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Effects of Culture Age on Symbiotic Infectivity of *Rhizobium japonicum*

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The infectivity of the soybean symbiont *Rhizobium japonicum* changed two- to fivefold with culture age for strains 110 ARS, 138 Str Spc, and 123 Spc, whereas culture age had relatively little effect on the infectivity of strains 83 Str and 61A76 Str. Infectivity was measured by determining the number of nodules which developed on soybean primary roots in the zone which contained developing and preemergent root hairs at the time of inoculation. Root cells in this region of the host root are susceptible to *Rhizobium* infection, but this susceptibility is lost during acropetal development and maturation of the root cells within a period of 4 to 6 h (T. V. Bhuvaneswari, B. G. Turgeon, and W. D. Bauer, Plant Physiol. 66:1027–1031, 1980). Profiles of nodulation frequency at different locations on the root were not affected by the age of the *R. japonicum* cultures, indicating that culture age affected the efficiency of *Rhizobium* infection rather than how soon infections were initiated after inoculation. Inoculum dose-response experiments also indicated that culture age affected the efficiency of infection. Two strains, 61A76 Str and 83 Str, were relatively inefficient at all culture ages, particularly at low inoculum doses. Changes in infectivity with culture age were reasonably well correlated with changes in the proportion of cells in a culture capable of binding soybean lectin. Suspensions of *R. japonicum* in water were found to retain their viability and infectivity.

Previous studies from this laboratory have shown that the susceptibility of legume roots to nodulation by rhizobia is developmentally restricted and transient (4, 6). As a consequence of the developmental acquisition and loss of root cell susceptibility to rhizobia, nodules which develop higher up on the primary root result from infections initiated at earlier times than those of nodules which develop closer to the root tip (RT). The position of nodule development is thus potentially useful as a quantitative indicator of the rate and efficiency of infection initiation by *Rhizobium* cultures. The location of each nodule on a primary root can be determined with respect to a mark made on the transparent face of a plastic growth pouch (4, 6). This mark indicates the position of the RT at the time of inoculation. In soybeans, the portion of the primary root which remains above the RT mark is susceptible to nodulation for a period of no more than 4 to 6 h (6). Since the generation time of the soybean symbiont *Rhizobium japonicum* is generally 8 to 12 h, the narrow time window of root cell susceptibility means that the progeny of the rhizobia in the original inoculum should, statistically, make only a small contribution to nodulation above the RT mark. We report here that culture age is a factor which can substantially affect nodulation above the RT mark by *R. japonicum*, apparently by influencing the efficiency with which the bacteria in the original inoculum are able to initiate infections.

MATERIALS AND METHODS

Rhizobia. *Rhizobium japonicum* USDA I-110 ARS (referred to as strain 110 ARS), an isolate of USDA-I-110 that is resistant to azide, rifampin, and streptomycin, was obtained from D. Kuykendal, U.S. Department of Agriculture (USDA), Beltsville, Md. USDA strains 83, 123, and 138 were obtained from D. Weber, USDA Beltsville, and strain 61A76 was obtained from the Nitragin Co., Milwaukee, Wis. Spontaneous mutants of these latter strains, selected for resistance to streptomycin or spectinomycin (500 μg/ml), were used for the present studies (designated as Str and Spc strains, respectively). The rhizobia were shake cultured on yeast extract-mannitol-gluconate medium and harvested as described previously (6). Stock cultures were normally maintained in freeze-dried ampoules. Starter cultures (10 ml) were grown to early stationary phase, subcultured (1 ml of 1.0 absorbance unit at 620 nm per 100 ml), and grown to the desired growth phase for use as inocula. Inocula were prepared by straight dilution of cultures with sterile water. *Rhizobium* cell numbers were determined by direct counting in Pe-

troff-Hauser chambers and by viable cell counts after plating.

Plants. Seeds of soybean (*Glycine max* L. Merr cv. Williams) were obtained from Dewine and Hamma Seed Co., Yellow Springs, Ohio. The seeds were surface sterilized and germinated on yeast extract-mannitol-gluconate agar plates (6). Seedlings without visible microbial contamination were transferred to disposable seedling growth pouches (Northrup King Seed Co., Minneapolis, Minn.), inoculated with 250 μl of a *Rhizobium* suspension, marked to indicate the position of the RT at the time of inoculation, and maintained in a growth chamber as previously described (6). Nodulation on the primary roots was scored 7 to 9 days after inoculation as described (6). A computer-assisted method to facilitate nodule scoring and data analysis was developed. Data on the location of primary root nodules relative to the RT mark were entered directly into an Apple II Plus computer (Apple Computer, Inc., Cupertino, Calif.) with an Apple graphics tablet and pen mounted under a dissecting microscope. The program for data acquisition, storage, and analysis is available on request.

