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TRIP REPORT

Date submitted 11/30/78

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PERIOD OF TRAVEL (inclusive dates) 22 to 25 October 1978

ITINERARY Washington, D.C. to Ithaca, N.Y. to Washington, D.C.
(Use attachment for details, including time schedule)

PURPOSE To participate in the Workshop on Selecting and Breeding Legumes for Enhanced Nitrogen Fixation and to review the BNF work done under 211(d)AID/csd-2834

ORGANIZATIONS AND PERSONS CONTACTED:
(Use attachment for details.) Participant scientists in Workshop (list attached). Cornell University staff, esp. Dr. M. Alexander, D. Bouldin (List attached).

RESULTS/ACCOMPLISHMENTS:
1. Potential for improving BNF by Breeding Legumes is high for beans, Low for winged bean, intermediate for many crops
2. 211(d) activities are progressing satisfactorily, but some disruption due to uncertainty about extension for 3 more years. (See attached)

Etc.

FOLLOW-UP ACTION REQUIRED:
(Indicate what, by whom, when.) 211(d) grant proposals to be moved forward for action as soon as possible.

OTHER REMARKS:
(May include other information, observations, and impressions of general interest.)

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Attachments: (List)
1. Narrative
2. Participants in Workshop
3. M. Alexander group
4. Dr. Green Comments

Distribution: (List)

DS/AGR:D.Peterson
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D. Green, Iowa State U.

AGR Form No. 2

NARRATIVE:

The Workshop was very valuable in bringing microbiologists, plant physiologists, plant breeders and agronomists together to discuss the potential for improving BNF by breeding legumes. Uncertainty exists about how much of the variation in N uptake and BNF is due to "environment" and how much to "heredity". At least four specific genetic factors have been identified in soybeans, but only general genetic relationships have been found in beans and alfalfa. These preliminary results are encouraging, but indicate the complexity of the genetic relationships with BNF in the plant. Similar complexities have been noted among the root nodule bacteria (rhizobia). The availability of carbohydrate to the root nodules and their efficiency in fixing nitrogen were areas for research. Increased capacity for BNF can be reflected in yield only if the legume genotypes can respond to increased nitrogen. The area is a fertile one for research, but the progress is likely to be slow due to the complexities.

The 211(d) BNF activities are being conducted by several senior staff, especially Drs. M. Alexander, D. Bouldin, T. Scott along with junior staff, Drs. H. Lowendorf and S. Mughogho, and graduate students, especially Wei-Liang Chao, Lawrence Osa-Afiana, J. Jose Pena-Cabriales, Carlos Ramirez and Eugene Madsen. One of the three SOTA have been submitted in final, draft, one in working draft, and one will be submitted in December.

PARTICIPANTS

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Boyce Thompson Institute at Cornell
October 23 and 24, 1978

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M. ALEXANDER

Research Associates:

Alasdair M. Cook -- Effects of acid rain
Christian Daughton -- Alkyl phosphonate metabolism
Gary M. Dunny -- Degradation of synthetic chemicals
Mitiku Habte -- N_2 fixation in rice culture
T. C. Huang -- Ecology of protozoa
Henry S. Lowendorf -- N_2 fixation in tropical soils
Arthur Rosenberg -- Metabolism of insecticides
Richard F. Strayer -- Effects of acid rain
John T. Wilson -- N_2 fixation and establishment of algae
Jerry A. Yordy -- Formation and degradation of nitrosamines

Graduate Students:

Gene Bartholomew -- Microbial metabolism of CO
Wei-Liang Chao -- Competition among *Rhizobium* strains
Sarah Greene -- Nitrosamine formation
Kikuo Honda -- Sulfur transformations
Stuart N. Jacobson -- Cometabolism
Linda Lennox -- N_2 fixation in cereal rhizosphere
Francisco A. Mendez-Castro -- N_2 fixation in cereal rhizosphere
Michelle Thomas -- Survival of new genotypes
Lawrence Osa-Afiana -- Survival of *Rhizobium*
J. Jose Pena-Cabriales -- Drought resistance of *Rhizobium*
Carlos Ramirez -- Competition among *Rhizobium* strains
Stephen C. Simkins -- Microbial interactions in continuous culture
Eugene Madsen -- Rhizosphere microbiology

Technician:

M. Tony Lieberman -- N_2 fixation in cereal rhizosphere

Secretaries:

Dawn Marshall
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Workshop on Selecting and Breeding Legumes *
for Enhanced Nitrogen Fixation
Organized by
Boyce Thompson Institute at Cornell University
Ithaca, New York
October 23 & 24, 1978

The workshop in general was successful in achieving its primary goal of reviewing recent work, the related areas of nitrogen fixation and in considering the requirements for future research. I will comment on each of the major reports separately giving my impression of the possibilities of using the presented information or methods in a breeding program.

