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# Impact of leaf removal on initial survival and growth of container-grown and bare-root seedlings of Hinoki cypress (*Chamaecyparis obtusa*)

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ABSTRACT

As a part of improving forest management practices, we tested the idea that partial defoliation of container-grown and bare-root Hinoki cypress (*Chamaecyparis obtusa*) seedlings prior to outplanting can reduce transplant shock and enhance seedling survival during their first growing season after transplanting. For container-grown seedlings, the remaining leaves of defoliated seedlings showed photosynthetic capacity (Amax) about 1.5 times higher than the non-defoliated control in the first 3 weeks. For defoliated bare-root seedlings, Amax ranged 3.3–4.8 times higher than non-defoliated control in the first 4 weeks after transplanting. Thereafter, Amax of defoliated seedlings of both types began to decrease slowly. Amax of newly emerged leaves after transplanting was significantly higher than that of existing leaves for container-grown seedlings but not bare-root seedlings. For container-grown seedlings, both defoliated and non-defoliated plants showed high survival rate (>90%) and there were no significant differences among levels of defoliation. For bare-root seedlings, non-defoliated control showed highest mortality (30%) by the end of the growing season but seedlings that were 25%, 50%, 75% defoliated all suffered little mortality. In terms of initial growth, there was no significant difference between defoliated and non-defoliated control in container seedlings suggesting that defoliation treatment had no effect on reducing their growth. In comparison, bare-root seedlings showed marginally but significantly greater height growth in defoliated plants, suggesting that non-defoliated seedlings suffered greater transplant shock and reducing the amount of leaves may contribute to minimize water stress and mortality. Therefore, we found that for bare-root seedlings, partial defoliation can effectively reduce transplant shock resulting in high survivorship and growth performance; the lack of defoliation response in container-grown seedlings would suggest that defoliation is unnecessary for improving their transplant success.



## 1. Introduction

In reforestation, it is critical to maximize post-transplant seedling survival and growth and maintain seedling health. If bare-root seedlings are used, they often suffer from root damage and root loss that occurred when they were excavated from the tree nursery. Additionally, if field conditions vary significantly from those in the nursery, the transplanted seedling will experience stress during the acclimation process. Thus strategies for dealing with transplanting stress are crucially important to seedlings survival and growth. Stresses affecting seedlings are commonly called transplant shock and these may include photodamage due to excessive light, high or low temperature, drought, or insect attack (e.g., Close et al., 2005; Richardson-Calfee and Harris, 2005; Struve, 2009). A transplanted seedling that can recover quickly from transplant shock and survive under harsh field conditions during the initial stage would contribute to improving the efficiency of reforestation and reducing total cost. To raise such a transplant seedling, we need to consider the relationship between the amount of leaves and its effect on whole-plant water balance. If a seedling with a large amount of leaf area is transplanted, it may be less able to maintain water balance because of insufficient water absorption from reduced root mass during the transplanting

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process. In nursery, if seedlings are grown under favorable fertilization or irrigation, they can grow more shoot in proportion to root (Harris, 1992; Zainudin et al., 2003) and render these seedlings more vulnerable to desiccation. Given this rationale, it would seem feasible to minimize transplant shock and enhance the survival rate of transplanted seedlings by reducing the amount of leaf area especially for evergreen species such as *Chamaecyparis obtusa*.

Leaf removal may reduce seedling growth from the loss of photosynthetic area, leading to lower carbon gain. Other studies on deciduous trees have reported that leaf removal directly decreased the amount of photosynthetic tissue resulting in reduce growth, reproduction and survival (Harris and Bassuk, 1995; Harris and Fanelli, 1999). In addition, leaf removal risks disrupting the supply of auxin that would normally stimulate root development (Llewellyn, 2006). Therefore, excessively defoliated plants can also incur an increased risk of mortality due to loss of belowground functions. At the whole plant level, it is known that plants with a high leaf area mass ratio or high top to root ratio are not well suited to maintaining a favorable water balance in the field after transplanting (Close et al., 2005). A lower top to root ratio leads to a better balance between root water acquisition and shoot water demand following transplanting (Ledig, 1983) and a lower leaf area to plant mass ratio minimizes stomatal water loss (Wright et al., 2001). These findings suggest that appropriate leaf removal can contribute to maintaining better water balance and maintain carbon gain, and an improved survival rate in transplanted seedlings. However, there has been no experimental confirmation of these expectations. As far as we know, this is first study to validate the effect of defoliation on survival, physiological performance, and growth of conifer seedlings after outplanting.

