



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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Bozeman, Montana

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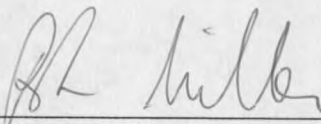
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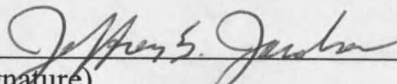
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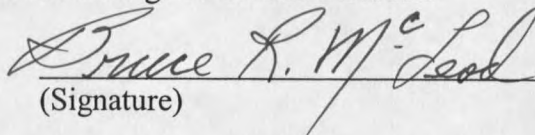
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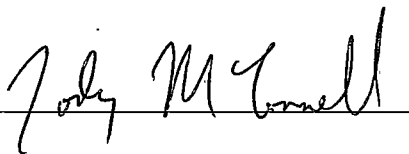
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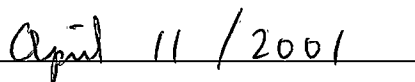
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ABSTRACT

Pulse crop production is increasing steadily throughout the northern Great Plains region and is dependent on routinely successful biological N_2 -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, N_2 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.

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).

MANAGEMENT OF N SYMBIOSIS AND PLANT N NUTRITION
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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 (Piekielk 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 Methods

Experimental 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

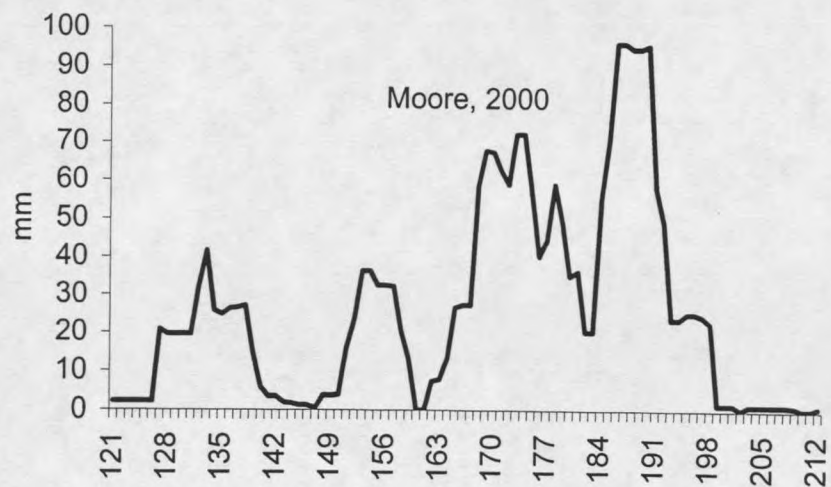
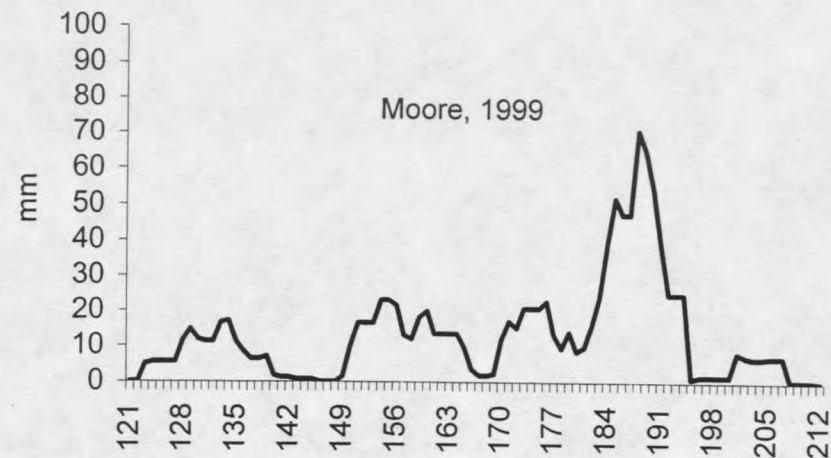
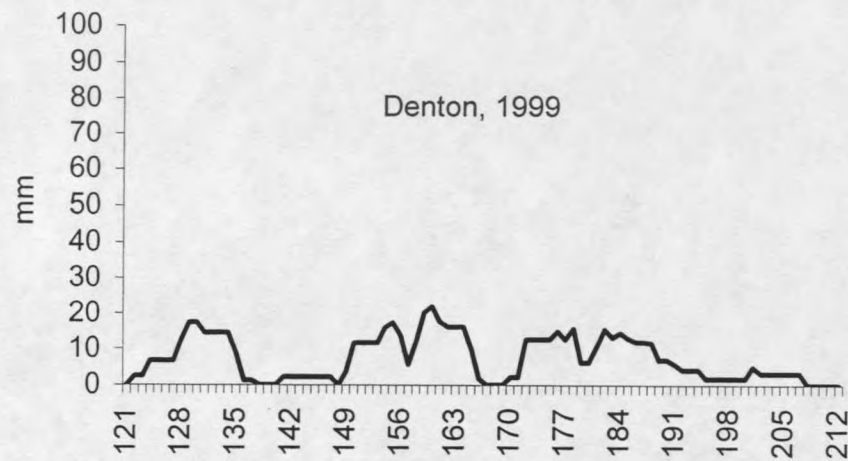


Figure 1. Weekly precipitation from 1 May to 31 July (Julian days) at Denton and Moore, 1999 and Amsterdam and Moore, MT, 2000. Values were recorded as running 7-day totals, indicating the total rainfall received for each date plus that received in the previous 6 days.

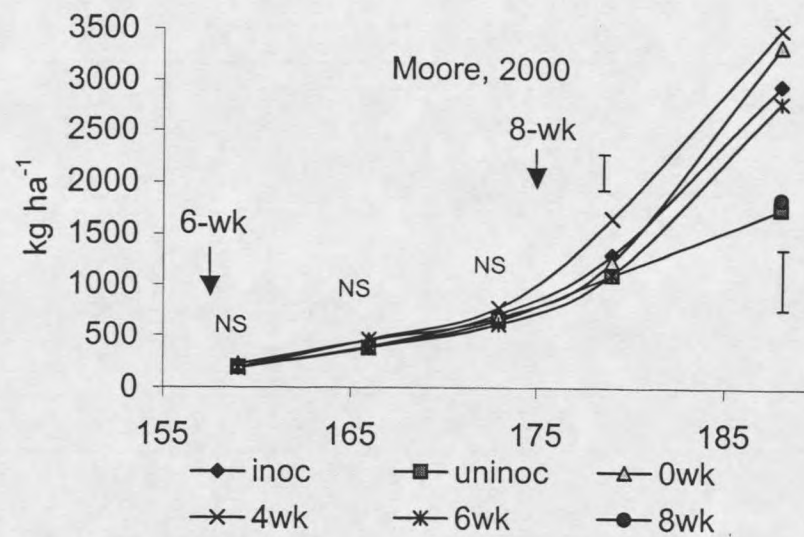
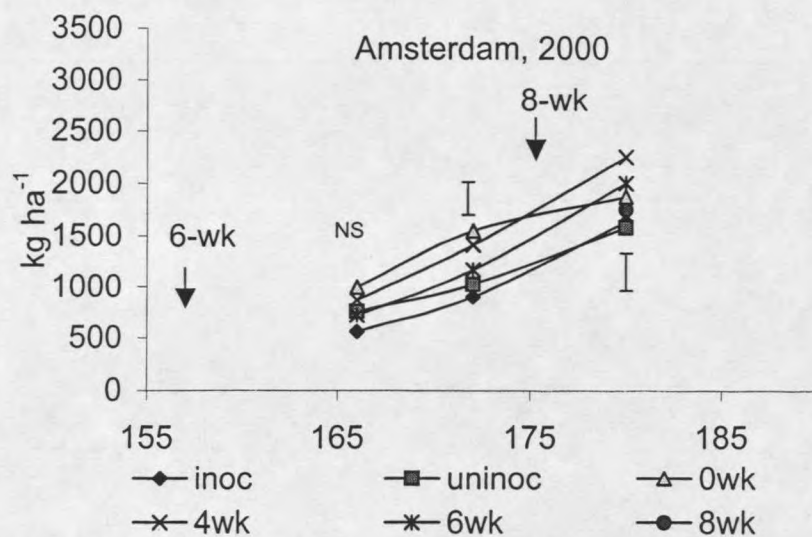
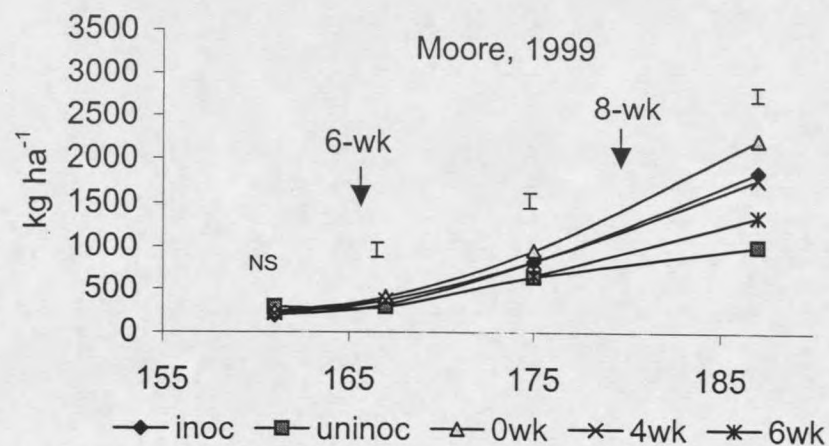
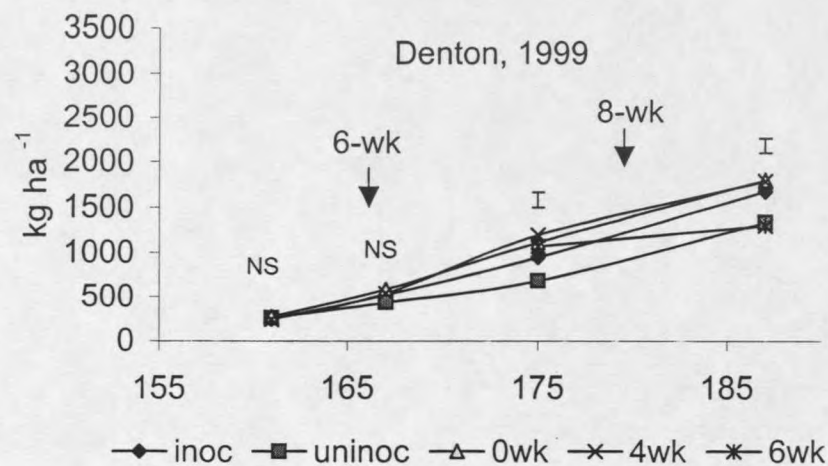


Figure 2. Shoot biomass accumulation from the 4 node stage to anthesis (Julian days) for field pea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000. Fertilizer was applied at the beginning of each interval indicated. Least significant differences indicated by vertical bars on graph (P -value ≤ 0.05).

biomass than the uninoculated and 8-wk treatments. The 6-wk fertilizer treatment produced a variable biomass response.

Shoot N concentration was more sensitive than shoot biomass to the N management treatments (Figure 3). After the 6th week of growth (7-10 node stage), treatments differed (P -value ≤ 0.05). In two of the three sites, biomass N at anthesis for the inoculated treatment was equal or greater than all fertilized treatments (P -value ≤ 0.05).

Chickpea Chickpea biomass accumulation was less than field pea and shoot biomass differences occurred two weeks earlier with field pea than chickpea. For chickpea, treatments did not differ until anthesis (P -value ≤ 0.01), where early fertilizer treatments were higher than the inoculated control for biomass accumulation in 2000 (Figure 4). Nitrogen concentration of the biomass differed among treatments during the 4 - 6 wk period at three of four sites (P -value ≤ 0.05) (Figure 5).

