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Formononetin accelerates mycorrhization and increases maize production at low phosphorus application rates

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Abstract: The formononetin biostimulant may be an option for reducing P fertilization once it stimulates mycelial growth of arbuscular mycorrhizal fungi and increases plant ability to take up nutrients through the roots, especially phosphorus. The objective of this study was to evaluate the effect of formononetin associated with phosphorus fertilization in maize. Field experiments were conducted in a randomized block design with a 3 × 4 factorial arrangement (0, 50 or 70, and 140 kg ha^{-1} P_2O_5; and formononetin application rates: 0, 25, 50, and 100 g ha^{-1}), with four replications. Formononetin (100 g ha^{-1}) increased the mycorrhizal colonization rate up to 30% in maize in the first four weeks after emergence when no P fertilizer was applied, and to 17% when 50 or 70 kg ha^{-1} of P_2O_5 were applied. The application of 50 and 100 g ha^{-1} of formononetin significantly increased plant height, ear height, and grain yield (22% - 76%) when no P fertilizer was applied. The use of formononetin in the field stimulates mycorrhizal colonization, has a positive effect on maize yield, and reduces the need for P fertilizer application in maize. However, this effect was evident only at low P soil contents.

Key words: arbuscular mycorrhiza, biostimulant, isoflavonoid, *Zea mays*.

INTRODUCTION

Arbuscular mycorrhizal fungi (AMF), belonging to the Glomeromycota phylum, are important components of soil microbiota, have a generalized presence in the soil, and contribute to the diversification and stability of natural ecosystems (Van der Heijden et al. 1998). These fungi colonize the roots of most known plant species, including those of agricultural interest, establishing a mutual symbiotic association called arbuscular mycorrhizas (AMs) (Schüßler et al. 2001). Prominent functions performed by the AMF may include reduction in the use of fertilizers, especially phosphorus (P) (Tawaraya et al. 2012, Cozzolino et al. 2013, Ribeiro et al. 2016), relief from the effects of diverse stresses (Siqueira et al. 1999, Rangel et al. 2013, Schneider et al. 2013, Zayed et al. 2013), and promotion of soil aggregation and stabilization of the aggregates (Rillig & Mummey 2006, Graf & Frei 2013). These studies have shown that mycorrhizal plants develop more rapidly, are much better nourished, and are more tolerant to water deficit and stresses caused by toxic factors and by diseases (Folli-Pereira et al. 2012, Cordeiro et al. 2015).

Over the past decades, AMFs have attracted the interest of researchers, who have been trying to exploit their practical potential, especially in tropical regions. Many soils in tropical regions have high P fixation capacity and, consequently, low P availability (Yamada & Abdalla 2004, Cordeiro et al. 2015). The AMFs have a very clear role in regard to expanding the capacity to take up not only insoluble P, but also other macro- and micronutrients (Miyasaka & Habte 2001, Govindarajulu et al. 2005). In spite of the proven benefits of AMFs, their widespread agricultural use is limited by the impossibility of producing infective propagules on a large scale. However, the flavonoid formononetin has emerged as one alternative to this limitation (Nair et al. 1991, Siqueira et al. 1991, Silva-Júnior & Siqueira 1997, Davies et al. 2005, Novais & Siqueira 2009, Cordeiro et al. 2015, Ribeiro et al. 2016, Santiago et al. 2017).

Formononetin stimulates sporulation, spore germination, and mycelial growth of AMF in the soil, which, associated with the plant root system, expands plant capacity to take up essential elements, especially P (Nair et al. 1991, Siqueira et al. 1991, Baptista & Siqueira 1994, Silva-Júnior & Siqueira, 1997, Davies et al. 2005, Novais & Siqueira 2009, Salgado et al. 2017, Silva et al. 2017). Formononetin mainly stimulates germination of native spores and mycelial growth, and can thus move up the process of fungal colonization in roots. Consequently, establishment of symbiosis can occur earlier. Earlier colonization by AMF makes the root system more effective in providing water and nutrients to plants (Gavito & Miller 1998, Grant et al. 2005, Bittman et al. 2006, Seguel et al. 2012). In addition, mycorrhizas can contribute to reducing disease, improving plant health, either because AMF compete with diverse root pathogens for the same sites of infection, or they induce defense genes in the plant (Vigo et al. 2000, Pozo et al. 2002 Pozo & Azcón-Aguilar 2007, Sikes et al. 2009, Wehner et al. 2010, Gallou et al. 2011, Jung et al. 2012, Cameron et al. 2013). Therefore, plants with extensive presence of mycorrhizas have competitive advantages over those with little presence, already in the first stages of development.

