

Article

# Effects of Light Intensity and Girdling Treatments on the Production of Female Cones in Japanese Larch (*Larix kaempferi* (Lamb.) Carr.): Implications for the Management of Seed Orchards

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**Abstract:** To ensure sustainable forestry, it is important to establish an efficient management procedure for improving the seed production capacity of seed orchards. In this study, we evaluated the effects of girdling and increasing light intensity on female cone production in an old *L. kaempferi* (Lamb.) Carr. seed orchard. We also evaluated whether there is a genotype-specific reproductive response to these factors among clones. The results showed that female cone production was augmented by girdling and increasing light intensity. There was a difference in the effectiveness of girdling treatment levels, and the probability of producing female cones increased markedly at higher girdling levels. At light intensities where the relative photosynthetic photon flux density was higher than 50%, more than half of the trees tended to produce female cones, even in intact (ungirdled) trees, and the genotype-specific response to light intensity was more apparent in less-reproductive clones. These findings suggested that girdling less-reproductive trees combined with increasing light intensity was an effective management strategy for improving cone production in old seed orchards.

**Keywords:** breeding; genotype × environment interaction; mast seeding; seed production; thinning

## 1. Introduction

Japanese larch (*Larix kaempferi*) is a major plantation species in central and northern Japan [1]. The species has been introduced widely in China, Europe and North America, because of its rapid growth and sparse branching characteristics. *Larix kaempferi* often shows superior growth compared to other larch species (e.g., *L. decidua* Mill. and *L. laricina* (Du Roi) K. Koch) and has therefore been widely used in breeding programs involving hybrid breeding [2,3]. As a result of these efforts, hybrids between *L. kaempferi* and other larches have been used commercially in North America [4], Europe [3] and Japan [5,6].

In Japan, a breeding program for *L. kaempferi* was initiated in the 1950s. As part of the program, more than 500 first-generation plus trees were selected and used to establish clonal seed orchards [1]. The selection of second-generation plus trees started in the 2010s, and this is ongoing [1,7]. Compared to traditional seed sources, the superior growth of seedlings derived from the clonal seed orchards of the plus trees has been demonstrated [1], and significant variations in growth and wood property traits among families were reported [7,8]. Data analyses based on several provenance tests clarified that genotype × environment (G × E) interactions in several of these growth traits were not small [9].

However, genotype-specific responses in the reproductive traits (e.g., cone production) to environmental conditions have not yet been clarified in detail for *L. kaempferi*.

Reforestation using *L. kaempferi* has increased over the last decade in Japan, and *L. kaempferi* is now the second most important forestry species for plantation in Japan and occupies about 25% of newly planted forest areas [10]. Owing to its high juvenile growth performance, the demand for improved seeds and seedlings of *L. kaempferi* has been increasing [11]. However, the clonal seed orchards of the second-generation plus trees are currently too young to produce enough seeds. On the other hand, the clonal seed orchards of the first-generation plus trees, which were established mainly in the 1960s and 1970s, are now more than 50 years old. Due to the high density of old stems and branches, these old seed orchards are too tall to be managed efficiently, and too dark to produce sufficient quantities of good-quality seeds. In many conifers, mast seeding is a limiting factor for sustainable seed production [12,13]. To overcome this limitation, numerous efforts have been made to enhance seed production, e.g., improving light intensity [14–17], girdling [18–23], fertilizer [19] and drought [24,25]. To establish an efficient management system for the old seed orchards of Japanese larch, it is necessary to quantitatively evaluate such treatment effects on seed production.

Clear reproductive responses to changes in light intensity have been reported in forest plants [26–28]. Previous studies that examined the effect of increasing light intensity found that thinning operations often offer increased flowering [14,29,30]. Trees located at well-lit sites tended to have more flowers, and the quality and quantity of their seeds were positively correlated with magnitudes of flowering [31]. Previous studies on larch orchards have also reported that increasing light intensity improved cone production [14–17]. Uchiyama et al. [14] quantified a relationship between light levels and female cone production in an old orchard of *L. gmelinii* var. *japonica*. However, quantitative estimation of the effect of light intensity is still limited for old seed orchards of *L. kaempferi*.

