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"WORLD RHIZOBIUM STUDY AND COLLECTION CENTER"

Nitrogen Fixation & Soybean Genetics Laboratory
Beltsville Agricultural Research Center
Beltsville, Maryland

ANNUAL PROGRESS REPORT
1986
AID-USDA RSSA
WORLD RHIZOBIUM STUDY
& COLLECTION CENTER

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INTRODUCTION

In 1976, the U.S. Agency for International Development (AID) contracted with the U.S. Department of Agriculture to establish the "World Rhizobium Study and Collection Center" within the Agricultural Research Service at Beltsville, Maryland. The purpose of the center is to provide a safe and perpetual depository and collection center for Rhizobium and Bradyrhizobium cultures, which are available to users worldwide, and to provide research and training opportunities in the area of legume-Rhizobium technology to scientists from developing countries. The overall goal of this project is to increase legume productivity through enhanced nitrogen fixation.

Since 1912 the Agricultural Research Service has maintained a collection of Rhizobium, and, despite limited funds, has continued to distribute this germplasm worldwide. The establishment of this project with supplemental funds from AID represents an investment on behalf of lesser developed countries. Benefits to them include increased collection, evaluation and distribution of rhizobial germplasm for legumes commonly grown in less developed countries, and opportunities for their scientists to work with the excellent rersearch staff in the Nitrogen Fixation and Soybean Genetics Laboratory. The combination of an existing Rhizobium collection (the USDA Rhizobium Culture Collection), experienced personnel and international connections makes the ARS, its facilities and programs, the best qualified instsitution for establishing and maintaining a truly international center for the collection and study of rhizobia. The center is housed within the Nitrogen Fixation & Soybean Genetics Laboratory, where a multi-disciplinary staff of research scientists working on nitrogen fixation is available for collaborative efforts.

The World Rhizobium Study and Collection Center is linked to numerous programs and institutions worldwide which promote research and utilization of symbiotic nitrogen fixation including NifTAL, INTSOY, the UNESCO/UNEP/ICRO MIRCENs, the International Research Centers sponsored by CGIAR, and the Limiting Factors in BNF Program (AID/CSRS).

SCOPE OF WORK

The specific objectives of the project are to:

1. Collect isolates of Rhizobium which fix nitrogen efficiently in association with legumes.
2. Collect other organisms which fix nitrogen when associated with other plants.
3. Characterize the performance of newly acquired microbial germplasm with reference to specificity of host, cultivar-Rhizobium interctions, nitrogen fixing efficiency and environmental variables.
4. Enumerate and cataloge germplasm accessions for distribution to researchers worldwide.
5. Serve as a depository of microbial germplasm.

6. Distribute upon request stock cultures for research and commercial inoculum production.

7. Train researchers and technicians from developing countries in techniques and subject matter for applied research and technology of Rhizobium.

8. Consult with and provide technical guidance to researchers in the area of symbiotic nitrogen fixation in developing countries.

9. Modify exisiting methods of preservation of rhizobia to determine the best inexpensive method which can be used in developing countries.

10. Study inoculation methods in relation to persistence and competition among strains of Rhizobium.

PROJECT PERSONNEL

Deane F. Weber, Ph.D.	Microbiologist, Principal Investigator
Harold H. Keyser, Ph.D.	Microbiologist, Curator
Richard F. Griffin, B.S.	Biological Laboratory Technician, Collection Manager
Mike Mendelsohn	Biological Aid (half time)
Joseph Manna	Biological Aid (half time)

* * * * * 1986 ACTIVITIES AND ACCOMPLISHMENTS * * * * *

GERMPLASM

In 1986 we distributed 666 cultures from 103 requests for germplasm of Rhizobium. Requests from foreign countries accounted for 40 % of the total. Cultures were sent to the following countries:

Holland	Syria
Pakistan	Canada
Poland	France
Senegal	India
Australia	Malawi
Norway	Uruguay
United Kingdom	Egypt
West Germany	Saudi Arabia
Zimbabwe	Mexico
Guatemala	Argentina
Rep. of China	Japan

A summary of the 1986 requests by Rhizobium species follows:

Bradyrhizobium japonicum	280 cultures
Bradyrhizobium sp.	126 cultures
Rhizobium meliloti	11 cultures
Rhizobium fredii	84 cultures
Rhizobium leguminosarum	
bv phaseoli	31 cultures
bv trifolii	79 cultures
bv viceae	27 cultures
Rhizobium loti	3 cultures
Rhizobium sp.	25 cultures

The current holdings of the accessioned collection are as follows:

Genus and species	Number of Cultures
Bradyrhizobium japonicum	457
Rhizobium meliloti	109
Rhizobium leguminosarum	
biovar *trifolii*	382
biovar *viceae*	121
biovar *phaseoli*	67
Rhizobium fredii	11
Bradyrhizobium spp.	1198
TOTAL	2345

In addition, we have 314 cultures which are uncharacterized and not in the accessioned collection, bringing the grand total to 2,659 cultures.

Accessioned cultures are permanently preserved by two parallel methods: frozen in 20% glycerin at -80°C, and lyophilized (freeze-dried) with subsequent storeage also at -80°C. Each culture is replicated 4 times in both conditions. Of the 2345 accessioned cultures, only about 600 remain to be permanently preserved. These are mostly cultures received last year from the Nitragin Co. These (along with some unaccessioned cultures) are preserved on agar slants in screw-capped tubes in a 4°C cold room.

In addition to servicing requests for germplasm and antiserum, in 1986 we provided a deposition and lyopohilization service to Dr. Eduardo Schroder of the University of Puerto Rico. Forty-six cultures of *Rhizobium* isolated from *Leucaena leucocephala* were received and lyophilized, 6 replicates of each. These lyophilized cultures were returned to Dr. Schroder and a culture of each was retained in our collection. The request was made due to problems with the survival of these cultures in a cold room and the lack of lyophilization equipment available to Dr. Schroder.

TRAINING

Mr. Hussein Kamel Abd El-Maksoud, from the National Research Centre in Cairo, Egypt, came to our project for intensive training and research in the serology, ecology and cultivar-strain interactions of *Rhizobium* with several host legumes. He arrived in late March, 1986, for a 10 month visit. His research efforts included the evaluation of restricted nodulation by selected soybean genotypes in the field, evaluation of alternate carriers for incoulants appropriate for Egyptian conditions, and the determination of the rhizobial requirement for the tuberous legume *Apios americana*.

ASSIATANCE AND COLLABORATION

Assistance and collaboration was provided to other programs, both national and international, dealing with the use and promotion of biological nitrogen fixation:

Dr. Keyser visited the West Africian Rhizobium MIRCEN, Bambey, Senegal, May 6 to May 9, 1986. The MIRCEN director, Dr. M. Gueye, indicated several ways in which our Beltsville MIRCEN could assist the activities of the West Africian MIRCEN. These would include financial assistance in sponsoring a taining course in BNF technology for the West African region, participation in the workshop by Drs. Weber and Keyser as instructors, training at the Beltsville center of personnel from West Africa interested in gaining research experience and specialized technique skills, and possible collaboration on future research grants. An interim-visit report was also prepared for BOSTID on Dr. Gueye's grant "Improving the yield and biological nitrogen fixation of Bambara groundnut". Meetings were also held with the USAID staff in Dakar and Senegal's CNRA staff regarding the activities of the Beltsville Rhizobium Study and Collection Center and our relationship with the West Africian MIRCEN.

Dr. Keyser also visited the East Africian Rhizobium MIRCEN at the University of Nairobi, Kenya, May 10 to 15, 1986. The MIRCEN director, Dr. S.O. Keya, expressed his thanks for the training provided to his employee, Beatrice Anyango. A visit was made of their inoculant production facility and field trials on Phaseolus vulgaris. We agreed that Beltsville would provide antiserum for their incoulant strains of Rhizobium phaseoli so they could pursue examination of competition in different environments using immunofluoresence. An interim-visit report was also prepared for BOSTID on Dr. Keya's grant "Effects of environmental factors on nitrogen fixation by beans in Kenya".

Dr. Keyser visited the research station at San Jose de La Rinconada, Sevilla, Spain. The Rhizobium inoculant for Spain is produced here under the supervision of Francisco Temprano and Rafael Orive. This is an excellent small scale facility, with emphasis on faba beans and lupines. We discussed collaboration on competition studies using antibiotic and serological markers in Spanish soils. Support for this study is being provided by the U.S.-Spain Joint Committee for Scientific and Technological Cooperation. We also discussed reserach strategies regarding introduction of white lupines and their rhizobia to alkaline soils of Spain.

Dr. Keyser was an invited speaker at the BOSTID Research Grants Program BNF Third Coordination Meeting, Rio de Janeiro, Brazil, August 4-8, 1986. A presentation was given on the influence of the host in determining nodulation and competition in soybean, and a collaborative short presentation was made with Dr. E.L. Schmidt on strain identification with Rhizobium.

