STUDIES ON VARIATION WITH *OPHIOBOLUS GRAMINIS*

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Summary

Variations were studied in the monosporous F_1 progeny of an isolate of *Ophiobolus graminis* from wheat. All F_1 ascospores from perithecia on living host tissue were strongly pathogenic, but half from a perithecium in culture were weakly pathogenic. Perithecia were formed in culture and on host tissue only by strongly pathogenic progeny. Strongly pathogenic progeny survived well on artificially infected wheat straw buried in unsterilized soil, but weakly pathogenic progeny did not. Survival of all isolates was increased by nitrate enrichment of soil.

I. INTRODUCTION

Many data are available on the effects of soil conditions, particularly soil fertility, on saprophytic survival of *Ophiobolus graminis* in wheat straw (Garrett 1938, 1940, 1944; Butler 1953, 1959; Lucas 1955; Macer 1961; Van der Watt 1965). However, most workers have given little or no information about isolates used for survival studies and no one has determined whether different isolates survive equally well under the same conditions. Thus there is no information on whether survival is affected by specific morphological or physiological variations. Heritable variations in colony colour and pathogenicity of monosporous isolates from a single ascus of *O. graminis* were reported by White (1942); he found that four monosporous isolates were strongly pathogenic and the remaining four were weakly pathogenic; four also formed light-coloured colonies and the others dark-coloured colonies. There are no other published accounts of heritable variations in *O. graminis*. This paper, therefore, describes some variations within the F_1 progeny of a monosporous isolate of *O. graminis* and a comparison of the saprophytic survival of variants on wheat straw in soil differing in fertility.

II. MATERIALS AND METHODS

(*a*) *Isolates*

Wheat isolate W1 (Chambers and Flentje 1967) and its progeny were used exclusively in these studies. Ascospores were cultured singly to give a range of monosporous isolates. These were designated by numbers followed by the letter A, C, or R, depending on whether they were obtained from a single ascus (A), a perithecium in culture (C), or a perithecium formed on an oat plant (R).

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(*b*) *Methods of Isolation*

Monosporous cultures were obtained by placing a mature perithecium on a flamed microscope slide and adding a drop of water to induce ejection of ascospores. The spores were collected in a capillary pipette and after dilution in sterile water were scattered over the surfaces of freshly poured plates of potato–Marmite–dextrose agar (PMD) containing 33 p.p.m. aureomycin hydrochloride. After incubation for 2 days at 20°C, pieces of agar each containing a germinated ascospore were cut out and transferred to fresh plates of PMD. For the isolation of eight ascospores from a single ascus, a mature perithecium was split open on a flamed microscope slide and transferred to a Petri dish containing water agar covered by Cellophane. A needle with a curved tip was used to move the perithecium over the Cellophane surface so as to scatter the asci. Under a dissecting microscope any perithecial debris was cleaned from around an isolated ascus. A piece of Cellophane (4 mm^2) bearing the ascus was cut out and placed on the surface of a freshly poured plate of PMD containing aureomycin. A drop of sterile water was added to the ascus which was then kept under microscopic observation until the ascospores were ejected. More water was then added and the ascospores were dispersed by rotary movement of the dish. After incubation for 2 days, pieces of agar each containing a germinating ascospore were cut out and transferred to fresh plates of PMD.

For hyphal isolations from infected straw, small fragments of tissue were immersed in 1% silver nitrate for 30 sec. After three 10-sec rinses in sterile water, the fragments were placed on to fresh plates (three fragments per plate) of PMD containing aureomycin. After incubation for 2–4 days at 20°C, hyphal tips of the fungus were transferred to PMD slopes. A 30-sec immersion period in silver nitrate gave more satisfactory results than the 2-min period originally suggested by Davies (1935).

(*c*) *Soil*

A calcareous sandy soil from Moonta, S.A., was used for pathogenicity and survival studies. The physical characteristics of this soil were as follows: particle size distribution—1–2 μ, 8%; 2–20 μ, 2%; over 20 μ, 90%; total nitrogen (Kjeldahl) 0·041%; nitrate nitrogen, 7·5 p.p.m.; ammonium nitrogen, 3·9 p.p.m.; organic carbon, 0·7%; pH 8·5. The drying boundary curve is shown in Figure 1.

(*d*) *Pathogenicity Tests*

The method described by Chambers and Flentje (1967) was used.

