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Survival and Early Growth of *Gliricidia sepium* Fodder Trees in Subhumid Tropical Pasturelands: Contrasting Effects of NPK Fertilizer Salts vs. Organic Ammendments

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Abstract: Research on silvopastoral systems has suggested that combining grasses and fodder trees can make cattle production more sustainable. Establishing fodder trees in degraded pasture land confronts important challenges in subhumid tropical mountain slopes. Poor soils, dry weather and competition from grasses and other herbs can compound to impede proper sapling establishment. Better nutrition could help seedlings overcome weed competition with less weeding (a costly practice). In this study, researchers evaluate the effects of organic and synthetic fertilization on survival and early growth of *Gliricidia sepium* in weeded and unweeded conditions. The research was carried out, in the buffer zone of La Sepultura Biosphere Reserve, in Chiapas, Mexico. The experiment used a split-plot design. The main factor was the kind of fertilizer (i.e., vermicompost, NPK, organic foliar fertilizer) and secondary factor was weeding. We evaluated survival rate, above-ground dry-matter production and nodulation. We carried out Analyses of Variance (ANOVA) and multiple comparisons of the means of each variable with a Generalized Linear Model and Tukey test. Weeding had a greater effect than fertilization on survival and above-ground dry-matter production. In the un-weeded treatments, vermicompost was found to increase survival, base diameter and above-ground dry-matter production compared with control plots. Unweeded plots fertilized with NPK industrial salts (with NPKcontent equivalent to that of the vermicompost) resulted in a very low survival rate of saplings. Initial vermicompost application moderately reduced the interference of weeds with the tree samplings as reflected in better plant growth.

Key words: Silvopastoral system, weeds, interference, organic fertilization, facilitation, Mexico

INTRODUCTION

Deforestation and loss of forests have been particularly acute in Mexico. In 2005, Mexico's deforestation ranked third among countries in the tropics (FAO, 2006). In the early 1990s, cattle occupied 49% of Mexico's deforested land whereas agriculture occupied 14% (Masera *et al*., 1997). Today, agricultural expansion is the primary cause of deforestation in tropics developing tropical countries. In Latin America, livestock production actually occupies more of 27% of the land. The widespread introduction of cattle raising in tropical and subtropical areas of most Latin American countries as a result of the neoliberal model of development has brought about expansion of pastures at the expenses of forest and jungles, loss of biodiversity, low animal production, soil degradation and socioeconomic polarization. Nahed *et al*. (2010) and driven significant land use changes in Mexico. This change in land use has had a substantial impact on the soil's physical and chemical properties. In this study area, preliminary sampling confirm this soil degradation. Some basic analyses showed low pH (5.02), total Nitrogen 0.11%, organic mater 2.64%, apparent density 1.49 g mL^{-1}. In the mountainous Upper Tablon River Basin (UTRB),

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96% of cattle producers own open pasture plots (Barnhard *et al*., 2009). Open pasture accounts for 41% of total hectares allocated to cattle production. These lands are characterized by the absence of trees and high levels of weeds as well as insufficient fodder during the dry season. Many researchers have pointed out the potential positive externalities of shifting from extensive cattle production systems to silvpastoral systems. Both ecological and economic benefit can be derived from Silvopastoral Systems (SPS), Establishing SPS for a productive rehabilitation can provide ecosystem services, sustainable productivity and landscape-level restoration. Enhancing landscape connectivity with multi-purpose trees has positive effects on biodiversity conservation of wild and agricultural species. Some researchers have reported considerable milk and meat production increases when cattle are feed with fodder trees (Yamamoto *et al*., 2007; Jimenez-Trujillo *et al*., 2011).

Transforming the current patchwork of grasslands into silvopastoral systems could create a more suitable, biodiverse and sustainable landscape in the UTRB and the REBISE buffer zone. Since 2006, researchers are developing the project Participatory Design and Establishment of Sustainable Silvopastoral Systems for the conservation of soil, water and tree species, in the Buffer Zone of La Sepultura Biosphere Reserve.

