STUDIES ON OAT-ATTACKING AND WHEAT-ATTACKING ISOLATES OF *OPHIOBOLUS GRAMINIS* IN AUSTRALIA

By S. C. CHAMBERS*† and N. T. FLENTJE*

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Summary

Isolates of an oat-attacking strain of *Ophiobolus graminis* from Western Australia were differentiated from other isolates by comprehensive pathogenicity tests. Differential criteria for the two recognized varieties of *O. graminis* indicated that the Western Australian oat isolates were similar to var. *avenae*; South Australian isolates from oats were pathogenically similar to the type variety although some resembled var. *avenae* in their tolerance of oat extract. Pathogenicity on oats was the most reliable differential criterion and size of asci and ascospores the least; ascospore size was influenced by substrate, being generally greater in culture than on host tissue. Nuclear distribution in mycelia of wheat- and oat-attacking strains was similar but attempts to induce hyphal anastomosis between the two were unsuccessful.

A relation was observed between virulence of isolates and perithecial production on host tissue and in culture.

I. INTRODUCTION

Although oats are resistant to infection by the type variety of *Ophiobolus graminis*, an oat-attacking form, var. *avenae*, has been recorded in Wales (Turner 1940*b*), England (Garrett and Dennis 1943), Scotland (Dennis and Foister 1942), United States of America (Gould, Goss, and Eglitis 1961), Norway (Hansen 1963), and Northern Ireland (Anon. 1964). Turner (1940*a*, 1940*b*, 1957) differentiated between the two varieties on the basis of pathogenicity, size and septation of ascospores, and growth on oat sap and on media containing cysteine or cystine. Little other comparative information is available, particularly on nuclear distribution in their hyphae. Jones (1926) reported that the cells of *O. graminis* were uninucleate but Garrett (1942) suggested Jones' data referred to var. *avenae* and that some of the structures described were of another fungus. Nothing is known concerning the compatibility of the two varieties nor whether they combine readily to form further variants.

In Australia *O. graminis* has occasionally been recorded on oats (Darnell-Smith and McKinnon 1915; Osborn 1919; Scott 1948) and Hynes (1937) reported susceptibility of oats to two isolates in pathogenicity tests; Turner (1940*b*), however, discounted Hynes' results, suggesting that a toxic effect from excessive inoculum may have caused the observed stunting. Butler (1961), in his comprehensive review, stated that an oat-attacking strain had not been recorded in Australia. Chambers (1964) has since reported an oat-attacking strain in Western Australia but no pathogenicity tests

* Department of Plant Pathology, Waite Agricultural Research Institute, University of Adelaide.

† Present address: Victorian Plant Research Institute, Burnley Gardens, Burnley, Vic.

were conducted and no attempt was made to establish whether or not it was var. *avenae*. This paper therefore describes an examination of some Australian isolates of *O. graminis* from wheat and oats using Turner's differential criteria for the two varieties. Some studies on the compatibility of these isolates and on the distribution of nuclei in their hyphae are also described.

II. Materials and Methods

(*a*) *Isolates*

The isolates listed in Table 1 were used.

Table 1

ORIGIN OF ISOLATES OF *O. GRAMINIS*

Isolate No.	Source	Locality	Date
O1	Oats	Forest Hill, W.A.	December 1963
O2	Oats	Williams, W.A.	October 1965
O3	Oats	Woogenellup, W.A.	November 1962
O4	Oats	Mt. Gambier, S.A.	October 1962
O5	Oats	Mt. Gambier	October 1962
O6	Oats	Mt. Gambier	October 1962
O7	Oats	Mt. Gambier	October 1962
O8	Oats	Munabella, S.A.	September 1961
W1	Wheat	Alford, S.A.	February 1965
W2	Wheat	Glen Osmond, S.A.	October 1965
W3	Wheat	Glen Osmond	October 1965
W6	Wheat	Glen Osmond	October 1965
W7	Wheat	Glen Osmond	October 1965
W8	Wheat	Swan Hill, Vic.	November 1965

(*b*) *Pathogenicity Testing*

The technique for assessing pathogenicity of isolates was adapted from that described by Garrett (1936); the experimental design was a simple randomization of treatments within three replications.

A range of hosts was used. Seed was sown in thin plastic pots containing a calcareous sandy soil which had been partially sterilized with aerated steam at 160°F for 30 min (Baker 1962). Each seed was planted between two thin 8-mm disks taken from a 14-day-old potato–Marmite–dextrose agar (PMD) culture of a test isolate. Five seeds were sown in each pot, but the number of plants was reduced to four after emergence. Moisture content of the soil was adjusted to 15% (pF 1·6) and the pots were watered daily to a constant weight. Pots were placed in a growth cabinet adjusted to a 10-hr "day" with a light intensity of 1500 f.c. and day and night temperatures of 20 and 18°C, respectively.

After 4 weeks, the roots of plants other than oats were washed free from soil and the tops cut off and weighed. The oldest three seminal roots from each plant

were examined under a dissecting microscope (×50) and the length of runner hyphae of *O. graminis* measured. Measurements were also taken of the overall length of these roots together with the lengths discoloured by infection. Oat plants were allowed to grow for 8 weeks before harvesting as above and their three oldest secondary roots were examined instead of the seminal roots.