(*e*) *Sources and Measurement of Asci and Ascospores*

Perithecia were formed in culture by some isolates when the technique of Weste and Thrower (1963) was used, and a random selection of asci and ascospores measured (Turner 1940).

(*f*) *Estimation of Saprophytic Survival*

The technique of Garrett (1938) as modified by Butler (1953) was used for estimating survival of monosporous isolates on artificially infected wheat straw in

nitrate-enriched and unamended soil; the nitrate was added as sodium nitrate at the rate of 100 mg nitrogen per kilogram soil and soil moisture was adjusted to 8·5% (p*F* 1·8).

The inoculated straws were buried in screw-cap jars and stored in a growth cabinet adjusted to a 10-hr "day" with day and night temperatures of 20 and 18°C respectively. The soil surface in each jar was covered with thin polythene to reduce moisture loss. After 4, 8, 12, and 24 weeks of storage, 100 straws of each treatment were unearthed and tested for viable hyphae by the wheat seedling test (Garrett 1938).

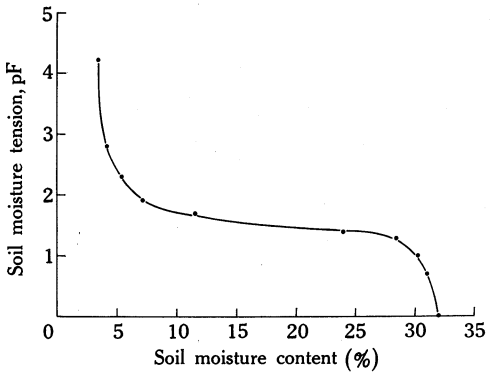

Fig. 1.—Drying boundary curve of experimental soil.

III. Experimental and Results

(*a*) *Colony Characteristics and Pathogenicity of Monosporous Isolates*

(i) *From a Single Ascus*

Eight monosporous isolates were obtained from a single ascus of a perithecium which developed on the roots of a living oat plant. Initially the isolates were similar in cultural appearance, each colony being flat and composed of hyaline hyphae which gave them an off-white colour. However, by 3 weeks all showed slight differences in strand development and colour.

Mature perithecia developed in all cultures by 21 days. The mean lengths of asci and ascospores from these perithecia in culture were greater than those of the asci and ascospores from field material from which the parent W1 was originally isolated (cf. Chambers and Flentje 1967).

When tested, the parent culture and its progeny were all found to be strongly pathogenic on wheat seedlings. Furthermore, there was no significant difference in the extent of hyphal growth along the roots nor in the mean length of root discoloured by infection by different isolates.

(ii) *From Perithecia Produced on Different Substrates*

A random selection of 100 monosporous isolates was made from a perithecium formed in culture and from another on the roots of a living oat plant.

Isolates from the perithecium on the oat plant were all similar in cultural appearance. Twelve isolates were selected at random and tested for pathogenicity on wheat seedlings; all proved to be strongly pathogenic and there were no significant differences between the extent of hyphal growth along roots nor in mean lengths of root discoloured by different isolates. Fertile perithecia were formed in culture by the same 12 isolates. Mean lengths of their asci were greater than those of asci on field material from which parent W1 was originally isolated. Mean lengths of their ascospores were either equal to or greater than those of ascospores on the original field material.

TABLE 1

PATHOGENICITY ON WHEAT OF 12 MONOSPOROUS ISOLATES FROM ONE PERITHECIUM OF *O. GRAMINIS*

Isolate	Fresh Weight of Host (mg)	Length of Discoloured Root (mm)	Length of Runner Hyphae along Root (mm)	Length of Whole Root (mm)	Analysis of Root Measurements using Transformation $\text{Log}_e X$:		
					Discoloured Root	Runner Hyphae along Root	Whole Root
2C	278	29	51	170	3·3958	3·9403	5·1154
4C	249	27	41	132	3·3142	3·7433	4·8774
25C	391	22	40	191	3·1467	3·6924	5·2538
62C	388	26	45	158	3·2657	3·8146	5·0666
92C	333	22	39	179	3·1460	3·6855	5·1867
98C	327	26	47	167	3·3061	3·8767	5·1012
11C	550	3	7	253	1·1945	2·0897	5·5322
31C	458	3	9	252	1·2904	2·2445	5·5330
53C	573	2	6	291	0·8283	1·8661	5·6735
69C	496	2	6	235	0·9986	1·9390	5·4587
74C	490	2	8	264	0·9027	2·1351	5·5782
100C	477	2	10	249	1·0594	2·3404	5·4992
W1	316	29	47	139	3·3864	3·8391	4·9298
Control	522	0	0	270			5·5970
Differences for significance at $P = 0·05$					0·7843	0·4054	0·2747
Differences for significance at $P = 0·01$					1·0678	0·5520	0·3705

