

Needle removal favors the survival of *Oreocallis grandiflora* in *Pinus* plantations in southern Ecuador

La remoción de acículas favorece la supervivencia de *Oreocallis grandiflora* en plantaciones de *Pinus* al sur del Ecuador

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Abstract

Pine plantations have negatively impacted biodiversity and the establishment of native flora in Ecuador. We evaluated whether needle removal facilitates the establishment of *Oreocallis grandiflora* in a plantation in the south of the country; we performed direct sowing of seeds in plots with and without needles. Our results showed that removal had no statistically significant effect on emergence ($p > 0.05$), but did have a significant effect on survival ($p < 0.0001$). After 22 weeks, we recorded $>86\%$ survival without aculeus and 7% with aculeus. Two years later, survival was close to 80% for the treatment without aculeus, and the growth of these plants was significant ($p < 0.0001$). We conclude that direct seeding is viable, but we recommend removing the needles monthly during the first months.

Keywords: direct seeding, emergence, establishment, gañal, native flora, pine needles.

Resumen

Las plantaciones de pino han impactado negativamente la biodiversidad y el establecimiento de flora nativa en Ecuador. Evaluamos si la remoción de acículas facilita el establecimiento de *Oreocallis grandiflora* en una plantación del sur del país; realizamos siembra directa de semillas en parcelas con y sin acícula. Nuestros resultados mostraron que la remoción no tuvo efecto estadísticamente significativo sobre la emergencia ($p > 0.05$), pero sí sobre la supervivencia ($p < 0.0001$). Después de 22 semanas, registramos una supervivencia $>86\%$ sin acícula y 7% con acícula. Dos años después, la supervivencia fue cercana al 80% para el tratamiento sin acícula, asimismo, se evidenció que el crecimiento de estas plantas fue significativo ($p < 0.0001$). Concluimos que la siembra directa es viable, pero recomendamos remover mensualmente las acículas durante los primeros meses.

Palabras clave: acícula de pino, emergencia, establecimiento, flora nativa, gañal, siembra directa.

1. Introduction

Pine is a native species of the northern hemisphere, belonging to the Pinaceae family, with both arboreal and shrubby habits (Richardson, 2000). It has valuable timber characteristics, fast growth, and easy cultivation, which is why it has been introduced and spread to several countries around the world for several decades (Hofstede et al., 2002). In Ecuador, the first plantations were established in the second half of the 20th century for both commercial and recreational purposes (Narváez et al., 2017), primarily using species such as *Pinus patula* and *Pinus radiata* due to their adaptation to diverse climatic and edaphic conditions (Quiroz et al., 2019). However, these plantations have caused problems in biogeochemical and hydrological processes, as well as in fire regimes, and have modified the availability of soil nutrients, affecting both surface and subsurface biotic communities (Richardson, 2000; Simberloff et al., 2010).

For example, the accumulation of pine needles in the soil inhibits the emergence, germination, and development of other understory species due to the natural oils present in these tissues and the dense canopy cover, which leads to a reduction in plant diversity, generating a less complex and more homogeneous ecosystem (Chacón et al., 2003; Yirdaw & Luukkanen, 2004). Additionally, it has been discovered that the dense layer of needles on the soil can have adverse effects on the germination process, acting as both a physical and chemical barrier. Therefore, it has been suggested that their removal can benefit natural restoration (Bueno & Baruch, 2011).

As a consequence, it has been a challenge to restore native vegetation within the pine plantations. Therefore, potential species have been sought, such as *Oreocallis grandiflora* (gañal), which, in previous research, is a species with the capacity to adapt to adverse conditions (Pretell et al., 1985; Vinuela et al., 2018a) and to provide suitable conditions for plant succession (Cotler & Maass, 1999). This species stands out for its

ecological importance (Crespo & Inga, 2020); its shrubby life form and abundant flowers are the functional traits that give it a key role in mutualistic networks (Crespo et al., 2021) since it is visited by birds, insects, bats, and even rodents have been recorded feeding on its nectar (Cárdenas et al., 2017). In addition, it has potential for use in the cosmetics industry and food industry due to the amount of flavonoids it has (Vinueza et al., 2018b). It is also known that rural communities use their flowers in traditional medicine and the creation of handicrafts (Minga & Verdugo, 2016).

