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Controlling Asian soybean rust (*Phakopsora pachyrhizi*) with *Bacillus* spp. and coffee oil

Dalton Vinicio Dorighello [a], Wagner Bettiol [b, *], Nilson Borlina Maia [c], Regina Maria Vilas Bôas de Campos Leite [d]

[a] Universidade Estadual Paulista "Júlio de Mesquita Filho", FCA/Campus Botucatu, 18.610-307, Botucatu, SP, Brazil
[b] Embrapa Environment, CP 69, CEP. 13820-000, Jaguariúna, SP, Brazil
[c] Instituto Agronômico de Campinas, Centro de Horticultura – Plantas Aromáticas e Medicinais, Campinas, SP, Brazil
[d] Embrapa Soybean, CP 231, CEP 86001-970, Londrina, PR, Brazil

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ABSTRACT

Asian soybean rust is currently the main soybean disease in Brazil and its control is primarily accomplished with fungicides. This study evaluated the potential of *Bacillus* spp. and coffee oil, alone and combined with fungicide, to inhibit the germination of *Phakopsora pachyrhizi* uredospores and control the disease on detached leaves and plants in greenhouse and field conditions. The trials were carried out using the BRS 316 RR soybean cultivar. *Bacillus subtilis* (QST-713) and *Bacillus pumilus* (QST-2808) isolates, roasted and crude coffee oils used individually, as well as coffee oils combined with half dose of fungicide, inhibited uredospore germination by 100%. In tests with detached leaves, *B. subtilis* (isolates QST-713, AP-3, and AP-51) and *B. pumilus* (QST-2808) reduced disease severity by 98.6, 75.3, 61.2, and 97.7%, respectively. The reductions resulting from crude and roasted coffee oils were 80.1 and 87.7% compared to 77.5, and 84.4%, respectively, at concentrations of 2 and 1%. Under greenhouse conditions, all treatments, except *B. subtilis* AP-51, reduced the area under the disease progress curve (AUDPC), while under field conditions, the *B. subtilis* QST-713 isolate and roasted coffee oil at 1 and 2% reduced disease severity by 23, 18, and 23%, respectively. The results indicate that *B. subtilis*, *B. pumilus*, and coffee oils exhibit the potential to control Asian soybean rust disease.

1. Introduction

Brazil is the second largest soybean producer worldwide. During the 2011/2012-crop season, 66.38 million tons were produced (CONAB, 2012). Asian soybean rust (*Phakopsora pachyrhizi*) is currently the main disease of the crop and can reduce production by 90% (Godoy et al., 2009). In the 2012/2013-crop season, US$1.186 million was spent on fungicides for use on soybean (SINDAG, 2013), primarily to control the rust. Despite the efficacy of fungicides to control the disease, the intensive use of fungicides can select pathogen-resistant populations, hindering management in consecutive crop seasons (Cook, 2001; Miles et al., 2005) and causing biological imbalances and environmental contamination.

Despite the fact that the majority of the fungicides recommended for control of Asian soybean rust are highly effective in reducing disease severity and increasing yield, results show a clear difference in efficacy among active ingredients alone or in combination, critical information that must be taken into account when choosing a fungicide for rust control (Scherm et al., 2009). According Godoy (2012), the efficiency of the triazoles in controlling the Asian soybean rust has decrease, enhancing the necessity of new alternatives to arrange a management program for the reduction the fungicides dependency.

The biocontrol of Asian soybean rust lacks thorough studies (Goellner et al., 2010). However, Ward et al. (2012) found that *Simplicillium lanosoniveum* reduced the number of uredia on soybean leaves by four times and increased the number of reddish-brown (RB) injuries, i.e., less sporulation and reduced spore germination. Reduced disease severity in the presence of *S. lanosoniveum* was reported by Ward et al. (2012), under field conditions. The hyperparasites *Verticillium psalliotae* (Saksirirat and Hoppe, 1991) and *Trichothecium roseum* (Sangit and Jha, 2002) have been discussed as potential biocontrol agents for Asian soybean rust. Biocontrol has shown effectiveness in controlling rust in

* Corresponding author. Tel.: +55 19 33112662.
E-mail addresses: wagnerbettiol@gmail.com, wagner.bettiol@embrapa.br (W. Bettiol).

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different crops: *Hemileia vastatrix* in coffee (Shiomi et al., 2006; Haddad et al., 2009), *Uromyces appendiculatus* in common bean (Bettiol et al., 1992; Yuen et al., 2001; Wagacha et al., 2007), *Puccinia pelargonii-zonalis* in geranium (Rytter et al., 1989) and *Puccinia striiformis* f. sp. *tritici* in wheat (Li et al., 2013).

According to Ongena and Jacques (2008), *Bacillus* spp. could be one of the major sources of potential microbial biopesticides due to their valuable characteristics. *Bacillus subtilis* can control several plant diseases, including rusts. Cell suspensions of *B. subtilis* strains AP-3 and AP-150 inhibited *H. vastatrix* urediniospore germination. The spraying of sterilised and unsterilised *B. subtilis* AP-3 and AP-150 cell suspensions on the detached leaves of coffee reduced the number of lesions by 72–87% (Bettiol and Várzea, 1992). The same trend was observed when these strains were sprayed on coffee plants. Under commercial conditions, Haddad et al. (2009, 2013) showed that *Bacillus thuringiensis* strain B157 can be considered a potential biocontrol agent for coffee leaf rust for reducing spore germination and disease severity. Given the severity of soybean rust, the combined application of biocontrol agents with fungicides should also be considered. Peng et al. (2014) suggested that the combination of *B. subtilis* NJ-18 and fungicides represents a promising tool for the control of sharp eyespot of wheat.