The roots of bare-root seedlings are sensitive to desiccation and damage during handling, storage, and transport from the nursery to the field (Garriou et al., 2000), which can lead to poor post-planting performance and high rates of mortality. But if survival rate of bare-root seedlings can be improved through "appropriate treatment" of leaf removal before transplantation, then this becomes a better alternative to using container-grown seedlings due to the latter's higher cost (Japan Forestry Agency, 2012).

Contrary to the negative impact of leaf removal on plant performance, many studies have also reported that following defoliation, a positive response such as increased photosynthetic activity in the remaining leaves and compensation growth (e.g., Zheng et al., 2011; Erbilgin et al., 2014). Such compensatory responses are attributed to increased above- and belowground sink demand driving a higher production of photoassimilates facilitated by resources reallocated from source organs such as roots, stem and branches (Bazzaz et al., 1987; Donaldson et al., 2006; Barry and Pinkard, 2013). Therefore, it is possible that appropriate levels of defoliation may lead to desirable seedling physiological response and compensatory growth during first growing season after transplanting.

Hinoki cypress (*C. obtusa*) is one of the most important conifer species and plantation tree in Japan with plantation areas of about 26,000 $km^2$ covering 25% of total plantation forest in Japan (Fujitake, 2007; Japan Forestry Agency, 2012). These plantation trees have mostly reached maturity and are ready for harvest, however, harvesting and reforestation are not progressing significantly partly due to high reforestation cost (Japan Forestry Agency, 2012). Currently, container-grown cuttings or seedlings are favored over bare-root seedlings for reforestation in Japan because of their high survival rate in year-round planting (e.g., Yamagawa et al., 2013; Yagihashi et al., 2015), but there is insufficient data to evaluate the field performance and cost-benefits of container-grown seedlings against bare-root seedlings.

To confirm whether reducing the amount of leaf area is effective in minimizing transplant shock, we investigated the effect of different degrees of leaf removal on growth, survival, and physiological responses of Hinoki seedlings after transplanting. We addressed the following questions: (1) Is photosynthetic performance of the remaining leaves different among seedlings with different degrees of leaf removal? (2) Does the degree of leaf removal affect seedling survival and growth differently after transplanting? (3) Is plant response to leaf removal different between container-grown seedlings and bare-root seedlings?

## 2. Methods

### 2.1. Plant material

Two-year-old container-grown and bare-root Hinoki seedlings (acquired from Toyonami tree nursery Ltd, Okayama, Japan) were used in the experiment (Photo 1). Based on tree nursery information, Hinoki seed were sowed in nursery beds composed of forest soil (mainly andosol) mixed with chicken manure at 300 kg per 1000 $m^2$, chemical fertilizer (N:P:K = 14:14:14) at 40 kg/1000 $m^2$ and phosphate fertilizer at 40 kg per 1000 $m^2$. When seedlings reached one year old, they were either transplanted directly in the ground at 15–18 cm interval between seedlings or in plastic multi-cavity trays with 40 (5 × 8) cavities per tray (JFA-150). Each cavity is 150 ml (45 mm dia. × 130 mm depth) and 5.5 cm center to center between cavities. For the container-grown seedlings, the containers were filled with a mixture of 3:3:4 cocopeat, perlite and forest soil, and about 1 g of slow release fertilizer (N:P:K: Mg = 10:10:10:1) was added in each container.

### 2.2. Experimental design

On the day of transplanting, bare-root seedlings were dug out at the tree nursery in the morning, and together with container-grown seedlings, transported and planted in an experimental field in Kansai Research Center, Forestry and Forest Products Institute in Kyoto on May 27, 2014. There were 20 seedlings for each stock type by defoliation treatment; they were planted in a completely randomized design in the experimental field. Seedlings were watered once at planting with no further watering applied. Weeding around each plant was applied once 3 months after planting. Seedling survival was recorded 45 days, 104 days and 149 days after planting in the first growing season. Air temperature, soil moisture, and light condition at the experimental field were monitored every 5 min during the experimental periods using data loggers. Mean air temperature, mean soil moisture, and daily maximum photon flux density in experimental field was 23.6 °C, 20.4%, 2506.2 $\mu mol\ m^{-2}\ s^{-1}$, respectively. At the time of


**Photo 1.** Container-grown (A) and bare-root (B) Hinoki seedlings at the time of transplanting (May, 2014).