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# Evaluation of Cotton Polyphenols as Factors of Resistance to Root-Knot Nematode and Fusarium Wilt

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The terpenoid aldehyde content of cotton (*Gossypium hirsutum* L.) roots of a root-knot nematode (*Meloidogyne incognita* Chitwood and Otiefa) resistant strain (A623) was higher initially and increased faster after inoculation than that of a susceptible strain (M-8). The presence of gossypol and five other terpenoid aldehydes in root exudates was confirmed. These terpenoid aldehydes were also found in the soil surrounding roots where cotton plants had grown; highest concentrations occurred in soils around the resistant inoculated strain. There was not a significant negative correlation of terpenoid aldehyde concentrations in healthy roots with root-knot nematode egg masses in infected roots of 10 other strains of cotton. However, the terpenoid aldehyde concentrations of roots of 17 cotton strains were significantly negatively correlated with fusarium (*Fusarium oxysporum* f. *vasinfectum* (Atk.) Snyd. & Hans) wilt incidence.

Root-knot nematode (*Meloidogyne incognita* Chitwood and Oteifa) and fusarium wilt caused by *Fusarium oxysporium* f. *vasinfectum* (Atk.) Snyd. & Hans (FOV) can significantly limit cotton (*Gossypium hirsutum* L.) production (Smith, 1953). The root-knot nematode (RKN) alone retards plant growth by attacking the root system, causing galling of the roots and initiating other debilitating effects. The incidence and severity of many diseases of seedlings and fusarium wilt (FW) of mature plants are increased in the presence of the nematode (Cauquil and Shepherd, 1970; Martin et al., 1956).

Since 1965, work to breed cotton genotypes resistant to this nematode has resulted in the development and release of Auburn 623 RNR and the identification of several other promising strains that are now being evaluated (Shepherd, 1974, 1979a,b, 1982). These strains typically limit root-knot nematode reproduction to less than 1000 eggs per plant in 40 days following inoculation of seedling plants with 8000 eggs per plant. Progress in incorporating nematode resistance into cotton has been slow largely because identifying resistant plants in segregating populations is a laborious process. The need is evident for a rapid chemical screening procedure.

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A number of secondary plant constituents in cotton have been attributed to impart resistance to pests. Those constituents include gossypol and a number of gossypol-related triterpenoids, sesquiterpenoid quinones, hemigossypols, and heliocides (Bell and Stipanovic 1977), flavonoids (Hedin et al., 1968), an anthocyanin (Hedin et al., 1967), and condensed tannin (Chan et al., 1978). Veech (1978) reported that RKN infection induced synthesis in cotton roots of some methoxy-substituted terpenoid aldehydes, methoxyhemigossypol, methoxygossypol, and dimethoxygossypol. Veech (1979), in a continuation of this study, investigated the histochemical localization and nemotoxicity of the terpenoid aldehydes in cotton. The terpenoid aldehydes were toxic to nematodes, and they accumulated most rapidly and intensely around the head of the nematode in the pericycle of resistant cultivars. Mace et al. (1974) reported on the histochemistry and isolation of gossypol and related terpenoids in healthy roots of cotton seedlings.

Mace and Howell (1974) and Mace et al. (1978) identified the flavonols catechin and gallocatechin and hypothesized that these were condensed proanthocyanidin (tannin) precursors in the roots and stem steles of healthy and verticillium-wilt inoculated cottons. The flavonols increased upon infection. Howell et al. (1976) found that the concentrations of catechin, gallocatechin, isoquercitrin, and condensed tannins were higher in *Verticillium dahliae* Kleb. infected resistant cotton leaves than in infected, susceptible leaves.

Bugbee (1970) studied vascular responses of cotton to infection by FOV. Harrison and Beckman (1982) studied

time/space relationships in FW-susceptible and -resistant cottons inoculated with FOV. Kaufman et al. (1981) reported on the apparent involvement of phytoalexins in the resistance response of cotton plants to FOV, and Kumar and Subramanian (1980) studied the role of gossypol in disease resistance.

