TERPENOID ALDEHYDE ACCUMULATION IN ROOTS OF COTTON SEEDLINGS IN RESPONSE TO INFECTION BY PYTHIUM ULTIMUM AND RHIZOCTONIA SOLANI

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Abstract

The accumulation of terpenoid aldehydes in seedling roots of four Upland cotton cultivars, Maxxa, Prema, Royale and GC 510, and two Pima cotton cultivars, S - 7 and OA 337, in response to infection by *Pythium ultimum* and *Rhizoctonia solani* was determined. The terpenoid aldehyde content of Pima cotton was initially higher than that of Upland cotton. Inoculation with both *P. ultimum* and *R. solani* stimulated terpenoid production in all cultivars with the exception of *R. solani* infection in the cultivar Prema. In Upland cotton, levels of terpenoids increased with plant age whether or not the roots were infected with a pathogen. There was no correlation between the accumulation of terpenoid aldehydes and the relative susceptibility of the cultivars to both *P. ultimum* and *R. solani.*

Introduction

Planting resistant cultivars is one of the most attractive strategies for the control of cotton (*Gossypium* spp.) diseases. Among the factors that may play a role in resistance are secondary plant constituents, including gossypol and related terpenoid aldehydes. These compounds, which have been intensively studied due to their toxicity to insects (9) and microorganisms (2), generally accumulate in lysigenous pigment glands in cotyledons of cotton seeds and in the epidermal cells of cotton roots.

Terpenoid phytoalexins were studied regarding cotton resistance to *Verticillium* wilt (*Verticillum dahliae*) (5), *Fusarium* wilt (*Fusarium oxysporum* f. sp. *vasinfectum*) (12), and the root-knot nematode - *Fusarium* wilt complex (6). In those studies terpenoid phytoalexins were present in greater concentrations in resistant cotton cultivars. Furthermore, desoxyhemigossypol (dHG) was identified as one of the most toxic terpenoids to both *Verticillium* and *Fusarium* wilt (11, 12).

Based upon the fungistatic properties of terpenoid compounds, Hunter (7) tried to relate synthesis of terpenoids in cotton seedlings with *Rhizoctonia*-induced seedling disease. In his study, inoculation with *R. solani* always increased the concentration of total terpenoids. Hunter *et al.* (8) also found that the terpenoid level in older cotton seedlings was generally higher than that in younger seedlings. Accordingly, they hypothesized that terpenoids might be involved in the age-related increased resistance to *R. solani*.

This study examined the accumulation of total terpenoid aldehydes in roots of cotton seedlings of different cultivars after inoculation with *Pythium ultimum* and *Rhizoctonia solani*, and explored the possible connection between terpenoid compounds and resistance to seedling diseases caused by *P. ultimum* and *R. solani*. The cultivars ranged from susceptible to resistant to seedling disease (*author, unpublished data*).

Materials and Methods

Four Upland cotton (*Gossypium hirsutum* L.) and two Pima cotton (*G. barbadense* L.) cultivars were used in this study. The Pima cultivars (OA 337 and S - 7) were considered resistant to both *Pythium* and *Rhizoctonia*, Maxxa and Prema resistant to *Pythium* but susceptible to *Rhizoctonia*, and Royale and GC 510 susceptible to both pathogens (*author, unpublished data*). Seeds were pre-germinated in a moist chamber on the top of 3 - 5 layers of water soaked paper towels for three days, and then placed in 30 x 48 cm seed germination papers (Anchor Paper Company, St. Paul, MN). The germination paper with emerging seeds was folded in half, loosely rolled, and placed upright in 1 - liter wide mouth jars containing 100 - 150 ml of distilled water. The jars were kept in the dark at room temperature.
Emerging seeds were inoculated when they were transferred from the moist chamber to the germination paper. Two-week-old cultures of *Pythium ultimum* (isolate from soil from the Shafter Cotton Research Station in Kern County, CA) or *Rhizoctonia solani* AG4 (isolate from a cotton seedling from the Kearney Agricultural Center in Tulare County, CA) were blended and diluted with 5% potato-dextrose agar (PDA) broth (prepared by blending one 9 cm-diameter-plate of culture with 200 ml distilled water, then diluting with 300 ml PDA broth). Each piece of germination paper contained 15 emerging seeds (one replication), which were sprayed with 10 ml of inoculum or 5% PDA broth only (noninoculated control) directed on the roots. Seedling roots were collected 24, 48, 72, and 96 hr after inoculation. Each treatment was replicated three times. The experiment was repeated once.

Terpenoid aldehydes were extracted by methods adapted from the procedures of Mace *et al.* (10) and Hunter *et al.* (8). Plants were removed from the germination paper and placed on ice. One gram of fresh roots was collected from each treatment by severing roots at the root-stem transition zone. Samples were macerated in a mortar in 10 ml of a cold solution of 0.25% $NaHSO_3$ in 95% ethanol, then filtered through two-layers of cheesecloth. The resulting homogenate was centrifuged at 15,000 g for 15 min (4 ^oC). The supernatant fluid was saved, and the pellet was resuspended in cold $NaHSO_3$-ethanol solution and

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recentrifuged. Two supernatant fluids from each sample were combined and stored at -20 °C.

