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Survival of *Gaeumannomyces graminis* var. *tritici* in Artificially Colonized Straw Buried in Naturally Infested Soil

G. C. Mac Nish

Department of Plant Pathology, Waite Agricultural Research Institute, University of Adelaide, Glen Osmond, S.A. 5064.
Present address: Department of Agriculture, South Perth, W.A. 6151.

Abstract

A study was made of the survival of *G. graminis* var. *tritici* in artificially colonized straws buried in naturally infested soil. The fungus already present in the naturally infested soil generally survived better than the fungus in the straw. A comparison was made of the survival of two isolates of *G. graminis* var. *tritici* in artificially colonized straws stored in similar soils from the same area, but with different cropping histories. Generally, survival decreased with increasing numbers of consecutive crops; this suggested that some 'factor' reducing saprophytic survival of *G. graminis* var. *tritici* in artificially colonized straws is induced in soil that is continuously cropped to wheat. It was also shown that the isolates (which had similar levels of virulence) had different survival abilities. This appeared to be related to differences in production of dark hyphae. When either isolate survived, it maintained its original virulence and also maintained its inoculum potential at an approximately constant level after an initial drop during the first 10 weeks of survival.

Introduction

Garrett (1938) introduced the use of standardized, artificially colonized straws as a convenient method of studying the survival of *Gaeumannomyces graminis* var. *tritici* Walker (hereafter referred to as *G. graminis*). Since then the technique has been widely employed in the study of the survival of *G. graminis* (Garrett 1940; Butler 1953; Chambers and Flentje 1967; Petersen and Christensen 1968). However, studies in the field (Mac Nish and Dodman 1973) and studies using relatively undisturbed naturally infested field soil (Mac Nish 1973*b*) have indicated that the survival of native *G. graminis* in undisturbed field soil tended to be greater than that commonly reported in studies using artificially colonized straw. The investigation reported in this paper was undertaken to study the survival of *G. graminis* in artificially colonized straws buried in soils known to be naturally infested and to compare the relative survival of the fungus in the straw and in the naturally infested debris. The conditions of storage for the soils were chosen to give an environment similar to that expected in the field and to give a controlled environment similar to cool wet conditions employed by Mac Nish (1973*b*) in other survival experiments.

Gerlagh (1968) found that continuous cropping led to a build up within the soil of an 'antagonism' to *G. graminis*. This 'antagonism' was induced in soil in the glasshouse by adding *G. graminis* inoculum (colonized wheat-seed and wheat-straw mixture) to the soil on each occasion that a consecutive crop was sown. He also found that the survival of *G. graminis* in artificially colonized straws stored in soil with induced 'antagonism' was reduced with increasing numbers of consecutive crops. The only other reference to cropping history affecting survival is in Van der

Watt (1965, Table 3), but there are insufficient data to draw firm conclusions from his results. A study on the effect of the cropping history of the soil on the survival of *G. graminis* is reported in this paper.

Gerlagh (1968, Tables 33 and 34) produced results which, although not conclusive, indicated that there was a tendency for surviving *G. graminis* to maintain its ability to affect the host (measured in Gerlagh's case in terms of average degree of root attack of infected plants). A more detailed investigation of the virulence and inoculum potential of surviving *G. graminis* is reported in this paper. In the investigations reported, the fungus and the straw were treated as a unit with the potential to cause disease to a wheat seedling. Better growth of a seedling with increasing period of burial of the unit would indicate a reduction in inoculum potential, a loss of virulence, or both. Lack of infection would indicate that the fungus had failed to survive in the straw, that the inoculum potential had fallen below a minimal level or that the fungus had become avirulent.

Chambers and Flentje (1967) have shown that isolates of *G. graminis* which differ in virulence may also differ in their ability to survive. In the same paper they reported differences in the development of hyphae in straws colonized by highly virulent and weakly virulent isolates. Other work of Chambers and Flentje (1968, 1969) suggests that the production of dark hyphae and survival are related. An investigation made into the production of hyphae in straws colonized by two isolates of *G. graminis* with similar virulence but differing survival abilities is described in this paper.

