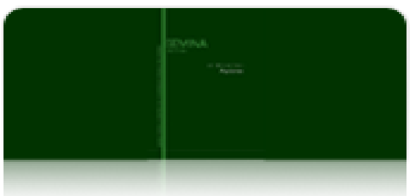

Semina: Ciências Agrárias
ISSN: 1676-546X
semina.agrarias@uel.br
Universidade Estadual de Londrina
Brasil

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Semina: Ciências Agrárias, vol. 36, núm. 1, 2015, pp. 1875-1893
Universidade Estadual de Londrina
Londrina, Brasil

DOI: 10.5433/1679-0359.2015v36n3Supl1p1875

Application of herbicides as growth regulators of emerald Zoysia grass fertilized with nitrogen

Doses de nitrogênio e aplicação de herbicidas como reguladores de crescimento em grama esmeralda

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Abstract

Nitrogen (N) is essential for nutrition and for the maintenance of the intense green color of lawns. However, this element affects shoot growth and, therefore, mowing frequency, which is a key factor of lawn-maintenance costs. Accordingly, this study aimed to evaluate the use of nitrogen fertilization in combination with the use of potential herbicides as growth regulators to promote the maintenance of the high visual (intense green) and nutritional quality of lawns of emerald Zoysia (*Zoysia japonica* Steud.) grown in Ultisol soil while reducing their leaf growth. The experiment was conducted at the Teaching, Research and Extension Education Farm (Fazenda de Ensino, Pesquisa e Extensão) of São Paulo State University (Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP), Ilha Solteira Campus/ São Paulo (SP), from June/2012 to June/2013. The experimental design consisted of randomized blocks with 20 treatments established in a 5 x 4 factorial arrangement with four replicates, including four herbicides (glyphosate, imazaquin, imazethapyr, and metsulfuron-methyl, applied at doses of 200, 420, 80, and 140 g ha^{-1} active ingredient (a.i.), respectively) and a control (without herbicide), and four N doses (0, 5, 10, and 20 g m^{-2}), split into five applications delivered throughout the year. The following items were evaluated: length, LCI (leaf chlorophyll index), leaf dry matter production and leaf N concentration and the percentage of phytotoxicity on lawn grass shoots. Doses from 10 to 20 g m^{-2} N provided sufficient N concentrations to maintain the emerald Zoysia. The herbicides metsulfuron-methyl and glyphosate were superior in the control of lawn leaf growth. While the former was phytotoxic, the latter had no effect on the aesthetic quality of the lawn, standing out as an herbicide that may be used at a dose of 200 g ha^{-1} to regulate the growth of emerald Zoysia.
Key words: Nitrogen fertilization, lawn, *Zoysia japonica* Steud

Resumo

O nitrogênio é essencial para a nutrição e a manutenção da coloração verde intensa dos gramados. No entanto, influencia o crescimento da parte aérea e, consequentemente, a frequência de cortes, principal

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Recebido para publicação 07/04/14 Aprovado em 22/09/14

fator do custo de manutenção em gramados. Neste contexto, objetivou-se avaliar a adubação nitrogenada e o uso potencial de herbicidas como reguladores de crescimento em grama esmeralda (*Zoysia japonica* Steud.), em Argissolo Vermelho, visando manter o gramado com boa qualidade visual (verde intenso) e nutricional bem como reduzir o seu crescimento foliar. O experimento foi conduzido na Fazenda de Ensino, Pesquisa e Extensão da UNESP, Campus de Ilha Solteira/SP, de junho/2012 a junho/2013. Utilizou-se o delineamento em blocos casualizados com 20 tratamentos dispostos num fatorial 5 x 4 com quatro repetições, sendo quatro herbicidas: glyphosate, imazaquin, imazethapyr e metsulfuron-methyl aplicados na dose de, respectivamente, 200, 420, 80 e 140 g ha^{-1} do ingrediente ativo (i.a.) e a testemunha - sem herbicida; e quatro doses de N: 0, 5, 10 e 20 g m^{-2}, parceladas em cinco aplicações durante o ano. Avaliaram-se: o comprimento, o índice ICF (índice de clorofila foliar) e a produção de matéria seca das folhas, determinaram-se também a concentração foliar de N e a porcentagem de fitointoxicação da parte aérea do gramado. Doses de 10 a 20 g m^{-2} de N proporcionaram concentração de N suficiente para a manutenção da grama esmeralda. Os herbicidas metsulfuron-methyl e glyphosate destacaram-se no controle do crescimento foliar do gramado, sendo que o primeiro mostrou-se mais fitotóxico e o segundo não prejudicou a qualidade estética do gramado, destacando-se como herbicida que possa ser utilizado como regulador do crescimento de grama esmeralda na dose de 200 g ha^{-1}.
Palavras-chave: Adubação nitrogenada, gramado, *Zoysia japonica* Steud

Introduction

Lawn areas are used for several purposes, whether leisure or commercial, as sports fields, public spaces for the population, residential gardens, slopes and embankments, median strips, and areas intended for grass production. Fertilization and irrigation are essential to all forms of use, and a key factor contributing to maintenance costs is the mowing required to maintain the appropriate heights of lawns (RODRIGUES et al., 2004; VILLAS BÔAS; GODOY, 2004).

The area of the United States cultivated with grass was 166 thousand hectares (ha) in 2007 (UNITED STATES DEPARTMENT OF AGRICULTURE, 2009), significantly larger than that in Brazil, which is approximately 17 thousand ha and has been reported to be increasing, although it is not ranked among the world's main producers of grass (GODOY et al., 2012a; ZANON; PIRES, 2010). A total of 10 thousand ha are produced per year (sales of BRL 300 million), and 80% of the sales are attributed to emerald Zoysia (CANAL RURAL, 2013).

Emerald Zoysia (*Zoysia japonica* Steud.) is the species used in most Brazilian residential gardens and is also frequently used for slope stabilization in areas with potential erosion problems. The emerald green color and rhizomatous-stoloniferous growth pattern produce a perfect lawn sod given the entanglement of stolons with leaves and dense soil cover. Emerald Zoysia grows well in areas with full insolation, has a high resistance to trampling, requires high nitrogen (N) fertilization, and has an optimal mowing height of 1.25 to 3.0 cm (GURGEL, 2003; GODOY et al., 2012a).

There is no official fertilization guideline for sod production or lawn transplantation and maintenance in the State of São Paulo (GODOY et al., 2012b). Furthermore, most studies published by Brazilian researchers have been directed towards lawn grass production (GODOY, 2005; BACKES, 2008; LIMA, 2009), with an existing shortage of research studies on transplanted lawns.

The correct management of N fertilization is a requirement for maintaining a high-quality lawn (GODOY et al., 2012a). Appropriate doses of N, the most highly required nutrient by lawn grasses, provide a more intense green color, which is aesthetically desirable but result in faster plant growth (GODOY; VILLAS BÔAS, 2003). This increased growth rate consequently increases the mowing frequency and, thus, both the nutrient uptake and lawn-maintenance costs (GODOY et al., 2012a). Therefore, the use of a plant growth regulator in lawns could be recommended, and a regulator that reduces height while maintaining the quality of the area treated, that is, without reducing the density or causing visible damage to plants, including necrotic spots of phytotoxicity, discoloration or thinning (RODRIGUES et al., 2004), would be optimal and viable for use around highways and airports, in industrial parks, cemeteries, and golf courses and along fences, sloped areas, and sites where mowing is difficult (SHAWN; HIPKINS, 2013).

