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Physiological and anatomical responses of *Acacia koa* (Gray) seedlings to varying light and drought conditions

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ABSTRACT

True leaves of *Acacia koa* (Gray) seedlings play a vital role in the early stages of seedling establishment, particularly in the Hawaiian archipelago where regeneration occurs under very heterogeneous light and moisture conditions. Given the importance of understanding seedling regeneration in the highly disturbed forests of Hawaii, we studied the growth, biomass allocation, physiological, and anatomical characteristics of *A. koa* seedlings with true leaves in response to three levels of light (low, intermediate, and high sunlight) and two levels of moisture regimes (100% field capacity, 20% field capacity) in a greenhouse experiment. With increasing light intensity, seedlings exhibited greater growth, biomass accumulation, intrinsic water-use efficiency, more foliar C, and higher stomatal density. Their leaflets were thicker, containing more palisade and spongy parenchyma. Low light intensity ameliorated drought-related effects of carbon assimilation on *A. koa* seedlings, while seedling growth of drought-stressed seedlings was reduced under intermediate and high sunlight. Our results suggest that true leaves of *A. koa* seedlings are adaptive when light is the only limiting environmental variable. The low adaptive capacity of true leaves to drought stress under high light intensity negatively affected seedling performance, which possibly explains environmental conditions under which phyllodes are likely to emerge.

1. Introduction

The current spatial extent of Hawaiian forests in which *Acacia koa* (Gray) occurs has declined over the past century due to timber extraction and land clearance. Agriculture and cattle grazing in deforested areas resulted in the fragmentation and degradation of forested ecosystems in Hawaii (Elevitch et al., 2006). Similar to forests in other tropical regions, these activities have altered the species composition and abundance of woody seedling communities by reducing soil fertility and structure, seed availability, and increasing light intensity (Uhl, 1987; Guariguata et al., 1995; Parrotta et al., 1997; Zimmerman et al., 2000; Loik and Holl, 2001). Natural regeneration of the two most ecologically and economically important native tree species in Hawaii, *A. koa* and *Metersideros polymorpha*, remains inhibited by persistent exotic grass species that outcompete seedlings for light, water, and soil nutrients and cattle grazing (Cabin et al., 2002; Brooker, 2006). The inability of seedlings to regenerate in areas invaded by grasses, especially shade-tolerant grass species, perpetuates forested grasslands in areas that were occupied previously by closed-canopy forests (Denslow et al., 2006).

The replacement of *A. koa* true leaves with phyllodes helps seedlings exploit the changing abiotic conditions that accompany forest succession. This morphological change possibly reflects how the adaptive value of certain traits changes with environmental conditions, from a situation where light is the principal limiting resource to one where water is the principal limiting resource (Hansen, 1996; Yu and Li, 2007). Bi-pinnately compound, horizontally oriented true leaves enable *A. koa* seedlings to maximize interception of the high intensity light in newly formed forest gaps (Walters and Bartholomew, 1984, 1990). True leaves display greater surface area per unit mass, photosynthesize at similar rates, have more chlorophyll per unit mass, and are thinner than phyllodes (Walters and Bartholomew, 1984; Atkin et al., 1998). These traits of true leaves increase the susceptibility of *A. koa* seedlings to drought stress, which is thought to cue the change from true leaves to phyllodes (Walters and Bartholomew, 1984). Myriad physiological and anatomical traits of phyllodes increase their drought tolerance, the most salient of which are: the steep inclination of phyllodes to incident radiation, lower transpiration and stomatal conductance rates relative to true leaves, and high trichome density (Walters and Bartholomew, 1984). Consequently, *A. koa* phyllodes are more physiologically responsive than juvenile true leaves to changes to moisture availability and are more efficient in their use of plant-available water (Hansen, 1986; Hansen and Steig, 1993).

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In response to varying amounts of light and water resources, seedlings undergo a series of structural, anatomical, and physiological adaptations (Holmgren, 2000; Sack and Grubb, 2002; Sack, 2004; Quero et al., 2006). Various 'trade-off' hypotheses have been tested to improve current understanding of seedling response to simultaneous light and drought stress (Kubiske et al., 1996; Holmgren, 2000; Mediavilla et al., 2001; Valladares and Pearcy, 2002; Hastwell and Facelli, 2003; Sack, 2004; Duan et al., 2005; Quero et al., 2006). Under the antagonistic version of the trade-off hypothesis, the drought effects on seedlings in full shade should intensify, as compared to those in full sun, since the proportionally greater C allocation to aboveground biomass reduces their ability to uptake water from the soil and accelerates water loss due to a relatively larger transpirational area (Kubiske et al., 1996; Ashton et al., 1999; Valladares and Pearcy, 2002). Other researchers have hypothesized that shade improves plant water relations of drought-stressed seedlings by lowering air temperatures and evaporation (Holmgren, 2000; Sack, 2004; Duan et al., 2005; Quero et al., 2006). Lastly, the relationship between drought stress and deep shade has been posited as being independent, which would allow for niche differentiation of tree species along either light or water gradients within forests (Sack and Grubb, 2002; Sack, 2004; Markesteijn and Poorter, 2009).

