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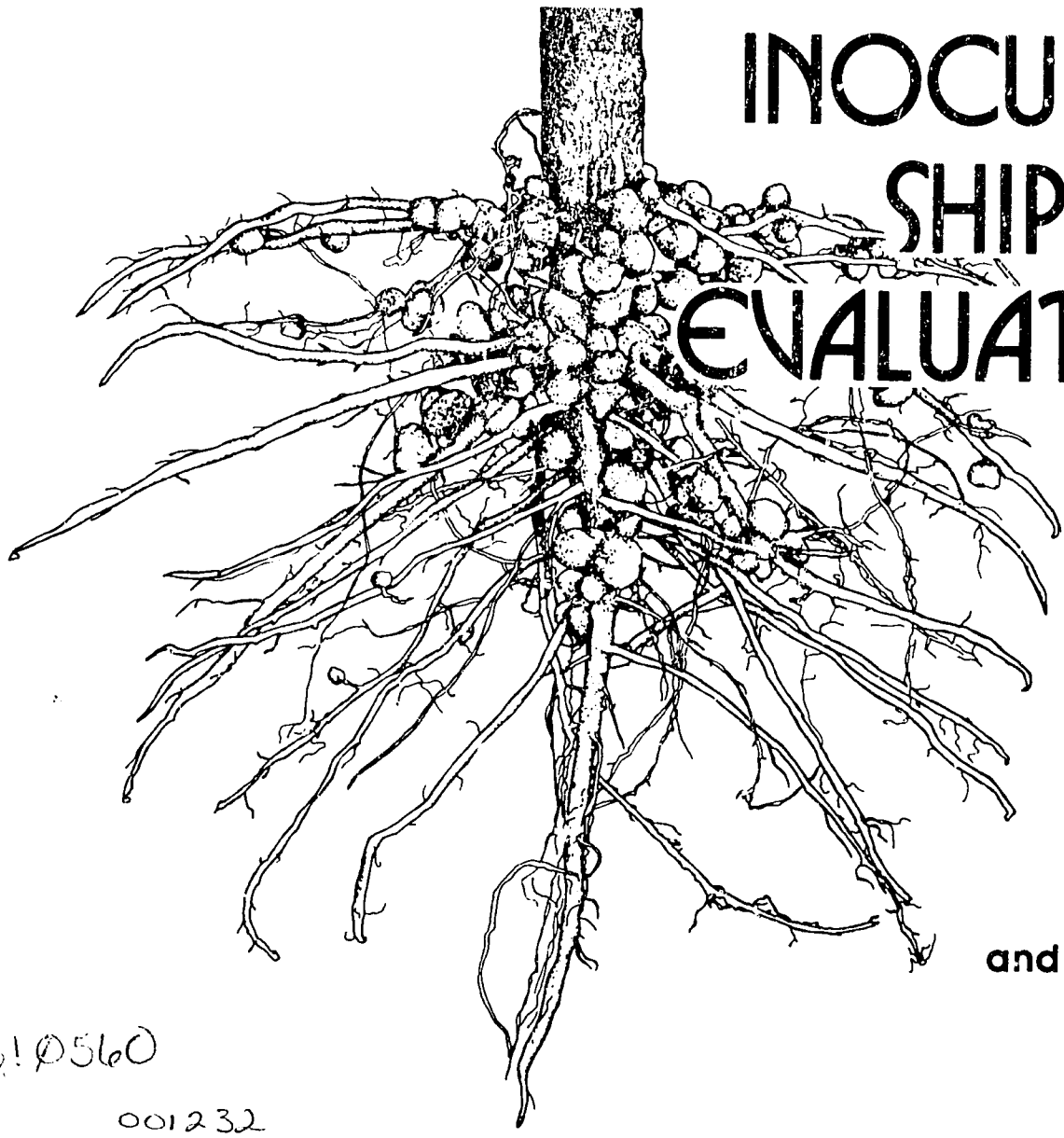
# INTERNATIONAL INOCULANT SHIPPING EVALUATION



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# INTERNATIONAL INOCULANT SHIPPING EVALUATION (IISE)

*by R.S. Smith, W.H. Judy, and W.C. Stearn*

## INTRODUCTION

The International Soybean Program (INTSOY) was established at the University of Illinois and the University of Puerto Rico in order to expand the use of soybeans for human food. Geographically, INTSOY efforts are oriented toward tropical and subtropical areas of the world where nutrition deficiencies in protein and calories tend to be concentrated.

In 1973, the International Soybean Variety Experiment (ISVEX) was initiated by INTSOY as the first element in the genetic improvement program. The objectives are to evaluate soybean cultivars (varieties) for widespread environmental adaptability and to develop improved cultivars for direct introduction or for use in breeding programs worldwide. ISVEX trials are conducted annually at 200 to 300 sites in 80 to 90 countries.

ISVEX trials are sent from the University of Illinois to cooperators around the world. They include recommended instructions for the management of the ISVEX field trial, a data collecting booklet, sufficient seeds of the various soybean cultivars, and granular soybean inoculant. The ISVEX units are shipped by various methods that result in their spending undetermined periods of time in uncontrolled conditions.

The International Inoculant Shipping Evaluation (IISE) trial was conducted in order to evaluate the quality of granular soybean inoculant after it was exposed to shipping and storage conditions during international transport. Stress factors such as high temperature, loss of moisture, time, and exposure to sunlight decrease the quality of inoculants by reducing the number of viable rhizobia bacteria. The granular soybean inoculant enclosed with the ISVEX trial may have encountered all of the above stress factors except direct sunlight. The IISE trial was designed to:

1. evaluate soybean inoculant quality after exposure to international shipping and storage conditions;

2. estimate the quality of the inoculant used with the ISVEX trial;

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3. determine temperatures encountered in international shipping and their effect on inoculant quality;

4. evaluate inoculant moisture loss and the resulting effect on quality after international shipping; and

5. determine the time required to ship inoculants internationally and its impact on inoculant quality.

The IISE was enclosed with the 1979 ISVEX trials and sent through the existing international network of ISVEX cooperators.[a] This simplified implementation of the trial and substantially reduced project costs.

## MATERIALS AND METHODS

### *IISE Unit*

The inoculant unit was prepared for international shipping in the following manner: Fifty grams of granular soybean inoculant (SOIL IMPLANT™ from The Stragin Co., Inc., Milwaukee, Wis.[b]) was sealed in a polyethylene bag. The inoculant contained 1.0 x $10^9$ *Rhizobium japonicum* cells per gram with 35 percent moisture content. An adhesive strip of 4 temperature-sensitive discs was affixed to the bag containing the inoculant. The appropriate discs on the Tempilabel Temperature Monitor (Tempil Division, Big Three Industries, Inc., South Plainfield, N.J.[b]) changed irreversibly from gray to black when temperatures of 38°, 43°, 49°, and 54°C were experienced.

