

# Fertilization of Imperial® Zoysia grass in different substrates compositions

## Abstract

The lawn may receive attention in very different ways. Regarding sports lawns, these are carefully handled from installation to maintenance. On the other hand, ornamental lawns may be treated as a background, especially in civil constructions in which they are the last to be worked. In this case, the soil that remains for its installation is severely compacted and without fertility. In any case, the base for lawn installation is very important and also, fertilization should be taken into account, as if made in wrong way may culminate in excess grass growth which is undesirable, since it leads to increase of cuts, burdening the system. Thus, The objective of this work was to evaluate Imperial® Zoysia grass (*Zoysia japonica*) development cultivated in different substrates compositions and fertilizations applications. Grass was planted in black plastic containers. The experimental design was factorial scheme 3×3 (substrates x types of fertilizers) with four replicates. Plots were submitted to three substrate combination: soil; soil + sand (1:1) and soil + sand + soil conditioner (1:1:1). Fertilization application was made using three commercial grass fertilizers incorporated into the substrates (30 g m<sup>-2</sup>) and one control without fertilizer. At 15 days after planting it was evaluated leaves fresh and dry mass, Falker Chlorophyll Index (FCI) and leaf analysis of N and S. The best result obtained was the cultivation of Imperial® Zoysia grass in substrate formed by soil, sand and soil conditioner without the necessity of fertilization.

**Keywords:** *Zoysia japonica*, sports lawn, ornamental lawns

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## Introduction

Zoysia grass (*Zoysia japonica*) is a species native from Japan, which have adapted well to Brazilian climate, due to its rhizomatous and branched root system. This species presents slender leaves, fast growing and intense green colour, important characteristic to turfgrass formation; with different leaf textures and shades of green, forming a perfect rug. In this case, the Imperial® variety, selected and developed by a Brazilian private research centre, is the first grass cultivation protected in Brazil by the Ministry of Agriculture, Livestock and Supply. Comparing to ordinary Zoysia grass, the Imperial® presents slenderly wider leaves, more vigorous root system among all grasses of this family, superior drought tolerance, reduced nutritional requirement and slow growth, which reduce the number of cuttings.<sup>1,2</sup>

It is remarkable the use of grass for many occasions as it is a fundamental element in landscaping projects. The gardens, no matter how small, become more harmonious when is occupied by green carpet of natural grass and the reason is due to it being fundamentally responsible from the perspective of landscaping for the depth of other elements that form the green area.<sup>3</sup>

For this establishment, it is necessary to verify the location where grass will be planted. The lawn implantation will depend on soil/substrate composition and analysis whose preparation is extremely important, observing all physical and chemical attributes.<sup>4</sup>

When lawn is established on substrate, it is important to notice that a single component may not achieve all requirements, in relation to plant nutrition or physical conditions, therefore, it is necessary to mix two or more materials.<sup>5</sup> Furthermore, the substrate considered ideal for seedlings production, whether forest species as well as ornamental species, needs to be uniform in its composition, being free from pests, pathogenic organisms and weeds.<sup>6</sup>

The use of substrate for lawns implantation is also justified by lack of soil preparation when the grass planting. Generally the grassy areas

are around buildings that have gone through the compaction process, in addition to removing the soil fertile layer. The substrate would replace this soil, restoring the area's ability to grow plants.

Sometimes, the fertilization of lawns is neglected or left aside in a landscape project. It is necessary to remember that a lawn, when established, tends to remain for many years and the removal of nutrients from the soil is constant, without the introduction of these elements through fertilization, the plant will not reach its full development. Therefore, mineral fertilization needs to be a common practice for maintaining lawns.

