Reproduction of Crown Rot of Wheat Caused by *Fusarium graminearum* Group 1 in the Greenhouse

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ABSTRACT

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Crown rot of wheat (*Triticum aestivum* L.) caused by *Fusarium graminearum* group 1 was reproduced in artificially infested field soil in the greenhouse. A technique was developed to study the relationship between the quantity of inoculum and disease development. Two hard white spring wheat cultivars, Cook (tolerant to crown rot) and Songlen (low tolerance to crown rot), were sown in galvanized bins. Three quantities of inoculum (artificially colonized wheat chaff) were used. The inoculum was spread as a thin layer in the surface soil, midway between the seed and the soil surface. Soil moisture was maintained at near field capacity. The plants were observed for symptoms periodically and harvested after 130 days, when they were rated for disease. Symptoms and yield loss were similar to those observed on field plants. The technique is useful for studies on infection, colonization, and tolerance of various lines of wheat.

Crown rot of wheat is a serious disease caused by *Fusarium graminearum* Schwabe group 1 (2,7) in Australia (1,3) and is particularly common on clay soils in the northern regions of the eastern wheat belt (1). It has also been reported from the Pacific Northwest of the United States (4). Precise studies on the various factors that affect disease development have not been possible because of the lack of a greenhouse technique for reproducing typical symptoms over a time scale similar to that of field-grown crops.

Purss (9) used inoculated seed in an attempt to reproduce the disease in the greenhouse, but the plants usually died at an early postemergent stage and consequently did not develop whiteheads and browning of the culm bases that occur toward maturity. Furthermore, the fungus is not normally seedborne and does not cause serious seedling death in the field (1). *F. graminearum* group 1 survives in field soil predominantly as hyphae in infested host residue (10,12). Colonized wheat chaff (fragments of stem internodes and nodes) was therefore selected as an appropriate form of inoculum for greenhouse studies.

A technique for reproducing typical symptoms of crown rot of wheat using artificially colonized wheat chaff and the influence of different quantities of inoculum on the development of the disease are reported.

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MATERIALS AND METHODS

Soil containers. Plants were grown in 60-L galvanized garbage bins (50 × 40 cm in diameter), which were scrubbed and steamed for 3 min before use. Bins were lined with new, polyethylene garbage bags and had no drainage holes.

Soil preparation. Soil was collected from the top 15 cm of a black earth at the I. A. Watson Wheat Research Centre, Narrabri, NSW. *F. graminearum* group 1 could not be isolated from this soil with the debris-plating and dilution-plating techniques described by Burgess and Liddell (2). Moreover, crown rot had not been observed in previous wheat crops on this soil. The soil (pH 7.4 in a 1:5 soil suspension in 0.01 M $CaCl_2$) is a high smectite, dark plains soil. Specific physical properties of the soil used in this experiment are given in Table 1, and further general characteristics may be obtained from Stace et al (11). The soil was air-dried, crushed to break down aggregates, and sieved (10-mm mesh).

Inoculum production. *F. graminearum* group 1 was isolated from the basal internode of a wheat culm (cultivar Cook) collected in November 1981 from the Darling Downs of southeastern Queensland. The culm base was surface-sterilized (1.8% sodium hypochlorite in 10% EtOH for 1 min) and plated on carnation leaf-piece agar (CLA) (6). A single, germinated macroconidium was then transferred to CLA. This isolate was deposited in the Biology Branch Herbarium, N.S.W. Department of Agriculture, Rydalmere, under the accession number DAR 52453. A conidial suspension from this isolate was used to inoculate sterile, moistened wheat chaff (fragments of stem internodes and nodes) in clear, sterile polyethylene bags. The chaff cultures were incubated under fluorescent lights with a photoperiod of 12 hr and a day temperature of 25 C and a night temperature of 20 C (2). The cultures were shaken every 2 days for 4 wk to promote uniform colonization. The colonized chaff was then air-dried, crushed, and sieved (710 μm diameter).

Experimental design. The experiment was a split-plot factorial design with two treatments replicated three times. The treatments were 1) inoculum quantities: three quantities of inoculum (0.5, 1.0, and 3.0 g of inoculum per bin) were added as a thin layer in the surface soil of the bins (control bins had no inoculum added); and 2) cultivars: two hard white spring wheat cultivars, Cook (tolerant to crown rot) and Songlen (low tolerance to crown rot), were grown. Inoculum quantities were assigned to the whole plots (bins) and cultivars to the subplots (half-bins). There were 12 bins total.

