Production of Oxalic Acid by *Mycena citricolor*, Causal Agent of the American Leaf Spot of Coffee

D. V. Rao and J. P. Tewari

Postdoctoral fellow and associate professor, respectively, Department of Plant Science, University of Alberta, Edmonton, Alberta, Canada T6G 2P5.

This research was supported by grants from the International Development Research Centre, Ottawa, Ontario, Canada, and the Natural Sciences and Engineering Research Council of Canada. Thanks are due to E. Vargas for supplying a culture of *Mycena citricolor*, G. Braybrook for help in the scanning electron microscopy and energy-dispersive microanalyses, W. A. Ayer and C. DuFresne for supplying ^{13}C nuclear magnetic resonance data and useful discussions, and S. Brezden for technical assistance.

Accepted for publication 9 October 1986.

ABSTRACT

Rao, D. V., and Tewari, J. P. 1987. Production of oxalic acid by *Mycena citricolor*, causal agent of the American leaf spot of coffee. Phytopathology 77:780-785.

Light and scanning electron microscopy revealed numerous prismatic crystals in agar and broth cultures of *Mycena citricolor*. Based on their shape, solubility, energy-dispersive X-ray emission spectra, and ^{13}C nuclear magnetic resonance studies, they were shown to be composed of calcium oxalate. Histochemical study of infected coffee leaf tissue revealed calcium oxalate crystals at the site of infection and in the necrotic lesion tissue. The latter contained more than 2 times the number of calcium oxalate deposits and more than 3 times the amounts of oxalic acid relative to healthy controls. In addition, necrotic lesions similar to those incited by *M. citricolor* developed when drops of oxalic acid solutions were placed on coffee leaves. In broth culture, increases in oxalic acid levels followed the growth curve of the fungus. The results suggest a key role for oxalic acid in the pathogenesis of *M. citricolor*, and the demonstration of calcium oxalate formation in this study provides concrete evidence for the host-calcium sequestration hypothesis. It is postulated that oxalic acid, by lowering the pH, stimulates indoleacetic acid-oxidase and macerating enzyme systems resulting in tissue disintegration and premature leaf drop.

Additional key words: *Coffea arabica, ojo de gallo.*

The American leaf spot of coffee or *ojo de gallo* is a disease of serious economic concern in Latin America (3,26). In Costa Rica, for example, this disease was reported to cause average annual yield losses of up to 20% in coffee crops (28). The causal fungus, *Mycena citricolor* (Berk. & Curt.) Sacc., a basidiomycete, has a wide host range. It incites brownish spots on coffee leaves and causes extensive defoliation resulting in severe yield losses. Young twigs and berries are also infected. The fungus spreads during the rainy season through repeated production of gemmae, the asexual propagules (3).

In spite of its recognition at the beginning of the century (5) and its economic importance, very little is known about the mechanism of pathogenicity of this fungus on coffee. More than three decades ago, Sequeira and Steeves (23) reported an indoleacetic acid (IAA)-oxidase to be responsible for some of the pathogenic effects of this fungus. The authors suggested that the enzyme inactivates host IAA, resulting in abscission and premature leaf drop. The enzyme was presumed to be a peroxidase (22).

This paper describes the production of oxalic acid by *M. citricolor* in cultures and infected coffee leaf tissue. The possible key role of oxalic acid is discussed in relation to pathogenicity by way of sequestration of host calcium and stimulation of IAA-oxidase and macerating enzyme systems. A brief report on part of this work was published earlier (21).

MATERIALS AND METHODS

Culture of *M. citricolor*. A Costa Rican isolate of *M. citricolor* (deposited at the Biosystematic Research Institute, Ottawa, Ontario, Canada, as DAOM 191786) was used for all studies reported here. The cultures were grown by transferring agar plugs containing actively growing hyphae to plastic petri plates (100 × 15 mm) containing potato-dextrose agar supplemented with 0.2% yeast extract (PDA). The plates were incubated at 22 C under continuous fluorescent lighting of approximately 2,000 lux. Gemmae from 3-wk-old cultures were used for leaf inoculations.

Subculturing was done at weekly intervals, and to maintain vigor and virulence of the pathogen it was occasionally transferred to coffee leaves and recovered in culture through new gemmae formed on the leaves. For studies on crystal morphology and composition and for time-course studies on oxalic acid production, the fungus was similarly grown on 1% water agar or in potato-dextrose broth with 0.2% (w/v) yeast extract (PDB). Each 250-ml Erlenmeyer flask containing 50 ml of broth was inoculated with an 8-mm agar hyphal plug and incubated in still culture as described above. In some experiments, PDB was supplemented with 0.1% (w/v) calcium carbonate (PDBC).

Coffee plants and leaf inoculations. Coffee seedlings (*Coffea arabica* L. cv. Caturra) were planted in plastic pots (14 cm in diameter) containing sterilized soil, peat, and sand (1:2:1) and grown in a greenhouse at 20 C. During winter months, the plants received supplementary lighting provided by high-pressure sodium lamps at 10,000 lux for 18 hr each day.

