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# SEED INOCULATION OF WHEAT AND BARLEY WITH AZOTOBACTER CHROOCOCCUM IN QUEENSLAND

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## SUMMARY

**The effectiveness of seed inoculation with *Azotobacter chroococcum* for increasing the grain yield of wheat and barley was assessed in glasshouse and field trials on the Darling Downs, Queensland. Inoculation significantly increased both the grain yield and straw yield of wheat grown in the glasshouse. There was a significant interaction for grain yield between inoculation and wheat cultivar, and the yield component most influenced by inoculation was the number of grains per ear.**

**Under field conditions, artificial inoculation increased the populations of *A. chroococcum* in the rhizosphere of wheat by no more than several thousand cells per root system even when heavily inoculated seed ($10^6$ cells/seed) was sown in soil favourable for this micro-organism. The most effective *A. chroococcum* inoculum significantly ($P < 0{\cdot}05$) increased grain yield above sterile-control inoculation three times and above untreated seed only once in 29 comparisons of inoculation treatments applied to different cultivars of wheat and barley grown at different sites. The mean percentage yield response of any cultivar to inoculation was slight (<5%).**

## I. INTRODUCTION

Seed inoculation of non-leguminous crops with bacteria has been practised on farms in the U.S.S.R. since the 1930's, the annual production of inoculum amounting to 25 million hectare portions (Mishustin and Naumova 1962). Careful assessments of research and on-farm results (Cooper 1959; Mishustin and Naumova 1962) showed that the most effective inoculant, *Azotobacter chroococcum*, increased the yields of field crops by ca 10% and of cereals sown in chernozem soils by ca 15–20%. Although this microorganism was originally used because it fixes nitrogen, inoculation did not improve the nitrogen nutrition of the plants and several other modes of action, each with some experimental evidence, were proposed.

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Subsequent research in England (Brown, Burlingham and Jackson, 1964) and South Australia (Ridge and Rovira 1968) generally supported these assessments and showed that very high percentage yield increases can sometimes be obtained. Brown, Burlingham and Jackson (1962*b*, 1964) found that the seed inoculum colonized the rhizosphere most effectively in soils with naturally occurring *A. chroococcum* and that good colonization was necessary for plant responses. Since the black earths and grey clays used for cereal culture in Queensland support relatively high populations of *A. chroococcum*, the potential use of this organism for seed inoculation was investigated.

## II. MATERIALS AND METHODS

*Bacteria.*—Three wheat cultivars of diverse parentage (Macindoe and Walkden Brown 1968) were grown in a number of black earth and grey clay soils from the Darling Downs. *Azotobacter* species were isolated from the wheat rhizoplanes by enrichment in Jensen's (1955) media, and strains producing non-diffusible brown-black pigments were identified as *A. chroococcum*. The sources of and the isolation media for the strains used in this investigation are listed in Table 1.

**TABLE 1**

SOURCE OF *Azotobacter chroococcum* STRAINS

| Strain Numbers | Source | | Enrichment Medium |
|---|---|---|---|
| | *Soil Association | Wheat Cultivar | |
| 1, 2 | Mywybilla | Spica | Mannitol |
| 3 | Waco | Festival | Ethanol |
| 4 | Group 1 | Festival | Mannitol + benzoate |
| 5 | Anchorfield | Spica | Mannitol + benzoate |
| 6 | Group 1 | Gabo | Ethanol + benzoate |
| 7 | Group 2 | Gabo | Ethanol |

* Names refer to black earth associations (Beckmann and Thompson 1960); group numbers refer to grey clay associations (Isbell 1962).

*Seed inoculation.*—Inocula were either whole liquid cultures (1969 field trials) or aqueous suspensions of agar-grown cells (glasshouse and 1971 field trials). The respective control inocula were sterile medium with organic components omitted or sterile distilled water. All cultures were grown aerobically at 28°C of 7–14 days. Liquid cultures were grown in modified $M_9$ medium (Parker 1955) in progressively larger volumes (Lee and Burris 1943) with forced aeration of the 2*l* final generations. Agar cultures for field trials were grown as described by Ridge (1970), using replica-plating (Lederberg and Lederberg 1952) to inoculate the large numbers of plates required. Composite inocula were prepared by mixing equivolume cell suspensions of individual strains.

