Black oats (*Avena strigosa* Schreb) solubilize rock phosphate and provide phosphorus to the successive crop, grapevine (*Vitis labrusca* L. 'Red Niagara')

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Abstract

In grape production, cover crops play a key role in soil protection and nutrient cycling. Thus, since grapevine requirements for P are low, adequate management of cover crops may provide sufficient P for growth. This paper evaluates the effect of black oats, as a cover plant, on the P solubilization in rock phosphate and its availability to the vine cultivated in succession. To test this hypothesis, we evaluated the plants growth parameters and P uptake in grapevines grown in succession to black oats in pots in a greenhouse experiment. The results showed that the soil sterilization decreased P availability to black oats, but not to grapevine. The soil fertilization with rock phosphate increased the P accumulation in oat and grapevine plants, although no further effect was observed from the use the cover crop. The plant height and dry mass contents were higher for grapevine in soils fertilized with rock phosphate and covered with black oats.

Keywords: cover crop, rock phosphate, Vitis labrusca, Avena strigose, successive cropping

INTRODUCTION

Phosphorus (P) is an essential plant nutrient, acting in many metabolic pathways, including respiration, photosynthesis, energy transfer, nucleic acids and protein synthesis (Grant et al., 2001; Jobi, 2013; Lim et al., 2014). Phosphorus fertilization is performed to provide an adequate supply of the nutrient to meet the crop demands, generally using soluble phosphates obtained from the hydrolysis of rock. However, large portions of the applied fertilizer can become unavailable to the plants after soil incorporation (Alves et al., 2002), thus making it necessary to provide a constant or superior than the required supply, as observed for grapevines, whose P requirements are low (Porro et al., 2009). One of the consequences of the frequent application of high dosages of P, known as excessive soil fertilization, is the increased potential of P transfer by run-off, leading to eutrophication of water springs (Chien et al., 2009; Melo et al., 2012; Yuan et al., 2013). Moreover, there are many reports demonstrating that excessive P usage, coupled with the low efficiency of plants to use different forms of P in the soil, leads to an increased concentration of P in crop lands, which is far higher than the optima for crops, at the same time promoting a strong depletion in the world's phosphate resources (Melo and Zalamena, 2016; Messiga et al., 2010; Schröder et al., 2011). Besides environmental pollution, excessive soil fertilization with P increases the production costs and negatively affects the uptake of micronutrients such as Zn (Singh et al., 1988), because increasing P doses lead to a reduction in Zn availability. This scenario may induce Zn deficiency symptoms and/or a reduction in the nutrient concentration in the plant.

Therefore, it is necessary to increase the efficiency of phosphate fertilization (Suh and Yee, 2011), and the industry is particularly focused on developing fertilizers that allow the nutrient to remain available to the plants for long periods (Karamanos and Puurveen, 2011;

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Sakurada et al., 2016), by incorporating new technologies of higher aggregate value. Research to increase the efficiency of P fertilization employing inexpensive sources, such as rock phosphate, is still scarce, and there are also reports of increased efficiency of P fertilization due to the use of cover crops, mainly annual ones (Esteves and Rosolem, 2011; Janegitz et al., 2016; Rodrigues et al., 2009; Rosolem et al., 2014).

Rock phosphates used as P sources to plants present as a main advantage lower costs in comparison to soluble phosphates, whereas having a better residual effect. In contrast, certain disadvantages are also known, such as the contamination by heavy metals, and to have their low solubility in the soil and its dependency of soil pH, Ca and moisture (Chien et al., 2009; de Oliveira et al., 2014). Several recent publications have demonstrated that the inoculation of P-rock with solubilizing microorganisms (MOs) may bypass some these drawbacks (Alves et al., 2002; Barroti and Nahas, 2000; de Carvalho Mendes and dos Reis Júnior, 2003; Sharma et al., 2013). However, it has been reported that the results field trials after MOs inoculation were not satisfactory in comparison to those from the greenhouse, likely due to the large diversity of soil microorganisms, and consequently the occurrence of highly competitive environments (de Carvalho Mendes and dos Reis Júnior, 2003).

