Sustainable Practices to Boost Tree Seedling Performance

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Tens of thousands of native Hawaiian trees are being outplanted every year in a variety of restoration and production-oriented projects. By far the most common outplanted species is *Acacia koa* Gray, the famous koa tree. The beautiful, durable heartwood of old koa trees is important culturally for canoes, surf boards, weapons, and other uses. Modern woodworkers turn koa wood into finely crafted furniture, musical instruments, and even more common items like jewelry boxes, pens, and picture frames. Ecologically, koa plays important roles in our native forests. As a nitrogen (N) fixer, koa adds this important nutrient to the soil through the turnover of leaves and roots, enriching the growth of all the plants of the forest. Its canopy moderates the understory environment, sheltering the seedlings of other native species and protecting them from extremes of temperature, humidity, and light. Its vertically oriented leaves intercept mist from passing clouds but allow the rain to fall through to the understory. The dead wood that accumulates from dying branches and suppressed trees provides a food source for native insects, which in turn supplies food for native birds. Downed logs and stumps and even the hollows of broken branches in the canopy provide a nursery site for germination of native plants that root in the rotting organic matter, avoiding competition with established plants on the ground and out of the reach of non-native ungulates like feral pigs. There is also a more practical reason that koa is the most common and usually the first native species outplanted in upland pastures and abandoned agriculture areas: it is one of the few native species that can compete successfully with introduced pasture and other grasses that often dominate these sites.

Another N-fixing native species is māmane, *Sophora chrysophylla* (Salisb.) Seem. Māmane is found in higher and drier habitats than koa, and thus grows more slowly. Māmane seeds are the primary food source for the palila (*Loxioides bailleui* Oustalet), a critically endangered forest bird. While māmane itself is not endangered, intact māmane forests have been decimated by a combination of grazing, wildfire, and herbivory by feral goats and sheep. The cold and dry environment typical of māmane forests makes restoration very challenging. Despite this, there are efforts to outplant māmane on Hawai‘i Island in order to expand the suitable habitat for the remaining populations of palila on Mauna Kea (Mauna Kea Forest Restoration Project, Three Mountain Alliance, Hakalau Forest National Wildlife Refuge, Hawai‘i Volcanoes National Park).

The successful establishment of seedlings after outplanting, especially in stressful sites, depends upon good seedling quality, including an appropriate root:shoot ratio, good leaf area, and leaves with sufficient nutrient concentration. A koa outplanting trial showed, as might be expected, that larger seedlings establish sooner than smaller seedlings, and that this early advantage is maintained as the trees grow together to form a closed-canopy forest (Jacobs et al. 2008). However, larger seedlings usually require larger containers (from dibble tubes to tree tubes). In remote field locations, this limits the number of seedlings that can be transported to the site at any one time and significantly increases outplanting effort.

Two alternatives to growing seedlings in larger containers are site preparation to control grasses or other vegetation and better greenhouse management of seedlings to maximize size and vigor in any given container size. Dr. James Leary of NREM has been exploring various low-dose herbicide application techniques and chemical combinations to control pasture grasses and other vegetation to prepare a site for outplanting of seedlings (Dr. James Leary...[updated 2010]). However, there are plenty of situations where a single application is insufficient to control the grasses or chemical use is imprudent or unfeasible, e.g. if existing native species are present on site or costs are prohibitive. Even where site preparation is feasible, there are likely to be additional benefits from improved nursery practices.

Fortunately, a lot of practical research has gone into improving nursery practices for tree seedling production. Two of the approaches that have been applied to koa are encouraging root symbioses and nutrient loading through exponential fertilization. Koa roots form symbiotic associations with soil bacteria in the genus *Bradyrhizobium* (Fig. 1). These bacteria colonize the roots of host plants, and once established, begin converting atmospheric nitrogen gas (N_2) into ammonia (NH_3). This is passed along to the roots and converted into amino acids. The nitrogen fixation process is energy-intensive, so the plant supplies carbohydrates to the bacteria through photosynthesis to drive the process (Postgate 1982). While *Bradyrhizobia* are abundant in healthy forests, open pastures may lack healthy populations of the optimal strains of these bacteria. Inoculation of seedlings with cultures of the appropriate strains ensures adequate root colonization prior to outplanting and also improves growth and N content in the nursery phase (Dumroese et al. 2009).