RESEARCH ACCOMPLISHMENTS

The Staff of the World Rhizobium Study and Collection Center conduct research with other scientists of the Nitrogen Fixation & Soybean Genetics Laboratory, using the collection as an exploitable base for research on important issues. Highlights of the research in 1986 follow:

Four Bradyrhizobium japonicum from sub-tropical Australia gave yield responses on 30 U.S., Canadian and Malaysian soybean varieties that were comparable to USDA 110 in Leonard jars or field soil in greenhouse testing. Host-strain interactions were evident on cultivars Hardee and Douglas. Competition between these strains and USDA 110 was variable; two were competitive and one was completely non-competitive. This demonstrates the wide range adaptiveness between diverse B. japonicum and diverse soybean germplasm.

Genotypes of soybean which restrict nodulation of strain USDA 123 have been identified. A group of 20 recent field isolates of serogroup 123 were compared with USDA 123 for nodulation on two of the restricting genotypes, results showing that 25% of the field isolates were restricted. Subsequent competition studies with restricted strains showed that they were not able to block nodulation of the effective strain USDA 110. Further screening revealed other soybean genotypes which may restrict nodulation of the field isolates of serogroup 123 not previously restricted. Also, a 2-year field trial demonstrated that two of the restricting genotypes were nodulated almost completely by the applied inoculant strain (USDA 110) in competition with USDA 123, while just the opposite was found with the commercial cultivar Williams.

Tests were performed in sand culture and field soil to evaluate the effect of prior infection of the plant, prior inoculation of the plant medium, and inoculant mixtures on competitive interactions of rhizobia on soybean varieties. The six varieties infected and/or nodulated with rhizobia of varied effectiveness, exhibited responses from inhibition of nodulation to increased nodulation and plant yield when re-inoculated with competitive and effective rhizobia or when these were part of inoculant mixtures. Six general inoculant responses were defined. These responses suggest that re-inoculation of field soils populated with indigenous rhizobia of questionable effectiveness might be valid despite generalizations of no response in yield by applied inoculants.

ABSTRACTS AND PUBLICATIONS

Abstracts and publications by the center's staff in 1986 follow:

Cregan, P.B., H.H. Keyser, and H.K. Abd. El-Maksoud. 1986. A field study of rhizobia strain competition as influenced by soybean genotype. Agronomy Abstracts p. 61.

Sadowsky, M.J., H.H. Keyser, and B.B. Bohlool. 1986. Serological characterization of *Rhizobium fredii*. Agronomy Abstracts p. 187.

Kinkle, B.K., J.S. Angle and H.H. Keyser. 1986. Long-term effects of sewage sludge application on soil populations of *Bradyrhizobium japonicum*. Amer. Soc. Microbiology, Abstracts of the Annual Meeting, p. 243.

Keyser, H.H. 1986. Preparation and use of legume inoculants. Appendix for procedural manual for research in nitrogen-fixing, multiple-use trees. BOSTID, Committee on Research Grants, National Research Council, Washington, D.C.

Cregan, P.B., and H.H. Keyser. 1986. Host restriction of nodulation by *Bradyrhizobium japonicum* strain USDA 123 in soybean. Crop Science 26:911-916.

APPENDIX

Attached are reports, abstracts and publications from 1986.

pastures in the extensive neotropical regions of the world. Top growth increased significantly with soil K and P amendment and with mycorrhiza colonization. Nodulation and nitrogenase activity were correlated with highly significant increases from G. fasciculatum, P treatments and K additions to 300 mg K kg^{-1} soil. Growth and peduncles of nonclipped plants increased about 4 fold from 90 to 225 day age with mature seed yield increasing about 10 fold; nodule mass and nitrogenase activity levels approximately doubled. Regrowth response of plants defoliated at 45 day intervals, following their initial 90 day age, was constant between clippings for regrowth, development in number of peduncles, seed yield, nodulation, and nitrogenase activity. Multiple regression for Nitrogenase = 0.55 g top wt. + 0.63 g nodule wt. + 1.91 day age - 0.07 peduncle no., R^2 = 0.85 and C. V. = 14.3%

Interactions of Mycorrhiza and Phosphorous Sources in Clover. Sharon G. RAINES* and J. J. MADDOX, TVA, Muscle Shoals, Alabama.
Clover was grown in the greenhouse to test the effectiveness of vesicular-arbuscular mycorrhiza (VAM) to increase the availability of phosphorous supplied in slowly soluble materials. The low-solubility P-sources were Tennessee, Florida, North Carolina, and Missouri rock phosphate (TN-RP, FL-RP, NC-RP, and MO-RP) and impure monoammonium phosphate (MAP). Conventional fertilizer P sources used for comparison were K_2HPO_4/KH_2PO_4 solution and CSP. VAM isolates evaluated were Gigaspora coralloidea, G. margarita, Glomus etunicatum, and the indigenous soil species. G. coralloidea and G. etunicatum produced the largest dry matter accumulation of clover tops with all P-sources. NC-RP and MAP were as effective in promoting vegetative growth as CSP or PO_4 solution. Top yield with TN-RP and FL-RP was increased 9% and 22% by G. coralloidea while top growth was increased 17% by G. etunicatum when MO-RP was the P-source. Total P uptake by clover tops was also increased by G. coralloidea and G. etunicatum. Uptake patterns for Mg, Ca, K, and Mn also varied with VAM isolate and P-source indicating that plant-VAM interactions are strongly affected by different VAM isolates and soil conditions.

Effects of soil moisture and available carbon on temporal and spatial variability of denitrification. G.W. RICE, S. SIMKINS, L. KLEMEDTSSON, and J.M. TIEDJE. Michigan State Univ. and Swedish Univ. of Agriculture.
Denitrification rates were measured from intact soil cores sampled from a 1.5-ha watershed planted to no-till corn in Michigan and from grass leys in Sweden. Soil moisture and CO_2 evolution rate explained only 13.1% of the variability in denitrification rates in the watershed. In grass, soil moisture and NO_3^- explained 36.7% of the variability of the denitrification rates. At sample intervals of 5 m, denitrification rates were not autocorrelated within the watershed. Denitrification in the grass leys showed evidence of temporal autocorrelation. Using an exponential model for the temporal semivariogram, 58.5% of the variability in denitrification rates could be accounted for by geostatistical jackknifing. Kriging, especially through time, can supplement the predictive abilities of models based on mechanistically important soil variables.

C and N bioavailability of soil particle size and density fractions.
J. S. ROGERS * and H. E. DONER, Univ. of California.
Soils from a California oak-grass savannah (mollic haploxeralf) and a nine year old mixed conifer site (ultic haploxeralf) were size and density fractionated to facilitate the study of mineral-organic matter complexation on soil C and N bioavailability. Soil samples from A and B horizons were ultrasonified and separated into 2mm - 250µm, 250-50, 50-5, 5-2, and <2µm size fractions by sieving and centrifuging. Whole soil density fractions of <1.8, 1.8 - 2.0, 2.0 - 2.6, and >2.6 x 10^{-3}kg m^{-3} using bromoform were obtained after ultrasonic dispersion. 2M KCl extractable N, hot water soluble, 12M H_2SO_4 hydrolyzable and non-hydrolyzable, and total C and N of the separates were determined. Size and density fractions were aerobically incubated, and CO2 evolved and N mineralized with time measured. Microbial availability of C and N was related to the nature of the organo-mineral complexes of the soil separates.

Laccase Positive Basidiomycete from Forest Residue Mulches. F. M. ROTHWELL, USDA-FS, Berea, KY
Qualitative procedures were used to determine the wood-decomposing capabilities for a basidiomycete that was isolated in axenic culture from mixed hardwood chip and bark materials used as a mulch on minesoils. The culture gave a positive Bavendamm reaction when grown on tannic acid plates; was able to decompose Kraft lignin when xylan or Walseth cellulose were added as cosubstrates in a mineral nutrient agar medium; and was laccase positive with syringaldazine following 4-months growth on whole-tree maple chips incubated in stationary flask culture. Because of the association of laccase activity with ligninolytic capabilities, the basidiomycete isolate in this study undoubtedly has an essential role in the catabolism of forest residue mulch materials. Though important early in minesoil stabilization woody residues may well be of greater significance as long-term reservoirs of aromatic compounds involved in humus formation on surface-mined lands.

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Serological Characterization of Rhizobium fredii. M.J. Sadowsky, H.H. Keyser*, USDA-ARS, Beltsville, MD, and B.B. Bohlool, University of Hawaii. *
Several isolates of Rhizobium fredii were examined to determine their serological relatedness to each other, to Bradyrhizobium japonicum, and to other fast- and slow-growing rhizobia. Immunofluorescence, agglutination, and immunodiffusion analyses indicated that, based on their somatic antigens, R. fredii could be divided into at least three separate serogroups, USDA 192, 194, and 205. With the exception of serogroup 122 there was no cross-reaction between any of the R. fredii isolates and 13 B. japonicum somatic serogroups. Strains from each of the other Rhizobium species examined (with the exception of R. meliloti and Rhizobium sp. for Leucaena, Sesbania, and Lablab) did not cross-react with antisera from the three R. fredii serogroups. Since antisera from R. fredii cross-reacted with several R. meliloti strains, the serological relationship between the two species was examined in more detail. Of the 23 R. meliloti strains examined, 8 shared somatic antigens with all 3 R. fredii serogroups while 3 of the strains reacted only with antiserum from USDA 192. The serological relatedness of these two species appears unique since the strains are known to be genetically diverse.

