Glyphosate sprayed on the pre-existing vegetation reduces seedling emergence and growth of forage species

La aplicación de glifosato sobre la vegetación pre-existente reduce la emergencia y el crecimiento de las plántulas de especies forrajeras

Adriana M. Rodríguez [1*], Elizabeth J. Jacobo [1], Agustín A. Grimoldi [2], Rodolfo A. Golluscio [2]

Originales: *Recepción:* 10/07/2020 - *Aceptación:* 12/04/2022

Abstract

Seeding pastures or forage crops by no-tillage methods usually involves the spray of glyphosate to suppress the existing vegetation. While many studies found detrimental effects of glyphosate on seed germination and seedling growth of the subsequent crop, others found negligible effects. This study aimed to evaluate the effect of glyphosate spraying on germination, seedling emergence and seedling growth of four forage species: *Trifolium repens*, *Lotus tenuis*, *Festuca arundinacea* and *Paspalum dilatatum*. The experiment was carried out spraying glyphosate on the pre-existing vegetation and on bare soil 1, 30, 60 and 90 days before sowing, and a control treatment sprayed with water. Glyphosate sprayed on pre-existing vegetation 1 to 60 days before seeding reduced emergence, while sprayed 1 to 30 or 1 to 60 days before seeding reduced seedlings belowground biomass and root length of all species and aboveground biomass of legumes respect to sprayed 90 days before seeding, sprayed on bare soil, and control treatment. This herbicide would remain active in the soil environment for at least 60 days after spraying when it was previously absorbed by plants, causing a severe damage to seedlings emergence and growth.

Keywords
herbicide • germination • legumes • grass • vegetation cover • bare soil • elapsed-time

1 Universidad de Buenos Aires. Facultad de Agronomía. Cátedra de Forrajicultura. Av. San Martin 4453, Ciudad Autónoma de Buenos Aires. Argentina. C1417DSE.
* arodrigu@agro.uba.ar

2 Universidad de Buenos Aires. Facultad de Agronomía. Consejo Nacional de Investigaciones Científicas y Técnicas. Instituto de Investigaciones Fisiológicas y Ecológicas Vinculadas a la Agricultura (IFEVA).

Resumen

La siembra directa de pasturas o verdeos involucra la pulverización con glifosato para eliminar la vegetación pre-existente. Si bien algunos estudios encontraron que el glifosato provocó efectos negativos en la germinación y el crecimiento de plántulas del cultivo, otros no encontraron efectos significativos. El objetivo de este trabajo fue evaluar el efecto de la pulverización con glifosato sobre la germinación y el crecimiento de las plántulas de cuatro especies forrajeras: *Trifolium repens, Lotus tenuis, Festuca arundinacea* y *Paspalum dilatatum*. Se pulverizó glifosato sobre la vegetación o sobre suelo desnudo 1, 30, 60 o 90 días antes de la siembra y se pulverizó con agua al tratamiento control. El glifosato pulverizado sobre la vegetación 1 a 60 días antes de la siembra redujo la emergencia, pulverizado 1 a 30 o 1 a 60 días antes de la siembra redujo la biomasa subterránea y la longitud de raíces de todas las especies y la biomasa aérea de las leguminosas respecto de la pulverización 90 días antes de la siembra, sobre el suelo desnudo y del tratamiento control. Este herbicida se habría mantenido activo en el suelo durante al menos 60 días luego de la pulverización, cuando fue previamente absorbido por la vegetación, causando un severo daño en la emergencia y el crecimiento de las plántulas.

Palabras clave

herbicida • germinación • leguminosas • pastos • cobertura vegetal • suelo desnudo • tiempo de carencia

Introduction

Glyphosate is a non-selective broad-spectrum herbicide, widely used on global scale (3, 12). The extended use of glyphosate has been attributed to its efficacy on control weed species (26), but an increasing number of reports suggest negative side effects on non-target plants (5). Many studies have found detrimental effects of glyphosate on seed germination and seedling growth of several crop species (1, 4, 8, 28, 31). However, the results are not conclusive because other studies have found little or negligible effects on crop species (7, 18, 27).

In cattle or sheep grazing systems, glyphosate is sprayed to suppress the existing vegetation before seeding or reseeding pastures or forage crops by no-tillage methods as direct drilling or broadcasting seeds (2, 34). However, little information is available about the effect of glyphosate on seed germination and seedling growth of forage species because most studies about the exposure of glyphosate on the root-zone were carried out on crop species and rarely in a real soil substrate.

Numerous studies consider that glyphosate toxicity to non-target plants in soils is marginal because it is inactivated by microbial degradation and adsorption by soil constituents (3). Glyphosate is a polar compound which adsorbs strongly to soil iron and aluminum oxides and clays (24). Therefore, the adsorption of glyphosate and aminomethylphosphonic acid (AMPA) increased strongly with iron and aluminum content of soils and decreased with increasing soil organic carbon content. This would indicate that glyphosate and AMPA are mainly adsorbed by clay minerals, while soil organic matter competes for adsorption sites and inhibits adsorption (14). Such soil binding is reversible, and after desorption glyphosate is degraded by various bacteria to AMPA, its main secondary metabolite, and ultimately to inorganic phosphate, ammonia and carbon dioxide (3, 4). Therefore, the labile pool of glyphosate in soils to be absorbed by plant roots could be dramatically reduced and, glyphosate toxicity over non-target plants would be negligible (15). Supported by these evidences, it was globally widespread that glyphosate is inactive in the soil, and therefore its phytotoxic effect could be considered as negligible. However, other studies detected residues of glyphosate and AMPA in soil solution after six months after spraying (32). A similar degradation process of glyphosate to AMPA also occurred in some plant species (29), a portion of these residues in soil would come from the glyphosate absorbed by plants, translocated towards the roots and underground organs and then exuded to the surrounding solution (21). Therefore, an important source of soil glyphosate or AMPA is root exudation from sprayed plants and its release from dead plants, which implies a toxicity risk to non-target plants due to rhizosphere transfer (16).

