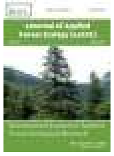
eJournal of Applied Forest Ecology (eJAFE), Vol 10, No. 2 (2022) 26 – 35
Available online at www.ejafe.com
ISSN: 2347-4009



Can forest management and slope orientation affect seed germination of secondary species? Experiences from a temperate forest in Mexico.

Yuriana Martínez-Orea, Leticia Bonilla-Valencia, Roberto Lindig-Cisneros[1], Cesar Flores-Ortíz[2], and Silvia Castillo-Argüero*

Universidad Nacional Autónoma de México, Facultad de Ciencias, Departamento de Ecología y Recursos Naturales, Av. Universidad 3000, Coyoacán, Cd. de México, C.P. 04510, México.

[1]*Universidad Nacional Autónoma de México, Instituto de Investigaciones en Ecosistemas y Sustentabilidad IIES, Michoacán, México.*

[2]*Universidad Nacional Autónoma de México, Facultad de Estudios Superiores Iztacala, Cd. de México, México.*

Received on October 22,2022;Accepted on December ;4,2022.

Abstract

Forest management includes technical aspects for forest conservation. Therefore, knowledge of the effects of silvicultural activities on secondary species seed germination should be considered, because they provide resilience. Seeds germinate differentially according to slope orientation, gap size and silvicultural practices such as weeding. With the objective of knowing the effect of weeding and slope orientation on seed germination, 24 microsites were established, 12 in north and 12 in south oriented slopes (half of each weeded, half not-weeded) in a fir forest. In microsites, seeds of *Cestrum thyrsoideum* and *Solanum cervantesii*, two common secondary species were germinated, environmental variables of soil light, moisture, temperature, pH, and nitrogen were registered. We also explored germination under different light types in chambers. The highest values of light at soil level and of *S. cervantesii* germination corresponded to not-weeded north-oriented microsites because canopy gaps were bigger than in weeded ones and because they were the most humid. South-oriented microsites received higher solar radiation and they registered the highest temperatures, specially weeded ones. The highest values of soil nitrogen and where pH was the lowest corresponded to weeded microsites-north oriented. For both species, germination percentages were the highest in white-light, the lowest germination percentages corresponded to far-red light conditions. Secondary vegetation might buffer drastic light changes at the level of soil and protect against soil moisture loss, for this reason weeding should not be practiced specially in south oriented microsites, since soil moisture is required for germination and the seeds of some secondary species respond differently to light qualities.

Keywords: Forest gaps, safe microsites, secondary species, soil moisture, silviculture.

Introduction

The destruction of primary forests has brought the expansion of secondary forests. According to Emrich *et al.,* (2000), there is an increasing interest in this type of vegetation because it is expanding in many countries, but even secondary vegetation has been reduced as a result of disturbances, such as deforestation. Furthermore, many ecological attributes of secondary species are still unknown. Some shrub species provide connectivity between forest fragments and others act as nurse species for conifer seedlings. Therefore, there is a need of knowing about their ecology, such as how disturbances affect their seed germination (Martínez-Orea *et al.,* 2019), since studies on this matter are still scarce. It has been reported that the availability of safe microsites for germination determine population dynamics (Crawley, 2012).

They are a result of the interaction between seed germination requirements and environmental variables such as soil moisture, soil temperature and light (Lindig-Cisneros and Zedler, 2002). Light has an effect on germination through several mechanisms and seeds can respond to spectral qualities such as a high or low red/far red light ratio, a mechanism mediated by phytochrome, which absorbs light of different wavelengths (Orozco-Segovia and Sánchez-Coronado, 2013), however light quality modification as a consequence of silvicultural activities, slope orientation and canopy features, needs particular studies that haven't been carried out in temperate ecosystems especially at a fine scale, since there can be a large variation in terms of different variables that shape microsites. Microsite variables are also the result of forest characteristics such as slope orientation, which can make south and north oriented slopes different in light quality, temperature and moisture (Méndez-Toribio *et al.,* 2016). These variables are affected by disturbances such as deforestation, and by some types of forest management and silvicultural practices. For example, weeding (suppression/control of secondary vegetation) is carried out in central Mexico forests for minimizing the competition between understory species and conifer seedlings. However, it has been suggested that this practice could

---

* Corresponding author : Silvia Castillo-Argüero
Email: silcas@ciencias.unam.mx

negatively affect soil moisture as well as modify light quality at the scale of the microsite, affecting seed germination of secondary species (Martínez-Orea *et al.,* 2019).

Whether secondary species are more or less abundant under certain perturbation regimes or silvicultural practices; the identification of their safe germination microsites is important in terms of restoration/conservation plans (Martínez-Orea *et al.,* 2019), above all considering their ecological roles. Few studies have related the effect of certain silvicultural practices such as weeding on safe microsite limitation, especially for secondary species germination, and especially in urban forests. Species of Solanaceae are present in most habits: trees, shrubs, and herbs. Despite their worldwide distribution and diversity, some species are restricted to particular ecological conditions. Little research has been conducted to document their role as colonizers of disturbed areas such as forest edges. Furthermore, this forest is disturbed mainly by deforestation, urbanization, agriculture and cattle, therefore canopy gaps are common. Plant colonization under gaps by secondary species (such as plants belonging to the family Solanaceae) may, therefore, play an important role in temperate forest resilience.

