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Alterations in the Phenols of Papaya Fruits Infected by *Colletotrichum* spp.

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The phenolic metabolism of unripe papaya fruits inoculated with *Colletotrichum gloeosporioides*, *C. dematium* I (both virulent) and *C. dematium* II (less virulent) was altered. Total phenols accumulated rapidly and remained in high concentrations for 4 days in *C. dematium* II-host. Thereafter, total phenols markedly decreased in the virulent pathogen-host combinations. Flavonoid glycosides, (+) catechin and O-dihydroxy-phenols accumulated for a longer period in the less virulent pathogen-host combinations. Chlorogenic acid accumulated for a longer period in *C. dematium* I-host than in the fruits inoculated with *C. gloeosporioides* or *C. dematium* II. In the fruits inoculated with *C. dematium* I and *C. gloeosporioides*, phenols declined faster than in the fruits inoculated with *C. dematium* II. The significance of phenols in the resistance of papaya fruits to *Colletotrichum* is discussed.

Key Words: Phenols, Resistance, Alteration, Phenol metabolism, *Colletotrichum*, Chlorogenic acid, Papaya fruits

Introduction

Colletotrichum gloeosporioides and *C. dematium* caused serious anthracnose disease at Jabalpur (Nema & Agarwal 1960 and Srivastava et al. 1964). Unripe papaya fruits were more susceptible than the ripe ones. *C. gloeosporioides* and *C. dematium* I were more virulent than *C. dematium* II. The virulent isolates caused brown, water soaked lesions whereas the less virulent isolate induced brown but dry lesions. Development of brown pigment suggested the participation of phenolic metabolism in the fruits.

Activation of aromatic metabolism is a characteristic feature of plants under stress and its role in disease resistance has been reviewed time to time (Farkas & Kiraly 1962 and Mahadevan 1973, 1980). The phenolic metabolism of cultivars differing in resistance to different races of pathogen is yet to be fully understood.

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In this paper, we report our results on the response of papaya fruits to different races of *Colletotrichum*.

Materials and Methods

Unripe papaya fruits weighing 100 g were inoculated (3 fruits/sp) after 36 hr of collection (fresh fruit contained high amount of latex which interfered during inoculation) with 2-day-old mycelial mat of *C. dematium* I, *C. gloeosporioides*, *C. dematium* II grown on PDA slants, and incubated at 27±1°C. Lesions and surrounding tissues were separated from the fruits after different hours of inoculation and used to extract phenols (Mahadevan 1975). Total phenols (Bray & Thorpe 1954), chlorogenic acid (Zucker & Ahrens 1958), O-dihydroxyphenols (Johnson & Schaal 1957), flavonoid glycosides (Geissman 1955) and (catechin +) (Goldstein & Swain 1963) present in the extract were estimated.

Suitable control was maintained.

Results and Discussion

The pattern of phenol accumulation of papaya fruits infected by different species of *Colletotrichum* is strikingly different. Both rapid and higher accumulation of flavonoid glycosides, (+) catechin, O-dihydroxyphenols and total phenols occurred in the avirulent combination (*C. dematium* II-fruits). Preliminary results (Agrawal 1978) showed that *C. dematium* II required 9 days to cause typical symptoms of anthracnose. The inability of *C. dematium* II to cause early symptoms must be due to high phenols at the infection site. However, in later stages (after 9 days), the concentration of phenols declined although it was higher than the virulent species—host combination.

Flavonoid glycosides (FG) accumulated in the papaya fruits inoculated with the isolates of *Colletotrichum* (figure 1). In the avirulent *C. dematium* II-host interaction, FG accumulated up to 4 days and as much as 4050 μg flavonoids was detected. In contrast, in virulent *C. dematium* I-papaya interaction, the maximum amount was 2900 μg by the end of 36 hr while the *C. gloeosporioides* infected fruits contained 2880 μg of flavonoids subsequently, FG decreased drastically in the fruits inoculated with the virulent strain, however, the concentration of FG was more than in the control.

The accumulation of FG indicates the availability of precursors at the site of infection. Infected fruits contained high concentrations of polyphenol oxidase

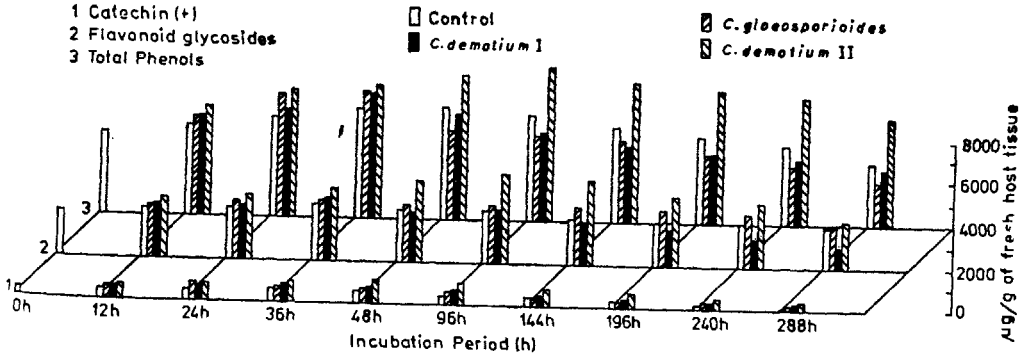

Figure 1 (+) Catechin: Flavonoid glycoside and total phenols metabolism of papaya fruits infected by *Colletotrichum* spp.

