Further Studies on the Relationship Between Cultural Characteristics and Pathogenicity in Ceratocystis ulmi

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ABSTRACT

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Cultural variability among *Ceratocystis ulmi* isolates of known pathogenicity was studied. Four agar media were used, and radial growth, aerial mycelium production, formation of a striate radial growth pattern, and dry weight of mycelium production per square centimeter of colony area were measured. The more aggressive isolates generally grew faster, produced a striate radial growth pattern, and produced less dry weight of mycelium per square centimeter of colony area than less aggressive ones. Aerial mycelium production was associated with pathogenicity only on two of the media. Although some isolates within each pathogenicity type possessed combinations of cultural characteristics intermediate to those typical of either the more or less aggressive isolates, multivariate discriminant analyses on these cultural characteristics accurately classified the isolates in their respective pathogenicity types.

Cultural characteristics and pathogenicity vary among isolates of *Ceratocystis ulmi* (Buis.) C. Moreau, the Dutch elm disease fungus (4,10,16,17). Recently in Great Britian and the United States (2,3,7,13,14), variation in cultural characteristics was associated with variation in pathogenicity. The cultural characteristic most consistently associated with pathogenicity has been the ability of more aggressive isolates to grow faster than less aggressive ones on agar media. Brasier and Gibbs (2,6) demonstrated that, on an Oxoid malt extract agar medium, more aggressive isolates produced more aerial mycelium than less aggressive ones, and only more aggressive ones produced a striate radial growth pattern. Schreiber and Townsend (13,14) found no relationship between pathogenicity and aerial mycelium production when *C. ulmi* isolates from the United States were tested on a malt extract or a potato dextrose agar medium.

Our preliminary studies showed that more aggressive isolates did not consistently produce more aerial mycelium on agar media than less aggressive ones, and the dry weight of mycelium produced per square centimeter of colony area appeared to be associated with pathogenicity. Because of the apparent inconsistencies in the relationship between pathogenicity and aerial mycelium production on agar media and our preliminary observations on mycelial dry weight, additional work on cultural variability in *C. ulmi* was undertaken. The purpose of this report is to define further the relationship between pathogenicity in *C. ulmi* and certain cultural characteristics produced on agar media A preliminary report on a portion of this work has been published (8).

MATERIALS AND METHODS

The pathogenicity of all isolates of *C. ulmi* used was determined previously by inoculation trials (7,14). For clarity only two categories, "more aggressive" and "less aggressive," are used to describe isolate pathogenicity (Table 1). Twelve isolates were supplied by L. R. Schreiber, USDA-SEA, Shade Tree and Ornamental Plants Laboratory, Delaware, OH 43015 (14). Seven isolates were supplied by H. S. McNabb Jr., Botany and Plant Pathology, Iowa State University, Ames, IA 50010 (7) (Table 1).

All isolates were maintained in screw-top tubes at 5 C on a glucose and yeast extract (GYE) agar medium (12). Before transfer to experimental media, each isolate was incubated on the GYE medium for 5 to 7 days at 25 C. Agar plug inocula were taken from the advancing margins of cultures of each isolate and transferred to 10 replicate plates of each experimental medium. Cultures were incubated on the experimental media for 6 days in total darkness at 20 C.

Four cultural characteristics were recorded: radial growth, aerial mycelium production, formation of a striate radial growth pattern

TABLE 1. Origin, code, and pathogenicity of isolates of *Ceratocystis ulmi* used in cultural studies

