SHORT COMMUNICATIONS

PATHOGENIC VARIATION IN *OPHIOBOLUS GRAMINIS**

By S. C. CHAMBERS†

Differences in pathogenicity of various *Ophiobolus graminis* isolates have been observed frequently (Davis 1925; Padwick 1936; Hynes 1937; White and McIntyre 1943; Henry and Gilpatrick 1947; Henry and McKenzie 1959). However, most workers give little or no information about the interval between isolation and testing of pathogenicity, although it is evident some isolates have been in culture for long periods. As virulence may vary considerably with repeated subculturing (Russell 1934, 1939), it is possible that some studies have been made with cultural variants which do not exist in nature. This communication, therefore, describes tests using the initial growth from fresh monosporous field isolates of *O. graminis*.

Materials and Methods

(i) *Isolates.*—Details of the isolates used are given in Table 1.

(ii) *Method of Isolation.*—Monosporous cultures were isolated by the technique of Chambers and Flentje (1967*b*). However, viable ascospores were obtained only from asci which discharged within 2 hr of being placed in water. Ascospores ejected beforehand did not germinate whilst asci which failed to discharge appeared to be immature.

(iii) *Soil.*—A sandy loam from the Mallee Research Station, Walpeup, Vic., was used. The physical characteristics of the soil were as follows: particle size distribution: 1–2 μm, 6%; 2–20 μm, 1%; over 20 μm, 93%; total nitrogen (Kjeldahl), 0·033%; organic carbon, 0·8%; bicarbonate phosphorus, 12 p.p.m.; pH 7·2. The drying boundary curve is shown in Figure 1.

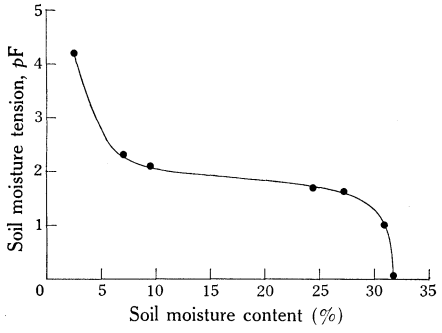

Fig. 1.—Drying boundary curve of soil used.

(iv) *Pathogenicity Tests.*—Tests were conducted in growth cabinets adjusted to 10-hr days of "natural" light using the technique outlined by Chambers and Flentje (1967*a*).

Experimental Details and Results

(i) *Tests with Initial Growth from Ascospores.*—Fifty monosporous isolates were made, the spores being taken at random from a perithecium on a naturally infected wheat straw. Agar disks containing the initial growth from a spore were used to inoculate wheat seeds. Five seeds were sown in each pot, but the number of plants

* Manuscript received May 11, 1970.

† Victorian Plant Research Institute, Burnley, Vic. 3121.

TABLE 1

PATHOGENICITY OF MONOSPOROUS FIELD ISOLATES OF *O. GRAMINIS* ON WHEAT

Isolates		Fresh Weight of Foliage (mg)		Length of Discoloured Roots (mm)		Length of Roots with Runner Hyphae (mm)		Whole Root Length (mm)	
Source	Nos.	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Perithecium from Curlewis, Vic.	1–50	83·6±6·2	32–218	25·7±0·9	9–39	36·0±1·6	9–60	50·4±3·4	9–121
	Control	642·0						306·0	
	51–100	119·8±8·9	41–309	29·2±0·9	13–42	40·2±1·4	15–70	59·4±3·1	15–119
	Control	647·5						306·0	
Perithecium from Ceres, Vic.	101–150	89·8±6·0	34–219	32·2±1·2	17–53	39·4±1·7	20–66	47·5±2·4	23–90
	Control	716·5						339·0	
	151–200	151·0±6·9	37–247	26·1±0·9	11–39	31·4±1·1	12–44	65·3±2·4	17–105
	Control	590·5						219·5	
Perithecium from Sutherlands Creek, Vic.	201–250	162·6±7·6	78–269	39·3±1·2	15–58	45·3±1·5	17–67	58·5±2·5	21–100
	Control	825·5						413·0	
	251–300	74·7±4·7	28–163	27·4±1·2	12–47	33·5±1·6	13–59	44·9±2·8	13–92
	Control	713·5						394·5	

was reduced to four after emergence. Each isolate was tested in one pot only because of limited space in the growth cabinets.

