THE INFLUENCE OF CERTAIN PHENOLS UPON THE GROWTH OF EIGHT PHYSIOLOGIC RACES OF *PHYTOPHTHORA FRAGARIAE* HICKMAN

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ABSTRACT.—The effect of nine phenols, caffeic acid, D-catechin, catechol, chlorogenic acid, cinnamic acid, p-cresol, gallic acid, protocatechuic acid, and tannic acid upon the growth of 8 races (A-1 to A-8) of *P. fragariae* was studied. Chlorogenic acid, slightly stimulated the growth of races A-4 and A-5. Growth of races A-6 and A-7 was slightly enhanced by protocatechuic acid, which inhibited growth of the other races. Races A-5, A-6 and A-8 produced the maximum amount of mycelium when grown in media containing gallic acid, catechol, and protocatechuic acid, respectively. Further, for reasons not presently known, the p-cresol-medium became pink during the growth of races A-2, A-4, A-6 and A-7. With the other races the same medium remained almost colorless. The medium decreased from the adjusted pH 6.5 to 4.5 depending upon the race and phenol involved.

Several workers have implicated phenols either directly or indirectly as possible causes of disease resistance (Allen, 1959). Jarvis (1956) in a preliminary study on the mechanism of resistance of strawberry cultivars to *P. fragariae,* isolated a melanic pigment from diseased strawberry roots but was unable to isolate it from either resistant or immune varieties. He suspected that a change in the polyphenol metabolism was a factor in disease resistance.

In view of this information the authors wondered if the effect of phenols on the growth of different races of *P. fragariae* would be significantly distinct enough between races to supply a means of *in vitro* differentiation of them.

Differentiation of fungi *in vitro* has been of growing interest. Davidson et al. (1938) divided 210 species of wood-decaying fungi (growth in the presence of gallic and tannic acids) into ten growth and color reaction groups. Levisohn (1959) reported on the *in vitro* means of separating strains of root-infecting fungus *Boletus scaber.* Jarvis (1961) studied the effect of phenols on the growth of 7 isolates representing 3 races of *P. fragariae.* Gill and Powell (1966) showed that the growth characteristics of races A-1 and A-3 of *P. fragariae* were distinct from each other and also from A-2 and A-4 when grown on agar made from frozen lima-beans. Further distinguishing characteristics of these races have also been shown by determining their C and N requirements (Gill and Powell, 1965).

MATERIALS AND METHODS

Subcultures of the eight races were obtained from R. H. Converse, U.S.D.A. as follows: A-1 (BDC3 SS-1); A-2 (104a SS-1); A-3 (11374 SS-1); A-4 (93 SS-1); A-5 (9); A-6 (31 SS-1); A-7 (W-6-2); A-8 (W-44-1). Converse and Scott (1962), and Converse (1966) have sub-

stantiated the fact that these are valid races. Stock cultures were maintained on an agar medium made of frozen lima-beans (Gill and Powell, 1966) and sub-cultured at intervals of approximately 14 days.

The races were grown in a basal medium as follows: sucrose, 5.0 g; KH_2PO_4, 0.5 g; $MgSo_4.7H_20$; 0.25 g; vitamin-free casein hydrolysate (acid) 10% solution (obtained from N.B.Co., Cleveland, Ohio), 2.5 ml; $CaC1_2$, 30.0 mg; thiamine hydrochloride, 100 ppm; microelement stock solution, 1.0 ml; distilled-deionized water to make 1 liter. The microelement stock solution consisted of $FeC1_3.6H_20$, 2.0 mg; $ZnSO_4.7H_20$, 1.6 mg; $CuSo_4.5H_20$, 0.4 mg; $MnSO_4.H_20$, 0.2 mg; MoO_3. 0.14 mg; distilled-deionized water to make 100 ml of solution.

Nine phenols (TABLE 1 and FIG. 1) were added singly at the rate of 0.001 M to the basal medium. The media were adjusted to pH 6.5 with either NaOH or HC1. It was determined again after autoclaving at 15 lbs psi for 20 min, and at the end of the growth period.