The inoculant bag with the attached temperature label was stapled to the inside of the flap of a 19- x 27-cm manila envelope. After examining the temperature label, each cooperator was instructed to seal the inoculant bag in the manila envelope and post it to the following address preprinted on the front of the envelope:

Dr. R. Stewart Smith
Soil Microbiologist/INTSOY
Department of Agronomy
University of Puerto Rico
Mayaguez, Puerto Rico 00708

To remind the cooperators that the inoculant must be returned for analysis, the legend, *Inoculant Sample, Please Return,* was stamped on the back of the envelope.

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[a]Five IISE trials were sent out with Soybean Preliminary Observation Trial (SPOT) experiments and can be identified in Appendix D by the letter *S* after the trial number.

[b]Mention of a proprietary product does not constitute a guarantee or warranty of the product by the University of Illinois and does not imply its approval to the exclusion of other products that may be suitable.

*Shipping of the IISE Units*

An IISE unit, accompanied by the two-page instruction sheet shown as Appendix A and a one-page data sheet, Appendix B, was enclosed with all ISVEX variety trials sent to cooperators during 1979. The ISVEX trials were shipped in cardboard boxes. The IISE unit was the last item placed in the box and the first to be seen when it was opened. The assigned ISVEX Trial Number was recorded on the top of the IISE data sheet. This served as cross reference to the cooperator for both the ISVEX and IISE trials.

IISE units were returned to Puerto Rico according to the following methods:

| Shipping methods | Number of trials |
|---|---|
| USAID Pouch | 28 |
| FAO Pouch | 6 |
| UNDP Pouch | 3 |
| Air Freight | 30 |
| Air Parcel Post | 5 |
| Total | 72 |

Note: Shipping methods for 4 of the 76 samples are undetermined.

All trials were sent from Urbana, Illinois. ISVEX trials with the enclosed IISE units were mailed to cooperators so they would arrive in advance of the scheduled soybean variety trial planting date.

*Cooperator Instructions and Contributions*

Each ISVEX cooperator first became aware of the IISE trial upon opening the ISVEX shipment and discovering the IISE unit. The accompanying instructions described the nature and objectives of the IISE trial. The cooperator was requested to fill out the data sheet and return it with the inoculant sample to Puerto Rico for quality analysis.

The temperature label affixed to each sample had recorded the maximum temperature the inoculant had experienced in shipment from Urbana, Illinois. Cooperators were asked to circle each temperature reading whose center had turned from gray to black, both on the data sheet and on the label. The name and address of each cooperator and the date of completion of the data sheet were also requested.

*Analysis of Returned IISE Units*

Upon receipt of IISE units at the INTSOY microbiology laboratory at the University of Puerto Rico, samples were analyzed and the data sheet shown in Appendix C was completed. Information recorded at this time included the trial number, name of the cooperator, the cooperator's address, and the date that the unit was received in Puerto Rico. The temperature label was examined and temperature readings from the blackened circles were recorded.

Each inoculant sample was refrigerated the day it was received and analyzed within one week. From the original 50 grams of granular inoculant, 10 grams were

used to determine the pH of the sample. Another ten-gram sample was removed and placed on an O'Haus moisture balance to determine the moisture content of the inoculant. The percentage of moisture was expressed on a wet weight basis. Another ten-gram portion was serially diluted and plated in triplicate on yeast extract mannitol agar with congo red. After approximately 7 days the *R. japonicum* population was counted.

## RESULTS AND DISCUSSION

### *Overview of the Study*

A total of 160 IISE units were sent to cooperators in tropical and subtropical countries. Seventy-six samples were returned for analysis to the INTSOY microbiology laboratory in Puerto Rico. Occasional incomplete information on some of these samples prohibited full analysis. Samples were returned from 43 countries, 12 of which are in Africa, 11 in Asia, 5 in Europe, 5 in Meso-America, 3 in the Middle East, and 7 in South America. They are listed below and their distribution is illustrated in Figure 1.

| Region | Country | Number of trials | Region | Country | Number of trials |
|---|---|---|---|---|---|
| Africa | Algeria | 2 | Europe | Austria | 1 |
| | Botswana | 2 | | Azores | 1 |
| | Ethiopia | 1 | | Czechoslovakia | 1 |
| | Gambia | 1 | | Italy | 2 |
| | Ghana | 3 | | Portugal | 1 |
| | Malawi | 1 | | | |
| | Morocco | 2 | | | |
| | Somalia | 2 | Meso-America | Belize | 1 |
| | Sudan | 2 | | Cuba | 1 |
| | Tanzania | 1 | | Guatemala | 1 |
| | Zambia | 2 | | Mexico | 4 |
| | Zimbabwe | 1 | | Puerto Rico | 2 |
| Asia | Bangladesh | 3 | Middle East | Iraq | 2 |
| | Burma | 1 | | Syria | 1 |
| | China, Taiwan | 2 | | Paraguay | 1 |
| | India | 1 | | Turkey | 2 |
| | Korea | 1 | | | |
| | Malaysia | 1 | | | |
| | Nepal | 1 | South America | Argentina | 5 |
| | Pakistan | 2 | | Chile | 2 |
| | Philippines | 1 | | Colombia | 6 |
| | Sri Lanka | 1 | | French Guiana | 1 |
| | Thailand | 1 | | Peru | 4 |
| | | | | Surnam | 2 |
| | | | TOTAL | | 76 |

*Figure 1. Countries returning samples in the International Inoculant Shipping Evaluation (IISE).*

Detailed information on the IISE cooperators is provided in Appendix D, and available data for each of the 76 returned shipping trials are shown in Table 1.

The initial *Rhizobium japonicum* population in the granular inoculant at 25 days after mixing and before shipping was $1.0 \times 10^9$ cells per gram. The *R. japonicum* population of 75 of the returned samples ranged from $1.39 \times 10^8$ to $2.00 \times 10^6$ cells per gram. The various rhizobia population ranges in the samples are summarized in Table 2. Only 5 samples, or 7 percent, had populations greater than $1.0 \times 10^8$ cells per gram, and only 4 samples, or 5 percent, had populations less than $1.0 \times 10^7$ cells per gram. Thus, in 88 percent of the samples the *R. japonicum* count had decreased to between $1.0 \times 10^7$ to $9.9 \times 10^7$ cells per gram. The mean population for all trials was $3.6 \times 10^7$ cells per gram. This constitutes an appreciable loss in viable rhizobia.