Giebel (1974) discussed four general mechanisms that render plants resistant to nematodes: (1) the plant may produce toxins that kill the nematode, (2) the plant may not contain sufficient nutrients for development and reproduction of the nematode, (3) the plant may not attract the nematode, and/or (4) the resistance may be based on plant tissue hypersensitivity to the nematode.

The objectives of the present study were to (1) identify chemical components that correlate with high resistance to RKN and FW in cotton roots, (2) determine what changes occur in chemical components after root invasion by RKN, (3) determine whether chemical content of cotton rhizosphere soil is affected by plant resistance in the presence of RKN, and (4) determine the effect of gossypol on in vitro growth of the FW fungus.

## MATERIALS AND METHODS

Analysis for terpenoid aldehydes was performed on cyclohexane/ethyl acetate/acetic acid, 500/500/1 (CHEA), extracts of root tissue by the phoroglucinol reaction (2% in 1/1 absolute ETOH/concentrated HCl, stand 1 h) with subsequent spectrometric analysis at 550 nm. The percent content was determined by comparison with data obtained from authentic gossypol and is expressed as gossypol equivalents. Condensed tannin analysis was performed on 70% aqueous methanol (MW) and acetone extracts of root tissue. The chromophore was developed by boiling 1 h with 1-butanol/HCl, 95/5. The percent content was determined by comparison with the color obtained at 550 nm from a purified cotton condensed tannin sample, the structure of which has recently been elucidated by Collum et al. (1981). Catechin analysis was performed on alcoholic extracts. The chromophore was developed with 2,4-dimethoxybenzaldehyde in HCl (1 mL of extract plus 3 mL of 0.5 g of DMB in 150 mL of cold ETOH and 50 mL of concentrated HCl), read at 510 nm, and compared with that obtained from an authentic sample of catechin. Phenol analysis was performed on solvent extracts of root tissue that were reacted with sodium tungstate and phosphomolybdic acid (via the Folin Dennis method; AOAC Methods 9.098–9.100, 1975). The chromophore was read at 725 nm and compared to that obtained with a tannic acid standard.

**Identification and Analysis of Terpenoid Aldehydes in Roots.** Terpenoid aldehydes were analyzed by methods adapted from the procedures of Bell et al. (1974) and identified by the procedures of Stipanovic et al. (1974). Chloroform/methanol, 2/1 (CM), extracts of roots of susceptible (M-8) and resistant (A623) strains collected 7 days after inoculation (and noninoculated roots) were concentrated and applied to silica gel TLC plates for irrigation with benzene/methanol/acetic acid, 45/8/4. The plates were sprayed with the phloroglucinol reagent, the bands were scraped from the plates, and the pigment was eluted from the silica gel powder with ethanol. The absorbancies were read at 550 nm and compared with that obtained from a gossypol standard. A second plate was edge-sprayed to locate the bands that were subsequently scraped from the plate and eluted with ethyl acetate. The eluate was analyzed by mass spectrometry via a solid probe.

**Experiments 1–3: Analysis of Polyphenols in Roots of Two Cottons Inoculated and Noninoculated with RKN.** Cotton breeding lines Auburn 623 RNR (A623) and M-8 were used in these experiments. Auburn 623 RNR is a breeding line highly resistant to RKN and FW (Shepherd, 1974), and M-8, a doubled haploid of "Deltapine 14", is highly susceptible to both diseases.

Production of RKN eggs, collection of the eggs for inoculum, and inoculation of plants were done as described previously (Shepherd, 1979b). To inoculate plants, seeds were planted in sand, and soon after emergence, one seedling per pot was transplanted into 7.6-cm pots filled with soil previously fumigated with methyl bromide. Seven to ten days before transplanting seedlings into pots, approximately 8000 RKN eggs were incorporated into the soil of each pot. Noninoculated plants were transplanted into pots treated in the same manner but without RKN eggs.

Seedlings in four pots of a treatment constituted a replication for chemical analysis. Pots were arranged in a split–split plot design with cultivars as whole plots, inoculations (plus and minus) as subplots, and roots (primary and lateral) as subsub plots. Each treatment was replicated 4 times. At harvest, roots were washed clean with tap water and then were excised.