Yield

Field Pea Early fertilizer treatments (0, 4-wk) and the inoculated treatment had higher seed yields than the 8-wk fertilizer treatment and the uninoculated control (P -value ≤ 0.05) (Table 4). Uninoculated control yields were $< 50\%$ of the inoculated control at the three rhizobia-responsive sites. In 1999, full yield recovery was attained by remedial N fertilizer at only the 0-wk and 4-wk treatments. In 2000, full yield recovery was attained with these treatments and the 6-wk treatment (Table 4). The 8-wk fertilizer treatment failed to recover yields equal to the inoculated control in both years, coinciding

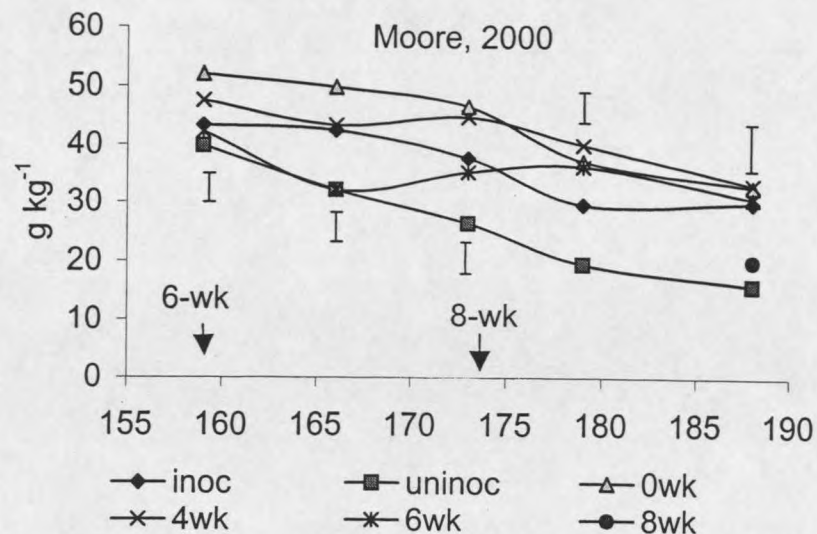
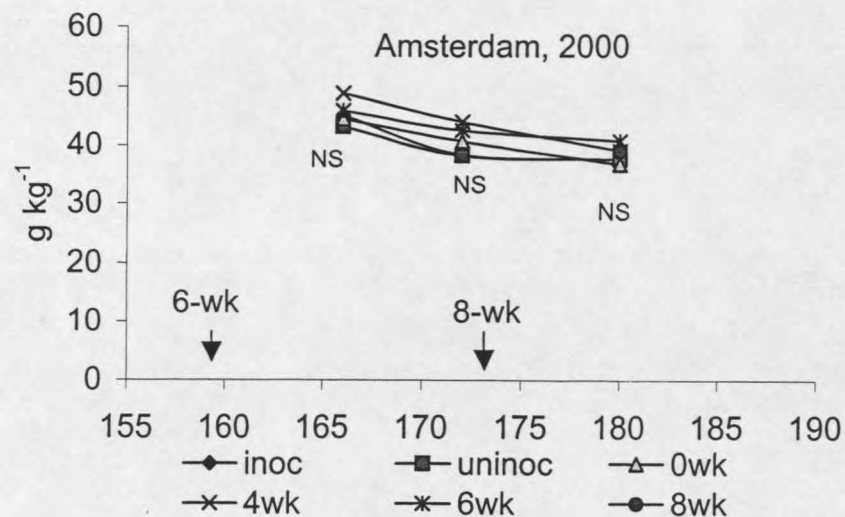
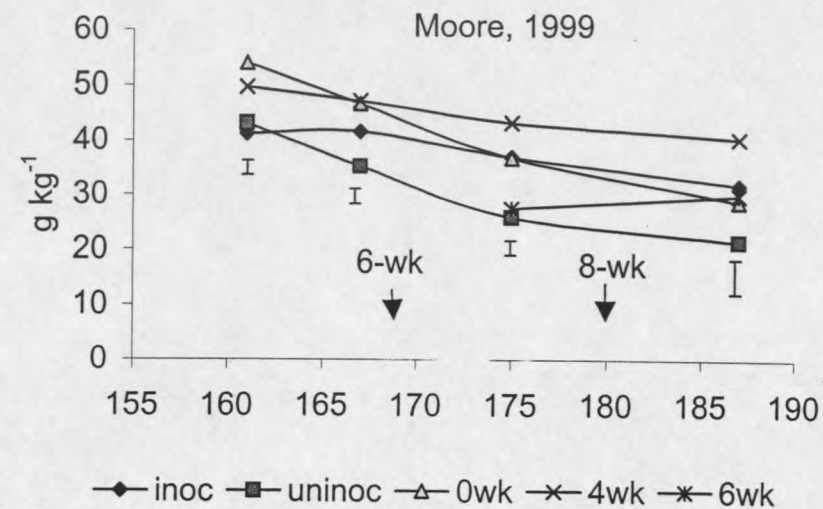
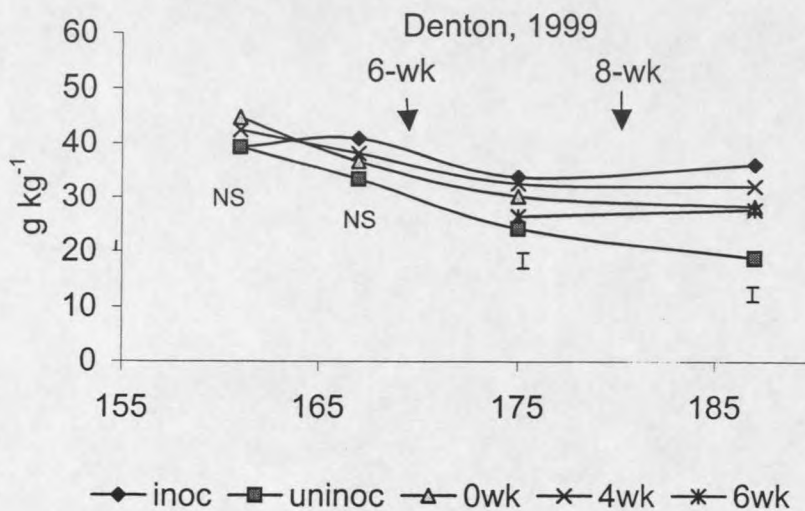


Figure 3. Shoot biomass N concentration from the 4-node stage to anthesis (Julian days) for field pea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000. Fertilizer was applied at the beginning of each interval indicated. Least significant differences indicated by vertical bars on graph (P -value ≤ 0.05).

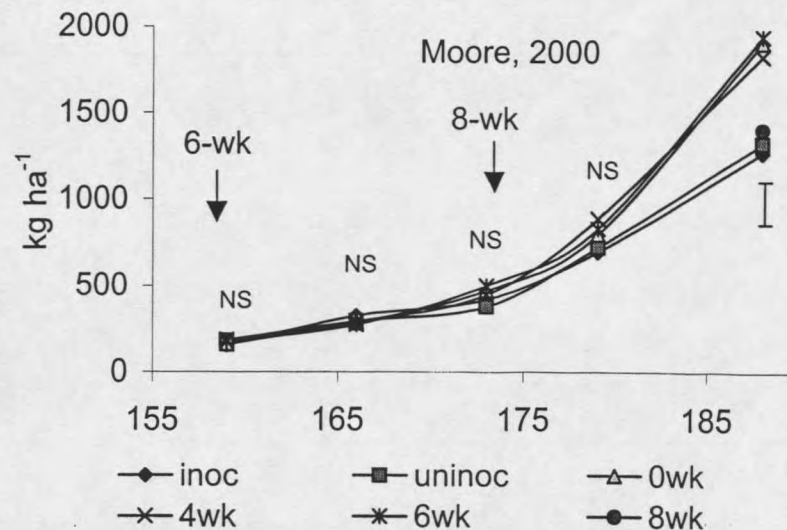
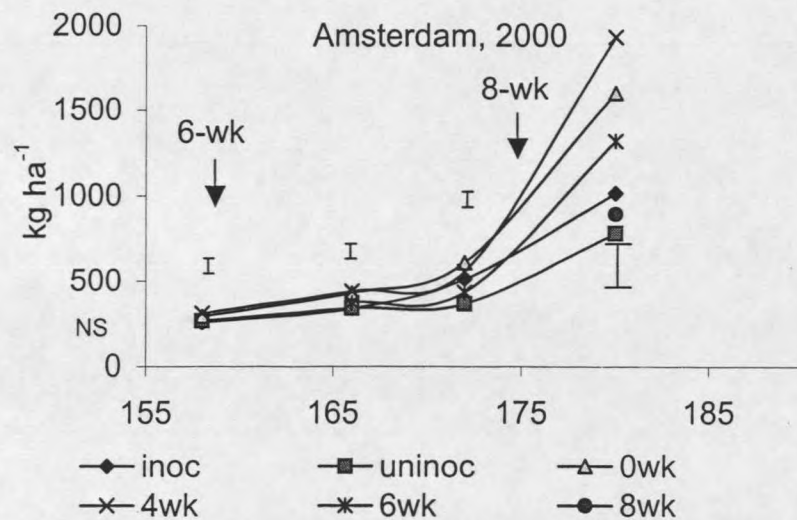
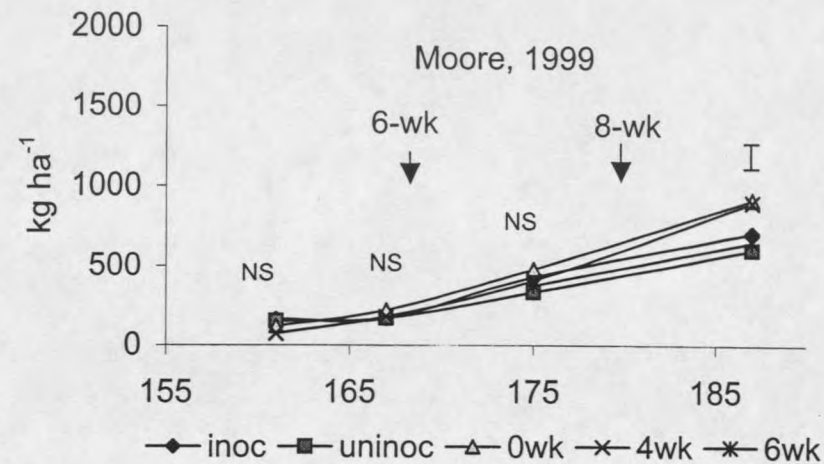
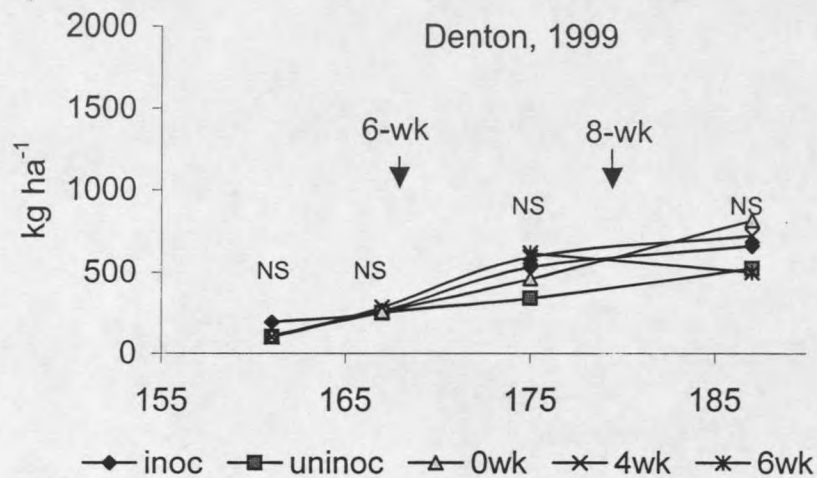


Figure 4. Shoot biomass accumulation from the 3 node stage to anthesis (Julian days) for chickpea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000. Fertilizer was applied at the beginning of each interval indicated. Least significant differences indicated by vertical bars on graph (P -value ≤ 0.05).

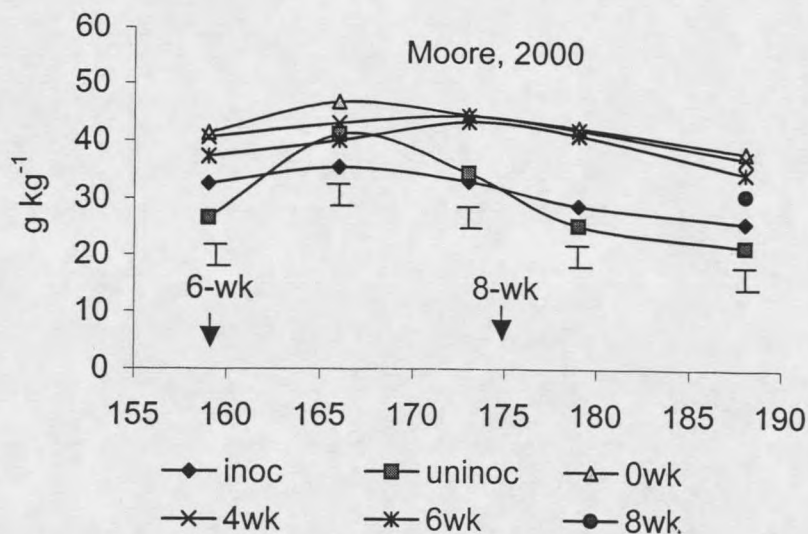
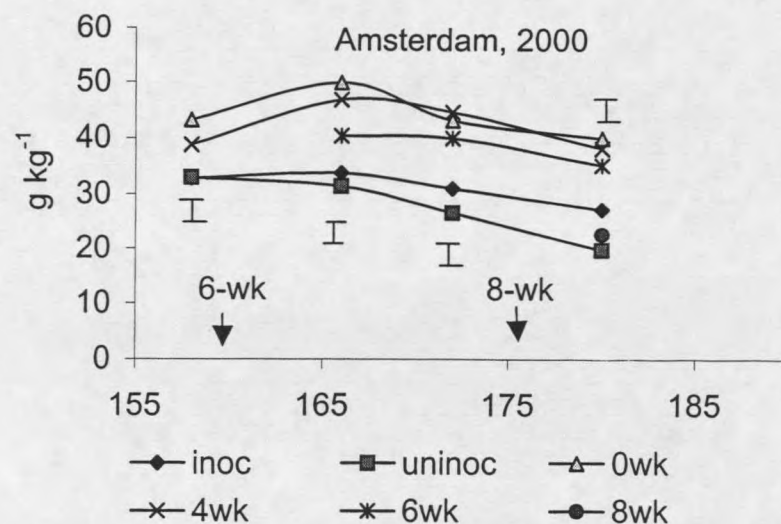
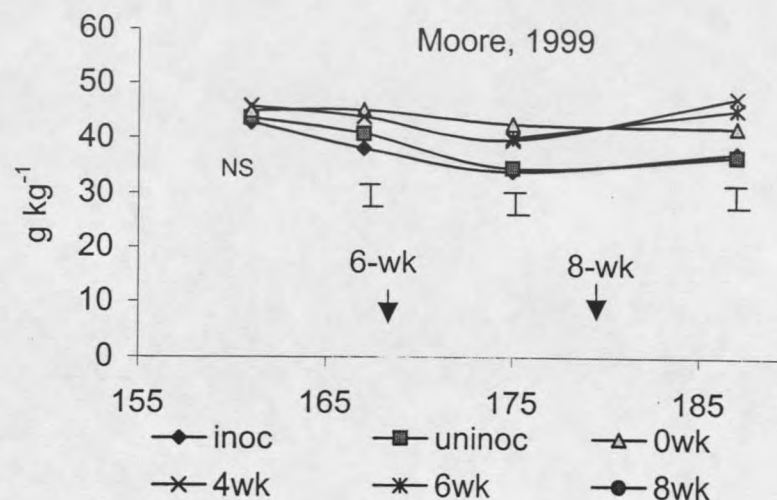
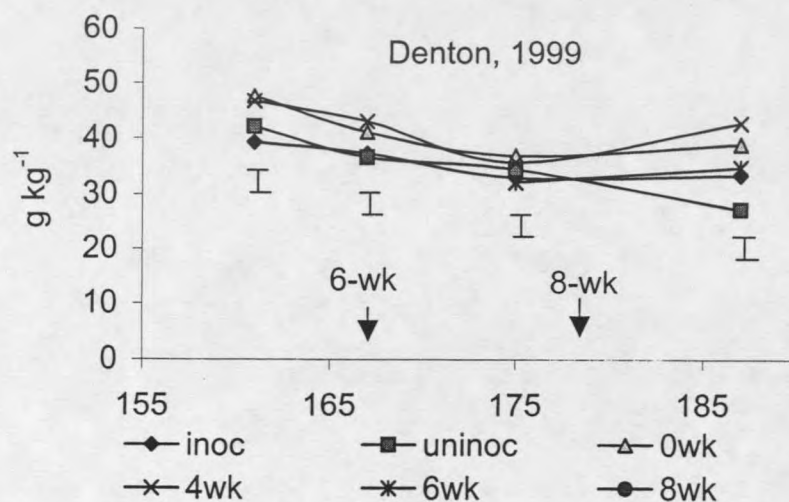


