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Response of *Commelina erecta* L. to glyphosate formulations, and role of starch and waxes in glyphosate sensitivity

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Abstract. The aim of this study was to evaluate the effects of two formulations of glyphosate on adult plants and seedlings of *Commelina erecta L.,* and to determine whether stem starch storage and epicuticular waxes of leaves are involved in plant sensitivity to this herbicide. The formulations used were isopropylamine salt (IPA) and diammonium salt (DA). All measured growth parameters and chlorophyll were negatively affected by both formulations. The IPA formulation showed a higher decrease in the branching rate and phyllochron, whereas the DA formulation showed a higher decrease in the elongation rate, chlorophyll content at 48 hours after application in adult plants, and the starch proportion in the stem basal section, and caused more leaf injuries. All plants of *C. erecta* presented starch storage in the both stem section. After glyphosate application, stem starch proportion of the basal section evolved differently in both growth stages, whereas stem starch proportion of the apical section showed an irregular variation. The amount of epicuticular waxes was higher in adult plants. These results show that both glyphosate formulations are ineffective for the management of *C. erecta*. The low sensitivity to glyphosate in *C. erecta*, especially in plants higher than five leaves, could be related to starch storage in stem and epicuticular waxes on leaves.

[Keywords: weed, dayflowers, herbicide, growth stage]

Resumen. Respuesta de *Commelina erecta* L. a dos formulados de glifosato, y rol del almidón y de las ceras epicuticulares en la sensibilidad al glifosato. El objetivo de este estudio fue evaluar los efectos de dos formulados de glifosato en plantas adultas (originadas de rebrotes) y plantas jóvenes (originadas de semillas) de *Commelina erecta* L. y determinar si el almacenamiento de almidón en el tallo y las ceras epicuticulares de las hojas juegan un rol importante en la sensibilidad a este herbicida. Se utilizaron dos formulados de glifosato: uno, a base de la sal isopropilamina (IPA), y otro, a base de la sal diamónica del ácido (DA). Todos los parámetros de crecimientos y la clorofila fueron afectados negativamente por ambos formulados. El formulado IPA redujo más la tasa de ramificación y el filocrono, mientras que el formulado DA redujo más la tasa de elongación, el contenido de clorofila a las 48 horas post-aplicación en las plantas adultas, la concentración de almidón en la parte basal del tallo, y causó más lesiones visibles en hoja. Todas las plantas de *C. erecta* presentaron almidón en ambas secciones del tallo. Luego de aplicar glifosato, la proporción de almidón en la sección basal del tallo evolucionó diferente en las plantas adultas que en las jóvenes. En la sección apical del tallo, la proporción de almidón mostró un patrón de variación irregular. La cantidad de ceras epicuticulares fue mayor en plantas adultas. Estos resultados muestran que ambos formulados no son eficientes para el manejo de *C. erecta*. La baja sensibilidad a glifosato en plantas de *C. erecta* con más de cinco hojas podría relacionarse con el almacenamiento de almidón el tallo y con la cantidad y la distribución de las ceras epicuticulares en las hojas.

[Palabras clave: maleza, flor de santa Lucía, herbicida, rizoma, edades de plantas]

Introduction

Glyphosate is globally considered the most important herbicide because it can effectively manage a broad spectrum of weeds (Travlos et al. 2017). Glyphosate exist in several chemical mixtures and/or forms, primarily as either glyphosate technical acid or as various salts of glyphosate (Cuhra et al. 2016). In Argentina, there are about 74 trademarks of glyphosate (Casafe 2020). Argentine farmers focus their attention on economic yields (Scursoni et al. 2019), so they generally choose the most economical herbicides. In troublesome weeds, it is important to determine whether one or the other formulation is more effective (Li et al. 2005) to minimize environmental risk, the evolution of weed resistance and production cost.

In Argentina, *Commelina erecta* L. is a troublesome weed in spring and summer crops (Dellaferrera et al. 2007; Nisensohn et al. 2011; Panigo and Nisensohn 2018). Control with glyphosate can be very well exerted on seedlings having less than 5 leaves, but not in adult plants (Rainero 2004; Panigo et al.

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2012). This glyphosate tolerance was related to the presence of basal axillary meristems not affected by the application of the herbicide (Panigo et al. 2019) and likely to rhizome size (Panigo et al. 2012). In other *Commelina* species, Tuffi Santos et al. (2004) found that the ability to tolerate glyphosate application is positively related to the starch proportion in their stem, which mobilizes quickly and allows leaf surface recovery. In *Commelina benghalensis* L., the mechanism of glyphosate tolerance is related to characteristics of epicuticular waxes and to the presence of differential metabolism (Monquero et al. 2004). In *C. erecta*, these traits have not been studied.