The herbicides imazaquin and imazethapyr (imidazolinones) and metsulfuron-methyl (a sulfonylureas) act by inhibiting acetolactate synthase (ALS) or acetohydroxyacid synthase (AHAS), enzymes involved in the biosynthesis process of the branched-chain amino acids valine, leucine, and isoleucine. This inhibition stops protein synthesis, which, in turn, affects DNA synthesis and cell growth (FERREIRA et al., 2005). Glyphosate is a glycine derivative and acts in the shikimic acid pathway; it is classified as a 5-enolpyruvylshikimate-3-phosphate (EPSP) synthase enzyme-inhibitor herbicide, thus inhibiting the formation of the aromatic amino acids tryptophan, tyrosine, and phenylalanine, which are essential for plant development and growth (VELINI et al., 2009; OLIVEIRA JUNIOR, 2011).

The use of herbicides as growth regulators of lawns is a method that has been well studied in the United States; conversely, scarce research has been conducted on this topic in Brazil. Studies that examine growth regulators and the dosages of herbicides required for the cultivated grass species are required to broaden the knowledge base of the interaction between lawn selectivity and maintenance, thus enabling efficient and secure recommendations based on scientific data generated under Brazilian conditions. The doses and timings of applications targeting the best growth control should also be evaluated because there are no official and safe recommendations for lawn management through the use of plant regulators for Brazil (RODRIGUES et al., 2004; MACIEL et al., 2011).

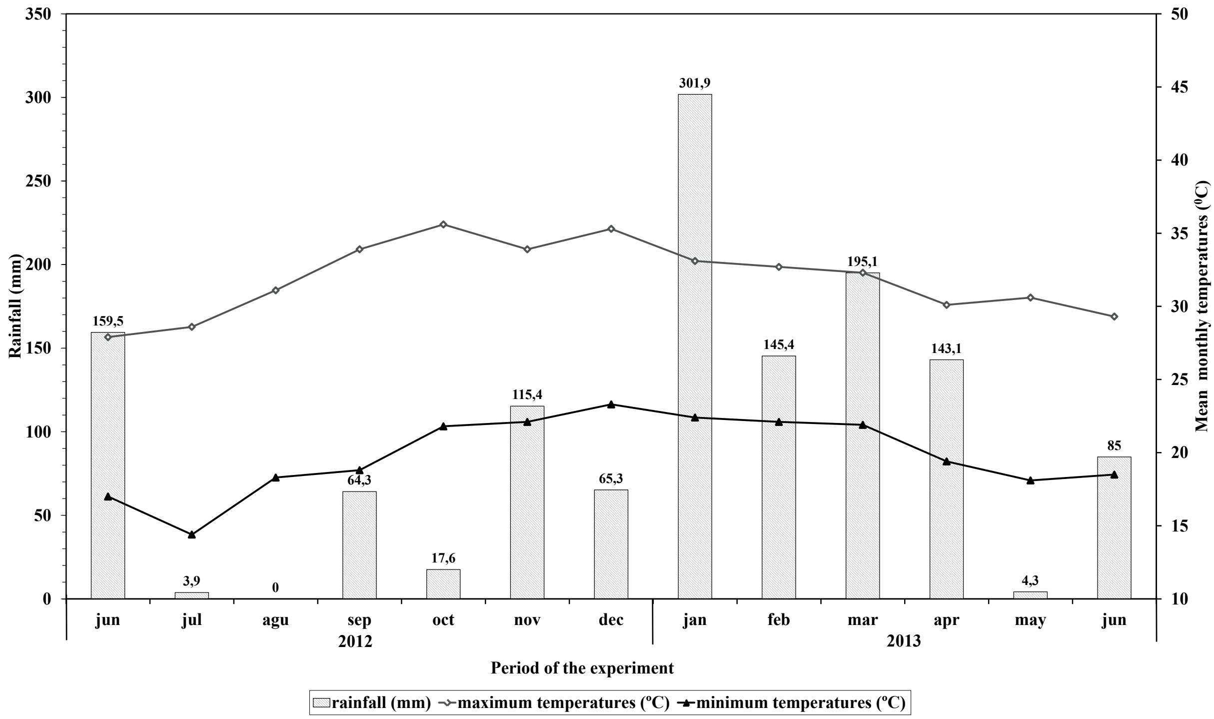
This study aimed to evaluate the use of nitrogen fertilization in combination with herbicides used as growth regulators for the production of lawns of emerald Zoysia with high visual and nutritional quality and reduced leaf growth.

Materials and Methods

The experiment was conducted from June 2012 to June 2013 at the Teaching, Research and Extension Education Farm (Fazenda de Ensino, Pesquisa e Extensão) of São Paulo State University (Universidade Estadual Paulista Júlio de Mesquita Filho, UNESP), Ilha Solteira Campus, located at 20º 22’ 23.5’’S latitude and 51º 22’ 12.6’’W longitude, with an altitude of 330 m, in the municipality of Ilha Solteira/SP, in a eutrophic sandy-clayey Ultisol. The climate data (rainfall and mean monthly minimum and maximum temperatures) corresponding to the experimental period were collected at the weather station of Ilha Solteira, using the CLIMA channel of the Unesp, Ilha Solteira, Hydraulics and Irrigation Section (Área de Hidráulica e Irrigação) and are outlined in Figure 1.

The control of weeds in the site was conducted on 06/01/2012 before planting the emerald Zoysia using glyphosate at a 2-kg active ingredient (a.i.) dose, delivered as 200 L spray solution ha^{-1}. Tillage was conducted on 06/08/2012 using disc plowing and mild harrowing, with subsequent leveling to avoid large variations in relief. The results from the initial soil (0-20 cm) chemical analysis of the experimental area, conducted in June 2012 according to the methodology of Raij et al. (2001), were 16 mg dm^{-3} P (resin), 18 g dm^{-3} organic matter (O.M.), pH 4.8 ($CaCl_2$); K, Ca, Mg, and H+Al concentrations of 2.3, 12.0, 4.0, and 22.0 $mmol_c$ dm^{-3}, respectively, and 45% base saturation (BS). The liming (06/09/2012) was conducted by broadcasting a dose of 1.2 t ha^{-1} dolomitic limestone (85% PRNT) on the soil surface and incorporating to 20 cm with the aim of increasing the BS to 70% (GODOY et al., 2012a). Phosphorus (P) and potassium (K) fertilization was conducted after the liming process (07/10/2012) and before establishing the lawn, using triple superphosphate (45% P_2O_5) and potassium chloride (60% K_2O) as the sources, respectively. A dose of 10 g m^{-2} $year^{-1}$ P_2O_5 and K_2O was delivered in a single application. The soil (0-20 cm) chemical analysis was conducted on 08/01/2012 according to the methodology by Raij et al. (2001), with the following results: 26 mg dm^{-3} P (resin), 17 g dm^{-3} O.M., pH 6.6 ($CaCl_2$); K, Ca, Mg, H+Al = 2.7; 28.0; 22.0, and 11.0 $mmol_c$ dm^{-3}, respectively, and 83% BS.

Figure 1. Mean monthly rainfall and the minimum and maximum temperatures during the experimental period. UNESP, Ilha Solteira/SP, 2012/13.



The management of the sprinkler irrigation was performed according to the evapotranspiration and the Penman-Monteith method, employing a Kc of 0.94 (SILVA, 2004).

