Effects of grasses on sapling establishment and the role of transplanted saplings on the light environment of pastures: implications for tropical forest restoration

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Abstract

Question: Does the presence of grasses affect the establishment (survival and growth) of tree saplings in an abandoned tropical pasture, and what is the potential of established saplings to modify pasture microenvironmental conditions, particularly light incidence?

Location: Abandoned cattle pasture, in the humid tropics of SE Mexico (18°25’–18°45’ N, 95°00’–95°18’ W).

Methods: The establishment of four native fast-growing tree species in a tropical abandoned pasture was evaluated. Saplings of Cecropia obtusifolia, Hamps nutricia, Omphalea oleifera and Erythrina folkersii were transplanted using a split-plot design. Plant survival and growth were evaluated over 12 mo under two experimental treatments: presence and absence of grasses. Light incidence at ground level was used to evaluate microclimate conditions under the grass matrix, compared to light incidence under saplings established in the pasture.

Results: Saplings had high survival (49–88%) regardless of the presence of grasses. In the presence of grasses, growth of surviving saplings either increased or was not impacted, and in only one out of eight comparisons did grasses negatively affect growth. The impact of saplings on light incidence in abandoned pastures depended on sapling species architecture: species with large total leaf area and canopy area (H. nutricia and E. folkersii) significantly reduced light incidence (12–29% of total incidence), while narrow-canopy species (C. obtusifolia and O. oleifera) generated less intense shade (37–89% of total incidence).

Conclusions: Grasses did not seem to represent a crucial limiting factor in sapling establishment of fast-growing native species in the short term. In addition, shading by recruited saplings of some native species could be important to out-compete grasses in the future, as well as for ‘facilitating’ regeneration of more shade-tolerant species. Transplantation of fast-growing native saplings into abandoned pastures, even without manipulation of the grasses, could be a useful practice for landscape-scale restoration programmes in tropical areas. Evaluation of the longer-term consequences (beyond the 1-yr span of this study) warrants further research.

Introduction

Tropical forests contain a disproportionate amount of the global biological diversity and provide essential environmental services for human societies, both locally and globally (Dirzo 2001; Dirzo & Raven 2003). However, they are threatened by extensive conversion into pastureland for cattle grazing (hereafter pastures) and agricultural use, as well as by the disruption caused by various extractive activities (Dirzo 2001). Pastures are rarely sustainable for long periods of time and, typically, the land is eventually abandoned (Aide & Cavelier 1994). Rates of subsequent recovery following abandonment depend on the degree of forest degradation and availability and establishment of
propagules (Aide & Cavelier 1994). Regeneration can potentially occur and when it does, it is often slow (Posada et al. 2000). Some important factors that limit recovery include poor seed dispersal, an absent or reduced seed bank (Holl 1999; Wijdeven & Kuzee 2000; Zimmerman et al. 2000), seed predation (Holl & Lulow 1997), herbivory (Holl & Quiros-Nietzen 1999) and grass competition (Holl 1998; Holl et al. 2000; Midoko-Iponga et al. 2005; Parsons et al. 2007).

Management for forest recovery involving sapling planting overcomes dispersal limitation and seed predation impact, as well as pre- and post-germination mortality due to herbivore and pathogen attack or dessication. Saplings are established juvenile plants that have developed beyond the cotyledon stage (Russell-Smith 1996). They are typically recognized as young trees with a well defined stem and branches, forming a distinct crown, and at this stage are taller and less vulnerable to herbivory and harsh microclimate conditions than seedlings, and also less affected by competition with grasses (Flory & Clay 2010). Thus, the use of saplings may help in restoration programmes.

