Effects of Catechin in Culture and in Cotton Seedlings on the Growth and Polygalacturonase Activity of Rhizoctonia solani

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ABSTRACT

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Cotton seedlings became more resistant to soreshin disease, which is incited by *Rhizoctonia solani*, as they aged from 5 to 14 days after planting. The concentration of catechins in hypocotyls of seedlings also was directly related to seedling age. The growth of three *R. solani* isolates was inhibited by (+)-catechin in a basic salts solution containing either sucrose or sodium polypectate (NaPP); inhibition of growth was directly related to catechin concentration. Thus, catechins present in seedling hypocotyls of cotton may contribute to the age-related resistance to soreshin through inhibition of pathogen growth. Polygalacturonase (PGase) activity in the NaPP medium was inhibited by (+)-catechin in cultures of the slightly virulent isolates C70 and W18, but not in cultures of the more virulent isolate 63SD2. When PGase activity of 63SD2 was expressed per unit of fungal growth, PGase activity was directly correlated with catechin concentration of the culture medium. This suggests that catechin may have induced PGase synthesis.

Additional key words: seedling disease, pectic enzymes, polyphenols, disease resistance, *Gossypium hirsutum*.

Polyphenols are present in appreciable quantities in various parts of the cotton (*Gossypium* sp.) plant (1, 14) including the hypocotyl (9). Catechins are the major components of the polyphenols found in cotton tissues (1, 7, 11). As cotton seedlings age, the concentration of catechins in hypocotyls increases and the seedlings become more resistant to the soreshin disease, which is incited by *Rhizoctonia solani* Kühn. The relationship between catechin concentration, seedling age, and soreshin resistance has been investigated relative to the polygalacturonase (PGase) activity of the causal organism. Following infection, the catechins in the seedling are oxidized and the tannins formed inhibit PGase activity of *R. solani* (9).

In addition to the possibility that condensed polyphenols may inhibit the hydrolytic enzymes of plant pathogens, the polyphenols, or their quinones, may inhibit the growth of pathogens (6). This second possibility suggested the present study in which the effect of (+)-catechin on the growth of three *R. solani* isolates is reported. The conditions of the study also provided an opportunity to examine the production and activity of PGase produced by the fungus in the presence of (+)-catechin.

MATERIALS AND METHODS

Catechin content of seedling hypocotyls.—Seedlings of *Gossypium hirsutum* L., 'Acala G 9098' were grown in rolls of germination paper in growth chambers as previously described (10) and sampled at eight different ages. The chambers had a diurnal cycle of 14 hr of light (26,000 lux) at 28 C and 10 hr of dark at 18 C. The catechin contents of hot 50% ethanolic extracts from healthy seedling hypocotyls (9) were determined colorimetrically with the dimethoxybenzaldehyde (DMB) reagent (1, 7, 11). The DMB reagent when used with extracts from cotton tissues is specific for (+)-catechin, (+)-gallocatechin, and small oligomeric condensed tannins derived from the catechins (1, 7, 11). Relationships between seedling age and catechin content were determined by regression analysis.

Fungus cultures.—*Rhizoctonia solani* was isolated from diseased cotton stem samples placed on water agar and was maintained by weekly transfer to potato-dextrose agar. For inoculum production the fungus was grown for 4 days in a basic salts solution (BSS) plus 20 g of sucrose/liter. The BSS contained 10 g KNO_3, 0.5 g KH_2PO_4, 0.25 g $MgSO_4 \cdot 7H_2O$, 0.72 mg $Fe(NO_3)_3 \cdot 9H_2O$, 0.44 mg $ZnSO_4 \cdot 7H_2O$, and 0.20 mg $MnSO_4 \cdot 4H_2O$ per liter of solution at pH 6.0. The mycelial mat was separated from the medium on a Büchner funnel and blended for 30 sec in a Waring Blendor (50 ml water/g fresh weight of mycelium).

