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Phosphorus-Zinc Interaction and Iron and Manganese Uptake in the Growth and Nutrition of Phalaenopsis (Orchidaceae)

Sarah Vieira Novais[(1)*], Roberto Ferreira Novais[(2)], Víctor Hugo Alvarez V.[(3)], Ecila Mercês de Albuquerque Villani[(3)] and Mariana Delgado Oliveira Zenero[(1)]

[(1)] Escola de Agricultura 'Luiz de Queiroz', Departamento de Ciência do Solo, Programa de Pós-graduação em Ciência do Solo, Piracicaba, São Paulo, Brasil.
[(2)] Universidade Federal de Viçosa, Instituto de Ciências Agrárias, *Campus* de Rio Paranaíba, Rio Paranaíba, Minas Gerais, Brasil.
[(3)] Universidade Federal de Viçosa, Departamento de Solos, Viçosa, Minas Gerais, Brasil.

ABSTRACT: Visual symptoms of Zn deficiency, induced by excessive P applications, have been observed in commercial orchid nurseries. The supply of other metallic micronutrients, such as Fe and Mn, may also be inadequate in the plant due to high application rates of P. The aim of this study was to investigate this interaction in the nutrition of *Phalaenopsis* plants. Experimental treatments consisted of three P rates (0.0, 0.5, and 1.0 g L^{-1}) and three Zn rates (0.00, 0.35, and 0.70 g L^{-1}), as well as fertilization with other basic nutrients, and a control treatment with the fertilizer B&G Orchidée®, at 1.0 g L^{-1}. Dry matter production was evaluated, as well as the levels of P, Zn, Fe, and Mn in both shoots and roots. Higher P rates induced higher shoot dry matter production. However, symptoms of Zn deficiency were observed in plants treated with the highest P rate in the absence of Zn. With increasing P rates, Zn concentrations in the shoots decreased more markedly than in roots, with accumulation of the element in the roots, indicating low Zn translocation to the shoot. A much higher Mn content in shoots (661 mg kg^{-1}) than in roots (75 mg kg^{-1}) suggested that the species is highly tolerant to this micronutrient. The Fe concentrations in the plant were much higher than those indicated in the literature as critical levels for this genus.

Keywords: ornamental plants, nutritional balance, micronutrient.

*** Corresponding author:**
E-mail: sarahnovais@usp.br

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INTRODUCTION

Studies on orchid nutrition involve some difficulties, including slow growth that is compatible with low nutritional demands and reduced responses to the addition of nutrients compared to responses seen in plants with rapid growth, such as annual agricultural crops (Arditti, 1992). Another difficulty is the scarcity of studies on the interaction among nutrients and their ideal balance within this plant, which will be dealt with in this study. However, producers and orchid lovers are aware of the need for more research on adequate fertilization of these plants to obtain better growth and development, as well as greater quantity and better quality of the flowers produced (Wang and Gregg, 1994; Wang, 1996, 2000).

The effect of nutrition on this diverse family that grows on varied substrates (Assis et al., 2008; Schnitzer et al., 2010) and under different fertilization regimes, chemical and/or organic (Bernardi et al., 2004; Lone et al., 2010; Rodrigues et al., 2010), has not been extensively studied. Most substrates used for cultivation of orchids are poor in nutrients and/or present low capacity for supplying nutrients, making fertilization necessary under these conditions (Rego et al., 2000; Araujo et al., 2007). In addition, nutritional studies on orchids are more frequently about *in vitro* cultivation (Kanashiro et al., 2007; Stancato et al., 2008; Moraes et al., 2009; Rodrigues et al., 2012a,b).

Problems occur in locations where there is low availability of Zn and other metallic micronutrients, particularly Fe and Mn, and deficiency of these micronutrients is induced by continuous application of high rates of P (Reis Júnior and Martinez, 2002; Imtiaz et al., 2006; Dechen and Nachtigall, 2007; Carneiro et al., 2008).

There are controversies about the reason for the phenomenon called “phosphorus induced zinc deficiency”. Some authors affirm that excess P can lead to a reduction in the rate of Zn diffusion (Rose et al., 2015), while others conclude that this deficiency occurs due to the ability of some phosphate fertilizers to raise substrate pH, increasing the availability of variable negative charges and consequently the adsorption of Zn (Carneiro et al., 2008). Another group of authors believes that excessive P leads to rapid growth that is not accompanied by Zn levels, resulting in low levels, caused by the dilution effect (Ova et al., 2015).

