**Project Update: June 2023**

1. *Herbicide efficiency experiment*

In order to prove chemical control efficiency on the mortality of Douglas fir individuals, we designed an experiment in which we tested two different systemic herbicides: (i) glyphosate (Roundup Full II®; composition: glyphosate 66.2 g (54% glyphosate acid equivalent) and (ii) Tocón® Extra; composition: aminopyralid 4,0 g + Triclopyr butoexyl ester 16,69 g. We also tested two different concentrations per herbicide: for glyphosate we used a 5% (low concentration) and 100 % (high concentration) solution; for aminopiralid + triclopyr we used a 3% (low concentration) and 7.5 % (high concentration) solution. We included a third control treatment in which no herbicides were applied to the trees. Herbicide application was carried out in April 2022 using the stem injection method. For this purpose, holes were drilled with a 8 mm drill bit around the tree trunk at ~1.5 m height and at a downward angle to avoid spillage. The number of holes per tree was based on DBH: < 10 cm, one hole; between 10 and 20 cm, two holes; between 20 and 30 cm, three holes. Each Douglas fir tree within a DBH category was randomly assigned to one of the four herbicide treatments or to the control treatment. Once assigned, each tree hole was filled with 6 ml of herbicide (maximum volume for the size of the hole). We obtained five replicates x two type of herbicides x two concentrations x three DBH classes = 60 trees + five control trees per three DBH classes = 75 trees in total. The first mortality control was carried out 6 months after application and the second one at 12 months. In each event, the percentage of crown defoliation was observed and other signs such as the presence of chlorosis or deformations were recorded. The results of this experiment after 6 months of treatment application (November 2023) clearly showed that glyphosate at high concentrations is the most effective herbicide for killing invasive trees. The trees injected with this chemical substance showed a relatively fast and effective death (complete defoliation) without generating disturbances in the treated area or affecting non-target species.

2. *Comparison between chemical and mechanical removal*

To enrich and complement the previous experiment, we compared the actual working hours between herbicide injection method and mechanical tree removal.

For this, we selected 30 pines covering the following DBH categories: 10 individuals < 10 cm DBH; 10 between 10 and 20 cm DBH and 10 > 20 cm DBH. We took the amount of time it takes a single person to fell the 10 trees in each DBH category with a chainsaw.

3. *Scientific communication*

We showed and discussed the above results with the national parks conservation department, which expressed the great importance of these findings. They emphasised the importance of publishing our results in order to start designing an exotic control plan. For this reason, we quickly began working together on a manuscript that we plan to submit next month to the journal *Management of Biological Invasion*. In addition, we plan to present those outcomes at the Argentine Ecology Meeting (RAE, for its acronym in Spanish) to be held in October 2023 in the city of San Carlos de Bariloche.

4. *Effect of mortality on plant regeneration*

In April 2022, six 40 x 20 m paired plots invaded by Douglas fir individuals were selected. Within the pair, one plot received a herbicide treatment and the other plot works as a control (no treatment). Both plots of the pair were separated from each other by a physical barrier of nylon (i.e., trenching; *Photo 1. Trenching treatment*) in order to cut all fungal connections that might exist between them (Fig. 1). Herbicide application was done by stem injection to all individuals (saplings and adults) of Douglas fir found within the 'herbicide' plot (*Photo 2. Stem-injection*). As we needed to ensure the death of all individuals, we used two types of herbicides on the same tree: glyphosate at 5% and Tocón® (triclopyr + aminopyralid) at 1.5%. The number of holes per tree (i.e., the dose) was based on the DBH, as explained above in the previous section. In addition, the corresponding number of holes for each DBH class was multiplied by each herbicide. Thus, a tree with DBH less than 10 cm had one hole for glyphosate injection and one hole for Tocón injection, i.e., a total of two holes were made, and so on. Each tree hole was filled with 6 ml of herbicide. During the same field trip, all woody seedlings (native and exotic) found within each paired plot (herbicide treatment and control) were recorded and closed with cages (*Photo 3. Selected seedlings*). The total number of seedlings marked was 243, of which 90% were exotic (*Pseudotsuga menziesii* and *Juniperus communis*) and 10% native (*Nothofagus dombeyi, Berberis darwini, Berberis microphylla, Schinus patagónicus, Maytenus boaria* and *Luma apiculata*). Growth and survival of all seedlings in the paired plots were recorded at 6, 9 and 12 months after the herbicide application campaign. During each field trip, plots were maintained by renewing seedling cages and tags, removing newly emerged and unmarked exotic seedlings, reapplying herbicides on trees with poor signs of mortality, among others. In all but one field trip, canopy opening data was collected from each plot using a light sensor that measures photosynthetically active radiation (PAR radiation) reaching the ground.

5. *Educational activities*

In April 2023, we had the opportunity to offer an educational activity in the kindergarten of school number 324 (Villa Los Coihues, Bariloche) where children observed specimens of native and exotic mushrooms that we collected from the forest. They learned the main morphological characters to identify them and then made drawings in which they reflected what they had learned. At the end, we shared ideas on how to care for and conserve our native ecosystems.