The nutritional requirement of grasses may vary according to species and cultivars, requiring all macronutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulfur (S) as micronutrients: iron (Fe), manganese (Mn), boron (B), copper (Cu), zinc (Zn) and molybdenum (Mo), always in correct quantities to provide their aesthetic quality (colour and soil cover), all of them are essential for their development.<sup>7</sup>

Nitrogen (N) is the most demanded nutrient by grass, influencing several relevant characteristics in lawns management. Due to being a structural nutrient, it is part of several biomolecules such as proteins, nucleic acids and hormones, the concentration of n in leaves may range from 20 to 50 g kg<sup>-1</sup> (2% to 5%).<sup>8</sup> In addition, this nutrient is responsible for the colour, vigour and proper lawns development.<sup>9</sup>

The management of nitrogen fertilization during all lawn phases and correctly way provides the formation of a quality site,<sup>8</sup> with a deep and vigorous root system, which causes greater efficiency in water and nutrients absorption. Furthermore, the same authors reported, regular applications of nitrogen fertilizers are necessary, as the amount of N available in the most soils are insufficient to supply the lawn requirement.

Potassium (K), another important element, is present in the plant in ionic form. It plays a key role in regulating the osmotic potential of

plant cells, and also activates many enzymes involved in respiration and photosynthesis.<sup>10</sup>

This mineral has great participation in nutritional processes, controlling cells water retention, which controls and regulates the sap circulation speed and, consequently, of almost all nutrients,<sup>11</sup> which guarantees an excellent enzymatic activity.<sup>12</sup> In addition, K acts on metabolic exchanges and transpiration by controlling the opening and closing of leaves stomata<sup>11</sup> and resistance of plant to pests and diseases incidence due to permeability of plasma membrane.<sup>13</sup>

Sulphur (S) is absorbed by plants in the form of anionic sulphate ( $\text{SO}_4^{2-}$ ), being found a large part in organic matter, and a small proportion in the atmosphere, in the form of sulphuric gas. When there is a deficiency of this secondary macronutrient, protein synthesis is inhibited because s is a participant in two essential amino acids (cystine and methionine), as a result of which plants have less chlorophyll content and less developed roots.<sup>14</sup>

The objective of this work was to evaluate Imperial® Zoysia grass (*Zoysia japonica*) development cultivated in different substrates compositions and fertilizations applications.

## Material and methods

The experiment was conducted in full sun and cemented area, in a region whose climate was classified by Köppen as Aw type,

characterized by tropical humid with rainy season in summer and dry in winter. The annual mean precipitation was 1,044.2 mm. The relative air humidity varied from 47.9% to 96.2% (annual minimum and maximum humidity, respectively) and annual temperature means was minimum of 19.4 °C and maximum of 32.9 °C.<sup>15</sup>

Imperial® Zoysia grass was implanted in black plastic containers (47.5×17.5 cm – top; 41.5×11.3 cm – bottom; 15.5 cm high, 8.46 L volume), using donated carpets from Itograss®, located in municipality of Pereira Barreto-SP.

The experimental design was factorial scheme 3×3 (substrates x types of fertilizers) with four replicates. Plots were submitted to three-substrate combination: S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1).

The soil used for substrates composition is classified as Dystrophic Red Latosol, sandy loam-clay texture according to the Brazilian Soil Classification System,<sup>16</sup> thick sand with 0.6 to 2.0 mm particle size was purchased from building materials store and soil conditioner was composed of pine bark naturally decomposed with addition of ash, of a mash-like physical nature.

Chemical and physical analysis for substrates were performed according to specialized literature for each attribute. The results were shown in Table 1, 2.

**Table 1** Chemical analysis of substrates according to specialized literature for each attribute [S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1)]

Substrates	P – resin	Organic Matter	pH	K	Mg	H + Al	SB	CEC
	(mg dm <sup>-3</sup> )	(g dm <sup>-3</sup> )	(CaCl)	----- (mmolc dm <sup>-3</sup> ) -----				
S1	8.25	9.25	5.15	1.23	2.25	10.5	4.48	14.98
S2	9.5	8.25	5.3	0.9	2	9	4.15	13.15
S3	72.25	24	6.4	5.3	31.75	9	165.5	175.05
Substrates	S-SO4	V	m	B	Cu	Fe	Mn	Zn
	(mg dm <sup>-3</sup> )	(%)	(%)	----- (mg dm <sup>-3</sup> ) -----				
S1	5.25	30	0	0.04	0.43	9.75	3.95	0.65
S2	5	33.75	0	0.05	0.30	22.25	6.58	0.6
S3	250.5	95	0	2.25	1.18	24.75	1.93	3.45