In experiment 1, 2 cm of tips of all roots were excised, combined by entry within replications and analyzed for gossypol equivalents (GE) on the seventh day after inoculation. In experiment 2, primary and lateral root tips were prepared as in experiment 1 and then analyzed separately for GE each day from the fourth day through the ninth day after inoculation. An analysis of variance of GE was made. In experiment 3, primary roots were prepared as in experiment 1 and then analyzed for GE and phenols separately each day for the first through the eight day after inoculation.

**Experiment 4: Analysis for Polyphenols in Roots of 20 Cottons.** In this experiment, seedlings of 20 cotton strains ranging in resistance to RKN and FW were planted in sand. Strains were planted in a completely randomized design with four replications. Seven days after emergence, primary root tips (2 cm long) from four plants per replication were excised and combined for analysis.

Twelve of the 20 strains were evaluated for root-knot resistance in a greenhouse test. Four replications were arranged in a randomized complete block design with 12 plants of a strain constituting a replication. Numbers of RKN egg masses were used as the criterion of resistance rather than actual numbers of RKN eggs. The rating scale was one of visual comparison so that "1" equaled numbers on A623 and "5" equaled those on M-8 with the others falling between. The rating 1 was equivalent to 5–10 egg masses per plant, whereas the rating 5 was equivalent to 150–200 egg masses per plant, and the size of the egg masses was larger with the susceptible plants. The mean RKN egg mass index (EMI) was calculated by averaging egg mass indices for a strain over replications. Linear correlations between GE found in root tips and the RKN EMI were calculated.

Field resistance to FW of 17 of the 20 genotypes had been determined previously (Kappelman, 1978) and the ratings are listed in Table III. Eleven of the 20 genotypes were evaluated for FW resistance in a greenhouse test by employing 4 replications of 12 inoculated plants, following procedures reported by Bugbee and Pressley (1967). Correlation coefficients were calculated between gossypol equivalents in root tips and wilting percentages in field and greenhouse experiments.

Finally, the 20 cotton genotypes were analyzed for content of tannins, catechin, and phenols in the roots.

**Experiment 5: Growth of FW Fungus in Media with Different Gossypol Concentrations.** Fusarium fungus isolate no. 352, which originated from a severely wilted plant obtained in 1976 at Tallassee, AL, was used in this experiment because of its known high virulence in past studies. It was obtained from the same field where the above 17 genotypes were tested for FW resistance in experiment 4. The fungus was grown on potato dextrose agar (PDA) (Difco 0013) to which gossypol acetic acid was added to attain gossypol concentrations of 0, 20, 40, 80, 160, and 320 ppm. After autoclaving and then cooling the media, we poured about 20 mL of the gossypol and amended agar into each of 12 Petri plates per gossypol concentration. Plates were cooled overnight at ambient temperature and inoculated the next day with 9 mm diameter cores (made with a cork borer) of the fungus growth on gossypol-free PDA (Difco 0013). Following inoculation, plates were maintained at temperatures of 27 ± 0.5 °C. Fungal growth was determined by measuring the diameter (mm) of the fungal colony in each Petri dish 7 days after inoculation.

**Experiment 6: Analysis of Polyphenols in Soil from Resistant and Susceptible Cotton Roots.** Seeds of A623 and M-8 were planted in 20.3-cm pots containing soil fumigated with methyl bromide. One week after emergence, seedlings were thinned to two per pot and 8000 RKN eggs/plant were pipetted into 3 cm deep holes in the soil around each plant. Pots were arranged in a completely randomized design with six per treatment replication; each pot was considered a replication. After about 3 months, above-ground plant parts were removed without disturbing the roots, and A623 and M-8 seedlings were reestablished in their respective pots. The only inoculum present was residual inoculum on old roots remaining in pots. This cycle of establishing seedlings in the same pots and growing them for 3 months was repeated 6 times. After the last cycle, a representative soil sample (675–1055 g) was taken from each pot for analysis. In order to minimize contamination of the soil sample by roots, the soil was moistened before removal of the root system. The soil was subsequently spread in a pan to facilitate the removal of remaining visual root parts. Soils were extracted exhaustively with CHEA and MW solvents, and extracts were analyzed for phenols and GE.