Young, turgid, fully expanded leaves obtained from 4- to 6-mo-old plants were used for inoculation with the gemmae. The leaves were detached and placed in a petri plate (150 × 20 mm) containing moistened sterilized vermiculite. The interveinal area of both halves of the upper leaf surface was scratched with a hypodermic needle, and each injury point was inoculated with three gemmae in a small droplet of sterile water. Points of injury on the left half served as control. The leaves were incubated at 20 C at a 12-hr light cycle of 1,800 lux.

When authentic oxalic acid solutions were used, serial dilutions of 0.8, 0.4, 0.2, and 0.1%, with pH values of 1.3, 1.6, 1.9, and 2.0, respectively, were prepared in sterile demineralized water, and a drop of each dilution was placed on a prescratched right half of the leaf. The left half of the leaf served as control. In addition, for comparison, serial dilutions of sulfuric acid (H_2SO_4) with pH values similar to those of oxalic acid solutions (pH values 1.2, 1.5, 1.6, and 1.8 for 1, 0.5, 0.25, and 0.125% H_2SO_4, respectively, in demineralized water) were prepared. Drops of each dilution were placed on prescratched leaves as described above.

Mycelium dry weight determination. The time-course of fungal growth and oxalic acid accumulation was studied in broth cultures over a 17-day incubation period. The culture fluid from each flask was individually filtered through a tared Whatman No. 1 filter paper, and the paper with mycelium was dried at 75 C for 48 hr. Dry weight of mycelium was recorded for each of the five replicates. The experiment was repeated two times.

Oxalic acid isolation and analysis. Isolation and analysis of oxalic acid in the culture filtrate obtained from the above experiment were carried out according to Bateman and Beer (2). Fifteen milliliters of filtrate was incubated overnight with 10 ml of 0.1 M calcium chloride-acetate buffer, pH 4.5, and centrifuged. The sediment was washed twice with 5% acetic acid saturated with calcium oxalate; after centrifugation, it was dissolved in 4 *N* H_2SO_4 and titrated against 0.025 *N* potassium permanganate ($KMNO_4$). Calculations were made as grams of oxalic acid per liter of broth.

The leaf tissue was sampled for oxalic acid isolation. Brownish lesions that formed 1 wk after inoculation with the gemmae were carefully cut out with a razor blade, and the leaf material was dried in an oven at 75 C for 48 hr, ground into a fine powder, and weighed. Comparable tissue from scratched areas on the other half of the leaf served as control.

Isolation of oxalic acid from leaf tissue was carried out by the conventional ether extraction procedure (2). Dry leaf powder (0.2 g) was mixed with 0.5 g of asbestos and ground with 1 ml of 4 *N* H_2SO_4 using a mortar and pestle. The contents were transferred to an extraction thimble and extracted with 50 ml of diethyl ether in a Soxhlet apparatus for 24 hr. Five milliliters of sodium hydroxide was added and ether flash evaporated. After the addition of 4 ml of calcium chloride-acetate buffer, overnight incubation, and centrifugation, the sediment was washed twice with 5% acetic acid saturated with calcium oxalate. Finally, the sediment was dissolved in 4 *N* H_2SO_4, heated to 60 C in a water bath, and filtered through Whatman No. 1 filter paper; the filtrate was analyzed for oxalic acid by $KMNO_4$ titration as described earlier. Two batches of samples with two replicates for each batch were analyzed. Oxalic acid amounts were expressed as milligrams per gram (dry weight) of leaf sample.

Histochemical studies. Crystals on the inoculated leaf surface and inside the leaf tissue were stained with silver nitrate-dithiooxamide for the identification of calcium oxalate (29). Scratched controls were included.

Gentle washing procedures were followed to stain surface crystals in order to minimize their dislodging. This was carried out by alternatively placing and retracting drops of fresh 5% acetic acid solution every 3 min for several times. Later, the crystals were similarly washed with sterile double-distilled water and drops of 5% aqueous silver nitrate solution were placed for 15 min. After washing with water, drops of saturated dithiooxamide solution were placed for 2 min on the leaf surface containing the crystals. The surface was successively washed with 50%, 70%, and absolute ethanol, mounted in glycerol, and examined with a light microscope.

Transverse hand sections of the brownish lesion area of 1-wk-old infected leaf tissue were cut with a razor blade and cleared in acetic-ethanol (glacial acetic acid and 95% ethanol in 1:1 ratio). They were washed in 5% acetic acid, stained with silver nitrate-dithiooxamide as described earlier, mounted in glycerol, and examined in a light microscope. Five sections each of infected and control tissues were used for quantitation of calcium oxalate deposits. A segment 2 mm in length (two per section) was chosen at random and the mesophyll cells containing the deposits were counted with an ocular micrometer; the number was expressed as percentage in relation to total number of cells in the segment.

Purification of crystals formed in PDBC. The PDBC culture fluid, incubated for 2 wk after inoculation with *M. citricolor*, was centrifuged at 4,000 *g* for 10 min. The sedimented crystals were washed in 5% acetic acid and centrifuged twice with alternate washings with sterile demineralized water. The final crystal sediment was suspended in sterile water. This preparation was used for scanning electron microscope observations and ^{13}C nuclear magnetic resonance (NMR) studies.