Fig. 1. Root nodules of Bradyrhizobium on a koa seedling produced in the nursery. Photo courtesy of JB Friday.

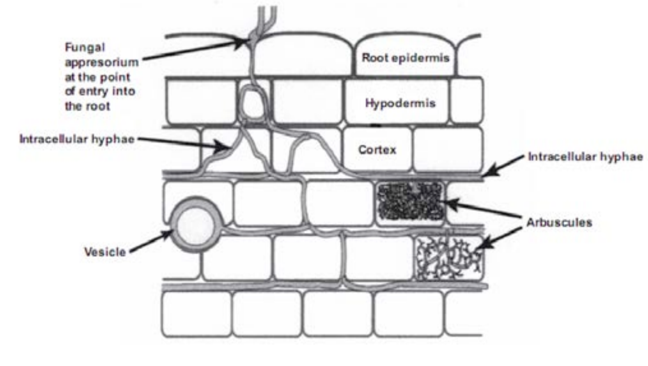


Fig. 2. Diagram of arbuscular mycorrhizal colonization of a plant root. Reprinted from Habte and Osorio (2001).

The other important symbiosis is with mycorrhizal fungi. These soil fungi also colonize the roots of many plant species (Fig. 2). The fungus receives carbohydrates from the plant that drives the growth of fine threads of fungal *mycelia* that greatly expand the effective root surface area of the plant. These mycelia are especially important for the uptake of immobile nutrients like phosphorus, iron, and zinc, and they may also protect against diseases and nematode infection (Habte and Osorio 2001). As with *Bradyrhizobia*, soils of open pastures or degraded sites may not support healthy populations of appropriate mycorrhizal fungi, and so inoculation in the greenhouse may benefit the seedling. Early work with inoculation of koa seedlings with mycorrhizae demonstrated short-term benefits (Habte et al. 2001), which may be important to help seedlings establish in stressful sites.

Optimal fertilization in the greenhouse is the other general approach to producing vigorous seedlings. Conventional approaches either add a single dose of granular fertilizer at the beginning of the nursery phase or lower doses of liquid fertilizer throughout the nursery phase. Both approaches suffer from a mismatch of nutrient availability vs. plant nutrient demand. Nutrient demand and uptake scale with plant size and growth rate. High nutrient availability early in the nursery phase is subject to leaching from the containers or even “burning” of the plant due to the high salt concentration. It also tends to inhibit colonization of plant roots with symbiotic organisms like mycorrhizae or *Bradyrhizobia*. By contrast, nutrient demand is highest near the end of the nursery phase, and nutrient availability may actually be too low, especially with a single-dose application. Ideally, nutrient supply should increase in an exponential fashion to match the pattern of plant growth.

One alternative to conventional fertilization techniques is the addition of a controlled-release fertilizer (CRF) in which the nutrient salts are encapsulated in a material that breaks down over time, slowing the release and thus availability of the nutrients. A common product sold in garden shops is Osmocote ®, which has an expected release time of 2-3 months. This overcomes the problem of over-dosing young seedlings. With koa, CRF addition was compatible with *Bradyrhizobium* inoculation, resulting in an optimal balance of plant size and symbiosis (Dumroese et al. 2009).

A more sophisticated approach is to ramp up doses of liquid fertilizer over time to match plant growth rate. A total desired amount of nutrient (usually N) is added in several doses, each greater than the last, according to a standard formula (Eq. 1).

$$N_t = N_s(e^{rt} - 1) - N_{t-1} \qquad [1]$$

This is called "exponential fertilization" because that is the form of the equation (and also the general trend in seedling growth in the nursery). The formula requires specification of the total N to be added (N_t), average seed N content (N_s), the expected growth rate (r), and the number of fertilizer additions (t), based on total time in the nursery and fertilizer frequency, e.g. weekly. This will obviously vary with species chosen, container size, nursery environment, and grower preferences, so initial trials that vary the total N added or total time in the nursery are necessary. As with use of CRF, exponential fertilization should be compatible with root symbioses. This should also result in much less leaching than conventional fertilization, especially early in the nursery phase. This reduces the environmental impacts of nursery production and improves the efficiency of fertilizer addition, requiring less fertilizer to produce a plant of a certain size and nutrient content (Dumroese et al. 2005). Another advantage of this fertilization regime is that it can support nutrient uptake in excess of growth requirements, called "luxury consumption." This results in a seedling that has a higher nutrient content than a comparably sized seedling grown under conventional fertilization (Fig. 3). This "nutrient loading" effect has been shown to improve establishment and early growth of seedlings after outplanting (Salifu and Timmer 2003a). Thus, exponential fertilization is both more effective and more efficient than conventional approaches.

