

EFFECT OF MIXING AND SIEVING ON INCIDENCE OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI* IN FIELD SOIL

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Abstract

A bioassay was employed to compare the effect of various treatments on the level of *G. graminis* var. *tritici* inoculum in soil cores taken from a take-all patch. In a comparison of undisturbed soil and mixed soil, mixing caused a small reduction in incidence, possibly due to a dilution of the inoculum in the surface soil. Graded degrees of sieving from 5 to 0.5 mm mesh size caused a significant reduction in inoculum levels, with the latter reducing incidence to 3% in seedlings at 4 weeks. However, it was also shown that increasingly finer sieving caused an increase in disease incidence if the seedlings were allowed to grow to maturity. It was not established whether the sieving affected the soil in such a way as to favour the pathogen, lower the resistance of the plant, or both.

I. INTRODUCTION

The core bioassay described by Mac Nish *et al.* (1973) is based on the assumption that roots explore the soil mass sufficiently for an inoculum unit of *Gaeumannomyces graminis* var. *tritici* Walker (hereafter referred to as *G. graminis*) to have an opportunity to infect a root. If this assumption is correct, mixing the soil in the core should not affect the percentage of roots infected. As infective units within field soil will vary from whole crowns to small pieces of debris (Hornby 1969), sieving infected field soil through increasingly smaller-size mesh should reduce the amount of inoculum remaining in the soil. The investigations described below study the effect of mixing and sieving on the incidence of *G. graminis* in seedlings. Also investigated is the correlation between the incidence of *G. graminis* on seedlings, and disease incidence and grain yield at maturity.

II. MATERIALS AND METHODS

(a) *Effect of Mixing and Sieving on Incidence of G. graminis*

Undisturbed soil cores were obtained from a take-all patch in an experimental area at Ceduna, S.A. (Mac Nish *et al.* 1973). The 25 cores removed consisted of five cores taken side-by-side in five consecutive drill rows to form a latin square for the five treatments. The treatments were undisturbed cores, thoroughly mixed cores, and three degrees of sieving: mesh sizes of 5, 2, and 1 mm. Prior to sieving, each core was thoroughly mixed. Any material remaining after the sieving was discarded. The cores were then bioassayed for the presence of *G. graminis* (Mac Nish *et al.* 1973). Dry weights of tops and roots of seedlings grown in the cores were also recorded.

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(b) Effect of Sieving on Incidence of G. graminis on Seedlings and Mature Plants

This experiment was designed to find out if there was a correlation between the incidence of *G. graminis* on seedlings in cores at 4 weeks and incidence and grain yield at maturity. Cores were taken over plant remains within a take-all patch in the experimental area at Ceduna. Undisturbed cores and cores sieved through one of three mesh sizes (5, 1, or 0.5 mm) were used. There were seven randomized replications of each treatment. Two series of all treatments were grown simultaneously for 4 weeks in a controlled environment (Mac Nish *et al.* 1973). One series of cores was bioassayed for the presence of *G. graminis*. In the remaining series of cores the number of plants was reduced to six to remove some variation in emergence. Each core was removed intact from its can and placed in an 11.5-litre plastic pot containing potting mixture (1:1 mixture of John Innes potting soil and a loamy sand). An additional treatment of seedlings growing in potting mixture only was included to act as a control. All pots were placed in an open-sided glasshouse and watered once a week to 17% soil moisture content (which is equivalent to a matric potential of about -0.5 bar). The plants were placed in the glasshouse in early July and reached maturity in late November. Five weeks after transfer to the glasshouse all pots received fertilizer (superphosphate, sulphate of ammonia, and potash, 42:30:3) at a rate equivalent to 90 kg/ha.

The only parameters that could be measured (at 4 weeks) for both the bioassayed and the potted series were leaf length (longest leaf) and number of "healthy" seedlings (i.e. those showing no yellowing or less than 0.5 cm of leaf-tip yellowing).

When the plants in the glasshouse had reached maturity they were rated for severity of disease incidence using the following scale:

Very low—plants large and vigorous, root infected by *G. graminis*, but no obvious symptoms (presence detected by crown bioassay, Mac Nish 1973).

Moderate—plants large and vigorous, *G. graminis* infection on roots easily observed.

Severe—reduction in plant size, stem blackening, head formed.

Very severe—plants very reduced in size, considerable stem blackening, no head formed.

Number of tillers, plant height (to the base of the uppermost florescence if head present or to the highest ligule if head absent), and grain yield (total yield and amount unable to pass a 2-mm sieve) were recorded.

