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Oils as adhesives for seed inoculation and their influence on the survival of *Rhizobium* spp. and *Bradyrhizobium* spp. on inoculated seeds

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Mineral oil, peanut oil and soybean oil were compared with water and gum arabic for their suitability as **adhesives for seed inoculaljon with peat inoculants. Inoculated seeds wore stored at 4, 28 and 34·'C. and - sampled after 1, 3 and 9 davs to determine the survival of rhizobia. Germination and nodulaton tests were performed on the inoculated seeds. Results showed that oils were suitable adhesives for peat inoculants. Although the oils initially bound less inoculant to the seed, the number of surviving rhizobia was similar to that obtained by the gum arabic treatment after storage at 28 and 34°C for 3 and 9 days. An interesting finding of this experiment was that peanut and soybean oils were superior to gum arabic in supporting significantly higher number of chickpea rhizobia at 34°C. Inoculated seeds tested for germination and nodulation showed no adverse effects from the oil treatments. Oils hold good potential as adhesives for seed application in inoculation technology.**

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Legume seed inoculation with rhizobial inoculants is widely practiced to cause symbiotic biological nitrogen fixation which leads to an increase of yields in leguminous crops. Inoculation is the bringing of rhizobia into contact with legume seeds or roots (Food and Agricultural Organization, 1984). There are various methods to accomplish this. Most commonly the seeds are coated with a powdered peat-based inoculant. Dry application by dusting the inoculant onto the seed is the simplest way of inoculation, but also the least efficient and therefore no longer recommended (Brockwell 1977; Food and Agricultural Organization 1984). Using, a sticking agent with the proper type and amount of inoculant greatly increased the number of rhizobia adhering to the seed (Green *et al.* 1984). High numbers of rhizobia are important for successful inoculation, especially in soils when competitive native strains are present (Weaver & Frederick 1972). Ideally, the gum should provide a pre-tested environment for the inoculant before and after sowing. Moisture loss is a major cause of rapid dying of rhizobia on the seed (Vincent *et al.* 1962; Burton 1979). This is especially true where the inoculant is exposed to elevated temperatures (Scudder 1975).

Various sticker materials have been used for seed inoculation. The most inexpensive of these is water. When a water slurry inoculation was used with cowpea, the rhizobia survived well (Date & Cornish 1968). However, although water provides good adhesion initially, the inoculant tends to crumble off during handling. Furthermore, water does not prevent desiccation of the inoculant (Elegba & Rennie 1984). There is an advantage in using a weak adhesive solution to ensure that the peat adheres firmly to the seed (Norris & Date 1976). Sugar is one of the adhesives used. It has good moisture retention but lacks tenacity (Brockwell 1977) and may also increase the chances of attracting microbial attack of the seed. Gum arabic is more tenacious and provides good adhesion. It has the additional advantage in that it promotes root hair infection by the rhizobia and growth (Subba Rao *et al.* 1971). However, gum arabic is awkward to use, not readily available and is too expensive for most farmers. Vegetable oil has been

shown to protect against desiccation of rhizobia when used with lyophilized cultures in oil-based carriers (Kremer & Peterson 1982). However, their potential properties as adhesives for seed inoculation are unknown. This study was conducted to assess several oils for their suitability as inoculant adhesives for various economically important legumes.

MaterialsandMethods

Inoculants

NifTAL's triple strain inoculants (Halliday & Somasegaran 1984) were prepared for each legume species as follows. The rhizobial strains were grown separately in yeast extract mannitol broth (Vincent 1970) at 28°C. Strains of bradyrhizobia for soybean and peanut were harvested after 7 days. Rhizobial strains for chickpea and common bean were harvested after 5 days. The three strains of each species were combined and injected into gamma-irradiated peat, incubated at 28°C for 2 weeks and the quality determined (Hoben & Somasegaran 1982).

