Tolerance of pedunculate oak (Quercus robur) saplings to herbicides

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 (FLUM₁₀₀), glyphosate (GLIF₂₀₀₀, 4₀₀₀, 6₀₀₀), metsulfuron-methyl (METS₈), dicamba (DIC₁₂₀), propaquizafop (PROP₁₀₀₀, 1₅₀₀₀, 6₀₀₀), flurochloridone (FLUO₁₀₀₀, ₁₅₀₀₀, ₄₀₀₀₀), atrazine (ATR₂₀₀₀, ₄₀₀₀), acetochlor (ACET₂₀₀₀), imazethapyr (IMAZE₅₀₀, ₁₀₀₀) and imazapyr (IMAZA₃₃, 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. ATR₂₀₀₀ and FLUO₁₅₀₀ treatments advanced the re-sprout phases. In terms of growth, saplings sprayed with IMAZE₁₀₀₀, IMAZA₁₀₀ and GLIF₄₀₀₀ 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, FLUO₁₅₀₀ 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 FLUM₁₀₀, ACET₂₀₀₀, ATR₂₀₀₀ or METS₈ 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...
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; Vinctil 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 (Rosa sales Robles and Esqueda Esquivel, 2008), and when it is possible, it is combined with mechanical removal (Vasic et al., 2013; Vinctil 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, cycloxydim alone or combined with graminicides (cyloxydim, 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 regen erated 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 thia-zopyr, or oxyfluorfen, and (iii) absence or very mild symptoms with simazine, fluopyram, and flufenacet-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, flufenacet-P-butyl, 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 Argudoll. 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

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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).
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 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 apart from GLIF and DIC 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_SPRING 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 and FLUM was more advanced than that of CT saplings, while it was more delayed for those treated with METS. 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^-2), stem (p=0.000125), roots (p=0.00044) and for total dry weight (p=2.0x10^-3). IMAZE treatments showed the lowest dry weight of leaves, which were not significantly different from those treated with IMAZA, ATR and FLUM. The lowest values of dry weight of roots were found in GLIF and IMAZA treatments, with significant differences only with CT, ACET and ATR. The lowest values of dry weight of roots were found in GLIF and IMAZA treatments, with significant differences only with CT, ACET and ATR. For the dry weight of the whole plant, GLIF presented the lowest value, but not significantly different from IMAZA, FLUO and IMAZE. In E_SPRING 2013, no treatment effects were found, and as for E_SPRING 2013 only FLUO 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^-4) and total plant height (p=1.3x10^-4). The plants treated with GLIF and IMAZE showed the lowest diameters, significantly different from CT. IMAZE and GLIF treatments presented the lowest total
plant height, significantly different from CT. The combined variable diameter$^2 \times$ height also showed treatment effects ($p=1.97 \times 10^{-6}$), 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$^2 \times$ 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...
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, fluorchloridone, 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-
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$_{4000}$, GLIF$_{4000}$, IMAZA$_{100}$, IMASE$_{100}$, 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 Macháčkova, 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
the responses were not distinctly 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$_{2000}$, ACET$_{2000}$, ATR$_{2000}$ and METS$_{50}$ behaved suitable to be applied to nearby crops, while IMAZA$_{1000}$, IMAZE$_{2500}$, GLIF$_{2000}$ 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 (i.e. acetochlor) were more phytotoxic. Among the combinations of herbicides used, winter applications would not significantly affect the growth of the oak saplings.

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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.

**ACKNOWLEDGEMENTS**

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