The evaluation of the effect of glyphosate formulation on morpho-physiological parameters of *C. erecta* is of great importance to understand its persistence in agroecosystems. Based on the above, the aims of the study were a) to evaluate and compare the effects of two glyphosate formulations on growth and physiological parameters in seedlings and adult plants of *C. erecta*, and b) to determine whether stem starch storage and epicuticular waxes are involved in plant sensitivity to this herbicide.

Materials and Methods

Plant material and growth conditions

The response of *C. erecta* was analyzed by comparing plants at two growth stages: seedlings and adult plants (AP). The seedlings are juvenile individuals and were obtained from the seeds germination. Adult plants were obtained from regrowth of rhizome fragments by vegetative propagations. This choice ensures different levels of starch storage in the rhizomes. Plants and seeds were collected from populations located in the city of Esperanza (31°26′ S - 60°55′ W), Santa Fe, Argentina. The APs and seedlings of *C. erecta* were transplanted to 1 L plastic pots and placed in a growth chamber, following the conditions used by Panigo et al. (2012).

Herbicide treatments

Two formulations of glyphosate plus a control group without application were used. The first formulation was glyphosate diammonium salt (DA) (GLIFOTOP ZAMBA®, 428 g/L salt; 356 g/L acid equivalent; Nidera S.A., Argentina). The other formulation was glyphosate isopropylamine salt (IPA) (ESTRELLA®, 480 g/L salt; 360 g/L acid equivalent; Asociación de Cooperativas Argentinas Coop. Ltda., Argentina).

Herbicides formulations were applied evenly to the plant surface using a handmade air-pressurized laboratory track sprayer (speed: 1 m/s) delivering 200 L/ha (flat fan nozzle pill, 270 kPa, and medium fine droplets). The dosage used was 1800 grams of acid equivalent per hectare of glyphosate, applied as an aqueous solution (Panigo et al. 2012). The application day was day 0, the moment when both growth stages presented 5-8 expanded leaves.

Measured parameters

On the one hand, 30 plants were used per treatment (growth stage x formulations) to quantify growth and physiological parameters (biomass on 10 plants, chlorophyll on 5 plants, the stem starch proportion on 5 plants, and height, numbers of branches and leaves on 10 plants). On the other hand, epicuticular waxes were determined in 15 plants (5 plants for distribution analysis and 10 for wax quantification) per growth stage, without herbicide treatment and with 5-8 expanded leaves.

At 0 (application day), 7, 14 and 21 days after application (DAA), the shoot height, the total number of branches and the total number of leaves per plant were recorded. Height measurements were transformed into natural logarithms and plotted against DAA to calculate axis relative elongation rates (RER). Linear regression equations were fit in the interval comprising four successive sampling dates, and RERs were the slopes of those lines. Likewise, branching relative rate (BRR) and leaf appearance rates (PC) were calculated from the slopes of branch and leaf number vs. DAA for each plant, respectively. At 21 DAA, leaf area (LA) per plant was estimated according to the methodology described by Lauri and Terouanne (1991). The linear regression equation used to calculate LA in *C. erecta* was as follows:

$$y = 0.6223x + 0.7134 \ (R^2=0.9938)$$

where y (cm^2) is the LA and x is the product of leaf length (cm)and leaf width in each leaf (cm).

Visible leaf injury level caused by glyphosate application was evaluated at 7, 14 and 21 DAA using a qualitative scale (0-100%), in which 0% corresponds to uninjured leaves and 100% to total leaf injury (or leaf death). Shoot biomass accumulation was measured at 21 DAA. Plants were harvested and washed with deionized water until a constant weight was achieved and then biomass was recorded. Chlorophyll content was extracted in fully expanded last leaves on the main axis, according to the methodology proposed by Arnon (1949). The extraction of photosynthetic pigment was at 24, 48 and 168 hours after application (HAA) of herbicide and was calculated as milligrams chlorophyll (a+b) per gram of leaf biomass.

Stem starch proportion was determined indirectly over the photomicrographs of the apical and basal sections of five main stems by formulation and growth stages. Stem cross-sections were cut at 7, 14 and 21 DAA with a freezing microtome (Leitz, 1310, Wetzlar, Germany), according to the technique proposed by D´ambrogio de Argüeso (1986). For the display of starch granules, all cuttings were submerged in lugol solution at 1% for 5 minutes, and afterwards were washed and mounted using water as a medium (Tuffi Santos et al. 2004). Digital photomicrographs of twenty cuttings, by section of stems and plants, were taken. The image capture system was composed of digital camera (Canon Inc, EOS REBEL T2i) and microscope (Leica Microsystems AG, DM 1000). With these digital photomicrographs, the starch proportion was quantified indirectly by the reaction with lugol using ImageJ ® software (Rasband 2019). The options oval, brush selections and wand tool in ImageJ were used to estimate the total surface of the transversal section (TS) of the stem and the surface tinted with lugol (LS). Both areas were measured, and the proportion of surface with starch was estimated as the ratio between them (LS/TS*100).

