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# *Bradyrhizobium japonicum* Survival in and Soybean Inoculation with Fluid Gels

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**The utilization of gels, which are used for fluid drilling of seeds, as carriers of *Bradyrhizobium japonicum* for soybean (*Glycine max* (L.) Merr.) inoculation was studied. Gels of various chemical composition (magnesium silicate, potassium acrylate-acrylamide, grafted starch, and hydroxyethyl cellulose) were used, although the hydroxyethyl cellulose gels were more extensively investigated. Gel inocula were prepared by mixing gel powder with liquid cultures of *B. japonicum* (2% [wt/vol]). The population of *B. japonicum* USDA 110 did not change in each gel type during 8 days of incubation at 28°C. These fluid gels were prepared with late-exponential-growth-phase cells that were washed and suspended in physiological saline. Mid-exponential-growth-phase *B. japonicum* USDA 110, 123, and 138 grew in cellulose gels prepared with yeast extract-mannitol broth as well as or better than in yeast extract-mannitol broth alone for the first 10 days at 28°C. Populations in these cellulose gels after 35 days were as large as when the gels had originally been prepared, and survival occurred for at least 70 days. Soybeans grown in sand in the greenhouse had greater nodule numbers, nodule weights, and top weights with gel inoculants compared with a peat inoculant. In soil containing $10^3$ indigenous *B. japonicum* per g of soil, inoculation resulted in increased soybean nodule numbers, nodule weights, and top weights, but only nodule numbers were greater with gel than with peat inoculation. The gel-treated seeds carried $10^2$ to $10^3$ more bacteria per seed ($10^7$ to $10^8$) than did the peat-treated seeds.**

Carriers of microbial inoculants need to supply sufficiently large populations of viable, beneficial microorganisms in order to positively affect plant growth. Plants most commonly inoculated with microorganisms are legumes with the appropriate rhizobia. Although peat is the most common commercial carrier medium, a variety of inoculant carriers (e.g., liquid growth medium, vermiculite, and humus) and adhesion agents (e.g., gum arabic, methyl cellulose, and oil) have been used for microbial inoculation of seeds (19, 20, 22, 25). This paper describes research on the feasibility of applying beneficial microorganisms with seeds in gels which are used in fluid drilling of crop seeds. *Bradyrhizobium japonicum* and soybean served as the test system.

Fluid drilling refers to the process of planting seeds or pregerminated seedlings coated in a gelatinous material. The gels used in fluid drilling must have rheological properties and have, therefore, a flowable consistency. Advantages of fluid drilling include more rapid emergence, greater germination synchronization, the ability to plant in cooler soils, and the possibility of incorporating additives, such as pesticides and nutrients, in the gels (5, 7, 8, 17). Practical use of fluid drilling has been generally restricted to small-seeded horticultural plants such as celery (*Apium graveolens* L.), carrot (*Daucus carota* L.), and flowers, although large-seeded crops such as field beans (*Phaseolus vulgaris* L.) have been fluid drilled (9). The development of a fluid drill (Kamterter, Inc., Lincoln, Nebr.) that more readily accommodates large seeds may result in increased utilization of the fluid drilling technique for plants such as corn (*Zea mays* L.) and soybean.

Gelatinous substances previously have been used as carriers for rhizobia (3, 11, 13, 18). Advantages of gels include the protection of microorganisms from desiccation and good microorganism-seed contact (13, 16). Dommergues et al. (3) showed that *B. japonicum* entrapped in a polyacrylamide gel could be used to inoculate soybeans. They used solidified wet blocks and wet crushes of the gel, air-dried gel blocks, and gel powder as inoculant carriers. These gel-based inoculants compared favorably with a peat-based inoculant in pot experiments. Jung et al. (13) successfully used dried and semidried polyacrylamide, alginate, and a xanthan and carob gum mixture as carriers for *Rhizobium* inoculants. The gels used in these previous studies, however, were not of a fluid consistency during seed inoculation and were not used in conjunction with fluid drilling. Pot-grown dwarf french (field) beans were successfully inoculated with *R. phaseoli* by using a wet alginate gel (18). Field beans inoculated with *R. phaseoli* in an alginate gel during fluid drilling had more nodules per plant 28 days after sowing than did beans inoculated with a peat carrier (11). The survival characteristics of rhizobia in the gel carriers were not reported in any of these studies.

