CONDITIONS FOR ESTABLISHMENT OF A KEY RESTORATION SPECIES, *Lupinus elegans* Kunth, in a Mexican Temperate Forest

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**Abstract:** Seedling establishment is essential to ensure the persistence of most plant populations. When the establishment of early successional species is hampered, the regeneration dynamics of plant communities may be altered, thus becoming an obstacle for ecological restoration practice. This is the case of *Lupinus elegans* (Fabaceae), a pioneer leguminous shrub of temperate forests that facilitates the establishment of other plant species. In this experiment, *L. elegans* seeds were planted in the same density within a landscape of abandoned agricultural fields, to determine site characteristics that favor the establishment of this species. By analyzing classification trees, the variables that explain *L. elegans* establishment were determined, before and after herbivory by *Zygogeomus trichopus*, an endemic gopher known as tuza de Nahuatzen, that prevented establishment of this species by 40%. The results showed that S-SW and SW-W orientations, a soil density > 0.8047 g/cm³, a vegetation cover < 110%, and slopes > 11.5° favored *L. elegans* establishment and minimized mortality caused by the gopher.

**Key words:** Fabaceae, germination, herbivory, interactions, population.

**Resumen:** El establecimiento de plántulas es esencial para garantizar la permanencia de la mayoría de las poblaciones de plantas. Cuando el establecimiento de especies sucesionales tempranas se encuentra limitada, la dinámica de la regeneración de las comunidades de plantas puede alterarse, volviéndose un obstáculo para la práctica de la restauración ecológica. Este es el caso de *Lupinus elegans* (Fabaceae), una leguminosa arbustiva que facilita el establecimiento de otras especies vegetales. En este experimento se sembraron semillas de *L. elegans* en la misma densidad en un paisaje formado por campos agrícolas abandonados, para determinar las características del sitio favorables para establecimiento de esta especie. Con base en el análisis de árboles de clasificación, se determinaron las variables que explican el establecimiento de *L. elegans*, antes y después de eventos masivos de herbivoría por *Zygogeomus trichopus*, la cual es una tuza endémica, conocida como tuza de Nahuatzen, que redujo el establecimiento de *L. elegans* en un 40%. Los resultados indicaron que las orientaciones S-SO y S-O, una densidad de suelo > 0.8047 g/cm², una cobertura vegetal < 110% y pendientes > 11.5° favorecen el establecimiento de *L. elegans* y minimizan la mortalidad causada por la herbivoría por la tuza.

**Palabras clave:** Fabaceae, germinación, herbivoría, interacciones, poblaciones.

Seedling establishment is an essential step in the survival of plant populations (Zobel et al., 2000). Successful seedling establishment depends not only on the particular seed characteristics but also requires a specific combination of biotic and abiotic conditions that will function as filters, thus defining the species that will colonize a particular site (Zobel et al., 2000; Bustamante-Sánchez et al., 2011). Abiotic filters include light, soil texture, nutrient availability, water availability, among others (Harper et al., 1965; Harper and Benton, 1966; Titus and del Moral, 1998; Holl, 1999; Germino et al., 2002; Jones and del Moral, 2005; Peters et al., 2008); biotic filters include competition, herbivory, and facilitation (Connell and Slatyer 1977; Callaway and Walker 1997). It has thus been proposed that seeds and seedlings require safe sites that favor germination and initial seedling establishment (Eriksson and Ehrlén, 1992), that in turn has
a fundamental role in the distribution of plant species and population viability (Münzbergová and Herben, 2005). It is noteworthy that few authors mention that environmental conditions that foster seed germination are not always the most suitable for seedling establishment of the same species (Harper et al., 1965; Harper and Benton, 1966; Titus and del Moral, 1998; Nathan and Muller-Landau, 2000; Jones and del Moral, 2005).

Among biotic interactions is of particular importance the characteristics of the plants already present in the site, since they might facilitate or hamper seedling establishment (Germino et al., 2002; Holl, 2002; Jones and del Moral, 2005). Likewise, animal species contribute to seedling recruitment by dispersing seeds, or prevent it, by consuming seedlings or seeds or removing the soil, particularly if they are burrowing species (Dhillion, 1999; Edwards and Crawley, 1999; Holl, 1999).

In short, following the sequence of events that leads to successful plant establishment, the main factors that have been identified as hindering germination and seedling establishment are the presence and intensity of disturbance, and the characteristics of the site where the seeds are deposited (Eriksson and Ehrlén, 1992; Holl, 1999; Nathan and Muller-Landau, 2000; Zobel et al., 2000). Afterwards, once a seedling has established, herbivory may significantly affect species distribution and consequently the composition of the community (Ashton et al., 1997; Edwards and Crawley, 1999; Edwards et al., 1999; Nathan and Muller-Landau, 2000).