Zinc has a fundamental role in the growth and development of plants as it is a cofactor in enzymes involved in protein synthesis and energy production, as well as in the maintenance of biomembrane structural integrity and production of IAA (Indole Acetic Acid) (Marschner, 2005; Hänsch and Mendel, 2009). Deficiency can also produce an increase in the level of reactive oxygen species (ROS) that interfere with the mechanisms of cellular detoxification by reducing the activity of anti-oxidative enzymes such as Cu/Zn superoxide dismutase and carbonic anhydrase (Cakmak, 2000; Hacisalihoglu and Kochian, 2003), resulting in reduced growth and production. A similar effect on growth (leaf elongation) is observed when synthesis of IAA is limited (Marschner, 2005).

Negative interaction can result in large losses in orchid cultivation, such as low flower production, plants more susceptible to pests and diseases, and even plant loss (Arditti, 1992). Roberto F. Novais (personal information) reported that fertilization of a commercial orchid nursery using an equal mixture of NPK 20-05-20 and simple super-phosphate, via fertigation (1 g L^{-1}), initially promoted intense growth, but after a few applications, growth ceased. Shortly afterwards, some plants from a few genera of orchids, for example *Phalaenopsis*, showed clear symptoms of Zn deficiency, reduced inter-node distance, multiple shoots, and reduced leaf size, as cited by Römheld (2001), and symptoms caused by limited IAA synthesis. These symptoms were attributed to deficiency induced by excessive accumulation of P in the plant tissues.

The hypothesis of this study is that with the increase in the P rate there is restriction in the absorption of Zn, Fe and Mn in *Phalaenopsis* sp. plants. The aim of this study is to analyze the effect of the P × Zn interaction on accumulation of plant dry matter and nutrition of *Phalaenopsis* sp. in regard to P, Zn, Fe, and Mn.

MATERIALS AND METHODS

The experiment was conducted in a greenhouse in *Viçosa*, MG, Brazil. The plant material consisted of three-month-old *Phalaenopsis* Blume (1825), produced by *in vitro* propagation and made available by a commercial nursery. These plants were placed in black polypropylene pots (0.5 dm^3) containing a layer of gneiss gravel (5 mm) over an expanded clay base (50-50 % by volume) and arranged on wooden tables.

The experimental unit was one pot, with one plant per pot. The plants were kept in a greenhouse with a metal frame and covered with a polypropylene net that allowed retention of 70 % of incident solar radiation.

Treatments consisted of the application of three rates of P (0.0, 0.5, and 1.0 g L^{-1}, as triple super phosphate, AR reagent) and three rates of Zn (0.0, 0.35, and 0.70 g L^{-1} as $ZnSO_4$, AR reagent), as well as base fertilization with N 8.0, K 6.0, Ca 5.0, Mg 1.25, S 3.8, B 0.09, Cu 0.05, Fe 0.56, Mn 0.20, and Mo 0.007 (mg L^{-1}), AR reagents. The commercial product B&G Orchidée® was used as reference fertilization at 1.0 g L^{-1}. B&G Orchidée® is a "Mixed Mineral Fertilizer", total content: 8.00 % N (soluble in H_2O), 11.0 % P_2O_5, 7.00 % K_2O (soluble in H_2O), 7.52 % Ca, 1.25 % Mg, 3.76 % S, 0.09 % B, 0.05 % Cu, 0.56 % Fe, 0.20 % Mn, 0.007 % Mo, and 0.35 % Zn (http://www.begflores.com.br/).

Treatments were applied weekly by fertigation, using a watering can. Each experimental unit was watered with sufficient solution to completely moisten the substrate and allow drainage of any excess. This procedure was adopted to simulate that used in commercial orchid nurseries for plant fertigation.

The experimental design was randomized blocks with six replications, and treatments in a (3 × 3) + 1 factorial arrangement, consisting of three application rates of P and three of Zn, plus the treatment B&G Orchidée® as a reference.

After six months, the plants were removed from the substrate and the roots washed with distilled water. The plants were separated into shoots and roots, and plant dry matter was determined after drying in a forced-air circulation laboratory oven at 70 °C until achieving constant weight.