Inoculum was mixed with the seed in the ratio 1:6 (w/w), using a clean cement mixer for larger quantities. Inoculated seed for machine planting was dried back to original weight in a forced-draught dehydrator operating at ambient temperature. Seed was sown within 2 days of inoculation in 1969 but in 1971 it was stored in sealed polyethylene bags until required for sowing up to 1 month after inoculation.

*Assay methods.*—Viable populations of *A. chroococcum* were assayed by dilution-plating (Brown, Burlingham and Jackson, 1962*a*). For rhizosphere samples, randomly excavated plants collected from all plots were composited by field blocks to give two or three laboratory blocks each of 10 plants. The seed plus first 0·3 cm of roots and the remaining roots plus closely adhering soil (the rhizosphere) were assayed separately. Impressions of whole root systems on agar were used to qualitatively assess the distribution of *A. chroococcum* cells along the roots.

*Pot techniques.*—Five plants were grown per pot containing 1 kg soil. The pots were set out in Wisconsin tanks in a glasshouse and the soil temperature maintained at 24°C. During the growth of the plants the soil was frequently returned to pF2 with distilled water, and a total of 400 ml plant nutrient solution (Hoagland and Arnon 1938) per kg soil supplied in split applications.

*Field techniques.*—Plot sizes were 12 m x 4 rows in 1969 and 15 m x 9 rows in 1971. Plots were sown with a cone planter on 18 cm row spacings. Sowing rate was ca 40 kg/ha, corrections being made for 100-grain weight and for slight differences in moisture content between untreated and inoculated seed to achieve the same seed density per length of row. All phosphorus-deficient sites were fertilized with superphosphate. Grain was harvested with a plot combine-harvester (PAM model 150S).

*Experiments.*—All experiments were designed to test seed inoculation with *A. chroococcum* under different conditions of soil type and plant cultivar.

Experiment 1 was conducted in the glasshouse. Two inoculation treatments (control and *A. chroococcum* strain 1) were applied to two wheat cultivars (Gabo and Spica) grown in two soils (Mywybilla and Waco). The layout of this $2^3$ factorial was a randomized complete-block design with six replicates. The plant attributes determined were: height at early tillering, time from sowing to ear-peep, number of ears per plant, number of plants with crown rot caused by the fungus *Gibberella zeae,* weight of straw, weight of grain, number of grains per ear, and 100-grain weight.

Experiments 2–15 were conducted in the field during 1969 and 1971 at eight sites on the Darling Downs (details of sites are given in Table 5). Four seed inoculation treatments, namely untreated control, sterile-control inoculation, *A. chroococcum* strain 1, and *A. chroococcum* composite of strains 2–7 were tested in all experiments.

In experiments 2–6 conducted in 1969 the inoculation treatments were applied to two wheat cultivars (Gamut and Timgalen) and to one barley cultivar (Prior). Each of these experiments was a split plot design with the three cultivars in nine randomized blocks as whole units and the four inoculation treatments as subunits.

In each of experiments 7–15 conducted during 1971 the four inoculation treatments were tested on a single cultivar of either wheat or barley in a randomized complete-block design with eight replicates. Two experiments with wheat cultivars (Gamut and Timgalen or Gala) and one experiment with barley (cv. Clipper) were conducted on each site. On site H, which had a history of crown rot disease, the inoculation treatments were tested on the disease-susceptible cultivar Gamut (experiment 12) and on the disease-tolerant cultivar Gala (experiment 15).

In addition to the weight of grain harvested, the following plant attributes were determined in some of these experiments: shoot and root lengths at early tillering (experiments 2, 3, 6, 7, 8, 9); dry weight and number of tillers per plant at late tillering (experiments 2, 3); potential yields at anthesis, i.e. the number of spikelets per square metre (experiments 2, 3, 4); grain protein percentage (experiments 7–15); and 100-grain weight (experiments 7–15). Assays for *A. chroococcum* on the seed at sowing and in the rhizosphere at early tillering were made on cv. Gamut in experiments 2, 3, 6, 7, 8 and 9.

