Managing inoculation failure of field pea and chickpea based on spectral responses

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McConnell, J. T., Miller, P. R., Lawrence, R. L., Engel, R. and Nielsen, G. A. 2002. **Managing inoculation failure of field pea and chickpea based on spectral responses.** Can. J. Plant Sci. **82**: 273-282. Pulse crop production is expanding in semiarid regions of the Northern Plains, and depends on successful biological N_2-fixation. Inoculation failure, resulting in plant N deficiency and economic crop loss, might be alleviated by remedial N fertilizer application. The experiment was conducted using no-till management at two dryland sites in Montana in 1999 and 2000, where field pea and chickpea were grown in cereal stubble. Shoot biomass, shoot biomass N concentration, seed yield and seed N concentration were measured for uninoculated and inoculated controls and compared with remedial fertilizer N applied 0, 4, 6, and 8 wk after seeding. Spectral reflectance was compared for the inoculated and uninoculated controls. For field pea and chickpea, the critical period for fertilizer N application to prevent yield loss occurred within 6 wk of seeding ($P \leq 0.05$). Logistic regression models derived from spectral reflectance had overall accuracies of 84 and 60% for detecting uninoculated control treatments in field pea and chickpea, respectively. The field pea model had a high degree of accuracy 6 wk after seeding, indicating it was capable of assisting a decision to apply remedial N fertilizer. Spectral reflectance provided a window of opportunity of 1 wk to apply remedial N fertilizer to attain full yield potential.

Key Words: Chickpea, field pea, inoculant failure, nitrogen, spectral reflectance

McConnell, J. T., Miller, P. R., Lawrence, R. L., Engel, R. et Nielsen, G. A. 2002. **Remédier à l'échec de l'inoculation d'après la réaction spectrale chez le pois de grande culture et le pois chiche**. Can. J. Plant Sci. **82**: 273-282. La production de légumineuses à graines ne cesse de s'accroître dans les régions semi-arides des Plaines du nord et dépend d'une bonne fixation biologique de l'azote. On remédie habituellement à l'échec de l'inoculation, qui entraîne une carence en N chez la plante et réduit le rendement de la culture, en appliquant un engrais azoté. Dans le cadre de cette expérience, le pois de grande culture et le pois chiche ont été cultivés sur du chaume de céréale à deux endroits désertiques du Montana, sans travail préalable du sol, en 1999 et 2000. Les auteurs ont mesuré la biomasse des pousses, la concentration d'azote dans cette biomasse, le rendement grainier et la concentration d'azote dans les semences des plants non inoculés et des témoins inoculés, puis ont comparé ces valeurs à celles obtenues après l'application d'un engrais azoté, 0, 4, 6 et 8 semaines après l'ensemencement. La réflectance spectrale des plants inoculés a été comparée à celle des plants qui ne l'étaient pas. Pour le pois de grande culture et le pois chiche, la période cruciale où l'on doit épandre l'engrais pour éviter une baisse du rendement survient dans les six semaines suivant les semis ($P \leq 0,05$). Les modèles à régression logistique de la réflectance spectrale sont exacts à 84 % et à 60 %, respectivement, pour ce qui est de déceler les plants témoins inoculés des autres, pour le pois de grande culture et le pois chiche. Le modèle du pois de grande culture se caractérise par un grand degré d'exactitude six semaines après les semis, signe qu'il peut faciliter la décision d'appliquer l'engrais ou pas. Dans ce cas, les producteurs disposeront d'une semaine pour épandre l'engrais s'il ne veulent pas que le rendement de leur culture diminue.

Mots clés: Pois chiche, pois de grande culture, échec de l'inoculant, azote, réflectance spectrale

Pulse crop production is increasing in the northern Great Plains (Miller et al. 2002). Soils in this region typically have insufficient levels of plant-available N for optimum growth. Biological N_2-fixation by *Rhizobia* species can make up for this deficit by supplying the majority of N required for a successful pulse crop (Mahon and Child 1979). Successful inoculation of pulse crops is important for optimizing plant N nutrition, seed yield, seed quality and economic return. There are no reliable estimates of the area of pulse crops in the northern Great Plains that are affected annually by inoculation failure, but when it occurs it can be financially harmful for individual producers (P. Miller, personal observation).

Environmental conditions and management practices influence the frequency of inoculation failure. The primary environmental factor in inoculation failure is dry seedbed conditions, a common occurrence in semiarid regions of the northern Great Plains. Tillage operations and erratic spring precipitation can produce dry, warm soils that desiccate and destroy rhizobia before the root infection can occur (Chatel and Parker 1973; Evans et al. 1980). Too often, human errors also cause inoculation failure through improper storage or application of inoculant, mechanical failure of inoculant delivery, or the inadvertent selection of improper rhizobial strains.

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

Table 1. 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 texture	Very fine sandy loam	Clay loam	Clay loam
Soil depth (cm)	150+	60-120+	45-90+
Soil N (kg N ha^{-1})^z	14	20	20, 28 (1999, 2000)
Annual temp. (°C)^y	6.6	6.6	6.1
Annual precip. (mm)^y	300-350	350-400	350-400

^{z}NO_3-NH_4 to a depth of 30 cm, determined by 1 M KCl extraction.
^{y}MAPS climate atlas of Montana, (Caprio et al. 1994).

nomic losses from inoculation failure might be corrected by applying N fertilizer after crop emergence. Remedial application of N after seeding was successful in recovering potential yield losses in soybean due to inoculation failure (Gault et al. 1984). Similar studies have not been conducted for cool-season pulse crops such as field pea and chickpea.