^{13}C NMR. A sample of the purified preparation was dissolved in 0.5 ml of deuterated HCl-methanol (1:1) containing tetramethyl silane as an internal standard. The ^{13}C NMR spectrum was obtained at 100.62 MHz and the control ^{1}H NMR spectrum at 400 MHz.

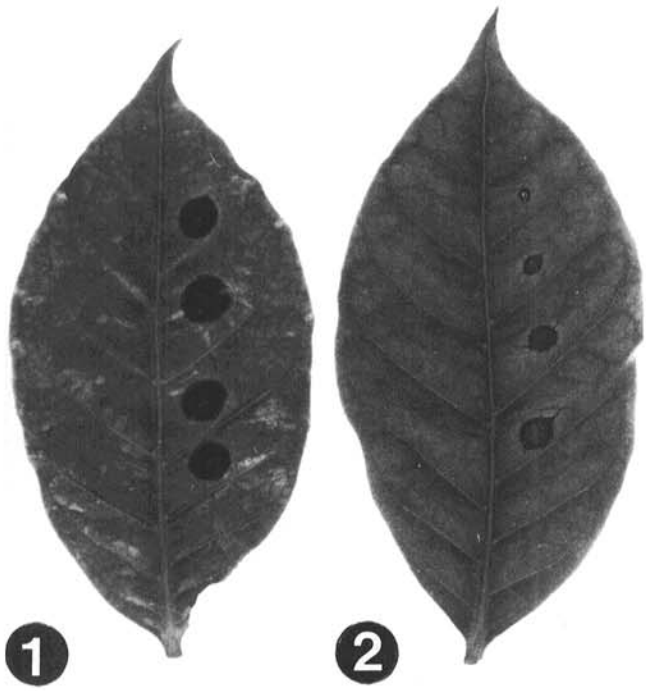
Scanning electron microscopy and energy-dispersive X-ray microanalysis. Crystals formed in culture and on the leaf surface were examined for their shape and size and for presence of major cations. The mycelial fragments from PDB, drops of culture fluid from PDBC, or purified PDBC crystals were placed on carbon disks and air-dried. The carbon disks were glued to metal stubs with conductive silver paint. Pieces of lesions with crystals present on the surface were gently washed with 5% acetic acid, air-dried, and glued to a stub by Scotch double-stick transparent tape. For studies on crystal morphology, the specimens were coated with gold in a sputter coater. Energy-dispersive X-ray microanalyses were made prior to gold coating. Micrographs were taken in a Cambridge Stereoscan 250 scanning electron microscope equipped with a Kevex Micro-X 7000 analyzer.

RESULTS

Symptoms on coffee leaves. *M. citricolor* incited chocolate-brown necrotic lesions with somewhat irregular margins (Fig. 1). Similar lesions were formed on leaves when oxalic acid solutions were applied. The size of lesion was directly proportional to the concentration of acid applied (Fig. 2). All concentrations of H_2SO_4 tested also caused brownish lesions.

Crystal morphology. Numerous crystals were observed in cultures, i.e., water agar, PDA, PDB, and PDBC (Figs. 3-8). They were prismatic tetragonal crystal forms described for calcium oxalate polyhydrate (8). Mainly two crystal patterns were observed: type 1 was bipyramid (Fig. 6), and type 2 was a prismatic column with a pyramid face on each side of the column (Fig. 5).

In water agar and PDA, bipyramids of different sizes were



Figs. 1–2. Symptoms on coffee leaves. **1,** Right half, brownish necrotic lesions incited by *Mycena citricolor* 1 wk after inoculation. Left half, scratched uninoculated control. **2,** Right half, similar lesions as above induced by drops of oxalic acid solution at (from top) 0.1, 0.2, 0.4, and 0.8%, respectively; note increase in lesion size with increase in concentration of oxalic acid solution applied. Left half, scratched control.

observed in association with the hyphae (Fig. 3). Bipyramids formed in PDBC were conspicuously larger than those formed in agar or PDB where there was no supplementation with calcium carbonate (Figs. 4 and 6). Pure crystals obtained from PDBC were not soluble in 5% acetic acid, indicating that they were neither calcium carbonate nor phosphate (11,29). These bipyramids also exhibited the paramorphic corrosion (Fig. 7) characteristic of calcium oxalate polyhydrates. They were apparently corroded upon prolonged exposure to aqueous solutions during the purification process.

On the leaves, crystals were observed on *M. citricolor*-incited lesions as well as on lesions induced by oxalic acid solutions (Figs. 9-14). On the lesion surface infected with the pathogen, crystals were seen as shiny masses around the gemmae near scratches and dispersed toward the periphery of lesions (Fig. 9). They were predominantly of calcium oxalate polyhydrate form, type 2, as described earlier (Fig. 10). Crystals on lesions induced by oxalic acid appeared to be monoclinic, which is characteristic of calcium oxalate monohydrate. Several of them had pseudorhomboedric symmetry (Fig. 12). Crystals were absent in the controls.