1. Natural nitrogen isotope ratios as indicators of N fixation - C. C. Delwiche.

   The basic information presented by this paper, such as the atom percent excess found in using ^{15}N, that ^{15}N is a little more slowly nitrified, and that there is a tendency for ^{15}N to be fixed a little more slowly was a good attempt in developing "confidence" in the method. The fact that only 10 to 12 samples can be done per day would not allow researchers to quantify differences among a large number of lines or cultivars.

2. Measurement of N fixation rates in soybean by <u>in situ</u> acetylene reduction - T. R. Sinclair.

   I am not really able to assess the acetylene reduction method per se, but have the impression that it may not really be measuring "what we think it is".

   The approach of using a preliminary screen (size and number of nodules and N content increase) to narrow the number of lines before using the intensive screen (acetylene reduction) is good. It appeared to me that their promising lines were those that had already been selected as good varieties (eg. Amsoy).

   The in situ flow through method, using a low concentration of C_2H_2 appears to me to be a good idea.

   It is my opinion that most of the variation (rates ranging from 2.0 to 8.0) within pure lines is due to "environment" rather than genetics. He will find out which it is when they select plants from the "pure lines" and test the progeny.

3. The importance of the H uptake system in rhizobium on the N fixing process of legumes - R. J. Maier.

   The work with H evolution differences has been very interesting and my opinion is that the rate of H evolution does indicate to some degree the "efficiency of fixation". Although the amount of energy saved is not great (25% $\pm$) and is "peanuts" when compared to the total energy in the plant system, it could make a difference in a crucial place and time. "Efficient strains" have been identified, but differences in seed or dry matter yields under field conditions are hard to demonstrate.

*Comments by Dr. Detroy Green, Prof. of Crop Breeding. Iowa State University, Ames, IA 50011

The production of efficient and non-efficient "isolines" of rhizobium and measuring a difference in legume dry matter (13.96:11.00) was interesting -- good basic information but how to use their information to breed for enhanced N fixation at present escapes me.

4. Estimating the biological cost of fixation - J. Mahon.

He did a good job of trying to estimate (or clarify) the energy use situation in N fixation.

His casual remark that, with peas, the least efficient strain out performed the more efficient strain reemphasized, to me, the importance of the total symbiotic situation. It possibly also reinforced my bias that, in breeding, the plant is the organism to improve at present production levels.

5. C supply and N fixation - W. Brun.

Several points were made that were of interest:

a) The tops of plants were very important in determining N fixation, possibly reemphasizing the importance of photosynthesis and/or top reservoir for remobilization during rapid seed fill.

b) Depodded plants did not drop in N fixation as much as control, but complete depodding did not alleviate the situation -- so, senescence did occur without leaf yellowing in the depodded plants.

This work plus other work continues to cause one to think that leaf senescence is involved in N fixation - There may be a possibility of breeding in this area.

6. N inputs into soybean and directions for future research - J. E. Harper.

He restated the N nutrition problem especially during seed filling in soybeans. No information was presented that really related to breeding for enhanced N fixation.

7. N fixation during soybean development and strategies for genotype selection - D. W. Israel.

Selection strategies included:

a) Greater photosynthetic rates

b) Slower rate of pod fill to spread the pod fill over a longer period.

c) Slower senescence

Item "b" above may be logical but I have seldom seen a lazy plant or a lazy man produce a large quantity of a quality product. A longer pod filling period may be helpful.

8. N fixation, source-sink relations, and photosynthetic capacity of alfalfa - G. H. Heichel.

They had selected 20 clones from a population of 800, based on C_2H_2 and yield in the greenhouse. When grown in the field, 7 of those selected were in the top ten.

There was a good relationship between yield and N fixation up to 2 g/plant. From 2 to 6 g/plant there was no relationship between N fixation and yield, but all high yielders had high N fixation. Their data may have been better than Heichel indicated because he appeared to be referring to % N from fixation rather than total N fixation. If this is the case, there may be differences among clones in N fixation <u>if</u> the high fixers were also high in take-up of N from the soil.

With 60 day old plants, defoliation resulted in a decline in N-fixation within 24 hours and then a gradual increase for 18-22 days. With older plants, defoliation did not result in a sudden decline in N fixation, possibly because of CHO in the nodule (in the form of starch) or some other CHO pool.

The amount of allantoic acid in the plant stem sap, as an indication of N fixation, was interesting from the standpoint of N being transported with one carbon with allantoic acid, but with 2 carbons with others. One comment was that this system may be a "carbon saver" but allantoic acid may be energy expensive to produce. I cannot evaluate this possibility.

My question following this and other presentations is: -- Is there a relationship between N fixation and "yield" in older plants?