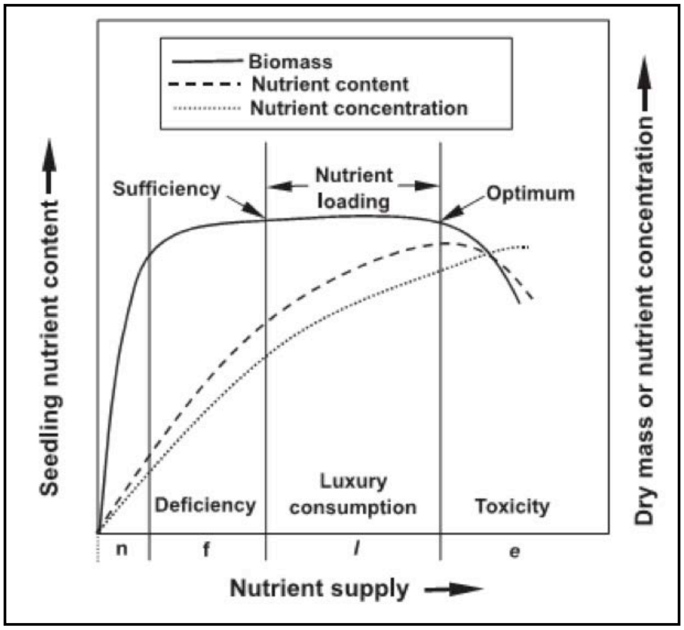


Fig. 3. Diagram of seedling response to increasing fertilization rate. Reprinted from Salifu and Timmer (2003b).

We tested exponential fertilization and mycorrhizal inoculation for koa and māmane in order to determine the optimal N addition rate. For both species, an initial dose of CRF followed by exponential fertilization of 1.5-2.0 g N/seedling was optimal for seedlings grown in D-40 size dibble tubes (656 cm^3).

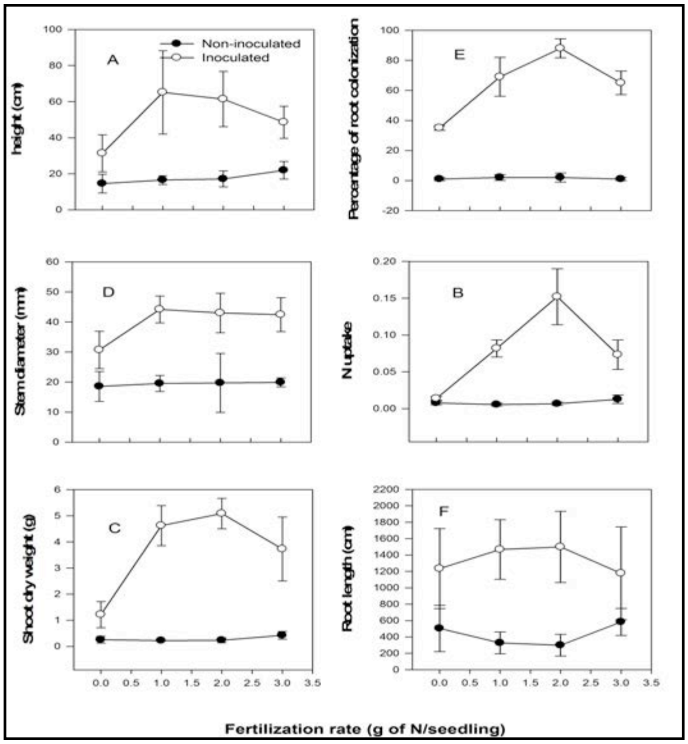


Figure 4. Growth of māmane seedlings at different levels of exponential fertilizer addition of N.

For koa, the nursery period and thus fertilization regime was 12 weeks; for māmane, it was 26 weeks. Seedlings of both species performed better at the optimal fertilization rate when they were inoculated with mycorrhizal fungi; for māmane, response to fertilization *required* mycorrhizal inoculation (Fig. 4). Thus, not only is mycorrhizal symbiosis compatible with exponential fertilization, it actually improves the seedling response.

