Universidade
Estadual de Londrina

MARCOS JAVIER DE LUCA

FIXAÇÃO BIOLÓGICA DO NITROGÊNIO, ESTADO NUTRICIONAL, RENDIMENTO E QUALIDADE DE GRÃOS DE SOJA SOB DIFERENTES DENSIDADES DE PLANTAS

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Tese apresentada ao programa de Pós-Graduação em Microbiologia da Universidade Estadual de Londrina, como critério para obtenção do titulo de doutor em Microbiologia.

Orientadora: Drª Mariangela Hungria.
Co-orientador: Dr. Marco Antonio Nogueira.

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2014

Catalogação elaborada pela Divisão de Processos Técnicos da Biblioteca Central da Universidade Estadual de Londrina.

Dados Internacionais de Catalogação-na-Publicação (CIP)

D278f De Luca, Marcos Javier.
Fixação biológica do nitrogênio, estado nutricional, rendimento e qualidade de grãos de soja sob diferentes densidades de plantas / Marcos Javier de Luca. – Londrina, 2014.
50 f. : il.

Orientador: Mariangela Hungria.
Coorientador: Marco Antonio Nogueira.
Tese (Doutorado em Microbiologia) – Universidade Estadual de Londrina, Centro de Ciências Biológicas, Programa de Pós-Graduação em Microbiologia, 2014.
Inclui bibliografia.

1. Nitrogênio – Fixação – Teses. 2. Soja – Rendimento – Teses. 3. Soja – Semeadura – Densidade – Teses. 4. Plantas – Nutrição – Teses. I. Hungria, Mariangela. II. Nogueira, Marco Antonio. III. Universidade Estadual de Londrina. Centro de Ciências Biológicas. Programa de Pós-Graduação em Microbiologia. IV. Título.
CDU 631.461.5

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AGRADECIMENTOS

Para mim, esta é uma das partes mais difíceis da tese e gostaria de começar com uma reflexão bem conhecida: “as pessoas e as comunidades colhem o que semeiam e suas alegrias e tristezas são produto de como elas utilizam o seu livre arbítrio.” Digo isto porque o que aparece resumido em 54 páginas é produto do esforço de muitas pessoas, de muitos recursos, de duas enormes Instituições, como Embrapa e INTA. Digo isto porque meu presente é produto da minha história pessoal, onde apareceram pessoas maravilhosas, cada uma num determinado momento, aportando um tijolo para construir o conhecimento que hoje posso oferecer a quem é o destinatário final e ao mesmo tempo a razão de que eu esteja aqui, “o produtor agropecuário, a família agropecuária”.

Mas... é merecido dar nomes.

Mariangela, minha orientadora, uma mãezona, é um anjo nesta terra e eu tive a bem-aventurança de trabalhar com ela, para quem não conhece... é um privilégio que poucos têm.

Marco Antonio, pensem uma pessoa que está sempre bem predisposta, gente boa.

Minha família completa, pais... que perderam o sono por mim, irmãos, cunhados, meus sobrinhos... filhotes que a vida me deu.

Quero fazer uma menção especial a Carlitos Lopez, técnico do INTA, que foi como um pai, com ele aprendi a “caminhar o campo” e comprender o que significa “servir a comunidade”, valores que hoje são praticados por poucas pessoas.

Não quero deixar de mencionar a Roberto Racca, também do INTA, ele tem muito a ver com meu doutorado e em definitiva foi ele quem me incentivou a vir ao Brasil, sábia decisão.

Outra grande parceira que tenho é a empresa Rizobacter, representada por Gonzalez Anta e Fabian Noguera, levam em frente experimentos na Argentina que muito aportaram a este trabalho.

Agradeço a todos meus companheiros tanto do INTA quanto da Embrapa, o dia a dia sem eles não seria possível e que alguns deram seu esforço me ajudando.

Laborsolo, dirigido por os professores Vieira e Fioretto, ofereceram suas instalações e conhecimento de forma desinteressada aportando uma parte fundamental deste trabalho, as análises nutricionais.

E por último... a Deus, que me deu todo o que tenho e que algum dia vai tirar... o que me lembra que só estou de passagem... até a próxima!!!

DE LUCA, Marcos Javier. **Fixação biológica do nitrogênio, estado nutricional, rendimento e qualidade de grãos de soja sob diferentes densidades de plantas**. 2014. 50 f. Tese (Doutorado em Microbiologia) – Universidade Estadual de Londrina, Londrina, 2014.

RESUMO

A crescente demanda mundial por alimentos tem resultado em pressões para o incremento na produção de grãos, sendo a soja (*Glycine max* (L.) Merr.) uma cultura amplamente difundida no mundo por causa da sua plasticidade e potencialidade de rendimento. Dentre os fatores críticos para obter altos rendimentos, existe um interesse renovado em arranjos diferenciais na densidade de plantas. Hoje, altas densidades de plantas são recomendadas, mas em menores densidades pode haver uma menor competição por água, nutrientes e luz solar, o que pode conduzir à maior sustentabilidade do sistema, particularmente em condições de estresses bióticos e abióticos. O objetivo deste trabalho foi o de determinar se a fixação biológica do nitrogênio (FBN) da soja é capaz de fornecer o nitrogênio (N) necessário para a obtenção de altos rendimentos; para isso, foi avaliada a capacidade de FBN da planta sob diferentes densidades. A hipótese do estudo é de que a capacidade de FBN é regulada pelo mecanismo de fonte:dreno, com taxas mais elevadas quando existe maior demanda pela planta. Foram conduzidos, por quatro anos consecutivos, ensaios a campo em Londrina, Estado do Paraná, com soja sob diferentes densidades, avaliando-se os parâmetros de FBN, nutrição das plantas, rendimento e qualidade dos grãos. No primeiro ensaio, foram avaliadas quatro densidades, variando de 40.000 a 320.000 plantas ha^{-1}. Em menores densidades verificou-se um estímulo da fotossíntese e da FBN por planta. Rendimentos de grãos semelhantes foram obtidos nas diferentes densidades, com redução apenas na densidade mais baixa, de 40.000 plantas ha^{-1}, que também foi o único tratamento com diferenças nos teores de óleo e proteína nos grãos. Nas três safras seguintes, foram realizadas avaliações em densidades variando de 80.000 a 320.000 plantas ha^{-1} e os resultados obtidos no primeiro ano foram confirmados. Nesses três ensaios, embora a densidade de plantas tenha sido reduzida em até 75%, o rendimento de grãos foi inferior em apenas uma safra, na menor densidade, e da ordem de 16%. Esses resultados indicam alta plasticidade da soja em adaptar a capacidade de fotossíntese e FBN a diferentes densidades. Além disso, o plantio em densidades mais baixas traz como vantagens o menor custo de implementação da cultura e menor suscetibilidade a estresses ambientais e nutricionais.

Palavras-chave: Densidade Planta. Nodulação. Nutrição Nitrogênio. Óleo. Proteína.

DE LUCA, Marcos Javier. **Biological nitrogen fixation, nutritional status, grain yield and quality in soybean under different plant densities**. 2014. 50 p. Thesis (Doctor in Microbiology) – Universidade Estadual de Londrina, Londrina, 2014

ABSTRACT

The increasing global demand for food has resulted in pressures to increase grain production, with soybean (*Glycine max* (L.) Merr.) representing a widespread crop due to its plasticity and grain yield potential. Among the critical factors to achieve high yields, there is a renewed interest in different arrangements of plant densities. Nowadays, high plant densities are recommended, but lower densities may reduce competition for water, nutrients and sunlight, leading to increased sustainability, particularly under biotic and abiotic stressing conditions. This study aimed at evaluating if biological nitrogen fixation (BNF) with soybean is capable of supplying the nitrogen (N) needed for achieving high yields; for that, the capacity of BNF under different plant densities was evaluated. The hypothesis is that the capacity of BNF is regulated by source:sink mechanisms, with higher rates when the plant's demands are higher. Field experiments were performed for four consecutive years in Londrina, state of Paraná, Brazil, with soybean growing under different plant densities, evaluating parameters of BNF, plant nutrition, grain yield and quality. In the first trial, four densities were evaluated, ranging from 40,000 to 320,000 plants ha^{-1}. Under low densities, photosynthetic and BNF rates per plant were stimulated. Similar yields were obtained for the different plant densities, with decreases only for the very low density of 40,000 plants ha^{-1}, also the only treatment with differences in seed protein and oil contents. In the following three crops, evaluations were performed in densities ranging from 80,000 to 320.000 plans ha^{-1}, and the results from the first year were confirmed. In these three field experiments, although plant density was reduced by up to 75%, yield was decreased in only one out of three cropping seasons, by 16%. These results indicate high plasticity in soybean to adapt photosynthesis and BNF to different densities. Furthermore, planting at lower densities has the advantages of lower input costs and less susceptibility to environmental and nutritional stresses.

Kewords: Plant density. Nodulation. Nitrogen nutrition. Oil. Protein.

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1 INTRODUÇÃO

A crescente demanda mundial por alimentos tem resultado em pressões para o incremento na produção de grãos, o que, por sua vez, tem repercutido com frequência em impactos ambientais que afetam a sustentabilidade agrícola. O nitrogênio (N) representa o nutriente exigido em maior quantidade para o incremento na produção de grãos e, no caso da soja [Glycine max (L.) Merr.], a leguminosa mais cultivada no mundo, o teor elevado de proteína nos grãos resulta em uma demanda por N bastante importante, estimada em cerca de 80 kg de N para cada 1.000 kg de grãos produzidos. Em maior ou menor proporção, esse N pode ser fornecido pelo processo de fixação biológica de nitrogênio atmosférico (FBN) (HUNGRIA et al., 2005; HUNGRIA et al., 2006a; HUNGRIA et al., 2006b). O cenário agrícola no Brasil é muito favorável, a produção nacional de grãos estimada para safra 2013/14 é de 185,0 milhões de toneladas (t), dos quais 86,08 milhões de t correspondem à soja, com uma área estimativa de plantio de 30 milhões de há., (CONAB, 2013); colocando a Brasil como o segundo maior produtor mundial.

Contudo, o país importa a maior parte dos fertilizantes químicos, sendo mais de 70% do N, com custos e logística críticos para a produção agrícola. Consequentemente, a viabilidade econômica da cultura da soja está diretamente relacionada a taxas elevadas de FBN - resultado de mais de cinco décadas de melhoramento vegetal e seleção de estirpes de Bradyrhizobium - que, para os patamares atuais de rendimento de grãos, da ordem de 3.000 a 4000 kg ha^{-1}, pode contribuir com taxas de 300 kg de N ha^{-1} ou superiores, representando 90% ou mais das necessidades de N da planta (HUNGRIA et al., 2005; HUNGRIA et al., 2006a; HUNGRIA et al., 2006b; HUNGRIA, CAMPO; MENDES, 2007). O potencial genético da soja, inicialmente estimado em 8.000 kg ha^{-1} (SPECHT; HUME; KUMUDINI, 1999), já foi superado, com relatos de agricultores norte-americanos produzindo mais de 10.000 kg ha^{-1} em competições “Kip Cullers” (KIP..., 2010). Esses altos patamares de rendimento, porém, ainda representam um grande desafio para o Brasil e para a América do Sul em geral, e implicam na necessidade de incrementar em mais de três vezes o aporte atual da FBN.