To determine the role of epicuticular waxes in herbicide sensitivity, the distribution and amount of waxes in leaves of seedlings and AP not treated was evaluated. Epicuticular wax distribution was evaluated on the middle part of the second and third leaves. These leaf samples were air-dried and adhered with silver paint on metal sample holders and then coated with gold deposited by sputtering using a VEECO Evaporator, model VE-300 operated in an Argon atmosphere. Then, the samples were photographed in a JEOL Scanning Electron Microscope, model JSM-35C with a SemAfore digital image acquisition system. Epicuticular waxes were extracted from 50 leaves of 10 plants per growth stage. Five groups of 10 fully expanded leaves from two individuals were immersed separately in containers with chloroform and methanol (90:10 mL) for 30 seconds, with gentle shaking to avoid leaf breakage and release of chlorophyll and other compounds. The extracts obtained were filtered and evaporated on a plate at 55 °C until the volume was reduced to approximately 15 mL. This solution (chloroform+wax) was transferred to 25 mL tubes of known weight. The chloroform was evaporated at room temperature. The tubes were weighed 2 and 4 days until constant weight was reached. The amount of epicuticular waxes was expressed per unit leaf area (µg/cm^2). The leaf area of all leaves was determined on photographs using ImageJ ® software (Rasband 2019).

Statistical design and analysis

Statistical analyses were performed with the software InfoStat ® (InfoStat 2018 version, Córdoba, Argentina). A complete randomized design was applied. Data, except for injury levels and amount of wax, were analyzed through ANOVA using the Fisher´s least significant difference (LSD) test for comparisons of means at 5% level of significance. A factorial design was applied following two selection criteria: growth stages (seedlings and AP) and herbicide formulation (control group, DA and IPA). In the case of total chlorophyll content, the analysis was carried out on each sampling date separately. Stem starch proportion data were square-root- transformed to reduce the heterogeneity of variances, and the analysis was carried out on separate dates and stem sections. Then, data were retransformed for graph representation. Injury levels at each growth stage and herbicide formulation applied were analyzed using the chi-square test on each sampling date separately. The amount of epicuticular waxes was analyzed using general linear models (GLM). The main effect of the factor (growth stage) was evaluated by analysis of variance and using Fisher's least significant difference (LSD) at 5% level of significance.

The experiment was conducted twice, and data were combined for the analyses because the interaction was null. Data variance was visually inspected by Levene's test to confirm the homogeneity of variance before the statistical analysis.

Results and Discussion

Axis relative elongation rate

Axis relative elongation rate significantly varied between growth stages and treatments. Under control and herbicide treatment conditions, all seedlings showed the highest RER (Table 1). Both formulations had a negative impact on RER but to a different degree. In both growth stages, DA decreased this rate by approximately 70%, whereas IPA only decreased it by about 30% to the control plants. Axis relative elongation rate of *C. erecta* was low, even in the control group, which can be a consequence of the plagiotropic growth of its axes. These results are similar to the ones obtained by Nisenshon et al. (2011), who found that, although *C. erecta* has a low growth rate, it can dominate in the weed community. After application, the decline in shoot elongation was consistent with previous works on this species (Panigo et al. 2012).

Branching relative rate

Seedlings plants had a higher BRR than AP (P<0.05) (Table 1). Exposure to both formulations caused a reduction in BRR respect to the control group, although the response was uneven between formulations. In plants treated with IPA formulations, 50% of the plants (Seedlings and AP) stopped branch production after herbicide application and presented a BBR twelve times lower than the control plants. On the other hand, plants treated with DA formulations showed a BRR approximately two times lower than the control plants. The herbicide had only a significant effect on the seedlings treated with IPA formulations.

Differences in responses between the formulations may be related to differences in absorption and translocation. The higher BRR in plants treated with DA was not related to a lower herbicide effect since at the end of the sampling (21 DAA) most of their branches were found dead. That is, the differences in the composition of the formulations at the plant level resulted in a different distribution of resources and therefore in a different BRR. When testing different glyphosate formulations, Li et al. (2005) observed slight differences in absorption or translocation between formulations.