Temporal Changes in Urease Activity in Floodwater of Submerged Soil. N. K. SAVANT*, A. F. JAMES, and G. H. MCCLELLAN, IFDC.
Urea broadcast in wetland paddy is largely hydrolyzed by urease in the floodwater and at the floodwater-soil interface, and serious NH_3 volatilization losses can occur. Two greenhouse experiments (without rice plants) were conducted to study temporal changes in urease activity in the floodwater and at the interface. Urease activity in the floodwater, as well as at the interface, was markedly influenced by sunlight and its positive effect on the development of algal biomass. Redox potential of soil at the time of submergence and the addition of powdered rice straw to oxidized soil followed by submergence influenced the temporal changes in urease activity in the floodwater and at the interface.

Survival of Inoculant Bradyrhizobia in Sterile and Non-Sterile Soils. M. M. SAVOY*, E. P. DUNIGAN, and G. A. BREITENBECK, Agronomy Dept., La. Agric. Exp. Str., LSU Agricultural Center.
The ability of bradyrhizobia to survive when added as peat-based inocula to autoclaved (sterile) and field-moist (non-sterile) samples of three Louisiana soils was studied by determining the number of streptomycin- and rifampicin-resistant B. japonicum (AN6a) recovered from soils incubated at 30°C for 0, 3, 10, or 30 days after inoculation. The data obtained showed that recovery of rhizobia at the time of inoculation from a sand, a silt loam, and a silty clay receiving 6.4 x 10^7 cells g^{-1} soil was similar for the three soils and averaged 54% of the cells added. Incubation of autoclaved soils for 3, 10, and 30 days after inoculation led to average increases of 105%, 300%, and 102%, respectively, in the number of inoculant rhizobia recovered, whereas incubation of field-moist soils for 3 or 10 days did not lead to a significant increase, and incubation for 30 days led to a 10- to 100-fold decrease in the number of inoculant rhizobia recovered. Results of experiments to assess the potential of a peat carrier for promoting the survival of inoculant rhizobia subject to dessication or predation in soils are also reported.

N-9 Microbial Immobilization and Remobilization of Cadmium in Soil Suspensions. P. CHANMUGATHAS*, J.-M. BOLLAG, and A. J. FRANCIS, The Pennsylvania State University, University Park, PA 16802 and Brookhaven National Laboratory, Upton, NY 11973.

The effect of microorganisms on the fate of cadmium was investigated in two different soil suspensions. Soils were suspended (5 g per 100 ml) in a medium amended with Cd (20 µg/ml) and incubated at 28°C. At various time intervals aliquots were withdrawn and Cd concentration in the aqueous phase was analyzed by atomic absorption spectrometry. During the first two weeks of incubation 11 µg/ml and 19 µg/ml of Cd were bound in non-sterile suspensions of Morrison sandy loam and Hagerstown silt loam soil, respectively, whereas the amounts of Cd bound in the corresponding gamma-irradiated sterile soil suspensions were 2 µg/ml and 17 µg/ml, respectively. During additional 12 weeks of incubation approximately 20 µg/ml and 10 µg/ml of Cd were released in the non-sterile suspensions of Morrison sandy loam and Hagerstown silt loam soil, respectively, while no release occurred in the sterile samples. The pH of both non-sterile suspensions was near pH 6.0 and remained constant after two weeks of incubation. The results suggest that soil microbial activity is responsible for both binding of Cd to and its subsequent release from soil.

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N-10 Long-term Effects of Sewage Sludge Application on Soil Populations of Bradyrhizobium japonicum. B. K. KINKLE,* J. S. ANGLE, and H. H. KEYSER. Univ. of Maryland, College Park, and USDA-ARS, Beltsville, MD.

The application of sewage sludge to land may increase the concentration of heavy metals in soil. Because the bioavailability of sludge-borne heavy metals may increase over time, the potential exists for the soil microbial population, including Bradyrhizobium japonicum, to be affected. To examine this problem, the minimum inhibitory concentrations for Pb, Cu, Al, Fe, Ni, Zn, Cd, and Hg were determined in minimal media using lab isolates representing eleven common serogroups of B. japonicum. Marked differences were found among the strains for sensitivity to Cu, Cd, Zn, and Ni. A silt loam soil amended 10 years ago with 0, 56, or 112 Mg ha^{-1} digested sludge was next examined for total B. japonicum numbers using the MPN method. Nodules from soybeans grown in the soil were typed serologically. The number of rhizobia in the sludge amended soils was found to increase with increasing rates of sludge. No serogroup distribution differences were found between the soils. The application of heavy metal-containing sewage sludge to soil does not appear to have a long-term detrimental effect on soil rhizobial numbers, nor does it result in a shift of the distribution of serogroups.

N-11 Phage Effects on Nodulation and Soybean Growth: Field Studies. FAWZY M. HASHEM* and J. SCOTT ANGLE, Univ. of Maryland, College Park, MD.

Previous laboratory and greenhouse studies have shown that phage significantly reduce homologous soil rhizobial populations. Reductions in nodulation and nitrogen fixation have also been observed. The purpose of the current field experiments was to examine the effect of a phage specific for Bradyrhizobium japonicum USDA 117 on nodulation, nodule occupancy, nitrogen fixation, and soybean growth. The phage plus combinations of USDA 117 and the nonhomologous USDA 110 were inoculated into a rhizobia-free, sandy loam soil and planted to Glycine max "Williams". When the phage was applied to soil inoculated with USDA 117 only, significant reductions in nodule weight and number, shoot weight, nitrogenase activity, and seed yield were observed. When, however, the soil also contained USDA 110, no significant effect on any of these parameters was found. Nodule occupancy by USDA 110 and 117 was affected by the phage. In soil which did not contain the phage, 46 and 44% of the identified nodules were occupied by USDA 110 and 117, respectively. When the phage was present in the soil, nodule occupancy by USDA 117 was decreased to 23%, while occupancy by USDA 110 was increased to 71%. These results suggest the potential to partially restrict nodulation by selected strains of rhizobia.

N-12 Effect of Iron and Rhizosphere Bacteria on Nodulation Competition Among Bradyrhizobium japonicum Strains. J. FUHRMANN,* and A. G. WOLLUM, II, North Carolina State University, Raleigh, NC 27695-7619.

Four fluorescent Pseudomonas spp. and one Serratia sp., isolated from soybean plant (Glycine max (L.) Merr.) rhizospheres, were tested for an ability to alter nodulation competition among Bradyrhizobium japonicum strains under two levels of iron availability. Three B. japonicum strains (USDA 31, 110, and 123) were combined in various ratios and used to inoculate soybean seed, either alone (control) or in combination with a rhizosphere isolate co-inoculum. Plants were cultured under greenhouse conditions in vermiculite with and without added ferric hydroxide. Nodule occupancy was determined after 28 days. Three Pseudomonas co-inoculants consistently increased occupancy by USDA 110 relative to the control at the low-iron treatment. This confirms similar studies previously conducted in our laboratory. Two of these isolates produced similar, although less pronounced, effects when ferric hydroxide was added to the rooting medium. The results suggest that iron availability may be an important factor modifying microbe-microbe and microbe-plant interactions involving B. japonicum in the host rhizosphere.

N-13 Survival of Fast and Slow-Growing Soybean Rhizobia in Soil Amended with Soybean Root Exudates. SALAH M. MAHMOUD* and J. SCOTT ANGLE, Univ. of Maryland, College Park, MD.

Root exudates are known to affect the soil population of rhizobia. To examine this problem, root exudates from Glycine max were tested to determine their effects on fast (Rhizobium fredii USDA 191) and slow-growing (Bradyrhizobium japonicum USDA 110) soybean rhizobia. Root exudates from nodulating and nonnodulating isolines of "Clark" were examined. A nonsterile silt loam soil was amended with the exudates at concentrations of 0.00, 0.10, 0.25, and 0.50%. The survival of the rhizobia was determined for 14 weeks. All treatments exhibited a significant decline in rhizobial numbers during the study. Exudates from the nodulating plants supported a higher population of rhizobia when compared to nonnodulating exudates and an unamended control. The survival of B. japonicum USDA 110 was enhanced to the greatest extent by the 0.1% root exudate concentration. Increasing the exudate concentration to 0.25 or 0.50% reduced the survival of this rhizobia. The greatest soil population of R. fredii USDA 191, however, was found with an exudate concentration of 0.50%. The increased growth of R. fredii USDA 191 on the highest root exudate concentration was most likely related to relative salt tolerance.