The use of cover crops is a viable alternative to increase the efficiency of P-rock fertilization, considering their conditioning action on the soil physical, chemical and biological properties (Barros et al., 2013; Janegitz et al., 2016). Moreover, they are also beneficial to other soil aspects, such as maintenance of edaphic fauna, structural preservation and increases in porosity, nutrient cycling and organic matter. This is generally reflected on the improved performance of the successive crop, which benefits from the increased solubilized P-rock by root exudates, along with retention and cycling of inorganic phosphate (Pi) that incorporates to the organic fractions of the plant tissue.

Therefore, this work was aimed to evaluate the effect of black oats as a cover crop, to solubilize rock phosphate and to provide available P to grapevines cultivated successively in the same pots.

MATERIAL AND METHODS

The experiment was carried out in a greenhouse at Embrapa Uva e Vinho, Bento Gonçalves, located at 29°09′44″ south latitude, 51°31′50″ west longitude and at an altitude of approximately 640 m. The soil used is classified as Sandy Typic Hapludalf (Embrapa, 2013), collected from the top layer (0 to 20 cm) in the municipality of Bom Princípio (RS). The soil was air dried and passed through a 8 mm sieve. Soil composition (June 2014) was pH 4.7, 140 g dm⁻³ clay, 6 g kg⁻¹ organic matter, 0,03 cmol_c dm⁻³ available P and 0,05 cmol_c dm⁻³ K (extracted by Mehlich-1); exchangeable cations (in cmol_c dm⁻³) Al 1,1, Ca 0,2 and Mg 0,9 (extracted by KCl 1 mol L⁻¹); 0,3 mg dm⁻³ B; 2,5 cmol_c dm⁻¹ cation exchange capacity and 140 g kg⁻¹ base saturation. Corrective fertilization consisted of the application of 90 kg ha⁻¹ K₂O (K chloride, 60% K₂O), 45 kg ha⁻¹ N (Urea, 45% N) and 1 kg ha⁻¹ chelated micronutrients (2% B, 0.8% Cu-EDTA, 5.6% Fe-EDTA, 0.32% Mo, 3.2% Mn-EDTA, and 2.0% Zn-EDTA). After fertilization, the soil was incubated for 30 days at 80% field capacity.

The experiment was designed as random blocks with four repetitions, and the treatments distributed as a 3-way factorial ($2 \times 2 \times 2$): doses of P-rock (zero and 1000 kg ha⁻¹), cover crop (with/without) and soil sterilization (with/without). Arad P-rock (32.3% total P₂O₅, 11.3% soluble in citric acid) and black oats (*Avena strigosa* Schreb) 'Comum' were used. Soil sterilization was performed by autoclaving for 45 min at 120°C and 1 atm. The experiment was divided in two stages. During the first stage, black oats were sown (30 seeds per pot), and thinned to 15 plants pot⁻¹ after germination. The remaining pots were kept at 80% field capacity. The cover crop was cultivated for 45 days, subsequently harvested, and returned to the soil surface of the original pots immediately? after determination of the biomass fresh weight. The second experimental stage consisted in planting the grapevine cv. Red Niagara in each pot, with and without the black oat mulch. Grapevine plants were obtained by in vitro micropropagation and gradually acclimated to horticultural substrate in the greenhouse. Before transplantation (July 14), aerial parts and roots were trimmed, keeping only two buds and 4 cm of roots. Grapevines were then transplanted, grown and

harvested after 80 days.

For black oats, fresh biomass production was evaluated by weighing with 0.1 g precision. For grapevines, from the 40th day onwards, four evaluations were carried out for relative index of total chlorophyll (RITC) and plant height at 13 day intervals. RITC was determined using a chlorophyll meter chlorofi*LOG*[®] (Falker[®], model CFL1030) that reads transmittance at wavelengths close to chlorophyll peaks (635 and 660 nm) and at near infrared (880 nm). The leaf is pressed in a capsule during the measurement of the radiation transmitted through the leaf structure. The result is a chlorophyll index, a non-dimensional unit, obtained from the average of three readings of the adaxial surface of the third basal leaf. Plant height was measured with regular measuring tape.

