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INOCULATION OF WHEAT WITH BEIJERINCKIA DERXII

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SUMMARY

The effects of inoculation with *Beijerinckia derxii* on the yield, yield components and phenological development of wheat were assessed in glasshouse trials. Plants of one cultivar whether grown under conditions of nitrogen limitation or sufficiency, or in the presence of an unidentified phyto-toxin produced by autoclaving soil, did not respond to seed inoculation. Likewise six cultivars of wheat grown with adequate mineral nutrients did not respond to either seed inoculation or seed plus soil inoculation.

I. INTRODUCTION

Although seed inoculation of cereals with the nitrogen-fixing bacterium *Azotobacter chroococcum* is used on farms in the U.S.S.R., the benefits to be gained from this practice are not large. The percentage yield responses obtained in the U.S.S.R. and elsewhere have averaged from <5% to 15% in different investigations (Cooper 1959; Mishustin and Naumova 1962; Brown, Burlingham and Jackson 1964; Ridge and Rovira 1968; Thompson 1974). Associated research has been directed towards methods for obtaining larger responses from seed inoculation more consistently. Because *A. chroococcum* has often been effective on plants supplied with complete mineral nutrients, mechanisms other than nitrogen fixation have been proposed and some bacterial genera from other families tested as inocula (Cooper 1959; Ridge and Rovira 1968). However, with but one exception, the potential of other *Azotobacter* species and other genera in the Azotobacteraceae has not been explored. Dobereiner and Ruschel (1961) found that inoculation of rice seed with an unidentified species of *Beijerinckia* increased yield by 55% in a nitrogen-deficient soil. This paper reports the results of experiments on inoculation of wheat with a strain of *Beijerinckia*.

II. MATERIALS AND METHODS

Bacterial Strain.—A strain of *Beijerinckia* was isolated by enrichment in acidic nitrogen-free medium (Becking 1961*a*), from a lateritic krasnozem soil, pH 5·8, at Toowoomba, Queensland. In pure culture it fixed 13 mg nitrogen per g glucose. In a recent taxonomic investigation (Thompson, unpublished) this strain was identified as a member of the species *Beijerinckia derxii* Tchan.

Inoculation.—Cultures of *B. derxii* in 500 ml McCartney bottles containing 40 ml of Becking's (1961*b*) medium were incubated aerobically at 28°C for 15 days. After incubation the cultures were shaken with sterile glass beads on a wrist-action shaker to homogenize the visco-elastic growth.

Seed was inoculated by mixing with undiluted inoculum and was sown moist. Control seed was treated similarly with sterile distilled water. Soil was inoculated by initial wetting with a 1:140 dilution of inoculum in sterile water. Assays of *Beijerinckia* by dilution-plating on Becking's (1961*b*) agar showed that inoculated seed carried 7 x 10^5 cells/seed at sowing and inoculated soil received 10^3 cells/g.

Pot techniques.—Ten plants were grown per pot containing 4·5 kg of lateritic krasnozem soil in a glasshouse. The air-dried soil was initially brought to pF2 with distilled water or *Beijerinckia* suspension and frequently returned to this moisture content with distilled water. During the growth of the plants, complete plant nutrient solution (Hoagland and Arnon 1938) or a similar solution lacking inorganic nitrogen was supplied in split applications totalling 300 ml/kg soil.

Experiments.—In experiment 1, two seed inoculation treatments (control and *Beijerinckia*) were tested on wheat, cv. Gabo, grown in three soil treatments (unautoclaved + nitrogen-free nutrient solution; unautoclaved + complete nutrient solution; and autoclaved + complete nutrient solution). The autoclaved soil was treated at 126°C for 90 min. on the day before sowing. The layout of this experiment was a 6 x 6 latin square design.