**Table 2** Physical analysis of substrates according to specialized literature for each attribute [S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1)]

Substrates	Substrate Density	Microporosity	Macroporosity	Total Porosity
	(kg dm <sup>-3</sup> )	----- % -----		
S1	1.1	24	20.9	44.8
S2	1.52	15.2	41.9	57.1
S3	1.31	18.4	32.4	50.8
Substrates	Granulometric Analysis (%)			
	Clay	Sand	Silt	
S1	6.2	93.6	0.2	
S2	3.6	96.1	0.3	
S3	5.9	93.9	0.2	

Table Continued...

Substrates	Substrate Density	Microporosity	Macroporosity	Total Porosity
	(kg dm <sup>-3</sup> )	-----%-----		
	Retained Water (cm <sup>3</sup> cm <sup>-3</sup> )			
	0 hour	24 hours	48 hours	72 hours
S1	0.607	0.533	0.523	0.511
S2	0.413	0.431	0.413	0.389
S3	0.461	0.492	0.461	0.44

In relations to treatments with fertilization application, it was used three commercial grass fertilizers (Maxgreen®) as described in Table 3 and one control without fertilizer.

**Table 3** Description of chemical composition of the fertilizers (Fertilization) used

Fertilization	N	P	K	Mg	S	B	Mo	Mn	Fe	Cu	Zn
	g kg <sup>-1</sup>										
F1	30	0	5	0.0	1.0	0.06	0.005	0.08	0.2	0.05	0.2
F2	24	0	15	0.0	1.0	0.06	0.005	0.08	0.2	0.05	0.2
F3	15	0	28	1.0	3.0	0.06	0.005	0.08	0.2	0.05	0.2

All fertilizers were incorporated into the substrates according to the treatments in the amount of 30 g m<sup>-2</sup>, and the treatments considered controls, did not receive any amount of fertilizer.

Irrigation management was daily performed, manually, receiving water until saturation to ensure field capacity of each substrate and manual weed control was performed.

At 15 days after planting (DAP) were evaluated fresh and dry mass of leaves – cutting leaves of each plot were removed using a pruning shears one centimetre from the ground and weighed on a precision scale to obtain the fresh mass. Posteriorly they were allocated into identified paper bags and dried in forced air ventilation oven at 60 °C. After stabilization the bags were weighted to obtain dry mass.

The Falker Chlorophyll Index (FCI) was determined by reading with chlorophyll meter, ClorofiLog CFL 1030 model. Four or six grass leaves from each container were taken and placed in the chlorophyll meter in order to cover the detector entirely, without overlapping the leaves.

Leaf analysis of N and S contents were performed, according to procedure described by Malavolta et al.<sup>12</sup> The means were submitted to Tukey test at 5% of probability and regression analysis using SISVAR program.<sup>17</sup>

## Results and discussion

The fresh mass means are shown in Table 4 and it is possible to notice that the only fertilizer which presented significant means was F1 in relation to substrates, producing the greatest amount of fresh mass at S1 (soil) and the smallest amount in S3 [soil + sand + soil conditioner (1:1:1)]. The interaction between substrates and fertilizers was not significant for fresh mass.

The F1 fertilizer, according to Table 3, was the one which contained more quantities of Nitrogen (N) and less of Potassium (K).

Santos et al.<sup>18</sup> also found difference in Zoysia grass cultivated with fertilizers in relation to treatments without (controls) and N also was

the element that increased the development.