The aim of this work is to analyze the germination response and viability of seeds of two common shrubs of Solanaceae (*Cestrum thyrsoideum* Kunth and *Solanum cervantesii* Lag.) to different light qualities under controlled conditions (germination cameras - devices where temperature and light are provided artificially-), and in microsites that differed according to slope orientation (south/north) and weeding (with/without secondary vegetation) in a fir forest in Mexico City. Due to the fact that anthropogenic disturbances affect the environmental variables at the level of microsite, our hypotheses suggest that the interaction between slope orientation and silvicultural practices such as weeding will have an effect on *Cestrum thyrsoideum* and *Solanum cervantesii* seed germination, showing higher percentages in south oriented slopes, due to the fact that they receive more light and the study species are secondary, thus they have a preference for germination in sites with higher solar radiation. We expect to contribute on the knowledge of the biology of seed germination of secondary species and how different variables shape safe microsites for their germination in a managed forest. We also expect that the seeds of both species will respond with higher percentages under white and red light in germination cameras. With respect to viability, we expect that seeds of both species will lose some viability in two years of storage.

Materials and Methods

Study site

Present study was carried out in the Magdalena river basin (MRB), which is located SW of Mexico City at an altitude between 2,570 and 3,870 a.m.s.l. The climate in this area is temperate sub humid type C(w2)(w)b(i'), mean annual temperature is 14°C (minimum 6°C, maximum 20°C), with a mean annual precipitation between 950 and 1,300 mm. It has an area of 3,100 ha and harbors rivers that provide most of the superficial water for Mexico City, therefore the importance of conserving the forests established in this area, mostly if we take into account that 67% of these forests were considered as conserved not so long ago. Nevertheless, they are constantly affected by deforestation, cattle rising, tourism, induced fires and farming. The three main vegetation types in the MRB are: oak, fir (*Abies religiosa* (Kunth) Schltdl. and Cham.) and pine forests. The fir forest has the greatest extension (1,071 ha) and is located at an altitude of 2,900-3,650 m.s.l. It is highly heterogeneous in topography because it is characterized by numerous differently oriented slopes (Ávila-Akerberg, 2002).

While *Abies religiosa* is the dominant tree species in the fir forest, other trees are also present such as *Sambucus nigra* L. and *Garrya laurifolia* Benth.Understory species as shrubs are abundant mostly in sites affected by deforestation, under large canopy gaps, or where cattle is practiced (Ávila-Akerberg, 2002), for example *Acaena elongata* L., *Ageratina glabrata* (Kunth) R.M. King and H. Rob., *Roldana angulifolia* (DC.) H. Rob. and Brettell, *Cestrum thyrsoideum* Kunth and *Solanum cervantesii* Lag. In different areas of this forest, especially where reforestation activities are practiced, the owners of this land carry out an elimination of the understory vegetation (weeding) in order to provide light availability for *Abies religiosa* seedlings. However, this management is practiced without considering site characteristics such as slope orientation, tree density or gap size, but Martínez-Orea *et al.,* (2019) have reported that this practice may be considered as a disturbance because it may change some variables at the fine scale such as soil moisture content. This is a conservation area in the city, managed by the city government, however, people in the community are included in restoration or environmental services payment programs.

Microsite characterization

In June 2016 two areas were chosen, one weeded and another one non-weeded (figure 1A and 1B, respectively). In these sites twenty-four microsites each of 1 m^2 were established in the *Abies religiosa* forest of the MRB, these differed in slope orientation

(South-S-/North-N-) and in the use or not of weeding (this practice was considered as a disturbance). In this way there were six microsites US (undisturbed/south oriented), six UN (undisturbed/north oriented), six PS (perturbed/south oriented), and six PN (perturbed/north oriented), they were all located between 2,932 and 3,170 m. Microsite environmental variables were characterized including: light at the soil level, soil temperature, moisture, total nitrogen (N) and pH. The quantification of these variables was carried out because they can have an influence on germination and can be modified by weeding and slope orientation (Martínez-Orea *et al.,* 2019).

Figure 1. A) Weeded and B) Non-weeded areas in the Magdalena river basin, Mexico City, Mexico.

Light was quantified by hemispheric photography; in each microsite nine photos were taken 1 m above the ground (NIKON D80 equipped with an EX SIGMA 4.5 fisheye lens, 1.28 DC DSM, Tokio JP) following Messier and Puttonen (1995). They were analyzed using the Hemiview canopy analysis software (ver. 2.1) for estimating the global site factor (GSF, $MJm^{-2}yr^{-1}$), which is defined as the quantity of light transmitted per unit area. Red/far-red (R/FR) light ratios from 10:00 to 17:00 were registered with a radiometer (Skye Instruments, SKR 100, United Kingdom), through five measurements/ microsite, this was carried out during one day after the sowing of seeds. For measuring soil moisture and temperature at each microsite, one data logger (easy LogUSB-ONSET, Massachusetts, USA) registered data every hour every day during June. Three soil samples were collected at each microsite, they made up a mixed sample of 200 g, which were analyzed for determining pH and nitrogen content. pH was obtained through the relation of soil and water at 1:2 and nitrogen (N) percentage through the moist digestion with H_2SO_4 Kjeldahl distillation (0.05).

