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FINAL REPORT

ON

ECOLOGICALLY BASED MODELS FOR PREDICTION OF LEGUME INOCULATION REQUIREMENT

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PROJECT PROFILE

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Executive Summary

NifTAL had developed a Maui-Net model for prediction the survival of rhizobia under field and environmental conditions of Maui, Hawaii NifTAL work indicated that our present effort were to expan on existing knowlege of the behavior of rhizobia in tropical soil to include factors that were relevant to farm system management Specifically, our aim was to integrate crop manage.nent practice with soil and climate to improve our ability to predict the ultimate performance of legume in the field

To achieve this goal, 4 regions representing different climatic conditions were chosen as north, northeast, central and south Twelve sites in each region representing 4 cropping systems of fallow, legume mono cropping, legume in rotation with other crops and nonlegume mono cropping were chosen It was found under fallow condition that the population of rhizobia varied from 0 to more than 1000 cells of rhizobia per gram soil, depending on the density of indigenous legume plants presented On farming with continuous legume cultivation high density of rhizobial populations were found compared to nonlegume mono cropping systems Under legume rotation the population were intermidiate and variable depending on soil temperature and moisture condition

In areas where soil were hot and dry during summer the rhizobial population were quite low Dynamic equilibrium of rhizobial populations that was apparent within a cropping system Enrichment of the population during legumes cultivation was usually matched by population decline during fallow periods between crop cycles Despite varying population fluctuations between time in the crop cycle but over time, system with greater intensity of legume cultivation had larger population of rhizobia

Based on the changing in population of rhizobia under different cropping system we developed a model for prediction the population of rhizobia and the response of legume to inoculation Estimation of rhizobial population used the following model -

Rhizobia (log 10) = 0 48 + 0 33 (% legumes) + 0 0016 (mean annual rainfall) + 0 28 (sum of extractable bases)

The second is a first order logistic equation that is used to describe the enrichment phase as following -

Rhizobia (log 10) = maximum rhizobia/$i+e^{-b \cdot kt}$(b&T will be derived)

Essentially, if there are no legumes in the system, we would not expect a significant increase in rhizobial numbers, hence the prevailling equilibrium population level would be the expected output When legumes are grown, the curve describes the population increase over the cropping

phase The enrichment is limited to a 100 fold increase over the initial count The increase is dampered by critical environmental conditions such as drought, acidity, low organic matter and high temperature

The third is the Mitcherich decline function for *Bradyrhizobium spp* persistence developed by Woomer et al 1988 This equation takes the form

$$\text{Rhizobia (log 10)} = A + (P^{*} \exp^{c\text{-days}})$$

Again, the extent of decline will vary depending on severity of the environmental conditions and length of time out of legume cropping Eventually, an equilibrium population will be riched and the cycle begins again

Given informations concerning cropping history, rainfall distribution, soil temperature at a specific site, by using the above model We are able to recommend the enusers, namely agricultural extension, people inoculant producers and farmers as to whether the said area need inoculation and to what extent that legumes will respond to inoculation The policy makers from other countries having similar climatic condition of Thailand can also benefit from our findings

Background and Rationale

There are many factors limiting the expression of the response of legumes to inoculation 1) poor quality of inoculant, 2) failure to overcome the native soil rhizobia by inoculated strains (Boonkerd *et al*, 1978, Johnson *et al*, 1965), 3) failure in inoculation due to high soil temperature (Marshall, 1964) and dessiccation (Vincent *et al.*, 1962), 4) environmental factors affecting plant yield potential (Singleton, *et al*, 1985), 5) indigenous population are effective and sufficient to nodulate and fix enough N for host (Singleton and Tavares, 1986), and 6) soil N status (Hatfield *et al*, 1974, Harper, 1974)

It is generally accepted that indigenous rhizobia maintain a low population density in fallow soil Under cultivated area, however, the number of indigenous rhizobia vary depending on soil and cropping system (Roughley et al, 1976, Weaver et al, 1987)

Rhizosphere effects of host legumes and other legumes on populations of rhizobia in the soil were studied by several investigators (Bushby, 1981, Robinson, 1967, Rovira, 1961, Tuzimura and Watanabe, 1962) They found rhizobial populations can be increased significantly if appropriate hosts are cultivated When rhizobia infect legume roots and nodules are formed, the microbes proliferate and increase their number in the nodules Upon decaying a large number of rhizobia are released into soil Bushby (1984) reported that nodule decay was consistently associated with large increase in the number of rhizobia per root system This was confirmed by Weaver *et al*, (1987) Crozat *et al*, (1987) studied the survival kinetics of *B japonicum* and they found that the introduced rhizobia reached the same equilibrium level of 10^3-10^4 rhizobia/g soil regardless of the initial populations

Corman *et al*, (1987) has developed a model to predict population equilibrium of rhizobia in the soil and found that the estimated numbers were quite well correlated with the actual number Their model is

$$\frac{dx}{dt} = Ax\left(1 - \frac{X}{M}\right)$$

Where x = the population size, rhizobia/g soil, at time t
A = exponential growth rate unit per time
M = asymtotic value which is the same unit as x

Without the host plant, rhizobia are similar to other soil bacteria Their ability to survive depends on their genetic and ecological advantage In the field environment, soil moisture and temperature are important factors that strongly influence survival of rhizobia and accounting for the dynamic fluctuation between wide ranges Many countries have distinct wet and dry season; soil

temperature may fluctuate in subtropical climate, but remain relatively high in tropical countries Most rhizobia are sensitive to low soil moisture Survival in soil maintained at near field capacity was much

superior to survival under dry conditions (Jensen Van Renburge and Strijdom, 1980, Marhler and Wollum, 1980, Boonkerd and Weaver, 1982) Flooding condition however, did not affect the survival of rhizobia (Weaver *et al*, 1986)

Survival of rhizobia in soil is also adversely affected by high soil temperatures Most rhizobia are generally less tolerant to high temperature However, no critically high temperature limit can be given for survival of rhizobia because one temperature level may kill one strain but may not harm other strains (Boonkerd and Weaver, 1982, Day *et al*, 1978, Wilkins, 1976) This effect of high temperature on rhizobial survival is also dependent on soil type (Marshall, 1964, Bushby, 1982, Bushby, 1984) Rhizobial growth and survival in soils is not usually limited by nutrient availability However, multiplication of rhizobia on the legume rhizosphere is reported to be limited by low calcium (O'Hara *et al*, 1978)

One of the most important factors affecting the response of legume to inoculation is the effectiveness of the particular rhizobia strains used Strains within a species of rhizobia vary in their effectiveness (Boonkerd *et al*, 1978, Kucey *et al*, 1988) The effectiveness of rhizobia is due to their genetic interaction with the host plants which is known as host-strain specificity In the case of host specificity a strain of rhizobia which is effective on one legume cultivar may not effective on others Thus, in producing legume inoculant the selection of strains of rhizobia for cultivated legumes is necessary

In addition to genetic compatibility, the amount of symbiotically fixed N_2 in a legume - rhizobia system is also determined by the two important environmental factors, namely soil moisture (Kucey *et al*, 1988) and mineral nutrients in soil (O'Hara *et al*, 1988) Thus, to evaluate the inoculation response in the field, records of soil moisture and the status of soil minerals of each location must be taken into consideration

The role of inorganic sources of N on the response to inoculation has not been consistently defined Nitrate has been shown to both inhibit and enhance N_2 fixation, depending on the level and timing of the application (Hatfield, *et al*, 1974, Olsen *et al*, 1975, Harper, 1974) While the availability of "starter" N prior, to the onset of fixation may benefit the symbiosis (Hatfield *et al*, 1974) further uptake of mineral N apparently replaces fixed N on a one to one basis (Johnson *et al*, 1975) In experiments conducted at several sites in the MauiNet Thies *et al*, (1991) found that the responses to inoculation in several legumes were attenuated or eliminated in soils of moderate N availability

In summary, the population dynamics of rhizobia are controlled by environmental conditions affecting rhizobial abundance and survival, such as

- soil type and the presence of mineral nutrients
- soil temperature and moisture
- host legumes

In studies on 14 sites on the island of Maui, Hawaii (Maui-Net), the abundance of native rhizobial populations were significantly correlated with mean annual rainfall, legume cover and shoot biomass, soil temperature, soil pH, and phosphorous retention (Woomer, *et al*, 1988) A regression model was developed from these studies which describe the abundance of indigenous rhizobia and include terms for the host rhizosphere, soil moisture, volume and fertility of the soil solution

$$r_1 = 0\ 48 + 0\ 33\ L + 0\ 0016\ P + 0\ 028\ EB$$

where r_1 = $\log_{10}$ indigenous rhizobia/g soil,
L = legume component of vegetation (%),
P = annual precipitation (mm/yr) and
EB = extractable bases in soil (c mol /kg)

From the above review it is apparent that response of legume to inoculation depends on the following conditions

1 density of indigenous population
2 effectiveness and host compatability of the rhizobial population
3 environmental conditions
4 soil N status

The aim of our study was to develop a model based on those parameters to predict the need for inoculating legumes in traditional crop management systems. Thies *et al*, 1991 found that yield increase due to inoculation was described by a hyperbolic function in which the response to inoculation was correlated to N mineralization and inversely affected by the numbers of indigenous rhizobia We are confident that the construction of this model is possible as we integrate the data collected in Thailand into the preliminary constructs developed in the MauiNet, and validate and refine those equations for the developing tropics.

Materials and Methods

Overview: Sites representing diverse agro-ecosystems were selected from four distinct climatic zones in Thailand The indigenous rhizobial populations were quantified and charaterised, and responses to inoculation measured in greenhouse and field experiments The growth and survival of the introduced inoculant strains were quantified under selected management and environmental

regimes. In collaboration with the NifTAL Project, data obtained from these sites were used to construct a response to inoculation model for tropical cropping systems

Specific objective 1:

To characterize and quantify the indigenous populations of rhizobia from diverse agro-ecosystems in relation to crop response to inoculation

Experimental Methods: We chose different climatic conditions in Thailand as the case study The Central plain, North, Northeast and South of Thailand comprises 4 distinct climatic zones The Central plain is a low land area having good soil fertility, good rainfall distribution of about 1,000 mm per year and is the site of intensive cropping with corn, sugar cane, rice, leguminous crops and fruit trees The North is a high land with mountainous areas, medium soil fertility, good rainfall distribution about 1,000 mm per year, with rice, leguminous crops, vegetable, tobacco, corn and fruit trees as the major cultivated crops The Northeast plateau is a region having generally poor soil fertility, very poor rainfall distribution but relatively high rainfall average annual rain of 1,500 mm Crops are rice, cassava, corn, leguminous crops The Southern part is partly peninsula with high rainfall of more than 2,000 mm per year Rubber, oil palm and fruit trees are mainly cultivated and they are intercropped with cover legumes Rice, pineapple and leguminous crops are also cultivated, but only to a small extent

Task 1

Site Selection

Experimental sites in each of the four mentioned parts of Thailand were selected In each site soil samples were collected to represent different types of cropping management Three different cropping areas, 1) continuously cropped in legumes, 2) continuously cropped in non-legumes, 3) rotationally cropped in legumes and 4) fallow were chosen In each cropping area, three specific sites were selected for soil sample collection At each site, where soil was collected, we took notes of soil temperature, moisture, legume plants within that area, recent cropping practice, and rainfall records from the meteorological station near by Thus, for each part of Thailand 12 specific sites were chosen (4 cropping x 3 soil sampling) Then total sites were 48.