**Experiment 7: Growth of Susceptible Cotton Exposed to RKN in Soil Amended with Different Concentrations of Soil Extracts and Gossypol.** Soil for filling 75 mm diameter pots was prepared as follows: (1) extracts obtained with CHEA solvents in experiment 6 were reconstituted and mixed with sterile soil in the same proportions as originally extracted from soil in A623 and M-8 pots; (2) gossypol was incorporated into serile soil at the levels 0, 1, 6, 10, and 100 μg/g of soil. After incorporation of 8000 RKN eggs into each pot, one M-8 plant was transplanted into each pot. Forty days later, soil was washed from roots and numbers of egg masses per plant were rated by using the scale of 1–5 described in experiment 4.

## RESULTS

**Experiment 1.** Extracts from M-8 and A623 roots, collected at 7 days after inoculation, contained gossypol, hemigossypol, methoxyhemigossyphol, methoxygossypol, dimethoxygossypol, and heliocide $H_1$ (Table I). Earlier, Veech (1978) had found that these same terpenoid aldehydes, except heliocide $H_1$, in cotton roots. Gossypol content per root tip was nearly 3× greater in A623 than in M-8. Total terpenoid aldehyde content per root tip was greater in the inoculated than in the noninoculated roots

Table I. Phloroglucinol-Reactive Compounds from Root Tips of Susceptible (M-8) and Resistant (A623) Cotton Breeding Lines 7 Days after Inoculation with (+) and without (−) RKN

| | | strain + and − RKN, μg/root | | | |
|---|---|---|---|---|---|
| | | M-8 | | A623 | |
| TLC band | compound[a] | + | − | + | − |
| 1 | HG | 2.5 ab[b] | 2.3 bc | 2.6 a | 2.0 c |
| 2 | MHG | 4.5 c | 4.1 c | 6.1 a | 5.6 b |
| 3 | G | 4.9 b | 4.7 b | 13.9 a | 13.5 a |
| 7 | MG | 2.9 b | 2.7 b | 5.8 a | 5.4 a |
| 5 | | 1.6 c | 1.4 c | 3.1 a | 2.7 b |
| 6 | DMG | 1.2 c | 1.1 c | 3.3 a | 2.9 b |
| 7 | | 1.0 a | 0.9 a | 1.2 a | 1.0 a |
| 8 | $H_1$ | 0.2 c | 0.2 c | 1.0 a | 0.7 b |
| | total: | 18.8 | 17.4 | 37.0 | 33.8 |

[a] G = gossypol; HG = hemigossypol; MHG = methoxyhemigossypol; MG = methoxygossypol; DMG = dimethoxygossypol; $H_1$ = heliocide. [b] Means within compounds not followed by the same letter differ significantly ($P < 0.05$) as determined by Duncan's multiple range test.

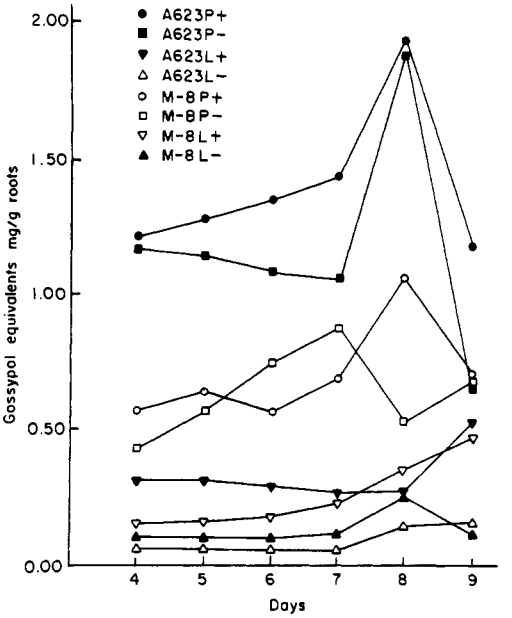


**Figure 1.** Gossypol equivalents from primary (P) and lateral (L) root tips of resistant (A623) and susceptible (M-8) seedlings at different days after inoculation with (+) and without (−) RKN.

of both cottons; the increase was more in A623 then in M-8 when inoculated roots were compared with noninoculated ones.