Histochemical studies. Histochemical staining of *M. citricolor*-infected lesion surfaces with silver nitrate-dithiooxamide showed black staining of all the crystals (Fig. 11), a reaction indicative of the presence of calcium oxalate (29). Hand sections of infected tissue contained darkly stained crystalline aggregates in palisade and spongy mesophyll cells (Figs. 13 and 14). The lesion area had more than 2 times the number of crystal-containing cells (294/894 = 33%) seen in the controls (96/690 = 14%).

Energy-dispersive X-ray microanalyses. The X-ray emission spectra of crystals from PDB and PDBC cultures and from surfaces of lesions induced by *M. citricolor* and oxalic acid all contained only calcium cation. A representative emission spectrum of crystals from *M. citricolor*-infected lesions is shown in Figure 8. In contrast, calcium-containing crystals were not observed on any of the lesions induced by H_2SO_4.

^{13}C NMR. The ^{13}C NMR spectrum of purified crystals showed a single peak at 162.4 ppm, whereas ^{1}H control did not show any signals. The spectral peak was indicative of the presence of oxalic acid, which was confirmed by comparing it with the ^{13}C shift of a known concentration of authentic oxalic acid. Thus, the ^{13}C NMR together with X-ray emission spectrum demonstrated that the crystals were composed of calcium oxalate.

Time-course of mycelial growth and oxalic acid accumulation in culture and infected leaves. Oxalic acid accumulation followed the growth curve of the fungus in broth cultures. Figure 15 shows the results of a representative experiment. Both dry weight (144 mg) and oxalic acid (0.32 g/L of broth) reached peak levels 17 days after incubation. Analysis of infected leaves showed oxalic acid levels (14.75 ± 2.6 mg/g dry weight) that were more than 3 times those of the controls (4.3 ± 1.0 mg/g dry weight).

