Carbohydrate Specificity for Fusaric Acid Synthesis

David Davis

Research Associate, The New York Botanical Garden, Bronx, New York 10458.

Supported by Public Health Service Research Grant AI-03527 from the National Institute of Allergy and Infectious Diseases and General Research Support Grant 5-SO1 FRO 5621-03.

The author thanks Antoinette Peragine and Grace Groves for technical assistance.

Accepted for publication 12 August 1969.

ABSTRACT

Fusaric acid is not produced by *Fusarium oxysporum* ff. sp. *conglutinans, dianthi, lycopersici, niveum,* or *vasinfectum* on Richard's medium containing pectin, polypectate, or carboxy-methylcellulose as the sole carbon source. Large quantities are produced on 2% glucose, but only trace amounts on 0.05% glucose. Since the fluids in vessel elements of tomato, for example, contain no more than 0.01% combustible solids, and since cell-wall constituents cannot be used for fusaric acid synthesis, it is probable that the xylem parenchyma is the major source of nutrient for fusaric acid synthesis. Phytopathology 60:111-113.

Wilting, vascular discoloration, and chlorosis, classical symptoms of fusarial wilt diseases caused by the special forms of *Fusarium oxysporum* Schlecht., are apparent when the pathogen is still chiefly confined to the vessel lumina following initial invasion through the root tip (10). Profuse fungal growth in vessel lumina does not necessarily induce disease symptoms, since vessels in roots of resistant Pan America tomato may be heavily infected with mycelium of *F. oxysporum* f. sp. *lycopersici,* yet plants are symptomless (9). Symptoms in tomato are, however, caused by fungal toxic products (1, 4, 5). Gäumann and his co-workers (5) have evidence that fusaric acid promotes disease. Fusaric acid, however, cannot be the sole determinant in pathogenesis, since it is also present in resistant plants shortly after inoculation (3). If fusaric acid is a contributing factor in disease development, sufficient amounts and kinds of nutrient must be present in the vessels for synthesis of toxic concentrations of this metabolite.

Potential sources of substrate for fusaric acid synthesis are fluids normally flowing through vessels of healthy plants, polysaccharide components of the vessel wall, and leakage of nutrient from toxin or enzyme-damaged xylem parenchyma contiguous to infested vessel lumina. The objective of this investigation was to determine the amounts and kinds of carbohydrate and nitrogen compounds (potentially available to the pathogen in the vessel lumina) which can be used for fusaric acid synthesis.

MATERIALS AND METHODS.—Richard's (4) medium, or modifications thereof, were used in all experiments. Two per cent glucose, pectin, sodium polypectate, or sodium carboxymethylcellulose were compared as carbohydrate sources, while equimolar (.088 M) DL-aspartic acid, ammonium sulfate, and potassium nitrate were compared as nitrogen sources for fusaric acid production. In nitrogen source experiments, 5% glucose was substituted for 5% sucrose, the prescribed carbohydrate in Richard's medium. The initial pH of all media was adjusted to 5.0, with KOH and agar added to make a 1.5% concentration. Ten-ml aliquots were placed in 25 × 100 ml tubes and autoclaved 15 min at 121 C. The tubes were inoculated with a 4-mm-diam plug removed with a cork borer from the margin of a 7-day-old *Fusarium* colony growing in petri dishes (100 × 20 mm) containing 20 ml of Richard's agar medium with glucose as the carbon source. The inoculated tubes were incubated at 28 C for 14 days, then placed in a freezer at −15 C for storage. For work-up, the agar cultures were melted in a boiling water bath and the mycelial mats removed with a forceps and dipped in boiling distilled water. The washed mats were transferred to porous crucibles where free water was extracted under suction. The remaining water was evaporated overnight in a vacuum oven at 80 C. Immediately following the removal of mycelial mats, the pH of the melted agar was adjusted to 4.0 with 1 N KOH or 1 N HCl, and the hot agar medium extracted three times with equal volumes of ethyl acetate. The ethyl acetate extract was concentrated in vacuo and the dried residue analyzed for fusaric acid content as previously described (3).