Most studies have focused on the microclimatic characteristics of safe sites as the determinant factors for seedling establishment (Harper et al., 1965; Harper and Benton, 1966; Titus and del Moral, 1998; Holl, 1999; Germino et al., 2002; Jones and del Moral, 2005). Nevertheless, it is important to consider other large scale factors that influence seedling establishment, such as topography, since they have a direct effect on variations at smaller scales (Germino et al., 2002).

Our study model, *Lupinus elegans* (Fabaceae), is a leguminous shrub growing naturally in the temperate pine and oak forests of Mexico and Central America, mainly in regions where soil use has shifted to agriculture or livestock grazing (Sánchez, 1980; Medina et al., 2000; Medina-Sánchez and Lindig-Cisneros, 2005; Alvarado-Sosa et al., 2007). The positive effect of *L. elegans* has already been evaluated under environmental restoration conditions and it has been shown that it induces an increase in plant species richness (Blanco-García and Lindig-Cisneros, 2005; Blanco-García et al., 2011; Diaz-Rodriguez et al., 2012); other species of the genus act as nurse-plants on the Neotropics (Vargas et al., 2009). It is therefore, important to determine the factors that limit the establishment of *L. elegans* in disturbed sites within its natural distribution range in North America, particularly in areas with low-nitrogen soils. The requirements for propagation of this species have already been tested under greenhouse conditions. Greenhouse propagated plants perform better than those sprouting from seeds planted on-site (Alvarado-Sosa et al., 2007). However, nursery propagation is expensive and limits the use of this species for restoration purposes. Consequently, it is useful to find out which factors affect its establishment from seed under natural conditions that is to define the establishment limitations for *L. elegans*, to minimize costs of restoration efforts and improve our ecological understanding of this species. Nevertheless, it is difficult to discern which environmental variables are relevant for seedling establishment from a statistical perspective. A promising strategy is the use of classification trees. Classification tree analysis is a non-parametric technique (De’ath and Fabricius, 2000) used mainly when there are several explanatory variables and guidance is needed to know which of these should be included in a model (Crawley, 2007). Thus, the objectives of the present study were to determine through the use of classification trees analysis, the factors (filters) that determine *L. elegans* seedling establishment. For this, the herbaceous richness in sites where *L. elegans* could be established was evaluated as well as the cover of the dominant herbaceous species, plot orientation, slope, soil density, and herbivory.

**Materials and methods**

**Study site.** The indigenous community of San Juan Parangaricutiro (CINSJP) is located in the state of Michoacán, Mexico at 2,750 m a.s.l., with an average temperature of 15.1 °C, and an annual average rainfall of 1,200 mm. The landscape consists of pine (*Pinus pseudostrobus* and *Pinus montezumae*), pine-oak forests (being the most common species: *Quercus crassifolia*, *Q. crassipes* and *Q. rugosa*) and at higher altitudes *Abies religiosa* forests (Medina et al., 2000; Velázquez et al., 2003). The study site is characterized by a mosaic of induced grasslands, mostly dominated by native species, established on abandoned agricultural fields, pine plantations from past reforestation programs, and fragments of natural forest. Patches of naturally grown *Lupinus elegans* are found mainly in the areas that have suffered some type of natural disturbance.

**Field experiment.** As part of a long-term experiment, *Lupinus elegans* seeds were sown at the site in June 2008. The CINSJP allowed us to start up the experiment, in two hills in the process of reforestation (trees were planted in 2002 and 2003). A total of 42 plots of varying sizes of *L. elegans* were randomly established (16, 32, 64, and 128 m²). The different sizes responded to the need of determining long term effects that are not reported in this study. All plots, regardless of size, were sown with the same density of seeds per square meter, 0.32 gr/m² (Gómez-Romero, 2006), by dropping individual seeds following an equally spaced grid. In September 2008 (during the rainy season) plant species richness in the four areas was assessed, and presence as well as percent...
cover of the three most abundant species in each plot were recorded. In addition, slope and orientation were measured, and soil samples were taken to quantify soil density in order to characterize the initial conditions. Furthermore, the number of *L. elegans* seedlings that sprouted from the seeds sowed were also counted. Seedlings were counted again in January, March and June 2009 (during the dry season). Seedling establishment was assessed as the number of plants in 10 m², both before and after herbivory by *Zygogeomys trichopus* (the Nahuatzen gopher); taking into account the following variables for each of the 42 plots: plot orientation, slope, soil density, and plant cover. Plant cover was obtained by adding the cover percentages of the three most abundant species and as a result in some cases percentages greater than 100% were recorded that reflect canopy overlapping.