The emerald Zoysia was planted using sod (63 x 40 cm) on 08/03/2012. A randomized-block experimental design was employed, with 20 treatments arranged in a 5 x 4 factorial arrangement with 4 replicates and a 10-m^2 area per plot. The treatments consisted of 4 herbicides (growth regulators), glyphosate, imazethapyr, imazaquin, and metsulfuron-methyl, which were applied at doses of 200, 80, 420, and 140 g a.i. ha^{-1}, respectively, and a control (without herbicide application), and four doses of N (0, 5, 10 and 20 g m^{-2}), divided among 5 applications throughout the year.

The doses of N used in this experiment were based on the graduate study by Dinalli (2011), who used and determined that the dose of 10 g m^{-2} N provided nutrient concentrations suitable for the development of emerald Zoysia. Urea (45% N) was used as the source of N and was manually applied, immediately after mowing, on 09/16/2012, 10/30/2012, 01/06/2013, 02/23/2013, and 04/14/2013, in the first, second, third, fourth and fifth evaluations, respectively. The lawn was irrigated after each fertilization to decrease losses by N volatilization.

The herbicides were applied 15 days after fertilization in the spring/summer months and 30 days later in the autumn/winter months. That significant difference in time reflects the slower rate of grass growth in the autumn/winter compared to the spring/summer under the Brazilian conditions. The doses of herbicides applied were based on the scientific literature. The applications were conducted in the morning during mild temperature conditions using a CO_2-pressurized backpack sprayer fitted with a 2-L tank (disposable bottles) and a bar with four 0.50-m-spaced, anti-drip spray nozzles (model 80.02 model), using a 3-psi working pressure to deliver 200 L spray solution ha^{-1}. The plots were laterally protected with tarpaulin during the application to avoid spraying the solution onto adjacent plots. The herbicides were applied on 09/30/2012, 11/13/2012, 01/21/2013, 03/08/2013, and 05/14/2013 to the first, second, third, fourth, and fifth evaluations, respectively. The first four evaluations corresponded to spring/summer and the last to autumn.

Evaluations were conducted every 30 days following the herbicide applications. Mowing was performed after the collection of plant material using a gasoline mower with a grass catcher to even out the size of emerald Zoysia in the treatments and to always maintain a height of approximately three cm from the soil level. The data presented in this study include the results for the first, second, third, fourth, and fifth evaluations conducted on 10/30/2012, 12/15/2012, 02/22/2013, 04/08/2013, and 06/14/2013, respectively.

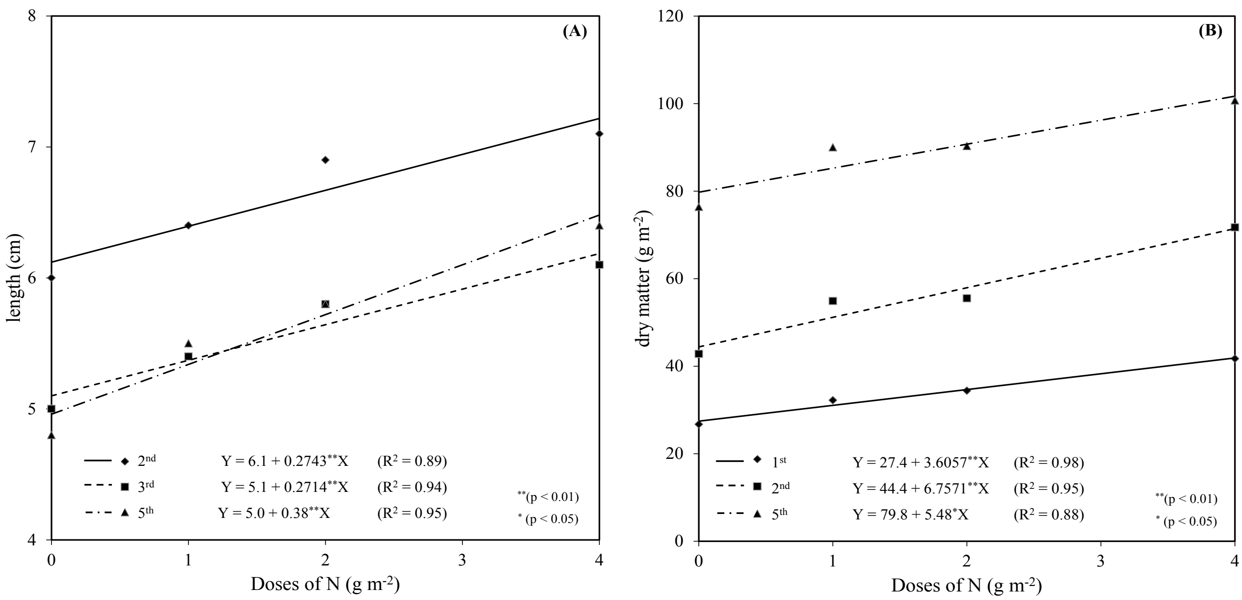
The following items were evaluated: a) Leaf length was measured on 15 leaves collected per plot using a millimeter ruler to obtain the mean value; and b) Chlorophyll meter readings of the leaf chlorophyll index (LCI) were collected on leaves using a portable, manual chlorophyll meter (Falker) to measure 15 leaves per plot, which were manually harvested. The measurements were conducted in a laboratory given the small size of grass leaves and the associated difficulty with handling them (GODOY, 2005; LIMA, 2009). The leaves were collected in the morning, placed in labeled, brown paper bags, and stored in styrofoam boxes with ice to prevent the leaf blades from rolling (which would impair the measurement). Each leaf was placed in the measurement area of the chlorophyll meter, and one measurement was collected per leaf from the middle of the leaf blade; c) leaf dry matter was assessed by manually collecting material using scissors near the soil surface to remove the green matter of leaves from the plants contained in one square meter, with three replicates collected per plot to form a composite sample that was stored in a labeled, brown paper bag and dried in an oven at 65°C for 72 hours; d) the leaf N concentration was assessed according to the methodology reported by Malavolta et al. (1997); and e) The phytotoxicity on lawn grass shoots was evaluated using a visual grading scale (0-100%), with "0%" corresponding to the "absence of injuries" and "100%" corresponding to the "total death" of the lawn grass (SOCIEDADE BRASILEIRA DA CIÊNCIA DAS PLANTAS DANINHAS, 1995). The methodology by Costa et al. (2010), who considered mild, moderate, and severe and aesthetically non-acceptable symptoms to correspond to grades lower than or equal to 10.0%, from 10.1 to 20.0%, and higher than 20.0%, respectively, was adopted to interpret the phytotoxicity to emerald Zoysia. The criteria used in the present study to establish these phytotoxicity grades were the number and evenness of injuries (yellowing, loss of the intense green color), the onset of dead plants, and lawn grass density (absence of patches with exposed soil and/or leaf thinning).

Using the SISVAR software (FERREIRA, 2008) for the statistical analyses, the data were subjected to analyses of variance and Tukey's tests, at the 5% probability level, for the comparisons of means of values among herbicide treatments, and polynomial regressions were performed to evaluate responses to N doses.

Results and Discussion

The values of emerald Zoysia leaf length increased linearly with the increase in N doses in the second, third and fifth evaluations (Figure 2A). Backes et al. (2010) also reported that the highest N doses (among 0, 100, 200, 300 and 400 kg ha^{-1} treatments supplied in a single application) from sewage sludge promoted greater emerald Zoysia growth compared to the treatment without N. However, the increase in leaf growth of ornamental lawns associated with greater N doses is not desirable from an economic standpoint because it increases the mowing frequency and, thus, both nutrient uptake and lawn-maintenance costs (BACKES, 2008; GODOY et al., 2012a).

