Terpenoid Aldehydes in Cotton Roots Susceptible and Resistant to the Root-Knot Nematode, Meloidogyne incognita

J. A. VEECH and M. A. MCCLURE[1]

Abstract: We investigated the role of terpenoid aldehydes in the resistance of cotton (*Gossypium hirsutum*) to the root-knot nematode (*Meloidogyne incognita*). Three-day-old, root-knot-resistant ('Auburn 623') and -susceptible ('Deltapine 16') seedlings were inoculated with *M. incognita*. Comparable portions of inoculated and noninoculated roots were harvested 2, 4, 7, and 10 days later. Terpenoid aldehydes were extracted, separated by thin-layer chromatography, eluted as their phloroglucinol derivatives, and measured colorimetrically. In noninoculated seedlings of each age, the susceptible cultivar contained more total and more of each of five specific terpenoid aldehydes (hemigossypol, methoxyhemigossypol, gossypol, methoxygossypol, dimethoxygossypol) than did the resistant cultivar. In both cultivars, the concentration of terpenoid aldehydes increased as seedlings aged. After inoculation, the concentration of terpenoid aldehydes was usually highest in the noninoculated, followed by the infected susceptible, infected resistant, and noninfected resistant seedlings in that order. The changes in concentration that occurred in response to infection, particularly at 7 and 10 days after inoculation, did correlate with host resistance, i.e., there was a net loss of total and each specific terpenoid aldelhyde in the susceptible cultivar, and a net gain in the resistant. Our data do not exclude the possibility that localized synthesis of terpenoid aldehydes is involved in resistance to root-knot nematodes. *Key Words: Gossypium hirsutum*, physiology, resistance.

The cotton (*Gossypium hirsutum* L.) cultivar 'Auburn 623 RNR' (A623), from a cross between 'Clevewilt 6-3-5' and 'Mexican Wild', is a transgressive segregate for resistance to the root-knot nematode, *Meloidogyne incognita* (Kofoid and White) Chitwood (12). This resistance, which is the highest among the various cultivars of cotton, is apparently incompletely dominant and is controlled by multiple genes. It interferes with ability of the parasite to reproduce. The root-knot index, size and number of egg masses, and the number of eggs per egg mass are all lower in A623 than in less resistant cultivars of cotton. Unfortunately, some of the agronomic qualities of A623 are less than desirable. An understanding of the biochemical and physiological nature of the resistance, however, might facilitate its incorporation into other cultivated varieties of cotton.

Gossypol and gossypol-related terpenoid aldehydes (TA) are toxic compounds that occur naturally in cotton. Their synthesis can be induced by external stimuli, including pathogens (1). Because many TA are reported to be fungitoxic (13, 15), they are considered to be phytoalexins (1). Gossypol contributes to the resistance of cotton to insects and is toxic to bollworm and tobacco budworm (5). The TA apparently contribute to the resistance of cotton to verticillium wilt (2, 7, 15), and their concentrations (3) and distribution (6, 7) in various cotton tissues have been studied. Cotton stems from new sites of TA accumulation in response to infection with *Verticillium dahliae* Kleb. (7). We found nothing in the literature, however, about the TA responses in cotton infected with nematodes. This paper reports changes in concentrations of TA in root-knot-susceptible and -resistant cotton roots infected with *M. incognita*. A preliminary report has been published (14).

MATERIALS AND METHODS

Seeds of root-knot-susceptible 'Deltapine 16' (DP16) and -resistant A623 cotton were germinated in the dark at 30 C in rolls of paper germination towels ("ragdolls") as described previously (4). After 3 days, the "ragdolls" were unrolled and the seedlings removed. A 2-cm-wide strip of Miracloth (Chicopee Mills Inc., Milltown, N.J.) was placed lengthwise across and near the

Received for publication 26 January 1977.

[1]Respectively Research Plant Physiologist, U.S. Department of Agriculture, Agricultural Research Service, National Cotton Pathology Research Laboratory, P. O. Drawer JF, College Station, Texas 77840; and Professor, Department of Plant Pathology, University of Arizona, Tucson, Arizona. The authors gratefully acknowledge the gift of the 'Auburn 623' cottonseed from Dr. Ray Shepherd. Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

middle of the germination paper. Twenty-five uniform seedlings were placed on the germination paper so that the terminal 5 mm of each root tip were on top of the Miracloth strip. Another Miracloth strip was placed directly over the first so that the root tips were sandwiched between the two strips and the nematodes were then added.

