RESTORATION ECOLOGY
The Journal of the Society for Ecological Restoration
SER SOCIETY FOR ECOLOGICAL RESTORATION

PRACTICE AND TECHNICAL ARTICLE

Assaying techniques to improve dry season plantings in eastern Madagascar

Chris M. Logan[1,2,3], D. Matthew Hill[1,2], Jean F. Solofoniaina Fidy[4], Jean C. Robertho[2], J. Leighton Reid[1,2]

Rampant deforestation in eastern Madagascar highlights the urgency of protecting and restoring its unique rainforests. This experiment addresses a restoration bottleneck: the precipitous drop in seedling survival during dry season plantings. We assayed nine soil, water, and shade treatments that were codeveloped with local farmers to improve survival and growth of 17 native tree species planted on a denuded hillside during one dry season. Most treatments had no effect; however, rustic shade structures decreased transplant shock mortality (i.e. mortality within the first 2 months after planting) by 75%. This improvement is broadly accessible; it can be accomplished with materials freely and easily accessed in most farmlands in eastern Madagascar. Also, survival varied among species and seedling size, suggesting that carefully selecting species that are resistant to the harsh dry periods, improving shade availability, and planting larger seedlings of each species could lead to further gains in native tree performance in dry season plantings. Short-lived dry season resistant species (e.g. *Uapaca* sp., *Trema orientalis*, *Psiadia* sp., *Macaranga* sp., and *Pittosporum ochrosiifolium*) could also provide shade as nurse trees for less resistant canopy trees, which have the potential to be established through enrichment plantings in years following the initial planting.

Key words: dry season, local ecological knowledge, shade structures, soil amendments, species selection, tropical forest restoration

Implications for Practice

- Shading and careful species selection are more likely than soil amendments or one-time watering to improve seedling survival in dry season plantings in central eastern Madagascar.
- Planting larger seedlings directly into the ground with no additives could decrease short-term seedling mortality.
- Planting hardy, drought-tolerant species (e.g. *Uapaca* sp., *Trema orientalis*, *Psiadia* sp., *Macaranga* sp., and *Pittosporum ochrosiifolium*) as nurse trees for other less-resistant trees is a potential avenue for future restoration.
- Codeveloping restoration knowledge with rural farmers may constrain technical success compared to expensive interventions but ensures that recommendations are actionable for regional practitioners.

Introduction

Ongoing deforestation is an existential threat to Madagascar's unique biodiversity. This island nation contains 5% of the world's known plants and animals, >80% of which are found nowhere else on Earth (Goodman & Benstead 2005). From 1953 to 2014, 44% of Madagascar's forests were cleared, often to expand low-yield farming practices (Klanderud et al. 2010). Beyond outright habitat loss, remaining forests have become increasingly fragmented and more vulnerable to further degradation via edge effects, invasive species, and wildfires (Manjaribe et al. 2013; Hill 2018). If current deforestation rates continue, models project there will be no remaining forest in eastern Madagascar outside of protected areas by 2080 (Morelli et al. 2020).

Conserving remaining intact habitats is paramount for preventing further extinctions, but ecological restoration is also needed to expand habitat cover and reconnect degraded fragments (Brancalion et al. 2019; Strassburg et al. 2020). To this end, Madagascar has committed to restore 4 million hectares by 2030 for the Bonn Challenge, an area representing approximately 6.9% of Madagascar's national territory (IUCN 2020). Meeting this ambitious target will be challenging with limited resources (Fagan et al. 2020). The most successful strategies

Author contributions: DMH, JFSF, JCR, JLR designed the experiment; DMH, JFSF, JCR led the implementation and field measurements; CML led data analysis and writing with editing from JLR, DMH.

