Effect of Organic manure and Mycorrhizal on the Growth and Yield of *Capsicum annum* (Hot Pepper).

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Abstract: A screen house experiment was conducted to investigate the growth and nutrient uptake as influenced by organic manure and arbuscular mycorrhizal fungi (AMF) in Capsicum annum. The treatments were inoculated with arbuscular mycorrhizal (M+), without arbuscular mycorrhizal (M-) and with application of organic manure; with pig dung (PD+), without pig dung (PD-), with poultry manure (PM+), without poultry manure (PM-). The experiment was laid out in factorial combination using complete randomized design with three replicates. The result of this study showed that at 5 weeks after planting plant height increased with the application of pig dung and poultry manure. Stem diameter increased across the weeks but at 5 weeks after planting mycorrhizal inoculation, with pig dung and poultry manure increased. The application of Pig dung increased the root and shoot biomass of plant when compared with the inoculated plants. The combined application of pig dung and mycorrhizal inoculation significantly increase Mg uptake when compared with control. The concentration of nutrients especially phosphorus must be considered in the soil before inoculation with AMF and application of organic manure maintained the soil fertility.

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Introduction.

Degradation of agricultural lands is escalating worldwide and most especially in the tropics. Mbagwu *et al.,* (2003) described it has temporary or permanent destruction of the soil productive capacity which is caused by man-induced activities. As a result of fertility decline, there is a low level of organic matter in soil associated with physical and biological properties and buildup of toxic concentrations in the soil. However, Swift and Palm (2000) suggested that it is more helpful to describe soil fertility as an ecosystem concept integrating the diverse soil functions, including nutrient supply which promotes crop production. These can be achieved by the use of appropriate management practices such as green manure, mulching, cover cropping and organic manure which have continuous effect on the soil in the long-run. Hence, there is need to understand the soil environment, recognize the limitation of that environment and ameliorate where possible without damaging the soil quality for efficient crop production.

Duruigbo *et al.,* (2013) described Organic agriculture as the use of natural resources and focus on environmental protection including the maintenance of soil structure and fertility, water resources and biodiversity). Application of organic residues to soils increase soil organic matter, buffer soil, improve aggregate stability and enhance water retention capacity of soils (Spaccini *et al*., 2002). Organic agriculture is environmental friendly and excludes use of inorganic fertilizer. Manure has been used effectively as organic fertilizer for centuries and their nutrient content varies depending on source and nutrient content (Egbuchua and Enujeke, 2013). Organic manures are efficient in increasing soil nutrient contents, ensuring positive residual effects and enhancing soil's physical and chemical characteristics (Ayeni *et al*., 2010). The utilization of organic manures is an integral part of agriculture that has been exploited overtime and across ages because of its ability to restore soil fertility, supply major plant nutrients, such as N. P. K., Ca, Mg and also stabilizer soil pH (Sanchez and Miller, 1986).

Many agricultural and horticultural crop species form a mutualistic symbiotic relationship with AMF in the root of 95% of all vascular plant (Smith and Read, 1997). Plant host benefits from increase AMF abundance and or functionality due to soil amendments (Rillig and Mummey, 2006; Warnock *et al*., 2010). Plants inoculated with AMF are more efficient in acquisition of nutrients which results into improved plant growth (Oseni *et al*., 2010), improve P uptake (van der Heijden *et al.,* 2006; Smith *et al.,* 2015), and under certain conditions they can also contribute to nitrogen (N) uptake (Hodge *et al.,* 2010; Whiteside *et al.,* 2012) and micronutrients (Perner *et al,*. 2007). Fagbola *et al*., (1998) also reported increased in P absorption due to more efficient mining of soil P. According to Javaid and Riaz (2008), root colonization with AMF enhances plant tolerance to growth inhabitation factors, thus improving crop growth and productivity. Hence, amended soil improves AMF functionality (Warnock *et al.,* 2010). Mycorrhizae are of immense importance in maintaining soil fertility and these are influenced by climatic and seasonal changes in the physico-chemical properties of soil.