However, grass competition remains a difficult practical problem, and it is not clear when and where it is advantageous to remove the existing vegetation (largely dominated by grasses) in a pasture (Gómez Aparicio 2009). In tropical abandoned pastures, grasses have been found to compete with trees for resources in some situations (Sun & Dickinson 1996; Holl 1998) but they can also buffer harsh abiotic conditions and even facilitate tree recruitment in others (Aide & Cavelier 1994; Vieira et al. 1994; Holl 1999). In highly degraded sites, grasses can increase the gravimetric water content of the soil (Aide & Cavelier 1994; Holl 1999), but they are also associated with higher air temperatures, vapour pressure deficits and photon flux densities (Holl 1999). Furthermore, grasses are in general known to inhibit tree establishment in some instances (Midoko-Iponga et al. 2005; Stevenson & Smale 2005). If regeneration of at least some tree species is achieved via establishment of saplings, this could potentially facilitate the establishment of other, more shade-tolerant plants and thereby act to improve microenvironmental conditions (Vieira et al. 1994; Parrotta et al. 1997), not only by attracting seed dispersers (Slocum & Horvitz 2000; Slocum 2001), but also by reducing cover of (typically) shade-intolerant grasses (Aide et al. 2000; Holl et al. 2000; Zimmerman et al. 2000).

In this study we evaluate the effects of grass competition on the survival and growth of tree saplings of four fast-growing native species transplanted into abandoned pastures. The specific questions addressed were: (1) how does grass presence affect establishment, in terms of survival and growth, of native saplings? (2) what is the potential of saplings to modify microenvironmental conditions, particularly light incidence? Our main aim was to determine the importance of grass competition as a limitation in developing a primary canopy and to assess the potential role of native saplings in shading the pasture and, in so doing, facilitating colonization of shade-tolerant species. The answers to these questions should help us to understand how to manage one of the factors that potentially limits forest recovery in abandoned pastures of the humid tropics.

Methods

Study site

The study was conducted in the Los Tuxtlas region, on the coastal plain of the Gulf of Mexico (18°25′–18°45′ N, 95°00′–95°18′ W). The climate, which is warm and wet, has mean annual precipitation of 4,725 mm, with most rain between June and February, and a dry season that nevertheless frequently includes months with precipitation above 100 mm; temperature ranges between 24 and 26 °C (Soto & Gama 1997). Soils are volcanic in origin and predominantly Ultisols and Alfisols. They vary from clay to sandy in texture and normally have a layer of organic matter present in the first few centimeters (Álvarez Sánchez et al. 2007).

Due to its geographical position and complexity in terms of altitude, slope aspect and geomorphology (García-Aguirre et al. 2010), Los Tuxtlas comprises several distinct ecosystems, although tropical rain forest dominates in the lowlands (<550 m a.s.l.). This region constitutes the northernmost limit of rain forest distribution in the continent (Dirzo & Miranda 1990) and contains a remarkable biodiversity, including tropical and temperate species, with a high incidence of endemism (Dirzo et al. 1997). However, its forested area has been drastically reduced through deforestation (Dirzo & García 1992) and fragmentation (Mendoza et al. 2005).

Species selected and collection methods

We selected four native species based on their high growth rate and ability to grow in the high-light conditions of the pastures of the study area: *Cecropia obtusifolia* Bertol., *Hamprea nutricia* Fryxell., *Erythrina folkersii* Krukoff & Moldenke and *Omphalea oleifera* Hemsl. (Table S1). Growth rate decreases in the order *C. obtusifolia* > *H. nutricia* ≥ *E. folkersii* > *O. oleifera*. All these species are frugivore-dispersed.

Transplanted saplings were 30–50-cm tall, and were collected in several forest fragments (2 km from the study site). Removal of saplings did not affect the natural regeneration of these species, given their relatively high abundances in the remaining forest tracts of Los Tuxtlas.
All saplings were kept in a plant nursery under a half-shade cloth for 2 mo preceding transplantation, in order to improve acclimatization to a grassland environment.

**Experimental design**

The study was conducted in a 20-yr-old pasture that had been fenced immediately prior to the experiment. At the time of abandonment, it was dominated by native grasses (*Paspalum conjugatum, P. notatum, Panicum spp.*, *Digitaria discolor*) mixed with several Fabaceae (*Mimosa pudica, Desmodium sp.*, *Trifolium sp.*) and the exotic stargrass (*Cynodon plectostachyus*) (Meli 2004). Topsoil (0–30 cm), pH (6), and compaction (0.8 g cm$^{-3}$) were similar to those recorded within the forest, while total nitrogen (8.97 mg g$^{-1}$) was higher (cf. Sommer-Cervantes et al. 2003).