The humid montane scrubland of southern Ecuador is currently covered by hundreds of hectares of pine plantations that could be restored. Information on the restoration of native biodiversity in pine plantations is scarce. For example, it has not been reported which native species persist and develop within these plantations, nor have the management strategies that facilitate their successful establishment under these conditions been identified. However, to reduce the costs of these programs, it is essential to optimize the use of available resources (Shoo & Catterall, 2013). An effective strategy in this regard is the implementation of no-tillage methods (Engel & Parrotta, 2001), which have proven to be a viable alternative. This technique is particularly suitable for the financial and geographic reality of farmers in the south of the country, as it makes it possible to dispense with the costs associated with the production and maintenance of seedlings in nurseries, thus facilitating its application on a larger scale and with less initial investment (Crespo Inga, 2020). In this study, we evaluated (i) whether needle removal influences the emergence and survival of *Oreocallis grandiflora* seedlings and (ii) whether the growth of *Oreocallis grandiflora* seedlings after 24 months of establishment is significant under experimental conditions in a pine plantation. This work constitutes an initial contribution to understanding the viability of restoring native Andean vegetation in forest contexts dominated by exotic species.

2. Methodology

2.1. Study site

The study was carried out in pine plantations in southern Ecuador, within the El Gullán Scientific Station ($3^{\circ}19' \text{ S}$, $79^{\circ}09' \text{ W}$), located in the parish of Las Nieves, in the canton of Nabón, on the southern border of the province of Azuay (Figure 1). The average annual temperature ranges between 7°C and 21°C , while annual precipitation varies between 400 mm and 600 mm (MAE, 2013). The area corresponds to a humid montane scrub ecosystem, located between 2,500 and 3,000 m above sea level, characteri-

zed by high floristic diversity. The predominant vegetation includes species such as *Hesperomeles ferruginea* (Rosaceae), *Myrcianthes rhopaloides* (Myrtaceae), and *Myrsine* depends (Myrsinaceae). In addition, important species such as *Miconia aspergillosis* (Melastomataceae), *Rhamnus granulosa* (Rhamnaceae), *Maytenus verticillate* (Celastraceae), *Piper barbatum* (Piperaceae), *Oreocallis grandiflora* (Proteaceae), *Oreopanax andreanus* (Araliaceae), and *Myrsine Andina* (Myrsinaceae) are also found (Minga et al., 2021).