N-14 Root Exudate Treatment Enhances Soybean Nodule Occupancy by Bradyrhizobium japonicum. M.J. SADOWSKY*, R.A. HAUGLAND, K.D. WEILAND, A. AYANABA, R.G. UPCHURCH, R.M. ZABLOTOWICZ, Allied Corp., Syracuse, NY 13209.

The effects of root-exudate (RE) treatment on nodule occupancy by Bradyrhizobium japonicum strains were investigated. Logarithmic or stationary phase cultures of the refe-rence strains, L-110 nal or 61A101c, were used in paired-competition assays with RE-treated or untreated cells of several challenge strains. Based on field and greenhouse competition studies, these challenge strains were placed into 3 competitiveness groups: high, AN-11, 61A101c, and AN-6; intermediate, AN-3a and 122SR; and low, I-110ARS and AN-B. Nodule occupancy of Glycine max cv centennial was determined using either an immunofluorescence technique or a rapid colored nodule assay. On this cultivar, L-110 nal forms small, dark-red nodules while the other strains form large, beige nodules. When L-110 nal was the reference, two of the strains responded positively to RE-treatment. Logarithmic and stationary phase cultures of AN-6 showed increased occupancy from 22-38% ($p < 0.10$) and from 23 to 39% ($p < 0.05$), respectively. While the maximum increase for stationary phase cultures of 61A101c was from 34 to 47% ($p < 0.05$), logarithmic phase cultures failed to respond to RE-treatment. The results of these studies indicate that RE-treatment increases the nodule occupancy of some, but not all B. japonicum strains.

initiated at the squaring stage in the glass boxes and at the flowering stage in pots. An index was developed and used to identify the differential response of cultivars to drought susceptibility. In the glass front box experiment, a high tap root dry weight was the most important and consistent trait associated with drought susceptibility. A short distance from the transition zone to the first main lateral root was highly correlated with drought susceptibility in the pots. Other traits associated with a resistant response to drought were a long root system, whether lateral or tap, and a high proportion of lateral roots in the total root system. The results indicated that some currently grown cultivars possess root traits that allow them to perform well under drought conditions.

Response to 12 Cycles of Mass Selection for Prolificacy in Maize. J. G. COORS, Univ. of Wisconsin-Madison.
Progress from 12 cycles of mass selection for early maturity and prolificacy in the Golden Glow maize population was evaluated. Cycles 0, 3, 6, 9, and 12 along with testcrosses of cycles 0, 6 and 12 with three inbreds, W9, A632, and W64A, were evaluated over two years, two locations, three stand densities (38271, 53597, and 68887 plants ha^{-1}), and two fertility levels (0 and 168 kg N ha^{-1}). The average number of ears per plant has increased by 3.0% per cycle. Grain yields have increased by approximately 2.8% per cycle. Correlated responses for pollen and silk dates, root and stalk lodging, and kernel characteristics were calculated. The advanced cycles appear more able to sustain elevated yields in high stress environments although stalk and root lodging have increased.

Introgression Theory.
N.M. COWEN, United AgriSeeds, Inc.
When an exotic equilibrium population is crossed and backcrossed one or more times to an adapted inbred the mean of any backcross generation can be predicted as a linear function of the means of the inbred line and the BC_0 and the number of generations of backcrossing. The genetic variance expressed among individuals in any backcross generation can be predicted as a function of the genetic variance among BC_0 families and the squared difference between the means of the BO_0 and the adapted inbred. The mean of selfed lines obtained from any backcross generation can be predicted as a function of the means of the corresponding progeny in the BC_0 and the adapted inbred. If the mean of the BC_0 is greater than the mean of the adapted inbred, then random mating in any backcross generation will reduce the mean, the converse is true as well.

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A Field Study of Rhizobia Strain Competition as Influenced by Soybean Genotype. P. B. CREGAN,* H. H. KEYSER, and H. K. ABD EL-MAKSOUD, USDA-ARS, Beltsville, MD.
Strains of Bradyrhizobium japonicum serogroup USDA 123 occupy a high percentage of the nodules of soybeans grown in the Midwest U. S. Displacement of USDA 123 in favor of more effective strain(s) should increase total N_2 fixed. We identified soybean genotypes which exclude strain USDA 123 under greenhouse conditions. The objective of this work was to test one of these genotypes under field conditions. In 1984 and 1985, the USDA 123-excluding genotype PI 377578 and 'Williams' soybean were planted in B. japonicum-free soil and inoculated with liquid inoculum of USDA 123 before planting followed by granular inoculum of USDA 122 and 138 at planting. Nodules were collected and strain nodule occupancy was determined using strain-specific fluorescent antibodies of USDA 123, 122, and 138. In 1984, 76% of the Williams nodules contained USDA 123 vs. 1% of the PI 377578 nodules. In 1985, Williams nodules contained 76, 20, and 11%, respectively, of USDA 123, 122, and 138, whereas the nodules of PI 377578 were occupied by 9, 60, and 48%, respectively, of these strains. PI 377578 appeared to exclude USDA 123 in favor of the highly effective strains USDA 122 and USDA 138.

Breeding for Improved Relative Growth Rates in Early Maize. W. Z. CROSS, North Dakota State Univ.
Research was undertaken to evaluate the effectiveness of a method of estimating relative growth rate (RGR) in early maize (Zea mays L.). RGR's were estimated as (leaf number x length x width x 0.5)/silk date measured on single S2 plants in unreplicated ear-to-row plots. Based on their RGR's, 32 S2 parental lines with fast and slow RGR's were paired by source population, year evaluated, and maturity and crossed to produce four sets of partial diallels for field evaluation at four locations in 1985. Entries were evaluated for early vigor, yield, agronomic traits, and yield components across environments using a factorial analysis. Results indicated that high RGR parents produced hybrids with increased early vigor which silked earlier, yet were taller with more, wider, and longer leaves and which yielded 9.3% more grain which was 2.8 points lower in moisture than hybrids from low RGR parents. The yield gain was due to increased kernel rows/ear, kernels/row, and kernel weight. Hybrids from low RGR parents had less stalk breakage. For most traits, GCA effects predominated over SCA effects indicating that simple mass selection should be effective in these populations.

A New Technique for Crossing Mungbeans. T. B. CUPKA* and L. H. EDWARDS, Oklahoma State University
Improved crossing techniques are needed to exploit the genetic potential of mungbean [Vigna radiata (L.) Wilczek]. In our technique, only the tip of the bud is opened to expose the stigma and style. After the anthers are gently removed with forceps, the emasculated bud is then ready to be crossed. When pollination is completed, cellophane tape is placed over the opening to seal the bud and the process is complete. An experienced technician can complete an emasculation and pollination in 1 min. The success rate using this technique averaged 60% with an average of six seeds per pod per successful cross. This technique differs from those presently being used in other legume crops because most other crossing techniques open the entire dorsal edge of the bud in order to make an emasculation and pollination. This technique appears to reduce moisture and temperature fluctuations within the bud and reduces bud abortion subsequent to artificial pollination.

Diallel Analysis of Verticillium Wilt Tolerance and Earliness of Plant Maturity in Cotton and Associations Between These Traits.
M. E. DEVEY* and M. L. ROOSE, Univ. of California.
A diallel cross involving nine cotton (Gossypium hirsutum L.) genotypes was used to study the inheritance of tolerance to Verticillium wilt, caused by Verticillium dahliae Kleb., and several measures of earliness. Estimates of genetic parameters following the method of Hayman and Jinks, showed Verticillium wilt tolerance to be controlled by predominantly additive genetic effects with some degree of partial dominance. Narrow-sense heritability estimates were high, 0.61 and 0.69 for F_1 and F_2 generations, respectively. Tolerance to Verticillium wilt in these crosses appeared to be controlled by recessive alleles. Combining ability analyses also indicated the importance of additive gene effects for Verticillium wilt tolerance as well as for the measures of earliness. General combining ability effects were of greater importance than specific combining ability effects for all traits. The measures of earliness used were generally related to each other but, except for earliness as defined by percentage of total lint yield in first harvest, did not appear to be related to Verticillium wilt tolerance.

Host Genetic Systems for Control of Rhizobial Specificity.
T.E. DEVINE* and J.J. O'NEILL, USDA-ARS, Beltsville, MD.
Once a rhizobial population has been established in the field, it has rarely been possible to displace the indigenous rhizobia from symbiosis with a legume crop. Though agronomically more desirable rhizobia are known and genetically engineered strains are being constructed, it is not now economically feasible to establish these rhizobia in fields against the competition of indigenous rhizobia. Therefore, two approaches may be taken: the host plant may be bred for adaptation to the indigenous rhizobia, or one could attempt to control the genotype of the rhizobia admitted to symbiosis in order to utilize highly efficient strains. Using isogenic lines, we have recently found that the Rj4 allele in soybean prevents normal nodulation with Bradyrhizobium japonicum strains USDA 61, 62, 83, 94, 238, and 259. Five of these six strains produce the chlorosis inducing phytotoxin, rhizobitoxine. This suggest that the Rj4 allele has a selective value to the plant carrying it in preventing nodulation with these rhizobia. Compared to the control, a 10-fold reduction in nodule occupancy of genetically marked strain USDA 61 was achieved through the use of the Rj4 carrying soybean cultivar 'Hill', over four years in field plot

Procedural Manual for Research in Nitrogen-fixing, Multiple-use Trees. 1986.
BOSTID, Committee on Research Grants, National Research Council, Washington, D.C.