The experimental area, of 3, 600 m$^{2}$ (60 m × 60 m), was divided into 7.5 m × 4.5-m plots ($n$ = 24) (Fig. 1). We transplanted the saplings in June 2003, when the experiment started. Each species was represented by six replicated plots, in which we planted 24 individuals per species in each plot in a 1.5 m × 1.5 m grid (0.7 individuals m$^{-2}$) (a total of 144 individuals of each species). Grasses were removed in one half of each plot (hereafter removal) while the other half acted as control (hereafter no removal) in a split-plot design.

Above-ground grass biomass was totally removed to ground level using a machete, taking care not to disturb the soil. Grass removal was repeated every 20 d within a 1-m diameter circle surrounding the individual experimental plants (cf. Holl 1998). The competition effect was estimated by comparing sapling survival and growth in removal and no removal plots. Species and treatments were randomly allocated to all plots. Sapling survival and growth was monitored seven times on an irregular basis between June 2003 and June 2004 (cf. Parsons et al. 2007). We estimated the total leaf area (TLA) and canopy area (CA) of each individual at the end of the study. TLA was estimated from the total number of leaves multiplied by the mean leaf area (MLA), while MLA was previously calculated from regression equations calculated for each species. To this effect, we measured the length, width and area of 40 leaves per species with a scan image analysis system (Delta-T Devices Ltd, Cambridge, UK) and the program WinDIAS 3.0 (Delta-T Devices). Regression analyses between length and width vs. leaf area were conducted for each species, retaining the linear function that provided the highest proportion of explained leaf area. All regressions were highly significant, with $r^2 > 0.95$.

Canopy area is a useful indicator of rain interception, light transmission, litter accumulation, loss of soil moisture and transpiration rate (Husch et al. 1982). We therefore calculated CA from two canopy measurements as: $CA = [(d_1 + d_2)/4]^2 \pi$, where $d_1$ and $d_2$ are two perpendicular crown diameters.

To evaluate shading produced by sapling canopies, prior to and 1 yr following the transplantation, we measured light incidence (660 nm) at ground level with a radiometer (SKR–100, Skye Instruments, UK) on a clear day, between 11:00 and 13:00 h. In removal plots we measured light under every sapling, while in no removal plots we measured it under the grass matrix. We also measured bare soil as a control. We measured plants from the different plots in a randomly selected sequence, to account for possible temporal variations occurring during the time of measurements.

**Statistical analyses**

Tree survival was quantified as the proportion of living saplings relative to the total number of trees planted in each plot. Proportional data were arcsine-transformed prior to conducting the statistical analysis. Growth was...
estimated as the relative growth rate (GR) in terms of height (HGR) and diameter (DGR) as: 
\[ GR = \frac{\ln x_f - \ln x_i}{\Delta t}, \]
where \( x_i \) and \( x_f \) are the initial and final values of the analysed variables, and \( \Delta t \) is the duration of the period of study, in this case 12 mo.

Statistical analyses were performed using JMP-IN (v 3.2.1; SAS Institute, Inc., Cary, NC, US). Grass effects on survival and growth of saplings were evaluated by comparing them in the removal and no removal plots, using a multivariate analysis of variance (MANOVA) with repeated measures (Potvin et al. 1990; Von Ende 1993) in a split-plot design (see experimental design). The model included the effect of the variance generated by the randomization of the species (\( n = 4 \)) in the different plots (\( n = 6 \)). This effect can be considered a random factor nested within species. We checked the assumptions of normality and homogeneity of variances with the Shapiro–Wilk (Shapiro & Wilk 1965) and Levene (Milliken & Johnson 1984) tests, respectively. We evaluated in detail the effect of grass presence on tree survival and growth in different stages (each measurement period) through a profile analysis (Von Ende 1993), by transforming the data into \( P - 1 \) contrasts (where \( P \) is the number of steps over time) (Potvin et al. 1990) that were generated between two consecutive steps. TLA, CA and light incidence were not included in the repeated measures analysis. Instead, we analysed them at the end of the experiment with a two-way MANOVA matching the split-plot model. In all analyses, when interaction terms between main factors were significant, we developed individual pair-wise comparisons of least-square means using Student’s \( t \)-tests (LSD, least significant difference).