Eggs of *M. incognita* were harvested from greenhouse-grown tomatoes or chili peppers by the method of McClure et al. (9). The eggs were hatched overnight at 28 C in 133 μg/ml aqueous Aretan (Plant Protection Ltd., Yalding, Kent, England). The freshly hatched larvae were washed in distilled water, concentrated by centrifugation, and suspended in water at 2,000 larvae/ml. Seedlings prepared as described previously were each inoculated with 0.1 ml of suspension (ca. 200 larvae). The inoculated seedlings and noninoculated controls were incubated for 2, 4, 7, or 10 days in controlled-environment chambers at 28 C during 14 h light and 18 C during 10 h of dark.

At each sampling time, the seedlings from 15 "ragdolls" of each cultivar, noninoculated and inoculated, were harvested. The portions of the roots sandwiched between the Miracloth strips were excised and immediately frozen in liquid nitrogen. The tissue was lyophilized and stored at –20 C for later fractionation.

The roots of some seedlings harvested 7 days after inoculation were stained with acid fuchsin in an attempt to determine the degree of infestation of *M. incognita* larvae. The larvae were counted in the root tissues beneath the Miracloth strips and in the rest of the root basipetal to the Miracloth strips.

The lyophilized root tissue was ground to a powder, and the TA extracted by constantly stirring the powder for 1 h in hexane-ethyl acetate (75:25), 100 ml/g dry wt of tissue. Distilled water, 0.5 ml/gm dry wt of tissue, was slowly added to the resulting suspension. The extract was filtered through Whatman No. 1 paper, and the filtrate was saved. By identical procedures, the residue was suspended and refiltered. The filtrates were pooled and centrifuged at 20,000 *g* for 20 min; the supernatant was reduced to dryness under vacuum. The dry residue, containing the TA, was dissolved in ethyl acetate (2.0 ml/gm dry wt of tissue) and stored at –20 C for later chromatography.

Known volumes of the TA samples in ethyl acetate were streaked onto 20- x 20-cm Polyamide 11 F thin-layer chromatography plates and separated by ascending chromatography in benzene:chloroform:methanol:acetic acid (75:23:3:1.5). A qualitative TA standard containing hemigossypol (HG), methoxyhemigossypol (MHG), gossypol (G), methoxygossypol (MG), and dimethoxygossypol (DMG) was cochromatographed adjacent to the unknown samples. The developed chromatograms were air-dried and then sprayed twice, 20 min apart, with phloroglucinol-HCl reagent (1 vol of 5% phloroglucinol in absolute ethyl alcohol and 1 vol of concd. HCl). Sprayed plates were air-dried, and the polyamide containing the individual TA-phloroglucinol derivatives were scraped from the plates. The derivatives were eluted from the polyamide with 5.0 ml absolute ethyl alcohol and constant agitation overnight.

The absorptions of the eluted phloroglucinol derivatives of the individual TA were measured at their λ_{max}^{EtOH} (HG, 553; MHG, 548; G, 550; MG, 551; and DMG, 545 nm), and their concentrations were calculated from their ϵ values (HG, 21,500; MHG, 20,000; G, 46,725; MG, 31,812; and DMG, 26,881) (3). The concentration of total TA (TTA) in each sample in ethyl acetate was determined by the reaction of 0.1 ml of sample solution with 4.9 ml of phloroglucinol-HCl reagent. The absorption at 550 nm was measured, and the concentration was calculated and expressed as gossypol equivalents. The entire experiment was repeated twice.

RESULTS

More larvae were observed in the susceptible DP16 roots (mean 5.1 ± standard error 0.7 in the inoculated zone and 11.7 ± 1.0 from the basipetal remainder) than in the resistant A623, for which the corresponding figures were 0.2 ± 0.2 and 1.8 ± 0.6.

The experiment to determine the concentrations of TA in roots gave variable results in the three replicates, although the trends were generally similar from replicate to replicate. Therefore, only the results from the third run are presented (Fig. 1).

