



Management of nitrogen symbiosis and plant nitrogen nutrition of field pea (*Pisum sativum*) and chickpea (*Cicer arietinum*) with spectral reflectance
by Jody Todd McConnell

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Land Resources and Environmental Sciences
Montana State University
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Abstract:

Pulse crop production is increasing steadily throughout the northern Great Plains region and is dependent on routinely successful biological N₂-fixation. Nodulation failure in grain legumes, resulting in plant N deficiency and economic crop loss, might be alleviated by a remedial N fertilizer application. The objectives of the study were to: (1) determine the potential period for yield recovery by fertilizer N application for simulated nodulation failure of dry pea and chickpea, (2) characterize spectral signatures for nodulated vs. non-nodulated dry pea and chickpea, and (3) determine if plant N deficiency can be detected through spectral analysis sufficiently early to permit yield recovery by remedial N fertilizer application.

Two grain legume crop types (semi-leafless field pea, kabuli chickpea) were evaluated for potential yield recovery from a nodulation failure with fertilizer applications occurring at 0, 4, 6, and 8 wk after seeding. The fertilizer application rates were: 102 kg N ha⁻¹ for field pea and 69 kg N ha⁻¹ for chickpea, in 1999, and increased to 183 kg N ha⁻¹ and 115 kg N ha⁻¹, respectively in 2000. Field pea displayed N deficiency clearly and revealed an application window for partial to complete yield recovery of 0 to 6 wk after seeding. Seed N content was increased by 0, 4 and 6 wk fertilizer treatments (P-value < 0.05). Chickpea yields were low due to predation and drought stress during the pod filling stage. Consequently, few yield differences were observed, but chickpea seed N concentrations of 0 to 6 wk after seeding fertilizer treatments resulted in plant N status equal to an inoculated, N₂ fixing crop.

Significant logistic regression models were derived for both pulse crops from reflectance values for inoculated and uninoculated control treatments. The model for field pea had an overall accuracy of 84% and was capable of assisting a management decision in a timely manner. The model for chickpea had a reduced accuracy of 60% and, as a result, was not useful for detecting nodulation failure.

MANAGEMENT OF NITROGEN SYMBIOSIS AND PLANT NITROGEN NUTRITION
OF FIELD PEA (*PISUM SATIVUM*) AND CHICKPEA (*CICER ARIETINUM*)
WITH SPECTRAL REFLECTANCE

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A thesis submitted in partial fulfillment
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MONTANA STATE UNIVERSITY
Bozeman, Montana

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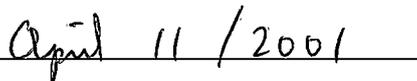
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ABSTRACT

Pulse crop production is increasing steadily throughout the northern Great Plains region and is dependent on routinely successful biological N₂-fixation. Nodulation failure in grain legumes, resulting in plant N deficiency and economic crop loss, might be alleviated by a remedial N fertilizer application. The objectives of the study were to: (1) determine the potential period for yield recovery by fertilizer N application for simulated nodulation failure of dry pea and chickpea, (2) characterize spectral signatures for nodulated vs. non-nodulated dry pea and chickpea, and (3) determine if plant N deficiency can be detected through spectral analysis sufficiently early to permit yield recovery by remedial N fertilizer application.

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Significant logistic regression models were derived for both pulse crops from reflectance values for inoculated and uninoculated control treatments. The model for field pea had an overall accuracy of 84% and was capable of assisting a management decision in a timely manner. The model for chickpea had a reduced accuracy of 60% and, as a result, was not useful for detecting nodulation failure.

INTRODUCTION

Background

Grain legume or 'pulse' crops fix atmospheric dinitrogen (N_2) gas through a symbiotic relationship with soil bacteria (*Rhizobium spp.*). This symbiosis can supply the majority of the N required for a successful crop, with the N acquired from the soil, seed reserves, and seed-placed fertilizer (Mahon and Child, 1979). Environmental conditions and management practices influence the development of this biological N_2 symbiosis.