**Results**

**Sapling survival and growth**

Average sapling survival was relatively high, at 69.4 ± 1.9%, ranging between 49.3% (E. folkersii) and 88.2% (C. obtusifolia) (Table S2). Sapling survival was significantly dependent on species (Fig. 2), treatment and on the interaction between these factors (Table 1). Although effects of grasses were significant in the general model, the analysis showed that E. folkersii was in fact the only species affected by grasses (\( F_{1,20} = 17.37, P < 0.01 \)). The effect of time depended on the species but not on the presence/absence of grasses. In particular, the survival trajectory was found to be species-specific, and with a specially marked decline between 150 and 270 d (150–240: \( F_{3,20} = 9.47, P < 0.01 \); 240–270: \( F_{3,20} = 4.06, P < 0.05 \)).

Surviving plants grew considerably in the pasture, even though average height of saplings after 1 yr did not exceed the mean height of grasses (\( \approx 120 \) cm). There was a gradient of inter-specific variation in height growth through time, in the direction E. folkersii > H. nutricia > C. obtusifolia > O. oleifera (with monthly growth rates of 7.9 ± 1.2, 5.7 ± 3.2, 4.3 ± 3.4 and 1.1 ± 1.0 cm, respectively). One year after planting, this trend was reflected in higher final heights (and stem diameter) which were higher for E. folkersii and H. nutricia than for C. obtusifolia and O. oleifera (Table S2). The corresponding MANOVA showed significant

![Fig. 2](image-url)
After 1 yr of growth, CA of Canopy area and light incidence good indicator of species differences. production is independent of height, and that TLA is a similar pattern to TLA, which suggests that leaf variability, it was significantly different across species (Table S2). Although CA showed strong intra-specific variability, it was significantly different across species (F_{3,20} = 9.35, P < 0.01), but was unrelated to the presence/absence of grasses.

Light incidence at the beginning of the experiment was, as expected, statistically indistinguishable across plots or species. However, after 1 yr we observed that light incidence through the grass matrix in the no removal plots had been reduced by 90% compared to the removal plots (Fig. 5). Light incidence beneath saplings was significantly different across species (F_{1,3} = 8.12, P = 0.01) and treatments (F_{1,3} = 20.61, P < 0.01), and the interaction

### Table 1. Results of repeated measurements MANOVA on sapling survival in abandoned pastures in Los Tuxtlas.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>df</th>
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<td>Grasses</td>
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<td>1,20</td>
<td>0.0226</td>
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<td>Time × Grasses</td>
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<td>5,16</td>
<td>0.6382</td>
</tr>
</tbody>
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Canopy area and light incidence

After 1 yr of growth, CA of E. folkerisii and H. nutricia was larger than that of C. obtusifolia, followed by O. oleifera (Table S2). Although CA showed strong intra-specific variability, it was significantly different across species (F_{3,20} = 9.35, P < 0.01), but was unrelated to the presence/absence of grasses.

Differences in HGR among species (F_{3,20} = 42.28, P < 0.01) and between treatments (F_{1,20} = 5.02, P = 0.02), but no interaction between them (F_{3,20} = 2.12, P = 0.10). Interestingly, saplings showed higher HGR in the no removal plots (Fig. 3a). MANOVA also showed significant differences in DGR among species (F_{3,20} = 19.20, P < 0.01) but not between treatments in general (F_{1,20} = 0.10, P < 0.75). However, we did find significant differences in the interaction species × treatment (F_{3,20} = 4.25, P < 0.01). Contrasts showed that O. oleifera and H. nutricia were affected by removal, but in contrasting ways: in removal plots DGR was lower in O. oleifera (t = 8.54, P < 0.01) and higher in H. nutricia (t = -2.10, P < 0.05) (Fig. 3b). In summary, the growth of surviving saplings either mostly increased or was not impacted in the presence of grasses. The only negative impact of the presence of grasses on sapling growth was on DGR of O. oleifera.

