BIOASSAY OF UNDISTURBED SOIL CORES FOR THE PRESENCE OF *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*

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Abstract

The presence of viable *G. graminis* var. *tritici* in field soil was detected by a bioassay. Wheat seedlings were grown in undisturbed soil cores maintained under standard conditions for 4 weeks. The percentage of roots infected per core was the main parameter chosen to give an estimate of the level of *G. graminis* var. *tritici* inoculum in the core. Some variability between cores from the same site was observed, but this could be reduced by taking cores over plant remains within take-all patches. In this way, high and reasonably uniform levels of inoculum could be obtained to study the effect of various treatments on the incidence of *G. graminis* var. *tritici*.

I. Introduction

To study the incidence and survival of *Gaeumannomyces graminis* var. *tritici* Walker (hereafter referred to as *G. graminis*) in field soil, methods of estimating the level of the fungus in the soil are needed. Recently, two approaches to this problem have been reported. Hornby (1968, 1969*a*, 1969*b*) studied the form and distribution of inoculum, estimating the number of "infective units" per unit volume of soil by growing seedlings in a series of volumetric dilutions of debris or soil. Slope *et al.* (1969) used an assay that measured the intensity of attack on seedlings grown in soil from the field in terms of an "infection index". For their index, soil cores (5 cm by 15 cm deep) were taken at random from the field and assayed for the presence of *G. graminis*. It is not specifically stated that the soil was removed from the core, but from the wording in the report, i.e. "each core filled one assay pot", it seems likely that the soil was removed and presumably mixed. Although there was variability between cores from the same location, Slope *et al.* (1969) found that the infection indices usually reflected the estimated crop infection. However, Hornby (1969*a*) mentioned that it was considered unreliable for advisory purposes because of lack of correlation with the incidence of *G. graminis* in the crop.

Investigations leading to a standardized method of bioassaying relatively undisturbed soil cores for the presence of *G. graminis* are described below. This

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technique has overcome some of the problems encountered by Slope *et al.* (1969) and has subsequently been employed to study the incidence and survival of *G. graminis* in the field (Mac Nish and Dodman 1973*a*, 1973*b*) and in soil removed from the field and maintained in controlled environments (Mac Nish 1973*b*).

II. Materials and Methods

(*a*) *Collection and Use of Soil Cores*

Undisturbed soil cores were obtained by driving a steel cylinder (9·8 cm internal diam. by 14·5 cm long) into the soil to a depth of approximately 10·5 cm. Removal of a small quantity of soil from one side allowed the soil column to be broken at the base of the cylinder and the intact soil core to be removed. The core was then pushed into a can by a piston (Fig. 1). The cans (internal diam. 9·9 cm, 12·4 cm deep) were previously painted on the inside with black bituminous paint to prevent rusting.

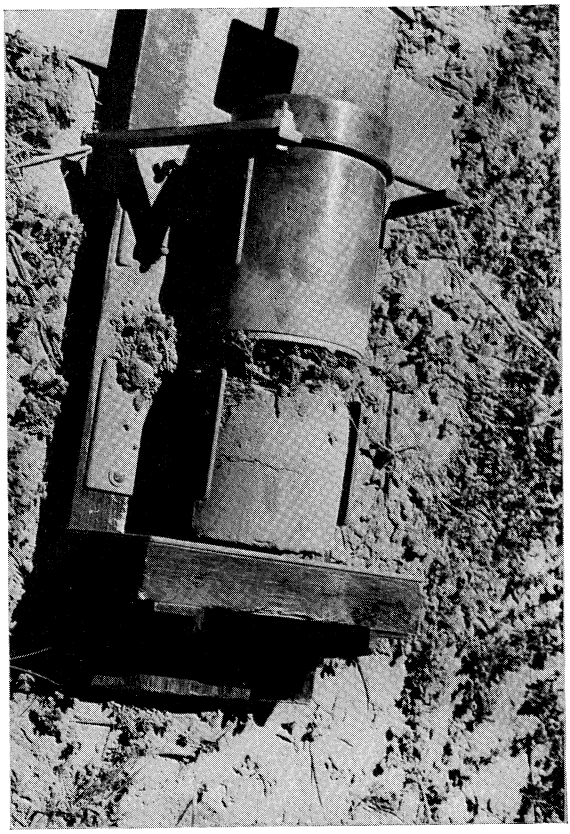

Fig. 1.—A piston was used to press the soil core from the steel cylinder into a can. In the above instance, where the can was not placed in position, the core can be seen to have remained intact.