The following formae speciales of *F. oxysporum* were used in this investigation: *conglutinans* (Wr.) Snyd. & Hans.; *dianthi* (Prill. & Del.) Snyd. & Hans.; *lycopersici* (Sacc.) Snyd. & Hans.; *niveum* (E.F.Sm.) Snyd. & Hans.; and *vasinfectum* (Atk.) Snyd. & Hans. Isolates of these forms were received from various sources and tested upon arrival and again at irregular intervals for pathogenicity on plants grown aseptically in test tube culture (2). Within 1 year of this study, at least one isolate of each form listed in Table 1 was tested (except *F. oxysporum* f. sp. *vasinfectum*) and found to be selectively pathogenic to either cabbage, carnation, tomato, or watermelon. Most of the other isolates were pathogenic to their respective suscepts within 2 years or less of these experiments. All stock cultures were grown on potato-carrot agar slants and stored at 10 C under mineral oil. Many isolates preserved in this manner were still highly pathogenic after 8 years.

RESULTS.—With two exceptions, neither pectin, polypectate, nor carboxymethylcellulose were suitable carbon sources for fusaric acid synthesis by the 20 *Fusarium* isolates examined (Table 1). Only one isolate of *F. oxysporum* f. sp. *niveum* and one of *F. oxysporum* f. sp. *conglutinans* produced fusaric acid on pectin. Milligram quantities were produced on modified Richard's medium containing 2% glucose. Mycelial growth was greatest and about equal on glucose and

TABLE 1. The effect of various carbohydrates and nitrogenous compounds on in vitro fusaric acid production and mycelial growth of isolates of *Fusarium oxysporum*

		mg Fusaric acid/g mycelium dry wt[a]						
		Carbohydrate source (2%)				N source (0.088 M)		
F. oxysporum f. sp.	Isolate no.	Glucose	Pectin	Poly-pectate	Carboxy-methyl-cellulose	Aspartic acid	Potassium nitrate	Ammonium sulfate
lycopersici	1	2/.12	t/.13	t/.05	t/.01	5/.4	2/.3	1/.4
	2	1/.13	t/.12	t/.03	t/.05	4/.3	3/.2	2/.1
	3	2/.14	t/.15	t/.04	t/.01	4/.5	4/.4	1/.4
vasinfectum	1	1/.14	t/.13	t/.04	t/.02	2/.3	2/.3	<.5/.3
	2	2/.12	t/.18	t/.05	t/.02	10/.4	2/.4	<.5/.4
	3	1/.12	t/.14	t/.05	t/.01	3/.3	5/.3	<.5/.2
niveum	1	<.5/.22	t/.15	t/.04	t/.01	<.5/.7	2/.4	1/.3
	2	<.5/.17	t/.14	t/.04	t/.01	1/.6	1/.4	<.5/.4
	3	1/.12	t/.11	t/.05	t/.01	7/.3	1/.2	1/.2
	4	2/.09	t/.12	t/.04	t/.01	13/.4	2/.3	1/.1
	5	2/.09	1/.07	t/.02	t/.01	1/.2	10/.2	10/.1
	6	<.5/.23	t/.17	t/.06	t/.01	<.5/.5	1/.5	1/.3
conglutinans	1	2/.07	t/.09	t/.04	t/.01	3/.3	3/.2	<.5/.2
	2	9/.08	t/.09	t/.04	t/.02	2/.3	2/.2	3/.1
	3	t/.10	t/.04	t/.02	t/.01	1/.3	<.5/.2	<.5/.1
	4	3/.06	1/.06	t/.04	t/.01	2/.2	2/.2	2/.1
dianthi	1	2/.16	t/.13	t/.05	t/.01	2/.6	2/.4	2/.3
	2	<.5/.10	t/.10	t/.04	t/.01	<.5/.4	1/.3	2/.2
	3	2/.19	t/.15	t/.09	t/.02	2/.7	2/.4	2/.1
	4	<.5/.12	t/.12	t/.04	t/.01	2/.4	2/.3	1/.2

[a] Amounts obtained from three 25- × 100-mm tubes each containing 10 ml modified Richard's medium. t Denotes less than 20 μg fusaric acid.

pectin, but sharply reduced on polypectate and carboxymethylcellulose (Table 1). The sparse growth on the latter substrates cannot in itself explain why only a trace of fusaric acid was produced on carboxymethylcellulose or polypectate. Appreciable fusaric acid (130 μg/30 ml) was produced by *F. oxysporum* f. sp. *lycopersici* on a 2% glucose supplemented Richard's medium when mycelial growth was still as sparse (after 48 hr of incubation) as growth on a medium containing 2% carboxymethylcellulose or 2% polypectate after incubation for 2 weeks. All isolates examined produced only trace amount (<5 μg/30 ml) fusaric acid on Richard's medium containing less than 0.05% glucose, while fewer than 50% of these isolates produced more than trace amounts on a 0.5% glucose medium.