Total leaf area (TLA) was markedly higher in H. nutricia than in the other species, with O. oleifera having the lowest value (Fig. 4). TLA was significantly different across species (F_{3,20} = 20.24, P < 0.001) and treatments (F_{1,20} = 7.34, P < 0.01), and the interaction between these factors was also statistically significant (F_{3,20} = 10.25, P < 0.001). Two species, C. obtusifolia (t = 2.05, P = 0.0412) and O. oleifera (t = 8.86, P < 0.0001), had higher TLA in the no removal plots, while H. nutricia (t = -1.98, P = 0.0484) had a lower TLA (Fig. 4). Since TLA is dependent on individual size, we calculated relative leaf area as RLA = TLA/total height. The RLA showed a similar pattern to TLA, which suggests that leaf production is independent of height, and that TLA is a good indicator of species differences.
species × treatment was also significant ($F_{3,3} = 4.45$, $P < 0.01$). Contrast analyses showed that light incidence was similar in removal and no removal plots but only under *H. nutricia* ($t = -0.85$, $P = 0.399$) and *E. folkersii* ($t = -0.74$, $P = 0.4659$), suggesting that the shading from these species (12% and 29%, respectively) is comparable to that of grasses.

**Discussion**

Sapling survival varied across species, but was close to 50%. Beyond survival, the established saplings experienced a negligible negative impact of grasses: growth was either indistinguishable between plots with and without grasses, or was even enhanced in the presence of grasses, and only DGR of one species (*O. oleifera*) revealed a negative impact of grasses. Furthermore, our results show some evidence of light attenuation by saplings, an aspect of potential significance, given the shading intolerance of grasses and the potential facilitation for the colonization of other shade-tolerant species.

We found sapling survival was not negatively affected, except for *E. folkersii*. However, it is worth noting that the hispid pocket gopher (*Orthogeomys hispidus*) foraged on the roots of 30% of the saplings of this species during month 7 of the study. Although survival rate at this time was 34%, it was never <91% in other periods, suggesting that survival could be much higher in the absence of the gopher. There is also evidence that the interaction between exotic plants and rodents can alter the establishment and growth of native species via apparent competition (Orrock et al. 2010), particularly when exotic species may provide refuge for small mammals such as voles and mice (Flory & Clay 2010). Although we found that the effects of grasses on sapling survival could be species-specific, herbivory could be an important factor, at least for some species (Holl & Quiros-Nietzen 1999), and therefore it should be evaluated, as should its interaction with competition (see Koch et al. 2004; Midoko-Iponga et al. 2005).

On the other hand, the presence of grasses was positively related to height growth in all sapling species, although the effect was significant only in two of them (*C. obtusifolia* and *O. oleifera*), whereas the effect of grasses on diameter growth was more species-specific. Grasses tend to have negative effects on seedling survival and growth, and their removal could have positive effects on tree establishment (Sun & Dickinson 1996; Hooper et al. 2005; Midoko-Iponga et al. 2005). However, the opposite could be expected in planted tree saplings because they already had a well-developed root system for accessing water and nutrients and often become tall enough to avoid shading (Flory & Clay 2010). The outcome of plant–plant interactions is highly variable, as it implies a balance of positive and negative effects that may change depending on factors such as micro-environmental conditions (Callaway & Walker 1997; Gómez Aparicio 2009). In other studies (González-Montagut 1996; Loik & Holl 2001), trees also reached larger final heights and showed higher growth rates in the presence of grasses. In these pastures, the seemingly positive effects of grasses on height may be related to competition for light, since saplings had not emerged from the grass canopy (except *E. folkersii*). However, apparent positive effects of grasses on height could be due to different allocation patterns in removal plots, with higher irradiance, where saplings suffered from water limitation or high leaf temperatures (Baoli et al. 2005). Diameters were not significantly different in three of four species, suggesting that differences in allocation might explain taller plants in no removal plots. This could result in less biomass investment in leaf material and more biomass to roots for water and nutrient uptake to compensate for transpiration losses (King 1994; Valio 2001). A number of studies suggested that light-demanding species commit resources to new growth, with low allocation to storage (Cornelissen et al. 1996). Hence, taller plants could have smaller roots, which could potentially translate into lower survival and growth in the long term (after 1 yr), but additional experimental comparison is needed to elucidate this aspect.

