Nutrición inicial en una reforestación de *Juniperus deppeana* Steud. y su capacidad de rebrote posincendio

Initial nutrition and post-fire resprout of a *Juniperus deppeana* Steud. reforestation

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Resumen

Las especies forestales de lento crecimiento son las menos estudiadas. Con un experimento de fertilización (N-P-K) se evaluó el caso de brinzales de *Juniperus deppeana* y su capacidad de rebrote posincendio. Se aplicaron tres niveles de N y P y dos de K para un total de 18 tratamientos con seis repeticiones. Adicionalmente, se comparó en plantas de uno y dos años de edad el incremento en diámetro a la base (IDB). Seis meses después de la fertilización solo la interacción N-P-K fue significativa (P<0.06) para IDB. Los tratamientos con niveles más altos de N presentaron el mayor IDB. A los siete meses de establecido el experimento, se presentó un incendio, y a partir de ese momento se determinó la capacidad de rebrote y concentración de macronutrientes (N-P-K-Ca-Mg-S), para ello se consideró el antecedente de la fertilización. Los efectos evaluados en el experimento fueron la fertilización, edad de la planta y daño por incendio. El porcentaje de rebrote posterior al incendio fue de 84 %. Contrario a lo esperado, los rebrotes de brinzales con antecedentes de fertilización presentaron menor concentración de nutrientes. La recuperación de estos en los individuos de *J. deppeana* posincendio está determinada por la redistribución de las reservas de nutrientes en el suelo y la planta. Para especies de lento crecimiento, como *J. deppeana*, la planta de dos años de edad es más recomendable para el establecimiento en plantaciones, por sus mayores tasas de incremento.

Palabras clave: Contenido nutrimental, crecimiento inicial, fertilización, *Juniperus deppeana* Steud., resistencia al incendio, táscate.

Abstract

Low growth rate forest species are rarely studied. The initial growth of *Juniperus deppeana* was evaluated through a field fertilization experiment (N-P-K). The post-fire regrowth capacity of the seedlings was also evaluated. There levels of N and P and two levels for of K were combined for a total of 18 treatments, using six plants as replicates. Additionally, the increase in the root collar diameter (IBD) was evaluated for one and two-year-old plants. Six months after fertilization, only the N-P-K interaction was statistically significant (P<0-06) for IBD. The highest levels of N matched to highest IBD. After seven months of the establishment of the field experiment, a forest fire occurred, after which the regrowth capacity and the concentrations of macronutrients (N-P-K-Ca-Mg-S) were evaluated, taking into account the fertilization background. The main effects tested in the experiments were, fertilization, seedling age and fire damage. Most of the trees (84 %) resprouted after the fire. Plants with a legacy of fertilization had lower nutrients in plant tissue. Post-fire nutrient recovery of *J. deppeana* is determined by the redistribution of nutrients reserves in the soil and the plant. For slow growing species *J. deppeana*, plants aged two years are more suitable for reforestation due to their higher growth rates.

Key words: Nutrient content, initial growth, fertilization, *Juniperus deppeana* Steud., fire resistant species, *Táscate*.

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Introduction

There are 68 known species of the *Juniperus* L. (Cupressaceae) genus in the world (Adams, 2014); around 20 exist in Mexico, and half of these are endemic. Their habits are varied, from shrubs to 20 m tall trees (Gernandt and Pérez-de La Rosa, 2014). *Juniperus deppeana* Steud. is native to the United States of America and Mexico (Adams *et al.*, 2007). Its ecological importance is evident in different ways; for example, its cones (strobili) are food for such wildlife as the endemic mountain rabbit (*Sylvilagus cunicularius* Waterhouse) (Lezama-Delgado *et al.*, 2016), the gray fox (*Urocyon cinereoargentus* Schreber) and the coyote (*Canis latrans* Say) (Rubalcava-Castillo *et al.*, 2020); while its foliage is preferred by the mule deer (*Odocoileus hemionus* Rafinesque) (Schwartz *et al.*, 1980).

Juniperus deppeana has been used to restore mining areas, as it is highly resistant to drought and grows in soils with low fertility, or to problems caused by the presence of heavy metals, such as mine waste substrates (Osuna-Vallejo *et al.*, 2017; Osuna-Vallejo *et al.*, 2020). It is a taxon with great ability for bioextraction of heavy metals: Hg (Osuna-Vallejo *et al.*, 2019), Cr, Cd, Cu, Hg, Ni and Pb (Mendoza-Hernández *et al.*, 2016); as well as soluble fractions of Mn and Zn from the soil solution (Morton-Bermea *et al.*, 2014). These characteristics make this species an alternative for the restoration of physically and chemically degraded soils, in which phytoremediation is relevant.