The following calculations compare the rates of rhizobia applied by the standard, seed-applied and granular inoculating methods. A standard, seed-applied peat inoculant containing $1.0 \times 10^8$ cells per gram applied at a recommended rate of 3.1 grams per kilogram of soybean seed would deliver $3.8 \times 10^4$ rhizobia per seed. A granular soil inoculant containing the same population per gram, $1.0 \times 10^8$ cells, and applied at its recommended rat of 3.4 grams per 6 meters of row would deliver $5.6 \times 10^5$ cells per centimeter. With a population of $1.0 \times 10^7$ cells per gram, the granular soil inoculant would provide $5.6 \times 10^4$ cells per centimeter. Both of these populations are higher than that delivered with a standard, seed-applied inoculant containing $1.0 \times 10^8$ cells per gram, which would

Table 1. Test Characteristics of Returned International Inoculant Shipping Evaluation (IISE) Samples

| Trial number | Shipping days | Temperature exceeded | Moisture | *Rhizobium japonicum* |
|---|---|---|---|---|
| | | °C | *percent* | *x 10⁶ cells per gram* |
| 535C | 98 | No change | 27.3 | 139 |
| 532C | 98 | No change | 29.4 | 125 |
| 466A | 36 | No change | 31.7 | 115 |
| 599B | 56 | No change | 23.5 | 115 |
| 351S | 10 | No change | 31.1 | 108 |
| 641C | 93 | No change | 28.1 | 93 |
| 639C | 69 | 38 | 26.3 | 91 |
| 602C | 53 | No change | 30.3 | 87 |
| 696C | 38 | No change | 30.2 | 87 |
| 576B | 10 | No change | 33.4 | 74 |
| 409A | 82 | 38 | 26.6 | 72 |
| 560B | 60 | No change | 28.3 | 66 |
| 643C | 103 | No change | 27.3 | 62 |
| 697C | ... | No change | 27.1 | 60 |
| 601C | 36 | No change | 31.1 | 58 |
| 642C | 99 | No change | 31.8 | 50 |
| 649C | 58 | 38 | 25.9 | 46 |
| 563B | 93 | No change | 27.9 | 45 |
| 568B | 76 | No change | 30.5 | 44 |
| .... | ... | 38 | 23.0 | 43 |
| 486A | 96 | 43 | 25.9 | 38 |
| 653C | 92 | No change | 23.6 | 37 |
| 699C | 117 | No change | 22.0 | 37 |
| 483A | 71 | 38 | 26.6 | 35 |
| 583B | 70 | 54 | 27.9 | 34 |
| (3) | ... | 38 | 21.0 | 31 |
| (2) | ... | 38 | 26.8 | 30 |
| 479A | 98 | No change | 25.7 | 29 |
| 450A | 125 | 49 | 24.8 | 27 |
| 484A | 154 | No change | 31.3 | 27 |
| 661C | 81 | 38 | 21.5 | 27 |
| 449A | 131 | No change | 24.2 | 26 |
| 471A | 67 | 38 | 27.5 | 26 |
| 482A | 111 | No change | 28.0 | 26 |
| 570B | 37 | 38 | 23.2 | 26 |
| 590B | 74 | No change | 25.7 | 26 |
| 638C | 85 | 38 | 26.2 | 26 |
| 652C | ... | 38 | 16.9 | 26 |
| 452A | 68 | 49 | 26.3 | 25 |
| 464A | 61 | 49 | 26.0 | 25 |
| 463A | 98 | No change | 24.3 | 24 |

Note: Missing values for trial numbers, shipping days, or exceeded temperature readings occurred because of the improper completion or absence of the data sheet.

Table 1, continued

| Trial number | Shipping days | Temperature exceeded | Moisture | *Rhizobium japonicum* |
|---|---|---|---|---|
| | | °C | *percent* | *x 10⁶ cells per gram* |
| 434A | 108 | 38 | 24.7 | 23 |
| 565B | 97 | No change | 16.8 | 23 |
| 574B | 91 | No change | 25.8 | 23 |
| .... | ... | No change | 22.8 | 23 |
| 472A | 224 | 38 | 20.9 | 22 |
| 441A | 114 | 38 | 21.0 | 21 |
| 470A | 268 | 43 | 13.5 | 21 |
| 625C | ... | 38 | 16.0 | 21 |
| 654C | 69 | 38 | 24.6 | 21 |
| (1) | ... | 38 | 24.3 | 21 |
| 438A | 76 | No change | 20.4 | 20 |
| 578B | 69 | 49 | 23.1 | 20 |
| 447A | 131 | No change | 19.9 | 19 |
| 350S | 83 | No change | 25.5 | 18 |
| 442A | 133 | 38 | 17.4 | 18 |
| 446A | 150 | No change | 23.8 | 18 |
| 462A | 180 | 38 | 18.9 | 18 |
| 637C | 116 | No change | 21.3 | 18 |
| 644C | 131 | 38 | 18.3 | 18 |
| 657C | 81 | 38 | 18.3 | 17 |
| 457A | 69 | 49 | 21.7 | 16 |
| 359S | 110 | No change | 24.4 | 15 |
| 455A | 231 | 38 | 17.9 | 15 |
| 461A | ... | 38 | 16.6 | 14 |
| 569B | 134 | 38 | 13.7 | 14 |
| 575B | 149 | No change | 28.4 | 14 |
| 358S | 110 | 38 | 19.5 | 13 |
| 431A | 231 | 43 | 12.4 | 12 |
| 473A | 103 | .. | 15.1 | 12 |
| 582B | 93 | 43 | 19.5 | 12 |
| 435A | 68 | No change | 22.3 | 9 |
| 448A | 225 | 43 | 14.5 | 6 |
| 489A | 373 | 38 | 15.4 | 3 |
| 593B | 143 | 49 | 14.6 | 2 |
| 354S | 110 | No change | 19.8 | .. |

Table 2. *Rhizobium japonicum* Counts in Returned IISE Samples

| *Rhizobium japonicum* cells per gram | Number of samples | Percentage of samples |
|---|---|---|
| Less than $10^7$ | 4 | 5 |
| 1.0 to 4.9 x $10^7$ | 55 | 73 |
| 5.0 to 9.9 x $10^7$ | 11 | 15 |
| Greater than $10^8$ | 5 | 7 |
| TOTAL | 75[a] | 100 |

Note: Initial *Rhizobium japonicum* count = 1.0 x $10^9$ cells per gram.
[a]Data were available from 75 of the 76 samples returned.

provide 3.8 x $10^4$ cells per seed. Therefore, the shipped samples that contained at least 1.0 x $10^7$ cells per gram would deliver more rhizobia per centimeter than would a seed-applied inoculant with a population of 1.0 x $10^8$ cells per gram.

*Factors Affecting Inoculant Longevity*

After shipping and storage, the quality of the inoculant as measured by the number of viable rhizobia per gram appears to have been influenced by the temperature to which the inoculant was exposed, the length of exposure, and the moisture loss. Each of these parameters was evaluated in the IISE samples.

*Temperature.* The inoculant was kept under refrigeration while at the University of Illinois. Therefore, potentially harmful temperatures were experienced only during the time of shipping to the cooperator or during the samples' return trips to Puerto Rico for analysis.