At the end of the experiment, grapevine aerial parts were harvested, dried under forced air circulation at 65°C for 72 h, weighed and finely ground to a powder. Phosphorus contents were determined in the soil and for the plant aerial parts, as described by Tedesco et al. (1995).

The resulting data were submitted to analysis of variance to test double and triple interactions. When interactions were significant, factor comparisons were carried out within each factor level. For non-significant interactions, the variables were compared using an F-test and the factors average.

RESULTS AND DISCUSSION

According to the results of the analysis of variance (Table 1), there was a significant effect of soil sterilization on black oat fresh biomass weight (p<0.01), soil pH (p<0.01), and RITC (p<0.05). The addition of P-rock was highly significant (p<0.01) for all investigated variables except soil pH, and the effect of soil cover was significant for all parameters (at p<0.05) except for RITC. Moreover, the interaction between soil sterilization and P-rock addition was also significant (p<0.05) for black oat fresh biomass, and the interaction between P-rock fertilization and soil cover was significant for P contents in grapevine aerial parts and in the soil.

	Factors					
Variable	Sterilization (S)	P-Rock (P)	Black oats (C)	S×P	S×C	P×C
Black oats fresh biomass weight	**	**	-	*	-	-
P content in grapevine aerial parts	ns	**	*	ns	ns	*
P contents in the soil	ns	**	*	ns	ns	*
Grapevine plant height	ns	**	*	ns	ns	ns
Total chlorophyll relative index	*	**	ns	ns	ns	ns
Soil pH	**	ns	*	ns	ns	ns
Grapevine dry matter	ns	**	*	ns	ns	ns

Table 1. Analysis of variance for P contents in the soil and accumulated in grapevine aerial part, grapevine plant height (GPH), relative index total chlorophyll (RITC), black oat roots fresh weight (SFW) and grapevine dry matter (GDM).

ns non-significant, - not determined, * and ** Significant according to F test at 5 and 1% probability, respectively.

At the first stage of the experiment, the addition of P-rock increased significantly the black oat fresh biomass yield (Table 2), leading to yields 21 and 13.5 times higher in comparison to the treatments with and without soil sterilization, respectively (Table 2). The differences in black oat biomass yield are likely due to delayed cell growth under severe P deficiency (Grant et al., 2001).

Microorganism population influenced the availability of P to black oats, as shown in Table 2, since sterilization improved significantly fresh biomass yield for treatments with Prock, whereas in the absence of P-rock fertilization yields were not significantly different



with and without sterilization. The results support that, under conditions of low availability of P, the microorganisms did not have the capacity to increase the availability of P. However, several authors report that microorganisms may increase the P availability from rock phosphate to plants, due to the large diversity of organisms such as fungi, bacteria and some algae with the aforementioned ability (de Carvalho Mendes and dos Reis Júnior, 2003; Sharma et al., 2013). In particular, this applies to fungi, which are known to possess the most prominent activity in solubilizing P-rock, since these organisms in general produce higher levels of organic acids than bacteria (Venkateswarlu et al., 1984).

Sail starilization	P-rock fe	Significance	
Soli sterilization	Present	Absent	- Significance
Present	46.2	2.2	**
Absent	55.6	4.1	**
Significance	**	ns	

Table 2. Black oats fresh biomass production (g pot⁻¹) with sterilized or non-sterilized soil and in the presence or absence of rock phosphate (P-rock) fertilization.

** = significant and ns = non-significant according to the F-test (p<0.05).

As shown in Table 3, soil pH was significantly affected by sterilization and the presence of the cover crop, with lower pH in the absence of sterilization and the soil cover presence, whereas fertilization with P-rock has no significant effect. These results support the hypothesis of competitive adsorption between organic acids and P in soil colloids. Besides solubilizing P-rock by acidification, the hydrolysis of insoluble P to available forms may occur by the action of phosphatase enzymes, reflecting the action of plants and soil MOs, which secrete these enzymes mainly under low P availability (Kapri and Tewari, 2010; Nahas, 2002).

Table 3. Soil pH after grapevine plant removal.