Figure 5. Shoot biomass N concentration from 3 node stage to anthesis for chickpea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000. Fertilizer was applied at the beginning of each interval indicated. Least significant differences indicated by verticals bars on graph (P -value ≤ 0.05).

with the 17 node stage. In 1999, this late fertilizer treatment was equal to the uninoculated control, while in 2000, this late fertilizer treatment was intermediate between the control treatments.

Table 4. Field pea seed yield, seed N concentration, and seed yield N at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

Treatment	Dent1999	Moor1999	Amst2000 ^z	Moor2000
<i>Seed yield (kg ha⁻¹)</i>				
Inoculated	960a	1930a	1430a	1450a
Uninoculated	420c	990c	1470a	710c
Fert N 0 wks	850a	1830a	1470a	1400a
Fert N 4 wks	860a	1870a	1430a	1550a
Fert N 6 wks	640b	1370b	1440a	1430a
Fert N 8 wks	290c	920c	1420a	1200b
Inoculated+fert	-	-	1380a	1410a
<i>Seed N concentration (g kg⁻¹)</i>				
Inoculated	40a	41a	44a	44a
Uninoculated	28d	25c	44a	26b
Fert N 0 wks	35bc	32b	43a	41a
Fert N 4 wks	37b	39a	43a	42a
Fert N 6 wks	34c	34b	45a	44a
Fert N 8 wks	29d	25c	46a	44a
Inoculated+fert	-	-	44a	43a
<i>Seed yield N (kg ha⁻¹)</i>				
Inoculated	38a	79a	64a	64a
Uninoculated	12d	26d	65a	20c
Fert N 0 wks	30b	59b	63a	58ab
Fert N 4 wks	32b	77a	62a	65a
Fert N 6 wks	21c	46c	65a	63a
Fert N 8 wks	8d	23d	65a	52b
Inoculated+fert	-	-	60a	64a

Means within a column followed by the same letter do not differ according to Protected LSD (P -value ≤ 0.05).

^z Amsterdam site was contaminated with native soil rhizobia.

The treatment response for seed N concentration differed from the yield response (Table 4). In 1999, all but one fertilizer treatment had lower seed N concentration values

than the inoculated control, while in 2000 at Moore seed N concentration values were equal to the inoculated control for all fertilizer treatments. Seed N yield (seed yield x N concentration) was examined to integrate the full N nutrition effect. In 1999, the 4-wk treatment caused the fullest N yield recovery, while in 2000 both the 4-wk and 6-wk treatments recovered plant N status fully (Table 4).

Table 5. Field pea shoot biomass, harvest index, and seed weight at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

Treatment	Dent1999	Moor1999	Amst2000 ^z	Moor2000
<i>Shoot biomass (kg ha⁻¹)</i>				
Inoculated	2860a	2990b	3760a	4050a
Uninoculated	1480b	1950c	4000a	3510a
Fert N 0 wks	2620a	3290a	4420a	3870a
Fert N 4 wks	2750a	3880a	4060a	3830a
Fert N 6 wks	2270a	3080b	4060a	4250a
Fert N 8 wks	1950b	2130c	3500a	2650b
Inoculated+fert	-	-	3790a	4100a
<i>Harvest index (seed yield/total shoot biomass)</i>				
Inoculated	0.25a	0.40a	0.28a	0.27b
Uninoculated	0.22a	0.37a	0.27a	0.25b
Fert N 0 wks	0.25a	0.36a	0.25a	0.28b
Fert N 4 wks	0.24a	0.34ab	0.24a	0.27b
Fert N 6 wks	0.22a	0.31b	0.26a	0.27b
Fert N 8 wks	0.14b	0.30b	0.29a	0.32a
Inoculated+fert	-	-	0.27a	0.26b
<i>Seed weight (mg seed⁻¹)</i>				
Fert N 0 wks	197c	214a	164a	152a
Fert N 4 wks	201bc	228a	162a	154a
Fert N 6 wks	204b	215a	166a	156a
Fert N 8 wks	215a	214a	154a	147a
Inoculated	203b	222a	160a	154a
Inoculated+fert	-	-	166a	155a
Uninoculated	216a	209a	162a	159a

Means within a column followed by the same letter do not differ according to Protected LSD (P -value ≤ 0.05).

^z Amsterdam site was contaminated with native soil rhizobia.

Harvest index values ranged from 0.14 to 0.40 with no consistent pattern observed in response to treatments (Table 5). Differences among treatments for seed weight occurred only at one site (Table 5).

Chickpea Chickpea seed yield was approximately 35% of field pea and yields did not differ between the inoculated and uninoculated control treatments at 3 of 4 site-years (Table 6).

Table 6. Chickpea seed yield, seed N concentration, and seed N yield at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

Treatment	Dent1999	Moor1999	Amst2000	Moor2000
<i>Seed yield (kg ha⁻¹)</i>				
Inoculated	380b	670a	510a	400a
Uninoculated	320bc	640a	510a	260bc
Fert N 0 wks	390b	620a	480a	210c
Fert N 4 wks	400ab	600a	500a	400a
Fert N 6 wks	460a	600a	490a	310abc
Fert N 8 wks	250c	470b	540a	380ab
Inoculated+fert	-	-	450a	330abc
<i>Seed N concentration (g kg⁻¹)</i>				
Inoculated	41a	41ab	46a	45ab
Uninoculated	31c	33d	33c	33c
Fert N 0 wks	39ab	40b	46a	47a
Fert N 4 wks	40a	43a	45a	44b
Fert N 6 wks	37b	41ab	44a	46ab
Fert N 8 wks	33c	36c	40b	46ab
Inoculated+fert	-	-	46a	46ab
<i>Seed N yield (kg ha⁻¹)</i>				
Inoculated	16a	27a	23a	18a
Uninoculated	10b	22ab	15b	12b
Fert N 0 wks	15a	25ab	22a	10b
Fert N 4 wks	16a	20b	21a	18a
Fert N 6 wks	17a	25ab	22a	14ab
Fert N 8 wks	9b	22ab	22a	14ab
Inoculated+fert	-	-	20a	12b

Means within a column followed by the same letter do not differ according to Protected LSD ($P \leq 0.05$).

Pod browsing by antelope (*Antilocapra americana*) reduced observed yields at Moore (visually estimated at 5-10% in 1999 and 45-50% in 2000), and hail injury affected yields at Denton (visually estimated at 25-30%).

Seed N concentration for the uninoculated control averaged 76% of the inoculated control. In 1999, N concentration was recovered equal to the inoculated control as late as the 4 to 6-wk treatment, for all but one location [1999, Denton].

Table 7. Chickpea shoot biomass, harvest index and seed weight at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

Treatment	Dent1999	Moor1999	Amst2000	Moor2000
<i>Shoot biomass (kg ha⁻¹)</i>				
Inoculated	1890a	3120a	2820b	3040a
Uninoculated	1460a	1920c	1740c	2440a
Fert N 0 wks	1870a	3340a	2570bc	3110a
Fert N 4 wks	2210a	3900a	3150ab	3730a
Fert N 6 wks	2090a	3280a	3130ab	3270a
Fert N 8 wks	1460a	2440b	2100c	3070a
Inoculated+fert	-	-	3610a	3270a
<i>Harvest index (seed yield /shoot biomass)</i>				
Inoculated	0.17a	0.18b	0.16bc	0.13a
Uninoculated	0.19a	0.25a	0.24a	0.12a
Fert N 0 wks	0.17a	0.14c	0.16bc	0.06a
Fert N 4 wks	0.16a	0.11c	0.14c	0.08a
Fert N 6 wks	0.18a	0.16bc	0.14c	0.09a
Fert N 8 wks	0.17a	0.20ab	0.21ab	0.11a
Inoculated+fert	-	-	0.11c	0.10a
<i>Seed weight (mg seed⁻¹)</i>				
Inoculated	407a	437ab	342bc	377a
Uninoculated	391a	429ab	347ab	367ab
Fert N 0 wks	392a	404c	323de	347c
Fert N 4 wks	396a	401c	316de	362abc
Fert N 6 wks	394a	417bc	329cd	358bc
Fert N 8 wks	391a	450a	363a	367ab
Inoculated+fert	-	-	312e	364ab

Means within a column followed by the same letter do not differ according to Protected LSD (P -value ≤ 0.05).

Seed N yield (seed yield x N concentration) integrated the overall treatment effects (Table 6), but due to the wildlife grazing seed N concentration was considered the best indicator for describing the response period for remedial N application in chickpea.

Harvest index values did not differ among treatments at two of the four sites, likely due to interference from hail damage and severe antelope predation at those sites. Where they differed, the uninoculated control and the 8-wk fertilizer treatment had the highest values, and the 0, 4 and 6-wk fertilized treatments had the lowest values with intermediate values for the inoculated control (Table 7). Seed size differed among treatments at three sites, though response patterns were difficult to interpret (Table 7). Generally, early fertilizer treatments had smaller seed size, as compared to control treatments and the 8-wk treatment.