[1]School of Plant and Environmental Sciences, Virginia Tech, 185 Ag Quad Lane, Blacksburg, VA 24061, U.S.A.
[2]Green Again Restoration, PO Box 4362, St Paul, MN 55104, U.S.A.
[3]Address correspondence to Chris M. Logan, email chrisl51@vt.edu
[4]Parc Ivoloina, Madagascar Fauna and Flora Group, BP 442, Morafeno, Toamasina 501, Madagascar

© *2022 The Authors.* Restoration Ecology *published by Wiley Periodicals LLC on behalf of Society for Ecological Restoration.*
This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.
doi: 10.1111/rec.13715
Supporting information at:
http://onlinelibrary.wiley.com/doi/10.1111/rec.13715/suppinfo

are likely to be those that minimize costs and are codeveloped with local communities (Holl 2017; Hua et al. 2022).

As in much of the seasonal tropics, minimizing seedling mortality is among the most important technical challenges for Malagasy forest restoration (Miandrimanana et al. 2019). Along the central east coast, the best season for planting native trees is during the mild rainy season (May–July). Outside of this brief window, seedling mortality nearly triples due to either too much or too little moisture in the dry season (Fig. 1). Dry season mortality is a problem in Madagascar as in other parts of the Tropics (e.g. McLaren & McDonald 2003; Bader et al. 2006; Thaxton et al. 2012). For Madagascar in particular, the dry season creates a bottleneck for native tree producers who cannot stock more tree seedlings than can be planted in a given year. It also limits the work of restoration organizations, which face diminishing returns on plantings after the start of the dry season. Extending the planting season, even at the margins, would allow more forest to be restored for a lower cost.

This experiment's purpose was to assay a set of restoration strategies codeveloped with rural Malagasy farmers to improve native tree seedling survival and growth during early dry season plantings in eastern Madagascar. We expected that weathered soils and poor access to limiting nutrients hinder seedling performance throughout the year, but we hypothesized that insolation and drought limit seedling survival the most during dry season plantings. As such, we predicted that moisture-holding soil amendments, watering, and shade would additively augment seedling survival and growth for native trees.

Methods

This experiment was conducted at Parc Ivoloina (18.0593°S, 49.3579°E), a forestry station and zoological park near Toamasina, Madagascar (Fig. 2). This area has a tropical rainforest climate (Af in the Köppen classification), with average temperatures ranging from 21°C in August to 27°C in January. Annual rainfall in Toamasina ranges from 3,000 to 3,500 mm/year, with a dry season in August–December and a cyclone season from January to March (Fig. 1). Soils are well-drained, highly weathered orange laterites. Our site was a recently burned 0.25-ha tract of regenerating forest on a south-facing hillside 25–77 m above sea level.

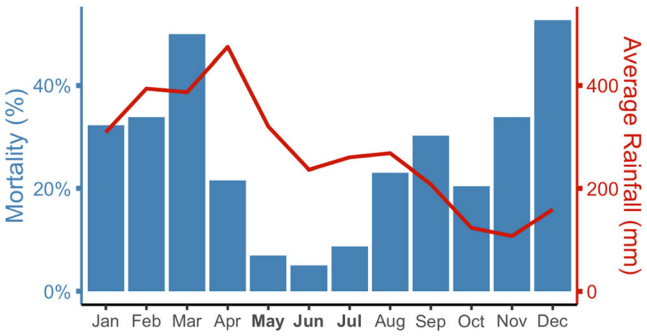


Figure 1. Native tree seedling mortality by planting month, based on a sample of 2,614 trees of 32 unique species planted in 2018–2019 (Hill unpublished data). Seedling mortality is shown with a one-month time lag to highlight the effect of rainfall on tree survival after planting. The best months for planting trees are May, June, and July (highlighted in bold); outside of those months seedling mortality rises significantly in response to rainfall patterns. Rainfall data obtained from: https://en.climate-data.org/africa/madagascar/toamasina/toamasina-4029/#climate-table.