The Tempilabel Temperature Monitor indicated the maximum temperature to which each sample was exposed. Four temperature-sensitive discs on each sample could change irreversibly from gray to black as each of the following ambient temperatures was encountered: 38°, 43°, 49°, and 54°C (see Appendix A). Table 3

Table 3. Temperature Changes in Returned IISE Samples

| Recorded temperature | Number of samples | Percentage of samples |
|---|---|---|
| Less than 38°C | 36 | 48 |
| 38°C to 42°C | 27 | 36 |
| 43°C to 48°C | 5 | 7 |
| 49°C to 53°C | 6 | 8 |
| 54°C or higher | 1 | 1 |
| TOTAL | 75[a] | 100 |

[a]Data were available from 75 of the 76 samples returned.

summarizes the samples that experienced temperature changes. Nearly one-half of the samples, or 48 percent, did not exceed 38°C and thus registered no color change on the disc. Thirty-six percent exceeded only the 38°C indicator. Temperatures higher than 43°C, 49°C, and 54°C were experienced by 7 percent, 8 percent, and 1 percent of the samples, respectively.

The relationship between the exceeded temperature and the final population of *R. japonicum* is presented as a linear regression in Figure 2. The regression coefficient of the equation for viable rhizobia after exposure to the indicated temperatures was significant but small. This equation indicates an inverse relationship between the number of rhizobia and exposed temperature. However, temperature alone does not fully explain the final population of rhizobia in the inoculant samples.

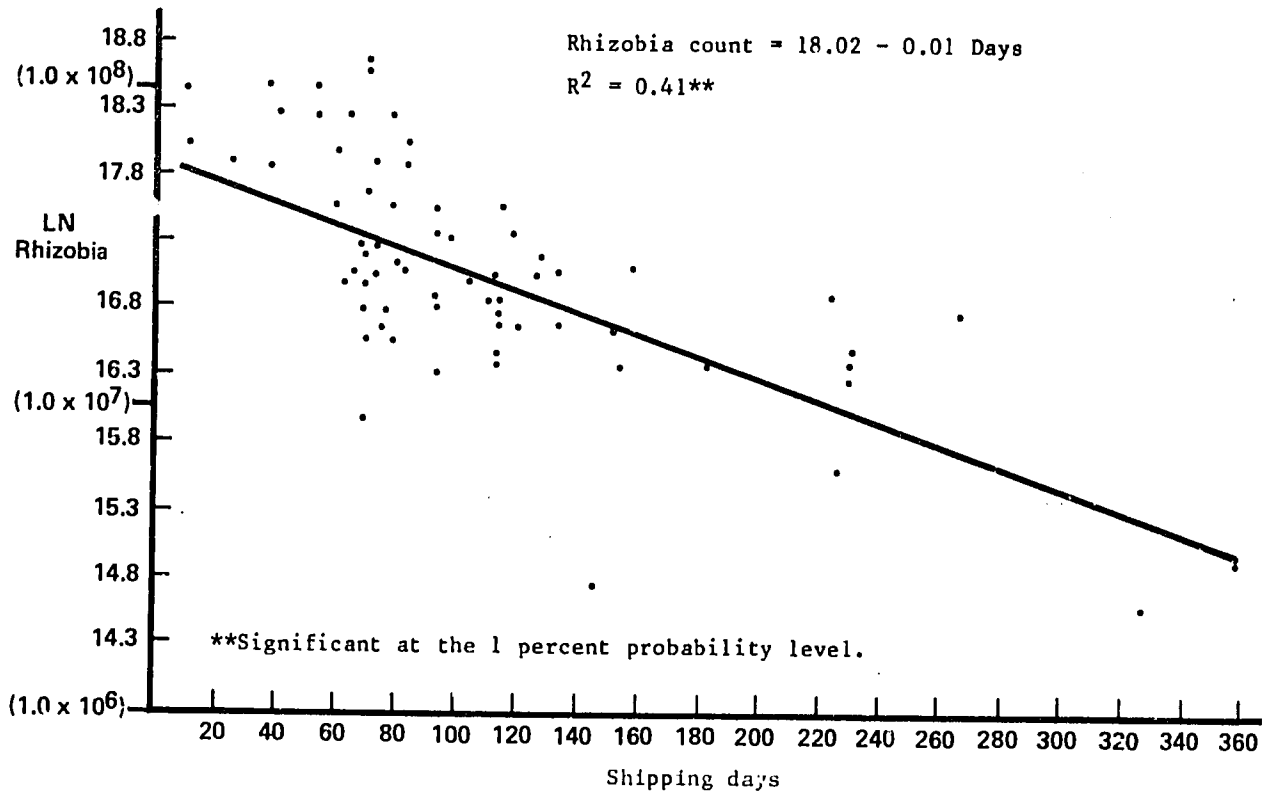


*Figure 2. Effect of temperature on the* R. japonicum *population in IISE samples.*

*Time.* The date each sample was sent to the cooperator from Urbana, Illinois, and the date it was received in Puerto Rico were recorded and used to calculate the number of days in transit. The length of shipment time for the samples is given in 30-day increments in Table 4. The majority of the samples were returned from 31 to 150 days after the date they were originally sent. The length of time in shipment ranged from 10 to 373 days, with a mean of 105 days.

Table 4. Shipping Time of Returned IISE Samples

| Shipping days | Number of samples | Percentage of samples |
|---|---|---|
| 0 to 30 | 2 | 3 |
| 31 to 60 | 8 | 12 |
| 61 to 90 | 18 | 27 |
| 91 to 120 | 22 | 33 |
| 121 to 150 | 9 | 14 |
| 151 to 180 | 2 | 3 |
| 181 to 210 | 0 | 0 |
| 211 to 240 | 4 | 6 |
| 241 to 270 | 1 | 1 |
| 271 to 300 | 0 | 0 |
| Over 300 | 1 | 1 |
| TOTAL | 67[a] | 100 |

[a]Data were available on 67 of the 76 samples returned.

Figure 3 illustrates the linear regression equation expressing the relationship between shipping days and rhizobia count. The regression coefficient of this equation is significant and larger than that obtained with the temperature parameter. This equation demonstrates an inverse relationship between time, the number of shipping days, and the final population of rhizobia.

