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Soybean Inoculant Types and Rates Evaluated under Dry and Irrigated Field Conditions[1]

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ABSTRACT

Effects of high soil temperature and low soil moisture for 7 days following planting were utilized in evaluating inoculant types and inoculating rates with soybeans (*Glycine max* (L.) Merr.) grown in a soil free of *Rhizobium japonicum* in Puerto Rico. Granular soil inoculant applied at 10 times the standard application rate provided significantly more tap root and total number of nodules than all other treatments. This increase was observed in sampling at 32 and 98 days after planting under both dry and irrigated conditions. The total of all inoculating types applied at 10 times the rate provided a significant increase in number of tap root nodules, total number of nodules and nodule dry weight per plant only at 32 days when compared with the total for application at the standard rate. Sampling at both 32 and 98 days indicated a significant increase in number of tap root nodules, total number of nodules and nodule dry weight per plant with irrigation when compared to the dry section.

INTRODUCTION

High soil temperatures and desiccation from low soil moisture are possible causes of nodulation failure. Daitloff (4) recorded 40°C soil temperatures for 4 hours in a moist soybean seed zone with some decline in nodulation, although this was by no means critical to subsequent nodulation. However, if high temperatures are experienced simultaneously with another stress factor, such as desiccation, a nodulation failure becomes more likely. Iswaran et al. (6) observed a rapid death of *Rhizobium japonicum* at 40° C when added to either soil or peat, with no worthwhile recovery after 4 weeks of storage. In terms of bacterial

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survival, Bowen and Kennedy (1) considered 40° C critical to the survival of rhizobia. When *R. japonicum* was applied with peat to the seed, Davidson and Reuszer (2) recovered from 0.1 to 1.6% of the original population if stored at only 30°C for 3 weeks. Kaul and Sekhon (7) found that mulching lowered the soil temperature by 10° C, and resulted in early and more uniform nodulation and improved soybean yields.

Since soil moisture is known to modify soil temperatures, these two factors are closely related in rhizobial survival. Diatloff (4) stated that desiccation of nodule bacteria in seed inocula was unlikely in soil moist enough to germinate seed. Diatloff (3) also reported that an absence of nodules was recorded during excessively wet or dry conditions. Worrall and Roughley (10) reported that moisture stress and method of inoculation greatly affected the number and distribution of infected root hairs and nodules of young seedlings of *Trifolium subterraneum*. A reduction of soil moisture potential from -0.36 to -3.6×10^5 Pa significantly decreased the number of infection threads and completely inhibited nodulation, although the number of rhizobia in the rhizosphere was unaffected.

Methods of inoculation such as placement of inoculant below the seed (5, 8, 9) have been investigated and reported to have potential value in protecting the applied rhizobia from temperature and moisture stresses occurring in the seed zone.

This paper examines soybean nodulation under dry and irrigated field conditions with an evaluation of different inoculant types, rates of inoculation and inoculant placement.

MATERIALS AND METHODS

Broth of *Rhizobium japonicum* strain 8-0, with a count of 1.6×10^9 cells/ml, was utilized in the preparation of the inoculants. Seed-applied peat powder had a count of 7.8×10^8 cells/g and was applied at a rate of 3.1 g/kg seed which supplied 3.0×10^5 cells/seed. Sucrose solution was used to adhere the inoculant to the seed. Granular soil inoculant was added on top of the seed in the furrow. It had a count of 1.2×10^9 cells/g and was applied at a rate of 3.4 g/6 m of row, which supplied 7.2×10^6 cells/cm row. Liquid soil inoculant was placed on the seed in the furrow, and at 2.5, 5.0 and 7.5 cm below the seed. Liquid inoculant was prepared by diluting 2.7 ml of the broth with 72.3 ml of water, and applied at a rate of 75 ml/6 m of row. This supplied 7.2×10^6 cells/cm row. Each inoculant type was utilized at the above standard rate (× rate) and 10 times the standard rate (10× rate).