Of course, the real advantages of these improved nursery production techniques are in seedling performance. Many studies of nutrient-loaded seedlings have shown they perform better in the first months and years after outplanting (Quoreshi and Timmer 2000, Salifu and Timmer 2003b). But these were mostly on sites that were well-suited to seedling establishment. We outplanted koa seedlings at sites that were much more challenging in terms of drought and grass competition, the Kanakaleonui Bird Corridor (~2100 m elevation) and Puu Mali (~1600 m elevation) along the slopes of Mauna Kea. At these high elevation sites, koa seedlings are susceptible to damage or mortality from night-time freezing temperatures (Scowcroft and Jeffrey 1999), which is common during the fall and winter months.

Our findings from both trials demonstrate significantly increased survival of seedlings produced with both mycorrhizal inoculation and exponential fertilization (Fig. 5). And, as in the greenhouse, the combination of these two practices yielded the best growth of surviving seedlings (Fig. 6). Both of these outplanting trials included a pre-plant herbicide treatment on half of the plots to reduce grass competition, but we saw no additional benefit of the site preparation. While this likely represents insufficient control of grasses with a single herbicide application, the high rate of survival for nutrient-loaded seedlings at Puu Mali suggests grass control may not be necessary for successful restoration of koa. Future trials with māmane at even higher and drier sites are needed to test the limits of the benefits from improved greenhouse management techniques.