Erlenmyer flasks containing 50 ml of medium were inoculated with 5 mm agar disks taken from the margin of actively growing cultures and maintained as still cultures at 20° C for 25 days. After this period the mycelial mat from each flask was filtered off using Whatman's No. 42 filter paper, rinsed 2 to 3 times with distilled water to remove residual contaminants, dried to a constant weight at 80° C, cooled in a dessicator over $CaC1_2$, and weighed. Six replications were used for each treatment.

RESULTS AND DISCUSSION

The effects of the phenols on growth are shown in TABLE 1 and FIGURE 1. In general, they were inhibitory but some appeared to stimulate the growth of certain races. The most inhibitory were caffeic acid, catechol, cinnamic acid, and tannic acid. Chlorogenic and protocatechuic acids were the only ones to give stimulation to some races. These results are similar to the findings of other workers (Christie, 1965; Jarvis, 1957 and 1958; Le Tourneau et al., 1957). Jarvis (1961) found different growth responses of seven isolates representing three races of *P. fragariae* to certain polyphenols. He reported that gallic acid, protocatechuic acid, chlorogenic acid, D-catechin, and DL-tyrosine at 0.001 M increased growth. Growth was inhibited by cinnamic acid, pyrocatechol, and p-cresol. Christie (1965) correlated the toxicity of phenolic compounds with their molecular structures on the growth of *P. cactorum* (Lab. and Cohn) Schroet. and *P. parasitica* Dast. He pointed out that phenols were more toxic with the OH group in the *ortho* rather than *para* position.

While there was some stimulation to growth of races A-4 and A-5 by chlorogenic acid and to A-6 and A-7 by protocatechuic acid, the differences from the controls were not considered significant. There appears to be no conclusive reasons as to why certain phenols are toxic to the

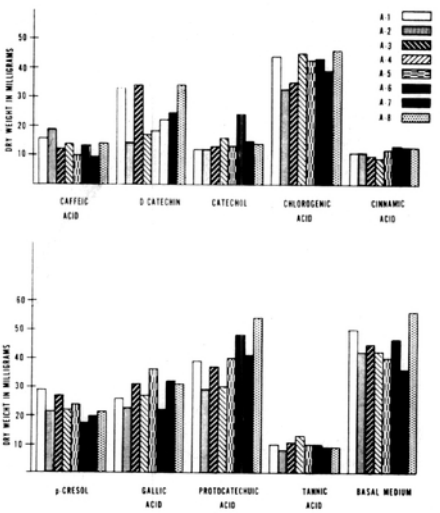

FIGURE 1. — Dry weight of the mycelium in mg after 25 days incubation at 20 C of eight races (A-1 to A-8) of *P. fragariae* in the presence of various phenols added to the basal medium.

TABLE 1. — Dry wt. of the Mycelium in mg of the races of *P. fragariae* in Basal Medium Incorporated with Various Polyphenols Separately and the Relative pH of the Media After Autoclaving; also After (in Parentheses) Growth Period of 25 Days at 20° C.

Phenol used	pH of the medium after autoclaving	A-1		A-2		A-3		A-4		A-5		A-6		A-7		A-8	
		mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH	mg	pH
Caffeic acid	6.6	15.7	(4.9)	19.2	(6.0)	12.0	(5.4)	13.7	(5.7)	9.9	(5.1)	13.4	(5.8)	9.4	(6.0)	13.9	(5.0)
D-Catechin	6.4	33.2	(4.5)	18.6	(5.6)	34.1	(4.7)	16.9	(5.0)	18.2	(4.7)	22.2	(5.4)	24.5	(5.1)	34.3	(5.0)
Catechol	6.4	11.8	(4.7)	11.9	(5.7)	13.0	(5.8)	15.7	(6.1)	12.7	(5.1)	23.4	(5.1)	14.8	(5.9)	13.4	(5.0)
Chlorogenic acid	6.5	44.2	(4.7)	32.5	(5.3)	35.2	(5.0)	45.0	(5.2)	42.5	(4.8)	42.7	(5.4)	39.1	(5.0)	46.0	(5.1)
Cinnamic acid	6.6	11.0	(5.6)	10.8	(5.9)	9.9	(6.0)	9.3	(6.0)	11.7	(6.0)	12.8	(6.2)	12.7	(6.1)	12.6	(5.9)
p-Cresol	6.5	28.7	(4.5)	21.4	(5.5)	27.0	(4.9)	21.7	(5.0)	23.7	(4.6)	17.5	(5.3)	20.0	(5.4)	21.5	(4.9)
Gallic acid	6.4	26.0	(4.7)	22.5	(5.7)	30.6	(4.9)	26.8	(5.2)	35.7	(5.1)	22.3	(5.6)	31.8	(5.2)	30.6	(5.0)
Protocatechuic acid	6.5	38.6	(4.7)	29.1	(5.5)	36.9	(5.0)	29.6	(5.5)	40.3	(4.9)	47.7	(5.5)	41.3	(5.4)	53.4	(5.0)
Tannic acid	6.0	10.0	(4.9)	7.0	(5.7)	10.5	(5.2)	13.3	(5.5)	10.0	(4.8)	9.8	(5.7)	8.3	(5.5)	8.4	(5.4)
Basal medium without phenol	6.6	49.9	(4.9)	42.3	(5.7)	44.5	(5.3)	41.8	(5.5)	40.0	(5.1)	46.5	(5.6)	35.4	(5.4)	56.1	(5.6)