**Table 4** Fresh mass (g m<sup>-2</sup>) of Imperial® Zoysia grass leaves after 15 Days after planted in different substrate compositions [S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1)] and fertilizers (Control: without fertilizer; F1; F2 and F3 fertilizers compositions are written in Table 3)

Substrates	Fertilizers			
	Control	F1	F2	F3
	----- g m <sup>-2</sup> -----			
S1	802.24 aAB	935.26 aA	697.46 aAB	601.99 aB
S2	710.46 aA	850.97 abA	749.64 aA	742.26 aA
S3	696.90 aA	620.27 bA	706.12 aA	536.98 aA
DMS line	294.21			
DMS column	266.35			
CV (%)	18.11			
F subs. x F fert.	1.29 <sup>ns</sup>			

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ at the 5% probability level by the Tukey test.<sup>ns</sup> not significant

Santos et al.<sup>18</sup> also found difference in Zoysia grass cultivated with fertilizers in relation to treatments without (controls) and N also was the element that increased the development.

According to Godoy et al.,<sup>8</sup> N is the main nutrient required by turfgrass and as observed in Table 3, the F1 contained in its formulations the greatest amount of N, developing grass in height increasing fresh mass production, as it is part some turfgrass essential physiological processes.<sup>9,10</sup> Although the plant nutrition being important, for turfgrass, in some cases, big growth is not recommended as it increase the maintenance, consequently increasing costs.<sup>20</sup>

Another important factor is in initial phase of tropical grasses, high N fertilization rates may cause increase in vegetative growth<sup>21</sup> due to plants allocate nutrients for vegetative biomass production,<sup>22</sup> corroborating to data found in these work.

In relation to dry mass, the same was found the only fertilizer which presented significant means was F1 in relation to substrates, producing the greatest amount of dry mass at S1 and the smallest amount in S3. The interaction between substrates and fertilizers was not significant for dry mass.

Silva et al.<sup>23</sup> working with Zoysia grass cultivated in different substrate formulations reported that treatment whit only soil presented the lowest amount of dry mass, the opposite found at present research. The possible explanation for this fact is due to its physical conditions, as shown in Table 2.

Even formed only by soil, this substrate have shown bigger retained water over time in relation to the other substrate, meaning that water was available to grass for more time (Table 2). Furthermore, the compaction of substrate may influence the reduction of fresh and dry mass. According to Barcelos et al.<sup>4</sup> substrate density influenced the accumulation of Zoysia grass mass, verifying that the most compacted substrate decreased values for both fresh and dry mas, corroborating to data presented in this research. At Table 2 it is possible to note that S1 presented the lowest values for substrate density and, in this case, also presented the highest means of fresh and dry mass.

It is important to remember that the exaggerated growth of the lawn is not interesting, as the costs for turfgrass maintaining may increase. Thus, if there is a lot of fertilizer added, there may be too much grass growth. With a great amount of dry matter on the lawn, after mowing, there is the formation of a layer that will prevent the entry of sunlight, which may stolate the plant and even death.

Falker Chlorophyll Index (FCI) of Imperial®Zoysia grass leaves are shown in Table 6. Substrates themselves presented interesting results analysing the control treatment. We noticed that S1 and S2 [S2 = soil + sand (1:1)] have not differed statistically from each other (30.27 and 32.60, respectively), however, S2 and S3 have differed (32.60 and 27.23, respectively).

Analysing each treatment of fertilization and control into substrate treatments, it is possible to note that in S1 the control (without fertilizer) have not differed statistically from F1 and F2 and this three treatments have differed statistically from F3, being the last one, the lowest result found. In S2 the best result found was control presented the best results for FCI in relation to all treatments with fertilizers. In S3 all treatments presented no difference statistically from each other.

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ at the 5% probability level by the Tukey test. \* significant at 5% of probability.

The measurement of chlorophyll content indirectly by devices is an up-and-coming way in scientific research. The traditional form is made in a laboratory performed by extraction using different organic solvents, such as acetone, ether, dimethyl sulfoxide or methanol<sup>24</sup> and after determining the pattern based on the absorbance of light by the pigments.<sup>25</sup> However, there is great variation in the results depending on solvent used,<sup>26</sup> in addition to being very laborious.

