ethylene inhibited overall root growth (Fig. 4A), it seemed to cause an increase in gall weight (Fig. 4B).

DISCUSSION

Ethylene is a plant hormone which has a profound effect on plant growth and development (1). We have demonstrated that *M. javanica*-infected root cultures and intact plants produced relatively high amounts of ethylene especially during the second week after inoculation. The role of increased ethylene production is highly

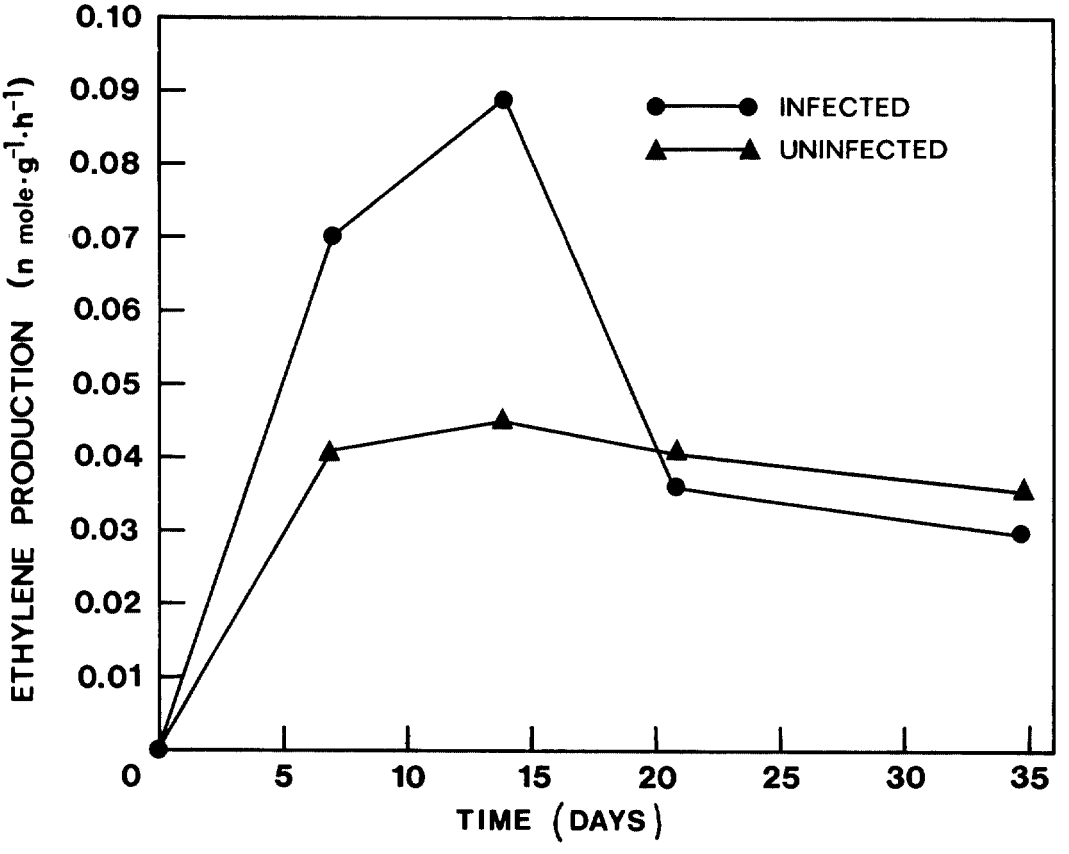

Fig. 3. Ethyelene evavluation in *Meloidogyne javanica*-infected and uninfected intact susceptible tomato plants.

correlated with an increase of the gall weight during that period of time. Moreover, infected roots exposed to ethylene at physiological concentration (1) had heavier galls than did infected roots not exposed to additional ethylene (Fig. 4B). Since increase in gall size and weight is mainly due to the hypertrophy of the gall parenchymal tissue (6), one can suggest that the ethylene hormone is related to this process. These findings support those of Orion and Minz (12), who showed that ethephon increases the size of galls by enhancing the cortical parenchyma growth.

Apelbaum and Burg (3) reported that at physiological concentration ethylene altered the orientation of cell wall microfibriles. This resulted in a change in growth direction in the elongation zone of shoots and roots, thereby inhibiting elongation and inducting expansion (4). Ethylene probably also postpones the termination of the growth period in this tissue by inhibiting differentiation and lignification (5). In addition, it probably induces an increase in RNA and protein synthesis (9) as well as additional incorporation of glucose and protein into cell wall cellulose (4) which allow accelerated growth in this region. The consequence of these events is the formation of large square-shaped cells in the elongation zone which form a bulb that keeps growing at an accelerated rate for a few days (4,5). The form of the galls in the elongation zone of tomato roots infected with *M. javanica* resembles those formed by ethylene. Therefore, it is conceivable that ethylene could be involved in controlling growth of the gall parenchymatous tissue.

The fact that no ethylene was found during the first 1–2 DAI (Fig. 1), indicates that ethylene is not involved in root penetration by the larvae, which occurs during this time.

