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CRESCIMENTO E PRODUTIVIDADE DA SOJA E PROGRESSO DE FERRUGEM ASIÁTICA EM DIFERENTES CONDIÇÕES DE DISPONIBILIDADE DE NITROGÊNIO

TESE DE DOUTORADO

Juliano Perlin de Ramos

Santa Maria, RS, Brasil.

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Juliano Perlin de Ramos

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elaborada por
Juliano Perlin de Ramos

Como requisito parcial para obtenção do grau de
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RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Agronomia
Universidade Federal de Santa Maria

CRESCIMENTO E PRODUTIVIDADE DA SOJA E PROGRESSO DE FERRUGEM ASIÁTICA EM DIFERENTES CONDIÇÕES DE DISPONIBILIDADE DE NITROGÊNIO

AUTOR: JULIANO PERLIN DE RAMOS
ORIENTADOR: JERÔNIMO LUIZ ANDRIOLO
Local e data: Santa Maria/RS, 31 de Março de 2014.

O objetivo deste trabalho foi determinar o efeito de taxas de adubação nitrogenada sobre a nodulação, crescimento da planta, rendimento e progresso da ferrugem asiática (*Phakopsora pachyrhizi* Sydow) de duas cultivares de soja de ciclo curto. Os experimentos foram realizados em casa de vegetação e no campo, em 2011/12 e 2012/13. Na casa de vegetação, as plantas foram cultivadas em areia com cinco soluções nutritivas e também em uma mistura de areia mais solo com seis doses de nitrogênio mineral. No campo, as plantas foram adubadas com seis doses de nitrogênio mineral. Para as determinações de ferrugem asiática, os experimentos foram duplicados e o patógeno (*Phakopsora pachyrhizi*) foi inoculado. Em todos os experimentos foi determinado o número de nódulos ativos, o crescimento, a produção de grãos e a concentração de N na planta. Nos experimentos de ferrugem da soja, a desfolha e a área abaixo da curva de progresso da doença (AACPF) foram determinados. No campo, as doses de nitrogênio aumentaram o crescimento das plantas apenas em 2011/12. O número de nódulos ativos por planta diminuiu em todos os experimentos. O rendimento de grãos no campo não foi afetado pelas doses de N. Na casa de vegetação, as doses de nitrogênio aumentaram o crescimento das plantas e o progresso da doença. Em 2011/12 no experimento de campo, houve um pequeno decréscimo no progresso da ferrugem e um ligeiro aumento na produtividade. Concluiu-se que a adubação nitrogenada mineral pode aumentar o crescimento da planta e a produção de grãos em condições ideais de água e nutrientes. No entanto, sob condições ambientais favoráveis para a ferrugem asiática, o aumento da produção de grãos obtido pelo uso de altas doses de N pode ser anulado pelos danos causados pela doença. Em condições de campo, os efeitos do N sobre o rendimento de grãos são de pequena importância em ambas as cultivares.

Palavras-chave: *Glycine max L.*. Adubação. Nutrição de plantas. *Phakopsora pachyrhizi.* Ferrugem asiática. Ferrugem da soja.

ABSTRACT

Doctor Thesis
Agronomy Post-graduation Program
Universidade Federal de Santa Maria

GROWTH AND YIELD OF SOYBEAN RUST AND PROGRESS OF ASIAN RUST IN DIFFERENT CONDITIONS OF NITROGEN AVAILABILITY

Author : Juliano de Perlin de Ramos
Advisor: Luiz Jerônimo Andriolo
Local and Date: Santa Maria/RS, March 31st, 2014.

The objective of this work was to determine the effect of mineral nitrogen fertilization rates on nodulation, plant growth, yield and the effect of nitrogen rates on the progress of Asian soybean rust (*Phakopsora pachyrhizi* Sydow) of two short cycle soybean cultivars. Experiments were carried out in greenhouse and in the field, in 2011/12 and 2012/13. In the greenhouse, plants were grown in sand and supplied with five nutrient solutions and also grown in a mixture of sand plus soil and supplied by six mineral nitrogen rates. In the field, plants were fertilized with six mineral nitrogen rates. For determinations of Asian soybean rust, experiments were doubled and *Phakopsora pachyrhizi* was inoculated. In all experiments, active nodules, growth, grain yield and plant N concentration was determined. In Asian soybean rust experiments, defoliation and the area under rust progress curve (AURPC) was determined. In the field, nitrogen rates increased plant growth only in 2011/12. Active nodules per plant decreased in all experiments. Grain yield was not affected by N rates in the field. In the greenhouse, nitrogen rates increased plant growth and the disease progress. In 2011/12 field experiment, a slight decrease of rust progress and a slight increase in yield was recorded. Mineral nitrogen fertilization can increase plant growth and grain yield under optimal supply of water and nutrients. However, under favorable environmental conditions for the Asian rust, the increase in grain yield by effect of high N rates might not be attained due to the damage caused by the disease.

Keywords: *Glycine max* L.. Fertilization. Plant nutrition. *Phakopsora pachyrhizi*.
Asian rust. Soybean rust.

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INTRODUÇÃO

A nutrição de plantas é processo crucial na produção vegetal, sendo um dos principais fatores que limita a produção das culturas (FAGERIA et al., 1998). Em soja (*Glycine max*), respostas positivas ao aumento das doses de fertilizantes têm sido encontradas sobre o crescimento e produtividade da cultura (GONÇALVEZ JUNIOR et al., 2010). Essas respostas sugerem que cultivares melhoradas geneticamente com o foco principal no aumento da produtividade geram materiais mais exigentes em fertilidade do solo. O nitrogênio é o nutriente mais requerido pela soja por estar associado também às proteínas e lipídios contidos nos grãos (SINCLAIR; HORIE, 1989) e sua demanda é suprida principalmente pela simbiose com bactérias do gênero *Bradyrhizobium spp.*, podendo atingir até 85% do total (HUNGRIA et al., 2006). Como consequência, a aplicação de fertilizantes minerais nitrogenados não faz parte das recomendações de adubação da cultura da soja no Brasil (REUNIÃO, 2012).

Várias pesquisas têm sido realizadas com o propósito de avaliar a resposta à aplicação de N inorgânico na cultura da soja. Ray et al. (2006) encontraram respostas positivas da aplicação de nitrogênio sobre a produtividade. Wesley et al. (1998) relataram que aplicações tardias de N aumentaram a produtividade, porém sem alterar os teores de N nas folhas, sugerindo que o nitrogênio aplicado tardiamente é diretamente carreado para o grão. Petter et al. (2012) observaram que a fertilização nitrogenada no período reprodutivo proporcionou incrementos no peso de mil grãos e no rendimento da cultura. Todavia, há controvérsias quanto aos reais benefícios da adubação nitrogenada na cultura. Mendes et al. (2008) não encontraram efeito significativo da aplicação tardia de nitrogênio na produtividade de soja. Barker & Sawyer (2005) observaram que a aplicação de N no período vegetativo aumentou os teores de N na massa das plantas, sem, no entanto, afetar a produtividade. Aratani et al. (2008)

não observaram aumentos significativos no rendimento de grãos com a aplicação de N em diferentes momentos durante o período de crescimento e desenvolvimento da planta.

Salvagiotti et al. (2008) analisaram seiscentos e trinta e sete conjuntos de dados publicados em revistas arbitradas (1966-2006) e observaram que existe vasta gama de resultados envolvendo a fixação biológica do N e a resposta à adubação nitrogenada em cobertura na soja. Esses resultados indicaram também que, em média, de 50% a 60% da demanda de N na cultura da soja é suprida pela fixação biológica. No mesmo trabalho os autores destacaram uma gama de fatores que podem interferir no nível de resposta à aplicação de N, como o potencial produtivo das cultivares, o solo e o clima.

Em relação às cultivares, destaca-se o fato que no Brasil e, principalmente, na Região Sul, cultivares de hábito de crescimento indeterminado e de ciclo precoce têm ganhado importância nos últimos anos. Estas cultivares prosseguem o crescimento vegetativo após o início da fase reprodutiva e podem atingir entre 30% e 50% de acréscimo no número de nós produtivos na haste principal após o início do florescimento (SEDIYAMA et al., 2005).

Os resultados da literatura não permitem concluir se a necessidade de N em soja precoce de alta produtividade e de hábito de crescimento indeterminado pode ser suprida exclusivamente por meio da fixação simbiótica de $N_{2.}$ Por outro lado, sabe-se que os fertilizantes minerais nitrogenados aplicados no solo afetam a nodulação nas raízes (HUNGRIA et al., 2006). Isso poderia reduzir ou retardar a absorção de N via simbiose.

Além dos efeitos diretos do nitrogênio sobre o crescimento e produtividade da soja, efeitos indiretos também podem ocorrer, como o desenvolvimento de doenças, que por sua vez afetam a produtividade da cultura. São escassos na literatura os trabalhos indicando relações entre adubação nitrogenada e intensidade de doenças na cultura da soja. Há trabalhos em outras culturas mostrando aumento das doenças com o aumento do fornecimento de N nos patossistemas, como por exemplo, em oídio (*Erysiphe graminis*) e ferrugem da folha

(*Puccinia striiformis*) em trigo (NEUMANN et al., 2004; DEBONA et al., 2009), míldio em melancia (SANTOS et al., 2009), antracnose *Colletotrichum* sp. em morango (WALTER et al., 2008) e também diminuição na síntese de compostos de defesa em tomate (MUZIKA; PREGITZER, 1992; STOUT et al., 1998; HOFFLAND et al., 2000 a) e no algodão (SCHMELS et al., 2003; CHEN et al., 2008). Em cenoura, há trabalhos que mostram diminuição de doenças com o aumento dos doses de N (HOFFLAND et al., 2000 b; WESTERVELD et al., 2008). Esses trabalhos indicam que a deficiência ou excesso de nutrientes pode influenciar diferentes reações das plantas à patógenos, podendo aumentar os níveis de defesa ou favorecer o aumento da doença.

Atualmente a ferrugem asiática da soja (*Phakopsora phakyrhizi S.*) vem sendo a principal enfermidade ocorrente nessa cultura no Brasil, havendo necessidade de investigar os efeitos da adubação mineral nitrogenada sobre o progresso dessa doença. A ferrugem da soja pode ocorrer em todos os estádios fenológicos, com alta capacidade de disseminação, o que a torna extremamente agressiva, com reduções de produtividade que podem chegar até a 89% (GODOY et al., 2009) no caso de epidemias severas.

Em relação a ferrugem, há trabalhos que indicaram variações na severidade da doença devido ao manejo nutricional. Balardin et al. (2006) relataram que adubações equilibradas entre fósforo e potássio proporcionaram reduções significativas na severidade. Doreto et al. (2012), observaram que o suprimento adequado de potássio para suprir unicamente a demanda da planta reduziu o progresso da doença. Pinheiro et al. (2011) mostraram que doses excessivas de potássio resultaram em aumentos na intensidade de ferrugem asiática, porém, quando este elemento foi fornecido em equilíbrio com cálcio, mesmo em doses consideradas altas, houve decréscimo na evolução da doença. Nos resultados de Debona et al. (2008), reduções na intensidade da ferrugem asiática foram obtidos apenas com o aumento na disponibilidade de cálcio. Esses resultados mostram a necessidade de realizar pesquisas para determinar os efeitos diretos e indiretos dos nutrientes frente às principais doenças da cultura.