**Experiment 2.** Primary and lateral root tips were analyzed separately for GE daily from the fourth through ninth day after inoculation. In Figure 1, which summarizes the data, the four higher traces represent the results from susceptible and resistant primary roots while the lower four represent the corresponding lateral roots. The GE concentration was usually higher in inoculated than in uninoculated roots of both genotypes. Increases in GE concentration as the result of infection were generally greater in both primary and lateral roots of A623 than in M-8. Increases in GE concentration as the result of infection of A623 were on average as great in lateral roots as in primary roots.

**Experiment 3.** Primary root tips were analyzed for phenols and GE separately each day from the first day after inoculation through the eight day (Figures 2 and 3). This experiment was similar to experiment 2, but gathering of data was commenced earlier to investigate any very early responses, and analysis of phenols was added. The phe-

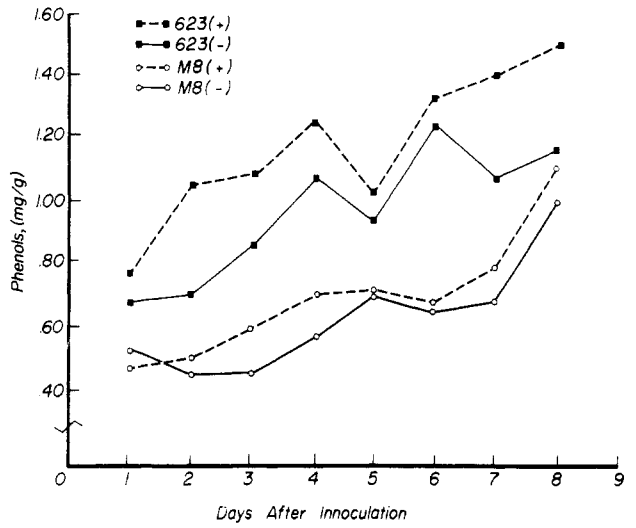


**Figure 2.** Cotton root phenols from primary root tips of resistant (A623) and susceptible (M-8) seedlings at different days after inoculation with (+) and without (-) RKN.

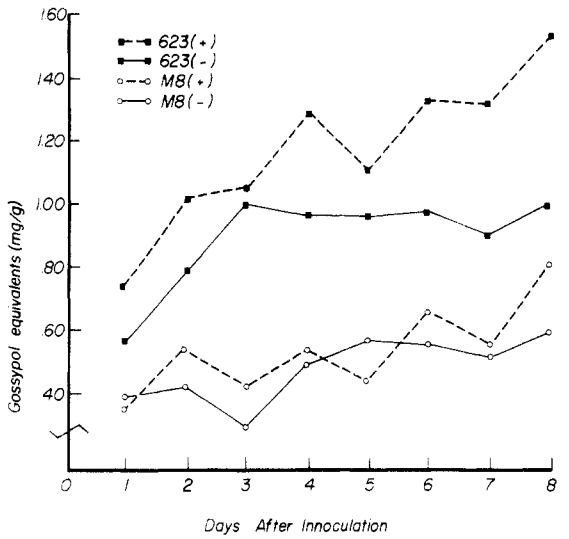


**Figure 3.** Gossypol equivalents from primary root tips of resistant (A623) and susceptible (M-8) seedlings at different days after inoculation with (+) and without (-) RKN.

nolic and GE concentrations of A623 primary roots were already higher after 1 day than those of uninoculated A623 roots (and M-8 roots) and continued to rise rapidly through the eight day. Although the phenol and GE concentrations of both inoculated and uninoculated M-8 roots rose through the 8 days, they never reached those of A623 roots. After 2 days, the phenolic GE content of inoculated M-8 roots had also increased relative to uninoculated M-8 roots, but the increment of increase was always less for M-8 than for A623. The increases in phenols and terpenoid aldehydes observed in experiments 1–3 evidently represent a phytoalexin-type response to the nematode that was greatest in the resistant genotype.