Additional evidence to support the in-

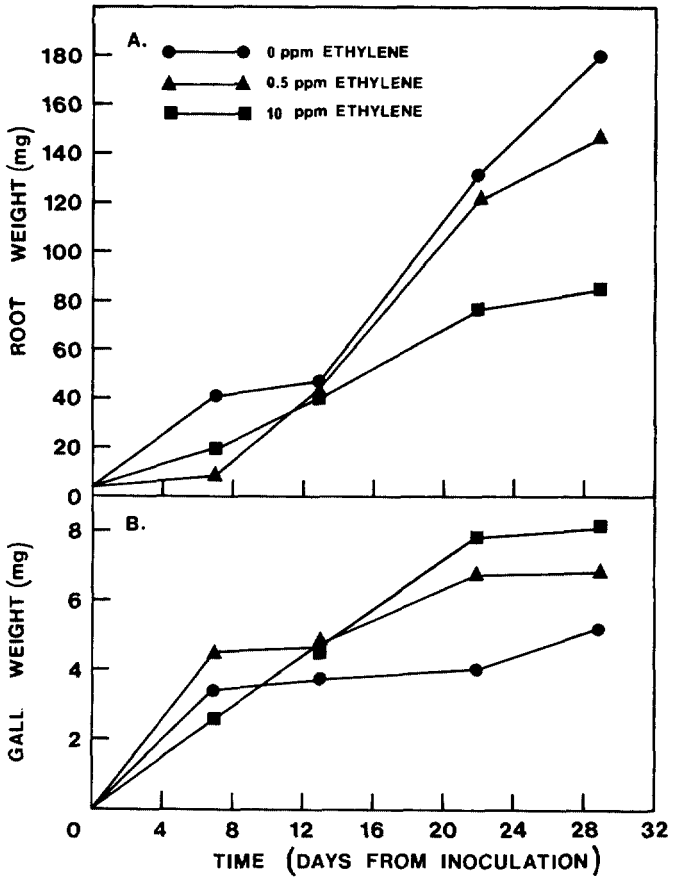

Fig. 4. Effect of applied ethylene on root and gall weights in *Meloidogyne javanica*-infected excised tomato root cultures. A) Root weights. B) Gall weights.

volvement of ethylene hormone in the gall formation was obtained when the pattern of ethylene production in inoculated *M. javanica*-resistant cultivars was studied (Fig. 1). The fact that low ethylene levels were produced in this tissue for a relatively shorter period of time, as compared with that in the susceptible cultivar, coincides with the hypersensitivity reaction (16) and the cessation of the giant cell formation gall.

Our findings in this study are in contrast to those of Akitt et al. (2), who reported that a significant decrease in ethylene concentration accompanied gall formation. This apparent contradiction can be explained, as suggested by Veech (16), if the comparison of the concentration of ethylene in galled and nongalled tissue were not calculated on the basis of ethylene amount per unit weight of tissue, but calculated instead on the basis of the total amount of ethylene produced by the entire root system. Ethylene measurements of monoxenic *M. javanica* cultures seem to be more reliable than those of intact plants, because in the former system only the nematodes and the parasitized tissue are involved, without the intervention of any other organism.

The large amount of ethylene produced in *M. javanica*-infected plants may affect not only the cortical hypertrophy of the gall, but also the whole pathogenic syndrome of nematode-infected plants by altering the normal balance of growth substances in the diseased host plant. Further experiments in this direction are now being conducted.

LITERATURE CITED

1. Abeles, F. B. 1973. Ethylene in plant biology. New York: Academic Press.

2. Akitt, D. B., A. W. Brown, and J. W. Potter. 1980. Role of ethylene in the response of tomato plants susceptible and resistant to *Meloidogyne incognita*. Phytopathology 70:94-97.

3. Apelbaum, A., and S. P. Burg. 1971. Altered cell microfibrillar orientation in ethylene-treated *Pisum sativum* stems. Plant Physiol. 48:648-652.

4. Apelbaum, A., and S. P. Burg. 1972. Effect of

ethylene and 2-4-dichlorophenoxyacetic acid on cellular expansion in *Pisum sativum*. Plant Physiol. 50:125-131.

5. Apelbaum, A., J. B., Fisher, and S. P. Burg. 1972. Effect of ethylene on cellular differentation in etiolated pea seedlings. Am. J. Bot. 59:697-705.

6. Bird, A. F. 1974. Plant response to root-knot nematode. A Rev. Phytopath. 12:69-85.

7. Hussey, R. S., and K. R. Barker. 1973. A comparison of methods of collecting inocula of Meloidogyne spp. including a new technique. Plant Dis. Rept. 57:1025-1028.

8. Jones, M. G. K. 1981. Host cell responses to endoparasitic nematode attack: structure and function of giant cells and syncytia. Ann. appl. Biol. 97:353-372.

9. Kang, B. G., and S. P. Burg. 1973. Influence of ethylene on nucleic acid synthesis in etiolated *Pisum sativum*. Plant Cell Physiol. 14:981-988.

10. Orion, D. 1973. Studies on plant root-knot nematode interrelationships. OEPP/EPPO (Eur. Mediterr. Plant Prot. Organ.) Bull. 9:67-71.

11. Orion, D., and H. Hostera. 1974. The effect of root-knot nematodes and etherel on Fusarium wilt of tomatoes. Neth. J. Pl. Path. 80:98-136.

12. Orion, D., and G. Minz. 1969. The effect of etherel (2-chloroethane phosphonic acid) on the pathogenicity of the root-knot nematode *Meloidogyne javanica*. Nematologica 15:608-614.

13. Orion, D. and D. Netzer. 1981. Suppresive effect of root-knot nematode on Fusarium wilt of muskmelons. Rev. Nematol. 4:65-70.

14. Sequeira, L. 1973. Hormone metabolism in diseased plants. Ann. R. Plant Physiol. 24:353-380.

15. S'Jacob, J. J., and J. Van Bezooijen. 1971. A manual for practical work in nematology (revised edition). Int. Postgraduate Nematology Course, Int. Agric. Center, Wageningen, The Netherlands.

16. Veech, J. A. 1981. Plant resistance to nematodes. Pp. 377-403 *in* B. M. Zuckermann, W. F. Mai, and R. A. Rohde, eds. Plant parasitic nematodes. Vol. 3. New York: Academic Press.