Pulse crop production is increasing steadily throughout the northern Great Plains region (Miller et al., 2001, in press) and is dependent on routinely successful biological N_2 -fixation. Dinitrogen fixation can fail to establish due to a number of production and soil-environmental influences. Nitrogen deficiency during the flowering and pod fill period has an adverse affect on most crops, especially pulses. Pod abortion and decreased seed size can be attributed to N deficiency during pod fill (Brevedan et al., 1977). Nodulation failure resulting in plant N deficiency and economic crop loss, however, might be alleviated by a remedial N fertilizer application. Early detection of nodulation failure and the characterization of remedial fertilizer application windows have not been addressed in regions of North America where cool-season pulse crops are grown.

Nitrogen Fixation

Nitrogen fixation is a biological process that is initiated soon after the pulse crop germinates, but takes 3-4 wk to become well established (LaRue and Weeden, 1992).

The plant provides energy, nutrients, and water to the rhizobia in the root nodules, and in return, the rhizobia provide N to the plant. The amount of atmospheric N₂ fixed varies with the type of pulse crop, the dry matter yield of the pulse crop, the supply of available soil N, and other environmental conditions.

Inoculation is the process of introducing an appropriate strain of *Rhizobium* to the soil in sufficient numbers to permit timely infection of legume roots. Different groups of pulse crops require different strains of rhizobia. For example, pea, lentil and fababean use the same strain (*Rhizobium leguminosarum*), while chickpea requires another (*Rhizobium ciceri*). Commercially available inoculants contain efficient N-fixing strains compatible with each crop (Bottomley, 1992). Inoculants are either coated on the seed or placed in the seed furrow to ensure immediate access to developing roots (Table 1)

Table 1. Rhizobium inoculant types and characteristics.

<u>Formulation Type</u>	<u>Liquid Inoculant</u>	<u>Powder Inoculant</u>	<u>Granular Inoculant</u>
Seed or soil applied	seed	seed	soil
Carrier type	water solution	peat or clay	peat or clay
Survivability in adverse conditions	poor	good	best

Seed coated inoculants require an adhesive solution to ensure adhesion of the inoculant to the seed surface. The inoculant is applied to the seed immediately prior to

planting by mechanically mixing the seed and inoculant, whereas soil-applied inoculants are applied in the seed furrow as granules during the seeding operation (Anonymous, 2000).

Nodulation Failure

The biological N fixation relationship might fail due to environmental conditions or management error. The primary environmental factor in nodulation failure is dry seedbed conditions, a common occurrence in the northern Great Plains. Tillage operations and variable spring moisture can produce dry, warm soils, which desiccate and destroy rhizobia before infection process can occur (Chatel and Parker, 1973; Evans et al., 1988). Other factors that inhibit nodulation include: cool soil conditions ($<5^{\circ}\text{C}$) (Rice et al., 1994), excessive levels of soil N ($> 39 \text{ kg/ha}$) (Allos and Bartholomew, 1955) and acidic soil ($\text{pH}<5$) (Bushby and Marshall, 1977, Coventry and Evans, 1989). Acidic and alkaline pH affects the receptivity of plant roots to rhizobial infection (Lie, 1974), and limits the availability of calcium and molybdenum (Bottomley, 1992). Calcium is a requirement in nodule cell division (Lie, 1974), and molybdenum is a nitrogenase constituent (Russell, 1973).

Management errors influencing nodulation can occur from the time inoculant is produced in the factory to furrow placement by the seeding operation. Rhizobia are sensitive to heat and desiccation. Common errors include improper storage at warm temperatures and use of incorrect strains of inoculant. Powder based inoculants-seed

mixes are frequently prepared without adhesives. During seeding the inoculant may fall to the bottom of the planter box. This leads to uneven inoculation rates throughout the field. Since N is required in high concentrations for pulse crops (Wery et al., 1993; Egli et al., 1978), nodulation failure can result in enormous economic losses due to yield reduction, poor grain quality and decreased N cycling.