**Experiment 4.** GE concentrations in primary root tips of 7-day-old seedlings of 20 cotton strains are shown in Table II. Mean RKN EMI for 12 of these genotypes and the mean FW percentages in field and greenhouse tests for 17 of the strains are also given. The correlation between GE content and RKN EMI ($r = -0.52$) was not significant. However, the correlation between GE contents of 17 strains and FW percentages in the field ($r = -0.59$, $P < 0.05$) and between gossypol contents of 11 strains and FW percentages in the greenhouse ($r = -0.77$, $P < 0.01$)

Table II. Gossypol Equivalent Content of Primary Root Tips, RKN EMI, and Mean FW Percentage of Cotton Strains

| strain | gossypol equivalents of root tip,[a] mg/g | RKN EMI[b] | FW %[c] field | FW %[c] greenhouse |
|---|---|---|---|---|
| HG-504 | 0.96 | | | |
| LA HG-83-7 | 0.92 | | 4 | |
| HG-469 | 0.85 | | 9 | |
| McNair 511 | 0.82 | 3.3 bc[d] | 3 b | 29 ab |
| Dixie King II | 0.72 | 5.0 g | 9 c | 34 |
| A623 | 0.70 | 1.0 a | 1 a | 27 a |
| Coker 201 | 0.67 | 4.2 dg | | 57 c |
| Delcott 277 | 0.65 | 4.9 g | 3 b | 30 ab |
| BW 7631 | 0.64 | | 4 | |
| Stoneville 213 | 0.63 | 4.3 dg | 17 c | 58 c |
| PD 695 | 0.62 | | 19 | |
| Coker 310 | 0.59 | 4.0 dg | 12 c | 44 bc |
| PD 8619 | 0.56 | | 4 | |
| Deltapine 16 | 0.56 | 4.1 dg | 6 bc | 41 b |
| Auburn 56 | 0.55 | 3.5 cd | 11 c | 48 bc |
| NC-177-16-C2 | 0.50 | | 9 | |
| Rowden | 0.49 | 4.9 g | 66 d | 85 e |
| Hancock | 0.48 | 4.9 g | 40 | |
| M-8 | 0.44 | 5.0 g | 59 d | 75 de |
| Tamcot-SP-21 | 0.43 | | | |

[a] Two-centimeter sections from ends of primary roots from 7-day-old seedlings. [b] Based on an index of 1-5, with 1 equal to the number of egg masses on A623 and 5 equal to the number on M-8 roots. [c] Means are averages of four replications in field and greenhouse experiments. [d] Means within columns not followed by the same letter differ significantly ($P < 0.05$) as determined by Duncan's multiple range test.

Table III. Diameter of *F. oxysporum* Colonies after 7 Days of Growth on PDA Medium Amended with Different Concentrations of Gossypol

| content of gossypol, ppm | diameter of fungal colonies, mm |
|---|---|
| 0 | 69 a[a] |
| 20 | 64 a |
| 40 | 53 b |
| 80 | 52 b |
| 160 | 49 b |
| 320 | 52 b |

[a] Means with no letters in common are significantly different ($P = 0.05$) according to Duncan's multiple range test.

were significant, indicating that gossypol in primary roots may be important in determining resistance to FW disease.

In experiment 4, analyses were also made on the same 20 cotton genotypes for content of tannins, catechin, and phenols in roots. The contents of these compounds averaged 8.82, 17.52, and 18.84 mg/g of root, respectively. Correlations between these compounds and RKN resistance or FW resistance were not significant.

**Experiment 5.** Diameters of 7-day-old colonies of fusarium wilt fungus, isolate no. 352 grown in PDA medium amended with different concentrations of gossypol, are given in Table III. The 20-ppm concentration had a limited effect on growth, but 40 ppm significantly decreased growth. Concentrations greater than 40 ppm did not further reduce growth. Bell and Stipanovic (1978) reported that hemigossypol, desoxyhemigossypol, and their methyl ethers (rather than gossypol) are usually the predominant compounds formed as phytoalexins. Therefore, the testing of these additional compounds may have been more informative.