Seeds

The seeds used were common bean *(Phaseolus migaris cv.* Bountiful), Kabuli chickpea *(Cicer arietinum),* soybean *(Glycne max cv.* Davis) and peanut *(Arachis hypogaea cv.* Burpee Spanish). Their average respective weights on a per seed basis were 0.15, 0.5, 0.2 and 0.5 g.

All seeds were surface sterilized in batches of 300 seeds by 5 min immersion in 3% hydrogen peroxide followed by five rinses with sterile deionized water. The seeds were then air dried overnight on sterile filter paper in the transfer chamber. Seeds were surface sterilized and dried before inoculation to eliminate micro-organisms that would interfere in the enumeration process later.

Seed Inoculation

Stickers used were deionized water, 40% (w/ v) gum arabic solution in water (Difco Laboratories, Detroit, MI), mineral oil (E.R. Squibb & Sons, Inc., Princeton, NJ), soy salad oil and peanut oil (Hollywood Foods, Los Angeles, CA). Batches of 300 seeds were placed into a previously unused, clean polyethylene bag, sticker was added and the bag was twisted shut in such a way that approximately 400 ml of air was trapped with the seeds and the sticker. The bag was then shaken for 1 min to allow uniform coating of the seed surface with sticker. The peat inoculant was then added and the bag was shaken again for 30 s to achieve uniform coating.

The volume of sticker or gum used varied with seed size and texture, and type of sticker. For peanut and chickpea seeds, 1 ml was used in the gum arabic and oil treatments except in the case of the water treatment where 2 ml was required. Soybean seeds received 0.5 ml in all treatments except for water, where 1.0 ml was used. The bean seeds were wetted with 0.3 ml sticker in all treatments. The amount of inoculant used was 1 g for each batch of 300 seeds. The inoculated seeds were allowed to dry on sterile filter paper in the transfer chamber and then dispensed into 25 ml glass vials.

Experimental Design and Enumeration of Rhizobia

Seed inoculation with each sticker was done in quadruplicate for each legume species. The inoculated seeds from each replication were grouped into three batches of 100 seeds. Each batch of 100 seeds was placed in a test-tube and the cap was kept loose to allow for gas exchange. One tube of seeds was taken from each replication to give a set of four tubes. Each set was stored at 4, 28 and 34°C. To determine the survival of rhizobia, 20 seeds were asceptically removed from each tube and enumeration was done as described elsewhere (Somasegaran &

Hoben 1985). The stored seeds were sampled at 0, 1, 3 and 9 days, and each sampling of 20 seeds was removed from each tube.

Plant Tests

Plant tests were undertaken to show that the sticker did not interfere with the seed germination and nodulation of the plants. Only seeds (peanut and soybean) stored at 34°C were tested for germination as it was expected that loss in seed viability was more likely at this temperature. For the germination test, 100 inoculated seeds of each legume species and sticker treatment were stored at 34°C for 3 days. Seeds were then sown in sterile vermiculite. Sterilized non-inoculated seeds were used as controls. Plant effectiveness tests were also conducted in the greenhouse. Two seeds were planted per modified Leonard jar (Vincent 1970) with four replications per treatment. The plants were harvested after 5 weeks. Parameters measured were nodule mass, nodule number and shoot dry weight.

Results and Discussion

We chose gum arabic and water as control treatments against which to compare the oils. Gum arabic was used because of its good qualities as it provides excellent adhesion and its presence enhanced the number of infected root hairs, promoted early nodulation and improved growth (Subba Rao *et al.* 1971). Water was chosen because we considered it a poor inoculant adhesive even though it is widely used and even recommended by inoculant companies.

The quantities of sticker materials required to coat 1 g of inoculant onto 300 seeds differed for each sticker treatment. This was because the adherence of the inoculant to the seed varied with seed surface, moisture absorption and seed size. In most cases, the glue-like quality of gum arabic allowed for maximum adhesion of the peat inoculant followed by water, which initially resulted in good coating and a rather brittle pellet after evaporation and absorption into the seed. The oils, being only moderately sticky and not absorbable by the seeds, bound less inoculant. This was especially true with the small, rather hard and smooth seed coat bean seeds. It was seen to a lesser extent with soybean and peanut seeds. An exception were the chickpea seeds. Their corrugated and somewhat rough surface gave the oil treatments no less sticking capability than gum arabic.