However, although it is a species native to Mexico's temperate and dry climates, little is known about the basic aspects of the management and use of its natural populations and reforestations, as well as of techniques to promote its initial growth. Information regarding the nutritional concentrations in its foliage and the effect of fertilization on its initial growth is equally scarce.

Chemical fertilization promotes the circulation of nutrients in the plantations and increases the productivity of the trees (Binkley, 1993). In addition, it is important to know the concentration of nutrients in *Juniperus deppeana*, as some research suggest the potential use of its extracts in medicine and in the cosmetic and food industries (Lascurain *et al.*, 2018).

In order to overcome the lack of information about the increase in root collar diameter and height and the effect of chemical fertilization on *J. deppeana* during the early stages of development, this work aims to determine the effect of field fertilization with N-P-K and the ability of saplings to resprout after a forest fire. Furthermore, it analyzes the growth of saplings established in a reforestation with one and two years of age. The hypotheses were: 1) *J. deppeana* saplings respond to increasing levels of N-P-K fertilization, with simple and combined effects; 2) in the field, two-year-old trees show greater survival and growth than trees aged one year; and 3) after a fire, individuals with a history of fertilization resprout more vigorously than those not fertilized.

Materials and Methods

The study was conducted within a *Juniperus deppeana* plantation, in the property known as *Los Linderos*, located in *Tepetlaoxtoc* municipality, State of Mexico, between 19°33'08" N and 98°43'02" W, and at an elevation of 2 900 m. The average annual temperature and precipitation are 12.7 °C and 550 mm, respectively, with more concentrated rainfall in September (IMTA, 2013). The plantation was established on July 21, 2017, combined with soil conservation works in the form of broad base terraces, which were previously used for cereal production. 614 one-year old and 499 two-year old individuals were planted. The planting was carried out in a regular-grid, in two polygons with a joint area of 0.6 ha (Figure 1).
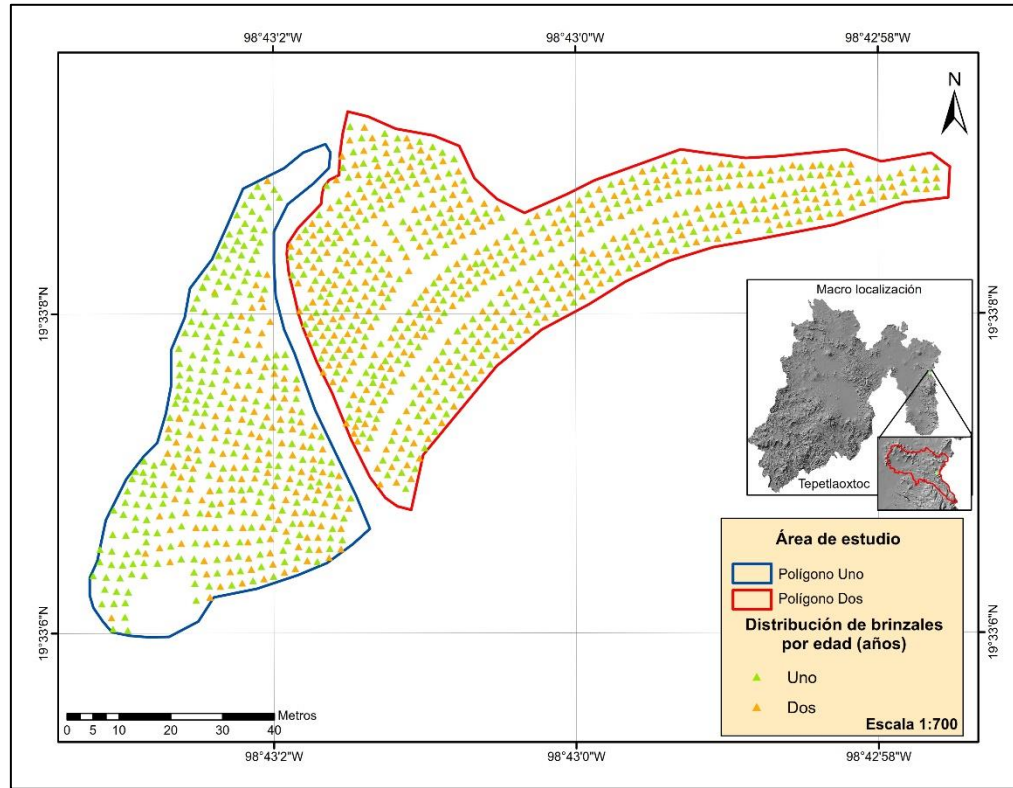
Macro localización = Macro localization; Área de estudio = Study area; Polígono Uno = Polygon One; Polígono Dos = Polygon Two; Distribución de brinzales por edad = Saplings distribution by age; Escala = Scale.