Os objetivos deste trabalho foram determinar o efeito da disponibilidade de nitrogênio sobre a nodulação, crescimento e produtividade de duas cultivares precoces de soja com hábito de crescimento indeterminado e sobre o progresso da ferrugem asiática. As cultivares foram selecionadas em função das características de precocidade e hábito de crescimento. A cultivar BMX Energia® RR apresenta porte médio, ciclo superprecoce, grupo de maturação 5.0; hábito de crescimento indeterminado e alto nível de engalhamento (BRASMAX, 2014). Possui resistência ao acamamento e alta exigência quanto à fertilidade do solo, não tolerando perdas de área foliar por ocorrência de pragas e doenças. A cultivar BMX Potência® RR é ereta, de porte alto, com elevado potencial de engalhamento, possui resistência ao acamamento e média a alta exigência em fertilidade do solo. O hábito de crescimento é indeterminado, o ciclo é semiprecoce, e pertence ao grupo de maturação 6.7. (BRASMAX, 2014).

Para atingir os objetivos propostos foram realizados experimentos em dois ambientes, casa de vegetação e à campo, no município de Itaara, estado Rio Grande do Sul – Brasil (latitude 29° 35’ Sul, longitude 53° 48’ oeste e altitude 444 m).

O Experimento 1 foi realizado de novembro de 2011 a abril de 2012, em casa de vegetação, para comparar o efeito da disponibilidade de N no crescimento da planta, produção de grãos e progresso da ferrugem asiática. As plantas foram cultivadas em vasos com areia e os nutrientes fornecidos através de soluções nutritivas em sistema fechado. Os tratamentos foram cinco concentrações de N nas parcelas principais e as cultivares BMX Potencia® RR e BMX Energia® RR nas subparcelas.

O Experimento 2 foi realizado no campo, de novembro de 2011 até abril de 2012, em sistema de plantio direto, em resteva de aveia. Os tratamentos foram seis doses N em kg ha^{-1}:

0 (T1); 30 (T2); 60 (T3); 120 (T4); 180 (T5) e 240 (T6) nas parcelas e as mesmas cultivares nas subparcelas. A fonte de N utilizada foi a ureia, aplicada em cobertura nos estágios de desenvolvimento V3, V5 e R1. Nesse período ocorreu estiagem e foi feita irrigação.

O Experimento 3 foi conduzido em casa de vegetação de dezembro de 2012 a abril de 2013, no mesmo local, mesmos dispositivos de cultivo e as mesmas cultivares do Experimento 1 realizado em 2011/12. Os vasos foram preenchidos com uma mistura a 1:2 de volume de areia (granulometria 1,2 - 2,4 mm) e solo proveniente da mesma gleba do experimento de campo (Experimento 2). Os tratamentos foram cinco doses de adubação de nitrogênio, em mg kg^{-1}: 0 (T1), 60 (T2), 120 (T3), 180 (T4), e 240 (T5), fornecido através da ureia. Apenas água foi fornecida diariamente às plantas através das fitas gotejadoras, quatro vezes ao dia, como feito no Experimento 1 em 2011/12.

O Experimento 4 foi realizado no campo de novembro de 2012 a março de 2013, na mesma área do experimento dois. Os tratamentos e o manejo da cultura foram realizados como descrito no Experimento 2, mas a irrigação não foi feita porque a chuva preencheu a demanda da de água da cultura.

Todos os experimentos foram conduzidos em duplicata para avaliar a ocorrência da ferrugem asiática. Para tal, cada um dos quatro experimentos foi instalado duas vezes de forma idêntica, no campo, os experimentos foram instalados lado a lado. Na casa de vegetação, os ambientes foram divididos por uma parede interna de polietileno, os dispositivos que continham os mesmos tratamentos foram alinhados para que mesmo com a separação física a solução nutritiva (Experimento 1), ou a água de irrigação (Experimento 3), circulasse em sistema fechado a partir do mesmo tanque. Após a instalação does experimentos, na condição com a doença foi feita a inoculação artificial do patógeno (*Phakopsora phakyrhizi S.*) e o controle químico não foi realizado. Na condição sem doença, o controle químico de doenças foi realizado com quatro aplicações de fungicidas, sendo a primeira aplicada de forma preventiva.

Os resultados dos quatro experimentos na condição sem doença compuseram o primeiro artigo da tese e os quatro com doença compuseram o segundo artigo.

Nitrogen on plant growth and yield of two soybean cultivars

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Abstract – The objective of this work was to determine the effect of mineral nitrogen fertilization rates on nodulation, plant growth and yield of two short cycle soybean cultivars and two growing periods. Two experiments were carried out in greenhouse and two in the field, in 2011/12 and 2012/13. In the greenhouse plants were grown in sand and in a mixture of sand plus soil and supplied with 5 N rates. In the field, plants fertilized with 6 Nitrogen rates. It was determined the dry mass of leaves, stem and roots, active nodules per plant, plant height, number of grains per plant, thousand grain weight, yield and plant N concentration. In the greenhouse N rates increased plant growth and yield linear or polinomially. In the field, it increased plant growth only in 2011/12. Active nodules per plant decreased in all experiments. Grain yield was not affected by N rates in the field. Mineral nitrogen fertilization can increase plant growth and grain yield under optimal supply of water and nutrients but in field conditions effects on grain yield are of minor importance.

Index terms: *Glycine max*; nitrogen fertilization; nodulation; mineral nutrition.

Nitrogênio no crescimento e rendimento de duas cultivares de soja

Resumo - O objetivo deste trabalho foi determinar o efeito das taxas de adubação nitrogenada sobre a nodulação, crescimento e produtividade de duas cultivares de soja de ciclo curto durante dois anos consecutivos. Dois experimentos foram conduzidos em casa de vegetação e dois no campo, em 2011/12 e 2012 /13. Na casa de vegetação as plantas foram cultivadas em areia e em uma mistura de areia mais do solo e foram nutridas com cinco doses de N. No campo, as plantas foram adubadas com seis doses de nitrogênio. Determinou-se a massa seca de folhas, caule e raízes, nódulos ativos por planta, altura da planta, número de grãos por planta, peso de mil grãos, produtividade e concentração de N nos tecidos. Na casa de vegetação, as taxas de N aumentaram o crescimento a produtividade das plantas linear ou polinomialmente. No campo, aumentou o crescimento das plantas apenas em 2011/12. O número de nódulos ativos por planta diminuiu em todos os experimentos. O rendimento de grãos não foi afetado pelas doses de N no campo. Adubação nitrogenada mineral pode aumentar o crescimento da planta e produção de grãos em oferta ideal de água e nutrientes em casa de vegetação, mas em condições de campo, os efeitos sobre o rendimento de grãos são de menor importância.

Termos para indexação: *Glycine max,* nitrogênio, nodulação, nutrição mineral.

Introduction

Soybean (*Glycine max* (L.) Merril) is nowadays one of the most important crop commodities. In Brazil, the cultivated area increased 10.7% in 2012/2013, reaching 27 million hectares and an average yield of 2937 kg ha^{-1} CONAB (2013). This increase was a consequence of new technologies being used mainly in crop protection, soil conservation, fertilization and new cultivars.

Short cycle cultivars with the indeterminate growth habit have been used last years in Southern Brazil. It has been argued that such cultivars have longer reproductive periods (Nogueira, 2007; Cober & Morrison, 2010) and they can reach higher grain yield under normal (Robinson & Wilcox, 1998) or dry wehther conditions (Niatami et al., 2013). Flowering begins early after planting and can proceed simultaneously with vegetative growth. A fraction from 40 to 50% of total plant growth can be attained after the beginning of flowering in these cultivars (Nogueira, 2007). The short cycle is a good trait for crop rotation with cereals or sugar cane and it has been a crop character took into account in soybean breeding programs (Câmara et al., 1998).

High fertilization rates can increase soybean plant growth and yield (Corrêa et al., 2007; Gonçalves Júnior et al., 2010) and also lipid and protein content in grains (Ray et al., 2006b). For nitrogen, it has been considered that up to 85% of crop requirements can be supplied by symbiotic bacteria of the genus *Bradyrhizobium spp*. (Hungria et al., 2006). This assumption has been the basis for the current crop protocol recommendation that mineral N fertilization is not necessary for this crop (Barker & Sawyer, 2005; Mendes et al.; 2008; Aratani et al., 2008). However, increases in plant growth and yield by effect of mineral N fertilization have been reported in the literature (Wesley et al., 1998; Barker & Sawyer, 2005; Ray et al., 2006b; Aratani et al., 2008; Petter et al., 2012). In a comprehensive revision in six hundred thirty-seven data sets published in refereed journals from 1966 to 2006, Salvagiotti et al. (2008) showed that in about half of papers reporting results of soybean nitrogen fertilization, nitrogen quantities from biological fixation were not enough to reach maximum plant growth and yield. Differences in cultivars, soils and crop management might be at the origin of such discrepancy. In short cycle cultivars with the indeterminate growth habit, a competition for carbon assimilates between shoot and roots can take place after the onset of flowering and afterwards. . As a consequence, root growth and the contribution of symbiotic nitrogen for plant growth might be reduced. Data about nitrogen fertilization in short cycle soybean cultivars with the indeterminate growth habit were scarce in the literature.

The objective of this work was to determine the effect of mineral nitrogen fertilization on nodulation, plant growth and yield of two short cycle soybean cultivars with the indeterminate growth habit.

Material and Methods

Four experiments were conducted at Instituto Phytus, Itaara city – Rio Grande do Sul State, Brazil (latitude 29°35' S, longitude 53°48' W, elevation 444 m), in greenhouse and in the field, in 2011/12 and 2012/13 growing periods.

Experiment 1 was conducted from November 2011 to April 2012. Sand previously washed with a 1% sodium hypochlorite solution was used as rooting media. Sand physical characteristics were 0.01-0.03 m gauge, 1.6 kg dm^{-3} bulk density and 0.243 L dm^{-3} maximum water retention capacity. Plants were grown in 4 dm^3 black polypropylene pots. There were 40 pots per bench in four lines, 30 cm apart between lines and 5 cm between pots, with one plant per pot.

Treatments were five nitrogen fertilization rates supplied to plants by means of nutrient solutions. Nitrogen concentrations in the nutrient solution of each treatment were, in mmol L^{-1}: 5.55 (T1), 8.05 (T2), 10.55 (T3), 13.05 (T4) and 15.55 (T5) and two cultivars, BMX Potência® RR and BMX Energia® RR. Concentrations of other nutrients in all nutrient solutions were the same, in mmol L^{-1}: 6.0 K^+; 4.0 $H_2PO_4^-$; 2.0 Ca^{++}; 1.0 Mg^{++} and of micronutrients were, in mg L^{-1}, 0.03 Mo; 0.42 B; 0.06 Cu; 0.50 Mn; 0.22 Zn and 1.0 Fe. A completely randomized split-plot (5x2) experimental design was used, with four replications, N concentrations in main plots and cultivars in subplot.

Treatments were settled by five closed soilless devices set up inside a polyethylene greenhouse. Each device consisted of a fiber cement tile, 0.85 m height, 3.00 m long and 1.10 m wide, at a 1% slope, covered by a 100 µm polyethylene film to avoid the contact with the nutrient solution. Pots were placed over gravel to assure drainage of the nutrient solution inside the rooting media.

Nutrient solutions were prepared in five 500L fiberglass tanks and supplied to plants by a submersible pump (8W) and dripper tubings, with one drip per pot. The nutrient solution drained off at the botton of pots. It was collected at the lower side of the cement tile and conducted back to the reservoir. Four daily 15 min fertigations were done, controlled by a timer, delivering 1.5 L of nutrient solution per pot. Electrical conductivity (EC) and pH were measured every 48 hours and corrected whenever a deviation higher than 10% from the initial value was recorded. Average values ranged from 0.9 to 1.2 dS m^{-1} and 5.5 and 5.8, respectively. Nutrient solutions were completely renewed when the volume inside the tank reached 250 L.

