RELATIVE EFFECTS OF SOIL NITROGEN AND SOIL ORGANISMS ON SURVIVAL OF *OPHIOBOLUS GRAMINIS**

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Introduction

All previous work on the effect of nitrate enrichment of soil upon the survival of *Ophiobolus graminis* on wheat straw has been with unsterilized soil (Garrett 1938, 1940, 1944; Butler 1953, 1959; Lucas 1955; Macer 1961; van der Watt 1965). While this has approximated field conditions, it has precluded any determination of the direct effect of the amendment as distinct from its effect through the growth of other soil organisms. Use of an initially sterile soil may provide a method of separating these effects. As ionizing radiation will completely sterilize soil with a minimum of side effects (van Groenewoud 1959; Bowen and Rovira 1961; Bowen and Cawse 1962), such a treatment was selected for studying the effects of soil nitrogen and soil micro-organisms on survival of *O. graminis*.

Materials and Methods

(i) *Soil.*—A calcareous sandy soil from Moonta, S.A., was used (Chambers and Flentje 1967). Sealed polythene plastic bags, each containing 200 g of air-dry soil, were sterilized by gamma-irradiation at a dosage of 5 Mrad at the Australian Atomic Energy Commission, Lucas Heights, N.S.W. Upon return, two bags were checked for microorganisms by placing some soil on each of 10 freshly poured plates of potato–Marmite–dextrose agar (PMD). No growth occurred on any of the plates after 10 days' incubation at 20°C.

(ii) *Inoculation of Wheat Straws with* O. graminis.—The method was based on that described by Butler (1953). Wheat straws were sterilized by adding 20 ml of distilled water to each batch of 50 straws in a 500-ml Erlenmeyer flask and autoclaving at 1·5 atm for 90 min. Each batch was inoculated with a 7-mm disk from a 14-day-old PMD culture of isolate W1 (Chambers and Flentje 1967); the flasks were incubated at 25°C for 14 days, shaken, and then left at the same temperature for another 21 days.

(iii) *Estimating Saprophytic Survival.*—Gamma-irradiated soil (200 g) was added to each flask by cutting the corner off a polythene bag and allowing the contents to run into the flask. Sterile water (10 ml per flask) was added to half the flasks and an equal volume of a sterile sodium nitrate solution (20 g nitrogen per flask) added to the remainder. This gave an initial moisture content of 8·3% (pF 1·8). Half the flasks in each series were then deliberately contaminated by adding 10–14 mg of air-dry unsterilized Moonta soil to each flask. After covering the cotton wool plugs with polythene to reduce loss of moisture, the flasks were stored in darkness at 20°C. After 4, 8, 12, and 24 weeks' storage, 100 straws of each treatment were unearthed and tested for viable hyphae by the wheat seedling test (Garrett 1938).

(iv) *Isolation of other Introduced Organisms.*—Before straws were used for the seedling test, isolations were made from each of 20 straws from the treatments containing introduced micro-organisms. No attempt was made to surface-sterilize the straws, but adhering soil was removed with Kleenex tissue. Part of the epidermis was cut away from the node in each straw and three small pieces of underlying tissue were removed and plated on PMD. The plates were incubated for 7–10 days at 20°C and the isolates from each sampling were recorded.

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Results

The percentages of straws containing viable hyphae of *O. graminis* at each sampling are given in Table 1; the fungi isolated from straws after deliberate contamination of soil are recorded in Table 2. Bacteria were recorded from all these straws.