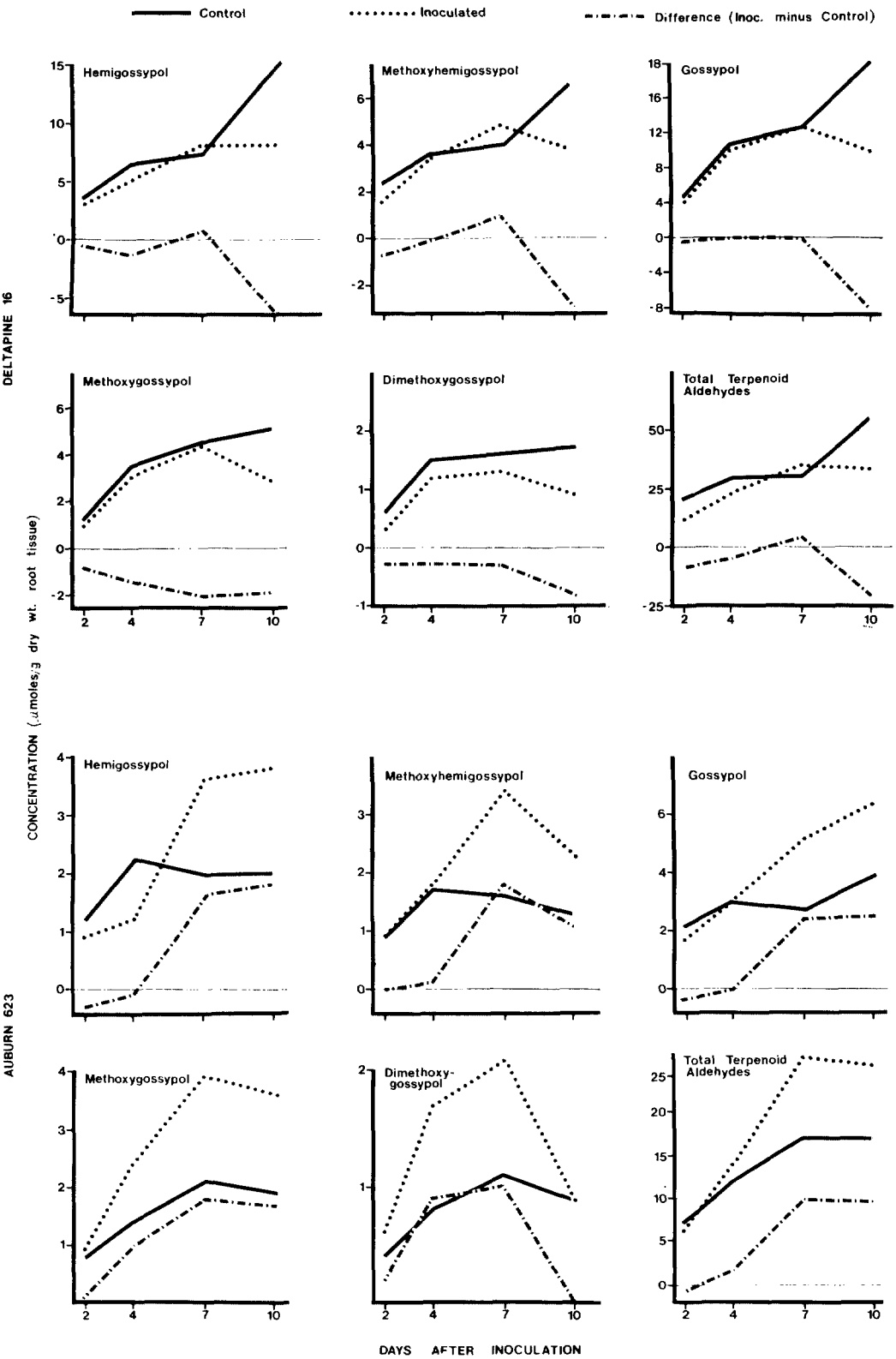

FIG. 1. The concentrations of five individual and total terpenoid aldehydes extracted from noninoculated and *Meloidogyne incognita* inoculated susceptible (Deltapine 16) and resistant (Auburn 623) cotton seedling at 2, 4, 7, and 10 days postinoculation. The changes in the concentrations (Diff) that occur as a result of infection are also shown.

Five TA, separated by thin-layer chromatography and chromogenically developed with phloroglucinol-HCl reagent, were identified as HG, MHG, G, MG, and DMG. These five compounds were found consistently in root extracts from both noninoculated and root-knot-nematode-inoculated seedlings of each cultivar at each sampling time.

In noninoculated seedlings of each age sampled, the concentrations of the individual and total TA were greater in the susceptible cultivar (DP16) than in the resistant cultivar (A623). Noninoculated seedlings of both cultivars had more of each TA and more total TA at the last sampling time than at the first, although the concentrations increased faster in the susceptible cultivar than in the resistant cultivar.

In *M. incognita*-inoculated seedlings of each age sampled, the total TA and individual TA (except DMG) were usually more abundant in the susceptible cultivar (DP16) than the resistant one (A623). Also, inoculated seedlings of both cultivars contained more of the individual and total TA at the last sampling time than at the first.

The effects of infection of TA concentrations in roots were calculated by subtracting the concentrations in the noninoculated controls from the concentrations in the inoculated seedlings. In almost every case, infection of the susceptible cultivar reduced the TA concentration, and the magnitude of the reduction increased with time. Infection of the resistant cultivar, however, nearly always increased the TA concentrations.