On the other hand, the differences in branching rates between growth stages could be related to different regrowth strategies between stages. At 60 DAA it has been observed that in AP bud activation occurs in the apical zone, increasing flower production, and in seedlings it occurs in the basal zone, generating an increase in the rhizome system (Panigo et al. 2019). Seedlings allocate their resources to increase their growth potential and AP plants allocate their resources to increase storage, as discussed below. This variety of morphogenetic responses is a part

Table 1. Growth parameters rate (±standard error) in *Conmmelina erecta* plants measured at two growth stages (seedlings and adult plants) and three treatments (untreated plants, plants treated with glyphosate diammonium salt and plants treated with glyphosate isopropylamine salt). RER: principal axis relative elongation rates, BRR: branching relative rates, PC: phylochrono, Seed: seedlings with five leaves, AP: adult plant; Control: untreated plants, DA: plants treated with glyphosate salt, IPA: plant treated with glyphosate isopropylamine salt. Note: means within a column followed by the same letter are similar at the 0.05 level of significance according to Fisher's LSD test.

Tabla 1. Tasas de crecimiento (±error estándar) en plantas de *Commelina erecta*, según dos edades (plantas jóvenes y planta adulta) y tres tratamientos (plantas control, plantas tratadas con glifosato formulado a base de la sal de diamónica y plantas tratadas con glifosato formulado a base de la sal isopropilamina). RER: tasa de elongación relativa del eje principal, BRR: tasa relativa de ramificación, PC: filocrono, Seed: plantas jóvenes con 5 hojas, AP: planta adulta; Control: plantas sin tratar, DA: plantas tratadas con glifosato formulado a base de la sal de diamónica, IPA: plantas tratadas con glifosato formulado a base de la sal isopropilamina. Nota: las medias dentro de una columna seguidas de la misma letra son significativamente similares al 0.05, según la prueba LSD de Fisher.

Herbicide treatment	Growth stages	RER (cm/day)	BRR (branch/day)	PC (leaf/day)
Control	Seed	0.04967±0.003 D	0.541±0.094 C	1.662±0.2830 C
	AP	0.03263±0.003 C	0.229±0.094 AB	0.7507±0.2830 AB
IPA	Seed	0.03299±0.003 C	0.043±0.094 AB	0.1467±0.2830 AB
	AP	0.02302±0.003 BC	0.018±0.094 A	0.1167±0.2830 A
DA	Seed	0.0165±0.003 AB	0.292±0.094 BC	0.9203±0.2830 BC
	AP	0.01218±0.003 A	0.119±0.094 AB	0.3718±0.2830 AB

of a general adaptation strategy, in which the growth of the plants is redirected to diminish stress exposure and constitutes the 'flight' response of plants (Potters et al. 2007).

Leaf parameters

Phyllochron (PC) was significantly greater in seedlings than AP regardless of the treatment (P<0.05) (Table 1). Like BRR, both formulations decreased PC (P<0.05), although the response was different between formulations. Within plants treated with IPA, the PC was 91% smaller in seedlings and 84% in AP, compared to the control group. Whereas, within the plants treated with DA, PC was only reduced by 50% in AP and 44% in seedlings respect to the control group. These results, together with those observed, showed a tendency towards a reduction in growth after application of both herbicide formulations. This reduction was not statistically significant.

The analysis of variance detected significant variations in LA for both factors studied as well as for the interaction between them (P>0.05). The control seedlings showed a significantly greater final LA compared to the rest of the treatment, which showed similar LA (Figure 1a). A greater LA could be indicative of a high growth potential, and this is important to determine the relative competitiveness of the weeds (Storkey et al. 2005). The reduction in the LA was similar with both formulations.

Seedlings control plants showed greater LA and PC than AP because they were in their establishment stage. To achieve its implantation, they need to develop a minimum occupation structure of the space (Barthélemy et al. 1997). That is why most of the parameters and rates analyzed in this work are higher in seedlings. On the contrary, the plants AP continue with their general growth strategy based on moderate growth (Panigo et al. 2012). Once again, it is evident that compensations and allocations of resources work differently between growth stages.

Biomass accumulation

The magnitude of reduction of shoot biomass was significantly higher in treated plants. The untreated seedlings showed three and seven times more accumulated biomass compared to the ones treated with IPA and DA respectively (Figure 1b). In AP the differences were higher, control plants presented five and eight more biomass compared to the ones treated with IPA and DA, respectively. This parameter was not significantly influenced by the growth stages. Biomass accumulation is one of the determining factors that increased the competition ability of *C. erecta* (Nisensohn et al. 2011). Thus, the reduction of biomass accumulation will critically affect the competition ability of the weeds (Tardif et al. 2006). In the field, the management of *C. erecta* with glyphosate must be complemented with

