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STUDIES OF VARIETAL RESISTANCE TO CROWN ROT OF WHEAT CAUSED BY FUSARIUM GRAMINEARUM SCHW.

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SUMMARY

Prior inoculation of wheat seed with a spore suspension of *F. graminearum* affected emergence, laboratory germination, colonization by the fungus, development of crown symptoms, "deadhead" production, yield, weight of individual grains and discoloration of the tillers.

The sub-crown internode tissue was shown to be a common site of invasion of plants by *F. graminearum*. The coleoptile and leaf base tissue are also frequently attacked. Direct root invasion is not considered very important. Infection occurs throughout the life of the plant.

Deadhead production and the development of crown symptoms prior to maturity are useful ways of comparing disease incidence. Yield must also be considered because of the effect of the disease on individual grain weight.

Experiments with and without inoculation offer a way of comparing varieties. Inoculation is shown to give results parallel with those from natural infection.

Early infection is suggested as being important in severe disease production.

Differences in varietal reaction were demonstrated, with Gala and Mengavi showing a reasonable level of field tolerance. None of the varieties tested gave any evidence of true resistance. Differences between varieties are considered to be due to a differential rate of development of the disease rather than to any difference in actual infection.

Seedling tests using seed inoculation and bulk inoculum methods are described. In general, there was no correlation between seedling blight in these tests and field reaction. It is possible that root rot may give an indication of field performance under some conditions.

I. INTRODUCTION

The fungus *Gibberella zeae* (Schw.) Petch has been widely recorded as a pathogen of wheat, causing head blight or scab, seedling blight and foot rot (Dickson 1956; Butler and Jones 1961; Butler 1961). All these phases have been

well described in the literature (Atanasoff 1920; Bennett 1931). The symptoms of foot rot caused by *Fusarium graminearum* Schw., the imperfect form of this organism, do not differ greatly from those produced by *Fusarium culmorum* (W. G. Sm.) Sacc. and *Fusarium avenaceum* (Fr.) Sacc., where discoloration of the stem generally does not proceed far beyond the first node (Butler and Jones 1961). The rather different and serious nature of the disease in Queensland has been recorded and the symptoms described (McKnight and Hart 1966). No record of occurrences as severe as those recorded in Queensland (McKnight and Hart 1966) and northern New South Wales (Magee 1957) can be found in the literature.

The perfect stage of *F. graminearum* was found for the first time on wheat affected by crown rot in Queensland in 1964. Descriptions of this will appear in a subsequent paper.

Considerable attention has been given to resistance to head blight in wheat. This work has been reviewed by Schroeder and Christensen (1963), who found that resistance is of two main types: resistance to initial infection and resistance to spread. McKnight and Hart (1966) describe experiments in which differing degrees of reaction to crown rot (*F. graminearum*) of various varieties are recorded. They have also observed good field tolerance in the variety Gala and have recommended its use.

Efforts to obtain resistance to take-all (*Ophiobolus graminis* Sacc.) and common root rot (*Helminthosporium sativum* P. K. and B. and *Fusarium* spp.) in wheat have been made for many years without any substantial progress. This work has been extensively reviewed by previous authors (Simmonds 1953; Butler 1961). Recently, however, Sallans and Tinline (1965) have reported success in selecting and breeding wheats resistant to common root rot caused by *Cochliobolus sativus* (Ito and Kurib) Drechs. ex Dastur.

In this paper, detailed field experiments comparing the reaction of 10 varieties of wheat to *F. graminearum* infection are outlined. Results of field experiments are compared with seedling reaction in the glasshouse. Methods of assessing differences between varieties are compared with a view to standardizing methods for future varietal comparisons. Detailed isolations were carried out throughout the season to record the progress of the disease in all varieties. Assessments of the effect of the disease on yield are also made.

II. METHODS AND MATERIALS

(a) Field Experiments

Sites and varieties.—Sites for field experiments were selected at Millmerran and Irvingdale on the Darling Downs in south-eastern Queensland. In each case the soil was black and heavy in texture, and had carried a crop of wheat severely affected by crown rot (*F. graminearum*) during the 1963 season.

A selection of 10 wheat varieties was made so as to give, as far as was known, a range in reaction to the disease. The varieties were Mengavi, Spica, Lawrence, Puseas, Festival, Gala, Hopps, Kenora, Puora and an unnamed hybrid of the parentage Kenya Governor x (Pusa x Flora), hereafter referred to as K.G.P.F., all of which have been at some time widely grown in Queensland. Varieties were compared with and without seed inoculation with *F. graminearum*.

Seed inoculation.—Seed inoculation has been used for *F. graminearum* on wheat by many workers (e.g. Atanasoff 1920; Dickson 1923). Simmonds (1928) and Greaney and Machacek (1934), working with *Fusarium culmorum,* established that subsequent drying of the seed did not impair the efficiency of the inoculation and made handling much easier.

On the basis of seedling blight production in the glasshouse, three virulent strains of *F. graminearum* which were also good spore producers were grown on a wheat/barley mixture (Schroeder and Christensen 1963) for 28 days. A spore suspension (400,000 spores per ml) was prepared from this mixture. Previous laboratory tests had established little difference in infection with levels of spore suspension as low as 20,000 spores per ml. Three pounds of seed of each variety was immersed for 1 hr in 1,000 ml of spore suspension and immediately dried quickly at room temperature. This was done 24 days prior to sowing at Millmerran and 14 days prior to the Irvingdale sowings. Control seed was treated in identical fashion except that sterile water was substituted for the spore suspension.

Layout and sowing.—The individual plots were 5 rows wide (7-in. spacing) and approximately 1 ch long and were 14 in. apart. The experiments were sown on a single face with three replications at Millmerran and four at Irvingdale.

Planting.—A set of five runs was reserved on one side of a 12-row combine for inoculated seed and another set on the other side for control seed. Consequently an inoculated plot was always adjacent to a control plot but otherwise the varieties were randomized.

Sowings were made at Millmerran on May 30, 1964, and at Irvingdale on July 1, 1964, at a depth of 3–4 in. The rate of sowing was calculated by counting the number of seeds delivered over a measured distance by each of the three centre runs on both the inoculated and the control side of the combine. Sowing rate of Millmerran was calculated to be 50 lb/ac and at Irvingdale 68 lb/ac.