Seed germination in microsites

Mature fruits of *Cestrum thyrsoideum* (4mm ± 0.01 long) and *Solanum cervantesii* (3mm ± 0.02 long) were collected from 30 individuals per species during the last week of May 2016. The fleshy cover was manually eliminated, and the seeds were disinfected for 10 minutes in a 10 % sodium hypochlorite solution, dried and then sown in the following design: three cloth bags (8 cm × 8 cm; 0.3 mm × 0.3 mm mesh) were filled with 50 seeds each (150 seeds/microsite/species), bags were sown randomly at microsites (2 cm depth). Three extra bags of 10 seeds were sown at each microsite; each one of these bags was exhumed every week to check for germination, these seeds were not included in the analysis. Once germination was registered (14 days after sowing), all bags were exhumed, and the germinated seeds (emerged radicles) were counted.

Seed germination in cameras

Three days after collection, six hundred seeds of each species were disinfected in sodium hypochlorite (10%) for three minutes. They were subjected to different light qualities (darkness D, white W, far red FR, and red R light) in Petri dishes in a germination chamber (Nuaire model I-36LL, USA, equipped with fluorescent lamps OSRAM de 17 watts and 60 % RH) at 22 °C / 20 °C, and 16 / 8 photoperiod. Three Petri dishes (9 cm diameter each) with humid absorbent paper (previously sterilized, as a substrate) per light treatment and with 50 seeds each were set in a growth chamber under: (1) white light (WL; photon flux density (PFD) = 33.21 $\mu moles\ m^{-2}s^{-1}$, R/FR = 1.73); (2), red light (RL; PFF = 5.18 $\mu moles\ m^{-2}s^{-1}$, R/FR = 3.39), (3) far-red light (FRL; PFF = 1.2 $\mu moles\ m^{-2}s^{-1}$, R/FR= 0.05), and (4) darkness (D). PFD between 400 and 700 nm was measured with a quantometer (Apogee, model MQ-200, Apogee Instruments, Inc., Logan, UT, USA), and the Red/Far Red ratio (R = 640 - 670 y RL = 690 - 748 nm) was measured with a radiometer SKR-100 (Skye Instruments, Scotland). For the treatment with Red Petri dishes were set inside a red plexiglass box (3 mm thick, 48 × 32 × 8 cm, Series 2424 Rohm and Hass, Mexico). For the Far-Red treatment, Petri dishes were set in a red plexiglass box with a cover of blue plexiglass (same dimensions of the red box, Series 2423). For the treatment in Dark, Petri dishes were covered with aluminum foil. For 30 days, every third day we registered germination under White, and one month after being sown we registered germination in the other light treatments, so seeds wouldn't receive other light qualities when dishes were opened. Another fraction of seeds (30) was subjected to a tetrazolium test immediately after collection. The rest of the seeds were stored in paper bags in the dark at 16 °C. Seed viability was evaluated in 2016, 2017 and 2018, following Moreno-Martínez (1996) method which consists in soaking thirty seeds in water for 12 hours at 15 ºC. Afterwards, water should be deleted, and seeds are cut transversely reaching the area where embryo is visible. Seeds are then placed in petri dishes with 2,3,5 triphenyl tetrazolium chloride solution 0.5%, these petri dishes should be kept in aluminum foil to protect them from light at 20 ºC for 24 hrs. After this time, solution should be rinsed. Those seeds-stained pink-red color are viable.

Data analysis

Microsite characterization was performed using the values of the environmental variables of light, soil temperature and moisture, nitrogen (N) and pH; microsites were grouped in microsite types for their analysis: US (undisturbed -non weeded-, south oriented), PS (perturbed -weeded, south oriented), UN (undisturbed -non weeded-, north oriented), PN (perturbed -weeded-, north oriented). In order to find differences in microsites according to their values of environmental variables and their significant effect on the study species germination, we carried out a generalized linear models (GLMs) (performed with package glm^2 in R software) where the response variable was the number of germinated/not germinated seeds; the type of distribution applied was binomial (error: binomial, link function: logit, Crawley, 2012) (v. 3.5.2; R Development Core Team, 2019). Variation coefficient values [VC] were calculated for the R/FR ratios for each microsite type; these values were then compared through a Z test (Zar ,1999). Kruskal-Wallis tests with multiple comparisons were performed (not normal distribution) to determine whether the microsite had an effect on the germination percentages of species and to determine statistical differences on the germination percentages between light quality types (Stat Soft STATISTICA 8.0 2007).