Our objectives were to determine the ability of *B. japonicum* strains to survive in fluid gels and to measure the response of soybeans to *B. japonicum* inoculation with fluid gel carriers. The survival of *B. japonicum* was measured in gels made with yeast extract-mannitol (YEM) broth cultures and in gels made with washed cell suspensions. Soybean responses to gel and peat inoculants were measured in greenhouse experiments. Cellulose-based gels were those principally used, although gels with other chemical compositions were also tested.

## MATERIALS AND METHODS

**Bacterial strains and carriers.** *B. japonicum* USDA 110, 123, and 138 were used. Strain 123 was resistant to rifampin at a concentration of 500 mg/liter. *B. japonicum* strains were maintained on YEM agar slants at 5°C.

Four different cellulose-based gels (Natrosol H4BR, MBR, and HHR [Hercules, Inc., Wilmington, Del.] and

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N-gel [Kamterter, Inc., Lincoln, Nebr.]) differing in the type and amount of side-chain substitution on the hydroxyethyl cellulose backbone were used. The side-chain composition imparts different relative viscosities and biodegradabilities to the cellulose gels. Other gels used were a magnesium silicate gel (Laponite) (Laporte, Ltd., Grimsby, South Humberside, England), a potassium acrylate-acrylamide copolymer gel (Viterra Agri-gel) (Nepera, Inc., Harriman, N.Y.), and a grafted starch gel (Waterlok) (Grain Processing Corp., Muscatine, Iowa). All these gel-forming substances are powders before liquid is added. The powders were sterilized by autoclaving them at 121°C for 15 min. Gel inocula were prepared with an equivalent of 20 g of powder per liter of solution containing *B. japonicum*, thus producing 2% (wt/vol) fluid gels. In general, fluid gels need to have a viscosity between 100 and 2,000 g/m per sec (centipoise [cP]), which corresponds to the range of 1.75 to 2.5% (wt/vol) mixtures for the gels used in this study (Kamterter). Peat inoculum was prepared by mixing *B. japonicum* solution with autoclaved, neutralized, sterilized peat (used in commercial inocula) and gum arabic. A mixture ratio of 10 ml of broth culture to 10 g of peat and 1 g of gum arabic was used.

**Survival in fluid gels.** Survival of mid-exponential-phase *B. japonicum* USDA 110, 123, and 138 was determined in N-gel and H4BR cellulose gels prepared with both YEM broth cultures and cultures of cells suspended in physiological saline solution. After incubation in separate flasks of YEM broth for 4 days at 28°C, the culture of each *B. japonicum* strain was divided into two equal portions. Broth was removed from one portion by centrifugation at 13,000 × *g* for 15 min at 5°C. The cells were washed twice by suspension in sterile physiological saline (8.5 g of NaCl per liter) followed by centrifugation as previously described. After the final wash, cells were suspended in a volume of sterile saline equivalent to the initial volume. The physiological saline and YEM cultures were each subdivided into three sterile beakers. An amount of H4BR cellulose gel powder sufficient to make a 2% (wt/vol) fluid gel was aseptically added to one of the beakers, an equal amount of N-gel cellulose gel was added to another beaker, and no gel additions were made to the third beaker. A total of six treatments (unaltered YEM broth culture, N-gel with YEM broth culture, H4BR gel with YEM broth culture, a physiological saline culture without gel, N-gel with saline culture, and H4BR gel with saline culture) were established for each strain. Cultures from each of the six treatments for a particular strain were aseptically dispensed into sterile, capped test tubes (3.0 ml/tube) in triplicate and incubated at 28°C. *B. japonicum* CFU were measured after 0, 1, 3, 6, 8, 10, 16, 23, and 35 days using standard dilution and YEM agar plate count procedures (20). The CFU in the cellulose gel treatments, except for strain 138 in N-gel made with YEM broth and in H4BR gel made with cells suspended in physiological saline, were also measured after 70 days. The initial dilution used to determine the *B. japonicum* CFU consisted of 1 g of gel inoculum in 99 ml of physiological saline. Colonies were counted after plates had been incubated for 10 days at 28°C. This study was analyzed as a completely randomized design, with analysis of variance conducted for each time period sampled. Factors evaluated were strains (three), carriers (three), and medium (broth or saline).