On return to the laboratory all plants present in the cores were clipped to surface level and any surface trash removed. The cores were watered to a soil moisture content equivalent to a matric potential of −0·6 bar. Except in the initial experiments, seven wheat seeds were sown at a depth of 2·5 cm in each core (one central hole surrounded by six holes all placed so that every seed was 3·2 cm from its neighbours). Cores were then placed in a controlled environment (16 hr of fluorescent light, 17,200–18,300 lumen/m^2, and 15°C constant temperature) and brought to constant weight with water every second day. After 4 weeks the seedlings were washed free of soil and the roots examined in water over a white background for the presence of *G. graminis* (Garrett 1941).

(b) Cores from the Keith Location

One experimental area was at Keith, S.A. The soil was a grey sand with the following physical characteristics: particle size distribution <2 μm, 3%; 2–20 μm, 2%; >20 μm, 95%; pH 6·9. The drying boundary curve is shown in Figure 2.

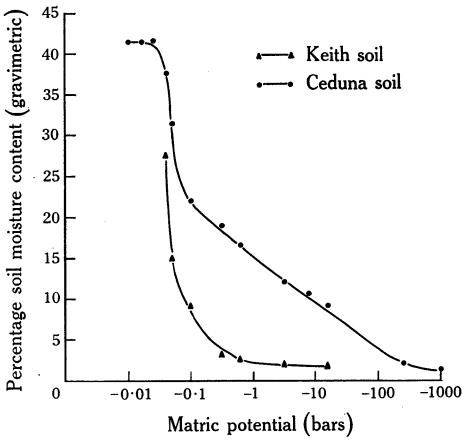

Fig. 2.—Drying boundary curve for soils from Keith and Ceduna.

A transect was made in late autumn in such a way as to cross a take-all patch observed in the previous spring. At each site on the transect, two cores were taken side-by-side and at right angles to the direction of the transect. Site 1 was on a bare, graded fire-break. Site 2 was on a small uncultivated area under a hoarding, and contained several species of grass (*Danthonia* sp. and *Vulpia* sp.) and *Erodium botrys* (Cav.) Bentol. Cereals had not been planted on this site for at least 4 years and possibly 9 years. The remainder of the transect cores were taken at 3-m intervals across the stubble. Sites 6, 7, and 8 were within the take-all patch recorded in the previous season.

In this initial experiment, nine seeds were sown per core and the soil moisture content was kept at 9% (equivalent to a matric potential of −0·1 bar). The parameters recorded are shown in Table 1.

(c) Cores from the Ceduna Location

The other experimental area was at Ceduna, S.A. At this location the soil is a dull brown sandy loam with the following physical characteristics: particle size distribution <2 μm, 17%; 2–20 μm, 8%; >20 μm, 75%; pH 8·5. The drying boundary curve is shown in Figure 2. Cores from this location were used in the following experiments.

(i) *Variability in Disease Incidence*

Cores were collected on three occasions for experiments studying the variability of incidence of *G. graminis*. On the first occasion a transect was taken at 3-m intervals across a field which had been generally infested with *G. graminis* the previous season. Three cores were taken at each of the eight sites as described above. Following a wheat crop in the same field a series of cores was taken along a drill row within a take-all patch. A total of 15 cores were selected at random and bioassayed. On a later occasion another series of cores was removed from the same take-all patch; each core was taken over a plant or its remains. Seven cores were selected at random and bioassayed.

For these experiments the percentage of seedlings and percentage of roots infected per core were recorded.

(ii) *Selection of Soil Moisture Conditions for Bioassay*

A preliminary experiment showed that there was no difference in moisture loss between cores containing diseased or healthy seedlings during the 48-hr period between waterings. In this time

the soil moisture content (gravimetric determination) fell from 16% to about 13%. This is equivalent to a change in matric potential from −0·6 to −2·2 bars. To determine the effect of soil moisture on disease incidence, cores taken over plant remains from a take-all patch at Ceduna were bioassayed as usual except that four soil moisture regimes (19%, 16%, 13%, and 10%) were used. To minimize variability in moisture content, the cores were watered every 24 hr rather than every 48 hr.*

Parameters recorded were percentage of seedlings and percentage of roots infected, top dry weight, and root dry weight per core.