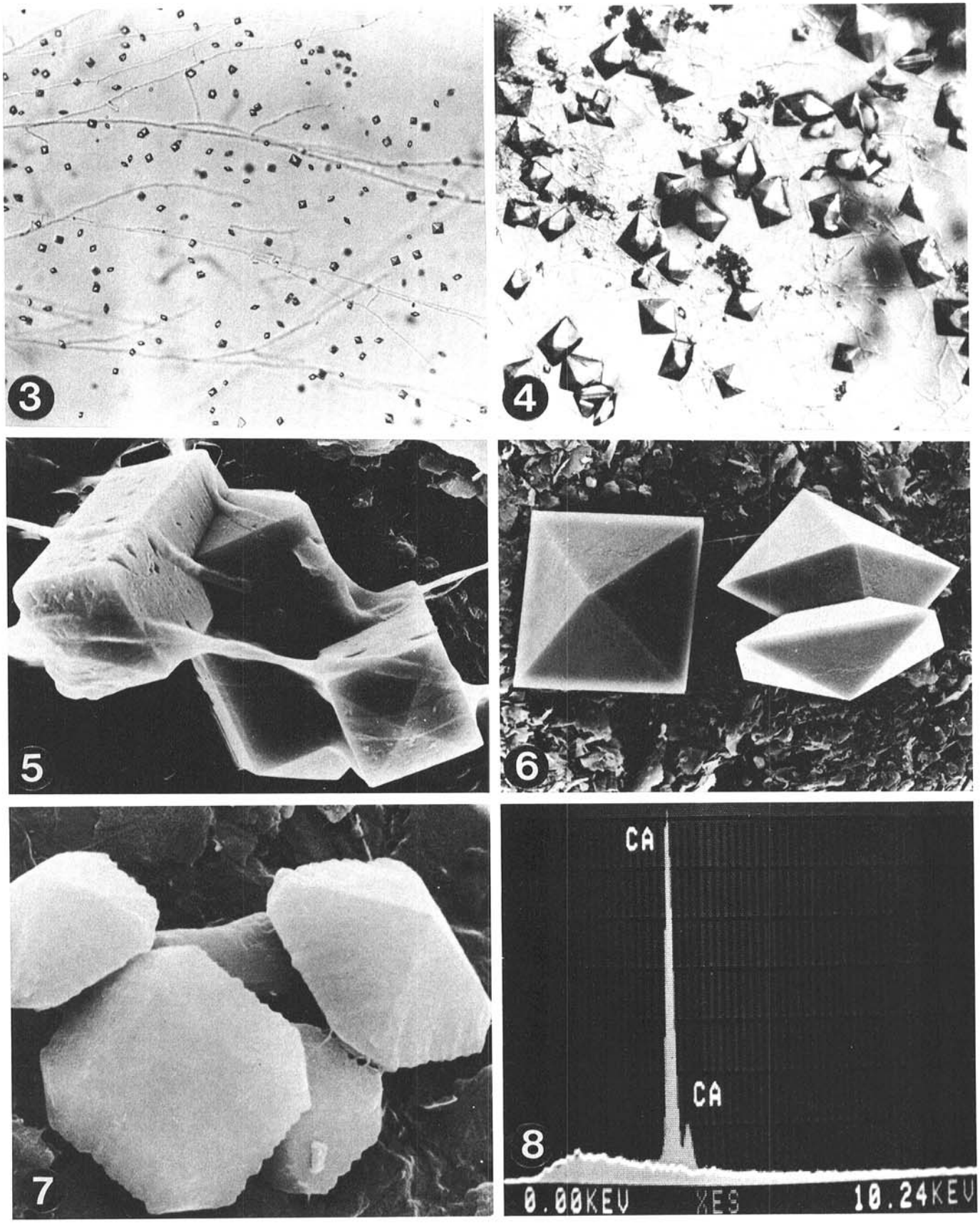
DISCUSSION

Calcium oxalate crystals may be either monohydrate (whewellite) or polyhydrate (waddellite) based on the amount of water present. Monohydrates show a monoclinic crystal pattern and polyhydrates show a tetragonal crystal system (1,8,24). Both forms are ubiquitous among higher plants and animals, and their accumulation in animals and humans may be associated with certain metabolic disorders (7,11). The information concerning their occurrence in association with fungi is limited. They are associated with ectomycorrhizal roots (16) and saprophytic fungi, and their formation in some soil and litter fungi is presumed to facilitate weathering and mobilization of cations in soil (4,12,14). They are present in plant tissue infected with *Sclerotium rolfsii* Sacc. (20).

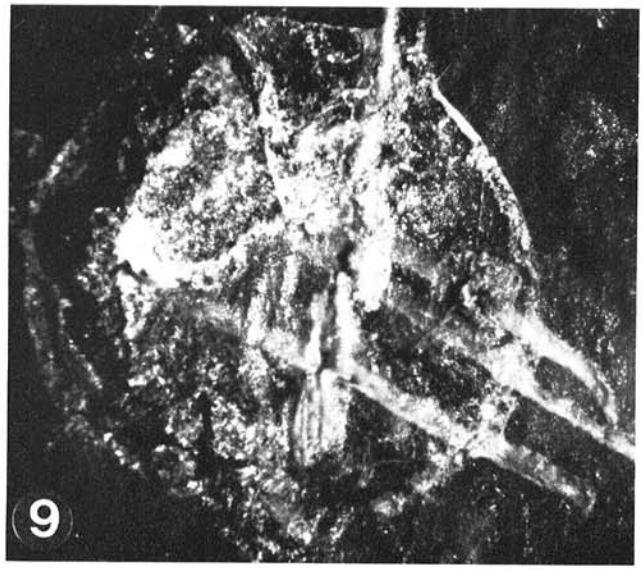
The results of this study indicated that crystals formed in culture subsequent to the growth of *M. citricolor* and in the leaf tissue upon infection with this pathogen were composed of polyhydrate calcium oxalate. Furthermore, the association of calcium oxalate crystals with symptoms induced by authentic oxalic acid solutions strongly suggests that oxalic acid may be playing a key role in the pathogenesis of this fungus on coffee. Consistent with this view, calcium-containing crystals were not observed on lesions induced by H_2SO_4. Calcium is an important constituent of pectates, the structural building blocks of middle lamella, and it is associated with membrane stability and activation of enzymes that take part in ATP synthesis (6,10). Oxalic acid secreted by the pathogen obviously must sequester calcium from these sources, resulting in the calcium oxalate formation and tissue disintegration observed in the infected tissue. Our observations support the host-calcium sequestration hypothesis for pathogenicity of oxalate-producing pathogens (2,9,13,15,18,25).

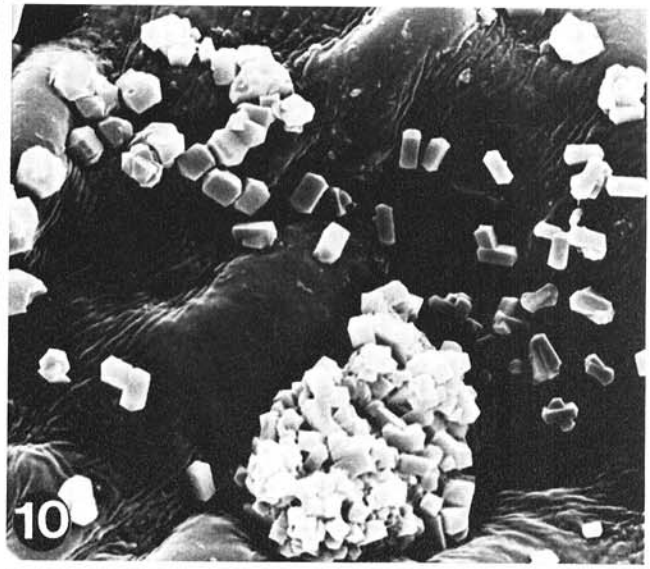
Oxalic acid is strong, the first dissociation constant (pKa = 1.23) being exceeded by only a few halogen-substituted carboxylic acids (11) such as trichloroacetic acid (pKa = 0.7). The similarity in symptoms induced by oxalic acid and H_2SO_4 indicates that lesion development may, at least in part, be the result of lowered pH brought about by oxalic acid secretion by the pathogen. It is also conceivable that oxalic acid, by lowering the cell pH, may activate enzymes such as IAA-oxidase and polygalacturonase and lead to further tissue disintegration. In fact, the optimal pH range for the activity of IAA-oxidase was shown to be 3.5–3.7 (22). Other studies with *S. rolfsii* (2) and *Sclerotinia sclerotiorum* (Lib.) de Bary (17,18) have indicated that the decrease in pH of infected tissue extracts during pathogenesis was attributed to the oxalic acid secreted by these pathogens. A similar system is thought to be operational in *Endothia parasitica* (Murr.) P. J. and H. W. Anderson as well (19). Studies are under way to demonstrate IAA-oxidase and macerating enzyme systems in *M. citricolor*-infected coffee tissue and to further understand the influence of oxalic acid on these enzymes.