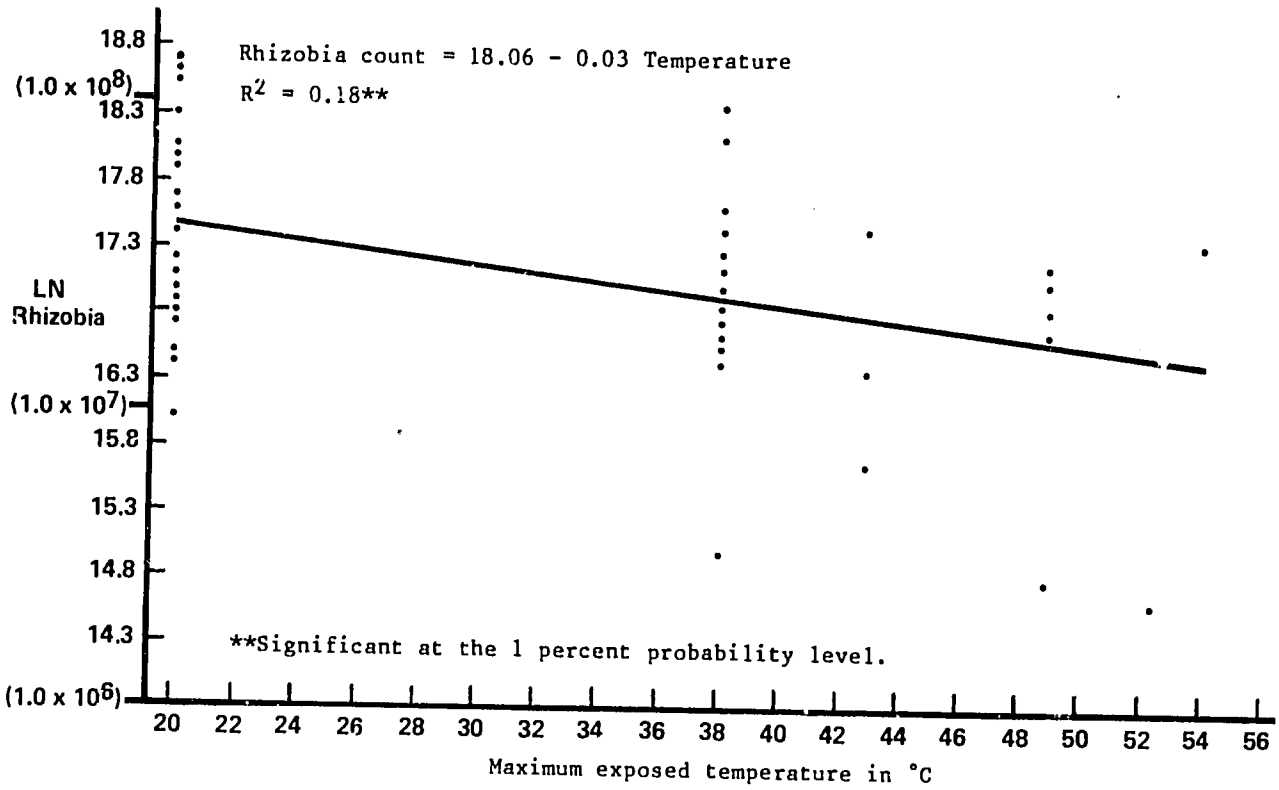


*Figure 3. Effect of time on the* R. japonicum *population in IISE samples.*

*Moisture.* The moisture content for each sample was determined upon receipt in Puerto Rico. Most of the samples had decreased from the initial 35 percent value to a moisture content below 30 percent, as shown in Table 5. Moisture content ranged from 33.4 to 12.4 percent, with a mean of 23.6 percent.

Table 5. Inoculant Moisture in Returned IISE Samples

| Percentage of moisture | Number of samples | Percentage of samples |
|---|---|---|
| 12.0 to 14.9 | 5 | 6 |
| 15.0 to 19.9 | 15 | 20 |
| 20.0 to 24.9 | 23 | 30 |
| 25.0 to 29.9 | 24 | 32 |
| 30.0 to 35.0 | 9 | 12 |
| Total | 76 | 100 |

Note: Initial moisture content = 35 percent.

The relationship between the final moisture content of the inoculant and the number of rhizobia is presented as a linear regression equation in Figure 4. The regression coefficient for this equation is significant and constitutes the largest effect of the three parameters evaluated. A direct relationship between the final moisture content and the number of rhizobia is indicated.

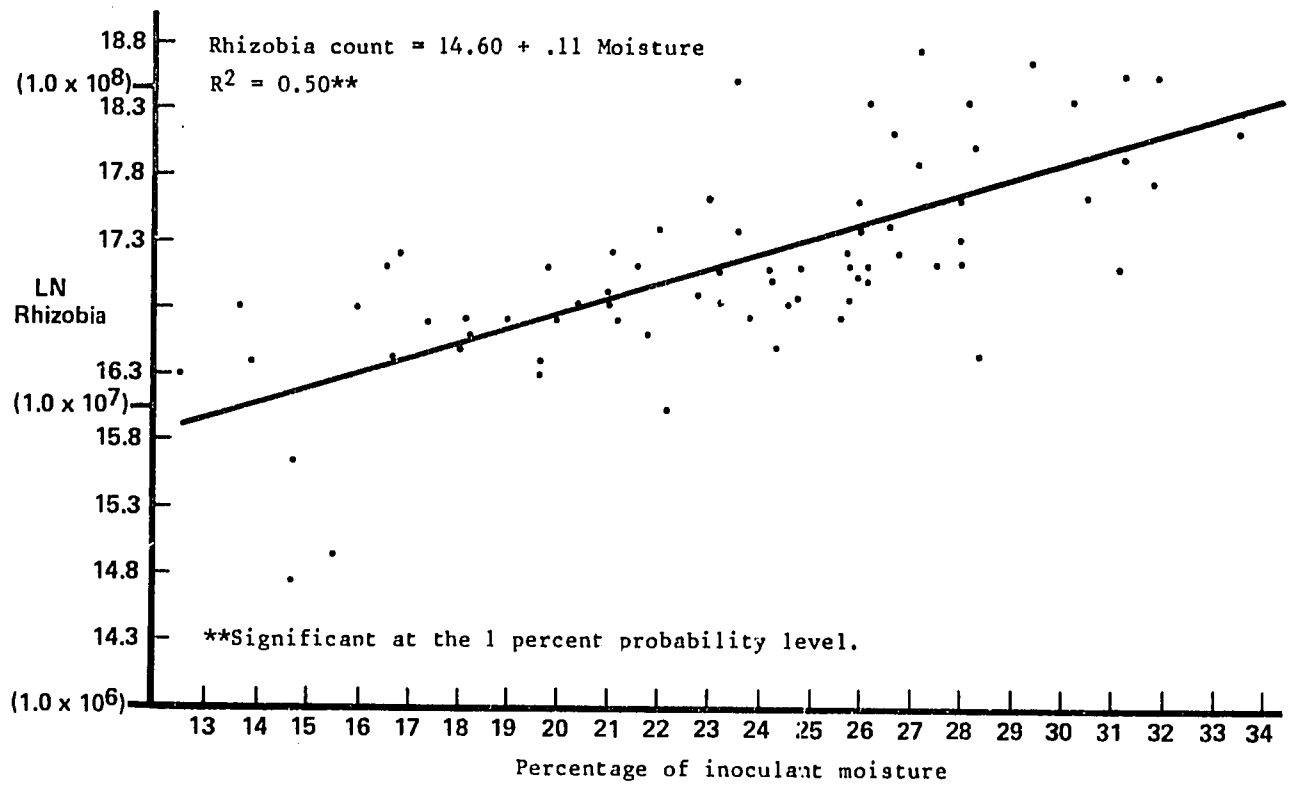


*Figure 4. Effect of final inoculant moisture on the* R. japonicum *population in IISE samples.*

A comparison of simple linear regression analysis with each of the independent parameters--temperature, moisture, and time--and multiple linear regression analysis with all combinations of these parameters is presented in Table 6. As indicated above, moisture exhibited the largest coefficient of determination for single regression analysis: $R^2 = 0.50$. When moisture and days were combined in multiple linear regression, the coefficient increased to 0.55.