Figure 1. Geographical location and spatial distribution of the *Juniperus deppeana* Steud. saplings, within the evaluated plantation.

Fertilization experiment

For fertilization purposes, a factorial experimental design of $3 \times 3 \times 2$ was proposed for the N-P-K factors, respectively. Each combination of treatments (Table 1) had six replications, with the tree as the experimental unit. The doses of N were 0, 1.13 and 2.26 g tree^{-1}; for P of 0, 1.13 and 2.26 g tree^{-1}; and for K of 0 and 0.63 g tree^{-1}. The fertilizers used were: Ammonium Sulfate (20.5-0-0), Triple Calcium Super Phosphate (0-46-0), and Potassium Sulfate (0-0-51), all applied in granulated form and selected according to their formula, in order to obtain the combinations proposed in the experiment. The fertilization doses were determined based on the preliminary

analysis of 10 composite samples of foliage from the experiment, and on what is cited in the literature (Miller *et al.*, 1991).

Table 1. Treatments applied in the *Juniperus deppeana* Steud. sapling fertilization test.

Treatment	Nitrogen (g tree^{-1})	Phosphorus (g tree^{-1})	Potassium (g tree^{-1})
1	0.00	0.00	0.00
2	0.00	0.00	0.63
3	0.00	1.13	0.00
4	0.00	1.13	0.63
5	0.00	2.26	0.00
6	0.00	2.26	0.63
7	1.13	0.00	0.00
8	1.13	0.00	0.63
9	1.13	1.13	0.00
10	1.13	1.13	0.63
11	1.13	2.26	0.00
12	1.13	2.26	0.63
13	2.26	0.00	0.00
14	2.26	0.00	0.63
15	2.26	1.13	0.00
16	2.26	1.13	0.63
17	2.26	2.26	0.00
18	2.26	2.26	0.63

The fertilizer was applied manually on August 31, 2018. For this purpose, the surface soil (5 cm) below the drip zone was removed, the product was spread and covered with the removed soil.

The variables of response to fertilization were increase in the root collar diameter at the ground level (IDB, for its acronym in Spanish) and increase in height (IH). Given the slow growth of the studied species, the increments are appropriate response variables, mainly because they minimize the effect of initial differences in diameter and height from one seedling to another.

The IH and IBD were obtained from the differences between the initial and final measurement of each sapling. Two evaluations were conducted: the first on June 6, 2018, and the second, on February 15, 2019 (six months after the application of the treatments). The height was measured from the soil surface to the tip of the main shoot, with a 2 m aluminum telescopic levelling stave, graduated in cm and accurate in mm. The diameter at the base of the soil was obtained with a digital Vernier caliper Steren with an accuracy of 0.1 millimeters.

The data were analyzed by means of an ANOVA test, with the statistical software R (R Core Team, 2018) in order to distinguish the differences in growth (IBD and IH), with a significance level of at least 0.1 %, because it was a field experiment (Hurlbert, 1984). The statistical model used was the following:

$$Y_i = \mu + \alpha_i + e_i$$

Where:

Y_i = Response variable

μ = Overall average of the data

α_i = Difference in the mean of the ith treatment

e_i = Error

Fire damage

Seven months after the fertilization experiment was established, a fire occurred that affected mainly the trees in polygon two. Therefore, there was an opportunity to evaluate the effect of fertilization on the survival, resprout and nutritional content of the saplings affected by the fire. The assessment of the regrowth was made through a classification into four qualitative regrowth categories: null (0 resprouts), low (1-6 resprouts), medium (7-15 resprouts) and high (>15 resprouts). In the analysis, all shoots at the base or at the top of the saplings were considered. A total of four evaluations of the resprout were carried out, on May 31st, 2019; August 7th, 2019; September 20th, 2019, and November 14th, 2019.