Sections of roots were also stained with lactophenol–cotton blue and examined for hyphal penetration by the test isolates.

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

Wherever possible, perithecia were obtained from the original field material, but this was not always available; plants used for pathogenicity tests were another source as some isolates formed perithecia on roots in contact with the sides of translucent pots; perithecia were also obtained in cultures of some isolates by using the technique of Weste and Thrower (1963).

Measurements for a particular isolate were based on a random selection of asci and ascospores from at least five perithecia from the same source (Turner 1940*b*).

(*d*) *Growth on Media containing Oat Leaf Extract*

An aqueous oat leaf extract was prepared (Janes 1947) and mixed with a glucose–asparagine liquid medium (Lilly and Barnett 1951) so as to give a series of nutrient solutions containing 0, 100, 150, 200, 300, 400, 500, and 1000 ml extract per litre. Each of the series was dispensed in 5-ml aliquots in 100-ml flasks and sterilized by autoclaving at 1 atm for 7 min. Three flasks of each medium were inoculated with 3-mm diameter disks from a 14-day-old culture of a test isolate and incubated at 25°C for 6 days. Mycelial mats were then washed thoroughly, dried for 24 hr at 95°C, and weighed.

(*e*) *Growth on Media containing Cystine, Cysteine, or Casein*

The method of Turner (1957) was used and mycelial dry weights were determined as above.

(*f*) *Preparations of Mycelium for Nuclear Studies*

Isolates were grown on Cellophane overlying PMD in Petri dishes. For determining nuclear distribution in mycelium, inoculum was taken from the periphery of an actively growing colony and placed centrally on the Cellophane. After incubation for 3–5 days at 20°C, a sector was removed and stained with HCl–Giemsa (Robinow 1945) adapted from the method described by Hrushovetz (1956). For studies on hyphal anastomosis, the inocula of two isolates were placed 25 mm apart and incubated at 20°C until the two colonies came into contact. The common segment of intermingling hyphae was cut out and stained with HCl–Giemsa.

III. Results

(*a*) *Pathogenicity*

Marked differences were observed in virulence of 11 isolates when their pathogenicity was tested on wheat and oats (Tables 2 and 3); some were avirulent;

TABLE 2

PATHOGENICITY OF 11 WHEAT AND OAT ISOLATES OF *O. GRAMINIS* ON WHEAT

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 Data using Transformations:			
					$\text{Log}_e X$ Fresh Weight of Host	$\text{Log}_e (X+1)$ Discoloured Root	$\text{Log}_e (X+1)$ Hyphae along Root	$\text{Log}_e X$ Whole Root
O1	252	14	20	127	5·5262	2·6870	3·0186	4·8339
O2	91	29	38	94	4·3906	3·4066	3·6463	4·5421
O3	477	2	4	194	6·1559	1·3648	1·4999	5·2572
O4	156	31	41	115	5·0393	3·4460	3·7254	4·7271
O5	367	5	9	142	5·9022	1·6566	2·2515	4·9503
O6	168	30	46	95	5·1210	3·4167	3·8350	4·5456
O7	265	19	26	127	5·5666	2·9679	3·2900	4·8422
O8	543	0	0	199	6·2964	0·0000	0·0000	5·2897
W1	188	23	33	99	5·2269	3·1633	3·5141	4·5932
W2	200	22	28	119	5·2430	3·1337	3·3520	4·7796
W3	178	26	37	108	5·1596	3·2865	3·6330	4·6607
Control	484			186	6·1731			5·2169
Differences for significance at $P = 0·05$					0·4300	0·3606	0·3355	0·2436
Differences for significance at $P = 0·01$					0·5812	0·4887	0·4546	0·3293

TABLE 3

PATHOGENICITY OF 11 WHEAT AND OAT ISOLATES OF *O. GRAMINIS* ON OATS

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 Data using Transformations:			
					$\text{Log}_e X$ Fresh Weight of Host	$\text{Log}_e(X+1)$ Discoloured Root	$\text{Log}_e(X+1)$ Hyphae along Root	$\text{Log}_e X$ Whole Root
O1	485	19	33	153	6·1822	2·9924	3·4950	5·0302
O2	451	32	48	127	6·1075	3·4944	3·8844	4·8412
O3	656	0	1	128	6·4826	0·0000	0·4621	4·8478
O4	691	0	3	157	6·5375	0·0000	1·2296	5·0561
O5	752	0	0	155	6·6218	0·0000	0·2311	5·0389
O6	776	1	2	130	6·6501	0·6931	1·1552	4·8696
O7	702	0	3	157	6·5528	0·0000	1·2689	5·0461
O8	743	0	0	162	6·6048	0·0000	0·2311	5·0630
W1	847	0	2	146	6·7405	0·0000	1·1945	4·9762
W2	847	0	5	151	6·7394	0·0000	1·6702	5·0137
W3	791	0	9	156	6·6720	0·0000	2·2959	5·0353
Control	798			158	6·6693			5·0633
Differences for significance at $P = 0·05$					0·1842	0·3075	0·6906	n.s.
Differences for significance at $P = 0·01$					0·2435	0·4659	0·9358	n.s.