During the dry season in October 2014–Jan 2015, we planted 369 seedlings representing 17 native tree species in 41 monospecific replicates (Table 1), where each replicate was composed of 9 trees of the same species. We used a circular planting design with a central tree seedling surrounded by a ring of eight seedlings, each separated by 2 m. Most seedlings were between 10 and 20 cm in height at the time of planting, so a distance of 2 m between each seedling was presumed as independent. Each tree was planted in an 8,000 cm^3 planting hole and assigned one of eight treatment combinations (Fig. 2). Controls were planted in the center position with no soil amendments, no watering, and no shade. All other seedlings were shaded with four fronds of a ruderal fern (*Dicranopteris linearis*) placed over the seedling in a cone shape to allow temporary shade without inhibiting growth, a practice used by Malagasy farmers (Fig. 3). We therefore expected apriori that shading was the most effective strategy for seedling performance and focused our design on watering strategies and soil amendments. Half of shaded seedlings were soaked in buckets of creek water prior to planting, and the other half were planted with only the moisture from their last watering at the nursery prior to being hand carried to the field site. Four of the eight shaded seedlings had their planting hole filled 33% with nutrient-rich compost, and four had their planting hole filled with one of three locally available absorbent materials (moss, coconut husk, or chopped petioles of traveler's palm [*Ravenala madagascariensis*]) to retain moisture.

We measured seedling survival and height at planting, and after 1–8 weeks, 1 year, and 6 years post-planting. For each set of nine seedlings, we recorded elevation using a Garmin GPS unit and hillslope position (bottom, side, or top). Height measurements were converted to relative growth prior to analysis using the formula: $height_{t2}/height_{t1}$. We estimated the effects of shade, water, compost, and absorbent materials on seedling survival and growth using generalized linear models, implemented in R version 4.0.4 (R Core Team 2020). Separate models were used to estimate the effects of alternate mechanisms including elevation, relative initial height (formula: initial height of each species/average initial height of each species), and hill-slope position; of these alternative models, only relative initial height was influential; and hence, it is the only one presented in the results. We then created a separate model to test if there was any interaction between shade or absorbent materials and species identity, of which there was no evidence of an interaction and is not presented in the results.

Results

Survival

The vast majority (98.7%) of seedlings survived initial transplant in the field, falling to 83.2% after 1 year and 40.9% after 6 years (Table 1). There was no evidence that soil amendments (compost and absorbent materials) or watering decreased tree