Origin	Code	Pathogenicity	Ref.[a]
Alabama[b]	ALA-1	More aggressive	(14)
Colorado[b]	COLO-1	More aggressive	(14)
Great Britain[c]	GB-1	More aggressive	(7)
Great Britain[c]	GB-2	More aggressive	(7)
Illinois[b]	ILL-1	More aggressive	(14)
Iowa[b]	IA-1	More aggressive	(7)
Massachusetts[b,d]	MASS-1	More aggressive	(14)
Missouri[b]	MO-1	More aggressive	(14)
North Dakota[b]	ND-1	More aggressive	(14)
Wisconsin[b]	WIS-1	More aggressive	(14)
Great Britain[c]	GB-3	Less aggressive	(7)
Great Britain[c]	GB-4	Less aggressive	(7)
Iowa[c]	IA-2	Less aggressive	(7)
Iowa[c]	IA-3	Less aggressive	(7)
Massachusetts[b]	MASS-2	Less aggressive	(14)
Maine[b]	ME-1	Less aggressive	(14)
North Carolina[b]	NC-1	Less aggressive	(14)
Ohio[b]	OH-1	Less aggressive	(14)
Tennessee[b]	TENN-1	Less aggressive	(14)

[a]The references cited refer to work that determined isolate pathogenicity.
[b]Isolates supplied by L. R. Schreiber, U.S. Dep. Agric., Sci. and Educ. Admin., Shade Tree and Ornamental Plants Laboratory, Delaware, OH 43015.
[c]Isolates supplied by H. S. McNabb, Jr., Botany and Plant Pathology, Iowa State University, Ames, IA 50010.
[d]This isolate was shown to be less aggressive by Schreiber and Townsend (14) but more aggressive in subsequent inoculation trials (9).

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(Fig. 1), and dry weight of mycelium. Radial growth values were obtained by averaging to the nearest millimeter the lengths of four radii per culture. Aerial mycelium production was determined visually as: 1 = none, 2 = limited, 3 = moderate, and 4 = abundant (Fig. 1). The striate radial growth pattern was recorded as: 1 = present or 2 = absent (Fig. 1). To determine dry weight of mycelium, all cultures were steamed until the agar melted. The mycelial mat of each culture was collected on a fine cloth (12) and washed with hot distilled water (5,12). The washed mats were dried at 100 C for 24 hr and dry weights were determined. The milligrams of dry weight of mycelium produced per square centimeter of colony area was calculated from the average radial growth values of each culture.

Four agar media were used. The defined agar medium (GA) contained 10.0 g of glucose, 2.0 g of L-asparagine, 1.0 g of KH_2PO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.2 mg of Fe^{++}, 0.2 mg of Zn^{++}, 0.1 mg of Mn^{++}, 0.1 mg of pyridoxine hydrochloride, 15.0 g of Difco Bacto agar, and 1,000 ml of distilled water (12). The initial pH was adjusted to 6.0 ± 0.1 with KOH or H_2SO_4 before autoclaving. A second medium (GYE) was similar to GA except that 2.0 g of Difco Bacto yeast extract replaced the L-asparagine and the initial pH was not adjusted. Other media that contained natural products were Difco potato dextrose agar (PDA) and Difco malt extract agar (MEA). All media were autoclaved and dispensed into sterile glass petri plates.

Analyses of variance (15) were used to determine whether the cultural characteristics produced on each medium differed between the more and less aggressive isolates. Multivariate discriminant analyses also were conducted to determine whether isolates could be classified by pathogenicity type based on cultural characteristics (11). Because of the nested structure of the experimental design and the requirements of the multivariate discriminant analyses, the 10 values for each isolate on each medium were averaged before the analyses were conducted (1).

RESULTS

The more aggressive isolates grew faster, produced less dry weight of mycelium per square centimeter of colony area, and produced more aerial mycelium on the PDA and GYE media than less aggressive ones. The more aggressive isolates also produced a striate radial growth pattern, but less aggressive ones generally did not. These differences were significant at the 0.01 level on each medium (Table 2). The production of aerial mycelium on the MEA and GA media was not associated with pathogenicity (Table 2). Isolates were correctly classified in their respective pathogenicity types from these cultural characteristics and the multivariate discriminant analyses (Table 2).