After weighing, the material was ground and a sample was digested with nitro-perchloric acid for later determination of P, Zn, Fe, and Mn content by Inductively Coupled Plasma - Atomic Emission Spectrometry (ICP-OES).

The results were submitted to analysis of variance and the means were compared by the Tukey test at 5 % using the R 3.2 program.

RESULTS AND DISCUSSION

The B&G® treatment promoted the greatest production of plant dry matter (dry weight) from the shoots ($p<0.01$) among the treatments, suggesting that this commercial fertilizer had the best nutritional balance (Tables 1 and 2).

There was a significant response for the mean of the Zn application rates ($p<0.01$) and the mean of the P application rates ($p<0.01$), highlighting the importance of supplying these nutrients as a general response of the experiment. However, a significant interaction

between Zn × P ($p<0.01$) was observed, indicating a differential response of each of these nutrients to varying application rates of the other, as is frequently documented in the literature (Imtiaz et al, 2006; Dechen and Nachtigall, 2007; Carneiro et al., 2008). While there was a linear response in production of phytomass (plant biomass) by the plant shoots in response to Zn application without the application of P ($p<0.05$), there was no significant response to increasing application rates of Zn in the presence of the highest application rate of P (Tables 1 and 2).

No significant interaction between Zn × P for root growth indicates an individual response for each of the nutrients. The gain in root dry weight, with a quadratic tendency, did not depend on the Zn application rate ($p<0.01$), which contradicts what was observed for shoots. As a general trend, there was a reduction in dry weight of the roots with an increase in the P application rate ($p<0.01$) at any of the Zn application rates (Tables 1 and 2).

Table 1. Dry matter production (DM) of shoots and roots of phosphorus, zinc, iron, and manganese in *Phalaenopsis* spp., for different phosphorus and zinc application rates

Treatment		DM	P	Zn	Fe	Mn
P	Zn					
g L^{-1}		g per pot	g kg^{-1}	mg kg^{-1}		
				Shoot		
B&G®(1)		0.24 a	5.12 a	87.85 cd	192.50 c	691.55 a
0.0	0.00	0.11 b	1.09 f	52.35 e	387.05 c	542.20 a
0.0	0.35	0.13 b	1.44 ef	160.75 b	235.70 c	725.80 a
0.0	0.70	0.12 b	1.52 ef	332.40 a	818.10 ab	643.85 a
Mean		0.12	1.35	181.83	480.28	637.28
0.50	0.00	0.13 b	2.44 de	60.80 de	918.70 a	617.00 a
0.50	0.35	0.09 b	2.42 de	107.10 c	432.70 bc	685.80 a
0.50	0.70	0.11 b	5.09 a	146.01 b	141.85 c	836.45 a
Mean		0.11	3.32	104.64	497.75	713.08
1.0	0.00	0.17 ab	3.97 bc	40.21 e	231.00 c	581.85 a
1.0	0.35	0.16 ab	3.04 cd	86.34 cd	525.35 abc	638.50 a
1.0	0.70	0.13 b	4.72 ab	149.20 b	316.70 c	648.20 a
Mean		0.15	3.91	91.92	357.68	622.85
				Root		
B&G®(1)		0.83 a	3.01 ab	303.50 bcd	450.06 a	108.28 a
0.0	0.00	0.96 a	1.03 c	94.79 e	595.0 a	99.62 a
0.0	0.35	0.89 a	1.22 c	413.21 bc	545.0 a	76.58 ab
0.0	0.70	0.77 a	1.14 c	661.72 a	558.0 a	53.97 b
Mean		0.87	1.13	389.91	566	76.72
0.50	0.00	1.03 a	2.70 ab	109.33 de	700.05 a	64.28 ab
0.50	0.35	0.91 a	2.18 bc	289.17 bcde	600.53 a	79.27 ab
0.50	0.70	0.93 a	2.19 bc	438.36 b	550.78 a	93.64 ab
Mean		0.96	2.36	278.95	617.12	79.06
1.0	0.00	0.96 a	3.63 a	102.42 de	489.08 a	58.37 ab
1.0	0.35	0.96 a	2.59 ab	219.75 cde	828.00 a	59.50 ab
1.0	0.70	0.89 a	2.71 ab	390.33 bc	550.15 a	64.58 ab
Mean		0.94	2.98	237.5	622.41	60.82

Means followed by the same letter do not differ statistically by the Tukey test at 5 %. B&G®: Commercial fertilizer for growing orchids, used as a reference (http://www.begflores.com.br/).