Soybean cultivars BMX Energia® RR and BMX Potência® RR were used. Characteristics of BMX Energia are medium height, early cycle, 5.0 maturity group, indeterminate growth habit, intensive branching and lodging resistant. Those of are BMX Potência® RR are high height, intensive branching, mid early cycle, 6.7 maturity group, indeterminate growth habit and lodging resistant (Brasmax, 2014).

Fungicides were applied for disease control. The first application was done at V3 stage, using Opera®, 0,75 L ha $^{-1}$ and Score®, 0,2 L ha^{-1}; the second at V5 stage, using Priori Xtra®, 0,3 L ha $^{-1}$ and Nimbus® 0,6 L ha^{-1}; the third 20 days later, using Fox®, 0,4 L ha^{-1} and Aureo® 0,3 L ha^{-1}; the 4th 15 days later using Priori Xtra®, 0,3 L ha $^{-1}$ and Nimbus® 0,6 L ha^{-1};

the 5th 15 days later using Priori Xtra®, 0,3 L ha $^{-1}$ and Nimbus® 0,6 L ha^{-1}. Pest control was done by insecticides. The first application was at V3 stage, using Ampligo® SC 0,03 L ha^{-1}; the second at V5, using Orthene® 750 BR WP 750, 0,3 L ha^{-1}; the third at R2 using Engeo Pleno® SC 0,2 L ha^{-1}; the 4th at R5.3 using Orthene® 750 BR WP 750, 0,3 L ha^{-1}. All controls were done using a CO_2 pressurized hand sprayer.

Experiment 2 was conducted in the field from November 2011 to April 2012. The soil belongs to the Franco class (EMBRAPA, 2013), with 286 g kg^{-1} sand, 470 g kg^{-1} silt and 244 g kg^{-1} clay. The chemical analysis done at the Soil Laboratory showed pH 5.6, organic matter 270 g kg^{-1}, phosphorus 20.8 mg dm-^{3}, potassium 160 mg dm^{-3}, calcium 5.3 cmol dm^{-3}, magnesium 2,0 cmol dm^{-3} and CTC 11.2 cmol 5.3 dm^{-3}. Phosphorus and potassium fertilization rates were 168 kg ha-^{1} (P_2O_5) and 120 kg ha^{-1} (K_2O), respectively (Reunião, 2009).

Treatments were six nitrogen fertilization rates, 0 (T1); 30 (T2); 60 (T3); 120 (T4); 180 (T5) and 240 (T6) kg ha^{-1} and two cultivars, BMX Potência® RR and BMX Energia® RR, respectively. Quantities were handly supplied in three equal fractions at V3, V5 e R1 developmental stages using a urea commercial fertilizer (Multifertil® (45-00-00)). The experiment was a (6x2) factorial randomized block design, nitrogen rates in plots and cultivars in split–plots with four replications. Plot size was 15m^2, with six 3m rows, border rows and border plants in rows were not used for determinations.

Crop water requirements during plant growth and development were estimated by (Matzenauer et al., 2003). Irrigation was done applying 12 mm water in December, 2011; 24 mm in January, 2012 and 12 mm in March, 2012.

Experiment 3 was conducted in the greenhouse from December 2012 to April 2013, using the same closed soilless device set up in the greenhouse for the 2011/12n experiment. Pots were filled with a 1:2 volume mixture of sand (1.2 to 2.4 mm gauge) and soil. The soil was collected in the 0-10 cm surface layer of the 2012 field experiment (Experiment 2). The mixture were fertilized by 168 mg Kg^{-1} P_2O_5 and 120 mg Kg^{-1} K_2O using the commercial fertilizers Multifertil® (00-21-30). Fertilizer quantities were estimated from the same fertilization rates used in the 0-10 cm soil surface layer in the field (Experiment 2).

Treatments were five nitrogen fertilization rates, in mg dm^{-3}: 0 (T1), 60 (T2), 120 (T3), 180 (T4) and 240 (T5), using a urea commercial fertilizer (Multifertil® (45-00-00)). Only water was supplied daily to plants in the closed soilless devices, four times a day as done in the Experiment 1 in 2011/12. Pest and disease control was done as in the Experiment 1.

Experiment 4 was a replication of Experiment 2. It was conducted in the same field, from November 4, 2012 to March x, 2013. Irrigation was not done during the growing period of this experiment because crop water requirements were fulfill by rainfall.

In all experiments seeds were treated before planting using Standak® and Maxim®, at 4 and 1 mL Kg^{-1}, respectively. Inoculation of *Bradyrhizobium japonicum* was also done using Bioagro® at 6 mL kg^{-1}, three hours before planting.

In plants growing in pots, four plants were collected in trials of each treatment at the R4 developmental stage. Roots were washed and number of nodules counted. Only active nodules were retained, identified by its internal redden color. In all experiments, four plants were collected at R6 developmental stage for biomass determinations. Plant height was measured with a rule, plant organs were separated, dried at 60°C and dry mass determined after constant weight was reached. At R9 developmental stage, four plants in each replication were harvested and threshed. Following grain yield (13% moisture), grain per plant and thousand grain weight (13% moisture) were determined. Dry mass of all plant organs was finely grounded and N concentration determined in the laboratory by the Kjeldahl method.

In all field experiments, four plants were collected of each replication in the R4 developmental stage. Roots were washed and number of nodules counted as described in greenhouse experiments. In each replication, four plants were collected at R6 and R9 developmental stages for biomass determinations, plant height and grain per plant respectively as already described. The four central rows of plots in each replication were harvested and threshed. Grain yield and thousand grain weight were determined at 13% moisture. Dry mass of all plant organs was finely grounded and N concentration determined as it did for greenhouse experiments.

Results were submitted to analysis of variance and regression analysis for quantitative variables using the software Assistat 7.7 (Silva & Azevedo, 2009) at 0.05 significant. The linear simple correlation matrix between variables coefficients was estimated using the same software at value<0.05 and <0.01.

Results

In greenhouse experiments (Experiment 1 and 3), the effect of nitrogen concentration was significant for all biomass variables and there were interactions only between N rates and cultivars for root dry mass (Figure 1). Total (A, B), shoot (C, D) and root (E, F) dry mass and plant height (G, H) fitted a polynomial model by effect of increasing N rates in plants grown in sand (Experiment 1, Figures 1 A, C, E, G) and sand plus soil (Experiment 3, Figures 2 B, D, F, H). Absolute values of these variables on plants grown in sand were on average two times higher than on plants grown in soil plus sand. Number of active nodules decreased linearly on both cultivars (Figures 2 A, B).

Number of grains (Figures 2 C, D) and yield (Figures 2 E, F) increased linearly on BMX Potência® plants by effect of N availability in both experiments. On plants of BMX Energia®, a second polynomial trend was fitted. On plants grown in sand and supplied by nutrient solutions (Experiment 1) number of grains and yield were 3.8 times higher than on plants grown in sand plus soil and fertilized with urea (Experiment 2). These differences can be attributed to a more uniform supply of nutrients along the growing period of plants by using nutrient solutions. It can be concluded that sand as rooting medium and fertigation by nutrient solutions can be successfully used to simulate plant growth responses in soil and it is a straightforward condition for better explicit nutritional effects.

In field experiments (Experiments 2 and 4), average air temperatures were in the range between 18 °C and 25.6 °C in both growing periods. The 2011/12 growing season was a dry weather period and rainfall was below crop water requirements in November, December, January and March (Table 1). In Experiment 2 (2011/12), shoot growth increased in BMX Potência® (Figure 3 A) while it decreased in BMX Energia®. In Experiment 4 (2012/13) any effect was recorded on plants of both cultivars (Figure 3B). Nevertheless, the number of active nodules decreased in both cultivars and growing periods (Figures 3 C, D). Grain yield differed between cultivars in 2011/12 (Figure 3 E), but any significant differences were recorded among nitrogen rates in both growing seasons 2011/12 and 2012/13 (Figures 3 E, F).

Any effects of nitrogen rates supplied by nutrient solutions or by mineral fertilizer were recorded in nitrogen concentrations of plant tissues and grains (data not shown).

Discussion

In plants grown in sand and fed with nutrient solutions (Experiment 1), the biomass and grain yield were about two and three times higher, respectively, than plants grown in pots with a soil/sand mixture (Experiment 3). These results highlight the benefits of the nutrient solution, providing a more stable and homogeneous availability of nutrients along the whole plant growing period. The sand porosity, higher than that of the sandy clay soil, might also have been a better condition for root growth and physiological activity. Results of the

Experiment 1 can be considered as the potential growth and yield of the two soybean cultivars in the environmental conditions of the experiment.

In the four experiments, nodulation has been decreased by mineral nitrogen, confirming data previously reported in the literature (Hungria et al., 2006; Ray et al., 2006a; Nogueira et al., 2010; Petter et al., 2012). Nevertheless, while the number of active nodules was higher on plants grown in the soil/sand mixture without N fertilization (T1, Experiment 3, Figure 2), it was not able to reach the maximum growth and yield of this experiment, nor the potential growth and yield recorded on plants of the first experiment supplied by nutrient solutions. These results agree with data of Petter et al. (2012), which concluded that moderate nitrogen rates of about 30 kg ha-^{1} can increase yield of soybean plants grown in the sandy Brazilian soil “cerrado”. This can be attributed to the time lag between shoot and root growth and nodulation activity. Although inoculation with *Bradyrhizobium* spp. being done at sowing, growth and physiological activity of nodules depends on the previous root growth, chemical signals between the plant and the bacteria and energy supply from the shoot (Schubert, 1995). While these events were not completed, plants can suffer from a temporary nitrogen deficiency, reducing growth. This hypothesis is supported by literature data from field experiments when plants were supplied with mineral nitrogen during early reproductive (Barker & Sawyer, 2005) and pod filling developmental stages (Imsande, 1998; Wesley et al., 1998; Petter et al., 2012). In contrast, it has been also reported in the literature any increase in grain yield by nitrogen fertilization at different developmental stages of soybean plants growing in soil (Aratani et al., 2008; Mendes et al., 2008). This discrepancy may be due to bad soil physical and chemical characteristics (Salvagiotti et al., 2008) and/or nitrogen losses affecting the soil nitrogen dynamics, as suggested by (Amado et al., 2002). In fact, nodulation and its physiological activity can be affected by environmental conditions, mainly the water availability in the root media (Ray et al., 2006a; Nogueira et al., 2010; Chafi et al., 2012).

This might be the case in Experiment 2 conducted in the field in 2011/12, a dry weather period.

The correlations among plant characters in greenhouse experiments (Table 2) indicate that biomass accumulation (DMR, DMS and TDM) was not related to grain yield, while plant height did. In fact, plant height affects the number of pods and grains per plant as it has been reported by Cober & Morrison (2010). Nevertheless, excessive shoot growth and plant height can lead to lodging, which can be at the origin of the lack of correlation between biomass accumulation and grain yield.

Present results showed that mineral nitrogen can increase soybean plant growth and grain yield (Figure 2E). Nevertheless, they were recorded from plants grown under optimal water and nutrient availabilities and physical characteristics of the root media. Besides being original in the literature, they can highlight the controversy on data of soybean nitrogen fertilization. Nevertheless, it has to be kept in mind that nitrogen and water have been supplied in a homogeneous way during the whole growing period. This is a condition that can be hardly attained in field grown crops, manly due to biological process and different soil conditions as suggested by (Amado et al., 2002). In the case of the mineral nitrogen supplied by fertilization being not entirely uptake by plants and simultaneously nodulation being inhibited, the effect would be a reduction in plant growth and yield.

Conclusions

- Mineral nitrogen fertilization reduces nodulation but can increase plant growth and grain yield of BMX Energia® RR and BMX Potência® RR soybean cultivars grown under optimal supply of water and nutrients.
- In field conditions, mineral nitrogen can increase shoot growth with minor effects on grain yield.