Field data.—Four parameters were measured as follows:

(1) Emergence counts: These were made on two 20-ft lengths of each of the three centre rows of each plot. Percentage germination was calculated using the base figures determined during rate-of-sowing calculation.

(2) Field ratings at approximate heading time: The number of plants showing discoloration of the tissues around the crown above ground level were recorded, together with the stage of maturity and the number of plants which failed to head. Two counts of 100 plants each were made in each plot.

(3) "Deadhead" counts just prior to maturity: Five sets of 100 successive heads at Millmerran and three sets of 100 at Irvingdale were selected at random in each plot. Only those with no obvious grain as determined by feel were recorded as "deadheads". Where a dead plant occurred, each advanced tiller present, whether it bore a head or not, was recorded as a deadhead.

(4) Yield determination: Plots were harvested and grain yield recorded. All varieties were dead ripe at Millmerran but at Irvingdale Lawrence was rather green and Hopps slightly immature.

Laboratory data.—The following information was collected in the laboratory:

(1) Within 10 days of sowing the germination of the seed used in the experiment was determined on seed trays after 3 and 7 days. A check was kept of the number of seeds, both germinating and not germinating, which were carrying obvious colonies of *F. graminearum*.

(2) The survival of *F. graminearum* on the inoculated seed was checked within 10 days of sowing. Twenty seeds from both inoculated and control samples of each variety were plated onto Czapek-Dox agar with streptomycin after washing in sterile water, and the number of seeds affected with *F. graminearum* subsequently recorded.

(3) Isolations were made from plant samples collected from the plots at various intervals prior to maturity. The sample from each plot consisted of 10 plants, 5 selected at random from each of the 2nd and 4th rows. In the laboratory, notes were made on the condition of the roots, scutellum remains at the base of the sub-crown internode, the sub-crown internode, crown, tillers and leaf sheaths. Isolations of all tissues suspected of being primarily infected were made onto Czapek-Dox agar with streptomycin. Where infection had obviously reached the crown, isolations were made from this tissue only. Surface sterilization was done with 1/1,000 mercuric chloride for 2 min. The number of positive isolations of *F. graminearum* was recorded. Results were used to calculate the percentage infection at various stages of maturity of the crop and to follow the progressive development of crown rot.

(4) Laboratory ratings were given to samples collected at approximate heading time when field ratings were being recorded. Plants were classified as healthy, slightly infected (in which case discoloration had proceeded beyond the outer leaf sheath in less than half the tillers on a plant), or severe (where more than half the tillers showed infection beyond the outer leaf sheath).

(5) Ten "grab" samples selected at random from each plot were bulked and used for the following determinations:

(*a*) Percentage infection at maturity: Isolations were made from the crown or lowest internode of one tiller from each of 15 plants.

(*b*) Percentage of tillers showing discoloration beyond the second node by visual count.

(*c*) Average number of tillers per plant: This was possible only with the Millmerran samples, because at Irvingdale crown rot was so severe that many plants broke off at ground level.

(*d*) Movement of the fungus up the stem: This was determined from the samples from one replication at each site. Ten tillers were selected, each from separate plants, from the Millmerran samples. Fifty tillers were taken at random from the composite Irvingdale samples and these were divided into those with empty heads, those in which the heads were obviously pinched, and those in which the heads were normally filled. Isolations were made from each internode above the crown.

(b) Seedling Tests

After exhaustive experimentation, three methods of testing the seedling reaction of wheat to *F. graminearum* were selected:

(1) Tube tests: These closely followed the procedure described by Simmonds and Sallans (1946), in which an inoculated seed was placed on a blotting-paper raft in a test-tube. Water was placed in the tube almost to the level of the raft and the tubes were kept at 25°C in a constant-temperature bath in the glasshouse. Forty inoculated tubes and 40 control tubes were set up for each variety.

Seed inoculation was carried out in a similar fashion to that used for the field plots. The spore concentration was approximately 350,000 spores per ml and the seed was held in a desiccator after treatment until used 3 days later. Control seed was again treated with sterile water. The seed was not surface-sterilized because every method of sterilization tried had some residual effect on the fungus following inoculation.

After 12 days' incubation the following data were recorded:

(*a*) Percentage emergence.

(*b*) Extent of seedling blight: Three categories were used here, namely healthy, slight (where infection had not proceeded beyond the coleoptile), and severe (where infection had proceeded into the plant beyond the coleoptile). Often it was difficult to distinguish between

slight infections and the natural colour of the coleoptile. Because of this the final seedling blight rating was calculated as the percentage of emerged plants showing severe symptoms.

(*c*) Extent of root rot: The only type of root rot encountered in these tests was that associated with the seed area. Accordingly three categories of numerical ratings were used—0 for healthy, 1 where only a trace of rot was present, and 2 where the rotting was severe. A root rot index was calculated using the formula devised by McKinney (1923):—

$$\frac{\text{Total numerical ratings} \times 100}{\text{No. of plants which emerged} \times \text{Highest rating}}$$

(*d*) Length of the longest leaf: This has been expressed as an average for the plants which emerged.

(2) Pot tests with seed inoculation: Non-sterile sand was used in these tests in preference to soil because of the relative ease of standardization. Seed was inoculated in identical fashion to the test-tube tests. In each 400-g plastic container, 25 seeds were sown 1½ in. deep. These containers were then brought close to their moisture-holding capacity (approximately 25%) with water and placed in temperature tanks set to run at 25°C in the glasshouse. While this temperature was maintained for most of the day, the midday peak varied from 28 to 38°C. Four replications were used. The moisture level was maintained by daily waterings with Hoagland's nutrient solution rather than water because it was found in previous experiments that better infection resulted. The seedlings were rated after 20 days and the results expressed as for the tube tests.