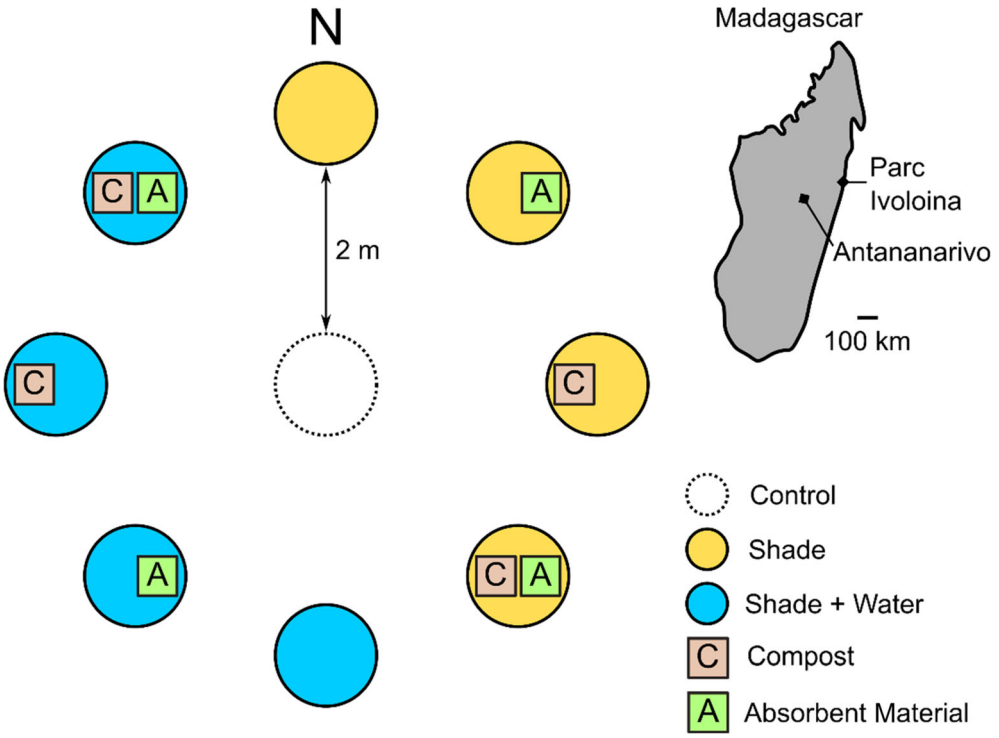


Figure 2. Experimental planting design. Controls received no shade, supplemental watering, compost, or absorbent material. The study area at Parc Ivoloina on the central east coast of Madagascar is shown in the inset map.

Table 1. Native tree seedling survival and growth, measured 1 and 6 years after a dry season planting on a burned hillside at Parc Ivoloina, Madagascar. * Indicates no data for growth due to 100% mortality after 6 years.

Species	*1-Year Survival (%)*	*6-Year Survival (%)*	*1-Year Growth (%)*	*6-Year Growth (%)*
Uapaca sp.	100.0	92.6	100.8 ± 70.1	1,429.7 ± 962.9
Syzygium jambos	100.0	100.0	61.7 ± 47.4	314.6 ± 270.2
Dichaetanthera crassinodis	97.8	60.0	288.5 ± 95.7	201.9 ± 175.9
Trema orientalis	94.4	0.0	432.1 ± 254.8	*
Psiadia sp.	91.7	8.33	276.5 ± 144.6	1,197.0 ± 340.1
Calantica sp.	88.9	88.9	196.5 ± 129.9	420.1 ± 349.6
Harungana madagascariensis	88.9	25.9	79.7 ± 71.4	582.1 ± 552.1
Macaranga sp.	88.9	36.1	153.2 ± 88.0	975.5 ± 366.2
Pittosporum ochrosiifolium	88.9	36.1	261.8 ± 85.2	952.0 ± 465.7
Hymenaea verrucosa	88.9	88.9	37.7 ± 20.8	183.5 ± 120.4
Intsia bijuga	83.3	44.4	15.0 ± 31.6	90.7 ± 136.6
Anisophyllea sp.	66.7	11.1	33.2 ± 42.5	189.5 ± 249.2
Ficus sp.	66.7	0.0	145.6 ± 83.3	*
Dypsis lutescens	55.6	38.9	−39.8 ± 12.6	−19.6 ± 36.6
Molinaea sp.	55.6	33.3	15.2 ± 37.3	−4.0 ± 17.0
Tambourissa sp.	51.9	33.3	80.2 ± 57.2	375.9 ± 185.1
Delonix regia	44.4	0.0	37.7 ± 74.4	*

mortality in the first few months, but absorbent materials were associated with a slight decrease in seedling survival after 1 year (Fig. 4, $p = 0.043$). Relative initial height was associated with greater seedling survival after both 2 months (Table S1, $p = 0.026$) and 1 year (Table S1, $p = 0.062$). This trend did not exist past 6 years (Table S1, $p = 0.945$). There was also moderate evidence that shade structures reduced initial transplant mortality by 75% (Fig. 3, $p = 0.026$); however, there was no evidence that this effect persisted 1 year after planting (Fig. 3, $p = 0.273$).