APPENDIX 5

Preparation and Use of Legume Inoculants

This appendix describes the preparation and use of legume inoculants containing the nodule bacteria *Rhizobium*. A good quality inoculant is a prerequisite for adequate nodulation and nitrogen fixation. Inoculant preparation requires aseptic procedures and access to microbiological facilities. If inoculant preparation is not feasible at your facility, specific inoculants (cultures or a peat-based carrier) can be obtained from the NifTAL Project and MIRCEN or from the *Rhizobium* Study and Collection Center at the U. S. Department of Agriculture.

Inoculation of legumes in the greenhouse or nursery can be accomplished with liquid broth culture (yeast-extract mannitol) or with a peat carrier. For use in the field with the direct seeding of legumes, the peat inoculant is recommended.

Preparation of Inoculum

While peat is the most common carrier for inoculants, bagasse, filter mud, charcoal, and lignite have also been used. The procedure described below relates specifically to peat as the carrier, and it would apply with minor variations to the others.

1. Particle size of the peat.

   The peat should be milled to particles of 200 mesh or finer (pass through a 0.075 mm sieve) for use as a seed coating.

2. Neutralization of the peat.

   Most peats are too acid for use as a carrier for rhizobia without neutralization with lime or dolomite. Finely divided $CaCO_3$ gives rapid reaction.

3. Sterilization of the peat.

   If possible, the peat should be autoclaved (120°C) for 2 hours, then allowed to cool for 24 hours, with provision for gas exchange.

4. Inoculation of peat.

a. Culturing of the rhizobia.

The strain(s) of rhizobia should be grown in yeast-extract mannitol (YEM) broth, the composition of which follows:

K_2HPO_4	0.5 g
$MgSO_4$	0.2 g
NaCl	0.1 g
Mannitol	10.0 g
Yeast extract	0.4 g
Distilled water	1 liter (adjust pH to 6.8 - 7.0)

Autoclave at 120°C for 15 minutes. After cooling to room temperature, 1 liter of broth can be inoculated with a loopful of culture from an agar slant. Fast-growing rhizobia will reach maximum cell density (about 10 cells per ml) in 3 to 5 days at 25°C, while slow growers require 7 to 10 days.

b. Addition of rhizobial broth to peat.

When the strains have reached maximum density, the YEM broth is added to the peat at a volume to obtain 35-40 percent moisture in the peat on a wet weight basis. The author routinely adds 100 ml of mature YEM broth to 180 g peat (36 percent moisture). With the wet peat in a plastic bag, gently mix the contents to ensure good wetting of the peat, which is initially hydrophobic.

5. Incubation and storage of peat inoculant.

Though the peat inoculant can be used after adequate mixing (step 4b), best results are obtained by allowing the peat to incubate at room temperature (25-30°C) for 2 to 3 weeks. This permits the growth of rhizobia in the peat to high population levels. During this period, the bag should not be closed tight, as exchange of gases is important for adequate growth of the rhizobia. Do not allow the moisture content to fall below 30 percent. If it should, add sterile YEM broth to a moisture level of 35 percent. The inoculant should not be kept at room temperature for more than 4 weeks, but should then be kept in a refrigerator (4°C). When stored at 4°C the peat can be used as an inoculant for up to 12 months. However, it is best to use the inoculant as soon as possible after the 2-3 week incubation.

Use of Inoculum

1. Inoculating directly with YEM broth culture in the greenhouse or nursery.

Under favorable greenhouse and nursery conditions, legume seed inoculation can be achieved with YEM broth culture. From step 4a above, the mature broth should contain about 10^9 cells per ml. The addition of one-tenth of a milliliter directly to each seed would

provide 10^8 cells of rhizobia, which is more than adequate. At this rate, one liter of broth will permit the inoculation of 10,000 seeds. After the seed is placed in a hole or furrow, drip the 1/10 ml directly onto the seed, and cover before the broth dries.

2. Inoculation of legume seed with peat.

The inoculation of seed with peat is best accomplished by the use of an adhesive. Excellent results are obtained with a 40 percent solution of gum arabic (=gum acacia). A 5 percent methyl ethyl cellulose solution can also be used. The gum arabic solution is prepared by heating 100 ml water and slowly adding 40 g with constant stirring. Neutralization of the solution may be needed.

The recommended rate of inoculation is about 10 g of peat for each kilogram of seed. While seed size varies, the object is to obtain seed which is visibly coated with peat. For each kilogram of seed, addition of 20-30 ml of gum arabic solution should coat the seed thoroughly. This is followed by the addition of the peat, with adequate mixing to give uniform coating of the seeds. Inoculated seed should be planted within 24 hours. In the field, the inoculated seed should not be exposed to direct sunlight for long periods of time. Once the inoculated seed is placed in the furrow or hole, it should be quickly covered.

Jseful References with Details of Legume Inoculant Preparation and Use. (for full references, see the bibliography above)

1. [J. Burton.] Legume Inoculants and Their Use.

2. R. J. Roughley, "The preparation and use of legume seed inoculants"

3. P. Somasegaran and H. J. Hoben. Methods in Legume-Rhizobium Technology

4. J. M. Vincent. A manual for the Practical Study of Root-Nodule Bacteria

Sources of Rhizobium Cultures and Peat Inoculants

Refer to Section 5, Table 3: Sources of Rhizobium, Frankia, and VA Mycorrhizal Fungi for Inocula.

Host Restriction of Nodulation by *Bradyrhizobium japonicum* Strain USDA 123 in Soybean[1]

P. B. Cregan and H. H. Keyser[2]

ABSTRACT

The displacement of indigenous *Bradyrhizobium japonicum* serogroup USDA 123 in soybean [*Glycine max* (L.) Merr.] nodules in favor of a more effective inoculant strain(s) has been suggested as an approach to enhance soybean productivity in the midwestern USA. Our objective was to determine if the host plant could serve as a vehicle to restrict nodulation by an indigenous serogroup thereby allowing nodulation by a more effective rhizobial strain. A total of 1278 soybean plant introductions and cultivars were screened in the greenhouse with *B. japonicum* strain USDA 123. Thirteen genotypes demonstrated restricted nodulation with USDA 123 when compared to five standard cultivars. Two of these genotypes, PI 371607 and PI 377578, were planted along with the cultivar 'Williams' in a soil free of *B. japonicum* and inoculated with USDA 123 and either USDA 110, 122, or 138. In each competition treatment, greater than 75% of the nodules of Williams were occupied by USDA 123 whereas, in all cases, less than 10% of the nodules of the PI genotypes contained USDA 123. The PI genotypes excluded strain USDA 123 in favor of the inoculant strain. In another experiment, the two PI genotypes and Williams were inoculated with either USDA 123 or 110. With USDA 123, nodulation and N accumulation was significantly less in the PI genotypes than in Williams. In contrast, with USDA 110, nodulation and N accumulation of the PI genotypes was similar to Williams. Thus, while the PI genotypes nodulated and fixed N_2 poorly with USDA 123, nodulation and fixation with an efficient strain such as USDA 110 was normal. Our results suggest that the trait identified in the PI genotypes could positively impact soybean productivity by excluding all or part of the indigenous serogroup 123 population in favor of more effective strains of *B. japonicum*.

Additional index words: Glycine max (L.) Merr., Dinitrogen fixation, Rhizobial competition.

A MAJOR emphasis of recent research in the field of N_2 fixation has been the development and application of genetic engineering techniques for the creation of rhizobial strains with enhanced N_2 fixation. Such strains may soon be available and, indeed, highly effective naturally occurring strains already exist. However, establishing an introduced strain of *Bradyrhizobium japonicum* in the nodules of soybean [*Glycine max* (L.) Merr.] grown in soils populated by indigenous rhizobia has been, and remains, a critical problem (Ham, 1976; Ham, 1980a; Vest et al., 1973; Cregan and van Berkum, 1984). A number of studies have demonstrated that even at high inoculation rates, the proportion of nodules formed by an inoculum strain is generally less than 20% (Caldwell and Vest, 1970; Ham et al., 1971a; Kuykendall and Weber, 1978). Although Johnson et al. (1965) recovered an inoculum strain in 82% of the nodules of 'Kent' soybean, the inoculum was applied at 700 times the recommended commercial rate and at least 12% of the indigenous population was serologically indistinguishable from the inoculum strain. Similarly, Kapusta and Rouwenhorst (1973) recovered an inoculum strain in up to 60% of the nodules after the application of ultra-high rates of inoculum. Weaver and Frederick (1974) indicated that in order to establish an inoculum strain in 50% of the nodules of a soybean plant, the rate of inoculum application would have to be at least 1000 times greater [per gram of soil (g^{-1} soil)] than the indigenous population. These studies suggested that with the rhizobial strains, soybean genotypes, and inoculation techniques currently available, the displacement of indigenous strains by those carried in inoculum was difficult and economically unfeasible.