Due to their chemical similarities, both glyphosate and AMPA can be taken up by roots from the soil solution (16), so affecting the growth of seedlings. In forage species, only one report stated that the incorporation of glyphosate before sowing into a *Vertic Argiudol* reduced chlorophyll content and above and belowground growth in seedlings of the legume *Lotus corniculatus* (8). In accordance, there is evidence that rootlets of soybean and maize seeds that germinate after spraying could absorb secondary metabolites of glyphosate, mainly AMPA exuded by roots of sprayed plants (31). Besides, glyphosate degradation resulted two to six times slower when it was previously absorbed by plants than when it was directly applied to the soil (10). The elapsed-time between glyphosate spraying and sowing was directly related to sunflower seedling growth and biomass production, and glyphosate had yet detrimental effects at the largest elapsed-time of 21 days (33). Glyphosate markedly inhibited growth of rootlets and shoots of *Raphanus sativus* seedlings exposed to the herbicide in the root-zone after 7 and 14-days of exposure (18). Negative effect on seed germination was found when seeds of legume species were in direct contact with glyphosate in water solution (17, 23). In summary, contrary to the idea that glyphosate is inactivated in soil, several studies suggest that glyphosate spraying on the pre-existing vegetation may cause deleterious effects on seed germination, seedling emergence and growth of the subsequent crop.

Therefore, the aim of this study was to evaluate the effect of glyphosate spraying on germination, seedling emergence and seedling growth of four greatly extended forage species. We predicted that negative effects of glyphosate on seed germination and establishment would be greater when glyphosate is sprayed on pre-existing vegetation than on bare soil, and that the elapsed-time between spraying and seeding will be positively related to germination, seedling emergence and growth of forage seedlings.

Materials and methods

Experimental design

We extracted soil from paddocks of a livestock farm located in the center of the Flooding Pampa region (36°40’12” S, 59°32’10” W, 80 m a. s. l.). We selected paddocks covered by native grasslands that had never been sprayed with any herbicide. The dominant soil belongs to the series General Guido, *typic Natraquoll*, characterized by an acidic (pH 6.8), non-saline A_1 horizon 12 cm-depth and a clayey and sodic B_{2t} horizon (pH 8.2). Soil was extracted from the top horizon, sieved to remove vegetation and seeds, and then distributed in 400 plastic containers of 13 x 17 x 25 cm. Containers were maintained in a greenhouse with controlled temperature (20-25°C), natural light and regular watering throughout the experimental period. In April 2010, 20 seeds of each forage species *L. tenuis*, *T. repens, P. dilatatum* and *F. arundinacea* were sown in 100 containers per species and covering them with a thin layer of the same soil.

To compare the effects of glyphosate sprayed on pre-existing vegetation cover (C+) or on bare soil (C-) on germination, seedling emergence and seedling growth of the four forage species, we generated a vegetation cover of *Lolium multiflorum* Lam plants. For this, we harvested seeds of *L. multiflorum* in December 2009 from the same grassland paddocks where soil was extracted. Twenty-five seeds of *L. multiflorum* per container were sown in 200 containers 45 days before glyphosate spraying. Plants of *L. multiflorum* were thinned to 25 plants per container and periodically cut to maintain a similar height of 12 cm. In the 200 containers under C+ level, *L. multiflorum* plants were cut to ground level two days after sowing to avoid confounding effects of competition between *L. multiflorum* plants and forage seedlings. Other 200 containers were not sown with *L. multiflorum* to obtain the C- level.

To evaluate whether the elapsed-time between glyphosate spraying and sowing was positively related to germination, seedling emergence and growth of seedlings, glyphosate was sprayed 1 (G1), 30 (G30), 60 (G60) and 90 (G90) days before sowing the forage seeds or was not sprayed (G0 - control treatment sprayed with water 1day before sowing) (9).

A monoammonium salt of glyphosate (1440 g ae ha^{-1}) was applied at a dose of 2.88 L of glyphosate ha^{-1} with a CO_2 pressurized backpack sprayer fitted with a TT11001 nozzle delivering 100 L ha^{-1} at 200 kPa (36). The levels G1, G30, G60 and G90 consisted in 40 containers that were kept without vegetation cover (C-) and 40 containers that were sown with 25 seeds of *L. multiflorum* (C+) 60 days before glyphosate spraying. Control level G0 consisted in 80 containers (40 C+ and 40 C-) sprayed with the same amount of water without glyphosate one day before the sowing of forage seeds.

In summary, a completely randomized factorial design with 5 replications was performed. The two main factors were 1) Cover on which glyphosate was sprayed (C), at 2 levels: presence (C+) or absence (C-) of vegetation cover; and 2) Spraying of glyphosate before sowing (G), at 5 levels: sprayed 1(G1), 30 (G30), 60 (G60) and 90 (G90) days before sowing or not sprayed (G0). The same experiment was performed for each forage species and we analyzed the responses of each forage species to glyphosate treatments but did not compare among species due the great morphological and physiological differences among them.

Plant material

We selected two forage legume species, *Lotus tenuis* Waldst. cv. Aguapé and Kit. Ex Willd and *Trifolium repens* L. *cv.* El Lucero and two forage grass species *Paspalum dilatatum* Poir. (cv. Relincho) and *Festuca arundinacea* (Schreb.). These species are worldwide used in mixed pastures and are also naturalized in native grasslands of temperate regions (13). *T. repens* and *F. arundinacea* grow during the cool-season period, while *L. tenuis* and *P. dilatatum* grow during the warm-season period. Seeds of *L. tenuis*, *T. repens*, *P. dilatatum* and *F. arundinacea* were provided by commercial seed breeders.