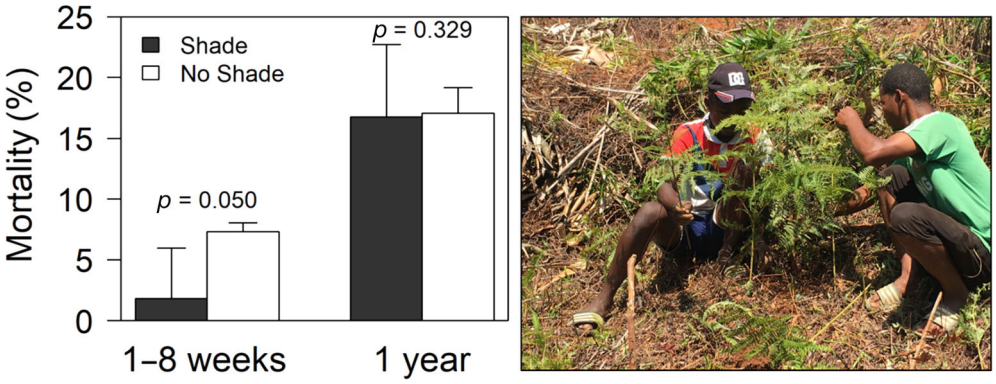


Figure 3. Native tree seedling mortality with and without shade structures created with *Dicranopteris* fern leaves. Error bars represent 1 SE.

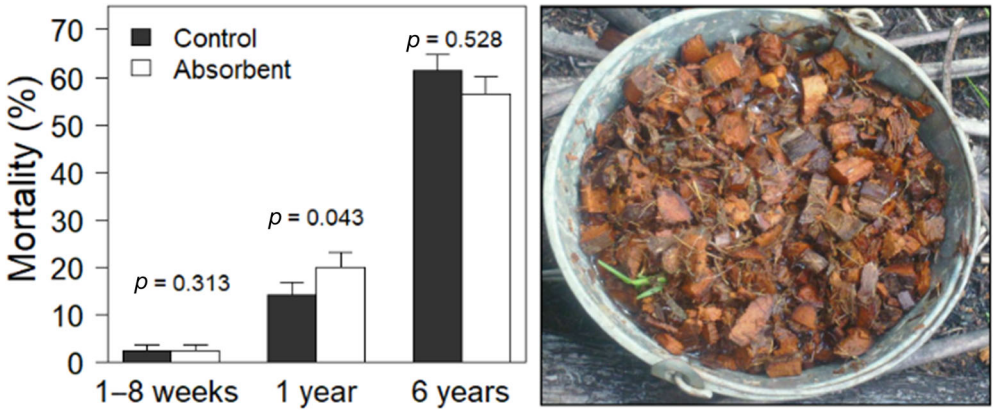


Figure 4. Native tree seedling mortality when treated with and without absorbent materials (coconut husks, *Ravenala* petioles, moss). The bucket in this photo consists of chopped petioles of traveler's palm (*Ravenala madagascariensis*), which were planted with seedlings to retain moisture. Error bars represent 1 SE.

The greatest variation in seedling survival occurred among species (Table 1). After 1 year, survival ranged from a maximum of 100% in *Uapaca* sp. to a minimum of 44.4% in *Delonix regia*. After 5 years, survival rates became more erratic as successful, short-lived pioneers died. For example, *Trema orientalis* experienced 0% survival after 6 years despite having 95% survival in the first year. Other initially high-surviving species displaying delayed mortality include *Dichaetanthera crassinodis*, *Harungana madagascariensis*, *Pittosporum ochrosiifolium*, *Intsia bijuga*, *Anisophyllea* sp., and *Ficus* sp. (Table 1). In contrast, longer-lived species like *Uapaca* sp. and *Syzygium jambos* had >90% 6-year survival.