In experiment 2, three inoculation treatments (control; seed inoculation with *Beijerinckia;* and seed plus soil inoculation with *Beijerinckia*) were applied to six wheat cultivars (Gamut, Gabo, Gala, Mendos, Festival and Spica) grown with complete nutrients. In this experiment the pots were set out in Wisconsin tanks and the soil temperature maintained at 24°C. The layout was a split-plot design with six cultivars in four randomized blocks as whole units and the three inoculation treatments as subunits.

In both experiments, many attributes of the plants were determined, the main ones being: time from sowing to anthesis, number of ears per plant, numbers of spikelets and grains per ear, and dry weights of grain and straw.

III. RESULTS

Experiment 1.—The results for grain and straw yields and some other attributes of the plants are given in Table 1. Seed inoculation with *Beijerinckia* did not significantly affect any attribute of the plants grown in any of the three soil treatments. The plants responded to the nitrogen in the nutrient solution as seen by the increased yield of grain and straw and particularly by the production of fertile secondary tillers. Autoclaving this lateritic krasnozem soil resulted in a toxic effect on the wheat plants. In autoclaved soil, the seedlings emerged 1 day later, became partially chlorotic 2 weeks after emergence and remained chlorotic and stunted for a further 8 weeks. This effect is illustrated in Figure 1. Subsequently the vigour of the plants improved and they eventually produced grain.

Fig. 1.—Appearance of wheat plants 6 weeks after sowing in pre-autoclaved lateritic krasnozem soil.

TABLE 1

MEAN EFFECTS OF SEED INOCULATION WITH *Beijerinckia* AND SOIL TREATMENT ON THE GRAIN YIELD AND OTHER ATTRIBUTES OF WHEAT (CV. GABO)

Treatment		Yield (g/Pot of 10 Plants)		Primary Tiller			Secondary Tillers
Soil	Seed Inoculation	Grain	Straw	Sowing to Anthesis (days)	Spikelet Number	Grain Number	Ears/Plant
Unautoclaved	Control	7·3	11·4	65·1	13·2	18·2	0
	Beijerinckia	7·6	11·5	64·8	13·3	18·7	0
	Mean	7·4	11·4	64·9	13·2	18·5	0
Unautoclaved + nitrogen	Control	19·8	16·6	67·6	15·8	26·7	1·07
	Beijerinckia	20·1	17·0	67·7	15·5	27·4	0·98
	Mean	19·9	16·8	67·6	15·7	27·1	1·03
Autoclaved + nitrogen	Control	8·3	9·5	95·3	8·5	17·3	0·72
	Beijerinckia	6·0	7·1	98·8	7·9	13·5	0·52
	Mean	7·1	8·3	97·0	8·2	15·4	0·62
Inoculation means . .	Control	11·8	12·5	76·0	12·5	20·7	0·59
	Beijerinckia	11·2	11·9	77·1	12·2	19·9	0·50
Necessary differences	*P*						
Soil treatment . .	<0·05	1·7	1·9	4·3	0·8	2·9	0·16
	<0·01	2·4	2·6	5·9	1·1	3·9	0·22
Inoculation . .	<0·05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Soil treatment x inoculation	<0·05	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

Experiment 2.—The results of this experiment are given in Table 2. The six cultivars were representative of wheats grown commercially in Queensland but were also reasonably diverse as gauged by their range in grain yield (15·2–23·6 g), grain to straw ratio (0·45–0·99) and time from sowing to anthesis (70–84 days). None of these cultivars was significantly affected by either seed or seed plus soil inoculation with *Beijerinckia* in either grain yield or any other attribute.

IV. DISCUSSION

In experiment 1, seed inoculation with *Beijerinckia* did not cause the wheat plants to respond in any way approaching the effects produced by the nitrogen in the plant nutrient solution. Thus the bacteria did not improve the nitrogen nutrition of the wheat plants. The direct cause of the phytotoxicity induced by autoclaving the soil was not determined but it may have been due to toxic concentrations of certain cations or organic compounds (Rovira and Bowen 1969) or to toxic metabolites produced by early re-colonizing microorganisms. Seed or soil inoculation with some microorganisms is reported to alleviate similar toxicities on other plant species (Cooper 1959; Rovira and Bowen 1969) but seed inoculation with *Beijerinckia* did not have that effect on the wheat plants in this experiment.