*Statistical analysis.*—An analysis of variance was done on the data for grain yield, and other plant attributes, from each experiment. Only when the F value for inoculation treatment was significant ($P<0\cdot05$) were specific inoculation treatments compared by L.S.D. (Steele and Torrey 1960, p. 106).

## III. RESULTS

*Experiment 1.*—Seed inoculation with *A. chroococcum* significantly ($P<0\cdot05$) increased the grain yield of wheat grown in the glasshouse (Table 2). There was a highly significant ($P<0\cdot01$) inoculation x cultivar interaction and the main effect of inoculation was entirely due to the substantial yield response (31%) of cv. Gabo. The cultivar Spica did not respond. Inoculation increased the yield of plants grown in Mywybilla soil proportionately more than in Waco soil but this inoculation x soil type interaction was statistically non-significant.

TABLE 2

EFFECTS OF INOCULATION WITH *Azotobacter chroococcum* ON MEAN GRAIN YIELD (g/POT OF FIVE PLANTS) IN RELATION TO WHEAT CULTIVAR AND SOIL TYPE

| Soil | Inoculation | Cultivar | | (Inoculation x Soil) Means |
|---|---|---|---|---|
| | | Gabo | Spica | |
| Mywybilla | Control | 4·59 | 6·50 | 5·54 |
| | *A. chroococcum* | 6·64 | 6·79 | 6·72 |
| Waco | Control | 5·14 | 7·15 | 6·14 |
| | *A. chroococcum* | 6·12 | 6·79 | 6·45 |
| | | | | Inoculation Means |
| (Inoculation x cultivar) means | Control | 4·86 | 6·82 | 5·84 |
| | *A. chroococcum* | 6·38 | 6·79 | 6·59 |
| Cultivar means | | 5·62 | 6·81 | |

| Necessary differences— | 5% | 1% |
|---|---|---|
| Inoculation means | 0·74 | NS |
| Cultivar means | 0·74 | 0·99 |
| (Inoculation x cultivar) means | 1·05 | 1·41 |

No other sig. diffs.

Analyses of some components of the grain yield of Gabo (Table 3) showed that inoculation significantly increased the number of grains per ear.

Inoculation also increased the weight of straw (Table 4) and although this effect was greater with Gabo than with Spica the inoculation x cultivar interaction was non-significant.

Neither the mean height of plants at tillering nor the mean time from sowing to ear-peep was significantly influenced by inoculation treatment. Approximately 5% of the plants in this experiment showed symptoms of crown rot but the incidence of this disease was not related to treatment.

**TABLE 3**

EFFECT OF INOCULATION WITH *Azotobacter chroococcum* ON GRAIN YIELD COMPONENTS OF GABO WHEAT

| Inoculation | Yield Components | | |
|---|---|---|---|
| | Ears/Plant | Grains/Ear | 100-Grain Weight |
| Control .. .. .. | 1·18 | 20·3 | 4·09 |
| Inoculated .. .. .. | 1·33 | 22·5 | 4·18 |
| Necessary differences 5% | N.S. | 1·5 | N.S. |
| 1% | .. | 2·1 | .. |

**TABLE 4**

EFFECTS OF INOCULATION WITH *Azotobacter chroococcum* ON MEAN STRAW YIELD (g/POT OF FIVE PLANTS) IN RELATION TO WHEAT CULTIVAR AND SOIL TYPE

| Soil | Inoculation | Cultivar | | (Inoculation x Soil) Means |
|---|---|---|---|---|
| | | Gabo | Spica | |
| Mywybilla .. .. .. | Control .. | 6·05 | 12·03 | 9·04 |
| | *A. chroococcum* | 8·51 | 12·10 | 10·31 |
| Waco .. .. .. .. | Control .. | 7·72 | 12·22 | 9·97 |
| | *A. chroococcum* | 8·58 | 13·38 | 10·98 |
| | | | | Inoculation Means |
| (Inoculation x cultivar) means .. | Control .. | 6·89 | 12·12 | 9·51 |
| | *A. chroococcum* | 8·54 | 12·74 | 10·64 |
| Cultivar means .. .. .. .. .. | | 7·71 | 12·43 | |

| Necessary differences— | 5% | 1% |
|---|---|---|
| Inoculation means .. .. .. .. | 1·06 | NS |
| Cultivar means .. .. .. .. | 1·06 | 1·42 |

No other sig. diffs.