Table 6. Summary of Linear Regression Analysis

| | A | (B) Temperature | (C) Moisture | (D) Days | $R^2$ |
|---|---|---|---|---|---|
| Temperature | 18.062 | -0.030 | | | 0.18** |
| Moisture | 14.596 | | 0.108 | | 0.50** |
| Days | 18.025 | | | -0.008 | 0.41** |
| Temperature, days | 18.492 | -0.018 | | -0.007 | 0.48** |
| Moisture, days | 15.744 | | 0.076 | -0.004 | 0.55** |
| Temperature, moisture | 15.089 | -0.009 | 0.099 | | 0.51** |
| Temperature, moisture, and days | 16.316 | -0.010 | 0.066 | -0.004 | 0.56** |

Note: *Rhizobia* count = A + B (temperature) + C (moisture) + D (days).
**Significant at the 1 percent probability level.

The largest coefficient of determination, 0.56, was obtained when all three parameters were evaluated in multiple linear regression. These results indicate that the combined effects of maximum temperature exposure, number of days in transit, and final inoculant moisture contribute significantly to the variation in the final *R. japonicum* population in the manner prescribed by the multiple linear regression equation. It appears that 56 percent of the total variation in population is explained by the estimated multiple linear regression of temperature, time, and moisture.

## SUMMARY

One hundred sixty samples of granular soil inoculant were sent from INTSOY, located at the University of Illinois in Urbana, Illinois, to cooperators around the world. Seventy-six were returned by the cooperators to the INTSOY laboratory at the University of Puerto Rico, Mayaguez, Puerto Rico, for quality analysis. The experiment was conducted during 1979 and designated as the International Inoculant Shipping Evaluation (IISE). The quality of the inoculant, which initially contained $1.0 \times 10^9$ *R. japonicum* cells per gram, decreased during shipment from the effects of time, temperature, and moisture. These factors were measured in terms of shipping days, maximum exposed temperature, and final inoculant moisture, respectively. The effect of these three factors on final inoculant quality was determined by agar-plate counting of the live, colony-producing units in the inoculant.

The rhizobia population in 88 percent of the samples decreased to a level between $1.0 \times 10^7$ and $9.9 \times 10^7$ cells per gram. However, it is estimated that a granular soil inoculant with a population of $1.0 \times 10^7$ cells per gram would deliver more rhizobia than would a seed-applied powder inoculant with a population

of $1.0 \times 10^8$ cells per gram. This suggests that most of the returned samples, when used as granular soil inoculants, would provide sufficient rhizobia to nodulate soybeans under favorable growing conditions even after being stressed by time, temperature, and desiccation.

The relationship between the *R. japonicum* population and the number of shipping days and temperature exposure were both significant. An inverse relationship was exhibited with each of these independent variables. While the relationship with the third independent variable, final inoculant moisture, was also significant, it was directly related to the final population of *R. japonicum*. While all three factors investigated--time, temperature, and moisture--affected the final population of rhizobia in the granular inoculant distributed in this IISE, the final inoculant moisture exerted the largest influence.

*APPENDIX A*

INSTRUCTION SHEET IISE 79

INTERNATIONAL INOCULANT SHIPPING EVALUATION (IISE)

International Soybean Program (INTSOY)
Departments of Agronomy
University of Illinois
Urbana, Illinois 61801
U.S.A.

University of Puerto Rico
Mayaguez, Puerto Rico 00708

The International Inoculant Shipping Evaluation (IISE) is included with the ISVEX supplies but is a separate trial in which we ask your cooperation. Please read this Instruction Sheet and participate in this evaluation by sending to Puerto Rico the attached inoculant sample and data sheet in the preaddressed envelope.

Soybeans inoculated with sufficient *Rhizobium japonicum* bacteria develop nodules on the root systems. Symbiotic dinitrogen fixation within the nodules provides the plant with nitrogen for protein production.

High temperature, lack of moisture, time, and exposure to sunlight are all stress factors which decrease the quality of inoculants by reducing the number of viable rhizobia bacteria. The 5-lb bag of granular soybean inoculant enclosed for use with the ISVEX trial may have encountered any or all of these stress factors. Therefore, INTSOY has initiated this IISE trial with the following objectives:

a) evaluate soybean inoculant quality after exposure to international shipping and storage conditions.
b) estimate the quality of the inoculant used with the ISVEX trial as an aid in the interpretation of ISVEX nodulation data.

Participation in the IISE only requires that you complete the IISE Data Sheet and return it, along with the attached inoculant sample, in the preaddressed envelope provided. In Puerto Rico, the INTSOY Microbiology Section will evaluate the quality of the returned inoculant samples and will determine the effect of shipping inoculants internationally.

Thank you for your assistance in this evaluation.

Address any questions or comments to:

Dr. R. Stewart Smith
Soil Microbiologist/INTSOY
Department of Agronomy
University of Puerto Rico
Mayaguez, Puerto Rico 00708

*APPENDIX A, continued*

PLEASE DO THE FOLLOWING:

No. 1: COMPLETE IISE DATA SHEET (page 3)
- Complete the IISE Data Sheet at the time of planting your ISVEX trial.

No. 2: NAME AND ADDRESS
- enter your name and address on the IISE Data Sheet.

No. 3: DATE
- enter the date you completed the IISE Data Sheet.

No. 4: CIRCLE BLACK CENTERS ON TEMPILABEL TEMPERATURE MONITOR
- One Tempilabel temperature monitor is attached to the inoculant sample. Circle with pen or permanent marker on the Tempilabel temperature monitor each centigrade temperature (38°C, 43°C, 49°C, 54°C) in which the center is black. Each indicator center turns from light grey to black when that specified temperature is exceeded.

Example:

| Tempilabel | | TEMPERATURE MONITOR | |
|---|---|---|---|
| 38°C | 43°C | 49°C | 54°C |

Only 38°C center is black.

No. 5: CIRCLE BLACK CENTERS OF TEMPILABEL ON IISE DATA SHEET
- circle on the IISE Data Sheet each centigrade temperature (38°C, 43°C, 49°C, 54°C) in which the center is black.

No. 6: ENCLOSE IISE DATA SHEET AND INOCULANT SAMPLE WITH TEMPILABEL IN PREADDRESSED ENVELOPE
- without detaching the inoculant sample from the envelope, fold the inoculant sample and tempilabel into the preaddressed envelope. Place the completed IISE Data Sheet in the same envelope.