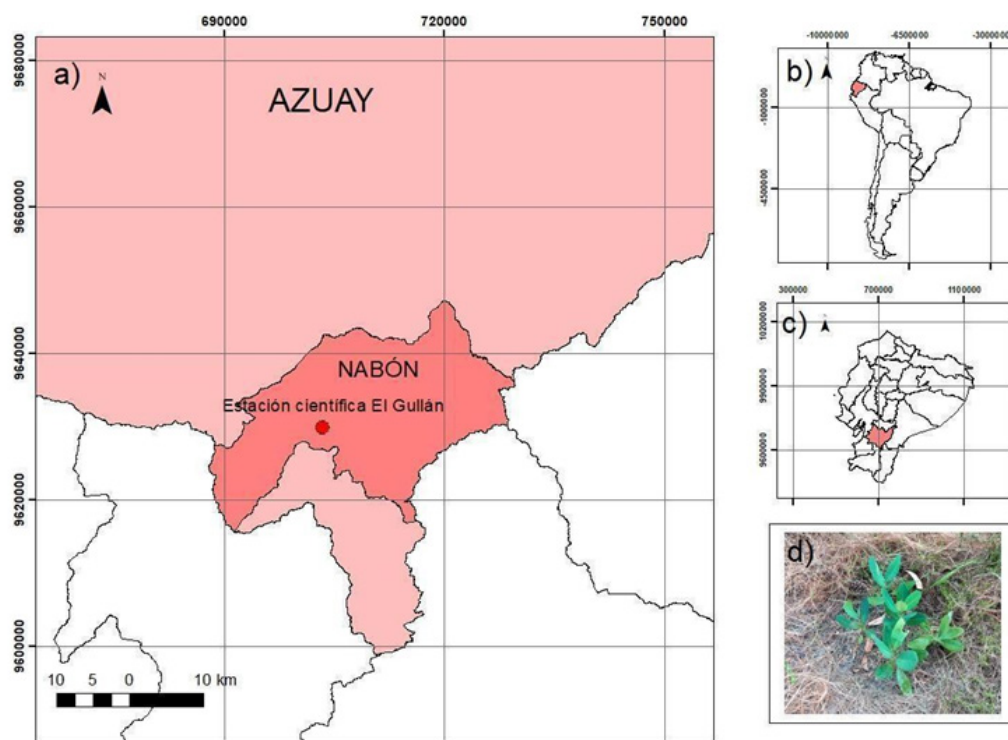


Figure 1: El Gullán Research Station, Azuay province, southern Ecuador. a) El Gullán Research Station ($3^{\circ}19' \text{ S}$, $79^{\circ}09' \text{ W}$); b) Map of South America; c) Map of Ecuador; d) Individual of *O. grandiflora*; e) Map of Ecuador; f) Individual of *O. grandiflora*.

Source: Own elaboration.2025.

2.2. Experimental design

We installed three experimental blocks in a *Pinus patula* plantation that had been thinned by 25% prior to the start of the experiment. We did not carry out previous physical-chemical analyses of the soil. However, for the establishment of the blocks, we considered elements such as slope, tree cover, and exposure, all of which were relatively homogeneous between plots. The vegetation cover consisted exclusively of the pine tree layer, along with mosses and epiphytic bromeliads, but lacked a native understory. The ground litter corresponded only to pine needles, with no other visible plant remains.

We positioned the blocks vertically with respect to the terrain's slope, ensuring a minimum distance of 50 m between each one to minimize interference. Each block was 24×10 m in size. Within these, we established experimental units of 2×2 m, with two treatments: direct seeding with and without pine needles, randomly assigned. Each treatment was repeated eight times per block to ensure replicability and minimize data loss due to external factors, such as accidental damage or environmental disturbances. Additionally, this distribution enabled a more accurate assessment of the effects of the presence or absence of aculeus on seedling emergence and initial growth.

For the experiment, we collected seeds of *O. grandiflora* between March and May 2022 from eight mother plants located in remnants of natural vegetation near the study area. For the selection of mother plants, we performed a randomized sampling, considering only individuals in the active fruiting stage and maintaining a minimum distance of 20 meters between them to reduce the probability of close kinship. In total, we sowed 240 seeds, five per experimental unit, distributed equally between the two treatments (120 per treatment).

Germination was recorded as the emergence of seedlings visible above the soil surface. We collected these data every 15 days for 18 weeks, while seedling survival was assessed monthly until week 22 post-sowing. To analyze initial growth, we measured seedling height at the end of the observation period (week 22) using a caliper, taking the distance from the base of the stem to the apex as a reference. In addition, we performed a second height measurement two years after planting using a flexometer under the same protocol to evaluate their long-term development. However, the number of plants monitored was 30, these belonging to the treatment without aculeus, due to the null survival of plants from the treatment with aculeus.

2.3. Statistical analysis

Both emergence and survival were evaluated in final percentages. Therefore, we considered the ratio of emerged seeds to sown seeds and multiplied by 100. Next, the normality and homogeneity of the variables were evaluated using the Shapiro-Wilk and Levene's tests, respectively. Since these statistical assumptions were not met, an attempt was made to achieve normality by transforming the data; however, these two assumptions were still not met. Therefore, to determine whether there are statistical differences in the emergence and survival of seeds sown in plots covered with or without aculeus, the Wilcoxon rank sum statistical test was performed ($p\text{-value} < 0.05$) (ISTA, 2002; Dao, 2022). Likewise, the same statistical test was applied to determine if the growth of these plants (without aculeus) was significant after two years of planting. However, it was necessary to consider the variables as paired samples. This analysis is represented through bar graphs with the standard error of the treatments (emergence and survival), as well as size (initial and current), and tables with measures of central tendency and dispersion. The analyses and graphs were conducted using the statistical program R (R Core Team, 2024).