(3) Pot tests with cornmeal/sand inoculation: 3% cornmeal/sand medium, prepared as described by Rao (1959), was inoculated with the same strains used for the seed inoculation work. This medium was approximately 8 weeks old when incorporated in non-sterile sand at the rate of 1 part of inoculum to 9 parts of sand. Previous tests had shown this to be a satisfactory level to use. Containers used were aluminium cylinders approximately 3½ in. in diameter and 6 in. deep. Control pots were prepared in identical fashion but were sterilized at 15 lb/sq in for 1 hr. The seed was sown 1½ in. deep and the containers again brought near to their moisture-holding capacity with water. These pots were subsequently maintained in the same manner as those in the seed inoculation experiment and ratings were made after 24 days.

III. RESULTS AND DISCUSSION

Field emergence.—A summary of the field emergence figures appears in Table 1. At Millmerran there was a significant reduction in emergence due to inoculation in only one variety, Festival (1%), while at Irvingdale significant

reductions occurred in Lawrence, K.G.P.F. (1%) and Puora (5%). The overall reduction in emergence due to inoculation was not significant at Millmerran but was highly significant at Irvingdale. Dickson (1923) found environmental factors to be critical in the production of pre-emergence and post-emergence blight caused by *F. graminearum,* so differences between sites as recorded in these experiments are not surprising.

TABLE 1

FIELD EMERGENCE AS A PERCENTAGE OF SEEDS CALCULATED TO HAVE BEEN SOWN

Variety	Mean Emergence (%)					
	Millmerran			Irvingdale		
	Control	Inoculated	Mean	Control	Inoculated	Mean
Mengavi	46·0	45·7	45·8	40·2	35·5	37·9
Spica	50·0	46·7	48·3	37·0	36·0	36·5
Lawrence	..	..	..	20·0	10·7	15·4
Puseas	37·0	35·3	36·2	33·2	34·0	33·6
Festival	60·3	48·7	54·4	44·5	45·0	44·7
Gala	33·3	36·7	35·0	28·7	28·5	28·6
Hopps	51·3	49·7	50·5	39·7	36·0	37·9
Kenora	49·3	53·3	51·3	40·5	41·0	40·7
Puora	13·3	13·0	13·2	21·5	12·2	16·9
K.G.P.F.	..	..	..	37·0	25·0	31·0
Mean	42·6	41·1	41·9	34·2	30·4	32·3
Necessary differences for significance	Individual means 1% 9·7 5% 7·0 Treatment means 1% 2·5 5% 3·4		1% 8·3 5% 6·0	Individual means 1% 9·6 5% 7·1 Treatment means 1% 3·0 5% 2·2		1% 6·3 5% 4·7

Laboratory germination.—A highly significant reduction in germination on laboratory trays was obtained following inoculation of Lawrence, Gala and Puora (Table 2). These three varieties with K.G.P.F. also showed the lowest overall germination after 3 days. The same varieties showed a much greater colonization of the seed by *F. graminearum* in the germination trays, thus clearly demonstrating the effect of seed quality on subsequent fungal development. Some varieties—Spica, Puseas, Festival and Kenora—showed a significant increase in germination due to inoculation after 3 days but these differences had largely disappeared after 7 days.

TABLE 2

GERMINATION AFTER 3 AND 7 DAYS AT 27·5°C ON TRAYS AND INFECTION WITH *F. graminearum* ON BOTH GERMINATED AND UNGERMINATED SEED

Variety	Germination After 3 Days (%)			Germination After 7 Days (%)			Mean Percentage Seeds Showing Obvious *F. graminearum* Infection					
							Germinated		Failed to Germinate		Total	
	Control	Inoculated	Mean	Control	Inoculated	Mean	Control	Inoculated	Control	Inoculated	Control	Inoculated
Mengavi	93·0	93·7	93·4	95·5	96·2	95·9	0·25	4·75	0·0	4·0	0·25	8·75
Spica	80·7	91·0	85·9	92·0	95·5	93·7	1·0	4·5	0·0	5·5	1·00	10·00
Lawrence	56·7	52·5	54·6	74·0	56·5	65·2	0·0	12·5	0·5	44·0	0·50	56·50
Puseas	81·0	90·0	85·5	85·5	92·0	88·7	0·0	5·0	0·0	5·75	0·00	10·75
Festival	88·5	95·5	92·0	93·2	98·0	95·6	0·0	3·0	0·0	1·5	0·00	4·50
Gala	61·2	45·5	53·4	88·7	63·0	75·9	0·0	10·25	0·0	20·25	0·00	30·50
Hopps	90·5	93·7	92·1	93·0	96·5	94·7	0·0	6·5	0·75	3·5	0·75	10·00
Kenora	82·2	91·5	86·9	86·2	93·7	90·0	0·25	4·5	0·5	5·0	0·75	9·50
Puora	43·0	33·2	38·1	50·0	38·2	44·1	0·0	16·75	3·0	58·5	3·00	75·25
K.G.P.F.	63·5	64·0	63·7	70·0	70·0	70·0	0·0	10·75	0·0	26·25	0·00	37·00
Mean	74·0	75·1	74·6	82·8	80·0	81·4	0·15	7·85	0·48	17·43	0·63	25·28
Necessary differences for significance	Individual means 1% 8·9 5% 6·7 Treatment means 1% 2·8 5% 2·1		1% 6·3 5% 4·7	Individual means 1% 9·7 5% 7·3 Treatment means 1% 3·1 5% 2·3		1% 6·8 5% 5·1						

TABLE 3

MEAN PERCENTAGE OF INFECTED TISSUES AS INDICATED BY ISOLATIONS OF *F. graminearum* (Millmerran)

Time After Sowing (days)	Treatment	No. of Plants Used in Determination	Roots‡			Sub-crown Tissue			Crown	
			†(a) Seminal	†(a) Secondary	*(b)	Scutellum Remains	Coleoptile‖	Sub-crown Internode§	Leaf Sheath Bases§	Crown or Tillers
38	Control	258	0	0	0	3·2	16·7 (18)	6·3	0·4	0·4
	Inoculated	267	12·0	1·1	1·5	55·9	51·2 (43)	59·4	6·7	9·0
67	Control	164	..	0	2·4	3·0	0 (27)	7·5	2·4	1·2
	Inoculated	180	..	2·2	9·4	38·8	7·4 (27)	31·0	13·6	12·8
100	Control	180	..	¶	3·3	12·9	8·3 (12)	19·7	3·2	20·0
	Inoculated	178	..	..	4·5	39·1	25·0 (8)	46·0	5·9	58·4
128	Control	177	..	..	¶	..	..	..	7·8	36·1
	Inoculated	89	..	..	..	..	..	..	16·0	56·2
163	Control	405	..	..	..	..	..	..	..	47·4
	Inoculated	405	..	..	..	..	..	..	..	63·0