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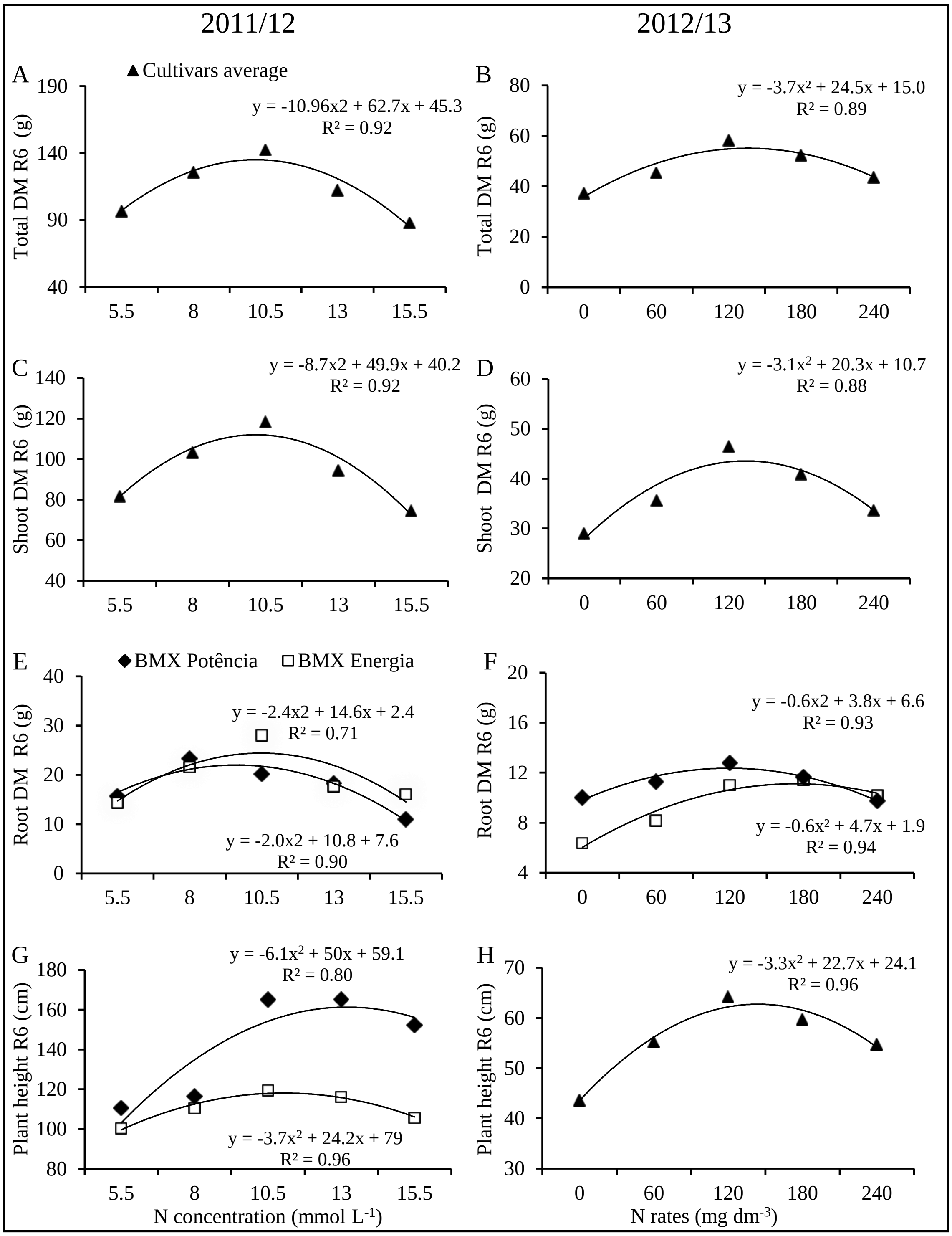

Figure 1. Dry mass (Total DM) (A, B), shoot dry mass (Shoot DM) (C, D), root dry mass (Root DM) (E, F), plant height (G, H) at R6 of soybean plantsgrown on sand with N concentrations of 5.5, 8.0, 10.5, 13.0, and 15.5 mmol L^{-1} (A, C, E) (Experiment 1, 2011/12) and grown in sand plus soil with N rates of 0, 60, 120, 180 and 240 mg dm^{-3} (B, D, F) (Experiment 3, 2012/13).

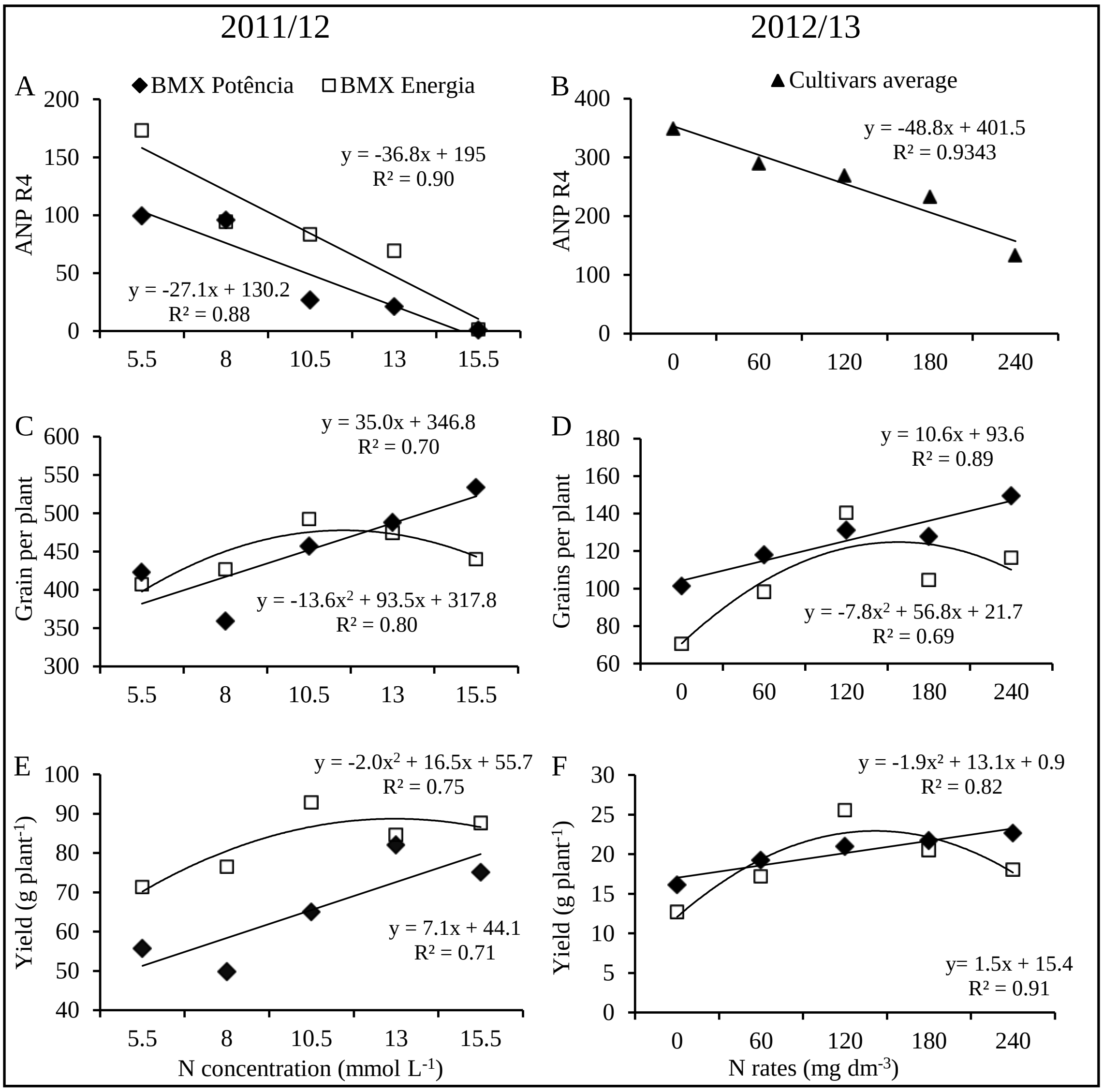

Figure 2. Active nodules per plant (ANP) (A, B), at R4 stage, grain per plant (GP) (C, D) and yield (E, F) at R6 stage of soybean plants grown on sand with N concentrations of 5.5, 8.0, 10.5, 13.0, and 15.5 mmol L $^{-1}$ (A, C, E) (Experiment 1, 2011/12) and in sand plus soil with N rates of 0, 60, 120, 180 and 240 mg dm $^{-3}$ (B, D, F) (Experiment 3, 2012/13).

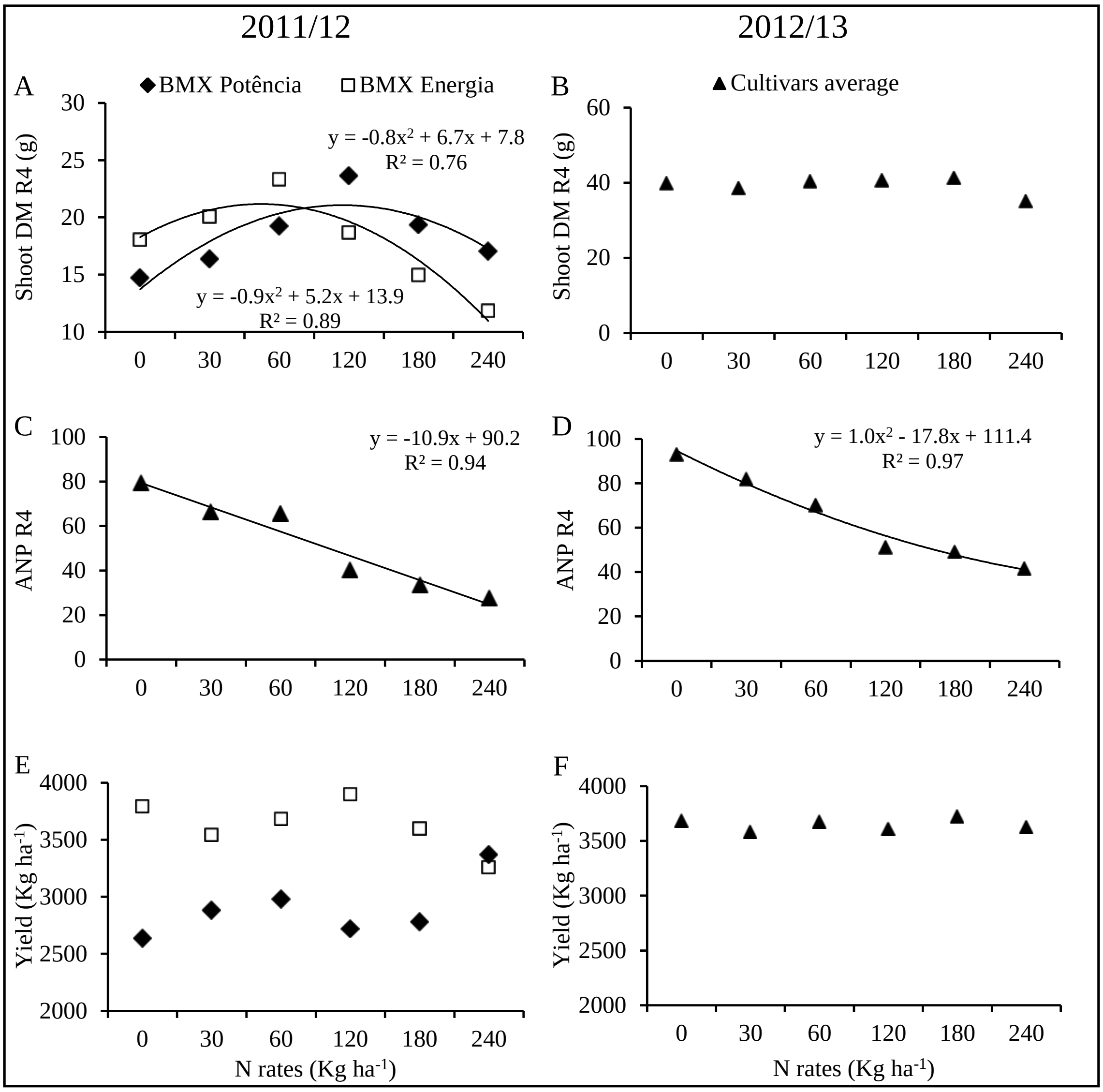

Figure 3. Shoot dry mass (Shoot DM) (A, B), active nodules per plant (ANP) (C, D) at R4 stage and yield (E, F), with N rates of: 0, 60, 120, 180 and 240 kg ha^{-1} in 2011/12 (Experiment 2; A, C, E) and 2012/13 (Experiment 4; B, D, F).