The monosporous isolates from the perithecium formed in culture showed slight variations in cultural appearance and they fell into four groups. The majority (58) were characterized by pronounced white strands and another 25 differed only in that the strands were dark brown to black in colour. Of the remainder, 13 had no pronounced strands, whilst the other four were characterized by slowness of growth. However, after the first subculturing, all isolates were similar in cultural appearance and in growth rate and indistinguishable from those obtained from the perithecium obtained from the oat root.

The parent W1 and three isolates of each of the four original groupings were tested for pathogenicity on wheat seedlings. Six F_1 isolates were strongly pathogenic, but the remainder were only weakly pathogenic. The strongly pathogenic isolates

were equally as virulent as the parent and there was no significant difference in the amount of root discoloration caused, nor in the extent of their runner hyphae along roots (Table 1). However, the six weakly pathogenic isolates produced only limited growth along roots and very little discoloration. Hand-sections of roots were stained with lactophenol–cotton blue and examined microscopically. In sections infected with strongly pathogenic isolates, infection hyphae frequently branched from the external runner hyphae, entered the cells of the cortex, also penetrated the endodermis, and invaded the vascular tissue. With weakly pathogenic isolates, however, penetration by infection hyphae occurred less frequently and was generally confined to the first two or three layers of cortical cells.

TABLE 2

ASCUS AND ASCOSPORE MEASUREMENTS OF SINGLE SPORE PROGENY AND PARENT ISOLATE OF *O. GRAMINIS*

Isolate	Mean Length of Asci (μ)	Mean Ascospore Length (μ)	Modal Ascospore Length (μ)	Range of Ascospore Lengths (μ)	Mean No. of Septa	Range of No. of Septa
2C	117 ($\pm$0·59)	95 ($\pm$0·53)	94	84–107	7·7	3–11
4C	120 ($\pm$0·76)	95 ($\pm$0·51)	92	81–107	7·6	5–11
25C	120 ($\pm$0·76)	94 ($\pm$0·55)	92	81–110	7·6	3–11
62C	120 ($\pm$0·69)	96 ($\pm$0·49)	100	81–113	7·9	5–11
92C	121 ($\pm$0·77)	96 ($\pm$0·45)	100	84–107	7·8	5–11
98C	121 ($\pm$0·81)	94 ($\pm$0·49)	92	81–107	7·4	5–11
W1*	120 ($\pm$0·72)	95 ($\pm$0·56)	92	81–110	7·7	5–11
W1†	106 ($\pm$0·91)	83 ($\pm$0·58)	81	68–94	7·0	5–9
W1‡	106 ($\pm$0·57)	84 ($\pm$0·51)	81	71–94	7·0	5–9

* Asci obtained from perithecium in culture.

† Asci obtained from original material.

‡ Asci obtained from pathogenicity test.

In culture, mature perithecia were produced by the six strongly pathogenic isolates, but not even rudiments of perithecia were formed by the six weakly pathogenic isolates. Mean lengths of asci and ascospores formed in culture were again greater than those of the original material from which the parent W1 was isolated (Table 2). During pathogenicity tests, the strongly pathogenic isolates irrespective of origin, formed perithecia on host tissue; none was produced on plants inoculated with weakly pathogenic isolates.

Three weakly pathogenic and three strongly pathogenic isolates, together with the parent W1, were also tested for pathogenicity on five other hosts, viz. *Bromus mollis* L. (soft brome), *Hordeum leporinum* L. (barley grass), *H. vulgare* L. (barley), *Lolium rigidum* Gaud. (Wimmera rye grass), and *Vulpia myuros* (silver grass). The results (Table 3) indicate isolates which were weakly pathogenic to wheat were also consistently weakly pathogenic on each of the other hosts. Barley, barley grass, and soft brome were susceptible to strongly pathogenic isolates; hyphal growth was less extensive along the roots of silver grass; and Wimmera rye grass appeared to have some resistance to strongly pathogenic isolates.