Pepper (*Capsicum species*) is one of the most varied and widely used foods in the world. Hot pepper (*Capsicum annum*) is widely cultivated and seen has traditional vegetable or spice (Grubben and El-Tahir, 2004). The major constraints of pepper production are infertile soil, weeds, pests and diseases. Reinder (2007) reported that the use of organic manure in horticultural crops production enhances genetic expression of such crop in term of quality and quantity of the crop. Comparatively, yield in developing countries is about 10-30% of that in developed countries (Grubben and El-Tahir, 2004). Capsicum in combination with herbs is believed by people to cure certain ailments and diseases such as measles, fever and cold. (Grubben and El-Tahir, 2004). The objective of this study is to determine the response of *Capsicum annum* (Hot Pepper) to organic manure and mycorrhizal inoculation in topsoil.

2.0 Materials And Methods

Soil collection and analysis.

The experimental soil was collected on the agricultural land site of the University of Ibadan, Nigeria (7°24'N; 3°48'E) and pot experiment conducted in the screen house of the Department of Agronomy, University of Ibadan, and Ibadan, Nigeria. The collected soil sample was collected at a depth of 0-15cm. These soils were air dried and sieved with 5mm mesh size to remove debris before weighing into 5kg pots. The bulk soil sample was air dried at room temperature and grounded to pass through 2mm and 0.5mm diameter sieve before analysis. The particle distribution analysis was done by Hydrometer method (Gee and Bauder, 1986). The pH was determined in a 1:2 soil/water suspension using digital pH meter. Organic carbon was determined by Walkley-Black dichromate wet oxidation method (Nelson and Sommers, 1982). Total nitrogen was by micro-Kjeldahl distillation technique as described by Bremmer and Mulvaney (1982). Available phosphorus was determined by Bray No 1 method as described by IITA (1982). Exchangeable K, Ca and Mg were extracted using Ammonium Acetate, K was determined using flame photometer, Ca and Mg determined using Atomic Absorption Spectrophotometer (AAS) (Table 1).

Table 1. Soil physicochemical properties

Soil depth (cm)	pH (H2O)	Organic C (g/kg)	Total N	Available P (mg/kg)	Exchangeable Bases K (cmol/kg)	Na	Ca	Mg	Sand (g/kg)	Silt	Clay
0-15	7.1	25.52	1.39	15.5	2	0.8	1.9	9.8	838	160	2

Experimental Design

The experiment was a factorial combination conducted in a completely randomised design with three replicates. The factors include: two levels of pig dung (with or without), two levels of poultry manure (with or without), and two levels of mycorrhizal inoculation (with or without).

Manure analysis and mycorrhizal treatments.

Pig and poultry manure were incorporated into the soil at the rate of 10t/ha. Air dried manure were characterized by digestion with nitric and sulfuric acid, analyzed for macronutrients by standard procedures (Kaira and Maynard 1991; Cater 1993) (Table 2). Arbuscular mycorrhizal *(Glomus clarum)* inoculum (20g) was applied at third-quarter central top of the soil 24 hours before transplanting pepper seedlings (Carling *et al*., 1978). Pepper seedlings (Roma) were obtained from Institute of Agricultural Research and Training Ibadan, Nigeria. Mycorrhizal colonization was quantified after clearing root sample in 10% KOH, acidified in 1% HCl for 30 min and then stained with 0.05% trypan blue in lactoglycerol (1: 1: 1 lactic acid, glycerol and water) at room temperature (Phillips and Hayman, 1970). The stained roots, inside a gridline plate (Giovanetti and Mosse, 1980) were observed under a dissecting microscope.

Table 2. Poultry and pig manure chemical properties

	pH (H_2O)	Organic C (%)	Total N (%)	Available P (%)	Exchangeable Bases K (cmol/kg)	Na	Ca	Mg
Poultry manure	9.1	68.26	0.52	0.32	0.8	0.2	0.2	1.9
Pig dung	5.6	63.61	0.31	0.06	0.4	0.0	0.3	0.1

Data collection and harvest.

The growth parameters observed on the seedlings were: height (cm) and stem diameter (cm). The number of fruit, roots and shoot dry weight biomass, N, P, K, and Ca, Mg of leaf and soil, and percentage mycorrhizal colonization were determined. Leaf, shoot and root dry weights were taken after oven drying the samples at 70°C for 48 h. The plant samples were digested in triple acid as described by Juo *et al,* (1974). The P in solution was determined by ascorbic acid (Murphy and Riley, 1962). Nitrogen in the leaf was determined by digesting dried and ground leaf samples in hot tetraoxosulphate (vi) acid solution with selenium catalyst (Novozamskey *et al.,* 1983). The exchangeable cations (K, Ca and Mg) were determined using Atomic Absorption Spectrophotometer.