Table 1. Average air temperatures and rainfall during the crop growing periods in 2011–2012 and 2012–2013. Itaara city – Rio Grande do Sul, Brazil.

Year	Month	Min (C°)		Max (C°)		Med (C°)		Rainfall (mm)	
2011 (2012)*	October	13.52	(15.26)	22.51	(23.63)	18.01	(19.45)	177	(294)
2011 (2012)	November	15.28	(20.64)	26.29	(26.81)	20.78	(23.72)	28	(79)
2011 (2012)	December	16.33	(19.60)	27.66	(30.20)	22.00	(24.90)	40	(329)
2012 (2013)	January	18.23	(18.01)	29.84	(29.92)	24.04	(23.97)	98	(152)
2012 (2013)	February	20.50	(18.44)	30.64	(27.48)	25.57	(22.96)	294	(102)
2012 (2013)	March	11.90	(15.64)	26.40	(24.14)	19.15	(19.89)	34	(219)

*Without parentheses, 2011/12; with parenthesis, 2012/13.

Table 2. Simple correlations among variables, Active nodules per plant (ANP), Root dry mass (Root DM), Shoot dry mass (Shoot DM), total dry mass (Total DM), plant height, grains per plant (GP) and yield, averaged over two crop seasons of plants grown in the greenhouse with sand (Experiment 1, 2011/12) and in the mixture of sand plus soil (Experiment 3, 2012/13).

VA\VA	ANP	Root DM	Shoot DM	Total DM	Plant height	Grains per Plant	Yield
ANP	1	0.08ns	0.05ns	0.06ns	-0.58**	-0.74**	-0.75**
Root DM	-	1	0.87**	0.92**	0.53*	-0.1ns	0.17ns
Shoot DM	-	-	1	0.99**	0.58**	-0.07ns	0.19ns
Total DM	-	-	-	1	0.59**	-0.08ns	0.19ns
Plant Height	-	-	-	-	1	0.61**	0.84**
Grains per Plant	-	-	-	-	-	1	0.89**
Yield	-	-	-	-	-	-	1

**Significant at 1% level of probability *Significant at 5% level of probability; ns: non-significant.

Nitrogen rates and progress of Asian soybean rust in two soybean cultivars

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Abstract – The aim this work is to evaluate the effect of nitrogen rates on the progress of Asian soybean rust (*Phakopsora pachyrhizi* Sydow) over two soybean (*Glycine max*) cultivars. Two experiments were carried out in greenhouse and two in the field, in 2011/12 and 2012/13. In the greenhouse plants were grown in sand and in a mixture of sand plus soil and supplied with 5 N rates. In the field, plants fertilized with 6 Nitrogen rates. *Phakopsora pachyrhizi* was inoculated in all experiments. It was determined defoliation and the Area Under Rust Progress Curve (AURPC), dry mass and total concentration of N in leaves, height of plants, number of grains, thousand grain weight and yield. Grain yield was not affected by N rates in the field. In the greenhouse, nitrogen rates increased plant growth and the disease progress. In 2011/12 field experiment, a slight decrease of rust progress and a slight increase in yield from 160 to 242 kg ha^{-1} were recorded. However, under favorable environmental conditions for the Asian rust, increases in grain yield obtained by using high N rates on both cultivars is minor than the damage caused by the disease.

Index terms: *Glycine max, Phakopsora pachyrhizi*, mineral nutrition, mineral nitrogen.

Doses de nitrogênio e o progresso da ferrugem asiática em duas cultivares de soja

Resumo – O objetivo deste trabalho foi avaliar o efeito de doses de nitrogênio sobre o progresso da ferrugem asiática (*Phakopsora pachyrhizi* Sydow) de duas cultivares de soja (*Glycine max*). Dois experimentos foram conduzidos em casa de vegetação e dois no campo, em 2011/12 e 2012/13. Na casa de vegetação as plantas foram cultivadas em areia e em uma mistura de areia mais do solo onde foram fornecidas cinco doses de N. No campo, as plantas foram adubadas com seis doses de nitrogênio. *Phakopsora pachyrhizi* foi inoculado em todos os experimentos. Determinou-se a desfolha e a Área Abaixo da Curva de Progresso da Doença (AACPF), massa seca e concentração total de N nas folhas, altura de plantas, número de grãos, peso de mil grãos e produtividade. O rendimento de grãos não foi afetado pelas doses de N no campo. Na casa de vegetação, as doses de nitrogênio aumentaram o crescimento das plantas e o progresso da doença. Em 2011/12 no experimento de campo, um ligeiro decréscimo no de progresso da ferrugem e um ligeiro aumento no rendimento foram registrados. No entanto, sob condições ambientais favoráveis para a ferrugem asiática, o aumento na produção de grãos pelo uso de altas doses de N pode ficar comprometido pelos danos causados pela doença, em ambas as cultivares.

Termos para indexação: *Glycine max*, *Phakopsora pachyrhizi*, nutrição mineral, nitrogênio mineral.

Introduction

The plant mineral nutrition is crucial to the plant development limiting the crop production (Fageria, 1998). In soybean (*Glycine max*), positive response to development and yield have been found as the fertilizer use increase (Corrêa et al., 2004; Gonçalves Junior et al., 2010). The Nitrogen (N) is the most nutrient requested by soybean. Prado et al. (2010) reported that disabled N soybean plants decreased the development of organs and shoot besides yellowing leaves. Also, the level of N availability can change the protein and lipid content in the grains (Ray et al., 2006).

In Brazil, 85% of total N available is supplied by symbiosis between soybean and *Bradyrhizobium spp* (Hungria et al., 2006). However, the development of high yield varieties might suggests the need of new sources of mineral N. Some studies showed positive response on soybean yield (Ray et al., 2006; Mendes et al., 2008; Nogueira et al., 2010; Petter et al., 2012). But others showed no significant response on N applications (Barker & Sawyer, 2005; Hungria et al., 2006; Aratani et al., 2008). The controversy about N and yield, identified by (Salvagiotti et al., 2008), it was poorly studied.

Soybean rust (*Phakopsora pachyrhizi* Sydow.), is the main disease in Brazil. This disease can occur in all soybean stages. High humidity and frequent rainfall causes the disease outcome (Tsukahara et al., 2008; Del Ponte et al., 2006). The soybean rust have high progress rate and damage up to 89% (Godoy et al., 2009), being very aggressive (Yorinori et al., 2005).

The plant nutrition can be an option to minimize the damage caused by rust in soybean. According Balardin et al. (2006), nutrient deficiency or excess can increase defenses or promote infections. Nutritional management has been related to the lower progress rate of rust. Balardin et al. (2006) reported that balanced fertilization between phosphorus (P) and potassium (K) resulted in significant reduction in rust severity. Similar response was observed for higher levels of K (Doreto et al., 2012). Pinheiro et al. (2011) showed that higher levels of

K promote an increase on rust rate. However, when it was supplied along with Calcium (Ca) a significant decrease of rust was observed as observed by Debona et al. (2008).

The effect of nitrogen rates on the progress of Asian soybean rust was the main goal of this research.

Material and Methods

The work was done at the experimental station of Instituto Phytus, Itaara – Rio Grande do Sul, Brazil (latitude 29 ° 35 'S, longitude 53 ° 48' W, and elevation of 444 m). Four experiments were carried out at greenhouse and field conditions, from October to March at two consecutive years 2011/12 (Experiment 1 and Experiment 2) and 2012/13 (Experiment 3 and Experiment 4).

Seeds were previously treated with Standak® and Maxim® XL (50 and 200 mL to 50 kg of seeds, respectively) and inoculated with *Bradyrhizobium japonicum* (Bioagro®) at the dosage of 300 ml 50 kg^{-1}. The inoculation took place three hours prior to sowing considering each experiment.

The BMX Energia® RR cultivar presents super short cycle, maturation group 5.0; indeterminate growth habit and high number of branches (BRASMAX, 2014). It has resistance to desiccation and high requirement on soil fertility, reduced tolerance to leaf area losses due to pests and diseases. The BMX Potência® RR cultivar is erect, tall-sized, with a high number of branches. It has resistance to desiccation and medium to high demand on soil fertility. The growth habit is indeterminate, medium cycle, and belongs to the Maturation Group 6.7 (BRASMAX, 2014).

At R1 stage, in all experiments, the soybean plants were inoculated using pathogen spore solution. Inoculation was performed using a CO_2 pressurized application bar. The used application bar contained 4 application tips (XR 11001). The rate applied was 150 L ha^{-1} at 33 psi pressure. The solution inoculated was obtained by adding 10 mL of tween (1%) per liter of the *Phakopsora pachyrhizi* spore solution, at 1x10^6 spores mL^{-1} concentration. The spores were directly collected from infected soybean plants. Spore concentration was determined by Newbauer chamber. After inoculation 150 pots containing infected soybean plants by *Phakopsora pachyrhizi* were uniform scattered into greenhouse, in order to simulate natural and constant inoculation.

The experiment 1 was conducted from November 2011 to April 2012. In the greenhouse five devices were built in a closed raised system (0,8 m height). The devices were made of a 3.00 m long and 1.10 m wide cement tile settled at a 1% slope. Polypropylene pots (4 dm^3 content) were placed on devices with a basaltic gravel layer at the bottom in order to drainage be easier. The pots were filled with washed sand (particle size between 1.2 to 2.4 mm), placed 30 cm apart each other in the row and 5 cm between pots.

Then fertilizers salts quantities were calculated and nutrient solutions applied. Treatments were five nitrogen concentrations in the nutrient solutions, in mmol L^{-1}: 5.55 (T1), 8.05 (T2), 10.55 (T3), 13.05 (T4) and 15.55 (T5) and two cultivars, BMX Potência® RR and BMX Energia® RR. The experimental design was completely randomized split-plot (5x2) and four replications. It was utilized 40 pots each treatment, the main plots were 5 N concentrations. The secondary factor was two cultivars.

Concentrations of other nutrients in all nutrient solutions were the same, in mmol L^{-1}: 6.0 K^+; 4.0 $H_2PO_4^-$; 2.0 Ca^{++}; 1.0 Mg^{++} and of micronutrients were, in mg L^{-1}, 0.03 Mo; 0.42 B; 0.06 Cu; 0.50 Mn; 0.22 Zn and 1.0 Fe. A completely randomized split-plot (5x2) experimental design was used, with four replications, N concentrations in main plots and cultivars in split - plot.