Because it is uncertain when and where inoculation failure might occur, it is likely that efficient survey techniques would be valuable for identifying fields or delineating areas within variable field landscapes where inoculation failure has occurred. Remote sensing has been used successfully as a method for diagnosing N nutritional status of other crops – sweet pepper (Thomas and Oerther 1971), corn (Piekielek and Fox 1992), rice (Turner and Jund 1994) – using hand-held radiometers as well as platform sensors (Yoder and Pettigrew-Crosby 1995). These previous studies hold that N deficiency and reflectance are related, and because nodulation failure results in N deficiency, it is possible that spectral reflectance could aid in identifying nodulation failure. Remote sensing has not been used to evaluate plant N nutritional status or inoculation success in cool-season pulse crops. For field pea and chickpea, the objectives of the study were (1) to determine the critical period for fertilizer N application to prevent yield loss due to inoculation failure, (2) to characterize spectral signatures for inoculated vs. uninoculated treatments, and (3) to determine if plant N deficiency can be detected through early measurements of spectral analysis to effectively assist a decision to apply remedial fertilizer N.

MATERIALS AND METHODS

The experimental sites occurred near Denton and Moore, MT, in 1999 and near Amsterdam and Moore, MT, in 2000 (Table 1). 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 at Amsterdam was a Manhattan very fine sandy loam (coarse, loamy mixed Typic Calciboroll) (DeYoung and Smith 1936). The treatments were two crop types (semi-leafless field pea – 1999 cv. Alfetta, 2000 cv. Espace; and kabuli chickpea - cv. Dwelley) and six N treatments (Table 2). The experimental was a randomized complete block design with four replications. Plot size was 15.2 × 3.6 m.

In 1999, the experiment was seeded into tilled cereal stubble with a Conserva-Pak (Conserva-Pak, Yorkton, SK, Canada) research drill with a seed furrow width of 30 cm, double shoot hoe-type openers and on-row packing. 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 (HarvesTechnologies, 2110 Park Ave., Brandon, MB, Canada.) hoe-type openers with single shoot capability and on-row packing. Planting depth was 4-5 cm. Targeted plant densities of 70–90 plants m^{-2} for field pea and 30–50 plants m^{-2} for chickpea were achieved at all sites. Seeding occurred between 28 April and 6 May, considered mid-spring seeding dates for the respective sites. Weeds were controlled effectively by pre-emergent glyphosate (480 g a.i. ha^{-1}) and ethalfluralin (1.5 kg a.i. ha^{-1}), and post-emergent quizalofop P-ethyl (50 g a.i. ha^{-1}) herbicide applications.

Strain-specific Liphatech Soil Implant (Lipha Tech Inc., 3101 West Custer Ave., Milwaukee, WI, USA.) peat granular inoculant was placed in the seedrow at 5.5 kg ha^{-1}. For the treatments, fertilizer application rates were 102 kg N ha^{-1} for field pea and 69 kg N ha^{-1} for chickpea in 1999. These rates were equivalent to the estimated N removed by seed, using yield targets of 1980 kg ha^{-1} for field pea and 1344 kg ha^{-1} 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. Nitrogen fertilizer (urea) was surface-broadcast with an air-delivery Valmar (Valmar Inc., Box 100, Elie, MB, Canada) granular applicator in 1999 and applied manually with a hand-held rotary spreader in 2000 to prevent plant damage from machinery traffic. Fertilizer P was applied each year at a rate of 10 kg ha^{-1}, placed as a separate band 2.5 cm to the side and 5 cm below the seed in 1999, or placed in the seed furrow in 2000.

Shoot biomass was measured weekly from the 4th node stage to anthesis by hand-clipping a 1.1-m^2 area on one-half of each plot (i.e., 1.8 × 15.2 m) reserved for destructive sampling. Growth stages were determined by enumeration of shoot nodes. Seed yields were harvested from 1.56 × 15.2 m and 1.52 × 15.2 m areas in each plot in 1999 and 2000, on the side (i.e., 1.8 × 15.2 m) where destructive sampling had not occurred, avoiding the edge row. Shoot biomass and harvest seed samples were dried for 72 h at 40°C to determine dry matter yields. Plant N concentrations were determined for subsamples taken from ground biomass and seed samples with an automated Dumas instrument [LECO CNS-2000 (LECO Corp., 3000 Lakeview Ave., St. Joseph, MI, USA.)]. At Moore and Amsterdam, rainfall was recorded by electronic tipping bucket recorders. At Denton, rainfall was recorded at a weather station within 10 km of the field site, and corroborated with producer records collected less than 1 km away.

Table 2. Treatments and fertilizer application timings

Treatment	Days after seeding
Inoculated/unfertilized control (Inoc)	–
Uninoculated/unfertilized control (Uninoc)	–
Uninoculated/fertilizer N applied 0 wk after seeding (0–wk)	8, 9, 0, 0[z]
Uninoculated/fertilizer N applied 4 wk after seeding (4–wk)	29, 30, 30, 28[z]
Uninoculated/fertilizer N applied 6 wk after seeding (6–wk)	40, 41, 41, 44[z]
Uninoculated/fertilizer N applied 8 wk after seeding (8–wk)	54, 55, 61, 56[z]
Inoculated/fertilizer N applied 0 wk after seeding (0–wk)	–, –, 0, 0[y]

[z] Denton and Moore, 1999, Amsterdam and Moore, MT, 2000, respectively.
[y] Additional control added in 2000 only.