Dentre os fatores críticos para obter altos rendimentos, existe um interesse renovado em arranjos diferenciais na densidade de plantas. Hoje, altas densidades de plantas são recomendadas, de até 400.000 plantas/ha (EMBRAPA, 2011), ou mesmo superiores (NATONAL SOYBEAN RESEARCH LABORATORY, 2012). Contudo, em menores densidades pode haver melhor distribuição das plantas, reduzindo a competição por água, nutrientes e luz solar e aumentando a radiação interceptada pela cultura (ANDRADE et al., 2002), o que pode conduzir a melhores rendimentos, particularmente frente aos crescentes relatos de períodos prolongados de seca (BLUMENTHAL; QUACH; SEARLE, 1988; ANDRADE et al., 2002). O conhecimento atual sobre os efeitos de diferentes densidades de cultivares com alto potencial genético na FBN, porém, ainda é incipiente.

Para maximizar a eficiência no uso da radiação solar incidente é necessário ajustar a distância entre as linhas de uma cultura e o número de plantas por linha. Maiores taxas de crescimento da cultura (TCC) podem ser atingidas em espaçamentos que permitam interceptar 95% da radiação fotossinteticamente ativa no momento do início da frutificação ou, o mais tardar, no início do crescimento linear das sementes (R5) (BOARD; HARVILLE; SAXTON, 1990; BOARD; KAMAL; HARVILLE, 1992). Cabe mencionar, ainda, que cerca de 87% das vagens da soja abortam antes de alcançarem 2 cm de comprimento e incrementos de luz na parte inferior do dossel, onde a radiação é baixa e a abscisão de flores e vagens é elevada, resultam em um aumento na retenção de vagens e na produção de soja (STOCKMAN; SHIBLES, 1986). Finalmente, existem efeitos qualitativos, pois há relatos de que a relação infravermelho/vermelho afeta a ultraestrutura dos cloroplastos, a partição de carboidratos para as células, a eficiência fotossintética e a concentração de vários metabólitos (KASPERBAUER, 1987), bem como a nodulação e a FBN (LIE, 1964, 1969).

Na simbiose estabelecida entre bactérias do gênero Bradyrhizobium e a soja, a bactéria supre a planta hospedeira com N como produtos da FBN, tais como aminas (asparagina) e ureídos (alantoína e ác. alantóico), que são exportados para a planta via xilema (SERRAJ; SINCLAIR; PURCELL, 1999) e, em troca, são fornecidos à bactéria substratos de carbono (C) provenientes da fotossíntese, requeridos como fonte de energia para o processo biológico e como moléculas receptoras do N reduzido (NEVES; HUNGRIA, 1987; WILLIAMS; DEJONG; PHILLIPS, 1982). Consequentemente, as interações entre densidade de plantas, radiação solar e fotossíntese, além de regularem o crescimento das plantas e o rendimento das culturas, são críticas para o processo de FBN.

Geralmente, se aceita que qualquer limitação no fornecimento de fontes de carbono (C) para o nódulo por fatores ambientais adversos, como seca, por exemplo, pode estar associada com diminuição na atividade da nitrogenase. Várias enzimas envolvidas no metabolismo do C em nódulos diminuem sua atividade juntamente com a inibição da atividade da nitrogenase por fatores abióticos. Uma delas é a sacarose sintase em nódulos, que resultou no incremento no teor de sacarose, correlacionando com uma queda na atividade da

FBN, aumentando a concentração de ureídos nos nódulos, bem como nos tecidos da raiz e do caule (SERRAJ; SINCLAIR; PURCELL, 1999).

Recentemente, Collier e Tegeder (2012) confirmaram que na exportação dos ureidos dos nódulos para a parte aérea, estão envolvidas proteínas transportadoras de membrana localizadas no córtex interno e endodermes vascular do nódulo denominadas UPS1. Quando estes transportadores são inibidos resulta num acúmulo de ureidos no interior dos nódulos, diminuindo a fixação de N_2.

Streeter (2003) observou, em plantas de soja em condições de seca, uma queda de 30-40% no acumulo de N nas folhas e vagens de plantas estressadas em relação às plantas controle, indicando uma diminuição acentuada na fixação biológica de nitrogênio. Ainda nesse ensaio, houve 50% de incremento no acúmulo de polissacarídeos bacterianos em nódulos e diminuição na atividade de FBN, indicando que o impacto negativo sobre a atividade de nódulos não foi causada por uma queda no fornecimento de C para os bacteroides. O tratamento com seca resultou em incremento estatisticamente significativo na concentração de N nas folhas e vagens, o que indica que não houve deficiência de N. Em plantas sob condições de seca observaram-se reduções no crescimento, mas considerando que a concentração de N em outros tecidos não diminuiu, o autor sugere que o impacto negativo da seca sobre a atividade do nódulo não foi a causa da redução do crescimento. No final do período de seca, a concentração de compostos de C, aminoácidos e ureídos foram significativamente maiores nos nódulos das plantas sob estresse hídrico. Esses resultados indicam que, sob condições de seca, a atividade de FBN nos nódulos pode ser limitada porque a demanda por N da FBN para suportar o crescimento das plantas é baixa.

No contexto acima mencionado, pode-se sugerir que incrementando a demanda de compostos de N, tais como o aumento do número de grãos por planta haveria uma redução no efeito de “feedback” negativo que os ureídos exercem sobre a atividade da nitrogenase aumentando, portanto, a FBN em soja. Para aumentar o número de grãos por planta deve ocorrer uma diminuição do aborto floral, muito comum em soja, ou aumentar o número de flores.

Considerando os rendimentos cada vez mais elevados de novas cultivares de soja, mas também o incremento na frequência de estresses bióticos e abióticos, torna-se essencial a procura constante por novas tecnologias abordando diferentes estratégias. Como exemplo, a seleção de novas estirpes de Bradyrhizobium visando incrementar a eficiência do processo de FBN, bem como de plantas e tecnologias que assegurem maiores taxas de FBN, resultando em uma agricultura com altos rendimentos.

As raízes das plantas são reconhecidas como a fonte dos sinais que influenciam as respostas fisiológicas das partes aéreas. Em um experimento conduzido por Gan et al. (2002) com o objetivo estudar o efeito da densidade de semeadura em diferentes genótipos de soja sobre a produção e partição da biomassa, entre outros aspectos, foi constatado que em uma cultivar de tipo de crescimento indeterminado a diminuição de plantas por unidade de superfície produziu alterações no quociente parte aérea/raiz, sendo de 5,2 em uma densidade padrão e 2,9 em densidade quatro vezes inferior, indicando que a partição de biomassa para raiz foi maior no último caso, ou seja, um menor número de plantas por m^2 favorece a massa radicular. Uma explicação para esses resultados pode ser a de que, sob condições controladas, o vermelho e o infravermelho atuam através do sistema fitocromo para regular a partição da massa seca entre a parte aérea e as raízes (KASPERBAUER, 1987). Espera-se que, como consequência do aumento da massa radicular, a absorção de nutrientes também seja alterada, além de resultar em maior número de sítios na raiz para a formação de nódulos.

Outro efeito conhecido das baixas densidades de plantas é que ocorre um incremento no fornecimento de luz na parte inferior do dossel de plantas, onde normalmente a radiação é baixa e a abscisão de flores e vagens pequenas é alta, resultando em um aumento na retenção de vagens e na produção da cultura de soja (JOHNSTON et al., 1969). Além disso, também pode ocorrer uma alteração na partição de proteína, óleo e ácidos graxos para os grãos, dependendo da posição dos mesmos ao longo do caule principal da planta, diferindo entre cultivares e com a intensidade da luz (PROULX; NAEVE, 2009; BELLALOUI; GILLEN, 2010). As concentrações de proteína e ácido oleico são maiores nos grãos produzidos nos nós superiores, enquanto que nos nós inferiores as concentrações de óleo e ácido linoleico são mais elevadas. Baseados nestes trabalhos, poder iam-se esperar mudanças nas concentrações de óleo e proteína nos grãos como consequência de alterações nas densidades de semeadura.

1.1 HIPÓTESE

Em condições de incremento na disponibilidade de luz por planta, como resultado de menores densidades, ocorre um incremento na taxa fotossintética, que, por usa vez, resulta em maior demanda de nitrogênio, suprida pelo incremento nas taxas de FBN. Desse modo, é possível que rendimentos semelhantes sejam obtidos em soja sob diferentes densidades, sem alterações na qualidade dos grãos.

1.2 OBJETIVO GERAL

Determinar a capacidade da fixação biológica do nitrogênio em soja, semeada em diferentes densidades, em fornecer o nitrogênio necessário para sustentar altas produções de grãos sem afetar sua qualidade.

1.3 OBJETIVOS ESPECÍFICOS

a) Esclarecer o efeito da densidade, considerando tanto o distanciamento entre linhas de semeadura como o número de plantas por metro, em parâmetros relacionados à fixação biológica do nitrogênio e à nutrição das plantas;
b) Determinar componentes do rendimento dos grãos de soja sob diferentes densidades;
c) Determinar a qualidade dos grãos em termos de teores de proteína e óleo.

2 ARTIGO 1

Plant densities and modulation of symbiotic nitrogen fixation in soybean

Abstract: Soybean nitrogen (N) demands can be supplied to a large extent via biological nitrogen fixation, but the mechanisms of source/sink regulating photosynthesis/nitrogen fixation in high yielding cultivars and current crop management arrangements need to be investigated. We investigated the modulation of symbiotic nitrogen fixation in soybean [*Glycine max* (L.) Merrill] under different plant densities. A field trial was performed in southern Brazil with six treatments, including non-inoculated controls without and with N-fertilizer, both with 320,000 plants ha^{-1}, and plants inoculated with *Bradyrhizobium elkanii* at four densities, ranging from 40,000 to 320,000 plants ha^{-1}. Differences in nodulation, biomass production and N accumulation and partition were observed at stage R5, but not at stage V4, indicating that quantitative and qualitative factors (such as sunlight infrared/red ratio) assume increasing importance during the later stages of plant growth. Decreases in density in the inoculated treatments stimulated photosynthesis and nitrogen fixation per plant. Similar yields were obtained for the different plant densities, with decreases only for the very low density of 40,000 plants ha^{-1}, also the only treatment with differences in seed protein and oil contents. Results confirm a fine tuning of the mechanisms of source/sink, photosynthesis/nitrogen fixation under lower plant densities. Higher photosynthesis and nitrogen fixation rates are capable of sustaining increased plant growth.

Introduction

Soybean [Glycine max (L.) Merrill] is an important agribusiness commodity globally. In Brazil, this legume is produced on 30 million hectares, including remote areas; yet it persists as one of the most profitable crops, mainly because its nitrogen (N) requirements are met by symbiotic nitrogen fixation (HUNGRIA et al., 2005; HUNGRIA et al., 2006a; HUNGRIA et al., 2006b). In the soybean-Bradyrhizobium symbiosis, the plant supplies the bacteria with photosynthates (C) via phloem and receives N from fixation via xylem (WILLIAMS; DEJONG; PHILLIPS, 1982; NEVES; HUNGRIA, 1987). Symbiotic nitrogen fixation consumes 6–12 g C g^{-1} of fixed N, representing about 20–30 % of the total plant photosynthesis; however, this strong sink for C does not necessarily reduce yield, because it may modulate source activity (photosynthesis) (KASCHUK et al., 2009; KASCHUK et al., 2010b; KASCHUK et al., 2012).

Soybean plant densities of 400,000 plants ha^{-1} (EMBRAPA, 2011) or even higher (NSRL, 2012) are recommended. However, at lower densities, interplant competition for water, nutrients and light could be mitigated (BLUMENTHAL; QUACH; SEARLE, 1988; ANDRADE et al., 2002). Conversely, under high densities shaded leaves may not contribute to canopy photosynthesis (BOARD; HARVILLE; SAXTON, 1990; BOARD; KAMAL;

HARVILLE, 1992), and will likely senesce and/or be susceptible to diseases (PONS; PEARCY, 1994). Furthermore, the lack of light penetration to deeper layers of the canopy may decrease yields (STOCKMAN; SHIBLES, 1986). Finally, changes in the red/infrared ratios through the canopy may deeply affect both photosynthesis (KASPERBAUER, 1987), and the onset of nodule formation (LIE, 1969).

