Establishing a Wild, Ex Situ Population of a Critically Endangered Shade-Tolerant Rainforest Conifer: A Translocation Experiment

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Abstract

Translocation can reduce extinction risk by increasing population size and geographic range, and is increasingly being used in the management of rare and threatened plant species. A critical determinant of successful plant establishment is light environment. Wollemia nobilis (Wollemi pine) is a critically endangered conifer, with a wild population of 83 mature trees and a highly restricted distribution of less than 10 km². We used under-planting to establish a population of W. nobilis in a new rainforest site. Because its optimal establishment conditions were unknown, we conducted an experimental translocation, planting in a range of different light conditions from deeply shaded to high light gaps. Two years after the experimental translocation, 85% of plants had survived. There were two distinct responses: very high survival (94%) but very low growth, and lower survival (69%) and higher growth, associated with initial plant condition. Overall survival of translocated W. nobilis was strongly increased in planting sites with higher light, in contrast to previous studies demonstrating long-term survival of wild W. nobilis juveniles in deep shade. Translocation by under-planting may be useful in establishing new populations of shade-tolerant plant species, not least by utilizing the range of light conditions that occur in forest understories.

Introduction

Extinction risk for a species is determined by two key traits: population size and geographic range [1]. Translocation, the intentional movement of an organism from one place to another [2], can reduce the risk of extinction in the wild by increasing a species geographic range and population size [3–5]. Translocation is increasingly being used to reduce extinction risk in rare and threatened plants [6,7], with the long-term aim that translocated individuals become...
established and produce seedlings of their own [6]. However, optimal conditions for establishment of rare and threatened plants are often poorly known [8].

For forest trees, a critical determinant of successful establishment is light environment [9]. The life history strategies of forest trees range from species with seedlings that require high light to survive and grow, and have high maximum growth rates (shade-intolerant species), to species with seedlings that can recruit in deep shade and have slower maximum growth rates (shade-tolerant species) [9,10]. Protection from direct sunlight, as well as the milder temperature and moisture conditions afforded by the understory, is crucial for the successful establishment of shade-tolerant species [11–14]. Under-planting has been primarily developed as a strategy for promoting regeneration of shade-tolerant species that are valued for their timber, such as oak in temperate deciduous forests USA [15,16], spruce in boreal regions in Canada [17] and Sweden [18], shade-tolerant conifer species in coastal temperate rainforest in Canada and the USA [19,20], and for reintroduction of high-value commercial species into degraded forests tropical Americas and Asia [21,22]. The silvicultural theory associated with under-planting for timber production can be applied in species translocation.

Here we report on an experimental translocation (with the aim of informing future translocations, *sensu* [23–25]) of *Wollemia nobilis* (Araucariaceae), a critically endangered, shade-tolerant rainforest conifer [26]. Fewer than 100 mature and 100–200 juveniles of *W. nobilis* exist in the wild, in a warm temperate rainforest community in the sandstone canyon landscape of Wollemi National Park in southeastern Australia [27]. Location information for the wild population of *W. nobilis* is confidential and protected by law [28]. No genetic variation has been detected in *W. nobilis*; genetic analysis revealed no polymorphism at 13 allozyme loci, more than 800 amplified fragment length polymorphism loci or 20 simple sequence repeat loci [29]. Trees 2–20 m tall are scarce, suggesting a serious bottleneck in recruitment from the seedling to mature stage [30]. The viability of *W. nobilis* seed is low (~10%; [31]) and seed dispersal is limited—no seedlings have been found >50 m from mature trees. Of those seeds that do germinate, subsequent establishment is also limited, although once established, juveniles can survive in the deep shade for 16 years (and potentially longer) growing very slowly (<2 cm/yr in height) [30]. The optimal conditions for *W. nobilis* establishment are unknown [30]. The small wild population and highly restricted distribution of *W. nobilis* have contributed to its classification as critically endangered, with the high risk of extinction due to a single event. For this reason, translocation of *W. nobilis* has been identified as highly desirable [28]. The understory of *W. nobilis* habitat is characterized by ~3% light, and greenhouse experiments have shown that stem growth of small (<0.7 m tall) *W. nobilis* increases with light (up to 50% light; [32]). In particular, we asked:

1. How does growth (stem length, branch number) and survival of translocated *W. nobilis* vary along a light gradient?

2. How does the effect of light on *W. nobilis* growth and survival vary with plant size?

The terminology used to describe plantings of rare and threatened species for conservation outcomes is often confusing [33–35]. Such plantings have been variously called reintroduction, reinforcement, ecological replacement, assisted colonisation/migration and community construction [33]. Often the distinction between these terms depends on the past distribution of the species. For *W. nobilis*, which is only known as living individuals from a single site, but is present in the region (and elsewhere) in the fossil record for hundreds of millions of years [36,37], these definitions are often ill-suited. Throughout this paper, we simply use the relatively broad term “translocation”, which is defined as the intentional movement of an organism from one place to another [2].
Methods

Study area

This study was carried out in warm temperate rainforest on land owned and managed by Blue Mountains Botanic Garden Mount Tomah (BMBGTM; -33°32' S, 150°25' E). BMBGTM gave permission for the study to be carried out on their land.

Study design

Site assessment for experimental translocation. Potential sites for the experimental translocation were formally assessed nine months before planting. We assessed the suitability of potential sites based on two criteria: (i) habitat characteristics and (ii) logistics, implementation and management [8]. A simplified ranking table was completed for each potential site (S1 Table, sensu [8]). The site that we selected for the experimental translocation was in a native warm temperate rainforest community located on land managed by the BMBGTM. The translocation site is in a deeply incised canyon and has environmental conditions that are similar to those of the natural W. nobilis sites. Mean annual rainfall at the experimental translocation site (1376 mm) was higher than at the wild site (952 mm). We tested soil for N, P, K, micronutrients and pH, to assess similarity to the wild site. In addition, we sampled for the presence of Phytophthora cinnamomi, a root fungus that causes dieback in juveniles and adults of many plant species [38] including W. nobilis [28,39]. Key soil variables at the experimental translocation site were similar to those of the wild site (S2 Table).