Response variables

The number of emerged seedlings was daily recorded until twenty consecutive days without new emergencies. This period averaged 40 days after sowing. After 60 days from sowing, all seedlings were carefully harvested and seeds remaining in the soil were exhumed by sieving the soil through two sieves of 2.0 and 0.2 mm and examined under an optical microscope to determine whether germination had initiated (*i.e.* rootlet appearance) (30). The exhumed seeds that did not germinate (*i.e.* without visible rootlet) were evaluated by the tetrazolium test to determine their viability (19). The response variables related to seed germination and seedling emergence were expressed in percentage and calculated as following: a) Seed germination (%) as the number of established seedlings plus the number of exhumed germinated seeds in relation to the number of seeds sown of each species, b) Viable seeds (%) as the number of established seedlings plus the number of exhumed germinated seeds plus the number of viable not germinated seeds in relation to the number of seeds sown of each species, and Seedling emergence (%) as the number of established seedlings in relation to the number of seeds sown of each species (30).

Seedling morphological traits were assessed after seedling removal by measuring the number of leaves of the unique tiller and the length of the third leaf (lamina plus sheath) in the grasses *P. dilatatum* and *F. arundinacea*, the number and average length of the stems in *L. tenuis* and the number of leaves and the average length of the petioles in *T. repens.* The length of the seminal root of the four species was also measured. All length measurements were carried out by using a mechanical caliber. Aboveground and belowground biomass of seedlings were determined by oven drying until constant weight.

Statistical analyses

Variables were analyzed separately for each species through a factorial two-way ANOVAs at $\alpha=0.05$, being the main factors C and G. Tukey tests were applied to *post-hoc* comparison among means. Variables that involve percentages (seed germination, seedling emergence and viable seeds) were arcsine square-root transformed before analysis to meet ANOVA assumptions. Normality and homogeneity of variances were previously checked performing Kolmogorov-Smirnov and Levene and Brown-Forsythe tests. The results that involve percentages are shown as non-transformed mean $\pm$ 1 SD. Statistical analyses were performed using the package STATISTICA for Windows (StatSoft,Tulsa, OK, USA).

Results

Effects of glyphosate spraying on seed germination and viability

Germination and seed viability were not affected by the main factors C and G or their interaction. Germination was 50.6 ±12.3% for *L. tenuis*, 57.3±5.9 % for *T. repens*, 77.3±13.0% for *P. dilatatum* and 77.1±11.5% for *F. arundinacea*, while seed viability was 84.96±7.37% for *L. tenuis*, 82.16±6.28% for *T. repens*, 87.69±6.81% for *P. dilatatum* and 90.17±3.25% for *F. arundinacea*.

Effects of glyphosate spraying on seedling emergence

Seedling emergence of the four forage species was clearly affected by the interaction between them. Seedling emergence showed a consistent pattern among species: it was lower under C+ at G1, G30 and G60, than under C+ at G90 and G0 or under C- at any G level (figure 1, page 40). Therefore, glyphosate sprayed on preexisting vegetation cover 1 to 60 days before sowing caused a negative effect on seedling emergence of different magnitude depending on the species. Seedling emergence of *L. tenuis* under C+ at G1, G30 and G60 was 45% lower than under C+ at G90 and G0 and C- at G1, G30, G60, G90 and G0 (26.1+10% *vs*. 47.4+11.5% respectively figure 1 a, page 40). *T. repens* showed the lowest seedling emergence under C+ at G1 and G30 (21.1±6.9%), which was almost 63% lower than under C+ at G90 and G0 and C- at any G levels (56.5±3.7%) (figure 1 b, page 40). Seedling emergence of *P. dilatatum* under C+ at G1, G30 and G60 was 35% lower than under C+ at G90 and G0 and C- at any G levels (49.9±13.9%vs 76.6±14.8% respectively, figure 1 c, page 40). Seedling emergence of *F. arundinacea* under C+ at G1, G30 and G60 was almost 40% lower than under C+ at G90 and G0 and C- at any G levels (45.9 ±14.5% *vs*. 75.7±12.1% respectively figure 1 d, page 40).

Effects of glyphosate on seedling biomass

Above and belowground biomass of legumes seedlings was affected by the main factors C and G or by the interaction between them. Under C+ at G1 and G30, aboveground biomass of *L. tenuis* was 83% lower and above and belowground biomass of *T. repens* seedlings was 51% lower than under C+ at G90 and G0 and under C- at any G level (0.0169 ±0,0069 g. *vs*. 0.0987±0.0393 g; 0.0105±0.0047 *vs*. 0.0213 ±0.0054 g and 0.0067±0,0037 *vs*. 0.0136±0.0042 g respectively, figure 2 a and b, page 41). Belowground biomass of *L. tenuis* seedlings was lower under C+ than under C- and at G1 and G30 respect to G90 and G0 (figure 2 a, page 41). Under C+ at G60, aboveground biomass of *L. tenuis* seedlings was higher than under C+ at G1 and G30 but lower than under C+ at G90 and G0 and under C- at any G level (figure 2 a, page 41), while above and belowground biomass of *T. repens* seedlings did not differ respect to the other G levels (figure 2 b, page 41). Belowground -but not aboveground- biomass seedling of grasses was also affected by the main factors C and G and by the interaction between them. Belowground biomass of *P. dilatatum* seedlings was 46% lower under C+ at G1 and G30 than under C+ at G90 and G0 and under C- at any G level (0.0122±0.0050 g *vs*. 0.0224 ± 0.0049 g, (figure 2 c, page 41). Belowground biomass of *F. arundinacea* was 40% lower under C+ at G1 and G30 than under C+ at G60, G90 and G0 and C- at any G level (0.0148 ±0.0033 g *vs*. 0.0248±0.0047 g, (figure 2 d, page 41).

Effects of glyphosate spraying on morphological variables

Morphological variables of legumes seedlings were affected by the main factors C and G and by the interaction between them (figure 3, page 42). The number of stems of *L. tenuis* (figure 3 a, page 42), the petiole length of *T. repens* (figure 3 c, page 42) and the root lengths of both species (figure 3 b and figure 3 d, page 42) were significantly reduced under C+ at G1 and G30 respect to under C+ at G90 and G0 and under C- at any G level. The number of leaves per tiller and the leaf length of grasses were not affected by any treatments, but the root length of both grasses *P. dilatatum and F. arundinacea* was lower under C+ at G1 and G30 respect to under C+ at G60, G90 and G0 and under C- at any G level (figure 3 e and figure 3 f, page 42).

