Development of American Isolates of Puccinia graminis f. sp. tritici on an Artificial Medium

W. R. Bushnell and D. M. Stewart

Plant Physiologist, Crops Research Division, and Research Plant Pathologist, Plant Protection Division, respectively, ARS, USDA, Cooperative Rust Laboratory, University of Minnesota, St. Paul 55101.

Cooperative Investigation, Crops Research Division, Plant Protection Division, ARS, USDA; and Department of Plant Pathology, University of Minnesota. Scientific Journal Series Paper No. 7324, Minnesota Agricultural Experiment Station.

The authors thank Martha Hubbeling Bailey and Dana Drennan for technical assistance.

Mention of a trademark name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA, nor imply its approval to the exclusion of other products also available.

Accepted for publication 10 November 1970.

ABSTRACT

Uredospores of 25 diverse American isolates of *Puccinia graminis* f. sp. *tritici* were seeded in dense populations on agar containing peptone, glucose, and mineral salts, then incubated under cool, moist conditions. Twenty isolates formed white mycelial mats, indicating development beyond the germ-tube stage. Eleven isolates grew slowly (6-41 μ/day) in subcultures made from the stromata. Growth rates usually declined through successive monthly subculturings until growth ceased, but the growth rates of two isolates increased to 42-64 μ/day by the sixth subculture. These two isolates have continued to grow until the present time (9 months or more). The results indicate that American isolates of the stem rust fungus vary widely in culturability, and that present techniques are not adequate for long-term culture of most of these biotypes. Phytopathology 61:376-379.

Additional key words: wheat stem rust, axenic rust culture.

Several North American isolates of *Puccinia graminis* Pers. f. sp. *tritici* Eriks. & E. Henn. failed to grow in preliminary trials in our laboratory, in contrast to the slow but definite growth of an Australian isolate in artificial culture (1). To find culturable isolates for use in North America, and to evaluate the growth potential of American biotypes more completely, we selected diverse isolates of *P. graminis* f. sp. *tritici* from available stored uredospore cultures. The isolates were chosen for diversity in race, date of collection, geographic origin, and the host species from which the culture had been collected.

MATERIALS AND METHODS.—The origin of the 25 isolates of this investigation are listed in Table 1. Eighteen of the isolates were obtained from field collections made in the USA, one each from Mexico and Colombia. Three were derived through hybridization on barberry (*Berberis vulgaris* L.) in the greenhouse at St. Paul, Minn.; one was a mutant induced artificially by Rowell et al. (6); and one was of unknown origin. Field collections were obtained during race surveys in 1953-65 from wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), or wild barley (*Hordeum jubatum* L.), except for one isolate of race 56 from rusted barberry. Four isolates (No. 7, 8, 17, and 23) were collected in areas in which barberry plants were known to occur (Table 1).

Uredospore cultures of each isolate had been increased when received in St. Paul on seedlings of Little Club wheat and stored in liquid nitrogen or in a vacuum-dried condition. For the present study, uredospores were removed from storage and increased 1 or more times on Little Club seedlings, and the physiologic race was determined according to Stakman et al. (8).

Contaminant-free uredospores were produced on surface-sterilized leaves as described earlier (1). Uredospores of each isolate were seeded thickly with a cotton swab onto a peptone-glucose-mineral salt medium (1) in four zones, 1.5-2.0 cm in diam, in each of three or four petri dishes. All culture dishes were sealed shut with tape (Autoclave tape, 3M Co., St. Paul, Minn.) to reduce microbial contamination. This procedure has had no effect on Australian race 126-Anz-6,7. Cultures were incubated at 13-15 C, about 3 C lower than in earlier work (1). The agar concentration was usually 1%. This agar concentration was used in earlier studies, but was erroneously reported as 2% (1).

Subcultures were prepared 4-6 weeks after the initial seeding of uredospores, depending on the rate of fungus development. If mycelial material was adequate, four inoculum pieces (1.5-2.0 mm in width) were transferred to each of four petri dishes. The subcultures were incubated, using the same medium and conditions as were used for seedings of uredospores.