Lectin binding. Binding of fluorescein isothiocyanate-labeled soybean lectin to *R. japonicum* cells was assayed as described earlier (5).

RESULTS

Nodulation by antibiotic-resistant mutants. The introduction of antibiotic resistance markers into rhizobia sometimes affects the ability of the marked strains to nodulate their host (15). Since the present studies are preliminary to investigations of the physiology of *Rhizobium* colonization and interstrain competition on the soybean root surface, antibiotic-resistant mutants of several common *R. japonicum* strains were isolated as an aid to identification and quantitation of different strains in mixtures from the root surface. The ability of each mutant isolate to nodulate soybean seedlings under growth pouch conditions was determined and compared with that of the corresponding parental wild-type strain. As shown in Table 1, each of the antibiotic-resistant mutants used in the present study nodulated as well as the corresponding wild-type strain.

TABLE 1. Relative nodulating ability of wild-type and antibiotic-resistant isolates of *R. japonicum* strains[a]

Isolate	% Nodulation above RT mark	Avg no. of nodules above RT mark
138 wild type	87	1.8
138 Str Spc	79	1.6
110 wild type	67	1.3
110 ARS	68	1.3
123 wild type	89	2.0
123 Spc	87	1.7
123 Nal[b]	88	1.9
123 Str	97	2.8[c]
61A76 wild type	44	0.5
61A76 Str	43	0.5

[a] Sets of 60 to 80 plants were inoculated with 1.0×10^4 to 1.4×10^4 *Rhizobium* cells of the indicated strain per plant.

[b] Resistant to nalidixic acid.

[c] Antibiotic-resistant strain that differed at the 95% confidence level from the parental strain with respect to nodulation above the RT.

Effect of culture age. To test the effect of culture age on nodulation by *R. japonicum*, small samples were withdrawn daily from a test subculture for preparation of inocula, determination of cell numbers, etc. Inoculum suspensions were prepared from the subculture by simple dilution in sterile distilled water to a concentration of 10^5 bacteria per ml. Each seedling was inoculated by adding a portion (250 μl) of the inoculum suspension dropwise over a 2- to 3-cm region of the root nearest the tip. A set of 50 to 60 seedlings was inoculated each day over a 5- to 7-day period. Both the average number of nodules above the RT mark made at inoculation and the percentage of inoculated plants that developed nodules above the RT mark were determined. Both of these measures of nodulation give comparable information (6). However, appreciable changes in the average number of nodules above the mark must occur for changes in the percentage of plants nodulated above the mark to be evident if the percentages involved are relatively high (85 to 100%).

For strains 110 ARS, 123 Spc, and 138 Str Spc, substantial changes were observed in the ability of cultures to initiate nodule formation above the RT mark at different culture ages (Fig. 1 and 2). Exponential-phase cultures of these strains were much more effective than stationary-phase cultures in their ability to induce nodule formation above the mark. Both the magnitude and the abruptness of this effect were more pronounced for strains 110 ARS and 138 Str Spc than for strain 123 Spc. In contrast, there was relatively little change in the ability of strains 61A76 Str and 83 to initiate nodulation above the mark at different culture ages (Fig. 3).

The majority of these experiments involved inoculation of a different set of plants each day over the course of 5 to 7 days. To circumvent variations in the handling and growth of the plants, some experiments were done with inocula prepared and used all on the same day. Subcultures were initiated at daily intervals from a given starter culture of the test strain. On the day of inoculation, the subcultures, each of a different age, were diluted to 10^5 cells per ml