Light measurements and determination of planting sites (gaps). We identified 30 potential planting locations in rainforest canopy gaps. We selected the 30 gaps to span the full range of canopy openness at the site. Light was measured from the heavily shaded rainforest (near the creek line) to beneath large canopy gaps at the rainforest-woodland boundary. Light, as a proportion of total available light, at each gap was estimated from hemispherical photography. Hemispherical photography is widely used in studies of canopy structure and transmission of light forest [40]. A camera (Canon 60D camera with a Sigma 4.5 mm hemispherical lens) was mounted on a tripod, levelled and placed so that the top of the photo was facing magnetic north. Three photos were taken for each gap using automatic exposure bracketing (F 14 and ISO 100). Additional photos, one for each plant, were taken at 12 months to capture within-gap light variability, using the same methods. Hemispherical photos were analysed first using the automatic thresholding algorithm in Sidelook [41]. In several instances where the automatic thresholding algorithm did not result in adequate partitioning of canopy and sky, the automatic threshold was noted, and the Gap Light Analysis (GLA 2.0, [40]) software was used to fix problem areas (e.g., sun flare and reflections from rock face). GLA was then used to calculate proportion of canopy and sky and light variables. All analyses were done using the blue channel.

Transmitted total light at the experimental translocation site ranged from 0 to 24%, compared to the wild site (0–6%, Fig 1; S3 Table). The experimental translocation site therefore provided a greater range of light availabilities.

Source material. A total of 191 plants were included in our experiment. One hundred and one plants were obtained from the Australian Botanic Garden Mount Annan (ABGMA), which maintains ex situ populations of W. nobilis. The plants were 2–3 years old, grown from cuttings from 20 individual wild parents, and were raised in a hygienic environment free from pathogens (e.g., Phytophthora cinnamomi). Plants from the ABGMA varied in size. There were 29 plants in 165 mm diameter pots, with a mean stem length of 272 (±13) mm; 55 plants in 250 mm pots, mean stem length 768 (±216) mm; 17 plants in 265 mm pots, mean stem length
1042 (±73) mm. Due to the limited availability of plants from botanic gardens, an additional 90 cutting-grown *W. nobilis* were sourced from a commercial supplier. These were in 100 mm pots and had a mean stem length of 456 (±6) mm. Seedlings (rather than plants grown from cuttings) are typically preferred in restoration because of their genetic diversity, and the potential long-term implications for populations [42,43]. However, it should be noted that translocated *W. nobilis* populations will have low genetic diversity because the small wild population of *W. nobilis* has extremely low genetic diversity [29]. We used plants grown from cuttings rather than seeds or seedlings because of the inadequate availability of seed.

**Planting.** Planting occurred over four days in August 2012 (late winter). Planting was timed to occur before the *W. nobilis* growing season (i.e., spring). A quarantine station was established outside of the site, and all equipment was sterilised with a methylated spirits solution to minimize potential *P. cinnamomi* spread. Individual plants were manually transported from the road end and placed at planting sites within the warm temperate rainforest. Plants were removed from pots and their roots were gently teased out immediately before planting. In each gap we planted six or seven plants, this included three plants from the commercial supplier (3 × 100 mm pots) and three or four plants from the ABGMA (1 × 165 mm pot, 2–3 × 250–265 mm pots). The experimental translocation site was approximately 120 m long, following a creek, and extending 60 m upslope from the creek (i.e., a total area of 120 m × 60 m).

![Fig 1. Total transmitted light in the experimental translocation site and the wild *Wollemia nobilis* site.](https://example.com/fig1.png)

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m). Gaps were spread along the site. Planting was undertaken in an area of approximately 4 m × 4 m within each gap, with at least 1 m between plants. Plant placement was dependent on ability to dig a planting hole (i.e., not impeded by rocks). Each plant was watered (~ five litres), its stem length was measured, and wallaby (and lyrebird) guards were erected. Clearing of ground-level vegetation was sometimes required. In these cases a consistent area (1 m in diameter) was cleared around each plant. Each plant was again given approximately five litres of water one month after planting (September 2012). A total of 191 *W. nobilis* were outplanted. Photographs of the plants and experimental translocation site can be found in S1 Fig.

**Monitoring.** Monitoring was undertaken at 1 month (September 2012; survival only), 2 months (October 2012), 5 months (January 2013; survival only), 6 months (February 2013), 11 months (July 2013), 16 months (December 2013), 18 months (February 2014; survival only) and 25 months (September 2014) after planting. Stem length was measured and number of branches counted. *Wollemia nobilis* only produces first-order plagiotrophic shoots (or branches); *W. nobilis* branches do not branch [44]. Branches are therefore limited in the size to which they can grow and, in mature trees, they are typically shed after 5–15 years [45]. Signs of insects and pathogens were recorded. The wallaby guards were removed in June 2013 as there were no signs of herbivory on exposed branches and the cages had the potential to restrict branch development as the trees grew.

**Statistical analysis**

Preliminary examination of the data showed that plants from the two suppliers had very different trends in growth and survival. Moreover, during planting we observed that all plants from the commercial supplier were root bound and chlorotic relative to the plants from the botanic garden. For this reason, we subset the data by supplier for analysis, and refer to plant origin as either "commercial" or "gardens".

**Stem growth and branch number.** Total stem growth and number of branches at 25 months was calculated for live plants (*n* = 157; not including those that had died back and then resprouted). We were interested in how growth varied with light availability, and if there were any interactions between light and stem length at *t*₀. Stem growth was analyzed according to light, initial stem length (stem length at *t*₀) and gap. The same analysis was completed for change in number of branches. We used linear mixed-effects models (lmer in the *lme4* package; [46]) so that a random effect for gap could be included. We fitted a full model: light and stem length at *t*₀, their interaction, and the random effect of gap on each, with the form:

\[
\text{Stem growth}_{ij} = (\beta_0 + b_{0j}) + (\beta_1 + b_{1j})\text{Light}_i + (\beta_2 + b_{2j})\text{Length}_i + (\beta_3 + b_{3j})\text{Light}_i \times \text{Length}_i + \epsilon_{ij}
\]

\[
b_{0j} \sim N(0, \sigma^2_{0j})
\]

\[
\epsilon_{ij} \sim N(0, \sigma^2)
\]

where light, length, and their interaction are fixed effects and gap is a random effect. The *β*ₙ are the fixed-effects coefficients, which are identical for each gap, and *b*₀ are the random-effects coefficients, which vary for each gap *j*.