Symbiotic nitrogen fixation is an overwhelming sink for photosynthate and can compete with other destinations, such as grains (NEVES; HUNGRIA, 1987; KASCHUK et al., 2009; KASCHUK et al., 2010b). However, plants can increase photosynthesis rates to support increasing sinks (PAUL; FOYER, 2001; KASCHUK et al., 2009; KASCHUK et al., 2012). It remains to be determined to what extent source/sink relationships can be up-regulated. In addition to the C requirements for nitrogen fixation and yield, soybean photosynthesis has to supply high C sinks from the seeds to lipid and protein accumulation (PENNING DE VRIES; BRUNSTING; VAN LAAR, 1974; KASCHUK et al., 2010b).

In this study we report results from a field experiment aiming at determining the effects of plant density on nitrogen fixation, yield, plus lipid and protein contents of soybean in connection with the relationship between source activity and sink strength. The hypothesis was that different plant densities might not affect grain yield and quality, and as a “compensatory mechanism” leads to increases in both photosynthesis and nitrogen fixation rates.

Materials and Methods

Field site description

The experiment was performed in the summer season of 2009/2010 in Londrina, state of Paraná (PR), Brazil (23º11′ S; 51º11′ W, 620 m a.s.l.). The soil is classified as Latossolo Vermelho Distroférrico (Brazilian classification system; Typic Haplustox, USA taxonomy). The average annual temperature in Londrina is 21 ºC, with an average maximum of 28.5 ºC in Feb and a minimum of 13.3 ºC in July. Average annual rainfall is 1,651 mm, with 123 days of rainfall per year; maximum rainfall occurs in the summer (Jan-Mar) and minimum in winter (June-Aug). According to Köeppen’s classification, the climate in Londrina is subtropical humid (Cfa: humid, subtropical, with hot summers). Daily average temperature and daily rainfall during the experiment and their averages between 1998-2012, are shown in Figure 1.

Lime had most recently been applied to the area in 2008. Chemical analysis of the soil (0-20 cm layer, samplings made 30 days before sowing) resulted in the following characteristics immediately before sowing: pH ($CaCl_2$ 0.01 mol L^{-1}), 5.21; H+Al, 37.1 ($mmol_c$ dm^{-3}); Al, 0.7 ($mmol_c$ dm^{-3}); P, 13.43 (mg dm^{-3}): K, 6.4 ($mmol_c$ dm^{-3}); C, 18 g dm^{-3}; Ca+Mg, 75.6 ($mmol_c$ dm^{-3}); base saturation, 69 %. The soybean bradyrhizobia population in the soil was estimated by the most probable number (MPN) method using soybean plants (VINCENT, 1970).

Treatments, experimental design and crop management

Before sowing, the soil was prepared with the traditional practices of ploughing and disking. At sowing, the area received 300 kg ha^{-1} of fertilizer of formulation 0-20-20. The commercial cultivar BRS 133 (genealogy: FT Abyara × BR83-147; maturity group 7.3, determinate type of growth) was sown on the 4th of November of 2009.

The experiment consisted of six treatments: T1) Non-inoculated control, with 50 cm between sowing lines and 16 plants m^{-1} (0.5 × 16 pl) (320,000 plants ha^{-1}) (C); T2) Non-inoculated control + N-fertilizer (200 kg N ha^{-1}, as urea, 50 % applied at sowing and 50 % at R2, broadcast, sown at 0.5 × 16 pl) (320,000 plants ha^{-1}) (C + N); T3) Inoculated, with 50 cm × 4 plants per linear meter (0.5 × 4 pl.) (80,000 plants ha^{-1}); T4) Inoculated, with 50 cm × 16 plants (0.5 × 16 pl) (320,000 plants ha^{-1}); T5) Inoculated, with 1.0 m between lines and 4 plants per linear meter (1.0 × 4 pl) (40,000 plants ha^{-1}); T6) Inoculated, with 1.0 m between lines and 16 plants per linear meter (1.0 × 16 pl.) (160,000 plants ha^{-1}). Each plot measured 4 m in width by 6 m in length. The experiment had a completely randomized block design, with six replicates.

Seeds were not treated with fungicides or insecticide. Inoculation in treatments 3 to 6 consisted of adding peat inoculant (10^9 CFU g^{-1}) containing B. elkanii commercial strains SEMIA 587 and SEMIA 5019 (= 29W). A 10 % sugar solution was used as adhesive and the inoculant was applied to supply a theoretical concentration of 1.2 million cells $seed^{-1}$, following the technical recommendation for the crop in Brazil (HUNGRIA; CAMPO; MENDES, 2007; EMBRAPA, 2011). At V4 stage all treatments were sprayed with 20 g ha^{-1} of Mo, as also recommended (HUNGRIA; CAMPO; MENDES, 2007; EMBRAPA, 2011). The following products were used: herbicides Clorimuron (50 g ha^{-1}) and Cletodim (0.4 L ha^{-1}); insecticides Diflubenzuron (80 g ha^{-1}), Thiametoxam + Lambdacihalotrina (200 cc ha^{-1}). Rainfall provided moisture as shown in Figure 1.

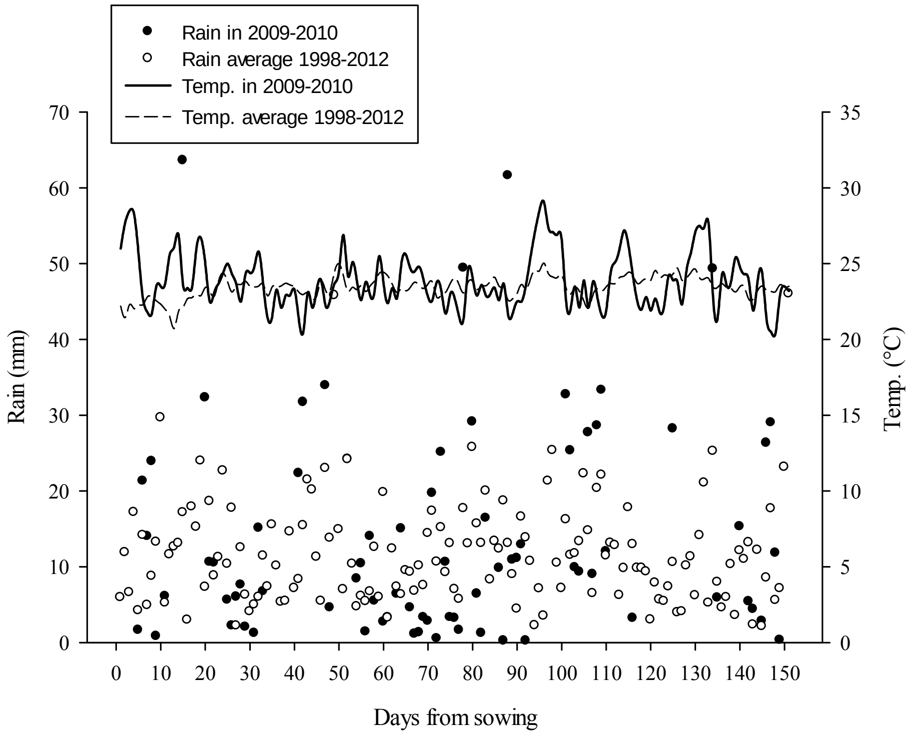

Figure 1 - Daily average temperature and daily rainfall during the experiment in 2009-2010 and their averages between 1998-2012.

Soil sampling, harvest, plant analyses and statistics

Samplings were performed at three growth stages (FEHR et al., 1971): V4 (four unfolded trifoliolate leaves), R5 (seeds are 3 mm long in the pod at one of the four uppermost nodes on the main stem) and R8 (full maturity). At stages V4 and R5, eight plants per replicate were randomly harvested, excluding the central area (8 m^2) of the plot determined to be used for yield evaluation. Harvesting of plants was carefully performed with a shovel to include most of the root system and verifying falling nodules; the whole plant was taken to the laboratory. Procedures at the laboratory to evaluate nodulation, shoot dry weight and total N in tissues were made as described before (HUNGRIA et al., 2006b). At V4 and R5, the evaluated parameters were nodulation (nodule number and dry weight), and shoot dry weight, while the dry weight of leaflets (presented apart from the whole shoot dry weight) was evaluated only in R5. Nitrogen content was evaluated by Kjeldahl's digestion at V4 in shoots (leaves + stems) and at R5 in shoots and in pods.

At the final harvest (8 m^2 harvested in the central part of each replicate), the parameters estimated were number of plants m^{-2}, yield (corrected to 13 % of moisture), dry weight of 100 grains (also corrected to 13 % of moisture), number of grains $plant^{-1}$, and N and oil contents of the grains. Oil content in grains was determined in milled seeds in a

Soxhlet extractor, using n-hexane as the solvent and following the methodology of Zenebon, Pascuet and Tigela (2008).

The data were analyzed using the statistical Infostat software (DI RIENZO et al., 2009). All assumptions required by the analysis of variance were verified. Means were analyzed using Fisher's test at $p<0.05$.

Results

The soil presented a high population of soybean bradyrhizobia, estimated at 2.871×10^4 CFU g^{-1} soil (MPN method). The naturalized bradyrhizobial population produced good nodulation [nodule number per plant (NN), dry weight per nodule (DWN) and nodule dry weight per plant (NDW)] even in the non-inoculated control (T1) (Table 1). Inhibitory effects of chemical N-fertilizer (T2) on nodulation parameters were clearly observed at V4 and R5 stages (Table 1).

In the comparison of the non-inoculated treatment (T1) with the inoculated treatment of the same plant density of 320,000 plants ha^{-1} (T4), there was an increase in dry weight per nodule ($p < 0.0001$) at V4 (Table 1), associated with large nodules at the root crown. At R5, no differences were detected in the comparison of the inoculated treatments with densities of 0.5 × 4 pl (T3) and 1.0 × 4 pl (T5), but these two treatments were superior ($p < 0.0001$) to the treatments with 0.5 × 16 pl (T4) and 1.0 × 16 pl (T6), both in nodule number and in dry weight parameters (Table 1). In addition, higher leaf production was observed in both treatments with 4 plants per linear meter (T3 and T5) (Figure 2).

Table 1 - Effects of plant density on nodulation [nodule number, NN; dry weight per nodule, DWN; nodule dry weight, NDW; plant growth (shoot dry weight, SWD); nitrogen absorbed (N abs) by soybean cultivar BRS 133 at the V4 and R5 stages. Experiment performed on an Oxisol with established population of bradyrhizobia (≥ 10^4 cells g^{-1}).