The objectives of the study were to: (1) determine the window of opportunity for yield recovery by fertilizer N application for simulated nodulation failure of dry pea and chickpea, (2) characterize spectral responses for nodulated vs. non-nodulated dry pea and chickpea, and (3) determine if plant N deficiency can be detected through spectral analysis early enough to permit yield recovery by remedial N fertilizer application.

Literature Review

Much of the research on N fixation in annual legumes was undertaken in the soybean (*Glycine max*) producing regions of the mid-west U.S. where nodulation failure is uncommon due to generally favorable soil-climatic conditions. One previous study examined the possibility of fertilizing unnodulated soybeans to recover yield (Gault et al., 1984). Application of 54 kg N ha⁻¹ as NH₄NO₃ 60 d after sowing, successfully recovered yield compared with a nodulated control. Split application treatments did not recover yield as successfully as did a single application 60 d after seeding. The study concluded that yield recovery was possible with in-crop N fertilizer application. There have been extensive studies investigating relationships between plant biophysical

characteristics and spectral reflectance. Remote sensing studies concerning agriculture have estimated crop biophysical properties pertinent for management decisions.

Estimation of sugar content in sugarbeets was attempted through spectral relationships with canopy N concentration (Humburg and Stange, 1999). Through the development of a Beet Difference Vegetation Index, an index consisting of spectral bands, Humburg and Stange (1999) predicted sodium, amino N, and recoverable sugar per ton, with an R^2 value of 0.52 for sugar content. The best model included spectral bands at 500, 550, and 830 nm and did not contain red band wavelengths (600-700 nm), commonly found in many other reflectance indices. The study concluded that farm management decisions could be influenced using remote sensing.

Nitrogen concentration was estimated in sweet pepper using spectral reflectance (Thomas and Oerther, 1971). Regression equations expressing reflectance as a function of leaf N concentration of sweet peppers, grown in the greenhouse, were used to estimate the N concentration of sweet peppers grown in the field. The regression equation using the 550 nm wavelength had an R^2 of 0.93. The study indicated that the N concentration of sweet pepper could be accurately predicted by using reflectance, albeit under greenhouse conditions.

Application of remote sensing for the purposes of predicting plant N status and nodulation failure in pulse crops has not been addressed. Previous investigation involving pulses, studied other biophysical parameters. The reflectance of pea and fababean was studied and the soil adjusted index (SAVI, SAVI2) predicted biomass and

radiation use (Ridao et al., 1996). Water deficits in fababean produced a change in leaf angle that lowered the fraction of photosynthetically active radiation intercepted by the canopy, when compared to irrigated fababean.

Spectral prediction of crop parameters is not limited only to N concentration and biomass. Phosphorus deficiency affected the spectral reflectance and morphology of soybean (Milton et al., 1989). Greenhouse and field studies documented the spectral and morphological changes in plants having anomalous concentrations of metallic elements in the soil or other growing medium (Milton et al., 1983). The shift in spectral signature was due to increased reflectance between 550 and 650 nm. Soybean plants deficient in phosphorus had similar reflectance responses, compared to soybean plants that were dosed with elevated concentrations of metallic elements (Milton et al., 1989). It was hypothesized that nutrient imbalances or anomalous metal concentrations set up physiological conditions at the soil/root interface that were responsible for the observed reflectance differences. The reflectance of plants grown in metallic-enriched substrates displayed reflectance patterns similar to plants deficient of a nutrient, in this case phosphorus. It was hypothesized that the metals react with phosphorus in the plant, rendering it unavailable for use and resulting in a signature displaying phosphorus deficiency.