The cultural characteristics of several isolates seemed to be intermediate to those typical of either pathogenicity type (Table 2). Isolate ILL-1 grew slower and produced more dry weight of mycelium on the GYE and GA media than other more aggressive isolates. The less aggressive isolates OH-1 consistently grew as fast as some of the more aggressive ones, and isolates GB-4 and ME-1 consistently produced less dry weight of mycelium than other less aggressive ones. Less aggressive isolates MASS-1, NC-1, and OH-1

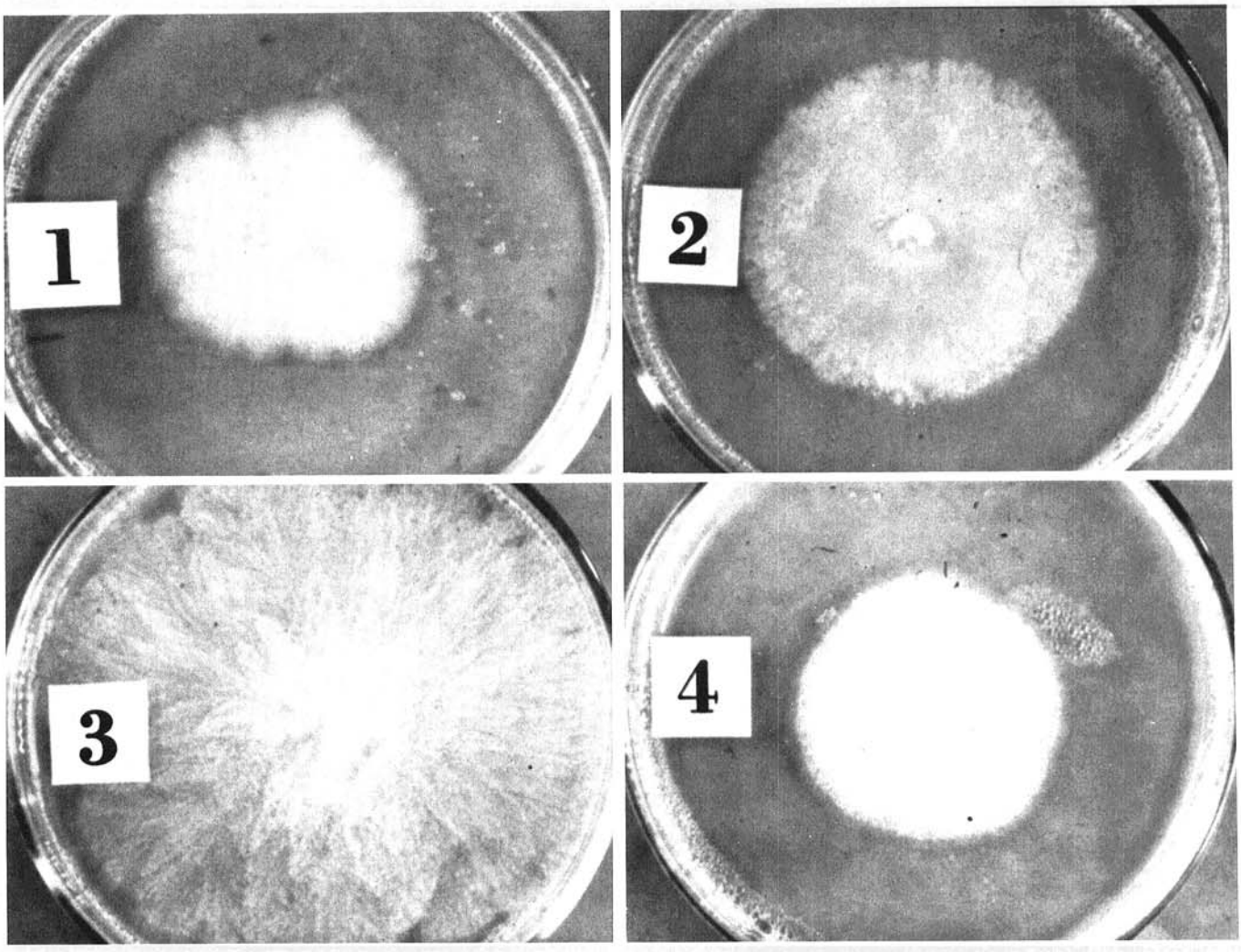

Fig. 1. Cultures illustrate rating scales for aerial mycelium production and for striate radial growth pattern formation. The numbers in the plates indicate the aerial mycelium ratings as: 1 = none, 2 = limited, 3 = moderate, and 4 = abundant. The culture in plate 3 produced striate radial growth, and those in plates labeled 1, 2, and 4 did not.

produced the striate radial growth pattern on some media, whereas other less aggressive ones did not (Table 2).