**TABLE 5**

EFFECT OF SEED INOCULATION WITH *Azotobacter chroococcum* ON GRAIN YIELD (kg/ha) IN FIELD EXPERIMENTS

| Cultivar | Inoculation | †Site A | B | C | D | E | F | G | H |
|---|---|---|---|---|---|---|---|---|---|
| WHEAT— | | ‡Exp. 2 | Exp. 3 | Exp. 4 | Exp. 5 | Exp. 6 | Exp. 7 | Exp. 8 | Exp. 9 |
| Gamut .. .. | 1. Untreated .. .. | 1 129 | 583 | 433 | 863 | 1 531 | 1 720 | 1 478 | 3 102 |
| | 2. Control inoculation | 1 104 | 549 | 514 | 852 | 1 558 | 1 727 | 1 514 | 3 073 |
| | 3. *A. chroococcum* | 1 049 | 541 | 368 | 873 | 1 654 | 1 764 | 1 497 | 2 956 |
| | 4. *A. chroococcum* (composite) | 1 117 | 566 | 410 | 830 | 1 561 | 1 731 | 1 627 | 3 226 |
| | | | | | | | Exp. 10 | Exp. 11 | Gala Exp. 12 |
| Timgalen .. .. | 1. Untreated .. .. | 1 166 | 355 | 957 | 1 527 | 1 493 | 1 346 | 1 742 | 3 097 |
| | 2. Control unoculation | 1 145 | 329 | 1 329 | 1 407 | 1 445 | 1 295 | 1 721 | 3 162 |
| | 3. *A. chroococcum* | 1 181 | 344 | 1 283 | 1 308 | 1 557 | 1 431 | 1 730 | 3 020 |
| | 4. *A. chroococcum* (composite) | 1 148 | 415 | 1 249 | 1 419 | 1 485 | 1 397 | 1 738 | 2 919 |
| BARLEY— | | | | | | | Exp. 13 | Exp. 14 | Exp. 15 |
| cv. Prior .. .. | 1. Untreated .. .. | 2 519 | 896 | 3 152 | 4 099 | 2 443 | 2 905 | 2 161 | 2 867 |
| sites A–E .. .. | 2. Control inoculation | 2 585 | 835 | 3 286 | 3 981 | 2 486 | 2 967 | 2 322 | 2 884 |
| cv. Clipper sites F–H | 3. *A. chroococcum* | 2 661 | 873 | 3 234 | 4 024 | 2 470 | 3 034 | 2 329 | 2 747 |
| | 4. *A. chroococcum* (composite) | 2 529 | 990 | 3 198 | 3 963 | 2 489 | 2 950 | 2 400 | 2 960 |
| All cultivars .. .. | 1. Untreated .. .. | 1 605 | 611 | 1 514 | 2 163 | 1 823 | .. | .. | .. |
| | 2. Control inoculation | 1 611 | 571 | 1 710 | 2 080 | 1 830 | .. | .. | .. |
| | 3. *A. chroococcum* | 1 630 | 586 | 1 628 | 2 069 | 1 894 | .. | .. | .. |
| | 4. *A. chroococcum* (composite) | 1 598 | 657 | 1 619 | 2 071 | 1 845 | .. | .. | .. |
| Significant differences ($P<0{\cdot}05$) between inoculation treatments | | N.S. | All cvs. 4>2 | N.S. | N.S. | N.S. | Exp. 10 Timgalen 4, 3>2 | Exp. 14 Clipper 4≫1 4>2 3>1 | N.S. |