In various countries, formononetin is already used in agriculture as a stimulant of mycorrhization; however, this product is not yet sold in most tropical countries, including Brazil. As a large portion of world grain production is concentrated in tropical regions and in soils with low P availability, the use of formononetin is an alternative to maximize the benefits of mycorrhizas in increasing uptake of this nutrient. In Brazil, the maize crop generates one of the largest demands for phosphorus fertilizers (IPNI 2018). As maize is a mycotrophic plant, evaluating the effect of formononetin associated with different application rates of phosphorus on this crop in a field experiment will provide useful information that may support its use on a wide scale in agriculture. Use of formononetin may optimize the use of P, whether by reducing the need for application of phosphorus fertilizers or by better exploiting the P retained in the soil.

Therefore, the aim of this study was to evaluate the effect of application of formononetin, associated with phosphorus fertilization, on the maize crop.

MATERIALS AND METHODS

Experiments were conducted in the municipalities of Lavras (21° 13’ 27’’ S, 44° 58’ 19’’ W, and 927 m altitude), Ijaci (21° 12’ 18’’ S, 44° 58’ 49’’ W, and 957 m altitude), and Uberlândia (18° 50’ 24’’ S, 48° 14’ 57’’ W, and 840 m altitude) in the state of Minas Gerais, Brazil, in the 2010/11 crop year. In Lavras and Ijaci, the experiments were repeated in 2011/12, for a total of five experiments. Climate in the region of Lavras and Ijaci in the

Köppen classification is mesothermal type with a dry winter (Cwb), mean annual temperature of 19.3°C, and rainfall of 1,411 mm. Climate in the Uberlândia region is type Aw, characterized as rainy tropical, megathermal, typical of savannas, with a dry winter. Mean annual temperature is 23.1°C, and main annual rainfall is 1,550 mm.

In Lavras, the two experiments were set up in a *Latossolo Vermelho Distrófico* [dystrophic Red Latosol (Oxisol)] with clayey texture. The soil chemical characteristics before setting up the experiment were pH (H_2O) 5.8, 4.9 mg dm^{-3} P (Mehlich-1), 125 mg dm^{-3} K, 4.6 cmol$_c$ dm^{-3} Ca, 0.4 cmol$_c$ dm^{-3} Mg, 0.0 cmol$_c$ dm^{-3} Al, 4.0 cmol$_c$ dm^{-3} H+Al, 410 g kg^{-1} clay, 240 g kg^{-1} silt, and 350 g kg^{-1} sand. Maize had been planted in this area in the last 10 years.

In Ijaci, the soil of the area of the two experiments was classified as a *Latossolo Vermelho-Amarelo Distrófico* [dystrophic Red Yellow Latosol (Oxisol)] with clayey texture and the following chemical characteristics: pH (H_2O) 6.5, 10.3 mg dm^{-3} P (Mehlich-1), 101 mg dm^{-3} K, 3.8 cmol$_c$ dm^{-3} Ca, 1.5 cmol$_c$ dm^{-3} Mg, 0.0 cmol$_c$ dm^{-3} Al, 2.3 cmol$_c$ dm^{-3} H+Al, 570 g kg^{-1} clay, 80 g kg^{-1} silt, and 350 g kg^{-1} sand. Up to 2009, this area was covered with pasture (*Brachiaria* spp.), and maize was grown from 2009 on.

In Uberlândia, the experiment was set up in a *Latossolo Vermelho Distroférrico* [Dystroferric Red Latosol (Oxisol)], with clayey texture and the following chemical characteristics: pH (H_2O) 5.8, 1.2 mg dm^{-3} P (Mehlich-1), 54.7 mg dm^{-3} K, 1.0 cmol$_c$ dm^{-3} Ca, 0.4 cmol$_c$ dm^{-3} Mg, 0.1 cmol$_c$ dm^{-3} Al, 2.9 cmol$_c$ dm^{-3} H+Al, 490 g kg^{-1} clay, 370 g kg^{-1} silt, and 140 g kg^{-1} sand. The area of the experiment had a *Brachiaria* grass cover for approximately the previous 20 years.