Not all media supported equal amounts of cultural growth and development (Table 2). Medium MEA supported the least aerial mycelium and mycelial dry weight production. Medium GA did not support as much radial growth as other media but generally supported the most extensive and variable aerial mycelium production (Table 2).

DISCUSSION

The association of radial growth and the striate radial growth pattern with pathogenicity was not surprising; other workers have observed similar relationships (2,6,7,13,14). The dry weight of mycelium produced per unit colony area, however, was an additional characteristic that could be associated with pathogenicity. Our results indicate an inverse relationship between radial growth and the amount of mycelium produced per unit of agar media. The combination of slower radial growth and greater dry weight of mycelium production per unit colony area by less aggressive isolates suggests that these isolates produce denser mycelial mats or mycelium composed of materials that contributes more to dry weight than those of the more aggressive ones. How these growth characteristics might be involved in the pathogenicity differences of these isolates is not known.

Although aerial mycelium production varied, only that produced on the PDA and GYE media was associated with pathogenicity (Table 2). These results are not consistent with the results of earlier work (2,3,6,13,14). The subjective nature of the evaluations for aerial mycelium production may partly explain these inconsistencies (Fig. 1); different workers may have evaluated production differently. In addition, Schreiber and Townsend (13,14) and Brasier and Gibbs (2,6) used different *C. ulmi* isolates, and isolate differences might explain the lack of agreement in their results. However, we used some of the same isolates as those used by both groups of workers (Table 1), so the effect of isolate differences should have been reduced. Variation in composition of the agar media tested also might explain these inconsistencies. In our work, differences in aerial mycelium production occurred on two quite similar media, GYE and GA. These media differed only in their source of nitrogen and initial pH. Because aerial mycelium production on agar media by *C. ulmi* is highly variable, a defined agar medium should be developed so that the association of pathogenicity and this cultural characteristic can be examined critically.

With the multivariate discriminant analyses (Table 2) we accurately classified isolates in their respective pathogenicity types. The classification was accomplished in spite of variability among isolates in each pathogenicity type. In fact, some cultural characters produced by some isolates (ILL-1, GB-4, MASS-1, ME-1, NC-1, and OH-1) were intermediate to those typical of either type. Of these isolates, ME-1 is of special interest because it has an intermediate degree of pathogenicity (14).

Brasier and Gibbs indicated there is more cultural variability among less aggressive isolates of *C. ulmi* than among more aggressive ones (2,3,6). Our results (Table 2) generally support their findings. They also emphasized that the use of cultural characteristics to classify isolates applies only to freshly isolated cultures, because cultural characteristics often change with time in culture (2,3,6). Even though the isolates we tested were in culture

TABLE 2. Cultural characteristics produced by *Ceratocystis ulmi* isolates grown on four agar media[a] and pathogenicity classifications for each isolate