Figure 2. Mean length (A) and leaf dry matter (B) values of emerald Zoysia, according to N dose.



Upon herbicide application, there was a difference between the grass leaf length values of the second evaluation and those of the control (Table 1). The lowest leaf length values were recorded when applying metsulfuron-methyl, an herbicide that proved efficient in controlling grass growth, glyphosate, or imazethapyr, with reductions of 26.9%, 20.5%, and 16.7% respectively, relative to the control. Rogers (1985) noted that the application of metsulfuron-methyl (140 g ha^{-1}) effectively controlled the height of Bermuda grass, corroborating the results from the present study for emerald Zoysia. Maciel et al. (2006) recorded height values of 5.54, 5.53, and 5.56 cm 28 days after application (DAA) of imazethapyr to lawns composed of bahiagrass at doses of 25, 75, and 125 g ha^{-1}, respectively; these values differed from the value recorded for the control in the same period, which was 6.64 cm. Glyphosate (600 g ha^{-1}) also suppressed the plant growth of centipedegrass (*Eremochloa ophiuroides*), with a 22% reduction in height compared to the control at 28 DAA (FRY, 1991). Leite et al. (2010) noted that the inhibition of bahiagrass growth at 31 DAA was 26.19% compared to the control when using glyphosate applied 4 days after mowing (216 g acid equivalent (a.e.) ha^{-1}). The results of previous studies and the present study demonstrate the potential of these herbicides as growth regulators of lawn grasses.

Table 1. Mean length and leaf dry matter of emerald Zoysia, for five evaluations, according to the herbicide treatment. UNESP, Ilha Solteira/SP, 2012/13.

Herbicidas	length (cm)					
	1st	2nd	3rd	4th	5th	Mean
Control	4.5 a	7.8 a	5.5 a	6.8	5.7 a	6.1 a
imazaquin	4.3 a	6.8 ab	5.9 a	5.9	6.0 a	5.8 ab
imazethapyr	4.6 a	6.5 bc	5.7 a	6.2	5.8 a	5.8 abc
glyphosate	4.6 a	6.2 bc	5.4 a	5.8	5.3 a	5.5 bc
metsulfuron-methyl	4.5 a	5.7 c	5.4 a	5.8	5.3 a	5.3 c
D.M.S. (5%)	0.6	1.0	0.8	0.7	0.8	0.4
C.V. (%)	10.83	13.47	11.78	10.14	12.30	6.18
	leaf dry matter (g m^{-2})					
Control	33.3 a	64.6 a	112.5	98.8	94.5 a	80.7 a
imazaquin	33.3 a	61.1 a	104.9	106.8	100.9 a	81.4 a
imazethapyr	31.2 a	59.7 a	113.9	98.5	95.6 a	79.8 ab
glyphosate	33.3 a	50.7 ab	97.9	81.8	81.4 a	69.0 bc
metsulfuron-methyl	37.5 a	43.7 b	100.7	82.8	74.5 a	67.8 c
D.M.S. (5%)	8.8	14.7	21.4	27.7	36.9	11.1
C.V. (%)	22.20	22.44	17.26	25.23	35.35	12.54

Averages followed by the same letter in the column do not differ by the Tukey's test, at 5% probability.
Evaluations: 1st: 10/30/2012; 2nd: 12/15/2012; 3rd: 02/22/2013; 4th: 04/08/2013 e 5th: 06/14/2013.

Goatley Junior et al. (1996) reported that imazaquin (420 g ha^{-1}) reduced the growth of bahiagrass by equal to or greater than 40% compared to the control at 28 DAA; this result differs from that recorded in the present study on emerald Zoysia because the application of imazaquin did not result in a value of leaf length lower than that of the control in this study.

An interaction between the N dose and the applied herbicide occurred in the fourth evaluation regarding the variable leaf length (Figure 3 and Table 2). A linear increase in the value of grass leaf length was observed with the increase in N dose in the control (without herbicide application) and after imazethapyr application (Figure 3). The highest leaf length of the control was 8.8 cm, that is, a 79.6% increase over the length of the non-fertilized grass (4.9 cm). The 7.1 cm length recorded for the imazethapyr application was a 39.2% increase over the length of the grass without nitrogen fertilization (5.1 cm). This smaller percentage of increase upon herbicide application indicates the existence of its potential use as a lawn grass growth regulator, as assessed by Maciel et al. (2006) and Johnson (1990), who found that imazethapyr applied twice at a dose of 80 g ha^{-1} controlled bahiagrass growth for 35 DAA.

A difference in leaf length values occurred at doses of both 2 and 4 g m^{-2} N upon herbicide application in the fourth evaluation (Table 2). The shortest lengths at a dose of 2 g m^{-2} N were recorded when applying the herbicides glyphosate and metsulfuron-methyl (both resulted in a 5.4-cm length) and imazaquin (6.0 cm) compared to the control value (7.5 cm). At this dose, the reduction in length relative to the control was 20% for imazaquin and 28% for glyphosate and metsulfuron-methyl. All of the herbicides proved effective in the control of grass growth relative to the control at the highest N dose applied (4 g m^{-2}). The percentages of reduction were 31.8 (imazaquin), 19.3 (imazethapyr), 27.3 (glyphosate), and 30.7 (metsulfuron-methyl).

Figure 3. Effect of N dose on the mean leaf length of emerald Zoysia in the fourth evaluation, for the imazethapyr herbicide treatment.

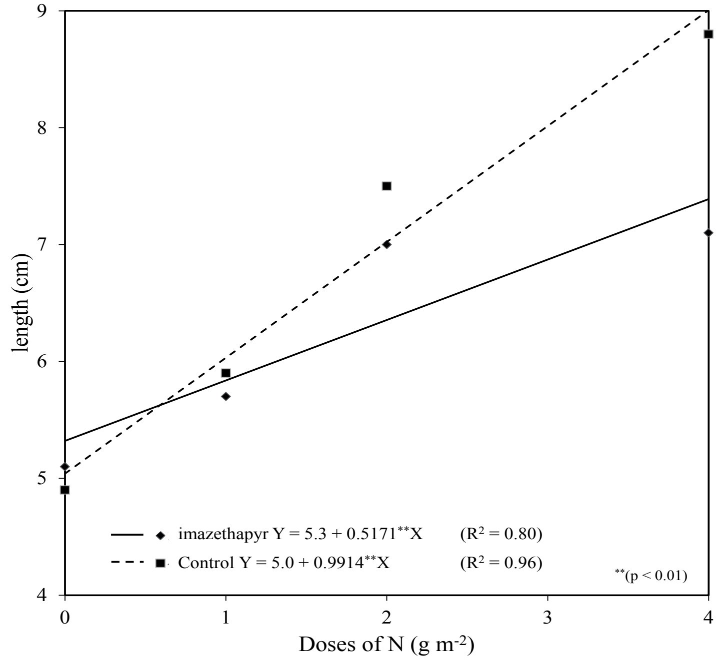


Table 2. Mean leaf length of emerald Zoysia in the fourth evaluation, according to the herbicide treatment and N dose. UNESP, Ilha Solteira/SP, 2012/13.

Doses of N (g m^{-2})	Control	imazaquin	imazethapyr	glyphosate	metsulfuron-methyl
0	4.9	6.1	5.1	5.1	5.5
1	5.9	5.7	5.7	6.4	6.2
2	7.5 a	6.0 bc	7.0 ab	5.4 c	5.4 c
4	8.8 a	6.0 b	7.1 b	6.4 b	6.1 b
D.M.S. (5%)			1.4		

Averages followed by the same letter in the line do not differ by the Tukey's test, at 5% probability.