9. Legume host effects on symbiotic N fixation - D. Phillips

The work with the soybean line that has delayed leaf senescence (DLS) is worthwhile. It is interesting that, although the leaves stay green, the:

a) N fixation drops, but drops "somewhat later" than with other cultivars.

b) There is a decline in the photosynthetic rate (as measured by both ^{14}C or IRGA - drop of 25 to 15).

In alfalfa, the relative efficiencies changed from plus values to minus values as time progressed (from 7 days after cloning to anthesis). There was a 2X greater H evolution in nodules that werc 6 weeks old than at 2 weeks.

In general, higher CHO in the roots resulted in greater H evolution (differences between Vernal and non-dormant alfalfas). Could this work mean that selecting for "efficient N fixation in alfalfa may result in less winter hardy types?"

10. Breeding for N fixation in soybean - T. Devine.

This work involved planting non-nodulating lines of soybeans in the field and searching for nodulated plants. They inspected 180,000 plants and found 123 nodules. To present, all <u>Rhizobium</u> that produced nodules on the rj_1 genotype also caused chlorosis.

To me, this work may be interesting but I think the work at other places in selecting for the substantial exclusion of indigenous Rhizobium strains and the selection of "receptiveness" to introduced strains will be more productive.

11. Plant improvement to enhance N fixation in cultivars of P. vulgaris - P. Graham.

They have made a single evaluation, immediately before flowering, of N fixation - plus measuring dry weight of 600 to 800 PI lines, inoculated and uninoculated.

a) Climbing cultivars fixed more N than determinate or "partially determinate" (weakly indeterminate) cultivars. The proportion of total CHO going into nodules in the climbing type was greater.

b) With the "weakly indeterminate" type, a delay in flowering of 33 to 40 days essentially doubled fixation for the season.

c) They suggest that, in breeding, the F_3 or F_4 be selected for yield under high N fertility and the F_4 or F_5 be grown in Rhizobium free soil and inoculated -- selecting for yield and N fixation. This appears to me to be a reasonable approach.

d) They think the lower leaves are transporting CHO to the roots, with little from the top leaves of plants.

e) The suggestion of dipping stems and roots in K iodide (Adam's technique) to estimate the amount of starch and/or the remobilization of starch could possible be a "fast technique" for preliminary screening.

Harold Evans says "There is a lot of nitrate reductase in nodules, much in the leaves, and little in the roots. There may be an effect on fixation (and soybean line differences??).

12. Breeding for improved N fixing potential in alfalfa - D. Barnes.

Dr. Barnes emphasized that N fixing problems in alfalfa (perennial forage producer) is different from soybeans (an annual seed producer).

In their work, increased N fixation as indicated by the acetylene method was associated with a more fibrous root system. They used simple recurrent selection and selected high and low groups for 5 traits with the following general results:

a) After 2 cycles of selection, the selections for high N fixation (acetylene reduction), nodule mass, and fibrous roots remained high and low selections remained low. This is seedling year data and does not indicate the 2nd and 3rd year potentials.

b) Nodule mass accounted for more variance in acetylene reduction than any other character, but when selecting for high and low, all traits were about equal in predicting acetylene reduction.

c) He said that selection for acetylene reduction was best in increasing all other plant characters, so acetylene reduction may be the best method for selection.

They have also made a 16 to 28% increase in N fixation rate of two synthetics over Saranac, using ^{15}N. This is seedling year data and there was no increase in dry matter production over Saranac. The total seasonal N production was 18 and 57% above Saranac. In the greenhouse the acetylene reduction was 10 and 16% greater than Saranac, nodule mass was not different, and top dry weight was greater.

This Minnesota work on alfalfa is well planned and executed. They are conducting an impressive program, but measurements are needed past the establishment year.

13. Results from the small-group discussion.

a) There is little information on effects of environmental stresses on N fixation such as dormancy due to drought-especially in range species, fertility, acidity, and air pollution.

b) Why is there a "pecking-order" in drawing CHO from pods-nodules-roots-stems-leaves? Why does this order change during different stages of growth?

c) There is little nodulation of soybeans at 12°C but adequate nodulation at 15°C.

1. Selection of host plants for germination and growth under cooler temperatures and selection of Rhizobium for effectiveness under cool temperatures may be important in some species and environments.

d) The percent of the total energy of the plant saved by lack of H evolution may not be great, but if energy becomes limiting in the nodule then it is important. (Not all energy is available to the nodule--what is in, or not in, the nodule is important.)

e) There was not much interest in Dr. Devine's work and also not much in the other work in selecting for soybean lines that would differentially select for "efficient strains" of Rhizobium.

f) Increased N fixation may not increase yield unless there are legume genotypes that will give increased yield under high N soil fertility or fertilizer rates.

1. So - should the first selection for high yield be under high N fertility with subsequent selection or alternate year selection under inoculated plots?