Nutritional content of resprout

After the fire, comparisons were made between the treatments, including those saplings that had been damaged by the fire in order to evaluate the regrowth capacity and the concentration of N-P-K-Ca-Mg-S macronutrients in the regrowth tissue. The following comparisons were made:

By age of the saplings. Nutritional content of the regrowth between one- and two-year old saplings.

Ability to regrow in fertilized saplings. Regrowth capacity and nutritional content of the regrowth according to the fertilization history and the pre-fire IBD.

Plant samples from the fire outbreak were collected on November 22nd, 2019. The tissue of the fire outbreak was obtained seeking to include the developed components that would allow the collection of at least 50 g of dry matter for chemical analysis purposes. The samples were sent to the *Salvador A. Blanco* laboratory of the *Colegio de Postgraduados* in order to estimate the N, P, K, Ca, Mg and S concentrations. The N concentration was determined in the dried and ground plant tissue with the Semimicro-*Kjeldahl* method (Bremmer, 1965). P, K, Ca, Mg and S concentrations in the extract resulting from the

digestion with HNO_3:$HClO_4$ (2:1, v:v) of dried and ground plant tissue were determined using an atomic emission spectrophotometer with inductively coupled plasma (ICP-OES 725 Series, Agilent), as described by Alcántara and Sandoval (1999). Based on the data of the nutritional content of the foliage, a Student's t mean comparison test with a significance level of 0.05 % was performed.

Increment in diameter, height and survival

Three measurements were made, in June 2018, May 2019, and September 2019. Two groups were considered: trees affected by fire (IH) and unaffected trees (NA). The mean and median of the IBD and IH were estimated. Since the data were not normally distributed, the Mann-Whitney U test (Mann and Whitney, 1947) was used for comparison between groups.

Results and Discussion

Effect of fertilization on *Juniperus deppeana* saplings

Increment in diameter at the base

The analysis of variance for the IBD calculated with data from June 2018 and February 2019, showed significant effects (P<0.06) by fertilization for the N-P-K interaction (Table 2).

Table 2. Analysis of variance for the increase in root collar diameter in the *Juniperus deppeana* Steud. sapling fertilization experiment.

Treatment	Degrees of freedom	Sum of squares	Mean squares	F-value	Pr > F
N	2	58.46	29.33	1.5606	0.21
P	2	9.16	4.58	0.2447	0.78
K	1	14.35	14.35	0.7663	0.38
N*P	4	117.45	29.35	1.5678	0.19
N*K	2	14.21	7.11	0.3793	0.69
P*K	2	11.26	5.63	0.3006	0.74
N*P*K	4	174.45	43.61	2.3287	0.06
Residuals	90	1 685.55	18.73		
Total	107	2 084.89			

