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EFFECT OF NITRIFICATION OF A BLACK EARTH SOIL ON LEGUME NODULATION

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SUMMARY

Black soil from the Darling Downs, Queensland, nitrified to 130-159 p.p.m. nitrate nitrogen in the glasshouse when subjected to 8 cycles of wetting and air-drying. The highest level in the field was 43 p.p.m. nitrate nitrogen.

Nodulation inhibition in soybeans by nitrate and ammonium ions was compared. The nitrate ion was more active and nitrate levels close to 168 p.p.m. N were necessary to prevent nodulation, while ammonium ions as high as 224 p.p.m. N were only partly inhibitory.

Interplanting soybean with sorghum in the field reduced the nitrate levels in the vicinity of the legume but did not overcome a nolulation failure from inoculated seed.

Nitrate inhibition is an unlikely explanation for soybean nodulation failures on black soils.

I. INTRODUCTION

Nitrate nitrogen can rapidly appear in agricultural soils with cultivation and rewetting after a dry spell. Martin and Cox (1956) showed that the black earth soils of the Darling Downs, Queensland, could nitrify *in situ*. This could lead to an accumulation of nitrates in the surface and subsoils (Waring and Teakle 1960). Nitrates are known to reduce nodulation of legumes, while Norris (1964) has recorded a complete inhibition of nodulation in phasey bean (*Phaseolus lethyroides*) by high soil nitrates.

A nodulation failure with inoculated soybean in black soils has been reported by Harty (1964). In field trials carried out by the author, 10 out of 25 inoculated plantings failed to nodulate. The present investigation was aimed at determining whether soil nitrate accumulation is responsible for legume nodulation difficulties on these black soils.

II. GLASSHOUSE STUDY ON SOIL NITRIFICATION

Materials and methods.—In order to investigate whether black soils are capable of nitrifying to inhibitory nitrate levels, soil from Jondaryan was incubated in the glasshouse in two shallow trays and covered with a loose polythene sheet to prevent moisture losses. The soil was subjected to eight cycles of wetting and air-drying with 1 week moistening and incubating and 2 weeks drying to intensify mineralization of soil nitrogen. The control soil was air-dried and stored in plastic bags until required. Artificial nitrate levels were created in the soil for comparison by the addition of varying amounts of calcium nitrates. The nitrate levels at the beginning of the experiment were 82, 176, 356 and 541 p.p.m. N for the addition series, 130 and 159 p.p.m. N for the incubated soils, and 38 p.p.m. N for the control. Ammonium levels were low and no nitrites were detected in any soil. Inoculated cowpea (*Vigna sinensis*) seeds were sown into plastic-lined pots with four replications of each soil treatment. Plants and nodules were harvested after 5 weeks' growth.

Results.—Nitrification of the soil by the method described produced a substantial rise in nitrate level; this was not sufficient to prevent nodulation but did inhibit nodulation to an extent.

Table 1 shows the number and size distribution of nodules from cowpeas. There was a reduction in the total number of nodules and the number of large nodules from the incubated soil as compared with the control soil; the reduction being similar to that of the comparable added soil nitrate treatment (176 p.p.m.).

TABLE 1

EFFECT OF SOIL NITRIFICATION ON NODULATION OF COWPEAS

Treatment	Soil Nitrates (p.p.m. N)	Mean Dry Weight per Plant (g)	Mean No. of Nodules and Size per 6 Plants				
			> 3·3 mm	3·3–2·4 mm	2·4–1·2 mm	< 1·2 mm	Mean Total Nodules
Black soil control ..	38	485	2·7	12·0	18·1	2·2	35·0
	82	527	1·7	8·0	19·2	8·2	37·1
	176	571	1·5	7·1	13·7	4·5	26·8
	356	568	0	0·2	2·2	5·0	7·4
	541	581	0	0	0·5	4·2	4·7
Incubated black soil I ..	130	565	1·5	7·0	16·0	1·2	25·7
Incubated black soil II ..	159	557	1·0	4·0	13·7	3·0	21·7

Under the conditions of the experiment, levels as high as 541 p.p.m. N did not completely prevent nodulation. Several small nodules of 1 mm diam. were observed at this level which could have resulted from the reduction in nitrate levels by plant utilization.