All isolates of *F. oxysporum* ff. sp. *lycopersici* and *vasinfectum* produced less fusaric acid (but not less growth) on ammonium sulfate than on aspartic acid or potassium nitrate containing Richard's medium. In contrast, the amount of fusaric acid produced on these nitrogen sources by isolates of *F. oxysporum* ff. sp. *niveum, conglutinans,* and *dianthi* followed no discernible pattern. Mycelial growth produced by these isolates was consistently less on ammonium sulfate than on aspartic acid and potassium nitrate (Table 1).

DISCUSSION.—There is insufficient utilizable carbohydrate in vessel elements of healthy plants for fusaric acid to accumulate in toxic concentrations after infection. Less than 0.01% carbohydrate is present in the root pressure exudate of tomato plants (8, 11); none can be detected shortly after decapitation (8). This investigation demonstrated that most isolates at best produce only trace amounts of fusaric acid on 10 times this level of glucose. Furthermore, relatively large amounts of fusaric acid are required for a phytotoxic effect (5). Since cell-wall constituents, namely pectin, pectate, and cellulose, are not substrates for fusaric acid synthesis, leakage of nutrient from membrane injured xylem parenchyma contiguous to the vessel element is the most likely source of carbohydrate for synthesis of this metabolite. If this hypothesis be correct, there is a self-perpetuating mechanism of fusaric acid production in susceptible plants. Parenchyma cells adjoining and slightly above invaded vessels would be damaged by accumulation of newly generated fusaric acid in areas of restricted water flow due to vessel plugging (6). As a consequence, more hexose would be released into vessel elements with a greater resulting potential for fusaric acid synthesis.

Dead vessel elements are probably incapable of active defense against disease. The contiguous xylem parenchyma of resistant varieties may, however, possess the necessary apparatus to inhibit disease. Either hyposensitivity or hypersensitivity of parenchyma cells to specific fungal toxins, high concentrations, and/or highly fungitoxic kinds of aglycones in the xylem parenchyma, and accumulation of parenchyma-derived tyloses in vessels could reduce the rate of disease development.

LITERATURE CITED

1. DAVIS, D. 1954. The use of intergeneric grafts to demonstrate toxins in the *Fusarium* wilt disease of tomato. Amer. J. Bot. 41:395-398.
2. DAVIS, D. 1963. Investigations on physiology of selective

pathogenicity in *Fusarium oxysporum* in test tube culture. Phytopathology 53:133-139.

3. DAVIS, D. 1969. Fusaric acid in selective pathogenicity of *Fusarium oxysporum*. Phytopathology 59:1391-1395.
4. FAHMY, T. 1923. The production by *Fusarium solani* of a toxic excretory substance causing wilting in plants. Phytopathology 13:541-550.
5. GÄUMANN, E. 1957. Fusaric acid as a wilt toxin. Phytopathology 47:342-357.
6. GOTTLIEB, D. 1944. The mechanism of wilting caused by *Fusarium bulbigenum* var. *lycopersici*. Phytopathology 34:41-59.
7. LUDWIG, R. A. 1952. Studies on the physiology of hydromatic wilting in the tomato plant. McDonald Col., McGill Univ., Tech. Bull. 20. 38 p.
8. OVERBEEK, J. VAN. 1942. Water uptake by excised root systems of tomato due to non-osmotic forces. Amer. J. Bot. 29:677-683.
9. SNYDER, W. C., K. F. BAKER, & H. N. HANSEN. 1948. Interpretation of resistance to *Fusarium* wilt in tomato. Science 103:707-708.
10. WALKER, J. C. 1969. Plant Pathology. 819 p. McGraw-Hill Book Co., N.Y.
11. WOOD, R. K. S. 1961. *Verticillium* wilt of tomato—the role of pectic and cellulolytic enzymes. Ann. Appl. Biol. 49:120-139.