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**Fungicide soybean seed treatment: interactions with inoculant, soil,
and plant**

Felipe Fadel Sartori

Thesis presented to obtain the degree of Doctor of
Science. Area: Crop Science

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Felipe Fadel Sartori
Agronomist Engineer

Fungicide soybean seed treatment: interactions with inoculant, soil and, plant
versão revisada de acordo com a Resolução CoPGr 6018 de 2011

Advisor:
Prof. Dr. **DURVAL DOURADO NETO**

Co-advisor:
Prof. Dr. **VALDEMAR LUIZ TORNISIELO**

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1. *Glycine max* 2. Ureídeo 3. Pré-inoculação 4. Translocação 5. Radiomarcado 6. *Phytophthora sojae* I. Título

DEDICATION

To my father and my mother. For all their efforts and support along my academic life, but most importantly, for their constant example of kindness and gratitude. Every time an unfortunate life event tried to push them back, they stood up and thrived, and I will always carry this lesson in my life.

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RESUMO

Fungicidas no tratamento de sementes de soja: interações com inoculante, solo e planta

O tratamento de sementes se tornou parte do sistema de produção de soja no Brasil, a ponto de que quase não se encontraram áreas que não utilizem essa modalidade de aplicação. Não obstante, apesar da sua sólida presença comercial, dúvidas aparecem safras após safra sobre questões que envolvem a interação dos produtos aplicados via sementes, e dos mesmos com o ambiente de produção. Assim, os objetos do presente estudo foram: (i) determinar como o tratamento de sementes com fungicidas associado à pré-inoculação de *Bradyrhizobium elkanii* em soja pode prejudicar a fixação biológica de nitrogênio (FBN) e a produtividade; (ii) como os fungicidas aplicados via tratamento de sementes são absorvidos e translocados nas plântulas, e como interagem com o solo e suas propriedades; (iii) e a longevidade da ação dos fungicidas aplicados via tratamento de sementes ao longo do desenvolvimento das plantas. Para investigar os objetivos propostos pelo projeto, quatro experimentos de campo e sete em casa de vegetação foram executados ao longo dos anos 2016, 2017, 2018 e 2019. Os experimentos de campo tiveram como propósito avaliar o efeito de fungicidas somado à pré-inoculação de *B. elkanii* em sementes de soja, tanto na FBN como na produtividade, avaliando-se a concentração de ureídeos, a eficácia da FBN e os componentes de produtividade da cultura. Já os experimentos em casa de vegetação buscaram elucidar o padrão de absorção e translocação de diferentes fungicidas aplicados via tratamento de sementes em plântulas de soja por meio do uso de moléculas radiomarcadas, bem como a longevidade do tratamento de sementes na eficácia do controle de *Phytophthora sojae*, avaliando-se a severidade de doença nas raízes e biometria das plantas. Os resultados mostram que tanto o tratamento de sementes como a pré-inoculação afetam a FBN, não acarretando, contudo, em impactos na produtividade. Ademais, o tratamento de sementes não resultou em produtividade superior ao controle com apenas inoculante em nenhum dos experimentos. Quanto à absorção e translocação de fungicidas aplicados via tratamento de sementes em plântulas de soja, nota-se que a maior parte dos produtos, quando absorvidos, concentram-se nos cotilédones das plantas, e que o teor de matéria orgânica do solo pode influenciar na absorção dos produtos. Por último, em relação a longevidade de ação de fungicidas ao longo do desenvolvimento das plantas, notou-se que o limite de eficácia satisfatória no manejo de *Phytophthora sojae* foi de até 14 dias após a semeadura.

Palavras-chave: *Glycine max*, Ureídeo, Pré-inoculação, Translocação, Radiomarcado, *Phytophthora sojae*

ABSTRACT

Fungicide soybean seed treatment: interactions with inoculant, soil, and plant

Seed treatment has become part of the soybean production in Brazil and, currently, almost a hundred percent of the areas use this type of pesticide application. Nevertheless, despite its solid commercial presence, questions regarding the interaction of pesticides with biological products and the environment arise year after year. Thus, the objectives of the present study were: (i) to determine how soybean seed treatment with fungicides associated with *Bradyrhizobium elkanii* pre-inoculation can be detrimental to the biological nitrogen fixation (BNF) and yield; (ii) how fungicides applied via seed treatment are absorbed and translocated in seedlings, and how they interact with the soil and its properties; (iii) and the longevity of fungicide action along plant development. In order to investigate these goals, four field and seven greenhouse experiments were carried out over the years 2016, 2017, 2018 and 2019. The field experiments aimed to evaluate the effect of fungicides plus pre-inoculation with *B. elkanii* on soybean seeds, both on BNF and yield, evaluating the concentration of ureides, the BNF efficiency and the productivity components of the crop. The greenhouse experiments sought to elucidate the absorption and translocation pattern of different fungicides applied via seed treatment in soybeans using radiolabels active ingredients, as well as the longevity of seed treatment efficacy on controlling *Phytophthora sojae*, assessing severity of root rot and plant development. The results showed that both seed treatment and pre-inoculation affect BNF, however, did not cause impacts on yield. In addition, seed treatment did not have superior yield than control with inoculant alone in any of the experiments. Regarding the absorption and translocation of fungicides applied via seed treatment in soybean, it was observed that most products, when absorbed, were concentrated in the cotyledons of plants, and that the soil organic matter content can influence the absorption of the products. Finally, regarding the longevity of action of fungicides during plant development, it was shown that the satisfactory efficacy in the management of *Phytophthora sojae* was up to 14 days after planting.