TABLE 2

MEAN VALUES FOR GRAIN YIELD AND OTHER ATTRIBUTES OF SIX WHEAT CULTIVARS INOCULATED IN TWO WAYS WITH *Beijerinckia*

Treatment		Yield (g/Pot of 10 Plants		Primary Tiller			Secondary Tillers
Cultivar	Inoculation	Grain	Grain/straw Ratio	Sowing to Anthesis (days)	Spikelet Number	Grain Number	Ears/Plant
Gamut	Control	21·7	0·96	72·3	20·0	39·8	0·03
	Seed	21·5	1·01	72·2	20·3	40·1	0·03
	Seed + soil	19·6	0·99	71·8	19·1	36·7	0·05
	Mean	20·9	0·99	72·1	19·8	38·9	0·03
Gabo	Control	23·4	0·98	71·5	16·7	37·3	0·43
	Seed	23·7	0·99	73·4	17·0	36·1	0·55
	Seed + soil	23·8	0·93	73·7	17·2	36·6	0·60
	Mean	23·6	0·97	72·8	17·0	36·7	0·53
Gala	Control	17·6	0·49	81·3	15·3	23·2	1·23
	Seed	17·5	0·45	86·5	15·3	25·4	1·03
	Seed + soil	17·6	0·46	85·3	14·7	23·8	1·08
	Mean	17·6	0·46	84·4	15·1	24·1	1·11
Mendos	Control	19·6	0·84	70·7	17·0	30·3	0·63
	Seed	21·8	0·89	70·6	16·9	31·4	0·63
	Seed + soil	20·8	0·90	69·3	17·1	31·6	0·28
	Mean	20·7	0·88	70·2	17·0	31·1	0·51
Festival	Control	19·2	0·76	70·0	18·9	33·8	0·58
	Seed	17·1	0·68	71·0	19·4	33·6	0·50
	Seed + soil	15·4	0·65	69·2	17·8	32·2	0·43
	Mean	17·2	0·70	70·1	18·7	33·2	0·50
Spica	Control	15·8	0·46	78·7	16·8	22·9	0·28
	Seed	16·0	0·46	81·2	16·9	20·9	0·55
	Seed + soil	14·2	0·42	78·1	16·3	19·7	0·33
	Mean	15·3	0·45	79·3	16·6	21·1	0·38
Inoculation means	Control	19·6	0·75	74·1	17·4	31·2	0·53
	Seed	19·6	0·75	75·8	17·6	31·2	0·55
	Seed + soil	18·6	0·73	74·6	17·0	30·1	0·46
Necessary differences	*P*						
Cultivars	$<0·05$	2·3	0·08	2·3	0·8	3·8	0·45
	$<0·01$	3·2	0·11	3·2	1·1	5·2	0·63
Inoculation	$<0·05$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Cultivar x inoculation	$<0·05$	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.

In a previous glasshouse experiment in which seed inoculation with *A. chroococcum* was tested on two wheat cultivars, the grain yield of one was increased by 31% while that of the other was not affected (Thompson 1974). Experiment 2 was conducted under similar conditions with six wheat cultivars to test for a similar inoculation x cultivar interaction, but *Beijerinckia* inoculation did not increase the grain yield of any of these reasonably diverse cultivars.

Ridge and Rovira (1968) found that inoculation of wheat with *A. chroococcum* often shortened the time to ear emergence, particularly for plants grown with complete nutrients. *Beijerinckia* inoculation did not have this effect on any of the cultivars in experiment 2 as gauged by the mean time from sowing to either ear-peep or anthesis.

In these experiments the strain of *Beijerinckia* used was assessed as an inoculum for wheat under different conditions and on a range of wheat cultivars. No indication that this organism improved the growth of wheat was obtained and it is unlikely to have any beneficial effect on wheat under any other test conditions.

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