**Table 5** Dry mass (g m<sup>-2</sup>) of Imperial®Zoysia grass leaves after 15 Days after planted in different substrate compositions [S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1)] and fertilizers (Control: without fertilizer; F1; F2 and F3 fertilizers compositions are written in Table 3)

Substrates	Fertilizers			
	Control	F1	F2	F3
	----- g m <sup>-2</sup> -----			
S1	300.83 aAB	331.19 aA	235.71 aAB	202.47 aB
S2	277.77 aA	319.08 abA	278.42 aA	213.25 aA
S3	261.09 aA	228.45 bA	264.42 aA	201.90 aA
DMS line		107.8		
DMS column		97.6		
CV (%)		18.43		
F subs. x F fert.		1.21 <sup>ns</sup>		

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ at the 5% probability level by the Tukey test.<sup>ns</sup> not significant

**Table 6** Falker Chlorophyll Index (FCI) of Imperial®Zoysia grass leaves after 15 Days after planted in different substrate compositions [S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1)] and fertilizers (Control: without fertilizer; F1; F2 and F3 fertilizers compositions are written in Table 3)

Substrates	Fertilizers			
	Control	F1	F2	F3
	----- FCI-----			
S1	30.27 abA	30.43 aA	27.37 aA	20.53 bB
S2	32.60 aA	19.80 cC	25.27 aBC	30.27 aAB
S3	27.23 bA	25.43 bA	27.87 aA	26.47 aA
DMS line		5.51		
DMS column		4.99		
CV (%)		9.07		
F subs. x F fert.		10.26*		

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ at the 5% probability level by the Tukey test.<sup>ns</sup> not significant

Thus, the improvement of the measurement of chlorophyll content indexes by devices is essential. The CloroflLOGFalker, used in the present research, uses three light frequency bands that allow an accurate analysis of the chlorophyll content with the obtaining of instantaneous measurements, visualized immediately by the device display. The optical measurement analyses the absorption of light by the leaf, indicating the presence of chlorophyll, and a lower sensor receives the radiation transmitted through the leaf structure. From this information, the device provides values called Chlorophyll Falker Index (ICF), which are proportional to the absorbance of chlorophylls.<sup>27</sup>

Chlorophyll molecules are formed by a central magnesium atom, in addition to other components.<sup>28</sup> This means that the plant needs to absorb this element in order to produce chlorophyll and continue with its metabolic processes. However, some factors may negatively influence the absorption of magnesium by the plant, such as the amount of potassium available in the substrate, in these case, greater the amount of K, less the absorption of Mg by the plant.<sup>29</sup>

In substrate S1, as seen previously, control and fertilizers F1 and F2, have not differed statistically from each other, but all of them have differed from fertilizer F3, which means that grass cultivated with only soil (S1) with no fertilization (control) or fertilized with F1 and F2 presented the highest amount of chlorophyll (FCI) (Table 6). Considering the amount of K in each treatment, already presented at substrate (Table 1) and added after fertilization (Table 3), grass fertilized with F3, which contains more K decreased the amount of chlorophyll.

At chemical analysis (Table 1), the substrate S2 was the substrate which presented the lowest value of K in its composition and grass cultivated with no fertilizer presented more chlorophyll. In these case, all treatments with fertilizers or not, except F2, presented FCI bigger than S1, even with more addition of K, which means that the K added after may have leached.

For present research, K amount was determined to chlorophyll content because in substrate S3, even presenting more quantity of Mg (Table 1), the FCI was the lowest in relation to S1 and S2 (Table 6). These substrate was formed by soil, sand and soil conditioner and the increment of K in its chemical analysis may have interfered in grass Mg absorption.



It is important to mention that for turfgrass, the intense green colour is extremely important to aesthetic for garden, sport fields. The chlorophyll present in the leaves has the green pigment, so the more chlorophyll the leaf has, the greener the lawn will be.

Regarding lawns fertilization, we may not generalize stating that the application of K will always negatively interfere with the amount of leaf chlorophyll. In this situation, using different substrates, which have different chemical characteristics, fertilization with K was harmful. Therefore, researches have great importance for the knowledge of the best management to be used in each situation.