Table 2. Analysis of variance of dry matter (DM) and macro- and micronutrient content in the leaves and roots of *Phalaenopsis* spp

Source of variation	DF	Mean Square								
		DM	P	K	Ca	Mg	Zn	Fe	Mn	Cu
						Shoot				
Treatment	9	0.008*	0.048*	0.124	0.135	0.005	14426.3*	307082.7*	13227.1	1783
Zinc (Zn)	2	0.031*	0.039*	0.119	0.034	0.007	37740.3*	72083.2	27915.6	41.5
Phosphorus (P)	2	0.042*	0.107*	0.315	0.318**	0.001	14197.5*	148334.5	13964.4	855.3
ZN × P	4	0.027*	0.012*	0.062	0.019	0.003	5833.3*	535635.4*	8303.1	1584.0
Zn/P = 1	2	0.005	0.001	0.041	0.009	0.000	39773.4*	789377.5*	16954.6	45.0
Zn/P = 2	2	0.001	0.048*	0.192	0.006	0.006	3659.6*	308328.9*	25000.3	1573.3
Zn/P = 3	2	0.000	0.014*	0.009	0.564	0.006	5973.9*	45647.6	2567	1591.2
P/Zn = 1	2	0.000	0.041*	0.141	0.567	0.002	209.2**	260491.3**	2786.3	1190.0
P/Zn = 2	2	0.006	0.013*	0.017	0.148	0.003	2993.9**	43636.1	3841	1315.6
P/Zn = 3	2	0.001	0.077*	0.279	0.151	0.001	22661.1*	915478*	23943.3	1517.7
Additional	1	0.193	0.093*	0.004	0.433*	0.019	2628.1*	180366.7	2071.3	7917.5**
Residue	10	0.002	0.001	0.334	0.080	0.007	61.2	70429.9	21600.7	2317.1
CV (%)		36.8	8.93	22.42	13.91	27.15	6.4	55.59	22.23	52.03
						Root				
Treatment	9	0.019*	0.049*	0.050**	0.046*	0.004*	193101.0*	800561.3	2164.4*	290.7
Zinc (Zn)	2	0.004*	0.009*	0.044	0.014**	0.007*	704158.0*	588228.5	71.6	274.6
Phosphorus (P)	2	0.006*	0.187*	0.104*	0.171*	0.006*	96287.7*	995121.5	1635.3*	101.4
ZN × P	4	0.255	0.005*	0.027	0.003	0.003**	34238.3*	904637.3	2216.6*	248.9
Zn/P = 1	2	0.613**	0.001	0.016	0.006	0.011*	484772.0*	3958.5	3071.5*	194.0
Zn/P = 2	2	0.001	0.005*	0.036	0.113	0.001	162240.7*	2197955.0	1364.0*	218.8
Zn/P = 3	2	0.063	0.013*	0.047	0.003	0.001	125621.7*	195589.7	69.3	359.6
P/Zn = 1	2	0.193	0.104*	0.011	0.035*	0.000	319.0	66644.5	2972.1 *	162.5
P/Zn = 2	2	0.336*	0.053*	0.040	0.768*	0.001	38271.3*	134655.7	628.1	144.8
P/Zn = 3	2	0.193	0.038*	0.108*	0.065*	0.011*	126174.0*	2603096.0	2468.3*	291.8
Additional	1	0.255	0.034*	0.047	0.029*	0.000	64.5	419803.0	7199.1*	869.1
Residue	10	0.023	0.001	0.029	0.006	0.001	5856.3	858341.4	395.2	1068.5
CV (%)		17.27	16.66	22.24	18.4	23.66	24.96	132.16	26.36	44.74

* and **: significant at 5 and 1 %, respectively. DF: degree of freedom.

The root/shoot dry weight ratio had a value of 6.6 in all the treatments, and 3.4 for the B&G® treatment, suggesting that the latter condition did not favor the highest allocation of carbohydrates into the plant roots in a more balanced nutritional state (Marschner, 2005). The high values of this ratio in *Phalaenopsis*, even with the B&G® treatment, indicate a specific morphological characteristic of plants from this genus (Arditti, 1992).