3. Results

Concerning emergence, the averages of the treatments were not similar, although their medians were. As shown in Table 1, the average emergence in plots with aculeus was higher, reaching 43.3% (with 51 plants emerging), while in plots without aculeus, it was 33.3% (with 39 plants emerging). Moreover, in plots with acicula,

emergence occurred in all replicates, whereas in plots without acicula, some replicates had no emerged seeds. On the other hand, the coefficient of variation in the treatment with needles was 43%, a lower value compared to the plots without needles, which reached 53%, suggesting greater variability in emergence in the absence of needles.

Table 1: Measures of central tendency and dispersion for emergence in the evaluated treatments. CAS treatment: with aculeus and SA: without aculeus
Source: Own elaboration (2025).

Treatment	Mean %	Median	Median	Minimum	Maximum	EE	CV
CAS	43.33	40	20	80	18.75	0.43	
SA	33.33	40	0	60	17.75	0.53	

SE: standard error. CV: coefficient of variation.

Survival showed the opposite behavior to emergence; in this variable, the treatment with needles recorded an average of 7.14% (14 plants), while the average for the treatment without needles was 86.6% (33 plants), which was higher than the

previous one. However, in both treatments, there were replicates with null and 100% percentages. Hence, the coefficient of variation of the plots with needles was higher (3.74) than that of the treatment without needles (0.4) (Table 2).

Table 2: Measures of central tendency and dispersion for the survival of *O. grandiflora* plants in the evaluated treatments. Treatment CAS: with aculeus and SA: without aculeus.
Source: Own elaboration (2025)

Treatment	Mean %	Median	Median	Minimum	Maximum	EE	CV
CAS	7.14	0	0	100	26.73	3.74	
SA	86.67	100	0	100	34.57	0.40	

SE: standard error. CV: coefficient of variation.

According to Figure 2a, the mean percentages of emergence for the two treatments were similar. After applying the Wilcoxon rank sum test, it was found that there were no statistical differences between the two treatments ($p\text{-value} > 0.05$), as the difference between the two treatments was only 10%. Meanwhile, in the bar graph for survival (Figure 2b), a clear difference between the means can be observed. Therefore, after applying the Wilcoxon rank sum test, significant differences were found between the evaluated treatments ($p\text{-value} < 0.0001$). In this case, the difference between treatments was 79%.

After 24 months, we measured the plants again to determine if their growth was significant over this period. The Wilcoxon rank sum for paired samples showed that the growth of the plants in the treatment without aculeus in the last two years was significant ($p\text{-value} < 0.0001$). The average initial height of the plants was 1.30 cm, which increased to 8 cm over the two years, indicating a growth of approximately 6.7 cm. This suggests that the species develops and grows slowly (Figure 3).