Inoculation[†]	Spacing[‡]	Density	NN	DWN	NDW	SDW	NN	DWN	NDW	SDW	N abs
			---------------- V4			--------------------	------------------------- R5				----------------------
		pl ha^{-1}	n° pl^{-1}	mg nod^{-1}	mg pl^{-1}	g pl^{-1}	n° pl^{-1}	mg nod^{-1}	mg pl^{-1}	g pl^{-1}	mg N mg nod^{-1} pl^{-1}
T1-Control	0.5 × 16	320,000	25.3 a[§]	4.3 b	110 a	2.09 b	79.7 bc	6.5 b	520 b	28.48 c	2.71 ab
T2-Control + N	0.5 × 16	320,000	16.5 b	2.6 c	40 c	2.62 ab	60.6 c	4.9 c	300 c	29.68 c	
T3-Inoculated	0.5 × 4	80,000	25.1 a	4.1 b	100 ab	2.91 a	171.4 a	7.8 a	1340 a	72.90 a	2.77 ab
T4-Inoculated	0.5 × 16	320,000	26.4 a	5.0 a	130 a	2.60 ab	89.4 b	6.2 b	540 b	36.04 bc	2.26 b
T5-Inoculated	1.0 × 4	40,000	24.2 a	4.0 b	100 ab	3.15 a	152.2 a	7.8 a	1180 a	84.58 a	3.56 a
T6-Inoculated	1.0 × 16	160,000	21.5 ab	4.3 b	90 b	2.58 ab	99.7 b	6.7 b	670 b	45.69 b	3.41 a

[†] Non-inoculated control, with or without N fertilizer (200 kg of N ha^{-1}, split 50 % at sowing and 50 % at flowering); and inoculation with *B. elkanii* strains SEMIA 587 + SEMIA 5019, at the rate of 1.2 × 10^6 cells seed^{-1}; [‡] Distance between lines (m) × number of plants per linear meter; [§] Means (n = 6) within a column followed by different letters are different ($p \leq 0.05$, Fisher's test).

Shoot dry weight (SDW) values are also shown in Table 1. Similarly to the nodulation data, at V4 differences between inoculated treatments under different plant densities were not significant, whereas, at R5 the same treatments, 0.5 × 4 pl (T3) and 1.0 × 4 pl (T5), were similar and higher than the treatments 0.5 × 16 pl. (T4) and 1.0 × 16 pl (T6) (Table 1).

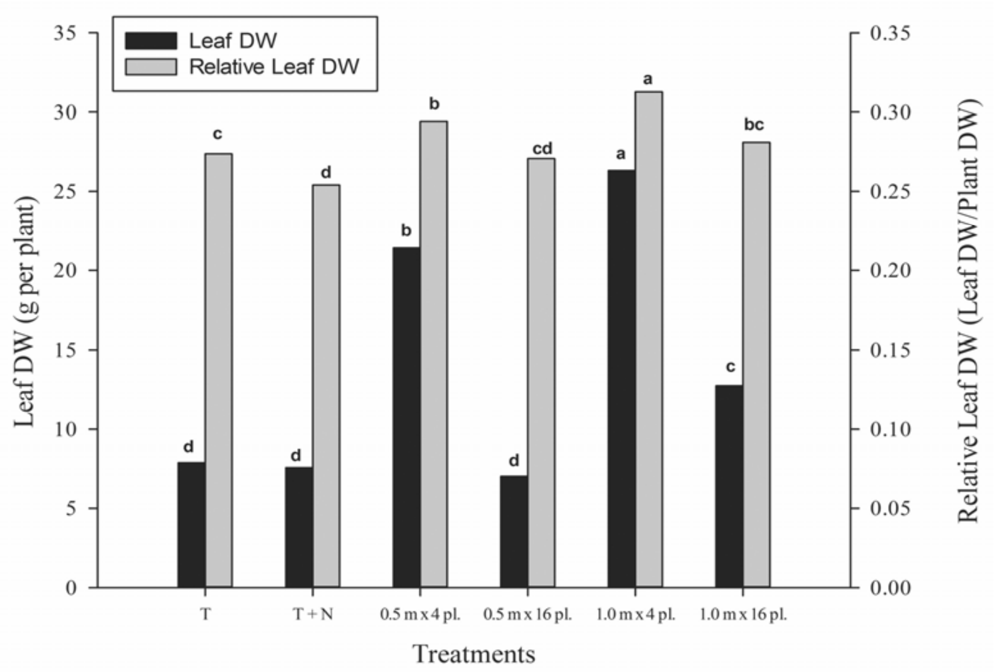

Figure 2 - Dry weight (DW) of leaflets per plant and relative dry weight of leaflet/total shoot dry weight at R5, as a function of plant density. Treatments: C, non-inoculated control; C + N, non-inoculated control + 200 kg of N ha^{-1}, both with plant density of 0.5 m × 16 pl linear m^{-1}. All other treatments were inoculated with *Bradyrhizobium* and sown at densities of 0.5 m × 4 pl, 0.5 m × 16 pl, 1.0 m × 4 pl and 1.0 m × 16 pl. Different letters indicate differences ($p \leq 0.05$, Fisher's test).

Patterns of N accumulation in shoots and pods at R5 (Figure 3) were similar to the results for nodulation and plant biomass production (Table 1). In Figure 3, emphasis should be given to the values of N in pods at R5, which confirm the superiority of the inoculated treatments with lower numbers of plants per meter, 0.5 × 4 pl and 1.0 × 4 pl. It was also possible to establish a linear relationship between the parameters of plant biomass and N accumulation in plants both at V4 ($R^2 = 0.95$) and at R5 ($R^2 = 0.99$) (Figure 4). However, the distribution of N in different organs was variable at R5 because, as already mentioned, the N content of the pods was higher in treatments with fewer plants per meter (Figure 3).

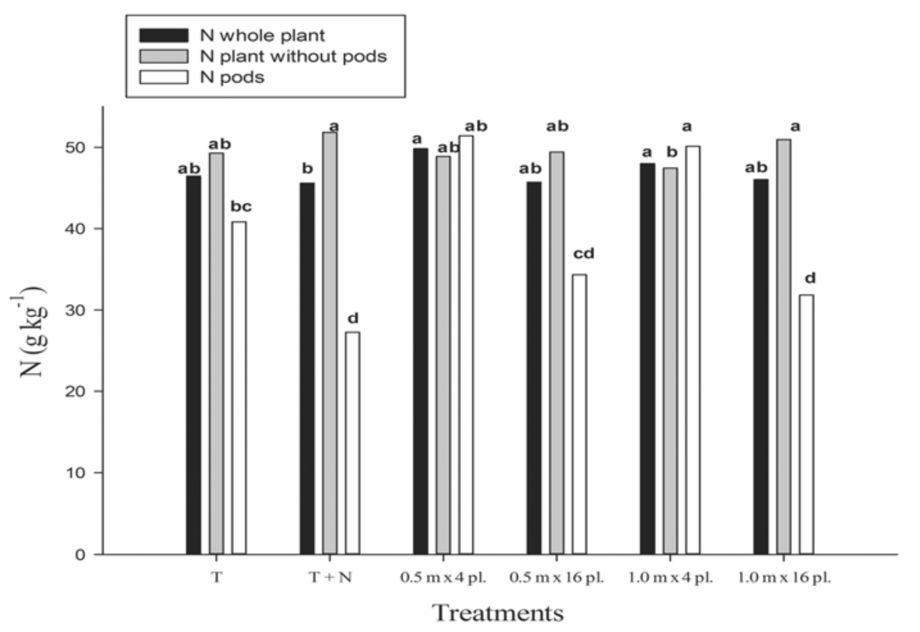

Figure 3 - Nitrogen (N) content per kg of dry weight at R5, considering the whole plant, the plant without the pods and only the pods. Treatments as described in Figure 2. Different letters indicate differences ($p \leq 0.05$, Fisher's test).

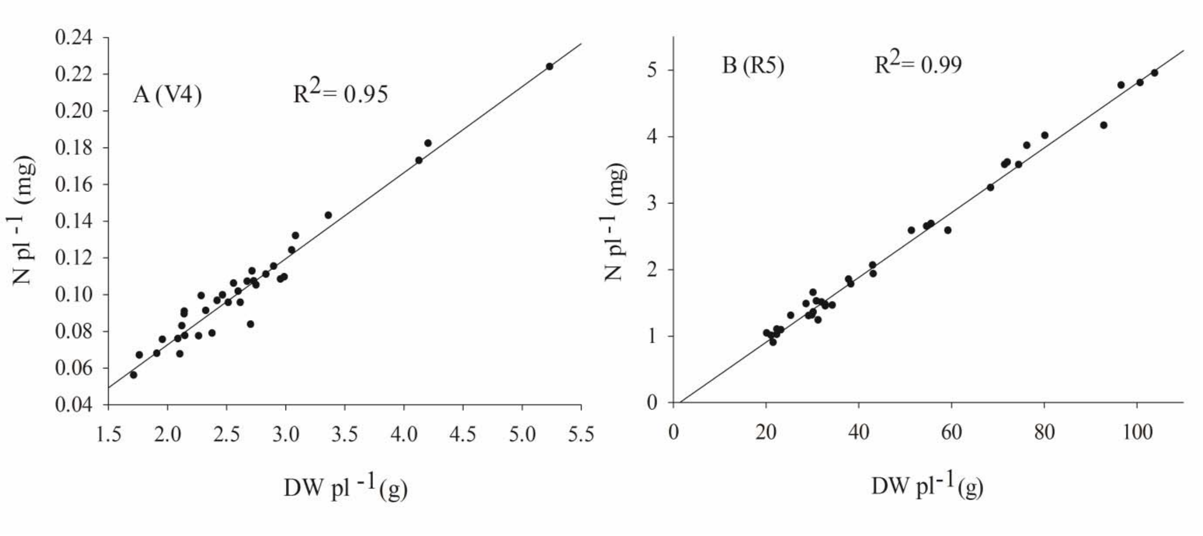

Figure 4 - Linear regression at V4 (A) and R5 (B) growth stages between shoot dry weight per plant, DW pl^{-1} (g); and N content per plant, N pl^{-1} (mg) of the plants from all treatments.

The highest yield was observed in the inoculated treatment with 0.5 × 16 pl (T4), followed by the non-inoculated control receiving N fertilizer at the same density (T2) and T3 and T6 (Table 2). Only at the lower density (T5) a significant decrease in total grain production was observed.

Table 2 - Effects of plant density on grain yield parameters of the soybean cultivar BRS 133.

Inoculation[†]	Spacing[‡]	Density[§]	Density[¶]	Yield	100 grains	No. grains	Oil content	Protein content
		------- pl ha^{-1} -------		kg ha^{-1}	g	n^{o} pl^{-1}	------- g kg^{-1} -------	
T1-Control	0.5 × 16	320,000	361,700	3,160 ab[††]	14.4 b	82.9 d	230.2 ab	361.3 a
T2-Control + N	0.5 ×16	320,000	328,400	3,240 a	14.5 b	87.6 d	229.8 ab	358.4 ab
T3-Inoculated	0.5 × 4	80,000	87,700	3,156 ab	16.2 a	277.4 b	227.5 ab	362.9 a
T4-Inoculated	0.5 ×16	320,000	361,900	3,334 a	14.4 b	76.6 d	216.7 b	361.5 a
T5-Inoculated	1.0 × 4	40,000	42,100	2,137 c	16.3 a	376.2 a	232.8 a	354.6 b
T6-Inoculated	1.0 ×16	160,000	177,100	3,004 b	14.5 b	135.8 c	228.1 ab	357.1 ab

[†] Non-inoculated control, with or without N fertilizer (200 kg of N ha^{-1}, split 50 % at sowing and 50 % at flowering); and inoculation with *B. elkanii* strains SEMIA 587 + SEMIA 5019, at the rate of 1.2 × 10^{6} cells seed^{-1}; [‡] Distance between lines (m) × number of plants per linear meter; [§] Theoeretical value; [¶] Evaluation at harvest time, based on number of plants m^{-2}; [††] Means (n = 6) from a same column followed by different letters are different ($p \leq 0.05$, Fisher's test).

