Root Residues: Substrates used by *Fusarium culmorum* to Infect Wheat, Barley and Ryegrass

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Fusarium culmorum was grown on different natural substrates and its subsequent pathogenicity towards wheat, barley and ryegrass studied. This fungus caused more inhibition of plant growth of all test species when grown on root residues than when present as a spore suspension in sand. More wheat seedlings were killed when sown into infected ryegrass root residues than when sown into infected wheat or barley residues. However, when ryegrass was sown into infected ryegrass roots no plant death occurred compared with over 50% when sown into infected wheat or barley. Dressing ryegrass seeds with a formulation containing calcium peroxide gave some control of *F. culmorum*. These results are discussed in relation to the problems that can sometimes occur following reseeded grassland.

INTRODUCTION

Establishment problems for a new grass crop can follow direct reseeded. Possible explanations include damage by insects (Clements, 1980), microbiologically produced phytotoxic compounds (Lynch, 1977; Lynch & Penn, 1980, Gussin & Lynch, 1981) and soil-borne pathogens (Labruyere, 1979). *Fusarium culmorum* has long been known as a potential pathogen of grasses; Bennett (1935) described its prevalence on British grasses and cereals and Gordon (1952) made similar observations in Canada. More recently Penn & Lynch (1982) indicated its importance as a pathogen of cereals in the presence of decaying couch grass (*Agropyron repens*). Following grassland establishment by direct seeding, we have always isolated *F. culmorum* from establishing grasses. However, although the isolates have always been potentially pathogenic the seedlings have not always been affected. The substrate base from which a fungus grows in soil can strongly influence its growth and pathogenicity (Garrett, 1981) and therefore we have investigated the potential of different root residues to act as substrates from which *F. culmorum* may infect wheat, barley and ryegrass.

The potential for calcium peroxide, which has antimicrobial properties (Lynch *et al.*, 1981), as a seed dressing to alleviate *F. culmorum* infection of ryegrass has also been studied.

Because of the variability of soil and the consequential difficulty in designing reproducible experiments, the present study in a controlled environment was performed using sand culture.

METHODS

Preparation of spore suspensions. *Fusarium culmorum* (W. G. Smith) Sacc. IMI 239950, was isolated from diseased ryegrass plants and maintained in sterile soil. The fungus was recovered from the soil by inoculating on to potato dextrose agar; spore suspensions were prepared 10 d later by adding distilled water (10 ml) to each dish and gently brushing the surface with a camel hair brush. This suspension was adjusted to 10^6 spores ml^{-1} as necessary.

Preparation of infected root materials. Barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) seeds were selected by weight (weight per seed was 35–40 and 40–45 mg, respectively) to decrease plant variability (Harper & Lynch, 1980). Ryegrass (*Lolium perenne*, cv. S24) seeds were selected for uniform seed size by eye, with large and small seeds likewise being rejected to decrease variability. Cereal seeds were grown for 9 d (5 plants per jar) and ryegrass for 14 d (10 plants per jar) in acid washed, coarse sand (particle size 0.5–1.5 mm diam.) moistened with nutrient solution in 0.5 kg Kilner jars (0.4 l capacity); each treatment was replicated four times. Inoculum (3 ml) of

a standard spore suspension (10^6 spores ml^{-1}) was added as a drench to each jar immediately after planting. Jars were covered with plastic bags until emergence to maintain high humidity, and the plants were grown in a controlled environment cabinet at 20°C ($\pm 1^\circ\text{C}$), 75% relative humidity ($\pm 10\%$) and 16 h photoperiods.

Plant culture solution. All jars were watered daily to maintain their initial weight with a plant culture solution of composition: 1.5 mM- $\text{Ca}(\text{NO}_3)_2$, 5 mM- KNO_3 , 1 mM- KH_2PO_4 , 1.5 mM- MgSO_4 , 2 mM- NaNO_3 , 0.01 mM- Fe^{3+} as ferric EDTA, 9.2 μM - H_3BO_3 , 0.16 μM - CuSO_4 , 14.1 μM - KCl , 3.6 μM - MnSO_4 , 0.016 μM - $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$, 0.77 μM - ZnSO_4 .