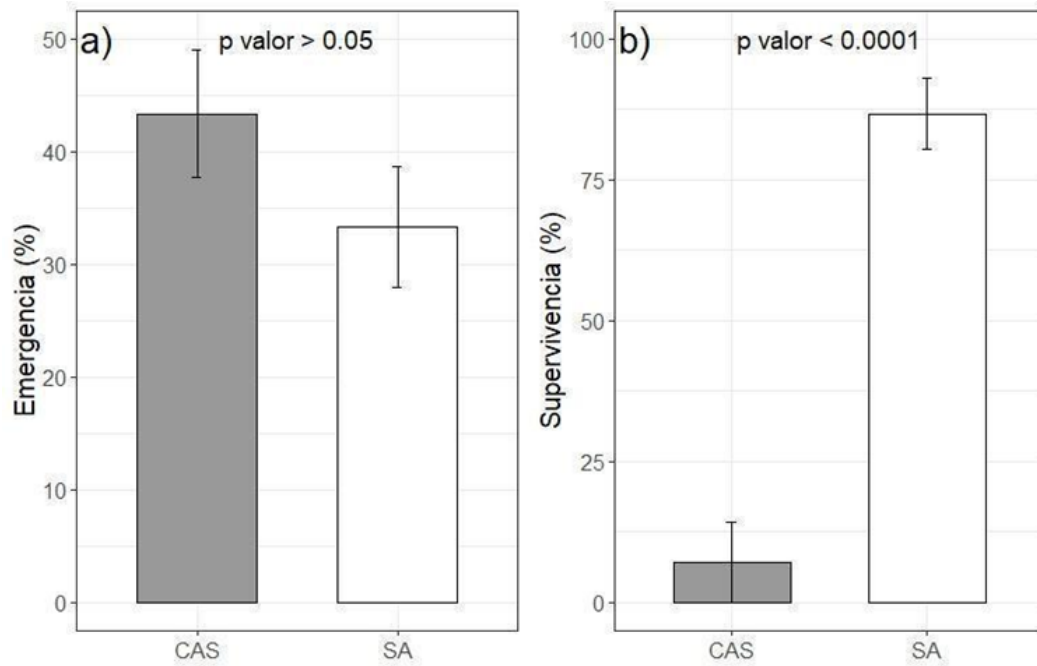


Figure 2: Final mean emergence (a) and survival (b) for *O. grandiflora* seeds after direct seeding into a pine plantation. Seeding treatments applied included keeping the aculeus intact (CAS) or removing the aculeus (SA). Figure 2a shows seedling emergence after 18 weeks of direct seeding, while Figure 2b shows seedling survival after 22 weeks of observation. Bars with different letters denote significant statistical differences.

Source: Own elaboration.2025.

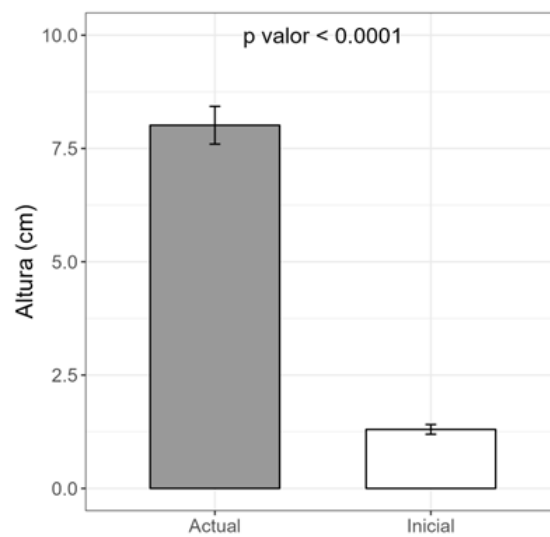


Figure 3: Comparison of means of *O. grandiflora* plant growth recorded in two years.ion. Bars with different letters denote significant statistical differences.

Source: Own elaboration.2025.

4. Discussion

In this study, we evaluated the hypothesis that the removal of *Pinus patula* needles favors the emergence, survival, and growth of *Oreocallis grandiflora* seedlings in plantations in southern Ecuador. The results partially support this hypothesis: although no statistically significant effect on emergence was observed, removal did improve survival, both in the short and medium term.

The findings of this study suggest that direct seeding of *O. grandiflora* is a viable strategy to recover native vegetation cover within pine forest plantations when needles are removed. It is recommended to complement this practice with management actions such as thinning, which was part of the experimental context, to facilitate adequate light conditions. Additionally, it is recommended to perform needle removal every month during the first 4 to 6 months after planting, based on the results observed in this study.

One of the most remarkable results was the marked contrast between relatively high emergence and low survival in the presence of needles. *O. grandiflora* seeds exhibited an emergence rate of over 40%, which is consistent with previous studies reporting high germination percentages under natural conditions, provided that the seeds have not been stored for more than three months (Palomeque et al., 2020). In this study, seeds were sown shortly after collection without exceeding this storage threshold. Emergence occurred in a staggered manner over 18 weeks, which is consistent with the presence of physiological dormancy in the species (Villena-Ochoa et al., 2024).

Emergence in plots with needles could be related to a phenomenon of herbicidal hormesis, where low concentrations of compounds present in the needle stimulate germination (Portuguez-García et al., 2020). Additionally, the needle cushion may have contributed to conserving moisture and temperature in the microsite. However, it has also been reported that when the accumulation is not controlled, these needles generate a

hydrophobic layer that prevents water infiltration (Chacón et al., 2003). Although we did not experimentally identify which of these mechanisms predominated, the positive effects observed are likely due to an initial favorable balance between moisture retention and chemical stimulus.

