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Efficiency of Nodule Initiation in Cowpea and Soybean[1]

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ABSTRACT

When serial dilutions of a suspension of *Bradyrhizobium japonicum* strain 138 were inoculated onto both soybean and cowpea roots, the formation of nodules in the initially susceptible region of the roots of both hosts was found to be linearly dependent on the log of the inoculum dosage until an optimum dosage was reached. Approximately 30- to 100-fold higher dosages were required to elicit half-maximal nodulation on cowpea than on soybean in the initially susceptible zone of the root. However, at optimal dosages, about six times as many nodules formed in this region on cowpea roots than on soybean roots. There was no appreciable difference in the apparent rate of nodule initiation on these two hosts nor in the number of inoculum bacteria in contact with the root. These results are consistent with the possibility that cowpea roots have a substantially higher threshold of response to symbiotic signals from the bacteria than do soybean roots. Storage of *B. japonicum* cells in distilled water for several weeks did not affect their viability or efficiency of nodule initiation on soybean. However, the nodulation efficiency of these same cells on cowpea diminished markedly over a 2 week period. These differential effects of water storage indicate that at least some aspects of signal production by the bacteria during nodule initiation are different on the two hosts. Mutants of *B. japonicum* 138 defective in synthesis of soybean lectin binding polysaccharide were defective in their efficiency of nodule initiation on soybean but not on cowpea. These results also suggest that *B. japonicum* may produce different substances to initiate nodules on these two hosts.

Rhizobium and *Bradyrhizobium* are soil bacteria able to symbiotically infect and nodulate a wide diversity of legumes, reducing atmospheric nitrogen in these nodules to ammonia for the plant in exchange for fixed carbon (1, 12). Specific isolates of rhizobia are able to infect and nodulate a limited range of host genotypes. This fact has been used as the basis for species designations among rhizobia (13). Within the normal host range, changes in host or bacterial genotype can significantly alter the compatibility of the resultant associations. Genetically determined incompatibility can affect the initiation of infections, the frequency of successful infection development, and the efficiency or persistence of nodule function (12, 13, 16, 21 22). It would be of considerable biological interest and agronomic relevance to identify the kinds of mismatches in development, metabolism, or signal exchange that cause such imcompatibility.

One measure of symbiotic compatibility is the relative efficiency of nodule initiation. This has been defined as the number of bacteria that must be added as an inoculum in order to generate a given number of nodules in the initially susceptible region of the root (6). The fewer cells of a given isolate that are required to generate a half-maximal response in a given host, the more efficient this combination is in the initiation of the first nodules. Defined in this manner, the efficiency of nodule initiation can provide a quantitative indication of the ability of the two symbiont species to respond rapidly and appropriately to each other in the establishment of the association.

Both bacterial multiplication on the root after inoculation and host-mediated feedback regulation of nodule formation (17, 24) can confound any attempt to determine the number of bacteria required to elicit nodulation responses on a particular host. Such problems can be avoided experimentally in soybean (*Glycine max* L. Merr.) and cowpea (*Vigna unguiculata* L. Walp). In these two hosts, the normal process of acropetal root development creates a narrow time window for the initiation of infections by rhizobia, a window that extends from the time that a root hair emerges until about 2 to 3 h later when it is fully elongated (5, 6, 8, 10, 25). Thus, the location of a nodule on soybean and cowpea roots provides an indication of the time after inoculation that it was initiated. The first nodules to appear will be located in that small region of the root where root hairs emerge and develop within a few hours after exposure to the bacteria. Operationally, Bhuvaneswari *et al.* (5, 6, 8) defined the initially susceptible region of the root as that region just above the mark made on a plastic growth pouch to indicate the position of the RT[4] at the time of inoculation. They showed that root cells above this mark lost their susceptibility to nodule initiation within about 6 h after inoculation. Analysis of nodulation in this initially susceptible region circumvents the feedback regulatory controls of the host that suppress emergence of nodules in younger regions of the root (17, 24). Furthermore, since the generation time of *Bradyrhizobium* isolates is relatively long (6–12 h), the nodules that develop in this initially susceptible region of the root will be generated by the added bacteria and not their progeny.