others weakly pathogenic on both wheat and oats; the remainder were strongly pathogenic on one or both hosts. Disregarding avirulent and weakly pathogenic isolates, the two virulent Western Australian isolates (O1, O2) discoloured secondary oat roots and significantly reduced top growth; secondary roots of oat plants inoculated with South Australian isolates O4, O6, O7, W1, W2, and W3 were almost entirely free from infection and top growth was not reduced significantly (Plate 1, Figs. 1 and 2). Hyphae of isolates O1 and O2 were observed in the vascular and inner cortical tissues, being more numerous in roots infected with O2; infections of secondary roots by South Australian isolates were fewer and restricted to the outer cortical cells.

All virulent isolates reduced top growth of wheat and discoloured seminal roots; hyphae of these isolates were observed in vascular tissues of roots.

A comparison of the pathogenicity of isolates O1 and W1 on the five hosts *Bromus mollis* L., *Hordeum leporinum* L., *H. vulgare* L., *Lolium rigidum* Gaud., and *Vulpia myuros* (L.) Gmel. is given in Table 4. Fresh weights of *B. mollis* and *H. leporinum* were reduced considerably more by isolate W1 than by isolate O1; the two isolates caused similar reductions to the fresh weight of *H. vulgare*, but they had little, if any, effect upon the fresh weights of *L. rigidum* and *V. myuros*. Isolate O1 caused more root injury than isolate W1 on *V. myuros*, but isolate W1 caused more root injury than isolate O1 on *B. mollis* and *H. leporinum*; there was no significant difference in the virulence of the two isolates on *H. vulgare* and *L. rigidum*.

(*b*) *Perithecial Formation and Virulence*

Mature perithecia were produced on host tissue and in culture by isolates O2, O4, O6, W1, W2, and W3 which were all strongly pathogenic on wheat; the moderately pathogenic isolate O1 produced one mature perithecium and several empty perithecia-like bodies in culture but none on host tissue whereas the moderately pathogenic isolate O7 formed immature perithecia-like bodies on both substrates; the weakly pathogenic and avirulent isolates O3, O5, and O8 produced none on either substrate.

Isolates W6, W7, and W8 produced perithecia in culture but were not tested for pathogenicity on wheat.

(*c*) *Measurements of Asci and Ascospores*

Ascus and ascospore measurements for each isolate which produced perithecia are given in Table 5; measurements based on the contents of mature perithecia on original field material are also included.

(*d*) *Growth on Differential Media*

The effect of increasing concentrations of oat leaf extract on the mycelial dry weight of six isolates from oats and three isolates from wheat is illustrated in Figure 1; all isolates from oats with the exception of isolate O8, were more tolerant than isolates from wheat to increasing concentrations of oat leaf extract.

The effect of cysteine, cystine, and casein amendments on the mycelial dry weight of the same isolates is set out in Table 6.