There are no other results for studies focusing on nitrogen fertilization associated with herbicide application as lawn grass growth regulators published in the literature, thereby hindering the comparison of data regarding the interactions (N doses and herbicides) observed in this study. However, the reduction in length resulting from herbicide applications may be explained based on their mechanisms of action in plants.

The herbicides imazaquin, imazethapyr, and metsulfuron-methyl inhibit the synthesis of the branched-chain amino acids valine, leucine, and isoleucine. These herbicides are transported via roots and leaves and are translocated by the phloem and xylem, accumulating in plant growth sites (meristems). They act by inhibiting the enzyme ALS, which is essential for the synthesis of the cited amino acids in plants, thus interrupting protein synthesis and, in turn, affecting DNA synthesis and cellular growth (FERREIRA et al., 2005; OLIVEIRA JUNIOR, 2011; RODRIGUES; ALMEIDA, 1998).

Glyphosate acts in the shikimic acid pathway and is classified as an inhibitor of the EPSP enzyme, which is involved in the formation of the aromatic amino acids tryptophan, tyrosine, and phenylalanine, essential for plant growth, development, and maintenance. Glyphosate rapidly translocates through the symplast when absorbed by leaves and other parts of plant shoots and usually follows the source-drain photoassimilate flow after reaching the phloem, accumulating in active growth areas (meristems) and thus blocking the growth of plants. The production of indolylacetic acid (IAA), a hormone responsible for growth, is completely dependent on the shikimic acid pathway. Changes in EPSP enzyme activity may significantly alter the concentrations of IAA (FERREIRA et al., 2005; VELINI et al., 2009; OLIVEIRA JUNIOR, 2011). These impacts can explain the reduced lawn grass growth upon glyphosate application.

A linear increase in leaf dry matter occurred with the increase in N doses in the first, second, and fifth evaluations (Figure 2B). Similar results were observed by Backes et al. (2010), who reported an increasing linear effect on the leaf + stem dry phytomass as a function of the dose of sewage sludge (0, 100, 200, 300, and 400 kg ha^{-1} N, delivered in a single application) in an area of emerald Zoysia production, and by Fonseca et al. (2002), who observed that the control (absence of N) produced the smallest amount of total (stem + leaves) and green (only leaves) dry matter compared to the other treatments (200 and 400 kg ha^{-1} N, delivered in a single application, using ammonium sulfate as the source) in a study on bahiagrass.

The results from this study confirm the N effect on plant shoot growth and the association between N fertilization doses and plant dry matter production (OLIVEIRA et al., 2010; FONSECA et al., 2002). However, a greater amount of dry matter is not a desirable result for lawns with ornamental purposes because it increases the demands for a greater mowing frequency to maintain the aesthetics (AMARAL; CASTILHO, 2012), thereby increasing the maintenance costs.

The dry matter values differed between the herbicide treatments and the control in the second evaluation (Table 1). The dry matter produced after metsulfuron-methyl application (43.7 g m^{-2}) was smaller than the control (64.6 g m^{-2}). A similar result was reported by Rogers (1985) for lawns of Bermuda grass at 28 DAA of metsulfuron-methyl (140 g ha^{-1}). Conversely, Costa et al. (2010) observed a dry matter increase of emerald Zoysia at 49 DAA of metsulfuron-methyl (2.4 g ha^{-1}) compared to the control (without application).

Interaction between the N dose and the applied herbicide occurred in the third and fourth evaluations (Figures 4 A and B, Table 3). In the third evaluation, the mass of leaf dry matter increased linearly with an increase in N in the control and after imazaquin application (Figure 4A). For the application of the highest N dose (4 g m^{-2}), leaf dry matter increased 43.7 and 65.6% in the control and imazaquin treatment, respectively, compared to the absence of nitrogen fertilization, indicating that the grass dry matter values were more affected by the nitrogen fertilization when combined with herbicide. A quadratic fit of the dry matter response to N dose was observed for imazethapyr, with the highest estimated yield of 144.1 g m^{-2} occurring at the dose of 2.2 g m^{-2} N.

In the third evaluation, dry matter values differed between herbicide applications and the control at the dose of 2 g m^{-2} N (Table 3). The lowest values of dry matter were recorded when applying the herbicides imazaquin, glyphosate, and metsulfuron-methyl. Imazaquin reduced leaf dry matter by 12.8% compared to the control value, while glyphosate and metsulfuron-methyl caused a 17.9% reduction. Thus, these herbicides demonstrated potential to reduce the leaf dry matter production of lawn grasses, as also reported by Leite et al. (2010), who found that glyphosate (216 g ha^{-1} a.i.) caused an 18.7% reduction of bahiagrass dry matter compared to the control at 71 DAA, by Costa et al. (2010), who applied the herbicide metsulfuron-methyl (2.4 g ha^{-1}) to Saint Augustine grass and recorded a reduction in plant dry matter of approximately 70% compared to the control at 49 DAA, and by

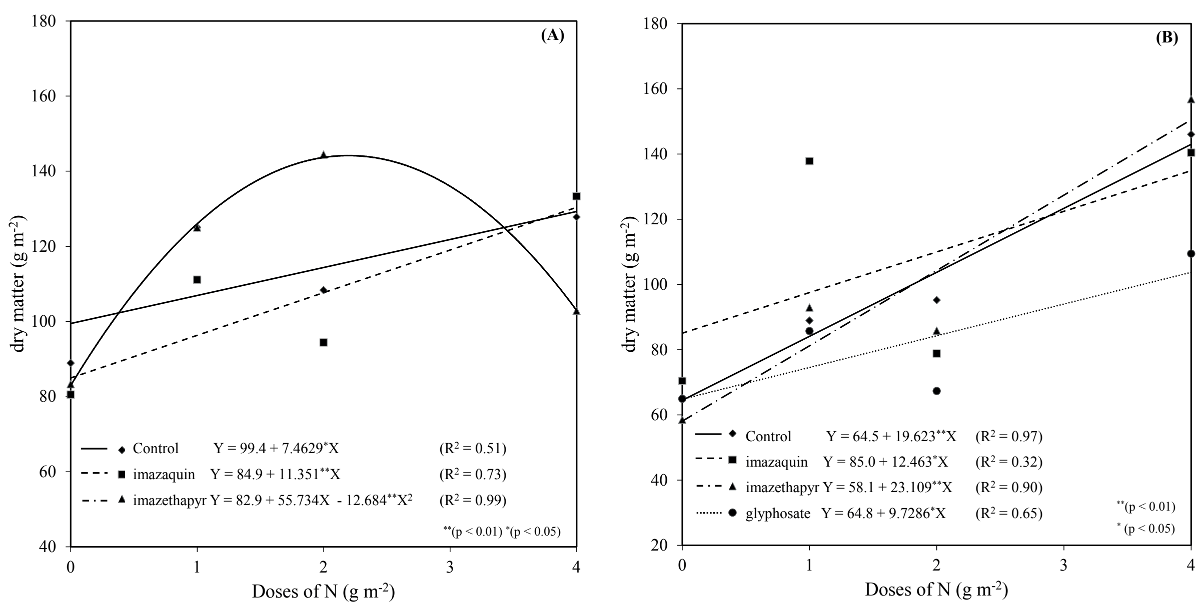
Rodrigues-Costa et al. (2011), who observed a reduction in *Brachiaria decumbens* dry matter of approximately 49.2% after imazaquin application (150 g ha^{-1}) at 45 DAA. Notably, none of the cited studies evaluated the application of those herbicides in combination with nitrogen fertilization.