Establishing fodder trees in degraded pasture land confronts important challenges in subhumid tropical mountain slopes. Poor soils, dry weather and competition from grass and other herbs can compound to impede proper establishment of fodder tree saplings. Silvopastoral systems are typically difficult to manage (Franzel and Scherr, 2002). It is difficult for plants to establish themselves, given the intense competition between grass/weeds and tree saplings for sunlight, water and nutrients (Holl *et al*., 2000; Hooper *et al*., 2005). Weeding in early stages of development is extremely important for the survival and growth of tree saplings in grasslands (Bendfeldt *et al*., 2001; Holl *et al*., 2000). However, weeding has proved to be a constraint for producers who have planted exploratory modules of the fodder tree *Gliricidia sepium* in the project. In many cases, weeds have out-competed the tree saplings.

It is important to determine whether application of fertilizer at the base of saplings sown in an open grassland can provide them with a competitive advantage over grass and other herbs and enable them to establish successfully. Various researchers have studied the fertilization of tree species planted in degraded grasslands. The results have been inconsistent and are difficult to interpret. Cobbina (1994) found that the application of phosphorus to *G. sepium* saplings did not increase the dry weight of stems. Cobbina (1994) also found that application of synthetic nitrogen and phosphorus to *G. sepium* saplings increased the height of plants and the dry matter of stems. Siemann and Rogers (2003) found that fertilization with nitrogen derived from chemical fertilizers increased growth of saplings for two tree species without increasing the growth of adjacent weeds. Empirical evidence suggests that there may not be a relationship between soil fertility and relative intensity of competition in the subsoil and topsoil (Casper and Jackson, 1997; Peltzer *et al*., 1998; Rajaniemi, 2002). Moreover, it is not easy to predict the outcome of interactions between plants because the competitive abilities of plants above and below ground are context dependent and change in complex ways according to resource gradients (Garcia-Barrios, 2003). Researchers contend that where higher soil fertility does not increase soil competition between grass and tree saplings but does increase aboveground growth of *G. sepium* saplings then organic and synthetic fertilization of *G. sepium* saplings could improve their growth and facilitate their establishment. If successful, this technique could reduce the labor-intensive weeding required of producers during the trees' establishment. The outcome could be sensitive to the form in which nutrients are made available (e.g., highly concentrated NPK salts vs. organic ammendments).

The objective of this study was to determine if application of fertilizer at the base of *Gliricidia sepium* sapling provides them with a competitive advantage over weeds and grasses and enables them to establish more successfully. More specifically, researchers investigate if organic fertilization results better than synthetic for reducing the competitive advantage of grass and weeds in terms of survival and growth which organic fertilizer results better for this purpose if nodulation increases in saplings amended with vermicompost and how the previous results change when saplings are weeded or not weeded.

Researchers compared survival rate, above-ground dry matter and root nodules of saplings that had been treated with synthetic fertilizers and with organic, locally produced fertilizers to determine whether local fertilizers offer the same or better results than synthetic fertilizers in treatments with and without weeding. To achieve this, researchers measured the response of *G. sepium* to different types of fertilizer: Vermicompost; synthetic NPK and foliar fertilizer known as Super Magro. The hypothesis was that un-weeded saplings amended with vermicompost + organic foliar fertilizer will survive, grow better and produce more nodules than with other organic or industrial fertilization schemes. Weeded saplings may or may not display the formerly described advantage of vermicompost + organic foliar fertilizer.

MATERIALS AND METHODS

Study area: This study was carried out in an open pasture land outside the town of Los Angeles, in the municipality of Villaflores at the center of the UTRB, Sierra Madre of Chiapas (latitude 16°16'08"N, longitude 93°36'14"W). The town's territory is mountainous with altitudes ranging between 880 and 1500 m above sea level. The soils are sandy (granite-based regosols) and original vegetation is an ecotone of oak-pine forest, tropical deciduous forest, evergreen subtropical forest and secondary vegetation. Average annual rainfall at the center of the ejido is 1200 mm and temperature is semi-calid.