**Experiment 6.** This study was conducted to determine whether there were differences between resistant and

Table IV. Concentrations of Gossypol and Total Phenols Extracted from Soil in Root Zone of RKN-Resistant and -Susceptible Cotton Strains Grown in Pots of Different Times

| growth interval and cultivar | soil content, μg/g gossypol | CHEA phenols | MW phenols | gossypol in roots, μg/g |
|---|---|---|---|---|
| 40 days | | | | |
| M-8 | 1.55 a[a] | 1.24 a | 3.04 a | 0.68 |
| Auburn 623 | 0.79 a | 1.44 a | 2.10 a | 1.08 |
| 6 90-day cycles[b] | | | | |
| M-8 | 1.50 b | 1.86 b | 2.23 b | c |
| Auburn 623 | 5.58 a | 6.36 a | 3.42 d | |

[a] Means in a chemical component within a time period with letters in common were not significant ($P = 0.05$) according to Duncan's multiple range test. [b] New seedlings were started in pots after each of the first five cycles. [c] Comparisons not made because M-8 roots were too decomposed after the sixth cycle (90 days) to make reliable analysis.

susceptible cotton roots in amounts of gossypol and other phenolic compounds secreted into soil. Soil from the root zone of A623 and M-8, analyzed 40 days after inoculation with RKN, had similar amounts of gossypol, and phenols (Table IV). However, when A623 and M-8 were grown for six 90-day cycles in the same pots, soil from the A623 root zone contained 3.7× more gossypol, 3.4× more nonpolar phenols (including gossypol), and 1.5× more polar phenols (flavonoids and tannins) than soil from the M-8 root zone. This result provides evidence of a greater buildup of root secretions in greenhouse soils from resistant A623 than from susceptible M-8. The difference may be due in part to the smaller root system of RKN-inoculated M-8 plants.

**Experiment 7.** This study was made to determine if gossypol or soil leachates from resistant plants had an inhibiting effect on RKN growth and reproduction. There were no differences in RKN reproduction on susceptible M-8 plants grown in soil amended with different levels of gossypol or in soil amended with extracts from soil in which resistant A623 and M-8 had been grown previously. By comparison, the mean diameter of FW fungal colonies was decreased significantly if the culture media was amended with gossypol (experiment 5, Table III). However, gossypol may be bound in soil so that it is not as readily available as in the culture medium.

## DISCUSSION

The terpenoid aldehydes of primary and lateral root tips increase with age, are present in greater concentrations in the roots of the RKN-resistant strain A623 as compared with the susceptible strain M-8, and increase fastest after inoculation of the resistant plant. This information is at apparent odds with the report of Mace et al. (1974), who reported that there were no (or low) terpenoid aldehyde concentrations in root tips of seedlings grown in the dark in paper germination towels. However, Mace et al. (1974) did not analyze roots grown in soil and natural light, nor did they expose roots to RKN. These treatments might elicit chemical responses in roots not detectable in their absence. Such a response results from the nematodes' physical entry and initial feeding in the root tip (Veech, 1978, 1979; Veech and McClure, 1977).

Effective techniques for detecting RKN-resistant plants must allow survival of individual plants for further breeding use. Plants could survive with samples of root tips removed but probably not with larger samples removed further up on primary and lateral roots. Root tips do not necessarily contain sedentary, developing RKN as does root tissue a few days older, because root elongation continues at the tip after feeding sites are established by RKN. However, nematodes could cause a systemic chemical response resulting in enhanced accumulation of terpenoid aldehydes and phenols in the root tips. Hunter et al. (1978) reported that several terpenoid aldehydes were exuded into the soil from cotton roots and that infection of hypocotyls by *Rhizoctonia solani* increased the quantity of terpenoid aldehydes exuded by roots.

Gossypol and several related terpenoid aldehydes were found in root extracts as expected and in corroboration of the report of Veech (1978). The results in Table I, which are reported on a "per root" basis, show the greatest terpenoid aldehyde concentrations in resistant inoculated roots, largely because the mass of susceptible inoculated roots was less. Consequently, subsequent measurements were determined on the basis of root weight.

Some caution should be used in interpreting the statistically significant negative correlations between GE contents and FW percentages in the greenhouse and field, because the inoculation procedure involves direct injection of fungal spores into the stem. Under these conditions, constitutive terpenoids in the roots of healthy plants may have only a limited effect on the resistance to FW, and consequently the significant correlation may not be entirely due to a direct cause–effect relationship.