Growth

There was moderate evidence that shade structures increased seedling growth (+25%) in the first few weeks after planting ($p = 0.045$). There was no evidence that other treatments impacted seedling growth at any time (all $p > 0.331$, Table S2). We found strong evidence that larger seedlings had slower growth rates through the first year (Table S2, $p < 0.001$). Many of the high-surviving species had higher growth rates, with some seedlings having 6-year growth rates over 1,000% (e.g. *Uapaca* and *Psiadia* sp.; Fig. S1). For *Trema orientalis*, *Psiadia* sp., *Delonix regia*, and *Ficus* sp., no living individuals were relocated after 6 years, so no growth calculations were possible for this time interval (Table 1). Based on survival and growth, we offer preliminary recommendations on

Table 2. Preliminary recommendations on tree species selection for dry season plantings based on a dry season planting on a burned hillside at Parc Ivoloina, Madagascar.

Species Capable of High Survival and Rapid Growth in Dry Season Plantings	*Species Better Suited to Wet Season Plantings or Experimental Planting Beneath Nurse Trees*
Trema orientalis	*Syzygium jambos*
Psiadia sp.	*Dichaetanthera crassinodis*
Uapaca sp.	*Calantica* sp.
Macaranga sp.	*Harungana madagascariensis*
Pittosporum ochrosiifolium	*Hymenaea verrucosa*
	Intsia bijuga
	Anisophyllea sp.
	Ficus sp.
	Dypsis lutescens
	Molinaea sp.
	Tambourissa sp.
	Delonix regia

species selection for dry season plantings in central, eastern Madagascar (Table 2).

Discussion

We assayed a suite of experimental strategies co-designed with Malagasy farmers to improve native tree species establishment during dry season plantings in eastern Madagascar. Out of nine treatment combinations, the most efficacious was a rustic shade structure constructed from ruderal fern leaves. Shade structures reduced transplant shock by 75%, suggesting that intense solar radiation is a primary limitation on initial establishment in dry season plantings. Miandrimanana et al. (2019) likewise observed greater native tree seedling survival beneath shade structures in the Malagasy highlands (but see Pareliussen et al. 2006). In contrast to our fern leaves, Miandrimanana et al. (2019) used more permanent wooden structures, which continued to impact tree establishment through the first year.

The increase in tree survival that we observed with rustic shade structures was relatively modest compared to more intensive interventions used elsewhere, but we view this outcome as a significant advancement on prior tree plantings without shade structures. Costlier interventions in more developed countries such as irrigation systems or hydrogel would produce better technical outcomes for plantings (Chirino et al. 2011) but are impractical in the Malagasy context. Methods and solutions that are not ubiquitously available locally end up being prohibitively expensive for farming families that often earn <$2 per day. In contrast, the shade structures described here can be freely and easily made from materials readily at hand at almost any restoration site in eastern Madagascar. Also, since these structures and the species they were planted with were co-developed with Malagasy farmers, they are more likely to be used (Fremout et al. 2021).

Tree selection is another low-cost technique to improve tree survival in dry season plantings. Of 17 native tree species tested, 10 survived their first year at rates ≥83%, and four maintained ≥83% survival through the entire 6-year experiment. Moreover, four species doubled or tripled in height within a year, indicating that these species are well-suited for dry season plantings. High-performing species like *Trema orientalis* and *Uapaca* sp. are early-successional plants; selecting hardy species for dry season plantings is a common way of overcoming drought pressure in restoration. Planting larger seedlings of these drought resilient species out of the nursery is also an effective way to mitigate seedling mortality in the dry season. Prior studies have likewise found high variance in seedling tolerance of harsh, dry conditions (e.g. Werden et al. 2018).