Seedlings grown as above were inoculated by pipetting 0.2 ml of the fungus suspension on to the hypocotyl of 5-day-old seedlings just below the top of the germination paper roll. Two days after inoculation, disease reaction was graded on a scale ranging from 1 = no infection to 5 = dead plant. The three isolates used in this study, 63SD2, W18, and C70, gave disease ratings of 3.8, 2.8, and 2.8,

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respectively.

Bioassay media and methods.—For the sucrose medium, 4 g of sucrose was dissolved in 20 ml of BSS and the solution was filter-sterilized. Solutions of various concentrations of (+)-catechin (Nutritional Biochemicals Corporation, Cleveland, OH 44128) were prepared in BSS. To each of five 50-ml flasks, 18 ml of each catechin solution was added and the flasks were autoclaved. After the contents had cooled, 2 ml of the sucrose solution was added aseptically to each flask, the contents were stirred, and 2 ml of medium was added to individual 50-mm diameter petri dishes.

For the sodium polypectate (NaPP) medium, 8 g of NaPP was added to 360 ml of hot (50-60 C) BSS and mixed thoroughly. Then, 18-ml samples were autoclaved in 50-ml flasks. Catechin (1.6 g) was dissolved in 20 ml of BSS, and appropriate dilutions were made with BSS. The diluted solutions were autoclaved (10 ml/flask), and after cooling, 2 ml of each solution was added aseptically to 18 ml of BSS containing the NaPP. Then the media were added aseptically to sterile 50-mm diameter petri dishes (2 ml/dish). The final catechin concentrations in both the sucrose and NaPP media were 0, 1, 2, 4, and 8 mg/ml.

Each dish of liquid medium was inoculated in the center with a 7-mm diameter disk cut from a 4-day-old fungal mat of *R. solani* grown on BSS containing 20 g of sucrose/liter. Cultures were incubated at 21 C. The diameters of colonies were measured 26 and 43 hr after inoculation. Mean radial growth of the fungus from the edge of the inoculum disk was calculated from two measurements of the colony diameter made at right angles to each other. Each treatment was replicated five times. Only the radial growth after 43 hr is presented in Fig. 1. The concentration of catechin required for 50% inhibition of radial mycelial growth (ED_{50}) was calculated for each fungal isolate and culture medium.

Pectolytic enzyme assays.—After cultures had grown for 43 hr in the NaPP medium, 1 ml of 0.05 M citrate buffer at pH 4.7 was added to each dish and the dishes were placed at 30 C for 1 hr. The liquid from the five replications of each treatment was then decanted and pooled, and the total volume was adjusted to 15 ml with citrate buffer.

Activity of PGase was measured viscometrically in duplicate as described previously (9). Relative PGase