TABLE 3

PATHOGENICITY ON VARIOUS HOSTS WITH SIX SINGLE SPORE ISOLATES FROM ONE PERITHECIUM OF *O. GRAMINIS*

Host	Isolate	Fresh Weight of Host (mg)	Length of Discoloured Root (mm)	Length of Runner Hyphae along Root (mm)	Length of Whole Root (mm)	Analysis of Root Measurement using Transformation $\text{Log}_e X$:		
						Discoloured Root	Runner Hyphae along Root	Whole Root
Bromus mollis	2C	49	13	30	77	2·6374	3·4121	4·3521
	25C	38	15	29	75	2·7362	3·4061	4·3233
	98C	47	14	29	69	2·7268	3·3997	4·2307
	69C	48	0	3	77	0·2310	1·1945	4·3437
	74C	43	0	3	74	0·2310	1·2904	4·3087
	100C	49	0	2	83	0·2310	0·8283	4·4315
	W1	51	13	26	74	2·6464	3·3026	4·2807
	Control	56			84			4·4372
Hordeum leporinum	2C	64	22	32	72	3·1120	3·4837	4·2904
	25C	63	22	33	73	3·1349	3·5319	4·2955
	98C	62	23	34	80	3·1093	3·5355	4·3723
	69C	81	1	5	95	0·6932	1·8431	4·5545
	74C	100	2	7	98	1·1945	2·0228	4·5895
	100C	94	2	6	89	0·8283	1·7918	4·5032
	W1	73	19	29	85	2·9769	3·4078	4·4540
	Control	99			98			4·5772
Hordeum vulgare	2C	334	19	40	165	2·9627	3·6954	5·1113
	25C	337	19	48	165	2·9949	3·8913	5·1093
	98C	313	21	45	153	3·1019	3·8282	5·0338
	69C	383	0	1	196	0·0000	0·8283	5·2836
	74C	378	0	3	234	0·0000	1·3648	5·4567
	100C	384	0	4	212	0·0000	1·6094	5·3610
	W1	291	21	39	148	3·0718	3·6880	5·0058
	Control	363			236			5·4659

TABLE 3 (*Continued*)

Host	Isolate	Fresh Weight of Host (mg)	Length of Discoloured Root (mm)	Length of Runner Hyphae along Root (mm)	Length of Whole Root (mm)	Analysis of Root Measurement using Transformation $\text{Log}_e X$:		
						Discoloured Root	Runner Hyphae along Root	Whole Root
Lolium rigidum	2C	27	3	14	45	1·2904	2·6734	3·8343
	25C	36	0	11	55	0·0000	2·4849	4·0278
	98C	38	2	16	52	1·1552	2·8501	3·9607
	69C	43	0	1	55	0·2310	0·4621	4·0240
	74C	36	0	1	54	0·2310	0·6932	3·9946
	100C	40	0	1	60	0·2310	0·8283	4·0913
	W1	37	1	12	47	0·6932	2·5383	3·8584
	Control	38			53			3·9903
Vulpia myuros	2C	24	5	15	34	1·8269	2·7875	3·5480
	25C	24	4	14	34	1·5351	2·7125	3·5358
	98C	35	4	18	38	1·6702	2·9331	3·6489
	69C	25	1	1	42	0·4621	0·8283	3·7644
	74C	33	0	1	44	0·2310	0·8283	3·8062
	100C	28	0	1	44	0·0000	0·8283	3·7980
	W1	23	4	12	36	1·6566	2·5897	3·6061
	Control	32			43			3·7977
Differences for significance at $P = 0·05$						0·2001	0·1868	n.s.
Differences for significance at $P = 0·01$						0·2657	0·2480	—

TABLE 4

PERCENTAGE OF WHEAT STRAW IN WHICH STRONGLY PATHOGENIC AND WEAKLY PATHOGENIC ISOLATES OF *O. GRAMINIS* SURVIVED WHEN BURIED IN UNSTERILIZED SOIL WITH (+N) OR WITHOUT (−N) ADDED NITROGEN

Survival Period (weeks)	Strongly Pathogenic Isolates								Weakly Pathogenic Isolates								W1	
	2C		25C		92C		98C		31C		69C		74C		100C			
	+N	−N	+N	−N	+N	−N	+N	−N	+N	−N	+N	−N	+N	−N	+N	−N	+N	−N
0	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
4	100	97	100	99	98	100	100	99	24	16	45	6	85	44	79	23	100	77
8	99	70	100	59	98	66	99	73	15	1	27	0	21	0	7	3	97	32
12	94	64	95	58	75	41	96	47	0	0	7	0	0	0	1	0	94	22
24	86	22	81	41	95	22	75	30	0	0	0	0	0	0	0	0	88	10

(b) Survival of Isolates Differing in Pathogenicity

The survival of four weakly pathogenic and four strongly pathogenic isolates, together with parent W1, was compared on wheat straw buried in soil with and without added nitrogen (Table 4).

