Tolerance of pedunculate oak *(Quercus robur)* saplings to herbicides

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ABSTRACT

Spraying herbicides to reduce weed competition is a usual practice in agricultural regions. However, tree tending under agroforestry or forest plantations is hampered by the extensive use of these pesticides. Here, we assessed the survival, symptoms expression and growth of pedunculate oak *(Quercus robur)* saplings after spraying different herbicides at recommended doses in two seasons: winter and spring. Three independent herbicide trials were carried out on oak sapling plots (E_WINTER 2013, E_SPRING 2012, E_SPRING 2013) in Ayacucho, province of Buenos Aires, Argentina. Plants were kept under uniform conditions in a nursery for about three months. We alternatively tested the herbicides (doses in sub-index, $cc.ha^{-1}$ or $g.ha^{-1}$) flumioxazin ($FLUM_{100}$), glyphosate ($GLIF_{2000,\ 4000,\ 6000}$), metsulfuron-methyl ($METS_{8}$), dicamba ($DIC_{120}$), propaquizafop ($PROP_{500,\ 1000}$), flurochloridone ($FLUO_{1000,\ 1500,\ 4000}$), atrazine ($ATR_{2000,\ 4000}$), acetochlor ($ACET_{2000}$), imazethapyr ($IMAZE_{500,\ 1000}$) and imazapyr ($IMAZA_{33,\ 100}$). At the end of each trial all plants survived, regardless of treatment, although the symptoms varied depending on the trial and product. Glyphosate associated with chlorosis, necrosis and deformation, propaquizafop with necrosis, and dicamba, flurochloridone, imazethapyr and imazapyr with chlorosis. ATR_{2000} and $FLUO_{1500}$ treatments advanced the re-sprout phases. In terms of growth, saplings sprayed with $IMAZE_{1000}$, $IMAZA_{100}$ and $GLIF_{6000}$ had lower total dry weight than the control (CT) ($p<0.05$), in E_SPRING 2012. In E_WINTER 2013 differences of dry weight were not significant, but in E_SPRING 2013, $FLUO_{1500}$ sprayed saplings had a lower dry weight of roots than CT ($p<0.05$). Total plant height and stem diameter presented similar trends. According to the whole set of symptoms assessed, the application of $FLUM_{100}$, $ACET_{2000}$, ATR_{2000} or $METS_{8}$ would be compatible with oak saplings production. The low effect of herbicides in wintertime might be related to different ways of avoidance to herbicide penetration: root depth, the absence of foliage or to the protective structure of buds (perulae); in spring, to root depth, leaf cuticle thickness and hairiness, and to the (high) capacity of the oaks to re-sprout. Although the results obtained are promising for oak plantation in sites exposed to common herbicides, further research is needed to assess long term responses, or to repeated applications over time.

Keywords: weed control, agroforestry, symptoms, growth, survival.

RESUMEN

El uso de herbicidas en áreas agrícolas es una práctica usual para reducir la competencia de malezas. Sin embargo, la plantación de árboles en sistemas agroforestales o en macizos es amenazada por la aplicación extensiva de estos agroquímicos. En este estudio se evaluó supervivencia, síntomas y crecimiento inicial de plantines de roble pedunculado (Quercus robur) tratados con distintos herbicidas bajo las dosis usualmente recomendadas y en dos épocas del año: invierno y primavera. Con este fin se llevaron a cabo tres ensayos

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independientes sobre plantines de roble (E_WINTER 2013, E_SPRING 2012, E_SPRING 2013) en la localidad de Ayacucho, provincia de Buenos Aires, Argentina. Las plantas se mantuvieron en el vivero durante aproximadamente tres meses bajo condiciones uniformes. Se probaron alternativamente los herbicidas (dosis en subíndices, en cc.ha^{-1} o g.ha^{-1}): flumioxazin ($FLUM_{100}$), glifosato, ($GLIF_{2000, 4000, 6000}$), metsulfuronmetil ($METS_8$), dicamba (DIC_{120}), propaquizafop ($PROP_{500, 1000}$), flurocloridona ($FLUO_{1000, 1500, 4000}$), atrazina ($ATR_{2000, 4000}$), acetoclor ($ACET_{2000}$), imazetapir ($IMAZE_{500, 1000}$) e imazapir ($IMAZA_{33, 100}$). Al finalizar los ensayos todas las plantas sobrevivieron, independientemente de los tratamientos, aunque los síntomas variaron según los ensayos y productos aplicados. El glifosato se asoció con clorosis, necrosis y deformación de las hojas, propaquizafop con necrosis, y dicamba, fluorocloridona, imazetapir e imazapir con clorosis. Los tratamientos ATR_{2000} y $FLUO_{1500}$ adelantaron las fases de rebrote. En cuanto al crecimiento, los plantines tratados con IMAZE1000, $IMAZA_{100}$ y $GLIF_{6000}$ alcanzaron un menor peso seco total que el control (CT) ($p<0.05$) en el ensayo E_SPRING 2012. En el ensayo E_WINTER 2013 no se observaron diferencias significativas en el peso seco, pero en E_SPRING 2013, con $FLUO_{1500}$ los plantines alcanzaron menor peso seco de raíces que el control ($p<0.05$). Las alturas totales y los diámetros de tallo presentaron tendencias similares. Según los síntomas observados en el total de los ensayos, $FLUM_{100}$, $ACET_{2000}$, ATR_{2000} y $METS_8$ serían compatibles con los plantines de roble. Los modos de evadir la penetración de los herbicidas en el período invernal pueden relacionarse con la profundidad de las raíces, con la ausencia de hojas y con la protección de las yemas por pérulas; y en la primavera con la profundidad de las raíces, con el espesor de la cutícula y con la pilosidad foliar, y con la alta capacidad de rebrote de los robles. Si bien los resultados alcanzados son promisorios para la implantación de robles en sitios expuestos a herbicidas de uso corriente, es necesario conducir otros estudios para evaluar la reacción de las plantas a largo plazo, o sujetas a aplicaciones repetidas en el tiempo.