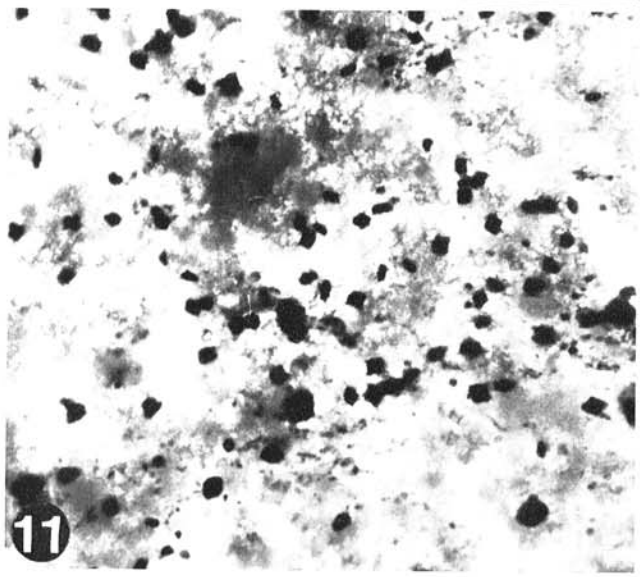
The presence of significant amounts of calcium oxalate deposits in the infected tissue is interesting. If we presume, as discussed earlier, that calcium sequestration during pathogenesis has significant pathological ramifications in the structure and physiology of the infected host, it is reasonable to assume that calcium, if present in the tissue in high amounts, may have a profound effect in conferring resistance to the host plant. Our tests

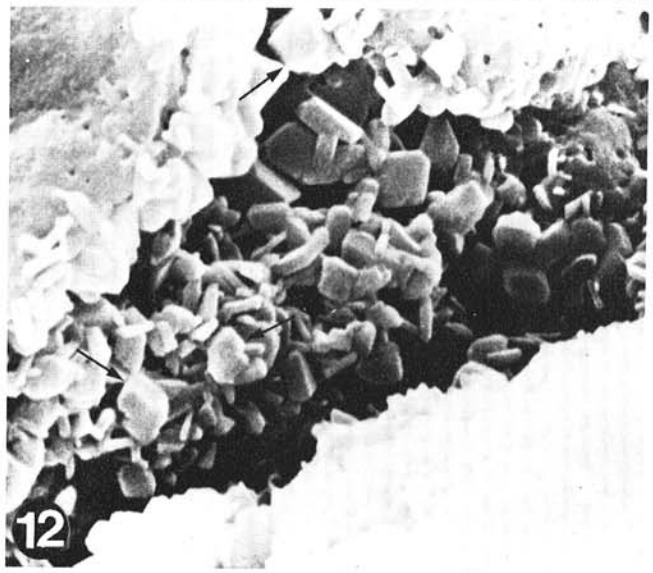


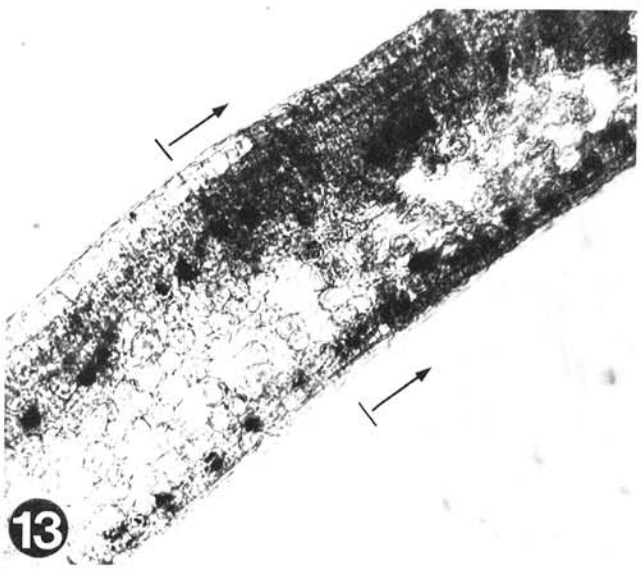
Figs. 3–8. Crystals in agar and broth cultures of *Mycena citricolor*. **3,** Light micrograph of water agar culture; note crystals adjacent to fungal mycelium (×400). **4,** Light micrograph of potato-dextrose broth (PDB) culture supplemented with 0.1% calcium carbonate ($CaCO_3$) showing bipyramids in association with mycelium (×400). **5,** Scanning electron micrograph of crystals in PDB (×3,400). **6,** Scanning electron micrograph of culture fluid from PDB supplemented with $CaCO_3$ showing bipyramids (×2,500). **7,** Bipyramids in purified preparation showing paramorphic corrosion (×2,500). **8,** Energy-dispersive X-ray spectrum of crystals on coffee leaf infected with *M. citricolor*; note K_α and K_β emission peaks for calcium. White baseline represents background spectrum.