DISCUSSION

In a study of 12- to 21-day-old fusarium-wilt-susceptible and -resistant cottons, Raj (11) found more gossypol in the noninoculated susceptible variety than in the noninfected resistant variety. Upon infection by *Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hanson, the concentration of gossypol increased more consistently in the resistant than in the susceptible variety but always remained lower in the resistant variety. Because Raj did not separate his extracts into individual TA, our data on total TA, expressed as gossypol equivalents, should be analogous to his gossypol data, and our results do somewhat parallel his. At each age sampled, our noninoculated nematode-susceptible variety contained more than twice as much total TA as the noninoculated resistant variety. Upon infection by *M. incognita,* however, the total TA content decreased in the susceptible cultivar and generally increased in the resistant cultivar.

The preferred site for penetration of the root by *M. incognita* corresponds well with the portion of the root that is devoid of TA. Mace et al. (6) histochemically demonstrated the absence of TA in the first 3 cm of the root tip, but found high concentrations in the epidermis of the rest of the root. Minton (10) reported that most root-knot nematode larvae penetrate cotton roots in the apical 2 cm. McClure and Robertson (8) showed that penetration of cotton roots by *M. incognita* was rarely more than 2 cm from the tip and never occurred 4 cm from the tip. Perhaps, TA inhibits the entry of *M. incognita* into parts of the cotton root other than the tips. However, *M. incognita* also penetrates the roots of most other hosts only in the apical region, even though TA do not occur in most other hosts. Thus, exclusion of the nematode by TA remains speculative.

Our findings, paralleling those by Raj (11), do not establish a role for preformed individual or total TA in the resistance of cotton to root-knot nematodes. However, the observation that the concentrations of TA increase upon infection of the resistant cultivar, but decrease upon infection of the susceptible cultivar, suggest a possible role. Additional susceptible and resistant cultivars should be studied to determine any correlation between host resistance and infection-induced changes in the concentrations of TA.

LITERATURE CITED

1. BELL, A. A. 1967. Formation of gossypol in infected or chemically irritated tissues of Gossypium species. Phytopathology 57:759-764.
2. BELL, A. A. 1973. Nature of disease resistance pages 403-462. *in* Verticillium wilt of cotton, U.S. Department of Agriculture ARS-S-17.
3. BELL, A. A., R. D. STIPANOVIC, and C. R. HOWELL. 1974. Terpenoid aldehydes of Gossypium: Isolation, quantitation and occurrence. Proc. Beltwide Cotton Prod, Res. Conf., p. 40-45.

4. CARTER, W. W., S. NIETO, and J. A. VEECH. 1977. A comparison of two methods of synchronous inoculation of cotton (Gossypium hirsutum L.) seedlings with Meloidogyne incognita Chitwood. J. Nematol. 9:251-253.
5. LUKEFAHR, M. J., and D. F. MARTIN. 1966. Cotton plant pigments as a source or resistance to the bollworm and tobacco budworm. J. Econ. Entomol. 59:176-179.
6. MACE, M. E., A. A. BELL, and R. D. STIPANOVIC. 1974. Histochemistry and isolation of gossypol and related terpenoids in roots of cotton seedlings. Phytopathology 64:1297-1302.
7. MACE, M. E., A. A. BELL, and C. H. BECKMAN. 1976. Histochemistry and identification of disease-induced terpenoid aldehydes in verticillium wilt-resistant and -susceptible cottons. Can. J. Bot. 54:2095-2099.
8. McCLURE, M. A., and J. ROBERTSON. 1973. Infection of cotton seedlings by Meloidogyne incognita and a method of producing uniformly infected root segments. Nematologica 19:428-434.
9. McCLURE, M. A., T. H. KRUK, and I. MISAGHI. 1973. A method of obtaining quantities of clean Meloidogyne eggs. J. Nematol. 5:230.
10. MINTON, N. A. 1962. Factors influencing resistance to cotton to root-knot nematodes (Meloidogyne spp.). Phytopathology 52:272-279.
11. RAJ, S. A. 1974. Changes in gossypol content of cotton plants susceptible to fusarium wilt. Annamalai Univ. Agric. Res. Annu. 1974 4-5:198-200.
12. SHEPHERD, R. L. 1974. Transgressive segregation for root-knot nematode resistance in cotton. Crop Sci. 14:872-875.
13. STIPANOVIC, R. D., A. A. BELL, M. E. MACE, and C. R. HOWELL. 1975. Antimicrobial terpenoids of Gossypium: 6-methoxygossypol and 6,6'-dimethoxygossypol. Phytochemistry 14:1077-1081.
14. VEECH, J. A., and M. A. McCLURE. 1976. Terpenoid aldehydes in root-knot-susceptible and -resistant cotton seedlings. J. Nematol. 8:304.
15. ZAKI, A. I., N. T. KEEN, and D. C. ERWIN. 1972. Implication of vergosin and hemigossypol in the resistance of cotton to Verticillium albo-atrum. Phytopathology 62:1402-1406.