RESULTS.—Growth was judged to have begun when the surface of the seeded zones became white, contrasting with the brown color of sporelings en masse which had not developed beyond the germ-tube stage. The extent of hyphal growth at this stage was not assessed microscopically, but experience with Australian race 126-Anz-6,7 suggested that hyphae in the white zones had branched abundantly. All but five of the 25 tested isolates had formed such white zones (Table 1). Ten of these growing isolates produced thick stromata 4-6 weeks after seeding comparable to the stromata produced by the Australian isolate. The 15 remaining isolates had not formed thick stromata 6 weeks after seeding.

Only 11 of the isolates grew in subcultures prepared by placing mycelial pieces cut from the seeded zones on fresh medium 4-6 weeks after the initial seedings (Tables 1, 2). Most, but not all, of the isolates which

TABLE 1. Development of 25 isolates of *Puccinia graminis* f. sp. *tritici* from dense seedlings of uredospores on a peptone-glucose-mineral salt-agar medium

Isolate no.	Culture	Race	Source			Development[a]	
			Location	Year	Host	Seeding[b]	First subculture[c]
1	61-54RUC	56	Greenhouse[d]		Barberry	++	+
2	61URN104	23	Minn.	1961	Barley	++	+
3	60-S21-36	38	Minn.	1960	Wheat	++	+
4	57-14-17	32B	Kans.	1957	Wheat	+	+
5	56-B20-49	56	Mich.	1956	Barberry	++	+
6	56-C-5	147	Colombia	1956	Wheat	+	+
7	59-45-6	29	Wash.	1959	Wheat[e]	++	+
8	60-30-6	Mixture[f]	N.Y.	1960	Wheat[e]	++	+
9	64-32-63	15B-2	N. Dak.	1964	Wheat	++	+
10	58-30-22	186	N.Y.	1958	Wheat	++	+
11	61-36-89	56	Penn.	1961	Wheat	+	+
12	56-35-7	275[g]	Ore.	1956	Wheat	++	Contaminated
13	17-53B	29	Mexico	1953	Wheat	++	—
14	111 × 36#5[h]	111	Greenhouse		Barberry	+	Contaminated
15	54-33-38	15B-1	Ohio	1954	Wheat	+	—
16	65-N41-6	17	Texas	1965	Wheat	+	—
17	56-44-2	56	Va.	1956	Wheat[e]	+	—
18		48	Unknown			+	—
19	65-24-6	87	Mont.	1965	Wheat	+	—
20	111-55A	111	Greenhouse[d]	Before 1955	Barberry	+	—
21	63-21-197	32A	Minn.	1963	Wheat	—	—
22	61-33-45	56	Ohio	1961	Wheat	—	Not sub-cultured
23	56-44-6	56	Va.	1956	Wheat[e]	—	Not sub-cultured
24	64-21-19	56	Minn.	1964	Wild barley	—	Not sub-cultured
25	111 × 36#5M82[h]	111	(Induced mutant)			—	Not sub-cultured

[a] Listed in order of decreasing growth as judged from growth in subculture, or from initial seedings if subculture failed to grow.
[b] — = Brown, without macroscopic evidence of growth beyond germ tubes. + = Branched growth of germ tubes sufficient to make the seeded zone appear white, but without sufficient growth to form a thick stroma. ++ = Thick dense stroma, white on the upper surface.
[c] + = Grew in subcultures as shown in Table 2. — = No growth in subculture.
[d] Source of parent isolates unknown.
[e] Culture collected from wheat in a barberry area.
[f] Mixture of: A, similar to race 40 except Kubanka susceptible; B, similar to race 194 except Vernal susceptible; C, similar to race 232 except Einkorn resistant.
[g] Similar to race 275 except infection type X on Mindum, Kubanka, and Spelmar.
[h] Near-isogenic line of Rowell et al. (6).

grew in the first subculturing had formed stromata in the seeded dish. Development in successive subculturings was generally poor as growth rates of most isolates declined to less than 25 μ/day, the approx minimum rate necessary to maintain an isolate through successive subculturings. Only six isolates grew after a third subculturing, and only two isolates (No. 1 and 2) survived subculturings for 9 months (Table 2). The growth rate of isolate No. 1 increased slowly over the first several subculturings, reaching a rate sufficient for continued maintenance by the fourth subculturing. The growth rate of isolate No. 2 increased abruptly in the sixth subculturing, after growing so slowly in earlier subculturings that only two mycelial pieces remained viable.