Reseeding technique. After 9 d (cereals) or 14 d (ryegrass), the shoots were removed at seed level (to prevent regrowth) without disturbing the root systems and wheat, barley or ryegrass seeds were 'reseeded' adjacent to the old seeds. Control jars contained uninfected root material or *F. culmorum* only. Ryegrass seeds were planted either undressed or dressed with 20% (w/w) of a calcium peroxide formulation bound to the seed with polyvinyl alcohol (Lynch *et al.*, 1981).

Assessment of plant growth and infection. Percentage germination and shoot heights were measured after a further 9 and 14 d for cereals and ryegrass, respectively. Plants were then taken from the jars and any adhering sand particles removed from the root systems. The longest roots of all plants were measured and were then examined macroscopically for infection and scored on an arbitrary scale such that any plant with lesions greater than 2.5 mm on one or more roots was recorded as infected. Plants with lesions less than 2.5 mm on only one root rarely showed any stunting of growth at 28 d.

These lesions were further examined microscopically to confirm infection and *F. culmorum* was reisolated from them on all occasions. Wheat seeds often showed pre-emergence death and these were examined by scanning electron microscopy. Seeds were bisected and fixed in glutaraldehyde (1%, w/v) for 1 h followed by glutaraldehyde (3%, w/v) for 24 h. They were then washed in distilled water and dehydrated to 20% (v/v) acetone in water. Further dehydration to pure acetone was achieved by dehydration/diffusion under vacuum. They were then dried using a CO_2 critical-point dryer.

RESULTS

The non-inoculated wheat root residues had no effect on germination or root and shoot growth of the cereals but did inhibit root and shoot growth of ryegrass. Both barley and ryegrass non-inoculated residues inhibited growth of all test plants but had little effect on germination (Table 1). *Fusarium culmorum* alone reduced wheat germination by 43% and severely inhibited root and shoot growth. Germination of barley and ryegrass was not inhibited and, whereas both shoot and root growth of barley were depressed, there was no effect on ryegrass. However, when

Table 1. *Effect of plant residues and Fusarium culmorum* infection on the germination and root and shoot growth of wheat, barley and ryegrass

Root residue	Germination and root and shoot extension*								
	Wheat			Barley			Ryegrass		
	Percentage germination	Root	Shoot	Percentage germination	Root	Shoot	Percentage germination	Root	Shoot
Non-inoculated									
Wheat	104	104	104	99	106	100	100	79	86
Barley	93	88	90	97	76	71	85	ND	ND
Ryegrass	100	86	88	87	74	77	81	81	83
L.S.D.†	2.3	4.4	3.9	3.0	8.2	7.6	5.0	6.7	5.2
Inoculated									
None	57	31	35	99	65	74	91	105	88
Wheat	21	11	12	88	66	78	49	39	47
Barley	21	8	11	78	63	65	34	36	38
Ryegrass	6	4	5	60	61	68	100	80	43
L.S.D.†	10.8	6.0	6.6	8.3	1.1	2.9	16.0	16.7	11.5

ND, Not determined.

* Percentage of controls where no plant residues or infection were present. Extension was measured as the total root or shoot system.

† Least significant difference ($P = 0.05$).

Fig. 1. Scanning electron micrographs of *Fusarium culmorum* on the wheat seed coat. (a) Appressorium-like structure on cuticle cells. Note the extensive covering of bacteria. (b) Young macroconidia.

infected wheat or barley residues were present as a substrate base for the fungus, germination and root and shoot growth of wheat and ryegrass were strongly inhibited but there was little additional effect on barley. Infected ryegrass residues caused severe inhibition of germination and root and shoot growth of wheat. However, ryegrass residues caused no inhibition of

Table 2. *Degree of infection by Fusarium culmorum as influenced by its growth on wheat, barley or ryegrass root material*

Root residue	Percentage infection and death*					
	Wheat		Barley		Ryegrass	
	Infection	Death	Infection	Death	Infection	Death
None	100	45	66	6	80	17
Wheat	100	66	100	25	92	52
Barley	95	90	95	25	95	62
Ryegrass	100	93	72	40	80	0
L.S.D.†	1.25	11.0	8.4	7.0	3.9	12.1

* Both values were zero when *F. culmorum* was not inoculated. Percentage death includes both pre- and post-emergence death. Percentage infection includes dead seeds.