Palabras clave: *control de malezas, sistemas agroforestales, síntomas, crecimiento, supervivencia.*

INTRODUCTION

Weed competition is a major issue for native regrowth or planted forests establishment in temperate regions (Vasic *et al.,* 2014, 2013; Willoughby *et al.,* 2006; Dixon *et al.,* 2005) and their control require combined strategies (Vasic *et al.,* 2014, 2013; Vencill *et al.,* 2013; Devine and Harrington, 2010; Tu *et al.,* 2001; Jiménez and Cabezuelo, 1995). In pedunculate oak *(Quercus robur)* saplings, competition results in lower sprouts growth, plant dry weight, stem diameter, nitrogen concentration, plant water potential and stem conductance (Venegas and Palazuelos, 2009; Lof, 2000). These effects are similar to those caused by low soil water potential, while weeds also reduce light, temperature and the availability of nitrogen in the soil (Venegas and Palazuelos, 2009; Lof, 2000). In trials with different European oak trees and other temperate hardwoods, Cogliastro *et al.* (1990) found, after three growing seasons, that the growth of the trees was higher in treatments with herbicide applications or mechanical control of weeds, which was associated to a larger availability of foliar nitrogen and phosphorus.

The use of pre or post-emergence herbicides is a usual and effective management technique to reduce competition from weeds, particularly in temperate cropping regions (Rosales Robles and Esqueda Esquivel, 2008), and when it is possible, it is combined with mechanical removal (Vasic *et al*., 2013; Vencill *et al*., 2012; Jiménez and Cabezuelo, 1995). However, direct spraying or the drift of herbicides can significantly affect the survival or growth of forest saplings (Matschke and Machačkova, 2002; Jiménez and Cabezuelo, 1995) of both natural forests and plantations. There is little information on the effects of herbicides on forest saplings (Matschke and Machačkova, 2002). In the Argentine pampas and other major agricultural regions of the world, the incorporation of trees under agroforestry systems or in plantations close to crops is hampered by the extensive application of herbicides.

Some post-emergence herbicides, such as cycloxydim, clopyralid and metazachlor have been tested on seedlings of *Q.robur* and *Prunus avium* with 6-8 expanded leaves, and on *Fraxinus excelsior* and *Acer pseudoplatanus* with 2-4 expanded leaves (Willoughby *et al.,* 2006). Survival was not affected in any case, although metazachlor reduced growth after repeated applications. In a test with a variety of coniferous and broadleaved species, clopyralid alone or combined with graminicides (cycloxydim, fluazifop-P-butyl, propaquizafop) did not affect the survival or growth of saplings (Dixon *et al.,* 2005). Another essay, on one to three years old pedunculate oak naturally regenerated plants monitored throughout three growing seasons, showed phytotoxicity from tribenuron-methyl, but not from nicosulfuron or imazamox (Vasic *et al.,* 2014). In controlled trials on two-year-old oak saplings *(Q. rotundifolia)*, Jiménez and Cabezuelo (1995) found (i) high toxicity of systemic herbicides like glyphosate, triclopyr or glufosinate-ammonium and residual herbicides as pendimethalin and diuron, causing mortality four months later, (ii) temporary or mild phytotoxicity of contact and residual herbicides as thiazopyr, or oxyfluorfen, and (iii) absence or very mild symptoms with simazine, flupoxan, and fluazifop-P-butyl. The application of simazine presented significant differences in height growth compared to the remaining treatments.

On the other hand, Matschke and Machačkova (2002) found, within one year after 2-4-D and glyphosate applications, a reduction of the concentration of indole acetic acid and cytokinins in oak *(Q.robur)* leaves, particularly with glyphosate. These authors highlighted that some morphological and physiological effects can be expressed in the next season, due to damages to dormant apical meristems. Also, in a trial with different sulfonylureas and asulam, damages of oak saplings occurred with metsulfuron-methyl and asulam (Lawrie and Clay, 1993). Iliev *et al.* (2013) searched the effects of the application of oxyfluorfen, fluazifop-P-buthyl, quizalofop-P-ethyl and fenoxaprop-P-ethyl in field tests on two-year-old saplings of *Q. cerris* during 100 days of the growing season. While survival was not significantly affected, oxyfluorfen caused less growth in height, while the remaining herbicides (from the arilofenoxi propionic family) induced similar or greater plant height.

In this study, we explored the survival, symptoms expression, and growth responses of pedunculate oak saplings to the application of recommended doses of different herbicides under two seasons conditions, late winter and late spring. Both seasons are related to the opportunity of application of herbicides on winter and summer crops in temperate areas of the pampas of Argentina.