In a dimly-lighted room, the supernatant fluid was mixed with 50%-saturated aqueous NaCl solution and ethyl acetate (1 : 2 : 1, v/v/v) in a separatory funnel. The lower aqueous phase was discarded, and the ethyl acetate phase was washed twice with a 50%-saturated NaCl solution and once with a saturated NaCl solution (1 : 1, v/v). The ethyl acetate extract was then dried with 10% (w/v) anhydrous Na_2SO_4 for 20 min and evaporated in a rotary evaporator at 30 °C. The residue was dissolved in 1.5 ml of dehydrated ethanol as the crude terpenoid sample.

The terpenoid aldehydes were measured by mixing 50 µl of the above ethanol extract and 50 µl of reagent (a freshly mixed solution of an equal volume of 5% phloroglucinol in 95% ethanol and concentrated HCl) for 45 min, diluting the mixture with 1.0 ml of dehydrated ethanol, and reading the absorbance with a spectrophotometer at 550 nm. Absorbance readings were converted to "gossypol equivalents" according to a standard curve prepared with gossypol (95% pure, Sigma Chemical Co., St. Louis, MO).

Data were collected and analyzed by analysis of variance and least significant difference (LSD) mean separation with MSTAT-C version 1.42 (Michigan State University, East Lansing, MI).

Results and Discussion

Disease development. A yellow discoloration of some roots was observed 24 hr after inoculation with *Pythium* and *Rhizoctonia*. On the second and third day post-inoculation, infected areas started to develop a brown discoloration; by the fourth day after inoculation, most infected roots were brown. The development of brown lesions was faster and more severe in *Pythium*-susceptible cultivars, i.e., Royale and GC 510, than that in *Pythium*-resistant cultivars. No differences in discoloration were discerned between cultivars inoculated with *R. solani*. No discoloration was observed in noninoculated seedlings.

Terpenoid aldehyde accumulation. There were significant differences ($P < 0.01$) in the accumulation of total terpenoid aldehydes among cultivars, treatments, and sampling times after inoculation. The concentrations of terpenoid aldehydes in the Pima cultivars OA 337 and S - 7 were generally higher than the concentrations in Upland cotton. In noninoculated seedlings, terpenoid aldehyde levels were 2.4 - 2.5 mg/10 g of fresh roots of Pima cultivars, and 1.0 - 1.6 mg/10 g roots of Upland cultivars. Terpenoid aldehyde levels increased in all the cultivars challenged with *Pythium*, while the levels increased in five cultivars challenged with *Rhizoctonia.* Terpenoid aldehyde levels remained constant in the cultivar Prema whether inoculated with *Rhizoctonia* or not (Figure 1).

In general, concentrations of terpenoid aldehydes in cotton roots increased as the seedlings aged. However, an exception was observed in Pima cotton. The terpenoid content declined as the seedlings of cultivar OA 337 aged, and terpenoids increased in cultivar S - 7 only when challenged by the pathogens. In cultivars of Upland cotton, the concentrations of terpenoid aldehydes kept rising similarly in both inoculated and noninoculated treatments as the seedlings aged. There was no correlation between the accumulation of terpenoid aldehydes and the relative susceptibility of the cultivars to *Pythium* or *Rhizoctonia* infection, and the nonsignificant ($P = 0.05$) coefficients of correlation ranged from -0.088 to -0.644 (Figure 2 and 3).

This study confirmed that seedling infection by *Pythium* or *Rhizoctonia* accelerates the accumulation of terpenoid compounds in most cotton cultivars. The accumulation of terpenoids was observed in both resistant and susceptible cultivars challenged with *P. ultimum* or *R. solani*, which is clearly different from the response of cotton to *Verticillium* or *Fusarium* wilt where intense terpenoid response is only displayed in wilt-resistant cultivars (4, 5). Higher concentrations of terpenoid aldehydes were detected in older cotton seedlings of most cultivars in this study, which supports the hypothesis that age-related increased resistance may be due at least in part to terpenoid accumulation (7). However, an exception was noted in this study. In cultivar OA 337, which is considered resistant to *Rhizoctonia*, levels of terpenoids decreased as the seedlings aged (Figure 1). Apparently, other factors that confer resistance are present.

Terpenoids are considered to be major phytoalexins to various microorganisms in cotton (4), and many different terpenoids have been isolated from cotton plants (2, 9, 13), but their toxicity to either *Pythium* or *Rhizoctonia* is unknown. Further studies of the toxicity of terpenoid phytoalexins to these pathogens are required before the contribution of terpenoids to cotton seedling disease resistance can be properly assessed.