OAT- AND WHEAT-ATTACKING ISOLATES OF *O. GRAMINIS*

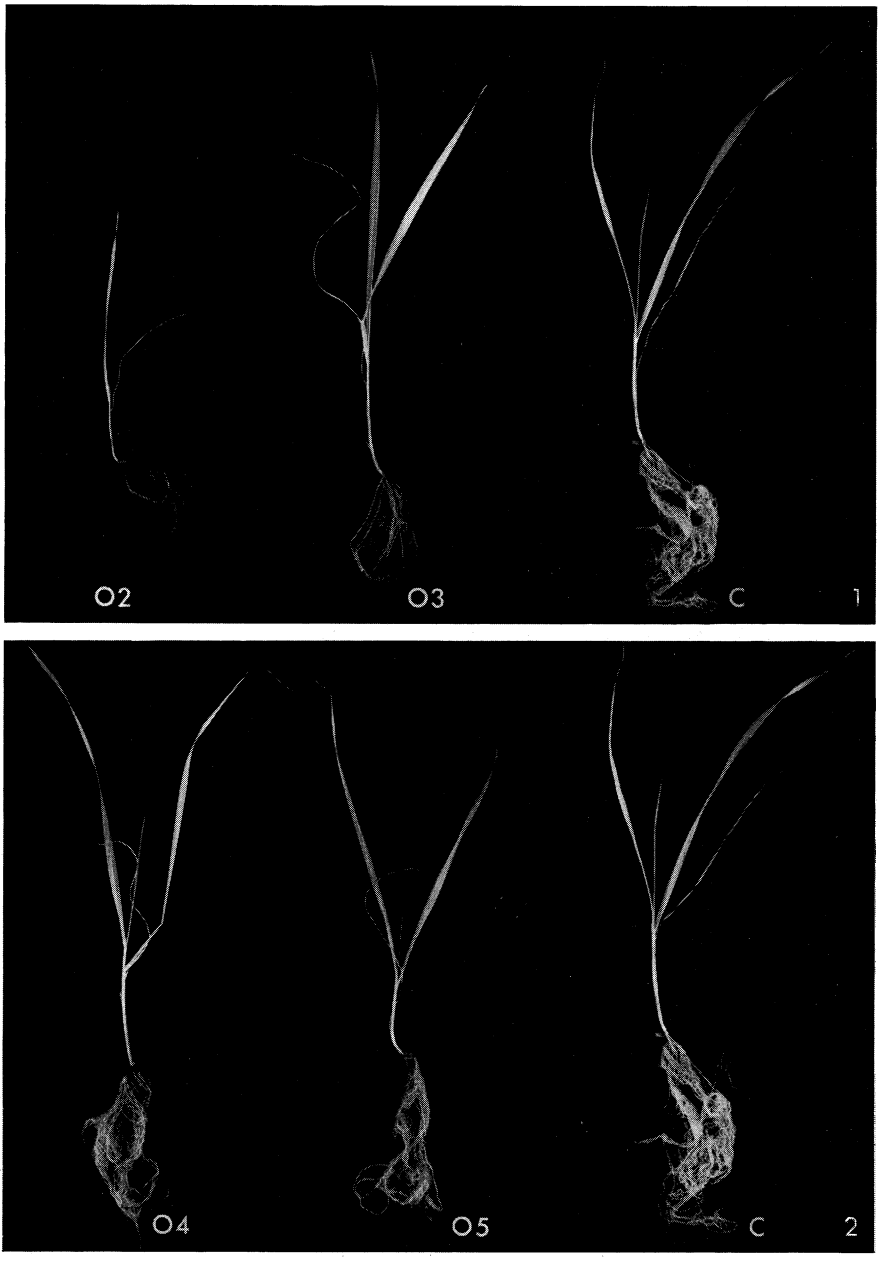
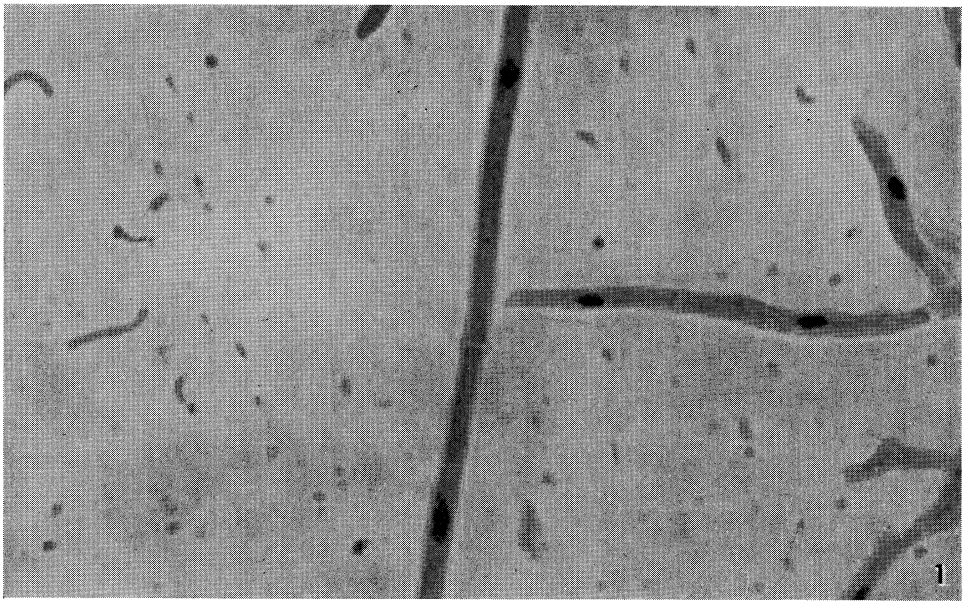


Fig. 1.—Root rotting and stunting of oat seedlings by isolate O2 but not by isolate O3 of *O. graminis* from Western Australia. *C*, control.

Fig. 2.—Failure of isolates O4 and O5 of *O. graminis* from South Australia to affect oat seedlings. *C*, control.