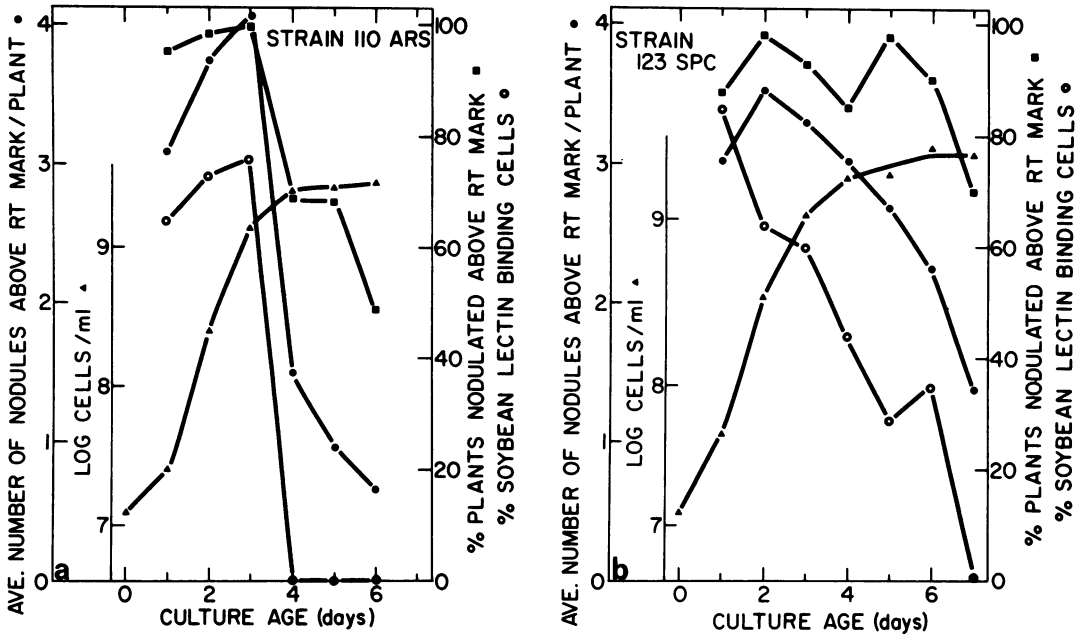

FIG. 1. Nodulation of soybean by *R. japonicum* strains 110 ARS (a) and 123 Spc (b) at different culture ages. The data points for strain 110 ARS (a) represent the average of two separate experiments, each with at least 40 plants per treatment. The viable cell number in the inocula ranged from 5×10^4 to 1×10^5 per ml. Data points for strain 123 Spc (b) are averages from at least 55 plants per treatment. Viable cell number in the inocula ranged from 6.0×10^4 to 3.5×10^5 per ml. Symbols: △, viable cell number per milliliter in the culture; ●, average number of nodules above the RT per plant; □, percentage of plants nodulated above the RT mark; ○, percentage of *Rhizobium* cells binding fluorescein isothiocyanate-labeled soybean lectin. Binding of labeled lectin was determined as described in the text.

with water and used to inoculate separate sets of 50 to 60 seedlings. The results of these experiments were not significantly different from those obtained by the original protocol. Although the general patterns obtained by either protocol were readily reproducible, individual points in given experiments sometimes varied (e.g., Fig. 2, day 2) for unknown reasons.

The time required to mark and inoculate several hundred seedlings was sufficiently long (2 to 4 h) to raise the question of whether the *Rhizobium* inocula, diluted in distilled water, were stable with respect to infectivity during the interval between dilution and inoculation. This was tested for strain 110 ARS by inoculating sets of seedlings at 0, 4, or 8 h after dilution in water. The results for both exponential- and stationary-phase cultures of strain 110 ARS are shown in Table 2. Incubation for several hours in distilled water had no measurable effect on the infectivity of exponential-phase cultures of strain 110 ARS and increased the infectivity of strain 110 ARS stationary-phase cultures to the level of exponential-phase cultures.

Nodulation profiles. It seemed possible that the culture age-dependent changes in the extent of nodulation above the RT mark (Fig. 1 through 3) could be accounted for by changes in the time required for rhizobia to initiate infections. Delayed initiation of infection could result in a shift of the peak of maximum nodulation frequency (6) to a position below the RT mark. Conversely, earlier initiation of infections might result in a shift of the peak of maximal nodulation frequency to a position further above the RT mark. Alternatively, changes in the time required to initiate infections might result in changes in the shape of the nodulation peak so that more or less of the total nodulation occurred above the RT mark.

To examine these possibilities, profiles of the nodulation frequency at different relative distances above and below the RT mark were determined for both exponential- and stationary-phase cultures of three *R. japonicum* strains, 110 ARS, 61A76 Str, and 123 Spc (Fig. 4). Nodulation frequency profiles for these three strains revealed no substantial differences between the nodulation profiles generated by exponential-phase cultures and stationary-phase cultures of a given strain, although on one occasion nodulation by an exponential culture of strain 110 ARS was significantly higher in the region above the smallest emergent root hair mark (see Fig. 4a).