Given the long retention time of true leaves by *A. koa* seedlings (3–5 months), the physiological and morphological adaptations of juvenile true leaves to drought and light stress likely play an important role in determining spatial and temporal patterns of *A. koa* seedling establishment. In contrast to previous studies on *A. koa* that compared true leaves with phyllodes (Walters and Bartholomew, 1984, 1990; Hansen and Steig, 1993; Hansen, 1996), in the present study we focus exclusively on the physiological, anatomical, and morphological responses of *A. koa* seedlings with true leaves to variable amounts of light intensity and drought stress. Herein, we investigate the response of *A. koa* seedlings to the interaction of light intensity and drought stress in a controlled greenhouse setting in terms of biomass allocation, height and root collar diameter, height and root collar diameter increment leaf mass ratio, root mass ratio, intrinsic water-use efficiency (iWUE), foliar C concentration, stomatal density, upper and lower epidermis thickness, palisade and spongy parenchyma thickness, and leaflet thickness. Using the theoretical framework of 'trade-off' hypotheses, we formulated the following hypotheses:

(1) We expected that *A. koa* seedlings would adapt to higher levels of light intensity by increasing growth and biomass, iWUE, foliar C, decreasing stomatal density, and increasing thickness of leaf, upper and lower epidermis, and palisade and spongy parenchyma. We expected seedlings exposed to increasingly intense light would respond by allocating relatively more C to root biomass and less to leaf biomass, thereby increasing root mass ratios and decreasing leaf mass ratios;

(2) We expected that *A. koa* seedlings would respond to drought stress by decreasing growth and biomass accumulation, stomatal density and LMR and increasing iWUE, foliar C concentration, and leaflet, upper and lower epidermis, and palisade and spongy parenchyma thickness;

(3) We expected that drought stress would significantly increase within each light treatment relative to non-droughted seedlings. To this end, physiological and structural responses of *A. koa* seedlings subjected to the drought treatment would grow more slowly and accumulate less aboveground, belowground and total biomass, have a higher root mass ratio and a lower leaf mass ratio, greater iWUE and foliar C concentration, and a lower stomatal density with thicker leaves, upper and lower epidermis, and palisade and spongy parenchyma.

2. Materials and methods

2.1. Light and drought treatments

Approximately 100 *A. koa* seeds were collected from The Nature Conservancy of Hawaii's Kona Hema Preserve located on the island of Hawaii (the Big Island), Hawaii, USA (19°12′N, 155°48′W) (P. Baker, personal communication). The greenhouse experiment was performed at the Yale University Greeley Memorial Laboratory Greenhouse (41°16′N, 72°55′W) in New Haven, CT, USA. Light and temperature were regulated to replicate natural conditions experienced by *A. koa* seedlings (Walters and Bartholomew, 1984). Natural light was augmented by artificial lighting in the greenhouse to increase day length and light quantity. Average daily maximum photosynthetically active radiation (PAR) in the greenhouse was $924.2 \pm 411.4\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$, yet reached values between 1200 and $1600\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$ on clear, sunny days, and the average temperature was $25.56 \pm 1.60\,^{\circ}\text{C}$ during March and April 2006–2008. There were no continuously logged PAR or temperature data available during the experiment (March–April 2005). PAR and temperature were logged at 30 min intervals using a HOBO Photosynthetic Light (PAR) Smart Sensor (Onset Computer, Bourne, MA, USA) connected to a Hobo Micro Station Data Logger (Onset Computer, Bourne, MA, USA).

A split-plot design was implemented to test for the effects of variable levels of light and drought. Shade enclosures were built to create the three light regimes used in the experiment—low, intermediate, and full sunlight. Two shade enclosures were constructed for each light regime. All enclosures measured $1.1\,\text{m} \times 0.82\,\text{m} \times 1.86\,\text{m}$ in size and were covered by variable R: FR transparent plastic to homogenize incident light quality and a $0.5\,\text{mm}^2$ mesh black shade cloth to alter incident light quantity. In the low sunlight enclosures, average instantaneous PAR was $21.28 \pm 13.06\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$ and transmittance was 3.1%. In the intermediate sunlight enclosures, average instantaneous PAR was $80.33 \pm 55.15\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$ and transmittance was 11.8%. In the full sunlight enclosures, average instantaneous PAR was $255.25 \pm 176.78\,\mu\text{mol m}^{-2}\,\text{s}^{-1}$ and transmittance was 37.4%. Average instantaneous PAR measurements were taken every other day during the experiment using a Li-90 quantum sensor (Li-Cor, Lincoln, NE, USA). Transmittance was calculated by dividing the average of three instantaneous PAR measurements in the greenhouse by the average of three instantaneous PAR measurements in each shade enclosure.

Seeds were scarified, imbibed with water, and sown into 9 in. pots containing Metro-Mix soil (Sun Gro Horticulture Canada Ltd., Vancouver, BC, Canada). Seeds were distributed equally across treatments and germinated in three different light treatments (Ashton and Berlyn, 1992). Seedlings remained in the same shade enclosures for 10 months until they were destructively sampled. All plants were watered daily prior to starting the drought treatment.

Before the experiment started, seedlings with only compound true leaves and no phyllodes were selected for height uniformity. A total of 82 seedlings was used for the experiment and were distributed evenly across experimental treatments. To facilitate the removal of the stomatal boundary layer, thereby enhancing the effect of the drought regime, 6 in. Air King Clip-On Air Circulators were installed within each enclosure (Air King America, West Chester, PA, USA). The air fans operated for 8 h each day during the experiment. The drought regime, conducted from March 20th to April 24th 2005, was designed to mimic an extended drought similar to the dry season that occurs on the leeward side of the island of Hawaii (Giambelluca et al., 1986). Within each light treatment, half the seedlings were randomly selected to receive the drought treatment (see Table 1 for treatment description). The drought regime consisted of lowering soil moisture content to 20% of field capacity,