III. Experimental Details and Results

(a) *Disease Development in Undisturbed Soil Cores*

Results for the transect at Keith are shown in Table 1. There was no *G. graminis* at site 1. This was to be expected since the site was a graded fire-break, devoid of

TABLE 1

PARAMETERS RECORDED FROM WHEAT SEEDLINGS GROWN IN CORES TAKEN FROM A TRANSECT ACROSS A FIELD AT KEITH INFESTED WITH *G. GRAMINIS*

Values are means for two replicates

Site	Vegetation	Emergence	No. of seedlings infected	Total leaf length (cm)	Total No. of seminal roots	Total No. of seminal roots infected	Total length of seminal root discoloured (cm)
1	Bare	8·0	0	231	30·0	0†	0†
2	Grasses and *Erodium*	9·0	8·5	220	39·5	30·0	79·0
3	1968 wheat stubble	8·5	7·0	244	33·0	13·0	26·0
4	As site 3	8·5	6·5	243	34·5	12·0	18·5
5	As site 3	8·0	6·5	303	32·5	11·0	18·5
6	As site 3	8·5	9·0	258	38·0	30·0	35·5
7	As site 3	8·5	5·5	254	34·5	9·5	12·5
8	As site 3	9·0	9·0	252	39·5	33·5	40·5
9	As site 3	8·0	7·0	244	30·0	13·0	15·5
10	As site 3	8·5	7·0	273	36·0	20·0	25·0
Standard error				37·9	5·1	3·6	8·6
L.S.D. at $P = 0·05$				n.s.	n.s.	8·3	19·8
$P = 0·01$						11·2	29·3

† Not included in analysis of variance.

plants. It was found that the grasses growing at site 2 when the cores were collected were infected with *G. graminis*. This would account for the high incidence of *G.*

* A problem with the use of cores is to express the soil moisture level in meaningful terms. The moisture-characteristic curve is the best method of expressing soil moisture (Griffin 1963), but the establishment of such curves with large cores is difficult. Theoretically, at matric potentials drier than −1 bar soil structure should have no effect on moisture characteristic (Salter and Williams 1965). Some time was spent in preliminary experiments trying to establish the drying boundary curve for matric potentials of 0 to −1 bar for soil in cores, but moisture equilibrium was not obtained. The drying boundary curve for disturbed soil was therefore used as an approximate estimate of the curve for the cores. It is also realized that surface watering will lead to uneven distribution of soil moisture throughout the soil profile. This will become greater the further the desired moisture level is below field capacity.

graminis at site 2. Although sites 3, 4, 5, 9, and 10 were outside the take-all patch recorded in the previous season, there was considerable *G. graminis* present in the crop debris surrounding the patch. Two of the sites (6 and 8) within the take-all patch had high levels of *G. graminis*, but the reason for the low level at site 7 is unknown.

(*b*) *Selection of Parameters*

Of the disease incidence parameters recorded in Table 1, the number of roots infected per core was more convenient to assess than length of root discoloured per core. As there is a highly significant correlation (Fig. 3) between these two parameters, the former was chosen as the main parameter. To eliminate the effect on the results of variability in emergence, the percentage of roots infected per core was used in all later experiments. In some later experiments the percentage of seedlings infected per core was also recorded. Top weight and root weight were recorded in those experiments where the treatments were likely to affect plant growth as well as the incidence of *G. graminis*.

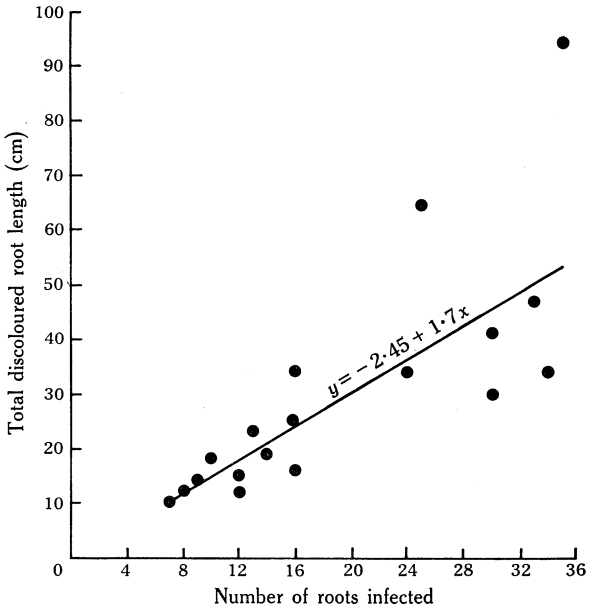

Fig. 3.—Relationship between the number of roots infected (x) and length of roots discoloured (y) for wheat seedlings grown in cores from a transect at Keith. Variance ratio 24·2***; correlation coefficient 0·7757.