Vertical bars indicate 1 SD of the means. Different letters indicate significant differences provided by the Tukey test of the interaction factor (P< 0.05). Insert, results of ANOVA test: Main factors are Cover on which glyphosate was sprayed (C, at two levels C+ and C-) and Spraying of glyphosate before sowing (G, at five levels G1, G30, G60, G90 or G0). Significant levels (SL) are indicated with an asterisk (*) when P<0.05 and with two asterisks (**) when P<0.01.

Las barras verticales indican 1 desvío estándar de las medias. Letras distintas indican diferencias significativas según el test de Tukey (P<0,05) para el factor interacción. Se insertaron los resultados del ANOVA: Los factores principales son Cobertura sobre la que se pulverizó glifosato (C, a dos niveles: C+y C-) y Pulverización de glifosato antes de la siembra (G, a cinco niveles: G1, G30, G60, G90 or G0). El nivel de significancia (SL) se indica con un asterisco (*) cuando P<0,05 y con dos asteriscos (**) cuando P<0,01.

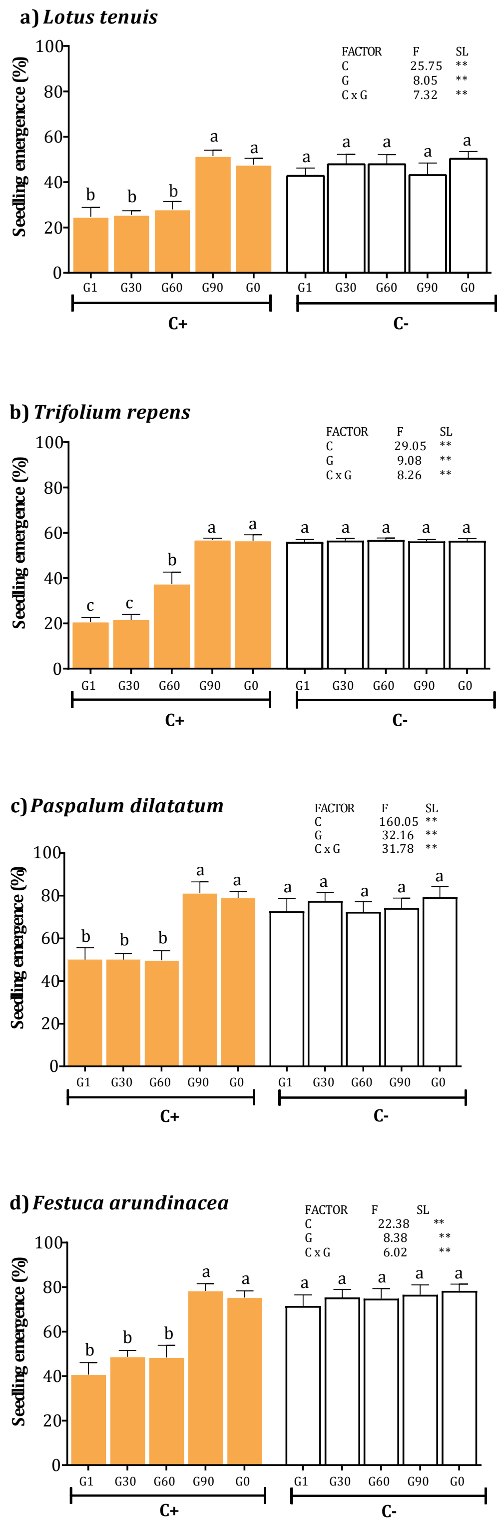

Figure 1. Seedling emergence (%) of *Lotus tenuis* (a), *Trifolium repens* (b), *Paspalum dilatatum* (c) and *Festuca arundinacea* (d) subjected to glyphosate spraying on the preexisting *L. multiflorum* vegetation cover (C+; orange bars) or bare soil (C-; white bars) at 1, 30, 60, 90 days before sowing and a control treatment not sprayed (G1, G30, G60. G90 and G0).

Figura 1. Emergencia de plántulas (%) de *Lotus tenuis* (a), *Trifolium repens* (b), *Paspalum dilatatum* (c) y *Festuca arundinacea* (d) pulverizadas con glifosato sobre la vegetación pre-existente de *L. multiflorum* (C+; barras naranjas) o sobre suelo desnudo (C-; barras blancas) 1, 30, 60, 90 días antes de la siembra y el tratamiento control no pulverizado (G1, G30, G60, G90 y G0).

Vertical bars indicate 1 SD of the means. Different letters indicate significant differences provided by the Tukey test of the interaction factor (P< 0.05). Insert, results of ANOVA test: Main factors are Cover on which glyphosate was sprayed (C, at two levels C+ and C-) and Spraying of glyphosate before sowing (G, at five levels G1, G30, G60, G90 or G0). Significant levels (SL) are indicated with an asterisk (*) when P<0.05 and with two asterisks (**) when P<0.01.

Las barras verticales indican 1 desvío estándar de las medias. Letras distintas indican diferencias significativas según el test de Tukey (P< 0,05) para el factor interacción. Se insertaron los resultados del ANOVA: Los factores principales son Cobertura sobre la que se pulverizó glifosato (C, a dos niveles: C+y C-) y Pulverización de glifosato antes de la siembra (G, a cinco niveles: G1, G30, G60, G90 or G0). El nivel de significancia (SL) se indica con un asterisco (*) cuando P<0,05 y con dos asteriscos (**) cuando P<0,01.