Finally, the number of grains per plant and the 100-grain weight in the inoculated treatments with lower number of plants per meter [0.5 × 4 pl (T3) and 1.0 × 4 pl (T5)] were higher than at the other densities ($p < 0.0001$) (Table 2). Significant correlations were found between the 100-grain weight parameter with nodule weight ($p \leq 0.0001$), leaflets ($p \leq 0.0001$), and pods ($p \leq 0.0004$) (data not shown). However, no differences were observed on oil and protein contents between treatments, except in the extreme case of 40,000 pl ha^{-1} (Table 2).

Discussion

Soybean is exotic to Brazil, where the soils were originally devoid of compatible rhizobial strains capable of nodulating the legume (SANTOS; VARGAS; HUNGRIA, 1999; FERREIRA; HUNGRIA, 2002; HUNGRIA et al., 2006a). However, the site where the experiment was conducted had been cropped for more than 20 years with soybean in the summer, always receiving inoculants containing soybean bradyrhizobia. Consequently, the high naturalized bradyrhizobia population resulted in good nodulation even in the non-inoculated control, but N-fertilizer clearly inhibited nodulation. The results from our study confirm previous reports in Brazilian soils (MENDES; VARGAS; HUNGRIA, 2004; HUNGRIA et al., 2006a; HUNGRIA et al., 2006b; HUNGRIA; CAMPO; MENDES, 2007; MERCANTE et al., 2011) that reinoculation (inoculation every year) with elite strains can improve nodulation, nitrogen fixation rates and grain yield in soybean.

When the naturalized population was compared to the inoculation in plants under the same density of 320,000 plants ha^{-1}, there was an increase in dry weight per nodule at V4 (Table 1), associated with large nodules at the root crown. At R5, no differences in nodulation were detected in the comparison of treatments differing in the distance between rows (0.5 × 4 pl and 1.0 × 4 pl), but on average, passing from 16 to 4 plants per linear meter in the inoculated treatments increased nodule dry weight at R5 by 108 %. The higher nodulation at R5 in the treatments with lower numbers of plants per linear meter, with no effect of the distance between planting rows could be explained by the higher driy leaflets weight (leaf blade without petiole) per plant, as well as by the partitioning of this plant biomass, consisting of a higher leaf production in both treatments with 4 plants per linear meter, implying in greater availability of photosynthates. In consequence, there might be greater availability of root exudates capable of promoting rhizobial growth in the rhizosphere, as well as of C sources for the formation and functioning of the nodules, maintaining higher nitrogen fixation rates. Furthermore, the increased availability of C skeletons per plant would also facilitate the transport of ureides, the major nitrogenous compounds with low C:N ratio synthesized in soybean nodules (NEVES; HUNGRIA, 1987; HUNGRIA et al., 2006b). Altogether, these results provide strong evidence for the source/sink links between photosynthesis and nitrogen fixation (NEVES and HUNGRIA, 1987; KASCHUK et al., 2009; KASCHUK et al., 2012).

The results obtained for biomass accumulation (SDW) and the patterns of N accumulation in tissues were similar to those reported for nodulation. These results indicate that plant biomass production at R5 was more affected by the number of plants per meter than by the distance between rows. Indeed, at R5, in the inoculated treatments, there was an average increase in SDW of 93 % in treatments with 4 plants per linear meter, when compared with 16 plants per meter.

Besides the mechanisms of source/sink, another explanation for the higher nodulation and plant biomass in treatments with 0.5 × 4 pl and 1.0 × 4 pl might be related to qualitative differences in light, particularly in terms of the infrared/red relationship. In a study about the absorption, reflection and transmission of light from individual leaves of soybean, it has been reported that the majority of the blue and of the red are absorbed, whereas much of the infrared is reflected or transmitted (KASPERBAUER, 1987). Consequently, plants that grow in fields with little space between lines, or otherwise with high plant densities, receive higher ratios of infrared/red than those growing with greater distances between rows, or otherwise at lower densities (KASPERBAUER, 1987). In turn, the infrared/red ratio influences various parameters of plant development, such as ultra-structure of the chloroplasts, the partitioning of carbohydrates to cells, photosynthetic efficiency, concentration of several metabolites, and partitioning between shoots and roots (KASPERBAUER; HAMILTON, 1984; KASPERBAUER; HUNT; SOJKA, 1984; KASPERBAUER, 1987). Furthermore, there is also evidence of the control of nodulation by the phytochrome system, similarly favored by red light and inhibited by infrared radiation (LIE, 1969).

Regarding the efficiency of the nodules, treatments with 40,000 and 160,000 pl ha^{-1} presented greater values of N per unit of nodule dry weight than plants with 320,000 pl ha^{-1}, probably due to increased supplies of photosynthates. Nodule dry weight was not correlated with plant biomass, or with the N accumulated at V4, but was significantly correlated at R5 (both with $p < 0.0001$) (data not shown), indicating that increased nodule mass in low-density treatments was related to higher rates of nitrogen fixation. In contrast, Kapustka and Wilson (1990) found that an increase in soybean plant density reduced nodule number and dry weight per plant, but kept high specific activity per nodule, which resulted in the same values of nitrogen fixation per plant.

In R5, N content of the pods was higher in treatments with fewer plants per meter, what could be explained by the higher content of RuBisCO enzyme (ribulose-1,5-bisphosphate carboxylase oxygenase, E.C. number 4.1.1.39), which comprises about 50 % of the total protein content in leaves and is also present in the pods, and thus represents an important source of N for mobilization (SCHILTZ et al., 2004). The amount and activity of RuBisCO are directly related to light quality, being superior in red light compared to infrared light (ESKINS; JIANG; SHIBLES, 1991). Consequently, especially at advanced growth stages such as R5, one might assume that the content and activity of RuBisCO are higher in lower plant densities.

Crop yield data highlighted that higher yields were observed in the inoculated or N-fertilized treatments with 0.5 × 16 pl (320,000 pl ha^{-1}) and, surprisingly, neither differed from the inoculated treatment with 0.5 × 4 pl, corresponding to only 80,000 pl ha^{-1}. The yield at 80,000 pl ha^{-1} was also not different from that with 1.0 m × 16 pl (160,000 pl ha^{-1}); only at the lowest plant density, of 40.000 pl ha^{-1} there was a decrease ($p < 0.0001$) in total grain production. Consistent with these results, in an experiment conducted by Board (2000) in which three soybean densities were investigated, of 80,000, 145,000 and 390,000 pl ha^{-1}, yield was not affected by plant density, which was attributed to an equilibrium in the crop growth rate (CGR) at the beginning of the reproductive period, producing equivalent numbers of pods per square meter. Interesting, the results from our experiment were obtained under adequate precipitation, and it is possible that an even better performance could have been obtained under water stress conditions; as pointed out before, at lower densities interplant competition for water might be mitigated (BLUMENTHAL; QUACH; SEARLE, 1988; ANDRADE et al., 2002). Another important comment is that nowadays there is a pressure for increasing soybean plant densities aiming at getting higher yields; however, our results indicate that this might not be the best approach.

Both the number of grains per plant and the 100-grain weight in the inoculated treatments were higher in the spacing of 0.5 m. BRS 133 is a high-yield cultivar ($p < 0.0001$). These results indicate that, under favorable C/N source/sink conditions, it is possible to improve expression of the genetic potential of the cultivar. The lower number of plants per meter allowed the largest individual plant growth and higher photosynthetic rate per plant which, in turn, demanded a greater supply of N through biological fixation. On the other hand, at higher densities, photosynthetic rates per plant were lower, as were nitrogen fixation inputs per plant. These results are consistent with studies of photosynthetic rate reduction, in which manipulations of the source, such as shading and defoliation, resulted in reductions in the number and dry weight of grains per plant (PROULX; NAEVE, 2009; EGLI, 2010). In addition, with decreases in the supply of photosynthates (source) caused by the same treatments there are decreases in the rates of nitrogen fixation (NEVES; HUNGRIA, 1987).

In our experiment, shading did not change oil content between treatments ($p = 0.4977$), except in the extreme case of 40,000 pl ha^{-1}, which resulted in higher values. Proulx and Naeve (2009) observed that shading caused greater decreases than defoliation, whereas Butler, De Bruin and Pedersen (2010) found no differences in the linoleic acid content at densities ranging from 185,000 to 556,000 plants ha^{-1}. Altogether, these results indicate that in general neither protein nor oil content are affected by density, except at very low plant populations, as shown in our study, and probably at very high populations.

Conclusions

Our study confirm a fine tuning between the C/N, source/sink mechanisms, i.e. between photosynthesis and biological nitrogen fixation. Under lower plant densities photosynthetic rate per plant increased and, consequently, higher C supply to the nodules resulted in increases in nodulation and in nitrogen fixation rates. The number of plants per linear meter was a stronger factor than the distance between rows, especially at R5, indicating greater importance of source/sink mechanisms at later stages of plant growth, when quantitative and qualitative factors (e.g. infrared/red ratio) affecting light become decisive. We conclude that soybean has the potential to at least quadruple both photosynthesis and biological nitrogen fixation under lower plant densities. It is also worth mentioning the implications related to the cost of using four times more seeds and inputs, particularly pesticides, at sowing, as similar yields can be achieved with much lower plant densities than those recommended today, with important environmental and economic implications.

3 ARTIGO 2

Feasibility of lowering soybean planting density without compromising nitrogen fixation and yield

Abstract: Adjusting density can be critical to reducing inter-plant competition for water, nutrients and sunlight, and to increasing intercepted radiation, photosynthesis and biomass production. The objective of this study was to evaluate the effects of plant-population size on soybean nodulation, nutrition, yield and grain quality. Three field experiments were performed in southern Brazil with soybean cultivar BRS 284, of indeterminate growth type and maturity group 6.6, at 80,000 and 320,000 plants ha^{-1}. For N supply plants were dependent either largely on biological fixation of atmospheric nitrogen-with a naturalized population of *Bradyrhizobium* or inoculated with *Bradyrhizobium japonicum* strain CNPSo 2050-or largely on N-fertilizer-200 kg N ha^{-1}, split at sowing and at the R1 growth stage. The lower density of plants resulted in increments in nodulation parameters, but plant nutritional status-evaluated by the DRIS method (Diagnosis and Recommendation Integrated System)-in general was not affected. Seed oil content was increased by 3.4%, but protein decreased by 4.5% at the lower density. The N source affected nodulation, but not nutritional status or yield. Although plant density was reduced by 75%, yield was decreased in only one out of three cropping seasons, by 16%. These results indicate high plasticity in soybean to adapt photosynthesis and nitrogen fixation to different densities. Furthermore, planting at the lower density has the advantages of lower input costs and less susceptibility to environmental and nutritional stresses.

Keywords: Plant density, Nodulation, Nitrogen nutrition, Photosynthesis.

Introduction

On a dry-weight basis, about 40 percent of the grain of soybean [Glycine max (L.) Merrill] is composed of proteins, resulting in high demand of N by the plants, most of which can be provided by the biological nitrogen fixation process (HUNGRIA et al., 2006a; HUNGRIA et al., 2006b). A major step in the soybean-Bradyrhizobium symbiosis is the translocation of C-rich compounds, generated by photosynthesis, to provide energy and C skeletons for the synthesis of N-rich compounds in the root nodules that, in turn, are transported to the host plant shoot (WILLIAMS; DEJONG; PHILLIPS, 1982). Under reasonable cropping conditions, both photosynthesis and biological nitrogen fixation are reciprocally up-regulated to support soybean´s demand on C and N needs (KASCHUK et al., 2010a; KASCHUK et al., 2012), such that not only higher yields but also more grain protein are produced when legumes mostly rely on symbiosis (HUNGRIA et al., 2006a; KASCHUK et al., 2010b).