*P*-values were estimated using the Satterthwaite approximation for degrees of freedom in the *lme4* package. Models were fit with REML to give unbiased parameter estimates. Effect sizes were estimated using both marginal and conditional R² [47,48] to differentiate between the proportions of variance explained by fixed effects, and the combination of fixed and random effects, respectively.

**Survival.** Plants were classified as alive (*n* = 162) or dead (*n* = 29) at 25 months. Live plants included those that had died back and resprouted (*n* = 6), and dead plants included two plants that had died back, resprouted, and then died (*n* = 2). We were interested in how survival...
varied with light conditions, and if there were interactions between light and plant size in determining survival, hence survival was analyzed as a function of stem length at $t_0$, light, and gap. We used a generalized linear mixed-effects model (GLMM; `glmer` in the `lme4` package) as follows:

$$\text{Survival}_{ij} = (\beta_0 + b_{ij}) + (\beta_1 + b_{ij}) \text{Light}_i + (\beta_2 + b_{ij}) \text{Length}_i + (\beta_3 + b_{ij}) \text{Light}_i \times \text{Length}_i + \epsilon_{ij}$$

$$b_{ij} \sim N(0, \sigma_{b_{ij}})$$

$$\epsilon_{ij} \sim N(0, \sigma^2)$$

where light, length, and their interaction are fixed effects and gap is a random effect. The $\beta_n$ are the fixed-effects coefficients, which are identical for each gap, and $b_{ij}$ are the random-effects coefficients, which vary for each gap $j$. To accommodate the binomial structure of the survival data (i.e., dead or alive), a logit link function was used.

Predictor data were centered (mean subtracted) and scaled (divided by two times the standard deviation). We fit full model as above, although this model, with random intercepts and slopes for both initial stem length and light, was affected by over-specification. For this reason, we present two models, one with a random intercept and slope for stem length, the other with a random intercept and slope for light. Effect sizes were estimated using marginal and conditional $R^2$ [47,48]. Predictions for mortality as a function of light were made without the gap random effect (i.e., as GLM), to give an overall picture of the relationship between mortality and light, regardless of gap.

Survival (time to event) analysis was performed using `survfit` in the `survival` package to investigate patterns in timing of mortality throughout the experiment. The advantage of survival analysis is that it can handle censored observations, where the event of interest (i.e., mortality) did not occur, and incorporate information from both censored and non-censored observations. Commercial and gardens plant data were grouped together in a single analysis. The data were treated as interval censored, as we did not have exact dates of plant mortality. All analyses were undertaken in R [51].

The individual in this manuscript has given written informed consent (as outlined in PLOS consent form) to publish these case details.

**Results**

**Stem growth**

The commercial plants grew much less than the gardens plants (Fig 2). Growth over the study period (25 months) was a mean of 14 ($\pm$22, 1 standard deviation [SD]) mm for the commercial plants and 160 ($\pm$84) mm for the gardens plants, equivalent to annual growth of 7 mm and 77 mm, respectively.

The full model for growth in stem length of gardens plants explained 39% of the variation in the data, 10% of which was explained by fixed effects (marginal $R^2 = 0.10$, conditional $R^2 = 0.39$; Table 1a). The only significant fixed effect was initial stem length ($t$-value = 2.337, $P = 0.026$; Table 1a), indicating higher growth in larger plants. The effect of light was not significant. The majority of variation was explained by random effects. The most important random effect was the random intercept for initial stem length, with variance of 2.079 (Table 1a), indicating the effect of initial stem length varied among gaps. However, this was much less than the variance of the residuals of 49.075 (Table 1a).

In contrast, the model for stem growth of the commercial plants explained only 16% of the variation in the data (marginal $R^2 = 0.06$, conditional $R^2 = 0.16$; Table 1b). No fixed effects were significant. The most important random effect was the random intercept for stem length,
Fig 2. Stem growth as a function of stem length at $t_0$ and total light. Commercial plant data are black points. Gardens plant data are white points and solid line. Line represents model estimates (fixed effects) for growth as a function of stem length at $t_0$.

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with variance of 21.972 and a standard deviation of 4.687 (Table 1b). This indicates the effect of initial stem length on stem growth varied among gaps.

Branch number

Gardens plants underwent greater change in branch number, with values ranging from -41 to 25, compared with commercially grown plants, which ranged from -16 to 1.

The model of change in branch number for gardens plants explained 67% of the variation in the data (conditional R² = 0.67; Table 2a), with only 23% explained by fixed effects (marginal R² = 0.23; Table 2a). No fixed effects were significant, although light had a P-value of 0.072 (Table 2a). The most important random effect was the random intercept for initial stem length, with variance of 9.712 (Table 2a). However, the residuals had a much larger variance of 62.65 (Table 2a).

The full model for change in branch number in commercial plants explained only 10% of the data (marginal R² = 0.10; Table 2b). None of the fixed effects were significant, and the variance within the random effect residuals (variance = 17.42; Table 2b) was greater than any captured by the random slopes or intercepts.

Survival

The majority of mortality occurred in gardens plants. Ninety-four percent of commercial plants survived, while survival of gardens plants was only 69%. Twenty-nine plants died in total. The native pathogen Botryosphaeria infected 17 of the 19 plants that died in <3% light, and 4 of the 10 plants that died in >3% light. Botryosphaeria species are opportunistic fungi that predominately affect trees that are injured or stressed [52,53]. Botryosphaeria species can
be pathogenic to *W. nobilis* seedlings [39], but can also exist as non-pathogenic endophytes [54].