Statistical analysis. To standardize the response variable the number of *Lupinus elegans* individuals in 10 m² was used because naturally occurring patches of this species are approximately of this size. As a first step, classification trees were obtained. The classification tree is formed by the repetitive division of the data, and these divisions are defined by simple rules based on the explanatory variables. Each one of the groups is characterized by the mean value of the response variable, the group size and the values of the response variable that define it (De’ath and Fabricius, 2000). This creates a structure similar to that of an inverted tree having a root node where all data are found, intermediate nodes, division points giving rise to each group to which a value has been assigned, and the terminal branches. Three classification trees were obtained: the first one assessing the variables that explained establishment prior to herbivory by the Nahuatzen gopher; the second to evaluate *L. elegans* survival after herbivory, and finally a classification tree to assess mortality caused by herbivory and to find out which of the explanatory variables were related to its occurrence. Nevertheless, this non-parametric technique only reduces data variation by grouping, and does not imply that the groups are statistically different. Accordingly, we conducted parametric tests to evaluate the differences between the classification tree groups using one-way ANOVA. It is important to note that the groups differ in the number of plots they contained, and therefore the degrees of freedom in the analyses may differ. Data presented are means and standard deviation unless noted otherwise. All statistical analyses were made using R (R Development Core Team, 2011).

Results

The plant community in the studied reforested areas does not include many herbaceous species. During the 2008 growing season 44 species were found, among these the following pioneer species, which are usually present in abandoned agricultural fields, were dominant: *Jaegeria hirta*, *Muhlenbergia macroura*, *Oenothera pubescens*, *Phacelia platycarpa*, and *Vulpia myuros*. The most abundant species in the four areas were *Vulpia myuros* (Poaceae) and *Trifolium amabile* (Fabaceae) with average percent cover of 20-60% and 5-41% respectively. All other species had percent cover lower than 10%.

There was a low percent establishment of *Lupinus elegans* with respect to the amount of seeds sown. Two factors greatly affected its establishment: the frost season in December and January 2008-2009 (six and seven months after seed sowing) that reduced the average survival by 20% and the herbivory by the Nahuatzen gopher during June 2009 further reduced survival by almost 40%. In June 2009 only 43.8% of the seedlings recorded in September 2008 survived (1,090 seedlings three months after planting). Mortality occurred mainly among lower height individuals (Figure 1).

Since plots were allocated randomly, they differed with regards to their physical characteristics. With respect to plot orientation, the following number of plots had each: N-NE (3), NE-E (14), E-SE (7), SE-S (5), S-SW (0), SW-W (3), W-NW (3), and NW-N (7).

Abiotic factors limiting *L. elegans* establishment. Classification-tree analysis of abundance data collected after the first growing season (and before herbivory), showed that out of the four variables assessed, only plot orientation and soil density grouped *Lupinus elegans* seedling abundance (Figure 2). The first variable influencing seedling abundance was orientation, which divided the data into two groups: the first group included those plots having S-SW, SW-W and W-NW orientations (to the left of the diagram), and the second in-

![Figure 1. Survival curve of Lupinus elegans seedlings in the study area. Two main events of mortality were observed, the frost season and herbivory by the Nahuatzen gopher.](image-url)
Figure 2. Classification tree of data for *Lupinus elegans* seedling abundance during the first growth season (2008-2009) before herbivory occurred. End nodes represent the mean seedling abundance (seedlings/10 m²) for each homogeneous group.

Table: Classification tree of data for *Lupinus elegans* seedling abundance after herbivory by *Zygogeomys trichopus*. The groups and subgroups defined by the classification tree are: the groups RA and S-SW_SW-W which arise as a result of the first division of data by orientation. Subgroups L, M, H arise as a result of the subdivision of the RA group, according to different ranges of soil density. The LVC and HVC subgroups arise from the subdivision of the S-SW_SW-W group data according to plant cover.