(*c*) *Variability in Disease Incidence between Cores from the Same Location*

Although there was some variability in the disease level between cores taken together at Keith (Table 2), the differences were not as large as some of those between cores taken together at Ceduna (Table 3). The transect at Ceduna established the presence of *G. graminis* in the field, but cores with a much more predictable level of *G. graminis* were required to study the effect of various treatments on the incidence of *G. graminis*.

The results of the bioassay of cores taken along a row within the take-all patch at Ceduna are shown in Table 4. These data were used to calculate the estimated

magnitude of differences between treatments necessary to obtain a significant difference at $P = 0{\cdot}05$. The following formula was employed for this purpose:

$$\text{difference} = t(2s^2/m)^{\frac{1}{2}}$$

where

$$s^2 = \text{estimate of variance} = \frac{\Sigma x^2 - (\Sigma x)^2/n}{n-1},$$

x = percentage plants or percentage roots infected per core,

t = the value of the distribution for the number of samples (n) at $P = 0{\cdot}05$, and

m = the proposed number of replications.

TABLE 2

COMPARISON OF THE INCIDENCE OF *G. GRAMINIS* IN DUPLICATE CORES FROM A TRANSECT MADE AT KEITH

Site	Percentage of plants infected		Percentage of roots infected		Site	Percentage of plants infected		Percentage of roots infected	
	Core 1	Core 2	Core 1	Core 2		Core 1	Core 2	Core 1	Core 2
1	0	0	0	0	6	100	100	79	79
2	89	100	62	87	7	44	88	19	38
3	100	71	47	31	8	100	100	83	87
4	63	89	25	43	9	86	89	52	38
5	83	89	35	33	10	67	100	42	70

TABLE 3

COMPARISON OF THE INCIDENCE OF *G. GRAMINIS* IN TRIPLICATE CORES FROM A TRANSECT MADE AT CEDUNA

Site	Percentage of plants infected			Percentage of roots infected		
	Core 1	Core 2	Core 3	Core 1	Core 2	Core 3
1	0	88	89	0	60	36
2	0	0	0	0	0	0
3	0	0	0	0	0	0
4	0	50	44	0	15	11
5	22	25	33	5	6	8
6	0	0	0	0	0	0
7	11	0	44	3	0	10
8	0	0	22	0	0	6

The available controlled environment space for later experiments dictated a maximum of seven replications. The estimated differences necessary to obtain a significant difference between any two treatments at $P = 0{\cdot}05$ with seven replicates are shown in Table 4.

The bioassay results for cores taken over plant remains within the take-all patch are shown in Table 5. The variability was reduced slightly by this procedure.

TABLE 4

COMPARISON OF THE INCIDENCE OF *G. GRAMINIS* IN CORES TAKEN ALONG A DRILL ROW IN A TAKE-ALL PATCH AT CEDUNA

Core	Percentage of seedlings infected	Value expressed as arcsin (deg)	Percentage of roots infected	Value expressed as arcsin (deg)
1	57	49·1	52	45·9
2	100	90·0	100	90·0
3	100	90·0	59	50·2
4	100	90·0	96	78·2
5	100	90·0	100	90·0
6	71	57·7	52	46·2
7	80	63·4	57	48·2
8	67	54·8	29	32·3
9	80	63·4	59	50·1
10	100	90·0	73	58·7
11	100	90·0	97	79·4
12	83	65·9	77	61·3
13	100	90·0	94	76·3
14	100	90·0	79	62·4
15	100	90·0	83	65·5
Mean	89	77·7	74	62·3
S.D.	14·9	16·2	21·8	17·4
Difference*	17·1	18·7	24·5	19·9

* Difference necessary for significance with seven replications (see text).

TABLE 5

COMPARISON OF THE INCIDENCE OF *G. GRAMINIS* IN CORES TAKEN OVER PLANT REMAINS IN A TAKE-ALL PATCH AT CEDUNA

Core	Percentage of seedlings infected	Value expressed as arcsin (deg)	Percentage of roots infected	Value expressed as arcsin (deg)
1	83	65·7	36	36·9
2	100	90·0	75	60·0
3	100	90·0	89	70·6
4	100	90·0	89	70·6
5	100	90·0	69	56·2
6	100	90·0	76	60·7
7	100	90·0	100	90·0
Mean	98	86·5	76	63·6
S.D.	6·4	9·2	20·7	16·2

(*d*) *Selection of Soil Moisture Conditions for Bioassay*

Soil moisture content in the range 13–19% had no effect on the percentage of infected seedlings or roots (Table 6). At 10% soil moisture content, crusting of the soil surface affected emergence, resulting in a reduction in the amount of root tissue available to explore the soil (Table 6). There was also a reduction in top growth

associated with a decrease in soil moisture. It is apparent that this is an effect of soil moisture on the host rather than the pathogen. Soil moisture content equivalent to a matric potential of −0·6 bar was employed in all further work with soil core bioassays.

