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Copper phosphite enhances efficacy of a strobilurin-triazole fungicide in controlling late season foliar diseases of soybean

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ABSTRACT

Septoria brown spot (*Septoria glycines*) and Cercospora leaf blight (*Cercospora kikuchii*) are late season foliar diseases (LSFD) that co-occur every year in the soybean growing regions of Argentina. Repeated use of commercial, formulated mixes of strobilurin-triazole fungicides to control LSFD has prompted the need for tactics to increase the efficacy of these fungicide groups, thereby reduce number of applications and help prolong their life use. The objective of this study was to determine the effect of copper phosphite (CuPhi), tank-mixed with a strobilurin-triazole fungicide, in the fungicide efficacy in controlling LSFD and protecting soybean yield. Field experiments were conducted during the 2014/2015 and 2016/2017 growing seasons in six different locations in the Pampas region of Argentina. At each location, treatments consisted of: i) one foliar application of a fungicide formulated as a mix of picoxystrobin and cyproconazole (60 and 24 g a.i. ha^{-1}, respectively), ii) one foliar application of the fungicide tank mixed with a CuPhi formulation, and iii) an untreated control. Treatment application timing in each trial was defined by an LSFD scoring system previously developed by the authors. All treatments were disposed to the experimental units in a randomized complete block design with four replications. Severity of LSFD was estimated visually as the percentage of diseased leaf area in 50 plants randomly selected in each plot at R6-R7. Grain yield (kg ha^{-1}) was determined at physiological maturity in all trials. Severity and yield analysis using a linear mixed-effect model indicated that addition of CuPhi to the fungicide tank mix significantly reduced LSFD severity and protected yield ($P < 0.05$). The observed effect on disease control, however, was influenced by location in the 2014/2015 growing season, but not in the 2016/2017 growing season. The net economic return with the addition of CuPhi to the fungicide tank mix was 66.1 and 85.5 USD ha^{-1} higher than the fungicide formulation alone, in the 2014/15 and 2016/17 crop seasons respectively. This study shows that the addition of CuPhi to a strobilurin-triazole fungicide tank mix had a synergistic and/or additive effect in controlling LSFD in soybean and protecting grain yield. Enhanced efficacy of these fungicide groups with the addition of CuPhi could reduce the number of applications, and in consequence, help prolong life use of these fungicide modes of action.

1. Introduction

Septoria brown spot (*Septoria glycines*) and Cercospora leaf blight (*Cercospora kikuchii*) are late season foliar diseases (LSFD) that co-occur every year in most soybean growing regions of Argentina. These diseases can reach high levels of intensity under favorable weather conditions, with the potential to reduce grain yield and seed quality (Carmona, 2011; Hartman et al., 2015). Control of LSFD is traditionally carried out with fungicides, predominantly with formulated mixtures of quinone outside inhibitors (QoIs) and demethylation inhibitors (DMIs), or of the newly introduced succinate dehydrogenase inhibitors (SDHIs). Associated to the need for repeated applications of QoIs and DMIs to control LSFD each season, there is an increased risk of fungicide resistance development to those mode of actions. Therefore, novel tactics that enhance fungicide efficacy and help prolong life of mechanisms of action are critical in LSFD management programs.

Phosphites (Phis: derivatives of the phosphorous acid) have been reintroduced in disease control as products having direct antifungal effect (Guest and Grant, 1991). Desirable characteristics of Phis include their high compatibility in tank mixtures, translocation in both xylem and phloem, and induction of systemic acquired resistance (Guest and Grant, 1991; Massoud et al., 2012; Burra et al., 2014; Eshraghi et al., 2014a). In addition, Phis are considered to posse lower risk of disease resistance development than fungicides active ingredients (FRAC,

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2018). No case of true fungi resistance to Phis has been reported to date.

Recent research studies confirm the effectiveness of Phis in controlling both oomycete and fungal diseases in a few crops (Silva et al., 2011; Carmona et al., 2017a; b; Simonetti et al., 2015; Gill et al., 2018). However, while Phis seem promising in disease management, their performance in actual disease control is still poorly understood and their effect in tank mixes with the most widely used fungicide modes of action for control of LSFD is unknown (Graham, 2011; Bock et al., 2012; Lobato et al., 2011; Gómez-Merino and Trejo-Téllez, 2015; Liljeroth et al., 2016). Moreover, multilocation trials with formal assessments of LSFD intensity and fungicide application timings based on LSFD risk are lacking. The objective of this study was to determine the effect of copper phosphite (CuPhi), tank-mixed with a strobilurin-triazole fungicide, in the fungicide efficacy in controlling LSFD and protecting soybean yield.

2. Materials and methods

Field trials were carried out during the 2014/2015 and 2016/2017 growing seasons at six different locations in Argentina. In the 2014/15 season, experiments were conducted at Bigand and Diego de Alvear in Santa Fe province, and Pieres in Buenos Aires province. In the 2016/17 season, experiments were conducted at Galvez and Oliveros in Santa Fe province, and Salto in Buenos Aires province.

At each location, experiments consisted of (i) one foliar application of a fungicide (F) formulated as a mix of picoxystrobin and cyproconazole and tank mixed with the manufacturer's recommended adjuvant (Quil Oil® 22 cc ha^{-1}), (ii) one foliar application of the fungicide tank mixed with a CuPhi formulation (F + CuPhi), and (iii) an untreated control (Table 1). The fungicide formulation was supplied by DuPont Agro (Rosario, Santa Fe, Argentina) and the CuPhi product was supplied by Spraytec Fertilizantes, LDTA (Rosario, Santa Fe, Argentina). While there are a few Phis-based formulations on the market, the CuPhi product used in the study was selected because of its confirmed compatibility with several strobilurin-triazole fungicides in preliminary experiments (Carmona et al., unpublished).