**Zygogeomys trichopus herbivory.** The classification tree obtained from the same four explanatory variables, but after herbivory by gophers had occurred – an action that drastically modified the number of survivors –, showed that orientation, soil density, and plant cover explained *Lupinus elegans* seedling abundance (Figure 3). The first variable influencing seedling abundance was orientation, which divided the data into two groups: the first including S-SW and SW-W, and another one for all the remaining orientations (RA). According to this analysis, on the left branch (RA group) the next factor influencing establishment was soil density, which divided it into three subgroups: the L subgroup, where soil density was lower than 0.83 g/cm³ and average seedling abundance was 1.12 ± 0.99 individuals/10 m²; the M subgroup, defined by soil density between 0.83 and 0.87 g/cm³, had an average abundance of 2.27 ± 1.57 individuals/10 m²; and the H subgroup, defined by densities greater than 0.87 g/cm³, where average seedling abundance was 0.95 ± 1.01 individuals/10 m² (Fig. 4a). Differences among these groups were marginally significant ($F_{(2,32)} = 3.20, P = 0.054$).

In the S-SW_SW-W group the next relevant factor to explain *L. elegans* seedling abundance was plant cover. In this case, two subgroups were formed: the LVC subgroup, having plant cover lower than 110% and average seedling abundance of 3.33 ± 1.33 individuals/10 cm³, the highest average for the dataset. If plant cover is greater than 110% (HVC subgroup) this average was 1.91 ± 1.82 individuals/10 m².
Figure 4. Seedling abundance (individuals/10m²) in relation with the variables that group the data in the classification tree after herbivory by Zygogeomys trichopus.

Once the variables that explained Lupinus elegans seedling abundance were identified, the effects of each were assessed. Thus, following the development of the classification tree, the first groups arising from the orientation explanatory variable are S-SW_SW-W and RA. According to the results obtained after herbivory in the foregoing analysis, the average number of plants per 10 m² for the S-SW_SW-W group is significantly greater (F(1,46) = 9.27, P = 0.003) than for the RA group (Figure 4b). Plots with S-SW and SW-W orientations (the S-SW_SW-W group) facilitate L. elegans

Figure 5. Classification tree of Lupinus elegans mortality after herbivory by Zygogeomys trichopus. Variables grouping the data are soil density, slope and plot orientation.
establishment since seedling abundance is greater (2.56 ± 1.71 individuals/10 m²) than in plots with other orientations (RA group 1.24 ± 1.18 individuals/10 m²).

Conditions for herbivory by *Zygogeomys trichopus*. Percent mortality of *L. elegans* seedlings was grouped using the same explanatory variables of the previous analysis to assess conditions that favor herbivory by *Zygogeomys trichopus*. Soil density, orientation and slope grouped the data in the classification tree of Figure 5. The first variable is soil density, creating two groups: one for values lower and the second for values greater than 0.80 g/cm³ (left branch, LSD and right branch, HSD, respectively). The difference between mortality of LSD (24.04 ± 30.3%) and HSD (55.2 ± 33.45%) was significant (F(1, 46) = 10.16, P = 0.002).

The left branch (LSD) divided further into two groups depending on slope: the group SS with slopes lower than 11.5° and a mortality of 44.7 ± 35.4%, and the group IS with a slope higher than 11.5° and mortality of 9.5 ± 15.1%, differences being significant (F(1, 15) = 7.92, P = 0.013). The right branch of the tree was also divided by slope forming two groups: VSS with slopes lower than 4.5° and a mortality of 32 ± 41%, and IS2 with slopes greater than 4.5° and a mortality of 59.65 ± 30.76% (F(1, 29) = 3.04, P = 0.091).

Group IS2 divided further by orientation: a first group (BF) including NE-E and SW-W plots with a mortality of 49.62 ± 30.46%, and a second group (TR) of all remaining plots that showed a mortality of 68.25 ± 29.35% (F(1, 24) = 2.51, P = 0.12). BF divided further by soil density into two branches: LD when soil density was lower than 0.89 g/cm³, with a mortality of 27.28 ± 24%, and HD with soil density higher than 0.89 g/cm³ and a mortality of 65.59 ± 24.54% (F(1, 10) = 7.21, P = 0.022).

Discussion

*Lupinus elegans* survival curve shows that there were two determinant factors for seedling survival: a severe winter and gopher herbivory, the latter having the most significant impact on species survival. Herbivory has been reported before as a cause of high mortality in plants of this species transplanted to restoration sites in the vicinity of the study area (Blanco-García and Lindig-Cisneros, 2005; Alvarado-Sosa et al., 2007). In addition to these two events, we identified abiotic and biotic factors that have an impact on plants established by seeding: plot orientation, slope, soil density, and plant cover. Soil and terrain features have been widely used to explain seedling establishment of other species, mainly because of the great diversity in edaphic conditions which provide seeds a variety of conditions for germination (Harper et al., 1965; Titus and del Moral, 1998; Jones and del Moral, 2005).