Six similar tests were carried out using a total of two perithecia from each of three localities (Table 1). These results indicate that all isolates were strongly pathogenic, although considerable variation occurred within and between tests.

(ii) *Test with the First Subcultures.*—A replicated comparison was made between four isolates which had caused either most or least reduction in plant size in three of the initial tests. The experimental design was a simple randomization of treatments within each of four replications and agar disks from the first subcultures of the selected isolates were used as inocula.

Results (Table 2) show that all 12 were strongly pathogenic with possibly only minor differences in the virulence of some isolates.

TABLE 2

INITIAL PATHOGENICITY OF 12 MONOSPOROUS ISOLATES OF *O. GRAMINIS* ON WHEAT

Logarithmically transformed values given in parenthesis

Isolate		Fresh Weight (mg)		Length of Discoloured Roots (mm)	Length of Roots with Runner Hyphae (mm)	Whole Root Length (mm)
Source	No.	Previous Tests	Present Tests			
Curlewis	9	43	29 (1·46)	6 (0·76)	6 (0·78)	7 (0·82)
	10	32	38 (1·58)	9 (0·95)	9 (0·96)	9 (0·96)
	15	186	69 (1·84)	18 (1·27)	19 (1·29)	21 (1·36)
	17	218	49 (1·69)	11 (1·04)	12 (1·07)	14 (1·15)
Ceres	122	219	86 (1·94)	20 (1·31)	22 (1·34)	31 (1·49)
	132	176	63 (1·80)	15 (1·16)	16 (1·20)	18 (1·25)
	143	34	109 (2·04)	23 (1·35)	25 (1·40)	39 (1·59)
	145	41	69 (1·84)	14 (1·16)	16 (1·20)	20 (1·30)
Sutherlands	203	78	89 (1·95)	18 (1·25)	20 (1·31)	28 (1·44)
Creek	227	83	83 (1·92)	19 (1·28)	21 (1·33)	28 (1·44)
	232	269	125 (2·10)	24 (1·38)	26 (1·41)	40 (1·60)
	238	269	80 (1·90)	17 (1·24)	18 (1·26)	26 (1·41)
Control			590 (2·77)*	0	0	285 (2·46)*
L.S.D. ($P = 0·05$):			(0·25)	(0·30)	(0·32)	(0·38)
L.S.D. ($P = 0·01$):			(0·33)	(0·40)	(0·43)	(0·51)

* Excluded from analysis.

(iii) *Test after Subculturing for 11 Months.*—A similar replicated comparison was made between the same isolates after 11-monthly subculturings. However, the experiment differed in that replications were reduced to three and the number of seedlings to one per pot. Results (Table 3) indicate that eight of the isolates had become either weakly pathogenic or avirulent.

Discussion

The work demonstrated that all isolates were strongly pathogenic when first obtained from field material, but a number lost virulence with repeated subculturing.

Some variability in pathogenicity occurred within and between the initial tests in naturally lighted cabinets (Table 1), but a subsequent replicated experiment suggested most variation was due to experimental factors. In another series of pathogenicity tests in which the same cabinets were used the author found that variability in natural light contributed to variability of results (Chambers, unpublished data).

The isolation of only strongly pathogenic cultures from field material raises further doubts as to whether weakly pathogenic variants have any role in nature. Previously, Chambers and Flentje (1967*b*) studied the survival of strongly and weakly pathogenic monosporous isolates from a perithecium formed in culture; they found that strongly pathogenic isolates survived well on straw buried in unsterilized soil, but weakly pathogenic variants did not. It, therefore, seems probable that weakly pathogenic variants arise in nature, but are unable to survive. Consequently, all field isolates are likely to be strongly pathogenic initially.