This experimental approach has been used recently in several laboratories to investigate the relative infectivity of rhizobia and possible signal production and transduction by the symbionts. Bhuvaneswari *et al.* (6) showed that bacterial strain and culture age could be important factors in determining efficiency of nodule initiation on soybean. Law *et al.* (19) showed that synthesis of soybean lectin binding exopolysaccharide by *B. japonicum* was correlated with efficiency of soybean nodulation. Bhagwat and Thomas (2–4) reported that root exudate-induced synthesis of a new capsular polysaccharide by *Bradyrhizobium* isolates may be important for initiation of nodules on cowpea. Similarly, Halverson and Stacy (14, 15) found that the nodulation efficiency

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[4] Abbreviation: RT, root tip.

of *B. japonicum* on soybean could be significantly increased by exposing the bacteria to soybean root exudate or purified soybean lectin before inoculation.

In the present studies, isolates of *B. japonicum* were inoculated onto two different hosts, soybean and cowpea, to compare the relative efficiencies of nodule initiation and to investigate questions of signal exchange. By definition, *B. japonicum* isolates are capable of nodulating soybean. *B. japonicum* isolates also form effective (*i.e.* nitrogen-fixing) nodules on cowpea and siratro (*Macroptelium atropurpureum*) (16, 20, 26). Keyser *et al.* (16) analyzed nodulation of these three hosts by several strains of *B. japonicum* and found significant differences in nodule effectiveness and hydrogenase activity in the nodules. Results from the present studies demonstrate that, for unknown reasons, nodule initiation on cowpea by cells of a given isolate of *B. japonicum* is much less efficient than nodule initiation on soybean. Evidence has also been obtained to suggest that a given isolate of *B. japonicum* may produce different substances in order to nodulate soybean than it does to nodulate cowpea.

MATERIALS AND METHODS

Bacterial Cultures and Preparation of Inocula. The following strains of *Bradyrhizobium japonicum* were used in this study: *B. japonicum* 3I1b138 (hereafter referred to as strain 138); 3I1b138ss (a spontaneous mutant of 138 selected sequentially for resistance to streptomycin and spectinomycin at 300 μg/ml); Cap6 and Cap2, which are mutants of 138ss that produce reduced amounts of soybean lectin binding polysaccharide in culture (19); and 61A76. The sources and maintenance of these *B. japonicum* strains have been described (8, 19). *Bradyrhizobium* sp. 176A28 was obtained from J. Burton, Nitragin Co., Milwaukee, WI. For preparation of inocula, bacteria were cultured in yeast extract mannitol gluconate medium as described earlier (6). Early log phase cultures ($A_{620\ nm}$ 0.1 to 0.3) were pelleted and washed once with sterile phosphate buffered saline. Washed cells were resuspended in sterile water to approximately 10^8 cells/ml. These suspensions were diluted serially with water to the required cell density for immediate use as inocula. In certain experiments, the initial stock suspensions in water were stored at room temperature as described by Crist *et al.* (11) for up to 5 weeks prior to dilution for use as inocula. Viable cell counts in diluted inoculum suspensions were routinely determined by plating.

Growth, Inoculation, and Nodulation of Plants. Seeds of soybean (*Glycine max* L. Merr. cv Williams) and cowpea (*Vigna unguiculata* cv California Blackeye) were obtained from Dewine and Hamma Seed Co., Yellow Springs, OH and Burpee Seed Co., Warminster, PA, respectively. Seeds were surface sterilized, germinated, and grown in plastic growth pouches as previously described (5). Seedlings 3 d old were inoculated by dripping 250 μl of the desired inoculum suspension onto a 3 to 4 cm length of the primary root near the tip, which includes the infectible region of the root. Nodule counts were determined under a dissecting microscope at ×10 magnification. The locations of individual nodules with respect to the mark made on the pouch at the time of inoculation to indicate the position of the RT were determined with the aid of a computer graphics tablet as described by Bhuvaneswari *et al.* (6). All experiments were repeated at least twice.

Number of Bacteria Associated with the Roots. Sets of 30 3-d-old soybean and cowpea seedlings were inoculated with 250 μl of *B. japonicum* 138ss as described above. After either zero or 6 h incubation in the growth pouches, a 2-cm segment was excised from the tip region of each root with a razor blade assembly. Sets of 10 segments were placed in sterile test tubes containing 2 ml water and the tubes sonicated for 5 min in a cup horn device to release any bacteria adhering to the roots without killing them, as described previously (28). The released bacteria were counted by plating onto yeast extract mannitol gluconate agar containing 300 μg/ml streptomycin.