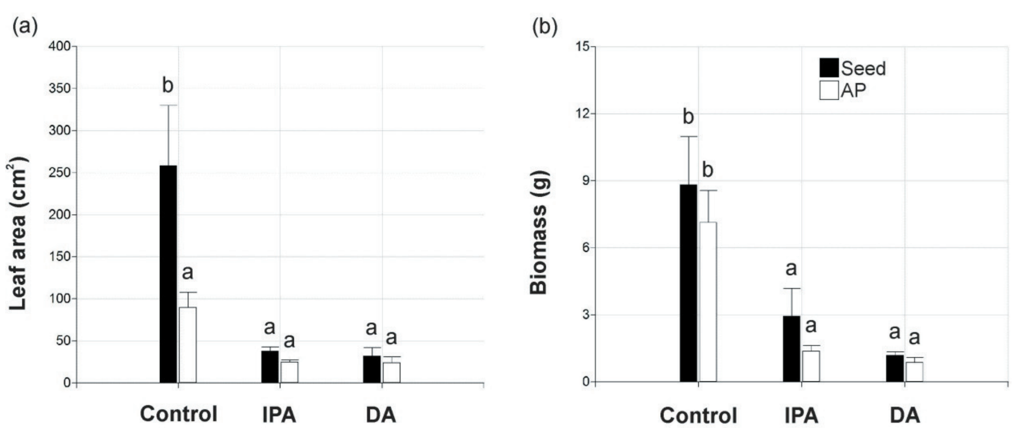

Figure 1. Leaf area (a) and biomass accumulation (b) of *Commelina erecta* plants. The graph shows plants treated with a glyphosate isopropylamine salt (IPA) and plants treated with a glyphosate diammonium salt (DA) at 21 days after application and plants without herbicide treatment (Control). Black and white columns represent mean values from two growth stages: seedlings with five leaves (Seed) and adult plant (AP), respectively. The vertical lines above the bars indicate the standard error. Means with a common letter are not significantly different (P>0.05), according to Fisher's LSD test.

Figura 1. Área foliar (a) y biomasa (b) en plantas de *Commelina erecta*. El gráfico muestra plantas tratadas con glifosato formulado a base de la sal isopropilamina (IPA), plantas tratadas con glifosato formulado a base de la sal diamónica del ácido (DA) a los 21 días post-aplicación y plantas sin tratamiento herbicida (Control). Las columnas negras y blancas representan los valores medios de dos edades: plantas jóvenes (Seed) y plantas adultas (AP), respectivamente. Las líneas verticales arriba las barras indican el error estándar. Las medias con letra común no son significativamente distintas (P>0.05), según la prueba LSD de Fisher.

other tactics, because although its biomass is affected, this weed persists.

Total chlorophyll

The chlorophyll content was similar between growth stages in all sampling dates. At 24 HAA seedlings control showed significantly greater chlorophyll content compared to the seedlings treated with both formulations (Figure 2a). At 48 HAA, AP treated with DA showed a significant lower content than the rest (Figure 2b). Glyphosate toxicity often results in a limitation in photosynthesis (Mateos-Naranjo and Perez-Martin 2013). In sensitive species, glyphosate reduce the chlorophyll content after application until the effective death of the plants (Suresh Kumar et al. 2019). In *C. erecta*, the glyphosate affected chlorophyll content in the leaf shortly after treatment. This corresponds with the findings in other problematic weeds (Fuchs et al. 2002; Kempenaar et al. 2010). After 48 HAA, the chlorophyll content was recovered, regardless of the growth stages.

Injury level

The types of injury observed on the plants were: mottled, chlorosis, totally or partially necrotic tissues, folded sheets, waved edges, prefoliations and chlorotic apexes, etc. These typical injuries are related to the mechanism of action of glyphosate (Shaner 2009). The extent of the injury caused by glyphosate formulations increased over time (Figure 3). At 7 DAA injuries were more obvious in the AP than in seedlings, whereas similar injuries between formulations were observed. At 14 and 21 DAA injury levels differed only between glyphosate formulations. Visual injury over 50% (to 90%) was recorded on the surface of leaves treated with DA, and necrosis was observed in stems and leaves. In general, plant treated with IPA showed slightly lighter injury levels (15-80%) and necrosis was only observed in leaves.

Stem starch proportion

In the apical section, stem starch proportion was always low (<15%) in all treatments, at all dates (Table 2). In this section, significant differences for growth stages were only found at 7 and 14 DAA, whereas significant differences between formulations were observed in the three sampling date analyzed (P<0.05). At 7 DAA, the AP and those plants exposed to DA formulations were the ones that showed the highest starch proportion. Meanwhile, at 14 and 21 DAA, the interaction between the factors was detected. Stem starch proportion was reduced in two growth stages treated with the DA formulation at both dates. Moreover, the stem starch proportion in seedlings treated with the IPA formulation was significantly higher than the rest at 14 DAA, whereas was the treatment with the smallest proportion at 21 DAA.