TABLE 3

PATHOGENICITY OF *O. GRAMINIS* ISOLATES ON WHEAT AFTER 11 MONTHLY SUBCULTURES

Logarithmically transformed values given in parenthesis

Isolate No.	Fresh Weight (mg)	Length of Discoloured Roots (mm)	Length of Roots with Runner Hyphae (mm)	Whole Root Length (mm)
9	1009 (3·00)	0	0	318 (2·50)
10	1026 (3·01)	2 (0·49)	4 (0·66)	251 (2·40)
15	1130 (3·05)	0	0	340 (2·53)
17	178 (2·25)	30 (1·50)	38 (1·59)	52 (1·71)
122	588 (2·77)	7 (0·88)	11 (1·08)	153 (2·18)
132	849 (2·93)	3 (0·56)	3 (0·65)	230 (2·36)
143	897 (2·95)	1 (0·36)	2 (0·46)	316 (2·50)
145	973 (2·99)	0	0	279 (2·45)
203	986 (2·99)	< 1 (0·10)	1 (0·20)	301 (2·48)
227	519 (2·72)	14 (1·19)	28 (1·47)	112 (2·05)
232	528 (2·72)	26 (1·43)	35 (1·56)	110 (2·04)
238	1028 (3·01)	0	0	322 (2·51)
Control	973 (2·99)	0	0	321 (2·51)
L.S.D. ($P = 0·05$)	(0·15)	(0·59)	(0·73)	(0·20)
L.S.D. ($P = 0·01$)	(0·21)	(0·82)	(1·01)	(0·27)

Acknowledgments

Grateful acknowledgment is made to Mr. R. Jardine for assistance with with statistical analyses. This work was supported by funds from the Wheat Industry Research Council of Australia.

References

CHAMBERS, S. C., and FLENTJE, N. T. (1967*a*).—Studies on oat-attacking and wheat-attacking isolates of *Ophiobolus graminis* in Australia. *Aust. J. biol. Sci.* **20**, 927–40.

CHAMBERS, S. C., and FLENTJE, N. T. (1967*b*).—Studies on variation with *Ophiobolus graminis*. *Aust. J. biol. Sci.* **20**, 941–51.

DAVIS, R. J. (1925).—Studies on *Ophiobolus graminis* Sacc. and the take-all disease of wheat. *J. agric. Res.* **31**, 801–25.

HENRY, A. W., and GILPATRICK, J. D. (1947).—Relative pathogenicity of single and mixed strains of *Ophiobolus graminis* Sacc. Abstr. in *Proc. Can. phytopath. Soc.* **15**, 14–15.

HENRY, A. W., and MCKENZIE, A. R. (1959).—Note on the comparative activity of monosporous and mycelial isolates of *Ophiobolus graminis* Sacc. from the same source as wheat pathogens in natural and sterilized soil. *Can. J. Pl. Sci.* **39**, 405–7.

HYNES, H. J. (1937).—Studies on take-all of wheat. I. *J. Aust. Inst. agric. Sci.* **3**, 43–8.

PADWICK, G. W. (1936).—Biologic strains of *Ophiobolus graminis* Sacc. *Ann. appl. Biol.* **23**, 45–56.

RUSSELL, R. C. (1934).—Studies in cereal diseases. X. Studies of take-all and its causal organism *Ophiobolus graminis* Sacc. Bull. Can. Dep. Agric. No. 170. (N.S.)

RUSSELL, R. C. (1939).—Pathogenicity tests with cultures of *Ophiobolus graminis* Sacc. *Sci. Agric.* **19**, 662–9.

WHITE, N. H., and MCINTYRE, G. A. (1943).—The pathogenicity of single spore isolates of *Ophiobolus graminis* under field conditions. *J. Coun. scient. ind. Res. Aust.* **16**, 93–4.