For N content, Table 7 shows the interaction between substrates and fertilizers/control have not differed statistically. In grass cultivated without fertilizers, more leaf N was found in substrate S1 (11.90 g of N kg<sup>-1</sup> of dry mass) and S3 (12.37 g of N kg<sup>-1</sup> of dry mass), which have not differed statistically from each other, but both have differed from S2 (11.27 g of N kg<sup>-1</sup> of dry mass).

**Table 7** Nitrogen (N) content (g kg<sup>-1</sup> of dry mass) in Imperial® Zoysia grass leaves after 15 Days after planted in different substrate compositions [S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1)] and fertilizers (Control: without fertilizer; F1; F2 and F3 fertilizers compositions are written in Table 3)

Substrates	Fertilizers			
	Control	F1	F2	F3
----- g kg <sup>-1</sup> of dry mass -----				
S1	11.90 abBC	12.60 aAB	13.30 aA	11.15 aC
S2	11.27 bA	11.62 bA	11.74 bA	10.94 aA
S3	12.37 aA	12.55 aA	11.27 bA	11.81 aA
DMS line	0.99			
DMS column	0.89			
CV (%)	3.66			
F subs. x F fert.	2.48 <sup>ns</sup>			

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ at the 5% probability level by the Tukey test.<sup>ns</sup> not significant

The same was found in fertilizer F1, which presented 30% of N in its formulation. At the time of grass leaf analysis, the leaf N content in grass cultivated in substrate S1 (12.60 g of N kg<sup>-1</sup> of dry mass) and S3 (12.55 g of N kg<sup>-1</sup> of dry mass) was higher than S2 (11.62 g of N kg<sup>-1</sup> of dry mass).

For fertilizer F2, which presented 24% of N in its formulation, higher leaf N was found in grass cultivated in S1 (13.30 g of N kg<sup>-1</sup> of dry mass), differing statistically from S2 (11.74 g of N kg<sup>-1</sup> of dry mass) and S3 (11.81 g of N kg<sup>-1</sup> of dry mass).

The substrate texture and quantity of organic matter may explain the reduction of leaf N accumulation in substrate S2. This substrate was formed by soil and sand, according to Table 2, it presented the highest percentage of sand (96.1%) and lowest content of organic matter (8.25 g dm<sup>-3</sup> – Table 1), and both attribute may intensify the N leaching.<sup>30</sup>

Leaching is the loss of nutrients from the surface layers to the deeper layers of the soil. This means that nitrogen fertilization needs to be done carefully on more sandy substrates to avoid this type of loss.

While on more clayey substrates there is greater retention of applied N, on sandy substrates there is not. In both cases, if the same amount of N is applied, the availability for plant will be different. For this reason, it is recommended to fractionate N fertilization when the substrate is sandy, avoiding the loss of nutrient and contamination of groundwater.

The organic matter present in the substrates may also have interfered with the availability of N for plants, in this case, the substrates that presented the highest amounts of organic matter (S1 and S2 – Table 1) also exported the largest amount of leaf N.

The decomposition of organic matter influences nutrients availability of soil. This influence is related to complexation or adsorption of competing ions, which inhibits the action of soil functional groups making nutrients free in soil solution. The decomposition of organic material must also be considered an important source of nutrients in the soil, as its decomposition results in mineralization of nutrients from plant tissues.<sup>31</sup>

The export of P to leaves is shown in Table 8, which shows the amounts of the element per kilogram of dry matter found in each treatment. In substrate S1, the leaf P content found have not differed statistically among treatments without fertilizer (3.97 g of P kg<sup>-1</sup> of dry mass) and fertilizer F1 (3.70 3.97 g of P kg<sup>-1</sup> of dry mass) and F3 (3.73 3.97 g of P kg<sup>-1</sup> of dry mass), however, all of them have differed from F2 (3.16 3.97 g of P kg<sup>-1</sup> of dry mass).