The two treatments with the highest BDI (treatments 13 and 15) had in common, the highest dose of N (2.26 g N tree^{-1}) and the absence of K (Figure 2). The three treatments with the lowest BDI corresponded to the control and the two that included application of 0.63 g K tree^{-1} (treatments 6 and 8). Although there is no complete explanation of the reasons for this result, the addition of K was not favorable and had negative effects on the BDI. This is similar to that reported by Balám-Che *et al.*, (2015) when they applied 15 g of K tree^{-1} to a one-year old teak plantation. The K in the fertilizer may temporarily compete for spaces in the soil exchange zone, for example, with assimilable forms of N (NH$_{4+}$), and therefore it may have a negative effect, especially if the soil is not K deficient.
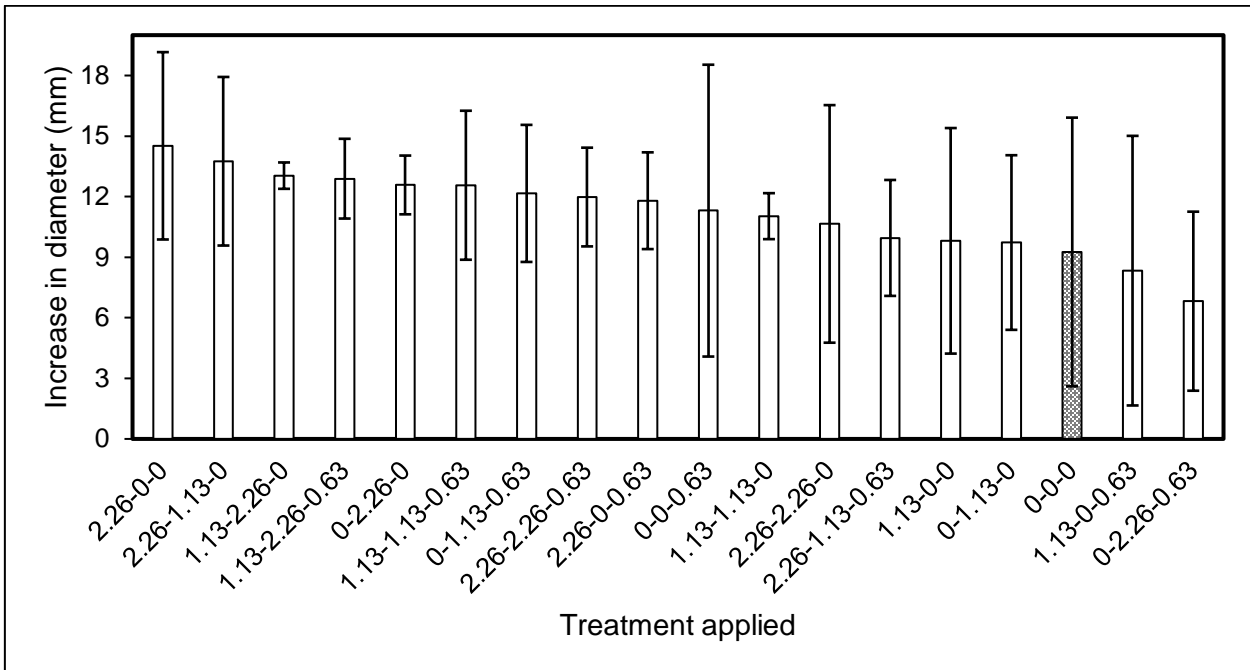
The numbers under the bar indicate the amounts of NPK applied in grams tree[-1] respectively. The lines above the bar correspond to the standard deviation.

Figure 2. Increase in the root collar diameter of *Juniperus deppeana* Steud. saplings per treatment applied. The dark bar indicates the control.

Lázaro-Dzul *et al.* (2012) record both the dominant, positive impact of N and the negative response to the addition of K, when they apply different doses of N (0, 138, 185 kg ha^{-1}) and K (0, 123, 164 kg ha^{-1}) in young plantations of *Pinus patula* Schiede ex Schltdl. & Cham. Whereas in fast growing species, the gain in K seems to be stimulated with the addition of micronutrients, such as B (Rodríguez-Juárez, *et al.*, 2014).

Increment in height

Fertilization produced differences in IH with statistical significance (P=0.1), particularly for treatments with P application (Table 3). When compared to the IBD, the variance of IH was three times higher.

Table 3. Analysis of variance for the increase in height in the *Juniperus deppeana* Steud. sapling fertilization experiment.

Treatment	Degrees of freedom	Sum of squares	Mean squares	F-value	Pr > F
N	2	89.8	44.90	0.6775	0.51
P	2	361.4	180.70	2.7270	0.07
K	1	2.3	2.30	0.0349	0.85
N*P	4	128.1	32.03	0.4833	0.75
N*K	2	74.7	37.35	0.5634	0.57
P*K	2	199.3	99.65	1.5042	0.23
N*P*K	4	512.4	128.10	1.9335	0.1
Residuals	90	5,963.0	66.26		
Total	107	7 331.0			

The high variation in IH is a reflection of external factors to the experiment: insect attack on the shoots and damage by wild herbivores at the top. On the other hand, the low growth rates in height influenced the lack of statistical strength of the fertilization effect in *Juniperus deppeana*. Osuna-Vallejo *et al.* (2020) estimated annual height growth rates of 11 cm year^{-1} in saplings of this species; however, when the soil presents harvest residues or surface organic material, height growth rates double (Wood *et al.*, 2012).

Growth of saplings of different ages

The increase in diameter was greater in the two-year old NA saplings (P<0.0001). The mean values were 2.9 and 7.8 mm year^{-1}, for one- and two-year old saplings, respectively. After 476 days, this difference remained statistically significant (P<0.0001), with values of 4.8 mm year^{-1} (one-year sapling) and 10.8 mm year^{-1} (two-year sapling) (Table 4).

Table 4. Increase in diameter at the ground level in *Juniperus deppeana* Steud. saplings not affected by fire.