OAT- AND WHEAT-ATTACKING ISOLATES OF *O. GRAMINIS*



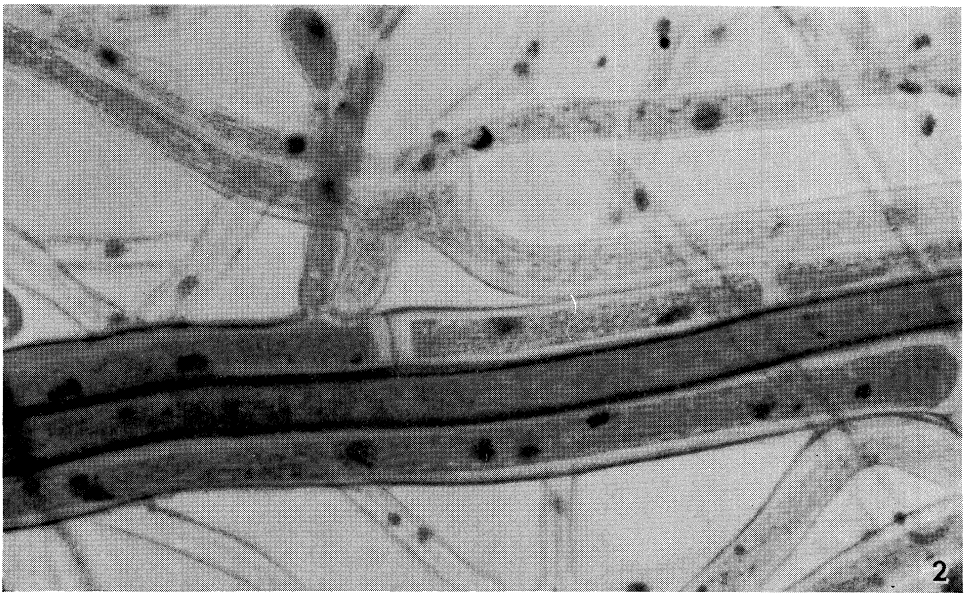


Fig. 1.—Uninucleate cells in hyaline hyphae of *O. graminis*.

Fig. 2.—Bi- or multinucleate cells in dark-coloured hyphae of *O. graminis* which have formed common strands.

TABLE 4

PATHOGENICITY OF OAT ISOLATE O1 AND WHEAT ISOLATE W1 OF *O. GRAMINIS* ON VARIOUS HOSTS

Host	Isolate	Fresh Weight of Host (mg)	Length of Discoloured Root (mm)	Length of Hyphae along Root (mm)	Length of Whole Root (mm)	Analysis of Data using Transformation:		
						$\text{Log}_e X$ Discoloured Root	$\text{Log}_e X$ Hyphae along Root	$\text{Log}_e X$ Whole Root
Bromus mollis	O1	68	0	9	69	0·0000	2·2236	4·2339
	W1	44	11	25	60	2·4470	3·2642	4·1455
	Control	73			78			4·3690
Hordeum leporinum	O1	198	13	36	136	2·5462	3·5810	4·8930
	W1	65	39	48	88	3·6821	3·8857	4·4714
	Control	258			193			5·2624
Hordeum vulgare	O1	355	28	44	141	3·3311	3·7984	4·9468
	W1	344	23	43	131	3·1768	3·7668	4·8635
	Control	467			188			5·2421
Lolium rigidum	O1	90	9	16	72	2·2232	2·7555	4·2805
	W1	86	5	15	64	1·7006	2·7340	4·1593
	Control	91			71			4·2631
Vulpia myuros	O1	37	9	18	34	2·2443	2·9110	3·5372
	W1	38	6	16	30	1·9702	2·8113	3·4049
	Control	40			40			3·7003
Differences for significance at $P = 0·05$						0·3250	0·3186	0·2376
Differences for significance at $P = 0·01$						0·4377	0·4290	—

(e) *Nuclear Distribution*

Segments, containing both hyaline and dark-coloured hyphae, from cultures of all isolates were examined after staining. In all segments hyaline cells were predominantly uninucleate and dark-coloured cells were generally bi- or multinucleate (Plate 2, Figs. 1 and 2). The number of nuclei in dark-coloured cells also appeared to be related to cell length; the longest cells contained most nuclei. Cells varied considerably in size and, in general, dark-coloured cells and peripheral tip cells were larger than the remaining hyaline cells. Strands of two or more hyphae were present in all cultures and were usually composed of dark-coloured cells.

A more detailed examination was made of 800 cells from each of isolates O1 and W1. Nuclei were counted in equal numbers of cells of the four sources: hyphal tips, single hyaline hyphae, hyaline strands, and dark-coloured hyphae. The hyphal tip cells were of two kinds, internal and peripheral, and only 100 cells of each were examined. All cells were also measured and details of nuclear counts and cell measurements are set out in Table 7. Counts were also made of the number of nuclei in cells of 100 ascospores of isolates O1 and W1; all cells were uninucleate.

(f) *Hyphal Anastomosis*

Five isolates from oats and three from wheat were opposed in all possible combinations to determine whether they would anastomose with each other. On meeting, opposing hyphae reacted in one of the following ways:

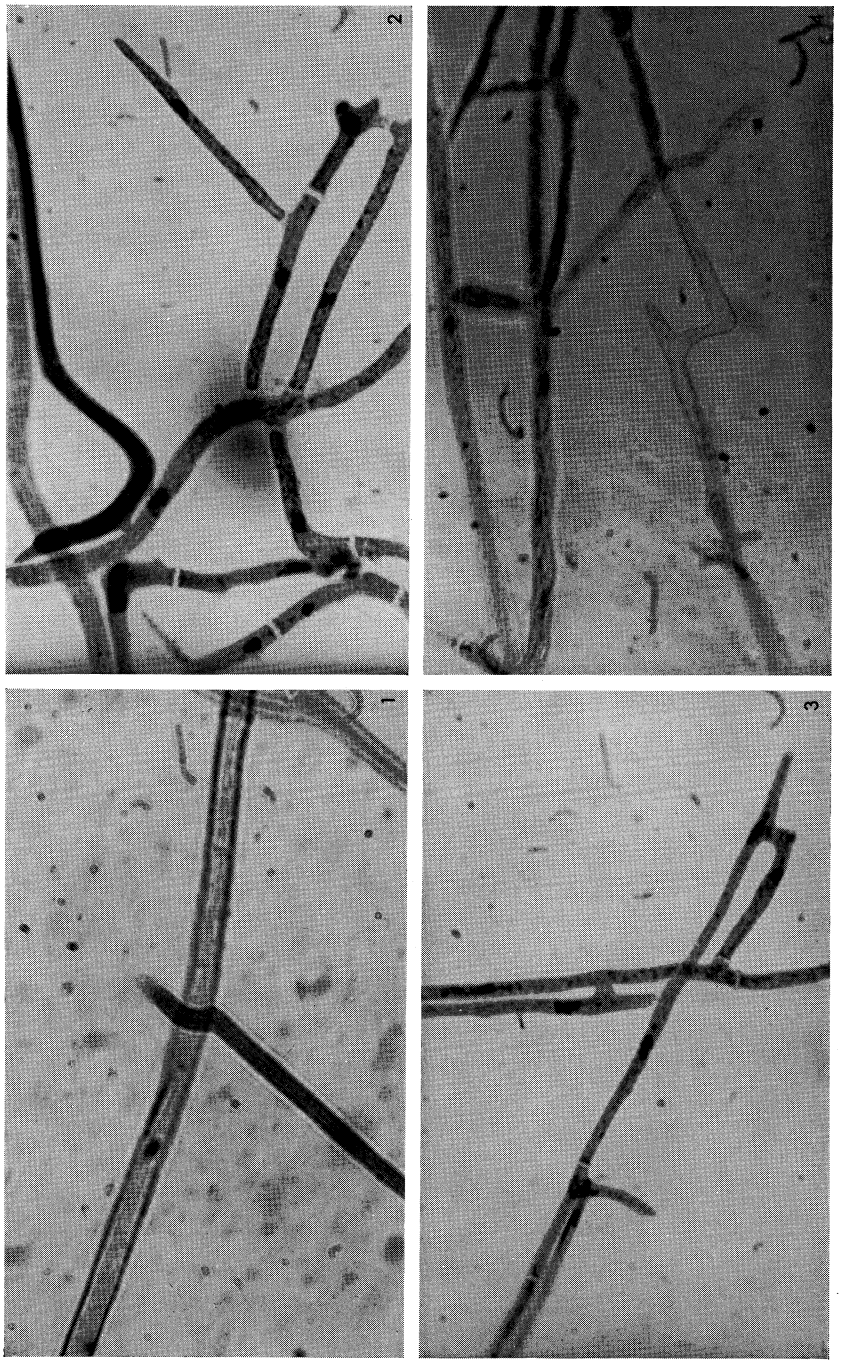
1. Intermingled freely, usually growing over and around each other (Plate 3, Fig. 1). Occasionally the opposing hyphae formed a relatively loose common strand, separating again after a short distance ..... Reaction N (Table 8)
2. Formed many common strands (Plate 2, Fig. 2) which were similar to those in pure cultures ..... Reaction ST
3. Formed peg-like outgrowths towards hyphae (Plate 3, Fig. 2) of the opposing isolate ..... Reaction P
4. Anastomosed but the cells collapsed and died soon afterwards (Plate 3, Fig. 4) ..... Reaction K
5. Anastomosed without any adverse effects upon the cells (Plate 3, Fig. 3) .... Reaction A

The reactions of the eight isolates towards each other are summarized in Table 8. Compatibility of isolates, expressed as successful hyphal anastomosis, was observed only between pathogenically similar isolates from the same locality. It occurred only occasionally in the two successful combinations despite the large masses of intermingling hyphae. Western Australian isolates from oats were incompatible with South Australia isolates from oats and both were also incompatible with isolates from wheat. Self-anastomosis, however, was relatively common in all preparations and occurred most frequently between hyphae near the advancing edge of the colony.

IV. Discussion

A comparison of the preceding data with that of Turner (1940*a*, 1940*b*, 1957) and Janes (1947) indicates similarities between Western Australian oat-attacking isolates and var. *avenae*; both stunted oats, discoloured secondary roots, and hyphae invaded the vascular tissue. No similar discoloration or penetration of secondary oat

OAT- AND WHEAT-ATTACKING ISOLATES OF *O. GRAMINIS*



Figs. 1–4.—Reactions between opposing hyphae from different isolates of *O. graminis*: *1*, Hyphae growing over each other with no special interaction. *2*, Formation of peg-like outgrowths towards opposing hyphae. *3*, Anastomosis between hyphae with no adverse effect. *4*, Anastomosis between hyphae with subsequent death of participating cells.