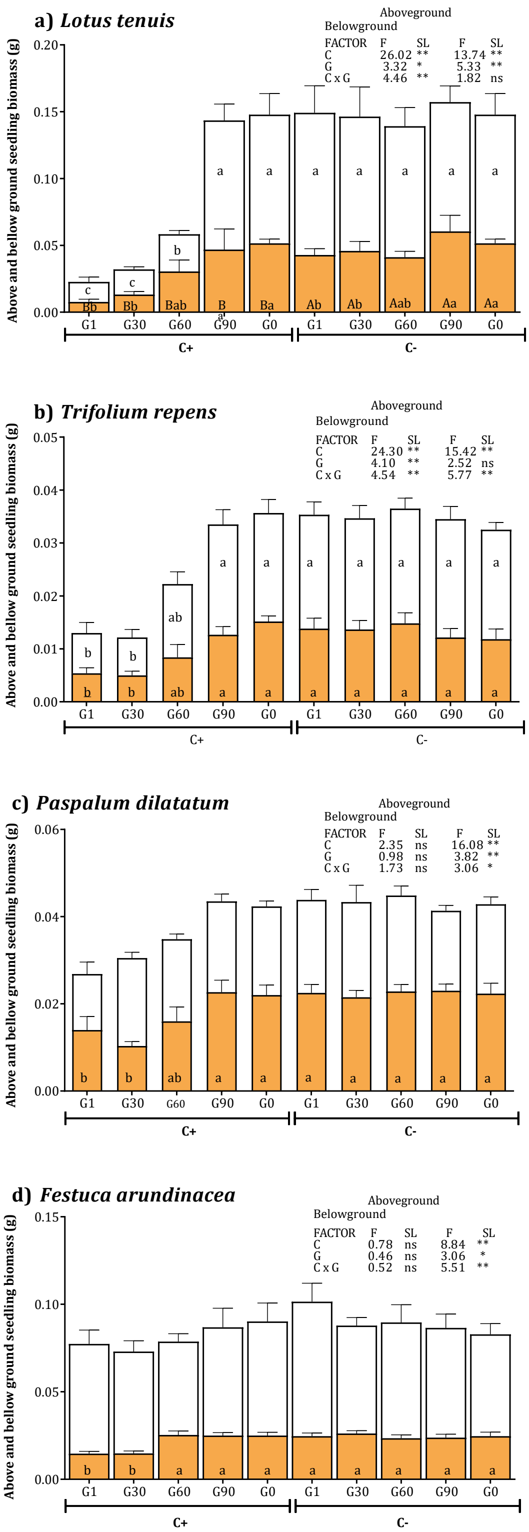

Figure 2. Above (white bars) and belowground (orange bars) biomass of seedlings of *Lotus tenuis* (a), *Trifolium repens* (b), *Paspalum dilatatum* (c) and *Festuca arundinacea* (d) subjected to glyphosate spraying on the preexisting *L. multiflorum* vegetation cover (C+; orange bars) or bare soil (C-; white bars) at 1, 30, 60, 90 days before sowing and a control treatment not sprayed (G1, G30, G60, G90 and G0).

Figura 2. Biomasa aérea (barras blancas) y subterránea (barras naranjas) de las plántulas de *Lotus tenuis* (a), *Trifolium repens* (b), *Paspalum dilatatum* (c) y *Festuca arundinacea* (d) pulverizadas con glifosato sobre la vegetación pre-existente de *L. multiflorum* (C+; barras naranjas) o sobre suelo desnudo (C-; barras blancas) 1, 30, 60, 90 días antes de la siembra y el tratamiento control no pulverizado (G1, G30, G60, G90 y G0).

Vertical bars indicate 1 SD of the means. Different letters indicate significant differences provided by the Tukey test of the interaction factor (P< 0.05). Insert, results of ANOVA test: Main factors are Cover on which glyphosate was sprayed (C, at two levels C+and C-) and Spraying of glyphosate before sowing (G, at five levels G1, G30, G60, G90 or G0). Significant levels (SL) are indicated with an asterisk (*) when P<0.05 and with two asterisks (**) when P<0.01.

Las barras verticales indican 1 desvío estándar de las medias. Letras distintas indican diferencias significativas según el test de Tukey (P< 0,05) para el factor interacción. Se insertaron los resultados del ANOVA: Los factores principales son Cobertura sobre la que se pulverizó glifosato (C, a dos niveles: C+y C-) y Pulverización de glifosato antes de la siembra (G, a cinco niveles: G1, G30, G60, G90 or G0). El nivel de significancia (SL) se indica con un asterisco (*) cuando P<0,05 y con dos asteriscos (**) cuando P<0,01.