Statistical analyses.

All data were analyzed using analysis of variance (ANOVA) with the GENSTAT and Duncan's Multiple Range Test (DMRT) at P<0.05 was used to separate the means.

3.0. Results.

Effect of organic manure and mycorrhizal inoculation on vegetative growth of pepper plant

Plant height under pig dung and poultry manure application was significantly high when compared with poultry manure thus contributing 25.7% increase to plant height at 5 WAP (Table 3). Similar treatment trend was observed at 7 WAP, with pig dung and poultry manure contributing 21.6% increase to plant height in soil. Pig dung application contributed 6.9% increase to plant height when compared with mycorrhizal inoculation and pig dung while with mycorrhizal inoculation and poultry manure application contributed 11.6% increase when compared with poultry manure application under screen house condition. There was no significant difference in plant height at 9 WAP. Although, pig dung and poultry manure application contributed 10.7% increase to plant height when compared with mycorrhizal inoculation, pig dung and poultry manure application. At 11 WAP, there was no significant difference although with pig dung and without mycorrhizal inoculation contributed 10.9% increase than with mycorrhizal inoculation and pig dung; with pig dung and poultry manure contributed 8% increase compared with mycorrhizal inoculation, pig dung and poultry manure in the soil. Stem girth of pepper plant at 5WAP indicated with pig dung and poultry manure was significantly high when compared with mycorrhizal inoculation and poultry manure; with pig dung and without mycorrhizal inoculation; with poultry manure and without mycorrhizal inoculation; control which were significantly low. There was no significant difference at 7WAP, although pig dung contributed (4.10%) an increase to stem girth when compared with mycorrhizal inoculation and pig dung whereas with pig dung contributed 25.0% increase to stem girth than with poultry manure. There was no significant difference at 9 and 11 WAP (Table.3).

Table 3. Plant height and Stem girth of pepper plant as affected by mycorrhizal inoculation, pig dung and poultry manure application

Mycorrhizal inoculation	Pig dung application	Poultry manure application	Plant Height				Stem Diameter			
			Weeks after Planting				Weeks after Planting			
			5	7	9	11	5	7	9	11
M+	PD+	PM+	9.03ab	11.43ab	14.60a	16.10a	0.15a	0.22a	0.34a	0.45a
M+	PD+	PM-	8.50ab	11.57ab	14.43a	16.26a	0.13ab	0.23a	0.42a	0.64a
M+	PD-	PM+	9.00ab	12.00ab	14.57a	16.28a	0.11b	0.16a	0.29a	0.51a
M+	PD-	PM-	9.60ab	12.07ab	14.20a	15.70a	0.12ab	0.22a	0.39a	0.57a
M-	PD+	PM+	10.36a	13.53a	16.36a	17.50a	0.12ab	0.17a	0.34a	0.52a
M-	PD+	PM-	9.13ab	12.43ab	15.86a	18.26a	0.11b	0.24a	0.38a	0.64a
M-	PD-	PM+	7.70b	10.60b	14.16a	15.56a	0.10b	0.18a	0.37a	0.56a
M-	PD-	PM-	9.83ab	13.03ab	15.53a	17.33a	0.10b	0.20a	0.41a	0.51a
ANOVA										
M			NS	NS	NS	NS	*	NS	NS	NS
PD			NS	NS	NS	NS	*	NS	NS	NS
PM			NS	NS	NS	NS	NS	NS	NS	NS
M*PD			NS	NS	NS	NS	NS	NS	NS	NS
M*PM			NS	NS	NS	NS	NS	NS	NS	NS
PM*PD			*	NS	NS	NS	NS	NS	NS	NS
M*PM*PD			NS	NS	NS	NS	NS	NS	NS	NS

Means in columns followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test at (P<0.05), (PD+)=With Pig dung; (PD-)= Without Pig dung; (PM+)=With Poultry Manure; (PM-)=Without poultry manure; (M+)=With mycorrhizal; (M-)=Without mycorrhizal; *P<0.05. NS=Not significant. M=mycorrhizal; PD=pig dung: PM=poultry manure

Organic manure and mycorrhizal inoculation on dry weight biomass (root and shoot) number of fruit yield and mycorrhizal colonization.