For the germination tests in growth chambers, germination percentages were calculated and analyzed for statistical differences between light quality types through a Kruskal-Wallis test. These germination percentages were also transformed with the arcsin of the square root (Zar, 1999) and were related to time using a sigmoid exponential function: y = a/(1+(b*xc)) for an adjustment to the curves with Table Curve 2D 5.01 (AISN Chicago IL USA) software to calculate the lag time and germination rate for each species. We applied a principal component analysis (PCA) on the set of environmental variables and germination percentages of each species to reduce the dimensionality of the environmental variables characterizing the study microsites. This analysis was performed using R statistical software (R Core Team, 2015) with the packages vegan (prcomp function) and ggplot2 for PCA plot.

Results

The Kruskal-Wallis (H) tests showed significant differences between microsites on the germination percentages of *S. cervantesii* (H = 46.58, p = 0.0038 ***), while no differences were found for *Cestrum thyrsoideum* (H = 30.41, p = 0.13). However, when grouping microsites per type we observed that both species germinated more in undisturbed microsites than in perturbed ones, but they differed between south and north oriented microsites. These results coincide with the values of the environmental variables registered in microsites showing clear patterns. For example, undisturbed south oriented microsites (US) were characterized by the highest values of light, in terms of the GSF and the R/FR ratio and also by the highest pH value and where the highest percentages of *C. thyrsoideum* were registered: $\overline{X}$ = 68.77±8.22. *S. cervantesii* germinated in the highest percentages in undisturbed north oriented microsites (UN): □ = 46.44±5.37, where the highest values of soil moisture content and the lowest values of soil nitrogen values were found (table 1).

Table 1. Mean values (± standard error, e.e.) of the environmental variables of pH, N (soil nitrogen content %), Temp (soil temperature °C), soil moisture content (%), GSF (global site factor $MJm^{-2}y^{-1}$), R/FR (red/far red ratios) during June 2016, G% (germination percentages of *Cestrum thyrsoideum* and *Solanum cervantesii* in four microsite types (M type, we considered weeding as a disturbance): US (undisturbed south oriented), UN (undisturbed north oriented), PS (perturbed south oriented), PN (perturbed north oriented) in the *Abies religiosa* temperate forest in the Magdalena river basin, Mexico.

M type	pH	N	Temperature	Moisture	FSG	R/FR	G % Cestrum thyrsoideum	G % Solanum cervantesii
US	6.38±0.22	0.60±0.04	11.03±0.31	86.51±1.58	0.495±0.04	1.72±0.14	68.77±8.22	37±1.46
UN	6.15±0.25	0.57±.06	11.7±0.48	94.75±0.21	0.261±0.02	1.28±0.06	46.77±4.28	46.44±5.37
PS	6.1±0.2	0.64±0.01	12.22±0.22	83.7±0.06	0.169±0.03	1.30±0.14	54±3.73	33±1.57
PN	5.75±0.15	0.65±0.04	11.32±0.16	86.81±0.9	0.158±0.01	0.72±0.05	57±3.35	45.88±2.45

Despite the differences in terms of germination percentages and of the environmental variables,only *C. thyrsoideum* showed significant differences in germination due to the effect of slope orientation, while *S. cervantesii* germination was not affected by slope orientation, disturbance or by their interaction according to the GLM analysis (table 2).

Table 2. Results of the generalized linear model (GLM) for the germination of *Cestrum thyrsoideum* and *Solanum cervantesii* in different microsite types of the *Abies religiosa* temperate forest, México. Perturbation (perturbed –weeded- microsites), OrientationS (south oriented microsites), OrientaciónN (north oriented), Perturbation:OrientationN (interaction between variables), *** significant differences.

Effects	Estimated	Std	Z value
Cestrum thyrsoideum			
Perturbation	0.7028	0.7794	-0.902
OrientationS	1.7734	0.7894	2.247
Perturbation:OrientationN	-2.0990	1.1065	-1.897
Solanum cervantesii			
Perturbation	-0.0274	0.4555	-0.6
OrientationN	-0.4232	0.4553	0.93
Perturbation:OrientationN	-0.1889	0.6443	-0.293

Looking at the particular effects of each variable on the germination of each species, in table 3 we can observe that the environmental variables that had a significant effect on the germination of *C. thyrsoideum* and *S. cervantesii*. In microsites UN (undisturbed north oriented microsites) and in PN (perturbed north oriented) all environmental variables had a significant effect on the germination of *C. thyrsoideum*, while in microsites US (undisturbed south oriented) pH, soil temperature and moisture had a significant effect on *S. cervantesii* germination and in UN (undisturbed north oriented) microsites soil nitrogen and moisture as well as GSF had significant effects on its germination (table 3). Light quality measured through the red/far red rate did not have a significant effect on the germination of these species in any microsite type.