The investigation of corn leaf chlorophyll concentration and canopy reflectance, and reinforced the idea that N content is closely linked with chlorophyll content (Daughtry et al., 2000), since most leaf N is contained in chlorophyll molecules (Yoder et

al., 1995). They used a spectroradiometer, similar to the one used for this study, except reflectance was simulated with fluorescence. They used pairs of reflectance indices to estimate chlorophyll concentrations, with minimal interference from background effects. The study offered a unique method of estimating chlorophyll with reflectance with existing indices.

Indices of spectral data were developed to minimize or maximize patterns and signals from canopy reflectance. Normalized Difference Vegetation Index (Rouse et al., 1973), Greenness Vegetation Index (Gitelson et al., 1996), Transformed Soil Adjusted Vegetation Index (Baret et al., 1989), Perpendicular Vegetation Index (Richardson and Wiegand, 1977), Soil Adjusted Ratio Index (Major et al., 1990), Soil Adjusted Vegetation Index (Huete, 1988), Ratio Vegetation Index (Jordan, 1969) and Difference Vegetation Index (Tucker, 1979) are the indices that can be used to study reflectance data. Most published research on crop canopy reflectance, involved crops other than pulses and collected data after canopy closure had occurred. Canopy closure ensures that a large proportion of the spectral data is derived from the plants, minimizing background noise. It has been discovered that many of the indices currently used unnecessarily restrict regression analysis related to biophysical parameters.

Multiple regression performed on the decoupled bands provided superior explaining power (Lawrence and Ripple, 1998). This is important because the time period that reflectance values were collected for the following study was during the early seedling to first flowering stage, when the reflectance signal emitted by the plant canopy

is a small portion of the total reflectance, and background soil reflectance interfered. Using indices that couple bands together will mask the different responses of individual bands.

Alternative methods for estimation of plant biophysical variables exist in addition to remote sensing. Hand-held SPAD (Soil Plant Analysis Development, Minolta Camera Corporation) meters have been developed to estimate N concentration in crop canopies. The strong positive relationship between photosynthetic rate and leaf N concentration permits the chlorophyll meter to determine a crop's need for additional fertilizer N (Turner and Jund, 1994). The SPAD meter does not measure leaf chlorophyll or N directly, rather it provides quantitative measurements of leaf greenness (Turner and Jund, 1994). The measurements have been correlated with limited success for maize N requirements ($R^2 = 0.59$) (Piekielek and Fox, 1992).

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SPECTRAL REFLECTANCE

Introduction

Pulse crop production is increasing steadily throughout the northern Great Plains region (Miller et al. 2001) and is dependent on routinely successful biological N₂-fixation. Dinitrogen symbiosis can supply the majority of the N required for a successful crop, with the remaining N acquired from the soil, seed reserves, and seed-placed fertilizer (Mahon and Child, 1979). Environmental conditions and management practices influence the development of this biological symbiosis, requiring optimal conditions for satisfactory N nutrition. The primary environmental factor in nodulation failure is dry seedbed conditions, a common occurrence in the northern Great Plains. Tillage operations and variable spring moisture can produce dry, warm soils that desiccate and destroy rhizobia before the infection process can occur (Chatel and Parker, 1973; Evans et al., 1980). Management errors also cause nodulation failure through improper storage or application of inoculant, mechanical failure of inoculant delivery or the inadvertent selection of improper rhizobial strains (P. Miller, 2000, personal communication).

Since N is required in high concentrations at for pulse crops (Wery et al., 1993; Egli et al., 1978), nodulation failure can result in large economic losses due to yield reduction, poor grain quality and decreased N cycling. Pod abortion and decreased seed size can be attributed to N deficiency during pod fill (Brevedan et al., 1977). Nodulation

failure resulting in plant N deficiency and economic crop loss might be alleviated by a remedial N fertilizer application. Previously, remedial N fertilizer application was successful as a means of recovering yield from nodulation failure in soybean (Gault et al., 1984). An application of 54 kg N ha⁻¹ as NH₄ NO₃ 60 d after sowing successfully recovered yield compared with a nodulated control. Split application treatments (32 and 60 d after seeding) did not recover yield as successfully as did a single application 60 d after seeding.