Survival of *B. japonicum* 110 in all seven gel formulations was determined by using late-exponential- to stationary-growth-phase cultures to prepare the gels. A YEM broth culture that had been incubated for 7 days at 28°C was centrifuged, washed, and suspended in physiological saline as previously described. Fluid gels were then prepared by using the saline cell suspension. Bacterial CFU were measured after 0, 2, 4, 6, and 8 days by using the dilution procedure previously described and standard YEM agar plate counts. This study was analyzed as a completely randomized design, and analysis of variance was conducted for each gel to evaluate the effect of time on *B. japonicum* survival.

The influence of temperature (5, 15, 25, 35, and 45°C) on the survival of *B. japonicum* strains 110, 123, and 138 in the HHR and H4BR cellulose gels was also determined. Gel inocula were prepared with YEM broth cultures that had been incubated at 28°C for 5 to 7 days. *B. japonicum* CFU were determined after 0, 1, 2, 3, 4, 6, and 8 days for two trials and after 0, 1, 2, and 3 days for another two trials by using the dilution procedures previously described and YEM plate counts. A completely randomized design was used, and analysis of variance was performed for each time period sampled. Factors evaluated were strains (three), gels (two), and temperature (five).

**Greenhouse studies.** Three greenhouse studies were conducted. Greenhouse temperatures were maintained between 20 and 30°C. The plants received approximately 14 h of light per day. When necessary, metal halide lamps were used to provide additional hours of light. No greenhouse experiments were conducted during the summer months. Soybean (cv. Woodsworth) nodule numbers, nodule weights, and top weights were measured. In the first study, all seven gel formulations and peat were used as carriers for *B. japonicum* USDA 110. In the second study, each *B. japonicum* strain (110, 123, and 138) was used in HHR and H4BR cellulose gel inocula and a peat inoculum. In both of these studies, the soybeans were grown in sand. The third study was identical to the second, except that a Sharpsburg silty clay loam soil (fine, montmorillonitic, mesic typic Argiudoll) was used instead of sand. The native bradyrhizobial population in this soil was estimated by a most-probable-number procedure (23).

For all the greenhouse studies, soybean seeds were surface sterilized by soaking them first in ethanol for 2 to 3 min and then in 10% bleach for 4 min. Seeds were rinsed at least five times with sterile distilled water. Each strain of *B. japonicum* was grown in a separate flask of YEM broth until mid- to late-exponential phase (5 to 7 days) and then split into the number of carrier treatments used. The fluid gel and peat inoculums were prepared as previously described. Fifty surface-sterilized soybean seeds were added per 50 ml of fluid gel inoculum (1 ml of fluid gel per seed) or per 0.2 g of peat inoculum (0.004 g of peat per seed) (20) and thoroughly mixed. Control treatments consisted of surface-sterilized seeds with fluid gel prepared with sterile YEM broth containing no *B. japonicum* and surface-sterilized seeds without any carrier applied.

The soybeans were grown in modified Leonard jar assemblies by using nitrogen-free nutrient solution (24). Autoclaved cotton ropes were used instead of cotton strips to connect the containers. Three seeds per pot (11 cm in diameter and 10 cm deep) were planted by using a separate sterile spatula for each treatment to transfer the seeds. The amount of inoculum transferred was that which adhered to the seed. Ten to 14 days after germination, soybeans were thinned to one per pot by cutting the extra seedlings just below the first node. Soybeans were harvested approximately 7 weeks after planting, and soybean top dry weights, nodule numbers, and nodule dry weights were measured.