Light quality and quantity directly affect photosynthesis and impact yield. In soybean, the most critical stage is from flowering to the middle of the reproductive cycle or even later; high growth rates in this period increase the number of grains per plant and hence optimize crop yield (ANDRADE et al., 2002). From flowering on, if availability of light in the lower part of the canopy is low due to mutual shading, abscission of flowers and developing pods can be high, reducing yield (JOHNSTON et al., 1969). Qualitative changes in the ratio of red:infrared light through the canopy also affect photosynthesis (KASPERBAUER, 1987), regulating properties such as the ultrastructure of chloroplasts, the partitioning of carbohydrates into the cells, photosynthetic efficiency and the concentrations of several metabolites (KASPERBAUER, 1987). It is also noteworthy that light intensity and quality can affect the partitioning of protein, oil and fatty acids in soybean grains (BELLALOUI; GILLEN, 2010).

Light quality and quantity also affect several steps of the symbiosis (LIE, 1969; BALATTI; MONTALDI, 1986; NEVES; HUNGRIA, 1987). Starting with the rhizosphere, light modifies root exudation both quantitatively and qualitatively (NEUMANN; RÖMHELD, 2000), affecting rhizospheric microorganisms. Red-light-treated plants also allocate more photosynthates to the roots (KASPERBAUER; HUNT; SOJKA, 1984), promoting nodule number. In addition, changes in the red:infrared ratio affect the onset of nodule formation (LIE, 1969).

The effects of plant density represent an intriguing subject of study, potentially with profound implications for farmers’ profits. There is a general recommendation of high plant density for soybean in order to achieve high yields, and in Brazil most farmers adopt the density of 300,000 plants ha^{-1}. However, decreased seeding rates may result in lower sowing costs, spatial distribution that reduces competition for water, nutrients and sunlight and increased radiation penetration to lower branches, altogether improving yield and farmer´s income (BOARD; HARVILLE; SAXTON, 1990; BOARD; KAMAL; HARVILLE, 1992). It is also important to consider that costs with soybean seeds takes 10-13% of overall costs of soybean crop systems in Brazil (CONAB, 2014), and any agronomical practice that decreases cost production without affecting productivity on a land area bases should be considered for an efficient farm planning.

The aim of this study was to evaluate the effects of plant density on nodulation, plant nutrition, yield and quality of soybean grains. The hypothesis was that, by reducing plant density there will be compensatory changes in the photosynthetic and biological nitrogen fixation processes that may positively impact per plant and total yields.

Materials and Methods

Field site description and procedures before sowing

The experiments were performed for three consecutive cropping seasons—2010/2011, 2011/2012 and 2012/2013—at the experiment station of Embrapa Soybean in Londrina, State of Paraná, southern Brazil (23°11´S, 51°11´W, elevation of 620 m). The soil is classified as Latossolo Vermelho Distroférrico (Brazilian classification system; Typic Haplustox, USDA soil taxonomy). The average annual temperature is 21°C, with an average high of 28.5°C in February and low of 13.3°C in July. Average annual rainfall is 1,651 mm, with 123 days of rainfall per year; maximum rainfall occurs in the summer (January–March) and minimum in winter (June–August). According to Köeppen's classification, the climate is subtropical humid (Cfa: humid, subtropical, with hot summers).

Lime was applied to the soil six months before the first experiment, based on the soil chemical analysis, aiming at elevation of base saturation to 70 percent (EMBRAPA, 2011).

About 40 days before sowing, 20 soil subsamples were collected from the 0–20-cm soil layer. Each set of 20 soil subsamples was pooled to constitute one composite sample, and four composite samples were taken for chemical, granulometric and microbiological analyses.

For chemical analysis (PAVAN et al., 1992), samples were oven-dried (60°C, 48 h) and sieved (2 mm). Soil pH was determined in 0.01 M $CaCl_2$ (1:2.5; soil:solution) after 1 h shaking. Ca, Mg, and Al contents were determined in 1 M KCl (1:10; soil:solution) extracts after 10 min shaking. P and K contents were determined in Mehlich-1 extract (0.05 M HCl + 0.0125 M H_2SO_4; 1:10 soil:solution) after shaking for 10 min. Al was determined by titration with 0.015 N NaOH, with bromothymol blue as indicator. Ca and Mg concentrations were determined in an atomic absorption spectrophotometer, K in a flame photometer, and P by colorimetry, by the molybdenum blue/ascorbic acid method. C was determined by dichromate oxidation. Soil-chemical characteristics are presented in Table 1.

Soil granulometric fractions were determined according to EMBRAPA (1997) and consisted of (g kg^{-1}): 710 (clay), 82 (silt), 208 (sand).

Soybean bradyrhizobia population was estimated by the most probable number (MPN) method using soybean cultivar BRS 284, indeterminate type of growth, as trap host (VINCENT, 1970), and the results for each year are shown in Table 3.

Table 1 - Soil chemical properties (0-20 cm) and soybean *Bradyrhizobium* population before sowing in 2010, 2011 and 2012.

Properties	**2010**	**2011**	**2012**
Ca (cmol$_c$ dm^{-3})	4.2	3.9	4.0
Mg (cmol$_c$ dm^{-3})	1.9	1.7	1.4
K (cmol$_c$ dm^{-3})	0.6	0.9	0.7
S-SO$_4$ (mg dm^{-3})	21	6.4	7.0
P (mg dm^{-3})	15.4	19.1	28.9
CEC pH 7 (cmol$_c$ dm^{-3})	12.8	11.0	10.6
CEC-effective (cmol$_c$ dm^{-3})	6.7	6.4	6.2
Base saturation (V%)	52.8	58.1	58.1
pH in CaCl$_2$	4.9	5.1	5.2
Potential acidity (H + Al)	6.0	4.6	4.4
Bradyrhizobium population (CFU g^{-1} soil)	1.79 x 10^4	9.17 x 10^3	3.85 x 10^4

Treatments, experimental design and crop management

The experiments consisted of six treatments, performed at the same site for three cropping seasons, as follows: 1) Non-inoculated (naturalized population of *Bradyrhizobium*), 80,000 plants ha^1 (T1); 2) Non-inoculated (naturalized population of *Bradyrhizobium*), 320,000 plants ha^1 (T2); 3) Non-inoculated control + N fertilizer, 80,000 plants ha^{-1} (T3); 4) Non-inoculated control + N fertilizer, 320,000 plants ha^{-1} (T4); 5) Inoculated with *Bradyrhizobium japonicum* strain CNPSo 2050, 80,000 plants ha^{-1} (T5); 6) Inoculated with *B. japonicum* strain CNPSo 2050, 320,000 plants ha^{-1} (T6).

The experiment had a completely randomized block design, with six replicates. Each plot measured 4 m in width by 10 m in length. Plots had 8 lines, with 0.5 m between lines. Sowing was performed to deliver 8 seeds m^{-2} + 10% (considering the seed germination rate of 90%) in the 80,000 plants ha^{-1} density and 32 plants m^{-2} + 10% in the treatment with 320.000 plants ha^{-1}; population densities were confirmed after germination. The plots were separated by 0.5 m-wide rows and 1.5 m-wide terraces to avoid cross contamination from surface flushes containing bacteria and/or fertilizers caused by heavy rainfall. The cultivar used was BRS 284 (genealogy Mycosoy-45 × Suprema), of indeterminate growth type and maturity group 6.6. Dates of sowing were 03/11/2010, 09/11/2011, and 29/10/2012. At sowing, 300 kg of fertilizer, with the formulation 0-20-20, were applied in bands in the sowing line.

Inoculated treatments received a liquid inoculant prepared with *B. japonicum* strain CNPSo 2050, an isolate from Cordoba´s region, Argentina. A peat inoculant was prepared at the concentration of 10^9 CFU g^{-1} and applied to supply 1.2 million cells $seed^{-1}$. Seeds were not treated with fungicides or insecticides. Non-inoculated treatments (with the naturalized population of *Bradyrhizobium*, as the soil had received inoculants in previous years) without and with N fertilizer were included; the latter consisted of 200 kg of N ha^{-1} as urea, 50% at sowing and 50% as topdressing at the R1 growth stage (beginning of flowering, *i.e.* plants with at least one flower on any node, (FEHR *et al.*, 1971). Two densities of plants were evaluated: 80,000 plants ha^{-1} and 320,000 plants ha^{-1}.

At the V4 stage (four unfolded trifoliolate leaves, scale of FEHR *et al*., 1971) Co (2.5 g ha^{-1}) and Mo (20 g ha^{-1}) were supplied by leaf pulverization. During plant growth cycle, the following products were used: herbicides Clorimuron (50 g ha^{-1}) and Cletodim (0.4 L ha^{-1}); insecticides Diflubenzuron (80 g ha^{-1}), Thiametoxam + Lambdacihalotrina (200 mL ha^{-1}).

The experiments were not irrigated, and water was provided only by natural rainfall.

Plant analyses and statistics

At the R1 growth stage, plants were evaluated for nodulation and nutrient content. For nodulation, eight plants per replicate were randomly collected, excluding the central area (8 m^2) of the plot that was left for grain yield-evaluation at physiological maturity. Plant collection was carefully performed with a shovel to include most of the root system and fallen nodules.

In the laboratory, roots and shoots were separated, and nodulated roots were carefully washed and oven-dried at 65°C for approximately 72 h. Nodules were then removed from roots and allowed to dry for another 72 h before counting and weighing. The parameters evaluated for nodulation were number of nodules per plant, dry weight per nodule and nodule dry weight per plant.

Also at the R1 growth stage, 30 recently matured leaves with petioles were collected per plot to evaluate plant-nutritional status. At the laboratory the leaves were carefully washed with distilled water, oven dried (65°C for approximately 72 h) and ground (35 mesh). Total-N content was determined in a sulfuric acid extract, while P, K, Ca, Mg, and S were determined in a nitric-perchloric extract. The nutritional status was evaluated by applying the Diagnosis and Recommendation Integrated System (DRIS) (BEAUFILS, 1973), using the database of the company Laborsolo, Londrina, Brazil, whose constant sensitivity is 1, adopted for the data to stay near zero. The C content was determined in a TOC analyzer (Elementary model Cube).

Further analyses performed in the same 30 recently matured leaves with petioles collected at the R1 stage included (μmol g^{-1} dry weight): ammonium, amide, nitrate, ureides and total nitrogenous compounds (ammonium + amide + nitrate + ureides), performed as described before (BODDEY et al., 1987; HUNGRIA; KASCHUK, 2014).

At physiological maturity, plants in the 8 m^2 central part of each plot were harvested. Parameters estimated were grain yield (corrected to 13% moisture content), dry weight per grain and number of grains per plant.

Oil and protein contents in grains were also determined. Lipid content (oil) was determined in milled grains (Tecnal, model TE-651) in a Soxhlet extractor (Tecnal, model sebelin TE-188), using n-hexane as solvent and following the methodology of Zenebon, Pascuet and Tigela (2008). Protein content was evaluated in a NIR analyzer Thermo brand (model FTIR Antaris II).

The data were analyzed using the statistical Infostat software (DI RIENZO et al., 2009). All assumptions required by the analysis of variance were verified. Means were compared using Fisher's test at $p<0.05$.

Results and Discussion

Nodulation, nitrogenous compounds and carbon content in leaves

Nodule number and dry weight were higher in the low plant density treatment, of 80,000 plants ha^{-1}, with both the naturalized soybean bradyrhizobia soil population (T1) and in the treatment inoculated with *B. japonicum* strain CNPSo 2050 (T5); in addition, as expected, nodulation was substantially reduced by the addition of N-fertilizer (Table 4). Considering the average of treatments T1 and T5, nodule number and dry weight were 52% and 24% higher, respectively, than the average of the treatments with high plant density, T2 and T6. However, specific dry weight per nodule was higher in the high density treatments (Table 2), probably reflecting a compensatory mechanism for the lower number of nodules.