The full model of survival of the gardens plants, with a random intercept and slope for light, explained 92% of variation in the data (conditional $R^2 = 0.92$; Table 3a). Fixed effects explained 31% of variation (marginal $R^2 = 0.31$; Table 3a) and there was a significant positive correlation with light ($P = 0.037$, Table 3a; Fig 3). In terms of random effects, the random slope for light

| Parameter | Value (95% CI) | SE | Z value | P-value | Variance | SD | Intercept variance | Intercept SD |
|-----------|----------------|----|---------|---------|----------|----|-------------------|-------------|
| **(a) Gardens plants** |
| Intercept | 10.34 (2.30, 18.38) | 4.10 | 54.45 | 2.522 | 0.015* | - | - | - |
| Light | 0.88 (-0.06, 1.82) | 0.48 | 60.00 | 1.829 | 0.072 | <0.001 | <0.001 | <0.001 |
| Stem length at $t_0$ | -0.11 (-0.26, 0.03) | 0.07 | 36.45 | -1.570 | 0.125 | 0.024 | 0.154 | 9.712 | 3.116 |
| Interaction | -0.01 (-0.02, 0.01) | 0.01 | 54.81 | -1.033 | 0.306 | - | - | - |
| Residuals | - | - | - | - | - | 62.65 | 7.915 | - | - |
| $R^2$ | 0.23 (marginal) | - | - | - | - | 0.67 (conditional) |

| **(b) Commercial plants** |
| Intercept | -4.51 (-19.19, 10.17) | 7.49 | 76.00 | -0.602 | 0.549 | - | - | - |
| Light | 0.93 (-0.75, 2.60) | 0.85 | 76.00 | 1.087 | 0.281 | <0.001 | <0.001 | <0.001 |
| Stem length at $t_0$ | -0.03 (-0.35, 0.28) | 0.16 | 76.00 | -0.210 | 0.831 | <0.001 | <0.001 | <0.001 |
| Interaction | -0.02 (-0.06, 0.01) | 0.02 | 76.00 | -1.242 | 0.218 | - | - | - |
| Residuals | - | - | - | - | - | 17.42 | 4.17 | - | - |
| $R^2$ | 0.10 (marginal) | - | - | - | - | 0.10 (conditional) |

* = $P > 0.05$,

** = $P > 0.01$.

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### Table 3. Full models of survival for garden plants.

| Parameter | Fixed effects | Random effects (parameter| gap) |
|-----------|----------------|---------------------------------|
| **(a) Random slope and intercept for light** |
| Intercept | 1.90 (0.63, 3.16) | 0.64 | 2.925 | 0.003** | 0.92 (conditional) |
| Light | 1.34 (-0.51, 3.18) | 0.94 | 1.420 | 0.156 | 1.687 | 1.299 | 2.890 | 1.700 |
| Interaction | -0.79 (-4.04, 2.45) | 1.65 | -0.478 | 0.633 | 1.687 | 1.299 | 2.890 | 1.700 |

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encapsulated more variance (84.433; Table 3a) than did the random intercept for light (5.196; Table 3a). This indicates that the survival response to light varied with gap. The alternative model, with random effects for initial stem length explained only 54% of the data (conditional R² = 0.54; Table 3b). In contrast, for the commercial plants the full model explained only 1% of variation in survival (marginal R² = 0.01, conditional R² = 0.01; Table 4, S2 Fig), indicating that

Table 4. Full model of survival for commercial plants.

| Parameter            | Fixed effects | Random effects (parameter| gap) |
|----------------------|---------------|--------------------------|
|                      | Value         | SE | Z value | Pr Z | Variance | SD | Intercept variance | Intercept SD |
| Intercept            | 2.84 (1.93, 3.75) | 0.47 | 6.104 | <0.0001*** | - | - | - |
| Stem length at t₀    | 0.37 (-1.38, 2.12) | 0.90 | 0.126 | 0.679 | <0.001 | <0.001 | <0.001 | <0.001 |
| Light                | 0.12 (-1.76, 2.00) | 0.96 | 0.414 | 0.900 | <0.001 | <0.001 | <0.001 | <0.001 |
| Interaction          | -0.22 (-4.48, 4.05) | 2.18 | -0.099 | 0.921 | - | - | - |
| R²                   | 0.012 (marginal) | 0.012 (conditional) |

*** = P > 0.001.

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variation in survival of commercial plants was not strongly associated with gap, light or plant size.

Survival analysis (time to event) revealed most of the mortality occurred between the first and second summer, although new mortality occurred at each survey (S1 File).

Discussion

Translocation is a key strategy to reduce extinction risk for *W. nobilis* [28], but the establishment requirements for *W. nobilis* were poorly known. While shade tolerance of *W. nobilis* had been demonstrated in the wild [30], vigorous growth in high light conditions had been shown in greenhouse studies [32]. We characterised the growth and survival of planted *W. nobilis* in a range of light conditions within a new warm temperate rainforest site through experimental translocation. Although it was not our intention, initial plant condition had a strong influence on growth and survival, so our results are considered for each group of plants separately. The commercially grown plants had high survival rates (94%), but also low growth (mean = 14 mm), whereas the plants supplied by the botanic gardens had higher growth (mean = 160 mm) and lower survival (69%).

Survival

Increased light was associated with increased survival in the gardens-supplied *W. nobilis*. Positive relationships between survival and light have been demonstrated in under-planting studies of shade-tolerant species in boreal, temperate and tropical forest types. In Ontario, in east-central Canada, survival of under-planted, shade-tolerant *Picea glauca*, was lowest in uncut (low light) sites, compared to all sites with some level of thinning [55]. Likewise, in the Oregon Coast Range in north-western US, planting seedlings of several conifer species (*Abies grandis, Picea sitchensis Tsuga heterophylla*) underneath un-thinned *Psudeostuga menzeiesii* stands was lethal, with all seedlings dead after two years [19]. Alternatively, survival rates increased in thinned stands with higher light [19]. Further, in guidelines for enrichment planting in mixed dipterocarp forest in Sri Lanka, Ashton [56] suggests the optimal conditions for establishment of the majority of species, shade-tolerant and intolerant, are within gaps.