Materials and Methods

Soil Cores

Undisturbed soil cores (Mac Nish *et al.* 1973) were collected from four sites on two adjacent farms 16 km west of Ceduna, South Australia. Although the sites were scattered over the farms all the soils were brown calcareous earths (Northcote 1960) and were very alike. The physical and chemical properties of soil A were as follows: particle size distribution $< 2\,\mu$m, 15%; 2–20 μm, 6%; $> 20\,\mu$m, 79%; pH, 8·7; NaCl equivalents, 0·41%; total nitrogen, 0·103%; total carbon, 1·38%; soil water matric potential (sieved soil) for $-0{\cdot}05$ bar, 25·7%; for $-0{\cdot}10$ bar, 16·0%; for $-0{\cdot}30$ bar, 12·8%; for $-0{\cdot}57$ bar, 11·4%; for -225 bars, 2·0%; for -980 bars, 1·3%. The other three soils had similar physical and chemical properties. The cropping histories of the soils are shown in Table 1.

Series I cores were collected along the drill rows in February 1970 and had soil moisture contents (gravimetric) of 1·3, 1·4, 1·5 and 1·8% respectively. A random sample of cores from each site were bioassayed (Mac Nish *et al.* 1973) prior to the commencement of the survival experiments. The percentage of seedlings infected in soils A, B, C and D were 81, 74, 48 and 58% respectively. Differences, however, were not significant. There was considerable viable *G. graminis* in all soils despite the fact that the farmers reported severe take-all on soil A, moderate take-all on soil B and no take-all on soils C and D.

Series II cores collected in November 1970 after harvest were taken from sites near those used for series I. The site where soil C was removed in February 1970 was resown to wheat, but an area a few metres away was left as pasture. Cores from this latter site are denoted as soil Cp. Although the crops on soils C and D were vigorous, crown bioassays (Mac Nish 1973*a*) revealed that 78 and 82% respectively of the plants carried viable *G. graminis*.

Isolates Used

Isolate 2C was a strongly pathogenic single-ascospore culture originally obtained by Chambers from isolate W1 (Chambers and Flentje 1967). Isolate 044 was obtained from Ceduna in August 1969. It produced perithecia on glucose–asparagine (Lilly and Barnett 1951) agar in the light at room

temperature (21–25°C). Ascospore length (average 83 μm, range 70–92 μm), pathogenicity and virulence on wheat seedlings identify this isolate as *G. graminis* var. *tritici*. Stock cultures of both isolates stored on glucose–asparagine agar maintained their virulence throughout the period of the investigations.

Colonization of Straws

Straws used were clean bright nodal sections (15 mm long by 3–5 mm diameter at the node) from the cultivar Gabo. For survival tests, batches of 150 straws and 30 ml of distilled water were autoclaved at 121°C for 1·5 h. The fungus was grown for 8 days on 10 ml of potato–'Marmite'–dextrose agar (PMDA) in 500-ml Erlenmeyer flasks. One batch of straws was added to each flask and colonized at 20°C for 8 weeks. Ten days after the addition of the straws, 20 ml of straw infusion (the broth from 15 g of straws autoclaved at 121°C for 1 h in 1500 ml of distilled water) was added to moisten the straws. Three weeks later the flasks were shaken to mix the straws. Prior to use the straws were washed in sterile water to remove adhering mycelium and blotted dry.

Straws employed in virulence tests were colonized as follows: 20 straws and 4 ml of distilled water were autoclaved at 121°C for 1·5 h. The fungus to be tested was grown on PMDA in a petri dish for 2–4 days at 20°C. The straws were placed on the colony and incubated for 2 weeks at 20°C. Prior to use straws were removed and shaken in sterile water to remove external mycelium.

Table 1. Cropping histories of the experimental soils

Year	Soil A	Soil B	Soil C	Soil D
1962	Unknown	Unknown	Wheat	Wheat
1963	Unknown	Unknown	Pasture	Wheat
1964	Pasture	Wheat	Wheat	Wheat
1965	Wheat	Pasture	Pasture	Wheat
1966	Wheat	Pasture	Wheat	Wheat
1967	Pasture	Pasture	Wheat	Wheat
1968	Pasture	Wheat	Wheat	Wheat
1969	Wheat	Wheat	Wheat	Wheat
1970	Pasture	—	Wheat	Wheat

Burial of Straws

In each core, 50 colonized straws were pressed vertically into the soil leaving about 1 mm of straw showing above the surface. This was to simulate the lower stem region of infected stubble standing in soil. Although the straws were not completely covered the term 'buried' is used to describe their position in the soil. Straws were inserted at approximately 1-cm centres on a square grid pattern. For all treatments four cores (i.e. four replications) were used. Five straws were removed at random from each core for each survival test. To provide a 'control' unaffected by micro-organisms, colonized straws were also buried in autoclaved coarse sand in McCartney bottles. Each bottle contained five straws.