Table 3. Results of the GLMM (Generalized lineal model of mixed effects) analysis for the environmental variables of pH, N (soil nitrogen content, %), Temp (soil temperature °C), Moist (soil moisture content %), GSF (global site factor MJm^{-2}y^{-1}) and their effect on the germination of *Cestrum thyrsoideum* and *Solanum cervantesii* in four microsite types: US (undisturbed south oriented), UN (undisturbed north oriented), PS (perturbed south oriented), PN (perturbed north oriented) in the *Abies religiosa* temperate forest of the Magdalena river basin, México, *** significant differences.

Species	Microsite type	pH	N	Temp	Moist	GSF
Cestrum thyrsoideum	US		3.3(p≤0,001) ***	3.7(p≤0,001) ***	3.6(p≤0,001) ***	
	UN	6.5(p≤0,001) ***	-3(p≤0,01) **	-9(p≤0,001) ***	10.9(p≤0,001) ***	-6(p≤0,001) ***
	PS		2.3(p≤0,05) *	5.1(p≤0,001) ***		
	PN	-5.1(p≤0,001) ***	-7.5(p≤0,001) ***	-4.1(p≤0,001) ***	-4.4(p≤0,001) ***	-6.2(p≤0,001) ***
Solanum cervantesii	US	3.6(p≤0,001) ***		2.1(p≤0,05) *	3.3(p≤0,001) ***	
	UN		4.1(p≤0,001) ***		-2.5(p≤0,05) *	10.2(p≤0,001) ***
	PS			3.07(p≤0,01)**		
	PN	5.9(p≤0,001) ***				

The principal component analysis (PCA) (figure 2) showed that *C. thyrsoideum* germination was associated to light variables and *S. cervantesii* germination was associated to soil nitrogen and temperature.

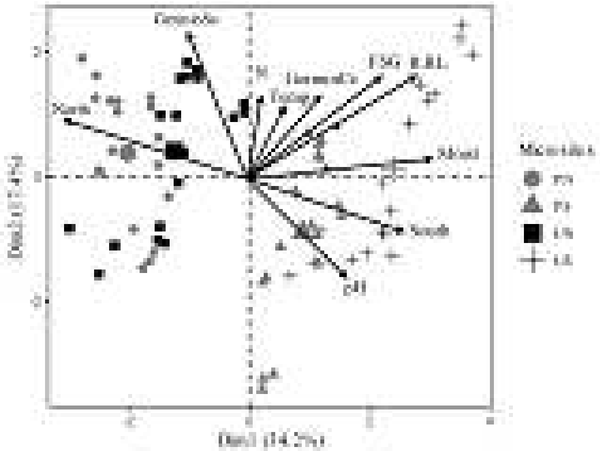


Figure 2.

During the day the mean values of R/FR (red/far red) light were the highest in US undisturbed south oriented microsites ($\overline{X}$ = 1.72±0.14), while the PN perturbed north oriented microsites showed the lowest value ($\overline{X}$ = 0.72±0.05). Around midday (13:30) values reached their highest R/FR values in all microsites, with the highest peaks in PS and US microsites. The highest variation of the R/FR values corresponded to the PS perturbed south oriented microsites [VC] = 1.02. The rest of the microsites showed smaller variations of this value: UN undisturbed north oriented [VC] = 0.85, US undisturbed south oriented [VC] = 0.82, PN perturbed north oriented [VC] = 0.67. However, in all comparisons of the [VC] values, we found statistical differences between microsites: UN and PN ($Z = 2.1 > 0.73$, $p < 0.05$), UN and PS ($Z = 1.3 > 0.95$, $p < 0.05$, PS and US ($Z = 2.4 > 0.95$, $p = 0.03$), PS and PN ($Z = 1.17 > 0.48$, $p < 0.05$), US and PN ($1.30 > 0.48$, $p = 0.01$), except for the comparison between UN and US microsites ($Z = 0.85 < 1.24$, $p = 0.31$).

In germination chambers the seeds of *Cestrum thyrsoideum* started germinating first (at the 7th day), while seeds of *Solanum cervantesii* started to germinate at the 12th day. For both species, germination percentages were the highest in White light (68.6 and 86.6 % for *S. cervantesii* and *C. thyrsoideum*, respectively), germination percentages under Red light conditions turned out as 52 and 50 % respectively. Germination in Darkness behaved as 69 and 26 %. Under Far Red-Light percentages resulted in 5 and 3 % respectively. For each species, significant differences between germination percentages for each light quality were observed (*Solanum cervantesii* $H = 16.45$, $p = 0.0009$, *Cestrum thyrsoideum* $H = 17.78$, $p = 0.0005$). Lag time and maximum germination rate values resulted as 12.4 ± 1.24 days and 6.3 ± 0.21 % day^{-1} for *Solanum cervantesii,* and6 ± 0.87 days and 6.9 ± 0.64 % day^{-1} for *Cestrum thyrsoideum*. Seed viability percentages showed slight decreases for both species for each year tested after storage. For *Solanum cervantesii* viability values were 90, 90 and 80 % for 2016, 2017 and 2018 respectively, while for *Cestrum thyrsoideum* they were 95, 92 and 88 %.