Moving a plant to a new location (i.e., translocation) is likely to influence growth and survival. In our study, the change in light conditions from nursery to experimental translocation site, was likely an important driver of growth and survival responses. Plants alter their photosynthetic apparatus in response to the irradiance that they experience as new leaves form [57,58]. Most of the leaf area of the gardens-supplied *W. nobilis* was established in greenhouse conditions of 50% full sunlight. As such, we suspect that higher mortality of gardens plants in darker sites was likely due to insufficient acclimatisation to lower light conditions prior to outplanting. Had the gardens plants been nursery grown in deeper shade, or placed in larger canopy gaps, survival may have been greater. Likewise, survival was enhanced at higher light sites. The ability of shade-tolerant species to respond to increased light availability is well known [59]. Indeed, few tree species recruit well in deep shade and nearly all species increase recruitment and growth with increasing light availability (e.g., [60,61]). For this reason we suggest that future growth and survival will be higher for the *W. nobilis* in higher light sites. The relationship between survival and light (in gardens plants) was influenced by gap. We are unsure of what drove this variation, although potential influences include soil variations across the site and, or spatial variation in *Botryosphaeria* infection. Notably neither initial plant size, light nor gap explained variation in mortality of commercially-grown plants.

Most *W. nobilis* mortality was associated with infection with a *Botryosphaeria* species (74%, 24 of 29), and occurred in deeply shaded sites characterised by cool and moist conditions. Such
conditions are favourable for *Botryosphaeria* growth [62–64]. Fungal diseases are a common problem for threatened conifers globally. In the US and Canada, a key threat to the endangered conifer *Pinus albicaulis* is White pine blister rust (*Cronartium ribicola*). *Cronartium ribicola* is found across the entire distribution of *Pinus albicaulis* and can result in mortality of >50% of infected trees, with mortality more likely in older trees [65,66]. *Torreya taxifolia*, also in North America, is listed as critically endangered because of widespread mortality of adult trees (but not juveniles) caused by infection with *Fusarium torreyae* [67,68]. While we had been vigilant about the *Phytophthora cinnamomi* threat to *W. nobilis*, the impact of *Botryosphaeria* was not expected.

Other factors commonly implicated in the survival of translocated plants are herbivory and water availability [69]. There was no evidence of herbivory at the experimental translocation site, and herbivore cages were removed after nine months to allow unimpeded growth. Watering was stopped one month after planting and there was no subsequent observed decline in survival, despite increasing temperatures into summer. The high overall survival of these translocated *W. nobilis* indicates that habitat rarity is unlikely to be the cause of the current limited distribution of *W. nobilis* (sensu [8]), instead disturbance history, or dispersal and establishment limitation (sensu [70]) are likely to be more important.

**Growth**

Growth of translocated *W. nobilis* was not correlated with light, a result that contrasts with greenhouse studies of *W. nobilis* [32]. Increasing growth with increasing light is the most typical result reported in under-planting studies from around the world. In temperate US forests, under-planted conifer seedlings, *Thuja plicata, Abies grandis* and *Tsuga heterophylla*, had higher growth rates where overstorey retention was lowest [20]. There was also a positive relationship between growth of under-planted (and naturally established) *Pinus strobus* seedlings in the understorey and light in the mesic hemlock hardwood forests of US mid-west [71]. In that study light levels measured at seedlings were not strongly related to gap size, and this was attributed to light interception within the understorey [71]. In a tropical lowland rainforest in Borneo, growth of planted non-pioneer dipterocarp seedlings was higher in gaps relative to seedlings planted in the untreated controls [72]. Similarly, in the Amazon, growth of six under-planted timber species was higher in gaps that were larger, and had higher light (both gap size and light were measured, [73]). Based on these studies we predict that future growth rates of *W. nobilis* will be higher in larger gaps. We found that stem growth of gardens plants was significantly correlated with initial plant size—larger plants grew more. The relationship between stem growth and initial plant size was varied with gap, as did the relationship between branch count and initial plant size. Despite early growth of translocated *W. nobilis* was not being correlated with light, mean stem growth of the translocated gardens-grown *W. nobilis* was much greater than stem growth observed in wild seedlings (77 cf. <10 mm/year; [30]).

The commercially grown plants were in poor condition compared with the garden-supplied plants, showing signs of chlorosis and root binding. Chlorosis can be a response to irradiance [74,75] and/or nutrient status (high or low; [76,77]). Root binding occurs when development of lateral- and/or tap-roots is impeded [78,79]. Restriction of rooting space, regardless of water and nutrient availability, can lead to reduced root growth [80] and whole plant growth after planting [81,82]. While the commercial *W. nobilis* had low mortality (i.e., 6% versus 31% in gardens plants), they also grew very little. Variation in the quality of nursery stock can have long-term effects on tree growth and stability [83,84]. In the north-western US, early losses of under-planted conifers (*Thuja plicata*) were attributed to the freezing conditions the seedlings were exposed to before planting [19]. Cole and Newton [85] similarly noted an impact of

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planting stock quality on the survival of under-planted *Pseudotsuga menziesii* and *Tsuga heterophylla*. Other studies have been established specifically to assess nursery conditions and treatments on outcomes (e.g., *Quercus rubra*, [86]). The chlorosis and root binding observed in commercially grown plants at time of planting may have limited plant growth, but also led to resource conservation and therefore higher survival. Notably, of the 37 *W. nobilis* that died back, eight (21%) *W. nobilis* subsequently resprouted (although two of these individuals then died). Interestingly, all except one of these resprouting plants were commercially grown.

Conclusion

Translocation offers the potential to buffer rare and threatened species from extinction by establishing new populations (*sensu* [87,88]). Translocation by under-planting is an approach that utilizes the range of environmental conditions that occur within forest understories, which may be particularly useful in establishing new populations of shade-tolerant plant species.

Management recommendations

To maximise survival of transplants, future translocations of *W. nobilis* should be undertaken within sites with light availabilities at the higher end of the range we tested (>20%), and further work should consider the suitability of even higher light levels. Practitioners may also consider using larger plants to maximize growth.

In this study we used cutting-grown plants because no seed-grown plants were available. Future translocations of *W. nobilis* should include seedlings when they become available, to account for the possibility that previously undetected genetic variation may emerge from seeds.

A companion study describing the microbial responses associated with Wollemi pine translocation is described by Rigg et al. [89].