The proportion of starch in stem apical section was reduced after glyphosate application, but

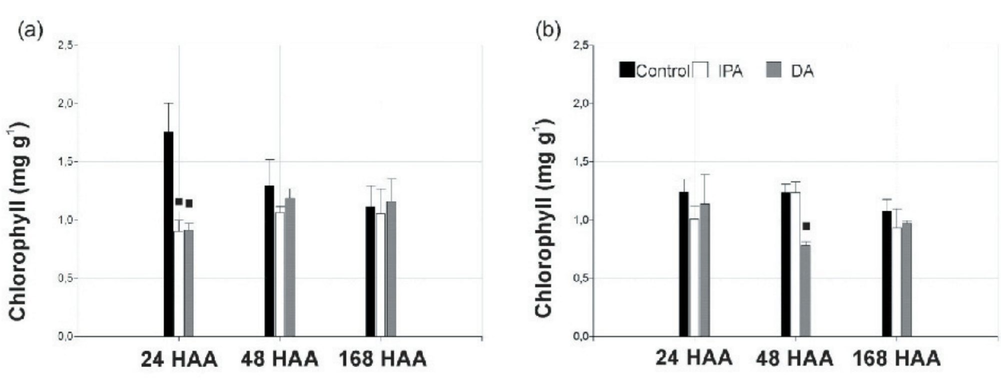

Figure 2. Total chlorophyll content in *Commelina erecta* leaves. a) Seedlings with five leaves; b) adult plant (b). The columns represent changes at 24, 48 and 168 hours after application (HAA) of glyphosate isopropylamine salt (IPA, white), plants treated with glyphosate diammonium salt (DA, gray) and in plants without herbicide treatment (Control, black). Vertical lines above the bars indicate the standard error of the mean. Squared above denote significant differences to the control group (P<0.05), according to Fisher's LSD test.

Figura 2. Contenido de clorofila total en hojas de *Commelina erecta*. a) Plantas de 5 hojas; b) plantas adultas. Las columnas representan los cambios a las 24, 48 y 168 horas después de la aplicación (HAA) de glifosato formulado a base de sal isopropilamina (IPA, blanco) y glifosato formulado a base de la sal diamónica (DA, gris), y en plantas sin tratamiento herbicida (Control, negro). Las líneas verticales sobre las barras indican el error estándar de la media. Los cuadrados arriba barras indican diferencias significativas con respecto al control (P<0.05), según la prueba LSD de Fisher.

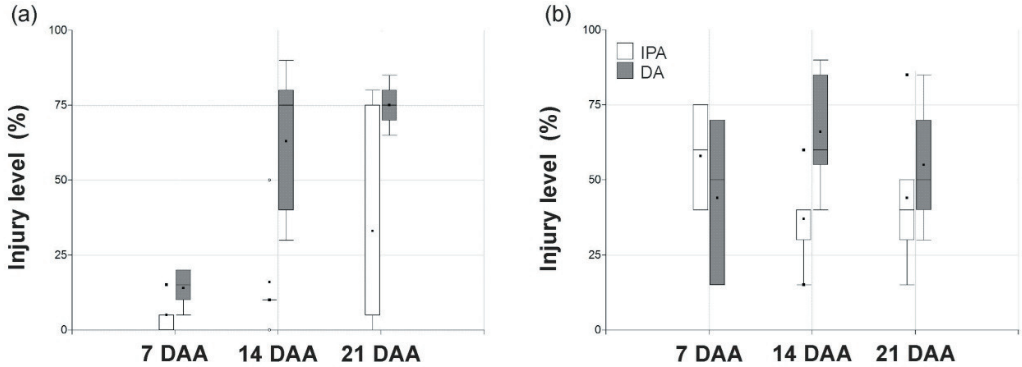

Figure 3. Visible leaf injury level in the plants of *Commelina erecta* at 7, 14, 21 days after application (DAA), caused by the application of glyphosate isopropylamine salt (IPA, white) and glyphosate diammonium salt (DA, gray). Two growth stages: a) seedlings with five leaves and b) adult plants, were compared. The boxes represent the range between 25% and 75% percentile. The line in the box represents the median and the whiskers mark the 5% and 95% percentiles. The black squares represent extreme values when they are outside the whiskers and the means when they are inside the box.