Micronutrients were supplied at 0.03 Mo, 0.26 B, 0.06 Cu, 0.50 Mn, 0.22 Zn (mg L^{-1}) concentrations, using a stock solution which was maintained in amber container. The iron was supplied separately using a chelate at 1.0 mg L-^{1} concentration. It was checked the electrical conductivity (EC) and pH solutions contained into the tanks twice a week. The EC and pH of initial solutions were recorded and ranged from 0.9 to 1.2 mS and 5.5 to 5.8 respectively. When EC showed a deviation greater 10% it was adjusted by adding water or new nutrient solution quantities, as it was required. The pH was maintained in the range 5.5 to 6.5 by addition of NaOH or H_2SO_4 1N concentration. The solution was completely renewed whenever the level reached 30% of tank content.

In device, the nutrient solution was pumped to pots through drip irrigation 4 times a day by 15 minutes controlled by a timer. The solution excess was conducted through rails installed in down device side, which lead nutrient solution to the 500 dm ^{3} content tanks settled under the pot banked.

The pest control was performed with four applications of insecticides: V3 (Ampligo® SC 0.03 L ha-1), V5 (Orthene® 750 BR 750 WP; 0.3 L ha-^{1} Nomolt® SC; + 0.05 L ha-^{1}), R2 (Engeo Pleno® SC; 0.2 L ha-1), and R 5.3 (Orthene® 750 BR 750 WP; 0.3 L ha-1). All applications were performed with pressurized sprayer (compressed CO_2), with 2 spray tips TJ 60 11002, spaced by 0.5 m, and gallonage of 150 L ha-^{1}.

The experiment 2 was carried out in field. The soil was a sandy, 286 g k^{-1} sand, 470 g k^{-1} silt and 244 g k^{-1} clay, it belongs to the Franco class (EMBRAPA, 2013). The soil pH was 5.6, organic matter (27 g kg^{-1}), phosphorus (20.8 mg dm-^{3}), potassium (160 mg dm-^{3}), calcium (5.3 cmol dm-^{3}), Mg (cmol 2 dm-^{3}) and CTC (11.2 cmol 5.3 dm-^{3}). Meteorological data was collected from a meteorological station located at the experimental field (Table 1).

Soybean was planted in November 19, 2011. The uniform seed rate, 14 seeds per meter, was supported by a precision seeder-fertilizer. The distance between rows was 0.5 m. The P – K was applied respectively at the rate 168 (P_2O_5) - 120 (K_2O) kg ha-^{1}. The experiment was a factorial randomized block design with split plots (6x2) and four replications. The main plots were 6 N rates (0, 30, 60, 120, 180 and 240 kg ha^{-1}) always in three roofing applications (V3, V5 and R1 stages). The subplots were 2 varieties, as already described. The plots were 6 rows (3 x 5 meters). The experiment was irrigated when needed (12.5 cm m^{-2}). The management of pests and weeds were according to the good field practices.

The pest control was carried out after emergency by four applications of insecticides: V3 (Belt® SC; 0.04 L ha-^{1} Nomolt® SC; + 0.05 L ha-1), V6 (Ampligo® SC 0.03 L ha-1), R2/R3 (Engeo Pleno® SC; 0.2 L ha-1), and R 5.3 (Orthene® 750 BR 750 WP; 0.3 L ha-1). All applications were performed with pressurized sprayer (compressed CO_2), with 2 spray tips TJ 60 11002, spaced by 0.5 m, and gallonage of 150 L ha-^{1}.

The experiment 3 was carried out in 2012 in the greenhouse. The pots were filled with substrate composed by washed sand and soil (1:2) from the same site in the field (experiments 2 and 4). It was kept the same proportions of P-K added at sowing in the field, considering the application of fertilizer in a proportional soil profile to 0-10 cm (1 m^{2} = 100 dm^{3}), so 30 kg ha-^{1} = 30 mg dm^{-3}, in order to provide similar nutrient concentrations in both field (Experiment 2) and greenhouse. It was observed the same rule to the N plots treatments. It was applied the total nutrient doses in the substrate preparation. The N concentrations were 0, 30, 60, 120, 180 and 240 (30 mg dm^{-3}). The drip irrigation was used at the same frequency as the Experiment 1. When the tanks levels reached 30% of total content, they were filled with water up to 50%. The pest control was performed with four applications of insecticides, as in experiment 1.

The experiment 4 was carried out in 2012/13, and presented the same treatments and management used at Experiment 2 in the field. The irrigation was not needed in 2012/13 crop season and the planting time was 10 days earlier than previous year.

It was evaluated the Area Under Rust Progress Curve (AURPC), defoliation (%), thousand grain weight (g) (TGW) and grain yield per plant (g) (yield) in both greenhouse (1 and 3), and field (2 and 4) experiments. Nitrogen concentration (g kg^{-1}) (NCL), dry mass of leaves (g) (DML) at R1 stage, number of grains per plant (GP) was obtained from the greenhouse experiments.

The AURPC was used to convert the disease progress in data which can be calculated, analyzed and compared according proposed by (Campbell & Madden, 1990). The AURPC was calculated based on 4 rust severity evaluations weekly done from 10 days after inoculation. It was used the diagrammatic scale suggested by (Godoy et al., 2006). It was evaluated the same 4 plants per treatment at each evaluation for greenhouse and 4 randomized points each replication on field. It was considered only the middle portion of the canopy. The rust severity caused early defoliation on soybean plants. The defoliation showed the direct rust effect on soybean leaves. Defoliation was calculated by counting the nodes on the main stem with and without leaves at the same time and sample in the last date severity rust evaluation.

For DML and NCL determinations 4 plants/treatment/trail were collected in the greenhouse and field experiments. The leaves were detached, conditioned in paper bag and dried. The leaves weight was gotten on analytical balance. They were sent to the Laboratório de Ecologia Florestal of Universidade Federal de Santa Maria, to determine total N concentration. The leaves were milled using a Willey mill and the N concentration was determined by the Kjeldahl method.

After maturation (R9), 4 plants were harvested from greenhouse experiments (8 m^2 were harvest from field experiments), yield and thousand grain weight were determined based on moisture content at 13%. The grain number per plant (GP) was counted using the Auto Meter grain. The replication was composed by 1 soybean plant.

Statistical analyzes were performed using the spreadsheet Office Excel Sigma Plot software and Assistat 7.7. The F test (p-value <0.05) was used for testing the main effects and interactions between N concentration and cultivars. When interactions or N concentration effects were significant data were submitted to regression analysis.

The linear simple correlation matrix with the coefficients between assessed variables was estimated through the (p-value <0.05) significance and (p-value <0.01) t test, in greenhouse experiments.

Results and Discussion

Greenhouse

The N content did not show any effect on Nitrogen concentration in leaves (NCL) as observed in the 2011/12 and 2012/13 experiments (Figures 1 E, F). The area under rust progress curve (AURPC) showed an increase three times higher on BMX Energia® cultivar and four times higher on BMX Potência® cultivar considering the increase of free Nitrogen in the 2011/12 experiments (Figure 1 A). Slight reduction on the area under rust progress on both cultivars was observed in the 2012/2013 experiments (Figure 1 B). Defoliation showed similar tendency as AURPC. The increase of availability of N produced a defoliation of 25% on cultivars average in 2011/12 (Figures 1 C). Defoliation seems to be affected by the increase of rust progress rate. The small difference on defoliation among treatments 2012/13 (Figure 1 D) was influenced by the rust progress rate (Figure 1 B).

The interaction between nitrogen concentration and cultivars was not significant for DML R1 2011/12, GP 2012/13, TGW 2012/13 and yield plant $^{-1}$ 2012/13 (Figure 2 A, D, F H). The DML increased 50% 2011/12 (Figure 2 A), it showed no significant interaction between N and cultivars. BMX Energia® showed significant decrease on DML for nitrogen concentration above 120 mmol L^{-1}, no differences were observed on DML in the cultivar BMX Potência® as de nitrogen concentration increase (Figure 2 B).

The GP decrease at nitrogen concentration above 10.5 mmol L^{-1}, both on BMX Energia® and BMX Potência® 2011/12 (Figure 2 C). The decrease of GP on both cultivars might be related to the mulching on the two higher N rates causing an increase of rust severity. In the 2012/13 (Figure 2 D) trials the GP increase on both cultivars with no mulching and slight seed weight increase. The TGW only in 2011/12 showed significant interaction between N and cultivars, it was observed decrease as the N concentration was above 10.5 $mmoL^{-1}$ on BMX Energia® cultivar (Figure 2 E). In the experiments carried out in 2011/12, on sand, the TGW on BMX Potência® showed an increase of 50 grams. No difference on TGW was observed in the 2012/13 experiments (Figure 2 F). The BMX Energia® cultivar decreases the yield due to higher N availability; the BMX Potência® cultivar increases the yield from 30 to 40 grams per plant (Figure 2 G, 2011/12). On mix sand plus soil experiments, it was not observed significant interaction between N rates and cultivars; an increase of five grams per plant was observed on cultivars average (Figure 2 H, 2012/13).

On the basis of the data submitted to the Experiment 1, 2011/12 on sand and Experiment 3, on mix sand plus soil, there are two factors that can explain the variation in the progress of rust. First, the increased rate of N in nutrient solution resulted in increased DML, with a large leaf area (data not shown), canopy microclimate conditions became more favorable for the development of rust. It is because nitrogen is coupled with photosynthetic apparatus, increases in it availability result in increases in biomass for soybean (Wood et al., 1993; Nunes-Nesi et al., 2010 and Nogueira et al., 2010). Second, in spite of the NLC was not significant, biochemical changes in the foliage may have happened depending on the increase of N. In this work biochemical changes were not determined, but would be the aim of the new studies. The analysis of simple correlation between variables (Table 2) helps to understand the relations between yield and the other variables in each of the two years, manly the relations between Biomass accumulation and soybean rust.

In Experiment 1, on sand, positive correlations were: AURPC and defoliation (r2 = 0.92), AURPC and NLC (r2 = 0.44), NLC and defoliation (r2 = 0.41), DML and AURPC (r2 = 0.59), DML and defoliation (r2 = 0.53), TGW and productivity (r2 = 0.65). Negative correlations occurred between: AURPC and GP (r2 = - 0.42), defoliation and GP (r2 = -0.59). In this case, the excess of leaves, depending on the increase of N, was lost with increasing defoliation. This loss was insignificant, for yield, the amount of 10.5 mmol L^{-1} in N solution (Figure 2 E, G). The concentration of 10.5 mmol L^{-1} N upward, increased the rust and aggravated the loss. As a consequence, the TGW and GP decreased from the concentration of 10.5 mmol L^{-1} N in the solution. As the progress of soybean rust is influenced by temperature and humidity, the increase of biomass favors the progress of rust. Lima et al. (2012) in cross planting, noted increased disease by increasing the population. Besides promoting changes of temperature, humidity and biomass of soybeans, increased biomass implies a lesser light penetration. According to Debona et al. (2008), soybean plants subjected to shading showed an increase of rust. However, the presence of light reduces the germination of spores of *Phakopsora phakyrhizi* (Furtado et al., 2009). This information leads us to believe that the excessive increase in the growth of soybeans can speed up the progress of soybean rust. Thus, the ideal biomass must be known for each variety of soy, so that the overgrowth should be avoided.

In experiment 3 (Table 2), the mix of sand and soil, and all the mineral nutrition provided in substrate, the plant had more difficulties to grow. So, in all treatments of Experiment 3 soybean plants were visually smaller than in the previous year, so the correlation between variables showed some changes. Positive correlations were: DML and defoliation (r2 = 0.65), GP and Yield (r2= 0.85). The negative correlations occurred between:

AURPC and defoliation (r2 = - 0.54), AURPC and NLC (r2 = 0.53), AURPC and NLC (r2 = 0.53), AURPC and GP (r2 = -0.67), AURPC and Yield (r2 = -0.81).