Storage of Cores and Sand

Series I cores were stored on benches outside at the Waite Institute or were maintained cool and wet (15°C with a matric potential between −0·1 and −0·2 bar). Sand was maintained cool and wet (15°C with a matric potential of −0·1 bar) in bottles with tightened screw tops to maintain constant matric potential and sterility.

Series II cores were maintained cool and moist (15°C with a matric potential between −0·1 and −0·5 bar). Sand was maintained cool and wet as above.

Determination of Survival and Virulence

The tests used to determine survival or virulence are similar. For the survival test, colonized straws were removed from the cores, washed with a jet of water to remove attached soil and then used. For the virulence test, straws were used immediately after colonization.

Five straws were placed on the surface of a potting mixture (air-dried 1 : 1 mixture of sand and loam) in a plastic cup and pressed horizontally to a depth of 2·5 cm. A seed was placed in contact with the straw and the soil was pressed around seed and straw. The cups were watered to a soil moisture content equivalent to a matric potential of −0·1 bar and placed in a controlled environment (16 h of fluorescent light, 17 200–18 300 lumen/m^2, and 15°C constant temperature). Cups were covered with plastic sheet for the first 5 days to minimize evaporation; thereafter they were watered daily to constant weight.

Cups were randomized within four replicated blocks. Within each block, two extra cups containing only soil and seeds were incorporated to act as uninoculated 'standards'. Standards quoted in the text are a mean of eight cups.

After 3 weeks, the seedlings were washed free of soil and the following parameters recorded per cup: (1) number of seedlings, (2) percentage of seedlings infected in the scutellar-node region, and (3) total root dry weight. Seedling emergence was recorded to ensure that differences in total weight were not due to differences in emergence.

As there is a possibility of cross infection of seedlings within a cup, levels of survival are based on the presence or absence of infected seedlings per cup. The average percentage of seedlings infected is included in the text (Table 2) but this should only be treated as an estimate of survival.

Root dry weight was chosen as the parameter to measure the effect of the fungus on the host because it was more easily obtained than length of discoloured root. A preliminary experiment demonstrated a highly significant correlation ($r = 0{\cdot}9136$) between root dry weight and length of discoloured root.

Isolation of Fungus from Straws

On some occasions four extra straws (one per replicate) were removed from series I storage treatments, washed, surface-sterilized with silver nitrate (Davies 1935), cut longitudinally and plated surface downwards on PMDA plus 30 ppm Aureomycin hydrochloride. The number of straws from which *G. graminis* was isolated after 12 days incubation at 20°C was recorded. Reisolates were stored on glucose–asparagine agar.

Preservation of Straws and Determination of Type of Hyphae Present in Straws

Straws buried in series II cores were removed after 4, 8 or 12 weeks and preserved in universal fixative (McLean and Cook 1941). At the end of the experiment the straws were homogenized in 75% glycerol and observed microscopically. The number of quadrats (0·375 by 0·375 mm) out of 40 containing the various hyphal types (i.e. thin hyaline, thick hyaline and thick dark) was recorded.

Experimental Details and Results

Survival of G. graminis

(i) *Series I, cores maintained outside at the Waite Institute*

Soils from different collection sites were separated by a distance of 4·5 m to minimize cross contamination by rain splash (M. V. Carter, personal communication). Cores were in cans (with drainage holes) placed in boxes and surrounded by fine gravel to minimize temperature fluctuations. For each soil, two extra cores were included and weighed to determine changes in soil moisture content. The screen air temperature and rainfall are shown in Fig. 1 together with changes in the soil matric potential for soil A. The other soils had similar changes. The cores were placed in the open on 1 May 1970 prior to the commencement of the main winter rains and received no treatment other than the removal of any seedlings that appeared.

Straws were removed after 5, 10, 15, 20, 27, 32 and 37 weeks of burial and used in survival tests. The results of the survival test and the number of straws from which *G. graminis* was isolated after 10, 15 or 20 weeks burial are shown in Table 2. The results show that survival estimates by both infection and isolation are similar.