Supporting Information

S1 Fig. Photographs of translocated *Wollemia nobilis* and experimental translocation site. (a) Translocation site showing *Wollemia nobilis* and warm temperate rainforest (Gap 11) (b and c) Translocated *Wollemia nobilis* new growth. (TIF)

S2 Fig. Survival of commercially grown *Wollemia nobilis*, according to height and light. (DOCX)

S1 File. Survival analysis. Fig A. Survival of translocated *W. nobilis* through time. Table A. Numerical results of survival analysis showing survival of translocated *W. nobilis* through time. (DOCX)

S1 Table. Simplified ranking table *sensu* Maschinski and Haskins 2012. (DOCX)

S2 Table. Comparison of soil characteristics at the translocation and wild sites. (DOCX)

S3 Table. Gap characteristics. Light conditions and relative elevation of the gaps where *Wollemia nobilis* were planted, and summary survival and growth of *W. nobilis* according to supplier and gap. (DOCX)
S4 Table. Parameter estimates for the glm that was used to generate predictions for *Wollemia nobilis* survival according to stem length and light (Fig 3).

DOCX

S5 Table. Wollemi pine translocation data. Includes plant origin, planting site light, survival and growth data.

XLSX

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**Author Contributions**

Conceived and designed the experiments: HZ CO TA PB. Performed the experiments: HZ CO TA PB. Analyzed the data: HZ PB. Contributed reagents/materials/analysis tools: CO TA. Wrote the paper: HZ CO TA PB.

**References**

1. Mace GM, Collar NJ, Gaston KJ, Hilton-Taylor C, Akcakaya HR, Leader-Williams N, et al. (2008) Quantification of extinction risk: IUCN's system for classifying threatened species. Conservation Biology 22: 1424–1442. doi:10.1111/j.1523-1739.2008.01044.x PMID: 18847444

2. World Conservation Union I (1987) IUCN position statement on the translocation of living organisms: introductions, re-introductions, and re-stocking. IUCN, Gland, Switzerland. Available: http://intranet.iucn.org/webfiles/doc/SSC/SSCwebsite/Policy_statements/IUCN_Position_Statement_on_Translocation_of_Living_Organisms.pdf.

3. Webber BL, Scott JK, Didham RK (2011) Translocation or bust! A new acclimatization agenda for the 21st century. Trends in Ecology & Evolution 26: 493–494.

4. Rout TM, Hauser CE, Possingham HP (2007) Minimise long-term loss or maximise short-term gain? Ecological Modelling 201: 67–74.

5. Müller H, Eriksson O (2013) A pragmatic and utilitarian view of species translocation as a tool in conservation biology. Biodiversity and Conservation 22: 1837–1841.

6. Vallee L, Hogbin T, Monks L, Makinson B, Matthes M, Rosetto M (2004) Guidelines for the translocation of threatened plants in Australia. Australian Network for Plant Conservation, Canberra, Australia.

7. Maschinski J, Haskins KE (2012) Plant Reintroduction in a Changing Climate. Island Press. 432 pp.

8. Maschinski J, Falk DS, Wright SJ, Possley J, Roncal J, Wendelberger KS (2012) Optimal locations for plant reintroductions in a changing world. In: Maschinski J, Haskins KE, editors. Plant reintroduction in a changing climate. Island Press. pp. 109–129.

9. Whitmore T (1989) Canopy gaps and the two major groups of forest trees. Ecology 70: 536–538.

10. Hubbell SP, Forster RB, O'Brien ST, Harms KE, Condit R, Wechsler B, et al. (1999) Light-gap disturbances, recruitment limitation, and tree diversity in a neotropical forest. Science 283: 554–557. PMID: 9915706

11. Parrott JA, Turnbull JW, Jones N (1997) Catalysing natural forest restoration on degraded tropical landscapes. Forest Ecology and Management 99: 1–7.
12. Paquette A, Bouchard A, Cogliastro A (2006) Survival and growth of under-planted trees: a meta-analysis across four biomes. Ecological Applications 16: 1575–1589. PMID: 16937819

13. Ashton P, Gamage S, Gunatilleke I, Gunatilleke C (1997) Restoration of a Sri Lankan rainforest: using Caribbean pine Pinus caribaea as a nurse for establishing late-successional tree species. Journal of Applied Ecology 34: 915–925.

14. Kobe RK (1999) Light gradient partitioning among tropical tree species through differential seedling mortality and growth. Ecology 80: 187–201.

15. Johnson PS (1984) Responses of planted northern red oak to three overstory treatments. Canadian Journal of Forest Research 14: 536–542.

16. Dey DC, Gardner ES, Schweitzer CJ, Kabrick JM, Jacobs DF (2012) Underplanting to sustain future stocking of oak (Quercus) in temperate deciduous forests. New Forests 43: 955–978.

17. Stewart JD, Landhäusser SM, Stadt KJ, Lieffers VJ (2000) Regeneration of White Spruce under Aspen canopies: seeding, planting and site preparation. Western Journal of Applied Forestry 15: 177–182.

18. Erefur C, Bergsten U, Lundmark T, de Chantal M (2010) Establishment of planted Norway spruce and Scots pine seedlings: effects of light environment, fertilisation, and orientation and distance with respect to shelter trees. New Forests 41: 263–276.

19. Maas-Hebner KG, Emmingham WH, Larson DJ, Chan SS (2005) Establishment and growth of native hardwood and conifer seedlings underplanted in thinned Douglas-fir stands. Forest Ecology and Management 208: 331–345.

20. Brandeis TJ, Newton M, Cole EC (2001) Underplanted conifer seedling survival and growth in thinned Douglas-fir stands. Canadian Journal of Forest Research 31: 302–312.

21. Khurana E, Singh JS (2001) Ecology of seed and seedling growth for conservation and restoration of tropical dry forest: a review. Environmental Conservation 28: 39–52.

22. Navarro-Carrillo RM, Griffith DM, Ramirez-Soria MJ, Pariona W, Goliche R, Palacios G (2011) Enrichment of big-leaf mahogany (Swietenia macrophylla King) in logging gaps in Bolivia: The effects of planting method and silvicultural treatments on long-term seedling survival and growth. Forest Ecology and Management 262: 2271–2280.

23. Drayton B, Primack RB (2012) Success rates for reintroductions of eight perennial plant species after 15 years. Restoration Ecology 20: 299–303.