The levels of P in the shoots of the plant increased linearly with an increase in the P application rate for each of the Zn rates tested, following the expected nutritional model ($p<0.01$). In a similar way, the levels of P in this part of the plant also increased with the Zn application rate for each P application rate, the only exception being zero application of P. This would indicate that the increase in availability of Zn did not favor P absorption, which confirms the significant negative interaction between Zn × P ($p<0.01$).

In the roots, responses to levels of P were observed, where the level of P increased with an increase in the P application rate for each of the Zn application rates tested. However, a decrease in P levels was observed with an increase in Zn rates for each P rate. This

was mainly observed in the two higher rates of Zn and confirms the negative interaction between Zn × P observed ($p<0.01$) (Tables 1 and 2).

The average level of P in the leaves was in the range of 1.0 to 5.5 g kg^{-1} and was considered adequate according to Jones Junior et al. (1991). The ratio between the means of the level of P in the shoots and in the roots was 1.34, indicating a reasonable similarity between the two parts of the plant (shoots and roots).

The Zn levels, both in the shoots and in the roots, were dependent on the application of P and Zn and their interaction and increased with an increase in the Zn application rate ($p<0.01$) (Table 1). The increase was around six times from the lowest to the highest rate of Zn tested with no additional application of P (0.0 rate), and much lower, around three times as high, at the highest P application rate. For the same rate of Zn, the leaf level of this nutrient decreased significantly with an increase in P ($p<0.01$) (Table 2), confirming the negative interaction between Zn × P observed by various authors (Reis Júnior and Martinez, 2002; Li et al., 2003; Marschner, 2005; Imtiaz et al., 2006). At the highest P application rate and an absence of applied Zn, typical symptoms of induced Zn deficiency were observed, characterized by limited growth of leaves at the tip and a root system (apical meristem) with limited growth and possible necrosis at the root tips (Figure 1), as previously observed in commercial orchid nurseries (Figure 2). In the shoots, the Zn levels were adequate (20-200 mg kg^{-1}) according to Jones Junior et al. (1991). The highest Zn level in the roots was 2.51 times that of the shoots and occurred along with the increase in P, which was probably due to reduced translocation of Zn to the shoots of the plant, previously observed by Lopez G and Malavolta (1974).

At the highest supply of P to the plant, the symptoms of induced Zn deficiency can become severe, even when the levels of this micronutrient are maintained high and stable and above the values considered adequate for plant nutrition. Under these conditions, although the total levels are elevated, lower physiological availability is observed, caused by the lower proportion of water-soluble Zn in the plant (active Zn) and the lower activity of anti-oxidative enzymes, such as superoxide dismutase (SOD) (Marschner, 2005).

The levels of Fe in the shoots were found to be dependent on the P × Zn interaction ($p<0.01$), with decreasing levels of Fe associated with the application of higher rates of P, particularly noticeable at the highest P rate (Tables 1 and 2). In general, the Fe levels found were well below the 75-200 mg kg^{-1} range recommended by Jones Junior et al. (1991).