The CFU of *B. japonicum* applied per seed was deter-

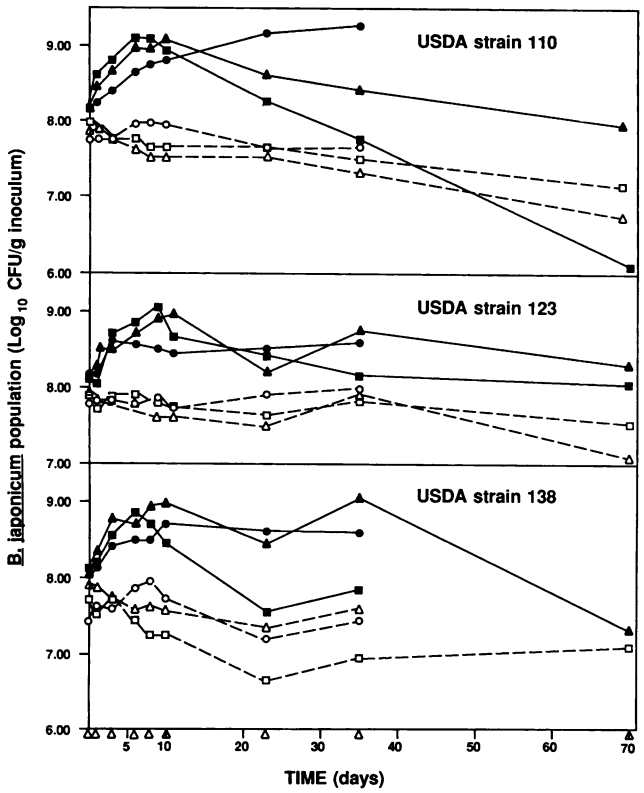


FIG. 1. Survival of *B. japonicum* USDA 110, 123, and 138 in YEM broth (●), in two cellulose gels prepared with YEM broth (H4BR [▲] and N-gel [■]), in physiological saline (○), and in two cellulose gels prepared with washed cultures suspended in physiological saline (H4BR [△] and N-gel [□]). Each point is the mean of three replicates of three trials.

mined for each treatment immediately after planting. Three seeds from each treatment were each placed in a separate dilution bottle by using the same procedure that was used in planting the seeds. Standard dilution and plate count procedures were used to determine the CFU per seed (20).

Duncan's multiple range test was used to analyze the data from the first study, in which inoculant was carried by each gel and by peat. This study had 15 replicates of each treatment and was repeated three times. Greenhouse studies using the three *B. japonicum* strains with two cellulose gels and peat in sand and soil were repeated three times by using a completely randomized design containing 15 replicates of each treatment (strain-carrier combinations). The study using the Sharpsburg soil was repeated an additional three times by using a completely randomized block design. Analyses of variance for each trial and for all trials combined were performed on the data. Factors evaluated were strains (three), carriers (three), and trials (three or six).

## RESULTS

**Survival in fluid gels.** *B. japonicum* USDA 110, 123, and 138 were able to survive at least 70 days in the N-gel and H4BR cellulose gels at 28°C (Fig. 1). Growth of *B. japonicum* in the fluid cellulose gel inocula was observed when YEM broth cultures in mid-exponential phase were used to prepare the gels. During the first 10 days, each strain's growth in the cellulose gels prepared with YEM broth culture was equivalent to or greater than its growth in YEM broth without gel. After 10 days, YEM broth without gel supported the highest strain 110 population, but at least one of the gels prepared with YEM broth contained populations equivalent to those in YEM broth alone for the other two strains. By day 35, higher populations were maintained for all three strains in the H4BR compared with the N-gel prepared with YEM broth.

Survival but no growth occurred when YEM was not present in the cellulose gels (cells suspended in physiological saline). The population of each strain was similar in physiological saline with or without gel, except for strain 138, which had a lower population in the N-gel than in the other two physiological saline mediums after 6 days.