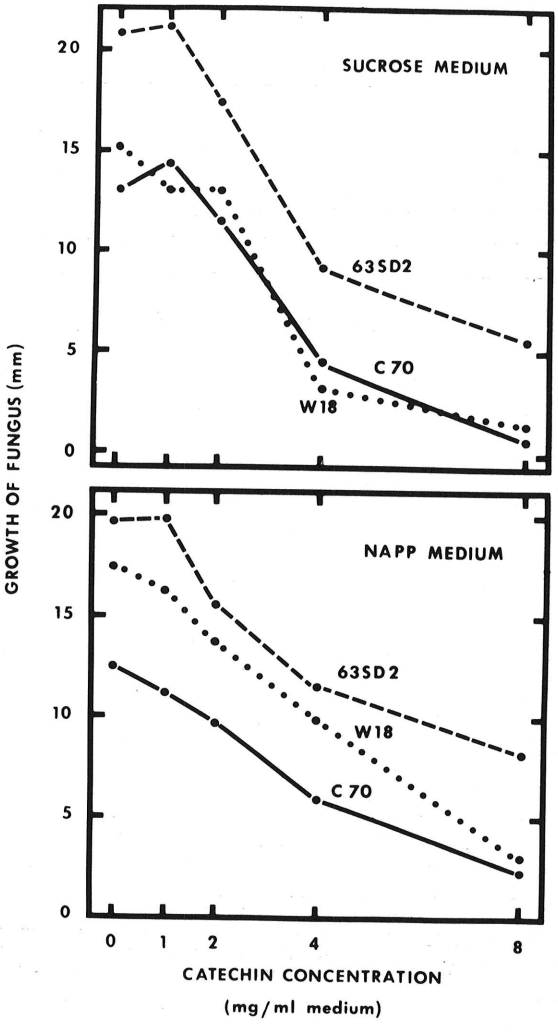

Fig. 1. Effect of various concentrations of catechin on growth of three *Rhizoctonia solani* isolates in a basal synthetic medium with sucrose or sodium polypectate (NaPP) as the carbon source. Radial growth of the colony was measured after 43 hr of growth in the medium at 21 C.

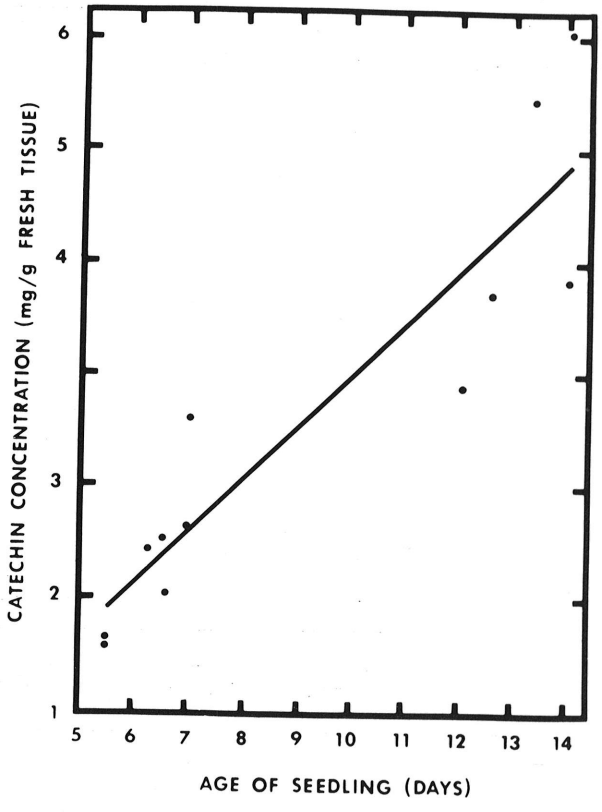

Fig. 2. Regression plot of catechin concentrations in cotton hypocotyl extracts vs. seedling age. Correlation coefficient = 0.91.

activity was expressed as 1,000 divided by the time in min at which 50% loss in viscosity of NaPP occurred. The mean relative PGase activities of each of the culture filtrates from the various catechin treatments then were divided by the mean areas of fungal growth to give PGase activity/mm^2 of fungal mat.

Activity of polygalacturonase (PGase) mixed with catechin or condensed tannins.—The culture filtrate from isolate 63SD2 in the NaPP medium containing 8 mg catechin/ml medium was stored at 4 C for 5 days. After this period, presumably because of enzyme denaturation by oxidized catechin (condensed tannins), PGase activity had disappeared from the filtrate. Then this filtrate without PGase was mixed with equal volumes of fresh culture filtrates from each of the isolates grown in NaPP medium without catechin, and the PGase activities were measured immediately. Controls containing fresh culture filtrates mixed with buffer solution also were assayed. In addition, a mixture of equal parts of the culture filtrate from 63SD2 in NaPP medium without catechin and fresh NaPP medium containing 8 mg catechin/ml was tested for PGase activity.

RESULTS

The catechin concentrations in extracts of seedling hypocotyls, when plotted against seedling age, gave a regression coefficient of 0.91, which was statistically significant, $P = 0.01$ (Fig. 2).

Media containing catechin were progressively more brown with higher concentrations of catechin. As the fungi grew, the media under the fungal mats darkened and brown precipitates formed. This darkening and precipitation was probably due to the oxidation and polymerization of catechin by oxidase activity of the fungus (15). No precipitate formation or darkening of the media occurred in sterile media containing catechin or in media without catechin in which the fungus was grown.