MATERIALS AND METHODS

Three independent trials of tolerance of oak saplings to herbicides were conducted in Ayacucho, province of Buenos Aires, Argentina. Different products and dosage (treatments) of herbicides were tested on a single application (table 1). One trial was initiated in August, late winter (E_WINTER 2013), and the remaining two in November, late spring (E_SPRING 2012, E_SPRING 2013). The trials lasted 98, 111, and 90 days, respectively. These seasons coincide with pre-sprouting (late winter) or unfurled leaves (spring) phenological phases of the oak saplings (Díaz-Maroto *et al.,* 1993) and match with the opportunity of herbicide application for winter and summer grain crops in the pampas. One-year-old saplings raised in the nursery from local seeds were used. Plants were chosen randomly from the nursery among the best qualified (unique and straight stems with no forks, upper buds healthy and flawless stems) and transplanted to individual plastic pots with 8 drainage holes around the bottom, 20 cm high and 12 cm diameter (volume: 2.26 liter). The substrate was ground dark soil from an A horizon of a local Typic Argiudoll. Each treatment plot was constituted by lots of 15 (E_SPRING 2012) or 30 saplings (E_WINTER 2013, E_SPRING 2013). The herbicides were sprayed using a Giber H® manual sprayer 1.5 kg.cm^{-2} previous pressure, and 3 kg.cm^{-2} maximum pressure. Each application was done in a single pass over the plants arranged in a row, simulating a sprinkler of a field spraying machine. The doses were prepared with neutral water, in the range recommended by the manufacturer on the basis of 100 l.ha^{-1} of the application broth. A precision scale and a graduated cylinder were used for the measurement of the products. Control plants were treated with neutral water only. Both lots of treated and control plants were labeled and distributed randomly in

TRIAL	**E_WINTER 2013**	**E_SPRING 2012**	**E_SPRING 2013**
PERIOD AUG-DEC 2013	PRE-SPROUT	UNFURLED LEAVES	
	NOV 2012-MAR 2013	NOV 2013-FEB 2014	
COMMON NAME/ commercial (ACRONYM)	**TREATMENTS (products x dose)**		
FLUMIOXAZIN / Sumisoya (FLUM)	$FLUM_{100}$		
GLYPHOSATE / Roundup (GLIF)	$GLIF_{4000}$	$GLIF_{2000}$, $GLIF_{6000}$	$GLIF_{4000}$
METSULFURON_METHYL/ MetsulfuronAgros(METS)	$METS_{8}$		
DICAMBA/ Hunter Agros (DIC)	DIC_{120}		
PROPAQUIZAFOP/ Agil (PROP)		$PROP_{500}$, $PROP_{1000}$	
FLUROCHLORIDONE/ Rainbow (FLUO)		$FLUO_{1000}$, $FLUO_{4000}$	$FLUO_{1500}$
ATRAZINE / Mazina 50(ATR)		ATR_{2000}, ATR_{4000}	ATR_{2000}
ACETOCHLOR / Acetoclor Helm (ACET)		$ACET_{2000}$, $ACET_{4000}$	$ACET_{2000}$
IMAZETHAPYR / Pivot (IMAZE)		$IMAZE_{500}$, $IMAZE_{1000}$	
IMAZAPYR / Clearsol(IMAZA)		$IMAZA_{33}$, $IMAZA_{100}$	

Table 1. Herbicides, treatments (product x dose) and time period of the trials. Treatments are identified by an abbreviation of the common name of the herbicides with a sub-index representing the dose of active product (cc.ha^{-1}, except for imazapyr and metsulfuron-methyl, in g.ha^{-1}).

an open sector of the nursery on a polyethylene mesh to prevent soil rooting. During the trial period, the plants were kept under uniform conditions of luminosity, undergoing regular irrigation by aspersion. The presence of the following symptoms of damage on the leaves of the upper third was visually monitored every 12-15 days: chlorosis (isolated), spread chlorosis, necrosis, outbreak burn, and leaf deformation. At the end of each trial, the total plant height and the collar diameter of the stem were measured in three (E_SPRING 2012) or nine (E_WINTER 2013, E_SPRING 2013) randomly picked plants per treatment. Also, the dry weight of these saplings was determined by oven drying (60°C, seven days) the foliage, stem and roots. Besides, in the last two trials the average phenological phase, total plant height, and stem diameter were recorded over time. Qualitative data were analyzed by hierarchical clustering and principal components analysis (PCA) in bi-plot graphics. Quantitative data were analyzed with ANOVA models considering the treatments as a source of variation of a completely randomized design. Phenology variation was analyzed assuming a divided plot design considering the treatment as main plot factor and the observation date as the subplot (repeated measures over time). Assumptions of normality and homoscedasticity were probed through Shapiro-Wilks and Levene tests. All analyses were performed by using version 3.0.3 of R® (R CoreTeam, 2014), and *lsmeans* package (Lenth, 2014) for multiple comparison tests.