Task 2

Soil Sampling

Before sampling, all equipment and containers used were cleaned to remove rhizobial contaminant. Especially spade or hoe was washed with water and soaked in alcohol then flamed This

had to be done every time samples were collected. The top 2 cm of soil was removed from a sampl site, and the soil was mined to a depth of 20 cm and passed through a 6 5 mm screen Making poole sample from 20 cores within the area of 20 x 20 m on a clean plastic sheet, a subsample of 5 kg wa taken The sample was then immediately put into an ice box and kept at low temperature until used Each soil sample was used for (1) analyses for type, texture, moisture, organic matters and nutrier elements (2) determination of rhizobial population (3) isolation and determination of rhizobial specie and effectiveness

Task 3

Determination of Rhizobial Population

About 500 g of each soil sample from task 2 was used for determination of rhizobi populations by most probable number (MPN) - plant infection technique (Weaver and Frederic 1974) Host plants which are known to have different specificity with strains of rhizobia were used The highly host-strain specific soybean, relatively specific *P. lunatus*, and promiscuous *Macroptilu atropurpureum* (siratro) were used All legumes were grown in plastic growth pouchs

50 g (oven dry basis) subsamples of soil was diluted in 150 ml of sterile water an placed on a wrist-action shaker for 15 minutes This was designated as part of a serial dilution Seve additional four-fold dilutions of the initial dilution were made

A 1 ml portion of each dilution was applied to four replication plants The plants wer placed in an air conditioning light room equipped with Sun-Brellar light source which provide flu density of light about 450/uEs^{-1} m^{-2} with a 12 hr-12 hr light-dark regime Plants was scored fo nodulation 21 days after inoculation

Task 4

Determination the changing in effectiveness population under continuous cultivation of the host

We hypothesize that host legume may influence on the change in effectivenes population with time To test the hypothesis we used effective and ineffective strains of rhizobi having different serogroups to inoculate the host The two strains of rhizobia were mixed in differen ratios, 1 0, 1 1, 1 10, 1 100 and 0 1 effective · ineffective strains and they were inoculated into the 1 kg pasturized soil contained in the clay pots The host plants (soybean and lima bean) were plante into the soil immediately after inoculation Ten plants were allowed to grow per pot For eac treatment, 3 plants were collected at 3 weeks after planting and the other 3 plants were collected agai at 5 weeks for determination of nodules accompany by effective and ineffective strains and the plan dry weight The rest of plants were harvested at full pod forming

Task 5

Determination the responses of different legume species to inoculation when there were different numbers of rhizobia in soil at planting.

Hypothesis : Legume species will response differently to inoculation when they are planted in the soil containing the number of a specific rhizobia

To test the hypothesis three legume species, soybean, lima bean, peanut were used The pasturised soil was used and it was filled in clay pot of 7 kg per pot Effective rhizobia strains specific to each legume were inoculated the soil containing in the pots to provide different amount of rhizobia of 0, 10, 100, 1000 and 10000 cells per gram soil Immediately after soil inoculation the soybean, lima bean and peanut with and without seed inoculation were planted into each pot. Treatments were arranged in completely randomized design with 3 replications Then, for each legume there were 30 pots

Field experiments :

Experiments were carried out in the field under various locations having various population of indigenous rhizobial. Basic treatments were (1) control, (2) rhizobium, (3) rhizobium + 56 kg P_2O_5 + 37 kg K_2O per hectare, (4) 75 kg N + 56 kg P_2O_5 + 37 kg K_2O per hectare

Rationale : A modeling of survival kinetics to predict the equilibrium population of a rhizobia in a soil is possible (Corman *et al*, 1987) Their predictive model was based on initial population of rhizobia and their growth rate in a soil Information from the MauiNet sites have been used to develop equations predicting rhizobial populations and responses to inoculation (Woomer *et al*, 1988, Woomer, *et al*, in preparation, Thies *et al*, in preparation) A predictive model for the growth response of *Azolla* to climatic variables was also made by Lumpkin and Bartholomew (1986) They used multiple regressions of the growth rate across the climatic variables to develop their predictive models In our study we will attempt to construct a model using environmental data and experimental data to predict the crop yield response to inoculation

Experimental procedure : NifTAL project, University of Hawaii our project collaborator was responsible for analyses of data and constructing saprophytic and symbiotic models Regression equations was developed for the prediction of soil populations and survival of introduced bacteria Response to inoculation data were used to validate and extend preliminary equations developed from trials at the Maui-Net sites

Results and Discussions

Site selection

Forty eight sites in 4 regions representing different climatic conditions as North, Northeast, Central and South were chosen Within each region was selected 12 sites consisted of uncultivated area, 3 sites, continuous cultivation of legumes, 3 sites, legumes rotation with other crops, 3 sites and continuous cultivation with nonlegumes, 3 sites Within each site cropping system and indigenous plants were described in Table 1-4 The analyses of soil samples in each are presented in Table 5-8

Population of indigenous rhizobia under different cropping system.

In the tropic the majority of indigenous rhizobia were slow growing bacteria which is classified as *Bradyrhizobium* Cowpea group, *Bradyrhizobium* spp, were the predominant group Determination of rhizobial population in this study we used siratro-MPN plants infection as counting the total population, soybean for counting *B. japonicum* and lima bean as *B.* spp lima bean

Approximately 45% sites soil contained indigenous *Bradyrhizobium* spp 100 cells per gram soil The range of population was 10 - 1000 cells per gram soil (Fig 1) For lima bean rhizobia we found that about 65% of sites soil contained no rhizobia and 30% sites contained 10 cells per gram indicated that lima bean required specific rhizobia (Fig 2) Populations of soybean rhizobia (*B. japonicum*) were similar to the lima bean that was most soil contained no indigenous rhizobia except, the area where soybean has bean cultivated continuously (Fig 3)

Cropping system had influence on rhizobial population Total rhizobia, as measured by siratro-MPN, were high under legume cultivation while under non legume cultivation the number of rhizobia was from 0-100 cells per gram soil (Table 4) The population of lima rhizobia was from 0-10 cells per gram under legume cultivation while under non legume and fallow the number was from 0-10 cells per gram soil (Fig 5) For soybean the number of rhizobia under legume cultivation and rotation was from 0-1000 cells per gram soil depending on soybean cultivation The number under non-legume cultivation or fallow was relatively low from 0-10 cells per gram soil (Fig 6)

It was indicated in this study that cropping system, environmental and climatic conditions had influence on the population of rhizobia In the area where legumes were not cultivated and uncultivated areas rhizobial population were relatively low Legume crops was also affected the specific rhizobial population The population of *B japonicum* were higher under soybean cropping system while cowpea type rhizobia were high under legume cultivation, including soybean Lima bean (*Phaseolus lunatus*) requires specific rhizobia The population of lima bean rhizobia were not correlated well with soybean and sirato rhizobia Lima bean rhizobia were relatively low in the North and Central but rather high in the South while soybean rhizobia were almost not found in the South

Table 1. Characteristics of sites in the North

Sites	Croping systems	Vegetation
N-1-A (Prao, FCRC)	Uncultivation	Short-grass savanna and trees
N-2-B (Prao, FCRC)	Continuous cultivation of legume	Soybean multiplication area
N-3-C (Prao, FCRC)	Legume rotation with other crops	Soybean-corn rotation
N-4-B (Prao-CM, km 82)	Continuous cultivation of legume	Peanut growing area
N-5-A (Prao-CM, km 82)	Uncultivation	Young forest trees
N-6-D (Prao-CM, km 82)	Continuous cultivation of nonlegume	Sugar cane cultivation with mimosa as weed
N-7-D (Mae Taeng)	Continuous cultivation of nonlegume	Rice-garlic rotation
N-8-C (Mae Taeng)	Legume rotation with other crops	Rice-soybean rotation
N-9-B (San Sai, FCRC)	Continuous cultivation of legume	Soybean
N-10-A (San Sai, FCRC)	Uncultivation	Short-grass
N-11-C (Lampang)	Legume rotation with other crops	Rice-soybean rotation
N-12-D (Lampang)	Continuous cultivation of nonlegume	Sugar cane with mimosa and sesbania as weeds

A = Uncultivation
B = Continuous cultivation of legume
C = Legume rotation with other crops
D = Continuous cultivation of nonlegume

Table 2 Characteristics of sites in the Central.

Sites	Croping systems	Vegetation
M-1-D (C P Farm)	Continuous cultivation of nonlegume	Corn cultivation
M-2-B (C P Farm)	Continuous cultivation of legume	Cover legumes
M-3-A (C P Farm)	Uncultivation	Short-grass savanna with shrubs
M-4-C (C P Farm)	Legume rotation with other crops	Soybean-corn rotation
M-5-A (Petchburi)	Uncultivation	Short-grass savanna with shrubs and trees
M-6-C (Petchburi)	Legume rotation with other crops	Soybean-corn rotation corn-cowpea rotation
M-7-D (Petchburi)	Continuous cultivation of legume	Pine apple
M-8-B (Petchburi)	Continuous cultivation of legume	Crotolaria, soybean, peanut
M-9-A (Prabhuthabat)	Uncultivation	Short-grass savanna with trees
M-10-B (Prabhuthabat)	Continuous cultivation of legume	Soybean cultivation
M-11-C (Prabhuthabat)	Legume rotation with other crops	Soybean-cotton rotation
M-12-D (Prabhuthabat)	Continuous cultivation of nonlegume	Corn cultivation

Table 3 Characteristics of sites in the South

Sites	Croping systems	Vegetation
S-1-D (Prajuob)	Continuous cultivation of nonlegume	Pine apple
S-2-B (RRC, Suras)	Continuous cultivation of legume	Peanut growing area
S-3-C (RRC, Suras)	Legume rotation with other crops	Corn-cover legume rotation
S-4-D (Chumporn)	Continuous cultivation of nonlegume	Cucumber, corn and banana
S-5-C (Forage Station)	Legume rotation with other crops	Centrocema-grass forage
S-6-D (Patthalung)	Continuous cultivation of nonlegume	Rice cultivation
S-7-A (Patthalung)	Uncultivation	Short-grass savanna
S-8-D (Patthalung)	Continuous cultivation of nonlegume	Pine apple - para rubber inter-croping
S-9-A (Hat Yai)	Uncultivation	Short-grass savanna with wild legumes
S-10-B (Patthalung)	Continuous cultivation of legume	Sesbania sp cultivation
S-11-B (Had Yai)	Continuous cultivation of legume	Mungbean growing area
S-12-C (Had Yai)	Legume rotation with other crops	Corn-peanut rotation

Table 4 Characteristics of sites in the Northeast.