Sowing and inoculation. Wheat seed (Cook and Songlen) was obtained from the I. A. Watson Wheat Research Centre. Germination rates were 95–100%. Fourteen seeds per bin was used as a standard sowing rate equivalent to 30 kg ha^{-1} (a common rate on black earth seed beds).

Table 1. Major physical properties of a black earth from the I. A. Watson Wheat Research Centre, Narrabri, NSW, Australia

	Particle size distribution	
Composition	**Size (mm)**	**%**
Clay	0.002	51.1
Silt	0.002–0.02	12.6
Fine sand	0.02–0.20	19.6
Coarse sand	>0.20	16.0
Moisture content at fixed moisture potential	**(g g^{-1})**	
Wetting curve		
−0.03 MPa (field capacity)	0.35	
−0.1 MPa	0.31	
Drying curve		
−0.1 MPa	0.33	
−0.3 MPa	0.28	
Bulk density	**(g cm^{-3})**	
Field	1.0–1.6	
Experimental bins (at ψ −0.1 MPa)	1.1	

Air-dry soil was placed in the bins to 15 cm below the rim, and this formed the subsoil (soil below the seed). It was gently watered, over a week, to approximate field capacity on the wetting moisture characteristic. The garbage bag was then closed over the top of the subsoil for a further 2 days to allow redistribution and equilibration of the water.

Seven seeds of each cultivar were sown in adjacent subplots on 21 June 1982, which coincided with the sowing of commercial crops in New South Wales and ensured that conditions of day length and light intensity were similar to field conditions. Seeds were sown on the moist subsoil and covered by a 2-cm layer of dry surface soil. A layer of finely ground inoculum of the appropriate weight was then scattered uniformly over the soil with a large, sterile sieve (710-μm aperture). Another 2 cm of dry soil was placed over the layer of inoculum. The surface soil was gradually watered to field capacity by a fine mist over 5 days to reduce surface slaking. Bins were maintained at field capacity by misting every second day for 17 days to ensure a high rate of infection (8).

Growth conditions. The bins were placed in a glasshouse with a night temperature of 10–12 C and a maximum day temperature of 22 C for the first 90 days (anthesis) and 27 C thereafter. The soil in all bins was maintained at near field capacity by measuring water loss with a mobile gantry and weighing crane (Fig. 1) and replenishing by gentle surface watering at 2- or 3-day intervals. Details of the design and construction of the gantry and weighing crane assembly have been published elsewhere (2). The gantry is mounted on two fixed and two swivel castors for easy maneuvering and spans three bins. The weighing crane assembly is mounted on a movable girder trolley and consists of a bin cradle suspended from a Shear Beam Load Cell with a weighing capacity of 100 ± 0.1 kg (EFM Load Cell Systems, Melbourne, Australia) attached to a pulley block. The cradle was designed to hold one bin containing mature wheat plants. The weighing system allows one operator to weigh 70 bins containing 70–80 kg of wet soil to an accuracy of 100 g in 2–3 hr. Plants were regularly assessed for disease symptoms and insect or other damage. At anthesis, 1.5 ml L^{-1} of 30% (w/v) dimethoate and 0.4 g L^{-1} of 50% (w/v) cyhexatin were applied to control aphids and mites, respectively. Each subplot was thinned to five plants at 39 days (late tillering), after the observation of postemergence death.

Disease assessment. Observations on symptom development and emergence were made daily for the first 25 days after sowing, and thereafter, observations were made on days 39, 51, 87, and 101. The