24. Murcia C, Aronson J (2014) Intelligent tinkering in ecological restoration. Restoration Ecology 22: 279–283.

25. Young TP, Petersen DA, Clary JJ (2005) The ecology of restoration: historical links, emerging issues and unexplored realms. Ecology Letters 8: 662–673.

26. Jones W, Hill K, Allen J (1995) Wollemia nobilis, a new living Australian genus and species in the Araucariaceae. Telopea 6: 173–176.

27. Benson J, Allen C (2007) Vegetation associated with Wollemia nobilis (Araucariaceae). Cunninghamia 10: 255–262.

28. New South Wales Government DOC (2006) Wollemi Pine Recovery Plan: 1–37. Available: http://www.environment.gov.au/system/files/resources/245df899-5818-44c1-a1f0-76fb70bb9fc6/files/wollemia-nobilis.pdf.

29. Peakall R, Ebert D, Scott LJ, Meagher PF, Offord CA (2003) Comparative genetic study confirms exceptionally low genetic variation in the ancient and endangered relictual conifer, Wollemia nobilis (Araucariaceae). Molecular Ecology 12: 2331–2343. PMID: 12919472

30. Zimmer HC, Auld TD, Benson J, Baker PJ (2014) Recruitment bottlenecks in the rare Australian conifer Wollemia nobilis. Biodiversity and Conservation 23: 203–215.

31. Offord C, Porter C, Meagher P, Errington G (1999) Sexual reproduction and early plant growth of the Wollemi pine (Wollemia nobilis), a rare and threatened Australian conifer. Annals of Botany 84: 1–9.

32. Offord CA, Meagher PF, Zimmer H (2014) Growing up or growing out? How soil pH and light affect seedling growth of a relictual rainforest tree. Annals of Botany: Plants 158: 263–276.

33. Seddon PJ (2010) From reintroduction to assisted colonization: moving along the conservation translocation spectrum. Restoration Ecology 18: 796–802.

34. Jørgensen D (2011) What’s history got to do with it? A response to Seddon’s definition of reintroduction. Restoration Ecology 19: 705–708.

35. Dalrymple SE, Moehrensclager A (2013) “Words matter.” A response to Jørgensen’s treatment of historic range and definitions of reintroduction. Restoration Ecology 21: 156–158.

36. Chambers TC, Drinnan AN, McLoughlin S (1998) Some morphological features of Wollemi Pine (Wollemia nobilis: Araucariaceae) and their comparison to Cretaceous plant fossils. International Journal of Plant Sciences 159: 160–171.
37. Macphail M, Hill K, Partridge A, Truswell E, Foster C (1995) Wollemi Pine—Old pollen records for a newly discovered genus of gymnosperm. Geology Today 11: 48–50.

38. Hardham AR (2005) Phytophthora cinnamomi. Molecular Plant Pathology 6: 589–604. doi: 10.1111/j.1364-3730.2005.00308.x PMID: 20565682

39. Bullock S, Summerell B, Gunn L (2000) Pathogens of the Wollemi pine, Wollemia nobilis. Australasian Plant Pathology 29: 211–214.

40. Frazer GW, Canham CD, Lertzman KP (1999) Gap Light Analyzer (GLA). Available: http://www.caryinstitute.org/science-program/our-scientists/dr-charles-d-canham/gap-light-analyzer-gla.

41. Nobis M, Hunziker U (2005) Automatic thresholding for hemispherical canopy-photographs based on edge detection. Agricultural and Forest Meteorology 128: 243–250.

42. Crutsinger GM, Collins MD, Fordyce JA, Gompert Z, Nice CC, Sanders NJ (2006) Plant genotypic diversity predicts community structure and governs an ecosystem process. Science 313: 966–968. PMID: 16917062

43. Reusch TBH, Ehtiars A, Hämmerli A, Worm B (2005) Ecosystem recovery after climatic extremes enhanced by genotypic diversity. Proceedings of the National Academy of Sciences 102: 2826–2831.

44. Hill KD (1997) Architecture of the Wollemi Pine (Wollemia nobilis, Araucariaceae), a unique combination of model and reiteration. Australian Journal of Botany 45: 817–826.

45. Burrows GE, Meagher PF, Heady RD (2007) An anatomical assessment of branch abscission and branch-base hydraulic architecture in the endangered Wollemia nobilis. Annals of Botany 99: 609–623. PMID: 17272303

46. Bates D, Maechler M, Bolker B, Walker S, editors (2014) lme4: Linear mixed-effects models using Eigen and S4. Available: online http://CRAN.R-project.org/package=lme4. Accessed 6 April 2015.

47. Lefcheck J, editor (n.d.) R-squared for generalized linear mixed-effects models. Available: https://github.com/jslefche/rsquared.glmer. Accessed 6 April 2014.

48. Nakagawa S, Schielzeth H (2012) A general and simple method for obtaining R$^2$ from generalized linear mixed-effects models. Methods in Ecology and Evolution 4: 133–142.

49. Gelman A (2008) Scaling regression inputs by dividing by two standard deviations. Statistics in Medicine 27: 2865–2873. PMID: 17960576

50. Therneau T, editor (n.d.) A package for survival analysis in R. Package version 2.37–4. Available: http://CRAN.R-project.org/package=Surv. Accessed 7 April 2013.

51. R: A language and environment for statistical computing. (n.d.) R: A language and environment for statistical computing. Available: http://www.R-project.org/.

52. Dreistadt SH (2008) Pests of landscape trees and shrubs: An integrated pest management guide. University of California Agriculture and Natural Resources, Davis, California.

53. Sinclair W, Lyon H (2005) Diseases of trees and shrubs. Comstock Publishing Associates, New York.

54. Slippers B, Summerel BA, Crous PW, Coutinho TA, Wingfield BD, Wingfield MJ (2005) Preliminary studies on Botryosphaeria species from Southern Hemisphere conifers in Australasia and South Africa. Australasian Plant Pathology 34: 213–220.

55. Man R, Rice JA, MacDonald GB (2009) Long-term response of planted conifers, natural regeneration, and vegetation to harvesting, scalping, and weeding on a boreal mixedwood site. Forest Ecology and Management 258: 1225–1234.

