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Effect of Inhibitors and Stimulators of Ethylene Production on Gall Development in *Meloidogyne javanica*-Infected Tomato Roots[1]

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Abstract: Excised tomato roots infected with *Meloidogyne javanica* produced ethylene at 3–6 times the rate of noninfected roots. This increase in ethylene production started 5 days after inoculation. Gall growth and ethylene production in infected roots were accelerated by 1-aminocyclopropane-1-carboxylic acid (ACC), indole acetic acid (IAA), and ethrel known as ethylene production stimulators. When inhibitors of ethylene production, like aminoethoxyvinylglycine (AVG) or aminoxyacetic acid (AOA), or inhibitors of ethylene action like silver thiosulfate (STS), were applied, gall growth and ethylene production were inhibited. Enhanced expansion of parenchymatous cells was observed in sections from nematode-induced galls and ethylene-treated roots. Lignification of xylem elements and fibers in the vascular cylinder was markedly inhibited in the gall, compared with noninfected root tissue. Because ethylene is known to induce cell expansion and to inhibit lignification, it is suggested that this plant hormone plays a major role in the development of *M. javanica*-induced galls. Ethylene affects gall size by enhancing parenchymatous tissue development and allows expansion of giant cells and the nematode body by reducing tissue lignification.

Key words: ethylene production, ethylene stimulators, ethylene inhibitors, parenchymatous tissue, cell expansion, lignification.

Gall formation induced by *Meloidogyne* spp. has been studied thoroughly for many years (8,17,23). Most of these studies have dealt with gall formation and induction of giant cells in the gall. Little attention has been given to the hypertrophied cortical parenchymatous tissue, although such tissue is the largest constituent of the gall. Plant growth substances, such as auxins and cytokinins, have been implicated in the gall formation process (8,9,17,20). We have recently shown (13) that increased ethylene production is closely associated with *M. javanica* infection and gall formation in susceptible tomato plants. However, the induction of ethylene production by nematode infection and the role of ethylene in gall formation remained obscure.

The pathway for ethylene biosynthesis has recently been elucidated and can be summarized in the following sequence: methionine → S-adenosyl-methionine (SAM) → 1-aminocyclopropane-1-carboxylic acid (ACC) → ethylene (1). In most of the cases studied, the rate-limiting step in this pathway is the formation of ACC (2,24). The mode of ethylene induction by some stress conditions consists of increased conversion of SAM to ACC (25) and, in some cases, increased conversion of ACC to ethylene as well (4,24). Aminoethoxyvinylglycine (AVG) and aminoxyacetic acid (AOA), two compounds that specifically inhibit the conversion of SAM to ACC, have been useful tools in investigating stress-induced changes in the pathway of ethylene biosynthesis (24).

The effect of ethylene on various plant physiological processes was elucidated by employing stimulators of ethylene production or inhibitors of its action (22). Silver thiosulfate (STS) inhibits ethylene action by blocking access of the hormone to its binding site. STS has served as a useful tool for studying effects of ethylene on various phenomena and has been shown to reverse growth inhibition induced by root-knot nematodes in tomato plants. On the other hand, supraoptimal concentrations of auxin induced ethylene production in vegetative tissue (11); ethylene produced in this manner inhibits root and shoot growth and causes swelling of the subapical region (2,3,12). Similar effects have been observed with application of ethylene, or the ethylene-releasing compound ethrel (2-chloroethanephosphonic acid) (3,10,18). The objectives of this study were to explore 1) the mode of induction of ethylene

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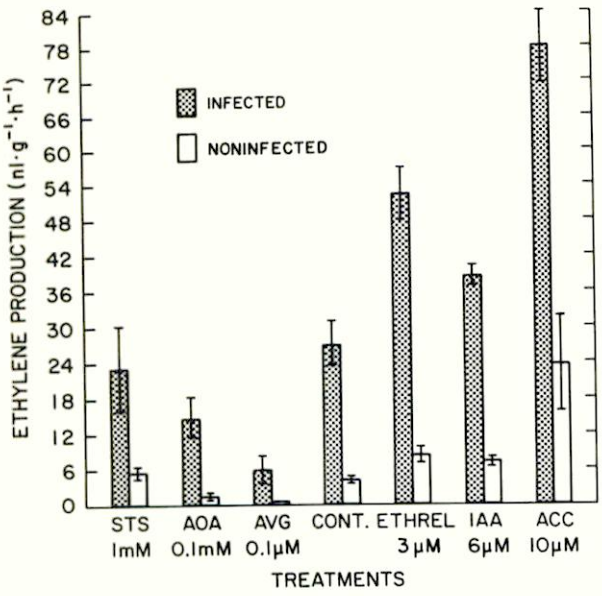

FIG. 1. Influence of ethylene stimulators or inhibitors on the rate of ethylene production in excised tomato root cultures with and without *Meloidogyne javanica* nematode inoculation.