[1] Average of four replications.

growth of fungi while others are stimulatory.

Autoclaving did not affect the pH of the media, except when tannic acid was used but following growth a number of the phenolic media had dropped to pH 4.5. (TABLE 1). There was no correlation between the mycelium production and post-growth pH.

A significant observation involved a change in color of the p-cresol medium during incubation. Races A-2, A-4, A-6 and A-7 caused a change in this medium from colorless to pink to cherry pink. The same medium remained almost colorless with A-1, A-3, A-5 and A-8. In the pink-colored media the pH ranged from 5.0 to 5.5 for the different races. In the colorless medium the pH ranged from 4.4 to 5.0 in the respective races. There is no explanation for this phenomenon at this time.

LITERATURE CITED

ALLEN, P. G. 1959. Physiology and biochemistry of defense. pp. 435-467 in Plant Pathology. An Advanced Treatise (Ed. by J. G. Horsfall and A. E. Dimond). Academic Press, New York.

CONVERSE, R. H. 1967. Physiologic races of *Phytophthora fragariae* on strawberry in California, Oregon and Washington. Phytopathology 57. (In Press)

————, and D. H. SCOTT. 1962. Physiologic specialization in *Phytophthora fragariae*. Phytopathology 52: 802-807.

CHRISTIE, T. 1965. The effects of some phenolic compounds upon the growth of two species of *Phytophthora*. New Zealand J. Agr. Res. 8: 630-650.

DAVIDSON, R. W., W. A. CAMPBELL, and D. J. BLAISDELL. 1938. Differentiation of wood-decaying fungi by their reactions on gallic acid and tannic acid medium. J. Agr. Res. 57: 683-695.

GILL, H. S. and DWIGHT POWELL. 1965. Differentiation *in vitro* of pathogenic races A-1, A-2, A-3, and A-4 of *Phytophthora fragariae*. Phytopathology 55: 1059-1060 (Abstr.).

————. 1966. Studies on growth characteristics of physiologic races A-1, A-2, A-3, and A-4 of *Phytophthora fragariae* Hickman on different media. Mycopathologia et Mycologia Applicata. 29:217-225.

JARVIS, W. R. 1956. Report of the Mycology Section. Ann. Rep. Scottish Hort. Res. Inst. 1955-56, p. 26.

————. 1957. In Fourth Annual Report, 1956-57, Scottish Hort. Res. Inst., p. 55.

————. 1958. In Fifth Annual Report, 1957-58, Scottish Hort. Res. Inst., p. 51.

————. 1961. Growth of isolates of *Phytophthora fragariae* Hickman in the presence of various polyphenols. Trans. Brit. Mycol. Soc. 44: 357-364.

LE TOURNEAU, D., J. G. MCCLEAN, and J. W. GUTHRIE. 1957. Effect of some phenols and quinones on growth *in vitro* of *Verticillium alboatrum*. Phytopathology 47: 602-606.

LEVISOHN, I. 1959. Strain differentiation in a root-infecting fungus. Nature, London, 183: 1065-1066.

Manuscript received May 19, 1966.