Table 3. Mean leaf dry matter of emerald Zoysia for the third and fourth evaluations, according to the herbicide treatment and N dose. UNESP, Ilha Solteira/SP, 2012/13.

			Third evaluation (22/02/2013)		
Doses of N (g m^{-2})	Control	imazaquin	imazethapyr	glyphosate	metsulfuron-methyl
0	88.9	80.5	83.3	83.3	100.0
1	125.0	111.1	125.0	119.4	119.4
2	108.3 a	94.4 b	144.4 ab	88.9 b	88.9 b
4	127.8	133.3	102.8	100.0	94.4
D.M.S. (5%)			42.7		
			Fourth evaluation (08/04/2012)		
0	65.1	70.4	58.5	64.9	81.9
1	88.9 ab	137.8 a	92.9 ab	85.7 ab	76.4 b
2	95.2	78.8	85.8	67.3	95.9
4	146.0 a	140.4 a	156.8 a	109.4 ab	77.1 b
D.M.S. (5%)			55.3		

Averages followed by the same letter in the line do not differ by the Tukey's test, at 5% probability.

Figure 4. Effect of N dose on the mean leaf dry matter of emerald Zoysia in the third (A) and fourth (B) evaluations, according to the herbicide treatment.



The leaf dry matter values in the control and after the applications of imazaquin, imazethapyr, and glyphosate all increased linearly with an increase in applied N in the fourth evaluation

(Figure 4B). It is noteworthy that the increase observed for the imazaquin application was also observed in the third evaluation (Figure 4A). Increases in the dry matter values of 124.3, 99.4, 168.0, and 68.6% were recorded in the control, imazaquin, imazethapyr, and glyphosate treatments, respectively, at the highest N dose (4 g m^{-2}) compared to the corresponding unfertilized treatments. The potential of glyphosate in controlling the increase in dry matter associated with N fertilization was notable in comparison to the other herbicides and the control because it allowed for the smallest percentage increase in dry matter.

Differences between the dry matter production of herbicide treatments compared to the control occurred at doses of both 1 and 4 g m^{-2} N in the fourth evaluation (Table 3). The lowest dry matter values were recorded when using metsulfuron-methyl, at both doses, albeit without differing from the control value at the dose of 1 g m^{-2} N. This herbicide promoted a 47.2% reduction in the yield of leaf dry matter compared to the control at the dose of 4 g m^{-2} N.

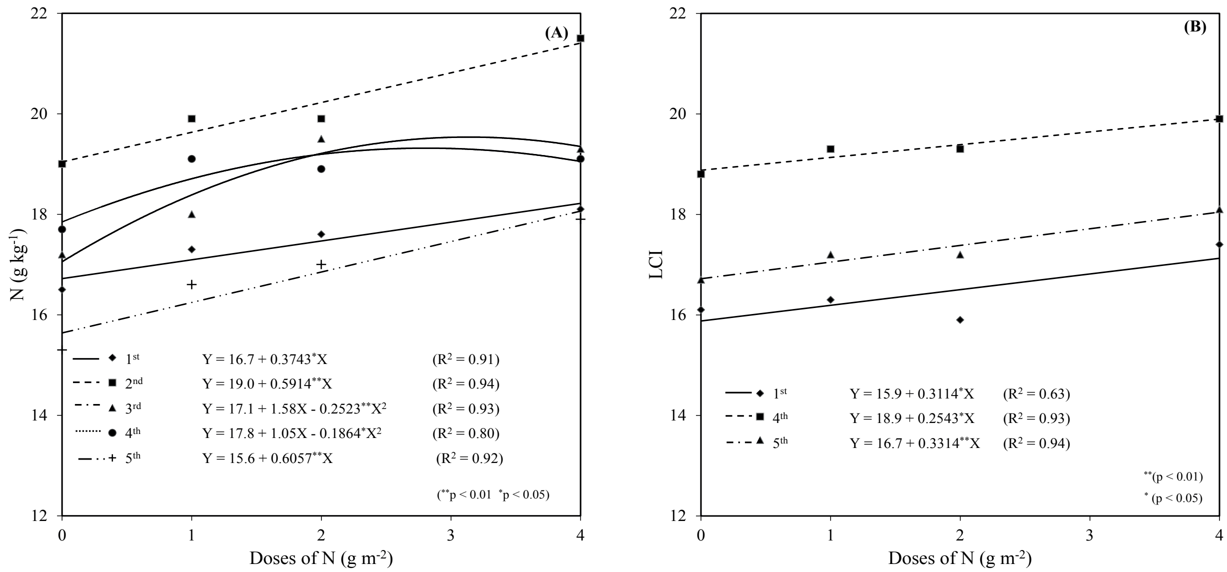
The reductions in leaf dry matter production of emerald Zoysia as a result of the application of the herbicides imazaquin, metsulfuron-methyl, and glyphosate may be related to their mechanisms of action, as previously reported (FERREIRA et al., 2005; OLIVEIRA JUNIOR, 2011; RODRIGUES; ALMEIDA, 1998). Other explanations may also be provided regarding glyphosate. The aromatic amino acids tyrosine, phenylalanine, and tryptophan are fundamental to plant protein synthesis. All enzymes require these amino acids; thus, all processes involving the participation of proteins/enzymes are indirectly affected by the reduction of these amino acids caused by the application of this herbicide (VELINI et al., 2009). Furthermore, the shikimate pathway accounts for 35% of the plant dry matter and processes 20% of the carbon fixed by photosynthesis (FURLANI JUNIOR et al., 2009; KRUSE et al., 2000).

Glyphosate and metsulfuron-methyl exhibited a superior performance regarding the reductions in the length and leaf dry matter values of emerald Zoysia when considering the overall mean of the five evaluations performed (Table 1), proving to be herbicides with potential for use as plant growth regulators of this species of grass. This growth regulation is a positive attribute because the mowing frequency is determined by the lawn growth rate (UNRUH, 2004) and greater grass growth and dry matter production are not desirable (AMARAL; CASTILHO, 2012; BACKES, 2008; GODOY et al., 2012a).

The N leaf concentrations of emerald Zoysia increased linearly with an increase in N dose in the first, second, and fifth evaluations (Figure 5A). These results corroborate those reported by Backes et al. (2010), who reported a liner increase in the leaf blade N concentration in a study conducted in a commercial area of emerald Zoysia; at 45 DAA of sewage sludge (0, 100, 200, 300, and 400 kg ha^{-1} N, delivered in a single application), the N concentration was 28 g kg^{-1} for the dose of 400 kg ha^{-1} N. In another study of a commercial area of emerald Zoysia fertilized with sewage sludge (0, 100, 200, 300, and 400 kg ha^{-1} N, delivered in a single application), Oliveira et al. (2010) also recorded a linear increase in the leaf blade N concentration for all four time-points evaluated (45, 130, 190, and 250 DAA), with maximum values of 20, 25, 24, and 19 g kg^{-1}, respectively.