Sites	Croping systems	Vegetation
NE-1-B (LID)	Continuous cultivation of legume	Peanut, mungbean
NE-2-B (KKU Farm)	Continuous cultivation of legume	Soybean
NE-3-B (KK-FCRC)	Continuous cultivation of legume	Peanut
NE-4-C (Ban Kaw)	Legume rotation with other crops	Soybean-rice rotation
NE-5-C (Ban Ton)	Legume rotation with other crops	Soybean-rice rotation
NE-6-C (Ban Kaw)	Legume rotation with other crops	Soybean-rice rotation
NE-7-D (Ban Samjan)	Continuous cultivation of nonlegume	Cassava
NE-8-D (KKU Farm)	Continuous cultivation of nonlegume	Cassava
NE-9-D (Ban Kaw)	Continuous cultivation of nonlegume	Sugar cane

Table 5. The analysis of soil samples of different sites in the North.

Sites	Moisture (%)	pH	OM (%)	N (%)	P (ppm)	K	Ca	Mg	Na
						(meq/100g)			
N-1-A	9 2	5 1	0 89	0 05	13 9	0 22	1 08	0 67	0 18
N-2-B	7 0	5 0	0 63	0 03	63.9	0.28	1 91	0.72	0 11
N-3-C	18 3	5 8	2 11	0 88	22 8	0 91	5 39	1 69	0.18
N-4-B	11 0	5 2	1.76	0 06	2 8	0 36	3 35	1 39	0 18
N-5-A	9 3	4.8	0 87	0 05	1 8	0 23	1 30	0 73	0.14
N-6-D	8 6	5.0	0 43	0 04	41 0	0 24	1.00	0.72	0 21
N-7-D	23.4	5.7	1 10	0 11	15 2	0.97	9 18	1 70	0.13
N-8-C	61.1	5.6	1 49	0 09	0 9	0 48	10 71	1 92	0 16
N-9-B	11 4	5.4	0 23	0 06	155 8	0 20	2 14	0 47	0 16
N-10-A	11 7	5 4	0 60	0 04	3 5	0 12	1 45	0 34	0 28
N-11-C	5 9	5 4	1 45	0 13	0 4	0 31	6 76	2 36	0 41
N-12-D	25 2	6 0	1 08	0 09	12 9	0 71	25 42	2 23	0 23

Table 6 The analysis of soil samples of different sites in the Central

Sites	Moisture (%)	pH	OM (%)	N (%)	P (ppm)	K	Ca	Mg	Na
						(meq/100g)			
M-1-D	19.2	7 6	1 64	0 08	31 9	1 23	30.76	4 74	0 41
M-2-B	13.9	7.3	2 63	0 10	12 2	0 61	26 42	3 17	0 30
M-3-A	13.6	4.6	0 81	0 05	2 3	0 49	0 84	0 36	0.12
M-4-C	4 3	7 2	1 56	0 10	125 4	1 74	20 76	5 30	0.31
M-5-A	4.9	7.0	2 27	0 13	13 4	1.97	23.49	8 20	0.57
M-6-C	10 2	5 9	0 73	0 05	8 4	0 58	2 34	0 58	0 18
M-7-D	6 7	7 6	0 50	0 03	4 1	0.19	2 01	0.42	0 12
M-8-B	9 2	7.4	0 79	0 03	14 5	0 76	6 41	1 23	0.18
M-9-A	4 4	6 6	2 13	0 11	9 7	0 22	11 26	1 19	0 07
M-10-B	5 9	6 1	1 10	0 05	35 5	0 16	4 11	0 65	0 07
M-11-C	12 6	6 3	1 05	0 05	65 3	0 11	4 55	0 54	0 05
M-12-D	25 2	5.9	0 68	0.05	20 8	0 20	4 65	0.72	0.16

Table 7 The analysis of soil samples of different sites in the South

Sites	Moisture (%)	pH	OM (%)	N (%)	P (ppm)	K	Ca	Mg	Na
						(meq/100g)			
S-1-D	8 8	5.5	0 4	0 05	7 8	0 54	2 79	1.30	0.19
S-2-B	11.0	6.5	0 5	0 03	7 1	0 12	1 25	0 72	0.15
S-3-C	10.2	5 6	0 4	0 05	17.9	0 11	1.25	0 43	0.14
S-4-D	3.0	4.9	0 7	0 04	63 7	0 31	2 36	0 47	0 12
S-5-C	3.4	5.4	1 6	0.06	4 8	0 29	1 49	0 65	0 12
S-6-D	22 7	4 4	1 0	0 06	3 0	0.13	1 78	0.25	0.26
S-7-A	11.9	4.2	1 1	0 03	2 1	0 14	1.01	0 21	0 13
S-8-D	8.3	5.9	0 6	0 03	6 7	0.12	2 14	0 21	0 15
S-9-A	8 0	4 5	0.8	0 02	12 2	0 09	0 65	0 11	0.08
S-10-B	10 3	4 3	0 6	0 03	2 9	0 08	0 78	0 11	0 18
S-11-B	3.8	7.0	0 4	0 02	24 7	0 07	2 27	0 11	0.14
S-12-C	8.3	5.9	0 8	0 02	91 2	0 15	1 88	0 43	0.17

Table 8 The analysis of soil samples of different sites in the Northeast

Sites	Moisture (%)	pH	OM (%)	N (%)	P (ppm)	K	Ca	Mg	Na
						(meq/100g)			
NE-1-D	9 4	5 2	0 40	0 04	97 0	0 13	1.05	0 29	0 16
NE-2-B	11.0	5.8	0 40	0 04	57 5	0 20	1 65	0 19	0 18
NE-3-B	13 3	5 2	0 40	0 02	212 1	0 14	1 38	0 22	0.17
NE-4-C	11 0	5 0	2 70	0 04	9 4	0 08	1 59	0 33	0 17
NE-5-C	18 3	5 1	1 70	0 09	2 3	0 29	1 04	1 73	0 29
NE-6-C	4 6	5 2	0 67	0 03	2 2	0 19	4 76	1 26	0 25
NE-7-D	16 0	5 1	0 36	0 03	5 2	0 13	0 59	0 18	0 18
NE-8-D	8.7	4 0	0 71	0 03	1.5	0 14	0 61	1.25	0.17
NE-9-D	8 5	4 7	0.73	0.03	7 7	0 11	0 96	0 23	0.15

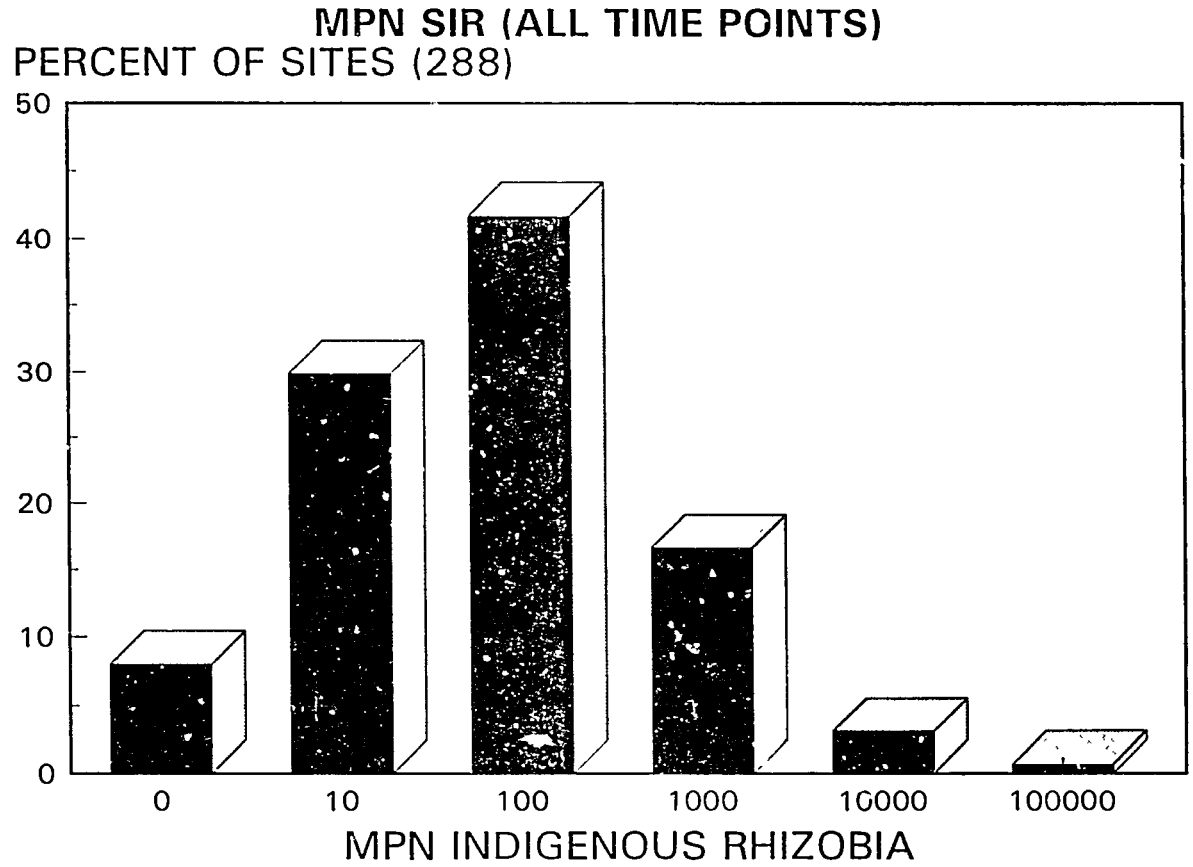


Figure 1. Population of total indigenous rhizobia as measured by siratro-MPN in relation to percent of sites.