Remote sensing studies have developed relationships between spectral reflectance responses and crop physical properties including: chlorophyll content (Daughtry et al., 2000; Yoder et al., 1995), N content (Piekielek and Fox, 1992; Thomas and Oerther, 1971; Turner and Jund, 1994), phosphorus deficiency (Milton et al., 1991), sugar content (Humburg and Stange, 1999), radiation use efficiency (Ridao et al., 1996), and soluble metal accumulation (Milton, et al., 1983). Remote sensing has not been used to evaluate plant N nutritional status or inoculation success in cool-season pulse crops.

The objectives of the study were to: (1) determine the potential period for yield recovery by fertilizer N application for simulated nodulation failure, (2) characterize spectral responses for nodulated vs. non-nodulated dry pea and chickpea, and (3) determine if plant N deficiency can be detected through spectral analysis early enough to permit yield recovery by remedial N fertilizer application.

Materials and MethodsExperimental Design

The experiment occurred at three sites in central and southwestern Montana in 1999 and 2000 where winter wheat production is common (Table 2). The soil type at Denton was a Winifred clay loam (fine, montmorillonitic, Typic Haploboroll), at Moore was a Bridger clay loam (fine, mixed Argic Cryoboroll) (Clark, 1988), and Amsterdam was a Manhattan very fine sandy loam (coarse, loamy mixed Typic Calciboroll) (DeYoung and Smith, 1936).

Table 2. Site characteristics at Amsterdam, Denton and Moore, MT.

Site	Amsterdam	Denton	Moore
Lat, Long	45.45 N, 111.25 W	47.30 N, 109.95 W	46.47 N, 109.43 W
Elevation (m)	1525	1100	1520
Soil Order	Manhattan very fine sandy loam	Winifred clay loam	Bridger clay loam
Soil Depth (cm)	150+	60-120+	45-90+
Soil N (kg N ha ⁻¹) ^z	14	20	24
Annual Temp (°C) ^y	6.6	6.6	6.1
Annual Precip.(mm) ^y	300-350	350-400	350-400

^z NO³⁻ NH⁴ KCL extraction

^y MAPS climate atlas of Montana, Caprio et al., 1994.

The experimental design was a four-replicate, randomized complete block. The experimental units were 15.3m x 3.7m. The treatments were two crop types (field pea -

1999 cv. Alfetta yellow semi-leafless, 2000 cv. Espace green semi-leafless, chickpea- cv. Dwelley Kabuli) and 6 N nutrition treatments (Table 3).

Table 3. Treatments and fertilizer application timings.

Treatment	Days applied after seeding
Unfertilized / uninoculated control (Uninoc)	-
Unfertilized / inoculated control (Inoc)	-
Uninoculated / fertilizer N applied 0 weeks after seeding (0 wk)	8, 9, 0, 0 ^z
Uninoculated / fertilizer N applied 4 weeks after seeding (4 wk)	29, 30, 30, 28 ^z
Uninoculated / fertilizer N applied 6 weeks after seeding (6 wk)	40, 41, 41, 44 ^z
Uninoculated / fertilizer N applied 8 weeks after seeding (8 wk)	54, 55, 61, 56 ^z

^z Denton and Moore, 1999, Amsterdam and Moore, MT, 2000, respectively.