Discussion

Forests are heterogeneous at different structural levels: canopy, understory and soil. Canopy gaps are critical for the recruitment and establishment of plant species with consequences in natural regeneration dynamics and therefore, in diversity. At the scale of forest soil, where seeds are, differences in environmental variables can arise due to several factors, for example, forest gaps and slope orientation can shape different microsites, and these, conform potential regeneration niches, resulting in safe sites for germination and/or establishment of new seedlings (Méndez-Toribio *et al,* 2016). In our results, global site factor was higher in U undisturbed (non-weeded) microsites than in P perturbed (weeded) microsites, therefore the higher germination percentages of both species could have been a result in part of the higher light availability associated to gap size, since gaps were larger in undisturbed microsites. It is worth to mention that this forest is affected by deforestation, and it occurs independently from the disturbance that was evaluated in this research as a factor: weeding (removal of secondary vegetation), which is practiced in this forest in order to favor the establishment of conifer trees planted by reforestation activities.

We observed different germination percentages of both species in microsites, with significant differences for *S. cervantesii* as a response to microsite, and according to the Generalized Linear Method(conventional linear regression models for a continuous response variable and/or categorical predictors)analysis *C. thyrsoideum* responded to microsites differently according to their orientation either south or north.

The response of seeds to light qualities is related to the functional diversity in germination within a species, meaning that in a population there are seeds that are responsive to light qualities in different proportions. Both species showed significant differences in germination percentages under different light qualities. In general, both species behaved as secondary in similar proportions, because they germinated in high percentages under red light, which is present under canopy gaps or in open sites after disturbance. Several Solanaceae species colonize open and disturbed forest areas in our study site and also in other ecosystems such as in Brazilian Savannas as reported by Oliveira *et al.,* (2004). In our study site, these species inhabit forest sites where disturbances have occurred and are common shrubs under canopy gaps of temperate forests in central Mexico. This was evident in our multivariate analysis, because both species germinated in higher percentages in microsites where the highest values of Global Site Factor and Red/Far-Redlight were registered, however *S. cervantesii* germinated in highest percentages in north oriented microsites compared to *C. thyrsoideum*, which preferred south oriented microsites. Probably, the requirements of soil moisture for germination are higher in *S. cervantesii* than those of its congener, because north oriented sites are often more humid than south oriented ones. This might be related to the fact that this species inhabits the center of forest fragments rather than border areas.

The seeds of both species started to germinate after two weeks of setting the experiments in growth chambers and in the microsites in the forest which matches Keeley and Thullen (1983) and allows us to suggest that a proportion of seeds possess little or no dormancy. However, a non-deep physiological dormancy has been found in a proportion of seeds of several *Solanum* species, sometimes showing seasonal cycles of dormancy/non-dormancy (Baskin and Baskin, 1998), these cycles are mainly driven by changes in temperature(Bithel *et al.,* 2002). In our north oriented microsites, the probability of occurrence of lower temperatures (than in south oriented slopes) combined with a higher soil moisture availability could have triggered higher germination percentages of *S. cervantesii* in north oriented microsites. A cold stratification can weaken dormancy (during wintertime) and high temperatures (during spring or summer) can make it deeper, probably cold stratification in northern microsites (more humid and less warm than south oriented microsites) weakened the dormancy of *S. cervantesii*, but as temperatures were rising up, close to the summertime when seeds were sown in June, not all seeds germinated. It has been reported that seeds of *S. nigrum* change their sensitivity to light types after a cold stratification period and alternating temperatures. Other species such as *S. physalifolium* germinate only in the presence of gibberellins and KNO_3, which makes seeds more sensitive to light (Bithell *et al.,* 2002). The relation between this forest management type, soil nitrogen content and seed germination of the study species was not very clear, but we expected significant effects of microsite type on soil nitrogen because of the sudden availability of plant material caused by weeding. Nitrites and nitrates have been identified as compounds that can also trigger germination in species of Hydrophyllaceae as well as in other Solanaceae species such as *Nicotiana attenuata* (Keeley and Fotheringham, 1997). However, this response is pH dependent and usually occurs at 4-5 values. We should note that the lowest pH values in our study site were found in the perturbed and north oriented microsites (5.7 ± 0.15). These nitrogen compounds can promote germination (Keeley and Fotheringham, 1997). This is why we also expected some effect of soil nitrogen on the germination of both species in north oriented perturbed microsites, where the highest percentages of soil nitrogen were found probably due to weeding and because they were also characterized by the highest soil moisture content. However, in the GLMM analysis results, there were significant effects of microsite type on soil nitrogen content in all microsites for *C. thyrsoideum* germination, and for *S. cervantesii* only in undisturbed and oriented to the north microsites. It has been observed that some forest management techniques can increase soil nitrogen content. For example, Johnson and Curtis (2000) reported that sawlog harvesting in coniferous forests produces significant increases (up to 20 %) in soil nitrogen. In this sense sawlog harvest is a little similar to weeding because in the former only logs are removed from the forest and the residues such as branches with leaves are left in the forest, as in the case of weeding in our study site. Burning after a harvest is another management practice that can increase soil nitrogen. However, changes in soil properties such as nitrogen content due to forest management are also time dependent, but they are often detected in periods close to 4 years, with increases of up to 18 % more nitrogen in soil caused by a sawharvest, for example.