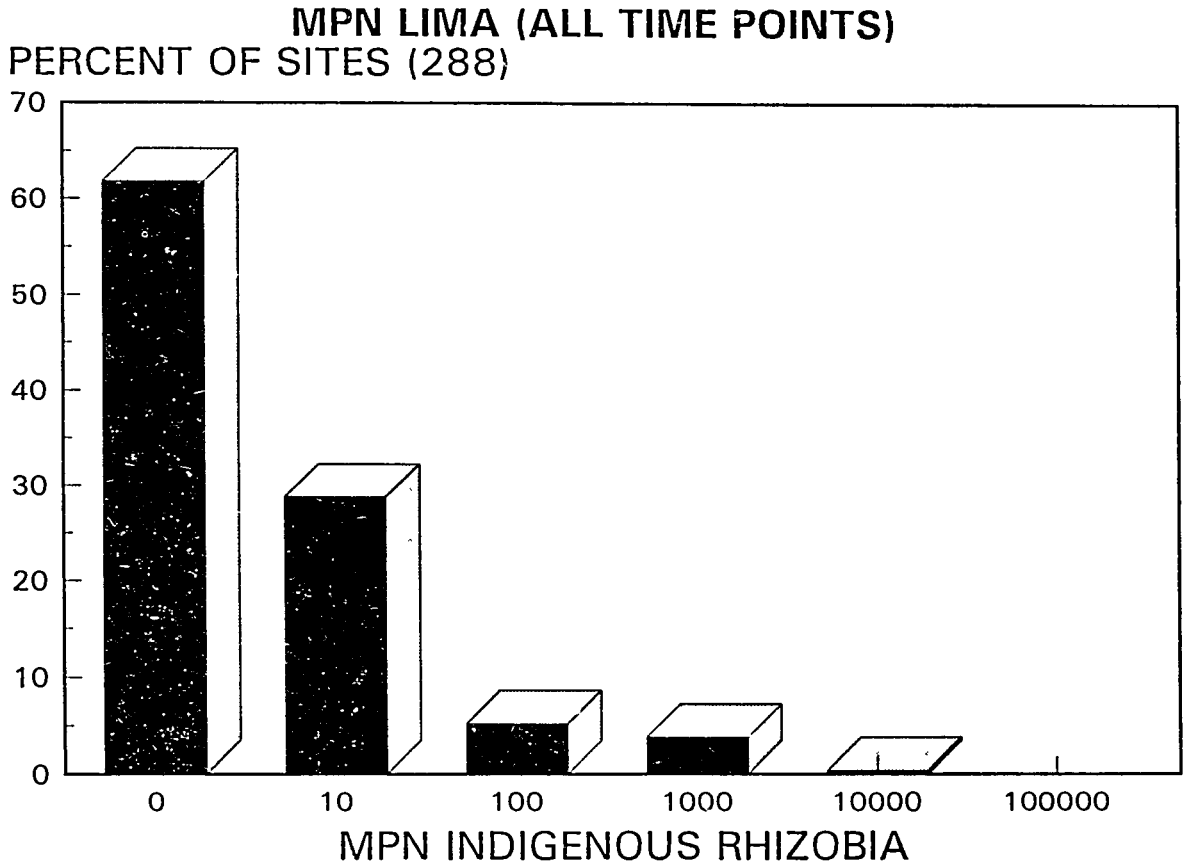


Figure 2. Population of indigenous lima-rhizobia in relation to percent of sites.

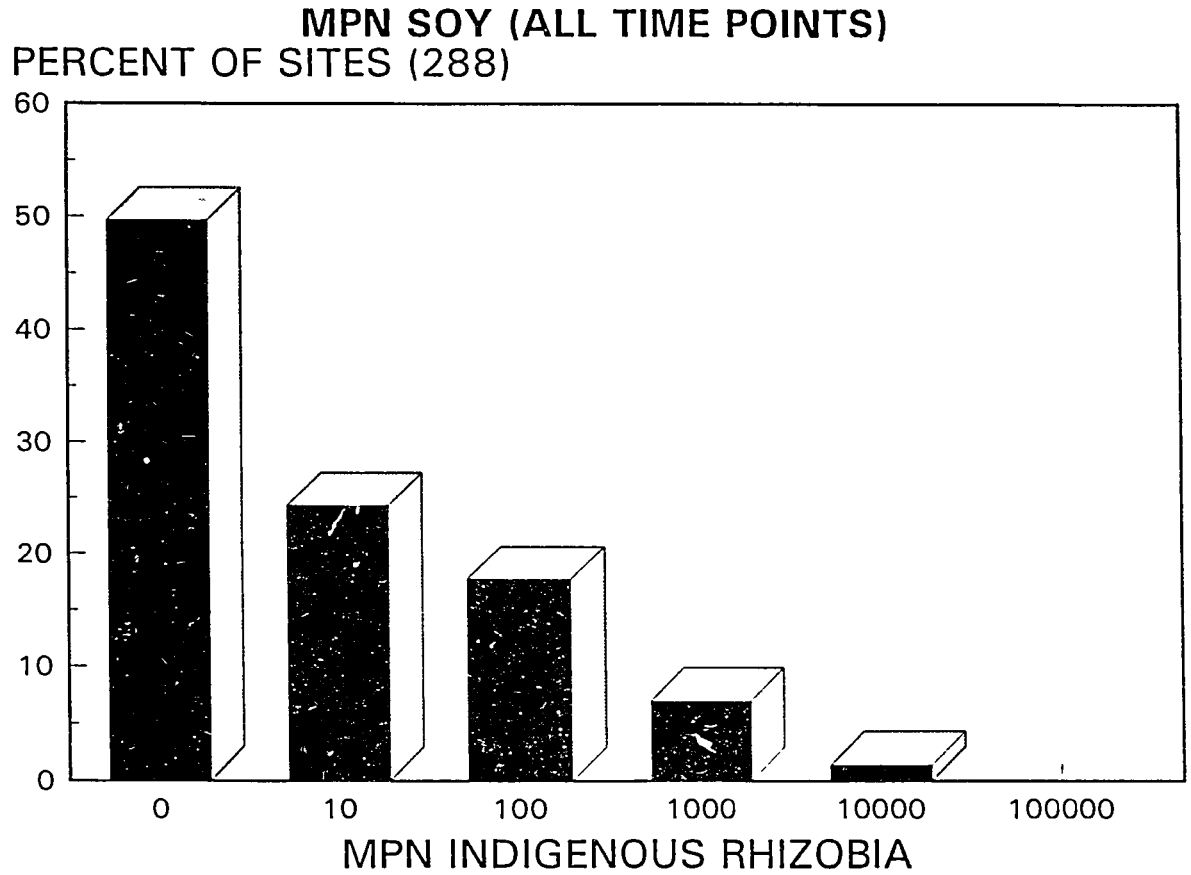


Figure 3. Population of indigenous *B. japonicum* in relation to percent of sites.

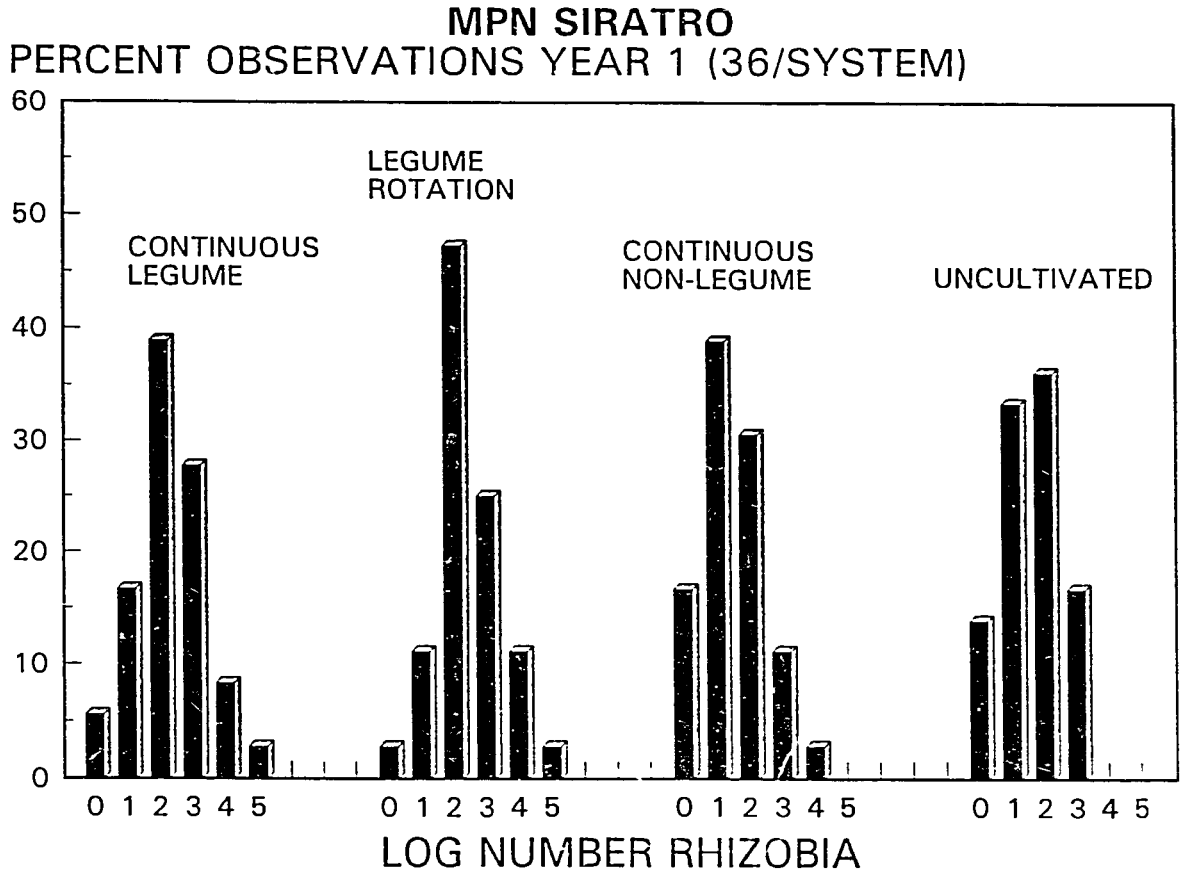


Figure 4. Population of total indigenous rhizobia as measured by siratro-MPN under different cropping system in relation to percent of observations.