Table 2. Survival of *G. graminis* in straws removed from series I cores

Experimental details			No. of cups containing infected wheat seedlings when grown in contact with straws (max. of four cups)							Percentage of infected seedlings per cup when grown in contact with straws (mean of four cups)							Isolation of *G. graminis* from straws (max. of four straws, one per replicate)			
Storage conditions	Isolate	Soil	Period of burial (weeks)							Period of burial (weeks)							Period of burial (weeks)			
			5	10	15	20	27	32	37	5	10	15	20	27	32	37	5	10	15	20
Outside at Waite Institute	2C	A	4	4	4	4	4	4	4	92	95	100	84	100	94	100	—	4	4	4
		B	4	4	4	4	4	4	4	90	94	100	100	85	95	74	—	4	4	3
		C	4	4	4	2	0	0	0	95	75	39	15	0	0	0	—	4	3	3
		D	4	4	4	4	4	4	4	100	100	95	84	80	79	82	—	3	4	4
	044	A	4	4	4	4	4	4	4	90	100	95	80	76	88	95	—	4	4	4
		B	4	4	4	4	2	2	0	100	84	85	45	38	18	0	—	4	4	3
		C	4	2	1	0	0	0	0	85	10	13	0	0	0	0	—	4	0	0
		D	4	4	1	0	0	0	0	100	64	25	0	0	0	0	—	4	3	1
Cool and wet	2C	A	4	4	4	4				95	100	100	100				4	4	4	4
		B	4	4	4	4				100	100	90	95				4	4	4	4
		C	4	4	2	1				100	55	38	10				4	4	3	0
		D	4	4	4	4				100	95	95	100				4	4	4	4
	044	A	4	4	4	4				100	95	100	83				4	4	4	4
		B	4	4	4	4				100	95	85	74				4	4	4	4
		C	3	0	0	0				40	0	0	0				3	4	0	0
		D	4	3	2	1				100	44	31	5				4	4	2	2
Cool and wet	2C	Sand	4	4	4	4	4	4	4	100	94	94	88	85	100	75	4	4	4	4
	044	Sand	4	4	4	4	4	4	4	90	95	68	61	82	63	60	4	4	4	4

There were marked differences in survival in the different soils. These differences did not appear to be associated with visual differences in breakdown of the straws. In all soils except soil A, isolate 2C survived better than isolate 044.

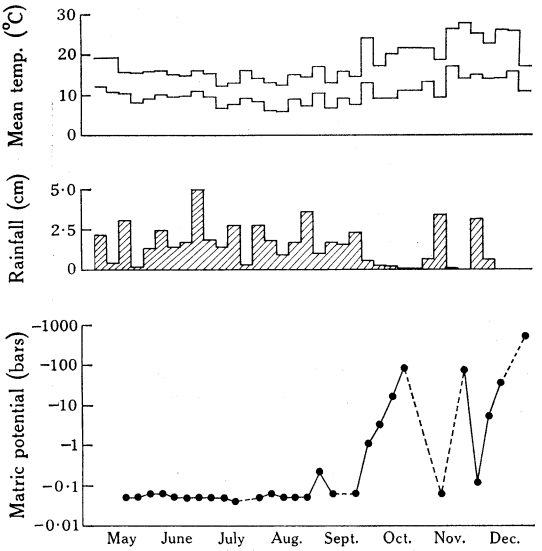

Fig. 1. Weekly air temperature maxima and minima, rainfall and soil matric potential for soil A cores, at the Waite Institute, 1970.

When the experiment was terminated (37 weeks), the cores were bioassayed for the presence of *G. graminis*. The top 2 cm of soil was removed and sieved (2-mm mesh) to remove any of the added straws that may have remained in the soil. The soil was then replaced. The results from the bioassay (Table 3) show that both the soil and the isolate used to colonize the straws affected the level of viable native *G. graminis* surviving in the soil.

Table 3. Incidence of *G. graminis* on wheat seedlings grown in experimental soils after storage outside at the Waite Institute for 37 weeks

Values are means for four replicates

Soil	Percentage seedlings infected		Value expressed as arcsin (rad)		Soil means (rad)	L.S.D. for soil means	
	Isolate 2C	Isolate 044	Isolate 2C	Isolate 044		$P = 0·05$	$P = 0·01$
A	57	11	0·86	0·24	0·55		
B	55	34	0·84	0·55	0·70	0·30	0·41
C	8	0	0·20	0	0·10		
D	55	12	0·84	0·25	0·55		
Isolate means (rad)			0·69	0·26			
L.S.D. for isolate means	$P = 0·05$		0·21				
	$P = 0·01$		0·29				
	$P = 0·001$		0·39				

In only one treatment had the native *G. graminis* completely disappeared by 37 weeks. This is in contrast to the survival of the fungus in artificially colonized straws where it failed to survive to 37 weeks in four treatments (Table 2).