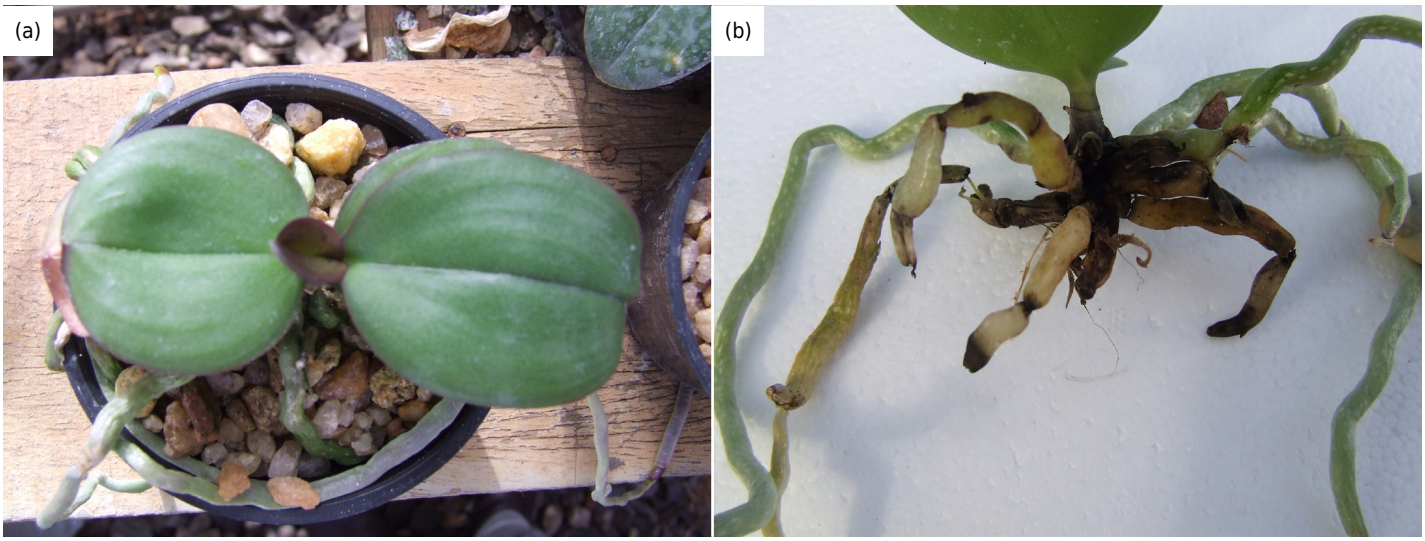

Figure 1. Initial visual symptom of Zn deficiency in *Phalaenopsis* spp. plant, characterized as limited growth of the last leaf, induced by application of the highest rate of P and a zero rate of Zn (a); and the root system characterized by limited growth and necrosis in the root tips (b) of the same plant with leaf symptoms.