In horticulture, girdling is commonly used to promote flower production [32] and improve fruit quality [33] and yield [32,34]. Girdling interrupts phloem transport by removing a part of the bark and cambium without affecting xylem transport [35]. When the main stem (trunk) of a tree is girdled, the translocation of assimilates to the below-ground parts of the tree is interrupted, resulting in a super-abundance of assimilates in the above-ground parts above the girdle [36,37]. Girdling has therefore been used to control the resource allocation, and to promote cone production [18–23]. It is, however, rarely quantified how different girdling levels (severity) affect the cone production of *L. kaempferi*.

To ensure the sustainability of forestries, establishing an efficient management procedure for improving the seed production in seed orchards is necessary. In this context, studies on the effects of light intensity and girdling manipulation on the reproductive performance of mast seeding conifer species, such as *Larix* and *Picea*, have attracted the interest of silviculturists and forest managers. In this study, we quantitatively evaluated the effects of girdling manipulation and increasing light intensity on female cone production in an old *L. kaempferi* seed orchard. We also evaluated genotype-specific responses in reproduction among *L. kaempferi* clones after making changes in light intensity. Finally, we examined the relationship between female cone production and tree sizes.

## 2. Materials and Methods

### 2.1. Study Site

The study site was the Fujisan Seed Orchard (35.42° N, 138.74° E; 1320–1350 m a.s.l.; total area 10 ha), managed by Yamanashi prefecture. The orchard, located on the northeastern slope of Mount Fuji, is divided into several plots and the largest plot (#9; 2.4 ha) was used for this study. The orchard was established between 1961 and 1962 and contains 526 stems (stem density: 223.8 stems/ha in 2017) comprising 44 first-generation *L. kaempferi* clones that were selected mainly from forests in Yamanashi and Nagano prefectures. The mean annual temperature and precipitation were 10.6 °C and 1568 mm,

respectively. The soil of the area is comprised primarily of weathered volcanic sediments derived from Mount Fuji.

## 2.2. Girdling Treatments

We examined whether girdling could be used to enhance the reproductive performance of *L. kaempferi*. Girdling is a procedure that involves removing a 2 cm-wide semilunar ring of bark and cambium at a height of approximately 80–100 cm on the main stem (trunk) using a knife (Figure A1). We clarified the effects of the following four types of girdling treatments: one semilunar ring of bark and cambium was removed (referred as to level 1); two semilunar rings of bark and cambium were removed, with each ring oriented in opposite directions (level 2); three semilunar rings of bark and cambium with the same orientation were removed (level 3); and no girdling was performed (level 0; i.e., intact). We randomly assigned 8, 14 and 12 trees to levels 1, 2 and 3, respectively, and all of the remaining trees were assigned to level 0. Girdling was conducted in May 2016. As two of 12 trees from the level 3 treatment group were dead in autumn, these trees were removed from the analysis.

## 2.3. Light Intensity

The photosynthetic photon flux density (PPFD) was used as an indicator of light intensity in this study. Measurements were conducted on a cloudy day in July using LI-250 light meters (LI-COR, Lincoln, Dearborn, MI, USA). The measurements were performed four times (in four directions) around the crown of each tree at the approximate midpoint of the height of the crown (5–6 m). The mean relative PPFD (rPPFD) was calculated for each planting position, as follows:  $rPPFD = \text{PPFD above the crown of each tree} / \text{PPFD above forest canopy (i.e., open sky)}$ .

## 2.4. Tree size and Reproductive Status

We scored the reproductive status of each tree based on the extent of female cone production, as follows: trees that did not produce female cones (hereafter referred to as index 1), trees with female cones that were very sparsely distributed within the crown (index 2), trees with female cones that were sparsely distributed within the crown, or trees produced numerous female cones on a few branches only (index 3) and trees producing cones abundantly on several branches (index 4).