Variable	Soil ster	Soil sterilization		P-rock fertilization		Soil cover (black oats)	
	Present	Absent	Present	Absent	Present	Absent	
рН	4.85	4.41	4.68	4.59	4.56	4.76	
Significance	*1	*	n	s		**	

** = significant and ns = non-significant according to F-test (p<0.05).

As shown in Figure 1A, grapevine aerial parts accumulate lower levels of P in the treatments without P-rock fertilization, regardless of the presence of the cover crop. In contrast, the presence the cover crop led to a higher P accumulation with P-rock fertilization.

The use of black oats as cover crop did not influence the levels of extractable P from the soils in the absence P-rock fertilization (Figure 1B). However, when P-rock fertilization was used the presence of the cover crop reduced somewhat the contents of extractable P. These observations are likely to be due to the larger accumulation of P in grapevine and black oats, suggesting that black oats may have contributed to increase P availability in the soil, since the cover crop accumulated a portion of extractable P and promoted nutrient accumulation in grapevine.

Our results corroborate the study by Pavinato and Rosolem (2008) which showed that an addition of plant residues can increase the phosphorus availability in the soil, due to P in the plant residue or the competition of residue-derived organic compounds for soil P adsorption sites. This contributes to an increased P availability in the soil solution, similar to the action of organic anions present in the rhizosphere, released from cover crops or by grapevines, which can also interfere with P status in the soil (Lopez-Hernandez et al., 1986).



Figure 1. Phosphorus content in grapevine aerial parts (mg plant⁻¹) (A) and available soil P content (mg dm⁻³) after the cultivation of grapevine (B), depending on the presence of P-rock fertilization and black oats soil cover.

The use of black oats as a cover crop and soil sterilization had no influence on grapevine RITC until day 53 (Figure 2); however, at days 66 and 80 soil sterilization and P-rock fertilization promoted slight (4.5 and 5.4%, respectively) increases in RITC (Figure 2). These effects may be due to the death of soil microorganisms, that can promote a decrease of soil P availability, consequently generating a deficiency of P in the plants, which is typically characterized by leaf darkening, from dark green to purple depending on symptom severity (Grant et al., 2001). Favela et al. (2006) report similar symptoms, coupled with possible irregular discoloration brown or purple spots, starting from the lower end of older leaves, due to the accumulation of anthocyanins. The changes in leaf color interfere with RITC readings.



Figure 2. Changes in relative index of total chlorophyll (RITC) throughout grapevine growth depending on the presence of soil sterilization (A) and P-rock fertilization (B). * = significant; NS = non-significant according to F-test (p<0.05).



The application of P-rock fertilizer, as expected, contributed to a significant increase in grapevine plant height (Table 4), since P is a critical element in plant metabolism, acting mainly on photosynthesis, respiration and energy transfer and therefore promoting growth (Gatiboni, 2003; Grant et al., 2001).

Days after grapevine	Soil cover	P-rock fe	Maan	
planting	(black oats)	Present	Absent	wean
40	Present	22.9	24.6	23.8A
	Absent	24.1	20.4	22.3A
	Mean	23.5a	22.5a	
53	Present	34.9	28.8	31.9A
	Absent	32.7	23.4	28.1A
	Mean	33.8a	26.1b	
66	Present	50.0	29.8	39.9A
	Absent	39.0	24.5	31.8B
	Mean	44.5a	27.2b	
80	Present	71.0	30.3	50.7A
	Absent	49.8	25.0	37.4B
	Mean	60.4a	27.7b	

Table 4.	Grapevine plant height (GPH), in cm,	cultivated in	n soil in the	presence a	nd absence
	of P-rock fertilization.					

Means followed by equal letters, small caps for the line and capitalized for the rows, are not significantly different according to F-test (p<0.05).

The use of black oats as a cover crop had no effect on the grapevines up to the 53rd day after planting, although from the third evaluation on, 13 days later, it did cause a significant increase on grapevine plant height (Table 4). The increased height can be explained by the decomposition and liberation of nutrients from the cover crop mulch, which guaranteed a better nutrient support for grapevine growth in the long term.