The radial growth of the three isolates was inversely related to the concentration of catechin in both media (Fig. 1). Although measurement of dry weights is generally considered to be the best method to determine growth differences (4), radial growth measurements were used in this study. The small amount of media (2 ml) in each 50-mm diameter petri dish restricted growth of the fungus to essentially a single plane radiating from the inoculum plug. In addition, no visual differences were observed in the type or density of mycelial growth. Therefore, I judge that the radial measurements were a reliable index of the relative effects of the treatments. The ED_{50} values for isolates 63SD2, W18, and C70 were 4.1, 2.9, and 3.3 mg catechin/ml of sucrose medium and 4.6, 4.3, and 4.1 mg catechin/ml of NaPP medium, respectively.

The PGase activity in the NaPP culture filtrates from the slightly virulent isolates C70 and W18 was inhibited when catechin was present in the medium (Table 1). At 8 mg catechin/ml medium, PGase activity was present, but was too low to cause a 50% loss in viscosity after an assay period of 4 hr. The activity of PGase in culture filtrates of 63SD2 was greater in the NaPP medium that contained 1 and 2 mg catechin/ml than in those that contained no catechin, but it was progressively lower with 4 and 8 mg catechin/ml medium. When the relative enzyme activities in the NaPP culture filtrates of 63SD2 were expressed per area of fungal growth, they were directly related to catechin concentrations (Table 1).

When the filtrate from 63SD2 in NaPP medium containing 8 mg catechin/ml was kept at 4 C for 5 days and then added to the filtrates from each of the isolates in NaPP medium without catechin, PGase activity was unchanged in fresh filtrates from 63SD2, but was inhibited 44% in those from C70 and W18 (Table 2).

TABLE 1. Polygalacturonase (PGase) activity in culture filtrates of three *Rhizoctonia solani* isolates grown in a sodium polypectate (NaPP) medium containing various concentrations of catechin

Isolate	Catechin concentration (mg/ml medium)	PGase activity[a]	
		Per volume of filtrate[b]	Per mm^2 of fungal growth ($\times 10^3$)
C70	0	28.3	35.1
	1	9.3	13.7
	2	7.5	12.1
	4	7.1	24.8
	8	Tr.	...
W18	0	27.3	20.1
	1	7.4	6.1
	2	5.7	6.2
	4	5.1	9.1
	8	Tr.	...
63SD2	0	62.5	37.7
	1	115.4	68.7
	2	78.6	70.6
	4	59.6	84.0
	8	52.5	121.4

[a]Activity of PGase was measured viscometrically (Hunter, R. E. 1974. Physiol. Plant Pathol. 4:151-159) and is expressed as 1,000 divided by time (min) at which 50% loss in viscosity of NaPP occurred.

[b]The abbreviation Tr. = trace activity; detectible, but too low to cause a 50% loss in viscosity after an assay period of 4 hr.

TABLE 2. Polygalacturonase (PGase) activity in filtrates prepared from three *Rhizoctonia solani* isolates grown in a sodium polypectate (NaPP) medium and diluted (1:1) with buffer or with filtrate from 63SD2 cultures grown in the medium containing 8 mg catechin/ml

Isolate	PGase activity[a]		
	When diluted with buffer (control)	When diluted with filtrate from culture containing 8 mg catechin/ml[b]	Percentage inactivation of PGase by filtrate with catechin
C70	18.5	10.3	44
W18	20.4	11.4	44
63SD2	59.5	61.8	0

[a]Activity of PGase was measured viscometrically (Hunter, R. E. 1974. Physiol. Plant Pathol. 4:151-159) and is expressed as 1,000 divided by time (min) at which 50% loss in viscosity of NaPP occurred.

[b]Cultural filtrate from isolate 63SD2. This filtrate was removed after 43 hr of growth and stored 5 days at 4 C; it had negligible PGase activity after storage.

When fresh NaPP medium containing 8 mg catechin/ml was added to the culture filtrate from 63SD2 in NaPP medium without catechin, PGase activity also was unchanged. Thus, the possibility that catechin or condensed tannins stimulated PGase activity was eliminated.