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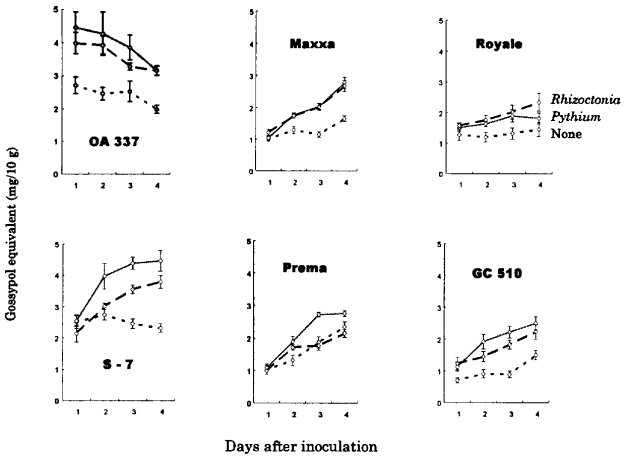

Figure 1. Terpenoid accumulation in cotton seedling roots in response to inoculation by *Pythium ultimum* or *Rhizoctonia solani*. OA 337 and S - 7 are considered resistant to both *Pythium* and *Rhizoctonia*, Maxxa and Prema resistant to *Pythium* but susceptible to *Rhizoctonia*, and Royale and GC 510 susceptible to both pathogens.

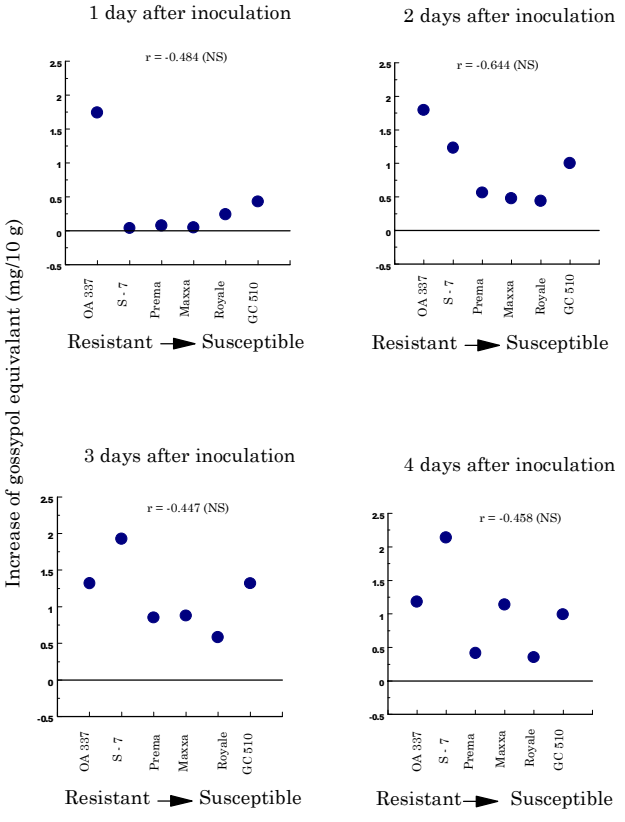

Figure 2. Increases of terpenoid level in response to inoculation by *Pythium ultimum* (NS =not significant at 5% level).

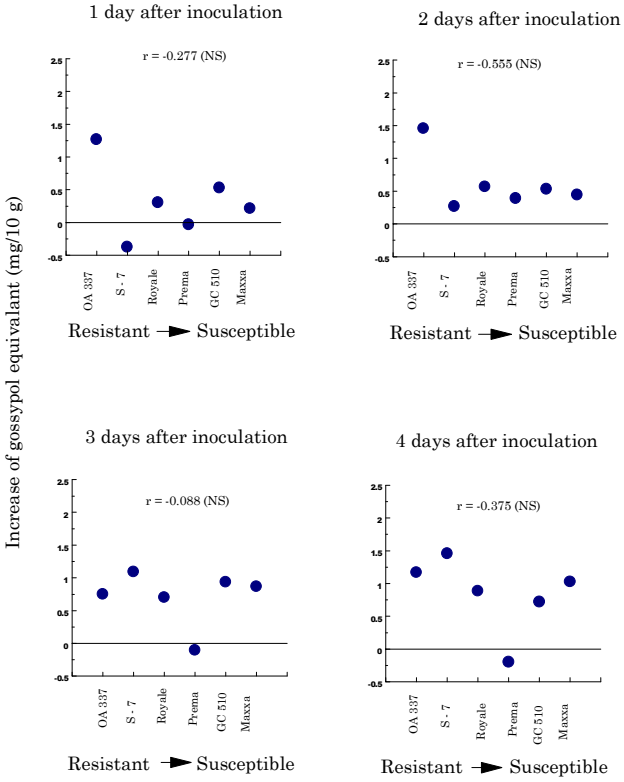

Figure 3. Increases of terpenoid level in response to inoculation by *Rhizoctonia solani* (NS =not significant at 5% level).