III. RESULTS

(a) Effect of Mixing and Sieving on Incidence of G. graminis

The results for this experiment are given in Table 1. Mixing the soil had no effect on the percentage of seedlings infected, but did significantly reduce the percentage of roots infected. As expected, sieving the soil reduced the amount of inoculum, although the use of a 5-mm sieve did not cause a significant reduction in infected roots when compared to the mixed soil. The reduction in mesh size to 2 and 1 mm significantly reduced the percentage of infected roots, but did not eliminate infection. Both top weight and root weight were significantly increased by sieving. Although the increase in plant growth correlates well with the reduction in disease incidence, it also correlates with increase in soil texture. Without further investigation it is impossible to be sure that the increase in growth was due to the decrease in the incidence of *G. graminis*.

(b) Effect of Sieving on Incidence of G. graminis on Seedlings and Mature Plants

The comparison between leaf length and number of "healthy" seedlings at 4 weeks for the two series of cores is given in Table 2. Also shown is the incidence of *G. graminis* in the bioassayed series. As there were only two parameters for comparison, analysis of variance was performed on the data for the number of "healthy"

seedlings. For the comparable parameters, only the seedlings in undisturbed cores were significantly different from those of other treatments. The similarity of the standard errors for both series (in both parameters) suggests that the results of the bioassay for percentage of infected roots may be similar in both series. As in the previous experiment, graded sieving reduced the incidence of *G. graminis*.

TABLE 1

EFFECT OF MIXING AND OF SIEVING SOIL ON THE INCIDENCE OF *G. GRAMINIS* AND ON TOP AND ROOT WEIGHT OF SEEDLINGS GROWN IN CORES FROM A TAKE-ALL AREA

Values are means of five replicates arranged in a 5×5 latin square

Core treatment	Percentage of seedlings infected	Value expressed as arcsin (deg)	Percentage of roots infected	Value expressed as arcsin (deg)	Dry weight per seedling (mg)	
					Top	Roots
Undisturbed	100	90.0	95	78.5	30	17
Mixed	100	90.0	85	67.8	31	24
Sieved (5 mm)	91	79.1	76	61.5	35	31
Sieved (2 mm)	67	58.3	35	35.9	43	38
Sieved (1 mm)	50	45.2	16	23.4	43	42
S.E.		4.8		4.0	2.4	3.2
L.S.D.						
$P = 0.05$		10.4		8.8	5.2	6.9
$P = 0.01$		14.6		12.3	7.2	9.7

TABLE 2

LEAF LENGTH AND NUMBER OF "HEALTHY" SEEDLINGS AFTER 4 WEEKS GROWTH IN CORES TAKEN FROM A TAKE-ALL PATCH

One series of cores (A) was bioassayed to determine incidence of *G. graminis* while the other (B) was grown to maturity. Values are means of seven replicates

Core treatment	Average leaf length (cm)		No. of "healthy" seedlings		No. of seedlings infected (%)*	Value expressed as arcsin (rad)*	No. of roots infected (%)*	Value expressed as arcsin (rad)*
	A	B	A	B				
Undisturbed	14.9	12.2	3.1	3.7	98	1.51	86	1.29
Sieved								
5 mm	16.8	15.3	5.1	5.4	90	1.36	66	0.99
1 mm	17.3	16.8	6.0	6.6	55	0.88	26	0.50
0.5 mm	18.0	16.7	6.1	6.4	7	0.18	3	0.10
S.E.	0.8	0.7	0.5	0.5		0.10		0.08
L.S.D.								
$P = 0.05$	1.7	1.5	1.0	1.0		0.21		0.17
$P = 0.01$	2.3	n.s.	1.4	1.4		0.29		0.23

* For bioassayed cores (series A).

For the series potted out and transferred to the glasshouse, considerable variation in plant height was observed between plants within pots (Fig. 1) and between

replications. In some instances one or two plants dominated, tillering and growing vigorously. By late September and early November whiteheads began to appear amongst the plants (Fig. 1). In the control pots all plants were vigorous and disease-free. Disease ratings for this experiment are shown in Table 3, while the number of tillers produced, plant heights, and grain yields are given in Table 4. The variability in grain yield between replicates is shown in Table 5.