Design of experimental parcel: The treatments were established following a split-plot design with 10 treatments and 6 replicates. The whole plot treatment was type of fertilizer (i.e., no fertilizer, synthetic fertilizer and three types of organic fertilizer). The sub-plot treatment was weeding (i.e., full weeding vs. no weeding). Each replicate covered 20×9 m and contained 10 experimental units of 2×9 m. The experimental plots were set on the gentle slope (9 degrees) of an alluvial terrace. The surface area was 1080 m^2 (a 20×54 m rectangle). Within that area 1440 *G. sepium* seeds were sown. Each experimental unit contained 8 planted points with 3 seeds each. The latter were thinned to one plant per sowing point, making a total of 480 saplings under study.

Preparation and application of fertilizers

Vermicompost: The main component of the vermicompost was cow manure. Decomposing coffee pulp, soil with dead leaves, cedar shavings and household food waste were added. The worm species used to process organic material was *Eisenia foetida* (redworm). Table 1 shows the properties of the vermicompost. About 2 kg of vermicompost with 52% moisture were applied to the corresponding plots.

Foliar fertilizer: Foliar fertilizer, commonly called Super Magro was prepared. Table 2 shows the microelement contents of the organic foliar fertilizer. During the dry months included in the experiment (October, November, and December) plants whose treatments did not include foliar fertilizer (i.e., NPK, vermicompost) and the control were sprayed with the same amount of (plain) water as plants whose treatment did include foliar fertilizer treatment to avoid a confounding effect of watering. The foliar fertilizer was applied at 24, 57, 90, 121, 151, 181 and 211 days after sowing. The concentration was 5% for the first application and 10% thereafter. The 80-100 mL of fertilizer were applied to each plant each month. Table 3 provides a list of the contents of Super Magro.

Table 1: Contents and properties of the vermicompost

Trials	Unit	Quantity
Extractable phosphorus	mg kg^{-1}	13.60
Organic matter	%	20.30
pH		7.30
Total nitrogen	%	1.70
Bulk density	g mL^{-1}	0.62
Electrical conductivity	dS m^{-1}	3.15
Cation exchange capacity	Cmol kg^{-1}	44.90
Humic acids	%	1.94
Fulvic acids	%	0.13
Total acids	%	2.06

Table 2: Concentrations of microelements in foliar fertilizer

Elements	Quantity (mg L^{-1})
Potassium	1697.6600
Magnesium	477.3300
Calcium	2691.8300
Copper	0.8867
Iron	27.3400
Zinc	3.3100
Manganese	25.7200

Table 3: ADM results and weed height, collected 211 DAS

Fertilizer	Average height	SE	ADM (g m^{-2})	SE
Control	72.25	6.09	817.4	29.64
NPK	75.73	8.13	756.2	6.65
Vermicompost	64.29	5.10	591.4	8.78
Foliar	62.26	6.14	537.8	8.52
Vermicompost + foliar	68.20	5.60	561.9	5.50

Chemical fertilizer: The same nitrogen dose was applied to chemically fertilized plots and vermicompost plots. Nitrogen was applied at 3 time points; 24, 57 and 90 days. In each chemically fertilized plot, 1.7, 4.6 and 17 g of urea (46N-00P-00K) were applied, respectively making a total of 10.27 g of nitrogen per plot. At 57 days, 0.03 g of diammonium phosphate (18N-46P-00K) was applied (0.136 g of phosphorus). Potassium was applied at the same time as phosphorus with a dosage of 3.2 g of KCl (00N-00P-60K) per plant.

Plot preparation: In total, 480 holes were dug measuring 0.15×0.15×0.30 m. The distance between holes was 1.1 m, perpendicular to the slope and 1.8 m parallel to the slope. The holes were arranged hexagonally. At each seedling site, a 30-40 cm diameter circle around each seedling was weeded. The experimental units in the weeding treatment were completely cleared with a machete (hoe) at 24 days. Subsequent weeding was carried out every month.