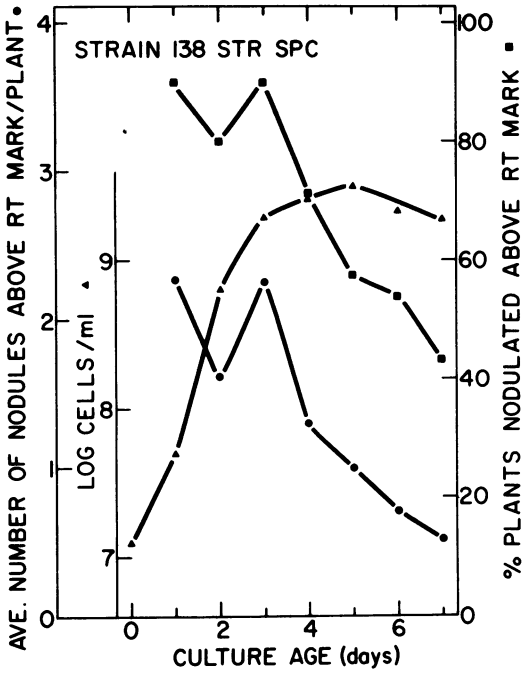

FIG. 2. Nodulation of soybean by *R. japonicum* strain 138 Str Spc at different culture ages. The data points are averages from at least 55 plants per treatment. The viable cell number in the inocula ranged from 6.0×10^4 to 3.5×10^5 per ml. Symbols: △, viable cell number per milliliter in the culture; ○, average number of nodules above the RT mark per plant; □, percentage of plants nodulated above the RT mark.

Some minor differences in nodulation patterns among the three different strains were evident. In particular, strain 61A76 Str developed nodules below the RT mark at a relatively higher frequency than did either strain 110 ARS or strain 123 Spc, and strain 110 ARS developed nodules above the smallest emergent root hair mark (6) at a somewhat higher frequency than did the other two strains.

Effect of inoculum dose. To further characterize the nature of the differences in infectivity observed in previous experiments, the dependence of nodulation above the RT mark on inoculum dose was investigated. The manner in which nodulation above the RT mark depends on inoculum dose can provide clues regarding possible threshold values required for the initiation of infections. Dose-response relationships can also provide information regarding the relative efficiency and regulation of infectivity (6; B. G. Turgeon and W. D. Bauer, Protoplasma, in press).

Dose-response curves for each test strain were determined for cultures in both exponential and stationary growth phases. Each plant in a set of 50 to 60 seedlings was inoculated with 250 μl of a suspension of a given cell concentration

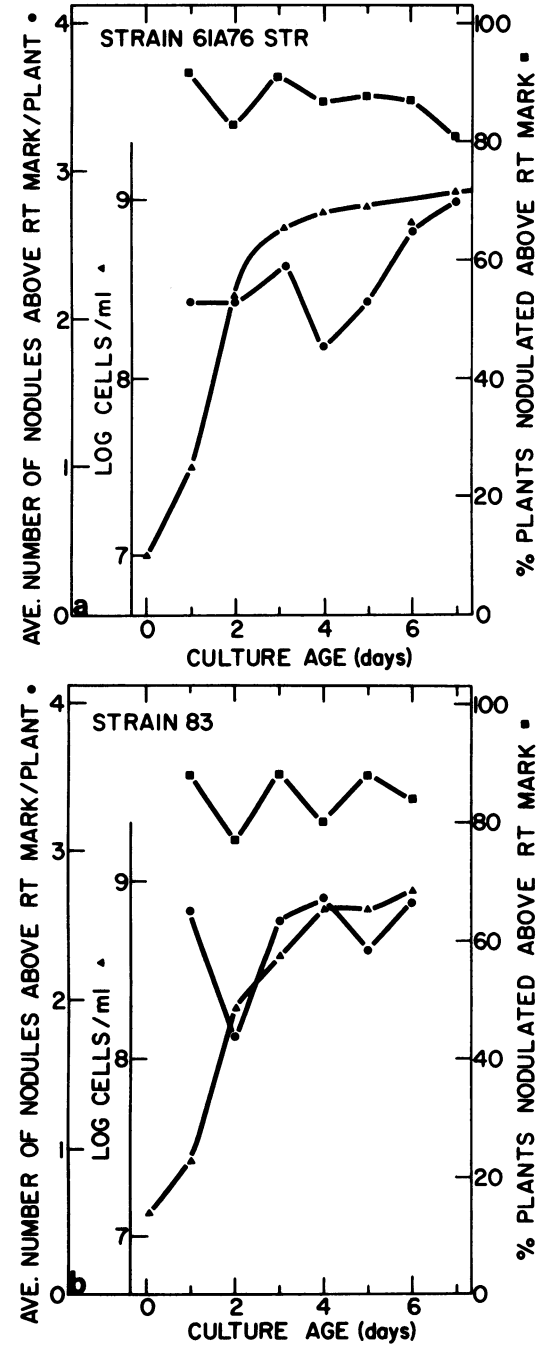

FIG. 3. Nodulation of soybean by *R. japonicum* strains 61A76 Str (a) and 83 (b) at different culture ages. The data points are averages from at least 55 plants per treatment. The viable cell number in the inocula ranged from 6.0×10^5 to 1.3×10^6 per ml for strain 61A76 str (a) and from 8.0×10^4 to 1.0×10^5 for strain 83 (b). Symbols: △, viable cell number per ml in the culture; ○, average number of nodules above the RT mark per plant; □, percentage of plants nodulated above the RT mark.