Soil pH was determined in a soil/H_2O suspension (1:2.5 w/v). The available potassium and phosphorus contents were extracted with Mehlich-1 solution (Mehlich 1953). Then K was determined by flame photometry and P by colorimetry. Exchangeable Ca, Mg, and Al were extracted with 1 mol l^{-1} KCl solution. Both Ca and Mg were measured by atomic absorption spectrophotometry, and Al by titration. Soil texture was determined by the hydrometer method, according to Bouyoucos (1951).

The experiments were set up in randomized blocks in a 3 × 4 factorial arrangement, consisting of 3 phosphorus application rates (P_2O_5): 0, 70, and 140 kg ha^{-1} in Lavras and Uberlândia and 0, 50, and 100 kg ha^{-1} in Ijaci; and 4 application rates of formononetin (potassium salt of 7-hydroxy, 4'-methoxyisoflavone – commercial product PHC-506): 0, 25, 50, and 100 kg ha^{-1}, in powder form. The experimental plot was composed of four crop rows of 6m length spaced at 0.80 m, with four replications (blocks). Planting occurred in December of the years 2010 and 2011, and harvest in April 2011 and 2012.

The P_2O_5 was applied in the form of Monoammonium phosphate (MAP), in the formulation 10-54-00 at planting, at which time 40 kg ha^{-1} of N in the form of urea was also applied, being careful to deduct the N content in the MAP, and 80 kg of K_2O in the form of KCl. Broadcast fertilization was carried out after 30 days at the following rates: 135 kg ha^{-1} N in the form of urea, 90 kg ha^{-1} K_2O in the form of KCl, and 60 kg ha^{-1} S in the form of Sulfurgran (90% S). After furrows were opened with a planter, fertilization and sowing was performed manually, leaving 4.8 plants per meter, aiming to obtain a final plant population of 60 thousand plants per ha.

Formononetin was directly applied on the seeds – seeds were placed in sterile plastic bags with the formononetin; the mixture was homogenized so that the product completely covered the seeds; and then the seeds were sown manually. In Lavras and Ijaci, the maize cultivar GNZ 2004 was used, and in Uberlândia, PIONEER® P3646H (Herculex®).

The following evaluations were made: mycorrhizal colonization rate (MCR), plant height (PH), ear height (EH), 100-grain weight (100GW), leaf nutrient concentrations, and maize grain yield (YIELD).

For evaluation of MCR, in Lavras and Ijaci in the 2010/11 crop year, roots were collected at 4 and 6 weeks after plant emergence. In Uberlândia in 2010/11 and in Lavras and Ijaci in the experiments of 2011/12, collections were made only in the sixth week after emergence. Fine roots from 8 plants per plot were collected and stored in sterile plastic bags. In the laboratory, the roots were washed and then cleared in 5% KOH solution. After that, the roots were washed in running water and acidified for 3-5 minutes in 1% HCl. The roots were then stained with trypan blue in 0.05% lactoglycerol (water:glycerol:lactic acid, 1:1:1) (Phillips & Hayman 1970). The MCR was quantified by the stained root grid line intersect method and observed in a stereoscopic microscope, according to Giovannetti & Mosse (1980).

During phenological stage 4, characterized by emergence of the flower tassel, leaf samples were taken for evaluation of nutrient concentrations. For that purpose, leaves opposite to and below the ears of four plants per plot were collected. The leaves were dried in a forced air circulation oven at 65°C, and after reaching constant mass, they were ground and then analyzed in regard to P, K, Ca, Mg, S, Cu, Fe, Mn, and Zn concentrations, according to the methodology described by Malavolta et al. (1997). N concentrations were determined by the Kjeldahl method (Sarruge & Haag 1979).

Plant height and ear height were evaluated during phenological stage 6 (~10 weeks after emergence). At the end of the maize cycle, the effects of the treatments on grain yield (corrected to 14% moisture) were evaluated.

The data were subjected to analyses of variance and Scott-Knott mean values tests using the SISVAR statistical program (Ferreira 2014). The data regarding percentage of mycorrhizal colonization were transformed by arcsine $(x/100)^{0.5}$.

RESULTS

The climatic data for the locations where the studies were carried out show that the mean monthly temperature was from 20-25°C, and rainfall in the period of full crop development was greater than 100 mm. This indicates conditions adequate for the crop (Figure 1).

The formononetin and phosphorus significantly ($p<0.05$) affected all the characteristics evaluated, except for 100-grain weight (Table I and II).