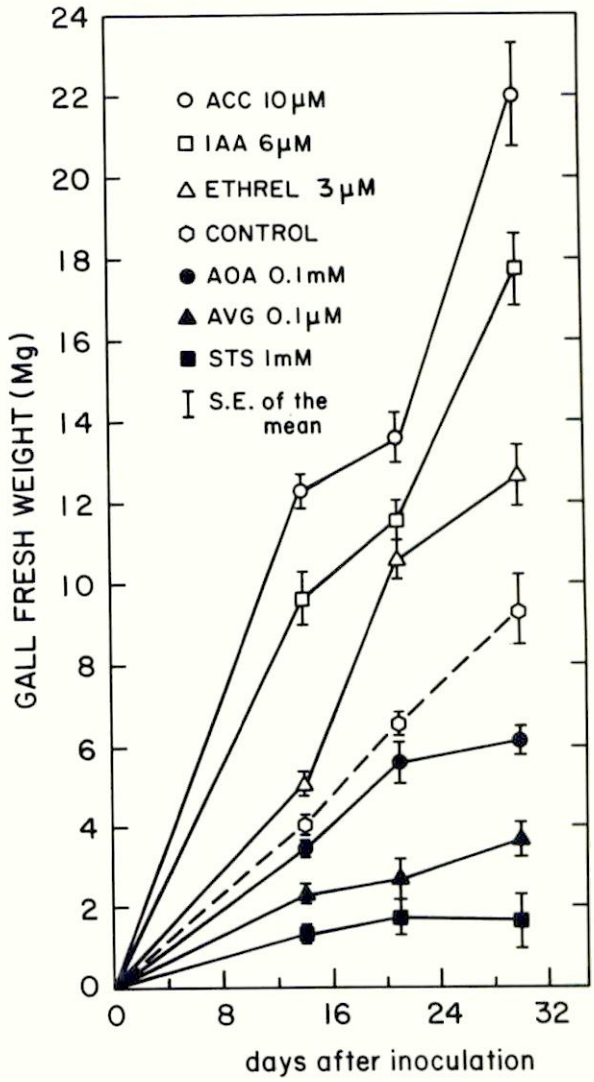

FIG. 2. Effect of ethylene stimulators or inhibitors on the rate of *Meloidogyne javanica*-induced gall growth in excised tomato root cultures.

resulting from *M. javanica* infection and 2) the role of ethylene in the host–parasite relationship.

MATERIALS AND METHODS

Excised tomato roots, *Lycopersicon esculentum* cv. Hosen-Eilon (susceptible to *M. javanica*), were grown in petri dishes or 25-ml Erlenmeyer flasks containing a chemically defined medium (21). Roots were inoculated with egg masses of *M. javanica* as described by Glazer et al. (13). Roots were treated with ethylene stimulators or inhibitors by mixing the chemicals under sterile conditions with the culture media. Tomato roots were exposed to ethylene by placing four petri dishes, each containing 10 excised roots, in airtight chambers and injecting ethylene through a vaccine cap.

Ethylene production by roots growing in cotton-plugged 25-ml Erlenmeyer flasks containing culture media was measured periodically by gas chromatography (13). Each treatment was replicated 10 times.

The effect of ethylene stimulators or inhibitors on gall growth was studied in similar root cultures in petri dishes. Each treatment was replicated five times. Twenty-five galls were removed from each replicate culture and their weights recorded at 2, 3, and 4 weeks after inoculation. Galls and root segments were processed for light microscope observation as follows: the tissue was dipped in 3% glutaraldehyde in phosphate buffer, pH 6.8, at 25 C. Segments of galls or roots were transferred to vials containing fresh fixing solution for 2 hours. Roots were then washed six times with buffer and postfixed in 2% buffered osmium tetroxide for 2 hours, dehydrated with acetone, and embedded in Epon. Sections were stained with toluidine blue and examined with a light microscope. The number of layers of parenchyma cells and the thickness of the parenchyma were determined. Roots from 4-week-old cultures were prepared for scanning electron microscope (SEM) observation by the procedure of Wergin and Orion (23).