No. 7: ADDRESS ENVELOPE
- write your name and address in the upper left corner of the preaddressed envelope.

No. 8: AIR MAIL TO PUERTO RICO
- air mail the envelope immediately to the address shown.

*APPENDIX B*

ISVEX 79
Trial No.____

RETURN WITH INOCULANT SAMPLE TO PUERTO RICO

IISE DATA SHEET

International Inoculant Shipping Evaluation (IISE)

Name_____

Address_____

_____

_____

Date _____

Circle on this Data Sheet each centigrade temperature in which the center is black.

38°C 43°C 49°C 54°C

Send in the preaddressed envelope provided, this Data Sheet and the small inoculant sample to:

Dr. R. Stewart Smith
Soil Microbiologist/INTSOY
Department of Agronomy
University of Puerto Rico
Mayaguez, Puerto Rico 00708

*APPENDIX C*

IISE - 1983
INTSOY Microbiology

I. Location

Trial No. _____

Country _____
Cooperator

Name _____

Address _____

_____

_____

_____

II. Time

Date sent from Illinois _____

Date on Data Sheet _____

Date Received in Puerto Rico _____

Days in shipment and storage _____

Method of Shipping: Outgoing _____ Return _____

III. Moisture

Moisture Analysis _____%.
Date Analyzed _____

IV. Plate Count

Date Plated _____

Date Read _____

Medium _____

| Dilution | Count/Plate Plate 1 | Plate 2 | Plate 3 | Average |
|---|---|---|---|---|
| _____ | _____ | _____ | _____ | _____ |
| _____ | _____ | _____ | _____ | _____ |
| _____ | _____ | _____ | _____ | _____ |

Adjusted Count = _____

V. Temperature Exceeded (circle)

38°C 43°C 49°C 54°C

*APPENDIX D*

Cooperators Participating in the International Inoculant Shipping Evaluation (IISE)

| Country | Trial number | Name of cooperator | Address |
|---|---|---|---|
| **AFRICA** | | | |
| Algeria | 532C<br>535C | Dr. Tayeb Ferhat Benabbad | IDCI<br>Station Regionale<br>Khemis-Milian, ALGERIA |
| Botswana | 565B<br>569B | Ms. B. Motalaote<br>Mr. G.J. Staring | c/o Dr. D.E. Gollifer<br>Agricultural Research Station<br>Private Bag 0033<br>Gaborone, BOTSWANA |
| Ethiopia | 574B | Mr. Alemu Mengistu | Debre-Zeit Agricultural Experiment Station<br>P.O. Box 32<br>Debre-Zeit, ETHIOPIA |
| Gambia | 462A | Mr. A.M. Pomfret | Ministry of Agriculture and Natural Resources<br>Rural Development Project<br>P.O. Box 739<br>Banjul, GAMBIA |
| Ghana | 490A | Dr. John K. Peprah | Crops Research Institute<br>P.O. Box 3785<br>Kumasi, GHANA |
| | 471A<br>472A | Mr. Heinz Burgin | Agro-Industries, Ltd.<br>P.O. Box 1950<br>Kumasi, GHANA |
| Malawi | 560B | Mr. P.K. Sibale | Chitedze Research Station<br>P.O. Box 158<br>Lilongwe, MALAWI |
| Morocco | 638C | Mr. Abid Abdelkader | ORMVAM<br>Basse Moulouya<br>Berkane, MOROCCO |
| | 699C | Mr. Nadah Driss | ORMVAT<br>Fouih Ben Salah,<br>MOROCCO |
| Somalia | 464A | Mr. Salad Guimale Ossoble | Central Agricultural Research Station<br>Ministry of Agriculture<br>Mogadishu, SOMALIA |

*APPENDIX D, continued*

| Country | Trial number | Name of cooperator | Address |
|---|---|---|---|
| **AFRICA, continued** | | | |
| | 489A | Mr. Faduma Husen Mohamed | Agricultural Research Institute<br>Mogadishu, SOMALIA |
| Sudan | 473A | Mr. Fathi Mohamad Khalifa | Abu-Naama Research Station<br>Abu-Naama, B.N.P.<br>Khartoum, SUDAN |
| | 486A | Dr. Osman A.A. Ageeb | Agronomy & Crop Physiology Section<br>Gezira Research Station<br>P.O. Box 126<br>Wad Medani, SUDAN |
| Tanzania | (3 trials without numbers) | Mr. Rainer H. Rothe | UNDP Rice and Food Crop Project<br>P.O. Box 159<br>Zanzibar, TANZANIA |
| Zambia | 441A | Dr. R.M. Singh | Private Bag 7<br>Chilanga, ZAMBIA |
| | 442A | Dr. C.R. Nissly | School of Agriculture<br>University of Zambia<br>P.O. Box 2379<br>Lusaka, ZAMBIA |
| Zimbabwe | 568B | Dr. J.R. Tattersfield | Crop Breeding Institute<br>Box 8100<br>Causeway<br>Harare (Salisbury),<br>ZAMBABWE |
| **ASIA** | | | |
| Bangladesh | 431A<br>455A | Dr. Peter R. Hobbs | IRRI<br>P.O. Box 64<br>Dacca, BANGLADESH |
| | 448A | Mr. Ataur Rahman | Plant Breeding Division<br>Institute of Nuclear Agriculture<br>P.O. Box 4<br>Mymensingh, BANGLADESH |

*APPENDIX D, continued*

| Country | Trial number | Name of cooperator | Address |
|---|---|---|---|
| ASIA, continued | | | |
| Burma | 470A | Dr. Myint Thein | Applied Research Division<br>Agricultural Corporation<br>Gyogon<br>Insein Post Office<br>Rangoon, BURMA |
| China, Taiwan | 359S<br>575B | Mr. S. Shanmugasundaram | AVRDC<br>P.O. Box 42<br>Shanhua, Tainan 741<br>Taiwan, CHINA |
| India | 582B | Mr. R.N. Trikha | Soya Production and Research Association<br>182 Civil Lines<br>Bareilly U.P., INDIA |
| Korea | 696C | Mr. Keun-Yong Park | Crop Experiment Station<br>Suweon 170, KOREA |
| Malaysia | 435A | Mr. Omar Ali Christ | Agricultural Research Station<br>P.O. Box 197<br>Tenom, Sabah, EAST MALAYSIA |
| Nepal | 583B | Mr. M.P. Bharati | Agronomy Division<br>G.P.O. Box 404<br>Khumaltar, Kathmandu, NEPAL |
| Pakistan | 593B | Mr. Altaf Hussain Chaudhry | Oilseeds Section<br>Agricultural Research Institute<br>Tandojam, PAKISTAN |
| | 652C | Dr. J.R. Lockman | Technical Services Association<br>23-2 Race Course Road<br>Lahore 3, PAKISTAN |
| Philippines | 483A | Mr. Benjamin M. Legaspi | BPI Economic Garden<br>Los Banos<br>Laguna, PHILIPPINES |
| Sri Lanka | (10) | Mr. C. Stanley Weeraratna | Faculty of Agriculture<br>University of Sri Lanka<br>Peradeniya, SRI LANKA |