Remote Sensing N₂ Fixation

Reflectance models Logistic regression analyses of reflectance were performed separately for each crop type. The best model determined for field pea was: $\text{logit}(\pi) = -4.12 + 0.43(560\text{nm}) + 0.75(613\text{nm}) - 2.89(660\text{nm}) + 3.83(706\text{nm}) - 1.70(830\text{nm})$

The best model determined for chickpea was:

$\text{logit}(\pi) = 2.69 - 0.58(680\text{nm}) + 1.06(706\text{nm}) - 0.37(760\text{nm}) + 0.92(813\text{nm}) - 1.07(830\text{nm})$. Both reflectance models were significant ($P\text{-value} \leq 0.05$) according to the drop-in-deviance test (Neter et al., 1996). The models differ by the absence of a green band (500 - 600 nm) in the chickpea model, and different bands within the red (600 - 700

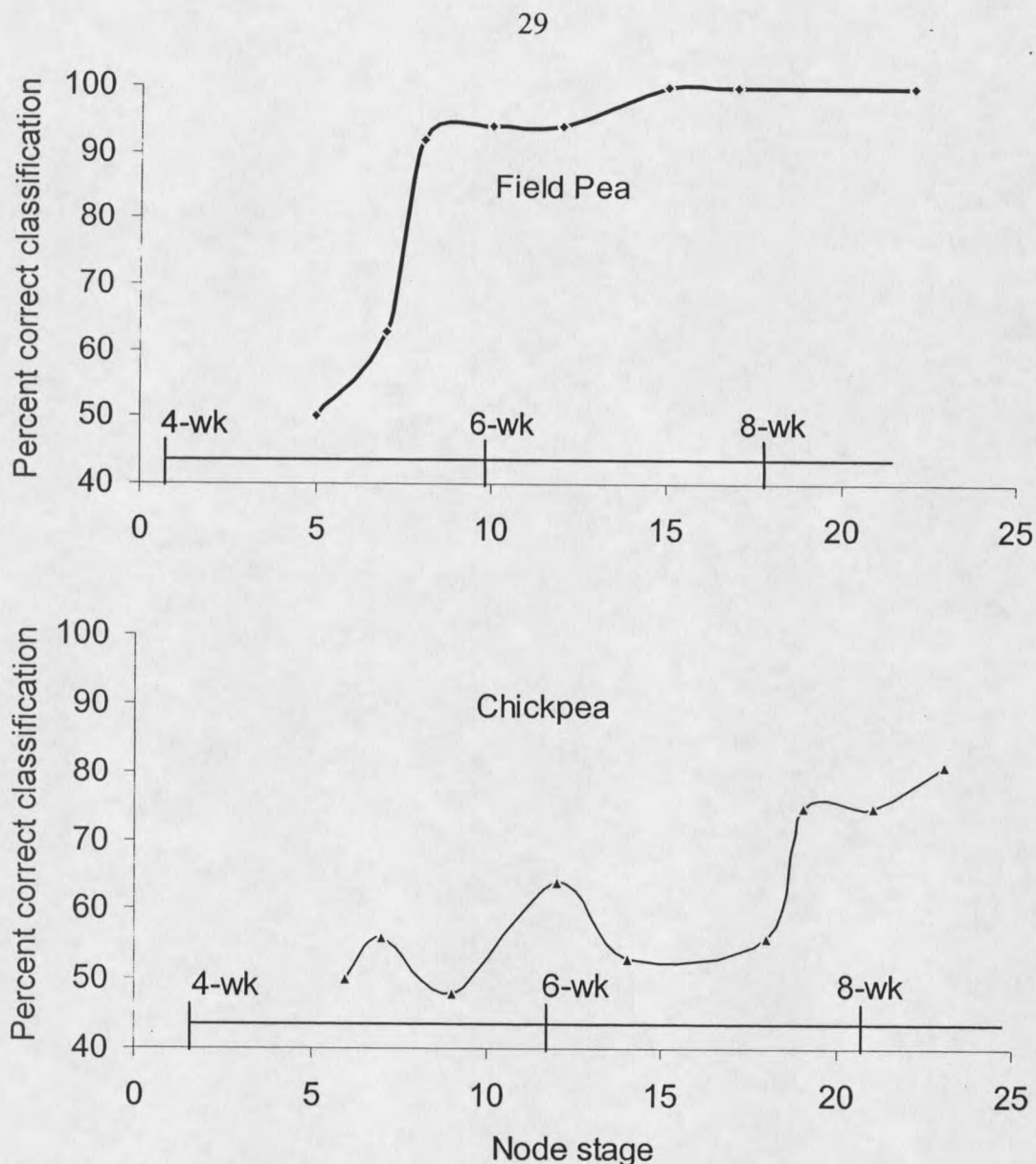


Figure 6. Logistic model accuracy for field pea and chickpea with increasing growth stage

nm) and near infrared (800 - 900 nm). The models also varied in classification accuracy by growth stage (Figure 6). The field pea model exceeded 90% accuracy by the 6th node stage (5 wk) while the chickpea model was inconsistent until anthesis. The field pea model had an overall accuracy of 84% for classifying a crop canopy as nodulated (actively fixing N₂, inoculated) or unnodulated (no N₂ fixation, uninoculated) (Table 8).

Table 8. Error matrix for the field pea and chickpea logistic regression models.

Field pea

Classified Data		Reference Data	
	Inoculated	Uninoculated	Row total
Inoculated	59	8	67
Uninoculated	16	67	83
Column Total	75	75	150
<u>Producer's accuracy</u>		<u>User's accuracy</u>	
Inoculated = 59/75 = 79%		Inoculated = 59/67 = 88%	
Uninoculated = 67/75 = 89%		Uninoculated = 67/83 = 81%	
Overall accuracy = (59+67)/150 = 84%			
<u>Khat = (150 (59+67) - (67*75)+(83*75))/(150)²-(67*75)+(83*75)) = 0.68</u>			

Chickpea

Classified Data		Reference Data	
	Inoculated	Uninoculated	Row total
Inoculated	50	31	81
Uninoculated	41	60	101
Column Total	91	91	182
<u>Producer's accuracy</u>		<u>User's accuracy</u>	
Inoculated = 50/91= 55%		Inoculated = 50/81 = 62%	
Uninoculated = 60/91 = 91%		Uninoculated = 60/101 = 59%	
Overall accuracy = (50+60)/182 = 60%			
<u>Khat = (182(50+60) - (91*81)+(91*101))/(182)² - (91*81)+(101*91)= 0.21)</u>			

For the inoculated class the field pea model had a producer's accuracy of 79% and a user's accuracy of 88%. The chickpea model had an overall accuracy of 60% and functioned differently from field pea as accuracy did not rapidly increase with node stage. Khat values showed the field pea model was 68% better at classifying nodulated versus unnodulated field pea than random assignment, while the chickpea model was only 21% better at classification than random assignment (Table 8).

SPAD meter

SPAD values in the logistic models were excluded due to insignificance during the model-building process. Models derived using only SPAD values were insignificant (P -value > 0.05).

Discussion

What was the potential period for yield recovery by fertilizer N application for simulated nodulation failure of dry pea and chickpea?

Shoot Biomass and Nitrogen Concentration

Field pea The sites were characterized by terminal drought, which had a detrimental effect on seed yield and harvest index. Field pea accumulated biomass similarly for all treatments until 6 wk post-seeding (10-12 node stage), suggesting N did not limit plant growth until after this point (Figure 2). It was concluded that N deficiency caused differences among treatments for biomass (P -value ≤ 0.01), since the uninoculated and inoculated controls differed markedly. Shoot N concentration were more sensitive than biomass accumulation to the applied treatments and is likely a better indicator of plant N status (Figure 3). The high mature shoot biomass for the inoculated control in 1999 means that the applied N rates limited yield, even under these moderate yield conditions. The higher N fertilizer rates in 2000 ensured that yield was not N-limited under the conditions of this study, and highlights the need for high remedial N

fertilizer rates even under modest yield potential.

Chickpea Chickpea was less responsive than field pea because inherently lower biomass accumulation required less N for sustained growth and N scavenging by an extensive rooting (Singh et al., 1989) likely lessened N deficiency. Since early fertilizer treatments were equal to or higher than the inoculated control for biomass accumulation (Figure 4) this might be attributed to the 'carbon cost' of N_2 fixation. Part of the photosynthate produced might have been used to sustain nodules in the inoculated treatment, while the fertilized treatments had a ready supply of N to use as moisture conditions allowed. This supported the hypothesis that legumes are more productive when grown on applied nitrogen (Allos and Bartholomew, 1955; Harper, 1974). Some researchers attribute the increased production to the energy cost of fixation (Ryle et al., 1978), while others contend that the applied N (at seeding) overcomes nitrogen stress that occurs as the seed nitrogen reserves are depleted and before fixation is sufficient to meet the demands of the growing plant (Pate and Dart, 1961; Hoglund 1973). Though severe N deficiency conditions were prevented by residual soil N levels for each site (14 and 27 kg N ha⁻¹), and 5 kg N ha⁻¹ applied as ammonium phosphate. *Rhizobia ciceri* are known to grow more slowly than *Rhizobia leguminosarum* (V.A.Biederbeck, 2001, personal communication). Slow development of the N_2 fixation symbiosis might have delayed plant growth until growing season conditions were less favorable for biomass accumulation.

Intermittent and sparse precipitation lessen the probability that late fertilizer treatments successfully leach into the soil profile for plant uptake. Examination of the N

concentrations of the biomass determined that the fertilizer treatments, up to the 6-wk treatment, had leached into the soil profile, available for plant uptake by the time of anthesis (Figure 5).

Effects of applied N on seed yield depended on the time from uptake to maturity and available soil moisture. This agrees, in part, with Gault et al. (1984), who was unable to recover soybean yields of uninoculated soybean with late fertilizer applications, as compared to successful recovery with earlier applications. Remedial fertilizer applications were successful up to 60 d (8.5 wk) after seeding (Gault et al., 1984). An earlier time period for yield recovery would be expected when considering chickpea, which is earlier maturing than soybean.

Yield

Field Pea Variable growing conditions caused differences among inoculated control treatments in 1999 and 2000. In 2000, rainfall was higher at Moore than in 1999 (Figure 1). Available spring moisture was also likely higher in 2000, due to increased snow catch in standing stubble [Moore, 2000] compared to tilled soil [Denton and Moore, 1999]. Further, seeding at Moore in 2000 occurred nearly 1-wk earlier than in 1999, which is known to influence pea yield strongly (Miller et al., 1998).

Uninoculated controls yielded 50% less than the inoculated control, a large decrease in light of the moderate pea yields achieved in this study (Table 4). This highlighted the need for producers to have the agronomic information to recover yield in the case of an inoculant failure. Full yield recovery was achieved with remedial N

fertilizer applications 4 to 6 wk after seeding, corresponding with the 0 to 11 node stage. The different response between 1999 and 2000 suggests that very high N applications are required to achieve full recovery of seed N yield (Table 4). It is unknown if this same recovery period would exist at high yield potentials, where N might be expected to limit plant growth at an earlier stage.

Flowering and seed set in field pea is induced by day-length and light intensity (Leopold and Kriedemann, 1975), considered a static stimulant for each treatment, regardless of node stage or biomass accumulation. Attainable yields in late fertilizer treatments, therefore, would be limited by fewer flower-pod sites caused from N-limited growth prior to fertilizer application. Even though favorable conditions during seed set encourage N uptake of late fertilizer application (8-wk+), yields would not be appreciably affected because of the static time period of flowering and limited seed-sites caused by limited growth prior to the N application.

The treatment response for seed N concentration differed from the yield response (Table 4). In 1999, fertilized treatments generally did not attain seed N concentration equal to the inoculated control. This might have been caused by inefficient use of broadcast N. In 2000 at the rhizobia-responsive site, seed N concentrations were equal to the inoculated control for all fertilizer timings, showing a recovery period for seed protein that was 2 wk greater than that for seed yield. In 2000, greater biomass was produced than in 1999 (Table 5), though during seed set dry conditions limited pod fill and likely increased pod abortion (Sionit and Kramer, 1977). The larger biomass production in 2000 was not sustainable based on the precipitation received, and as a result, pod abortion

and reduced yields occurred. Since legumes rely on N stored within the plant structure to translocate during seed fill (Egli et al., 1978), the increased biomass accumulation resulted in high N concentration in the seed in 2000.

Seed N yield (seed yield x N concentration) was examined to integrate the full N nutrition effect. The seed N yield can also be considered an indicator of fertilizer use efficiency or an estimate of the amount of applied N that was translated into seed yield. Subtraction of the seed N yield from the uninoculated treatment (soil available N) yielded the amount of N from fertilizer or fixation incorporated into seed yield. In 1999, the 4-wk treatment caused the fullest N yield recovery, while in 2000 both the 0, 4 and 6-wk treatments recovered plant N status equal to the inoculated control (Table 4). The window for yield recovery indicated by seed N yields agreed with that for yield with 0-wk to 6-wk application for partial-to-full seed yield recovery.

Harvest index values ranged from 0.14 to 0.40 with no consistent pattern observed in response to treatments (Table 5). These values were low compared to typical values (0.35-0.45) for peas grown in trials in the Canadian prairies (Miller et al., 1998). Low harvest index values are indicative that "haying off" occurred where drought stress limited seed production compared to vegetative biomass production, which occurred in 2000 in this study. "Haying off" is a condition where abundant shoot growth has occurred up to seed set, and then seed yield is reduced with the onset of drought stress, resulting in low harvest index values (Lehane and Staple, 1962). Differences among treatments (P -value ≤ 0.05) for seed weight occurred only at Denton and were unremarkable (Table 5).

Chickpea The low yield of chickpea relative to field pea was due to the longer maturity of chickpea, which was more affected by drought stress than early-maturing field pea (Ludow and Muchow, 1990). The lack of treatment differences between controls was unexpected but might have been due to a low yield potential for chickpea, which diminished the necessity for remedial N fertilizer applications. In addition to drought stress, pod browsing by antelope at Moore [2000] and hail injury (at Denton) decreased yields. As a result, seed N concentration was considered a more reliable indicator of the recovery period (Table 6). Using N concentration as the indicator, the fertilizer application window for yield recovery existed from 0 to 6 wk after seeding 90 to 13 node stage). Chickpea requires an additional 10 to 20 d to reach maturity compared to field pea (Anonymous, 2000). Thus, it is expected that the remedial N fertilizer application window for chickpea could be later than field pea.

During seed set, legumes translocate the N contained in plant tissues for seed development. This translocation can cause earlier maturing, abscission of leaves and senescence of nodules and roots due to the high demand of N during a short time period (Egli et al., 1978). Early maturity was observed in the uninoculated control and the 8-wk treatment.

Seed N yield integrated the overall treatment effects (Table 6), but due to the wildlife grazing effects on the yield portion of the parameter, seed N concentration was likely the best indicator for defining the window of opportunity for remedial N application in chickpea. Based on these results, it is recommended that remedial fertilizer treatments can be applied up to 6 weeks after planting.