With all legume seeds and sticker combinations, the initial (0 day) high numbers of viable rhizobia that survived on the seed (Tables 1 to 4) indicated that sufficient inoculant was initially bound to the seeds to comply with the standards of countries where minimum standards exist, for example Canada where large-seeded legumes are required to have 10^5 rhizobia per seed (Food and Agricultural Organization 1984). Except for the bean seeds (Table 1) high numbers were retained for all treatments exposed to 4 and 28°C even after 9 days of storage (Tables 2 to 4). At 34°C the decline in cell numbers became more obvious than at 4 and 28`C, and both adhesives and storage times became very significant sources of variation (Table 5). Rhizobial numbers on the bean seeds stored at 34°C (Table 1) dropped rapidly in all sticker treatments, except gum arabic where a 1.5 log drop after 9 days of storage resulted in still acceptable levels.

In the case of chickpea seeds, the oil treatments were clearly superior to gum arabic or water in supporting a high number of chickpea rhizobia (Table 2) and there was a significant interaction ($P = 0.001$) at 34°C between adhesives and storage time (Table 5). Both the mineral oil and the soybean oil treatments maintained viable rhizobia one log above gum arabic and 4×10^4 per seed; an increase of 1.5 log in the case of peanut oil. This is sufficient inoculant in some cases. When chickpea was grown in an Ultisol an inoculation rate of 10^4 rhizobia per seed was sufficient to obtain maximum shoot nitrogen in two chickpea genotypes and in contrast, a rate of 10^6 rhizobia per seed was necessary to realize

Table 1. Influence of adhesives, storage temperatures, and time on the survival of *R. leguminosarum* bv. *phaseoli* on inoculated bean seeds. Results given as $\log_{10}$ number of rhizobia per seed.

Adhesive		4°C†			28°C			34°C		
	0*	1	3	9	1	3	9	1	3	9
Gum arabic	6.54	6.71	6.55	6.13	6.08	5.85	5.63	5.85	5.37	5.08
Mineral oil	5.62	5.89	5.64	5.58	5.50	5.40	5.22	5.44	4.91	3.84
Peanut oil	5.50	5.46	5.12	4.85	5.06	4.45	4.82	4.62	4.49	3.10
Soybean oil	5.52	5.30	5.22	4.68	5.08	4.89	4.27	4.22	3.47	2.10
Water	6.18	6.03	5.73	5.68	5.47	4.92	4.90	5.17	4.69	3.89
LSD (0.05)	0.20	0.16	0.24	0.23	0.33	0.68	0.45	0.75	0.40	0.32

* Storage time in day(s). † Storage temperature. LSD—least significant differences.

Table 2. Influence of adhesives, storage temperatures, and time on the survival of *Rhizobium* spp. (chickpea) on inoculated chickpea seeds. Results given as $\log_{10}$ number of rhizobia per seed.

Adhesive		4°C†			28°C			34°C		
	0*	1	3	9	1	3	9	1	3	9
Gum arabic	6.65	6.68	6.66	6.61	6.76	6.28	6.01	5.83	4.75	2.73
Mineral oil	6.65	6.58	6.62	6.46	6.50	6.14	5.85	5.84	5.00	3.65
Peanut oil	6.70	6.59	6.41	6.46	6.65	6.25	5.53	6.20	5.62	4.43
Soybean oil	6.73	6.63	6.66	6.58	6.57	6.35	5.61	6.32	5.57	3.86
Water	6.30	6.28	6.37	5.94	6.21	5.95	5.72	5.32	4.59	2.99
LSD (0.05)	0.22	0.10	NS	0.21	0.17	NS	0.24	0.25	0.24	0.40

*† As in Table 1. LSD—least significant differences. NS—not significant.