The experiment was at the Isabela Substation, Agricultural Experiment Station of the University of Puerto Rico on an Oxisol (Coto kaolinitic clay) which was free of *R. japonicum*. Jupiter variety soybean (*Glycine*

max (L.) Merr.) was planted March 7, 1979, which is during the dry season in Puerto Rico. This allowed the establishment of all treatments in the field in both a dry soil (no water before and 7 days after planting, followed by irrigation to initiate seed germination and plant growth) and a moist soil (irrigation following planting and subsequently to maintain moist conditions). Plots were 4 rows 6 m long with 60 cm between rows, and were replicated four times in a randomized complete block design.

Seven-day cycle soil temperature recorders with probes at 2.5 cm and 10.0 cm were placed in both the dry and moist sections of the field. Three Bouyoucos moisture blocks were placed at 7.5 cm in both the dry and moist sections of the field to monitor soil moisture.

Because of poor emergence in the dry section, only five plants per plot were evaluated 32 and 98 days after planting for number of tap root nodules, total number of nodules, and nodule dry weight/plant.

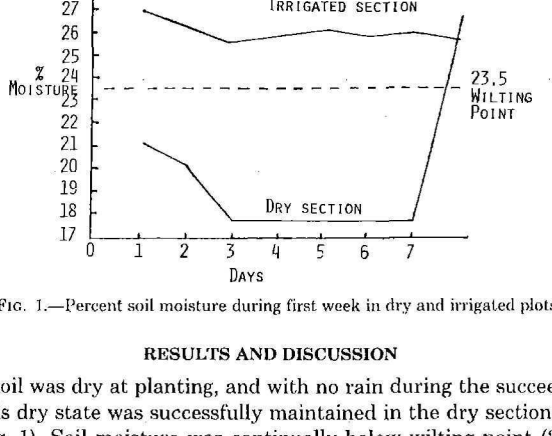

FIG. 1.—Percent soil moisture during first week in dry and irrigated plots.

RESULTS AND DISCUSSION

The soil was dry at planting, and with no rain during the succeeding 7 days this dry state was successfully maintained in the dry section of the field (fig. 1). Soil moisture was continually below wilting point (23.5%). After 7 days water was applied to the dry section to initiate seed germination, for without plant growth rhizobial survival and nodulation could not be evaluated. The irrigated section received water following planting and at frequent intervals to maintain soil moisture between wilting point and field capacity (fig. 1). The maximum soil temperatures at 2.5 cm in the dry plots were between 38 and 40° C, which was 3 to 8° C hotter than the maximum in the irrigated plots (fig. 2). Daily minimum

temperatures under both moisture regimes were below 26° C. Clouds during day 3 eliminated temperature differences. Temperatures at 10 cm ranged between 21 and 27° C with no significant difference between the two moisture conditions.

The inoculants' ability to provide early effective nodulation was evaluated by examination of the number of tap root nodules/plant at 32 days. Under dry conditions only the granular soil inoculant at 10× rate produced more than one tap root nodule/plant, and was significantly better than all other treatments (table 1). Under irrigation this treatment was also superior, with × rate of granular and liquid soil inoculant delivered in the furrow over the seed at 10× rate better than the remaining

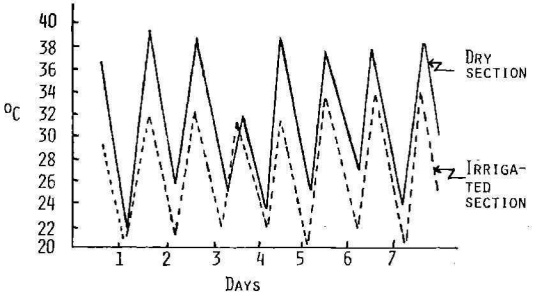

FIG. 2.—Soil temperature at 2.5 cm during first week in dry and irrigated plots.