The MCR ranged from 54% to 80% and was significantly affected ($p < 0.05$) by the P application rates, except in Uberlândia (Table II and III). The highest MCR ($p < 0.05$) mainly occurred at low P application rates (0, 50, and 70 kg ha^{-1} de P_2O_5); the values of these rates were ~14% greater than those observed at the higher P application rates (100 and 140 kg ha^{-1} P_2O_5).

Formononetin increased ($p < 0.05$) the MCR by ~30% in the first four weeks (2010/11-1) after maize germination in Lavras and in Ijaci when the application rate of 100 g ha^{-1} of the product was used without application of P_2O_5. Formomonotin increased MCR by ~17% in the presence of 50 and 70 kg ha^{-1} P_2O_5, compared to the treatment without application of formononetin (Table III). In the collections made six weeks after emergence, there was a significant effect ($p < 0.05$) of the formononetin only at the rate of 50 or 70 kg ha^{-1} P_2O_5 in Lavras in the two years studied and in

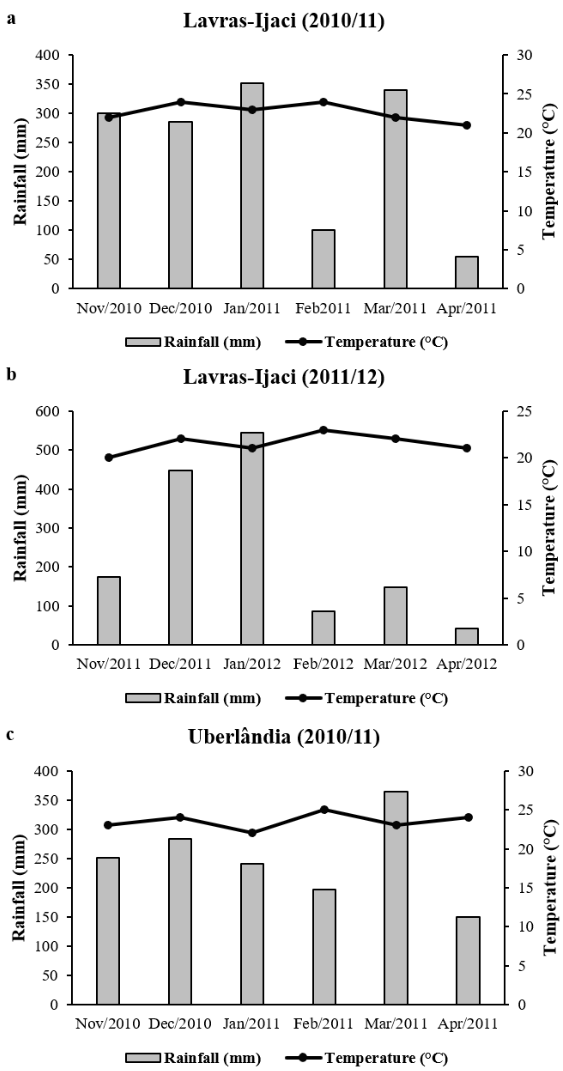

Figure 1. **Rainfall and temperature in Lavras, Ijaci, and Uberlândia in the period of conducting the experiments.**

Ijaci in 2010 (Table III). Comparison of MCR in the (2010/11-1) and sixth (2010/11-2) week post-emergence shows increases ($p < 0.05$) from 8% to 17% in this time interval.

Formononetin positively ($p < 0.05$) affected plant height in Lavras and Ijaci in 2011/12 and ear height in Ijaci 2011/12 (Tables I and IV), but did not have a significant effect on 100GW (Tables I and V) nor on leaf nutrient concentrations (Table SI - Supplementary Material). Regardless of the treatments, these factors were in the sufficiency ranges for crop development, as described by Malavolta et al. (1997) and Gott et al. (2014).

The mean maize grain yield was significantly ($p < 0.05$) affected by the P application rates in all the locations (Table VI). The highest maize yields occurred at application rates greater than 50 kg ha^{-1} of this element. When there was fertilization with 50 kg ha^{-1} or more of P_2O_5, the yields were around 13% - 51% higher than in the treatments without fertilization. At P rates above 50 kg ha^{-1}, effects ($p < 0.05$) were not observed from formononetin on maize yield. Nevertheless, in the absence of P application, the use of 50 and 100 g ha^{-1} formononetin increased maize grain yield from 22% – 76% in all locations (Table VI). Under these conditions, yield was increased an average of around ~3400 to 5600 kg ha^{-1} in Lavras 2010/11, ~4100 to 7100 kg ha^{-1} in Lavras 2011/12, ~4300 to 5600 kg ha^{-1} in Ijaci 2010/11, ~6300 to 7300 kg ha^{-1} in Ijaci 2011/12, and ~3900 to 5871 kg ha^{-1} in Uberlândia.