Soil characteristics are also known to influence the outcome of plant–plant interactions, either through increased soil compaction (Sun & Dickinson 1996; Holl 1999) or reduced availability of nutrients (Aide & Cavelier 1994;
Shade created by nurse plants appeared to be associated with secondary succession. In a tropical sub-humid forest, the varying levels of shade are an important factor in out-competing grasses (Haggar et al. 1997). At the same time, it can be an important factor in out-competing grasses (Haggar et al. 1997). In a tropical sub-humid forest, the varying levels of shade created by nurse plants appeared to be associated with variations in seedling establishment (Sánchez-Velasquez et al. 2004). Such differential effects are manifested in differences in plant survival and growth, leading consequently to differences in canopy cover and microhabitat conditions beneath the surviving trees. The microenvironmental conditions created by these species, as they grow, could determine regeneration trajectories occurring beneath them (Haggar et al. 1997). In the long term, it would be advisable to study the influence of these species on understorey microclimatic heterogeneity (spatial patterns) and on soil development (Parrotta et al. 1997), but also necessary to study their effects on seed rain (Slocum 2001) and the floristic composition of the regeneration that occurs below the canopy, as this could be associated with secondary succession.

Native species can have high survival in the presence of grasses, but their performance may vary depending on the relationship between the light demand and light incidence (Celis & Jose 2011). However, in this study the species performance seems to be more related to their growth rates than their light demand. For instance, Cecropia and Omphalea are at opposite ends of the light demand gradient, but their slower growth rates compared to Erythrina or Hampea seem to be associated with possible higher negative effects of grasses, and their trend to higher growth rates in no removal plots. At the same time, a higher growth rate can produce a larger canopy in less time and therefore higher shading. In this study, only two out of the four species produced dense shade, and one of these two species (E. folkersii) suffered from herbivory and subsequent reduced survival. However, saplings showed considerable survival, and thus the use of saplings as a restoration tool can overcome limitations of seedlings and seeds, but understanding the complexity of plant–plant interactions will influence the success of restoration. In our study, grass removal would not be essential to the development of a canopy; nevertheless, grass removal remains important in restoration plans involving seedling establishment (Midoko-Iponga et al. 2005; Stevenson & Smale 2005; Dañobeytia et al. 2007).

Neither soil conditions nor water availability appear to be limiting factors for sapling establishment in the studied pasture, at least for light-demanding species. However, herbivory should be further analysed in more detail, and also its possible interactions with grass competition (particularly below-ground), nutrient uptake and resources allocation.

Negative effects of grasses on trees tend to be stronger in terms of survival than for growth (Gómez Aparicio 2009). Therefore, restoration activities, at least during the early years, should probably focus on maximizing survival, despite the potential cost in terms of growth. Saplings, in addition to their higher survival rates, seem to be more resistant to grass competition and may facilitate colonization by more shade-tolerant, mature forest species. Therefore, their role as nurse plants in the restoration of those ecosystems not considered stressful, e.g. the rain forest, is promising (Gómez Aparicio 2009), but requires further research.

Acknowledgements

Support for fieldwork was provided by the Instituto de Ecología-UNAM. PM was supported by a Master’s fellowship from the Packard Foundation and the Red Latinoamericana de Botánica. We thank M. Aide, R. González Montagut and E. Del Val for helpful comments on an earlier draft. J. Campo provided support for the soil analysis and N. Mariano provided support for the statistical analysis.
References


Supporting Information

Additional supporting information may be found in the online version of this article:

Table S1. Species transplanted.
Table S2. Final condition of saplings after 1 yr.

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