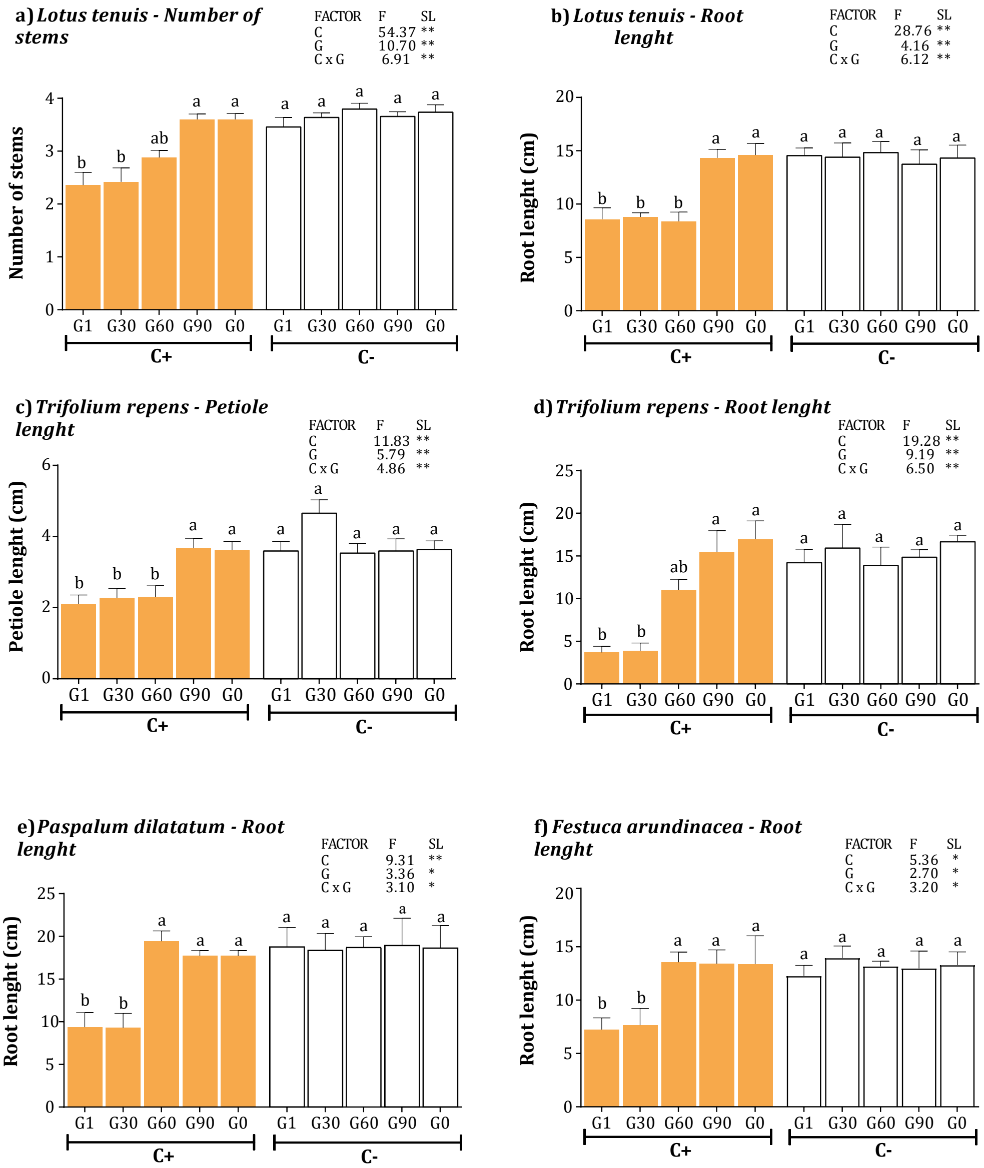

Figure 3. Number of stems (a) and root lenght (b) of *Lotus tenuis*, petiole length (c) and root length (d) of *Trifolium repens*, root lenght of *Paspalum dilatatum* (e) and root length of *Festuca arundinacea* (f) subjected to glyphosate spraying on the preexisting vegetation of *L. multiflorum* cover (C+; orange bars) or bare soil (C-; white bars) at 1, 30, 60, 90 days before sowing and a control treatment not sprayed (G1, G30, G60, G90 and G0).

Figura 3. Cantidad de tallos (a) y longitud de raíces (b) de *Lotus tenuis*, longitud de pecíolos (c) y longitud de raíces (d) de *Trifolium repens*, longitud de raíces of *Paspalum dilatatum* (e) y longitud de raíces de *Festuca arundinacea* (f) pulverizadas con glifosato sobre la vegetación pre-existente de *L. multiflorum* (C+; barras naranjas) o sobre suelo desnudo (C-; barras blancas) 1, 30, 60, 90 días antes de la siembra y el tratamiento control no pulverizado (G1, G30, G60, G90 y G0).

Discussion

Our results showed that glyphosate spraying on preexisting vegetation cover 1 to 60 days before sowing reduces seedlings emergence and growth of the forage legumes *Lotus tenuis* and *Trifolium repens*, and the forage grasses *Paspalum dilatatum* and *Festuca arundinacea*. The negative effect showed a threshold response because it prevailed during 30 or 60 days since glyphosate spraying until sowing, depending on the variable and the forage species.

We found that glyphosate greatly reduced seedling emergence and seedling growth when it was sprayed on pre-existing vegetation but not when it was sprayed on bare soil. Glyphosate mineralization rate in soil is much lower when it was previously absorbed by plants than when it is applied to bare soil (10, 21). Herbicides trapped in plant residues provides protection against microbial degradation (21), so glyphosate degradation in soil following their absorption by plants is much slower, which increase it persistence from two to six times respect to directly sprayed on bare soil (10). Glyphosate or AMPA are released from tissues of treated plants to the rhizosphere zone and transfer to non-target plants via contact contamination (5, 25, 33). On the contrary, when glyphosate is sprayed on bare soil, it suffers a rapid microbial degradation or inactivation by sorption to the soil matrix (15).

Our results indicated that the reduction of seedling emergence when glyphosate was sprayed on pre-existing vegetation can be attributed to the toxic effect on germinated seeds, after rootlet appearance, because seed germination and seed viability were not affected. The mortality of germinated seeds occurred before seedling emergence, suggesting a greater susceptibility to the herbicide in younger tissues and seedlings of small size, susceptibility that decreases as the size or the state of development of the plants increases (20, 35). Contrary to the studies that found reduction of seed germination when seeds germinated on boxes on filter paper and watered with glyphosate-water solutions (17, 22), seed germination was not affected when glyphosate was sprayed on vegetation cover or bare soil, not directly over the seeds.

Seedlings that survived to glyphosate sprayed on the pre-existing vegetation, suffered a decline of its growth. Both legume and grass seedlings showed lower belowground biomass and root length, and seedlings of legumes also showed much lower aboveground biomass. These are indirect symptoms, mainly expressed as limitations to root functioning like absorption of water (38) and nutrients (25), which result in lower above and belowground growth. Moreover, our results suggest higher susceptibility to glyphosate of legumes than grasses, as species, and even biotypes of the same species, differ in their susceptibility to glyphosate (3, 6).

Glyphosate persistence in soil is extremely variable as it is affected by soil properties, method of application, and environmental conditions, such as moisture and temperature (11). Our experiment showed that, in a *typic Natracuol* soil, the deleterious effect of glyphosate on seedling emergence and seedling growth had a threshold response pattern as it prevailed during at least 30 or 60 days between glyphosate spraying and sowing, with similar intensity along this period. Therefore, the recommendation of spraying glyphosate 7-30 days before seeding or overseeding pastures and forage species by no-tillage methods (1, 2, 37) would not be appropriate in order to avoid injury on seed emergence and seedling growth.