DISCUSSION

The lower MCR (Table III) at high rates of P occurred because, when plants are nutritionally provided for, especially with P, they diminish stimulation for mycorrhizal colonization (Nogueira & Cardoso 2007, Reis et al. 2008, Balota et al. 2012, Balzergue et al. 2013). In fact, in mycorrhizal symbiosis, host plants provide photoassimilates to the fungi, while they supply the plants with nutrients and water. However, when the soil has good fertility, the root system of the plants is able to take up nutrients and water adequately, dispensing with the need for the fungi. Thus, indirectly, the application of phosphorus tends to reduce establishment of AMs (Costa et al. 2005, Nogueira & Cardoso

Table I. Summary of individual analysis of variance for each location of maize cultivation for plant height (PH), ear height (EH), and grain yield.

Characteristic	Source of Variation	Lavras		Ijaci		Uberlândia
		2010/11	2011/12	2010/11	2011/12	2010/11
Plant height	Phosphorus (P)	**	**	**	**	NA
	PHC-506	ns	*	ns	**	
	P x PHC-506	*	**	ns	*	
	CV	10.15	6.92	6.45	4.93	
Ear height	Phosphorus (P)	**	**	**	**	NA
	PHC-506	ns	ns	ns	**	
	P x PHC-506	**	**	ns	**	
	CV	8.28	10.40	8.75	6.91	
100-grain weight (100GW)	Phosphorus (P)	ns	ns	ns	ns	ns
	PHC-506	ns	ns	ns	ns	ns
	P x PHC-506	ns	ns	ns	ns	ns
	CV	8.28	10.40	8.75	6.91	8.36
Yield	Phosphorus (P)	**	**	**	**	**
	PHC-506	**	**	**	ns	ns
	P x PHC-506	**	**	*	ns	*
	CV	13.02	12.01	13.43	13.85	20.54

****, *, and ns - significant at 1%, 5%, and not significant, respectively, by the Scott-Knott test; NA – parameter not evaluated. PHC-506 - commercial product (potassium salt of 7-hydroxy, 4'-methoxyisoflavone).**

2007, Balota et al. 2012, Balzergue et al. 2013). In contrast, low P concentrations tend to stimulate symbiosis, which is reflected in higher root colonization rates.

The higher MCR observed in the fourth week (Table III), at 0 and 70 kg ha^{-1} P_2O_5, when 50 and 100 g ha^{-1} formononetin were applied, occurred because this isoflavonoid favors earlier germination of AMF propagules, moving up the establishment of symbiosis (Nair et al. 1991, Siqueira et al. 1991, Silva-Júnior & Siqueira 1997, Davies et al. 2005, Novais & Siqueira 2009), compared to the treatments without the use of formononetin.

The difference in MCR of plants with and without application of formononetin after six weeks came to decline over time (Table III). This occurred because the plants that were not stimulated by the formononetin progressively came to have their roots colonized by AMF, later achieving values near those exhibited by the plants treated with formononetin, which already had high MCR values (~67% to 77%) from the fourth week on. This difference in the time of occupation of the symbiosis sites may be one of the key points of the benefit of formononetin for maize plants. Thus, plants with greater root colonization in the first stages have greater capacity for utilization of the soil and greater uptake of nutrients and water compared to plants with lower MCR.