Lignification was studied in roots taken

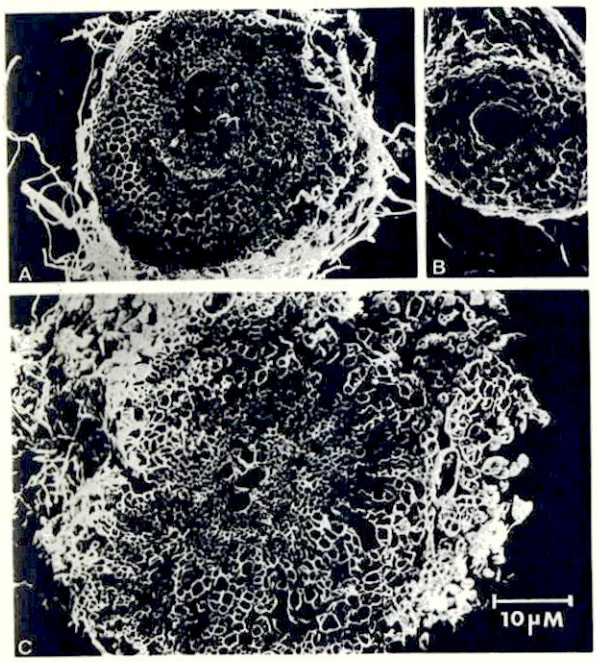

FIG. 3. Scanning electron micrograph of a cross section through a tomato root gall induced by *Meloidogyne javanica* from a nontreated root (A), a root treated with 0.1 μM aminoethoxyvinylglycine (B), and a root treated with 6 μM indole acetic acid (C).

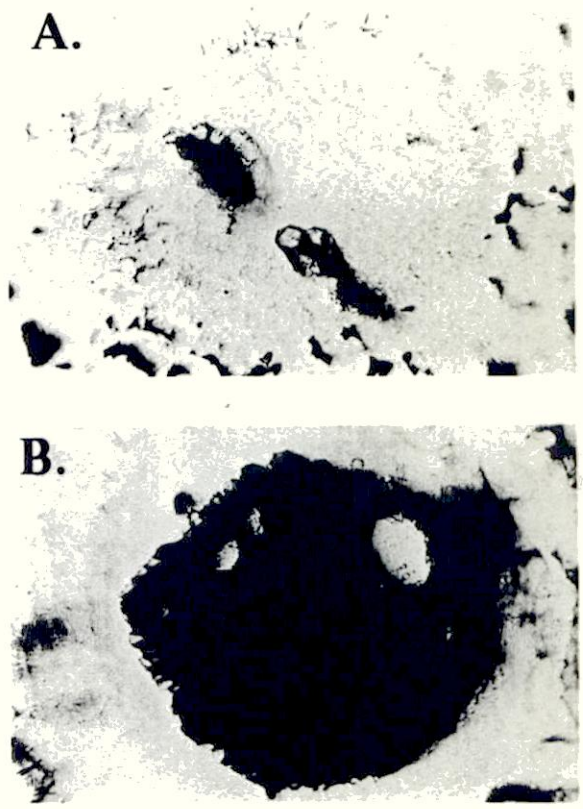

FIG. 4. Free-hand section of *Meloidogyne javanica*-induced tomato root gall (A) and noninfected control root (B) stained with phloroglucinol for lignin determination. Dark area indicates lignified elements.

from cultures at 14 and 28 days after inoculation. Free-hand sections were stained with phloroglucinol in 30% HCl (16) to detect lignin.

RESULTS

Effect of stimulators and inhibitors on ethylene production and gall growth: Ethylene inhibitors and stimulators did not alter normal growth and development of roots in culture. However, these compounds did affect ethylene production, gall growth, and the anatomy of roots. The effect of ethylene stimulators or inhibitors was determined on day 14 after inoculation, the time of maximum ethylene production. Application of 6 μM IAA increased ethylene production by 70% in noninfected roots and 40% in the infected ones, compared with noninfected or infected control treatments (Fig. 1). A similar but more pronounced effect was observed in cultured roots treated with 3 μM ethrel (Fig. 1). Treatment with ACC (10 μM) had the most stimulatory effect, increasing ethylene production 2.5-fold and 4-fold in infected and noninfected roots, compared with control roots.

Application of AVG and AOA reduced ethylene production in noninfected tissue by 80% and 60% and in infected tissue by 80% and 50%, respectively. Treatment with STS did not affect ethylene production in noninfected or infected roots in culture (Fig. 1).