A quadratic fit of the N leaf concentration response to N dose was observed in the third and fourth evaluations (Figure 5A). In the third and fourth evaluations, respectively, the highest leaf N concentrations were estimated to be 19.6 g kg^{-1} after the application of 3.1 g m^{-2} N and 19.3 g kg^{-1} after the application of a dose of 2.8 g kg^{-1} N. Therefore, the highest leaf concentrations in these two evaluations were obtained between the doses of 2 and 4 g m^{-2} N. Godoy (2005) also recorded a quadratic fit of leaf N concentrations as a function of the dose of N (0, 25, 50, 75, 100, 150, and 200 kg ha^{-1}, using urea as N source), at 90 days after cutting (DAC) the previous sod, when evaluating nitrogen fertilization in the production of emerald Zoysia sod. The author adopted the leaf concentration of 25 g kg^{-1} N when the grass was fertilized using 150 kg ha^{-1} N as the optimal value because it also promoted a faster rate of soil coverage by the grass.

Figure 5. Mean leaf N concentration (A) and leaf chlorophyll index (LCI; B) of emerald Zoysia, according to the N dose.



The highest values of leaf N concentration for the dose of 4 g m^{-2} N were 18.1, 21.5, and 17.9 g kg^{-1} in the first, second, and fifth evaluations, respectively (Figure 5A). These values are similar but slightly lower than the values reported by the authors cited. Notably, while the focus of the present study was to determine the level of maintenance fertilization that aims to maintain the intense green color of lawns, the studies cited were conducted in a grass production area, with the primary goals of increasing the rate of grass production (rate of soil coverage by grass) and maintaining sod quality (color and resistance to handling; GODOY; VILLAS BÔAS, 2003; GODOY et al., 2012a).

The concentrations of N in grass leaves, according to Godoy et al. (2012a), range from 20 to 50 g kg^{-1}, and a mean level of 14 g kg^{-1} N indicates a critical deficiency, while a level greater than or equal to 20 g kg^{-1} indicates sufficient N. Godoy et al. (2007) evaluated the effect of N doses (0, 200, 400, and 600 kg ha^{-1}, using urea as the N source) in the production of emerald Zoysia sod, applying 5% of the total dose of each treatment at 35 DAC and splitting the remainder among six applications every 45 days, and found that the grasses that did not received nitrogen fertilization contained N concentrations between 14 and 16 g kg^{-1}. This range in values may be considered as indicative of a severe deficiency. The grasses that received 600 kg ha^{-1} N contained concentrations between 24 and 26 g kg^{-1} N, which may be considered as optimal for the production of emerald Zoysia sod because the grasses with those concentrations formed sod more rapidly. The lowest concentration (15.3 g kg^{-1} N) in the present study was recorded for the control (0 g m^{-2} N), in the fifth evaluation; this value is within the interval reported by Godoy et al. (2007) for unfertilized grass. The highest concentration (21.5 g kg^{-1} N) recorded in the second evaluation (at a dose of 4 g m^{-2} N) is considered sufficient (GODOY et al., 2012a) and is within the range of values considered appropriate by Mills and Jones Junior (1996), i.e., 20 to 24 g kg^{-1} N. The other values were close to 20 g kg^{-1}, which is considered sufficient by Godoy et al. (2012a).

Regarding the herbicide applications, differences in leaf N concentrations compared to the control were observed in the first and third evaluations. A difference was recorded in the fifth evaluation between the glyphosate (highest concentration) and imazethapyr (lowest concentration) treatments, though these values did not differ from the control values (Table 4). The lowest leaf N concentrations of the lawn grass, compared to the control, were recorded in the first evaluation for the glyphosate and metsulfuron-methyl treatments. In the third evaluation, the glyphosate-treated grass contained the lowest leaf N concentration, albeit without differing from the control. Moreover, the highest concentration was recorded after imazaquin application, which differed from the control. In general, the leaf N concentrations in emerald Zoysia remained near 20 g kg^{-1} after herbicide application, levels considered sufficient by Godoy et al. (2012a).

Table 4. Mean leaf N concentrations and leaf chlorophyll index (LCI) of emerald Zoysia, for five evaluations, according to the herbicide and N treatments. UNESP, Ilha Solteira/SP, 2012/13.

Herbicidas	N (g kg^{-1})				
	1st	2nd	3rd	4th	5th
Control	18.5 a	19.9 a	17.7 b	18.4 a	16.4 ab
imazaquin	17.4 ab	20.5 a	19.7 a	19.2 a	16.7 ab
imazethapyr	17.7 ab	20.1 a	19.1 ab	18.5 a	15.8 b
glyphosate	16.3 b	19.5 a	17.5 b	18.4 a	17.5 a
metsulfuron-methyl	16.8 b	20.5 a	18.6 ab	19.0 a	16.9 ab
D.M.S. (5%)	1.6	1.6	1.8	1.6	1.6
C.V. (%)	7.74	6.67	8.38	7.51	8.17
			LCI		
Control	16.1 a	17.5 a	17.3 a	19.3 a	17.1 a
imazaquin	16.3 a	17.5 a	16.8 a	19.7 a	16.8 a
imazethapyr	16.4 a	16.2 a	16.8 a	19.3 a	17.4 a
glyphosate	17.2 a	16.6 a	16.1 a	19.2 a	17.2 a
metsulfuron-methyl	16.3 a	15.6 a	16.8 a	19.0 a	18.0 a
D.M.S. (5%)	2.0	3.4	2.5	1.6	1.4
C.V. (%)	10.70	17.40	12.91	6.96	7.15

Averages followed by the same letter in the column do not differ by the Tukey's test, at 5% probability.
Evaluations: 1st: 10/30/2012; 2nd: 12/15/2012; 3rd: 02/22/2013; 4th: 08/04/2013 e 5th: 06/14/2013.

No predominant effect of herbicide on leaf N was observed in the evaluations performed, complicating the determination of which herbicides positively or negatively affected the leaf N concentration in emerald Zoysia. The lower leaf N concentrations in the metsulfuron-methyl and glyphosate treatments compared to the control in the first evaluation are explained by the fact that these herbicides inhibit the synthesis of branched-chain amino acids and aromatic-chain amino acids, respectively (OLIVEIRA JUNIOR, 2011), which may indirectly affect the concentration of N, a nutrient that is part of the structure of several plant biomolecules (GODOY et al., 2012a), including amino acids. Glyphosate applications may alter the availability of specific plant macro and micronutrients, although, according to Meschede et al. (2009), few studies in the literature have reported an effect of the herbicide on nutrient uptake and transport in plants.

The mean values of the LCI increased linearly with an increase in N dose in the first, fourth, and fifth evaluations (Figure 5B). A similar result was reported by Backes et al. (2010), who found that the sewage sludge doses applied (100, 200, 300, and 400 kg ha^{-1} N, delivered in a single application) affected the green color intensity (GCI) of grass leaves at 45, 105, and 165 DAA of the sludge in a study conducted on a commercial area of emerald Zoysia, and by Godoy et al. (2012b), who recorded a linear increase in the GCI of Saint Augustine grass leaves with an increase in N (0, 150, 300, 450, and 600 kg ha^{-1}, split into three applications and using urea as N source) at 296 DAC.

The highest LCI values were recorded in the first, fourth, and fifth evaluations (17.4, 19.9 and 18.1, respectively) for the dose of 4 g m^{-2} N. Dinalli (2011) reported LCI values between 14.3 and 22.1 30 days after the fertilization of emerald Zoysia with 100 kg ha^{-1} N (source urea, split into five applications) in a study also conducted at Ilha Solteira/SP. The values recorded in the present study also fall within that interval mentioned and near the maximum value cited.