Figura 3. Lesiones foliares visibles en plantas de *Commelina erecta* a los 7, 14 y 21 días después de la aplicación (DAA) de glifosato formulado a base de la sal isopropilamina (IPA, blanco) y de glifosato formulado a base de la sal diamónica del ácido (DA, gris). Se comparan dos edades: a) plantas con cinco hojas y b) plantas adultas. Las cajas representan el rango entre el percentil 25% y el 75%. La línea de la caja representa la mediana y los bigotes marcan los percentiles 5% y 95%. Los cuadrados negros representan los valores extremos cuando están fuera de los bigotes y las medias cuando están dentro de la caja.

not uniformly. In seedlings treated with IPA stem starch proportion, was reduced between 14 and 21 DAA. In the rest of treatments, stem starch proportion decreased, compared to 7 and 21 DAA. This reduction can be related to the decrease in starch transport towards the apical meristems. With the senescence of the leaves, the plants must use the starch from the reserves to survive the effects of the glyphosate, and as this starch cannot be replaced, it reduces its transport towards destinies such as the apical meristems (Tuffi Santos et al. 2004).

Commelina erecta is a rhizomatous species (Panigo and Nisensohn 2018); therefore, the proportion of stem starch was high in the stem basal section. In this section, significant differences between glyphosate formulations at 7, 14 and 21 DAA were observed ($P<0.05$). In all sampling dates, plants treated with DA showed a lower starch proportion (Table 2). But at 14 DAA, as occurred in the apical zone, the effect of formulations and growth stages were not independent ($P<0.05$). In plants treated with DA, the proportion of starch was more affected in seedlings, whereas in the plants treated with IPA the proportion of starch was more affected in AP. On the other hand, ANOVA detected only significant variations between growth stages at 21 DAA ($P<0.05$). Starch proportion was significantly minor in seedlings than in AP.

Throughout the sampling, the basal section showed a different tendency between growth stages. Adult plants (AP) treated with both formulations first increased and then maintained the starch proportion in the basal section, whereas seedlings treated with

Table 2. Starch proportion in the apical and basal section of *Commelina erecta* stems. Two different growth stages (seedlings and adult plants) at 7, 14, and 21 days after application (DAA) of glyphosate isopropylamine salt (IPA) and of glyphosate diammonium salt (DA) were compared. Means within a column followed by the same letter are similar at the 0.05 level of significance according to Fisher's LSD test.

Tabla 2. Proporción de almidón en la sección apical y basal de tallos de *Commelina erecta*. Se compararon dos edades de plantas (jóvenes y adultas) a los 7, 14, y 21 días posteriores a la aplicación (DAA) de dos formulados de glifosato (uno a base de la sal isopropilamina [IPA] y otro a base de la sal diamónica [DA]). Las medias dentro de una columna seguidas de la misma letra son significativamente similares al 0.05 según la prueba LSD de Fisher.

	Formulation	Apical section			Basal section		
		7 DAA	14 DAA	21 DAA	7 DAA	14 DAA	21 DAA
Seed	IPA	0.25 A	14.99 B	0.89 A	34 AB	73.8 C	22.1 B
	DA	6.78 B	4.07 A	4.16 B	28.3 AB	35.2 A	7.91 A
AP	IPA	4.01 AB	1.91 A	3.35 B	35.4 B	51.5 B	52.9 C
	DA	13.9 C	2.14 A	2.29 AB	20.7 A	45.4 AB	42.7 C

both formulations first increased and then strongly decreased the starch proportion in the basal section. This different tendency in the stem starch proportion in the basal section could be related to the differential sensitivity to glyphosate between growth stages in *C. erecta*. In non-photosynthetic organs, starch is continuous turnover and this turnover can buffer short-term mismatches, ensuring maintenance and growth (Smith and Zeeman 2020). In treated seedlings, starch provided carbohydrates for growth. The starch proportion in the basal section increased and then decreased, and the growth rate was higher than AP. However, in the treated AP, starch provided carbohydrates for maintenance. The starch proportion in the stem basal section increased and was maintained high, with a moderate growth rate. In perennial weeds, the same species may combine different strategies to tolerate disturbance (Klimešová et al. 2008). In previous work, we had already observed that after 60 DPA *C. erecta* invested in a dual resource allocation strategy according to plant age (Panigo et al. 2019). The pathways of starch turnover are unknown in *C. erecta:* However, likely starch turnover can change profoundly with the growth stage.