‡ Expressed as % plants infected

†(a) Rot originating from base of rhizome in seminal roots or crown in secondary roots

*(b) Discrete root lesions

¶ Crown breakdown had commenced

‖ Isolations made only from discolored coleoptiles. No. used is indicated in brackets

§ Plants with severe crown infections not included

Following inoculation, all varieties except Mengavi (80%), Puseas (90%), and Hopps (95%) showed 100% infection of the grain with the fungus on isolation plates. Hopps showed a 10% infection of the control seed. This fungus is, of course, a well-known seed-borne parasite (Orton 1931) and has been encountered frequently in routine seedling tests in this laboratory.

Plant symptoms and isolation data.—In the examination of plant samples taken at regular intervals during the trials, symptoms were noted on many tissues from which *F. graminearum* was subsequently isolated. On the roots, two distinct types of infection were apparent. The most common was evident on the root tissue immediately adjacent to the sub-crown internode in the case of seminal roots and the crown in the case of secondary roots. It was characterized by a brown discoloration. The other lesions occurred as discrete entities, on both the seminal and the secondary roots, and were comparatively rare. This is in keeping with experience with *Fusarium* spp. and *Helminthosporium sativum* (Simmonds 1941). Another feature of the roots was their complete collapse in plants with severely affected crowns. All the evidence pointed here to infection coming back into the roots from the crown. Results of isolations from the roots, as with other tissues, are summarized in Table 3.

TABLE 4

PROGRESSIVE PERCENTAGE INFECTION OF VARIETIES WITH *F. graminearum* AS DETERMINED BY ISOLATION† (Millmerran)

Variety and Treatment	38 Days (After Sowing)	67 Days	100 Days	128 Days	163 Days (Maturity)‡
Mengavi	0*	5	25	30	34·3
Mengavi, Inoculated	57	55	75	30	57·3
Spica	0	15	30	62	44·3
Spica, Inoculated	83	60	75	80	72·0
Lawrence	0	30	10	35	56·7
Lawrence, Inoculated	..	25	45	30	60·0
Puseas	3	0	40	45	72·0
Puseas, Inoculated	63	75	75	80	84·7
Festival	4	11	30	35	43·3
Festival, Inoculated	70	55	85	80	67·0
Gala	11	0	25	25	17·7
Gala, Inoculated	63	50	80	40	32·3
Hopps	0	0	50	35	38·7
Hopps, Inoculated	66	75	65	80	44·3
Kenora	3	0	5	40	50·0
Kenora, Inoculated	60	75	85	90	70·0
Puora	4	16	30	88	70·0
Puora, Inoculated	83	60	75	67	79·0
Control (Av.)	2·8	8·6	27·2	43·9	47·4
Inoculated (Av.)	68·1	58·9	73·3	64·1	63·0

* Each figure is the mean of 2 or 3 determinations

† A plant was counted as infected if *F. graminearum* was isolated from any of the tissues

‡ Maturity isolations made from crown and bottom internode above the crown only

The sub-crown tissue commonly showed infection. This was evident as a brown discoloration of the scutellum tissue, the coleoptile and sub-crown internode tissue. The coleoptile was frequently badly discolored, with no obvious decay of the stem tissue beneath. It often was almost completely disintegrated. In many cases decay had appeared to reach the leaf bases of the developing tillers by way of this coleoptile tissue. Invasion of the tillers often appeared to proceed inwards from the leaf bases. In some cases the second internode above the crown showed discoloration before the first internode and this appeared to be the result of invasion of the former from an infected leaf base. Infected tillers were at first greenish brown in appearance but subsequently became dark brown. Occasionally there was a reddish discoloration of this tissue. Internally the affected internodes were generally filled with carmine to buff-coloured mycelium typical of *F. graminearum*.

From the summarized results in Table 3 it is evident that the roots have not played a very important role as a site for primary infection. This would appear to follow the results obtained by Colhoun and Park (1964). The sub-crown tissue and leaf bases were frequent sites of primary infection. The soft leafy tissues appear more prone to attack and when affected become a potent source of infection for the stem tissues below. As might be expected in inoculated seed, the scutellum tissue was a frequent site of primary infection, but it was not without significance in plants infected naturally from the soil.

So far as field symptoms are concerned, little real evidence of the presence of disease was evident in most plants until rather late in the life of the crop. Occasionally plants showed seedling blight or severe yellowing and even death prior to heading, but these were of no significance in the overall disease picture. This low incidence of seedling blight may be related to the low soil temperature experienced during the early life of the crop.

Crown discoloration became clearly evident generally just prior to heading time and premature ripening of affected plants was a frequent symptom.

Progressive infection.—Tables 4 and 5 give a summary of the isolations made from different varieties at the various sampling times. While there is considerable variation in the figures obtained, the trends for infection throughout the season are clearly evident. At Millmerran, in the control plots infection rose from 2·8% at 30 days to 43·9% at 128 days. The biggest rise, 18·6%, occurred between the 67-day and 100-day periods. At Irvingdale the level of infection early was much higher than at Millmerran. Infection was progressive throughout the life of the plant and was not restricted to any particular stage of development. There appeared to be no obvious relationship between moisture stress and infection. Indeed, the plots at Irvingdale experienced no lack of moisture throughout their life.