III. COMPARATIVE EFFECTS ON NODULATION OF AMMONIUM AND NITRATE NITROGEN

Materials and methods.—Nitrogen additions in the form of ammonium sulphate and calcium nitrate were made to autoclaved black soil in which soybeans were growing for 28 days to reduce the nitrogen level prior to superimposing the nitrogen treatments. A split application was made by injection into the soil on two occasions to give total nitrogen additions of 0, 56, 112, 168 and 224 p.p.m. N in both ammonium and nitrate series. The pots were inoculated with *Rhizobium* at the same time. The soybeans were grown for a further 15 days before harvesting. This method reduced the time of interaction of host, nitrogen source and *Rhizobium* necessary for a visible nodulation result. Ammonium ions were detected in all ammonium sulphate treatments at the end of the experiment, indicating that active nitrification had not occurred.

Results.—As shown in Table 2, the high nitrate and ammonium levels had a definite inhibitory effect on nodulation. Nodulation was prevented by the nitrate ion at levels close to 168 p.p.m. N, whereas the ammonium ion was still only partly inhibitory at 224 p.p.m. N.

TABLE 2

Comparative Effects of Ammonium and Nitrate Forms of Nitrogen on the Nodulation of Soybeans

N Additions (p.p.m. N)	Mean Number of Nodules per 5 Plants	
	Ammonium N Series	Nitrate N Series
0	23	23
56	27	33
112	25·5	16
168	25	1·5
224	11	0

IV. NITRATE LEVELS OCCURRING IN THE FIELD

Materials and methods.—Soil samples at a depth of 0–6 in. were collected on a number of occasions from field sites at Bongeen, St. Helens and Mt. Tyson where legume nodulation trials were being carried out, and submitted to the Agricultural Chemical Laboratory for nitrogen analysis.

Nitrate and ammonium estimations were made by a modified steam distillation method of Bremner (1964) and quick determinations by the Morgan method (Lunt, Jacobsen, and Swanson 1958). Nitrites were detected by the starch iodide test (Skerman 1959, p. 150), after extraction with 0·04% $BaCl_2$.

Results.—Of 10 field samples, 4 showed moderately high levels of nitrate (up to 43 p.p.m. N) but the remainder were low. The highest levels were recorded during the summer, particularly after a period of long fallowing. The levels of ammonium nitrogen were always low and did not exceed 8 p.p.m. while nitrates were not detected in any soils.

V. ATTEMPTS TO INFLUENCE NODULATION BY REDUCING NITRATE LEVELS IN THE FIELD

Gramineous plants interplanted with the legume could be expected to reduce the nitrate levels to a non-inhibitory level for nodulation (Norris 1964). To examine the possibility that nodulation failure in soybean is due to nitrate inhibition, inoculated soybean seeds were planted into an emerging 2-week-old sorghum crop sown on fallowed land at St. Helens. Controls were planted into an unsown headland. The soybeans were inoculated with a peat culture at the rate of $2 \cdot 0 \times 10^5$ viable bacteria per seed. Soil conditions at the time of planting were good and the growing conditions favourable. The soybeans and soil in the vicinity of the roots were examined at 7 weeks.

Results.—The nodulation and nitrogen levels recorded are shown in Table 3. While the results support the idea that a gramineous crop reduces the soil nitrate level, the complete failure of nodulation was apparently related to factors quite apart from nitrate level.

TABLE 3

NODULATION AND NITROGEN LEVELS

Treatment	No. of Plants Nodulated in 3 Replications	Soil N (p.p.m.) NO_3(N)	NH_4(N)
Soybean + sorghum ..	0/38	9	5
Soybean − sorghum ..	0/24	27	2

VI. DISCUSSION

The nitrate ion is clearly more active in inhibiting nodulation of soybeans than is the ammonium ion; this is in agreement with the work of Richardson, Jordan, and Garrard (1957). Glasshouse studies show that black soil is capable of nitrifying to inhibitory levels; however, the levels necessary to prevent nodulation far exceed any naturally occurring levels recorded in them at arable depth by the author or other workers (Waring and Teakle 1960; Martin and Cox 1956; Littler 1963). This is possibly due to the leaching of the nitrates to lower soil horizons (Skyring 1961). The observed nitrate levels could well influence the extent of soybean nodulation in black soil. However, nitrate inhibition is an unlikely explanation for the nodulation failures from inoculated seed. Furthermore, a reduction in nitrate level by the legume itself or associated gramineous plant did not bring about nodulation. Bacterial survival has been implicated in the failure; this will be reported in another paper.

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