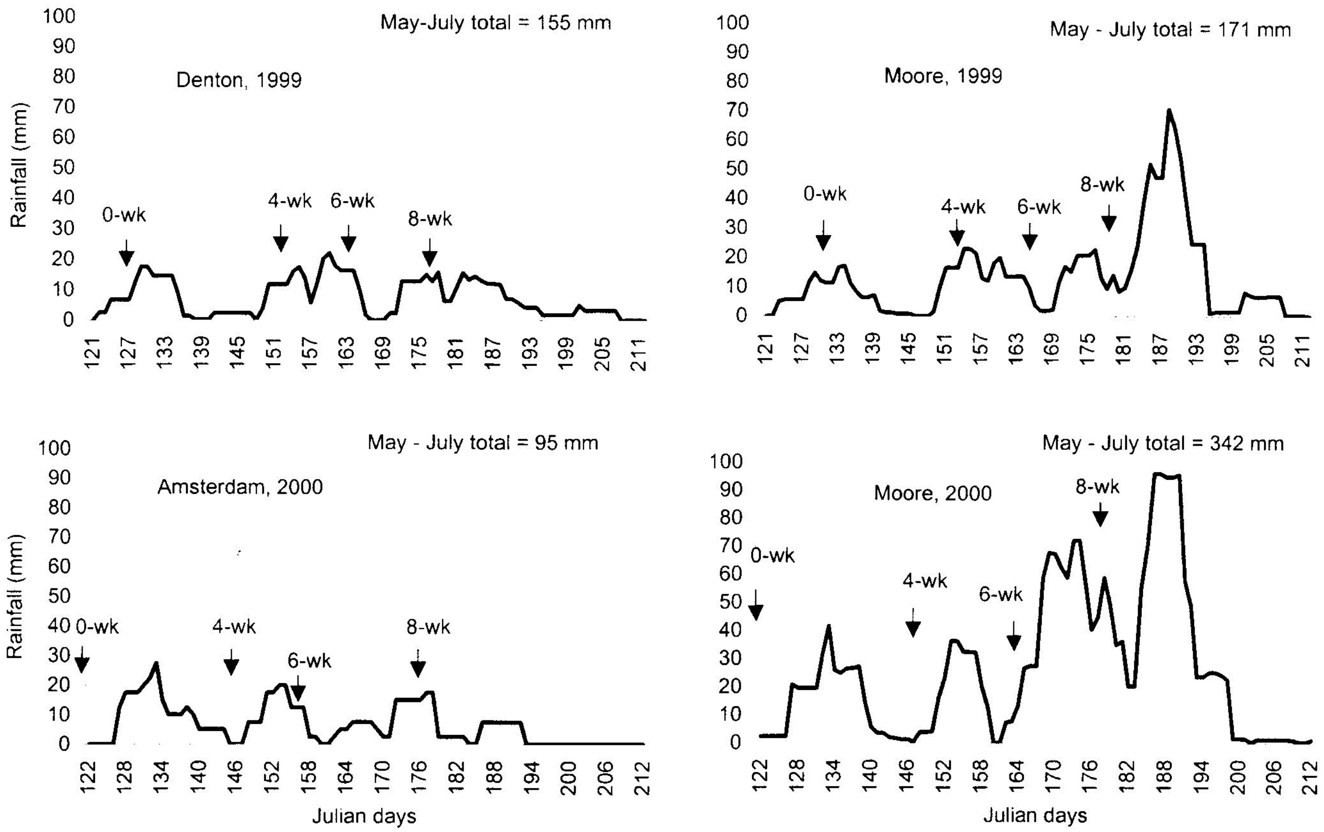

Fig. 1. Weekly precipitation from 1 May to 31 July (Julian days) at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000. Values were recorded as running 7-d totals, indicating the total rainfall received for each date plus the previous 6 d. The timing of fertilizer N applications is indicated by vertical arrows.

Radiometric data were recorded with a portable Cropscan (Cropscan Inc., 125 26th St. NW, Rochester, MN, USA.) radiometer during peak hours of sunlight (1000 to 1500 h). The radiometer received regular factory calibration and measurements were collected using matched upward and downward sensors, enabling conversion of measurements to percent reflectance for each spectral band. Readings were collected from two locations within each plot, at a height of 1.5 m, measuring an effective area of 3.1 m^2. The 10 spectral bands recorded were: 445–525 nm, 480–580 nm, 593–633 nm, 630–690 nm, 660–700 nm, 684–729 nm, 798–828 nm, 755–905 nm and 1500–1950 nm.

Analysis of variance as a randomized complete block design was performed separately for three field pea sites and four chickpea sites (SAS Institute, Inc. 1995) due to nonhomogeneity of error variances among sites for each crop. Treatment means were evaluated using the Protected LSD (Steel and Torrie 1980). For each crop type, reflectance band models were derived to differentiate between control treatments using a binary response (treated or control) and using spectral bands as potential predictor variables [vegetation indices were not used because they unnecessarily restrict the regression models (Lawrence and Ripple 1998)]. Models were fit using stepwise logistic regression for band selection (Neter et al. 1996; S-PLUS 2000). Logistic regression is appropriate for binary responses and in our models produced an estimate of the probability of uninoculated treatment (Ramsey and Schafer 1997). With the stepwise