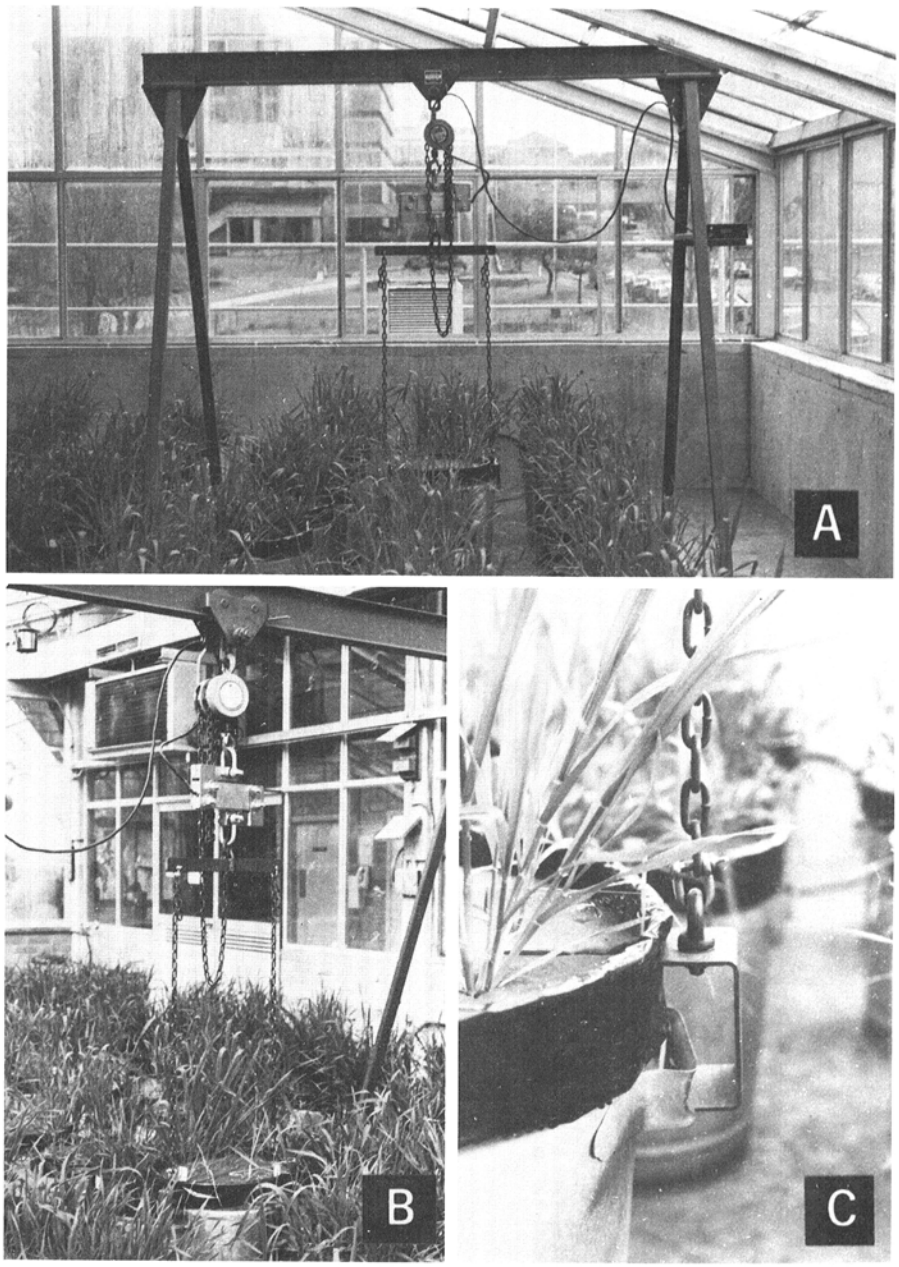

Fig. 1. Details of the mobile gantry and weighing crane used to measure water loss from bins throughout the experiments performed in the glasshouse. **(A)** Overall view of the gantry and crane showing a bin raised off the ground while being weighed. **(B)** Cradle used to lift bins without damaging plants. **(C)** Point of attachment of cradle to bin handles.

Table 2. Percentage of plants of two wheat cultivars displaying necrosis of the leaf sheaths 39, 51, and 101 days after sowing in uninfested soil and soil infested with three quantities of inoculum of *Fusarium graminearum* group 1

Quantity of added inoculum per plot (bin) (g)	39 Days		51 Days		101 Days	
	Cook (%)[a]	Songlen (%)	Cook (%)	Songlen (%)	Cook (%)	Songlen (%)
0.0	0	0	0	5	0	20
0.5	6	21	17	40	73	79
1.0	29	24	41	57	60	87
3.0	11	53	37	63	60	100

[a] Percent plants per plot.