Table 2 - Effects of plant density on nodulation (nodule number, NN and nodule dry weight, NDW) of soybean at the R1 stage. N treatments consisted of non-inoculated control, non-inoculated control receiving N-fertilizer (200 kg N ha^{-1} supplied as urea, split at sowing and at R1), or inoculated with *B. japonicum* strain CNPSo 2050.

Treatment	**Density** (pl ha^{-1})	**NN** (n^o pl^{-1})	**NDW** (mg $pl^{-1)}$	**Specific NDW** (mg $nodule^{-1}$)
T1-Non-inoculated	80,000	81.8 a[1]	264.9 a	3.6 c
T2-Non-inoculated	320,000	54.9 b	222.1 bc	4.1 ab
T3-N-fertilizer	80,000	48.9 b	123.1 d	2.7 e
T4-N-fertilizer	320,000	33.7 c	102.4 d	3.1 d
T5-Inoculated	80,000	71.9 a	254.0 ab	4.0 b
T6-Inoculated	320,000	46.5 bc	196.8 c	4.4 a

[1] Means of three crop seasons, each with six replicates. Different letters indicate significant difference (Fisher's test, $p<0.05$).

Plant density and architecture affect the quantity and quality of light available to the lower canopy, with consequences for nodulation. First, light availability and photosynthesis are directly related to the amount of C available for nodule formation (NEVES; HUNGRIA, 1987). Qualitatively, it has been long shown that nodulation is controlled by the phytochrome system, being favored by red light (R) and inhibited by infrared radiation (IR) (LIE, 1964). Recently, it was confirmed that phytochrome B (*phyB*) is part of a monitoring system that detects suboptimal light conditions (SUZUKI et al., 2011). In *Lotus japonicum phyB* mutants, nodulation by *Mesorhizobium loti* is significantly reduced and, in the same study, nodulation of wild-type plants was dramatically reduced when exposed to low R:IR ratio. In addition, the synthesis of jasmonic acid (JA) decreased in *phyB* mutants and also under low R:IR conditions. Altogether, the results suggest that JA is a positive regulator of nodulation in *L. japonicum*, being photomorphogenetically controlled by sensing the R:IR ratio (SUZUKI et al., 2011). Supporting these results, in the soybean-*Bradyrhizobium* symbiosis it has been shown that increases in methyl jasmonate result in higher nodulation (MABOOD; ZHOU; SMITH, 2006). Consequently, we may hypothesize that, in our study, low ratios of R:IR under high plant density were perceived by the phytochrome B and reduced the expression of JA-related genes, resulting in decreased nodulation.

In relation to the N compounds in recently matured leaves (collected according to the procedures specified for the DRIS analysis), for ammonium, amide and nitrate no differences were related to plant density (Table 3). Lower values were found in the non-inoculated plants, and nitrate content was higher in N-fertilized plants at low density. In contrast, N-ureides were about 100% higher under high density, whereas total-N values were higher in N-fertilized plants, independently of the plant density (Table 3).

Table 3 - Effects of plant density on the composition of N in soybean leaves (µmol g^{-1} dry weight) and carbon content (%) at the R1 stage. N treatments consisted of non-inoculated control, non-inoculated control receiving N-fertilizer (200 kg N ha^{-1} supplied as urea, split at sowing and at R1), or inoculated with *B. japonicum* strain CNPSo 2050.

N source	**Density**	**Ammonium**	**Amide**	**Nitrate**	**Ureides**	**Total N**	**C**
	(pl ha^{-1})	(µmol g^{-1} dry weight)					(%)
T1-Non inoculated	80,000	22.6 b[1]	33.1 c	225.5 bc	14.1 b	295.4 b	40.35 b
T2-Non inoculated	320,000	22.4 b	32.5 c	209.2 c	29.4 a	293.1 b	40.05 b
T3-N-fertilizer	80,000	26.6 ab	38.2 ab	255.7 a	17.4 b	337.9 a	43.40 a
T4-N-fertilizer	320,000	26.7 ab	38.7 a	244.0 ab	26.1 a	335.4 a	38.45 b
T5-Inoculated	80,000	27.6 a	36.0 abc	240.6 ab	14.2 b	318.4 ab	39.35 b
T6-Inoculated	320,000	23.9 ab	34.2 bc	208.8 c	29.1 a	296.0 b	39.55 b

[1] Means of three crop seasons, each with six replicates. Different letters indicate significant difference (Fisher's test, $p<0.05$).

One hypothesis for the higher N-ureides contents in plants under high population density is that the decrease in light density and the low R:IR ratio accelerated senescence in the lower canopy and favored remobilization to the upper leaves. Indeed, visual observation was that senesced leaves in the lower canopy were far more abundant under high density. Senescence can occur in the whole plant, at the organ and at the cellular levels, and light-mediated by phyB-is one of the specific signals affecting plant senescence (BALLARÉ; CASAL, 2000). This process could be orchestrated with the up-regulation of gene expression of xanthine dehydrogenase (XDH), a key enzyme in the synthesis of ureides. In Arabidopsis, it has been shown that XDHs also produce superoxide radicals, that may be involved in stress responses that require reactive oxygen species (JUVANY; MÜLLER; MUNNÉ-BOSCH, 2013). Therefore, increases in activity of XDHs may result in more ureides under high population density, representing an additional source of reactive oxygen species and helping in the photo-oxidative stress observed in lower leaves.

For all three cropping seasons, shoot dry weight (data not shown) and C contents in leaves were higher in the N-fertilized plants with 80,000 plants ha^{-1}, suggesting higher photosynthetic rates (Table 3). In 2011/2012 the plants suffered with severe drought, with the water deficit estimated at 132 mm from sowing to R1, whereas in 2010/2011 and 2012/2013 rainfall was regular (data not shown). In the year with drought stress, photosynthetic rates were reduced and so was C content in leaves; it was lower in 2011/2012 (mean of 39.0% for all treatments) than in 2010/2011 (40.3%) and 2012/2013 (40.5%).

Nutritional diagnosis

Based on the DRIS index for nutritional diagnosis, no deficiencies of P, K, Ca and S were observed, indicating that the status of these elements was not affected by plant density or N treatment; a slight imbalance was observed for Ca only in the T4 treatment, N-fertilized and with the high population density (Table 4). For Mg, imbalance was observed in all treatments and although without statistical differences, there was a tendency of being more drastic under the high population density (Table 4).

Table 4 - Diagnosis and recommendation integrated system (DRIS) evaluated in recently matured soybean leaves collected at R1 stage. N treatments consisted of non-inoculated control, non-inoculated control receiving N-fertilizer (200 kg N ha^{-1} supplied as urea, split at sowing and at R1), or inoculated with B. japonicum strain CNPSo 2050.

N source	**Density** (pl ha^{-1})	**P**	**K**	**Ca**	**Mg**	**S**	**N**
T1-Non inoculated	80,000	0.71 a[1]	1.22 bc	0.11 a	-1.00 ab	0.27 c	-1.85 a
T2-Non inoculated	320,000	0.71 a	1.25 abc	0.13 a	-1.08 ab	0.35 c	-1.90 ab
T3-N-fertilizer	80,000	0.50 a	1.03 c	0.09 ab	-0.87 a	0.76 a	-1.84 a
T4-N-fertilizer	320,000	0.68 a	1.44 a	-0.06 b	-1.15 ab	0.68 ab	-1.53 a
T5-Inoculated	80,000	0.73 a	1.20 bc	0.10 a	-0.96 ab	0.21 c	-2.85 b
T6-Inoculated	320,000	0.72 a	1.39 ab	0.02 ab	-1.29 b	0.40 bc	-1.49 a

[1] Means of three crop seasons, each with six replicates. Different letters indicate significant difference (Fisher's test, $p<0.05$).

Intriguingly, DRIS indicated imbalance of N in all treatments (Table 4), and this was observed in all three cropping seasons (data not shown). N imbalance detected by DRIS has been previously reported by Harger, Fioretto and Ralisch (2003) with four soybean cultivars-Embrapa 48, BRS 132, BRS 133 and BRS 134-belonging to different maturation cycles. It is worth mentioning that although N contents in leaves were higher in the dry year of 2011/2012, probably as a result of concentration in a lower biomass of leaves due to the severe drought stress (Figure 1), the N deficit detected by DRIS occurred independently of water availability (data not shown). However, we cannot disregard the possibility that the detection of N imbalance could result from limitations in the database. The use of DRIS is relatively recent in Brazil, and there is need for additions to the database-comprising more genotypes-with an emphasis on the introduction of genotypes of indeterminate growth type, under different soil and climatic conditions.

The sum of DRIS indices generates the nutritional balance index (NBI), and the higher the NBI, the greater is the nutritional imbalance, affecting yield negatively (SERRA et al., 2013). In our study, although some nutritional imbalance was observed, no consistent differences were observed between treatments, but they were observed between the dry season of 2011/2012 and the other two seasons with regular rainfall (Figure 2).

Overall, we should note that no indication emerged in terms of DRIS (Table 6) or NBI (Figure 6) that plant density affected nutritional balance.

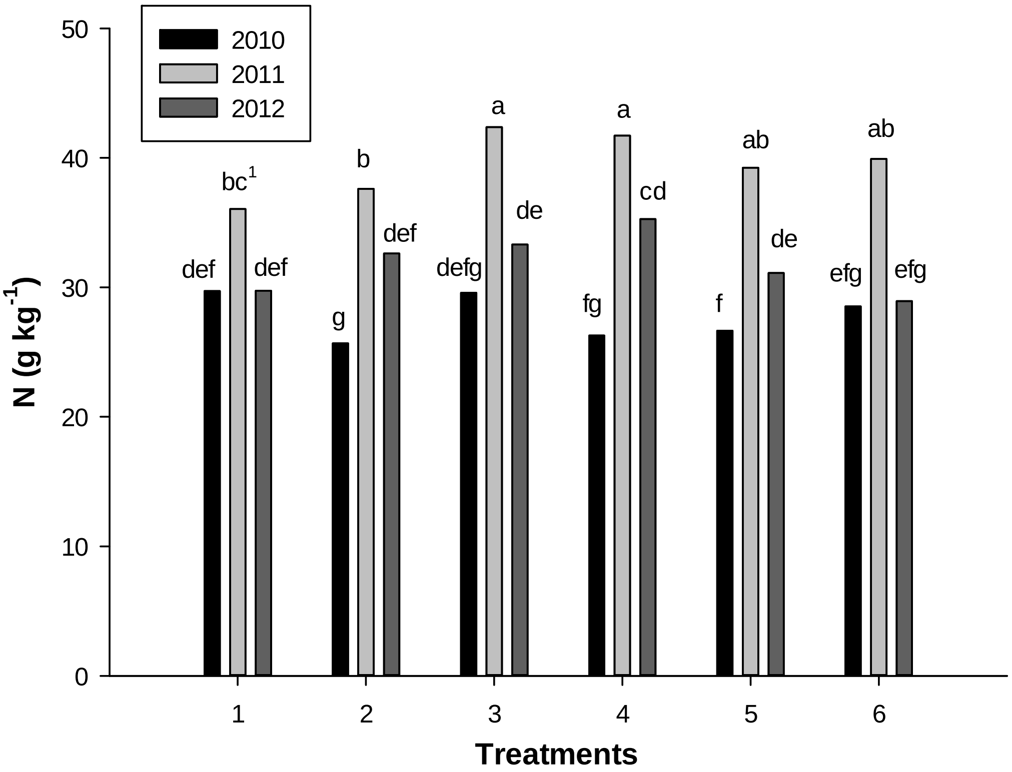

Figure 1 - N concentration (g kg^{-1}) in recently matured soybean leaves collected at R1, in three crop seasons. Treatments: 1) Non-inoculated control, 80,000 plants ha^{-1}; 2) Non-inoculated control, 320,000 plants ha^{-1}; 3) Non-inoculated control + N fertilizer, 80,000 plants ha^{-1}; 4) Non-inoculated control + N fertilizer, 320,000 plants ha^{-1}; 5) Inoculated with *B. japonicum* strain CNPSo 2050, 80,000 plants ha^{-1}; 6) Inoculated with *B. japonicum* strain CNPSo 2050, 320,000 plants ha^{-1}. Data represent means of six replicates. Different letters indicate significant difference (Fisher's test, p<0.05).