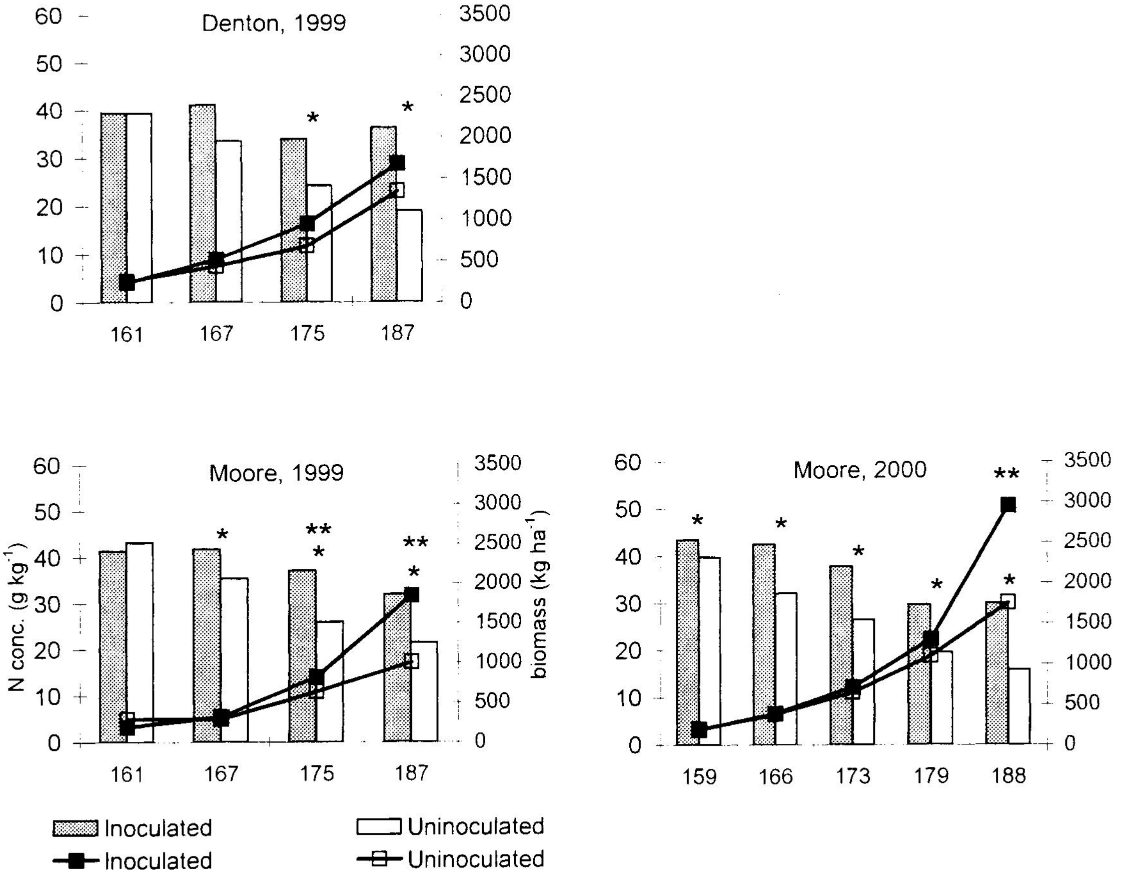

Fig. 2. Shoot N concentration and biomass accumulation from the four-node stage to anthesis (Julian days) for field pea at Denton and Moore, 1999, and Moore, MT, 2000. Single and double asterisks indicate differences for N concentration and biomass, respectively, at each sampling date ($P < 0.05$).

procedure, arrival at a significant model was indicated when the exclusion of any variable resulted in a significant drop-in-deviance statistic. To determine whether spectral distinctions could be detected for each crop, a regression model was built utilizing the full data set, excluding the 2000 Amsterdam site for field pea, since confirmed contamination from native rhizobia occurred at that site. The logistic models were applied to each observation to produce a predicted probability of nodulation. To test the accuracies of the models, observations greater than or equal to 50% probability were classified as nodulated and those below 50% as unnodulated.

The logistic regression models' accuracies were assessed using 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. 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 comission error or "user's accuracy" is calculated. 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 KHAT statistic was calculated and indicates a model's ability for classification compared to chance classification and is an estimate of KAPPA (Lillesand and Kieffer 1994).

RESULTS

Precipitation patterns at the experimental sites were characterized by terminal drought beginning in mid-July (~196 Julian days), which is typical for this region (Fig. 1). Field pea and chickpea were injured by hail at the Denton site on 22 June 1999 (173 Julian days), which temporarily interrupted plant growth. Field pea was contaminated by native rhizobia at Amsterdam in 2000, which was confirmed by a greenhouse plant trap experiment (Somasegaran and Heinz 1994). Browsing by antelope (*Antilocapra americana*) at Moore damaged chickpea, but not field pea in adjacent plots. Yield losses were visually estimated to be 5–10% in 1999 and 45–50% in 2000, and so the 2000 yield results were omitted.

Shoot Biomass and Nitrogen Concentration

For field pea, shoot biomass accumulation differences between the inoculated and uninoculated control treatments varied strongly by site (Fig. 2). At Denton, these control