RESULTS

Sapling survival and symptoms

Sapling survival at the end of each trial was 100%, regardless of treatment. However, the treatments induced different symptoms, like chlorosis (partial or spread), necrosis (total, in the edges or in spots), leaf deformation and burning. Fig. 1 shows dendrograms of hierarchical clustering of the treatments according to the observed symptoms in the 2nd - 3rd and the 8th - 9th weeks after the herbicide application. In E_SPRING 2012 (top graphs), 11 days after the treatments, the control (CT) clearly differed from $ACET_{4000}$ and from the group comprising the remaining treatments (cophenetic correlation 0.863). However, 58 days after herbicide applications, the control saplings (CT) presented similarity with the saplings treated with acetochlor (both doses), flurochloridone and atrazine (both only in low doses) (cophenetic correlation 0.782). In E_WINTER 2013, 64 days after treatments (at the stage of unfurled leaves) CT grouped with $FLUM_{100}$ and $METS_{8}$ and apart from $GLIF_{4000}$ and DIC_{120} (cophenetic corr. 0.872). In E_SPRING 2013, after 18 days of herbicide application, CT, ATR_{2000} and $ACET_{2000}$ formed a common group distinctive of $GLIF_{4000}$ and $FLUO_{1500}$ (cophenetic corr. 0.734) and after 62 days, this last treatment joined the $GLIF_{4000}$ group (cophenetic corr.0.631).

On the other hand, PCA with bi-plot graphics (fig. 2) shows the correspondence between treatments and types of symptoms observed in leaves and new shoots. Results of E_SPRING 2012 showed no alignment between CT and any of the observed symptoms for both dates. On day 58, the treatments $PROP_{1000}$, $ACET_{2000}$, $ACET_{4000}$ and ATR_{2000} reversed the initial symptoms while others, such as $GLIF_{2000}$, $IMAZA_{33}$ or $IMAZA_{100}$ showed symptoms not expressed before. In E_WINTER 2013, at day 64, CT saplings and those treated with $FLUM_{100}$, and $METS_{8}$, did not present symptoms in leaves. In E_SPRING 2013, CT, ATR_{2000} and $ACET_{2000}$ showed no symptoms at day 18, although at day 64 CT associated with necrosis and chlorosis.

Phenology

When analyzing the effects of treatments on the phenological phases of the saplings over the time elapsed since herbicide application, an interaction between treatment and date was found in experiment E_WINTER 2013 ($p<0.0001$) (fig.3a). Only in the last observation (day 98), there were significant differences between treatments ($p<0.05$); the phenological phase of saplings sprayed with $GLIF_{4000}$ and $FLUM_{100}$ was more advanced than that of CT saplings, while it was more delayed for those treated with $METS_{8}$. That date was associated with the change of the phase of unfurled leaves of the second regrowth to the start of the third regrowth (phases 6 and 7 of the *y*-axis of Fig.3). On the other hand, there were neither significant differences nor interactions between treatment and date in experiment E_SPRING 2013 ($p<0.06$) (fig.3 b).

Dry weight

Table 2 shows values of dry weight found in each trial, and differences among treatments ($p<0.05$, Tukey´s test). In E_SPRING 2012 there were treatment effects for the dry weight of leaves (ANOVA; $p=1.81x10^{-7}$), stem ($p=0.000125$), roots ($p=0.00044$) and for total dry weight ($p=2.02x10^{-5}$). $IMAZE_{1000}$ and GL_{6000} treated saplings presented the lowest dry weight of leaves, which were not significantly different from those treated with $IMAZA_{100}$, ATR_{2000}, $IMAZA_{33}$, $FLUO_{4000}$ and $IMAZE_{500}$. As for the dry weight of stem $GLIF_{6000}$ and $IMAZE_{1000}$ treatments showed the lowest values, with significant differences only with CT, $ACET_{4000}$ and ATR_{4000}. The lowest values of dry weight of roots were found in $GLIF_{6000}$ and $IMAZA_{100}$ treatments, with significant differences only with CT, $ACET_{4000}$, $PROP_{1000}$ and ATR_{4000}. For the dry weight of the whole plant, $GLIF_{6000}$ presented the lowest value, but not significantly different from $IMAZA_{100,}$ $FLUO_{4000}$, $IMAZE_{1000}$, and $IMAZE_{500}$. In E_WINTER 2013, no treatment effects were found, and as for E_SPRING 2013 only $FLUO_{1500}$ showed significant effects in the dry weight of roots, presenting a lower value than CT ($p<0.05$).

Diameter and height

In E_SPRING 2012 there were treatment effects for stem diameter ($p=2.38x10^{-8}$) and total plant height ($p=1.3x10^{-8}$). The plants treated with $GLIF_{6000}$ and $IMAZE_{1000}$ showed the lowest diameters, significantly different from CT. $IMAZE_{1000,}$ $GLIF_{6000}$ and $IMAZA_{100}$ treatments presented the lowest total