Light quality is different according to microsite characteristics, to some management techniques together applied in the site, and to the size of gaps, considering that under large canopy gaps there is usually a higher amount of certain light qualities such as red light. Martínez-Orea *et al.,* (2019) reported that secondary vegetation might function through buffering against drastic light changes at the soil level, as soil and litter do, probably this was the reason why the variation coefficient value was the highest in PS (perturbed south oriented microsites), where the elimination of secondary vegetation has been carried out and these differences were probably augmented in south oriented microsites, specially at midday when light entrance through the canopy is at its maximum. However, as Messier and Puttonen (1995) reported, light is a highly fluctuating variable in forests as our results showed as well as previous research for this study site has reported (Bonilla-Valencia *et al.,* 2017), because of many factors such as the year season, canopy gaps, slope orientation, sunflecks. This explains the significant differences found by the Z values between all microsites. However, for big scale (canopy) light dynamics, our values of Red/FarRed light (close to 1.15) have been reported by other authors such as Endler (1993) in forests with small gaps (like those in our P microsites), and also for large gaps (around 1.43, similar to the values our U microsites under larger gaps).

According to our GLM analysis the germination of both species was affected by the variables of the microsites which were located in different slope orientations. The variables that were different between slopes were soil moisture content (5 % more humid in north oriented microsites), nitrogen availability and a slightly more acid soil pH in north oriented microsites (6.24 versus 5.9). In terms of soil nitrogen there was a slightly higher (0.06 % higher) soil nitrogen content in perturbed (weeded) than in undisturbed microsites. It is known that water in soil is a requisite for seed imbibition, thus triggering germination (Baskin and Baskin, 1998). In the northern hemisphere, there is a higher soil moisture in north facing slopes than in south facing slopes, due to a higher irradiation in the latter ones (Méndez-Toribio *et al,.* 2016), which matches our results, and explains in part the fact that some species germinate and/or establish differentially between slopes Not only slope orientation is related to the differences in soil moisture content of microsites, but forest management can also modify the values of this variable according to Chen *et al.,* (1999). For example, Martínez-Orea *et al.,* (2019) reported that weeding reduces soil moisture content and negatively affects the germination of seeds of Caprifoliaceae and Asteraceae families.

Soil pH can affect germination of several species. For example, high pH values have been found to inhibit germination of some *Gossypium* species and of *Pinus halepensis* (Killi, 2004). In several species of cosmopolitan distribution families such as Asteraceae and Solanaceae, acid pH values are known to induce germination because acid soils can also lixiviate the seed coats promoting the entrance of water and radicle protrusion. Acidification of soils can occur in burned environments, but also in sites where there is a fast deposition of plant material under moist soil conditions, such as in our perturbed microsites because of the removal of secondary vegetation, however, the time needed for these changes to occur is not known for our study site, because the removal of secondary vegetation occurs constantly in some forest sites, and it has been going on for ten years, it is worth to mention that this plant material is left in the sites where it is cut.

Understory species are crucial for forest regeneration, resilience, and diversity, they can work as connectors between forest fragments and can also function as nurse species for conifer seedlings (Smith *et al.,* 1997). Therefore, the knowledge of their germination requirements in laboratory conditions and in the field by describing their safe sites for germination can be useful in reforestation programs.

Conclusions

Results of present investigation show that the interaction between slope orientation and weeding has effects on *Cestrum thyrsoideum* and *Solanum cervantesii* seed germination. Both species responded positively to light, since they are secondary, but they respond to soil moisture mostly. Therefore, even though they can germinate under certain disturbance regime, they still need some forest and understory cover in order to do it when soil moisture is available. Thus, we do not recommend secondary vegetation removal because it might reduce soil moisture content also, due to the important role of understory in buffering drastic light changes at the soil level, especially in south oriented microsites. Weeding might also change the values of soil pH and nitrogen content, even though the direction of these changes is still unknown in the study site, however, this silvicultural practice might produce structural changes in the understory that affect safe microsites, therefore causing low germination percentages in seeds of some secondary species. According to their viability times, seeds of both species can be stored in conditions that allow them to conserve high percentages for a time span of two years, which makes them good candidates for restoration/reforestation programs.

Acknowlwdgements

Special thanks to Beatriz Zúñiga-Ruíz for her assistance in germination tests and Marco A. Romero-Romero for figure edition. Thanks to Jorge Martínez-Villegas and Irene Sánchez-Gallén for the revision of a previous version of this manuscript.

References

Ávila-Akerberg, V. (2002). La vegetación en la cuenca alta del río Magdalena: Un enfoque florístico, fitosociológico y estructural. México. *Dissertation, Facultad de Ciencias, Universidad Nacional Autónoma de México.* 91p.