So far as individual varieties are concerned, the results in Table 4 and 5 show many discrepancies. However, it is clear that varieties such as Gala which show out best for disease reaction at maturity do not show lower levels of

TABLE 5

PROGRESSIVE PERCENTAGE INFECTION OF VARIETIES WITH *F. graminearum as* DETERMINED BY ISOLATION† (Irvingdale)

Variety and Treatment	57 Days (After sowing)	85 Days	142 Days (Maturity)‡
Mengavi	42*	30	45
Mengavi, Inoculated ..	84	90	68
Spica	30	40	68
Spica, Inoculated ..	79	80	59
Lawrence	25	60	58
Lawrence, Inoculated ..	82	50	66
Puseas	20	30	41
Puseas, Inoculated ..	85	90	47
Festival	11	70	48
Festival, Inoculated ..	63	70	65
Gala	35	40	66
Gala, Inoculated ..	60	80	72
Hopps	15	50	55
Hopps, Inoculated ..	85	60	20
Kenora	25	40	34
Kenora, Inoculated ..	80	70	22
Puora	15	30	33
Puora, Inoculated ..	89	60	42
K.G.P.F.	28	60	56
K.G.P.F., Inoculated ..	85	90	55
Control (Av.)	24·6	45·0	52·4
Inoculated (Av.) ..	79·2	74·0	51·6

* Each figure is the mean of 2 or 3 determinations

† A plant was counted as infected if *F. graminearum* was isolated from any of the tissues

‡ Crown and bottom internode above the crown only used for isolation at maturity.In many cases this tissue was badly decayed and may account for the poor isolation figures obtained

infection prior to maturity. Inoculated seed of all varieties has naturally shown a much higher early infection. However, the levels of infection at maturity are not as high in relation to the uninoculated as might be expected.

Field and laboratory ratings.—The field ratings made at approximate heading time (Table 6) show the difficulty of rating for crown discoloration where maturity differences are apparent. However, what proved to be the four most susceptible varieties—K.G.P.F., Puora, Puseas and Spica—showed the highest ratings, indicating that the disease was much more advanced in these varieties compared with other varieties of similar maturity, viz., Mengavi and Kenora. Inoculated plots again show a much greater overall development of crown symptoms at this stage. The laboratory ratings (Table 6) made from samples collected at the same time show that, while the disease may not be evident in the field, the tillers may still be carrying a high level of infection.

TABLE 6

COMPARISON OF FIELD RATINGS AND LABORATORY RATINGS MADE AT APPROXIMATE HEADING TIME (Irvingdale)

Variety	Stage of Maturity	Mean Percentage of Plants Showing Obvious Base Symptoms in Field			Mean Percentage of Plants Showing Symptoms of Disease in Laboratory Examination					
					Severe Disease			Total Disease		
		Control	Inoculated	Mean	Control	Inoculated	Mean	Control	Inoculated	Mean
Mengavi	Heading	4·33	18·67	11·50	23·10	63·57	43·33	35·53	80·47	58·00
Spica	Heading	9·67	31·33	20·50	22·17	56·90	39·53	45·50	79·57	62·53
Lawrence	Rosette	3·17	7·00	5·08	19·10	56·00	37·55	33·33	73·33	53·33
Puseas	Heading	14·83	37·50	26·17	22·23	72·57	47·40	32·47	79·23	55·85
Festival	Heading commencing	6·17	10·33	8·25	16·90	59·57	38·23	36·90	72·47	54·68
Gala	Heading commencing	3·67	12·00	7·83	12·00	22·67	17·33	24·43	54·23	39·33
Hopps	Heading commencing	6·17	13·33	9·75	10·23	62·23	36·23	32·00	80·00	56·00
Kenora	Heading	8·67	22·33	15·50	24·00	51·10	37·55	45·77	79·10	62·43
Puora	Heading	10·33	36·33	23·33	24·43	72·00	48·22	53·77	87·10	70·43
K.G.P.F.	Heading	17·67	35·17	26·42	17·33	57·77	37·55	39·57	83·53	61·55
Total mean		8·47	22·40	15·43	19·15	57·44	38·30	37·93	76·90	57·42
Necessary differences for significance		Individual means 1% 10·21 5% 7·48 Treatment means (Inoc. & control) 1% 3·23 5% 2·37		1% 8·05 5% 5·88	Individual means 1% 31·76 5% 23·28 Treatment means 1% 10·04 5% 7·36		1% 18·55 5% 13·54	Individual means 1% 30·74 5% 22·54 Treatment means 1% 9·72 5% 7·13		1% 21·29 5% 15·54

Variability here is high, as indicated by the large difference necessary for significance. The total disease figures, i.e. slight plus severe infections, vary little between varieties. The only significant differences are between Gala and all varieties except Lawrence and Festival at the 5% level of probability. The numbers of severe infections show more marked differences. Here Gala is highly significantly less than all other varieties. Inoculation again produced a highly increased expression of symptoms, significant at the 1% level in all varieties except Gala for both severe and total infection. Even in Gala the difference in total crown symptoms was significant at the 5% level. At this stage there was a pronounced brown discoloration of the leaf bases at and below ground level. This rot frequently extended into the crowns and tillers.

In the production of deadheads (Table 7), there are clear differences between varieties at both sites, with Gala being consistently lower at a significant level from all the other varieties except Mengavi. There is a highly significant increase in deadheads due to inoculation at both sites but the pattern between varieties remains similar. Variability is high and the figures for Lawrence have been excluded because of its much later maturity. Generally it was easy to distinguish by feel whether or not heads were empty. No attempt was made to classify partly filled heads as deadheads.

TABLE 7

MEAN PERCENTAGE OF DEADHEADS JUST PRIOR TO MATURITY

Variety	Millmerran			Irvingdale		
	Control	Inoculated	Mean	Control	Inoculated	Mean
Gala	3·07	5·27	4·17	6·2	6·0	6·1
Mengavi	5·00	15·93	10·47	11·0	13·2	12·1
Festival	12·87	18·00	15·43	12·2	27·0	19·6
Kenora	14·73	25·00	19·87	23·5	36·7	30·1
Hopps	14·33	26·53	20·43	23·5	38·0	30·7
Spica	14·67	29·40	22·03	13·7	32·7	23·2
K.G.P.F.	..	..	..	35·0	37·2	31·1
Puora	26·00	31·93	28·97	29·2	41·2	35·2
Puseas	34·33	53·07	43·70	28·0	52·0	40·0
Mean	15·62	25·64	20·63	20·6	33·1	26·9
Necessary differences for significance	Individual means 1% 14·17 5% 10·28 Treatment means 1% 5·01 5% 3·64		1% 6·87 5% 4·95	Individual means 1% 15·3 5% 11·4 Treatment means 1% 4·8 5% 3·6		1% 13·8 5% 10·2

Number of tillers.—No relationship between disease incidence and the number of tillers produced could be established.