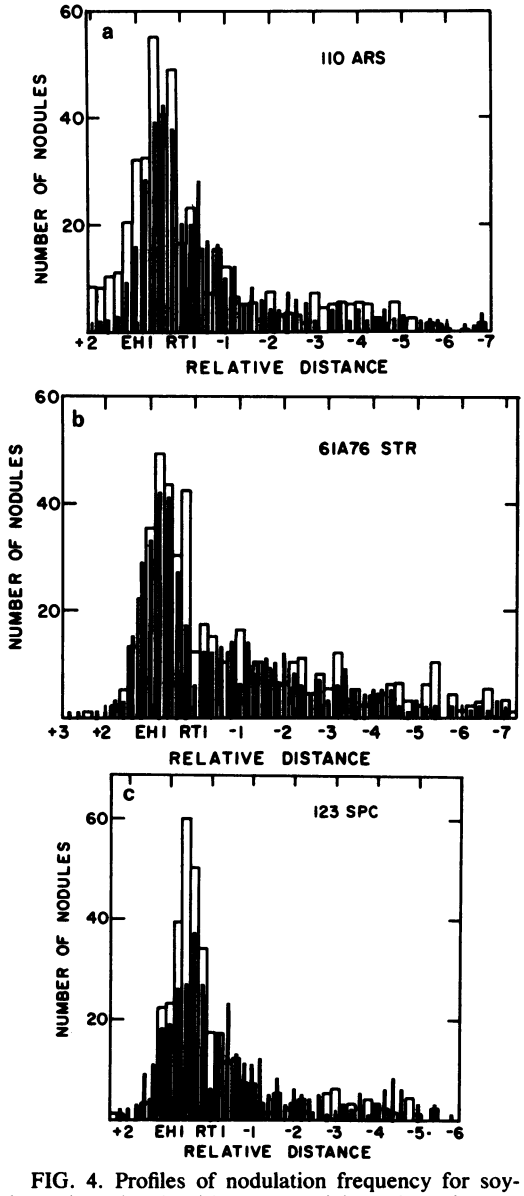

FIG. 4. Profiles of nodulation frequency for soybean inoculated with exponential- and stationary-phase cultures of *R. japonicum* strains 110 ARS (a), 61A76 Str (b), and 123 Spc (c). Open bars show profile data for exponential-phase cultures and solid bars show profile data for stationary-phase cultures. The positions of all nodules on the primary root of 100 to 150 plants were measured (±0.5 mm) relative to the RT mark made at inoculation (RT1). The position of the smallest emergent root hairs (EH1) was measured for each plant at the time of inoculation. The relative distance of each nodule on the primary root from the RT1 mark was calculated as a percentage of the RT1-to-EH1 distance determined for each plant. The average RT1-to-EH1 distance for the 672 inoculated seedlings was 13.9 mm. Since the average rate of root elongation has been reported as approximately 2.4 mm/h (6), one relative distance unit in this figure is equivalent to approximately 6 h of root growth. Positions on the root below the RT mark made at inoculation are indicated by negative relative distances. Viable cell counts in the inocula ranged from 6.5×10^4 to 1.4×10^5 per ml for strains 123 Spc (c) and 110 ARS (a) and from 8.7×10^5 to 8.8×10^5 per ml for strain 61A76 Str (b).

TABLE 2. Nodulating ability of *R. japonicum* 110 ARS after dilution in sterile distilled water[a]

Cell phase	Interval between dilution and inoculation (h)	% Plants nodulated above RT mark	Avg no. of nodules above RT per plant
Exponential (2 days)	0	76	1.2
	4	74	1.3
	8	74	1.6
Stationary (6 days)	0	42	0.5
	4	52	0.9[b]
	8	67	1.3[b]

[a] Sets of 45 to 50 plants were inoculated with 250 μl per plant of 1.3×10^5 to 1.5×10^5 cells per ml suspensions of exponential-phase (2-day) or stationary- phase (6-day) cells diluted in water.

[b] Nodulation above the RT mark differing from the zero time control at the 95% confidence level.

obtained by serial dilution of the test culture with sterile water. Viable cell counts of one of the test dilutions were made to establish the actual number of bacteria added to each plant. The dose-response curves for four *R. japonicum* strains are shown in Fig. 5 and 6.

The dose-response relationship for strain 110 ARS was approximately log-linear over the range of inoculum doses tested (Fig. 5a). Inocula of different culture ages produced dose-response curves of different slopes. The dose-response curves for strain 110 ARS all appear to have a common intercept on the inoculum dose axis. Mid-log-phase cultures of 110 ARS generated the greatest nodulation above the RT mark, whereas stationary-phase cultures generated the least nodulation. Late-log-phase cultures gave an intermediate response.

