Inoculating *Acacia koa* with *Bradyrhizobium* and applying fertilizer in the nursery

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Collaborators: This project is being undertaken through cooperation between Purdue University, University of Idaho, USDA Forest Service, State of Hawai'i, and US Fish and Wildlife Service.

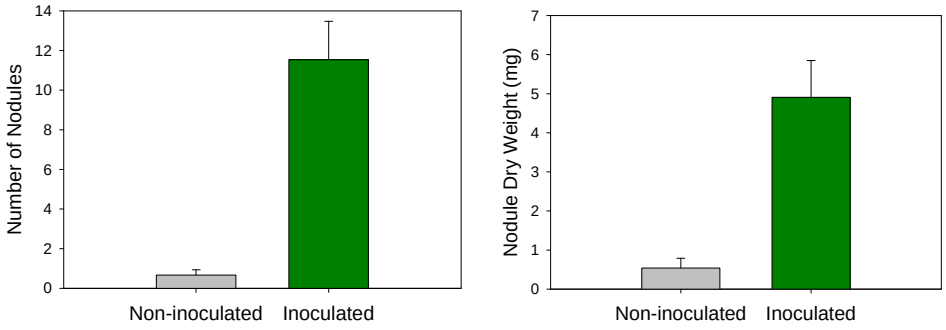
Background: Koa (*Acacia koa* A. Gray) is a valuable, endemic Hawaiian hardwood tree, planted extensively for forest restoration. A legume, koa has a symbiotic relationship with the bacteria *Bradyrhizobium*, which grows inside nodules that form on koa roots. Simply, koa supplies the bacteria with carbohydrates and the bacteria, through a chemical process, remove nitrogen from the atmosphere and make it available to koa. The relationship is an asset to a species such as koa that pioneer on sites with low, inherent fertility (such as lava flows). Debate continues as to the importance of developing symbiotic microorganism relationships on plants in the nursery, especially when plants are outplanted on sites that probably have the microorganisms naturally. Furthermore, the currently accepted convention is that in order to develop symbiotic relationships during nursery production, fertilization, especially nitrogen, must be drastically reduced or even eliminated. If inoculation and fertilization could be combined during nursery production, however, seedlings could still be grown rapidly to reduce nursery production time, while still providing seedlings with symbionts needed after outplanting. We grew seedlings at Waimea State Tree Nursery (Waimea, HI) either inoculated or not inoculated with *Bradyrhizobium* and given one of six different levels of fertilization. This handout represents the nursery component of this project; we will monitor growth after outplanting too. This is part of an ongoing commitment to Hawaiian native plant regeneration by the collaborating parties.

Procedures: We collected about 100 nodules from koa roots at Hakalau National Wildlife Refuge in December 2006, blended them in 1 liter of distilled water, and refrigerated the solution. In January 2007 at the Waimea State Tree Nursery, we transplanted germinating koa seeds collected at Hakalau into Deepot D-16 containers (262 cm^3) filled with medium containing one of six levels of controlled release fertilizer (0, 5 [equals the "medium" recommended rate on the fertilizer label], 10, 15, 20, and 25 kg m^{-3} Osmocote Plus 15-9-12 [5 to 6 mo]). Within two days of transplanting, we diluted our 1 liter of inoculum into 6 liters of tap water, and inoculated half of the seedlings with 10 ml of solution per seedling—that application rate delivered about 65,000,000 colony forming units of the bacteria to each plant. Our experiment was a 6 (nutrient levels) × 2 (inoculant levels) × 4 (blocks) randomized complete block design. In April 2007, we outplanted seedlings at Hakalau in a randomized design, with a group of 10 randomly selected seedlings from each of the treatments planted in each of 4 blocks. Within each block, we planted seedlings at a spacing of 2 m (120 seedlings per block). Immediately after outplanting, we measured seedlings for height and root-collar diameter. At the same time, we harvested 5 seedlings from each nutrient × inoculants × block combination and measured seedling infection, nodule number and weight, and seedling morphology. We re-measured outplanted seedlings in August 2007 and plan to measure again one year after outplanting.