The number of female cones produced per stem was counted manually, and all counts were performed in triplicate. To investigate the relationship between reproductive status and tree size, all living tree stems (trunks) within the orchard were mapped and their diameter at breast height (DBH), tree height and crown radius was measured.

## 2.5. Data Analysis

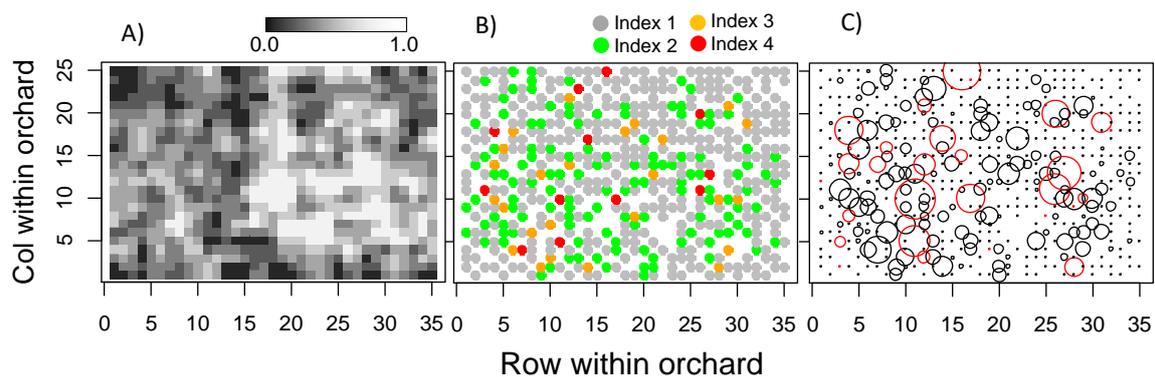
To analyze the reproductive performance of *L. kaempferi*, we used generalized linear mixed-effect models [38], by using R 3.2.5 [39]. Based on the reproductive score data, the effects of light intensity and girdling on the probability of female cone production were analyzed using ordered logit and binomial logit functions. The fixed-effect explanatory variables were rPPFD and girdling treatment; the rPPFD was used as a covariate, while the girdling was treated as a main factor. We treated “block within site” as a random-effect factor, to account for spatial pseudo-replication. To test whether the genotype-specific reproductive response to light intensity varied among clones, we included “clone” and the interaction with rPPFD (i.e., “clone:rPPFD”) as random effects.

The relationship between female cone production and tree size traits was analyzed by ANCOVA-like linear mixed models. The DBH, height, crown radius and height/crown radius ratio for tree stems were treated as fixed-effect covariates. We also included “clone” and the interaction terms with traits (e.g., “clone:DBH”) as random effects. In this study, each trait was analyzed separately because of the multicollinearity among size traits.

### 3. Results

#### 3.1. Status of the *L. kaempferi* Orchard

There was a marked variation in the light environment in the study orchard, and the mean rPPFD was  $50.1 \pm 15.2\%$  (Figure 1A). A total of 526 stems belonging to 44 clones were investigated. Of these stems, 31.3% (164/526) produced female cones (Figure 1B). Of the 164 trees that reproduced, reproductive scores of 76.8% (126) and 15.2% (25) were obtained for indices 2 and 3, respectively, while only 7.9% (13) was obtained for index 4. In total, 18,114 cones were produced in the orchard, with the mean and maximum numbers of cones per stem being 34.4 and 5960, respectively (Figure 1C). The mean ( $\pm$ SD) DBH, height and crown radius were  $38.6 \pm 6.2$  cm,  $10.3 \pm 2.2$  m and  $4.3 \pm 0.9$  m, respectively.



**Figure 1.** Within the orchard, spatial variation in (A) relative photosynthetic photon flux density. (B) Fruiting index of each tree. Index 1: trees that did not produce female cones, index 2: trees with female cones that were very sparsely distributed within the crown, index 3: trees with female cones that were sparsely distributed within the crown, or trees produced numerous female cones on a few branches only and index 4: trees that produced female cones abundantly on several branches. (C) Number of female cones produced per tree. Red circles indicate girdled trees and black circles indicate intact trees.