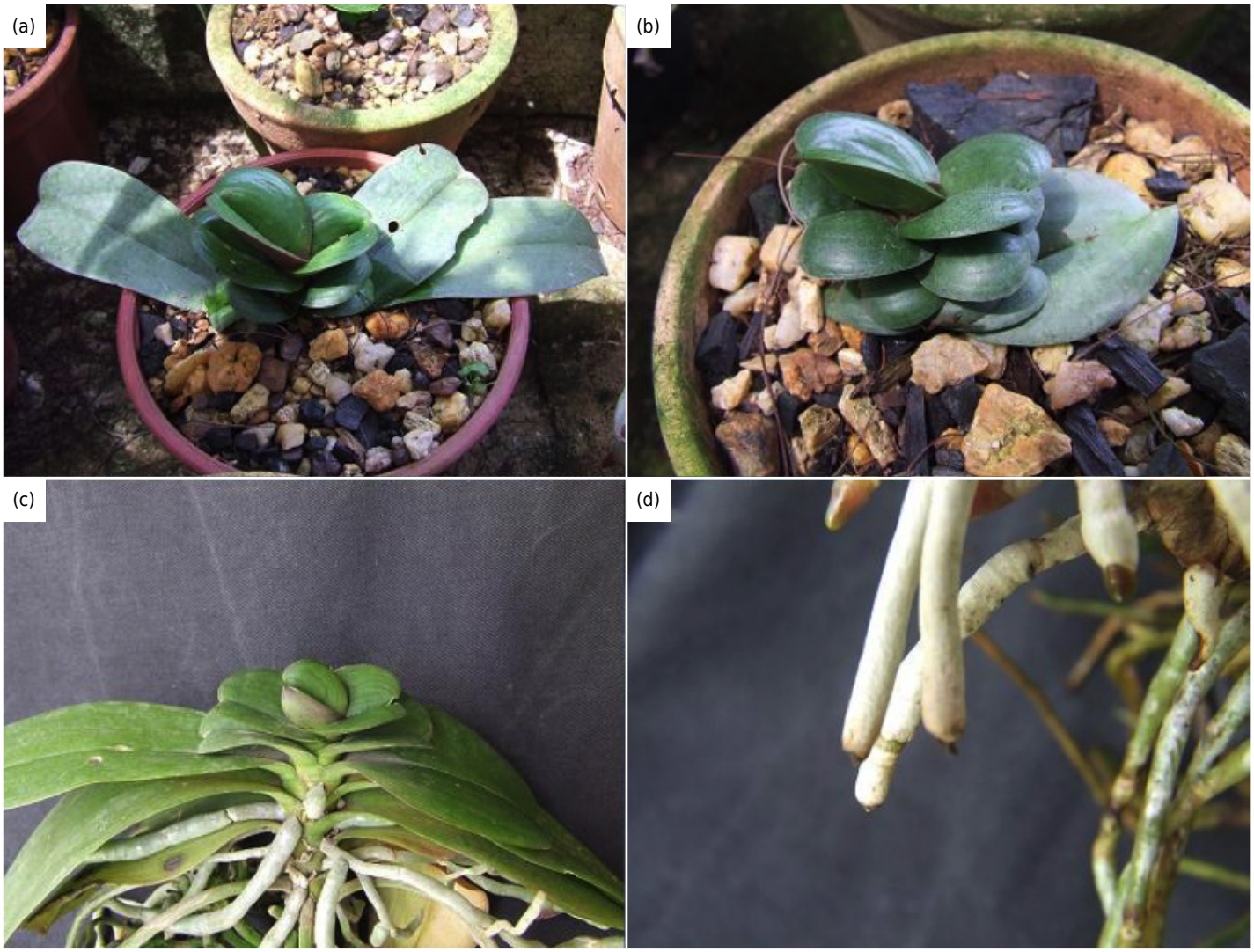

Figure 2. Visual symptom of severe Zn deficiency in plants of *Phalaenopsis* spp. induced by excessive supply of phosphorus in a commercial nursery, characterized by slower growth of the last leaf pair (a, b, c) and shortening of the roots (d). Source: R. F. Novais, personal communication.

In the roots, the treatments did not significantly alter the levels of Fe, even though the levels found were high. The addition of the fertigation solution to the Fe present in the expanded clay (with a red color), used as the substrate, facilitated the availability of high Fe concentrations, as corroborated by the observations of Breś et al. (2010) using two cultivars of *Phalaenopsis*. A situation similar to the mismatch of total Zn and active Zn in the plant is documented in the literature for total Fe and active Fe (Koseoglu, 1995; Marrocos, 1997; Isaakidis et al., 2002). Jones Junior et al. (1991) found high levels of Fe, and although they were above the ideal range, the plants were not nutritionally unbalanced.

The levels of Mn in the shoots were not significantly altered by the treatments (Tables 1 and 2), with values from 542 to 836 mg kg^{-1}, well above the range considered adequate (100-200 mg kg^{-1}) by Jones Junior et al. (1991) and Arditti (1992). However, in the roots, the levels of this micronutrient were below the range mentioned, representing an average of 11.3 % of the level in the shoots, and were significantly altered by P and the Zn × P interaction ($p<0.01$). A reduction in the level of Mn was observed in the roots with the increase in P rates without additional Zn application, but not after Zn application. A similar reduction in the absence of P was also observed. The treatment with the commercial fertilizer B&G®, which had the best results for plant growth, also had high Mn (692 mg kg^{-1} in the shoots and 108 in the roots).

Nurseries of the species *Cattleya* spp. (with levels from 100 to 1,000 mg kg^{-1}) and *Phalaenopsis* (100 to 2,000 mg kg^{-1}) are considered Mn accumulators, with leaf levels well above those found in other ornamental plants (Furlani et al., 2001; Liu et al., 2014). The slow growth of orchids (Arditti, 1992) appears to be one of the possible reasons for this accumulation of Mn. Other authors consider that the tolerance of the plants to Mn is related to various physiological mechanisms (Hauck et al., 2003; Wang et al., 2009). Excess Mn can accumulate in vacuoles (McCain and Markley, 1989; Marschner, 1995), the cell wall (Menon and Yatazawa, 1984), thylakoids (Lidon and Teixeira, 2000), and the Golgi complex (Hughes and Williams, 1988), indicating that this nutrient in inactivated in the plant to limit its negative effects, even at high levels. Therefore, it is recognized that more research is needed on the subject.

In the specific case of orchids with high commercial value, new studies should be considered on the way that the accumulation of these nutrients affects flowering and flower quality.

CONCLUSION

The levels of Zn in the shoots and roots were seem to be dependent on the negative interaction of P × Zn, as shown by the significant decrease in Zn levels with the increase in the P application rate. With the increase in the P application rate, there was a higher accumulation of Zn in the roots, indicating a lower translocation of this nutrient to the shoots of the plant. Initial symptoms of Zn deficiency were observed for the highest P application rate when no additional Zn was applied. Special care should be taken not to fertilize *Phalaenopsis* with excessive rates of P. The Fe levels in the plant were well above those considered to be critical levels for this genus. There was preferential accumulation of Mn in the shoots of the plants.

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