The results for root dry weights (as a percentage of the standard*) are shown in Figs 2*a* and 2*b*. Analysis of variance (factorial design) of the raw data showed that there was a highly significant ($P = 0{\cdot}001$) effect on root dry weight due to period of burial, soil cropping history and isolate used to colonize the straws.

The connection between survival and disease incidence is clearly seen in Fig. 2*a*. Root growth of seedling in contact with straws from soil D, where isolate 2C failed to survive after 20 weeks, was similar to the standard from week 20 to the end of the experiment. Root growth with straws from the remaining three soils, where isolate 2C had a high level of survival to the end of the experiment, reached a peak at about 10 weeks and continued thereafter at about the same percentage of the standard. For isolate 044 only those seedlings in contact with straws from soil A were consistently different from the standard from week 15 onwards.

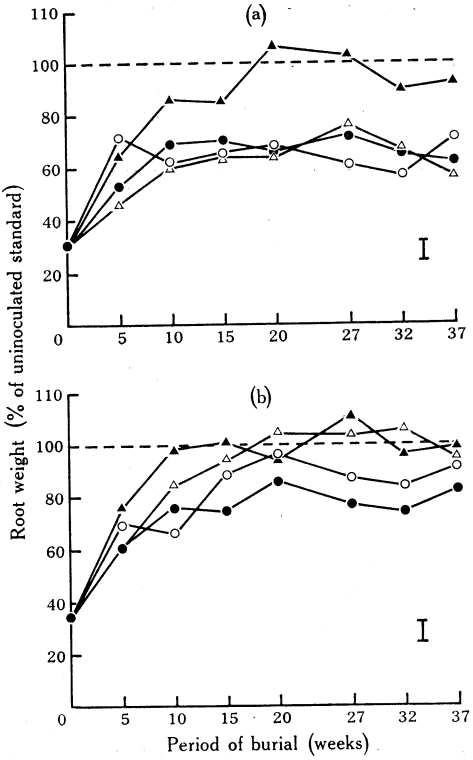

Fig. 2. Root dry weight (as percentage of uninoculated standard) of seedlings grown in contact with straws colonized by (*a*) isolate 2C and buried in each of four soils maintained outside at the Waite Institute, and (*b*) isolate 044 and buried under comparable conditions. ● Soil A. ○ Soil B. ▲ Soil C. △ Soil D. Approximate S.E. shown by vertical bars.

One reisolate of *G. graminis* per treatment from the 20-week straws was used in a virulence test (an exception was isolate 044 in soil D, where the reisolate used was one obtained on the last occasion *G. graminis* was isolated). Both isolates 2C and 044 maintained their virulence during burial (Table 4).

(ii) *Series I, cores maintained cool and wet*

Cores were watered fortnightly with distilled water to a constant weight equivalent to a soil moisture content of about 17%.† Average loss in weight between waterings was about 29 g per core, i.e. a change in matric potential from $-0{\cdot}1$ to $-0{\cdot}2$ bar.

* During the term of the experiment the root dry weights of the standard were not significantly different.

† The exact values varied slightly due to small differences between the four soils.

The results of the survival tests and the number of straws from which *G. graminis* was isolated are shown in Table 2, whilst the results for root dry weights were similar for the first 20 weeks to those shown in Figs 2*a* and 2*b*. As the results in this experiment resembled those obtained from storage of cores outside, the experiment was terminated at 20 weeks. The similarity of the moisture and temperature conditions in this experiment with those in the previous experiment during the first 20 weeks (Fig. 1) may account for the results for both experiments being alike.

No differences in the rate of breakdown of straws were observed between those colonized by isolates 2C or 044 and those buried in different soils. The virulence of the reisolates obtained after 20 weeks of burial (5 weeks for isolate 044 in soil C) was maintained (Table 4).

Table 4. Virulence of isolates of *G. graminis* before and after reisolation from straws buried for 20 weeks

Values are means for four replicates

Storage conditions	Soil	Root dry weight (mg)[A] Isolate 2C	Isolate 044
Outside at Waite Institute	A	31	24
	B	27	31
	C	30	31
	D	34	28
Cool and wet	A	28	27
	B	29	29
	C	36	36
	D	36	43
Cool and wet	Sand	18	31
L.S.D.		n.s.	