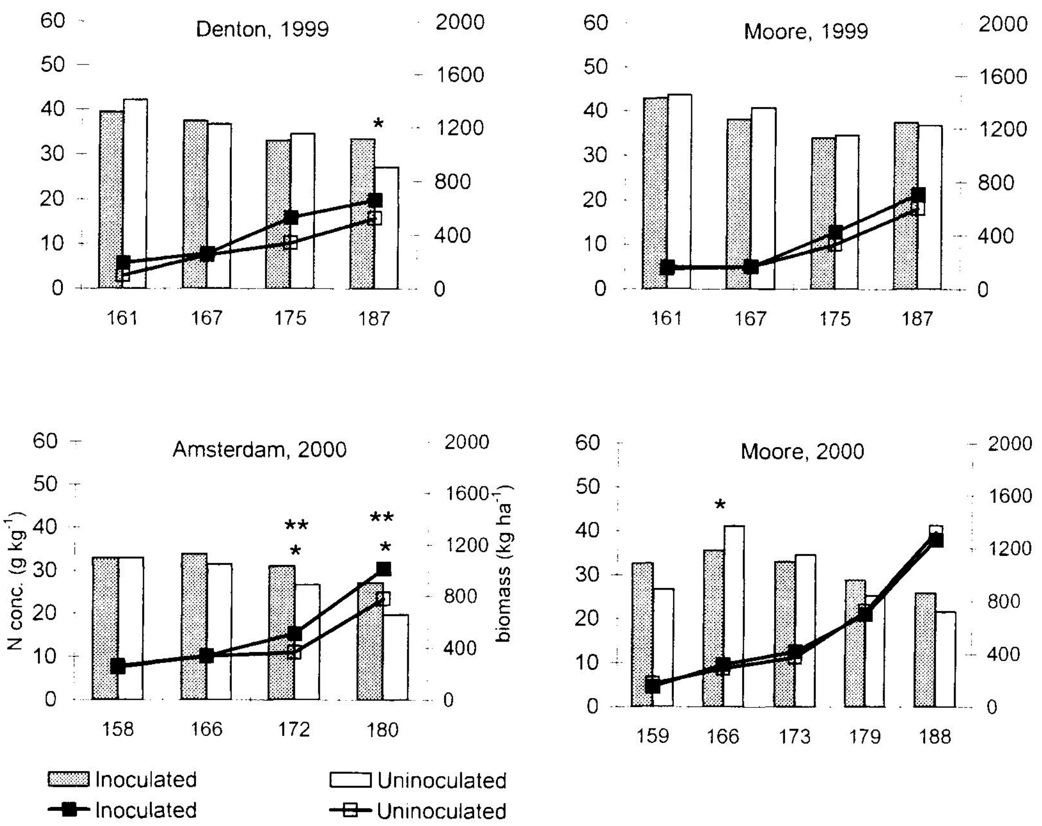

Fig. 3. Shoot N concentration and biomass accumulation from the three-node stage to anthesis (Julian days) for chickpea at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000. Single and double asterisks indicate differences for N concentration and biomass, respectively, at each sampling date ($P < 0.05$).

treatments did not differ for shoot biomass at any date. At Moore, shoot biomass for the inoculated control exceeded the uninoculated control after 7 wk in 1999 and 9 wk in 2000 ($P \leq 0.05$). The controls differed in shoot N concentration sooner than shoot biomass accumulation. Shoot N concentration for the inoculated control exceeded the inoculated control after 6 wk in both years at Moore, and after 7 wk at Denton ($P \leq 0.05$). For Chickpea, differences in shoot characteristics between the inoculated controls occurred infrequently and later than for field pea (Fig. 3). Shoot biomass for the inoculated control exceeded the uninoculated control only at Amsterdam, requiring nearly 8 wk of growth to become apparent ($P \leq 0.05$). Shoot N concentration for the controls differed at two of the four sites, after 9 wk at Denton and after 8 wk at Amsterdam ($P \leq 0.05$).

For field pea, where shoot growth differences occurred between the controls, N fertilizer maintained shoot biomass at anthesis equal to the inoculated control when applied within 4 and 6 wk of seeding at Moore in 1999 and 2000, respectively (Table 3; $P \leq 0.05$). Nitrogen fertilizer was even more effective at maintaining shoot N concentration at anthesis. At Moore in both years, N fertilizer applied within 6 wk of seeding produced shoot N concentrations that equaled or exceeded the inoculated control ($P \leq 0.05$). At Denton, N fertilizer did not produce shoot N concentration at anthesis equal to the inoculated control, but exceeded the uninoculated control for all N fertilizer treatments ($P \leq 0.05$). The N fertilizer response in chickpea differed markedly from that for field pea. By anthesis, shoot biomass and N concentration produced by N fertilizer applied within 4 wk of seeding exceeded the inoculated control in all cases, except for Denton, where no treatment differences were observed for shoot biomass (Table 4; $P \leq 0.05$). In 2000, when N fertilizer rates were increased from 1999, shoot biomass and N concentration exceeded the inoculated control when applied within 6 wk of seeding at both sites ($P \leq 0.05$).

Seed Yield and N Concentration

For field pea, seed yields for the uninoculated control were consistently < 50% of the inoculated control at the three rhizobia-responsive sites (Table 5). In 1999, seed yields where N fertilizer was applied within 4 wk of seeding were equal to the inoculated control ($P \geq 0.05$), while N fertilizer applied 6 wk after seeding produced yields intermediate between the control treatments. In 2000, at Moore, seed yields where N fertilizer was applied within 6 wk of seeding were equal to the inoculated control ($P \geq 0.05$), while the 8-wk N fertilizer treatment yielded intermediate between the control treatments. In 1999, only the 4-wk N fertilizer treatment had seed N concentration equal to the inoculated control at both sites, while in 2000 at Moore, seed N

Table 3. Field pea shoot biomass and N concentration at anthesis at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000

Treatment	Dent1999	Moor1999	Amst2000[z]	Moor2000
		Shoot biomass (kg ha^{-1})		
Inoculated	1680*ab*	1850*b*	1630*cd*	2960*ab*
Uninoculated	1340*b*	1010*d*	1580*d*	1750*c*
Fert N 0-wk	1800*a*	2220*a*	1870*bc*	3340*ab*
Fert N 4-wk	1790*a*	1770*b*	2250*a*	3500*a*
Fert N 6-wk	1300*b*	1350*c*	2000*ab*	2790*b*
Fert N 8-wk	–	–	1750*bcd*	1850*c*
		Shoot N concentration (g kg^{-1})		
Inoculated	36*a*	32*b*	38	30*a*
Uninoculated	19*d*	22*c*	38	16*b*
Fert N 0-wk	28*bc*	29*b*	37	33*a*
Fert N 4-wk	32*b*	41*a*	39	33*a*
Fert N 6-wk	28*c*	30*b*	41	31*a*
Fert N 8-wk	–	–	39	20*b*

[z]Amsterdam site was contaminated with native soil rhizobia.