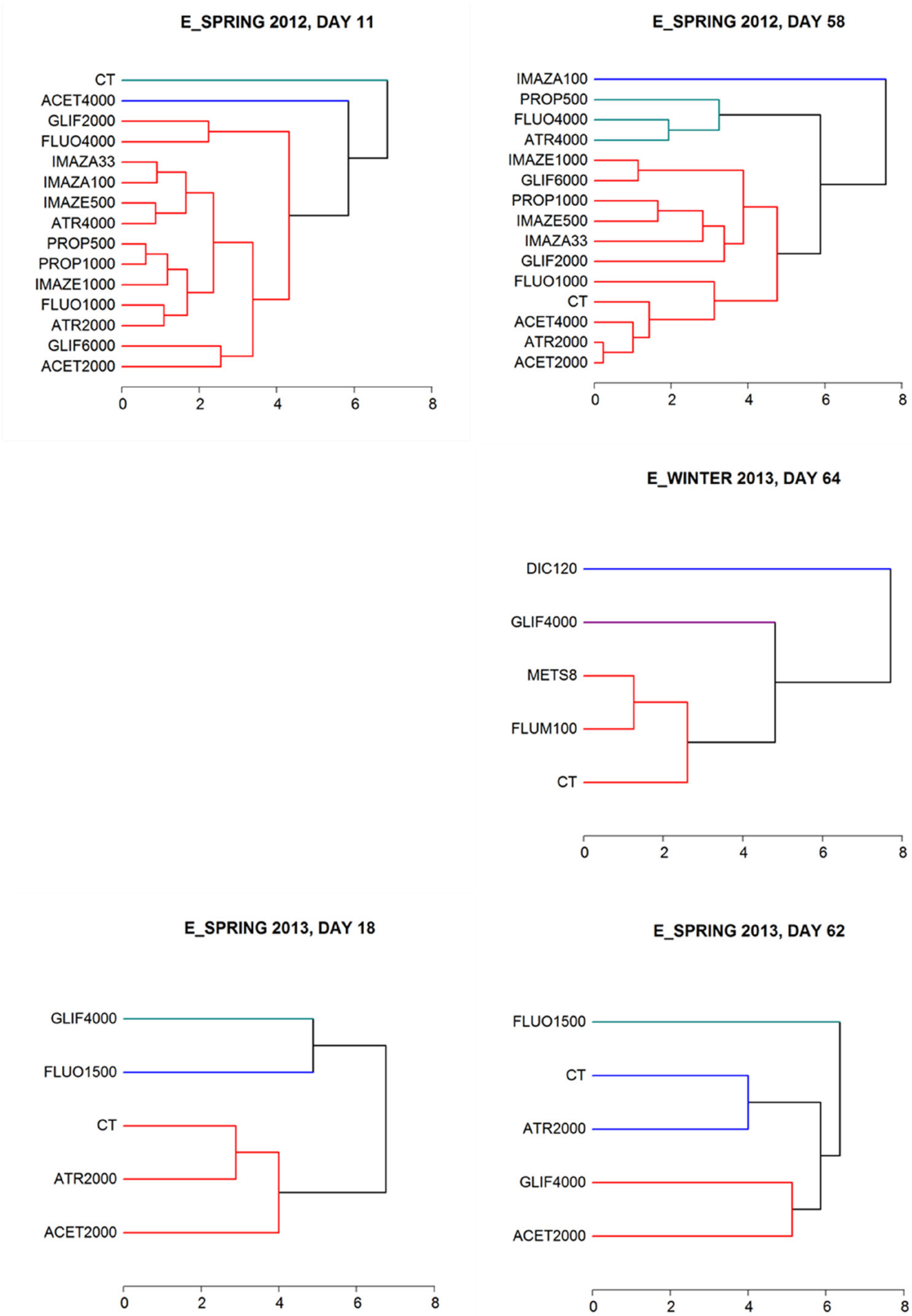

Figure 1. Cluster analysis (Manhattan distance) for testing: E_SPRING 2012 (above), E_WINTER 2013 (center) and E_SPRING 2013 (below), 11-18 days (left) and 58-64 days after treatment (right), with a three group leveling.

plant height, significantly different from CT. The combined variable diameter^2 x height also showed treatment effects (p=1.97x10^{-6}), and CT differed from $IMAZE_{1000}$ and $GLIF_{6000}$ treated plants, showing the highest value. There were no treatment effects in stem diameter or total plant height in E_WINTER 2013. In E_SPRING 2013, effects in total plant height were not found, but the stem diameter and the diameter^2 x height of CT and ATR_{2000} treated plants were significantly higher than those of $FLUO_{1500}$, $GLIF_{4000}$ and $ACET_{2000}$ treated plants.

DISCUSSION

Survival

All of the plants survived at the end of the trials, regardless of the treatment. The survival capacity of the saplings