Tiller discoloration.—Examination of the tillers at maturity for obvious discoloration beyond the second node above the crown revealed an abundance of the brown discoloration characteristic of infection with *F. graminearum* (Table 8). Sometimes the typical reddish-buff mycelium was found in great abundance in the lumen. A great deal of variation was found in the percentage of tillers showing this discoloration; nevertheless, Gala shows the least infection. It is in fact highly significantly less than all other varieties at Irvingdale and all varieties except Festival and Mengavi at Millmerran. Mengavi in this respect is not nearly as free of discoloration as Gala but is still better than other varieties. The overall effect of inoculation is to increase the amount of discoloration at both sites at the 1% level of probability.

TABLE 8

MEAN PERCENTAGE TILLERS DISCOLORED BEYOND THE SECOND NODE AT MATURITY

Variety	Millmerran			Irvingdale		
	Control	Inoculated	Mean	Control	Inoculated	Mean
Mengavi	20·90	18·40	19·65	33·20	57·65	45·52
Spica	38·90	53·33	46·12	50·50	61·97	56·24
Lawrence	..	..	..	40·77	55·05	47·91
Puseas	73·07	80·90	76·98	59·82	80·82	70·32
Festival	26·20	42·87	34·53	65·80	76·17	70·99
Gala	7·67	9·87	8·77	24·32	36·67	30·50
Hopps	27·30	44·67	35·98	47·70	63·02	55·36
Kenora	37·97	59·27	48·62	64·60	73·12	68·86
Puora	53·23	57·40	55·32	65·35	78·77	72·06
K.G.P.F.	..	..	..	74·40	85·10	79·75
Mean	35·65	45·84	40·74	52·65	66·84	59·75
Necessary differences for significance	Individual means			Individual means		
	1% 23·10		1% 27·13	1% 20·96		1% 14·03
	5% 16·77		5% 19·55	5% 15·57		5% 10·39
	Treatment means			Treatment means		
	1% 8·17			1% 6·63		
	5% 5·93			5% 4·92		

Movement of infection up the tillers.—The results of isolations from the various internodes above the crown at maturity are rather similar for both sites; the Irvingdale figures appear in Table 9. The fungus occasionally progresses upwards as far as the internode below the head but is commonly restricted to the first three internodes above the crown. There seems to be little difference between varieties in this regard, very susceptible varieties such as Puseas and K.G.P.F. reacting in similar fashion to tolerant ones such as Gala. It is conceivable, however, that assessments prior to maturity could indicate differences, but this is conjecture.

TABLE 9

TOTAL NUMBERS OF ISOLATES OF *F. graminearum* FROM 50 TILLERS OF EACH VARIETY AT MATURITY (Irvingdale)

Variety	Internode Above Crown					
	1	2	3	4	5	6
Mengavi	9	10	8	3	3	0
Mengavi, inoculated	17	19	23	6	3	1
Mengavi, total	26	29	31	9	6	1
Spica	16	15	9	4	0	0
Spica, inoculated	32	34	26	11	6	0
Spica, total	48	49	35	15	6	0
Lawrence	11	13	17	5	1	..
Lawrence, inoculated	26	26	19	5	5	..
Lawrence, total	37	39	36	10	6	..
Puseas	13	21	10	0	0	0
Puseas, inoculated	19	29	19	3	0	0
Puseas, total	32	50	29	3	0	0
Festival	19	12	6	2	1	0
Festival, inoculated	14	13	13	2	0	0
Festival, total	33	25	19	4	1	0
Gala	16	19	10	4	6	0
Gala, inoculated	28	22	16	5	0	0
Gala, total	44	41	26	9	6	0
Hopps	15	15	5	0	0	..
Hopps, inoculated	16	24	9	2	0	..
Hopps, total	31	39	14	2	0	..
Kenora	8	8	7	4	0	..
Kenora, inoculated	9	12	7	2	0	..
Kenora, total	17	20	14	6	0	..
Puora	9	16	14	5	2	0
Puora, inoculated	19	17	20	10	4	0
Puora, total	28	33	34	15	6	0
K.G.P.F.	22	25	8	6	8	..
K.G.P.F., inoculated	18	21	19	7	3	..
K.G.P.F., total	40	46	27	13	11	..
Control, total	128	154	94	33	21	..
Inoculated, total	198	217	171	53	21	1
Total	326	371	265	86	42	1

In Table 10 a summary has been made of isolations made from tillers carrying healthy heads, partly filled heads and deadheads. Tillers with ears classified as healthy have a remarkably high percentage of infection in the third internode. There is, however, a greater level of infection in the 3rd and 4th internodes in stems carrying deadheads. Inoculation has produced a greater overall infection but there is not a marked increase in progression up the stem (Table 10), although some increase is evident. The lower number of isolates from the first internode as compared with the second is probably a reflection of the advanced stage of disease. The badly decayed lower internodes did not offer good material for isolation. The possibility of some infection originating in the second internode

through leaf base infection as previously described cannot, however, be overlooked. The ready isolation of *F. graminearum* from the internodes of affected wheat plants contrasts with the experience of Colhoun and Park (1964). These authors, working with *F. culmorum* and *F. nivale* (Fr.) Ces., made successful isolations from the nodes only.