Seed size is an especially important consideration with Kabuli chickpea production as premiums are offered for large seed size. Seed size differed among treatments at 3 sites, though response patterns were inconsistent (Table 7). Generally, early fertilizer treatments had smaller seed size, as compared to control treatments and the 8-wk treatment. Seed size in grain legumes is reduced by drought stress during the time period from seed formation to maturity (Wery et al., 1993). The later fertilized treatments, and control (inoculated, uninoculated) treatments had growth rates that were limited by N availability, whereas the early fertilized treatments had rapid growth rates, due to abundant N and moist conditions early in the growing season. The early fertilizer treatments likely exhausted soil moisture reserves sooner, enhancing the effects of terminal drought, resulting in smaller seeds. Fertilizing chickpea prior to flowering was addressed previously with irrigated chickpea. The effect of applied N on a chickpea stand prior to flowering was examined, and it was concluded that seed yield and seed size were not affected by N applied prior to flowering due to the indeterminant growth pattern of the chickpea (Bonfil and Pinthus, 1995). The chickpea continued vegetative growth in response to the applied N and reduced photosynthate allocation to seed development. The vegetative structures of the plant out-competed the seed pods as a carbon sink, and resulted in reduced yield and small seed size.

Very low harvest index values indicated poor translation of shoot biomass to final seed yield (Table 7). Harvest index values for chickpea typically occur in the range of 0.35-0.50 (Brown, et al., 1989; Miller et al., 1998). Drought stress during seed set resulted in pod abortion and reduced yield potential (Sionit et al., 1977). Other grain

legumes, such as field pea and soybean, are most sensitive to drought stress during flowering and early pod filling (Hiler et al., 1972; Sionit et al., 1977).

Remote Sensing N₂ Fixation

Can nodulation failure be detected by spectral reflectance?

Reflectance models These models differed from Humburg and Stange (1999), who devised a model for sugar content estimation in sugarbeet containing only three bands (500, 550 and 830 nm), excluding any red band. The inoculated plant stands were considered 'healthy' and reflected near infrared light, absorbing blue, green, and red portions of the spectrum during photosynthesis. The uninoculated treatment for both crops reflected higher quantities of each band, except near infrared. The uninoculated treatments absorbed less radiation due to decreased photosynthetic activity and chlorophyll content. Red light is absorbed less in nutrient deficient plants, resulting in a yellowish spectral signature. Near infrared radiation is highly reflected by healthy plants and is affected by internal structures within the plant leaf and water content (Thomas and Oerther, 1972; Lillesand and Kiefer, 1994). Near infrared wavelengths have been used previously to indicate plant stress and were important bands in the models derived in this project. Thomas and Oerther (1972) used regression to estimate N content in sweet pepper using only the green band (500-600 nm). Though different parameters and crops were studied, plant physiological responses to stress are similar, thus, visible reflectance responses to stress are generally similar (Carter, 1993). The constituent bands of the models derived in this study support the hypothesis that leaf spectral reflectance is most

likely to indicate plant stress in the sensitive 500-600 nm, 600-700 nm, and NIR wavelength ranges (Carter, 1993).

The field pea model had a producer's accuracy of 79% for nodulated and more importantly a user's accuracy of 88% for nodulated stands. The user's accuracy is more important because a misclassification of inoculated instead of uninoculated would cause more problems than classifying an area as uninoculated rather than inoculated. The former case would recommend fertilizer application where it otherwise would not require it, while the latter case would not recommend a fertilizer application where it is required.

The chickpea model had a producer accuracy of 55% and a user's accuracy of 62% for nodulated plant stands. Thus, the model would not be useful due to limited accuracy. The difference in models was due to physiological differences between crop canopies. The two crops produce different amounts and rates of biomass accumulation, and as a result, would have different magnitudes of spectral reflectances between control treatments. Biomass accumulation rates influence the total reflectance signal emitted from the canopy / soil surface and different shoot biomass amounts would have different N requirements.

The field pea model had a khat value of 0.68, while the chickpea model had a khat value of 0.21, indicating the field pea model was 68% better at classifying nodulated versus unnodulated field pea than random assignment, while the chickpea model was only 21% better at classification than random assignment.

If a producer is aware of an inoculant-related problem during the planting operation, a remedial fertilizer application decision could be made without the need for

the reflectance models. The exact location that a problem occurred, however, is seldom determined accurately. In this instance, the reflectance models would aid in delineating the area where the inoculant product failed, providing an efficient use of remedial N fertilizer for recovery of crop growth.

Can spectral reflectance detect nodulation failure early enough to permit yield recovery by remedial N fertilizer application?

Figure 6 displayed the model's accuracy increased with increasing node stage, and was highly accurate at a sufficiently early growth period to influence a management decision for field pea. For example, the 6-node stage corresponded to the end of the 4-wk fertilizer treatment interval, indicating that the model could be used to monitor a field pea crop for N_2 fixation failure with accuracy, and sufficiently early to apply remedial N fertilizer. From the agronomic analyses, a successful window for fertilizer application existed up to the 4-wk treatment, with possible recovery up to the 6-wk treatment. Nitrogen fixation is a process that requires four weeks to become established, therefore, the model had a window of up to one week to successfully diagnose an inoculant failure and influence a management decision.

SPAD meter utility

The SPAD meter measured reflectance in the green band (500nm - 600 nm), commonly used for chlorophyll content estimation and indirect N concentration estimation. The field pea model contained one green band, while the chickpea model did not. SPAD measurements during model building for control treatment differentiation

were discarded due to insignificance, and models evaluated containing only SPAD values were also insignificant. Readings from the SPAD meter device were not found to be diagnostic for inoculant failure or success. Since the SPAD reading is taken from a single leaf sample, it can vary depending on the leaf position and portion of the leaf from which the sample is taken. The SPAD does not consider overall canopy reflectance.

Conclusions

Full yield recovery of field pea occurred with applications of fertilizer N 0 and 4 wk after seeding (0 to 5 node stage). While partial to full yield recovery occurred with N fertilizer applications 6 wk after seeding (9 to 12 node stage). Seed size in field pea was not affected by fertilizer timing, though seed N content was affected for a time period which extended beyond that for yield recovery. Chickpea seed N concentrations revealed an application window of 0 to 6 wk after seeding (0 to 13 node stage), resulting in seed N status equal to an inoculated, N_2 fixing crop.

Significant logistic regression models were derived for two pulse crops from reflectance values for inoculated and uninoculated control treatments. The model for field pea had a high degree of accuracy for classification between the two controls, and was capable of assisting a management decision in a timely manner. The model for chickpea was incapable of differentiating between inoculated and uninoculated control treatments to influence a management decision. For field pea the reflectance model identified nodulation failure sufficiently early to effectively apply remedial N fertilizer.

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Appendix A

REFLECTANCE RESPONSES AND CLIMATE DATA

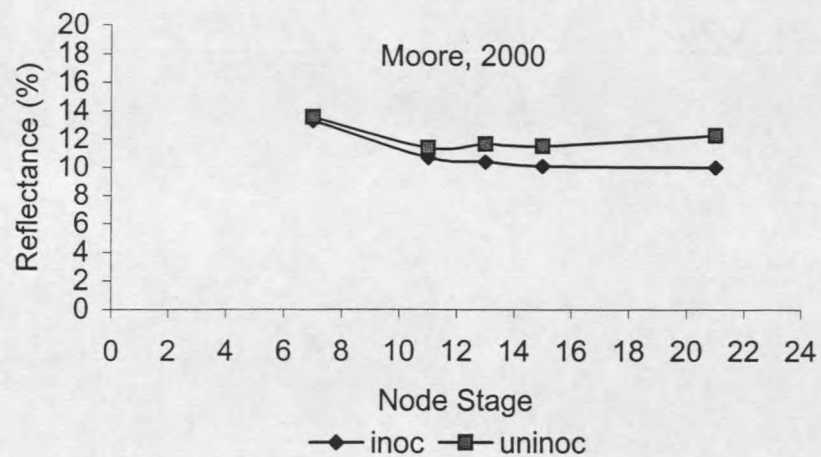
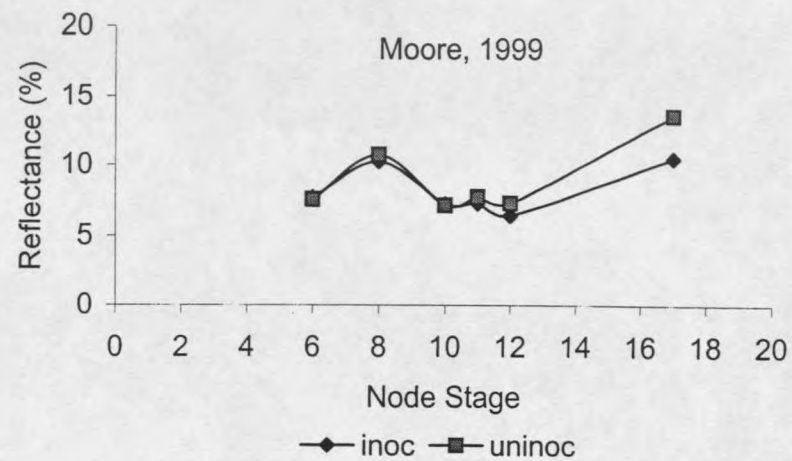
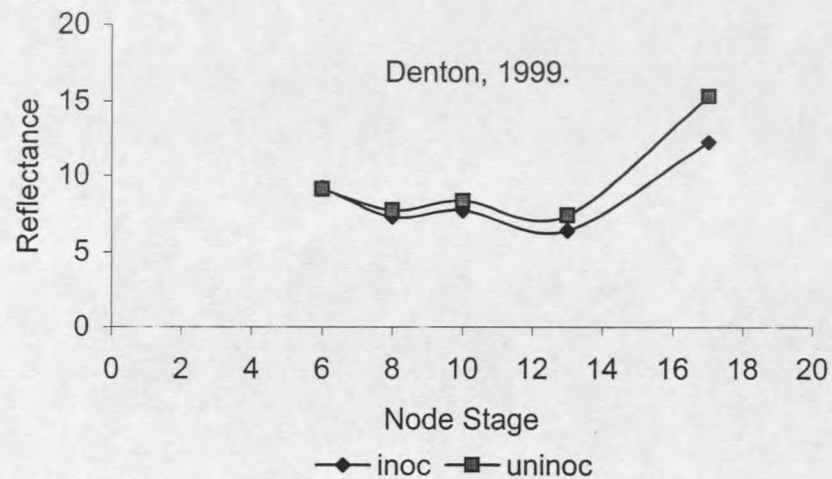


Figure 7. Reflectance in percent for the 560 nm band from seedling (6,7-node stage) to anthesis for field pea at Denton and Moore, 1999, and Moore, MT, 2000.

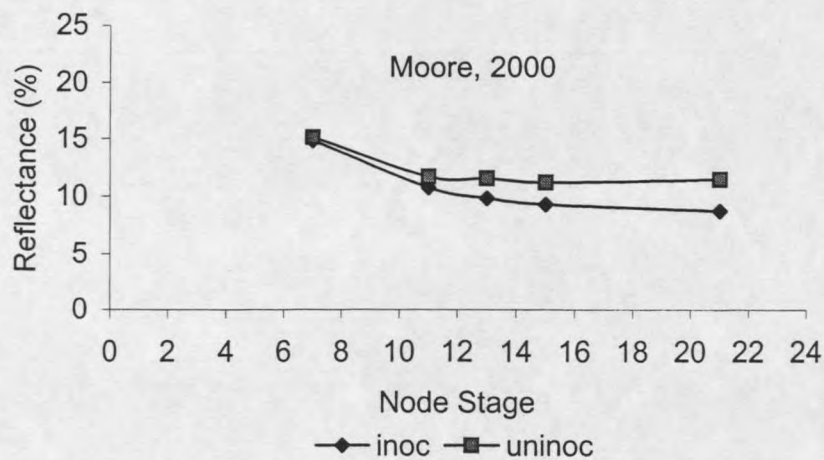
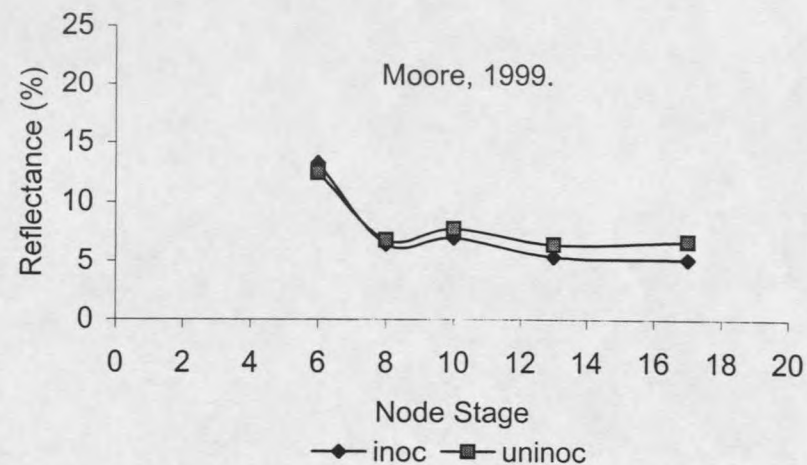
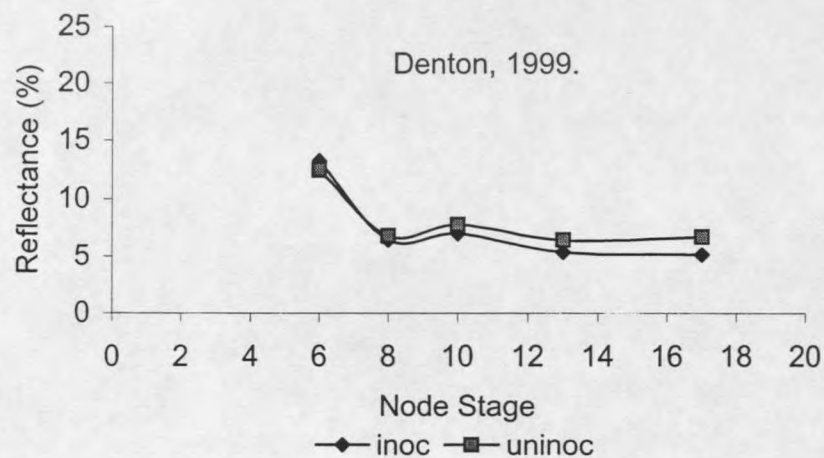


Figure 8. Reflectance in percent for the 613 nm band from seedling (6,7-node stage) to anthesis for field pea at Denton and Moore, 1999, and Moore, MT, 2000.