The root dry weight under soil amendments showed no significant difference although with pig dung contributed 17.1 % increase when compared with mycorrhizal inoculation and pig dung, 22.8% and 15.6% increase when compared with poultry manure; control respectively. The shoot dry weight showed no significant difference although; pig dung contributed 16.9% increase when compared with mycorrhizal inoculation and pig dung, 39.2% increase when compared with control (Table 4). Pig and poultry manure application contributed 10.5% increase compared with mycorrhizal inoculation, pig dung and poultry manure. There was no significant difference in number of fruit yield under screen house condition although control performed better with 57.8% increase when compared with mycorrhizal inoculation, pig dung and poultry manure application and 21% increase when compared with pig dung and poultry manure (Table 4). Pig dung and mycorrhizal inoculation contributed 26.6% increase when compared with pig dung whereas poultry manure contributed 71.5% increase when compared with mycorrhizal inoculation and poultry manure. Mycorrhizal colonisation was significantly high at with mycorrhizal inoculation, pig dung and poultry manure when compared with mycorrhizal inoculation and poultry manure application; with mycorrhizal inoculation; with pig dung and poultry manure; with pig dung; with poultry manure and control under screen house condition. However, with mycorrhizal inoculation, pig dung and poultry manure; with mycorrhizal inoculation and pig dung contributed 49.8% and 45.8% increase compared with control respectively.

Table 4. Effect of mycorrhizal inoculation, pig dung and poultry manure application on dry weight biomass of root and shoot, number of fruit yield and mycorrhizal percentage colonisation.

Mycorhizal Inoculation	Pig Dung application	Poultry manure application	Dry Root Biomass	Dry Shoot Biomass	Number of Fruit Yield	Mycorrhizal Colonization
			-----------------(g/plant)--------------------			(%)
M+	PD+	PM+	0.96a	1.70a	2.67a	58.73a
M+	PD+	PM-	2.43a	3.37a	5.00a	54.43ab
M+	PD-	PM+	2.56a	1.80a	1.33a	32.57de
M+	PD-	PM-	2.10a	2.46a	1.67a	40.33c
M-	PD+	PM+	1.36a	1.90a	5.00a	41.67c
M-	PD+	PM-	2.93a	4.06a	3.67a	36.5cd
M-	PD-	PM+	2.26a	2.63a	4.67a	50.9b
M-	PD-	PM-	2.47a	2.47a	6.33a	29.47e
ANOVA						
M			NS	NS	NS	**
PD			NS	NS	NS	NS
PM			NS	NS	NS	NS
M*PD			NS	NS	NS	*
M*PM			NS	NS	NS	NS
PM*PD			NS	NS	NS	NS
M*PM*PD			NS	NS	NS	NS

Means in columns followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test at (P<0.05), (PD+)=With Pig dung; (PD-)= Without Pig dung; (PM+)=With Poultry Manure; (PM-)=Without poultry manure; (M+)=With mycorrhizal; (M-)=Without mycorrhizal; *P<0.05,**P<0.01, NS=Not significant. M=mycorrhizal; PD=pig dung: PM=poultry manure

Nutrient uptake as influenced by organic manure and mycorrhizal inoculation.

N uptake showed that with pig dung and poultry manure was significantly high when compared with pig dung; with poultry manure; with mycorrhizal inoculation and pig dung application were significantly low. Exception was with mycorrhizal inoculation, pig dung and poultry manure; control; with mycorrhizal inoculation and without pig dung and poultry manure; with mycorrhizal inoculation and poultry manure which showed no significant difference (Table 5). There was no significant difference in P uptake of pepper plant although with mycorrhizal inoculation and pig dung contributed 68.7%

increase when compared with pig dung and 64.1% when compared with control. Similar trend was observed in K uptake, although poultry manure contributed 27.7% when compared with mycorrhizal inoculation and poultry manure in the soil and 64.1% increase when compared with control. With mycorrhizal inoculation, pig dung and poultry manure contributed 38.5% increase compared with pig dung and poultry manure increase in K uptake. Ca uptake showed no significant difference although with mycorrhizal inoculation and pig dung showed 18.8% increase when compared with control. With mycorrhizal inoculation, pig dung and poultry manure contributed 57.1% and 38.8% increase compared with pig dung and poultry manure application and control. Mg uptake indicated with mycorrhizal inoculation, pig dung and poultry manure application was significantly high when compared with control which was significantly low (Table 5).