TABLE 10

PERCENTAGE INFECTION RECORDED BY ISOLATION IN VARIOUS INTERNODES OF STEMS CLASSIFIED INTO THREE GROUPS ACCORDING TO CONDITION OF HEADS (Irvingdale)

Condition of Head	No. of Heads	Internode Above the Crown						Percentage of Tillers Infected
		1	2	3	4	5	6	
Healthy	545	36·1	35·0	23·3	7·5	3·1	0	54·5
Partly filled	278	30·6	37·0	24·1	6·1	5·4	0·4	59·4
Deadheads	157	31·8	40·1	42·7	15·9	3·2	0	70·7
Total	980	33·8	36·4	26·6	8·5	3·8	0·1	58·5

Yield.—While the overall grain yield figures (Table 11) reflect differences other than susceptibility to crown rot, the varieties showing the highest number of deadheads, Puora and Puseas, showed the overall lowest yield. Inoculation has caused a highly significant reduction in yield at both sites (Figures 1 and 2). At Millmerran the difference in yield between inoculated and control is highly

TABLE 11

MEAN YIELD (bus/ac) AT 12% MOISTURE

Variety	Millmerran			Irvingdale		
	Control	Inoculated	Mean	Control	Inoculated	Mean
Mengavi	35·23	32·10	33·67	36·67	30·42	33·95
Spica	29·77	23·60	26·68	35·27	20·77	28·02
Lawrence	..	..	..	15·32	10·35	12·84
Puseas	24·47	16·10	20·28	23·35	14·82	19·09
Festival	28·17	27·30	27·73	27·62	18·32	22·97
Gala	27·27	30·17	28·72	31·42	24·72	28·07
Hopps	28·93	18·47	23·70	25·27	13·05	19·16
Kenora	31·00	22·03	26·52	29·15	20·27	24·71
Puora	19·87	14·43	17·15	19·92	8·50	14·21
K.G.P.F.	..	..	..	26·80	13·45	20·12
Mean	28·09	23·02	25·56	27·08	17·47	22·30
Necessary differences for significance	Individual means			Individual means		
	1% 8·35		1% 4·04	1% 6·00		1% 5·18
	5% 6·06		5% 2·91	5% 4·60		5% 3·84
	Treatment means			Treatment means		
	1% 2·95			1% 1·96		
	5% 2·14			5% 1·46		

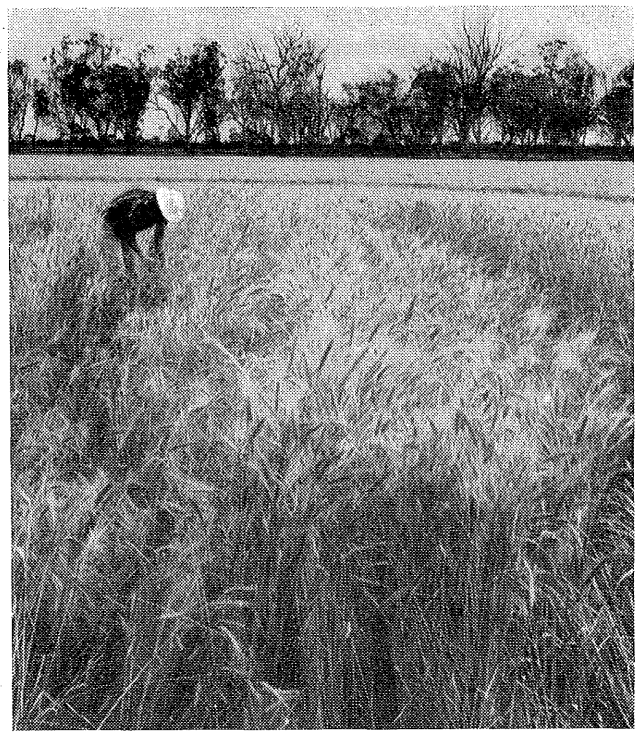

Fig. 1.—Effect of seed inoculation of the variety Hopps. Inoculated plot on the left; control plot on the right.

Fig.2.—Close-up of K.G.P.F. plots. Control plot on the left; inoculated plot on the right.

significant in Puseas, Hopps and Kenora. At Irvingdale the differences are highly significant in all varieties except Lawrence, which can again be discounted because of its late maturity. Field reductions in yield due to seed inoculation, in addition to soil inoculation, have been reported by Johnston and Greaney (1942) with species of *Fusarium*.

Grain weight.—The mean weight of seed samples from each variety show marked differences between varieties (Table 12). Moreover, there are highly significant differences between inoculated and control in all varieties except Mengavi. Hopps was excluded from this test because of the severe weevil infestation occurring in the samples prior to the test being carried out. The overall reduction in individual grain weight associated with inoculation demonstrates the insidious effect this disease can have on yield. The tolerant variety Gala is adversely affected in this manner.

TABLE 12

DRY WEIGHT OF SEED SAMPLES FROM INDIVIDUAL VARIETIES (Irvingdale)

Variety	*Mean Weight (g)		
	Control	Inoculated	Mean
Mengavi	15·690	15·702	15·696
Spica	18·817	17·980	18·398
Lawrence	13·954	12·153	13·053
Puseas	16·571	15·788	16·180
Festival	13·365	12·593	12·979
Gala	15·167	14·384	14·776
Kenora	14·103	13·570	13·837
Puora	13·239	12·846	13·042
K.G.P.F.	17·260	15·962	16·611
Mean	15·352	14·553	14·952
Necessary differences for significance ..	Individual Means		
	1% 0·301		1% 0·213
	5% 0·228		5% 0·161
	Treatment means		
	1% 0·100		
	5% 0·076		

* Each sample was of 500 seeds and each figure is the mean of 10 samples.

Maturity.—Observations made during the season indicate that in some varieties there was a marked delay in maturity associated with inoculation. This was particularly evident in K.G.P.F. and Hopps. The differences in moisture content recorded in grain samples collected at harvest time reflect best this difference in maturity. Thus in Hopps at Irvingdale control samples had a moisture content of 19·5% while the inoculated sample had 25·3%. Gala control contained 14·7% moisture while Gala inoculated had 16·4%.