[A] Root dry weights for isolate 2C, isolate 044 and standard at the commencement of the experiment were 38, 34 and 126 mg respectively. Standard for the virulence test at 20 weeks was 128 mg (not included in analysis of variance).

(iii) *Series I, sand maintained cool and wet*

The results (Table 2) show that the survival of isolate 2C in sterile cool wet sand was similar to its survival in soils A, B and D stored outside, and the survival of isolate 044 in sand was similar to its survival in soil A maintained outside. As noted previously there was a tendency for isolate 2C to survive better than isolate 044.

The pattern of change in root dry weight for both isolates was similar to that established by isolate 2C in soils A, B and D, or isolate 044 in soil A stored outside. Reisolates of both 2C and 044 maintained their virulence (Table 4).

(iv) *Series II, cores maintained cool and moist*

This experiment was conducted to determine whether the results obtained with series I cores could be confirmed. Another aim was to continue to study the effect of cropping history on survival using soil collected after another crop.

Cores were watered every 4 weeks to a constant weight equivalent to a soil moisture content of 17%. Average loss in weight per core was 108 g, i.e. a change in matric potential from −0·1 bar to near −0·5 bar. Cores received no treatments other than the removal of any seedlings that appeared.

Straws were removed and used in survival tests after 4, 8 and 12 weeks of burial. Results for the soils are shown in Table 5 (both isolates had 100% survival in sand). Survival was again influenced by the isolate used to colonize the straws and the soil used to bury the straws. As previously, isolate 2C survived better than isolate 044. The survival of both isolates in soil A was similar to that previously recorded in this soil in series I (Table 2). Survival of isolate 044 in both soil C and soil Cp was similar and was better than the survival for this isolate in soil C in series I. Both isolates did not survive as well in soil D as they had in soil D in series I.

Table 5. Survival of *G. graminis* in straws removed from series II cores

Isolate	Soil	No. of cups containing infected wheat seedlings when grown in contact with straws (max. of 4 cups)			% of infected seedlings per cup when grown in contact with straws (mean of 4 cups)		
		Period of burial (weeks)			Period of burial (weeks)		
		4	8	12	4	8	12
2C	A	4	4	4	100	100	100
	C	4	4	4	100	100	90
	Cp	4	4	4	100	100	100
	D	4	4	1	100	85	5
044	A	4	4	4	100	100	95
	C	4	3	3	90	59	20
	Cp	4	4	2	95	78	25
	D	2	0	0	15	0	0

Although there were some differences in survival between the series I and series II cores, the general pattern of survival being reduced with increased numbers of consecutive crops was maintained. Once this was established, the experiment was terminated.

Hyphal Production by Isolates Used in Survival Studies

Straws from series II soils were examined after 12 weeks burial. There were no consistent differences in the number of thin hyaline hyphae, but there were differences in the numbers of thick hyaline hyphae and dark hyphae. Straws stored in sand contained low numbers of dark hyphae but high numbers of thick hyaline hyphae. This may indicate that thick hyphae had formed but pigmentation had failed to develop to the same extent as in hyphae in the natural soil.

There were no observed differences in the number of dark hyphae in straws from three of the soils that would account for the differences in the survival of isolates 2C and 044. The exception was in soil D, where isolate 2C produced more dark hyphae than isolate 044. As survival of the two isolates within this soil was markedly different (Table 5), further detailed examinations of straws from this soil were made.

One preserved straw per replicate per time of burial was examined. The straws colonized by isolate 2C contained significantly more dark hyphae than those colonized by isolate 044 (Table 6). The number of dark hyphae in straws containing isolate 044 remained unchanged with time. There was no way of determining whether the dark hyphae counted were viable, but survival tests indicate that the hyphae of isolate 044 were no longer viable at 8 weeks (Table 5). If isolate 2C is able to produce more dark hyphae and produce them over a longer period than isolate 044, this may account for the differences in survival in soil D.

Table 6. Number of quadrats (out of 40) containing dark hyphae of *G. graminis*
Values are means for four replicates

Isolate	Period of burial (weeks)			Isolate	L.S.D. for isolate means		
	4	8	12	means	$P = 0{\cdot}05$	$P = 0{\cdot}01$	$P = 0{\cdot}001$
2C	13·5	19·0	24·0	18·8	3·8	4·9	6·7
044	10·5	9·5	8·8	9·6			
Period of burial means	12·0	14·2	16·2				
L.S.D. for burial means		n.s.					