† *Sites:* A–E sown 1969; F–H sown 1971.
A. Norwin (Mywybilla black earth, pH 7·7)
B. St. Helens (Waco black earth, pH 8·8)
C. Brookstead (Anchorfield black earth, pH 7·9)
D. Brookstead (Cecilvale grey clay, pH 7·3)
E. Oakey (red-brown earth, pH 6·3)
F. St. Helens (Waco black earth, pH 8·1)
G. Oakey (red-brown earth, pH 5·6)
H. Millmerran (Mywybilla black earth, pH 7·2)

‡*Experiments:* Experiments 2–6 were (3 x 9) x 4 split-plot designs. Experiments 7–15 were 4 x 8 randomized complete-block designs.

*Experiments 2–15.*—The grain yields from the field experiments are given in Table 5. In these experiments, which allowed a total of 29 comparisons between the four inoculation treatments, a statistically significant effect due to inoculation was obtained in only three instances (experiments 2, 10 and 14). Inoculation with the composite of *A. chroococcum* strains significantly increased yield above that obtained with sterile-control inoculation in all three instances, and above that obtained with untreated seed once (experiment 14). Inoculation with *A. chroococcum* strain 1 increased yield above sterile control inoculation once (experiment 10) and above untreated seed once also (experiment 14).

The mean percentage yield responses of the cultivars to the inoculation treatments are given in Table 6. The composite inoculum of *A. chroococcum* strains was the most effective but this did not increase the yield of any cultivar by more than 5%.

**TABLE 6**

MEAN PERCENTAGE YIELD RESPONSE OF WHEAT AND BARLEY CULTIVARS TO SEED INOCULATION WITH *Azotobacter chroococcum* IN FIELD EXPERIMENTS

| Seed Inoculation | Wheat Cultivars | | Barley Cultivars | | Inoculation Means |
|---|---|---|---|---|---|
| | Gamut | Timgalen | Prior | Clipper | |
| 1. Untreated .. .. | 100·0 | 100·0 | 100·0 | 100·0 | 100·0 |
| 2. Control inoculation .. | 100·4 | 101·0 | 100·5 | 103·0 | 101·4 |
| 3. *A. chroococcum* .. | 98·7 | 102·9 | 101·1 | 102·2 | 101·4 |
| 4. *A. chroococcum* (composite) .. | 102·1 | 103·0 | 100·4 | 104·8 | 102·6 |

There was no statistically significant effect of inoculation on shoot and root lengths at early tillering, dry weight and number of tillers per plant at late tillering, potential yield at anthesis, grain protein percentage and 100-grain weight in any of the experiments where these attributes were determined. There was no readily apparent difference between inoculation treatments in the time from sowing to ear emergence. The percentage of plants with crown rot in experiment 9 was not related to inoculation treatment (a mean of 35% of plants had symptoms of crown rot).

The soil at all sites except the two on red-brown earth (sites E and G) contained naturally occurring populations of *A. chroococcum*. The number of *A. chroococcum* cells placed on the seed by artificial inoculation and the number present in the rhizosphere of Gamut at early tillering on six of the sites are given in Table 7. Inoculated seed at sowing carried higher numbers of viable *Azotobacter* cells in 1971 than in 1969 and in both years the composite inoculum (treatment 4) gave higher numbers than the single strain inoculum (treatment 3). The particularly high level of $10^6$ viable cells/seed was achieved with the composite inoculum in 1971. The populations of *A. chroococcum* in the rhizosphere were increased to a degree related to the numbers on the seed at sowing but maximum increases were only several thousand cells per root system. Root impressions showed that these cells were established along the full length of the roots.