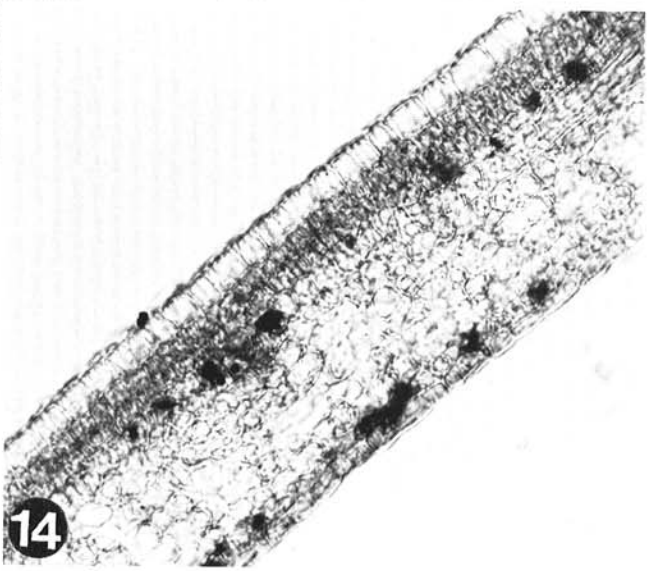












Figs. 9–14. Crystals on coffee leaf surface developed after inoculation with *Mycena citricolor*. **9,** Low-magnification view of scratched leaf surface showing shiny crystalline deposits (×10). **10,** Scanning electron micrograph with many crystals showing prismatic columns with pyramidal faces (×2,200). **11,** Crystals stained with silver nitrate-dithiooxamide. Black staining indicates that they are composed of calcium oxalate (×500). **12,** Leaf surface containing monoclinic crystals after application of 0.2% oxalic acid solution; note crystals (arrows) with pseudorhomboedric symmetry (×4,500). **13,** Transverse section of coffee leaf tissue infected with *M. citricolor*, shown after staining with silver nitrate-dithiooxamide; note lesion area (arrows) with many mesophyll cells containing black deposits of calcium oxalate. **14,** Transverse section of healthy control after staining with silver nitrate-dithiooxamide, showing only a few cells containing these deposits (×350).

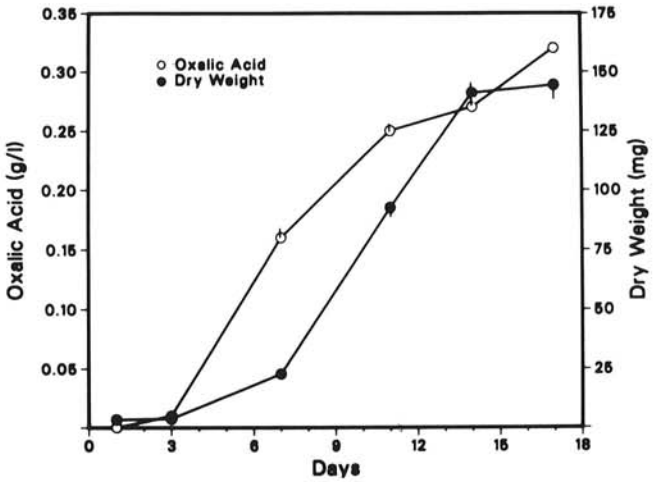


Fig. 15. Time-course of growth of *Mycena citricolor* and corresponding oxalic acid levels in potato-dextrose broth cultures. Half values of standard errors (SE) are shown as vertical bars; SE values that fall within circles were not shown.

(27) have indicated that external application of calcium to coffee leaves prior to infection with *M. citricolor* completely inhibits symptom development.