**Table 8** Phosphorus (P) content (g kg<sup>-1</sup> of dry mass) in Imperial® Zoysia grass leaves after 15 Days after planted in different substrate compositions [S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1)] and fertilizers (Control: without fertilizer; F1; F2 and F3 fertilizers compositions are written in Table 3)

Substrates	Fertilizers			
	Control	F1	F2	F3
----- g kg <sup>-1</sup> of dry mass -----				
S1	3.97 aA	3.70 cA	3.16 cB	3.73 bA
S2	3.94 aC	5.01 aA	4.54 aB	3.98 bC
S3	4.22 aB	4.69 bA	4.11 bB	4.67 aA
DMS line	0.31			
DMS column	0.28			
CV (%)	3.31			
F subs. x F fert.	24.65*			

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ at the 5% probability level by the Tukey test.<sup>ns</sup> not significant

In relation to grass cultivated in substrate S2, all treatments based on fertilizers or not, presented different behaviour, being the treatment with fertilizer F1 which export more P to leaves (5.01 3.97 g of P kg<sup>-1</sup> of dry mass). On the other hand, in substrate S3, F1 (4.69 3.97 g of P kg<sup>-1</sup> of dry mass) and F3 (4.67 3.97 g of P kg<sup>-1</sup> of dry mass) have not differed statistically between them, but both have differed among control (4.22 3.97 g of P kg<sup>-1</sup> of dry mass) and F2 (4.11 3.97 g of P kg<sup>-1</sup> of dry mass).

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ at the 5% probability level by the Tukey test. \* significant at 5% of probability.

Dias et al.<sup>32</sup> reports that the vast majority of tropical soils are highly weathered, with low levels of P. This low availability compromises plants establishment, mainly for Poacea family species. P is actively involved in many plant metabolic and enzymatic processes and, although the demand for this nutrient is low compared to N, its availability is very important, since it is a component of ATP molecule, which is necessary for all metabolic reactions within the cells. In this way, P accelerates and improves the metabolic development of the grass.

The leaf analysis, whether of micro or macronutrients, is of relevant importance to consider the plant nutritional situation and to verify if the fertilization that is being performed is responding. One situation is to do soil analysis and check the deficiencies of that place and another to know if plant is really able to absorb what is being applied. With the foliar analysis it is possible to understand if any factors outside the ideal conditions are interfering with the availability of nutrients.

In this case, several factors may have interfered with the absorption and exportation of P to the Imperial® Zoysia grass leaves. First of all, plant only may export what is available for absorption. Despite of not having P in fertilizers formulations, it is possible to note great difference at P content among substrates. In Table 1 substrate S3 showed the highest content of P (72.25 mg dm<sup>-3</sup>), followed by S2 (9.50 mg dm<sup>-3</sup>) and S1 (8.25 mg dm<sup>-3</sup>).

With more P, grass cultivated in substrate S3 have exported more P to leaves as seen in Table 8 for control and fertilizer F3, however, the same have not occurred to fertilizers F1 and F2. For these treatments, substrate S2 have provides more leaf P.

Fertilizers F1 and F2, according to Table 3, presented greater amount of N in their composition (30% and 24%, respectively) and, as reported by Barneix,<sup>33</sup> there are close relationships between the ability of root to assimilate N and plant mineral nutrition, associated with carbohydrates synthesis in leaves and the supply of assimilated to root. More precisely in relation to P, nitrogen has been shown to stimulate phosphate absorption by vegetables, depending on the intensity of this increase in the concentration and chemical form of the applied nitrogen.

To emphasise these idea, as seen previously, substrate S2 presented 96, 1 % of sand in its composition, which have not retain properly the N ions keeping them available for plant or for leaching. In this case, as there was a greater availability of N due to the constitution of the substrate S2 being predominantly formed of sand, the absorption of P by the plant was greater, even with reduced amount of P compared to the substrate S3.

The leaf S is reported in Table 9 and analysing each substrate into each fertilizer and control we may noticed that in all fertilizer treatment and without, S content in leaves of Imperial® Zoysia grass was higher in substrate S3.