RESULTS

Nodulation Efficiency of *B. japonicum* 138 with Soybean and Cowpea. Soybean and cowpea plants in growth pouches were inoculated with serial dilutions of the same suspension of *B. japonicum* 138. One week after inoculation, the number of nodules that developed in the initially susceptible region of the root was determined for each set of plants. Figure 1 shows the percentage of the maximum nodulation response obtained at various dosages for each host. The average number of nodules above the RT mark on soybean plants increased linearly with the log of inoculum dosage until it reached 3.3 ± 1 nodules/plant at a dosage of approximately 10^5 bacteria/plant. Nodule number then declined to about 80% of that level at dosages of 10^7 bacteria/plant. This dose-response behavior is quite similar to that reported earlier for the same cultivar of soybean inoculated with *B. japonicum* 3I1b I-110 ARS (24).

On cowpea plants, the average number of nodules generated above the RT mark by strain 138 was quite low at dosages up to 10^3 bacteria/plant. However, nodulation in this region increased abruptly at dosages greater than 10^3 bacteria/plant, reaching 19.8 ± 4.1 nodules/plant at dosages between 10^6 and 10^7 bacteria/plant (Fig. 1). Nodulation of cowpea plants above the RT mark decreased at higher inoculum dosages in a manner similar to soybean. Half-maximal nodulation above the RT mark on cowpea was observed at dosages of about 1 × 10^5 bacteria/plant. In

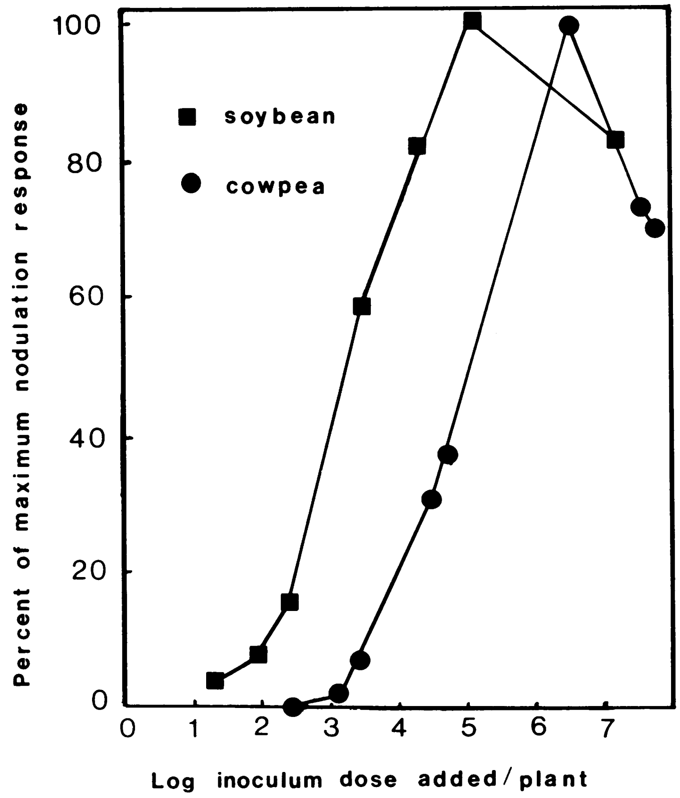

FIG. 1. Inoculum dose-nodulation response of *B. japonicum* 138 in combination with soybean (■) and cowpea (●). Plants were inoculated with 250 μl of a suspension of *B. japonicum* 138 diluted to give the indicated dosage per plant. Nodules on the primary root above the root tip mark were determined 1 week after inoculation as indicated in "Materials and Methods." Data points are from two experiments with each point representing population averages obtained from 60 to 70 plants.

various repetitions of these dose-response comparisons, half-maximal nodulation in the initially susceptible region of cowpea roots consistently required between 30- and 100-fold more cells of the same bacterium than were required to generate half-maximal nodulation responses with soybean.