Conclusion

Our results suggest that the elapsed-time between glyphosate spraying on pre-existing vegetation of *L. multiflorum* and sowing of *L. tenuis*, *T. repens*, *P. dilatatum* and *F. arundinacea* seeds in argillic and natric soils must be longer than 60 days to avoid a reduction of seedling emergence and to maximize seedlings size and biomass. This finding is relevant for management purposes because of the prevalence of argilic and natric soils such as *Argiudols* and *Natracuols* in the Pampa region, where seeding or overseeding pastures and annual forage crops by no-tillage methods after glyphosate spraying is an extended practice.

References

1. Allister C.; Kogan, M.; Pino; I. 2005. Differential phytotoxicity of glyphosate in maize seedlings following applications to roots or shoot. Weed Research. 45: 27-32. doi: 10.1111/j.1365-3180.2004.00424.x.
2. Bartholomew, P. 2005. Comparison of conventional and minimal tillage for low-input pasture improvement. Forage and Grazinglands. 3: 1-14. doi:10.1094/FG-2005-0913-01-RV.
3. Baylis, A. 2000. Why glyphosate is a global herbicide: strengths, weaknesses and prospects? Pest Management Science. 56: 299-308. doi: 10.1002/(SICI)1526-4998(200004)56:4<299:AIDPS144>3.0.CO;2-K.
4. Blackburn, L.; Boutin, C. 2003. Subtle effects of herbicide use in the context of genetic modified crops: A case study with Glyphosate (Roundup ®). Ecotoxicology. 12: 271-285. doi: 10.1023/A:1022515129526.
5. Bott, S.; Tesfamariam, T.; Kania, A.; Eman, B.; Aslan, N.; Römheld, V.; Neumann, G. 2011. Phytotoxicity of glyphosate soil residues re-mobilised by phosphate fertilisation. Plant and Soil. 342: 249-263. doi: 10.1007/s11104-010-0689-3.
6. Boutin, C.; Elmegaard, N.; Kjær, C. 2004. Toxicity testing of fifteen non-crop plant species with six herbicides in a greenhouse experiment: Implications for risk assessment. Ecotoxicology. 13: 349-369. doi: 10.1023/b:ectx.0000033092.82507.f3.
7. Claassens, A.; Rose, M. T.; Van Zwieten, L.; Weng, Z.; Rose, T. J. 2019. Soilborne glyphosate residue thresholds for wheat seedling metabolite profiles and fungal root endophyte colonization are lower than for biomass production in a sandy soil. Plant and Soil. 438: 393-404. doi: 10.1007/s11104-019-04023-y.
8. Clua, A.; Conti, M.; Beltrano, J. 2012. The effects of glyphosate on the growth of birdsfoot trefoil (*Lotus corniculatus*) and its interaction with different phosphorus contents in soil. Journal of Agricultural Science 4: 208-218. doi: 10.5539/jas.v4n7p208.
9. da Silva, P. V.; Rodrigues Milagres Viana, H.; Monquero, P. A.; Moraes Ribeiro, N.; Pereira Neto, W.; Inacio, E. M.; Christoffoleti, P. J.; de Carvalho Dias, R. 2021. Influence of sugarcane (*Saccharum officinarum*) straw on weed germination control. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 53(1): 220-233. https://doi.org/10.48162/rev.39.021
10. Doublet, J.; Mamy, L.; Barriuso, E. 2009. Delayed degradation in soil of foliar herbicides glyphosate and sulcotrione previously absorbed by plants: Consequences on herbicide fate and risk assessment. Chemosphere. 77: 582-589. doi: 10.1016/j.chemosphere.2009.06.044
11. Duke, S. O.; Lydon, J.; Koskinen, W.; Moorman, T.; Chaney, R.; Hammerschmidt, R. 2012. Glyphosate effects on plant mineral nutrition, crop rhizosphere microbiota, and plant disease in glyphosate-resistant crops. Journal of Agricultural and Food Chemistry. 60: 10375-10397. doi: 10.1021/jf302436u
12. Duke, S. O. 2018. The history and current status of glyphosate. Pest Management Science. 74: 1027-1034. doi: 10.1002/ps.4652
13. García, I. V. 2021. Lotus tenuis and *Schedonorus arundinaceus* co-culture exposed to defoliation and water stress. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 53(2): 100-108. https://doi.org/10.48162/rev.39.044
14. Gerritse, G.; Beltran, J.; Hernandez, F. 1996. Adsorption of atrazine, simazine, and glyphosate in soils of the Gnangara Mound, Western Australia. Australian Journal of Soil Research. 34: 599-607. doi: 10.1071/SR9960599.
15. Giesy, J.; Dobson, S.; Solomon, K. 2000. Ecotoxicological risk assessment for Roundup ® Herbicide. In Ware, G. W. (Ed.) Reviews of Environmental Contamination and Toxicology. Springer, New York. 35-120. doi: 10.1007/978-1-4612-1156-3_2.
16. Gomes, M.; Smedbol, E.; Chalifour, A.; Hénault-Ethier, L.; Labrecque, M.; Lepage, L.; Lucotte, M.; Juneau, P. 2014. Alteration of plant physiology by glyphosate and its by-product aminomethylphosphonic acid: an overview. Journal of Experimental Botany. 65: 4691-4703. doi: 10.1093/jxb/eru269
17. Gomes, M.; da Silva Cruz, F.; Bicalho, E.; Borges, F.; Fonseca, M.; Juneau, P.; Garcia, Q. 2016. Effects of glyphosate acid and the glyphosate-commercial formulation (Roundup) on *Dimorphandra wilsonii* seed germination: Interference of seed respiratory metabolism. Environmental Pollution. 220: 452-459. doi: 10.1016/j.envpol.2016.09.087.
18. Grzesiuk, A.; Dębski, H.; Oknińska, K.; Koczkodaj, D.; Szwed, M.; Horbowicz, M. 2018. Effect of rootzone glyphosate exposure on growth and anthocyanins content of radish seedlings. Acta Scientiarum Polonorum Hortorum Cultus. 17: 3-10. doi: 10.24326/asphc.2018.2.1
19. ISTA International Seed Testing Association. 2003. Working Sheets on Tetrazolium Testing. Volume I. The International Seed Testing Association. CH-Switzerland.
20. Koger, C. H.; Poston, D. H.; Reddy, K. N. 2004. Effect of glyphosate spray coverage on control of pitted morningglory (*Ipomoea lacunosa*). Weed Science. 53: 277-283. doi:10.1614/WS-04-098R
21. Laitinen, P.; Rämö, S.; Siimes, K. 2007. Glyphosate translocation from plants to soil: Does this constitute a significant proportion of residues in soil? Plant and Soil. 300: 51-60. doi: 10.1007/s11104-007-9387-1.
22. Mamy, L.; Barriuso, E.; Gabrielle, B. 2016. Glyphosate fate in soils when arriving in plant residues. Chemosphere. 154: 425-433. doi: 10.1016/j.chemosphere.2016.03.104.