The application of P-rock fertilization had no effect on grapevine mean height nor on RITC up to the 40 and 53 days after planting, respectively. However, 66 days after planting a significant increase in both variables was observed (Table 4; Figure 2B). The observations may be due to nutritional reserves in the root system of grapevines, which, although limited by trimming, could still be able to provide P for a relatively long period of time. Alternatively, the effect may be due to the time required by the grapevines to adapt to a novel concentration of P available in the soil. According to Vasconcelos and Raghothama (2006), the variation in soil nutrient contents may affect uptake by direct modulation of P transporters activity and gene expression induction.

The supply of P-rock as well as the use of black oats as a cover crop, favored an increase in the aerial part biomass of grapevines (Table 5). The observed response is likely to be associated to the functions performed by P in plants, since, besides its structural role, it also participates in the composition of organic compounds, such as ATP, amino acids and enzymes (Hendrickson et al., 2004; Nyoki and Ndakidemi, 2014). The increase in plant yield in the presence of the cover crop was possibly due to its effect on the P-rock fertilizer. Several authors have reported the possible mechanisms developed by the soil microorganisms and the plants (Deubel et al., 2000; Hoberg et al., 2005; Hoffland et al., 1989; Illmer and Schinner, 1995; Marschner, 2008; Richardson, 2001), by which the plants release organic acids to the soil via roots. These compounds contact with fertilizer particles, thus contributing to solubilize P, and consequently increasing P uptake by plants. Moreover, rhizodeposition, liberation of organic compounds, may contribute to raise the population of P solubilizing microorganisms, which is also responsible for the increase in P adsorption (Dobbelaere et al., 2003; Hoffland et al., 1989; Janegitz et al., 2016; Khan et al., 2010; Kolawole et al., 2013; Richardson, 2001; Saxena et al., 2015; Walpola and Yoon, 2013).

Table 5. Dry matter (DM) of the aerial parts of grapevine cultivated in presence or absence of black oats as a cover crop and the presence or absence of P-rock fertilization.

Variable	Soil cover	P-rock fe	Maan	
	(black oats)	Presence	Absence	wean
DM aerial parts (g)	Presence	6.55	3.08	4.82A
	Absence	4.93	2.78	3.86B
	Mean	5.74a	2.93b	

Means followed by equal letters, small caps for the line and capitalized for the rows, are not significantly different according to F-test (p<0.05).

Soil sterilization had no effect on mean GPH or GDM (Table 6). Several authors have reported beneficial and deleterious effects on plant development (De Deyn et al., 2004; Farzaneh et al., 2011; Luo et al., 2017; Schradin and Cipollini, 2012). The discrepancies may be associated to local microflora diversity, as plant-microbe relationship is affected by biotic and abiotic factors, such as plant species and soil physicochemical characteristics (Brimecombe et al., 2001). Moreover, the lack of significant differences between treatments in the presence and absence of soil sterilization for plant height and dry biomass may be due to the indirect effect of the sterilization method employed, since some reports by Berns et al. (2008), Katan and DeVay (1991) and Stromme (1962) have demonstrated an effective increase in N contents in the soil solution after autoclaving, which may have contributed to increase grapevine vigor on sterilized soil, even in the absence of MOs.

Table 6. Grapevine plant height and aerial part dry biomass in plants cultivated in the presence or absence of soil sterilization.

Soil starilization		Aprial parts DW			
Son Stermzation	1	2	3	4	Aerial parts Dw
Present	23.6a	31.4a	38.6a	46.3a	4.29a
Absent	22.4a	28.5a	33.0a	41.8a	4.37a

Means followed by equal letters, small caps for the line and capitalized for the rows, are not significantly different according to F-test (p<0.05).

FINAL CONSIDERATIONS

Phosphorus contents in the soil in the absence of P-rock fertilization were below grapevine and black oat physiological requirements, as demonstrated by the increase of P in plant tissues and enhanced growth.

In the current work, the presence of black oat as a cover crop and soil sterilization acted distinctly on Arad rock phosphate. The presence of soil sterilization had no influence on plant height and dry biomass production. In contrast, the use of black oats as cover crop led to higher P accumulation in grapevine aerial parts, indicating that black oats have mechanisms contributing to solubilize P-rock and provide available P to the succession crop grapevine.

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