All cottons that have been reported highly resistant to RKN have also shown high resistance to FW (Shepherd, 1974). There are also numerous reports that the FW disease reaction is inconsequential (Martin et al., 1956) in the absence of a debilitating or predisposing agent, such as RKN. Therefore, RKN-resistant cultivars may be more resistant or tolerant to FW disease than RKN-susceptible cultivars because of two processes. First, a degree of RKN resistance probably prevents some predisposition to FW disease. Second, in response to RKN attack, roots of RKN-resistant cotton may produce higher levels of gossypol than those of susceptible cotton, and the gossypol may retard FW disease.

In summary, the terpenoid aldehyde content of root tips was correlated negatively with the RKN egg mass index for the resistant inoculated A623 strain as opposed to the susceptible inoculated M-8 strain. However, a significant correlation was not obtained for a group including M-8, A623, and 10 other strains. Thus, at this time, the gossypol content of root tips cannot be considered a predictor of RKN resistance. Gossypol contents of root tips were significantly correlated negatively with fusarium wilt percentage.

**Registry No.** Gossypol, 303-45-7; hemigossypol, 40817-07-0; methoxyhemigossypol, 50399-95-6; methoxygossypol, 54302-42-0; dimethoxygossypol, 1110-58-3; heliocide $H_1$, 65024-84-2.

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# Volatilization of Surface-Applied Pesticides from Fallow Soil

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Various combinations of heptachlor, chlordane, lindane, trifluralin, and dacthal were sprayed as intimate mixtures onto bare soil and allowed to remain on the surface. Three field experiments were run on two soils. Volatilization losses were estimated from the measured decrease in soil residues and by vertical flux rates calculated by using the aerodynamic method and measured vapor density profiles over the field. Initial rapid volatilization rates from a moist silt loam soil were proportional to pure-compound saturation vapor densities. Half of all chemicals except dacthal disappeared in less than 3 days. Long-term losses were controlled by diffusion from a thin layer of soil. By contrast, loss rates from a very sandy soil were much slower, probably because the lack of capillary wetting created a dry soil surface: volatilization rates remained low until moisture was supplied to the surface, and peak rates coincided with dew formation after dark.

Volatilization and air transport is a major pathway of pesticide movement, and the scientific literature abounds with evidence that many different pesticides may be found at various times in the atmosphere (Lee, 1976). Some are evidently transported long distances and return to the surface as widespread environmental contamination (Eisenreich et al., 1981; Atlas and Giam, 1981; Zell and Ballschmitter, 1980; Harder et al. 1980). Factors controlling pesticide volatilization have been extensively studied in the laboratory and in microenvironmental chambers, but relatively few field measurements of post-application volatilization losses have been reported.

In pioneering work, Parmele et al. (1972) described various micrometeorological techniques for making field-scale measurements of pesticide volatilization rates. Although several techniques are feasible, only the aerodynamic, or momentum balance method has been widely used. Using this technique, Willis et al. (1972) estimated dieldrin losses from flooded, moist, or nonflooded fallow soil. Taylor et al. (1976) estimated seasonal losses of dieldrin and heptachlor incorporated into the soil of a growing corn crop. In a follow-up study, Taylor et al. (1977) compared losses of these same two pesticides from a 10-cm stand of orchard grass. Harper et al. (1976) studied soil and microclimate effects on volatilization of trifluralin from soil. White et al. (1977) estimated seasonal losses of trifluralin following shallow soil incorporation. Turner et al. (1978) compared volatilization losses of chlorpropham from applications of emulsion and microencapsulated formulations. Cliath et al. (1980) measured volatilization losses of eptam from water and wet soil following flood irrigation of alfalfa, and Willis et al. (1980, 1983) measured toxaphene and DDT volatilization from cotton fields.

We report the results of three field experiments. In each, we applied two or more pesticides to fallow soil as a homogeneous spray mixture. Their simultaneous volatil-

Table I. Soil Properties of the Beltsville and Salisbury Field Locations

| | % composition | |
|---|---|---|
| soil separates | Hatboro silt loam (Beltsville) | Norfolk sandy loam (Salisbury) |
| sand | 23 | 75 |
| silt | 57 | 8 |
| clay | 20 | 17 |
| organic matter | 1.2 | 0.6 |

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