Cultures of strain 123 Spc gave dose-response curves fairly similar in shape, slope, and intercept to those of strain 110 ARS (Fig. 5c). There was relatively little difference among dose-response curves for early-log-phase and stationary-phase cultures of strain 123 Spc.

The dose-response curves for strains 61A76 Str and 83 (Fig. 5b and 6), on the other hand, were substantially different from those obtained for strains 110 ARS and 123 Spc (Fig. 5). Both exponential-phase and stationary-phase cultures of strain 61A76 Str showed large increases in nodulation response only at inoculum doses

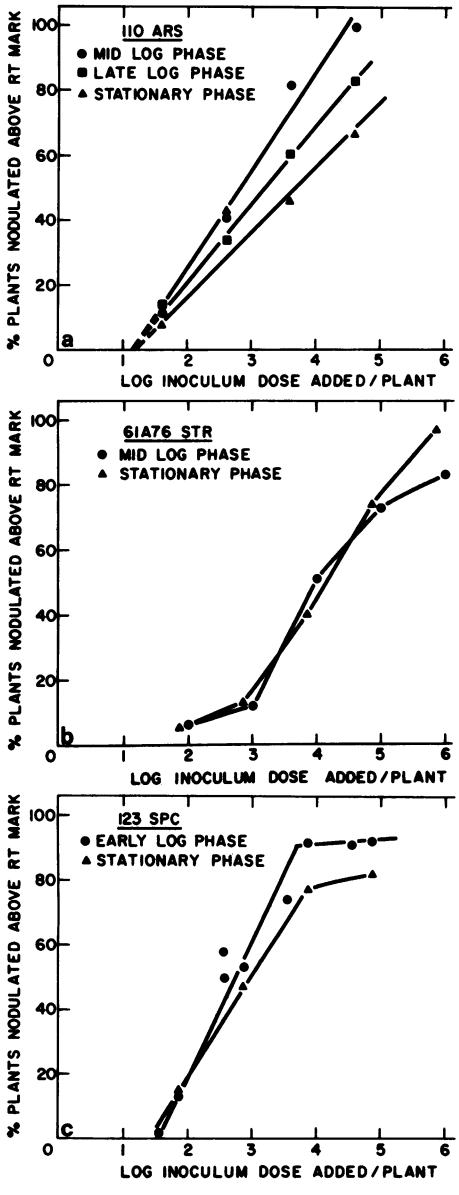

FIG. 5. Nodulation of soybean cv. Williams by cultures of *R. japonicum* strains 110 ARS (a), 61A76 Str (b), and 123 Spc (c) at different growth phases and at various inoculum dosages. Inocula were prepared by serial dilutions of 10^8-cell-per-ml suspensions. The numbers of viable cells per milliliter in the inocula were determined by plate counts. Sets of 40 to 60 plants were inoculated per treatment. Nodulation was scored as described in the text.

larger than 10^3 bacteria per plant. At higher inoculum doses, nodulation above the RT mark increased with dose at the same linear slope as mid-log-phase cultures of 110 ARS (Fig. 5a). However, nodulation by stationary-phase cultures of both strain 61A76 Str and strain 83 actually diminished in terms of the average number of nodules above the RT mark (data not shown) at the highest inoculum doses. Strain 61A76 Str generally infected and nodulated a substantially lower proportion of plants above the RT mark at a given inoculum dose than either exponential-phase 110 ARS or 123 Spc. Nodulation above the RT mark was comparable at all inoculum doses tested for both wild-type strain 83 and strain 83 Str.

Lectin binding. The ability of rhizobia to nodulate soybean is strongly correlated with their ability to bind soybean seed lectin (3, 5, 7, 8). Ultrastructural studies have shown that the capsules of *R. japonicum* serve as the receptor material for the binding of soybean lectin to the bacterial cell surface (1, 9, 13). Previous studies have demonstrated that the ability of *R. japonicum* cultures to bind soybean lectin is dependent on culture age (5, 11). This latter finding is of potential interest with regard to the effects of culture age on infectivity.