Sowing, thinning and nodule evaluation: On June 14, 1440 *G. sepium* seeds were placed in water for 48 h to speed up germination. The seeds had been collected locally during May. Sowing of the experimental plot occurred between June, 16 and 18. In each hole, 3 seeds were placed to guarantee germination of at least one of them. The holes had been previously filled with soil extracted from the same plot with the exception of the treatments that included vermicompost. In that case, 5 L of soil were mixed with 2 L of vermicompost and the mixture was used to fill the holes. The 960 g (dry weight) of vermicompost was thus used at each sowing site. Two months later saplings were thinned, leaving only the most vigorous seedling from each hole. The saplings were removed from the soil trying not to break the roots and the Rhizobium nodules were counted. The established experiment had a total of 480 *G. sepium* saplings at a density of 4440 saplings ha^{-1}. At the early stage of growth considered in this experiment, saplings interacted with grasses and other herbs but not among them. After the experiment another thinning will be applied to reduce the density to approximately 1000 young trees ha^{-1}.

Sampling methods

Survival: Data on survival were obtained counting live plants each month.

Above-ground dry matter: In January, 2011 at the end of the experiment (211 days after sowing), systematic sampling was carried out. In each experimental unit, the aerial parts of half the plants were harvested (those in positions 2, 4, 6 and 8) to obtain average weight of Above-ground Dry Matter (ADM) $unit^{-1}$. The stems, branches and leaves were dehydrated for 24 h at 55°C before weighing. Average ADM was estimated per experimental unit (i.e., replicate of a given treatment).

Nodulation: The plants eliminated during thinning (at 57 days) were extracted with roots intact and the number of *Rhizobium* sp., nodules was counted.

Weed dry matter: At the end of the experiment (211 days), grass and herb above-ground dry matter was harvested in a 10 cm radius around each unweeded *G. sepium* plant. In the case of stoloniferous grasses such as *Cynodon plectostachyus*, they were cut at two positions. The first cut was flush with the soil within the 20 cm range and the second cut was made before the stalk reentered the soil even if that occurred outside the 20 cm range. The above-ground dry matter of saplings and weeds was dehydrated for 24 h at 55°C.

Statistical analysis: Statistical analyses were done using a univariate Generalized Linear Model. The model specifically allows for the split-plot experimental design. Statistical analyses were carried out with SPSS (Version, 15.0). Survival graphs were created using the R software program (Version, 2.10.1). The Additive Linear Model for the split-plots design is as follows:

$$Yijk = \mu + \rho k + \alpha i + \varepsilon ik + \beta j + (\alpha\beta)i\,j + \varepsilon ijk$$

Where:

i	=	Levels of factor A (fertilizer)
j	=	Levels of factor B (weeding)
k	=	Repetitions or blocks
Yijk	=	The kth observation of the ith treatment
μ	=	Estimated population mean
ρk	=	Effect of the kth block
αi	=	ith-order effect of factor A (fertilizer)
εik	=	Error factor A, E(a)
βj	=	jth-order effect of factor B (weeding)
$(\alpha\beta)i\,j$	=	Interaction effect between factors A and B
εijk	=	Random variation or model error E(b)

Possible violation of variance homogeneity and independency were checked visually in; scatterplots of residuals versus fitted values, QQ-plots of the residuals and boxplots of residuals versus each explanatory variable. Normal distribution of numeric variables were also evaluated visually by means of QQ-plots (Zuur *et al*., 2009).

Above-ground dry matter: The average above-ground dry matter produced for each surface unit (i.e., experimental unit) was estimated from the total weight of leaves, branches and stems from individual plants that were collected. The ADM averages included a 0 value for plants 2, 4, 6 or 8 that had died during the experiment in order to avoid over-estimation of the production per surface unit.

Weed dry matter: The average above-ground dry matter of grass/herbs was compared among fertilization treatments. In addition, mean heights of grass were compared as grass was the main type of competitor in the majority of experimental units.

RESULTS

Survival: Survival at 24 days was 100% in all experimental units. The greatest mortality rate of saplings occurred between September and October (a total of 52 saplings). At 121 days, saplings that had not been weeded and had been fertilized with NPK had a low survival rate (<48%), significantly less than the control ($p\leq 0.0003$).