Table 5. Nutrient uptake in soil as affected by mycorrhizal inoculation, pig dung and poultry manure application

Mycorrhizal inoculation	Pig Dung application	Poultry Manure application	N	P	K	Ca	Mg
			(g/kg)	(mg/kg)	----------(mg/kg)----------		
M+	PD+	PM+	1.08ab	0.43a	1.66a	0.98a	0.31ab
M+	PD+	PM-	0.89b	1.31a	1.38a	1.17a	0.38a
M+	PD-	PM+	1.47ab	0.47a	1.25a	0.70a	0.22ab
M+	PD-	PM-	1.24ab	0.66a	1.35a	0.97a	0.28ab
M-	PD+	PM+	1.65a	0.36a	1.02a	0.42a	0.17ab
M-	PD+	PM-	0.95b	0.41a	1.34a	0.95a	0.25ab
M-	PD-	PM+	1.06b	0.74a	1.73a	0.82a	0.23ab
M-	PD-	PM-	1.22ab	0.47a	1.01a	0.60a	0.13b
ANOVA							
M			NS	NS	NS	NS	NS
PD			NS	NS	NS	NS	NS
PM			NS	NS	NS	NS	NS
M*PD			*	NS	NS	NS	NS
M*PM			NS	NS	NS	NS	NS
PM*PD			NS	NS	NS	NS	NS
M*PM*PD			NS	NS	NS	NS	NS

Means in columns followed by the same letter (s) are not significantly different according to Duncan's Multiple Range Test at (P<0.05), (PD+)=With Pig dung; (PD-)= Without Pig dung; (PM+)=With Poultry Manure; (PM-)=Without poultry manure; (M+)=With mycorrhizal; (M-)=Without mycorrhizal; *P<0.05, NS=Not significant. M=mycorrhizal; PD=pig dung: PM=poultry manure

4.0 Discussion

Effect of organic manure and mycorrhizal inoculation on vegetative plant growth.

The result obtained from our study showed that pig dung and poultry manure application increase plant height when compared with poultry manure application especially at 5 and 7 week after transplanting. This supports the observations of Adesina *et al,* (2014) who reported increase in plant height of pepper with manure application. Awosika *et al*, (2015) also reported that the application of pig manure only at low rates increased agronomic properties of tomato plant (*Lycopersicon ensculentum*). Inoculation with mycorrhizal and poultry manure application also showed increase when compared with poultry manure application. The growth increase is suggested to be attributed to mycorrhizal interactions which enhanced plant to acquire nutrients. According to Crews et al (1978) and Lovato *et al,* (1995) inoculation with AMF increases the survival and growth rates of plants and cuttings in screen houses and natural conditions. Olawuyi *et al,* (2014) reported that mycorrhizal inoculation with cow dung increased the agronomic characteristics of pepper plant. AMF aids in plant growth by increasing metabolic activities and accelerating transfer of nutrients from fungus to plants. Since, most crops species perform better with mycorrhizal inoculation, we suggest it enhanced the growth of pepper plants. Ewulo *et al,* (2008) and Ikeh *et al,* (2012) also reported increase in plant height of tomato and pepper respectively when soil was amended with poultry manure. Manure serves as source of organic matter which is easily mineralized and readily available for plant utilization. There was general increase in stem girth of pepper plant with organic manure application and AM in the soil. Our study indicated that soil treatments with pig dung and poultry manure application significantly increase stem diameter of pepper plant at 5 weeks after transplanting when compared with mycorrhizal inoculation and poultry manure application and control. This supports a report by Senjobi *et al,* (2010) who reported that addition of farmyard manure improved all the growth parameters of leafy vegetable. Similarly, Ewulo *et al*, (2007) reported that cow dung applied at 7.5t/ha increase stem diameter of pepper plant. Our study showed no significant effect between mycorrhizal inoculated plants and non-mycorrhizal inoculated plants for root dry weight biomass. However, application of pig dung showed increase when compared with mycorrhizal inoculation and pig dung for dry root biomass of pepper plant. Erinle *et al,* (2017) reported that application of manure significantly increase dry weight of root and shoot. This is in accordance with Fernado and Linderman (1997), who reported that root dry weight of mycorrhizal inoculated plants are not significantly different from each other. Grey and William (2006) also reported an increase in the supply of nutrients following organic manure application. The shoot dry biomass increased with application of manure. This may likely be as a result of initiating different conformational changes in growth of different plants varieties. There was no significant difference in number of fruit yield. In our findings, inoculated plants did not show a better yield performance. This must have been due to the concept of nutrient inbalance in tropical soils (Akinrinde, 2006). In addition, enhanced mycorrhizal infection may account for enhanced plant growth through improved nutrient uptake.