DISCUSSION

The toxicity of phenols to fungi was demonstrated by Cook and Taubenhaus (5) in 1911. One of the fungi that they tested was an unidentified *Rhizoctonia* spp. whose growth was retarded by 0.1-0.2% tannin. More recently Ross (13) found that the growth of two *R. solani* isolates was very slight in the presence of *p*-hydroxybenzaldehyde, ferulic acid, or vanillin at a concentration of only 0.01%. In the presence of 0.25% gallic acid, one of the isolates grew vigorously but the other grew poorly. Martin and Grossmann (12) found that a single isolate of *R. solani* grew well in the presence of 0.17% gallic acid but growth was inhibited 69.8% in the presence of 0.32% digallic acid. The results of these studies show: (i) that some phenolic compounds are effective growth inhibitors of *R. solani*, (ii) that the effective concentration for growth inhibition is different for each compound, and (iii) that isolates differ in response to the same compound. In my study, the range of ED_{50} values corresponded to 0.29-0.46% catechin in the culture media. These concentrations are close to the inhibitory concentrations of gallic acid used by Ross (13) and of digallic acid used by Martin and Grossmann (12). The three isolates of *R. solani* utilized in my experiments differed slightly in growth responses to catechin, but the differences were much less than those described by Ross (13) for two isolates cultured in the presence of gallic acid. Even though these three *R. solani* isolates showed similar patterns of inhibition, a much higher concentration of catechin was needed to inhibit their growth than that reported by Howell et al. (7) for *Verticillium dahliae*. Catechin concentrations of 1×10^{-4} M effectively inhibited growth of *V. dahliae* (7) but I found that concentrations in the range of 1×10^{-2} M were required to effectively inhibit growth of *R. solani*. However, in both my study and in that by Howell et al. (7), the concentrations of catechin that effectively inhibited growth in vitro corresponded to that present in host tissues. They found that (+)-catechin inhibited growth of *V. dahliae* at concentrations that coincided with those present in young symptomless leaves of *G. hirsutum* that had been inoculated with *V. dahliae*. In my study, the catechin concentration of seedlings between 5 and 12 days of age reached and went beyond the ED_{50} values for growth inhibition of *R. solani* isolates.

Thus, catechin concentrations in older seedlings may limit disease development by direct inhibition of *R. solani* growth. Furthermore, the concentrations of catechin in hypocotyls were based on extracts of whole hypocotyl segments, each segment being composed mainly of cortical parenchyma tissue. Previously, catechin was found to be localized primarily in epidermal and endodermal tissues of cotton seedling hypocotyls (8). Thus, cells in the epidermis that the fungus first contacts contain concentrations of catechin well in excess of the effective dose for inhibition of fungal growth.

Phenols, in addition to their effect on fungus growth, may also affect enzyme activity of the fungus. The inhibition of fungus pectic enzymes by oxidized phenols has been related to plant disease resistance in a number of instances (2). Byrde et al. (3) found that oxidized catechins were very effective inhibitors of PGase activity in culture filtrates of *Sclerotinia fructigena*. The age-related resistance of cotton seedlings to soreshin may be partially mediated through the inhibitory effect of oxidized catechin on PGase activity of *R. solani* (9).

The effect of phenolic compounds on enzyme activity usually is tested by adding the compounds to culture filtrates of the fungus. In the present study the fungus was grown in the presence of catechin and then the PGase activity was tested. The catechin, apparently oxidized by phenol-oxidizing enzymes of *R. solani* (15), reduced PGase activity of the filtrates from cultures of two of the three isolates. However, the PGase activity of one of the isolates, 63SD2, was not markedly reduced by the presence of catechin in the medium, and when the PGase activity was expressed per square millimeter of fungal growth, PGase activity increased with increasing concentrations of catechin. Neither catechin nor oxidized catechin stimulated PGase activity of this isolate; therefore, it is postulated that the presence of catechin or its oxidation products induced PGase production in culture. It is also possible that PGase production was induced in the cultures of the other two isolates. However, since the PGase from cultures of these two isolates was partially inactiviated by oxidized catechin, any possible increase in PGase production would not be evident due to inactivation of the enzyme. The present study confirms the inhibitory action of oxidized catechin on PGase activity (9) of some strains of *R. solani* but also suggests that the presence of catechin may induce synthesis of PGase in certain strains. Further studies will be needed to test this hypothesis.