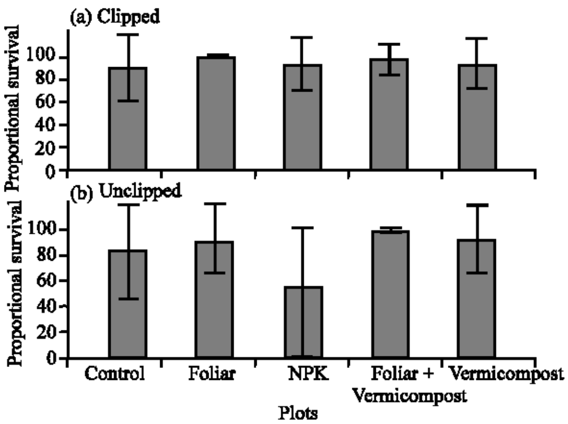

Fig. 1: Percentage survived±Standard deviation at 121 DAS; a) weeded and b) unweeded. An interaction was found only between fertilization and weeding in treatments with synthetic fertilizer. Clipped = Weeded

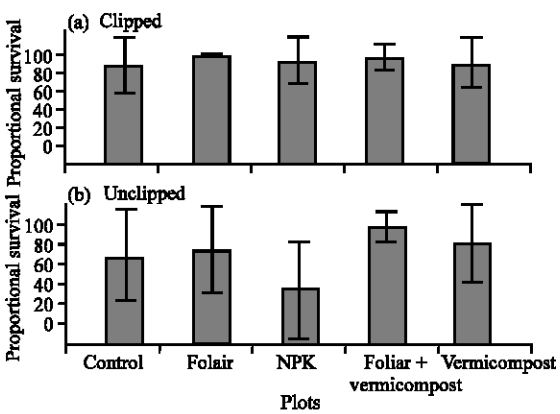

Fig. 2: Percentage survived±Standard deviation at 211 DAS; a) weeded experimental units and b) unweeded experimental units. Clipped = Weeded

Plants fertilized with vermicompost and foliar fertilizer showed a survival rate of 100%. Plants in the weeding treatment showed a high survival rate (≥89%), independent of the type of fertilizer applied. No significant differences were found. Survival rate was also high for plants in the weeded unfertilized control group (Fig. 1). In the weeded plots, survival did not decrease between October and January (i.e., during the dry season) but it did decrease in unweeded plots. Plants that received organic fertilization with a combination of vermicompost and foliar fertilizer showed a high survival rate (97%), greater than that of the control group (68%; $p = 0.004$). In turn, the latter survived longer than did those in plots with the synthetic fertilizer treatment (36%; $p \leq 0.0002$) (Fig. 2).

Above-ground dry matter: The factor that had the greatest influence on above-ground dry-matter accumulation at 211 days was weeding ($p<0.0001$). The interaction between weeding and fertilizer was not significant. In weeded plots, only the application of vermicompost moderately increased ADM production in comparison with the control (marginally significant $p = 0.058$). In unweeded treatments, the ADM of plants fertilized with vermicompost or a combination of vermicompost and foliar fertilizer was greater than that of the control ($p = 0.046$; $p = 0.036$, respectively) and that of plants treated with synthetic fertilizer ($p = 0.006$; $p = 0.013$, respectively). All comparisons were done with HSD Tukey test.

Nodulation: Sampling at 57 days in the vermicompost treatment showed twice as many native Rhizobium nodules as the control and as plants treated with synthetic ($p = 0.001$) or foliar fertilizer ($p<0.001$). The number of nodules on plants without vermicompost treatment ranged from 0.9-1.35 and from 4.89-5.03 on plants that received the vermicompost treatment. The comparison was done with HSD Tukey test.

Weeds: At the end of the experiment, no significant differences were found in height or ADM of grasses with respect to type of fertilizer applied to saplings. The control units also showed high values for ADM and height. Table 3 shows data on estimates for height and dry matter of weeds per square meter.