The nature of the indigenous population of *Bradyrhizobium* is of particular interest to those concerned with displacing this population in favor of an inoculum strain. In Minnesota, Ellis et al. (1984) found that in each of 2 yr, approximately 95% of the nodules of field-grown soybean plants contained strains of indigenous serogroup USDA 123. Further work in Minnesota by Moawad et al. (1984) documented the unique ability of the indigenous serogroup USDA 123 to form a large proportion of nodules even when equal populations of an inoculum strain were present in the soybean rhizosphere. Reyes and Schmidt (1979) noted the remarkable ability of serogroup USDA 123 to compete for nodule sites. High frequencies of strains of the USDA 123 serogroup in the nodules of field-grown soybean plants have not been limited to Minnesota soils. Indeed, reports from Illinois (Kapusta and Rouwenhorst, 1973), Iowa (Damirgi et al., 1967; Ham et al., 1971b), and Ohio (Miller, 1979) indicated the predominance of serogroup 123 in the nodules of field-grown soybean plants. Keyser et al. (1984) reported that 61% of the nodules collected from plants grown in Minnesota were occupied by strains of serogroup 123. In a survey conducted by D.F. Weber (1985, personal communication) an average of 64% of the soybean nodules collected from the northern midwest states of Ohio, Indiana, Illinois, Iowa, Nebraska, Minnesota, and Wisconsin contained strains of serogroup USDA 123. Together these states represented 52% of the 1984 U.S. soybean hectarage and 60% of the estimated soybean production (American Soybean Association, 1984).

The predominance of strains of serogroup USDA 123 in the soils of the upper midwest would be an asset if these strains offered the most highly effective N_2 fixation. There is, however, no evidence to suggest this to be the case. Caldwell and Vest (1970) compared the yield of five different soybean cultivars when inoculated separately with 30 soybean rhizobia in four field environments, three of these environments were free of *B. japonicum*. The yield of soybean cultivars inoculated with USDA 123 ranked 16th among the 30 strains. Calculations from data presented by Ham (1980b) indicated that, averaged across three soybean cultivars grown in soil free of indigenous soybean rhizobia, seed yield with USDA 123 ranked last among

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seven strains tested. Kvien et al. (1981) noted that whenever 50% or more of the nodules formed were occupied by the inoculant strains USDA 110 or USDA 138, rather than by strains of the indigenous serogroup 123, significant seed yield increases were detected. These reports suggest that the displacement of serogroup 123 in favor of more efficient strains should result in increased soybean productivity.

One approach to altering the strain of *Rhizobium* found in the nodules of soybean grown in the northern midwest would be the development of cultivars in which nodulation by serogroup USDA 123 is eliminated or substantially reduced. A reduction in nodulation by this highly competitive serogroup would allow nodulation by other strains and might provide a means of significantly increasing the proportion of nodules containing the inoculum strain. In soybean, genotypes exhibiting restricted nodulation with specific strains or serogroups of soybean rhizobia have been reported. The single dominant gene Rj_2 (Caldwell, 1966) conditions restricted nodulation with all available strains of the USDA 122 and c1 serogroups (Caldwell et al., 1966). The single dominant gene Rj_3 (Vest, 1970) conditions restricted nodulation with only strain USDA 33 but not with other strains that are serologically related. A third single dominant gene, Rj_4, conditions restricted nodulation with strain USDA 61 (Vest and Caldwell, 1972). The reaction of Rj_4 with strains serologically similar to USDA 61 has not been reported. The fact that there are genes in soybean that condition restricted nodulation with specific serogroups or strains suggests that genes might be found that restrict nodulation of serogroup 123. Thus, the objective of the work reported herein was: (i) to identify one or more soybean genotypes that restricted nodulation by *B. japonicum* strain USDA 123, and (ii) to test the capacity of these genotypes to preferentially nodulate with an inoculant strain other than USDA 123.

MATERIALS AND METHODS

Initial Screening Experiments

Samples of all the available accessions in the USDA soybean germplasm collection of Maturity Groups VI through X were obtained courtesy of Dr. E.E. Hartwig (USDA-ARS, Stoneville, MS 38776). In an initial experiment, seeds of 720 Maturity Group VI and VII accessions, which included 679 plant introductions and 41 cultivars, were screened for their ability to nodulate with *B. japonicum* strain USDA 123. An autoclaved mixture of equal volumes of vermiculite and coarse perlite was placed in 25-cm plastic pots that had been soaked overnight in a sterilizing solution of 800 μL L^{-1} Rocal II[3] (Dimethyl benzyl ammonium chloride) (National Laboratories, Montvale, NJ) followed by rinsing in tap water. Seeds of each accession were surface sterilized by immersion in acidified $HgCl_2$ (Vincent, 1970) for 2 min followed by five rinses in distilled water. Six different accessions were planted in separate hills at a depth of 2 cm in each pot using six seeds of each accession per hill. Before covering, each hill was inoculated with 2 mL of stationary broth culture (approximately 10^9 cells mL^{-1}) of USDA 123. Eight additional pots were planted with six hills each of the cultivar 'Lee'. Seeds in two of these pots were inoculated with USDA 123, two pots were inoculated with USDA 110, and the four remaining pots were left uninoculated. After covering the seeds, the vermiculite-perlite medium was covered with a 1-cm layer of sterile coarse perlite to serve as a dry barrier to prevent contamination by extraneous *B. japonicum*. Seedlings were thinned to two per hill at 4 days after emergence. At 2 weeks after planting, all pots received 500 mL of N-free nutrient solution. At 19 and 26 days after seeding, two of the uninoculated pots containing Lee soybean were given 15 mL of a 0.4 *M* $Ca(NO_3)_2$ solution. These latter pots served as the N control treatment. Plants were grown in the greenhouse with natural sunlight supplemented by incandescent light to extend the photoperiod to 18 h. Day and night temperatures were maintained at 25 ± 5°C. At 42 days after seeding, the plants were removed from the pots and the root systems were washed and examined. The nodulation of each root system was visually assessed by comparison with the nodulated root system of Lee. In those accessions in which nodule number was obviously less than Lee, the number of nodules per root system was determined. Accessions with less than 10 nodules (or that had germinated or grown poorly) were rescreened in the same manner as described above in order to confirm the nodule response obtained in the initial screening.

In a subsequent experiment, germplasm accessions from Maturity Groups VIII through X, which included 24 cultivars and 534 plant introductions, were seeded, inoculated, and otherwise treated and evaluated as described above. Again, those accessions with less than 10 nodules or that had germinated or grown poorly were rescreened.

Secondary Screening

Those accessions that appeared to have restricted nodulation with USDA 123 based upon the initial screening and rescreening tests described above were evaluated in a subsequent experiment using Monmouth fine sandy loam soil (Aric Normudult) from the University of Maryland Tobacco Farm at Upper Marlboro, MD. This soil had no detectable *B. japonicum* (<10 cells g^{-1} soil) as determined by a most probable number (MPN) test using the plant infection method (Vincent, 1970). The soil was limed to a neutral pH and placed in 25-cm plastic pots that had been sterilized with Rocal II as described above. Seeds were surface sterilized and groups of five germplasm accessions were planted in each of two duplicate pots using hills of five seeds for each accession. In addition, a sixth hill in each pot was planted with the cultivar 'Bragg'. Before covering, the seeds in one of the two duplicate pots were inoculated as described above with USDA 123 and the other duplicate pot was inoculated in a similar manner with an equal mixture of strains USDA 6, 110, and 136. Three additional pots were each planted with the cultivars Bragg, 'Williams', 'Corsoy', 'Hodgson', Lee, and 'Essex'. Seeds in one pot were inoculated with USDA 123, while the seeds in the second were inoculated with a mixture of USDA 6, 110, and 136. The third pot served as an uninoculated control. After planting, the soil surface was covered with a 1-cm layer of sterile gravel. Greenhouse photoperiod and temperature conditions were similar to those described above. Plants were thinned to two per hill. At 34 days after seeding the plants were removed from the soil and the number of nodules per root system was determined.

Two germplasm accessions that had demonstrated particularly limited nodulation with USDA 123 in the initial screening were examined in two separate experiments to obtain quantitative estimates of nodulation and N_2 fixation with USDA 123 in relation to *B. japonicum* strains normally considered to be highly effective. These experiments included:

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Experiment 1. A randomized complete block with four replications was employed in which each replication consisted of one 25-cm plastic pot sterilized as described above and filled with autoclaved vermiculite. Each pot was planted with one hill each of PI 371607, PI 377578, and Essex (a standard check cultivar) using five surface-sterilized seeds per hill. Before covering, the seeds were inoculated with *B. japonicum* strain USDA 123 as described above. After planting, the vermiculite surface was covered with a 1-cm layer of sterile coarse perlite. Nitrogen-free nutrient solution was applied as described above and greenhouse conditions were similar to those described previously. At 30 days after seeding, plants were harvested and the nodules removed, dried, and weighed. The plant tops above the cotyledonary node were removed and dried for subsequent determination of N content of duplicate samples using an Erba Nitrogen Analyser[1] (Carlo Erba Strumentazione, Milan, Italy).