The nodulation efficiency of the *B. japonicum* 138-cowpea combination was not measurably increased by preincubation of the bacteria in either cowpea or soybean root exudates, or by inoculation with low numbers of bacteria in the presence of higher numbers of bacteria separated from the inoculum cells by a 0.2 μm Nucleopore membrane (data not presented).

Dose-Response Behavior of Different *Bradyrhizobium* Isolates on Cowpea. In previous studies (6), inoculation with *B. japonicum* strain 61A76 resulted in little or no nodulation response in the initially infectible region of soybean roots at inoculum dosages below 10^3 to 10^4 bacteria/plant, very similar to the dose-response behavior of strain 138 on cowpea (Fig. 1). The dose-response behaviors of strains 138 and 61A76 on cowpea were therefore compared in order to learn whether this sigmoidal pattern of dose-response behavior was determined by the host, by the microbe, or both. The dose-response behavior and nodulation efficiency of a typical 'cowpea' isolate (*Bradyrhizobium* 176A28), which does not nodulate soybean, was also compared to that of the two *B. japonicum* isolates.

As shown in Figure 2, the dose-response behavior of the cowpea isolate 176A28 was similar, though not identical, to the behavior of *B. japonicum* strains 138 and 61A76 in nodulating cowpea. *B. japonicum* strain 61A76 generated a dose-response curve offset to somewhat higher dosages than the curve for strain 138, though not to dosages 2 or 3 orders of magnitude greater than strain 138, as with soybean (6). Half-maximal levels of nodulation on cowpea were attained with dosages of approximately 5 × 10^4 for isolates 138 and 176A28, and approximately 1 × 10^5 bacteria/plant for 61A76.

Distribution of Nodules on Primary Roots of Cowpea and Soybean. Nodules are not formed at a uniform frequency along the primary roots of soybean or cowpea. Acropetal root development prevents nodulation in the older region of the primary root where mature root hairs were present at the time of inoculation (5, 8, 10, 25). Delayed nodule initiation will result in a shift in the distribution of nodule formation to regions below the RT mark (2, 6, 14). Nodulation in younger regions of the root is substantially inhibited by the regulatory response to initial infections by rhizobia (17, 24). Therefore, the distribution of nodules on the primary roots of cowpea and soybean seedlings was examined in order to compare the relative rates of infection initiation and the regulatory responses elicited in the two hosts by a given isolate of rhizobia, *B. japonicum* 138.

Inoculum dosage had little effect on nodule distribution in the range from 10^3 to 10^6 bacteria/plant. Typical profiles of nodule distribution, obtained with dosages of 10^5 bacteria/plant, are shown in Figure 3. Despite the significantly larger number of nodules formed on cowpea roots, profiles of nodule distribution appeared to be essentially the same for both cowpea and soybean.

Number of Rhizobia Associated with Soybean and Cowpea Roots following Inoculation. Assays were performed to determine how many of the bacteria provided in the initial inoculum remained in contact with soybean and cowpea roots during the period that root cells above the RT mark were susceptible. Soybean and cowpea roots were inoculated with 1 × 10^4 cells of *B. japonicum* 138ss/plant. Immediately following these inoculations, an average of 1.9 ($\pm$0.7) × 10^2 bacteria were associated with the infectible zone of each cowpea root, corresponding to 1.9% of the initial inoculum, whereas an average of 2.3 ($\pm$1.5) × 10^2 bacteria were associated with each soybean root. Six h after inoculation,