Epicuticular waxes

Epicuticular waxes contribute to the regulation of foliar penetration (Schreiber 2010), affecting the bioavailability of foliar-applied herbicides in the plant (Gaba et al. 2017). The distribution and amount of epicuticular waxes vary within species with age, young leaves absorbing certain herbicides more readily because the cuticle wax deposits of young plants are lower than those of older plants (García Torres and Fernández Quintanilla 1991). *Commelina erecta*, showed a different distribution of epicuticular waxes between growth stages (Figure 4a,b). On AP leaves, a uniform layer was observed over the entire leaf surface with amorphous waxy protuberances emerging (Figure 4b). In seedling leaves, a lesser layer and fewer protuberances were observed. The amount of epicuticular waxes in *C. erecta* also varied between plant growths stages (P<0.05). A higher amount of epicuticular waxes was observed on the leaves of adult plants (Figure 4c). Like in other weeds, epicuticular waxes are related to low sensitivity to glyphosate (Malpassi 2006; Acosta et al. 2018) in *C. erecta*. As age advances, there is an increase in the deposition of different chemical compounds in the cuticle, leading to a reduction in the absorption of compounds by the leaf (Hull et al. 1975).

CONCLUSIONS

The formulations caused different effects on growth and physiological parameters in the *C. erecta* plants. The formulation of a glyphosate diammonium salt (DA) showed a higher decrease in the elongation rate and chlorophyll content at 48 hours after application in adult plants, caused more injuries and negatively affected the starch proportion in the stem basal section. The glyphosate isopropylamine salt (IPA) showed a higher decrease in the branching rate and phyllochron. Both

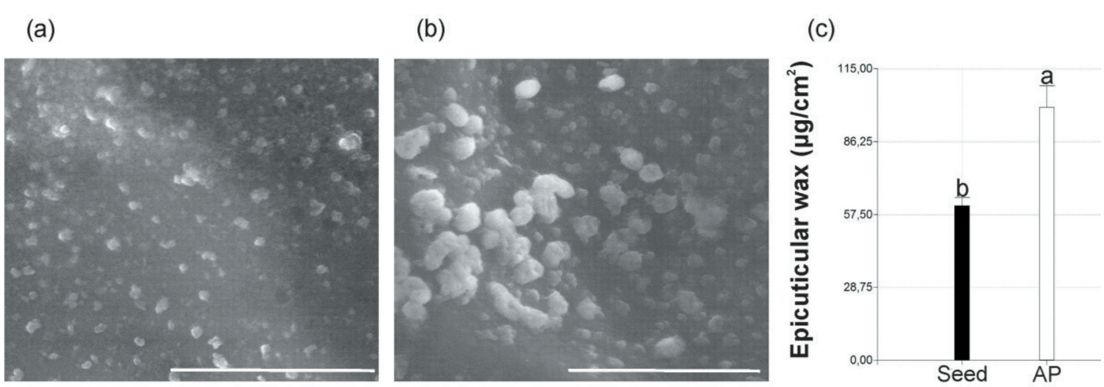

Figure 4. Epicuticular waxes on leaves of *Commelina erecta*. Distribution of waxes in leaves of two growth stages: a) seedlings with five leaves and b) adult plants (SEM, scale bar=25 µm). c) Amount of waxes (µg/cm^2) in both growth stages: seedlings (Seed) and adult plants (AP). The vertical lines above the bars indicate the standard error. Means with a common letter are not significantly different (P>0.05), according to Fisher's LSD test.

Figura 4. Ceras epicuticulares en hojas de *Commelina erecta*. Distribución de las ceras epicuticulares en hojas de dos edades: a) plantas con cinco hojas y b) plantas adultas (SEM, barra de escala=25 µm). c) Cantidad de ceras (µg/cm^2) en ambas edades: plantas jóvenes (Seed) y plantas adultas (AP). Las líneas verticales arriba las barras indican el error estándar. Las medias con letra común no son significativamente diferentes (P>0.05), según la prueba LSD de Fisher.

formulations decreased leaf area, biomass and chlorophyll content of seedlings at 24 hours after application. These results show that both glyphosate formulations are not efficient for the effective management of *C. erecta*.

Both growth stages presented starch storage in the stem sections. However, the results showed that the variation of starch in the basal stem section after herbicide application was different in the seedlings and adult plants. No constant pattern of variation in the proportion of starch in the stem apical section was detected. In addition, the amount of epicuticular waxes was higher in adult plants of *C. erecta*. The difficult management of older plants in fields with intensive glyphosate applications could be involved in the maintenance of starch proportion in the stem basal section after application and the higher amount the epicuticular waxes in the leaves.

The results showed that the growth, the reallocation of starch after application, and the amount of epicuticular waxes are different in the seedlings and adult plants. Consequently, the management strategy should be different between growth stages. For effective management chemical, mechanical, and flaming tactics (single or combined), should be used. According to our results, we recommend tactics that remove aboveground biomass in seedlings (during early spring and summer) and that disrupt starch storage in AP (during late summer).

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