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

Table 4. Chickpea seed shoot biomass and N concentration at anthesis at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000

Treatment	Dent1999	Moor1999	Amst2000	Moor2000
		Shoot biomass (kg ha^{-1})		
Inoculated	660	710b	1010*d*	1270*b*
Uninoculated	530	600b	780*e*	1320*b*
Fert N 0-wk	820	920a	1600*b*	1910*a*
Fert N 4-wk	730	900*a*	1930*a*	1830*a*
Fert N 6-wk	500	650*b*	1320*c*	1950*a*
Fert N 8-wk	–	–	900d*e*	1400*b*
		Shoot N concentration (g kg^{-1})		
Inoculated	33*c*	37*b*	27*c*	26*c*
Uninoculated	27*d*	37*b*	20*d*	22*c*
Fert N 0-wk	39*ab*	42*ab*	40*a*	38*a*
Fert N 4-wk	43*a*	47*a*	38*a*	37*a*
Fert N 6-wk	35bc	45*a*	35*a*	35*ab*
Fert N 8-wk	–	–	23*d*	31*b*

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

concentration values were equal to the inoculated control for all N fertilizer treatments ($P \geq 0.05$; Table 5).

Chickpea yields did not differ between the inoculated and uninoculated control treatments at two of three sites ($P \geq 0.05$; Table 6). However, seed N concentration for the uninoculated control was consistently lower than for the inoculated control ($P \leq 0.05$), ranging from 20 to 28% lower than the inoculated control. Nitrogen fertilizer applied within 6 wk of seeding generally produced seed N concentrations equal to the inoculated control ($P \geq 0.05$).

Spectral Reflectance

Logistic regression analyses of reflectance were performed separately for each crop type. The best model determined for field pea was:

$\text{Logit}(\pi) = -4.12 + 0.43(560\text{ nm}) + 0.75(613\text{ nm}) - 2.89(660\text{ nm}) + 3.83(706\text{ nm}) - 1.70(830\text{ nm})$, and 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 ($P \leq 0.05$) according to the drop-in-deviance test (Neter et al. 1996). The models differed by the absence of a green band (500 – 600 nm) in the chickpea model, and different bands within the red (600 – 700 nm) and near infrared (800 – 900 nm).

The models also varied in classification accuracy by growth stage (Fig. 4). The field pea model had an overall accuracy for all stages of 84% for classifying a crop canopy as inoculated (actively fixing N_2) or uninoculated (no N_2 fixation; Table 7). For the field pea model for all stages, the producer's accuracy was 79% and the user's accuracy was 88%. The chickpea model had a low accuracy of 60%. KHAT values showed the field pea model was 68% better at classifying inoculated versus uninoculated field pea than random assignment, while the chickpea model was only 21% better at classification than random assignment (Table 7).

DISCUSSION

Objective 1. What was the critical period for N fertilizer application to prevent yield loss due to inoculation failure?

Field pea and chickpea differed markedly in their response to inoculation in this study even though the same commercial brand and formulation type of inoculants were used, with the correct rhizobial strains for each crop. For field pea,

Table 5. Field pea seed yield and N concentration at Denton and Moore, 1999, and Amsterdam and Moore, MT, 2000

Treatment	Dent1999	Moor1999	Amst2000[z]	Moor2000
		Seed yield (kg ha^{-1})		
Inoculated	1140*a*	2290*a*	1650	1670*a*
Uninoculated	500*c*	1170*c*	1700	820*c*
Fert N 0-wk	1010*a*	2170*a*	1700	1620*a*
Fert N 4-wk	1020*a*	2210*a*	1650	1790*a*
Fert N 6-wk	760*b*	1620*b*	1660	1650*a*
Fert N 8-wk	340*c*	1090*c*	1640	1390*b*
Inoc + Fert N 0-wk	–	–	1590	1630*a*
		Seed N concentration (g kg^{-1})		
Inoculated	40*a*	41*a*	41	44*a*
Uninoculated	28*d*	25*c*	44	26*b*
Fert N 0-wk	35*bc*	32*b*	43	41*a*
Fert N 4-wk	37*b*	39*a*	43	42*a*
Fert N 6-wk	34*c*	34*b*	45	44*a*
Fert N 8-wk	29*d*	25*c*	46	44*a*
Inoc + Fert N 0-wk	–	–	44	43*a*

[z] Amsterdam site was contaminated with native soil rhizobia.

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

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

Treatment	Dent1999	Moor1999	Amst2000	Moor2000[z]
		Seed yield (kg ha^{-1})		
Inoculated	450*b*	790*a*	590	–
Uninoculated	380*bc*	760*a*	590	–
Fert N 0-wk	460*ab*	730*a*	550	–
Fert N 4-wk	470*ab*	710*a*	580	–
Fert N 6-wk	550*a*	710*a*	570	–
Fert N 8-wk	300*c*	560*b*	620	–
Inoc + Fert N 0-wk	–	–	520	–
		Seed N concentration (g kg^{-1})		
Inoculated	41*a*	41*ab*	46*a*	45*ab*
Uninoculated	31*c*	33*d*	33*c*	33*c*
Fert N 0-wk	39*ab*	40*b*	46*a*	47*a*
Fert N 4-wk	40*a*	43*a*	45*a*	44*b*
Fert N 6-wk	37*b*	41*ab*	44*a*	46*ab*
Fert N 8-wk	33*c*	36*c*	40*b*	46*ab*
Inoc + Fert N 0-wk	–	–	46*a*	46*ab*

[z] Yield losses due to antelope predation were estimated at 45–50%, so yield data were omitted.