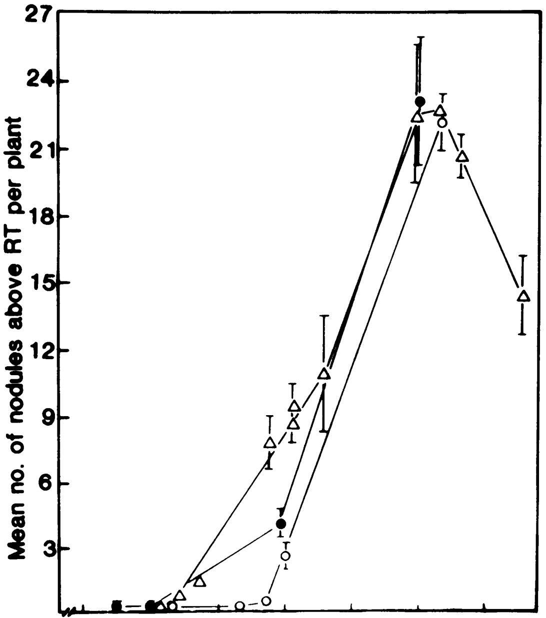

FIG. 2. Inoculum dose-nodulation response behavior of *B. japonicum* 138 (△), *B. japonicum* 61A76 (○) and *Bradyrhizobium* 176A28 (●) with cowpea. Plants were inoculated with 250 μl of a suspension of the rhizobia diluted to the indicated dosage per plant and scored for nodule formation 1 week later as described in "Materials and Methods." Data points are taken from two separate experiments and represent averages obtained from 45 to 70 plants. Error bars indicate the SD.

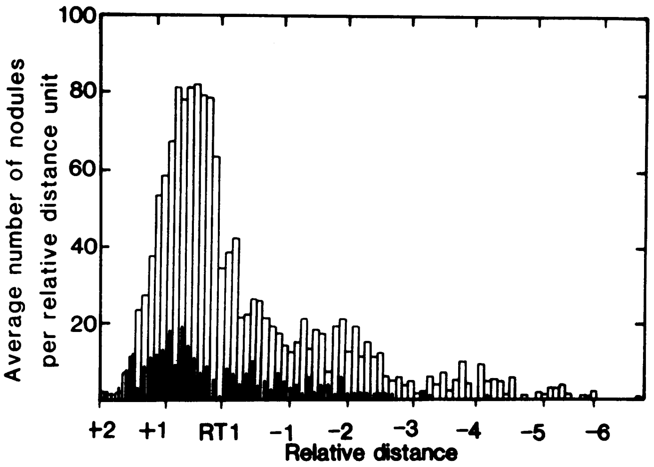

FIG. 3. Distribution of nodules on primary roots of soybean (dark bars) and cowpea (open bars) after inoculation with *B. japonicum* 138. Nodulation profiles are shown for the plants in Figure 1 inoculated with 10^5 bacteria/plant. The location of each nodule on the primary root of each plant was measured to the nearest 0.5 mm with a computer graphics tablet. The profiles of nodule distribution show the average number of nodules per relative distance unit at different locations along the primary root. A relative distance unit is the distance between the root tip and the shortest emergent root hairs visible with a dissecting microscope. This is measured independently for each plant and used to normalize nodule distribution for roots of different lengths. Younger regions of the root below the RT mark are indicated by negative distances. The profiles shown give population averages from 79 cowpea plants and 81 soybean plants and are representative of profiles obtained in various experiments with these hosts at inoculum dosages giving 10 to 100% of the maximum nodulation response in the region above the RT mark.

an average of 3.4% of the inoculated bacteria were associated with the infectible zone of each cowpea root and approximately 4.8% of the inoculated bacteria were associated with the infectible regions of the soybean roots. In repeated experiments of this kind, two-fold variations between averages were common, but the average number of bacteria associated with soybean was consistently a little higher than the average number associated with cowpea. The average number of bacteria associated with roots of either host was consistently two- to threefold higher 6 h after inoculation than immediately after inoculation.

Effect of Inoculum Storage in Water on Nodulation of Cowpea and Soybean. *B. japonicum* cells stored in distilled water at room temperature retain their viability and their ability to nodulate soybean for periods of months (11). It was of interest to determine whether water-stored suspensions of strain 138 cells retained their ability to nodulate cowpea as well as they retained their ability to nodulate soybean. Cells from early log phase cultures were harvested, washed once, and stored in water at a concentration of 1 × 10^8 cells per ml. At 1-week intervals, sets of cowpea and soybean plants were inoculated with cell suspensions obtained by dilution of the aged stock with water so as to contain approximately 1 × 10^5 cells per ml. The results of these experiments, shown in Figure 4, show that *B. japonicum* 138 cells stored in water for 5 weeks remained viable and formed as many nodules per plant on soybean as the freshly prepared inoculum, confirming the previous studies (11). Cowpea plants exposed to the same inocula developed progressively fewer nodules in the initially infectible zone.