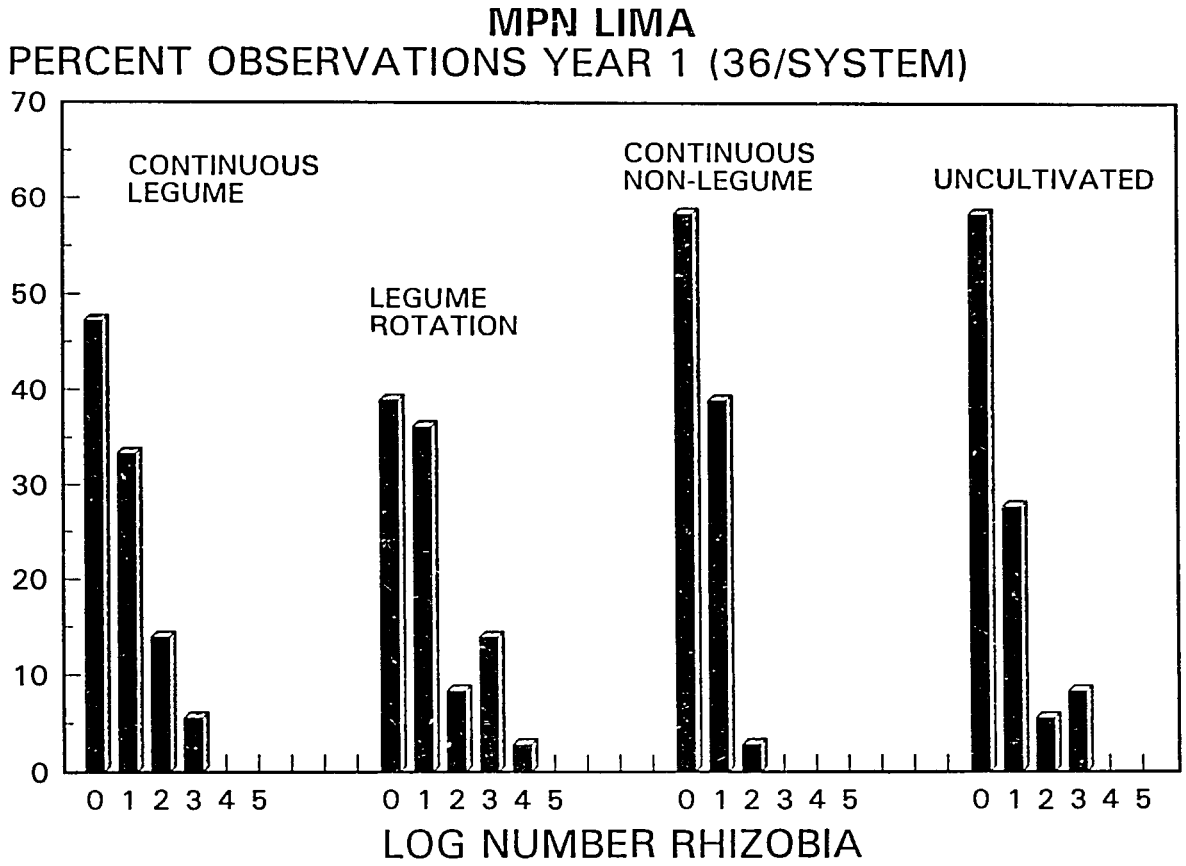


Figure 5. Population of lima-rhizobia under different cropping system in relation to percent of observations.

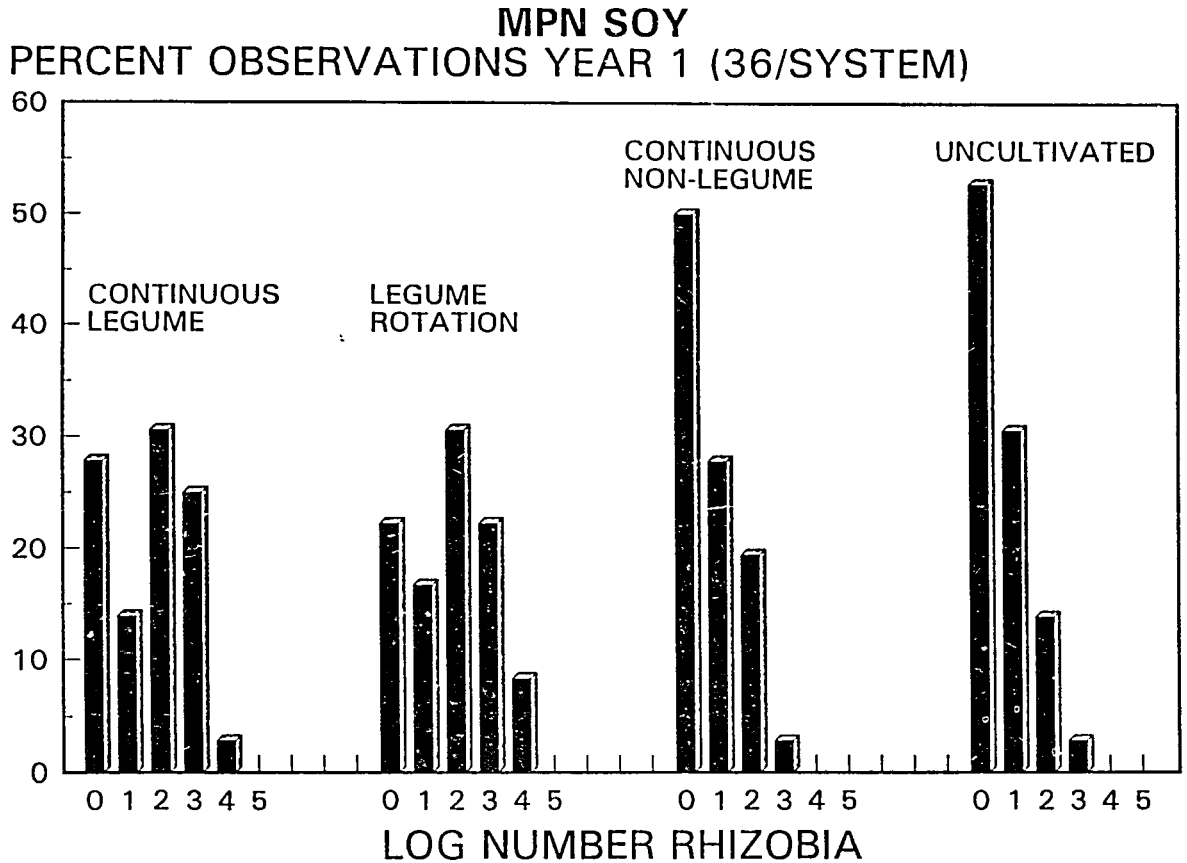


Figure 6. Population of *B. japonicum* under different cropping in relation to percent of observations.

Changing in effectiveness population under continuous cultivation of host plant.

The first experiment was conducted on soybean cultivar SJ 5 with two strains of *B. japonicum*, effective strain THA 5 and ineffective strain TAL 944 Results in Table 9 proved that strain TAL 944 is ineffective on SJ 5 soybean as plant dry weight and ARA were similar to the uninoculated plant in spite of the nodulation was high and they were formed by TAL 944. When the two strains were mixed in the ratio THA 5 TAL 944 of 1 100 plant dry weight was significantly greater than the uninoculated and inoculated with TAL 944 eventhough the nodule recovery was only 14% belong to THA 5 (Table 9) The plant dry weight was higher when inoculated with high number of THA 5 and the highest yield was inoculated with THA 100% It was noticed that the size of nodule formed by TAL 944 was about half of the THA 5

When soybean SJ 5 cultivar was planted again in the same plot following harvesting of the first planting, it was interesting to see from this experiment that the TAL 944 was still showed ineffective but more competitive (Table 10) The nodule recovery of the 1 1 ratio treatment was high with the TAL 944 as compared to the first planting was about 50 50 Again, the plant dry weight of the first planting was significantly higher when inoculated with the ratio 1 100 of THA 5 TAL 944 with the recovery of THA 5 only 14% but for the second planting at 45 days the plant dry weight was not significantly different eventhough the recovery were similar This might be due to the nodulation of the second planting was relatively lower than the first one resulted in the nodule tissue of bacteriod number of THA 5 were not high enough to compensate the nitrogen fixation (Singleton and Tavares, 1986)

The second experiment was done by using lima bean (*Phaseolus lunatus*) inoculated with different ratios of effective (lu 6) and ineffective (lu 12) strains Inoculation of lima bean with ineffective strain plant dry weight and ARA were similar to the uninoculated plant Increasing population of effective strain from 1-50% plant dry weight was increased but not statistically different eventhough the percent recovery higher with effective strain This might be due to the nodulation of lima bean was rather low

Responses of leguminous crops to inoculation under different population of rhizobia in soil.

The experiments were conducted in pot using 3 legumes, soybean, lima bean and peanut inoculated with their specific rhizobia

The first experiments were done at KKU and DOA The KKU experiment showed that dry matter yield and nodulation of soybean were increased with increasing the number of rhizobia in soil (Table 14) Inoculation with rhizobia on seed did not increase yield and nodulation when soil contained 10 cells of rhizobia per gram soil Similar results were found at DOA experiments (Table 15, 16).

Table 9. Responses of soybean cultivar SJ 5 to inoulation with effective THA 5 (E) and ineffective TAL 944 (I) rhizobia, pot experiment at 45 days (1st planting).