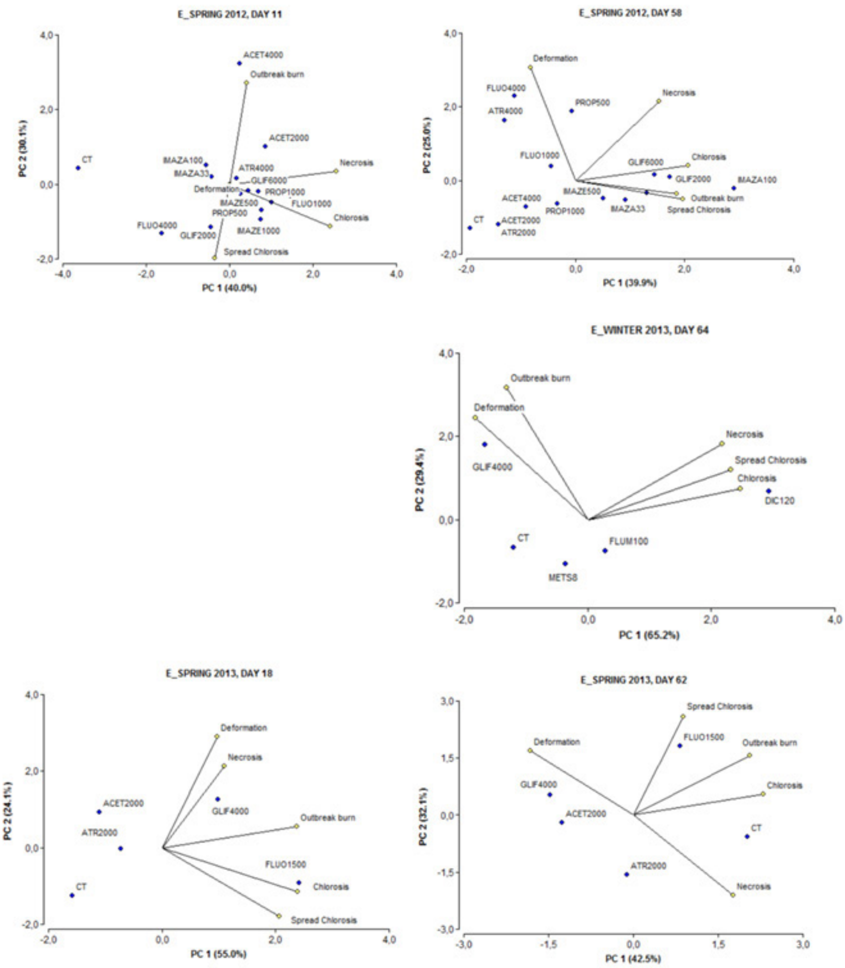

Figure 2. PCA with bi-plot graphics of E_SPRING 2012(above), E_WINTER 2013 (center) and E_SPRING 2013 (below),11-18days (left) and 58-64 days (right). Every inner radius represents the variation axis of the observed symptoms.

could be attributed to factors such as the spatial avoidance of underground biomass, with saplings developing fine roots away from the action zone of the pre-emergence herbicides (i.e. atrazine, acetochlor), or temporary avoidance, in case of winter applications. In winter, the absorption of contact herbicides would be restricted by the absence of leaves and the lignification of the stems. For example, glyphosate does not penetrate through the woody bark (Tu *et al.,* 2001). Also, aerial ingress through the buds would be limited by the protection of these meristems by scales (Feucht, 1988). On the other hand, tolerance and resistance to herbicides (*sensu* Vencil *et al.,* 2013) in actively growing saplings with expanded leaves would be given both by the extensive regrowth ability of white oaks (Qi *et al.,* 2006; Collet *et al.,* 1997) and by the leaf cuticle barrier, with thick adaxial palisade parenchyma (Qi *et al.,* 2006). While other barriers to herbicide penetration, such as the density and type of trichomes, are lower in *Q. robur* than those of other white oaks as *Q. petraea* or *Q. pubescens.* Doupuey and Badeu (1993) reported the presence of hairiness in leaf lamina, veins and petioles. Foliar trichomes of this species would be simple, serial and solitary in both sides (Penas *et al.,* 1994).

Symptoms and phenology

The analysis (cluster and PCA) of the observed symptoms in the upper leaves and sprouts showed variations between treatments (fig.1). Glyphosate treatments (different doses) associated rather with chlorosis, necrosis and deformation, propaquizafop with necrosis, and dicamba, flurochloridone, imazethapyr, and imazapyr treatments with chlorosis (imazapyr also caused outbreak burning) (fig.2). $FLUM_{100}$, $ACET_{2000}$, ATR_{2000} and $METS_8$ treatments grouped together with CT, thus they seem to be more compatible for oak saplings survival and growth than the rest of the evaluated herbicides (fig. 2).

On the other hand, in E_WINTER2013 the phenological curves differed from the control only in the last observa-