TABLE 5

ASCUS AND ASCOSPORE MEASUREMENTS OF ISOLATES OF *O. GRAMINIS* FROM WHEAT AND OATS

Isolate	Source of Asci	Mean Length of Asci (μ)	Mean Ascospore Length (μ)	Modal Ascospore Length (μ)	Range in Ascospore Lengths (μ)	Mean No. of Septa	Range in No. of Septa
O1	Original material	121±0·91	94±0·70	92	81–107	10·0	7–11
O2	Original material	123±0·78	103±0·56	105	92–118	9·6	5–11
	Culture	136±0·84	105±0·68	105	89–131	11·6	9–15
O3	Original material	124±0·84	91±0·55	92	84–105	10·0	7–13
O4	Pathogenicity test—oats	117±0·79	93±0·73	97	79–110	7·0	5–11
	Culture	125±0·67	102±0·44	102	92–113	7·9	5–11
O6	Pathogenicity test—oats	115±0·73	96±0·76	92	81–118	6·9	5–9
	Culture	128±0·58	99±0·49	97	92–113	8·0	5–11
W1	Original material	106±0·91	83±0·58	81	68–94	7·0	5–9
	Pathogenicity test—oats	108±0·80	85±0·52	85	73–97	6·9	5–9
	Culture	120±0·72	95±0·56	92	81–110	7·7	5–11
W2	Original material	113±0·91	91±0·60	92	79–105	6·9	5–7
	Pathogenicity test—oats	114±0·91	87±0·54	84	76–102	7·0	5–9
	Culture	118±0·65	99±0·53	100	89–113	7·0	5–9
W3	Original material	114±0·69	92±0·57	92	81–105	7·0	5–7
	Pathogenicity test—oats	114±0·74	88±0·85	84	71–110	7·0	5–9
	Culture	120±0·76	97±0·80	92	79–115	7·0	5–7
W6	Original material	106±0·56	86±0·56	89	73–100	6·9	5–9
	Culture	122±0·65	110±0·73	110	89–123	7·1	7–9
W7	Original material	113±0·71	90±0·53	92	79–100	7·0	5–9
	Culture	119±0·71	104±0·55	105	86–115	7·0	5–7
W8	Original material	96±0·73	76±0·54	79	66–86	6·4	3–9
	Culture	109±0·71	86±0·53	84	76–100	6·3	3–8

TABLE 6

EFFECT OF CYSTINE, CYSTEINE, AND SOLUBLE CASEIN ON MYCELIAL DRY WEIGHT OF *O. GRAMINIS* FROM WHEAT AND OATS

Isolate	Dry Weight (mg) of Mycelium on:				Analysis of Data using Transformation $\text{Log}_e(X+1)$:			
	Basal Medium (control)	Basal Medium plus Cystine	Basal Medium plus Cysteine	Basal Medium plus Casein	Basal Medium (control)	Basal Medium plus Cystine	Basal Medium plus Cysteine	Basal Medium plus Casein
O1	6	5	2	18	1·9904	1·7824	1·1945	2·9227
O2	10	7	2	14	2·3661	2·1135	1·0594	2·7268
O3	9	7	4	16	2·3026	2·0966	1·6472	2·8130
O4	13	12	16	35	2·6047	2·5570	2·8130	3·5825
O7	12	17	0	29	2·5467	2·9025	0·0000	3·3884
O8	16	13	11	35	2·8130	2·6589	2·5022	3·5924
W1	10	19	20	36	2·3867	3·0120	3·0557	3·6015
W2	15	19	18	37	2·7928	2·9778	2·9615	3·6273
W3	15	16	18	32	2·7713	2·8130	2·9615	3·4943
Differences for significance at $P = 0·05$							0·2101	
Differences for significance at $P = 0·01$							0·2786	

roots has been reported for type variety isolates. Both the Western Australian oat-attacking isolates and var. *avenae* were more tolerant than type variety isolates of oat extract. However, in contrast with Turner's observations on var. *avenae*, the

TABLE 7

SIZE AND NUCLEAR NUMBER OF CELLS OF ISOLATES O1 AND W1 OF *O. GRAMINIS*

Source	Isolate	Mean Length of Cells (μ)	Range in Cell Length (μ)	Width of Cells (μ)	Mean No. of Nuclei per Cell	Range in No. of Nuclei per Cell
Peripheral tip	O1	43·78±1·88	14·2–101·4	1·54±0·03	1·1	1–2
	W1	33·19±1·57	10·6– 86·1	1·63±0·04	1·1	1–2
Internal tip	O1	23·62±0·70	14·2– 41·3	1·77±0·04	1·0	1–2
	W1	16·32±0·59	7·1– 37·8	1·76±0·04	1·1	1–2
Single hyphae	O1	24·15±0·86	7·1– 97·9	2·05±0·05	1·1	1–2
	W1	17·56±0·61	5·9– 61·4	2·17±0·04	1·1	1–4
Hyaline strand	O1	18·60±0·73	10·6– 72·0	3·20±0·05	1·4	1–4
	W1	14·15±0·32	7·1– 33·0	1·87±0·03	1·2	1–2
Dark strand	O1	35·94±1·90	10·6–206·5	4·02±0·06	2·2	1–8
	W1	37·32±3·07	5·9–236·0	3·70±0·08	1·9	1–10

Western Australian isolate O1 was less virulent than the wheat isolate W1 on some alternate hosts (Table 4). This may have been due in part to a loss of virulence in culture by isolate O1 as it had been maintained for 3 years before testing pathogenicity.