To determine whether some inhibitor specific to cowpea nodulation accumulated in the stored suspensions, cowpea plants were inoculated with increasing dosages of the 5-week-old water-stored inoculum. Water-stored and fresh inocula generated comparable numbers of nodules above the RT mark at an inoculum dose of 2.5 × 10^6 cells/plant (Fig. 5), indicating that reduced nodulation on cowpea was not the result of inhibitor accumulation. The profiles of nodule distribution were essentially the same for sets of plants exposed to fresh and water-stored inocula at all dosages tested (data not shown), indicating that while nodule initiation was diminished, it was not appreciably delayed.

Nodulation of Cowpea and Soybean by *B. japonicum* Mutants with Reduced Synthesis of Soybean Lectin Binding Polysaccharide. Law *et al.* (19) isolated mutants of *B. japonicum* strain 138ss which produced reduced amounts of soybean lectin binding polysaccharide in culture. These mutants nodulated soybean less efficiently than did the parent strain. In order to determine whether reduced synthesis of the polysaccharide had a similar influence on nodulation of cowpea by *B. japonicum*, we compared *B. japonicum* 138ss and two of the mutants, Cap2 and Cap6, for their ability to nodulate both soybean and cowpea at various inoculum dosages.

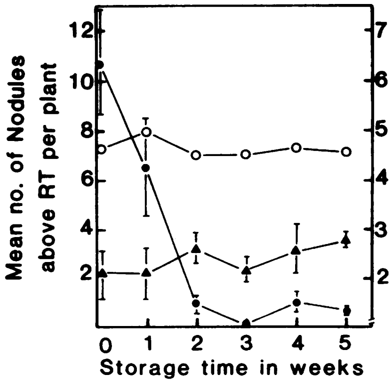

FIG. 4. Effect of storing *B. japonicum* 138 in distilled water suspensions on nodulation of soybean (▲) and cowpea (●). Suspensions containing 1 × 10^8 bacteria/ml of water were stored for various periods of time, then diluted 1000-fold with water and used to inoculate sets of 60 to 80 cowpea and soybean plants. The average number of cells in these inocula (○) was determined by plate counting of triplicate aliquots. Error bars indicate mean values ± SD.

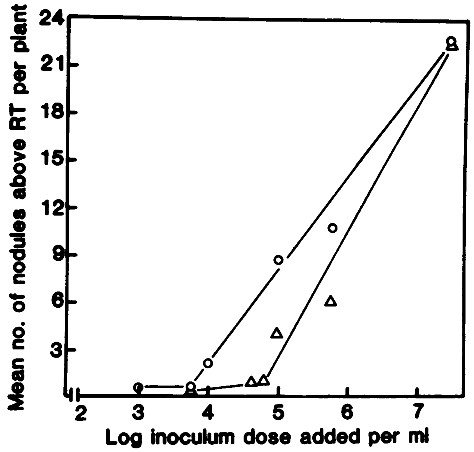

FIG. 5. Comparison of inoculum dose-nodulation response curves for cowpea inoculated with fresh (○) and 5-week-old water-stored (△) suspensions of *B. japonicum* 138. Inocula were prepared at the indicated dosages by dilution of either fresh or water-stored suspensions as described in "Materials and Methods." Each data point is an average from 45 to 60 plants.

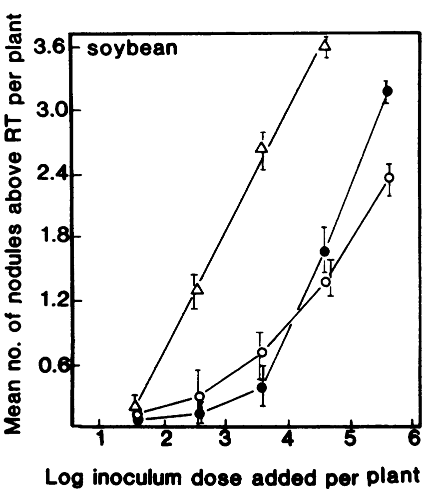

FIG. 6. Inoculum dose-nodulation response behavior of soybean inoculated with *B. japonicum* 138ss (△) and the capsule deficient mutants CAP2 (○) and CAP6 (●). Each point gives the average from at least 55 plants.