The effects of culture age on the percentage of cells capable of binding soybean seed lectin were determined for strains 110 ARS, 138 Str Spc, and 123 Spc. In general, changes in infectivity paralleled the changes in lectin binding for strains 110 ARS and 123 Spc (Fig. 1). No consistent results were obtained for strain 138 Str Spc. In preliminary experiments, strain 110 ARS cultured on succinate medium instead of yeast extract medium retained high nodulating activity into stationary phase even though lectin binding capacity was lost. Culture age-dependent changes in the percentage of cells capable of binding soybean lectin could not be readily measured for strains 61A76 and 83. Fewer than 1% of the cells of these strains normally bind the

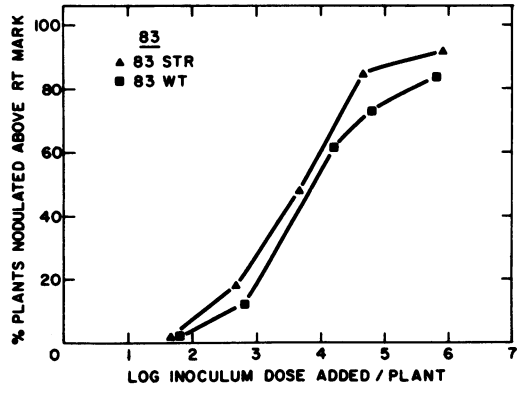

FIG. 6. Nodulation of soybean cv. Williams by stationary-phase cultures of *R. japonicum* 83 WT (■) and 83 Str (▲) at various inoculum dosages. Each data point is an average from 50 plants.

lectin (1, 5, 7) unless they are cultured in the presence of the host root (3).

DISCUSSION

The age of *R. japonicum* cultures had substantial effects on the ability of some strains of the bacteria to induce nodulation in those zones of the host root where developing and preemergent root hairs were present at the time of inoculation. These effects of culture age were quite reproducible from experiment to experiment. The effects could be observed whether the same culture of rhizobia was used to inoculate sets of plants on different days or whether *R. japonicum* cultures of different ages were used to inoculate sets of plants on the same day.

The rapid developmental acquisition and loss of root cell susceptibility implies that nodules which develop above the RT mark in soybean result from infections initiated within the 4- to 6-h period that root cells above the mark remained susceptible (6). Changes in nodulation above the RT mark thus result from changes in either the initiation of infections or the subsequent development and abortion of infections. Abortive infections occur at high frequencies in soybean. Examination of pouch-grown soybean seedlings inoculated with exponential-phase strain 110 ARS by serial sectioning revealed that 80 to 90% of the infections that developed above the RT mark were abortive (W. D. Bauer, unpublished observations). The proportion of abortive infections could thus be an important factor affecting the formation of nodules above the RT mark. However, steps in the infection process subject to detectable abortion, such as penetration, infection thread formation, induction of cortical cell divisions, and bacteroid formation, take place 24 to 96 h after inoculation (12, 14). It is thus difficult to see how the growth phase of cells in the original inoculum could affect the behavior of the progeny of these bacteria several generations later with respect to the proportion of abortive infections. We suggest, therefore, that culture age probably affects a short-term aspect of *R. japonicum* infectivity, specifically, the initiation of infections. Further studies involving quantitative analysis of serially sectioned roots inoculated with *R. japonicum* strains of different culture ages are needed to verify this suggestion.

Analysis of the infection process has not progressed to the point where the initiation of infections can be defined in physiological, biochemical, or cytological terms. However, since the normal acropetal development of root cells in the absence of rhizobia results in the loss of susceptibility to infection and nodulation, the initiation of an infection by *Rhizobium* is likely to involve the induction of some response in the host root cell (for example, root hair deformation) which alters the normal course of root cell development.

One tentative conclusion to be drawn from the culture age studies is that the ability of *R. japonicum* to initiate infections is not directly related to the rate of cell division of the bacteria. Differences in culture age caused nodulation above the RT mark to vary four- to fivefold in some strains (110 ARS, 138 Str Spc), whereas other strains showed variations of only 40% or so (61A76 Str, 83 Str). The different magnitudes of the culture age effect in different strains, as well as the different patterns of change (Fig. 1 through 3) and the stability of the infectivity of *R. japonicum* in water suspensions, all indicate that the ability to initiate infections is a property of *R. japonicum* that is not governed by the rate of cell division.

Another characteristic of the culture age dependence of infectivity is indicated by comparison of nodulation frequency profiles for exponential- and stationary-phase cultures of the various strains (Fig. 4). The closely parallel profiles provide evidence that both exponential- and stationary-phase cultures nodulated in the same parts of the root. There is no indication that nodule initiation was earlier in one than the other. Instead, it appears that the more infective cultures simply caused more nodules to be initiated in the same parts of the root.

In related work, Bhagwat and Thomas (2) have recently reported that major shifts in nodulation frequency above or below the RT mark in cowpea were dependent on preincubation of *Rhizobium* sp. 32H1 with substances in host root exudates. Preincubation of these rhizobia with host root exudates apparently induced changes in the bacteria which enabled them to infect at earlier times after inoculation.