TABLE 1.—*Effect of soil moisture, inoculant type and rate on number of tap root nodules at 32 days*

Treatment	Dry section		Irrigated section	
	× rate	10× rate	× rate	10× rate
	No.		*No.*	
Control	0 b[1]	—	0 c	—
Peat	0.1 b	0.3 b	0.9 c	1.4 c
Granular	0.9 b	2.5 a	3.3 b	5.3 a
Liquid-Seed	0.3 b	0.8 b	0.6 c	3.1 b
Liquid-2.5 cm	0.2 b	0.9 b	0.5 c	1.3 c
Liquid-5.0 cm	0 b	0 b	0 c	0.9 c
Liquid-7.5 cm	0.1 b	0 b	0.7 c	0.3 c
Rate total	1.6	4.5	6.0	12.3[2]
Moisture total	6.1		18.3[2]	

[1] Within each dry or irrigated section, values followed by one or more letters in common do not differ significantly at the 5% level according to Duncan's Multiple Range Test.

[2] Significant at the 1% level.

treatments. A comparison of × and 10× rates totaled among treatments in both dry and irrigated conditions (1.6 to 4.5 and 6.0 to 12.3, respectively) indicated more than twice as many tap root nodules formed with the inoculants at the 10× rate (table 1). Moisture totals between dry and irrigation (6.1 and 18.3, respectively) indicated that three times more tap root nodules were formed under the moist conditions (table 1).

The total number of nodules/plant at 32 days (table 2) followed a pattern similar to that of tap nodules, with granular 10× rate significantly better than all other treatments under both dry and irrigated conditions. Rate total under dry conditions (table 2) indicated that 10× rate was not significantly better than the × rate; however, under irrigation the rate

TABLE 2.—*Effect of soil moisture, inoculant type and rate on total nodule numbers at 32 days*

Treatment	Dry section		Irrigated section	
	× rate	10× rate	× rate	10× rate
	No.		*No.*	
Control	0[1]	—	0.2 d	—
Peat	0.2 **b**	0.8 b	2.1 d	4.8 **d**
Granular	4.1 b	11.3 **a**	16.6 **b**	33.1 a
Liquid-Seed	2.4 b	2.1 b	6.0 cd	13.8 bc
Liquid-2.5 cm	4.3 b	4.3 **b**	4.0 **d**	8.0 cd
Liquid-5.0 cm	0	0.1 b	0.4 d	3.6 d
Liquid-7.5 cm	0.3 b	0.2 b	2.7 d	4.4 **d**
Rate total	11.3	18.8	32.0	67.7[2]
Moisture total	30.1		99.7	

[1] Within each dry or irrigated section, values followed by one or more letters in common do not differ significantly at the 5% level according to Duncan's Multiple Range Test.

[2] Significant at the 1% level.

total for 10× was double the × rate and significant at the 1% level. As with tap nodules, an evaluation of moisture totals between dry and irrigated (30.1 and 99.7, respectively) showed that significantly more total nodules were formed under irrigated conditions (table 2).

The treatment relationships with nodule dry weight/plant (table 3) were very similar to those for total number of nodules; the granular treatment provided the most nodule weight, followed by liquid on the seed, and then liquid placed 2.5 cm below the seed. There was no difference between × and 10× rate totals under dry conditions; however, the 10× rate was more than double the × rate under irrigation and was significant at the 1% level. As with number of tap nodules and total number nodules, moisture totals between dry and irrigated (62.6 and 193.8, respectively) showed that more than three times the dry weight of nodules formed under the irrigated conditions.