In substrate S2, also in Table 9, the most important result found was grass leaves contained more S in fertilizer F2 (2.69 g of S kg<sup>-1</sup> of dry mass) than in control (2.03 g of S kg<sup>-1</sup> of dry mass) and in substrate S3, control and fertilizer F1 (4.64 and 4.68 g of S kg<sup>-1</sup> of dry mass, respectively) have not differed between each other, but have differed in relation to fertilizers F2 and F3 (3.60 and 3.56 g of S kg<sup>-1</sup> of dry mass, respectively).

Means followed by the same lowercase letter in the column and uppercase letter in the line do not differ at the 5% probability level by the Tukey test. \* significant at 5% of probability.

**Table 9** Sulphur (S) content (g kg<sup>-1</sup> of dry mass) in Imperial® Zoysia grass leaves after 15 Days after planted in different substrate compositions [S1 = soil; S2 = soil + sand (1:1) and S3 = soil + sand + soil conditioner (1:1:1)] and fertilizers (Control: without fertilizer; F1; F2 and F3 fertilizers compositions are written in Table 3)

Substrates	Fertilizers			
	Control	F1	F2	F3
----- g kg <sup>-1</sup> of dry mass -----				
S1	2.40 bA	2.30 bA	1.90 cA	2.03 bA
S2	2.03 bB	2.30 bAB	2.69 bA	2.17 bAB
S3	4.64 aA	4.68 aA	3.60 aB	3.56 aB
DMS line		0.56		
DMS column		0.5		
CV (%)		8.64		
F subs. x F fert.		8.60*		

The chemical analysis (Table 1) showed that substrate S3 had the highest amount of sulphate in its composition, being 47, 7 times bigger than in substrate S1 and 50 times bigger than substrate S2. The difference is related to the quantity of organic matter in the substrates, as seen in the same table. S3 contained 24.00 g dm<sup>-3</sup> of organic matter in its constitutions, against 8.25 g dm<sup>-3</sup> in substrate S2 and 9.25 g dm<sup>-3</sup> (Table 1) in substrate S1.

The increment of sulphate in S3 is due to the soil conditioner, as the three substrates are formed by the same soil. The literature reports that S has low availability in most Brazilians soils, especially the most weathered, such as Oxisols and Ultisols, ranging from 0.1% in mineral soils to 1% in organic soils. As mentioned, the same Oxisols was used, differing only sand and soil conditioner. Sand is an inert material, therefore, the soil conditioner increased the amount of sulphate.<sup>34</sup>

It is not possible to compare the results from substrates chemical analysis and leaf analysis for considering S in different types of element. In substrate analysis it was determined the S in sulphate form and at leaf in pure S. However, sulfate is readily available for plant absorption, the chemical analysis of substrate quantified exactly what plant could use, therefore, we may predict the comparison between them.

Interesting results should be considered, as in substrate S1 even the fertilizers having S in their composition, leaf S have not differed statistically from control, without fertilizer addition. The same was found in substrate S2 for fertilizer F3 comparing to control and even to substrate S3, the fertilization have not interfered in leaf S of Imperial® Zoysia grass.

A possible explanation for the fact is that S is extremely mobile in soil and may be lost by leaching.<sup>35</sup> Thus, there was a greater amount of nutrient than the plant's absorption capacity in relation to the S3 substrate.

S participate in the ferredoxins composition, enzyme complexes involved in photosynthesis and in the atmospheric nitrogen fixation and in chlorophyll formation, thus influencing the production of photoassimilates,<sup>36</sup> corroborating to found in this work, the highest leaf S found correspond to leaf N as seen in Table 7 (Substrate S3, control and fertilizer F1).

## Conclusion

The lawn fertilization needs to take into account the substrate composition on grass will be / was installed, since the dynamics of nutrients will depend on several factors such as physical and chemical attributes for its immediate or long-term availability.

In sporting or ornamental lawns the accelerated development of grass is not ideal, as it will reduce the mowing intervals, burdening the system. On the other hand, aesthetically the lawn needs to be as green as possible, as it is a conversion used by everyone.

Thus, according to data obtained in this research, the best result obtained was the cultivation of Imperial® Zoysia grass in substrate formed by soil, sand and soil conditioner without the necessity of fertilization.

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## Conflicts of interest

The authors declare no conflicts of interest.

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