Experiment 2. A randomized complete block design with four replications was used. The treatments consisted of a factorial arrangement of two soybean genotypes and three inoculation treatments. Leonard jar assemblies (Leonard, 1943) were filled with vermiculite, autoclaved, and planted with one hill of five surface sterilized seeds of either PI 371607 or PI 377578. Before covering, the seeds were either inoculated with 2 mL of stationary broth culture of USDA 123; 2 mL of a mixture of strains USDA 6, 110, and 122; or were left uninoculated. The experiment was conducted in a growth chamber with an 18-h photoperiod, 25°C day temperature, 20°C night temperature, and a photon flux density of 392 μmol $m^{-2}s^{-1}$ at seedling level. At 40 days after seeding, the plants were harvested and treated as described in Exp. 1.

Rhizobial Competition for Nodulation

Experiment 3. A randomized complete block experimental design was used that consisted of a split plot arrangement of treatments and four replications. Whole plots (pots) were inoculation treatments and subplots were soybean genotypes. Soybean-rhizobia-free Monmouth soil (5.2 kg pot^{-1}) was preinoculated with USDA 123 by alternately spraying and mixing the soil with a dilution prepared from a 5-day yeast-mannitol-salts solution (Vincent, 1970). A determination of viable cells showed that 1.4×10^6 cells were added g^{-1} soil. Each pot was then planted with two hills each of PI 371607, PI 377578, and Williams. Three competition treatments were developed by inoculating each hill of each pot with 1 mL of 6-day yeast-mannitol-broth culture containing 9.3, 3.4, or 4.0×10^6 cells of either USDA 110, 122, or 138, respectively. In this manner three treatments were established to allow a determination of the competition for nodulation between USDA 123 and 110, USDA 123 and 122, and USDA 123 and 138. After covering the seed, 1-cm layer of sterile gravel was placed upon the soil surface. Seedlings were thinned to one per hill. Greenhouse conditions were similar to those described above. At 28 days after planting, the plants were harvested and the nodules removed. The nodules from the two plants of each genotype within each pot were combined and oven dried. The serogroup of the strain occupying 15 nodules from each replication of each soybean genotype-competition treatment combination was determined using strain specific fluorescent antibodies as described by Schmidt et al. (1968). Fluorescent antibodies of USDA 123 and 110, USDA 123 and 122, or USDA 123 and 138 were used depending upon the competition treatment being analyzed.

Experiment 4. The experimental design was similar to that of Exp. 3. The inoculation treatments (whole plots) consisted of: (1) an uninoculated control, (2) preinoculation with USDA 123 to a level of 1.8×10^6 cells g^{-1} soil, (3) preinoculation with USDA 110 to a level of 4.9×10^6 cells g^{-1} soil, and (4) preinoculation with USDA 123 followed by seed inoculation using yeast-mannitol-broth (1.7×10^9 cells $hill^{-1}$) of USDA 110 at planting. Treatment 4 was established to allow a determination of competition for nodulation between USDA 123 and 110. Preinoculation was performed in the same manner as described for Exp. 3. The subplots were the genotypes PI 371607, PI 377578, and Williams planted as described for Exp. 3. At 42 days after planting, the plants were harvested and the nodules removed, dried, and weighed. The plant tops above the cotyledonary node were excised and dried for subsequent N analysis of duplicate samples using the Erba Nitrogen Analyser[1]. The nodules and plant tops from the two plants of each genotype within each pot were combined. The strain present in 30 nodules per replication of each soybean genotype receiving Treatment 4 was determined using strain specific fluorescent antibodies of both USDA 123 and USDA 110.

RESULTS

Initial Screening Experiments

The initial screening of germplasm accessions of Maturity Groups VI through X identified 22 genotypes with restricted nodulation when grown in artificial medium and inoculated with USDA 123. The selected genotypes produced from one to as many as eight nodules per plant. In contrast, the cultivar Lee consistently produced 30 or more nodules as did most of the germplasm accessions. In some instances when germination or growth was poor, the accessions were poorly nodulated as compared to Lee and other genotypes with vigorous healthy growth. The uninoculated control of Lee provided a basis for discrimination between poor growth resulting from N starvation and that resulting from other factors relating to poor vigor or disease. Thus, the 22 genotypes that were selected demonstrated healthy vigorous growth as well as restricted nodulation with USDA 123. Other accessions included in our screening may also restrict nodulation by USDA 123, but did not, in our judgement, grow adequately well to allow us to clearly distinguish between restricted nodulation and poor growth.

Secondary Screening Experiments

This experiment examined the nodulation response of the 22 genotypes that were identified in the initial screening experiments. All of the control genotypes produced greater than 30 nodules with USDA 123 (Table 1). Nine of the 22 test genotypes produced more than 5 nodules, whereas 13 accessions had two or less nodules per plant. In general, we noted that nodulation with USDA 123 on these 13 genotypes was more restricted in the *B. japonicum*-free soil than we had observed previously in the vermiculite-perlite mixture. When inoculated with a mixture of *B. japonicum* strains USDA 6, 110, and 136 these 13 accessions nod-

[1] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee or warranty by the USDA and does not imply its approval to the exclusion of other products that may be suitable.

Table 1. Number of nodules produced on soybean cultivars and plant introductions inoculated with *Bradyrhizobium japonicum* strain USDA 123 and a mixture of strains USDA 6, 110, and 136 (Secondary screening).

Soybean genotype	Maturity group	Mean nodule no. With USDA 123	Mean nodule no. With mixture of USDA 6, 110, 136	Nodulation phenotype‡
		nodules plant⁻¹		
Bragg	VII	>30	>30	N
Williams	III	>30	>30	N
Corsoy	II	>30	>30	N
Hodgson	I	>30	>30	N
Lee	VI	>30	>30	N
Essex	V	>30	>30	N
PI 371607	VI	1	>30	R
PI 376844	VII	2	>30	R
PI 377578	VII	0.5	>30	R
Acadian	VIII	0	>30	R
Arisoy	VIII	0	>30	R
Improved Pelican	VIII	1	>30	R
White Biloxi	VIII	1	>30	R
PI 263044	VIII	1	>30	R
PI 445842	VIII	0	>30	R
PI 324187	IX	0	>30	R
PI 325779	IX	2	>30	R
PI 341248	IX	0	>30	R
PI 163308	X	0.5	>30	R

† Values are the mean of two plants.
‡ N = normal nodulation with strain USDA 123, and R = restricted nodulation with USDA 123.

Table 2. Nodulation and N accumulation of PI 371607, PI 377578, and Essex grown in vermiculite and inoculated with *Bradyrhizobium japonicum* strain USDA 123 (Exp. 1).

Genotype	Nodule dry wt†	Nitrogen accumulation
	mg plant⁻¹	
PI 371607	4b‡	5b
PI 377578	5b	5b
Essex	34a	17a

† Values are the mean of four plants.
‡ Values within a column not followed by the same letter differ significantly at the 0.05 probability level as tested by Duncan's new multiple range test.

Table 3. Nodulation and N accumulation of PI 371607 and PI 377578 grown in vermiculite and inoculated with *Bradyrhizobium japonicum* strain USDA 123 or a mixture of strains USDA 6, 110 and 122 (Exp. 2).

Rhizobium treatment	Nodule dry wt†		Nitrogen accumulation	
	PI 371607	PI 377578	PI 371607	PI 377578
	mg plant⁻¹			
Uninoculated	0c‡	0b	3b	3b
USDA 123	120b	42b	9b	7b
USDA 6 + 110 + 122	185a	207a	29a	28a

† Values are the mean of four plants.
‡ Values within a column not followed by the same letter differ significantly at the 0.05 probability level as tested by Duncan's new multiple range test.

ulated at least as well as the control cultivars. The 13 genotypes selected included at least one representative from each of the five maturity groups and included cultivars as well as plant introductions.

Experiments 1 and 2. Of the 13 USDA 123 restricting genotypes, PI 371607 and PI 377578 were chosen for further testing because sufficient seed were available. When compared to the cultivar Essex, PI 371607 and PI 377578 nodulated to a limited extent (Table 2). Similarly, the N accumulation of the PI genotypes was significantly less than that of Essex. The nodule dry weight of PI 371607 and PI 377578, when inoculated with USDA 123, was significantly less than that obtained with a mixture of strains USDA 6, 110, and 122 (Table 3). When inoculated with USDA 123, the N accumulation of the two PI genotypes was not significantly different from the uninoculated control and was less than one-third of that obtained with the mixture of strains. These results supported our preliminary finding that nodulation by USDA 123 is restricted in PI 371607 and PI 377578.