Isolates	Cultural Characteristics[b]																Pathogenicity[f] classification (all media)
	Radial growth				Dry weight of mycelium[c]				Aerial mycelium[d]				Striate radial growth[e]				
	PDA (mm)	MEA (mm)	GYE (mm)	GA (mm)	PDA (mg)	MEA (mg)	GYE (mg)	GA (mg)	PDA	MEA	GYE	GA	PDA	MEA	GYE	GA	
More aggressive																	
ALA-1	35	31	39	30	0.47	0.25	0.47	0.36	3.0	1.6	2.0	3.0	1.0	1.0	1.0	1.0	M
COLO-1	33	30	36	24	0.44	0.22	0.39	0.36	2.5	2.0	2.4	3.0	1.0	1.0	1.0	1.0	M
GB-1	33	29	34	35	0.43	0.18	0.43	0.29	3.0	2.0	3.0	2.0	1.0	1.0	1.0	1.0	M
GB-2	34	30	34	29	0.56	0.12	0.44	0.36	3.0	2.0	2.5	3.5	1.0	1.0	1.0	1.0	M
ILL-1	29	29	29	22	0.38	0.17	0.76	0.63	3.0	2.0	2.5	3.3	1.0	1.0	1.0	1.0	M
IA-1	32	33	36	29	0.43	0.25	0.47	0.29	3.0	2.0	3.0	3.8	1.0	1.0	1.0	1.0	M
MASS-1	32	35	35	28	0.51	0.27	0.45	0.29	3.0	2.3	3.0	3.5	1.0	1.0	1.0	1.0	M
MO-1	36	34	35	31	0.52	0.25	0.48	0.37	3.0	2.0	3.0	3.0	1.0	1.0	1.0	1.0	M
ND-1	34	34	38	25	0.45	0.22	0.54	0.35	2.7	2.0	2.8	3.0	1.0	1.0	1.0	1.0	M
WIS-1	33	27	35	23	0.42	0.25	0.46	0.44	2.7	2.0	2.5	3.0	1.0	1.0	1.0	1.0	M
X	33	31	35	28	0.45	0.22	0.49	0.37	2.9	2.0	2.7	3.1	1.0	1.0	1.0	1.0	
Less aggressive																	
GB-3	22	22	23	18	0.88	0.32	0.75	1.08	2.0	2.0	2.0	4.0	2.0	2.0	2.0	2.0	L
GB-4	22	23	22	14	0.29	0.10	0.42	0.25	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	L
IA-2	15	16	14	14	1.87	0.91	2.25	0.45	2.0	1.0	3.3	4.0	2.0	2.0	2.0	2.0	L
IA-3	10	14	17	7	1.94	0.61	1.34	1.07	2.0	1.9	2.0	1.0	2.0	2.0	2.0	2.0	L
ME-1	16	21	14	11	0.58	0.21	0.76	0.38	2.6	3.0	2.4	2.4	2.0	2.0	2.0	2.0	L
MASS-2	26	29	27	21	1.34	0.33	0.81	1.31	1.0	1.0	1.0	3.3	1.0	1.0	1.0	1.4	L
NC-1	21	22	20	19	1.03	0.43	1.10	1.27	1.0	1.0	1.0	3.6	2.0	2.0	1.0	2.0	L
OH-1	32	30	31	30	1.80	0.63	1.10	1.12	2.0	1.0	2.0	3.0	1.0	1.0	1.0	2.0	L
TENN-1	25	20	15	17	0.98	0.33	0.56	0.66	2.6	1.9	2.0	1.3	1.8	2.0	2.0	2.0	L
X	21	22	20	17	1.19	0.42	1.02	1.06	2.0	1.6	1.9	2.7	1.8	1.8	1.8	1.9	
F values[g]	31.2*	23.8*	49.1*	19.2*	15.3*	7.2*	9.3*	11.4*	18.5*	2.4	9.2*	0.9	31.2*	31.2*	31.3*	188.7*	

[a]PDA = Difco potato dextrose; MEA = Difco malt extract; GYE = glucose-yeast extract; GA = glucose and L-asparagine.
[b]All data presented are means of 10 replicates.
[c]Per square centimeter of colony area.
[d]Production evaluated as: 1 = none; 2 = limited; 3 = moderate; 4 = abundant.
[e]Pattern formulation evaluated as: 1 = present; 2 = absent.
[f]Pathogenicity classification based on cultural characteristics and multivariate discriminant analyses. M = more aggressive; L = less aggressive.
[g]F values * = significant at the 0.01 level.

for some time, this additional source of variability did not affect the accuracy of the classification by the multivariate discriminant analyses (Table 2). Such successful classifications indicate that pathogenicity predictions for *C. ulmi* isolates of unknown pathogenicity should be possible with the use of selected agar media, proper combinations of cultural characteristics, and multivariate discriminant analyses.

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