Generally, the amount of growth from uredospores or subcultures appeared to be unrelated to the origin or race of the tested isolates. Although some isolates of race 56 failed to grow as we had indicated (7), other isolates of this race have grown in varying amounts (Tables 1, 2).

Large numbers of uredospores per unit area of agar surface were required to initiate growth. This was demonstrated for isolate No. 3 by pipetting 0.5, 1.0, 2.0, and 4.0 mg (fresh wt) of uredospores suspended in 0.1 ml water into glass rings, 1.5 cm in diam, on the surface of the agar medium. The agar medium had been dried slightly in covered petri dishes on the laboratory bench for 4-5 days so that water from the spore suspensions would be absorbed by the medium. Isolate No. 3 grew only at the two higher spore concentrations. In a similar test, Australian isolate 126-Anz-6,7 grew at all concentrations.

The new mycelial growth of subcultures was hard and compact, light brown or orange brown, contrasting with the aging inoculum piece which usually became dark brown. But isolate No. 1 started to produce white sectors in the 2nd year of successive subculturings.

TABLE 2. Growth by successive subcultures of *Puccinia graminis* f. sp. *tritici* on a peptone-glucose-mineral salt-agar medium

Sub-culture[a]	Days of sub-culturing[b]	Growth (μ/day)[c] Isolate no.[d]										
		1	2	3	4	5	6	7	8	9	10	11
1	37	26	13	33	41	21	31	24	26		21	6[e]
2	72	21	21	42	36	33	42	18	33	13	5[e]	
3	107	28	31	20	26	15	33	12	8	5[e]		
4	145	36	13	6	14	13	12	0	0			
5	189	42	20	12	8	0	0					
6	223	36	64[f]	0	0							
7	280	32	64[f,g]									
8	316	40										
9	366	47										
10	403	41[g]										

[a] Serial subculturings. First subculture from stroma produced by uredospores; each subsequent subculturing from the preceding subculture.
[b] Cumulative from the time dishes were seeded with uredospores, ±31 days.
[c] Growth rates from increase in width of growing colonies as measured 1-5 days from times of subculturing.
[d] Isolates as designated in Table 1.
[e] Not subcultured further.
[f] Total of only two mycelial pieces.
[g] Subcultures have continued to grow until the present time.

Thus, all or part of the new growth was white from one-twelfth of the inoculum pieces in the subculturing made 445 days after the original uredospore seeding, and from half the pieces subcultured at 529 days. Furthermore, ca. half of these white sectors consisted of filamentous hyphae which spread thinly over the agar surface at the periphery of the colony. Growth rates of subcultures from white sectors were 74-82 μ/day, somewhat faster than that of the parent culture. No sectoring was observed in other isolates.

Cultures were examined for sporulation after several serial subculturings. Most contained some teliospores as well as spores resembling uredospores. However, isolate No. 8 had large numbers of teliospores and isolates No. 6 and 9 each had small numbers of uredospores with no teliospores. Spores were generally found in the old inoculum pieces at the center of growing colonies (isolates No. 1 and 2). The old, dark-brown mycelium of serially subcultured isolates which had lost ability to grow sometimes contained spores and at other times did not. Thus, sporulation and growth were not in any way correlated.

Isolate No. 1 was applied to mesophyll tissues of seedling leaves of Little Club wheat in the manner used successfully to produce infections with in vitro cultures of Australian race 126-Anz-6,7 (1). No infections developed with subcultures prepared 167, 227, or 477 days after the original seeding of uredospores.

Scott & Maclean (7) and Kuhl et al. (5) reported wide variation in growth among trials with Australian race 126-Anz-6,7. Uncontrolled variations in method of spore production, seeding density, media, or other factors apparently favored growth only in certain trials. Such variation has not been great in our laboratory. Australian race 126-Anz-6,7 has produced stromata in all but one of seven uredospore seedings. The one failure was attributed to an insufficient density of spores. Isolate No. 3 (Table 1) formed thick stromata in all of five seedings over a 12-month period. Subcultures were made from four of these seedings, and three of the four grew in the first subculturing (as was the case for the trial listed for this isolate in Table 2). From these results, we conclude that most of the large differences in development shown in Tables 1 and 2 are due to genetic differences among isolates rather than differences in experimental conditions among trials.