Rhizobial Competition for Nodulation

The development of a genotype that restricted nodulation by strain USDA 123 would be of little use unless a corresponding increase in nodulation by strains of other more desirable serogroups was obtained. Thus, two experiments were conducted to test the effect of two genotypes restricting nodulation by USDA 123 in a situation in which both USDA 123 and a strain of another serogroup were present simultaneously in the rhizosphere.

Experiment 3. Monmouth soil containing no detectable *B. japonicum* was preinoculated to obtain approximately 10^6 cells of USDA 123 throughout the 25-cm pot in an effort to simulate the background USDA 123 population of a northern midwestern U.S. soil (Moawad et al., 1984). Both PI 371607 and PI 377578 preferentially nodulated with either USDA 110, 122, or 138 rather than USDA 123, when compared to the commonly grown cultivar Williams (Table 4). The difference in the proportion of nodules occupied by USDA 123 in the PI genotypes vs. Williams was substantial. Across the three rhizobial competition treatments, a mean of 80% of the Williams nodules contained USDA 123 in contrast to a mean of only 4% of the nodules of the PI genotypes. The inoculant strain appeared in 97% or more of the nodules of the PI genotypes but never exceeded 44% in Williams.

Experiment 4 In an attempt to quantify the effects of restricted nodulation by USDA 123 on nodulation and N accumulation when grown in soil, PI 371607, PI 377578, and Williams soybean were grown in *B. japonicum*-free soil. When uninoculated, top plant weight and N accumulation in the PI genotypes and Williams were not significantly different (Table 5). In soil preinoculated with USDA 123, the nodulation, plant weight, and N accumulation of Williams significantly exceeded that of the PI genotypes. In the case of preinoculation with USDA 110, no statistical differences were detected between Williams and the two PI genotypes for nodulation, plant weight, or N content. When treatment consisted of planting in soil preinoculated with USDA 123 followed by inoculation with USDA 110 at planting, nodule mass was similar in the three genotypes. In this treatment, top dry weight (but not N accumulation) was significantly greater in PI 377578 than in Williams. The percentage of nodules occupied by USDA 123 and 110 was nearly identical to that observed in Exp. 3 despite a difference greater than 100-fold in the amount of inoculant strain applied. Less than 10% of the nodules of PI 371607 and PI 377578 were occupied by USDA 123, whereas this strain was present in nearly 80% of the nodules of Williams soybean. Similar to the findings in Exp. 3,

Table 4. Strains recovered from the nodules of PI 371607, PI 377578, and Williams in three nodulation competition treatments established by preinoculation of Monmouth soil with strain USDA 123 followed by inoculation with either strain USDA 110, 122, or 138 at planting (Exp. 3).

Soybean genotype	Nodulation competition treatment†								
	USDA 123 vs. USDA 110			USDA 123 vs. USDA 122			USDA 123 vs. USDA 138		
	USDA 123	USDA 110	Other	USDA 123	USDA 122	Other	USDA 123	USDA 138	Other
	%								
PI 371607	0b‡	100a	2a	7b	98a	0a	7b	97a	0a
PI 377578	0b	98b	6a	2b	97a	0a	8b	97a	0a
Williams	83a	25b	7a	82a	44b	0a	76a	44b	0a

† Values of each genotype-treatment combination are the mean of 60 nodules (15 per replication) taken from the roots of a total of eight plants (two per replication). The total of USDA 123 and the corresponding competitor strain may be greater than 100% because of doubly occupied nodules.
‡ Values within a column not followed by the same letter differ significantly at the 0.05 probability level as tested by Duncan's new multiple range test.

Table 5. Nodulation, top dry weight, N accumulation, and bradyrhizobia strains recovered from the nodules of PI 371607, PI 377578, and Williams grown in Monmouth soil and inoculated as indicated (Exp. 4).

Inoculation treatment and soybean genotype	Nodule dry wt†	Top dry wt	Nitrogen accumulation	*B. japonicum* recovered from nodules‡	
				USDA 123	USDA 110
	mg plant^{-1}	g plant^{-1}	mg plant^{-1}	%	
Uninoculated					
PI 371607	0a§	1.98a	24a	–	–
PI 377578	6a	2.20a	28a	0	0
Williams	6a	1.98a	31a	0	0
Soil preinoculated with USDA 123					
PI 371607	22b	2.02b	29b	–	–
PI 377578	37b	2.18b	30b	–	–
Williams	211a	3.02a	66a	–	–
Soil preinoculated with USDA 110					
PI 371607	156a	3.32a	107a	–	–
PI 377578	180a	3.36a	106a	–	–
Williams	138a	2.95a	98a	–	–
Soil preinoculated with USDA 123 and inoculated with liquid USDA 110 at planting					
PI 371607	211a	3.18ab	85a	6b	99a
PI 377578	226a	3.84a	95a	9b	97a
Williams	184a	3.12b	81a	79a	45b

† Values are the mean of eight plants (two per replication).
‡ Values are the mean of 120 nodules (30 per replication) taken from the roots of a total of eight plants (two per replication). The total of USDA 123 and USDA 110 may be greater than 100% because of doubly occupied nodules.
§ Values within a column within an inoculation treatment not followed by the same letter differ significantly at the 0.05 probability level as determined by Duncan's new multiple range test.

USDA 110 was present in at least 97% of the nodules formed by the PI genotypes and only 45% of those formed by Williams.

DISCUSSION

Our results indicate the presence of soybean genotypes that restrict nodulation by *B. japonicum* strain USDA 123. These genotypes appear to noduiate and fix N_2 normally when inoculated with three other strains of *B. japonicum*. Of 13 germplasm accessions that restricted USDA 123 nodulation, two were tested further under conditions in which USDA 123 and an inoculant strain simultaneously competed for nodule sites. In contrast to the cultivar Williams, both the genotypes PI 371607 and PI 377587 preferentially nodulated with the inoculant strain. According to the most recent information available from the Statistical Reporting Service (Crop Reporting Board, 1983), Williams is the most commonly grown cultivar in the USA and occupies approximately 19% of the soybean hectarage in the north central production area. In addition, Williams has been commonly used as a parent in cultivar development programs. Our initial and secondary screening included the cultivars Lee, Bragg, Hodgson, Corsoy, and Essex, all of which demonstrated abundant nodulation with USDA 123. Thus, the 13 genotypes identified in our research (Table 1) appear to possess an unusual nodulation response with USDA 123 as compared to a representative group of soybean cultivars.

These results raise a number of questions. First, does the ability to restrict nodulation by the USDA 123 serotype strain also confer the ability to restrict nodulation by other strains of the USDA 123 scrogroup? In the case of the cultivar 'Hardee' that carries the Rj_2 gene and restricts nodulation by the USDA 122 serotype strain, all other USDA 122 serogroup isolates are also restricted (Caldwell et al., 1966). In the case of the Rj_3 gene, however, restriction is limited to only one strain and nodulation is normal with other serologically related strains (Vest, 1970). The USDA 123 serogroup is a complex one in which three serotypes have been reported (Date and Decker, 1965; Gibson et al., 1971). These serotypes include USDA 123, 127, and 129. Further tests must be conducted to determine the breadth of restriction of other USDA 123 isolates and serotypes. If restriction is limited to only a subgroup of the 123 serogroup, then the identification of genes that exclude other subgroups will be necessary. Whether these additional genes could be combined in a single genotype to offer exclusion of the whole 123 serogroup would require still further study. Such a genotype would be nodulated by other indigenous strains or by highly effective inoculum strains. A potential problem with this approach is that another indigenous serogroup of moderate effectiveness may become the dominant competitor in the rhizosphere and prevent nodulation by desired strains.

Another question raised by our findings concerns the effect upon competition for nodule sites of a genotype that restricts nodulation by the USDA 123 serotype strain when such a genotype is grown in a soil typical of those found in the midwestern USA. Our competition studies were conducted under controlled conditions in which competition was between strain

USDA 123 and a specific pure inoculant strain. Under these conditions about 80% of the nodules of Williams soybean were occupied by USDA 123, a proportion that would be anticipated in many soils of the northern midwest. The very high percentage of nodules of PI 371607 and PI 377578 occupied by the inoculant strain in preference to USDA 123 represents a shift, the magnitude of which has been difficult to accomplish by either manipulation of the host, rhizobia, or the rhizosphere environment (Reyes and Schmidt, 1979; Weaver and Frederick, 1974). Further research must be undertaken to examine the strains recovered from the nodules of these genotypes when grown in typical soils of the midwestern USA.

Rhizobial competition for nodulation in soybean has mainly focused upon competitive differences among rhizobia and upon soil or rhizosphere events. Little research has addressed the influence of the host upon competition. Our results indicate a significant effect of the soybean host in determining the strain of rhizobia that nodulates. This suggests that subsequent studies of rhizobial competition in soybean should consider the host as a variable that can significantly influence competition for nodule sites.

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