6. *Difficulties encountered*

After the SARS-CoV-2 pandemic, my PhD program was closer to its end, so I had to adapt the original proposal to an alternative one that would minimise potential failures and allow me to collect consistent results in the short term.

The original experiment was divided into two different trials. On the one hand, we chose a particular area invaded by Douglas fir on which we tested the efficiency of the chemical control (Section 1) and compared the cost-benefit ratio with the mechanical removal (Section 2). On the other hand, we chose another invaded location to analyse how tree mortality (and consequently the death of the symbiont fungi in their roots) combined with the trenching treatment affects the regeneration

*of native and exotic seedlings that were already growing in the forest (Section 3).*

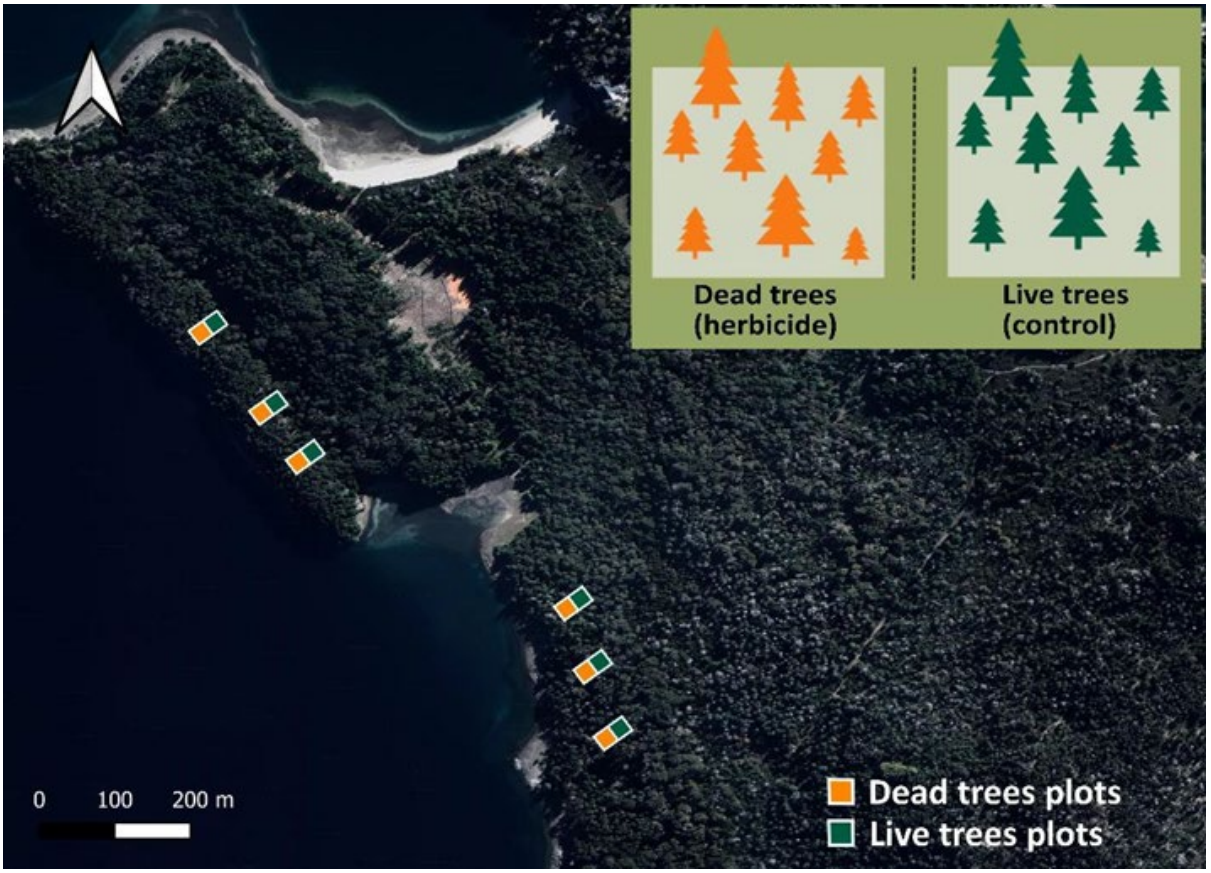


**Figure 1**. Site location at Isla Victoria, Bariloche, Argentina. The experimental design of paired patches is shown in the upper right corner. The dotted line represents the trenching treatment that restricts fungal connections between patches.

*Fundamentals* – Prior to April 2022, we had been working on an experiment in which we planted Douglas fir seeds (stratified and unstratified) on the forest floor and noticed that the germination rate was extremely low. Therefore, to ensure the collection of regeneration data, we decided that it would be best to select seedlings that were already present in the plots. Details of this experiment are given in section 4. This point also led us to reconsider the mechanical removal treatment since it generates a considerable disturbance and the fall of trees can damage the growing seedlings. However, as we were interested in evaluating the efficacy of this method, we conducted another trial as specified in section 2.