LITERATURE CITED

1. Arnott, H. J., Pautard, F. G. E., and Steinfink, H. 1965. Structure of calcium oxalate monohydrate. Nature 208:1197-1198.
2. Bateman, D. F., and Beer, S. V. 1965. Simultaneous production and synergistic action of oxalic acid and polygalacturonase during pathogenesis by *Sclerotium rolfsii*. Phytopathology 55:204-211.
3. Buller, A. H. R. 1934. *Omphalia flavida*, a gemmiferous and luminous leaf spot fungus. Pages 397-443 in: Researches on Fungi. Vol. 6. Longmans, Green, London. 513 pp.
4. Cromack, K., Jr., Sollins, P., Graustein, W. C., Speidel, K., Todd, A. W., Spycher, G., Li, C. Y., and Todd, R. L. 1979. Calcium oxalate accumulation and soil weathering in mats of the hypogeous fungus *Hysterangium crassam*. Soil Biol. Biochem. 11:463-468.
5. Delacroix, G. 1900. Les maladies et les ennemis des cafeiers. A. Challamel, Paris. 212 pp.
6. Doby, G. 1965. Plant Biochemistry. Interscience Publishers, London. 768 pp.
7. Franceschi, V. R., and Horner, H. T., Jr. 1980. Calcium oxalate crystals in plants. Bot. Rev. 46:361-427.
8. Frey-Wyssling, A. 1981. Crystallography of the two hydrates of crystalline calcium oxalate in plants. Am. J. Bot. 68:130-141.
9. Havir, E. A., and Anagnostakis, S. L. 1983. Oxalate production by virulent but not by hypovirulent strains of *Endothia parasitica*. Physiol. Plant Pathol. 23:369-376.
10. Hewitt, E. J., and Smith, T. A. 1975. Plant Mineral Nutrition. English Universities Press, London. 298 pp.
11. Hodgkinson, A. 1977. Oxalic Acid in Biology and Medicine. Academic Press, London. 325 pp.
12. Horner, H. T., Tiffany, L. H., and Cody, A. M. 1983. Formation of calcium oxalate crystals associated with apothecia of the discomycete *Dasyscypha capitata*. Mycologia 75:423-435.
13. Ikotun, T. 1984. Production of oxalic acid by *Penicillium oxalicum* in culture and in infected yam tissue and interaction with macerating enzyme. Mycopathologia 88:9-14.
14. Jones, D., McHardy, W. J., and Wilson, M. J. 1976. Ultrastructure and chemical composition of spines in Mucorales. Trans. Br. Mycol. Soc. 66:153-157.
15. Kritzman, G., Chet, I., and Henis, Y. 1977. The role of oxalic acid in the pathogenic behavior of *Sclerotium rolfsii* Sacc. Exp. Mycol. 1:280-285.
16. Malajczuk, N., and Cromack, K., Jr. 1982. Accumulation of calcium oxalate in the mantle of ectomycorrhizal roots of *Pinus radiata* and *Eucalyptus marginata*. New Phytol. 92:527-531.
17. Marciano, P., Di Lenna, P., and Magro, P. 1983. Oxalic acid, cell wall degrading enzymes and pH in pathogenesis and their significance in the virulence of two *Sclerotinia sclerotiorum* isolates on sunflower. Physiol. Plant Pathol. 22:339-345.
18. Maxwell, D. P., and Lumsden, R. D. 1970. Oxalic acid production by *Sclerotinia sclerotiorum* in infected bean and in culture. Phytopathology 60:1395-1398.
19. McCarroll, D. R., and Thor, E. 1985. Pectolytic, cellulytic and proteolytic activities expressed by cultures of *Endothia parasitica*, and inhibition of these activities by components extracted from Chinese and American chestnut inner bark. Physiol. Plant Pathol. 26:367-378.
20. Punja, Z. K., and Jenkins, S. F. 1984. Light and scanning electron microscopic observations of calcium oxalate crystals produced during growth of *Sclerotium rolfsii* in culture and in infected tissue. Can. J. Bot. 62:2028-2032.
21. Rao, D. V., Tewari, J. P., DuFresne, C., and Ayer, W. A. 1985. Calcium oxalate crystals in the cultures of *Mycena citricolor*, causal agent of the American leaf spot of coffee. (Abstr.) Phytopathology 75:1374.
22. Ray, P. M. 1960. The destruction of indole acetic acid. III. Relationships between peroxidase action and indole acetic acid oxidation. Arch. Biochem. Biophys. 87:19-30.
23. Sequeira, L., and Steeves, T. A. 1954. Auxin inactivation and its relation to leaf drop caused by the fungus *Omphalia flavida*. Plant Physiol. 29:11-16.
24. Sterling, C. 1965. Crystal structure analysis of weddellite, $CaC_2O_4(2+x)H_2O$. Acta Crystallogr. 18:917-921.
25. Stone, H. E., and Armentrout, V. N. 1985. Production of oxalic acid by *Sclerotium cepivorum* during infection of onion. Mycologia 77:526-530.
26. Tewari, J. P., Browne, L. M., and Ayer, W. A. 1984. The American leaf spot of coffee. Univ. Alberta Agric. For. Bull. 7:66-68.
27. Tewari, J. P., and Rao, D. V. 1986. Control of *Mycena citricolor*, the causal agent of the American leaf spot of coffee, by lime spraying. (Abstr.) Phytopathology 76:1140.
28. Wellman, F. L. 1961. Coffee: Botany, Cultivation and Utilization. Interscience Publishers, New York. 488 pp.
29. Yasue, T. 1969. Histochemical identification of calcium oxalate. Acta Histochem. Cytochem. 2:83-95.