DISCUSSION

Application of vermicompost at the time of sowing produced better rates of survival, nodulation and ADM of *G. sepium* than both non-application and the application of synthetic fertilizers in unweeded experimental units. Application of VC moderately reduced the negative effects of the interferences of grasses with the *G. sepium* saplings in weeded experimental units.

Competitive intensity, in terms of survival and production of sapling biomass, increased as a result of the effects of the synthetic fertilizer. This suggests that with this treatment, the tree-grass interaction follows the Grime-Hodgson Model which posits that in environments with greater abundance of resources (in this case, nutrients), productivity, biomass of the grasses and intensity of competition increase. This in turn suggests that the application of soluble NPK does not guarantee the net availability of resources for the *G. sepium* plants. Synthetic fertilization in some contexts can also generate positive effects for the growth of *G. sepium* as

Cobbina (1994) has reported. However, this is not a rule as the same researchers found contrasting results in the same experiment. Other studies have also reported null effects of the application of synthetic fertilizers on the establishment of fodder tree saplings in degraded pastures (Bendfelt *et al*., 2001). In contrast, the effects of vermicompost decreased competitive intensity in terms of production of ADM per surface unit and survival.

The tree-grass interaction with VC increased the survival rate and growth of *G. sepium* which follows the S-D Model of competitive intensity (Davis *et al*., 1998). The S-D Model suggests that competitive intensity should be inversely correlated to availability of resources. Vermicompost releases nutrients more slowly and locally than synthetic salts as a result, the availability of resources is lower as is competitive intensity. Vermicompost also improves the physical, chemical and biological properties of the soil (Atiyeh *et al*., 2002) in the rizosphere of the sapling such as porosity, aeration, drainage, moisture retention, mineral content and microbial activity (Edwards and Burrows, 1988; Atiyeh *et al*., 2000) which possibly allows for better root development in germinated seeds. Some researchers have emphasized the importance of the ecology of the soil from a holistic perspective that is for the restoration of ecosystems. Researchers share that perspective. In that sense, vermicompost produced better results than the control and than synthetic fertilizer as the association between *Rhizobium* sp. and Gliricidia increased in plants fertilized with that substrate. It has been documented that humic and fulvic acids present in vermicompost increase nodulation (Tan and Tantiwiramanond, 1983).

The contrasting results between the two forms of fertilizing the soil suggests that the way in which the nutrients (N, P, K) are applied either from synthetic or organic fertilizers has implications for the availability of the nutrients which determined the interaction model between grasses and tree seedlings.

It is not easy to predict the mechanisms of interaction between plants. The competitive abilities below and above the soil depend on the context and the way in which those abilities change depending on the amount of resources is complex (Garcia-Barrios, 2003). Once these interaction patters have been observed, researchers can answer the central question of this study. Application of vermicompost reduces the paradoxical effect of fertilizing and nourishing unwanted plants as occurred with the application of the N, P and K salts. This study contributes valuable information about the ecological knowledge of the interaction between *G. sepium* and grass for the productive rehabilitation of open pastures for extensive livestock grazing.

CONCLUSION

Researchers suggest that future research cross gradients of intensity of weeding with gradients of vermicompost dosage to investigate at which point the best results are obtained with the most economical combination. Oleta is finishing a participatory experimental evaluation of how local producers perceive the cost effectiveness of preparing and applying vermicompost to *G. sepium* saplings in small plantations.

IMPLICATIONS

- Direct sowing from seed of *G. sepium* in degraded plots is a viable alternative for the establishment of silvopastoral systems if the plots are kept cleared during the period of greatest weed growth
- Vermicompost improved growth and establishment of *G. sepium* in degraded grasslands. When weeding did not occur, survival increased but this did not prevent weeds from dominating in their competition with tree saplings
- Vermicompost stimulates the symbiotic association between *G. sepium* and Rhizobium during early stages of establishment
- Application of synthetic fertilizers was counterproductive with respect to the survival and growth of *G. sepium* without weeding and had no beneficial effect with weeding
- Further research into the interaction mechanisms between weeds and fodder trees in degraded areas is important as it will help guarantee success of more ecologically friendly silvopastoral projects as well as restoration of the study area and other ecologically and socially similar areas

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