Table 3. Influence of adhesives, storage temperatures, and time on the survival of *Bradyrhizobium japonicum* on inoculated soybean seeds. Results given as $\log_{10}$ number of rhizobia per seed.

Adhesive		4°C†			28°C			34°C		
	0*	1	3	9	1	3	9	1	3	9
Gum arabic	7.28	7.05	6.97	6.62	6.74	6.39	5.90	5.88	5.18	3.69
Mineral oil	6.58	6.50	6.40	6.12	6.22	5.78	5.33	5.62	4.99	3.82
Peanut oil	6.88	6.57	6.31	6.02	6.06	6.05	5.25	5.64	5.19	3.39
Soybean oil	6.71	6.58	6.51	6.07	6.36	5.88	5.21	5.39	5.03	3.58
Water	7.05	6.70	6.78	6.47	6.32	6.21	5.80	6.01	4.47	4.10
LSD (0.05)	0.11	0.10	0.17	0.17	0.22	0.22	0.28	0.28	NS	NS

*† As in Table 1. LSD—least significant differences. NS—not significant.

maximum shoot nitrogen for either chickpea genotype grown on an Oxisol (Somasegaran et al. 1988).

In the case of soybean and peanut seeds, gum arabic bound seven times as much inoculant to the seeds at the time of coating. The death rate was, however, more rapid at 34°C so that even after 1 day of storage, gum arabic offered no advantage over the oils. After 9 days, all treatments still showed approximately 10^4 rhizobia

Table 4. Influence of adhesives, storage temperatures, and time on the survival of *Bradyrhizobium* sp. on inoculated peanut seeds. Results given as $\log_{10}$ number of rhizobia per seed.

Adhesive	4°C†			28°C			34°C		
	0*	1	3	9	1	3	9	1	3	9
Gum arabic	6.31	6.19	6.19	6.19	6.28	6.10	6.08	6.10	5.53	4.73
Mineral oil	5.75	5.68	5.72	5.74	5.75	5.61	5.61	5.48	5.26	4.54
Peanut oil	5.77	5.50	5.37	5.23	5.36	5.19	5.19	5.40	4.89	4.04
Soybean oil	5.76	5.74	5.73	5.65	5.69	5.45	5.45	5.41	4.87	4.42
Water	6.00	6.05	5.65	5.85	6.06	5.93	5.93	5.69	5.44	4.56
LSD (0.05)	0.22	0.18	0.40	0.18	0.21	0.22	0.22	0.34	0.34	NS

*† As in Table 1. LSD—least significant differences. NS—not significant.

Table 5. Summary of significances of the sources of variation in the analysis of variance on the survival of soybean, peanut, bean and chickpea rhizobia on inoculated seed stored at 34°C.

Sources of variation	df	Rhizobia of			
		soybean	peanut	bean	chickpea
Replications	2	NS*	NS	NS	NS
Adhesives (A)	4	***	***	***	***
Storage time (T)	2	***	***	***	***
A × T	8	NS	NS	NS	**

* Not significant at the 0.05 level.
,* *F*-value significant at 0.01 and 0.001 probability levels, respectively.

per seed, which is sufficient for effective nitrogen fixation. Maximum total nitrogen in plant tops of soybean *[Glycine max (L.)* Merr.] was achieved at inoculation rates of 2×10^3 for soil-grown plants (Weaver & Frederick 1972). In the case of peanut, a 1 day storage of oil-coated seeds may be permitted as sufficient numbers of rhizobia survived to meet inoculation requirements (Table 4). Groundnut cultivars required 10^6 rhizobia per seed or more for maximum nodulation and nitrogen fixation (Nambiar *et al.* 1983).