On soybean, strain 138ss had a log-linear dose-response curve (Fig. 6) very similar to that of its parent, *B. japonicum* 138 (Fig. 1). The dose-response curves for the two capsular mutants were offset to substantially higher dosages. Half-maximal nodulation was attained with dosages of approximately 1 × 10^3 for the parent and with dosages of approximately 6 × 10^4 and 1 × 10^5 for Cap6 and Cap2, respectively (Fig. 6). On cowpea the dose-response curves for the two Cap mutants and the parent were very similar except for somewhat reduced nodulation by Cap2 at dosages of about 10^6 and 10^7 bacteria/plant (Fig. 7).

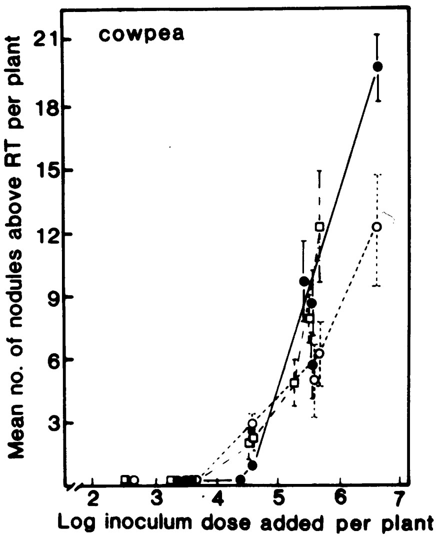

FIG. 7. Inoculum dose-nodulation response behavior of cowpea inoculated with *B. japonicum* 138ss (●) and the capsule deficient mutants CAP2 (○) and CAP6 (□). Each point is the average from at least 55 plants. Error bars indicate ± SD from the mean.

DISCUSSION

Differences in Efficiency between Soybean and Cowpea. The efficiency of nodule initiation for the combination of *B. japonicum* 138 with soybean was about 30- to 100-fold greater than the 138/cowpea combination (Fig. 1). The dose-response behaviors of *B. japonicum* 61A76 and *Bradyrhizobium* 176A28 with the cowpea host were quite similar to that of *B. japonicum* 138 with cowpea (Fig. 2). Thus, even though bacterial genotype can be an important determinant of dose-response behavior and efficiency (2, 6), it appears that the large differences between soybean and cowpea with respect to efficiency of nodule initiation are not peculiar to strain 138 or to *B. japonicum* isolates, but are instead characteristic of the host species.

It is not obvious in what way(s) the two hosts might differ so as to account for 30- to 100-fold differences in nodule initiation. Roots of cowpea and soybean seedlings are very similar in size, morphology, root hair development and growth rate under the conditions used here, and thus seem to have approximately the same number of sites where infections might develop. *B. japonicum* 138ss associated with both soybean and cowpea roots in comparable numbers following inoculation, so that the lower efficiency of cowpea cannot be readily explained in terms of reduced numbers of bacteria in contact with the root. As illustrated in Figure 3, both soybean and cowpea have similar distributions of nodule formation on the primary root at all inoculum dosages tested with these strains. Thus, the difference in number of bacteria required to initiate the first nodules does not appear to involve a delayed responsiveness of cowpea and the bacteria to each other. Nor does there appear to be a stronger feedback suppression of nodulation in cowpea than in soybean. Since the number of nodules that developed above the RT mark at optimal dosages was severalfold higher for cowpea than for soybean, cowpea is actually more susceptible to the bacteria than soybean, in contrast to what one would expect on the basis of its lower nodulation efficiency.

Perhaps the simplest explanation for this behavior is that cowpea might have a higher threshold of response to signal substances from the bacteria than soybean. This explanation is consistent with the failure of cowpea roots to respond to *Bradyrhizobium* cells at low inoculum dosages while forming more infections and nodules at high dosages of the same bacteria. Although this is an attractive explanation, we know of no direct measurements of responsiveness to signals from rhizobia in any host as yet, and other explanations are possible. For example, the two hosts may have the same sensitivity to bacterial signal molecules, but might differ in their ability to elicit synthesis of required nodulation signals in the bacteria (23). Recent studies have shown that soybean root exudates contain substances that induce synthesis of soybean lectin binding polysaccharide (7), that they contain a lectin that enhances efficiency of nodule initiation by *B. japonicum* (15), and that they contain phenolics that enhance expression of common *nod* genes in *B. japonicum* (18). Cowpea root exudates have also been reported to contain substances that enhance nodule initiation by *Bradyrhizobium* on cowpea (2–4). However, our preliminary studies provided no evidence of enhanced nodulation efficiency of *B. japonicum* 138 on cowpea following exposure of the bacteria to cowpea root exudate. A further possibility, consistent with the findings reported here, is that the bacteria may produce different signal substances to induce symbiotic responses in the two hosts. In this case, nodule initiation may depend on the relative concentrations of soybean-specific and cowpea-specific signals rather than the existence of different thresholds. Consideration of these possibilities indicates the need to isolate and identify active signal substances from the bacteria before alternate explanations can be rigorously tested.