and peroxidase (Agarwal & Agrawal 1980). Colewell and McCall (1945) reported that glycosidases release the aglycones, which may be oxidized by phenol oxidase, leading to the accumulation of quinones. These quinones, being fungitoxic in nature inhibit the development of pathogens. Furthermore, the quinones polymerize which adhere to the cell wall, imparting the characteristic brown colour to the developing lesions.

(+) Catechin accumulated up to 24 hr in *C. gloeosporioides*, up to 36 hr in *C. dematium* I and up to 96 hr in the avirulent strain-host combination. In the later stages, (+) catechin decreased rapidly in the fruits inoculated with virulent isolates than in the fruits infected by less virulent (figure 1). However, O-dihydroxyphenols accumulated up to 36 hr in less virulent isolates while only up to 24 hr in case of virulent isolates. But its level dropped rapidly in virulent isolates than in the less virulent isolate. Even on 8th day of infection, *C. dematium* II host combination contained almost 10 times more O.D. phenols compared with virulent spp. Both (+) catechin and O.D. phenols are the precursors of lignin and tannin (Oku 1960), which coat the cell-wall, and make it an effective barrier against fungal penetration and hydrolytic enzyme attack (Herrman 1962 and Cobb et al. 1967). Howell et al. (1976) reported that (+) catechin (1×10^{-5}M) inhibited the mycelial growth of *Verticillium dahliae* and at 5×10^{-6}M, inhibited the conidial formation. Old cotton leaves were susceptible because they contained reduced concentrations of (+) catechin.

Total phenols accumulated up to 4 days in *C. dematium* II infected fruits, but in *C. gloeosporioides*–infected fruits, up to 24 hr and in *C. dematium* I, up to

Table 1 *O-dihydroxyphenols and chlorogenic acid metabolism of papaya fruits infected by* Colletotrichum *spp.*

Incubation period (hr)	O-dihydroxyphenols				Chlorogenic acid			
	Host parasite combinations							
	Control	1	2	3	Control	1	2	3
0	1000	—	—	—	1000	—	—	—
12	56	75	1000	1480	91	1750	1380	1000
24	93	1050	1080	2400	1270	6000	2540	3950
36	89	1030	99	3580	1500	5250	3900	2700
48	75	1010	90	3450	1430	4000	5050	2250
96	55	1000	85	2500	1080	2500	2500	1900
144	40	98	75	1900	88	1860	2000	1500
192	32	78	71	1000	86	1230	1500	1000
240	26	65	65	90	76	78	90	75
284	22	55	13	90	66	70	85	50

1, *C. gloeosporioides*/host; 2, *C. dematium* I/host; 3, *C. dematium* II/host.
Oh reading shows the initial amount of O.D. phenols and chlorogenic acid.
Results are expressed in μg/g of fresh host tissue

36 hr (figure 1). Total phenols increased by 3 times in less virulent host combination than virulent-host combination. Further, in *C. dematium* II total phenols declined slowly. The significance of total phenols, FG, (+) catechin and O.D. phenols has been emphasised in different host-parasite combinations (Cole & Wood 1961, Matta et al. 1969, Olah & Sherwood 1973, Sridhar 1972, Sridhar & Ou 1974 and Walker 1970).

Chlorogenic acid accumulated up to 24 hr in *C. dematium* II papaya fruits compared with the 36 hr in *C. gloeosporioides* and 48 hr in *C. dematium* I. With disease development it markedly declined in less virulent pathogen-host combination (figure 1). The role of chlorogenic acid in the resistance of papaya fruits is not clear. For, it accumulated in large concentrations in the virulent isolates—host interaction compared with the less virulent-host combination.

Phenols accumulated in the fruits infected by all the isolates. According to Kuc (1963), phenols accumulated by the infected plants might inactivate the enzymes of parasites which in turn prevented further advance of the parasite by limiting its source of nutrients. The susceptible combination did not accumulate inhibitor(s); the pathogen remained active and ultimately killed the cells. In the *C. dematium* II-papaya fruits, the phenol concentration was high and it persisted for a longer period than in the compatible combination. Furthermore, cell wall degrading enzyme production was less while phenol oxidase and peroxidase activity was high compared with *C. gloeosporioides* and *C. dematium* I-host (Agrawal 1978). Presumably, the rate of inhibition or inactivation of cell wall degrading enzymes was fast in the incompatible interaction. In the compatible interaction because of reduced defence, the parasite actively proliferated and caused symptoms in the fruits within 4 days of inoculation.

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