56. Ashton MS, Gunatilleke C, Singhakumara B, Gunatilleke I (2001) Restoration pathways for rain forest in southwest Sri Lanka: a review of concepts and models. Forest Ecology and Management 154: 409–430.

57. Coopman RE, Reyes-Díaz M, Briceño VF, Corcuera LJ, Cabrera HM, Bravo LA (2008) Changes during early development in photosynthetic light acclimation capacity explain the shade to sun transition in Nothofagus nitida. Tree Physiology 28: 1561–1571. PMID: 18708338

58. Krause GH, Koroleva OY, Daling JW, Winter K (2001) Acclimation of tropical tree seedlings to excessive light in simulated tree-fall gaps. Plant, Cell & Environment 24: 1345–1352.

59. Canham CD (1988) Growth and canopy architecture of shade-tolerant trees: response to canopy gaps. Ecology 69: 786–795.

60. Delcamp M, Gourlet-Fleury S, Flores O, Garnier E (2009) Can functional classification of tropical trees predict population dynamics after disturbance? Journal of Vegetation Science 19: 209–220.

61. Rüger N, Huth A, Hubbell SP, Condit R (2009) Response of recruitment to light availability across a tropical lowland rain forest community. Journal of Ecology 97: 1360–1368.
Arauz LF, Sutton TB (1989) Temperature and wetness duration requirements for apple infection by Botryosphaeria obtusa. Phytopathology 79: 440–444.

Michailides TJ, Morgan DP (1992) Effects of temperature and wetness duration on infection of pistachio by Botryosphaeria dothidea and management of disease by reducing duration of irrigation. Phytopathology 82: 1399–1406.

Smith JP, Hoffmann JT (2000) Status of the White Pine Blister Rust in the Intermountain West. Western North American Naturalist 60: 165–179.

Smith CM, Shepherd B, Gillies C, Stuart-Smith J (2013) Changes in blister rust infection and mortality in whitebark pine over time. Canadian Journal of Forest Research 43: 90–96.

Smith JA, O’Donnell K, Mount LL, Shin K, Peacock K, Spector T, et al. (2011) A novel Fusarium species causes a canker disease of the critically endangered conifer, Torreya taxifolia. Plant Disease 95: 633–670.

Guerrant EO Jr., Kaye TN (2007) Reintroduction of rare and endangered plants: common factors, questions and approaches. Australian Journal of Botany 55: 362–370.

Jackson ST, Overpeck JT (2000) Responses of plant populations and communities to environmental changes of the late Quaternary. Paleobiology 26: 194–220.

Fahey RT, Lorimer CG (2013) Restoring a midtolerant pine species as a component of late-successional forests: Results of gap-based planting trials. Forest Ecology and Management 292: 139–149.

Romell E, Hallsby G, Karlsson A, Garcia C (2008) Artificial canopy gaps in a Macaranga spp. dominated secondary tropical rain forest—Effects on survival and above ground increment of four underplanted dipterocarp species. Forest Ecology and Management 255: 1452–1460.

Schulze M (2008) Technical and financial analysis of enrichment planting in logging gaps as a potential component of forest management in the eastern Amazon. Forest Ecology and Management 255: 866–879.

Mano J (2000) Early events in environmental stresses in plants. In: Inze D, van Montagu M, editors. Oxidative stress in plants. CRC Press, Boca Raton, Florida. pp. 217–245.

Muller P, Li X-P, Niyogi KK (2001) Non-photochemical quenching. A response to excess light energy. Plant Physiology 125: 1558–1566. PMID: 11299337

Mengel K (1995) Iron availability in plant tissues—Iron chlorosis on calcareous soils. Plant and Soil 165: 275–283.

Barya A, Carrier P, Franck F, Coulomb C, Sahut C, Havaux M (2001) Leaf chlorosis in oilseed rape plants (Brassica napus) grown on cadmium-polluted soil: causes and consequences for photosynthesis and growth. Planta 212: 696–709. PMID: 11346943

Ruehle JL (1985) The effect of cupric carbonate on root morphology of containerized mycorrhizal pine seedlings. Canadian Journal of Forest Research 15: 586–592.

Dumroese RK, Sung S-JS, Pinto JR, Ross-Davis A, Scott DA (2013) Morphology, gas exchange, and chlorophyll content of longleaf pine seedlings in response to rooting volume, copper root pruning, and nitrogen supply in a container nursery. New Forests 44: 881–897.

South DB, Harris SW, Barnett JP, Hainds MJ, Gjerstad DH (2005) Effect of container type and seedling size on survival and early height growth of Pinus palustris seedlings in Alabama, U.S.A. Forest Ecology and Management 204: 385–398.

Davis AS, Jacobs DF (2005) Quantifying root system quality of nursery seedlings and relationship to outplanting performance. New Forests 30: 295–311.

McConnaughay K, Bazzaz FA (1991) Is physical space a soil resource? Ecology 72: 94–103.

Halter MR, Chanway CP, Harper GJ (1993) Growth reduction and root deformation of containerized lodgepole pine saplings 11 years after planting. Forest Ecology and Management 56: 131–146.

Lindström A, Rune G (1999) Root deformation in plantations of container-grown Scots pine trees: effects on root growth, tree stability and stem straightness. Plant and Soil 217: 29–37.

Cole E, Newton M (2009) Tenth-year survival and size of underplanted seedlings in the Oregon Coast Range. Canadian Journal of Forest Research 39: 580–595.
86. Zaczek JJ, Steiner KC, Bowersox TW (1996) Northern red oak planting stock: 6-year results. New Forests 13: 175–189.

87. Maunder M (1992) Plant reintroduction: an overview. Biodiversity and Conservation 1: 51–61.

88. Maschinski J, Duquesnel J (2007) Successful reintroductions of the endangered long-lived Sargent’s cherry palm, *Pseudophoenix sargentii*, in the Florida Keys. Biological Conservation 134: 122–129.

89. Rigg JL, Offord CA, Zimmer H, Anderson IC, Singh BK, Powell JR. Conservation by translocation: establishment of Wollemi pine and associated microbial communities in novel environments. Plant and Soil. 2016; in press.