Results from the inoculum dose-response studies for *R. japonicum* also appear to be generally consistent with the notion that culture age affects the efficiency rather than the timing of infection initiation. In the simplest case, represented by strain 110 ARS (Fig. 5a), mid-log-phase cultures induced nodulation above the RT mark with the greatest efficiency; that is, fewer rhizobia from mid-log-phase cultures were required to generate a given nodulation response. Greater numbers of cells were required to achieve the same degree of nodulation if late-log-phase and stationary-phase cultures were used. Since the dose-response curves for strain 110 ARS are a family of straight lines with an apparently common intercept, it seems likely that only one variable is involved in the culture age effect for this strain. The significance of the intercept value obtained by extrapolation of the curves to zero nodulation is uncertain

(B. G. Turgeon and W. D. Bauer, Protoplasma, in press).

Inoculation methods of different efficiencies can cause changes in dose-response curves that are similar in both nature and magnitude to the culture age-dependent changes in *R. japonicum* infectivity. The dose-response curve for strain 138 (6) was similar to the curve shown in Fig. 5a for midexponential cultures of strain 110 ARS when a moderately efficient method of inoculation was used (employing Parafilm to prevent rhizobia from dispersing in the paper towel wick). However, the dose-response curve obtained for strain 138 was similar to the curve shown for stationary-phase cultures of strain 110 ARS when a relatively inefficient method of inoculation was used (dripping inoculum onto the paper towel between the roots). These results support the previous suggestion that the effects of culture age on *R. japonicum* infectivity involve changes in efficiency.

The dose-response curves for cultures of strain 61A76 Str and strain 83 (Fig. 5b and 6) represent a relatively complex case, so that interpretations must be correspondingly more limited and tentative. Since the response curves are not linear over much of the log dose range, it appears that two or more variables may be affecting infectivity. The number of cells of strain 61A76 Str or strain 83 Str required to achieve a given degree of nodulation above the mark is 10- to 40-fold greater than the number of exponential-phase strain 110 ARS, strain 138 Str Spc, or strain 123 Spc cells required. Strains 61A76 Str and 83 Str thus appear relatively inefficient in terms of initiating infections. This inefficiency is expressed almost exclusively at low inoculum doses. It appears that a relatively large number of bacterial cells (ca. 1,000) must be added to a root before normal efficiency is achieved.

The correlations between changes in lectin binding and infectivity with culture age are suggestive but not conclusive. The proportion of *R. japonicum* cells capable of binding soybean lectin in cultures of different ages generally paralleled the culture age-dependent changes in the number of nodules formed above the RT mark for strains 110 ARS and 123 Spc (Fig. 1) and strain 138 (5). Strains 61A76 Str and 83 Str showed relatively little variation in infectivity at different culture ages. These two strains have a very low proportion of cells capable of binding soybean lectin at any culture age and, perhaps correspondingly, they are relatively inefficient strains which require inoculation with many more cells to achieve the same degree of nodulation as log-phase cells of strains 110 ARS, 138 Str Spc, or 123 Spc.

Despite these general correlations between lectin binding and nodulation above the RT mark, there is need for caution in concluding that the two are causally related. The first point of caution is that acapsular mutants of strain 138 Str Spc, which cannot bind soybean lectin, are nonetheless able to nodulate soybean (10). The synthesis of a lectin-binding polysaccharide by *R. japonicum* appears to be correlated with ability to nodulate, but the polysaccharide need not be synthesized in the form of an insoluble capsule (10). If this is true, then the binding of lectin to the *R. japonicum* cell surface per se is not a very satisfactory basis for testing a cause-and-effect relationship with infectivity. A second point of caution is that the bacteria in the inocula do not generally retain their cell surface lectin binding capabilities. The difficulty is that the capsules of *R. japonicum* are lost rather easily unless the ionic strength of the suspending medium is relatively high (9). The cultures in the present studies were diluted with phosphate-buffered saline to preserve the capsules for determination of lectin-binding cells. However, the inocula were prepared by dilution of the cultures in distilled water. Assays have indicated that virtually none of the rhizobia in the inoculum preparations retained their ability to bind soybean lectin. Further studies are thus required to determine whether the correlations between lectin binding and infectivity are coincidental or relevant.

One of the most surprising and potentially useful findings from the present studies is the discovery that *R. japonicum* cultures retain their infectivity after extensive dilution in distilled water. The loss of symbiotic capabilities is a common problem commercially and in the laboratory when *R. japonicum* strains are repeatedly subcultured. Storage of *Rhizobium* suspensions in water at ambient temperatures may prove to be a convenient way of avoiding this problem. In preliminary tests, we have found that several *R. japonicum* strains retained full viability and full infectivity after 8 months of preservation in water.

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