DML was not excessive and mulching was not observed. But in the same way the previous year, DML presented positive correlation with defoliation, despite no significant defoliation (Figure 1 D). There was negative correlation between AURPC and the variables: defoliation, NLC, DML, GP and Yield. It should be observed that several factors may add to increased yield against the effects of rust, on the other hand, many conditions influenced by the availability of N may facilitate the progress of rust.

These results are in agreement with those observed in the literature about K (Pinheiro et al., 2011; Doreto et al., 2012). It indicates that excessive fertilization promotes an increase on rust rate. Thus, the balance between the positive and negative factors defines the supremacy of processes of defense or increase of the epidemic.

Field

Field data in 2011/12 (Figure 3), support the experiments in the greenhouse. Despite the low AURPC and defoliation, yield increased, as the Experiment 3 in the greenhouse. In Experiment 4, 2012/13, the absence of response to N was related to high rainfall frequency (Table 1, Figure 3) that occurred during the summer. So the mineral N applied in soil may have been requested by leaching (Robertson & Groffman, 2007).

Therefore, additional yield gained by increasing rates of N should not be seen as a management strategy against soybean rust, but rather as important information about nutrition condition for the N in plants of soybeans can help or hinder the control of soybean rust. In the 2012/13 experiments N doses did not produce any statistical significance on the evaluated parameters (Figures 3 B, D, F, H). The interaction between N doses and cultivars were significant for AURPC, defoliation and yield. The AURPC and defoliation showed linear decrease on both cultivars (Figure 3 A, C). No significant interaction between N doses and cultivars was observed on TGW on results obtained in the 2011/12, neither significant effect due to N rate in the 2012/13 experiments (Figure 3 E, F). In 2011/12, a linear increases of 162 kg ha^{-1}, from 0 to 240 kg of N ha^{-1} on yield (Figure 3G) of BMX Energia$^{®}$ as observed to BMX Potencia$^{®}$ up to 242 kg ha^{-1}.

The yield increase obtained with the higher N rates on both cultivars were minor than the damage caused by the disease.

Conclusions

1. The increase nitrogen availability for soybeans, when it is not in excess, can cause a small decrease in the progress of soybean rust.
2. This decrease was not related to the concentration of nitrogen in foliage.
3. When the availability of nitrogen caused an excessive growth, the progress of soybean rust increased and produced a higher damage caused by this disease.

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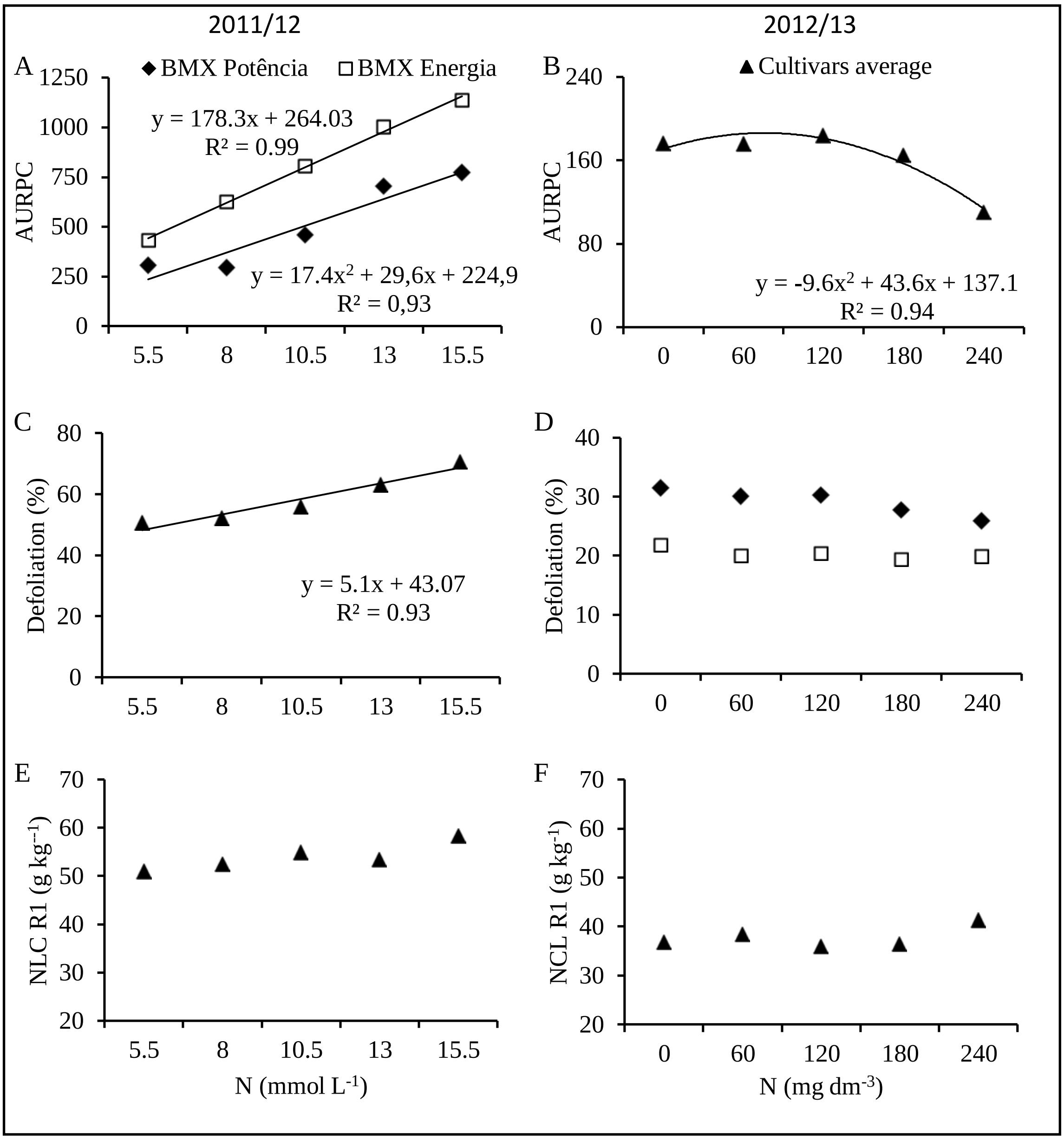

Figure 1. Area under rust progress curve (AURPC) (A, B), defoliation (C, D), at last date severity rust evaluation, nitrogen leaves concentration (NLC) (E, F) at R1 Stage grown on sand with N concentrations of 5.5, 8.0, 10.5, 13.0, and 15.5 mmol L^{-1} (A, C, E) (Experiment 1, 2011/12) and grown in sandy soil with lower concentrations of 0, 60, 120, 180 and 240 mg kg^{-1} (B, D, F) (Experiment 2, 2012/13).

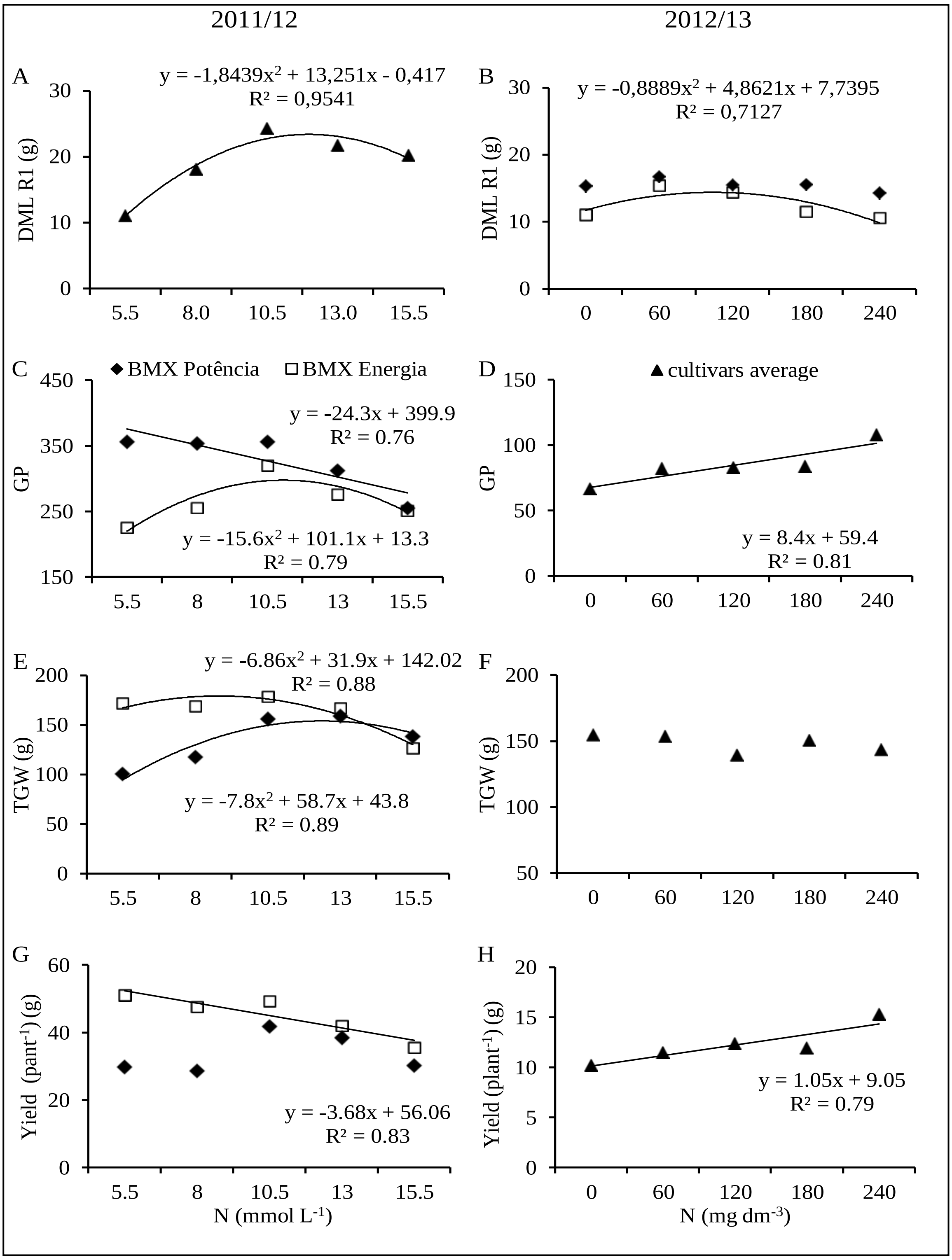

Figure 2. Dry mass of leaves (DML) (A, B) at R1 Stage. Grains per plant (GP) (C, D), thousand grain weight (TGW) (E, F), yield per plant (Yield plant-¹) (G, H) grown on sand with N concentrations of 5.5, 8.0, 10.5, 13.0 and 15.5 mmol L ¹ (A, C, E) (2011/12) and in sand plus soil with N rates of 0, 60, 120, 180 and 240 mg dm ³ (B, D, F) (2012/13).