plants were harvested on day 130, when the grain was hard but could be divided by thumbnail (hard dough stage). All plants were removed from the bin, and the total numbers of tillers, tillers bearing healthy heads, and tillers with whiteheads (heads with no grain or incompletely filled grain) were recorded. Symptoms were assessed, and each plant was given a disease rating according to the following scale: 0 = full yield: healthy, no necrosis, subcrown internode clear, coleoptile may be necrotic and sloughed off, few aborted tillers and then only aborted very early, no whiteheads, healthy root system; 0^+ = full yield: subcrown internode brown and coleoptile necrotic, one culm base brown to the first node, leaf sheaths clear, otherwise as for 0; 1 = full yield: more than one culm base brown to the first node or one culm base brown to higher than the first node, otherwise as for 0^+; 2 = reduced yield: more than 30% of culms with browning to the first node (at least one with browning above the first node), leaf sheaths with vascular necrosis or at least one necrotic, heads may be healthy but at least one with aborted florets or no aborted florets but grain pinched, otherwise as for 1; 3 = reduced yield: more than 70% of culms brown to the first node and many brown above the first node, leaf sheaths necrotic, most heads with aborted florets and pinched grain, whiteheads present, root system clear but reduced root hairs on crown roots, otherwise as for 2; 4 = nil yield: plant died prematurely; subcrown internode, coleoptile, leaf sheaths, and root system necrotic; all culm bases brown; heads all with aborted florets and pinched grain; more than three whiteheads, some with no grain at all; and 5 = nil yield: plant died prematurely; subcrown internode, coleoptile, leaf sheaths, and root system necrotic; all culm bases brown; no heads formed, or three or fewer whiteheads only with severely pinched grain.

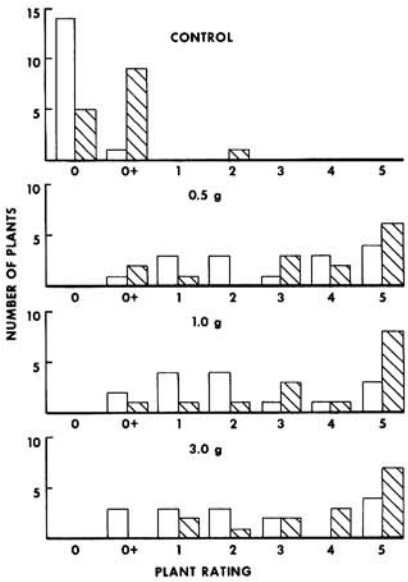

Fig. 2. Number of plants of two wheat cultivars (Cook and Songlen) with disease ratings based on symptom severity at harvest, 130 days after sowing in uninfested soil and soil infested with 0.5, 1.0, and 3.0 g of inoculum of *Fusarium graminearum* group 1 in a thin layer in the surface soil (Songlen indicated by hatched bars; total of 15 plants of each cultivar in each treatment).

Quantitative data on tiller number and head number were analyzed with two-way analysis of variance incorporating the split-plot design. Comparisons between control and infested means were made with a one-sided Dunnett's test (5). All data were transformed using a square-root transformation before analysis.

RESULTS

Emergence. Analysis of emergence data detected no differences between treatments, and all plants that finally emerged had done so by 17 days. Symptoms were not evident on any plants for the first 25 days, and no differences in growth rate or appearance were obvious up to this time.

At 39 days, Songlen generally displayed more leaf sheath necrosis than Cook in the bins containing inoculum (Table 2). One plant of Songlen in the plots receiving 0.5 g of inoculum per bin was obviously stunted and slightly chlorotic and wilted. However, no other plants showed obvious foliar symptoms at this time. No plants had died by 39 days (tillering completed), and the control plants were symptomless.

At 51 days, Songlen was showing some leaf sheath necrosis in all plots (Table 2). In the plots receiving 0.5 g of inoculum per bin, one plant of Cook and five plants of Songlen were stunted and appeared chlorotic and wilted. In the plots receiving 1.0 and 3.0 g of inoculum per bin, eight and six plants, respectively, of Songlen were stunted and becoming chlorotic. No plants had died by 51 days (stem elongation stage) and Cook in the control plots and in the plots receiving 1.0 g and 3.0 g of inoculum per bin remained symptomless.

By 87 days (anthesis), some plants of both cultivars had died in the plots with added inoculum, and Songlen was worst affected (Table 3). Songlen remained more severely affected than Cook in regard to leaf sheath necrosis and foliar symptoms. Cook remained symptomless and Songlen showed some leaf sheath necrosis in the control plots (Table 2).

By 101 days (milk ripe growth stage), several more plants of both cultivars, mainly Songlen, had died in the plots with added inoculum (Table 3).

By 130 days (harvest), Songlen was more severely diseased than Cook (Fig. 2), and all plants grown in infested plots were infected. Cook produced significantly more ($P = 0.01$) tillers than Songlen in the plots receiving 1.0 and 3.0 g of inoculum per bin. Cook produced significantly

Table 3. Percentage of dead plants of two wheat cultivars 87 and 101 days after sowing in uninfested soil and soil infested with three quantities of inoculum of *Fusarium graminearum* group 1

Quantity of added inoculum per plot (bin) (g)	87 Days		101 Days	
	Cook (%)[a]	Songlen (%)	Cook (%)	Songlen (%)
0.0	0	0	0	0
0.5	13	20	13	27
1.0	13	40	13	53
3.0	13	33	20	47

[a] Percent plants per plot.