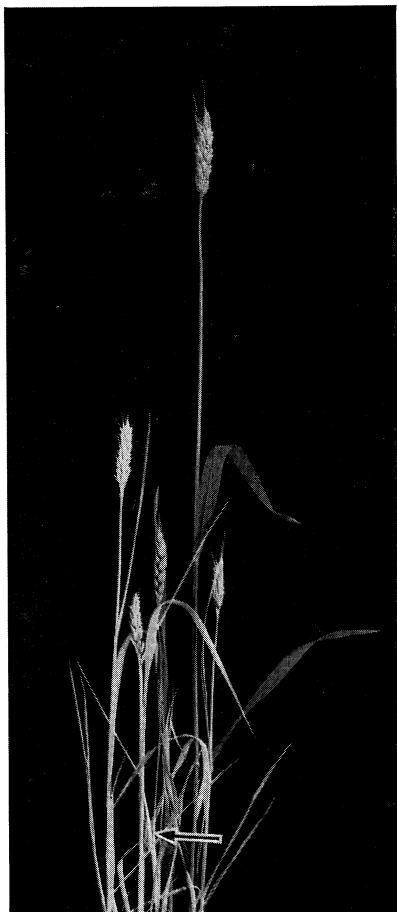


Fig. 1.—Plants growing in cores were transferred to large pots and grown to maturity in a glasshouse. Within pots, considerable variation in plant height was observed. In the above photograph plant heights ranged from 6 cm (arrow) to 57 cm. Some plants developed whiteheads while others remained green and vigorous.

The results for the undisturbed cores and the 0.5-mm-sieved cores are of particular interest. As anticipated, most of the plants in the former treatment were severely infected, but an unexpectedly high number of plants (72%) from 0.5-mm-sieved cores were in the severe category (Table 3). To a lesser extent the same is true of the results from the 1.0-mm-sieved cores. Examination of plant growth and grain yield (Table 4) indicates that the number of tillers produced followed the pattern expected, but plant height and yield (especially the yield of plump grain) were quite different from that expected. Although the differences in yield are large,

they are not significantly different because of the variability between replicates (Table 5) caused by scattered vigorous plants. However, the over-all impression was that disease severity in the sieved cores was much greater than expected.

TABLE 3
NUMBER OF PLANTS OUT OF 42 IN FOUR DISEASE RATING CATEGORIES
Plants were grown to maturity in cores transferred to large containers

Core treatment	No. of plants with disease rating:			
	Very low	Moderate	Severe	Very severe
Undisturbed	0	4	4	34
Sieved (5 mm)	7	16	18	1
Sieved (1 mm)	4	11	27	0
Sieved (0.5 mm)	0	12	30	0

TABLE 4
NUMBER OF TILLERS, PLANT HEIGHT, AND GRAIN YIELD FOR PLANTS GROWN TO MATURITY IN
CORES TRANSFERRED TO LARGE CONTAINERS
Values are means of seven replicates

Core treatment	No. of tillers per pot (6 plants)	Plant height (cm)		Grain yield	
		Av.	Range	Total (g)*	>2 mm (g)*
Control	17.5	67	47-84	11.6	11.5
Undisturbed	8.3	15	1-66	1.8	1.3
Sieved (5 mm)	13.3	44	7-83	7.6	6.7
Sieved (1 mm)	16.3	51	28-67	7.5	4.4
Sieved (0.5 mm)	16.0	38	24-74	5.6	2.6

* Standard errors were 1.5 for total yields and 1.6 for yields of grain >2 mm. Differences are not significant at $P = 0.05$ (control values not included in the analysis of variance).

TABLE 5
COMPARISON OF YIELD PER REPLICATE FOR PLANTS GROWN TO MATURITY IN CORES TRANSFERRED TO
LARGE CONTAINERS

Core treatment	Yield of grain (g) from replicate:							Mean yield of grain (g)
	1	2	3	4	5	6	7	
Undisturbed	0	3.9*	1.8†	0	0	3.5†	3.7†	1.8
Sieved (5 mm)	10.4	13.8	3.1	13.9	10.1	0.1	2.2	7.6
Sieved (1 mm)	9.7	7.3	8.6	4.2	2.4	12.3	8.1	7.5
Sieved (0.5 mm)	2.7	1.4	6.2	12.5	6.5‡	7.1	2.8	5.6

* 3.5 g from one plant.

† All from one plant.

‡ 3.0 g from one plant.

IV. DISCUSSION

Mixing soil in a core from a take-all patch resulted in a significant reduction in the incidence of *G. graminis* (Table 1). This could indicate that mixing, by scattering the inoculum throughout the soil, reduces the amount of inoculum that can be reached by the roots in 4 weeks. However, it may also suggest that where inoculum and seed are close together, as in undisturbed cores, some lesions may extend over more than one root. In the former case use of mixed cores would lead to an underestimate of the level of *G. graminis* inoculum present, while in the latter the use of undisturbed cores would lead to an overestimate of inoculum. As the difference in incidence between undisturbed cores and mixed soil was only just significant at $P = 0.05$, this matter has not been investigated further.