TABLE 13

MEAN PERCENTAGE EMERGENCE AND MEAN PERCENTAGE SEVERE SEEDLING BLIGHT IN THREE DIFFERENT SEEDLING TEST METHODS WITH *F. graminearum*

Variety	Tube Tests				Seed Inoculation/Sand Tests					Cornmeal Sand Inoculum/Sand Tests				
	Emergence		Severe Seedling Blight		Emergence		Severe Seedling Blight			Emergence		Severe Seedling Blight		
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated		Control	Inoculated	Control	Inoculated	
								Mean*	Equiv. Mean				Mean*	Equiv. Mean
Mengavi	90·0	85	13·9	85·3	88	87	0·0	52·9	0·818	96	85	0·0	44·2	0·726
Spica	97·5	70	12·8	96·6	99	91	2·0	31·1	0·585	94	95	3·4	38·4	0·661
Lawrence	72·5	22·5	3·4	66·7	44	19	8·7	61·5	0·852	30	22	0·0	57·7	0·893
Puseas	82·5	60·0	3·0	75·0	83	76	1·5	49·0	0·773	83	74	0·0	59·8	0·887
Festival	92·5	82·5	0·0	75·8	93	83	1·1	47·1	0·752	95	89	1·1	27·6	0·540
Gala	97·5	57·5	2·6	65·2	70	57	2·9	55·8	0·844	71	62	1·2	38·9	0·662
Hopps	92·5	80·0	0·0	56·3	86	83	0·0	38·3	0·666	95	92	4·3	39·7	0·675
Kenora	90·0	77·5	0·0	21·9†	90	75	4·3	59·1	0·882	85	75	0·0	34·0	0·606
Puora	87·5	35·9	0·0	64·3	51	26	0·0	65·5	1·014	57	29	0·0	31·9	0·580
K.G.P.F.	82·5	50·0	2·9	90·0	79	64	1·6	64·0	0·930	72	59	0·0	52·0	0·805
Mean	88·5	62·1	3·9	69·7	78·3	66·1	2·2	52·4		77·8	68·2	1·0	42·4	
Necessary differences for significance					Individual means 1% 17·4 5% 13·1 Treatment means 1% 5·5 5% 4·1		Not analysed		1% 0·583 5% 0·432	Individual means 1% 16·6 5% 12·4 Treatment means 1% 5·2 5% 3·9		Not analysed		1% 0·348 5% 0·258

† Kenora obviously less than all others

* Inverse sine transformation used

TABLE 14

ROOT ROT INDEX AND LENGTH OF LONGEST LEAF IN TWO METHODS OF SEEDLING TESTING

Variety	Seed Inoculation/Pot Tests				Cornmeal Sand Inoculum/Pot Tests			
	Root Rot Index		Length of Longest Leaf		Root Rot Index		Length of Longest Leaf	
	Control	Inoculated	Control	Inoculated	Control	Inoculated	Control	Inoculated
Mengavi	1·7	62·8	28·4	20·2	5·3	18·8	39·4	32·9
Spica	1·0	71·7	33·9	27·9	3·5	54·1	39·4	38·7
Lawrence	16·5	86·0	13·2	13·9	0·0	68·8	33·3	22·8
Puseas	7·3	68·2	31·4	20·5	7·6	49·7	39·9	34·5
Festival	2·1	83·1	34·0	25·5	2·5	54·6	41·5	40·7
Gala	5·0	62·4	23·3	15·5	4·2	22·8	36·5	30·6
Hopps	1·1	70·6	30·9	28·0	2·1	42·9	38·6	37·1
Kenora	4·4	71·0	28·5	23·4	0·0	57·9	37·6	35·8
Puora	0·0	44·7	26·7	13·3	1·4	67·8	35·3	26·8
K.G.P.F.	3·2	67·2	30·3	14·2	3·1	69·0	39·9	32·2
Mean	4·2	68·8	28·04	20·23	3·0	50·6	38·13	33·19
Necessary differences for significance	1% 15·15 5% 11·22	1% 20·71 5% 15·33	Individual means 1% 5·21 5% 3·91 Treatment means 1% 1·65 5% 1·24		1% 7·95 5% 5·89	1% 24·86 5% 18·41	Individual means 1% 6·00 5% 4·51 Treatment means 1% 1·90 5% 1·43	

Seedling tests.—In the seedling tests, results of which have been summarized in Tables 13 and 14, emergence was reduced by inoculation in most varieties. There was an overall reduction in emergence at the 1% level of probability in all three methods used. There appears to be variability between varieties, with generally those of overall lower germinability being most affected by inoculation. All varieties, including Gala and Mengavi, which have the highest field tolerance, are very susceptible to seedling blight. Varieties such as Spica, Hopps and Kenora in some of the tests show a comparatively low figure yet they were very susceptible in the field. There is no doubt that seed quality plays an important role in these seedling tests (Simmonds and Sallans 1946). This makes comparisons between varieties very difficult unless great care is exercised in seed selection. This would not always be possible in large-scale testing work.

The overall effect of inoculation was to reduce significantly the length of the longest seedling leaf. This is in keeping with results obtained by Ludwig *et al.* (1956) with *Helminthosporium sativum*. However, every variety was not affected to the same extent. For instance, Hopps showed little effect in this regard. Again there is apparently no connection between field tolerance to crown rot and effect of inoculation on leaf length in seedling tests. The root rot index gives no indication of any difference between varieties in the seed inoculation method. In the cornmeal/sand inoculum method, however, both Gala and Mengavi have the lowest figure and these are significantly less than all the other varieties except Hopps at the 1% level of probability and Hopps at the 5% level. These differences have been previously noted in other tests conducted in the glasshouse. In some tests, however, the differences were not nearly as pronounced and would appear to be affected by such critical factors as temperature and time of sampling.

General conclusions.—Wheat seed inoculation with a spore suspension of *F. graminearum* followed by rapid drying is a satisfactory method of inducing infection to crown rot.

A comparison between plots sown with inoculated and ordinary seed offers a useful method of determining the reaction of any variety. In this way different levels of disease incidence can be achieved, and hence a direct comparison obtained of the effect of the disease.

In assessing field reaction to crown rot, the production of deadheads, crown symptoms prior to maturity and yield should all be given consideration.

Seedling blight reaction in the glasshouse gives no guide to field tolerance. It could be of value if true resistance was found.

Gala, and to a lesser extent Mengavi, may be described as field tolerant. This tolerance does not appear to be related to the amount of infection occurring but rather to the rate of development of the disease in plants.

In these experiments, no high level of resistance was encountered. The differences in field tolerance were, however, great enough to encourage further search for better varieties and better testing techniques.

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