Malagasy practitioners are in the early stages of developing technical strategies for native tree planting, but efforts must ramp up quickly to successfully meet international commitments (Birkinshaw et al. 2013). Natural regeneration is one potential avenue for restoration, though regeneration is limited in eastern Madagascar due to invasive species like *Grevillea robusta* and *Leucaena leucocephala* as well as a lack of reliable seed dispersal by animals such as lemurs (Albert-Daviaud et al. 2020). Direct seeding is another option that could be further explored. However, by assaying dry season planting techniques, our work highlights that limiting solar radiation in restoration plantings may be a more productive avenue for further development than increasing access to water or soil nutrients. One promising application could be to select high-performing pioneer trees as nurse plants (Castro et al. 2002). Short-lived species could be planted in the dry season to provide persistent shade that would be expected to facilitate survival of more vulnerable hardwood seedlings when underplanted the following year. We therefore recommend using short-lived, high growth species during the dry season; in our assay, the species *Uapaca* sp., *Trema orientalis*, *Psiadia* sp., *Macaranga* sp., and *Pittosporum ochrosiifolium* were the most promising species for dry season plantings. They also offer potential services as nurse plants for less hardy species. Direct seeding of canopy species in young restoration plantations has found success in rainforests of Brazil (Bertacchi et al. 2016), though that concept remains unexplored in the Malagasy context. Such a system requires testing but could ameliorate an important seasonal bottleneck for native tree planting in eastern Madagascar and provide a practical solution to meet annual tree planting targets.

Acknowledgments

The authors thank M. Velo, the staff of Parc Ivoloina, and many local farmers and restoration practitioners without whom this work would not have been possible. The authors also thank S. Murphy, M. Guariguata and two anonymous reviewers for their feedback. Funding was provided by the Virginia Tech College of Agriculture and Life Sciences and by generous donations from supporters of Green Again Restoration.

LITERATURE CITED

Albert-Daviaud A, Buerki S, Onjalalaina GE, Perillo S, Rabarijaona R, Razafindratsima OH, Sato H, Valenta K, Wright PC, Stuppy W (2020) The ghost fruits of Madagascar: identifying dysfunctional seed dispersal in Madagascar's endemic flora. Biological Conservation 242:108438. https://doi.org/10.1016/j.biocon.2020.108438

Bader MY, van Geloof I, Rietkerk M (2006) High solar radiation hinders tree regeneration above the alpine treeline in northern Ecuador. Plant Ecology 191:33–45. https://doi.org/10.1007/s11258-006-9212-6

Bertacchi MIF, Amazonas NT, Brancalion PHS, Brondani GE, de Oliveira ACS, de Pascoa MAR, Rodrigues RR (2016) Establishment of tree seedlings in the understory of restoration plantations: natural regeneration and enrichment plantings. Restoration Ecology 24:100–108. https://doi.org/10.1111/rec.12290

Birkinshaw C, Lowry PP II, Raharimampionona J, Aronson J (2013) Supporting target 4 of the global strategy for plant conservation by integrating ecological restoration into the Missouri botanical Garden's conservation program in Madagascar. Annals of the Missouri Botanical Garden 99:139–146. https://doi.org/10.3417/2012002

Brancalion P, Niamir A, Broadbent E, Crouzeilles R, Barros F, Almeyda AM, et al. (2019) Global restoration opportunities in tropical rainforest landscapes. Science Advances 5:eaav3223. https://doi.org/10.1126/sciadv.aav3223

Castro J, Zamora R, Hódar JA, Gómez JM (2002) Use of shrubs as nurse plants: a new technique for reforestation in Mediterranean mountains. Restoration Ecology 10:297–305. https://doi.org/10.1046/j.1526-100X.2002.01022.x

Chirino E, Vilagrosa A, Vallejo VR (2011) Using hydrogel and clay to improve the water status of seedlings for dryland restoration. Plant and Soil 344: 99–110. https://doi.org/10.1007/s11104-011-0730-1

Fagan ME, Reid JL, Holland MB, Drew JG, Zahawi RA (2020) How feasible are global forest restoration commitments? Conservation Letters 13:e12700. https://doi.org/10.1111/conl.12700

Fremout T, Gutiérrez-Miranda CE, Briers S, Marcelo-Peña JL, Cueva-Ortiz E, Linares-Palomino R, et al. (2021) The value of local ecological knowledge to guide tree species selection in tropical dry forest restoration. Restoration Ecology 29:e13347. https://doi.org/10.1111/rec.13347