Similarly to biocontrol agents, natural products may be used for controlling plant diseases: the essential oils of thyme, lemon eucalyptus, citronella, and oil of neem inhibited the germination of *P. pachyrhizi* uredospores and reduced Asian soybean rust severity (Medice et al., 2007); eucalyptus essential oil reduced the severity of *Puccinia nakanishikii* in lemongrass (*Cymbopogon citratus*) (Lorenzetti et al., 2012); and the essential oils of *C. citratus*, *Ocimum gratissimum*, and *Thymus vulgaris* controlled seed-borne fungi in rice (Nguefack et al., 2008). Nguefack et al. (2013) concluded that the essential oil and extracts of *Callistemon citrinus* and *C. citratus* have potential as control agents against brown spot and other seed-borne fungal diseases in rice under both conventional and organic farming.

Despite the greater efficacy of fungicides, alternative products can be used in a disease management program and reduce dependence on these products. Thus, this study evaluated the potential of *Bacillus* spp. and coffee oils in inhibiting the germination of *P. pachyrhizi* uredospores and in controlling Asian soybean rust on detached leaves and plants in greenhouse and field conditions. The coffee oil was evaluated crude and after roasting, both alone and in combination with half doses of commercial fungicides.

2. Materials and methods

Plants of soybean cultivar BRS 316 RR (maturity group = 6.5) were grown in 2.5-L pots containing substrate (pine bark + charcoal) and soil (red dystroferric latosol – oxisol with clayey texture) (1:1). The plants were maintained in a greenhouse with a sprinkler system to ensure leaf wetness for eight hours overnight. Embrapa Soybean provided the initial *P. pachyrhizi* inoculum. Pathogen-infected soybean leaves were rubbed against healthy leaves maintained in the greenhouse, and then the sprinkler system was activated. After lesions sporulation, new soybean plants, at different growth stages, were periodically introduced into the greenhouse with the pathogen, ensuring a high quantity of inoculum within the greenhouse for spread of the pathogen among the plants.

The alternative products evaluated are shown in Table 1. Compressing roasted or crude coffee beans in a cold press expeller obtained the coffee oil. The oil was separated from the bean coffee mass, passed through a filter press, packaged and formulated using a patent pendent process (Fontal, 2007). The biofungicides Serenade® (*B. subtilis* QST-713) and Sonata® (*Bacillus pumilus* QST-2808) were provided by AgraQuest (Davis, CA, USA). Wettable powder products containing *B. subtilis* (BS) and *Bacillus licheniformis* (BL), together and alone, were also used. The products were manufactured by Chr. Hansen (Valinhos, SP, Brazil). In addition to these products, the AP-3 and AP-51 *B. subtilis* isolates were studied (Bettiol et al., 1992). These isolates were grown in 500-mL Erlenmeyer flasks containing 250 mL of GPL medium (glucose, 10 g; peptone, 10 g; yeast extract, 5 g; NaCl, 3 g; KH_2PO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; distilled water, 1000 mL; and pH 6.0) (Bettiol et al., 1992). The flasks were incubated on a shaker at room temperature for 10 days. The concentration of the products was 1.1×10^9 colony-forming units (CFUs). A fungicide mixture of epoxiconazole and pyraclostrobin (Ópera®, BASF, Schwarzheide, Germany) was used as a standard treatment.

2.1. Inhibition of uredospore germination

A uredospore suspension (10 μL) of *P. pachyrhizi* (1×10^5 uredospores/mL) was deposited on a glass slide, together with 10 μL of each product described in Table 1, but the product concentration was twice the one in Table 1. The slides were incubated in the dark for four hours at 22 ± 2 °C before the germination process was stopped by adding 10 μL of lactophenol cotton blue.

Table 1
Code, active ingredient, commercial name, company, and test concentration of the products evaluated for control of soybean rust.

Code	Active ingredient	Trade name	Company	Test concentration
SE	*Bacillus subtilis* QST-713	Serenade	AgraQuest	10^8
SO	*Bacillus pumilus* QST-2808	Sonata	AgraQuest	10^8
BS + BL	*B. subtilis* + *B. licheniformis*		Chr. Hansen	10^8
BS	*B. subtilis*		Chr. Hansen	10^8
BL	*B. licheniformis*		Chr. Hansen	10^8
AP3	*B. subtilis* AP-3		Embrapa[a]	10^8
AP51	*B. subtilis* AP-51		Embrapa	10^8
RCO 1%	Roasted coffee oil (RCO)		Embrapa	1%
RCO 2%	Roasted coffee oil		Embrapa	2%
CCO 1%	Crude coffee oil (CCO)		Embrapa	1%
CCO 2%	Crude coffee oil		Embrapa	2%
F	Fungicide (F)[b]	Ópera	BASF	0.66 + 0.25 g/L
0.5 F	Fungicide	Ópera	BASF	0.33 + 0.125 g/L
RCO 1% + 0.5 F	RCO + F			1% + 0.33 + 0.125 g/L
RCO 0.5% + 0.5 F	RCO + F			0.5% + 0.33 + 0.125 g/L
CCO 1% + 0.5 F	CCO + F			1% + 0.33 + 0.125 g/L
CCO 0.5% + 0.5 F	CCO + F			0.5% + 0.33 + 0.125 g/L

[a] Embrapa = Embrapa Environment.
[b] Fungicide = pyraclostrobin + epoxiconazole.