Maximum Number of Nodules Formed in the Initially Susceptible Region of Soybean and Cowpea Roots. Host species appears to be an important factor determining the maximum number of nodules generated in the initially infectible zone. *B. japonicum* 138 generated about six times as many nodules above the RT mark on cowpea than on soybean at optimal dosages. This difference in nodule number does not appear to be a peculiarity of *B. japonicum* 138. *Bradyrhizobium* 176A28 and *B. japonicum* strains 138 and 61A76 all formed about 20 nodules above the RT mark on cowpea at high dosages, while various *B. japonicum* strains generated only about two to four nodules above the mark on soybean at optimal dosages (6).

We have made a preliminary study of these differences at the level of infection initiation and development. Microscopic examinations using the serial section and staining methods of Calvert *et al.* (10) indicated that *B. japonicum* 138 initiated substantially more infections in cowpea roots than in soybean roots at dosages required for optimal nodulation, and that very few infections developed in cowpea roots inoculated with dosages of 10^2 to 10^3 bacteria/plant (data not shown). Infections in cowpea appeared to be initiated in short, curled root hairs, to proceed through infection threads, and to develop in the same manner as described for soybean (10). These observations indicate that the greater number of nodules that develop in the initially infectible zone of cowpea roots probably results from initiation of more infections, not from substantially reduced rates of abortive infection development.

Differential Effects of Water Storage and Mutations in Polysaccharide Synthesis on Nodulation of Soybean and Cowpea. The results in Figures 4, 5, and 6 provide evidence suggesting that *B. japonicum* 138 may produce one set of substances in order to initiate nodulation on soybean and a different set to nodulate cowpea. *B. japonicum* 138 retains full viability and nodulation efficiency on soybean, but drastically loses efficiency of nodule initiation on cowpea as a result of storage in water (Fig. 4). Higher dosages of these water-stored cells generated just as many nodules in the initially susceptible zone as generated by fresh inocula (Fig. 5). This result indicates that there was no accumulation of substances during storage in water that selectively inhibited nodulation on cowpea. If there was no accumulation of a selective inhibitor, then it seems likely that the loss of nodulation efficiency on cowpea resulted from diminished signal production by the bacteria. There is no evidence of correspondingly diminished signal production for nodule initiation on soybean. Therefore, it appears that different molecular signals may be involved in the initiation of nodules on these two hosts.

The dose-response behavior of the *B. japonicum* polysaccharide mutants demonstrates a considerably reduced nodulation efficiency on soybean but a relatively unchanged efficiency on cowpea (Figs. 6 and 7). The reduced nodulation efficiency on soybean confirms the earlier report by Law *et al.* (19) and is consistent with the notion that interactions involving soybean lectin and *B. japonicum* receptor polysaccharide are important to establishment of the symbiosis (1, 6, 7, 9, 14, 15, 19, 27). Since these mutants were just as efficient in nodule initiation as the parent on cowpea, the presence or absence of the soybean lectin binding polysaccharide was not important to cowpea nodulation, implying that nodule initiation on cowpea requires a different sort of signal substance from the cells. In this regard, it is of interest that *B. japonicum* strain 61A76 is deficient in synthesis of soybean lectin binding polysaccharide relative to stains 110, 123, and 138 (6, 7) and is considerably less efficient in nodule initiation on soybean than these strains (6) but is just about as efficient in nodule initiation as 138 on cowpea (Fig. 2). These observations clearly parallel the behavior of strain 138 and the Cap mutants on soybean and cowpea and strengthen the likelihood that *B. japonicum* uses different sets of molecules to recognize and respond to different host species.

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