Inoculation ratio E · I[1/]	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)	ARA (μ mole/C_2H_4 pl/hr)	Strain recovery (%) E	I
0 : 0	1.11 ab	10 a	54 a	0 66 a	0	0
0 : 1	1.78 a	50 b	220 ab	0.28 a	0	100
1 . 100	4 87 c	56 b	312 bc	1 85 ab	14	86
1 : 10	4.20 bc	48 b	475 c	5.64 c	21	75
1 : 1	5.26 c	42 b	354 bc	4.73 bc	48	52
1 0	5.87 c	16 a	189 ab	3 41 abc	79	5
F-test	* *	* *	* *	* *		
C V. (%)	36 3	39.1	4υ 5	73 4		

Numbers within the same column followed by the same letter are not significantly different at 5% level by Duncan's new multiple range test

Table 10 Responses of soybean to inoulation with different ratios of effective (E), THA 5 and ineffective (I), TAL 944 at 45 days (2nd planting)

Inoculation ratio E · I[1/]	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)	ARA (μ mole/C_2H_4 pl/hr)	Strain recovery (%) THA 5	TAL 944
0 . 0	0 5 a	5 0 a	70 a	0.7 ab	0	0
0 · I	0 9 ab	26 8 bc	20 ab	0 4 a	0	100
1 100	1 0 ab	26 6 bc	255 ab	1 6 abc	18 7	81 2
1 : 10	1 2 b	28 1 c	290 b	3.3 c	15 0	85 0
1 · 1	1 4 b	28 8 c	220 b	2 3 bc	28.7	70.0
1 0	1 4 b	13 0 ab	160 ab	3 4 c	98 0	0
F-test	*	* *	*	* *		
C V (%)	33 0	42 5	44 9	55 8		

Numbers within the same column followed by the same letter are not significantly different at 5% level by Ducan's new multiple range test

Table 11. Responses of soybean to inoulation with different ratios of effective (E), THA 5 and ineffective (I), TAL 944 at full pod filling (2nd planting)

Inoculation ratio E . I[1/]	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)	ARA (μ mole/C_2H_4/pl/hr)
0 : 0	1.1 ab	1 2 a	30 a	0 01 a
0 1	0 9 a	10 8 b	90 ab	0.07 ab
1 . 100	1 4 bc	12 0 b	110 ab	0 10 ab
1 10	1.7 c	6 1 ab	100 ab	0 07 ab
1 : 1	2 3 bc	5 0 ab	110 ab	0 09 ab
1 : 0	4 2 d	6.0 ab	180 b	0 18 b
F-test	* *	NS	NS	NS
C.V. (%)	16 7	76 7	54 4	82 6

Numbers within the same column followed by the same letter are not significantly different at 5% level by Ducan's new multiple range test.

Table 12 Responses of Lima bean (Phaseolus lunatus) to inoulation with effective Iu 6 (E) and ineffective Iu 12 (I) rhizobia, pot experiment at 45 days.

Inoculation ratio E : I[1/]	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)	ARA (μ mole/C_2H_4 pl/hr)	Strain recovery (%) E	I
0 0	7.50	1.00	46 6	1.33	0	0
0 1	8 83	1.10	84 0	1.76	0	22
1 100	12.10	2 12	188.0	5 06	44	19
1 . 10	12 44	4 37	156 0	3.87	56	9
1 : 1	10 60	5 25	196 0	4 05	51	15
1 : 0	10 62	9.12	196.7	2 50	76	0
F-test	NS	*	NS	NS		
C V (%)	29.3	102 7	126 7	125 6		

Numbers within the same column followed by the same letter are not significantly different at 5% level by Ducan's new multiple range test

Table 13 Responses of soybean to seed inoculation (THA 6) when grown in soil which was inoculated with different number of THA 5 rhizobia, DOA pot experiment at 45 days.

Soil inoc (THA 5) (cells/g soil)	Seed inoc (THA 6)	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)	Strain recovery (%)	
					THA 5	THA 6
0	-	0.72 a	1.5 a	30 a	0	0
10	-	3 08 bc	5.5 ab	123 ab	33	0
100	-	2 72 bc	8 5 abc	167 ab	79	0
1000	-	1.81 abc	13 0 cd	246 abc	98	0
10000	-	1.64 ab	15 1 cd	335 abcd	100	0
0	+	3 29 bc	15 5 cd	591 d	0	100
10	+	3.27 c	14.5 cd	364 bcd	14	81
100	+	2.09 abc	12 8 cd	345 abcd	29	71
1000	+	3 32 bc	19 0 d	538 cd	27	73
10000	+	3 54 bc	12 0 bcd	177 ab	34	66
F-test		*	* *	*	-	-
C V (%)		38 8	33 4	57 3	-	-

Numbers within the same column followed by the same letter are not significantly different at 5% level by Ducan's new multiple range test

Table 14. Responses of soybean to seed inoulation when grown in soil which was inoculated with different number of TAL 102 rhizobia, KKU pot experiment at 45 days.

Soil inoc (cells/g soil)	Seed inoc	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)
0	-	1.40	14	82.8
10	-	1 77	24	80.3
100	-	1.81	10	56.5
1000	-	2 04	15	105.2
10000	-	3.15	22	103.5
0	+	2 48	17	161.4
10	+	3.01	30	249.5
100	+	3.46	54	340 1
1000	+	2 62	31	194 2
10000	+	2 81	35	213.4

Table 15. Responses of soybean (Glycine max) to inoculation with rhizobial (THA 5, THA 6), glasshouse experiment, at 45 days

Treatment	Top dry wt (g/2 pl)	Nod no (/2 pl)	Nod wet wt (g/2 pl)	ARA (μ mole/plot/hr) C_2H_4	Recovery (%) THA 5	TAL 944
Control	1.44 a	3.00 a	0.06 a	1.09 a	0.00	0
R_1 10 cells	6 17 bc	11.00 ab	0 25 ab	1 43 a	33.35	0
R_1 100 cells	5 44 bc	17.00 abc	0.33 ab	4.27 a-d	79.17	0
R_1 1000 cells	3.63 abc	26 00 cd	0 49 abc	3 69 abc	98.11	0
R_1 10000 cells	3 29 ab	30.33 cd	0.67 a-d	2.73 ab	100.00	0
Control + R_2	6.58 bc	31 00 cd	1 18 d	7.33 d	0.00	100 00
R_1 10 cells + R_2	7 55 c	29.00 cd	0.73 bcd	5 08 bcd	13.75	81.25
R_1 100 cells + R_2	5 18 abc	25.67 cd	0 69 a-d	4.53 a-d	28.77	71.23
R_1 1000 cells + R_2	6 64 bc	38.00 d	1 08 cd	6.76 cd	26 67	73.33
R_1 10000 cells + R_2	7.09 bc	24 00 bcd	0.36 ab	4.99 bcd	33.93	66.07
Significant	*	* *	*	* *		
% C V	38 8 %	33 4 %	57.3 %	43 4 %		

Numbers within the same column followed by the same letter are not significantly diferent at 5% level by Duncan's new multiple range test.

Table 16 Responses of soybean to inoculation with rhizobial 2 strains (THA 5, THA 6), glasshouse experiment, at harvest.

Treatment	Dry wt (g/2pl)	Nod no (/2pl)	Nod fwt (g/2pl)	ARA (μ mole/plot/hr) C_2H_4
Control	8.19 a	11 69 a	0 45 a	0.70 a
R_1 10 cells	10.52 ab	22 33 a-d	0 67 ab	3.21 ab
R_1 100 cells	8 18 a	17 51 ab	0 54 ab	4.12 ab
R_1 1000 cells	9.29 ab	20 17 abc	0 68 ab	5.06 ab
R_1 10000 cells	10 79 ab	25 81 bcd	0 56 ab	6.30 bc
Control + R_2	10.11 ab	33 75 d	0 94 b	11.35 c
R_1 10 cells + R_2	14.49 b	31 89 cd	0 81 ab	8.26 bc
R_1 100 cells + R_2	7 70 a	21 21 abc	0 69 ab	6 26 bc
R_1 1000 cells + R_2	9.14 ab	27 94 bcd	0 72 ab	6.33 bc
R_1 10000 cells + R_2	7 12 a	17 23 ab	0 41 a	3 42 ab
Significant	NS	* *	NS	*
% C V	30 3 %	27 1 %	32 1 %	52 3 %

Numbers within the same column followed by the same letter are not significantly diferent at 5% level by Duncan's new multiple range test

The lima bean experiment done at KKU showed no differences in dry matter when plants were inoculated with different rates of rhizobia and reinoculation on seeds However, reinoculation on seed the plant were nodulated higher than the soil inoculation only (Table 17) For the DOA experiment, inoculation from 10 cells per gram soil dry matter yield and nodulation of lima bean were significantly higher than the uninoculation Inoculation with rhizobia 10 cells - 10,000 cells per gram soil were not significantly different It was also found that when soil contained from 10 cells of lima bean rhizobia per gram soil inoculation seeds with rhizobia could not increase yield of lima bean (Table 18)

The experiment on peanut at KKU was inoculated with strain of rhizobia TAL 1000. Results in Table 19 showed that there were no significant difference in dry matter yield, nodule number and weight This was probably due to soil contained high number of indigenous rhizobia as indicated by the uninoculated plant were nodulated in high number.

The DOA experiment using two different serogroups of peanut rhizobia found that inoculation could increase dry matter yield and nodulation higher than the uninoculation Inoculation soil from 10 to 10,000 cells per gram soil dry matter and nodulation of peanut were not different from one another It was also found that reinoculation on seed could not increase yield of peanut when soil contained 10 cells rhizobia per gram It was interesting to see that the peanut rhizobia strain NE-41-15 which was isolated from Norhteast of Thailand was more competitive than the strain 2212 A obtained from Israel (Table 20)

Another experiment done on peanut at DOA was inoculated with different number of rhizobia on seeds and plant in soil contained no indigenous rhizobia and in soil inoculated 2212 A strain at 10^9 cells per ml The results showing in Table 21 indicated that inoculation peanut at 10^4 to 10^5 could increase dry matter yield and nodultion significantly over the uninoculated plant. But inoculation at higher number at 10^6 to 10^7 could increase yield and nodulation significantly higher than the inocultion at 10^4 to 10^5 cells per seed This indicated that to obtain higher yield peanut should be inoculated with high number of rhizobia Moreover, when soil inoculated with strain 2212 A and seeds were inoculated with higher number of rhizobia from 10^5 to 10^7 cells per seed the dry matter yield of peanut was even higher than the others However, inoculation soil with strain 2212 A at 10^9 cells per ml gave dry matter yield comparable to peanut inoculated with strain NE-41-15 at 10 cells per seed indicating the two strains were comparable in effectiveness

Responses of soybean to inoculation under field condition

The field experiments were conducted in 1993 and 1994 in both dry and rainy seasons In 1993, 12 locations were conducted in Northeast and 6 locations in the North in rainy season. Results in the Northeast showed that yield of soybean was increased with inoculation only, but in some areas yield of soybean was much more increased with the application of P and K The application of N-P-K fertilizer gave yield comparable to inoculation but the cost of fertilizer is much higher than

Table 17. Responses of lima bean to seed inoulation when grown in soil which was inoculated with different number of TAL 22 rhizobia, KKU pot experiment.