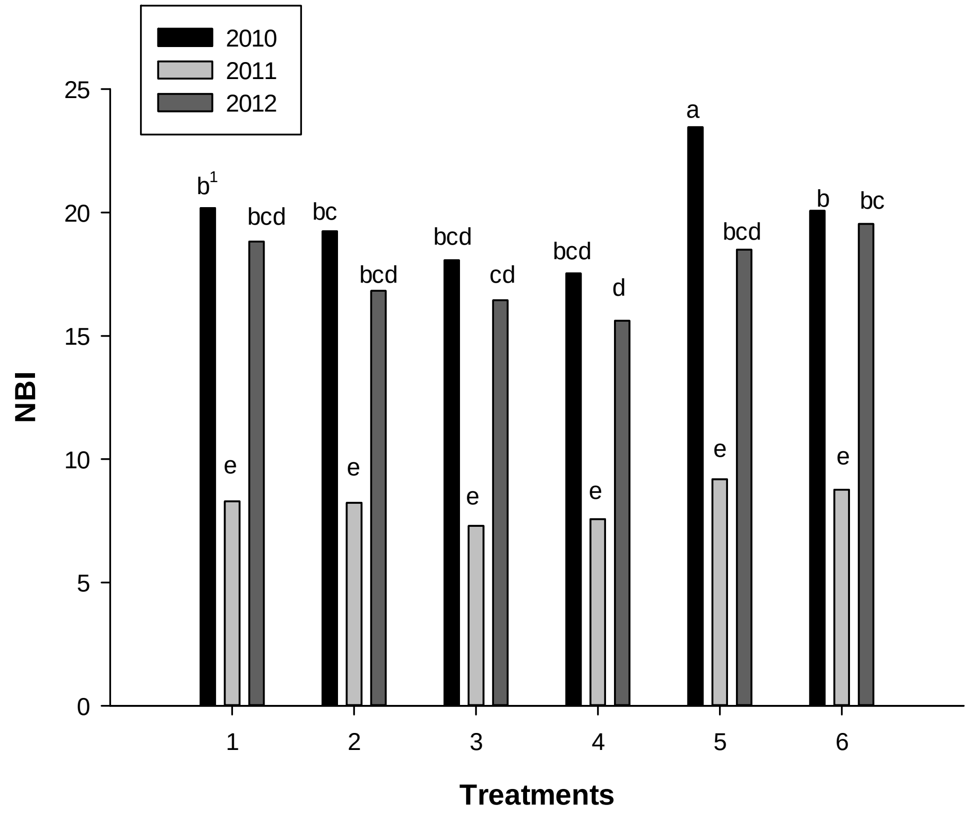

Figure 2 - Nutritional balance index (NBI) in recently matured soybean leaves collected at R1, in three crop seasons. Treatments: 1) Non-inoculated control, 80,000 plants ha^{-1}; 2) Non-inoculated control, 320,000 plants ha^{-1}; 3) Non-inoculated control + N fertilizer, 80,000 plants ha^{-1}; 4) Non-inoculated control + N fertilizer, 320,000 plants ha^{-1}; 5) Inoculated with *B. japonicum* strain CNPSo 2050, 80,000 plants ha^{-1}; 6) Inoculated with *B. japonicum* strain CNPSo 2050, 320,000 plants ha^{-1}. Data represent means of six replicates. Different letters indicate significant difference (Fisher's test, $p<0.05$).

Yield parameters and grain composition

There were no differences in yield among the treatments in the first two seasons (Table 5), and it is worth mentioning again that 2011/12 was very dry, resulting in low yields. In 2012/2013 grain yields were higher in the treatments with 320,000 plants ha^{-1} when compared with the density of 80,000 plants ha^{-1}, although statistically significantly only in the case of N-fertilized plants. Considering all treatments, high population density resulted in a yield increase of 16% in comparison to the low population density in this crop season. Therefore, the results confirm the high plasticity of soybean, once although there was a 4-fold difference in plant population (*i.e.*, 75% reduction), the mean decrease in yield, considering all three cropping seasons, was of 275 kg ha^{-1}, corresponding to only 10% (Table 5). Recently, in a field experiment with soybean at densities ranging from 40,000 to 320,000 plants ha^{-1}, De Luca and Hungria (2014) observed significant decreases in yield only for the very low density of 40,000 plants ha^{-1}, confirming the high plasticity of the plant to adapt photosynthesis and nitrogen fixation to different densities.

Table 5 - Effects of N source and plant density on soybean grain yield (kg ha^{-1}). N treatments consisted of non-inoculated control, non-inoculated control receiving N-fertilizer (200 kg N ha^{-1} supplied as urea, split at sowing and at R1), or inoculated with *B. japonicum* strain CNPSo 2050.

N source	**Density**	**Crop Season**			**Mean-3-year**
		2010/11	**2011/12**	**2012/13**	
	(pl ha^{-1})		(kg ha^{-1})		(kg ha^{-1})
T1-Non inoculated	80,000	3453 a	1338 a	2628 b	2473 cd^{2}
T2-Non inoculated	320,000	3661 a	1530 a	3205 ab	2799 a
T3-N-fertilizer	80,000	3704 a	1442 a	2638 b	2593 bc
T4-N-fertilizer	320,000	3723 a	1387 a	3308 a	2806 a
T5-Inoculated	80,000	3692 a	1321 a	2627 b	2372 d
T6-Inoculated	320,000	3558 a	1533 a	2875 ab	2656 ab

[1] Means of six replicates and different letters indicate significant difference (Fisher's test, $p<0.05$).

[2] Means of three crop seasons, each with six replicates. Different letters indicate significant difference (Fisher's test, $p<0.05$).

Low population density resulted in a 2.6-fold increase in the number of grains per plant (Table 6). Although in general there was a decrease in the dry weight per grain at low population density, considering all treatments the values were similar at 0.116 g per grain with 80,000 plants ha^{-1} and 0.119 g per grain with 320,000 plants ha^{-1} (Table 6).

In relation to the grain composition, the oil contents were higher in treatments T1 and T5 at the low population density, in comparison to treatments T2, T4 and T6 at high density (Table 8). The opposite occurred with protein content (Table 6), indicating an inverse relationship between the percentages of oil and protein in the grains (Pearson's correlation coefficient of –0.23, $p<0.0001$). No differences in oil and protein content in grains were related to N treatments (Table 8). Similar to our results, Butler, De Bruin and Pedersen (2010) reported an increase in protein and a decrease in oil content in grains of soybean grown under high population density.

Table 6 - Effects of N source and plant density on soybean grain yield-related parameters and grain quality. N treatments consisted of non-inoculated control, non-inoculated control receiving N-fertilizer (200 kg N ha^{-1} supplied as urea, split at sowing and at R1), or inoculated with *B. japonicum* strain CNPSo 2050.

N source	**Density** (pl ha^{-1})	**Number of grains** (n^{o} pl^{-1})	**Dry weight of grains** (g grain^{-1})	**Oil content** (%)	**Protein Content** (%)
T1-Non inoculated	80,000	344 a[1]	0.116 c	22.7 a	34.4 bc
T2-Non inoculated	320,000	128 b	0.121 a	21.7 b	36.1 a
T3-N-fertilizer	80,000	354 a	0.119 ab	22.0 b	34.8 b
T4-N-fertilizer	320,000	128 b	0.120 a	21.6 b	36.0 a
T5-Inoculated	80,000	334 a	0.112 d	22.7 a	34.0 c
T6-Inoculated	320,000	136 b	0.116 bc	21.9 b	36.0 a

[1] Means of three crop seasons, each with six replicates. Different letters indicate significant difference (Fisher's test, $p<0.05$).

A positive correlation between oil content and sugar availability-especially sucrose-in soybean seeds has been long recognized (HYMOWITZ et al., 1971). An increase in photosynthate production stimulated by light provides more ATP and C skeletons for fatty acid synthesis in soybean seeds (WILLMS; SALON; LAYZELL, 1999), and, in our experiment, plants under low population density received more light per plant, resulting in an increase in seed oil content.

Contrarily, protein content in seeds decreased at low density, consistent with several reports, again showing that oil and protein contents in soybean seeds are negatively correlated, attributed to the genetically-determined trade-off between the accumulation of protein and lipids in grain legumes (KWON; TORRIE, 1964; HYMOWITZ et al., 1971; BRIM; BURTON, 1979). It has also been known for a long time that protein and oil content in soybean seeds are quantitatively inherited traits, determined by a number of genes subject to genotype × environment interactions (HWANG et al., 2014). In our study, as light received per plant was far less limiting in the low-density treatments, we may assume that neither the photosynthates nor the nutrients (Table 6) were the limiting factors for the competitive synthesis of protein and oil contents. Therefore, differences should rather be attributed to genetic mechanisms controlling these traits. For decades it seemed impossible to breed for increases in both protein and oil contents; however, recently, in a genome-wide association study (GWAS) with 298 soybean germplasm accessions, Hwang et al. (2014) identified 40 SNPs (single nucleotide polymorphisms) associated with seed protein and 25 for oil content, seven of which had a significant association with both protein and oil. This knowledge may be useful in future efforts to improve both characters.

Conclusions

When compared to high density (320,000 plants ha^{-1}), plants under the lower density (80,000 plants ha^{-1}) resulted in increases in soybean nodulation and grain oil, but decreased grain protein content per plant. In general, the plant nutritional status was not affected by population density. Noteworthy was that a 75% reduction in the number of plants per ha resulted in a decrease in grain yield (16%) in only one out of three cropping seasons. Therefore, further studies should focus on rethinking the common recommendation of high plant density, as a decrease in the number of plants can match the performance of traditional cropping systems with lower risks of adverse effects of biotic and abiotic stresses.

4 CONCLUSÕES FINAIS

Os experimentos conduzidos permitiram comprovar que, em geral, o status nutricional da planta e as taxas de fixação biológica do nitrogênio (FBN) não foram afetadas pelas diferentes densidades, o que resultou em um incremento de mais de quatro vezes na produção de grãos por planta, no caso de 40.000 plantas ha^{-1} e 2,6 vezes com 80.000 plantas ha^{-1}A FBN foi capaz de suprir com êxito o N necessário para sustentar maiores demandas por planta em baixos rendimentos, conseguindo manter a produtividade em níveis comparáveis ao de altas densidades (320.000 pl/ha), Em geral, a qualidade dos grãos, avaliada pelo teor de óleo e proteína, também não foi afetada pela densidade. Confirma-se, portanto, a hipótese de fonte:dreno, pois sob maior demanda por planta em baixas densidades, houve um aumento nas taxas de fotossíntese e de FBN por planta.

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