Keywords: *Glycine max*, Ureides, Pre-inoculation, Translocation, Radiolabelled, *Phytophthora sojae*

INTRODUCTION

On 2018/2019 crop season, almost 98% of soybean seeds were treated with fungicides in Brazil (Richetti and Goulart, 2018). Specifically for soybean seeds, the first official recommendation for fungicide treatment was made by Embrapa Soja in 1981 (Henning et al., 1981). The treatment of soybean seeds with fungicides, which in the 1991/92 crop season did not reach 5% of the area (Henning et al., 2010), now approaches the 100%.

Currently, many different groups of fungicides have been used in soybean seed treatment to control both seed and soilborne pathogens (Dorrance et al., 2003; Broders et al., 2007; Ellis et al., 2010). Among these, benzimidazoles (which affects mitosis and cell division), strobilurins (which affects respiration), and phenylamides (which affects protein production) (Oliver and Hewitt, 2014) represent important molecules for fungicide seed treatment. Benzimidazoles (e.g., thiabendazole, carbendazim, and thiophanate-methyl), are effective against a wide range of ascomycetes and basidiomycetes, but not against oomycetes. On the other hand, phenylamides (e.g. metalaxyl and mefenoxam) are effective exclusively against oomycetes. Strobilurins (e.g. pyraclostrobin, azoxystrobin, and trifloxystrobin) are effective against some members of ascomycetes, basidiomycetes and oomycetes (Oliver and Hewitt, 2014). In addition to the mode of action of each active ingredient, other physicochemical characteristics may contribute to the classification of fungicides, such as the log K_{ow} (also known as log P), which indicates the partition coefficient between n-octanol and water and measures hydrophobicity/hydrophilicity of the molecule (Oliver and Hewitt, 2014).

Phytophthora sojae is one of the most important soybean pathogens across the world. This soilborne disease is essentially monocyclic. *P. sojae* produces sexual oospores in root tissue following infection, which can survive in the soil (Schmitthenner, 1985; Dorrance et al., 2007), and as a result, plants grown in fields infested with *P. sojae* are at constant risk. The primary strategies to manage *Phytophthora* root and stem rot are resistant cultivars (Rps genes and quantitative resistance), and seed treatment with systemic fungicides (Schmitthenner and Dorrance, 2015; Dorrance, 2018). The use of fungicides to control *Phytophthora* root and stem rot in fields with high disease pressure is a useful technique for both moderate susceptible and resistant cultivars, when compared to the non-treated control (Dorrance and McClure, 2001; Dorrance et al., 2009) thus soybeans grown in these high disease-risk environments should be treated with at least one active ingredient available in the market against oomycetes in their seed treatment mix (Dorrance, 2018).

More recently, seed producers have implemented a practice that has been very well accepted by soybean growers: Industrial Seed Treatment (IST). This technology relies on the

use of special and highly sophisticated equipment, which combines the application of fungicides, insecticides, nematicides, micronutrients, biological products and so on, with high rate accuracy (França-Neto et al., 2015).

In Brazil, *Bradyrhizobium* sp. inoculants are commonly applied in seed treatment or in furrow, the former being the most common. When applied via seed treatment, the inoculant is part of packages with chemicals such as fungicides, insecticides, nematicides, micronutrients and biostimulants, which sometimes are incompatible with the rhizobial survival (Campo et al., 2009). The possibility to deliver the inoculant along with the chemicals in the seed treatment triggered the development of the well-known “long life inoculants”, which, in theory, would allow seed pre-inoculation for periods of up to 60 days.

Therefore, the objectives of the present study were: (i) to determine how soybean seed treatment with fungicides associated with *Bradyrhizobium elkanii* pre-inoculation can be detrimental to the biological nitrogen fixation (BNF) and yield; (ii) how fungicides applied via seed treatment are absorbed and translocated in seedlings, and how they interact with the soil and its properties; (iii) and the longevity of fungicide action along plant development. In order to investigate these goals, four field and seven greenhouse experiments were carried out over the years 2016, 2017, 2018 and 2019.

CONCLUSIONS

Pre-inoculation of soybean seeds for 30 days associated with pesticides used in seed treatments may negatively affect the BNF in soybean even in areas with an established population of *Bradyrhizobium* spp. Nevertheless, seed treatment and storage reduced yield only in one out of four experiments, and in this case only for pyraclostrobin + thiophanate-methyl + fipronil. Furthermore, seed treatment did not increase yield in any of the experiments when compared to control treatment without pesticides.

The total amounts of fungicides absorbed by seedlings are frequently less the half of what is present on the seed, and most of the amount absorbed was concentrated in the cotyledons, not in seedling roots. Moreover, soil type affects the amount of fungicide absorbed by the plants for both lipophilic and hydrophilic molecule, and this effect includes the amount of fungicides in the roots.

The maximum satisfactory activity of fungicide seed treatment for *Phytophthora* root rot control goes until 14 days after planting (V_1), and that compound A was more efficient to manage *Phytophthora sojae* than others fungicides available in the market.

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