During the experiment, fragments of straws were examined microscopically for mycelium of *O. graminis*. At burial, mycelial development in straws was similar for both weakly and strongly pathogenic isolates; the leaf sheath was thoroughly coloured with many dark hyphae, whereas the tissue of the internodal segment contained mainly hyaline hyphae.

After burial, dark hyphae were produced in the tissue of the straw by strongly pathogenic isolates, development being greater and more sustained in the series buried in nitrate-enriched soil than in those in unamended soil. In straws with weakly pathogenic isolates, no development of dark hyphae occurred in the tissues after burial. Furthermore, many of the dark hyphae in the leaf sheaths were obviously empty and fragmented even after burial for 8 weeks. Straws of all treatments still cohered after burial for 24 weeks, but those buried in nitrate-enriched soil were darker and softer than those in unamended soil. The leaf sheaths tended to disintegrate in all treatments, but were more decayed in straws buried in nitrate-enriched soil.

Because of the low survival of weakly pathogenic isolates (Table 4) these determinations were checked by attempting to isolate the fungus from test straws which had given negative results by the wheat seedling test. The fungus was isolated from only 2% of these straws after 4 weeks burial and was not obtained from any after longer periods in soil.

During the "seedling" test for viable hyphae in straws, strongly pathogenic isolates produced perithecia in straw tissue exposed to light above the sand level. In straws from nitrate-enriched soil, perithecia were abundant after burial for 4 and 8 weeks, were present in moderate numbers after 12 weeks, but few formed after burial for 24 weeks. Perithecia were also produced in straws buried in unamended soil, but less abundantly and not after burial for 24 weeks; perithecia were not formed in any straws infected with weakly pathogenic isolates. The strongly pathogenic isolates caused severe symptoms of take-all in the test seedlings at the beginning of the experiment but, as viability declined, the symptoms on the seedlings became milder.

IV. Discussion

The results demonstrate that an isolate of *O. graminis* may produce only strongly pathogenic progeny on one substrate and progeny differing in virulence on another. The cause of this variability is unknown, but it appears to be determined by whether there is a parasitic or saprophytic relationship between the fungus and substrate at ascosporogenesis. Thus only virulent progeny are produced in perithecia on living roots, but half are weakly pathogenic when formed in saprophytic situations such as on dead roots (White 1942) or in culture. White deduced that his eight monosporous isolates were derived from a diploid primary ascus nucleus which was heterozygous for the pair of genes determining degree of virulence. Segregation of the two alleles occurred during reduction division and resulted in four strongly pathogenic

and four weakly pathogenic progeny; the same explanation seems applicable to ascospores formed in culture. However, the occurrence of only virulent progeny in perithecia on living roots suggests the diploid primary ascus was homozygous for virulence and that the plant may have selected the virulent allelomorph. It is not known whether the fungus is a parasite or a saprophyte when all or most perithecia are formed in nature and this would be a profitable avenue for further study.

The increased survival of *O. graminis* in infected straw buried in nitrate-enriched soil was in accordance with previous work (Garrett 1938, 1940, 1944; Butler 1953, 1959; Macer 1961). However, isolates of common origin differed in their ability to survive saprophytically and the apparent link between survival and virulence emphasizes the need for detailed studies on physiological variations which affect survival. The inability of weakly pathogenic isolates to survive in straw suggests they have a limited role, if any, in nature. At present there is no information available on the occurrence of weakly pathogenic strains in the field. It would, therefore, be of value to obtain monosporous isolates at intervals from the earliest appearance of perithecia until late autumn and determine the percentage of weakly pathogenic ascospores occurring before and after harvest.

As previously noted (Chambers and Flentje 1967) there was an apparent link between virulence and perithecial formation both in culture and on host tissue, but the reason is unknown.

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