Rhizobia dry rapidly under arid conditions, whether on the seed or in the soil (Vincent *et al.* 1962; Burton 1979). The death rate was slowed down by the addition of gum arabic or maltose (Vincent *et al.* 1962). Survival of rhizobia was improved when a sticker was made from methylcellulose (2%), sucrose (10%) and glutamate (10%) (Salema *et al.* 1982) and these researchers surmised that this protectant mixture could have improved survival by maintaining a sufficient bound water content in the cell envelope. It was also their conclusion that the protectant was able to functionally replace the water lost during dessication and thus prevented the formation of unfavourable conformations in labile structures.

Kremer & Peterson (1982) prepared rhizobial inoculants from vegetable oil and freeze-dried cultures. These oil-based inoculants promoted higher survival of rhizobia on seed than did peat-based inoculants. Apparently, oil acted as a barrier against evaporation.

Our results suggest that oil has the same protective effect when used as a sticker with a powdered peat inoculant. An oil envelope may possibly form around rhizobial cells preventing moisture from escaping. Since protection from dessication seems so important, it was interesting that the water treatment

supported the viability of large numbers of rhizobia after 1 day of storage at all three temperatures (Tables 1 to 4). The death rate during the drying of cells in water appeared to be inversely related to the concentration of the inoculum (Vincent *et al. 1962).* The numbers surviving on the seed in the water treatment were, in most cases, higher than in the oil treatments and the actual die-off rate was greater than in the other treatments. Although more moisture may have been lost, there was actually more water available since twice the volume was required to stick 1 g of inoculant to the seed than in any other treatment. The excess was absorbed by the seed and may have served as a protective source of moisture during the drying period. Nevertheless, water can be used as a sticker but we do not recommend it, because under field conditions the seeds are not handled as carefully as in this experiment and much less final adhesion is expected at the time of sowing.

Germination tests showed that oil had no adverse effect on seedling development. Germination rates equaled those of the controls which were *97%.*

An inoculation test for all sticker treatments stored for 1 day at 34°C was conducted in Leonard jars on soybean and peanut only, as greenhouse temperatures were not suitable for the more temperate climatic adapted beans and chickpeas. The plants grew normally and showed no significant differences amongst the various treatments including the control treatment of gum arabic which had been incubated at *28°C* (Table 6). Since all treatments had in excess of 10^5 viable cells per seed, differences would have been surprising although it has previously been reported that plants inoculated with oil-based inoculants have higher nodule numbers -and weights than plants inoculated with peat-based inoculants (Kremer & Peterson *1982).*

In conclusion, we submit that the oils tested have most of the qualities expected of a good adhesive for seed inoculation. They are reasonably sticky, inexpensive, readily available, non-toxic to seed and microbe, and most important, they protect the rhizobia from desiccation. These characteristics make oils uniquely suitable for application in developing countries where elevated temperatures are a problem and gum arabic cannot be considered because of high cost.

However, as has been shown with the bean seeds the oils tested were not equally good as inoculant adhesives for all legume seeds. More work is required to find suitable adhesives for other agriculturally important legumes. Perhaps more viscous and tenacious oils are required for the smaller, hard-coated varieties.

Table 6. Response of soybean and peanut to inoculation using oils, gum arabic and water and inoculant adhesives. Results given per plant.

Adhesives	Soybean			Peanut		
	Nod. no.	Nod. wt (mg)	Shoot wt (g)	Nod. no.	Nod. wt (mg)	Shoot wt (g)
Peanut oil	17	100	1.5	68	85	1.8
Soybean oil	17	100	1.4	44	42	1.0
Mineral oil	18	120	1.4	75	62	1.2
Gum arabic	15	110	1.4	64	75	1.3
Gum arabic*	18	150	1.5	64	65	1.5
Water	16	120	1.3	67	50	1.6
Uninoculated	0	0	0.63	0	0	0.7
LSD	NS	NS	NS	NS	50	NS

* Inoculated seeds were incubated at 28°C. All others were incubated at 34°C for 24 h before planting. Nod.—nodule. LSD—least significant differences. NS—not significant.

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