23. Mondal, S.; Kumar, M.; Haque, S.; Kundu, D. 2017. Phytotoxicity of glyphosate in the germination of *Pisum sativum* and its effect on germinated seedlings. Environmental Health and Toxicology. 32: 1-7. doi: 10.5620/eht.e2017011.
24. Morillo, E.; Undabeytia, T.; Maqueda, C.; Ramos, A. 2000. Glyphosate adsorption on soils of different characteristics. Influence of copper addition. Chemosphere. 40: 103-107. doi: 10.1016/S0045-6535(99)00255-6.
25. Neumann, G.; Kohls, S.; Landsberg, E.; Stock-Oliveira Souza, K.; Yamada, T.; Römheld, V. 2006. Relevance of glyphosate transfer to non-target plants via the rhizosphere. Journal of Plant Diseaces and Protection. 20: 963-969. doi: 10.1007/s11104-010-0689-3
26. Paiola Albrecht, A. J.; Moreira Silva, A. F.; Martins Barroso, A. A.; Paiola Albrecht, L.; Placido, H. F.; de Marco, L. R.; Baccin, L. C.; Victoria-Filho, R. 2021. Mixtures between glyphosate formulations and ACCase-inhibiting herbicides in the control of *Chloris elata*. Revista de la Facultad de Ciencias Agrarias. Universidad Nacional de Cuyo. Mendoza. Argentina. 53(1): 274-282. https://doi.org/10.48162/rev.39.026
27. Piotrowicz-Cieślak, A. I.; Adomas, B.; Michalczyk, D. J. 2010. Different glyphosate phytotoxicity to seeds and seedlings of selected plant species. Polish Journal of Environmental Studies. 19: 123-129.
28. Pline, W.; Wilcut, J.; Edmisten, K.; Wells, R. 2002. Physiological and morphological response of glyphosate-resistant and non-glyphosate-resistant cotton seddlings to root-absorbed glyphosate. Pesticide Biochemistry and Physiolgy. 73: 48-58. doi: 10.1016/S0048-3575(02)00014-7.
29. Reddy, K.; Rimando, A.; Duke, S. 2004. Aminomethylphosphonic acid, a metabolite of glyphosate, causes injury in glyphosate-treated, glyphosate resistant soybean. Journal of Agricultural and Food Chemistry. 52: 5139-5143. doi: 10.1021/jf049605v.
30. Roberts, H. A. 1981. Seedbanks in soil. Adv. Appl. Biol. 6: 1-55.
31. Rodrigues, J.; Worsham, A.; Corbin, F. 1982. Exudation of glyphosate from wheat (*Triticum aestivum*) plants and its effects on interplanted corn (*Zea mays*) and soybean (*Glycine max*). Weed Science. 30: 316-320. doi: 10.1017/S0043174500040601.
32. Simonsen, L.; Fomsgaard, I.; Svensmark, B.; Spliid, N. 2008. Fate and availability of glyphosate and AMPA in agricultural soil. Journal of Environmental Science and Health. 43: 365-375. doi: 10.1080/03601230802062000
33. Tesfamariam, T.; Bott, S.; Cakmak, I.; Römheld, V.; Neumann, G. 2009. Glyphosate in the rhizosphere. Role of waiting times and different glyphosate binding forms in soils for phytotoxicity to non-target plants. European Journal of Agronomy. 31: 126-132. doi: 10.1016/j.eja.2009.03.007.
34. Thom, E.; Fraser, T.; Hume, D. 2011. Sowing methods for successful pasture establishment: a review. In Mercer, C. F. (Ed) Pasture Persistence Symposium Grassland Research and Practice Series 15. New Zealand Grassland Association. Dunedin. New Zealand. 31-38.
35. Vanlieshout, L. A.; Loux, M. M. 2000. Interactions of glyphosate with residual herbicides in no-till soybean (*Glycine max*) production. Weed Technology. 14: 480-487. doi: 10.1614/0890-037x(2000)014[0480:iogwrh]2.0.co;2.
36. Vila Aiub, M.; Balbi, M.; Gundel, P.; Ghersa, C.; Powles, S. 2007. Evolution of glyphosate-resistant Johnsongrass (*Sorghum halepense*) in glyphosate-resistant soybean. Weed Science. 55: 566-571. doi: 10.1614/WS-07-053.1.
37. Welty, L. E.; Anderson, R. L.: Delaney, R. H.; Hensleigh, P. F. 1981. Glyphosate timing effects on establishment of sod-seeded legumes and grasses. Agronomy Journal. 73: 813-817. doi: 10.2134/agronj1981.00021962007300050016x.
38. Zobiole, L.; Kremer, R.; Oliveira Jr., R.; Constantin, J. 2011. Glyphosate affects micro-organisms in rhizospheres of glyphosate-resistant soybeans. Journal of Applied Microbiology. 110: 118-127. doi: 10.1111/j.1365-2672.2010.04864.x.

Acknowlegments

This research was funded by Universidad de Buenos Aires, Secretaría de Ciencia y Técnica (UBACyT 20020170100538BA). The authors acknowledge Prof. M. C. Plencovich for the edition of English language, to Prof. Julio Scursoni for his helpful comments to the original manuscript and to the anonymous reviewer for their valuable contributions that greatly improved this final version.