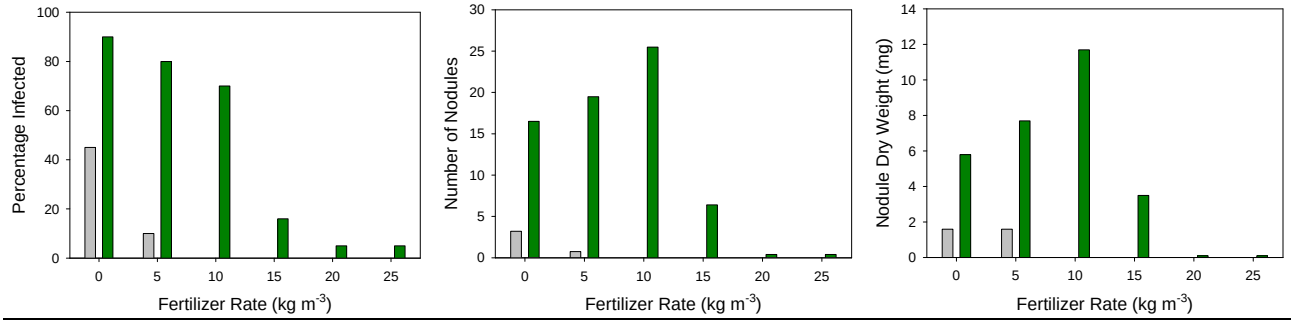
Figure 1. *Increasing rates of fertilizer increased seedling size. The control (no fertilizer) is at the far left; the highest rate on the far right.*

Findings to date

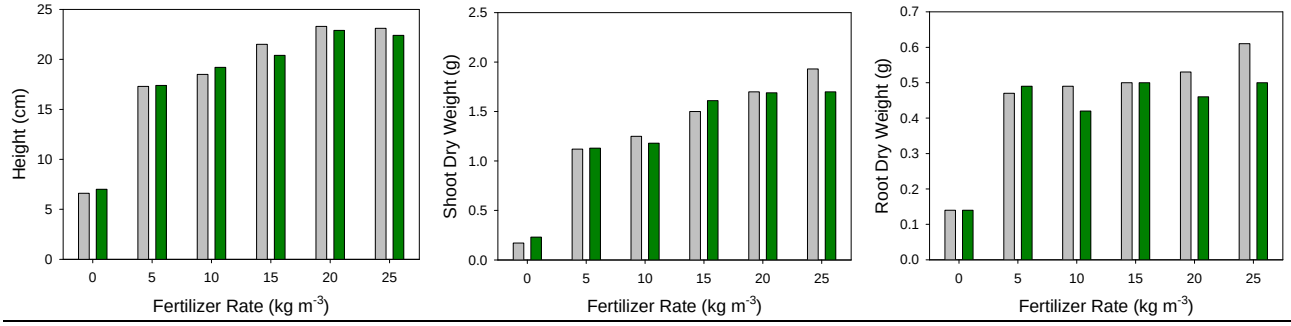
Inoculation works! – *inoculating koa seedlings increases the number and dry weight of nodules.*



Nodules – *the percentage of seedlings with nodules after inoculation decreases as fertilizer rate increases (left); nodule number (middle) and dry weight (right) increases as fertilizer rate increases from zero, but decreases drastically at the extreme fertilizer rates.* Gray bars = non-inoculated. Green bars = inoculated.



Seedling morphology – *more fertilizer yields taller seedlings (left) with larger shoots (middle), but not necessarily more roots (right).* Gray bars = non-inoculated. Green bars = inoculated.



Crude inoculum made from koa root nodules and applied to germinants in the nursery is an easy and effective way to inoculate seedlings. Rate of nodule formation and nodule size was greater with manufacturer recommended rates of fertilization compared to non-fertilized seedlings, but nearly absent at the highest (extreme) rates of fertilizer. Non-fertilized, inoculated seedlings had insufficient growth 3 months after outplanting, whereas inoculated seedlings given fertilization had sufficient growth, as did seedlings given the extreme rates of fertilizer. Our results suggest that seedlings can be inoculated and fertilized (at manufacturer rates) during nursery production without adversely reducing nodule formation and dry weight. Results from our outplanted seedlings should provide insight as to the benefit, or lack of benefit, of inoculating seedlings during nursery production.