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

the inoculated control exceeded the uninoculated control in shoot N concentration 6 to 7 wk (9–13 node stage) after seeding, which occurred 1 to 3 wk sooner than differences in shoot biomass accumulation (Fig. 2). This evidence indicates that the critical period for N fertilizer application occurred within 6 to 7 wk of seeding. Conversely, for chickpea, the control treatments differed infrequently and there were no important differences in the timing of the ability to detect differences in shoot N concentration and biomass prior to anthesis (Fig. 3). It is possible that the commercial strain of chickpea inoculant used in this study is poorly adapted to this soil-climatic region, as has been previously observed with commercially available chickpea inoculants in new chickpea production areas in Saskatchewan (Walley et al. 1997).

For field pea, N fertilizer applied within 6 wk of seeding was generally sufficient to recover shoot biomass and N concentration at anthesis equal to the inoculated control (Table 3). This agrees closely with the observation of shoot growth differences between the inoculated and uninoculated control treatments above. For chickpea, shoot biomass and N concentration at anthesis generally exceeded the inoculated control when N fertilizer was applied within 6 wk of seeding (Table 4), indicating a critical N fertilizer application period for optimal plant uptake similar to field pea.

For field pea, seed yield of the uninoculated controls was approximately 50% of inoculated controls reflecting the absence of indigenous inoculum and low soil N status (Table 5). This highlights the need for producers to have the agronomic information to recover yield in the case of inoculation failure. In addition, yield potentials observed in this study were comparatively modest. Economic losses from inoculation failure might be expected to be even greater under high yield conditions. For field pea in 2000 at Moore, a similar critical period was observed as that reported above, where N fertilizer applied within 6 wk of seeding maintained equal seed yield and N concentration compared to the inoculated control (Table 5). Though a similar pattern was

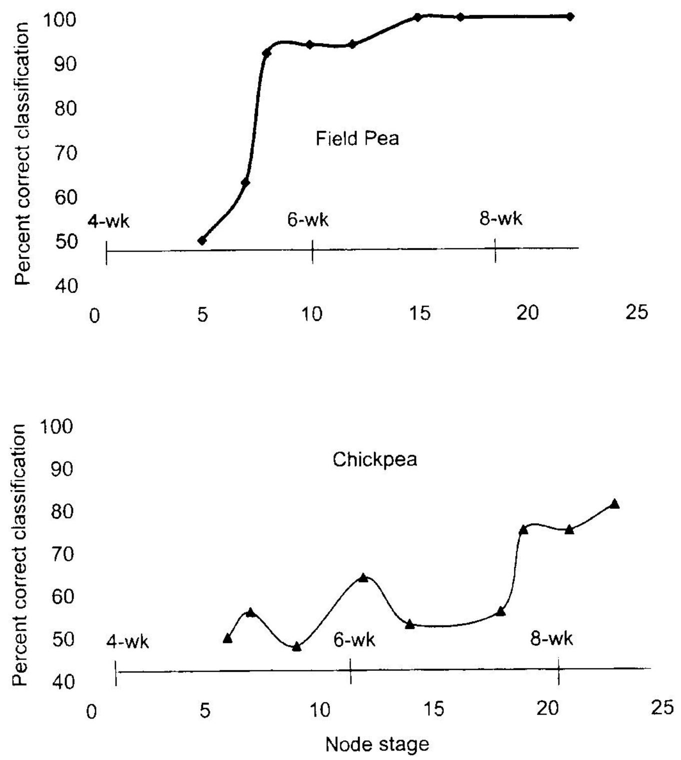

Fig. 4. Logistic regression model accuracy with increasing growth stage.

observed in 1999, seed yield and N concentration for the N fertilizer treatments generally did not equal the inoculated control. This might have been caused by insufficient N fertilizer rates or due to low rainfall (Janzen et al. 1984). Surface-applied fertilizer must await the next rainfall event to become available for plant uptake, which typically caused a delay between the timing of the N fertilizer application and plant access in this study, as would be the case in dryland farming. The interval between N fertilizer application and significant rainfall (5 mm) ranged from 1 to 9 d, averaged 5 d, and appeared random relative to timing of N fertilizer applications (Fig. 1). For example, at Moore in 1999, the interval between N fertilizer applied 6 and 8 wk after seeding and subsequent rainfall was 3 and 4 d respectively, indicating that the yield loss by the 8-wk N fertilizer treatment was not due to ill-timed rainfall. Comparing 1999 and 2000 at Moore, the interval between the 6-wk N fertilizer treatment and rainfall was 5 d in both years, again suggesting that the efficacy of N fertilizer timing was not strongly affected by the timing of post-application rainfall in this study.

For chickpea, it was evident that the higher shoot biomass and N concentrations at anthesis did not translate into increased seed yield or N concentration, as has been observed in past studies (Doughton et al. 1993; Walley and Hnatowich 1998). This suggests that chickpea might be inefficient at N translocation from vegetative to reproductive plant tissue. The results of seed yield and N concentration in chickpea also indicate a critical period within 6 wk of seeding for N fertilizer application (Table 6). Thus, all lines of evidence for both field pea and chickpea indicated that the critical period for N fertilizer application to prevent yield loss occurred within 6 wk of seeding. Chickpea requires an additional 10 to 20 d to reach maturity compared to field pea (Anonymous 2000). Thus, it was expected that the critical period for remedial N fertilizer application would be later than field pea, which did not occur here. Reasonably, the effects of applied N on seed yield depend on the length of the interval from plant uptake until maturity. Gault et al. (1984) reported uninoculated soybean [*Glycine max* (L.) Merrill] yields were recovered with fertilizer application 60 d after planting. An earlier time period for yield recovery, as is reported here, would be expected when considering field pea and chickpea, which require 400 to 600 fewer degree days (5°C) to reach maturity than even the shortest maturity classes of soybean (Miller et al. 2002).