Nodule characteristics were evaluated at 98 days to determine whether nodulation trends would change with time. At 98 days a similar order of treatments, as was observed at 32 days, was indicated for tap root nodules (table 4), total nodule number (table 5), and nodule dry weight/plant (table 6). Granular inoculant was consistently the best, with liquid on the seed and liquid at 2.5 cm overall better than the remaining treatments. Liquid at 5.0 and 7.5 cm below the seed in general produced less than one tap root nodule under both moisture conditions, apparently because

TABLE 3.—*Effect of soil moisture, inoculant type and rate on nodule dry weight (mg)/plant at 32 days*

Treatment	Dry section		Irrigated section	
	× rate	10× rate	× rate	10× rate
	No.		*No.*	
Control	0 c[1]	—	0.7 d	—
Peat	0.7 bc	1.4 bc	5.2 cd	18.8 bc
Granular	10.5 abc	17.1 a	21.1 bc	47.7 a
Liquid-Seed	5.6 bc	6.1 bc	16.7 bcd	28.0 b
Liquid-2.5 cm	11.0 ab	7.3 abc	9.0 cd	14.4 bcd
Liquid-5.0 cm	0 c	0.2 c	0.4 d	11.5 bcd
Liquid-7.5 cm	2.3 bc	0.4 bc	8.6 cd	11.7 bcd
Rate total	30.1	32.5	61.7	132.1[2]
Moisture total	62.6		193.8[2]	

[1] Within each dry or irrigated section, values followed by one or more letters in common do not differ significantly at the 5% level according to Duncan's Multiple Range Test.
[2] Significant at the 1% level.

TABLE 4.—*Effect of soil moisture, inoculant type and rate on number of tap root nodules at 98 days*

Treatment	Dry section		Irrigated section	
	× rate	10× rate	× rate	10× rate
	No.		*No.*	
Control	0 b[1]	—	0 d	—
Peat	0 b	0.1 b	3.2 bcd	3.2 bcd
Granular	0.7 b	2.9 a	7.6 ab	11.3 a
Liquid-Seed	0.2 b	0.6 b	6.1 bc	5.9 bc
Liquid-2.5 cm	1.5 ab	0.7 b	1.0 cd	1.5 cd
Liquid-5.0 cm	0.2 b	0 b	2.4 bcd	0 d
Liquid-7.5 cm	0 b	0 b	0.2 d	0.9 cd
Rate total	2.6	4.3	20.5	22.8
Moisture total	6.9		43.6[2]	

[1] Within each dry or irrigated section, values followed by one or more letters in common do not differ significantly at the 5% level according to Duncan's Multiple Range Test.
[2] Significant at the 1% level.

inoculant placed at these depths was below the tap root zone for nodule infection. However, these two treatments did produce more than 25 total nodules under irrigation (table 5), whereas fewer than 5 total nodules were formed in the dry soil. This suggests that inoculum placed at these depths can survive outside of the rhizosphere under moist but not under dry conditions.

The advantage of the 10× rate over the × rate under the irrigated condition at 32 days with all nodule parameters was no longer significant

TABLE 5.—*Effect of soil moisture, inoculant type and rate on total nodule numbers at 98 days*

Treatment	Dry section		Irrigated section	
	× rate	10× rate	× rate	10× rate
	No.		*No.*	
Control	1.5 d[1]	—	7.7 d	—
Peat	12.9 cd	2.6 cd	76.1 abcd	59.7 bcd
Granular	27.5 bc	63.5 a	96.3 abc	139.8 ab
Liquid-Seed	22.8 bcd	29.3 bc	80.5 abcd	146.3 a
Liquid-2.5 cm	46.3 ab	28.8 bc	73.4 abcd	76.4 abcd
Liquid-5.0 cm	1.5 d	1.9 d	25.4 cd	75.2 abcd
Liquid-7.5 cm	4.3 cd	1.2 d	50.4 cd	48.9 cd
Rate total	116.8	127.3	409.8	546.3
Moisture total	244.1		956.1[2]	

[1] Within each dry or irrigated section, values followed by one or more letters in common do not differ significantly at the 5% level according to Duncan's Multiple Range Test.
[2] Significant at the 1% level.