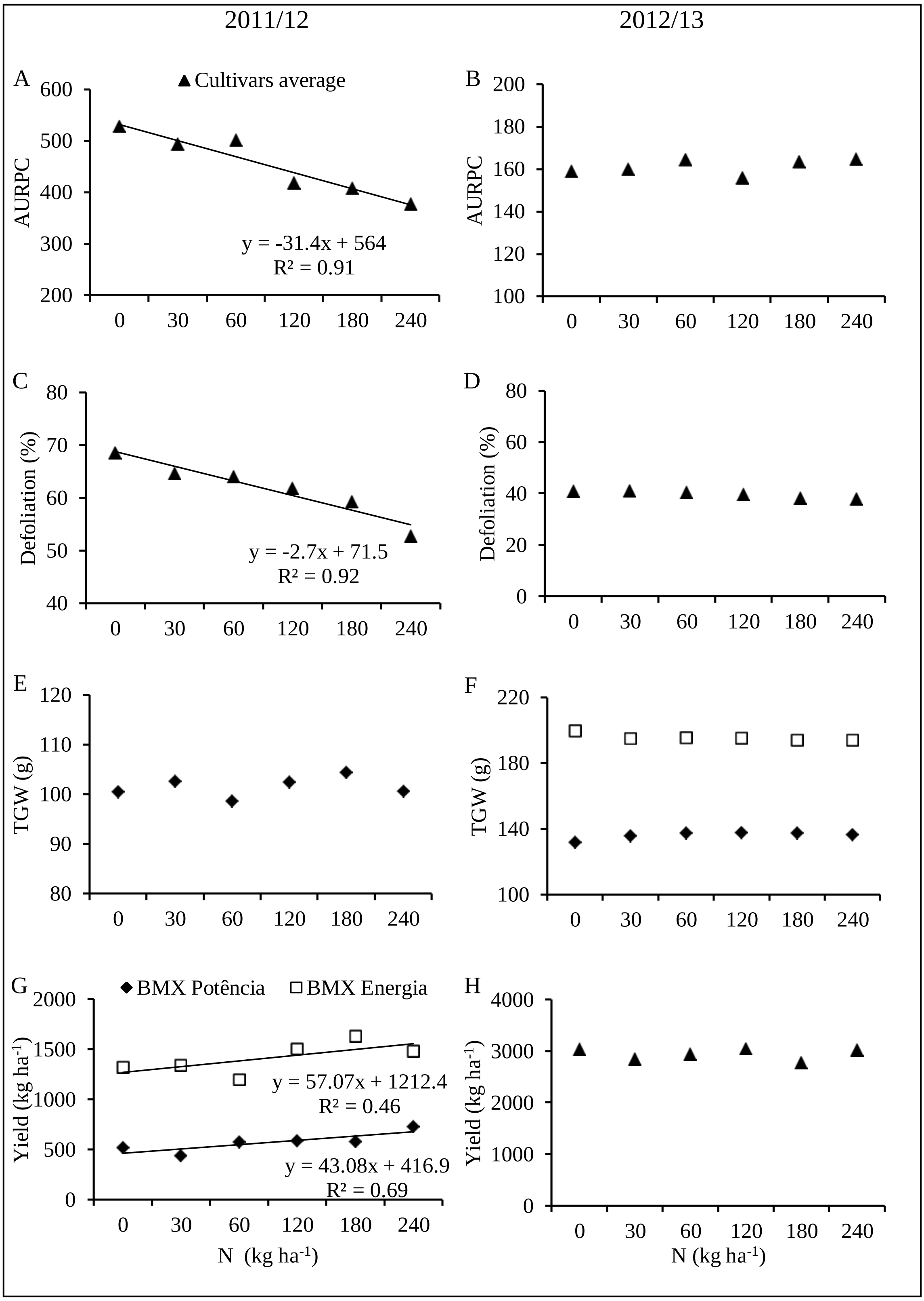

Figure 3. Area under rust progress curve (AURPC) (A, B), defoliation (C, D) at last date severity rust evaluation, thousand grain weight (TGW) (E, F), yield (G, H) grown in tillage with the N rates of 0, 60, 120, 180 and 240 kg ha^{-1} in 2011/12 (Experiment 2; A, C, E, G) and 2012/13 (Experiment 4; B, D, F, H).

Table 1. Averages of air temperatures and rainfall at the experimental site during the crop growing periods in 2011/2012 and 2012/2013. Itaara city – Rio Grande do Sul, Brazil.

Year	Month	Min (C°)		Max (C°)		Med (C°)		Rainfall (mm)	
2011(2012)	October	13.52	(15.26)	22.51	(23.63)	18.01	(19.45)	177	(294)
2011(2012)	November	15.28	(20.64)	26.29	(26.81)	20.78	(23.72)	28	(79)
2011(2012)	December	16.33	(19.60)	27.66	(30.20)	22.00	(24.90)	40	(329)
2012(2013)	January	18.23	(18.01)	29.84	(29.92)	24.04	(23.97)	98	(152)
2012(2013)	February	20.50	(18.44)	30.64	(27.48)	25.57	(22.96)	294	(102)
2012(2013)	March	11.90	(15.64)	26.40	(24.14)	19.15	(19.89)	34	(219)

With parenthesis: first experiment year. Without parenthesis: second experiment year.

Table 2. Simple correlation among variables, Area under rust progress curve (AURPC), defoliation (DEFOL), nitrogen centration in leaves, (NCL), dry mass of leaves (DML), plant height, grains per plant (GP), thousand grain weight (TGW) and yield, in two crop periods of plants grown in the greenhouse with sand (Experiment 1, 2011/12) and in the mixture of sand plus soil (Experiment 3, 2012/13).

Experiment – 1							
VA\VA	AURPC	DEFOL	NLC	DML	GP	TGW	YIELD
AURPC	1	0.92**	0.44*	0.59**	-0.42*	0.23ns	0.14ns
DEFOL	-	1	0.41*	0.53**	-0.59**	0.26ns	0.16ns
NLC	-	-	1	0.10ns	-0.18	0.02ns	-0.13ns
DML	-	-	-	1	-0.09	0.36ns	0.18ns
GP	-	-	-	-	1	-0.30ns	-0.19ns
TGW	-	-	-	-	-	1	0.65**
YIELD	-	-	-	-	-	-	1
Experiment - 3							
AURPC	1	-0.54*	-0.53*	-0.23ns	-0.67**	-0.25ns	-0.81**
DEFOL	-	1	0.29ns	0.65**	0.20ns	0.15ns	0.37ns
NLC	-	-	1	0.22ns	0.33ns	0.16ns	0.37ns
DML	-	-	-	1	0.10ns	-0.07ns	0.13ns
GP	-	-	-	-	1	0.10ns	0.85**
TGW	-	-	-	-	-	1	0.24ns
YIELD	-	-	-	-	-	-	1

**Significant at 1% level of probability *Significant at 5% level of probability; ns: non-significant.

DISCUSSÃO

Observando os resultados obtidos nos dois anos de experimento nota-se que a disponibilidade de N causa diversas alterações no crescimento e desenvolvimento da planta de soja e alterações no patossistema de *Phakopsora pachyrhizi* S. O aumento no acúmulo de biomassa nos diferentes órgãos da planta é benéfico e tem como consequência maiores valores de produtividade, mas devem ser observadas particularidades que podem tanto interferir de maneira direta na produtividade, como o crescimento e acúmulo de biomassa, assim como de maneira indireta, através de modificações microclimáticas que podem alterar a intensidade de doenças, neste caso, a ferrugem asiática da soja.

As varáveis do acúmulo de biomassa em função das diferentes disponibilidades de N (Artigo 1, Figura 1) não mostraram correlação direta com a produtividade (Artigo 1, Tabela 2). Nos resultados da literatura essa relação tem sido observada em plantas de soja (NOGUEIRA, 2007). Nos experimentos atuais essa relação pode não ter ocorrido devido à elevada disponibilidade de N, principalmente nas concentrações acima de 10,5 mmol L^{-1} e 120 mg dm^{-3} em 2011/12. Essas concentrações elevadas estimularam o crescimento vegetativo da planta. Folhas grandes e em número elevado reduzem a penetração da radiação solar no interior do dossel e a eficiência de intercepção, principalmente nas camadas inferiores. Além disso, o crescimento excessivo da parte aérea provoca acamamento, o qual reduz ainda mais a penetração da radiação solar no dossel e pode provocar mortalidade de plantas. A fração da área foliar que recebe baixa intensidade de radiação solar deixa de contribuir para a fotossíntese. Consequentemente, a relação entre o acúmulo de massa seca e a produtividade torna-se baixa ou deixa de ocorrer (Artigo 1, Tabela 2).

Os resultados da massa seca das raízes e da nodulação também mostraram particularidades interessantes. Sob disponibilidade de N baixa ou moderada, tanto em areia pura (Experimento 1) quanto em solo mais areia (Experimento 3), houve crescimento das raízes, enquanto sob disponibilidades elevadas esse crescimento foi reduzido. Essa relação entre a disponibilidade de nutrientes e o crescimento das raízes já foi demonstrada na literatura (KANG et al., 2011). Entretanto, a absorção de água também é um processo realizado pelas raízes. Em não havendo restrição hídrica durante o crescimento e desenvolvimento da planta, o reduzido crescimento das raízes decorrente de uma elevada disponibilidade de nutrientes pode não ter efeitos negativos sobre o crescimento da planta e a produtividade. Entretanto, no caso de uma restrição hídrica, principalmente na fase de floração e enchimento de grãos, o reduzido crescimento das raízes poderia trazer prejuízos severos ao crescimento e á produtividade. Quanto ao número de nódulos ativos, observaram-se valores mais elevados quando as plantas foram cultivadas na mistura de solo com areia em casa de vegetação. Mas mesmo em diferentes substratos de cultivo, tanto no ambiente controlado como no campo, a nodulação diminuiu com o aumento da disponibilidade de nitrogênio.

A altura de plantas apresentou alta correlação com a produtividade (Artigo 1, Tabela 2). Esta é uma particularidade que deve ser observada para cultivares precoces de soja de hábito de crescimento indeterminado. A altura de plantas mostrou ser uma variável importante por estar relacionada com o número de grãos por planta, em consequência do aumento no número de nós da planta (NOGUEIRA, 2007). Assim práticas de manejo que auxiliem o crescimento da haste principal favorecem diretamente a produtividade de grãos nestas cultivares.

Mas nem sempre aumentos da biomassa de plantas são favoráveis ao aumento da produtividade. Quando o crescimento é excessivo, pode ser aumentada a intensidade da ferrugem asiática mesmo em cultivares precoces e indeterminadas (Artigo 2).

As variáveis de produtividade em vários dos experimentos que foram realizados neste trabalho mostraram tendência de acréscimo na produtividade com o aumento da disponibilidade de nitrogênio. Entretanto, os dados de produtividade apresentados no Artigo 1 foram obtidos em condições de controle total de ferrugem, enquanto que aqueles relativos ao Artigo 2 forma obtidos em condição de epidemia não controlada. O fato de não ter sido feito controle da ferrugem asiática causou reduções próximas a 70% na produtividade de grãos e foi mais severa na cultivar BMX Potência®, a qual possui um ciclo em torno de 10 dias maior que a BMX Energia®. Estes dados comprovam o grande potencial de dano dessa doença, confirmando os dados de (GODOY et al., 2009). Isso significa que no manejo do nitrogênio na cultura da soja devem-se evitar quantidades de N que promovam crescimento excessivo. Tal crescimento gera condições favoráveis a epidemias da ferrugem asiática.

CONCLUSÃO

Com base nos resultados obtidos nesse trabalho pode-se concluir que a adubação nitrogenada mineral pode aumentar o crescimento da planta e a produção de grãos das cultivares de soja BMX Potência® RR e BMX Energia® RR em condições ideais de água e nutrientes. No entanto, sob condições ambientais favoráveis para a ferrugem asiática, o aumento da produção de grãos obtido pelo uso de altas doses de N pode ser anulado pelos danos causados pela doença. Em condições de campo, os efeitos do N sobre o rendimento de grãos são de pequena importância em ambas as cultivares. A expressão do potencial produtivo da soja através de uma disponibilidade mais elevada de N deve ser buscada preferencialmente pela fixação simbiótica. Uma nodulação mais eficiente poderia suprir as exigências de nitrogênio de forma gradual ao longo do ciclo, reduzindo os riscos do crescimento excessivo da planta que pode estar associado ao uso de fertilizantes nitrogenados em cobertura. Boas condições químicas e físicas do solo capazes de aumentar o número e a eficiência dos nódulos e/ou estirpes mais eficientes para o desenvolvimento de inoculantes são também alternativas a serem pesquisadas.

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