Increase in diameter at the ground level in one year			
Age of the saplings	**Mean (mm)**	**Median (interquartile range) mm**	**P-value**
One year	2.83	2.77 (1.55-3.82)	<0.0001
Two years	7.79	8.26 (4.54-10.90)	
Increase in diameter at the ground level after 476 days			
Age of the saplings	**Mean (mm)**	**Median (interquartile range) mm**	**P-value**
One year	3.60	3.60 (2.01-4.80)	<0.0001
Two years	8.23	8.23 (5.2-10.6)	

The two-year old saplings showed an advantage in their growth in diameter; however, survival was similar in both groups, with values above 98 %. This shows that *Juniperus deppeana* is a rustic species, with a higher survival rate than the average of taxa such as *Pinus greggii* Engelm. ex Parl. (46 %), *P. hartwegii* Lindl. (48.8 %) and *P. leiophylla* Schiede ex Schltdl. & Cham. (>70 %), which are used in Mexico for reforestation purposes (Burney *et al.*, 2015; Escobar-Alonso and Rodríguez, 2019). Osuna-Vallejo *et al.* (2020) indicate a survival rate of 98 % for *J. deppeana*, which is

similar to that recorded in this study. IH showed no statistical difference between the two sapling ages (P=0.63).

Saplings affected by the fire

In the diameter of the IH saplings, a very similar distribution to that of unaffected trees was observed. The fire damaged the branches and foliage to different extents, but because each sapling had an individual terrace of approximately 60 cm in diameter, the fire did not significantly affect the activity of the cambium at ground level; therefore, most of the trees resprouted. The two-year sapling RDI was higher than that of the one-year old (Table 5).

Table 5. Increase in diameter at the base in *Juniperus deppeana* Steud. saplings partially affected by fire.

Increase in diameter at the ground level in one year			
Age of the saplings	Mean (mm)	Median (Interquartile range) mm	P-value
One year	3.52	3.34 (1.64-4.69)	<0.0001
Two years	7.75	8.29 (4.54-10.9)	
Increase in diameter at the ground level in 476 days			
Age of the saplings	Mean (mm)	Median (Interquartile range) mm	P-value
One year	2.46	2.46 (1.07-3.74)	<0.0001
Two years	5.56	5.67 (2.96-8.36)	

In spite of fire damage, the saplings, kept their diametric growth, at least at the ground level. It was observed that after one year, the increase in IBD of saplings in the IH group exceeded that of the NA group (3.52 *vs* 2.83 mm, respectively). It is likely that the IH saplings, having less foliáge as a recovery strategy, concentrate their available reserves for growth in IBD.

The differences in the IBD between one- and two-year old saplings are related to the advantage of a larger and more developed root system. Two-year old saplings had the conditioning time and the opportunity to develop more leaf mass than saplings aged one year. Height and IH showed no difference between one- and two-year old saplings. The differences in plant IH in both ages disappeared in the first year.

Effect of fire and resprout

After four regrowth evaluations, during the eight months following the fire, it was determined that 84 % of the individuals resprouted, which denoted their fire resistance. This result is consistent with Rodríguez-Trejo *et al.* (2019), who confirm this characteristic. Wood *et al.* (2012) point out that the maximum regrowth of *J. deppeana* after a controlled burn is 50 %, which indicates that the percentage of resprout observed in the present study was high. The distribution in the fourth evaluation for low, medium and high regrowth was 6 % (44), 13 % (91) and 65 % (457), respectively.

Nutritional content of the foliage

The treatments with the highest increase in IBD, and which exceeded that of the control, were: 2.26-0-0, 1.13-2.26-0 and 2.26-1.13-0 g N-P-K tree[-1]. The application of N is evident in all three.

Statistical analysis indicated that the concentrations of N, P and K were higher in the control shoots than in those corresponding to the 2.26-0-0 treatment (Table 6). Although, the base fertilizer was ammonium sulfate, a source of S, the S content was

higher in the control regrowths. However, it is important to mention that regrowth concentrations themselves are affected by a dilution process; that is, an equal amount of an element is distributed over a larger amount of biomass and different levels of tree components (Montès *et al.*, 2002; Ávila-Angulo *et al.*, 2020).

Table 6. Statistical difference in nutrient content between control and treatment 2.26-0-0.