In our study, we observed increases in maize grain yield of 70% in Lavras, 25% in Ijaci, and 52% in Uberlandia when more than 50 g ha^{-1} formononetin were applied in the absence of phosphorus fertilization (Table VI). In these treatments, the higher rates of mycorrhization were observed from the fourth week on, indicating that the formononetin stimulated mycorrhization. Nevertheless, the mean yield achieved under these conditions, although quite expressive, in most cases was below the yields achieved when the soil was fertilized with more than 50 or 70 kg ha^{-1} P_2O_5. Ribeiro et al. (2016) observed that the application of formononetin at planting of soybean fertilized with half the recommended P application rate led to increases in grain yield equivalent to that of the plots that had received the full P application rate, indicating that formononetin can reduce the need for P fertilization. These results show that the effect of formononetin is related to the availability of phosphorus. Indeed, formononetin acts directly on the AMF propagules present in the soil, stimulating them during the process of root infection of the plants (Nair et al. 1991, Siqueira et al. 1991, Romero & Siqueira 1996). However, the mycorrhization process is controlled by the plant, which limits colonization by the fungus when it is well supplied with phosphorus. Reduction in mycorrhizal colonization due to greater availability of P is a well-known effect and is generally related to the nutritional state of the plants. It is understood that well-nourished plants would express mechanisms to reduce the development or activity of AMF in the roots, seeking to reduce the energy spent on maintaining the fungi (Smith & Read 2008, Balota et al. 2011). Siqueira et al. (2002) reported that, in part, to successfully obtain isoflavonoid-based products, nutritional or environmental conditions must impose some degree of stress on the crop in question. Indeed, the formononetin flavonoid was isolated and identified in clover plants (*Trifolium repens*) stressed by phosphorus deficiency (Nair et al. 1991, Siqueira et al. 1991), and the use of this stimulant in the synthetic manner on different plant species has shown positive results, including yield (Ribeiro et al. 2016, Salgado et al. 2017, Santiago et al. 2017).

Although a direct correlation cannot be made between mycorrhizal colonization and maize grain yield, we cannot disregard possible indirect benefits from the arbuscular mycorrhizas for this crop, especially because in our study, we observed that the effect of the formononetin in the absence of P fertilization was consistent in the two years studied. In addition, the fact that formononetin does not promote an increase in grain yield as high as the increases obtained by the addition of large amounts of phosphorus fertilizers does not make its use infeasible. The costs of transportation and application of the fertilizers must be taken into consideration. Formononetin is a powder and it can be applied directly in planters together with seeds at the time of planting, dispensing additional operations. In addition, few grams (50-100 g) of formononetin are applied per hectare, whereas the use of phosphorus fertilizers comes to dozens or hundreds of kilograms, requiring larger operations and machines for application in large crop fields.

Another relevant aspect is that in Brazil and in other tropical countries, maize grain yield can vary a great deal from one region to another. In the case of Brazil, for example, whereas in the South, Southeast, and Central West regions mean yield is higher than 6,000 kg ha^{-1}, in the North and Northeast regions, mean yield is below ~3,600 kg ha^{-1}, with some states with mean yield below 1,000 kg ha^{-1} (IBGE 2017). These discrepancies are related to the technological level used in each region, including low use or the absence of fertilizer application. Our studies showed positive responses to the application of formononetin in the absence of phosphorus fertilizer application, which may be a feasible alternative for these regions with low yields.

Table II. **Summary of analysis of variance for mycorrhizal colonization rate (MCR) in maize plants.**

Characteristic	Source of Variation	Lavras			Ijaci			Uberlândia
		2010/11-1[a]	2010/11-2[b]	2011/12[b]	2010/11-1[a]	2010/11-2[b]	2011/12[b]	2010/11[b]
MCR	Phosphorus (P)	**	**	**	**	**	**	ns
	PHC-506	**	**	**	**	**	ns	ns
	P x PHC-506	**	ns	**	**	ns	ns	ns
	CV	5.25	6.25	3.96	5.27	6.85	5.06	4.39

[a] - 4 weeks after emergence, [b] - 6 weeks after emergence; **, *, and [ns] – significant at 1%, 5%, and not significant, respectively, by the Scott-Knott test. PHC-506 - commercial product (potassium salt of 7-hydroxy, 4'-methoxyisoflavone).

Table III. **Mycorrhizal colonization rate (MCR) in maize plants cultivated under different application rates of formononetin associated with three application rates of P_2O_5 in the state of Minas Gerais in crop years 2010/11 and 2011/12.**