Sieving soil from a take-all patch through a 0.5-mm sieve failed to remove all the inoculum of *G. graminis* (Table 2). This supports Hornby's (1969) findings. He found that a 0.42-mm sieve removed most of the organic debris containing *G. graminis*, but even at this mesh size the fungus remained on some occasions. It is apparent, therefore, that the removal of large trash, including crowns, still leaves a small reservoir of *G. graminis* in the soil, and infection from this inoculum can eventually have a considerable effect on the mature plant (Table 3).

I was unable to correlate differences in incidence of *G. graminis* at the time of the bioassay with incidence on plants or grain yield from plants grown to maturity in the glasshouse. It was apparent that any level of infection at the time of the bioassay led to a marked reduction in yield at maturity. It is impossible to determine whether the infection on the plants grown to maturity in the 0.5-mm-sieved cores was due to all cores containing seedlings with at least one root infected at the 4-week stage, or whether with extra time the roots were able to explore more soil and thus come into contact with more inoculum. Passing the soil through a 0.5-mm sieve may allow a distribution of inoculum with a reduced inoculum potential. Thus, more than 4 weeks would be necessary to detect the inoculum by the bioassay. The possibility of cross-contamination in the glasshouse, as noted by Gerlagh (1968), can be discounted as check plants grown in potting soil were all free of *G. graminis*.

The possibility can be discounted that the low grain yield from plants grown in the 0.5-mm-sieved soil could be due to early vigorous growth causing a nutrient shortage at the end of the growing period. The control plants growing under the same conditions showed no signs of haying-off at the end of the season (Table 4).

It is possible that sieving affected soil texture and consequently aeration. Garrett (1936, 1937) demonstrated that improved aeration encouraged hyphal growth of *G. graminis* along the root surface, and it is known that take-all is favoured by light-textured soils (Griffiths 1933; Garrett 1934). On the other hand, while sieved soil is initially more "fluffy", after continued watering it compacts more than undisturbed soil, and this would reduce aeration. This compaction may have been unfavourable for plant growth and consequently may have lowered the resistance of the plant to the disease. The core transferred to the large pot only constituted approximately one-tenth of the total soil within the pot. However, as the seedlings were all in the core any adverse effects of sieving would be concentrated in the vital crown region of the plant. It is apparent that sieving the soil through a 0.5-mm

mesh and, to a lesser extent, a 1·0-mm mesh has caused a combination of factors favourable to disease development.

Uneven growth of plants and domination of the pot by one or two plants has been reported by Gerlagh (1968). In the experiment reported here there was a definite correlation between plant growth and incidence of *G. graminis*, but why some plants were so severely infected while others in close proximity had only mild infection remains unexplained.

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VI. REFERENCES

- GARRETT, S. D. (1934).—Factors affecting the severity of take-all. I. The importance of micro-organisms. *J. Dep. Agric. S. Aust.* **37**, 664–74.
- GARRETT, S. D. (1936).—Soil conditions and the take-all disease of wheat. *Ann. appl. Biol.* **23**, 667–99.
- GARRETT, S. D. (1937).—Soil conditions and the take-all disease of wheat. II. The relation between soil reaction and soil aeration. *Ann. appl. Biol.* **24**, 747–51.
- GERLAGH, M. (1968).—Introduction of *Ophiobolus graminis* into new polders and its decline. *Neth. J. Pl. Path.* **74**, Suppl. 2. 97 pp.
- GRIFFITHS, R. L. (1933).—“Take-all”. Incidence and control on the lighter soils of the Mallee. *J. Dep. Agric. S. Aust.* **36**, 774–8.
- HORNBY, D. (1969).—Quantitative estimation of soil-borne inoculum of the take-all fungus [*Ophiobolus graminis* (Sacc.) Sacc.]. Proc. 5th Br. Insectic. Fungic. Conf., Brighton, 1969. Vol. 1, pp. 65–70.
- MAC NISH, G. C. (1973).—Detection of *Gaeumannomyces graminis* var. *tritici* in wheat stubble. *Aust. J. biol. Sci.* **26**, 1285–8.
- MAC NISH, G. C., DODMAN, R. L., and FLENTJE, N. T. (1973).—Bioassay of undisturbed soil cores for the presence of *Gaeumannomyces graminis* var. *tritici*. *Aust. J. biol. Sci.* **26**, 1267–76.