TABLE 1

EFFECT OF SOIL ORGANISMS ON SURVIVAL OF *OPHIOBOLUS GRAMINIS* IN STRAW BURIED IN GAMMA-IRRADIATED SOIL WITH (+ N) AND WITHOUT (— N) ADDED NITROGEN

Survival Period (weeks)	Percentage of Straws with Viable Hyphae of *O. Graminis* in:			
	Uncontaminated Soil		Contaminated Soil	
	+ N	— N	+ N	— N
0	100	100	100	100
4	100	100	100	96
8	100	99	100	100
12	97	91	96	72
24	100	86	99	67

TABLE 2

FUNGI ISOLATED FROM WHEAT STRAWS INITIALLY COLONIZED BY *OPHIOBOLUS GRAMINIS* AND BURIED IN CONTAMINATED NITRATE-ENRICHED OR UNAMENDED SOIL

Fungi	Frequency of Isolation from Straws Buried in Nitrate-enriched Soil for:				Frequency of Isolation from Straws Buried in Unamended Soil for:			
	4 Weeks	8 Weeks	12 Weeks	24 Weeks	4 Weeks	8 Weeks	12 Weeks	24 weeks
Ophiobolus graminis	2	7	14	8	25	51	20	26
Rhizopus nigricans	23	11	39	2	5	1	35	11
Actinomucor repens	20		1	32			2	6
Gliocladium catenulatum	4	13	8	12				
Penicillium spp.		5	1	4	3	2		3
Mortierella sp.				3	5			
Fusarium oxysporum				2			1	
Gliocladium roseum			2				1	
Cladosporium sp.						1		
Fusarium roseum						1		
Unknown (sterile)		4		2	1			6

The soil surface in flasks which contained only *O. graminis* was covered by a dense mycelial growth within 4 weeks of storage. Two of these flasks were exposed to a light intensity of 210–290 f.c. at 20°C for 1 month and numerous perithecia developed on the sides of the flasks between the glass and the soil.

Throughout the experiment, straws were examined microscopically for mycelia of *O. graminis*. After burial for 4 weeks, mycelial development was similar in all

treatments, but by 12 weeks dark hyphae were more numerous in nitrate-enriched than in unamended soil. This difference was more obvious after 24 weeks' burial; after this period, dark hyphae were equally prevalent in straws in nitrate-enriched soil, irrespective of whether other organisms had been introduced or not. However, after 24 weeks in unamended soil, dark hyphae were fewer and more fragmented when other organisms were present. The straws of all treatments still cohered after 24 weeks, but were darker in colour when organisms other than *O. graminis* were present.

An unusual feature of the seedling test for viable hyphae was the low germination of the wheat seed. Germination ranged between 74 and 80% compared with 98% for the same seed when used for other tests in unsterilized Moonta soil (Chambers and Flentje 1967).

Discussion

Decline in viability of *O. graminis* was very slow in unamended irradiated soil, but was increased by introducing other soil organisms (Table 1). Nitrate enrichment of soil, however, outweighed the effects of introducing other soil organisms so that 99% of straws still contained viable hyphae of *O. graminis* after 24 weeks. Thus, survival of *O. graminis* appears to be affected more by availability of nitrogen than by competition and antagonism of other soil organisms. Nitrate enrichment of soil also increased the frequency of isolation of introduced organisms from straws, thereby making it more difficult to reisolate *O. graminis*, as evidenced by the lower frequency of isolation of *O. graminis* from the nitrogen-enriched soil (Table 2).

The experiment was planned to run for 24 weeks because previous tests in unsterilized soil had given positive results within this period (Chambers and Flentje 1967, 1968). However, it is obvious that to obtain critical data in irradiated soil, such tests should be continued for longer periods. The low germination of seed used for the seedling test indicates a possible weakness in the technique and should be studied further. Plant growth in irradiated soils has been examined by many workers and has been variously reported as slightly retarded (Bowen and Rovira 1961), unaffected (McLaren, Luse, and Skujins 1962), and consistently improved (Bowen and Cawse 1962). Bowen and Rovira (1961) also noted that irradiated soil became increasingly phytotoxic when stored at room temperature for 10 weeks prior to planting. The prolific growth by *O. graminis*, however, indicated that irradiation of soil had no deleterious effects upon the fungus. This growth is of particular interest in view of the occasional isolation of the fungus directly from soil (Warcup 1957).

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