Table 4. Mean number of tillers, yielding tillers, and whiteheads per plant produced by two wheat cultivars grown in uninfested soil and soil infested with three quantities of inoculum of *Fusarium graminearum* group 1

Quantity of added inoculum per plot (bin) (g)	Total tillers		Yielding tillers		Whiteheads	
	Cook[x]	Songlen[x]	Cook[x]	Songlen[x]	Cook[x]	Songlen[x]
0.0	5.74 a[y]	6.32 a	5.67 a	5.34 a	0.00 a	0.00 a
0.5	5.54 a	4.51 b	2.85 b*[z]	1.35 b*	1.13 b	0.99 b
1.0	6.93 a**	4.43 b**	4.15 a**	1.05 b**	0.72 b	0.95 b
3.0	5.86 a**	4.00 b**	3.48 b**	1.11 b**	0.66 b	1.07 b

[x] All means are retransformed.
[y] Same letters in columns indicate no significant different ($P = 0.05$) from control (Dunnett's procedure).
[z] Transformed LSD between cultivars significant at * = $P = 0.05$ and ** = $P = 0.01$.

more ($P = 0.01$) tillers bearing healthy heads in all treatments with added inoculum. Both cultivars produced the same number of whiteheads in all treatments with added inoculum.

There were no significant differences between the cultivars in the control plots with regard to the number of total tillers and yielding tillers (Table 4). No control plants were infected with *F. graminearum* group 1, although one plant (Songlen) was affected by common root rot caused by *Bipolaris sorokiniana* (Sacc.) Shoem.

DISCUSSION

The results of this experiment indicate that infection of wheat by *F. graminearum* group 1 and the subsequent development of symptoms are not linearly related to the quantity of inoculum applied as a thin layer in the surface soil. However, it is probable that below a certain threshold quantity of inoculum, the rate of infection would be lowered because of the reduced likelihood of contact between plant and inoculum. Above this threshold, all plants would become infected and, as this experiment shows, probably become diseased to a uniform level. Although the number of yielding tillers and whiteheads was uniform for each cultivar among plots with different quantities of inoculum, there was a tendency for the higher quantities of inoculum to result in earlier and more severe symptom development and premature death.

Hence satisfactory levels of disease can be obtained with relatively small quantities of inoculum as long as there is sufficient to ensure 100% infection. This is best achieved with an even layer of inoculum spread uniformly so that there are no gaps. A quantity of 0.5 g inoculum spread as a single layer on 1,260 cm^2 of soil surface (40-cm bin diameter) is equivalent to about 0.4 mg cm^{-2}. In untreated soil, this level appears to ensure 100% infection and a moderate rate of disease development.

Temperatures in the greenhouse were generally higher than in the field and would probably favor disease development (1). Cooler midwinter temperatures should therefore permit the most accurate reproduction of this disease.

A number of experiments involving moisture stress at some time through the growing season have been performed in our laboratory using this technique. This is simply achieved by withholding water so that the soil dries to a predetermined weight corresponding to a moisture content that is equivalent to the desired moisture potential on the drying curve of the soil. It is important to note that this method does not give an accurate measure of the moisture potential for the whole bin. Precise measurements of plant moisture stress must be made by determining the total and osmotic potential of the plant. The black earth used in this experiment is an ideal soil for these studies because it has a large water-holding capacity and does not need to be watered frequently to maintain a reasonably stable moisture potential. The fine texture of this soil also ensures that moisture stress develops slowly after withholding water, which is similar to the field situation.

For detailed studies on the behavior of the pathogen once it has successfully infected the host plant, it is advisable to use pasteurized soil. This ensures that the pathogen is relatively uninhibited by the soil microflora and that infection rates are high and disease development uniform so that the behavior of the diseased plant may be more easily studied. It has been shown that in pasteurized soil, as little as 0.25 g of ground inoculum per 40-cm-diameter bin is sufficient to ensure infection of all plants in a bin (C. M. Liddell, L. W. Burgess, and P. W. J. Taylor, *unpublished*) if layered in the manner described. This gives a moderate level of disease and allows detailed measurements of host growth and parasitic behavior of *F. graminearum* group 1.

ACKNOWLEDGMENT

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