Despite this initial positive effect, seedling survival was significantly lower in the presence of needles. Our results reveal that this same substrate affects the survival of *O. grandiflora* seedlings, which is in agreement with research evidencing phytotoxic effects of compounds released by needles, such as phenols (Fernandez et al., 2013; Portuguez-García et al., 2020; Valera-Burgos et al., 2012). The adverse effects on survival can be attributed to the leachates generated by the needle and their associated allelopathic effects (Panca-Jevera et al., 2024). Studies have shown that fresh needle exudates contain high concentrations of phenolic compounds that inhibit both germination and radicle elongation (Zhang et al., 2018). Although these effects are reduced with senescent needles (Santonja et al., 2019), in our experiment, we did not control for the age of the material present in the plots so that both types may have coexisted.

In addition, several studies suggest that the presence of needles in the soil may alter nutrient availability, partly by inhibiting nitrifying microorganisms (Tyukavina et al., 2019) and by contributing to substrate acidification (Fan et al., 2023; Oliva et al., 2014). This could have generated adverse edaphic conditions for *O. grandiflora*. Additionally, the mechanical effect of the needles may have hindered seedling rooting by limiting contact with the soil (Cano et al., 2008).

On the other hand, although needle removal had a positive effect on survival, seedling growth was slow. Two years after planting, plants reached a maximum height of 8 cm. This slowness may be due to the absence of continuous needle removal during that period, as this practice was only per-

formed during the first 22 weeks of the study. We consider that subsequent accumulation may have influenced the growth of individuals. However, this hypothesis still requires experimental verification. It is worth mentioning that *O. grandiflora* is adapted to acidic and shallow soils (Minga & Verdugo, 2016), which could explain its ability to persist despite restrictive conditions.

The morphological characteristics of native tree and shrub seedlings can also influence their initial growth. In the early stages, many species present functional traits that respond to the environment, modifying as they progress through their life cycle (Rocas, 2001). In this context, abiotic factors such as the availability of light, nutrients, and water, as well as competition with other species, are key determinants for the development of seedlings of woody species (Moreno & Cuartas, 2015).

Additionally, abiotic factors such as light incidence and topography are key determinants of seedling survival and growth in forest ecosystems (Park et al., 2018). For this reason, thinning is a complementary activity to needle removal, as it facilitates greater light input to the understory. Previous studies have shown that the amount and duration of light received by seed-

lings are key factors for their survival and development (García-Castro et al., 2018). In particular, the effects of light spans are critical in the early stages of growth; however, microenvironmental characteristics also vary according to the specific requirements of each species and its phenological stage (Moretti et al., 2019).

Despite these findings, studies on the interactions between pine plantations and native species regeneration in the tropical Andes remain limited, particularly in terms of long-term growth dynamics. This knowledge gap presents an opportunity for future research to explore the ecological mechanisms that influence the adaptation of *O. grandiflora* within forest plantations with exotic species. Understanding these processes will enable the design of more effective strategies to optimize its establishment, favor biodiversity, promote the recovery of degraded ecosystems, and ensure the sustainability of managed forest systems.

Ultimately, the findings of this study can serve as a basis for developing restoration protocols tailored to local conditions. The implementation of simple, sustainable practices supported by scientific evidence represents a crucial step toward effective ecological restoration and biodiversity conservation in highly disturbed landscapes.

5. Conclusions and recommendations

This study demonstrates that direct seeding of *Oreocallis grandiflora* is an effective strategy to facilitate the establishment of native flora within *Pinus patula* plantations in the southern Andes of Ecuador. Although the presence of needles did not significantly affect seedling emergence, it did reduce seedling survival, which highlights the importance of properly managing the surface substrate. The removal of needles during the first months after planting significantly increased survival, so it is recommended to perform this practice at least monthly during the first 4 to 6 months. It was also confirmed that, in the

treatment without needles, plant height after two years of establishment was significantly higher. Likewise, these practices should be implemented on a larger scale in restoration programs, prioritizing the immediate sowing of fresh seeds and conducting continuous follow-up to adjust strategies according to local conditions. Finally, it is recommended that we continue to investigate the long-term effects of these interventions and explore the responses of other native species in contexts dominated by exotic plantations in order to strengthen ecological restoration in high Andean ecosystems.

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