LITERATURE CITED

1. BELL, A. A., and R. D. STIPANOVIC. 1972. Chemistry and nature of fungitoxic compounds in diseased cotton. Pages 87-88 *in* Proc. Beltwide Cotton Prod. Res. Conf., 10-12 January, Memphis, Tennessee. 105 p.
2. BYRDE, R. J. W. 1963. Natural inhibitors of fungal enzymes and toxins in disease resistance. Pages 31-47 *in* S. Rich, ed. Perspectives of biochemical plant pathology. Conn. Agric. Exp. Stn. Bull. 663. 191 p.
3. BYRDE, R. J. W., A. H. FIELDING, and A. H. WILLIAMS. 1960. The role of oxidized polyphenols in the varietal resistance of apples to brown rot. Pages 95-99 *in* J. B. Pridham, ed. Phenolics in plants in health and disease. Pergamon Press, New York. 131 p.
4. COCHRANE, V. W. 1958. Cultivation and growth. Pages 1-34 in V. W. Cochrane, Physiology of fungi. Wiley & Sons, New York. 524 p.
5. COOK, M. T., and J. J. TAUBENHAUS. 1911. The relationship of parasitic fungi to the contents of the cells of the host plants. I. The toxicity of tannin. Delaware Agric. Exp. Stn. Bull. 91. 77 p.
6. GOODMAN, R. N., Z. KIRÁLY, and M. ZAITLIN. 1967. Phenol metabolism. Pages 187-231 *in* R. N. Goodman, Z. Király, and M. Zaitlin. The biochemistry and physiology of infectious plant disease. Van Nostrand, Princeton, New Jersey. 354 p.

7. HOWELL, C. R., A. A. BELL, and R. D. STIPANOVIC. 1976. Effect of aging on flavonoid content and resistance of cotton leaves to Verticillium wilt. Physiol. Plant Pathol. 8:181-188.
8. HUNTER, R. E. 1972. Relationship of plant constituents to seedling diseases. Pages 86-87 *in* Proc. Beltwide Cotton Prod. Res. Conf., 10-12 January, Memphis, Tennessee. 105 p.
9. HUNTER, R. E. 1974. Inactivation of pectic enzymes of polyphenols in cotton seedlings of different ages infected with Rhizoctonia solani. Physiol. Plant Pathol. 4:151-159.
10. HUNTER, R. E., J. M. HALLOIN, J. A. VEECH, and W. W. CARTER. 1978. Terpenoid accumulation in hypocotyls of cotton seedlings during aging and after infection by Rhizoctonia solani. Phytopathology 68:347-350.
11. MACE, M. E., and C. R. HOWELL. 1974. Histochemistry and identification of condensed tannin precursors in roots of cotton seedlings. Can.J. Bot. 52:2423-2426.
12. MARTIN, J., and F. GROSSMANN. 1972. Über die Hemmung pektischer und zellulolytischer Enzyme von Rhizoctonia solani Kühn und den Einflüss einiger Hemmstoffe auf den Krankheitsprozess. I. Auslese wirksamer Enzyminhibitoren. Phytopathol. Z. 75:38-51.
13. ROSS, D. J. 1960. Physiological studies of some common fungi from grassland soils. N. Z. J. Sci. 3:219-257.
14. SADIKOV, A. S. 1965. Recent advances in the chemistry of the cotton plant. J. Sci. Indust. Res. 24:77-81.
15. TOLMSOFF, W. J. 1970. Metabolism of Rhizoctonia solani. Page 104 *in* Parmeter, J. R. Jr., ed. Rhizoctonia solani, biology and pathology. University of California Press, Berkeley. 255 p.