Soil inoc (cells/g soil)	Seed inoc	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)
0	-	2 00	22	41
10	-	1.55	17	63
100	-	1.77	13	37
1000	-	1.99	68	28
10000	-	2 41	17	49
0	+	2.92	42	276
10	+	3.06	125	469
100	+	4 20	93	712
1000	+	3 89	72	244
10000	+	3.57	47	129

Table 18 Responses of lima bean to different rates of soil inoculation with rhizobial strain TAL 22 and seed inoculation with rhizobia isolate Iu 6 at planting, DOA pot experiment, 45 days after planting

Soil inoc (cells/g soil)	Seed inoc	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)	Strain recovery (%)	
					TAL 22	Iu 6
0	-	0.55 a	1 0 a	0 5 a	0	0
10	-	2 31 b	15.1 ab	63 5 ab	55	0
100	-	1.86 b	16.1 ab	69 0 ab	85	0
1000	-	2.49 b	15.5 ab	107 0 cd	100	0
10000	-	1 78 b	30.1 bc	135 0 bc	100	0
0	+	2.40 b	25 6 bc	110 0 bc	0	100
10	+	2 40 b	11.6 ab	115 0 bc	0	97
100	+	1.83 b	77 6 e	173.0 c	1	99
1000	+	2 58 b	58 0 de	144.0 bc	1	99
10000	+	2 24 b	41 6 cd	108.5 bc	1	99
F-test		*	* *	* *	-	-
C V. (%)		30 4	43 8	42 0	-	-

Numbers within the same column followed by the same letter are not significantly different at 5% level by Duncan's new multiple range test

Table 19. Responses of peanut to seed inoulation when grown in soil inoculated with different number of TAL 1000 rhizobia, KKU pot experiment.

Soil inoc (cells/g soil)	Seed inoc	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)
0	-	6.76	303	211.3
10	-	7 50	316	217.3
100	-	10 17	313	250.4
1000	-	8.31	280	297.6
10000	-	10.88	315	226.0
0	+	11.09	536	191.5
10	+	10.40	06	155.8
100	+	9 42	503	264.1
1000	+	12 90	576	271.1
10000	+	10.02	536	188.1

Table 20. Responses of peanut to different rates of soil inoculation rhizobial strain 2212 A and seed inoculation with rhizobia isolate NE-41-15, DOA pot experiment

Soil inoc (cells/g soil, 2212 A)	Seed inoc (NE-41-15)	Top dry wt (g/pl)	Nod no (/pl)	Nod fwt (mg/pl)	Strain recovery 2212 A	Strain recovery NE-41-15	Plant dry wt (g/pl)
0	-	1.26 a	2 3 a	25.5 a	0	0	4 24 a
10	-	2.79 bc	7.5 a	77 0 ab	38	0	4 53 a
100	-	2.96 bc	18.7 ab	117.5 bc	38	0	6 32 ab
1000	-	2.79 bc	11.1 a	62.0 ab	83	0	6.68 ab
10000	-	3.88 c	25 8 abc	120.5 bc	88	0	9 42 c
0	+	2.92 bc	52 9 de	210 5 d	0	88	5 75 ab
10	+	2.82 bc	71.3 e	226.5 d	8	88	5 78 ab
100	+	2.73 bc	47.1 cde	129 5 bc	31	69	6 01 ab
1000	+	2.15 ab	37 7 bcd	123 5 bc	36	64	6 68 ab
10000	+	2.72 bc	44 5 cd	175.0 cd	39	61	7 05 b
F-test		*	* *	* *	-	-	*
C V. (%)		2 64	42 5	32.7	-	-	20.4

Numbers within the same column followed by the same letter are not significantly different at 5% level by Duncan's new multiple range test.

Table 21. Responses of peanut to seed inoculation with different rates of rhizobia strain NE-41-15 while there was another strain. 2212 A in soil.

Treatment	Top dry wt (g/pot)	Nod no (/pot)	Nod fresh wt (g/pot)	ARA (μ mole C_2H_4/pot)	Strain recovery (%) 2212 A	NE-41-15	Plant dry wt (g/pot)	Hull (g/pot)	Seed (g/pot)
Control	2 09 a	0 a	0 a	0 a	0	0	32 46	3.30 a	5 19 a
Control	3 20 ab	0 a	0 a	0 a	0	0	40 45	4 08 ab	8.12 ab
NE-41-15-10^4	4 11 b	26 ab	0 17 b	6 85 ab	0	100	39 48	4 70 abc	9 47 cd
NE-41-15-10^5	3 72 b	34 ab	0 37 c	12 96 bcd	0	100	41 79	6 36 cd	12 55 cd
NE-41-15-10^6	6 42 c	81 bc	0 44 cde	14 64 b-e	0	100	35 00	6 26 cd	11.64 bc
NE-41-15-10^7	6 64 c	95 c	0 52 c-f	19.97 de	0	100	40 36	8 78 e	12 58 cd
2212A+NE-10^4	7 02 cd	231 e	0.57 def	20 04 de	75	25	33.95	5 81 bcd	12 57 cd
2212A+NE-10^5	7 71 cde	180 de	0.60 ef	17 33 cde	75	25	36 07	5 30 abc	12 09 bcd
2212A+NE-10^6	8.38 de	231 e	0 64 f	19.57 de	51	49	34.28	7 65 de	16 15 d
2212A+NE-10^7	8.71 e	191 de	0.59 def	23.15 e	44	56	36.42	5.28 abc	13.79 cd
2212A 10^9	6.21 c	141 cd	0 43 cd	14 26 b-e	100	0	33 64	7 40 de	12 83 cd
F-test	**	**	**	**	-	-	NS	**	**
% CV.	14 40 %	30.60 %	21 90 %	37 80 %	-	-	17.50 %	18 20 %	19.60 %

Numbers within the same column followed by the same letter are not significantly diferent at 5% level by Duncan's new multiple range test.

inoculant. In the North of Thailand yields of soybean with inoculation only and with P, K were higher than uninoculated and fertilizer N-P-K (Table 22).

In dry season 8 locations were conducted in the Northeast and 5 locations the North. The average yield of soybean in dry season was relatively lower than that in the rainy season However inoculation always gave higher yield than uninoculation Inoculation with application of P and K fertilizer gave yield similar to the application of complete fertilizer N-P-K. Field trial in 4 locations in the North found that there were no differences in inoculation and uninocultion because high number of indigenous soybean rhizobia was found (Table 23)

In 1994, experiments were conducted in 13 locations in Northeast and 5 locations in the North in dry season Yield of soybean was higher when soybean inoculated with inoculant and with inoculant plus P and K fertilizer The application of N-P-K fertilizer was similar to inoculation but the cost of production was much higher than inoculation. Similar results were obtained from 5 locations done in the North (Table 24)

Results of rainy season were confirmed the dry season in the Northeast and in the North (Table 25)

It could be concluded from field experiment that inoculation of soybean seeds before planting always gave higher yield than uninoculation. The application of N-P-K complete fertilizer gave the same yield as inoculation but higher cost of production, thus inoculation is recommended

Model for recommendation of legume inoculation requirement.

Additional time over the course of the last year has been spent on developing basic model structures for both the field and regional scale population flux models and establishing links with agencies that could provide the environmental data necessary to run the models.

Population flux at the field scale : This model is being developed by use of STELLA software STELLA is a continuous modular modeling program in which a basic structure can be developed and information integrated over time Appendix A gives the first approximation and explanation of the model structure APPENDIX A An initial population level can be entered (if known) or estimated from environmental data (if unknown). The rhizobial population flux over time is derived from information on population growth and death rates which are determined by environmental influences and agronomic practices Rhizobia are heterotrophs capable of persisting in soil in the absence of host legumes When in association with legume roots, an increase in the numbers of this organism occurs in the legume rhizosphere. Overall, on a field scale, total numbers of soil rhizobia are increased Host compatible organisms initiate nodule formation and fix nitrogen within nodules formed on legume roots The extent to which this occurs is largely host controlled At the end of the cropping

Table 22 Soybean seed yield of the field trials in rainy season (July-November) 1993 in different locations

Location	Control	Inoc	Inoc +P+K	N+P+K
	kg/ha			
KALASIN				
Mr Tonglaw	356	550	594	550
Mr Tongbai	400	700	869	881
Mr Prasert	338	538	606	581
Mr Paiboon	588	931	1050	1100
Mrs Somchai	262	612	594	469
Mr Supote	275	531	675	656
Average	370	644	731	706
UDORNTHANI				
Mr Chy	1818	2457	2935	2804
Mr Bai	2002	2817	3529	2926
Mr Sakorn	888	1860	2199	296
Mrs Lamduan	651	1572	1849	1470
Mr Sood	590	1636	1726	1275
Rice Expt	1411	2482	1941	2396
Average	1227	2137	2363	1861
LAMPANG				
B Tong Pueng	838	1225	1150	1025
B Lao	825	1225	1138	1075
B Sermsai	788	1175	1125	1088
B Na-iang	838	1062	1100	1075
B Huay He	1100	1825	1412	1300
B Maesaikam	850	1275	1388	1238
Average	873	1298	1219	1133
All average	823	1360	1438	1234

Control = neither rhizobium nor chemical fertilizer applied
Inoc = rhizobium seed inoculation
N = applied with 75 kg of N per ha
P = applied with 56 25 kg of P_2O_5 per ha
K = applied with 37 5 kg of K_2O per ha

Table 23 Soybean seed yield of field trial in dry season 1993

Location	Control	Inoc	Inoc + P+K	N+P+K	MPN total	MPN soybean
	kg/ha				cells/g soil	
KALASIN						
Nooman	1050	2200	2650	2225	0	0
Prapass	912	1138	1175	1300	0	0
Paiboon	650	1662	2012	1675	60 66	0
Croot	850	1775	2312	1938	4 9	0
Average	866	1694	2038	1784		
LAMPANG						
Glang	1700	1750	1812	1838	377 285	26.695
Napaw	1338	1412	1600	1512	39.63	8.36
Tontong	1225	1400	1338	1512	323.89	178 67
Tungfai	1300	1612	1575	1512	143 485	0
Average	1391	1544	1581	1594		
UDORNTHANI						
Rice Re	675	1402	1644	968	87 765	2 615
Chy	1152	1872	2271	2647	ND	
Sudjai	1245	2331	2913	3003	12 945	12.945
Sargorn	946	1774	2362	1188	ND	
Average	1004	1845	2298	1702		
All average	1087	1694	1972	1693		

Control = neither rhizobium nor chemical fertilizer applied
Inoc = rhizobium seed inoculation
N = applied with 75 kg of N per ha
P = applied with 56 25 kg of P_2O_5 per ha
K = applied with 37 5 kg of K_2O per ha

Table 24 Soybean seed yield of the field trials in dry season (January - April) 1994, in different locations