TABLE 6

EFFECT OF SOIL MOISTURE ON THE INCIDENCE OF *G. GRAMINIS* AND ON WEIGHT OF SEEDLINGS GROWN IN CORES FROM A TAKE-ALL PATCH AT CEDUNA

Values are means for seven replicates

Soil moisture content (%)	Matric potential (bar)	Emergence	No. of seedlings infected (%)	Value expressed as arcsin (rad)	No. of roots infected (%)	Value expressed as arcsin (rad)	Dry weight per seedling (mg) Top	Root
19	−0·2	5·9	100	1·57	90	1·36	46	15
16	−0·6	6·4	100	1·57	85	1·21	38	18
13	−2·2	5·3	97	1·50	77	1·15	25	17
10	−8·8	3·7	47	0·76	25	0·49	20	15
Standard error				0·09		0·10	2·4	1·8
L.S.D. at $P = 0·05$				0·18		0·21	5	n.s.
$P = 0·01$				0·25		0·28	7	

IV. DISCUSSION

The parameters chosen for the bioassay of cores need to indicate the relative amount of *G. graminis* present. As the roots are exploring the soil mass and are likely to come into contact with scattered inoculum, the number of lesions on the root system would appear to be the best method of estimating the number of propagules. However, the possibility of lesions coalescing with time makes it difficult to assess this parameter if enough time is given for the roots to fully explore a considerable volume of soil. For this reason, the use of the length of discoloured root is possibly a reasonable compromise. Unfortunately, the length of discoloured root has the disadvantage of being very tedious to measure, since areas of discoloured tissue may be scattered over the entire root system.

In a similar situation, Garrett (1941) recorded the number of seminal roots infected. Experience in the assessment of seedlings from cores has shown that the latter parameter can be determined quickly. The percentage of seedlings infected per core gives an indication of the possible result at maturity. As any infection on seedlings less than 4 weeks old is likely to cause severe take-all later in the growing period (Mac Nish 1973*a*), this parameter is of interest, although of limited value because of the small number of results possible from only seven seedlings.

In the transect at Keith there was no significant difference in the leaf length of seedlings grown in cores from any of the sites (Table 1). In later experiments there were obvious relationships between leaf growth and incidence of *G. graminis*, but leaf growth was affected significantly only in those cores where there was severe infection.

The use of soil cores to study the effect of different treatments on *G. graminis* has many possibilities. However, when using naturally infested soils rather than artificially inoculated ones, there is the problem of lack of uniformity in levels of inoculum. The investigations at Ceduna show that taking cores over plant remains can minimize variability of inoculum level (Tables 2 and 3). This allows high, and reasonably uniform, levels of inoculum to be obtained.

The core bioassay is based on the assumption that the roots of wheat seedlings will explore a large part of the soil mass within the core. However, it seems unlikely that there would have been enough root exploration in 4 weeks to ensure contact with every piece of debris containing viable *G. graminis*. This would be especially so if *G. graminis* is unable to grow from debris as suggested by Lucas (1955). Even if *G. graminis* can grow from its habitat to meet approaching roots (Brown and Hornby 1971), the bioassay should be treated as a relative estimate of *G. graminis* incidence in the soil.

Hornby (1969*a*, 1969*b*) separated infested soil into its various components, and estimated the number of "infective units" present. Although this approach will give a comparison of the numbers of infective units in various soils, the usefulness of the comparison is limited by the large variation in size of the units colonized by the fungus; these range from whole crowns to small pieces of debris. In the field a large infective unit may provide the infection point for several roots or even several plants. In the core bioassay no attempt is made to calculate the number of infective units present, but rather each core is treated as a unit with a potential for causing a certain level of disease. The results of the bioassay should be similar to those obtained by growing seedlings in the same unit of soil *in situ*, although in the field roots from seedlings outside the unit may also explore the soil. The environment will also influence the results, but the differences between the level of infection in the field and in the bioassay may not be very large in a short period of growth (4 weeks). However, the effect of the environment on symptom expression between the time of the bioassay and maturity could be considerable.

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