The results found in this study confirm the assertion that higher N doses promote a more intense green color of lawns, desirable from an aesthetics standpoint. Moreover, plants with a more intense green color, physiologically, have a greater carbohydrate photosynthetic capacity given the greater concentration of chlorophyll, the molecule responsible for capturing light energy from solar radiation (GODOY et al., 2012a). There were positive correlations between the leaf N concentrations and the LCI values for the five evaluations ($r= 0.95^*$), as reported by Backes et al. (2010) and Oliveira et al. (2010) for emerald Zoysia. The leaf green color index has been used for estimating the N concentration in various species because of the relationship between chlorophyll levels and plant N concentrations, according to Oliveira et al. (2010).

The LCI values did not differ between the herbicide treatments and the control (Table 4); the LCI values ranged from 15.6 to 19.7 for herbicide treatments. These values are within the interval reported by Dinalli (2011).

Differences in the phytotoxicity of herbicide applications relative to the control were recorded in all evaluations. The highest percentages of phytotoxicity were recorded when using metsulfuron-methyl, and the impact of glyphosate in the first evaluation was also notable (Table 5).

Table 5. Mean percentage phytotoxicity on emerald Zoysia leaves, for five evaluations, according to the herbicide and N treatments. UNESP, Ilha Solteira/SP, 2012/13.

Herbicidas	phytotoxicity (%)				
	1st	2nd	3rd	4th	5th
Control	0.0 b	0.0 c	0.0 d	0.0 d	0.0 c
imazaquin	0.8 b	1.7 c	3.1 c	2.7 c	0.0 c
imazethapyr	0.0 b	2.9 c	0.0 d	0.0 d	0.0 c
glyphosate	6.7 a	7.1 b	6.7 b	7.5 b	3.3 b
metsulfuron-methyl	8.7 a	11.2 a	11.6 a	14.6 a	9.7 a
D.M.S. (5%)	2.8	3.1	2.6	2.3	1.2
C.V. (%)	63.93	55.85	47.47	39.26	38.19

Averages followed by the same letter in the column do not differ by the Tukey's test, at 5% probability.
Note: Transformed data $(x + 1)^{0,5.}$
Evaluations: 1st: 10/30/2012; 2nd: 12/15/2012; 3rd: 02/22/2013; 4th: 04/08/2013 e 5th: 06/14/2013.

When used as a growth regulator, an herbicide should inhibit grass growth without affecting the beauty and characteristic green color (RODRIGUES et al., 2004). When analyzing the mean values of all evaluations, the performances of metsulfuron-methyl and glyphosate were superior for the control of grass growth and dry matter production (Table 1) but did not produce a difference from the control in the LCI, similarly to the other herbicides (Table 4). However, metsulfuron-methyl affected the grass aesthetics more intensely than glyphosate (Table 5). Intense yellowing was recorded in the plots receiving metsulfuron-methyl, with the leaves acquiring a burnt appearance (more opaque green color), and the grass density was also impaired (the lawn contained bare spots). Only mild yellowing occurred upon glyphosate application, without compromising the visual quality (intense green color) of the lawn. The symptoms caused by metsulfuron-methyl would be classified as moderate for the second (11.2%), third (11.6%), and fourth (14.6%) evaluations but as mild in the first (8.7%) and fifth (9.7%) evaluations, according to Costa et al. (2010); in contrast, the symptoms caused by glyphosate would be classified as mild in all evaluations (3.3 to 7.5%).

Grichar and Havlak (2010) also reported that metsulfuron-methyl (14.17 g ha^{-1}) caused significant injury (yellowing) at 10 DAA of the herbicide when evaluating the effects of herbicide application on Saint Augustine grass. The opposite was reported by Rogers (1985), who noted that the herbicide application (140 g ha^{-1}) was effective at controlling bahiagrass height and caused no significant visual damage for a period of up to 77 DAA. Costa et al. (2010), when evaluating metsulfuron-methyl application (2.4 g ha^{-1}) to Saint Augustine grass, recorded symptoms of mild phytotoxicity (< 10%) for a maximum period of 14 DAA, after which the symptoms disappeared. The phytotoxicity caused by metsulfuron-methyl may also be explained by its mechanism of action, previously described by Ferreira et al. (2005) and Oliveira Junior (2011). The latter author reported the development of interveinal chlorosis and/or leaf purpling and observed growth arrest within 7 to 10 DAA of applying this herbicide to susceptible plants.

In contrast to the results recorded in the present study, Fry (1991) found that although glyphosate reduced centipedegrass (*Eremochloa ophiuroides*) height by 22% at 28 DAA (when applied at 600 g ha^{-1} - a dose three times greater than the dose used in the present study), the grass exhibited severe chlorosis (intense yellowing) and grew at a low density with an uneven appearance. The mild yellowing reported in the present study when applying glyphosate may be related to chloroplast degeneration, recorded by Campbell et al. (1976) in *Agropyron repens*, 24 hours after herbicide application (560, 1120, 1680, 2240, and 4490 g ha^{-1}). Another explanation may be linked to the effects of glyphosate on the inhibition of chlorophyll synthesis (COLE et al., 1983) and its mechanism of action, which causes reduced photosynthetic efficiency and decreased production of aromatic amino acids (OLIVEIRA JUNIOR, 2011).

The applications of imazaquin and imazethapyr caused no injuries (phytotoxicity) to the grass compared to the control in the first, second, and fifth evaluations. The imazethapyr treatment (absence of phytotoxicity) also did not differ from the control in the third and fourth evaluations (Table 5). Although imazethapyr and imazaquin produced no significant injuries to grass shoots, when considering the mean of the five evaluations, these herbicides failed to perform well in terms of controlling lawn growth and reducing leaf dry matter production (Table 1). Grades from 0 to 3.1% were assigned to the injuries caused by imazaquin, which were classified as mild symptoms. For imazethapyr, only in the second evaluation was a grade of 2.9% assigned, also considered mild symptoms (COSTA et al., 2010). Goatley Junior et al. (1996) reported mild discoloration to bahiagrass 14 DAA of imazaquin (420 g ha^{-1}), although the discoloration disappeared by 56 DAA. Mild discoloration was also recorded in the present study, albeit at 30 DAA. Johnson (1990) noted that imazethapyr (80 g ha^{-1}) caused severe injuries (unacceptable injury with moderate to severe necrosis) to greater than 30% of bahiagrass at 14 and 28 DAA. However, that percentage decreased to 6 and 3% (mild discoloration) at 42 and 56 DAA, similar to the magnitude of symptoms observed in this study because the herbicide was also applied at the dose of 80 g ha^{-1} and caused the onset of mild symptoms at 30 DAA in only one evaluation and no injuries in the other evaluations.

Although symptoms were absent or mild in this study, the phytotoxic effects of imidazolinones (imazaquin and imazethapyr) is caused by their mechanism of action, according to Kraemer et al. (2009), and the development of interveinal chlorosis and/or leaf purpling, which are symptoms similar to those caused by metsulfuron-methyl, may occur because imidazolinones can inhibit the same enzyme (OLIVEIRA JUNIOR, 2011).

Conclusions

Doses from 10 to 20 g m^{-2} N, split into five applications throughout the year, provided sufficient N concentrations for the maintenance of emerald Zoysia lawn quality.

The performances of the herbicides metsulfuron-methyl and glyphosate for the control of grass growth were superior to the other examined herbicides. The former caused greater phytotoxicity, while the latter did not impair the aesthetic quality of the grass and, thus, is notable for its value as an herbicide that may be used as a growth regulator of emerald Zoysia at a dose of 200 g ha^{-1}.

Acknowledgements

To ITOGRASS for donating of emerald Zoysia sods, to FAPESP for research funding (process number 2012/04602-2) as well as the translation of the article (process number 2013/17935-2).

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