Goodman SM, Benstead JP (2005) Updated estimates of biotic diversity and endemism for Madagascar. Oryx 39:73–77. https://doi.org/10.1017/S0030605305000128

Hill DM (2018) Forest restoration in eastern Madagascar: post-fire survival of select Malagasy species. Master's Thesis, University of Minnesota

Holl KD (2017) Restoring tropical forests from the bottom up. Science 355:455–456. https://doi.org/10.1126/science.aam5432

Hua F, Bruijnzeel LA, Meli P, Martin PA, Zhang J, Nakagawa S, et al. (2022) The biodiversity and ecosystem service contributions and trade-offs of forest restoration approaches. Science:eabl4649. https://doi.org/10.1126/science.abl4649

IUCN (International Union for Nature Conservation) (2020) Madagascar. https://www.bonnchallenge.org/pledges/madagascar (accessed 10 Nov 2020)

Klanderud K, Mbolatiana HZH, Vololomboahangy MN, Radimbison MA, Roger E, Totland Ø, Rajeriarison C (2010) Recovery of plant species richness and composition after slash-and-burn agriculture in a tropical rainforest in Madagascar. Biodiversity and Conservation 19:187–204. https://doi.org/10.1007/s10531-009-9714-3

Manjaribe C, Frasier C, Rakouth B, Louis E (2013) Ecological restoration and reforestation of fragmented forests in Kianjavato, Madagascar. International Journal of Ecology 2013:726275. https://doi.org/10.1155/2013/726275

McLaren KP, McDonald MA (2003) The effects of moisture and shade on seed germination and seedling survival in a tropical dry forest in Jamaica. Forest Ecology and Management 183:61–75. https://doi.org/10.1016/S0378-1127(03)00100-2

Miandrimanana C, Reid JL, Rivoharison T, Birkinshaw C (2019) Planting position and shade enhance native seedling performance in forest restoration for an endangered Malagasy plant. Plant Diversity 41:118–123. https://doi.org/10.1016/j.pld.2018.09.005

Morelli TL, Smith AB, Mancini AN, Balko EA, Borgerson C, Dolch R, et al. (2020) The fate of Madagascar's rainforest habitat. Nature Climate Change 10:89–96. https://doi.org/10.1038/s41558-019-0647-x

Pareliussen I, Olsson EGA, Armbruster WS (2006) Factors limiting the survival of native tree seedlings used in conservation efforts at the edges of forest fragments in upland Madagascar. Restoration Ecology 14:196–203. https://doi.org/10.1111/j.1526-100X.2006.00121.x

R Core Team (2020) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org

Strassburg BBN, Iribarrem A, Beyer HL, Cordeiro CL, Crouzeilles R, Jakovac CC, et al. (2020) Global priority areas for ecosystem restoration. Nature 586:724–729. https://doi.org/10.1038/s41586-020-2784-9

Thaxton JM, Cordell S, Cabin RJ, Sandquist DR (2012) Native grass removal and shade increase soil moisture and seedling performance during Hawaiian dry forest restoration. Restoration Ecology 20:475–482. https://doi.org/10.1111/j.1526-100X.2011.00793.x

Werden LK, Alvarado JP, Zarges S, Calderón ME, Schilling EM, Gutiérrez LM, Powers JS (2018) Using soil amendments and plant functional traits to select native tropical dry forest species for the restoration of degraded Vertisols. Journal of Applied Ecology 55:1019–1028. https://doi.org/10.1111/1365-2664.12998

Supporting Information

The following information may be found in the online version of this article:

Table S1. *p*-values for effects of soil amendments, watering, and shade on native tree survival.

Table S2. *p*-values for effects of soil amendments, watering, and shade on native tree relative growth.

Figure S1. Growth and survival over 6 years for the five native species that have the greatest potential for dry season plantings.

Coordinating Editor: Manuel R. Guariguata

Received: 15 December, 2021; First decision: 29 January, 2022; Revised: 25 April, 2022; Accepted: 26 April, 2022