Baskin, C. and Baskin, J. (1998). Germination Ecophysiology of Herbaceous Plant Species in a Temperate Region. *American Journal of Botany* 75(2):286-305. DOI: 10.2307/2443896

Bithell, S., Mckenzie, B.A., Bourdot, G.W., Hill, G.D., and Wratten, S.D. (2002). Germination requirements of laboratory stored seeds of black nightshade and hairy nightshade. *Arable and Horticultural Weeds* 55:222-227. DOI: 10.30843/nzpp.2002.55.3894

Bonilla-Valencia, L., Martínez-Orea, Y., Castillo-Argüero, S., Barajas-Guzmán, G., Romero-Romero, M.A., and Díaz-López, T. (2017). Reproductive phenology of understory species in an *Abies religiosa* (Pinaceae) forest in the Magdalena River Basin, Mexico City. *Journal of the Torrey Botanical Society* 144(3):313-327. DOI: 10.3159/TORREY-D-16-00024.1

Chen, J., Saunders, S., Crow, T., Naiman, R., Brosofske, K.,Mroz, G., Brookshire, B., and Franklin, J. (1999). Microclimate in Forest Ecosystem and Landscape Ecology Variations in local climate can be used to monitor and compare the effects of different management regimes. *BioScience* 49(4): 288-297. https://doi.org/10.2307/1313612

Crawley, M.J. (2012). *The R book.* John Wiley and Sons. United Kingdom.

Emrich, A., Pokorny, B., and Sepp, C. (2000). The significance of secondary forest management for development policy. *Deutsche Gesellschaft für Technische Zusammenarbeit (GTZ) GmbH* Eschborn Germany.

Endler, J.A. (1993). The color of light in forests and its implications. *Ecological Monographs* 63(1):1-27. DOI: 10.2307/2937121

Johnson, D.W., and Curtis, P.S. (2001). Effects of forest management on soil C and N storage: meta analysis. *Forest Ecology and Management* 140(2-3):227-238. DOI: 10.1016/S0378-1127(00)00282-6

Keeley, J.E., and Fotheringham, C.J. (1997). Trace gas emissions and smoke-induced seed germination. *Science* 276(5316):1248-1250. DOI: 10.1126/science.276.5316.1248

Keeley, P.E., and Thullen, R.J. (1983). Influence of planting date on the growth of black nightshade (*Solanum nigrum*). *Weed Science* 31(2):180-184. DOI: 10.1017/S004317450006879X

Killi, F. (2004). Effects of potassium humate solution and soaking periods on germination characteristics of undelinted cotton seeds (*Gossypium hirsutum* L.). *Journal Environmental Biology* 25(4):395-398.

Lindig, C.R., and Zedler, J.B. (2002). Relationships between canopy complexity and germination microsites for *Phalaris arundinacea* L. *Oecologia* 133:159-167. DOI: 10.1007/s00442-002-1020-7

Martínez-Orea, Y., Orozco-Segovia, A., Bonilla-Valencia, L., Zavala-Hurtado, J.A., Vega E., and Castillo-Argüero, S. (2019). Germination response of two shrub species under controlled conditions and to secondary vegetation removal (weeding) in the Magdalena River basin Mexico. *Polibotánica* (48):135-150. DOI: 10.18387/polibotanica.48.11

Méndez-Toribio, M., Meave, J.A., Zermeño-Hernández, I., and Ibarra-Manríquez, G. (2016). Effects of slope aspect and topographic position on environmental variables, disturbance regime and tree community attributes in a seasonal tropical dry forest. *Journal of Vegetation Science* 27(6):1094-1103. DOI: 10.1111/jvs.12455

Messier, C., and Puttonen P. (1995). Spatial and temporal variations in the light environment of developing Scots pine stands: the basis for a quick and efficient method of characterizing light. *Canadian Journal of Forest Research* 25:343-354. DOI: 10.1139/x95-038

Moreno-Martínez, E. (1996). *Análisis físico y biológico de semillas agrícolas. 3ª edición*. Universidad Nacional Autónoma de México.

Oliveira, S., Ferreira, A., and Borghetti, F. (2004). Efeito alelopático de folas de *Solanum lycocarpum* A. St.-Hill. (Solanaceae) na germinacao e crescimento de *Sesamum indicum* L. (Pedaliaceae) sob diferentes temperaturas. *Acta Botanica Brasilica* 18:401-406. DOI: 10.1590/S0102-33062004000300001

Orozco-Segovia, A., and Sánchez-Coronado, M.E. (2013). Germinación. In: Márquez J, M Collazo Ortega, M Martínez-Gordillo, A Orozco-Segovia, S Vázquez-Santana (coord.). *Biología de angiospermas.* Universidad Nacional Autónoma de México. México. 602 p.

Smith, J., Sabogal, C., de Jong, W., and Kaimowitz, D. (1997). *Bosques secundarios como recurso para el desarrollo rural y la conservación ambiental en los trópicos de América Latina* (No. 13). Bogor: CIFOR. 36 p.

Zar, J.H. (1974). *Biostatistical Analysis.* Prentice Hall. Englewood Cliffs USA. 620 p.