Phosphorus (P_2O_5) (kg ha^{-1})[a]	PHC-506 (g ha^{-1})	Lavras			Ijaci			Uberlândia
		2010/11-1[b]	2010/11-2[c]	2011[c]	2010/11-1[b]	2010/11-2[c]	2011[c]	2010/11[b]
					%			
0	0	59.5 c	68.9 a	77.0 a	58.8 c	69.6 a	74.0 a	68.6 a
	25	64.0 b	72.7 a	79.1 a	61.8 c	74.9 a	76.9 a	69.1 a
	50	68.1 b	73.4 a	78.5 a	70.3 b	76.4 a	77.5 a	66.9 a
	100	76.9 a	77.3 a	80.5 a	76.2 a	77.8 a	79.3 a	71.7 a
	Mean	67.1 A	73.3 A	78.8 A	66.8 A	74.6 A	76.9 A	69.1 A
70 or 50	0	64.5 b	68.7 b	64.4 b	60.2 b	68.6 b	68.1a	66.2 a
	25	63.0 b	67.0 b	64.8 b	62.7 b	68.8 b	68.4a	67.9 a
	50	67.4 a	73.1 a	75.6 a	67.0 a	75.6 a	70.3a	71.0 a
	100	71.6 a	75.8 a	79.1 a	71.6 a	76.2 a	72.6a	71.2 a
	Mean	66.6 A	71.1 A	71.0 B	65.4 A	72.3 A	70.0 B	69.1 A
140 or 100	0	56.8 a	64.9 a	59.5 a	56.1 a	61.5 a	66.5 a	66.1a
	25	56.6 a	66.4 a	60.2 a	57.5 a	60.8 a	66.7 a	67.5a
	50	54.6 a	66.9 a	56.5 a	54.3 a	64.0 a	67.7 a	67.7a
	100	57.1 a	66.6 a	58.5 a	54.9 a	63.0 a	64.0 a	66.8a
	Mean	56.3 B	66.2 B	58.7C	55.7 B	62.3 B	66.2 C	67.0 A

[a]70 kg ha-1 in Lavras and Uberlândia and 50 kg ha-1 in Ijaci; [b]4 weeks after emergence; [c]6 weeks after emergence; lowercase letters compare MCR within formononetin; uppercase letters compare mean values of MCR in the phosphorus application rates by the Scott-Knott test at 5% probability. PHC-506 - commercial product (potassium salt of 7-hydroxy, 4'-methoxyisoflavone).

Table IV. **Plant height and ear height of maize cultivated in the field under different application rates of formononetin associated with three application rates of P_2O_5 in the state of Minas Gerais in crop years 2010/11 and 2011/12.**

Phosphorus (P_2O_5) ($kg\ ha^{-1}$)[a]	PHC-506	Lavras				Ijaci			
		2010/11		2011/12		2010/11		2011/12	
------ m ------									
0	0	1.80 a	1.52 c	0.86 a	0.64 a	1.60 a	1.63 b	0.85 a	0.88 b
	25	1.84 a	1.75 b	0.93 a	0.80 a	1.72 a	1.73 b	0.90 a	0.91 b
	50	2.02 a	1.91 a	1.03 a	0.85 a	1.77 a	1.70 b	0.96 a	0.93 b
	100	2.03 a	2.02 a	1.00 a	0.94 a	1.79 a	1.90 a	0.99 a	1.09 a
	Mean	1.92 B	1.80 C	0.96 B	0.81 B	1.72 B	1.74 B	0.92 B	0.95 C
70 or 50	0	2.24 a	2.10 a	1.19 a	1.05 a	1.90 a	1.91 a	1.04 a	1.08 a
	25	2.13 a	1.93 a	1.14 a	0.90 a	1.81 a	1.86 a	0.99 a	1.03 a
	50	2.25 a	2.14 a	1.23 a	1.01 a	1.84 a	1.92 a	1.00 a	1.12 a
	100	2.21 a	2.02 a	1.14 a	0.99 a	1.85 a	2.01 a	1.00 a	1.10 a
	Mean	2.20 A	2.05 B	1.18 A	0.99A	1.85 A	1.93 A	1.01 A	1.08 B
140 or 100	0	2.09 b	2.13 a	1.07 a	1.02 a	1.75 a	1.78 a	0.94 a	0.95 b
	25	2.55 a	2.22 a	1.22 a	1.13 a	1.88 a	1.97 a	1.05 a	1.11 a
	50	1.98 b	2.04 a	0.99 a	0.98 a	1.76 a	1.79 b	0.94 a	0.98 b
	100	2.23 a	2.22 a	1.17 a	1.07 a	1.87 a	1.92 a	1.04 a	1.06 a
	Mean	2.21 A	2.15 A	1.11 A	1.05 A	1.82 A	1.87 A	0.99 A	1.03 A

[a] 70 $kg\ ha^{-1}$ in Lavras and Uberlândia and 50 $kg\ ha^{-1}$ in Ijaci; lowercase letters compare plant and ear heights in formononetin application rates; uppercase letters compare in phosphorus application rates by the Scott-Knott test at 5% probability. PHC-506 - commercial product (potassium salt of 7-hydroxy, 4'-methoxyisoflavone).