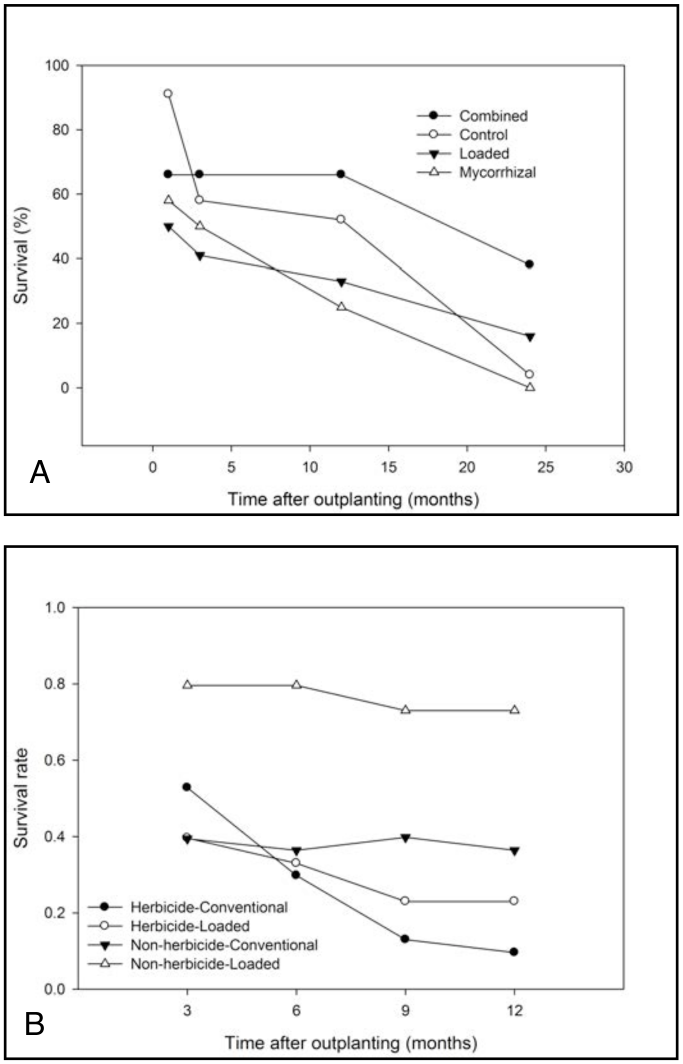
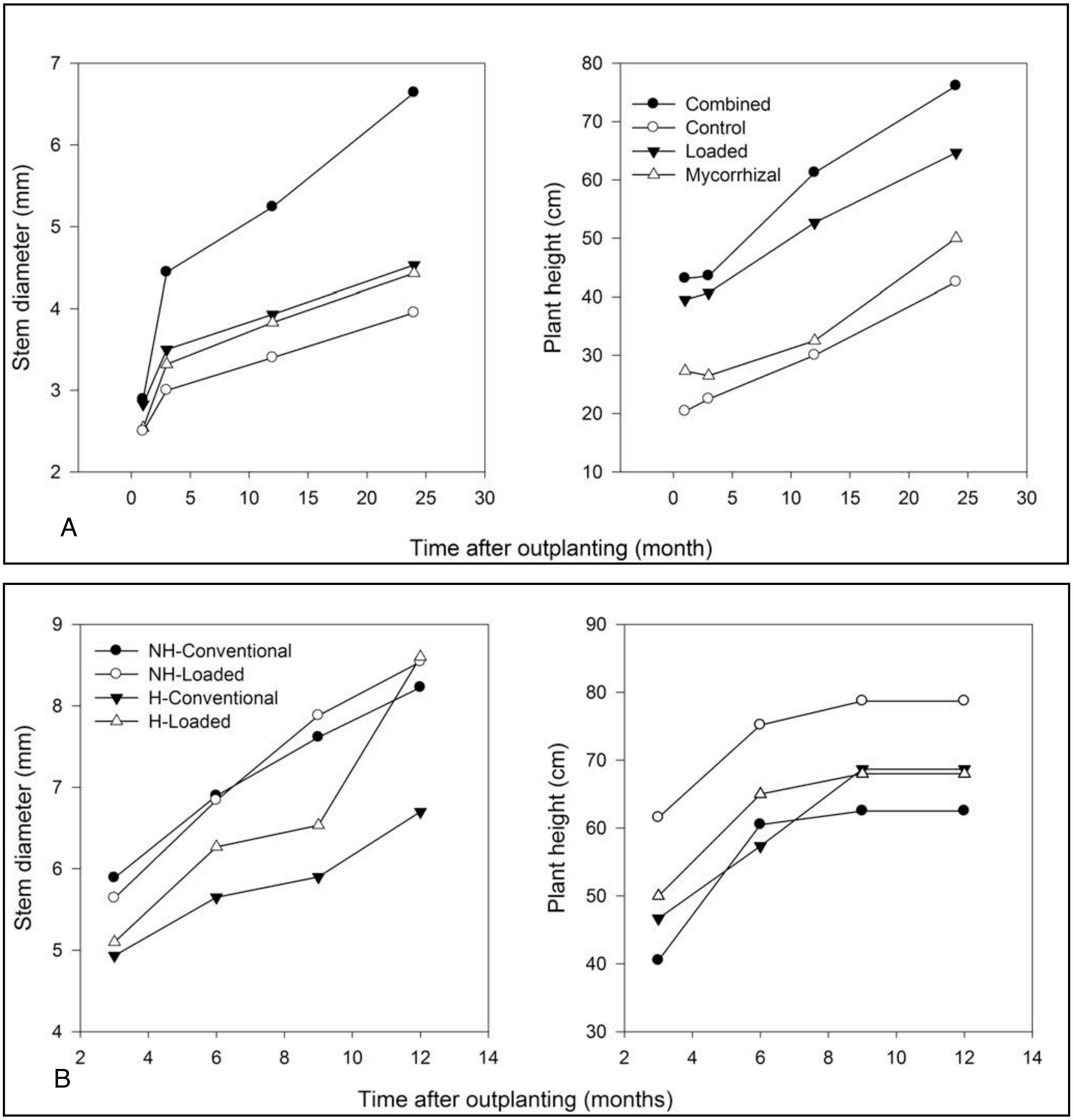


Figure 5. Survival of koa seedlings outplanted at the Kanakaleonui Bird Corridor (a) and Puu Mali (b) on Mauna Kea.

In summary, improved greenhouse management techniques, such as inoculation with symbiotic microorganisms and exponential fertiliza-

Figure 6. Growth of koa seedlings outplanted at Kanakaleonui Bird Corridor (a) and Puu Mali (b).



tion, can not only increase efficiency of fertilizer application and reduce waste but also produce larger, more vigorous seedlings that perform better in the field. These techniques can complement or even substitute for site preparation to control grasses, providing further cost savings and flexibility. Under stressful conditions, this improved performance can mean the difference between success and failure of restoration outplantings. We strongly recommend adoption of these techniques for these types of projects.

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