There were differences in the growth and survival of *B. japonicum* which depended on the strain, the medium, and the gel used. These interactions reflected growth and survival differences of each strain in the two gels, depending upon the culture medium. Despite differences in survival patterns, populations for all gel treatments (except for strain 138 in N-gel prepared with cells in physiological saline) at 35 days were equivalent to or greater than those present initially. The viscosity of the gels did not visibly change during the course of this study.

All seven of the gel formulations tested supported the survival of *B. japonicum* 110. Late-exponential- to stationary-growth-phase cultures suspended in physiological saline survived for at least 8 days with no statistical change in population over time at 28°C (data not shown). Again, there was no observable change in viscosity for any of the gels. An *Azospirillum brasilense* strain was also able to survive for at least 8 days with no significant change in population over time in the HHR and H4BR cellulose gels.

The effect of temperature on the growth and survival of bradyrhizobia was not altered by the fluid gels. For all strains there was little change in population at 5°C, some growth at 15°C, greatest growth at 25°C, and a large decrease in population at 45°C (data not shown). The only difference between strains (at the 0.05 probability level) was the ability of strain 110 to survive at 35°C. It grew slightly for the first 3 days before decreasing in population thereafter, whereas strains 123 and 138 slowly and steadily decreased in population.

**Greenhouse studies.** Increased soybean growth and nodulation were observed with the gel carriers for soybeans grown in sand and inoculated with *B. japonicum* 110 (Table 1). Some nodules were observed on the uninoculated soybean roots, but healthy plants did not develop. Soybeans inoculated with the peat carrier appeared to be healthy but had fewer nodules and lower nodule and top weights than those inoculated with the gel carriers. Soybeans inoculated with the grafted starch gel had lower nodule and top weights than did those plants inoculated with any of the other gels. Both gel and peat carriers contained $10^7$ to $10^8$ bradyrhizobia per g of carrier. However, the gel-coated seeds carried $10^7$ to $10^8$ CFU of *B. japonicum* 110 per seed, whereas the peat-coated seeds carried $10^4$ to $10^5$ CFU per seed.

Differences in nodule number, nodule weight, and top weight in soybean plants inoculated with *B. japonicum* strains 110, 123, and 138 in HHR and H4BR cellulose gels and peat are shown in Table 2. Again, some nodules formed on uninoculated soybean roots, but as before, these plants appeared unhealthy and had significantly fewer nodules and lower nodule and top weights than did inoculated plants (Table 3). The soybeans formed significantly more nodules with gel-carried inoculant than with peat-carried inoculant for each strain. Gel inoculation also resulted in greater

TABLE 1. The effects of inoculation with *B. japonicum* 110 in fluid gel and peat carriers on Woodsworth soybean after 7 weeks of greenhouse growth in sand[a]

| Carrier | Nodule no. (nodules/plant) | Nodule dry wt (g/plant) | Top dry wt (g/plant) |
|---|---|---|---|
| HHR-cellulose gel[b] | 94a | 0.40a | 2.46a |
| MBR-cellulose gel | 92a | 0.40a | 2.50a |
| H4BR-cellulose gel | 92a | 0.41a | 2.57a |
| N-gel cellulose gel | 89a | 0.41a | 2.52a |
| Mg-silicate gel | 92a | 0.41a | 2.49a |
| Acrylate-acrylamide gel | 92a | 0.42a | 2.39a |
| Grafted starch gel | 84a | 0.32b | 2.09b |
| Peat | 48b | 0.23c | 1.58c |
| Noninoculated controls | 24c | 0.10d | 0.67d |

[a] Values are means of 45 replications (15 plants per treatment, three trials). Numbers in the same column followed by the same letter are not significantly different at the 5% level of probability according to Duncan's new multiple range test.