Trials were conducted in commercial soybean fields planted to LSFD susceptible cultivars. Application timing of the treatments at each location and growing season was defined by an LSFD scoring system developed by Carmona et al. (2015, 2018). The system guides fungicide applications based on risk of LSFD rather than on fixed crop developmental stages. This system has proven to be effective in identifying a crop yield response to fungicide applications targeting LSFD in Argentina (Carmona et al., 2015, 2018).

The experimental design at each location was a randomized complete block with four replications per treatment, with the experimental unit being a 10 x 2-m plot with soybean rows spaced at 52 cm. The fungicide and the fungicide + CuPhi treatments were applied with a carbon dioxide pressurized sprayer equipped with four 50-cm apart, full-cone spray nozzles (Lurmark HCX4 30) and providing an overall pattern swath width of 2 m. The sprayer was operated at a pressure of 3.16 kg/cm^2 and at a water volume rate of 150 l/ha. Daily rainfall was measured *in situ* and all risk factors suggested by the LSFD scoring system were recorded for each trial. Severity of Cercospora leaf blight (*C. kikuchii*) and Septoria brown spot (*S. glycines*) was assessed visually as the percentage of diseased area of all leaves of 50 plants examined at R6-R7 in each plot (Carmona et al., 2015). The estimated severity of all leaves was averaged to obtain a single value per plot. Grain yield (kg/ha) was measured at physiological maturity in each plot and adjusted to 130 g/kg moisture content.

Table 1
Description of the products used in the treatments evaluated in the present study.

Product Trade Name	Fungicide active ingredient/s and concentration in g 100 mL^{-1}	Formulation type	Application rate	
			Milliliters of product ha^{-1}	g a.i. ha^{-1}
Stinger®	Picoxystrobin: 20 Cyproconazole: 8	Suspension concentrate	300	60 24
CUBO®	–	Suspension concentrate	200	–

Grain yield response (kg ha^{-1}) to the treatment (fungicide or fungicide + CuPhi) was calculated for each trial and growing season as yield in the treatment minus yield in the control. Net economic return was calculated as value (in USD ha^{-1}) of the yield advantage in the treatment (fungicide or fungicide + CuPhi) minus cost of treatment (product cost plus application cost in USD ha^{-1}). Soybean grain price was 214.8 USD t^{-1} and 233.4 USD t^{-1} in the 2014/2015 and 2016/2017 crop seasons, respectively. Fungicide cost was 11.4 USD ha^{-1}, CuPhi cost 6.6 USD ha^{-1} and application cost was 4 USD ha^{-1} for both crop seasons. Pricing of the grain, product cost and application cost were obtained from corresponding average prices in the region for the years of study according to the Rosario Board of Trade (RBT, 2018).

LSFD severity and soybean grain yield data were analyzed by a linear mixed-effect model implemented in the lme4 package (Bates et al., 2013, 2015) of R (R version 3.4.3, R Development Core Team, 2018). In the model, block within location was entered as a random effect. The final model representing all effects and sources of variation was:

$$Y_{ijk} = \mu + \tau_i + Loc_j + \tau Loc_{ij} + Block\,(Loc_j)_k + \varepsilon_{ijk}$$

$$E_{ijk} \sim N\,(0,\ \sigma^2)$$

$$Block\,(Loc_j)_k \sim N\,(0,\ \sigma^2)$$

Where Y_{ijk} is the soybean grain yield (kg/ha) or the LSFD foliar severity (%) of the i*th* treatment at the j*th* location for the k*th* replicate, μ is the yield or severity mean (=model intercept), τ_i is the main fixed effect of treatment, Loc_j is the main fixed effect of location, $Block\,(Loc_j)_k$ is the random effect of the k*th* block within the j*th* location and ε_{ijk} is the experimental error present in the k*th* observation on the i*th* treatment in the j*th* location, which is a random variable with mean zero and variance σ^2.

3. Results

Raw means of LSFD severity were consistently lower in the F + CuPhi treatment than in the fungicide formulation alone or the control (Table 2). Yield in the F + CuPhi treatment was also higher than that in the fungicide formulation alone or the control. Those differences were consistent across all locations and growing seasons (Table 2).

For the 2014/2015 growing season, there was a significant interaction between treatment and location ($P < 0.001$). Therefore, only simple effects were further examined. Contrasts of disease severity in the F + CuPhi treatment (averaged across the three locations) vs. that in the fungicide treatment alone or the control (also averaged across the three locations) indicated no statistical significance differences. However, the same simple effect contrasts for yield showed that the addition of CuPhi to the fungicide tank mix significantly protected yield compared to the fungicide alone or the control ($P < 0.05$; Table 3). The mean net economic return, calculated as previously described, was 63.2 USD ha^{-1} for the fungicide treatment alone and 129.3 USD ha^{-1} for the F + CuPhi treatment (Table 3).

For the 2016/2017 growing season, there was not a significant interaction between treatment and location. In consequence, only main effects were examined. Main effect analysis showed that in the three locations, addition of CuPhi to the fungicide tank mix significantly decreased LSFD severity and protected yield compared to the fungicide