Objective 2. Can inoculation failure be detected by spectral reflectance?

In this experiment, inoculated (N_2-fixing) plants were considered "healthy" and reflected proportionally more radiation in the near-infrared region and less in the blue, green, and red portions of the spectrum than uninoculated plants. The uninoculated (non-N_2-fixing) control for both crops reflected higher quantities of each band, except near infrared, potentially due to reduced radiation absorbance resulting from decreased leaf chlorophyll concentration and/or biomass (Takebe et al. 1990). Red radiation is absorbed less in nutrient-deficient plants, resulting in a yellowish spectral signature, as well as in canopies with lower biomass. Near-infrared radiation is highly reflected by healthy plants and is affected by internal structures in the plant leaf and by water content (Thomas and Oerther 1971; 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. Although some previous studies have found N responses, primarily in the green portion of the spectrum (Thomas and Oerther 1971), we did not find the green bands significant, perhaps indicating that the primary response being detected was total biomass. 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% and a user's accuracy of 88% for identifying the nodulated or positive control treatment (Table 7). The user's accuracy is more important because it represents the error that would be experienced by an actual user of a remotely sensed classification map. In this case, 12% of the time an area that was actually an inoculation failure would be classified as 'inoculated'. It would be economically desirable to minimize the frequency of this occurrence since remedial N fertilizer application would not be recommended where it was required to prevent economic yield loss. This result shows

Table 7. Error matrix for the field pea and chickpea logistic regression models

	Reference data		
Classified data	Inoculated	Uninoculated	Row total
Field pea			
Inoculated	59	8	67
Uninoculated	16	67	83
Column total	75	75	150
Producer's accuracy		User's accuracy	
Inoculated = 59/75 = 79%		Inoculated = 59/67 = 88%	
Uninoculated = 67/75 = 89%		Uninoculated = 67/83 = 81%	
Overall accuracy = (59+67)/150 = 84%			
KHAT = (150*(59+67) - (67*75)+(83*75))/(($150)^2$-(67*75)+(83*75)) = 0.68			
Chickpea			
Inoculated	50	31	81
Uninoculated	41	60	101
Column total	91	91	182
Producer's accuracy		User's accuracy	
Inoculated = 50/91= 55%		Inoculated = 50/81 = 62%	
Uninoculated = 60/91 = 66%		Uninoculated = 60/101 = 59%	
Overall accuracy = (50+60)/182 = 60%			
KHAT =(182*(50+60) - (91*81)+(91*101))/(($182)^2$ - (91*81)+(101*91) = 0.21			

good potential for spectral reflectance to be used to detect inoculation failure in field pea. The model, however, was based on field radiometer data and was not validated against an independent dataset. Additional research should be conducted to determine whether (1) similar results can be achieved using airborne or space-borne remote sensing and, (2) using independent validation data, models are robust across sites or need to be built on a site-specific basis.

Conversely, the chickpea model had a producer's accuracy of 55% and a user's accuracy of 62% for inoculated plant stands. Thus, the model would not be useful due to limited accuracy. The difference in models was attributed to physiological differences between crop canopies. Field pea and chickpea produce different amounts and rates of biomass accumulation and, as a result, would have different magnitudes of spectral reflectance 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.

Objective 3. Can spectral reflectance detect inoculation failure sufficiently early to assist a decision to apply remedial N fertilizer?

Since leaf chlorophyll concentration is positively linked to N concentration (Takabe et al. 1990; Turner and Jund 1994; Yoder and Pettigrew-Crosby 1995; Daughtry et al. 2000), and spectral reflectance is highly dependent on photosynthetic activity, these results show that plant N deficiency might be spectrally visible prior to negative departures in plant growth rates. For field pea, the model's accuracy increased with increasing node stage (Fig. 4) and was highly accurate at a sufficiently early growth stage to influence a management decision. The field pea model exceeded 90% accuracy by the 8th node stage (~5 wk after seeding), whereas the chickpea model was inconsistent until anthesis. The critical period for N fertilizer application to prevent yield loss in field pea lasted until 6 wk after seeding (corresponding to the 9 to 12-node stage), 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. In this study the window of opportunity to diagnose inoculation failure and apply remedial N fertilizer to prevent yield loss in field pea was at least 1 wk. The results for chickpea were inconclusive due to the small growth responses to inoculant or N fertilizer, and the subsequent lack of a reliable relationship between N-deficient chickpea and spectral reflectance.

CONCLUSIONS

Potential yield loss from inoculation failure can be prevented by remedial N fertilizer applied within 6 wk of seeding, coinciding with the 9–12 node stage of field pea and the 10–13 node stage of chickpea. The spectral reflectance model for field pea had a high degree of accuracy for classification between inoculated and uninoculated controls, while the reflectance model for chickpea was inaccurate. For field pea, the reflectance model identified inoculation failure sufficiently early to trigger a decision to apply remedial N fertilizer, and thus might offer a cost-effective way of monitoring N nutritional status in field pea. Spectral reflectance provided a window of opportunity of 1 wk to apply N fertilizer to attain full yield potential.

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