CONCLUSIONS

At high phosphorus application rates (greater than 50 kg ha^{-1} of P_2O_5), the rate of mycorrhizal colonization declined and the effects of formononetin were inhibited.

Formononetin did not have an effect on 100-grain weight and leaf nutrient concentrations in maize.

The application of formononetin at rates greater than 50 g ha^{-1} increased the mycorrhizal colonization rate and the grain yield of maize grown in the field at low soil phosphorus contents.

Table V. 100-grain weight (100GW) of maize, data obtained from plants cultivated under different application rates of formononetin associated with three application rates of P_2O_5 in the state of Minas Gerais in crop years 2010/11 and 2011/12.

Phosphorus (P_2O_5) (kg ha^{-1})[a]	PHC-506	Lavras			Ijaci			Uberlândia
		2010/11	2011/12		2010/11	2011/12		2010/11
-- g --------------------------------								
0	0	27.8 a	29.1 a		28.8 a	29.2 a		17.7 a
	25	28.9a	28.4 a		27.0 a	28.1 a		17.8 a
	50	28.7a	32.0 a		29.7 a	28.4 a		15.7 a
	100	29.9a	30.2 a		29.0 a	28.4 a		17.0 a
	Mean	28.9A	29.9A		28.6A	28.5A		17.1A
70 or 50	0	29.5a	30.2 a		29.4 a	28.7 a		17.4 a
	25	30.4a	30.5 a		29.0 a	29.1 a		17.9 a
	50	29.0a	30.5 a		29.2 a	28.3 a		17.2 a
	100	29.3a	29.4 a		29.1 a	29.5 a		17.7 a
	Mean	29.4A	30.15A		29.2B	28.9A		17.6A
140 or 100	0	29.9a	29.8 a		28.4 a	27.6 a		18.8 a
	25	30.4a	31.0 a		29.4 a	28.2 a		18.3 a
	50	29.6a	28.6 a		28.7 a	28.3 a		17.3 a
	100	28.7a	29.5 a		29.1 a	28.5 a		17.6 a
	Mean	29.6A	29.7A		29.3A	28.2A		18A

[a]70 kg ha^{-1} in Lavras and Uberlândia and 50 kg ha^{-1} in Ijaci; lowercase letters compare mean values of 100-grain weight in formononetin application rates, and uppercase letters compare mean values in phosphorus application rates by the Scott-Knott test at 5% probability. PHC-506 - commercial product (potassium salt of 7-hydroxy, 4'-methoxyisoflavone).

Table VI. Maize grain yield in a field experiment under different application rates of formononetin associated with three application rates of P_2O_5 in the state of Minas Gerais in crop years 2010/11 and 2011/12.

Phosphorus (P_2O_5) ($kg\ ha^{-1}$)[a]	PHC-506	Lavras		Ijaci		Uberlândia
		2010/11	2011/12	2010/11	2011/12	2010/11
------------------------------- kg ha^{-1} -------------------------------						
0	0	3472b	4160c	4272b	6322b	3918b
	25	2869b	4554c	4443b	5567b	3778b
	50	5853a	7699a	5493a	6954a	5283a
	100	5354a	6573b	5647a	7590a	6462a
	Mean	4387C	5747B	4964B	6608B	4860B
70 or 50	0	7441a	7033a	5005a	7163a	6619a
	25	7934a	6328a	5271a	7468a	5612a
	50	7067a	6576a	5663a	7917a	5834a
	100	7098a	6326a	5551a	6497a	6915a
	Mean	7385A	6565A	5373B	7261A	6245A
140 or 100	0	5786a	6057b	5423b	7652a	8935a
	25	6579a	6403b	6248a	7957a	8438a
	50	4206b	5996b	5071b	7865a	9417a
	100	6913a	7435a	6745a	7741a	7224a
	Mean	5871B	6473A	5872A	7804A	8504A

[a] 70 kg ha^{-1} in Lavras and Uberlândia and 50 kg ha^{-1} in Ijaci; lowercase letters compare yields in formononetin application rates; uppercase letters compare mean yields in phosphorus application rates by the Scott-Knott test at 5% probability. PHC-506 - commercial product (potassium salt of 7-hydroxy, 4'-methoxyisoflavone).

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SUPPLEMENTARY MATERIAL

Table SI

How to cite
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