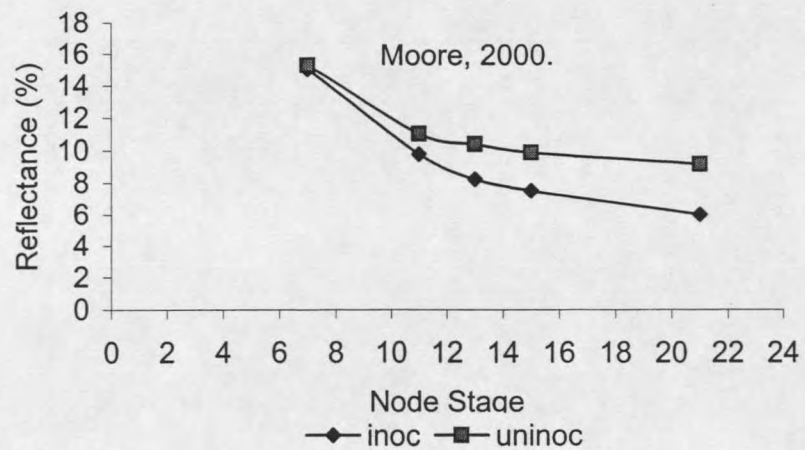
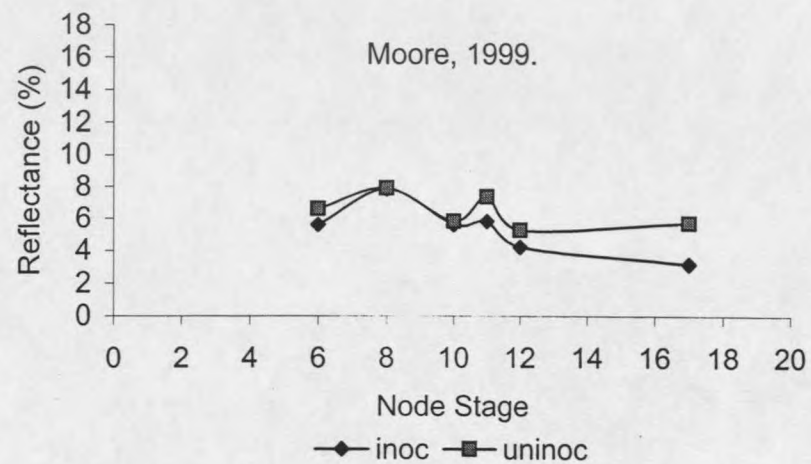
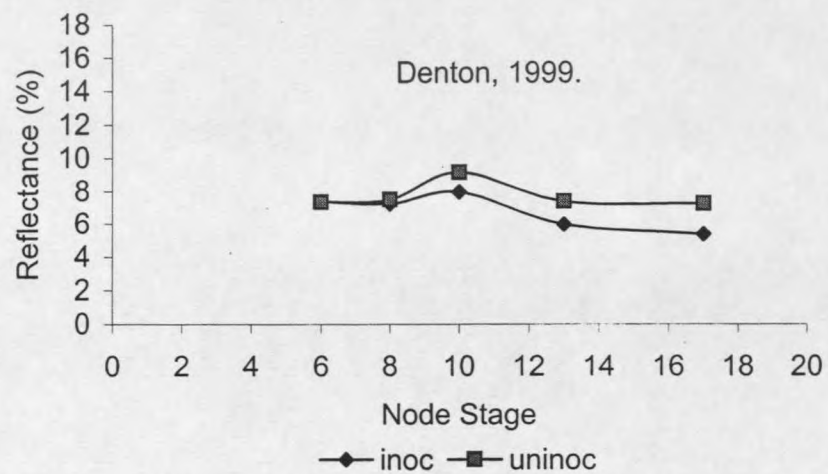


Figure 9. Reflectance in percent for the 660 nm band from seedling (6,7-node stage) to anthesis for field pea at Denton and Moore, 1999, and Moore, MT, 2000.

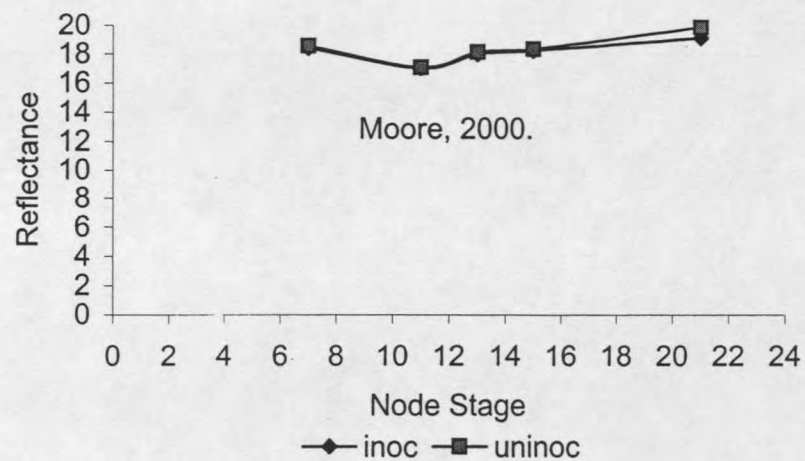
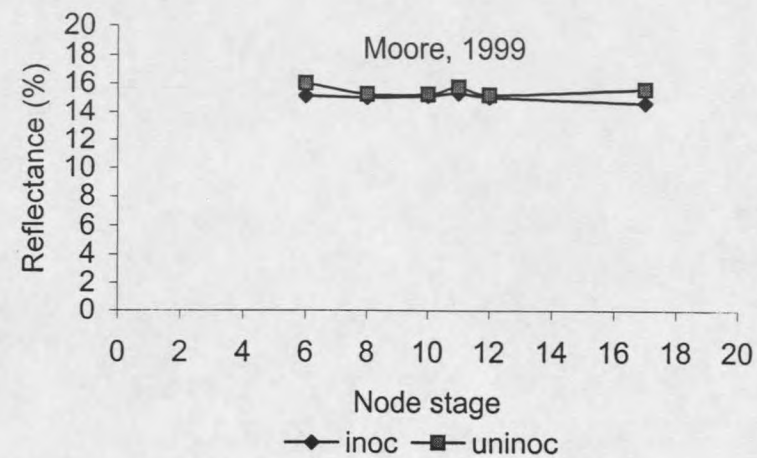
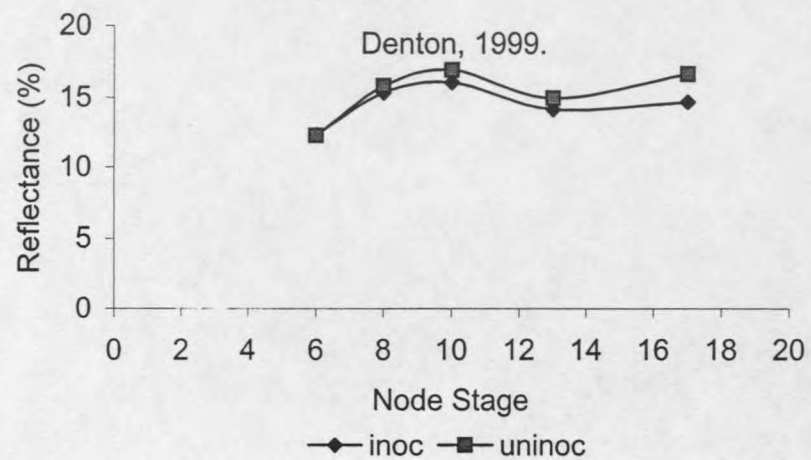


Figure 10. Reflectance in percent for the 706 nm band from seedling (6,7-node stage) to anthesis for field pea at Denton and Moore, 1999, and Moore, MT, 2000.

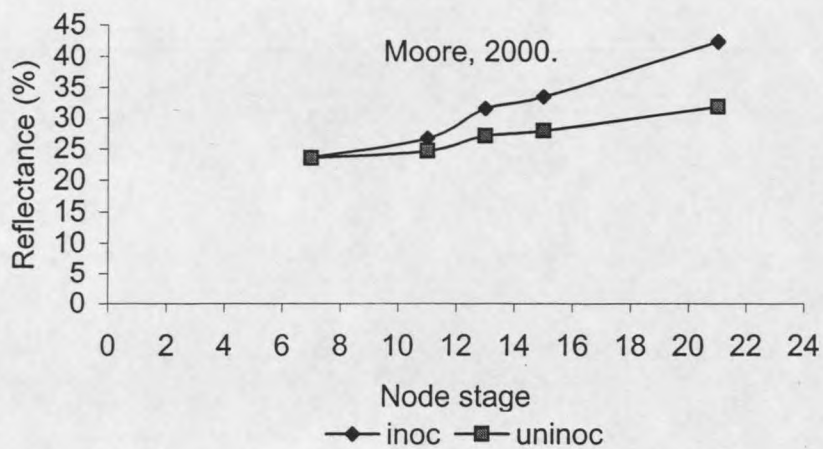
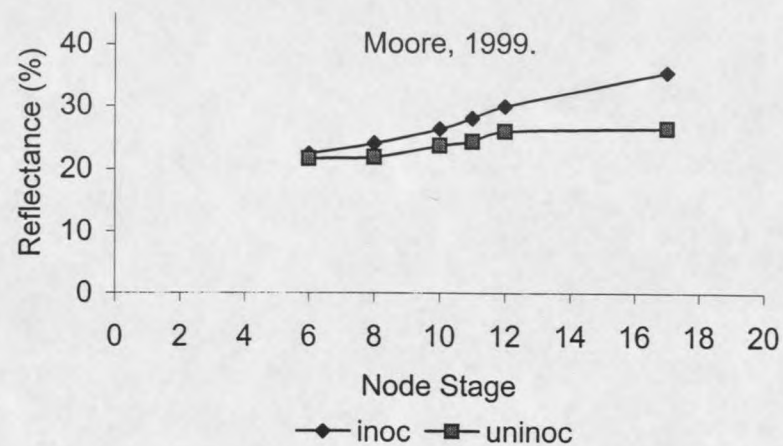
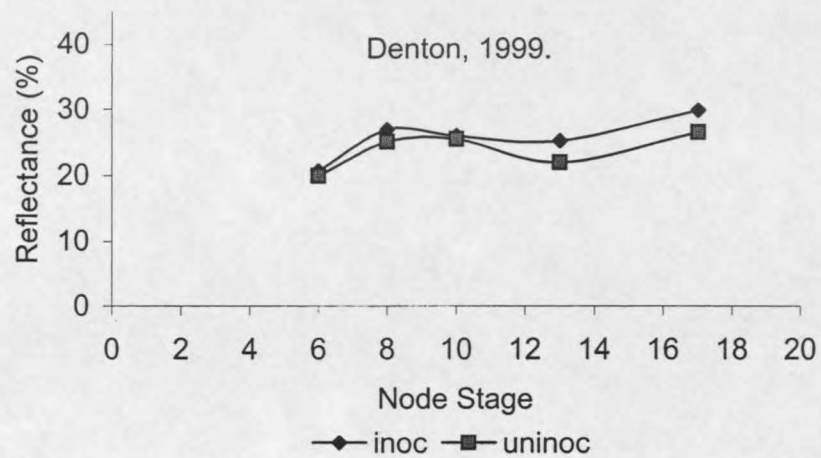


Figure 11. Reflectance in percent for the 830 nm band from seedling (6,7-node stage) to anthesis for field pea at Denton and Moore, 1999, and Moore, MT, 2000.