[b] Cellulose gel designations denote different side-chain substitutions.

nodule and top weight than did inoculation with peat for each strain in at least one of the cellulose gels, so that when the data for all the strains were pooled, the gel inoculants outperformed the peat inoculant for each soybean parameter measured. Whereas soybean nodule and top weights were greater with strain 110 in both gels compared with peat, nodule and top weights were greater with strains 123 and 138 in only one of the two cellulose gels (HHR and H4BR) for the two strains, respectively, compared with peat. No difference in soybean responses between the two gels was observed, but carrier effects on nodule weight were not consistent among strains. Relatively high overall variations were obtained, in part due to variability between trials. Also, including a control treatment contributed significantly to the overall variation (CV) due to the significant response to inoculation. Differences between the strains for nodule and top weight were detected. Strains 110 and 123 produced more soybean top growth and nodule weight than did strain 138. As in the previous study, the seeds inoculated with gels had more *B. japonicum* ($10^7$ to $10^8$ per seed) than did the peat-inoculated seeds ($10^4$ to $10^5$ per seed).

When soybeans were grown in soil containing approximately $10^3$ indigenous *B. japonicum* per g of soil instead of in sand, inoculation still resulted in soybeans with increased nodule numbers, nodule weights, and top dry weights (Tables 4 and 5). However, gel inoculation only increased nodule numbers relative to peat inoculation. No differences in soybean responses were detected between the two gels. Although soybean nodule numbers did not differ among the three strains, inoculation with strains 123 and 110 resulted in increased nodule weight, and especially top dry weight, compared with inoculation with strain 138. Furthermore, nodule weight was greater with strain 123 inoculation compared with strain 110 inoculation. Carrier effects were consistent among strains for overall means in soil, but as with sand there were differences in carrier effects among strains for individual trials (not shown).

The noninoculated soybeans grown in this soil did develop into healthy plants and were well nodulated. These soil-grown uninoculated plants had greater top growth and more nodules than did the successfully inoculated soybeans grown in sand (Table 2). However, the pattern of nodule formation on the roots of these soil-grown soybeans was strikingly different for gel-inoculated and uninoculated soybeans. The gel-inoculated plants had most of their nodules clustered around the tap root, while the nodules of the uninoculated plants were distributed more uniformly along their roots. The nodulation pattern with the peat inoculum was intermediate between the gel and uninoculated patterns.

TABLE 2. The effects of inoculation with *B. japonicum* USDA 110, 123, and 138 in H4BR and HHR cellulose gels and peat carriers on Woodsworth soybeans after 7 weeks of greenhouse growth in sand[a]

| Strain and carrier | Nodule no. (nodules/plant) | Nodule dry wt (g/plant) | Top dry wt (g/plant) |
|---|---|---|---|
| 110 | | | |
| H4BR-cellulose gel | 62 | 0.307 | 1.89 |
| HHR-cellulose gel | 72 | 0.324 | 2.11 |
| Peat | 31 | 0.206 | 1.36 |
| 123 | | | |
| H4BR-cellulose gel | 67 | 0.280 | 1.70 |
| HHR-cellulose gel | 76 | 0.344 | 1.96 |
| Peat | 48 | 0.272 | 1.70 |
| 138 | | | |
| H4BR-cellulose gel | 75 | 0.254 | 1.52 |
| HHR-cellulose gel | 76 | 0.183 | 1.23 |
| Peat | 40 | 0.188 | 1.22 |
| Pooled strains | | | |
| H4BR-cellulose gel | 68 | 0.280 | 1.70 |
| HHR-cellulose gel | 75 | 0.283 | 1.77 |
| Peat | 40 | 0.221 | 1.43 |
| None (noninoculated) | | | |
| H4BR-cellulose gel | 27 | 0.074 | 0.59 |
| HHR-cellulose gel | 25 | 0.093 | 0.61 |
| None | 24 | 0.079 | 0.59 |
| Coefficient of variation (CV) (%) | 40 | 49 | 47 |

[a] Values for each strain-carrier combination are averages of 45 replications (15 plants per treatment, three trials).