Field Operations

In 1999, the experiment was seeded into tilled cereal stubble with a *Conserva-Pak*¹ research drill with a seed furrow width of 30 cm, double shoot hoe-type openers and on-row packing. Seed and fertilizer products were placed in separate bands. In 2000, the sites were seeded into standing cereal stubble with a custom manufactured plot drill with a seed row width of 25 cm, with *Atom Jet*² hoe-type openers and on-row packing with single shoot capability. Planting depth was 4-5 cm and target seeding rates were 88 plants m² for peas and 44 plants m² for chickpea. Plant stand density varied between 78-88 plants m² for field pea and 35-52 plants m² for chickpea among site-years. Seeding occurred between 28 April and 6 May, considered normal seeding dates for the respective

¹ Conserva-Pak, Yorkton, SK, Canada

² HarvesTechnologies, 2110 Park Ave., Brandon, MB, Canada.

sites. Weeds were controlled effectively by pre-emergent glyphosate (480 g a.i. ha⁻¹) and ethylfluralin (1.50 kg a.i. ha⁻¹), and post-emergent quizalofop P-ethyl (50 g a.i. ha⁻¹) herbicide applications were applied according to recommended practice for both crops.

Inoculant and Fertilizer

Strain-specific *Liphatech Soil Implant*³ peat granular inoculant was placed in the seedrow (5.5 kg ha⁻¹). The 1999 fertilizer application rates were: 102 kg N ha⁻¹ for field pea and 69 kg N ha⁻¹ for chickpea. The fertilizer urea (46-0-0) was applied with an air-delivery *Valmar*⁴ granular applicator. The fertilizer rates were established by estimating the removal by plant and seed targeting a 1980 kg ha⁻¹ yield for field pea and 1344 kg ha⁻¹ yield for chickpea (Anonymous, 1992). In 2000, the rates were increased to: 183 kg N ha⁻¹ for field pea and 115 kg N ha⁻¹ for chickpea, to ensure N rates did not limit yield. Phosphate was applied each year at a rate of 22 kg ha⁻¹ in the form of ammonium phosphate (11-55-0), and placed in the seed furrow during the planting operation.

Crop Data

Biomass samples were removed weekly to monitor dry matter accumulation and N concentration from the 4th node stage to anthesis. Growth stages were determined by enumeration of plant nodes. Shoot biomass samples for each treatment were removed by hand-clipping an area 1.1 m², coinciding with radiometric readings. Additional

³ Liphatech Inc., 3101 West Custer Ave., Milwaukee, WI, U.S.A.

⁴ Valmar Inc., Box 100, Elie, MB, Canada

parameters measured were: plant height, mature biomass and harvest seed samples. Biomass and seed weights were reported on a dry matter basis. Plant N concentration was measured by automated dry combustion analysis using a LECO CNS analyzer⁴. Rainfall data was recorded by electronic tipping bucket recorders at Moore, and Amsterdam. Temperatures were recorded by a portable weather station on-site at Moore. Amsterdam temperatures were recorded by a state weather station at Belgrade, MT, 15 km east of the site. Temperatures and precipitation were recorded by a weather station at Denton, MT, 10 km north of the field site.

Spectral Data Collection

Radiometric data was recorded with a portable *Cropscan*⁵ radiometer during peak hours of sunlight (1000 to 1500 h). Measurements consisted of incoming solar radiation and reflected canopy radiation. Readings were collected from two locations within each plot. Each reading measured an effective area of 3.1 m². Cropscan software was used to convert spectral data into percent reflectance for each band recorded. The 11 bands and accompanying band width were as follows: 485 (80), 530 (50), 560 (80), 613 (40), 660 (60), 680 (40), 706 (45), 813 (30), 830 (150) and 1650 (300) nm. Five of the Cropscan bands are similar to the first five Landsat Thematic Mapper satellite bands which are: Band 1 - 450 to 520 nm, Band 2 - 520 to 600 nm, Band 3 - 630 to 690 nm, Band 4 - 760 to 900 nm, Band 5 - 1550 to 1750 nm.

⁴ LECO Corp., 3000 Lakeview Ave., St. Joseph, MI, U.S.A.

⁵ Cropscan Inc., 125 26th St. NW, Rochester, MN, U.S.A.

An additional plant N assessment tool (Minolta SPAD meter⁶) was introduced in the second year of the study. Readings were taken from the uppermost and lowest leaves and coincided with weekly biomass sampling.