Fresh weight of *M. javanica*-induced galls in root cultures increased linearly during days 14–30 after inoculation (Fig. 2). Application of ACC, IAA, or ethrel increased gall weight by 150%, 100%, and 50%, respectively, by day 30 after inoculation. The compound most inhibitory to gall growth was STS. On day 14 after inoculation, AVG had inhibited growth by 50%, AOA by 20%, and STS by 70%, and on day 30 inhibition was 60%, 40%, and 80%, respectively.

Histological observation: Sections of 4-week-old galls examined with a light microscope revealed that diameters of the vascular cylinder and especially the cortical parenchyma were enlarged by 4-fold and the number of cells in cross section had increased by 50%, as compared with noninfected roots of similar age. The size of the vascular cylinder in root tip sections treated with 10 ppm ethylene for 3 days

remained the same as in nontreated controls, whereas the cortical parenchyma diameter increased by 80% in the treated root tips.

Examination of fractured galls with SEM revealed that the hypertrophied cortical parenchyma of the galls grown in the presence of the ethylene production inhibitor AVG in the culture media (Fig. 3b) was less developed than the nontreated control (Fig. 3a). On the other hand, the parenchyma of galls developed in the presence of IAA (Fig. 3c) was larger in diameter than that of the control. Regardless of the chemical treatment, the vascular cylinder was not affected in size or structure.

Effect of nematode infection on xylem lignification and structure: Xylem elements and fiber in galls contained little lignin at day 14 after inoculation (Fig. 4a). In contrast, the entire vascular cylinder of noninfected roots of the same age was heavily lignified (Fig. 4b).

Discussion

Increased ethylene production stimulated by application of ACC, supraoptimal concentration of auxin, and ethrel to various plant organs has been reported (10,19,24). Excised tomato roots in culture, with or without *M. javanica* infection, produced elevated levels of ethylene in the presence of these ethylene stimulators. In addition, AVG and AOA, known specific inhibitors of ethylene production (24), inhibited ethylene production significantly. On the other hand, STS, a compound that inhibits ethylene action (15) by blocking access to its site of action (22), did not inhibit ethylene production in cultured roots.

Induction of ethylene production could occur at two sites along the hormone biosynthetic pathway: 1) increased formation of ACC from SAM where the enzyme ACC synthase is involved (24), and 2) increased conversion of ACC to ethylene where ethylene synthase is involved (24). The fact that ethylene production induced by *M. javanica* infection was inhibited by AVG and AOA provides information on the site of its induction by the nematode. Since these two specific ethylene inhibitors block the formation of ACC (24), it could be postulated that the nematode infection induced an increase in the rate of ethylene production by accelerating the conversion of SAM to ACC, probably via increasing the activity of ACC synthase (7). This hypothesis is supported by the finding that the ACC content of tomato plants was increased by *M. javanica* infection (14). A similar mode of induction was reported for some cases of stress-induced ethylene production (6,24,25). Swelling occurred in both nematode-induced galls and ethylene treated roots. The swelling in gall tissue could be attributed in part to rapid cytokinesis incited by high levels of kinetin (9,20), whereas swelling in ethylene-treated roots is attributed only to cell expansion (3,4). The finding that stimulators of ethylene production increased parenchyma cell size while inhibitors prevented parenchyma cell enlargement in galled tissue suggests that ethylene controls gall size by inducing parenchyma cell expansion. Therefore, the rate of ethylene production induced by the nematode infection may determine the rate of gall growth and, consequently, final gall size.

Support for the notion that ethylene plays a role in root-knot nematode-induced gall formation is provided by the fact that little of the vascular cylinder is lignified in the galls. Apelbaum et al. (5) showed that ethylene inhibited xylem formation and prevented fiber lignification in pea seedlings.

Deformed xylem elements in nematode-galled tissue, as seen in this study and by many others (8,17,23), could be attributed to the lack of lignification. Inhibition of lignification in the gall might allow rapid expansion of the giant cells with little resistance from the surrounding tissue.

We suggest that in *M. javanica*-infected roots, ethylene plays a major role in controlling gall size by inducing cell expansion in the cortical parenchyma and inhibiting xylem lignification in the vascular cylinder. These findings raise the question whether the increase in ethylene production serves the nematode or the host plant as a mechanism of defense. Our data suggest that the hormone allows the nematode and the giant cells to expand and develop freely in nonlignified tissue and, by increasing the volume of the parenchymatous tissue, the nematode is protected by the external environment.