TABLE 3. Analysis of variance of data in Table 2

| Comparison (degrees of freedom) | Level of significance ($P$)[a] | | |
|---|---|---|---|
| | Nodule no. (nodules/plant) | Nodule dry wt (g/plant) | Top dry wt (g/plant) |
| Noninoculated vs inoculated (1) | 0.01 | 0.001 | 0.001 |
| Among inoculated | | | |
| Gels vs peat (1) | 0.001 | 0.01 | 0.05 |
| H4BR vs HHR (1) | NS | NS | NS |
| Strains 110 and 123 vs 138 (1) | NS | 0.001 | 0.01 |
| Strain 110 vs 123 (1) | NS | NS | NS |
| Carrier × strain (4) | NS | 0.1 | NS |

[a] NS, Not significant.

## DISCUSSION

Bradyrhizobia were able to survive in gels used for fluid drilling. The cellulose gels tested were apparently not utilized as a substrate and did not hinder bacterial growth when nutrients (YEM) were supplied. The high water-holding capacity of the gels and their ability to suspend nutrients in forms available for rhizobial growth are desirable properties of suitable carriers (19, 25). These fluid gels are similar to soft agar in terms of texture. As long as beneficial gel properties are maintained, fluid gels should be able to serve as carriers for bradyrhizobia as well as for other microor-

TABLE 4. The effects of inoculation with *B. japonicum* USDA 110, 123, and 138 in H4BR and HHR cellulose gel and peat carriers on Woodsworth soybeans after 7 weeks of greenhouse growth in soil[a]

| Strain and carrier | Nodule no. (nodules/plant) | Nodule wt (g/plant) | Top dry wt (g/plant) |
|---|---|---|---|
| 110 | | | |
| H4BR-Cellulose gel | 91 | 0.299 | 2.73 |
| HHR-Cellulose gel | 95 | 0.279 | 2.49 |
| Peat | 91 | 0.291 | 2.52 |
| 123 | | | |
| H4BR-Cellulose gel | 100 | 0.315 | 2.56 |
| HHR-Cellulose gel | 105 | 0.318 | 2.69 |
| Peat | 86 | 0.307 | 2.65 |
| 138 | | | |
| H4BR-Cellulose gel | 102 | 0.282 | 2.30 |
| HHR-Cellulose gel | 100 | 0.283 | 2.29 |
| Peat | 87 | 0.293 | 2.42 |
| Pooled strains | | | |
| H4BR-Cellulose gel | 98 | 0.299 | 2.52 |
| HHR-Cellulose gel | 100 | 0.293 | 2.49 |
| Peat | 91 | 0.297 | 2.53 |
| None (noninoculated) | | | |
| H4BR-Cellulose gel | 76 | 0.261 | 2.16 |
| HHR-Cellulose gel | 78 | 0.266 | 2.22 |
| None | 79 | 0.284 | 2.22 |
| Coefficient of variation (CV) (%) | 34 | 28 | 26 |

[a] Values for each strain-carrier combination are averages of 90 replications (15 plants per treatment, six trials).

TABLE 5. Analysis of variance of data in Table 4

| Comparison (degrees of freedom) | Level of significance (*P*)[a] | | |
|---|---|---|---|
| | Nodule no. (nodules/plant) | Nodule dry wt (g/plant) | Top dry wt (g/plant) |
| Noninoculated vs inoculated (1) | 0.001 | 0.01 | 0.001 |
| Among inoculated | | | |
| Gels vs peat (1) | 0.001 | NS | NS |
| H4BR vs HHR (1) | NS | NS | NS |
| Strains 110 and 123 vs 138 (1) | NS | 0.1 | 0.001 |
| Strain 110 vs 123 (1) | NS | 0.1 | NS |
| Carrier × strain (4) | NS | NS | NS |

[a] NS, Not significant.

ganisms. Gel carriers may be most useful for microorganisms that are incompatible with peat.

There was concern that decreased oxygen availability may limit microbial survival in these gels. The rate of plant seed germination usually increases after short-term (24 to 36 h) immersion in gels but decreases thereafter, partially because of oxygen limitations (9). Frazier et al. (6) found that snapdragon (*Antirrhinum majus* L.) seed germination decreased after a 48-h submergence in gel, which was similar to our observations with soybean seeds (data not shown). We did not observe inhibition of bradyrhizobia after 48 h. The factors inhibiting plant seed germination apparently did not affect bradyrhizobia survival in fluid gels. The survival time of bradyrhizobia in the fluid gels easily exceeded that suggested by Strijdom and Deschodt (21) as being at least equivalent to the germination period.