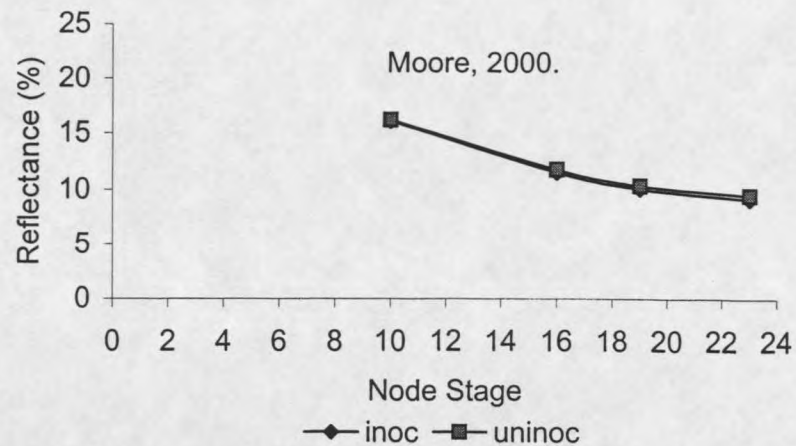
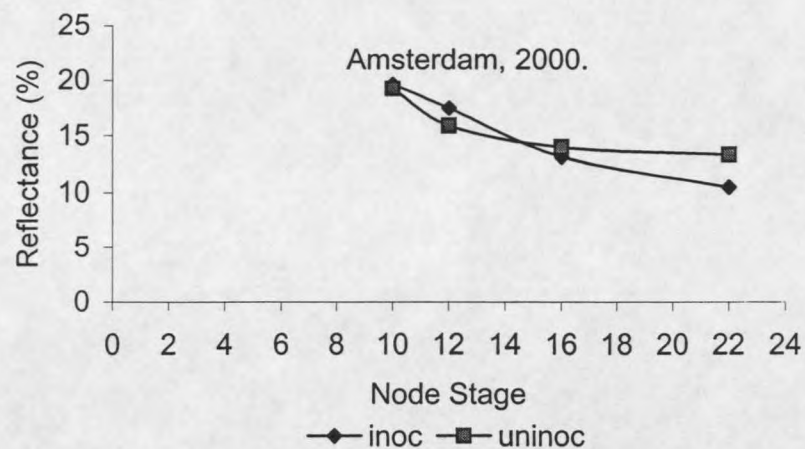
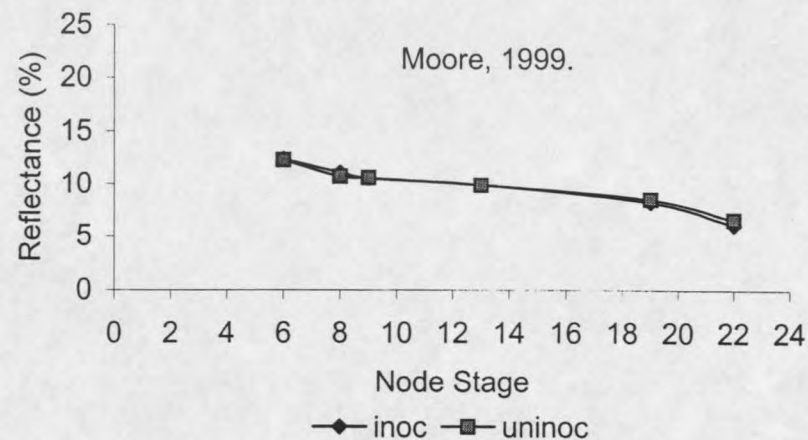
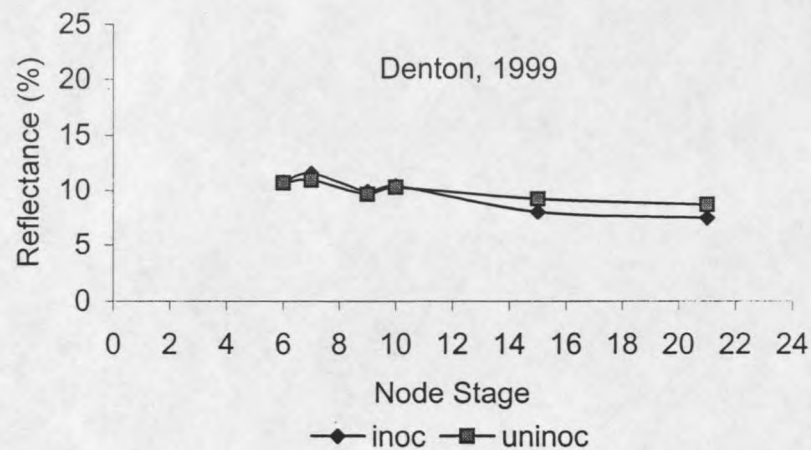


Figure 12. Reflectance in percent for the 680 nm band from seedling (8,9-node stage) to anthesis for chickpea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

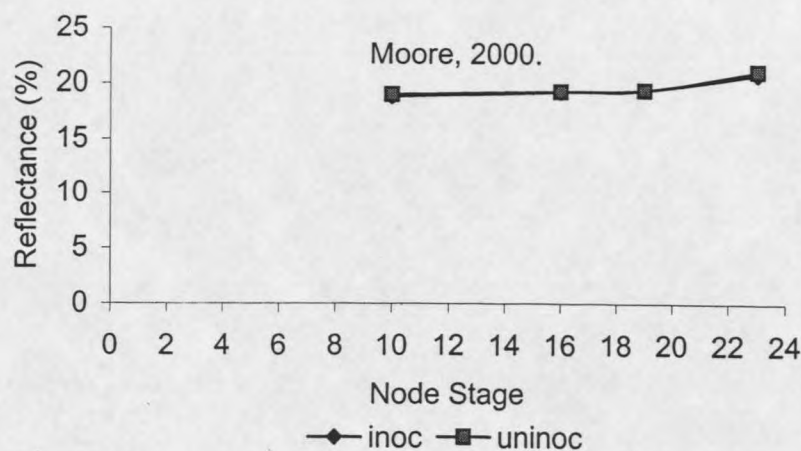
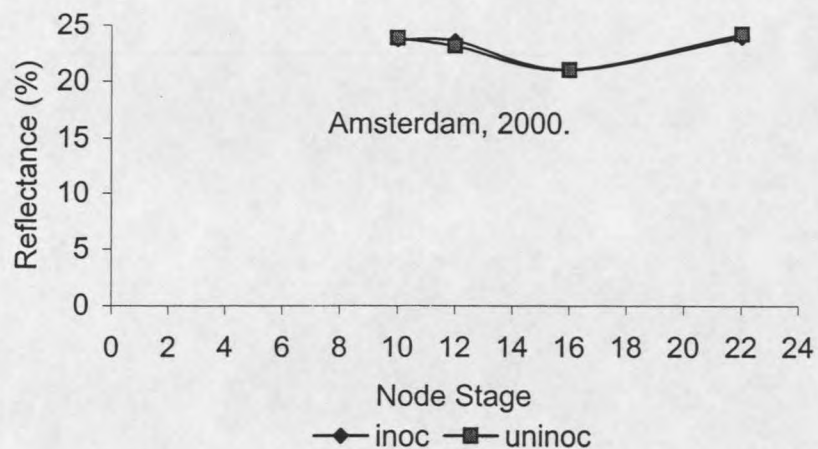
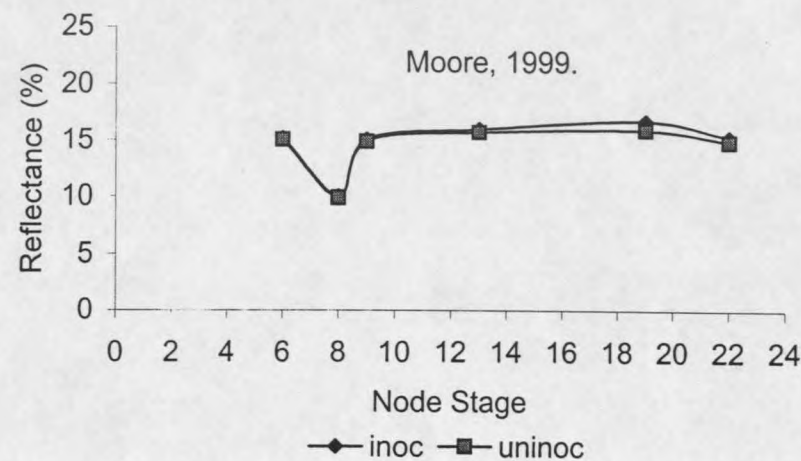
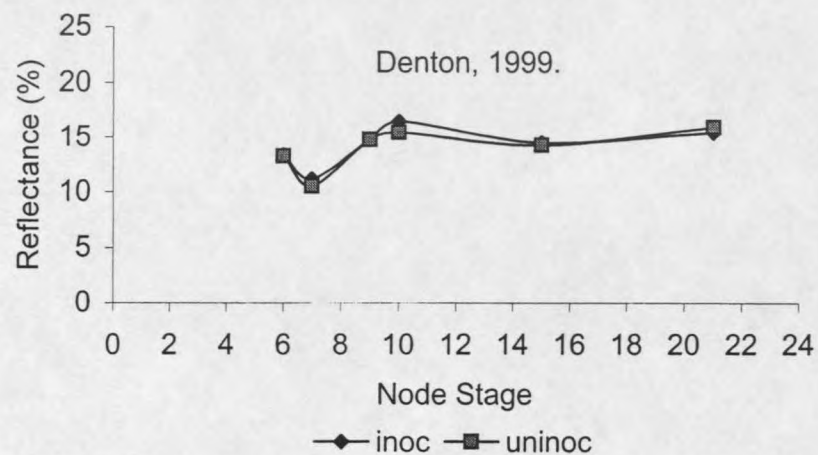


Figure 13. Reflectance in percent for the 706 nm band from seedling (8,9-node stage) to anthesis for chickpea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

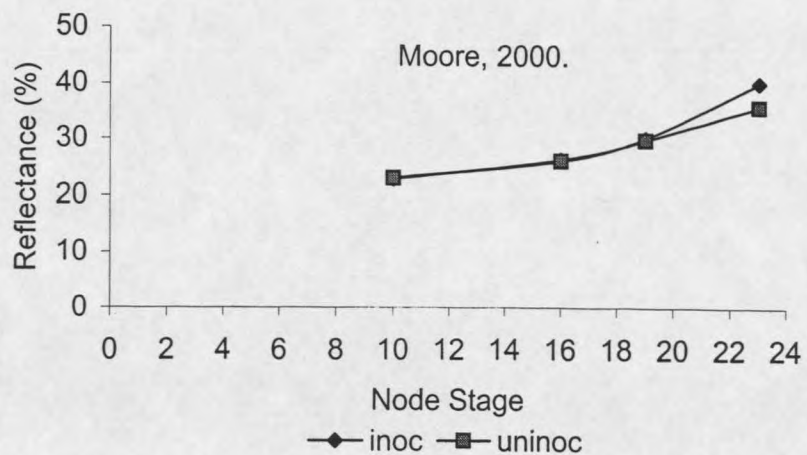
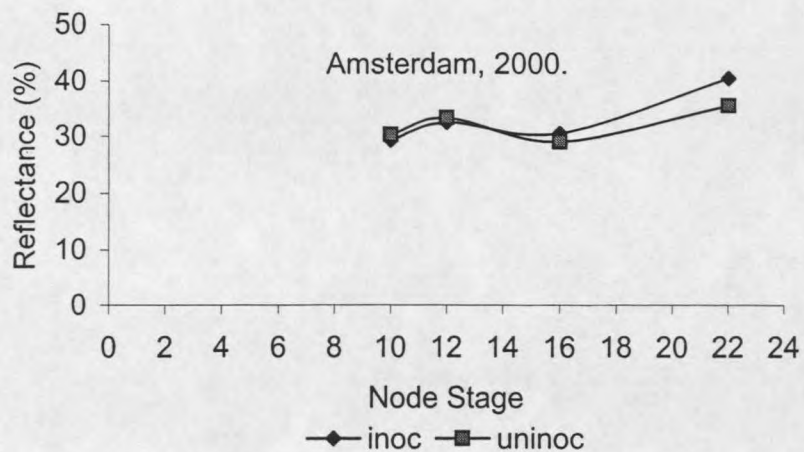
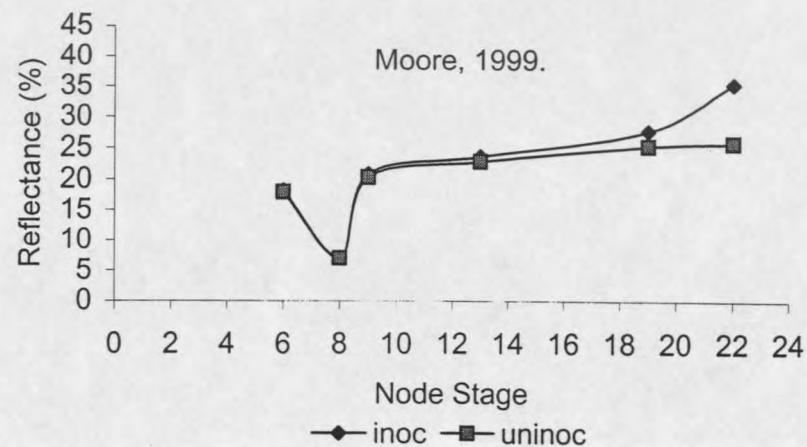
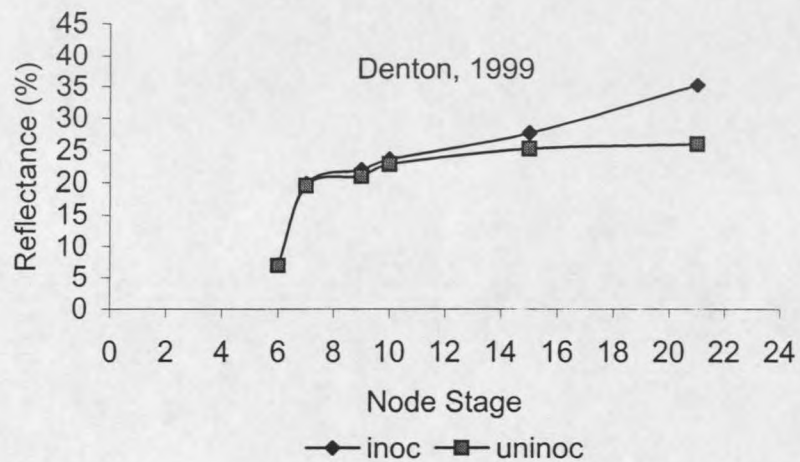