DISCUSSION.—American isolates of *P. graminis* f. sp. *tritici* generally grew poorly in artificial culture in the experiments described here. Only two of the isolates grew as rapidly as has Australian race 126-Anz-6,7 of *P. graminis* f. sp. *tritici* (1, 3, 11) or as have several North American races of *Melampsora lini* (Ehrend.) Lev. (4, 9). Data are too fragmentary to establish whether the American population of *P. graminis* f. sp. *tritici* is less suited to artificial culture than the Australian population, but the present evidence does indicate that isolates of this species vary widely in ability to grow. Furthermore, culturability did not correlate with any of the known characteristics of the tested isolates (race, geographical origin, or host species).

The slow and declining rate of development of most isolates indicates that the techniques used here were marginal for growth by *P. graminis* f. sp. *tritici*. The requirement for dense spore populations, and the usual decline in development through successive subculturings, suggest that uredospores release substances required by the rust mycelium for growth. Kuhl et al. (5) have evidence that diffusates from uredospores promote in vitro growth by sporelings at low population densities. Incorporation of such substances into the culture medium might improve development by North American isolates. Likewise, growth might be enhanced by one or more of the several additives recently found to improve growth of Australian race 126-Anz-6,7 such as gelatin (3), bovine serum albumin (5), casein hydrolysate (2), cysteine, or acid-hydrolyzed peptone (7).

Half the isolates which grew beyond the germ-tube

stage had not grown earlier in our laboratory (1). The low temperature used here may have enhanced rust fungus development, especially in view of Turel's report (10) that the flax rust fungus requires temperatures of 16 C or lower. Denser spore populations may also have favored growth here, although population density and other factors such as spore age and amount of medium per dish were not strictly controlled.

The few isolates of *P. graminis* which have survived serial subculturing apparently adapted slowly to the medium in use. Thus, the growth rates of isolates 1 and 2 increased between the first and sixth subculturings following the original seeding of uredospores (Table 2). Further adaptation was indicated by the eventual sectoring of isolate No. 1. Fragmentary data from our laboratory suggest that the growth rate of Australian race 126-Anz-6,7 also increased during the first few months of subculturing. Routine culture of *P. graminis* will be possible only after adaptation is either facilitated or made unnecessary through improved culture methods.

LITERATURE CITED

1. Bushnell, W. R. 1968. In vitro development of an Australian isolate of Puccinia graminis f. sp. tritici. Phytopathology 58:526-527.
2. Bushnell, W. R., & R. B. Rajendren. 1970. Casein hydrolysates and peptones for artificial culture of Puccinia graminis f. sp. tritici. Phytopathology 60: 1287 (Abstr.).
3. Coffey, M. D., A. Bose, & M. Shaw. 1969. In vitro growth of gelatin suspensions of uredospores of Puccinia graminis f. sp. tritici. Can. J. Bot. 47:1291-1293.
4. Coffey, M. D., A. Bose, & M. Shaw. 1970. In vitro culture of the flax rust, Melampsora lini. Can. J. Bot. 48:773-776.
5. Kuhl, J. L., D. J. Maclean, K. J. Scott, & P. G. Williams. 1971. The saprophytic culture of Puccinia species from uredospores: experiments on nutrition and variation. Can. J. Bot. 48: (in press).
6. Rowell, J. B., W. Q. Loegering, & H. R. Powers, Jr. 1963. Genetic model for physiologic studies of mechanisms governing development of infection type in wheat stem rust. Phytopathology 53:932-937.
7. Scott, K. J., & D. J. Maclean. 1969. Culturing of rust fungi. Annu. Rev. Phytopathol. 7:123-146.
8. Stakman, E. C., D. M. Stewart, & W. Q. Loegering. 1962. Identification of physiologic races of Puccinia graminis var. tritici. USDA, ARS. E617. 53 p.
9. Turel, F. L. M. 1969. Saprophytic development of the flax rust Melampsora lini, race No. 3. Can. J. Bot. 47:821-823.
10. Turel, F. L. M. 1969. Low temperature requirement for saprophytic flax rust cultures. Can. J. Bot. 47: 1637-1638.
11. Williams, P. G., K. J. Scott, J. L. Kuhl, & D. J. Maclean. 1967. Sporulation and pathogenicity of Puccinia graminis f. sp. tritici grown on an artificial medium. Phytopathology 57:326-327.