Discussion

Previous studies on the survival of *G. graminis* in naturally infested soils (Mac Nish 1973*b*) and the studies reported here on the survival of this fungus in artificially colonized straws buried in naturally infested soil raise the question of why the 'native' fungus generally survives better than the 'introduced' fungus. Survival of *G. graminis* appears to be linked to the type of hyphae present. An examination of naturally infected plant remains from the field showed that they contained mainly dark hyphae, while freshly prepared, artificially colonized straws contained few or no dark hyphae. The fungus established as resistant dark hyphae (Padwick 1936) prior to treatment, would have an advantage over the fungus that had to form dark hyphae after the treatment commenced. Differences in hyphae and in the way the habitats are colonized, could be expected to cause differences between the survival of *G. graminis* in artificially colonized straws and in natural debris. Use of artificially colonized straws may reveal survival patterns that do not apply in the field. Consequently experiments where only artificially colonized straws have been used may need to be re-examined.

The marked differences in survival in the different soils did not appear to be associated with visual differences in breakdown of the straws or with differences in the physical and chemical properties of the soils. Unless there were subtle interactions of physical or chemical properties, differences in survival associated with the soils appear to be due to the cropping history of the soils.

In an experiment reported by Butler (1959) where straws colonized by *G. graminis* were stored in natural soil, differences in survival were attributed to differences in soil fertility. Although the fertile soil (6 years of legume ley) had a total nitrogen content of 0·13% compared to 0·07% in the adjacent infertile area (50 years of cereal pasture rotation), the C:N ratio for both areas was similar. There is the possibility that the

reduced survival in the infertile soil was associated with cropping history rather than fertility.

The hypothesis is advanced here that continuous cropping with wheat (in these instances in the presence of 'natural' *G. graminis*) causes a 'factor' to develop in the soil which affects the saprophytic survival of *G. graminis* in artificially colonized straws. This 'factor' may be antibiotic antagonism as suggested by Gerlagh (1968) but could be something else. For instance, Lapierre *et al.* (1970) have found a virus that affects the parasitic phase of *G. graminis*. The same or other viruses may affect saprophytic survival. The 'factor' causing a reduction in survival in the present experiments is not necessarily removed by a 1-year break in the crop (soils C and Cp in Table 5). Results from these experiments indicate that the 'factor' may not continue to increase in intensity. It reached a maximum after three or four consecutive crops and thereafter varied slightly in effect between seasons.

The importance of this 'factor' is unknown, but possibly it has no significance in the field. The factor may only affect the survival of hyaline hyphae as found in artificially colonized straws. It may have no effect on 'parasitic' hyaline hyphae. By the time the survival phase is reached in the field, hyaline hyphae will have been replaced with dark hyphae.

The above hypothesis may explain the differences in survival between the soils, but there remains the question of why isolate 2C survived better than isolate 044. Although part of the explanation appears to be related to production of dark hyphae, a completely satisfactory explanation for the differences has not been found. Further, there may be a link between the poor survival of isolate 044 (compared to isolate 2C) and the effect of this isolate on the survival of native *G. graminis* (Table 3), but this matter was not investigated.

The drop in inoculum potential of infection units [e.g. the increase in root growth of seedlings grown in contact with straw colonized by isolate 2C and buried in soils A, B, D or autoclaved sand, or isolate 044 buried in soil A or sand (Fig. 2)] during the first 10–15 weeks after burial in some soils did not appear to be due to a lack of survival (Table 2), a reduction in virulence of the surviving fungus (Table 4) or microbial activity. The explanation could be related to the effect of the ratio of thin hyaline hyphae to dark hyphae on inoculum potential. I propose that the initial high inoculum potential obtained with freshly colonized straws was due to the hyaline hyphae. During the first 10–15 weeks after burial in wheat-field soil there was a natural reduction in the number of viable hyaline hyphae in the straws. This was accompanied by a reduction in inoculum potential. During this period the production of resistant dark hyphae commenced, and the drop in inoculum potential was halted as the dark hyphae took over from the hyaline hyphae. In the autoclaved sand the same sequence of events took place, with the thick hyaline hyphae acting in the same manner as the dark hyphae.

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