The adherence of the gel to the seed allowed higher *Bradyrhizobium* populations to be carried per seed compared with the peat. The ratio of peat to seed was within recommended amounts (20, 25). Because gel and peat carriers were prepared simultaneously with the same *Bradyrhizobium* cultures, the peat inocula were not cured. Nevertheless, the *Bradyrhizobium* populations per g of peat were within United States standards (19). Methyl cellulose has been used successfully as an adhesive for peat inocula (2, 4). Apparently, adhesive and carrier properties are combined in the fluid gels.

In general, the more bacteria carried by an inoculant, the more likely it will be successful (1, 2, 19, 22, 25). The ability of gels to carry more rhizobia per seed was used by others to explain the positive results obtained with gel inoculation (11, 13) and most likely accounts for the increased soybean growth we observed in sand. This research was intended to evaluate the feasibility of using fluid gels for bradyrhizobial inocula. We did not attempt to optimize comparisons with peat inoculant. Additional research, including field testing, is needed to assess the efficiency of fluid gel inoculation relative to peat inoculation.

All three *B. japonicum* strains used are native to Midwestern U.S. soils and are representative infective and effective strains (10). The better performance of strains 110 and 123 compared with that of 138 probably reflect the greenhouse experimental conditions (e.g., soybean cultivar) used.

Burton (1) reported that multiplication of rhizobia in the rhizosphere favored early nodulation. This may explain the difference in nodulation patterns between the gel and peat inocula and the native soil bradyrhizobia observed in soil. The gels used in the greenhouse studies were prepared with YEM broth cultures, which were shown to support *B. japonicum* growth. Whether gel inoculants enhance the competitiveness of inoculated strains over native soil strains was not addressed in this research. Gels were capable of delivering high microbial populations with seeds, which is necessary for inoculum strains when competing with native soil strains (12, 14).

Storage properties and the capacity for convenient distribution also determine the suitability of a carrier (19, 20, 25). Jung et al. (13) found that semidried or dried alginate and a mixture of xanthan and carob gum were successful *B. japonicum* inoculum carriers, provided that their storage time was less than 90 days. Other media and storage techniques could be combined with fluid gel inoculation. Peat inoculant, concentrated frozen pastes, or lyophilized rhizobia cultures could be added to the fluid gel before planting, since gels can carry particulate matter as long as their fluidity is maintained. Lyophilized bacterial cells could be combined with powdered nutrients and the gel powder and then stored dry until activated with water before planting. An advantage of lyophilization is the ability to concentrate cells. Kremer and Peterson (15) used lyophilization to concentrate rhizobia and combined these with an oil carrier to develop an inoculant with storage and heat-resistance properties superior to those of peat. Unlike oil, the cellulose gels used in our research did not increase the ability of *B. japonicum* to survive at high temperatures.

Both gel powders and fluid gels can be autoclaved for at least 30 min. Dispersal of the gel powder into solution after autoclaving becomes more difficult because of added moisture. The gel powder could not be sterilized with dry heat, since breakdown ("baking") of the powder occurred at temperatures greater than 50°C.

Contamination of the gels by microorganisms, especially

those capable of directly degrading the gel or attacking seeds or seedlings, is a concern. However, we have not experienced problems when sterile conditions were not maintained after fluid gels were prepared and planting occurred within 48 h.

The observance of medium by strain interactions in both the survival and greenhouse studies indicates that even among the cellulose gels, the gel that was best for each strain differed. The differences in bacterial growth between the cellulose gels may reflect dissimilarities in nutrient depletion (including oxygen) or toxin accumulation or both within the gels. The determination of the best gel, cultivar, seed treatment, and microbial combinations warrants further research. Fluid gels have good potential as carriers of beneficial microorganisms in the inoculation of seeds.

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