† Least significant difference ($P = 0.05$).

Table 3. *Response of calcium peroxide-dressed ryegrass seeds to Fusarium culmorum infection*

Root residue	Germination, root and shoot extension, and infection of ryegrass							
	Undressed seed				Dressed seed			
	Germination*	Percentage infection	Root*	Shoot*	Germination*	Percentage infection	Root*	Shoot*
None	84	80	80	60	97	75	86	78
Wheat	44	92	39	47	62	83	62	59
Barley	32	95	36	32	66	79	67	68
Ryegrass	51	80	52	46	67	81	62	66
L.S.D.†	11.0	3.9	10.0	5.7	8.1	3.4	5.7	3.9

* Values are expressed as the percentage of controls when no plant residues or infection were present.

† Least significant difference ($P = 0.05$).

germination of ryegrass and only depressed root growth slightly. Shoot growth was more strongly inhibited. The response from barley to ryegrass residues was intermediate between that of wheat and ryegrass (Table 1).

More wheat plants were killed than barley or ryegrass and much of this death was pre-emergent, seemingly a result of infection via appressoria (Fig. 1). All wheat plants became infected (Table 2). More barley plants died when sown into infected ryegrass residues than when sown into infected wheat or barley residues although fewer seedlings became infected. When ryegrass was sown into infected ryegrass residues, although 80% became infected, there was no plant death. This contrasts with the 52% and 62% plant death observed when sown into wheat and barley, respectively (Table 2).

Dressing ryegrass seeds with calcium peroxide improved plant growth. Germination was improved by 16% for ryegrass, 18% for wheat and 34% for barley. Root and shoot growth was improved in all cases, although there was still some inhibition compared with the control (Table 3). The degree of infection by the fungus was slightly decreased, except for the ryegrass residue where there was no effect.

DISCUSSION

Fusarium foot and root rots often lead to a stunting of plant growth (Fitt & Hornby, 1978; Weste, 1975; Cook, 1980) and this was the major symptom in this study for barley, whereas for wheat and ryegrass there was up to 90% and 60% plant death, respectively. The degree of infection on all three types of root was broadly similar, but barley plants in particular could support an extensive fungal biomass with little obvious effect on the plant.

The formation of appressoria, which is a normal prerequisite for infection in the fungi that produce them, is often enhanced in the presence of microorganisms, sometimes because the saprophytes deprive the pathogens of nutrients (Lockwood & Filonow, 1981). The extensive covering of bacteria observed on the appressoria of *F. culmorum* in the present study may reflect this.

At the time of seeding, the root residues were only partially dead with large areas remaining white and turgid with an intact cortex, as shown by light microscopy. The fungus was therefore probably living parasitically on the root residue. Cook & Bruehl (1968) showed that in the soil wheat straws are rarely colonized saprophytically by *F. culmorum* even when large populations are present, but more usually colonization is by the parasitic mode prior to death of the host. This study shows that the nature of the substrate base is important. Sowing ryegrass into infected ryegrass root residue did not lead to any plant death compared with the high death rates when sown into infected wheat and barley residues. It is possible that ryegrass roots may not be a particularly good substrate base for *F. culmorum* under these conditions. In soil, other stress factors acting on both the plant and the microflora will alter the results, and the significance of these results to soil-grown plants needs to be evaluated. The method employed did not allow for standard amounts of substrate to be used in each treatment, but the small variation in these would be unlikely to account for the results obtained.

Calcium peroxide gave some control of the *Fusarium* damage, although this was usually evident as a reduction in the extent of infection rather than a prevention of infection. This effect could be due to the coating preventing or slowing spore germination. The treatment could have other advantages in the neutralization of phytotoxic organic acids produced by saprophytes and the provision of oxygen when this is limiting (Lynch *et al.*, 1981); its potential for use in soil seems worth evaluating.

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