Effect of AM fungi, organic manure on nutrient uptake and mycorrhizal colonisation.

AM fungi have been considered to have wide range of host in which species are more effective with particular host plant in increasing nutrient uptake. According to Smith and Read (1997), AM fungi are able to colonize most crop species by improving plant growth and nutrient uptake. The contribution of AMF to plant nutrient uptake is often particularly evident in plant that are deficient in certain nutrients mainly phosphorus. In our study, inoculation with mycorrhizal and pig dung application aided uptake of P. This agrees with George *et al,* (2000) that AM symbiosis increases P uptake and growth of their plant host and mycorrhizal inoculated roots have higher phosphorus absorption capacity compared to non-mycorrhizal root (McArther and Knowles 1993; Ortas *et al.,* 1996). Subbarao *et al*, (2001) reported that organic manure sustained food production and P released from organic manure mineralization increases its availability in soil (Gopalakrishnan, 2007). This increase in uptake may be due to increase surface area of soil contact, increased movement of nutrients into mycorrhizae, a modification of the root environment and increased storage. Mycorrhizae can therefore be much more efficient than plant roots at taking up phosphorus in the soil. The application of organic manure contributed to N uptake in the soil which was significantly high with pig dung and poultry manure application. Hedge (2001) stated that plants seem to be better than fungi at short circuiting nitrogen cycle. Manures applied to soil enhance N concentration in the soil and tend to initiate different conformational changes in growth of different plants varieties. This shows the effectiveness of organic manure application in the soil. Ca and Mg uptake showed inoculated plants increase than the control. The mycorrhizal inoculation and pig dung application showed an increase in Ca and Mg uptake. This is in agreement that AM fungi is not a fertilizer base but can only aid nutrient uptake such as N, P, K, Ca and Mg from soluble pool of nutrients available for plant absorption (Bagayote *et al.,* 2000; George *et al.,* 2000 and Ortas *et al.,* 1996). Manure handling and storage has being widely studied, however the quality is difficult to quantify due to differences in the quality of the sources. Mycorrhizal colonization rates differed depending on the host plant and the AMF species. Our study indicated a strong colonisation level with mycorrhizal inoculation, pig dung and poultry manure when compared with pig dung and poultry manure application and control. Faisal *et al,* (2010) reported that the concentration of P in plant shoots increase with increasing level of inoculum. Studies have proven that AM colonization is influenced by nutrient availability (especially P) in soil. Hence, percentage colonization of roots is as a result of increased acquisition of P by the mycelial network. We suggest that AMF becomes less effective in colonisation of roots when P concentration in the soil is high. However, mycorrhizal colonization rates differed depending on the host plant and the AMF species.

5.0 Conclusion.

The role of organic manure with or without mycorrhizal inoculation in this study resulted to increase in plant height and stem girth of pepper plant. The organic soil amendments have strategically different approach that relies on long-term solutions in the soil. The combined application of pig dung and mycorrhizal inoculation increase uptake of nutrients most especially in uptake of P. The application of organic materials to nutrient depleted soils goes beyond just increasing or replenishing nutrient element content of amended soils. Thus, a concomitant use of mycorrhizal fungi with organic amendments is required to enhance flow of nutrients to crops. Lastly, high quality AM fungal strains with effective potential is required to have appropriate symbiotic association.

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