TABLE 8

REACTION OF WHEAT AND OAT ISOLATES WHEN OPPOSED ON CELLOPHANE OVERLYING POTATO–MARMITE–DEXTROSE AGAR IN PETRI DISHES

For explanation of symbols, see text, p. 934

Isolate	O1	O2	O4	O7	O8	W1	W2	W3
O1	A	ST	N	N	N	P	ST	N
O2		A	ST	ST	ST	K	N	N
O4			A	A	ST	K–ST	ST	N
O7				A	ST	K	N	N
O8					A	K	P–ST	N
W1						A	K	K
W2							A	A
W3								A

South Australian isolates from oats were pathogenically similar to the type variety (Tables 2 and 3), but some were equally tolerant as the Western Australian isolate O2 and var. *avenae* (Janes 1947) of oat extract (Fig. 1). This suggests some South Australian isolates may be intermediate for some characters between the two recognized varieties. In the present studies pathogenicity on oats was the most

reliable of Turner's criteria for distinguishing between the type variety and var. *avenae*. Measurements of asci and ascospores were the least reliable; thus most measurements for ascospores produced on host material were intermediate between the ranges recorded by Turner for var. *avenae* (101–117 μ) and the type variety (79–86 μ). Furthermore ascospore size was influenced by substrate and was usually greater in culture than on host tissue (Table 5); in the case of wheat isolate W6, the mean ascospore lengths on host tissue (86 μ) and in culture (110 μ) were within the type variety and var. *avenae* ranges respectively. Although there appeared to be

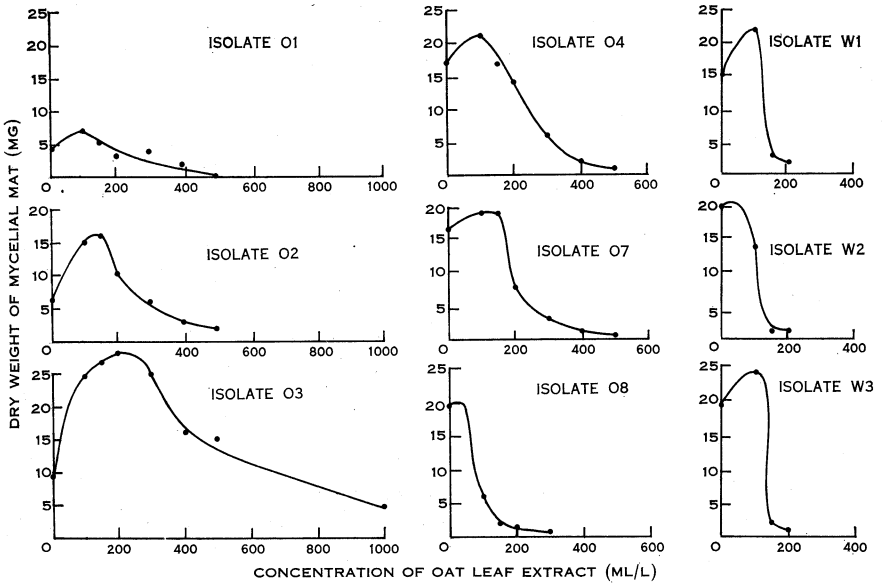


Fig. 1.—Effect of various concentrations of oat leaf extract on mycelial dry weight of oat and wheat isolates of *O. graminis*.

a general tendency for cystine and cysteine to increase the mycelial dry weight of isolates from wheat and to suppress that of Western Australian oat isolates (Table 6), statistical analysis showed that only some increases and decreases were significant. The effect of the two amino acids on South Australian isolates were variable, thus providing no information for classifying them as either variety of *O. graminis*.

No explanation can be given for the apparent link between virulence and perithecial formation by isolates from wheat and oats. Davis (1925) only obtained perithecia in cultures of the "New York" strain which was the most pathogenic of his three test isolates. Willetts (1961), when examining six isolates from wheat and oats, noted that two weakly pathogenic oat isolates failed to produce perithecia on either wheat or oats; the remaining four formed perithecia on host tissues on which they were actively parasitic.

Nuclear distribution was similar in the mycelium of wheat and oat isolates. A point of interest is the difference in the numbers of nuclei in hyaline and dark-coloured cells, especially in view of the different functions of hyaline "infection" hyphae and dark "runner" hyphae in nature. Wheat and oat isolates were apparently

incompatible, thereby indicating that there is little chance of variants arising from combinations of the two strains. However, these isolates were from different districts or States and successful anastomosis was only observed between pathogenically similar isolates from the same locality. It would therefore be of value to study the compatibility of a wide range of wheat- and oat-attacking strains if they could be obtained from the same property or district.

The oat-attacking strain in Western Australia is restricted to a narrow zone along the western fringe of the main southern wheat belt (Chambers 1964). However, there is insufficient evidence to determine whether it is indigenous or was introduced and also whether it is of recent origin. If its origin was due to recent genetic changes followed by selection, one might reasonably expect to find oat-attacking isolates widely distributed in Australia. This may be the case, but to date there are no other substantiated reports of its occurrence. The above studies, however, show there is considerable variation between different isolates of *O. graminis* and Turner's four criteria are not applicable for all oat-attacking isolates. It is probable that other oat-attacking isolates differing from both var. *avenae* and the isolates described above, will be found in Australia; it is also probable that isolates with other pathogenic specificities will be found as well.

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