Statistical Analyses

Analysis of variance as a randomized complete block design was performed separately for field pea and chickpea to examine differences between treatments (SAS, 1995). Sites were analyzed separately, due to non-homogeneity of error variances among sites. Treatment means were evaluated using the Protected LSD (Steel and Torrie, 1980). Reflectance band models were derived to differentiate between control treatments, utilizing a non-automatic, backward, stepwise logistic regression approach (Neter et al., 1996; S-PLUS, 2000). The logit link function is the regression structure for a binary response, where logit (π) is the probability of an uninoculated classification (Ramsey and Schafer, 1997). Regression analysis of single bands has been proven to have superior explanatory power as compared to numerous band indices (Lawrence and Ripple, 1998). The regression models were built utilizing the full data set, excluding the 2000 Amsterdam site for field pea, since confirmed contamination from native rhizobia occurred at that site. Arrival at a significant model was indicated when the exclusion of any variable resulted in a significant drop-in-deviance statistic. The logistic models were used to classify the reflectance data into nodulated and unnodulated groups using a cutoff value of 50% probability. The logistic regression models' accuracy were assessed using

⁶ Minolta Soil and Plant Device, Minolta Corp., Nippon, Japan.

an error matrix or contingency table (Congalton, 1991; Lillesand and Kieffer, 1994).

Overall accuracy, the simplest inference statistic, was calculated by dividing the total number of correct classifications by the total number of classifications. Divisions among the treatments can be performed as well, to examine how the model performed for the different treatments (controls). Dividing the total correct per category by the total number of samples for that category is referred to as the "producer's accuracy" or measure of omission error, indicating the probability of a sample being correctly classified (Congalton, 1991). Conversely, if the total number of correct samples in a category is divided by the total number of samples that were classified in that category, then the measure of commission error or "user's accuracy" is calculated (Congalton, 1991).

Producer's accuracy statistics were calculated using the column values of the error matrix, while the user's accuracy statistics were calculated using the row values. The difference in the two statistics is explained as; the author of a map can claim that 79% (producer's accuracy) of the time an area that was nodulated was identified as such, a user of the map will find that 88% of the time will an area visited that the map says is nodulated will actually be nodulated. The user's accuracy is of particular interest to a farmer who might utilize a derived field map for management applications. The KHAT statistic indicates a model's ability for classification compared to chance classification and is an estimate of KAPPA (Lillesand and Kieffer, 1994). The KHAT statistic is often used to differentiate between different methods of classification in remote sensing (i.e., supervised versus unsupervised classification).

Greenhouse Experiment

A greenhouse experiment was conducted to determine whether the rhizobial contamination witnessed with field pea at Amsterdam was due to a native population or equipment contamination. Soil was collected from 4 locations 20 m from the perimeter of the plots, where plot machinery had not travelled. Seeds were surface sterilized with hydrogen peroxide (3%) and incubated to hasten germination, on sterile yeast mannitol agar plates (Somasegaran and Heinz, 1994). The uncontaminated seedlings were planted in pots of the collected soil. The pots were watered to maintain moist conditions and incubated in a research greenhouse. Plant roots were observed for the presence or absence of nodules 5 wk after planting (Somasegaran and Heinz, 1994).

Results

Shoot Biomass and Nitrogen Concentration

Field Pea The precipitation patterns at sites in this study were characterized by terminal drought beginning in mid-July, typical of this region (Figure 1). Shoot biomass varied among sites due to moisture conditions (Figure 2). A hail storm occurred in late June at Denton retarding crop growth after the 6-wk fertilizer application. In 2000 at Amsterdam field pea data were omitted due to contamination by native rhizobia, which was confirmed by a greenhouse plant trap experiment (Somasegaran and Heinz, 1994). By anthesis, the 0-wk and 4-wk treatments, and the inoculated control had higher shoot