TABLE 6.—*Effect of soil moisture, inoculant type and rate on nodule dry weight (mg)/plant at 98 days*

Treatment	Dry section		Irrigated section	
	× rate	10× rate	× rate	10× rate
	No.		*No.*	
Control	21.9 d[1]	—	49.4 e	—
Peat	25.5 d	32.9 d	247.2 cd	307.7 bcd
Granular	170.9 b	258.7 a	432.5 abc	519.5 a
Liquid-Seed	73.2 cd	148.2 bc	383.4 abc	460.0 ab
Liquid-2.5 cm	218.5 ab	181.0 ab	381.0 abc	305.0 bcd
Liquid-5.0 cm	14.0 d	31.6 d	162.0 de	247.7 cd
Liquid-7.5 cm	24.4 d	13.2 d	257.3 cd	313.2 bcd
Rate total	548.4	665.6	1912.8	2153.1
Moisture total	1214.0		4065.9[2]	

[1] Within each dry or irrigated section, values followed by one or more letters in common do not differ significantly at the 5% level according to Duncan's Multiple Range Test.
[2] Significant at the 1% level.

at 98 days (tables 4, 5 and 6). Only a consistant slight advantage was noted for the 10× rate.

Comparing the moisture totals between dry and irrigated sections at 98 days indicated that the total number of nodules (table 5) and nodule dry weight/plant (table 6) were still three times larger under irrigation (956.1 and 4065.9, respectively) when compared to dry conditions (244.1 and 1214.0, respectively). These increased values under irrigation were similar to those observed at 32 days. However, the tap root nodule total under irrigation (43.6) was now six times larger than under dry conditions (6.9). This was because the number of tap nodules under dry conditions did not increase between the 32 and 98 day sampling, while under irrigation the number of tap nodules more than doubled.

In summary, the granular soil inoculant was superior under both dry and moist conditions, with the liquid placed on the seed in the furrow and liquid 2.5 cm below the seed better than the remaining treatments. Standard seed-applied peat inoculant was unsatisfactory under dry conditions and significantly less effective than granular under irrigated conditions. Liquid placed 5.0 and 7.5 cm below the seed was not effective in protecting the rhizobia from the moisture and temperature stresses.

The 10× rate of each inoculant provided a significant increase in nodulation characteristics only at the 32-day sampling period under irrigation. Dry soil conditions for the first week provided a strong stress to rhizobia with all inoculant types and rates. The amount of nodulation under irrigation was at least three times greater than in the dry soil at both sampling periods. Irrigation eliminated the moisture stress and reduced maximum temperatures.

This study indicated that to establish effectively soybean nodulation in a *R. japonicum*-free soil in the semi-tropics an inoculant of high quality is essential and that soil moisture above the wilting point during the first week is critical.

RESUMEN

Condiciones de alta temperatura y baja humedad del suelo durante los siete días siguientes a la siembra, fueron utilizadas para evaluar tipos y dosis de inóculo en soya (*Glycine max* (L.) Merr.) cultivada en un suelo libre de *Rhizobium japonicum* en Puerto Rico. La mitad del experimento, sin riego durante los primeros siete días, tuvo un contenido de humedad inferior al punto de marchitez y temperatura máxima del suelo entre 38 y 40° C a 2.5 cm de profundidad. La parte regada tuvo un contenido de humedad cercano a la capacidad de campo y temperaturas máximas del suelo entre 31 y 35° C a 2.5 cm de profundidad. El inóculo granular aplicado al suelo fue significativamente mejor que el inóculo líquido colocado en el surco a profundidades de 2.5, 5.0 y 7.5 cm debajo de la

semilla y que el inóculo de turba aplicado a la semilla. Solamente el inóculo granular aplicado al suelo produjo un aumento significativo en la nodulación con una dosis diez veces la recomendada, al comparar cada tratamiento con su dosis respectiva normal. El número de nódulos en la raíz primaria, el número total de nódulos y el peso de la materia seca de los nódulos por planta fueron significativamente mayores en la sección regada que en la no regada.

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