**TABLE 7**

EFFECT OF SEED INOCULATION WITH *Azotobacter chroococcum* ON THE POPULATIONS OF THIS ORGANISM IN THE RHIZOSPHERE OF GAMUT WHEAT AT TILLERING (CELLS/ROOT SYSTEM)

| Soil | *Seed Inoculation | Year and Site | |
|---|---|---|---|
| | | 1969 | 1971 |
| | | Site B | Site F |
| Waco black earth .. .. | 1, 2. Controls .. .. .. .. | 4 700 | 2 900 |
| | 3. *A. chroococcum* .. .. .. | 3 400 | 3 200 |
| | 4. *A. chroococcum* (composite) .. .. | 8 800 | 9 500 |
| | | Site A | Site H |
| Mywybilla black earth .. | 1, 2. Controls .. .. .. .. | 200 | 50 |
| | 3. *A. chroococcum* .. .. .. | 300 | 80 |
| | 4. *A. chroococcum* (composite) .. .. | 500 | 2 500 |
| | | Site E | Site G |
| Red-brown earth .. .. | 1, 2. Controls .. .. .. .. | 0 | 0 |
| | 3. *A. chroococcum* .. .. .. | 0 | 40 |
| | 4. *A. chroococcum* (composite) .. .. | 10 | 600 |

| *Cells/seed at sowing were: | Inoculation Treatment | 1969 | 1971 |
|---|---|---|---|
| | 1, 2 | 0 | 0 |
| | 3 | $<50$ | $10^3$ |
| | 4 | $10^4$ | $10^6$ |

## IV. DISCUSSION

The results of experiment 1 showed that different cultivars of wheat may respond to inoculation with *A. chroococcum* to different degrees and confirmed responses obtained previously with Gabo (Rovira 1965). Rovira (1965) found that inoculation decreased the time from sowing to ear emergence of wheat and suggested this may result from effects exerted by the bacteria on the early growth stages of the plants. No effect of inoculation on the time to ear emergence was noted in this experiment. However, inoculation increased the number of grains per ear and this may have been due to effects exerted by the bacteria in the earlier phenological stages soon after double-ridge formation.

Brown, Burlingham and Jackson (1964) found that diseased plants frequently responded proportionately more to inoculation with *A. chroococcum* than healthy plants even though inoculation did not reduce the level of infection. A similar mechanism may have been operative in this experiment since a small proportion of the plants were infected with *Gibberella zeae* causing crown rot. However, although both Spica and Gabo were infected, only the latter cultivar responded to inoculation with *A. chroococcum*.

In the field experiments, inoculation was tested on cultivars of wheat and barley recommended for commercial production of these grains in Queensland. Inoculation significantly ($P<0{\cdot}05$) increased grain yield in only a few instances. Other investigators have used less rigorous criteria for judging the results of inoculation experiments, e.g. significant differences at $P<0{\cdot}1$ or percentage yield response without statistical analysis. To justify use of the latter, the argument has been advanced that average yield increases of 5–10% may be biologically real and economically worthwhile but difficult to prove statistically. However, the average percentage yield response of any cultivar in this investigation did not exceed 5%. These results show that little benefit can be gained by inoculating winter cereals with *A. chroococcum* in Queensland.

Some effects of inoculation on plant growth may result in increased grain yield only through interaction with the environment (Ridge and Rovira 1968). If such were the case one might expect that differences due to inoculation could be detected in other plant attributes, in particular the time to ear emergence. Other attributes of the plants were determined in some of the experiments to check this possibility but no differences due to inoculation were obtained.

In experiment 9, inoculation with *A. chroococcum* did not alter the percentage of Gamut plants with crown rot and did not significantly affect the grain yield of either Gamut or of the disease-tolerant cv. Gala grown on the same site. Thus no indication that inoculation improved the performance of disease-affected plants was obtained..

While seed inoculation with *A. chroococcum* increased the populations of this bacterium in the rhizosphere of wheat the best inocula resulted in no more than several thousand cells per root system. Ridge and Rovira (1968) obtained similar results in South Australia and considered this was because the soils did not naturally contain *A. chroococcum* and presented an unfavourable environment to the seed inoculum. This cannot explain the present results because the soils on most of the sites contained naturally occurring *A. chroococcum*. Increasing the number of bacterial cells on the seed to the high rate of $10^6$ cells/seed did not greatly improve the numbers established in the rhizosphere even on soils which contained naturally occurring *A. chroococcum,* and it is difficult to conceive how greater numbers can be achieved in the rhizospheres of winter cereals grown under field conditions.

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