As mentioned, classification trees allow the ranking of the assessed variables without implying that the groups formed are significantly different. Nevertheless, they provide a predictive tool that can be used to establish groups to be compared afterwards by parametric methods (Vayssières et al., 2000). The combination of these two analytical approaches showed that orientation and soil density explain *Lupinus elegans* establishment. By using them in combination it was possible to define the optimal features of the establishment sites for this species in the study area, i.e., hillsides with S-SW and SW-W, orientations with a soil density close to 0.80 g/cm³, which have a larger density of individuals. A possible explanation could be that very loose soils do not provide sufficient support for plants to take root and establish (Goodman and Ennos, 1999). The study site has many areas where the soil had been removed by the burrowing activity of the Nahuatzen gopher, thus limiting the establishment of *L. elegans*. In regard to orientation, it is common knowledge that in the northern hemisphere, southern and western oriented hillsides are warmer because of their longer exposure to sunlight; therefore, early successional species that show greater resistance to unfavorable environmental conditions are more common than those that are sensitive to extreme conditions, as is the case of many late successional species (Olivero and Hix, 1998; Schlatter, 1994; Enciso et al., 2000; Ramírez-Contreras and Rodríguez-Trejo, 2004).

Gopher activity and its effect on survival of *Lupinus elegans* were detected in the study area since September, causing direct damage to the seedlings and indirectly by soil removal. However, the greater damage caused by *Zygogeomys trichopus* herbivory occurred later. The classification tree after gopher herbivory showed that S-SW and SW-W orientations continued to be determinant for the establishment of the species. In contrast, the W-NW orientation was no longer found among those favoring establishment. Soil density continued to be important and denser soils continued to hold a greater number of seedlings.

The results obtained by classification tree analysis showed that soil density had a determinant role in seedling mortality. This could be explained by the fact that gophers require firm soils to prevent their burrows from collapsing (Lacey et al., 2000), thus they limit the establishment of *Lupinus elegans*, since seedlings and saplings of this species thrive better in the dense soils where gopher activity takes place. Herbivory is a limiting factor in the establishment of seedlings and survival of fully-grown plants, and has a strong impact on plant mortality rates (Alverson et al., 1988; Benítez-Malvido, 1998; Crawley, 1997; Edwards and Crawley, 1999; Holl, 1999; del-Val et al., 2007). Herbivores can be particularly harmful for restoration when they remove the whole plant (Allen et al., 2005; Blanco-García and Lindig-Cisneros, 2005; Sweeney et al., 2007). It has been shown that herbivores are a limiting factor for plant survival under restoration conditions at many sites, showing an overall damage of 64% in the restoration of tropical forests in Costa Rica (Holl and Quiros-Nietzen, 1999) and of 78% of seedlings of *Quercus rugosa* at restora-
tion sites located close to Mexico City (Bonfil and Soberón, 1999), major damage to restorations has been reported by browsing by wild boars (Mayer et al., 2000), deer (Miller et al., 1982), and elk (Johnston et al., 2007).

In this study, the herbivory by *Zygogeomys trichopus* was determinant in the establishment of *Lupinus elegans*, since it clearly reduced the number of individuals observed within a three-month period. However, *Z. trichopus* is an endemic and endangered species of the temperate regions of the state of Michoacán (IUCN, 2012) and therefore if a temperate forest restoration effort is contemplating the use of *L. elegans*, it is important to take into account that it will not be possible to take measures to control the Nahautzen gopher population. Consequently, the number of seeds sown will need to be increased to compensate for herbivory losses, and it is advisable to sow them in slopes with SW-W orientations (with plant cover lower than 100%), and choosing high density soils. In many instances greater efforts are needed to reactivate natural environmental processes (Hobbs and Norton, 1996; Ashton et al., 1997). Thus the use of facilitating plants such as *L. elegans* can be a good strategy for the restoration of a site, since many leguminous plants enrich degraded soils due to their nitrogen-fixing capability, while providing establishment microsites for other species, therefore contributing to their restoration (Carrillo-García et al., 1999; Monroy-Ata et al., 2007; Blanco-García et al., 2011).

In order to make use of facilitating plants in restoration projects, it is first necessary to ensure the establishment of their seedlings and saplings. Even though *Lupinus elegans* is a species that grows naturally in disturbed sites, it requires certain conditions for seedling establishment. Trial tests such as the one conducted in this study allow us to set criteria for the establishment of plant species in restoration projects.

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**Literature cited**


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