Nutrient		Treatment	
		0-0-0	**2.26-0-0**
Nitrogen	Mean	1.428 %	1.008 %
	P-value*	0.006	
Phosphorus	Mean	0.154 %	0.11 %
	P-value*	0.007	
Sulphur	Mean	0.094 %	0.073 %
	P-value*	0.0206	

*Student's t P-*value*

There were statistical differences in N-P-S concentrations between the control and treatment 0-2.26-0.63, with higher values in the control (Table 7). The 0-2.26-0.63 treatment showed the lowest average increase in IBD of all. Given that the highest P level was applied in this treatment and the fertilizer contained S (potassium sulfate), the nutritional content of the leaves was expected to exceed that of the control.

Table 7. Statistical difference in nutrient content between the control and treatment 0-0.26-0.63.

Nutrient		Treatment	
		0-0-0	**0-2.26-0.63**
Nitrogen	Mean	1.428 %	0.980 %
	P-value*	0.003	
Phosphorus	Mean	0.154 %	0.109 %
	P-value*	0.022	
Sulphur	Mean	0.0944 %	0.0729 %
	P-value*	0.027	

* Student's t P-_value_

The leaves of the control saplings exhibited a higher content of the nutrients N and S than those of treatment 1.13-2.26-0 (Table 8).

Table 8. Statistical difference in nutrient content between control and treatment 1.13-2.26-0.

Nutrient		Treatment	
		0-0-0	**1.13-2.26-0**
Nitrogen	Mean	1.428 %	0.952 %
	P-value*	0.0004	
Sulphur	Mean	0.094 %	0.071 %
	P-value*	0.014	

* Student's t P-_value_

Treatment 1.13-2.26-0 was statistically different from the control group in terms of N content of the resprout (Table 9). The treatment was given the highest dose of P, although this was not reflected in regrowth.

Table 9. Statistical difference in nutrient content between the control and treatment 1.13-2.26-0.

Nutrient		Treatment	
		0-0-0	**1.13-2.26-0**
Nitrogen	Mean	1.43 %	1.02 %
	P-value*	0.004	

* Student's t P-*value*

The higher concentration of nutrients in the resprout tissue of the control saplings, compared to the fertilized ones, is due to the fact that fertilization promoted a higher translocation of nutrients in the aerial biomass of the fertilized saplings. Especially N, which is an element that is lost during forest fires (Binkley, 1993). It is also an essential nutrient in proteins, promotes the development of leaves and stems and produces rapid development in the initial phases of growth (Binkley, 1993). Likewise, the N provided with the fertilization may have modified the biological activity in the soil, possibly generating its immobilization, which limited its use for increasing in mass and foliage; for this reason, its foliar content is lower than that of the control.

Regarding fire damage, although in the long term, fire does not affect the nutrient reservoirs in the soil, it does have an important impact in the short term on the microbial biomass in the soil, particularly the assimilable forms of N (Fultz *et al.*, 2016). Therefore, the lower concentrations of nutrients are a combined reflection of their redistribution within the plant and their availability in the soil.

The age of the saplings did not present differences in the nutritional content of the post-fire shoots. However, the higher increase in the IBD of the two-year old saplings, which implies higher biomass, indicates that for slow-growing species, such as *J. deppeana*, the two-year old plants are more successful at overcoming fires at ground level, due to their higher growth rates.

Conclusions

The combined fertilization with N-P-K containing the highest levels of N (2.26 g tree^{-1}) has a significant effect on the increase of the diameter at the ground level (P<0.06). Two-year old *Juniperus deppeana* saplings have a greater increase in IBD than one-year old ones. This result is consistent, despite the negative effects derived from the fire disturbance.

Juniperus deppeana saplings have a great capacity for post-fire regrowth, mainly in the root collar area. This quality can be seen in both control and fertilized saplings. The age of the plant did not show statistical differences in the nutritional content of shoots, but it does in IBD. In the case of *J. deppeana*, a slow-growing species, a two-year old plant should be considered, since it has a good probability of success in the event of forest fires.

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Conflict of interest

The authors declare no conflict of interest.

Contribution by author

Pedro Sinai Rivera Torres: drafting of the manuscript, collection and analysis of field data; Armando Gómez Guerrero: planning and direction of the research, drafting of the manuscript; Arnulfo Aldrete: drafting of the manuscript, advice and follow-up on the research; Saraí Montes Recinas: obtaining economic resources, establishment of plantation and drafting of the manuscript.

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