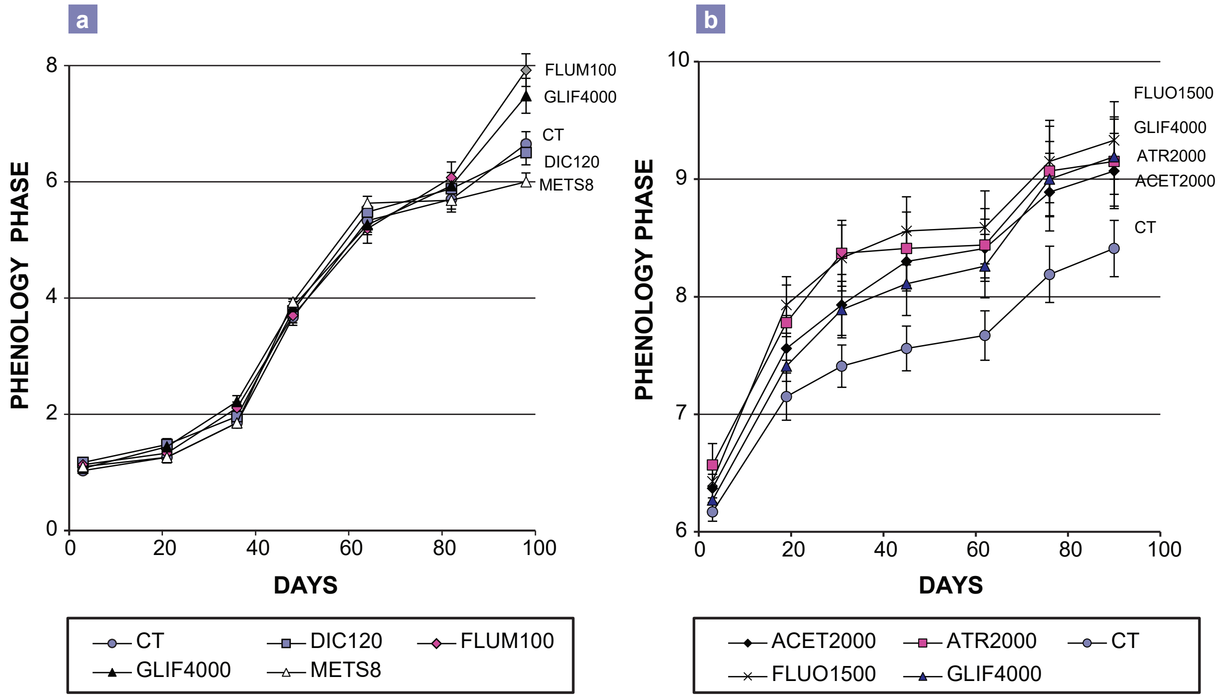

Figure 3. Phenological phases of saplings by treatment overtime (mean ± SE) of E_WINTER 2013 (a) and E_SPRING 2013 (b). Phases (y-axis) are: (1) leaves unfurled (first outbreak of the year), (2) upper swollen bud, (3) elongated green bud, (4) beginning of sprouting (leaf primordia of the second regrowth), (5) first unfurled leave, full sized (second regrowth), (6) final sprouting of the upper third (all leaves developed), (7) swollen bud (third regrowth), (8) elongated green bud (third regrowth), (9) leaf primordia of the third regrowth.

tion date (fig.3); at that time, $GLIF_{4000}$ and $FLUM_{100}$ treated plants advanced the third regrowth flush when compared to CT (p=0.06), while plants under $METS_8$ treatment remained in the previous stage (phase 6, all leaves of the second regrowth developed) (fig. 3, left). In the E_SPRING 2013 trial, the CT curve separated from the all the rest at phase 7 (swollen bud of third regrowth), and differences clearly aroused from the third flush of growth (phase 8) (fig. 3, right), showing premature aging of leaves caused by herbicides spraying. Both trials results suggest a phenological response to some herbicides at late flushing stages due to previous harm to foliage.

Dry weights, diameters and heights

No significant differences between treatments were found in dry weight, stem diameter or total plant height in the winter trial (E_WINTER 2013) (table 2), suggesting that the above mentioned late effects in foliar damage and phenology would have not affected early plant growth under any treatment. Instead, some treatments in any of the springtime trials somehow affected the growth of saplings (table 2), showing total or partial depression of growth in weight or size of plants treated with different products and doses ($GLIF_{6000}$, $GLIF_{4000}$, $IMAZA_{100}$, $IMAZE_{1000}$, $FLUO_{1500}$, $ACET_{2000}$).

The phytotoxicity of glyphosate we found for *Q. robur* has also been reported for plants of the genus *Quercus* and other hardwoods in previous studies (Matschke and Mačkova, 2002; Jiménez and Cabezuelo, 1995). On the contrary, reported damage caused to oaks by metsulfuron (Lawrie and Clay 1993) was not observed in our winter trial. The effect of the herbicides we tested, grouped by their mechanism of action (Papa, 2007; Tu *et al.*, 2001; CIAT, 1982), was not consistent with results previously published. For example, among the inhibitors of lipid synthesis (Group I), it was reported that quizalofop-P, fluazifop-P or cycloxydim did not damage and even promoted the growth of lateral roots (Lawrie and Clay,1993; Willowghby *et al.,* 2006) while in the present study propaquizafop, of the same pole group, caused leaf damage. Among the destroyers of cell membranes (Group II), all reported herbicides (metsulfuron-methyl, asulam, oxidazon, oxyfluorfen) expressed different levels of phytotoxicity (Jiménez and Cabezuelo,1995; Lawrie and Clay, 1993) although here, flumioxazin and metsulfuron-methyl appeared to be compatible with the oaks.

CONCLUSIONS

Oak seedlings behaved tolerant to various agrochemicals during early growth stages, underpinning the possibility of herbicide management in alley crops or in traditional crops adjacent to tree plantations. The adverse effects observed here, after direct spraying of the saplings, would be smaller under field conditions following preventive techniques such as directed crop spraying, use of protective screens or anti-drift sprinklers (Leiva, 2015). On the other hand, most of