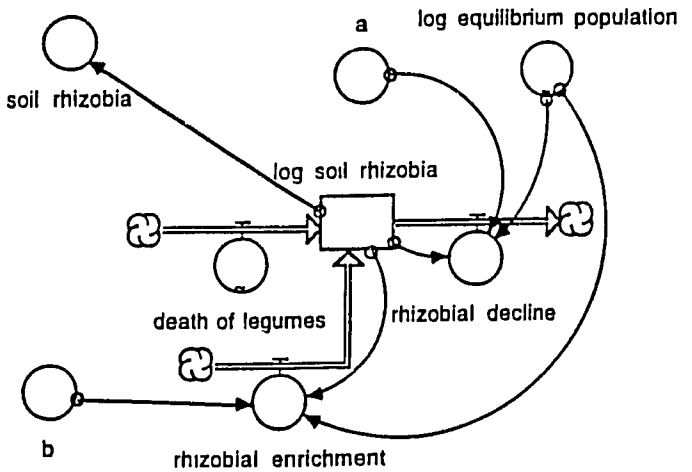
Location	Control	Inoc	Inoc +P+K	N+P+K
	---------- kg/ha ----------			
NONGKAI & UDORNTHANI				
Mr Daeng Mittsri	150	611	1470	1359
Mrs Tongsathid	506	1006	1656	1654
Mrs Sunithi Yarong	923	1323	1455	3090
Mr Thip Sawasweung	629	921	1463	1211
Mr Graithong Thamjom	720	1290	1341	1577
Mr Suwan Leusing	542	962	1461	1639
Average	578	1019	1474	1755
KALASIN				
Mr Pich Nonwiboon	431	894	1231	1488
Mr Sook Saranan	469	1156	1225	1375
Mr Ma Ketmontri	519	1044	1250	1281
Mr Somporn	212	994	1069	1062
Mr Bhan Saiwijit	222	694	1269	1303
Mr Somjit Gudwites	300	1038	1031	1088
Mr Thanorm	275	462	675	738
Average	347	897	926	1191
LAMPANG				
B. Na Jang	712	875	900	825
B Sermsai	775	1012	1000	988
B Wongsa	700	1075	1150	1038
B Mae Teun	762	1100	1200	1062
B Na Paw	1062	1438	1462	1550
Average	802	1100	1142	1093
All average	576	1005	1181	1346

Control = neither rhizobium nor chemical fertilizer applied
Inoc = rhizobium seed inoculation
N = applied with 75 kg of N per ha
P = applied with 56 25 kg of P_2O_5 per ha
K = applied with 37 5 kg of K_2O per ha

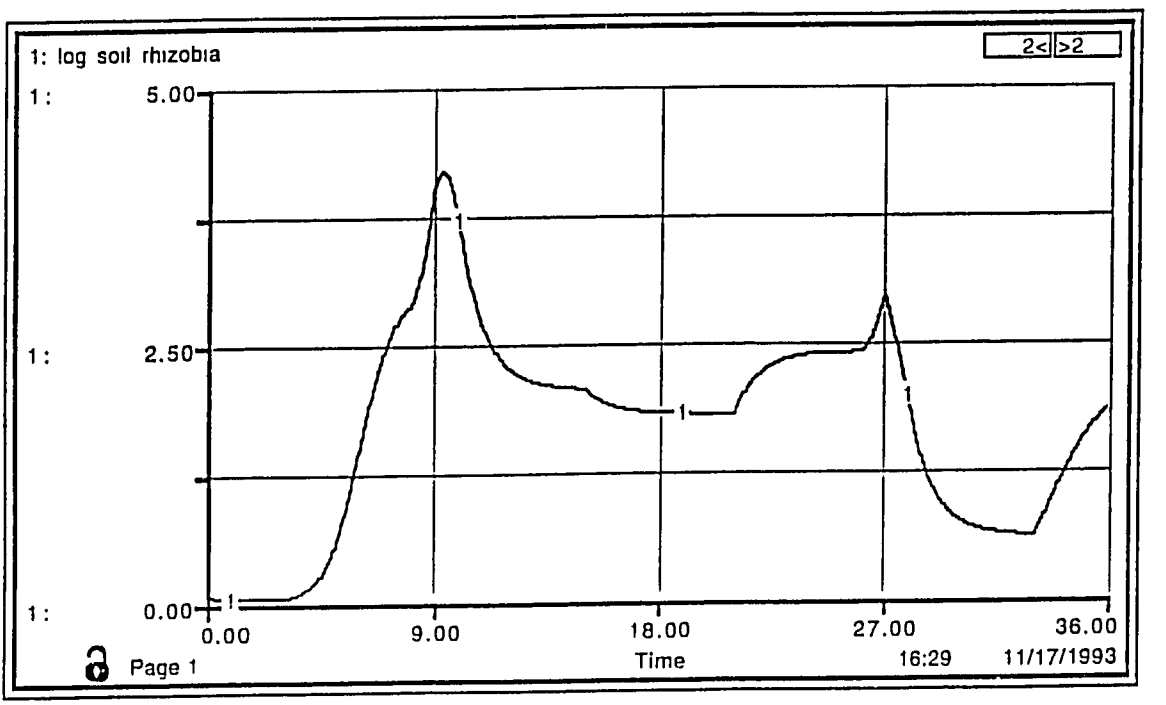
Table 25. Soybean seed yield of field trials in rainy season 1994.

Location	Control	Inoc	Inoc + P+K	N+P+K
	kg/ha			
UDORNTHANI				
B Numppon	553	1272	2022	1162
M. 5, Meungfia, Gudjab	436	946	1289	907
M 12, Meungfia, Gudjab	419	1334	1860	1479
T. Numppon	407	1238	1750	1048
Average	454	1198	1730	1149
KALASIN				
Mr Funt Poocoke	1525	2450	2750	2300
Mr Tonglaw Poonsomneug	2400	2850	3300	3100
Mrs Siya Naprom	2700	2950	3250	3150
Average	2208	2750	3100	2850
LAMPANG				
B Tone Tong Chai	1075	1350	1450	1450
B Napawtai	1425	1800	1600	1775
Average	1250	1575	1525	1612
All average	1304	1841	2118	1870

Control = neither rhizobium nor chemical fertilizer applied
Inoc = rhizobium seed inoculation
N = applied with 75 kg of N per ha
P = applied with 56 25 kg of P_2O_5 per ha
K = applied with 37 5 kg of K_2O per ha



Appendix A. Model of population flux at the field scale as developed by use of STELLA software.



Appendix B. Sample of regional scale population estimation.

cycle, nodules degenerate and bacteria are released into the soil. Which is again "enriched" with respect to this organism. These two processes are referred to as "enrichment". Survival and persistence of the bacteria released from nodules into the soil now depends on prevailing environmental factors Typically, populations decline rapidly over the first few days and weeks, but eventually reach a new equilibrium value

The basic model structure developed reflectes these aspects of the *Bradyrhizobium* " life-cycle". The extent to which each of these processes occur depends strongly on both environmental variables and agronomic management In order for the model to be used to generate population estimates, the impact of these two groups of factors needs to be incorporated

Regional scale population estimation : For population information to be useful in planning inoculant requirements country-wide and making appropriate decisions regarding resource allocation, we must eventually be capable of providing population estimates on a regional scale To this end, we have been investigating the possibility of using the Geographical Information System (GIS) for this purpose Together with Drs Simon Cook and Rob Corner (CSIRO, Floreat park, W A, Australia), a prelimary model structure has been developed Appendix B contains a copy of the paper we have written in which the basic structural components are described and model output capability to date is given The model we have developed is divided into aspects that influence rhizobia as soil inhabiting organisms and those that influence the symbiosis between rhizobia and their host legumes that ultimately impact populations levels of these organisms in soil Dr Hari Eswaran and Russell Almarez of the World Resources Institute, Soil Conservation Service, Washington, D C have cooperated in this effort by provided their GIS files that contain the soil map of Thailand and by providing weather data information Dr Theis has also been in touch with Mr Taweesak Verasilp of the Thailand Department of Agriculture to assess the availability of the more localized environmental information that will be required to carry this effort forward We were encouraged to learn that a considerable portion of what we believe we'll need is already available in the GIS language required, with additional information becoming available all the time We are currently in the process of developing the sematic network required to evaluate relevent environmental information at the catchment scale We envision that the outcome of this effort will be regional maps that will provide a management tool for determining inoculant requirements and their distribution country wide.

We can say with confidence that the zones difference significantly The Northeast is significantly different from the rest of the country. The North and Central different significantly from the South but are not different from each other

Significant increase in rhizobial numbers, hence the prevailling equilibrium population level would be the expected output. When legumes are grown, the curve describes the population increase over the cropping phase The enrichment is limited to a 100 fold increase over the initial count. The increase is dampered by critical environmental conditions such as drought, acidity, low organic matter and high temperature.

The third is the Mitcherich decline function for *Bradyrhizobium* spp. persistence developed by Woomer et al., 1998. This equation is :-

$$\text{Rhizobia (log 10)} = A + (B^{*} \exp^{c\text{-days}})$$

Again, the extent of decline will vary depending on severity of the environmental conditions and length of time out of legume cropping. Eventually, an equilibrium population will be riched and the cycle begins again.

Conclusion

Responses of legume to inoculation depended on the population of indigenous rhizobia, their effectiveness and soil nitrogen. The population of indigenous rhizobia were also influenced by soil temperature, moisture and cropping system In the areas where soil was dry and hot population of rhizobia in soil declined rapidly With host legumes grown continuously the population of rhizobia maintained in high number where the area without host legumes indigenous rhizobial population were quiite how

In soil contained more than 100 cells of effective rhizobia inoculation of rhizobia on seed may not necessary In consiideration of mixed population between effective and ineffective rhizobia, when soil contained effective ineffective at the ration of 1:10 growth of legume plant was not affected by ineffective strain eventhough more nodules were formed by ineffective strain because the small number of nodules formed by effective strains were larger than the ineffective nodules resulted in hiigher bacteriods of number were from effective strain

Evaluation the use of legume inoculation under field condition in different locations and seasons confirmed that soybean inoculated with effective strains of rhizobia yield was higher than the uninoculated control In soil which found to be low in available phosphorous, application of phosphorous fertilizer together with inoculation could greatly increase yield of soybean Upon analysing of economic impact of rhizobial inoculation we found that maximum profit was obtained from inoculation or inoculatiion with application P fertilizer

Data obtaining from this research including population of rhizobia under different cropping system, ration of effective and ineffective rhizobia and field evaluation on legume responded to inoculation we could develop a model for prediction legume inoculation requirement

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