*APPENDIX D, continued*

| Country | Trial number | Name of cooperator | Address |
|---|---|---|---|
| **ASIA, continued** | | | |
| Thailand | 438A | Dr. Prasan Yingchol<br>Mr. Ed Sarobol | Division of Agronomy<br>Department of Plant Science<br>College of Agriculture<br>Kasetsart University<br>Bangkok 9, THAILAND |
| **EUROPE** | | | |
| Austria | 601C | Dr. Ralph Gretzmacher | University of Agriculture<br>Gregor Mendel Street, 33<br>A-1180<br>Vienna, AUSTRIA |
| Azores | 697C | Mr. Antonio da Fronseca Carvao | Servicos Agricolas da Ilha Terceira<br>Terceira, AZORES |
| Czechoslovakia | 602C | Ing. Teodor Sinsky | Vyskumny Ustav Rastlinnej Vyroby<br>Bratislavska Cesta 122<br>921 68 Piestany,<br>CZECHOSLOVAKIA |
| Italy | 637C | Prof. Mauro Deidda | Centro Regionale Agrario Sperimentale<br>Via L.B. Albertini, 22<br>Cagliari, ITALY |
| | 642C | Dr. Giovanni Porreca | Laboratori Produzione Alimentare di Base, CNEN<br>Centro di Studio Nucleari della Casaccia<br>Rome, ITALY |
| Portugal | 643C | Mr. Abilio Silva | Instituto Nacional de Investigacao Agraria<br>Rua das Janelas Verdes 92<br>Lisbon 1200, PORTUGAL |
| **MESO-AMERICA** | | | |
| Belize | 570B | Dr. P. Collins | C.A.R.D.I.<br>Central Farm<br>Cayo District, BELIZE |
| Cuba | 461A | Ing. J.M. Alvarez | Director Nacional Cultivos Varios<br>Avenida R. Boyeros y Armada<br>Ciudad Habana, CUBA |

*APPENDIX D, continued*

| Country | Trial number | Name of cooperator | Address |
|---|---|---|---|
| **MESO-AMERICA, continued** | | | |
| Guatemala | 590B | Mr. Alan Praskin<br>Mr. Craig Bialick | Plenty<br>Molina Belen<br>Solola, GUATEMALA |
| Mexico | ---<br>457A<br>578B | Ing. Jaime Mascareno Castro<br>Ing. Hector Lopez Garcia<br>Ing. Jesus Rocha Agramont | Pronase<br>Blvd. E. Zapata<br>1809-Desp. 4<br>Culiacan, Sin., MEXICO |
| | 452A | Mr. Riza Aleman Rafael | Programa Leguminosas Comestibles<br>Campo Agricola Experimental Rosario Izapa<br>Apartado Postal 96<br>Tapachula, Chiapas, MEXICO |
| Puerto Rico | 351S<br>576B | Mr. James H. Oard | INTSOY<br>University of Puerto Rico<br>Isabela Agriculture Experiment Station<br>Isabela, PUERTO RICO |
| **MIDDLE EAST** | | | |
| Iraq | 599B | Dr. S.S. Rajan | UNDP/FAD<br>c/o United Nations Development Programme Office<br>P.O. Box 2048 (Alwiyah)<br>Baghdad, IRAQ |
| | 639C | Dr. Akhtar W.I. Khwaja | Oil Seed Section<br>Department of Field Crops<br>Abu Ghraib Research Station<br>P.O. Box 27016<br>Baghdad, IRAQ |
| Syria | 644C | Mr. Zaki Blanga | Ministry of Agriculture and Agrarian Reform<br>P.O. Box 113<br>Douma, SYRIA |
| Turkey | 641C | Dr. Yasar Bilgin | Seker Pancari Arastirma Enstitusu<br>Agronomi Subesi Sef Uzmani<br>Etimesgut, Ankara, TURKEY |

*APPENDIX D, continued*

| Country | Trial number | Name of cooperator | Address |
|---|---|---|---|
| **MIDDLE-EAST, continued** | | | |
| Turkey | 649C | Mr. H. Halis Agioglu | Cukurova University<br>Ziraat Fakultesi<br>Tarla Bitkileri Yetishirme ve Islahi Baskanligi,<br>TURKEY |
| **SOUTH AMERICA** | | | |
| Argentina | 652C | Mr. Raul Vicentini | INTA<br>Estacion Experimental Agropecuaria de Parana<br>Casilla Correo 128<br>3100 Parana<br>Entre Rios, ARGENTINA |
| | 657C<br>661C | Ms. Nora Mancuso | INTA<br>Rivadavia 1439<br>1033 Capitol Federal<br>Buenos Aires, ARGENTINA |
| Chile | 653C | Mr. Patricio C. Parodi | Department of Plant Science<br>School of Agriculture<br>Catholic University of Chile<br>Cassilla 114-D<br>Santiago, CHILE |
| | 654C | Mr. Vital A. Valdivia | Estacion Experimental La Platina<br>Casilla 5427<br>Santiago, CHILE |
| Colombia | 354S<br>447A<br>450A | Dr. Gilberto Bastidas R.<br>Mr. Carlos Arturo Varoy R. | ICA<br>Centro Experimental Palmira<br>Apartado Aereo 233<br>Palmira, Valle, COLOMBIA |
| | 358S<br>449A | Mr. Guillermo Arrieta Pico | ICA Motilonia<br>Apartado Aereo 13821<br>Codazzi, Cesar, COLOMBIA |
| | 446A | Mr. Miguel Angel Munoz P. | Apartado Aereo 206<br>Monteria, COLOMBIA |
| French Guiana | 466A | Mr. R. Vanbercie | IRAT<br>B.P. 60 Cayenne<br>97301 Cayenne Cedex,<br>FRENCH GUIANA |

*APPENDIX D, continued*

| Country | Trial number | Name of cooperator | Address |
|---|---|---|---|
| SOUTH AMERICA, continued | | | |
| Paraguay | 563B | Mr. Roberto Casaccia | Instituto Agronomico Nacional<br>Ministerio de Agricultura y Ganaderia<br>Caacupe, PARAGUAY |
| Peru | 350S<br>482A | Dr. Luis H. Camacho | Avenida Salaverry 674<br>Oficina 802, 8° Piso<br>Lima 11, PERU |
| | 434A | Ing. Manuel Guerrero Renteria | Avenida Cayetano Heredia 402<br>Castilla, Piura, PERU |
| | 484A | Dr. Dale E. Bandy | N. Carolina State University<br>Yurimaguas, PERU |
| Surinam | 463A<br>479A | Mr. E. Rellum-Vervhhrt | Agricultural Experiment Station<br>P.O. Box 160<br>Paramaribo, SURINAM |