TREAT	DRY WEIGHT (g.pl-1)									HEIGHT (cm)		DIAMETER (mm)		D^2x HEIGHT (cm^3)	
	LEAVES		STEMS		ROOTS		TOTAL DW		R/S						
E_SPRING 2012															
CT	2.36	ab	5.67	ab	12.69	a	20.72	a	1.58	38.1	ab	10.6	a	42.8	abc
$ACET_{2000}$	2.84	a	5.30	abc	10.04	abc	18.18	ab	1.23	36.1	abc	9.3	abc	31.2	abcd
$ACET_{4000}$	2.62	a	7.09	a	10.95	ab	20.67	a	1.13	39.7	a	10.9	ab	47.2	a
$PROP_{1000}$	2.43	ab	4.43	abc	11.08	ab	17.94	ab	1.62	35.7	abcd	9.1	abc	29.6	abcd
$PROP_{500}$	3.12	a	4.89	abc	10.05	abc	18.05	ab	1.25	33.9	abcd	8.6	abcd	25.1	abcd
ATR_{2000}	2.08	abc	5.18	abc	10.30	abc	17.56	abc	1.42	39.5	a	10.3	ab	41.9	abc
ATR_{4000}	2.58	a	6.14	ab	10.52	ab	19.24	a	1.21	37.6	abc	9.9	a	36.9	abc
$IMAZA_{100}$	1.06	bc	3.42	bc	4.45	c	8.93	cd	1.00	29.1	cde	8.7	abcd	22.0	bcd
$IMAZA_{33}$	1.96	abc	4.56	abc	10.19	abc	16.71	abc	1.56	32.5	abcde	9.7	ab	30.6	abcd
$FLUO_{1000}$	2.32	ab	4.77	abc	10.07	abc	17.16	abc	1.42	31.5	abcde	8.9	abc	25.0	abcd
$FLUO_{4000}$	1.87	abc	4.09	bc	9.82	abc	15.77	abcd	1.65	31.0	abcde	8.9	abc	24.6	bcd
$GLIF_{2000}$	3.21	a	4.92	abc	9.35	abc	17.49	abc	1.15	31.8	abcde	7.7	abcd	18.9	cd
$GLIF_{6000}$	0.88	c	2.42	c	4.36	c	7.66	d	1.32	25.5	e	6.7	d	11.4	d
$IMAZE_{1000}$	0.75	c	2.77	c	5.98	bc	9.49	bcd	1.70	27.3	de	7.4	cd	14.9	d
$IMAZ_{E50}0$	2.18	abc	3.91	bc	9.72	abc	15.81	abcd	1.60	30.5	bcde	8.8	abcd	23.6	bcd
E_WINTER 2013															
CT	2.54	a	4.63	a	5.46	a	12.63	a	0.76	44.4	a	5.8	a	14.99	a
DIC_{120}	2.44	a	4.93	a	5.19	a	12.55	a	0.70	45.5	a	5.9	a	15.89	a
$GLIF_{4000}$	2.53	a	4.96	a	6.27	a	13.76	a	0.84	45.7	a	5.9	a	15.69	a
$METS_8$	1.84	a	4.34	a	5.29	a	11.47	a	0.86	41.8	a	6.2	a	16.21	a
$FLUM_{100}$	2.42	a	4.96	a	5.07	a	12.45	a	0.69	46.3	a	6.0	a	16.40	a
E_SPRING 2013															
CT	2.83	a	6.62	a	8.92	a	18.37	a	0.94	46.9	a	6.7	a	22.7	ab
$ACET_{2000}$	2.70	a	6.14	a	7.87	ab	16.71	a	0.89	47	a	6.2	b	19.29	bcd
ATR_{2000}	3.16	a	6.70	a	8.73	ab	18.59	a	0.89	48.9	a	6.9	a	24.71	a
$FLUO_{1500}$	1.92	a	5.08	a	5.49	b	12.49	a	0.78	45.3	a	6.2	b	18.9	cd
$GLIF_{4000}$	2.21	a	6.26	a	6.38	ab	14.84	a	0.75	46.1	a	6.2	b	18.9	cd

Table 2. Mean dry weight (g.pl^{-1}) of leaves, stems, roots and total, root / shoot ratio, height (cm), stem diameter (mm), and diameter^2 x height (cm^3) by treatment of oak saplings at the end of each test. Same letters show no significant differences (Tukey test $p<0.05$) in each variable (columns).

the responses were not distinctively clear, and even with the experimental control and randomization imposed, other (genetic and environmental) factors (i.e. leaf insect attack) could influence results.

From all treatments, $FLUM_{100}$, $ACET_{2000}$, ATR_{2000} and $METS_8$ behaved suitable to be applied to nearby crops, while $IMAZA_{100}$, $IMAZE_{1000}$, $GLIF_{4000}$ and $GLIF_{6000}$ would not be advisable due to harm risks to the oak seedlings.

The observed differences on herbicides effect on growth between winter and springtime applications also highlight the importance of the opportunity of spraying herbicides. Winter applications would not significantly affect the growth of the oak saplings. The development of roots in-depth, growing away from the action radio of preemergence herbicides (a few centimeters under the ground, i.e. atrazine, acetochlor), the absence of leaves during the period of activity of contact or systemic herbicides (i.e. metsulfuron-methyl, imazethapyr, dicamba), or bud protection by lignified scales, might be some of the mechanisms that prevent phytotoxicity. During that season, flumioxazin and metsulfuron-methyl herbicides did not cause symptoms or loss of growth. Neither dicamba nor glyphosate affected plant growth, although in the last this case the saplings showed symptoms of phytotoxicity. On the contrary, post-emergence herbicides imazethapyr, glyphosate, propaquizafop, imazapyr and flurochloridone sprayed in spring time caused negative effects on the dry weight, plant height or stem diameter of the oak saplings. All these herbicides caused visible leaf symptoms and some anticipated regrowth flushes. In the same sense, atrazine (pre-emergent) would have not affected the saplings, which under the usual nursery practice develop roots of at least 10 cm in depth, much deeper than the activity zone of the herbicide, of about 5 cm (Devine and Harrington, 2010; Aldhous, 1975).

Although the results achieved are highly promising for oak plantation in environments exposed to some commonly used herbicides, further studies are needed to assess the reaction of treated plants in the long term and (or) after repeated applications.

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