Literature Cited

1. Adams, D. O., and S. F. Yang. 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proceedings of the National Academy of Sciences U.S.A. 76:170–197.
2. Apelbaum, A., and S. P. Burg. 1971. Altered cell microfibrillar orientation in ethylene-treated *Pisum sativum* stems. Plant Physiology 48:648–652.
3. Apelbaum, A., and S. P. Burg. 1972. Effect of ethylene and 2-4-dichlorophenoxyacetic acid on cellular expansion in *Pisum sativum.* Plant Physiology 50: 125–131.
4. Apelbaum, A., A. C. Burgoon, J. D. Anderson, T. Solomos, and M. Liberman. 1981. Some characteristics of the system converting 1-aminocyclopropane-1-carboxylic acid to ethylene. Plant Physiology 67:80–84.
5. Apelbaum, A., J. B. Fisher, and S. P. Burg. 1972. Effect of ethylene on cellular differentiation in etiolated pea seedlings. American Journal of Botany 59: 679–705.
6. Apelbaum, A., and S. F. Yang. 1981. Biosynthesis of stress ethylene induced by water deficit. Plant Physiology 68:594–596.
7. Boller, T., R. C. Herner, and H. Kende. 1979. Assay for enzymatic formation of an ethylene precursor, 1-aminocyclopropane-1-carboxylic acid. Planta 145:293–303.
8. Bird, A. F. 1974. Plant response to root-knot nematode. Annual Review of Phytopathology 12:69–85.
9. Bird, A. F., and B. R. Loveys. 1980. The involvement of cytokinins in a host-parasitic relationship between the tomato (*Lycopersicon esculentum*) and a nematode (*Meloidogyne javanica*). Parasitology 80: 497–505.
10. Burg, S. P. 1973. Ethylene in plant growth. Proceedings of the National Academy of Sciences U.S.A. 70:591–597.
11. Burg, S. P., and E. A. Burg. 1966. The interaction between auxin and ethylene and its role in plant growth. Proceedings of the National Academy of Sciences U.S.A. 55:262–266.
12. Chadwick, A., and S. P. Burg. 1980. Regulation of root growth by auxin-ethylene interaction. Plant Physiology 45:192–200.
13. Glazer, I., D. Orion, and A. Apelbaum. 1983. Interrelationships between ethylene production, gall formation, and root-knot nematode development in tomato plants infected with *Meloidogyne javanica.* Journal of Nematology 15:539–544.
14. Glazer, I., A. Apelbaum, and D. Orion. 1984. Reversal of nematode-induced growth retardation in tomato plant by inhibition of ethylene action. Journal of American Society for Horticultural Science 109: 886–889.
15. Halevy, A. H., and A. M. Kofranek. 1977. Silver treatment of carnation flowers for reducing ethylene damage and extending longevity. Journal of American Society for Horticultural Science 102:76–77.
16. Jensen, W. 1962. Botanical histochemistry. San Francisco: W. H. Freeman.
17. Jones, M. G. K. 1980. Host cell response to endoparasitic nematode attack: Structure and function of giant cells and syncytia. Annals of Applied Biology 97:353–372.
18. Kang, B. G., and S. P. Burg. 1973. Influence of ethylene on nucleic acid synthesis in etiolated *Pisum sativum.* Plant and Cell Physiology 14:981–988.
19. Orion, D. 1973. Studies on plant root-knot nematode interrelationships. European Plant Protection Organization Bulletin 7:67–71.
20. Orion, D., and W. P. Wergin. 1982. Chloroplast differentiation in tomato root galls induced by the root-knot nematode *Meloidogyne incognita.* Journal of Nematology 14:77–83.
21. Orion, D., W. P. Wergin, and B. Y. Endo. 1980. Inhibition of syncytia formation and root-knot nematode development of cultures of excised tomato roots. Journal of Nematology 12:196–203.
22. Sisler, E. C., and R. Goren. 1981. Ethylene binding—the basis for hormone action in plants. What's New in Plant Physiology 12:37–40.
23. Wergin, W. P., and D. Orion. 1981. Scanning electron microscope study of the root-knot nematode *Meloidogyne incognita* on tomato root. Journal of Nematology 12:358–367.
24. Yang, S. F. 1980. Regulation of ethylene biosynthesis. Journal of Horticultural Science 15:238–243.
25. Yu, Y. B., and S. F. Yang. 1980. Biosynthesis of wound ethylene. Plant Physiology 66:881–885.