Figure 14. Reflectance in percent for the 760 nm band from seedling (8,9-node stage) to anthesis for chickpea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

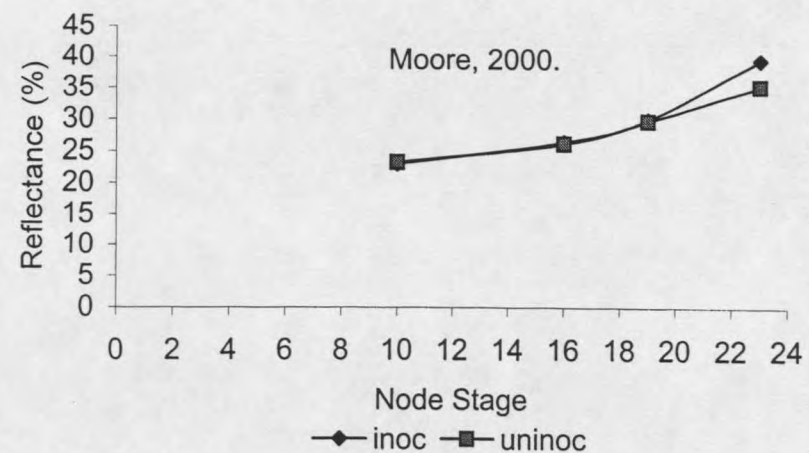
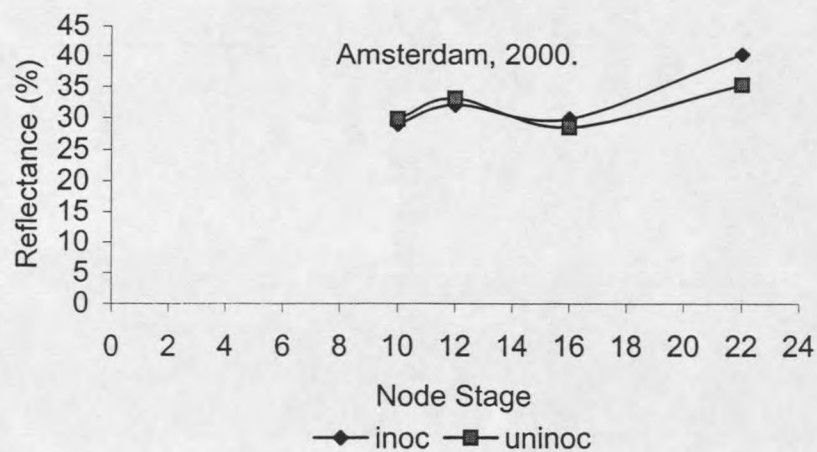
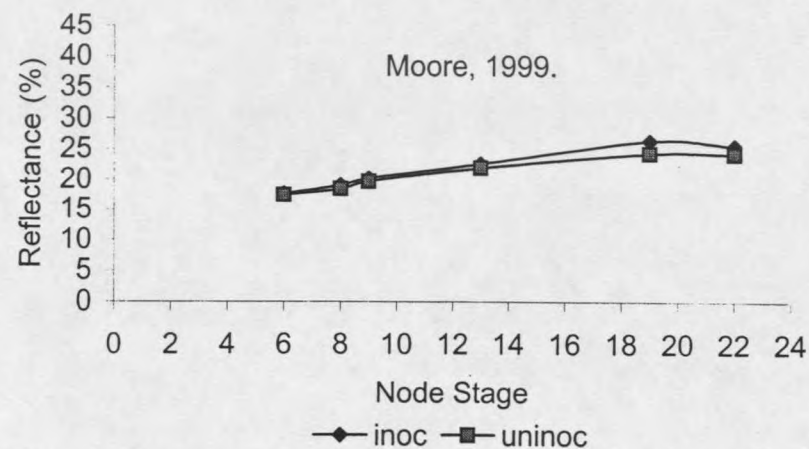
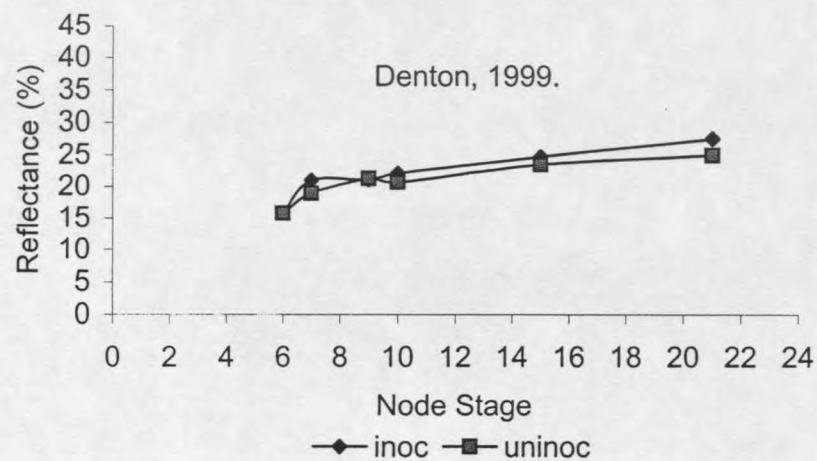


Figure 15. Reflectance in percent for the 813 nm band from seedling (8,9-node stage) to anthesis for chickpea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

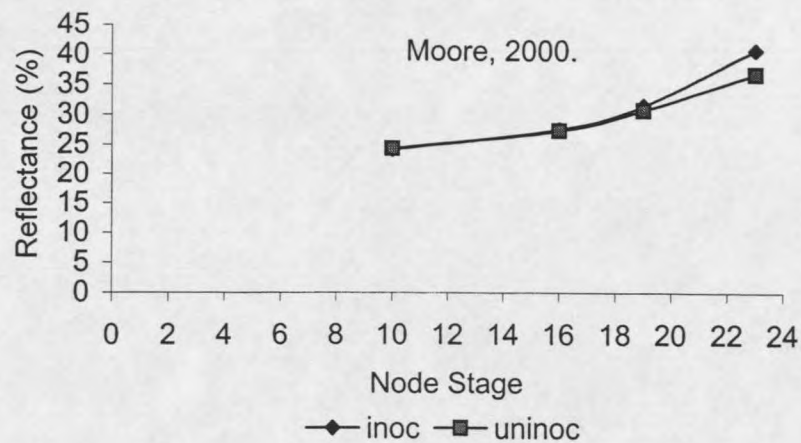
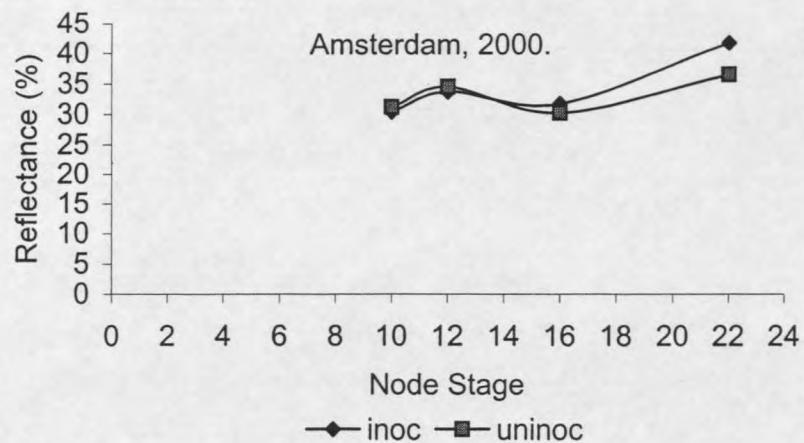
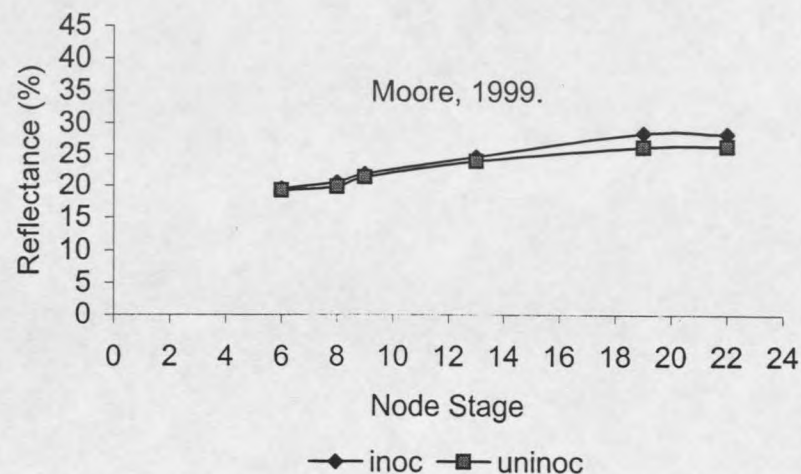
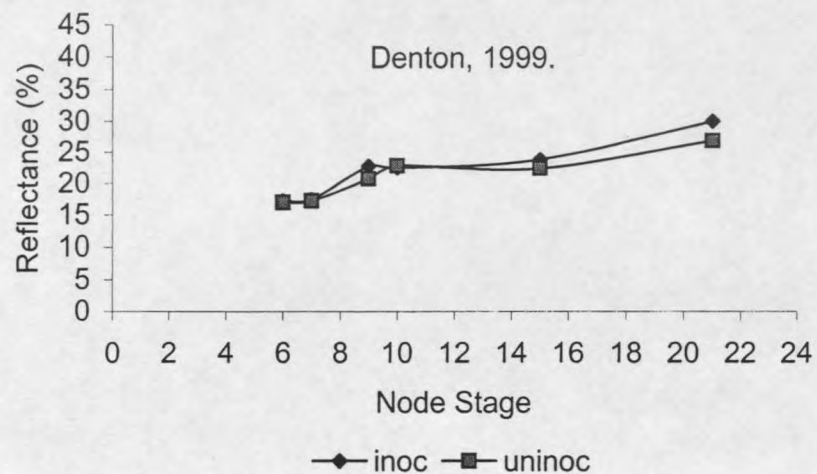


Figure 16. Reflectance in percent for the 830 nm band from seedling (8,9-node stage) to anthesis for chickpea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

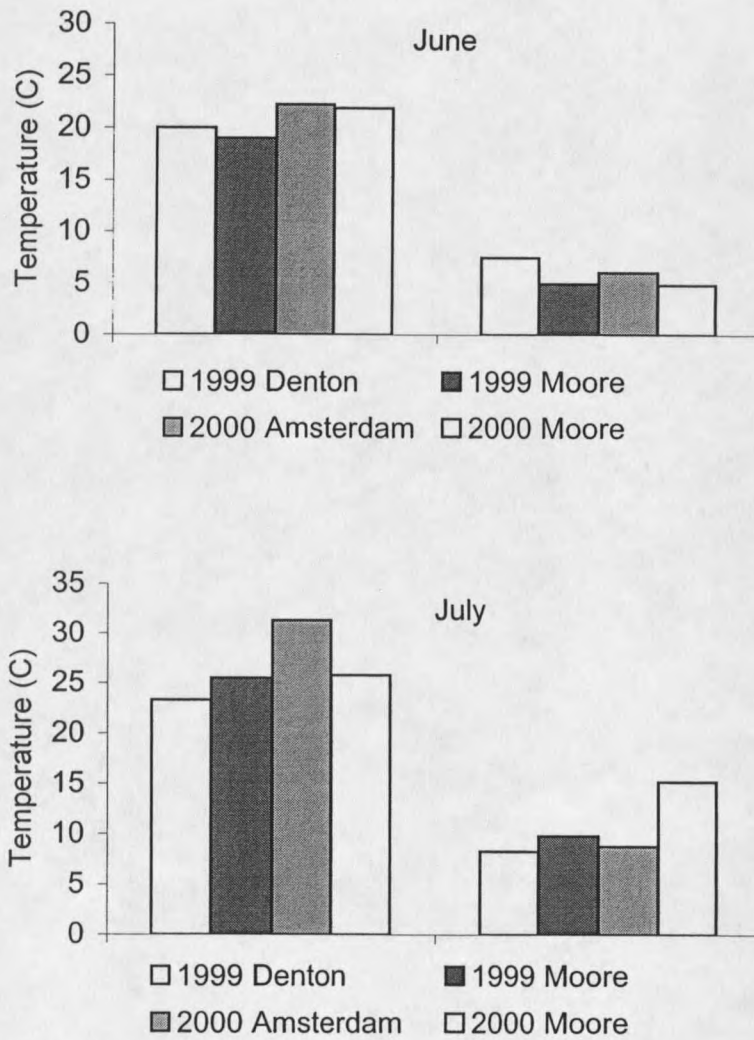


Figure 17. Maximum and minimum average temperatures for June and July at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000.

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