#### 3.2. Relationship between Female Cone Production and Light Intensity or Girdling Treatments

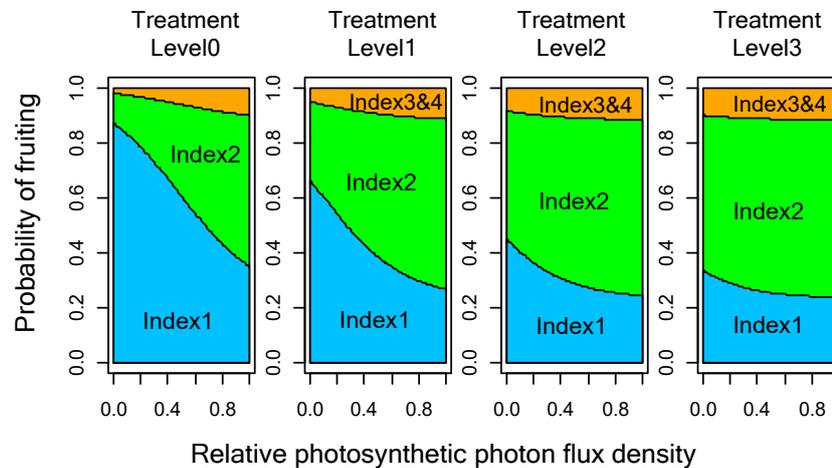
The probability of female cone production varied markedly in response to light intensity (rPPFD) across different girdling treatments (Figure 2, Table 1). In girdling level 0 (i.e., intact stems), the probability of producing female cones (green: index 2 and orange: indices 3 and 4) increased markedly with increasing light intensity. In the situation where rPPFD was greater than approximately 0.5 (i.e., 50% sunlight), the probability of producing female cones (green and orange) was larger than that of not producing cones (blue: index 1). Two out of 12 trees in girdling level 3 died, but none of the trees in girdling levels 0–2 died.

**Table 1.** Summary of generalized linear mixed-effect model for estimating the probability of reproduction in Japanese larch. Relative photosynthetic photon flux density (rPPFD) was used as a fixed-effect covariate, while the effect of girdling treatment was used as a fixed-effect main factor.

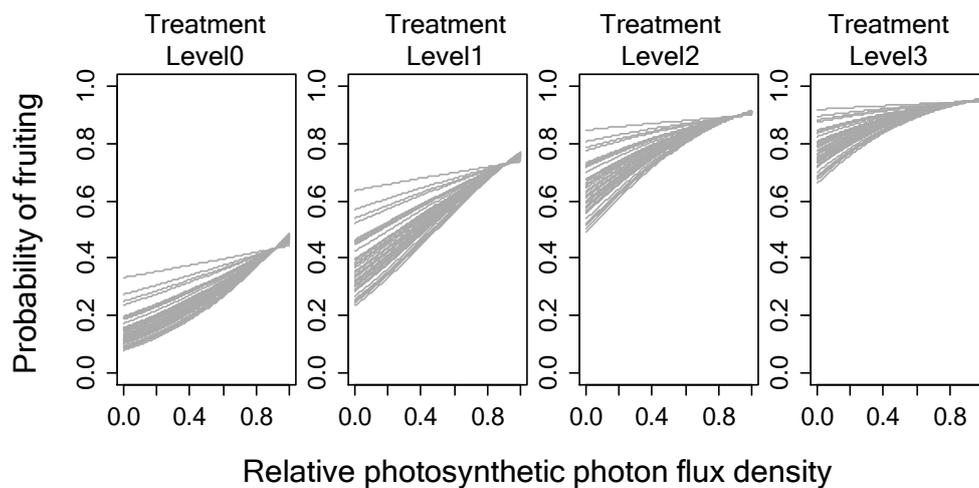
	Coef.	S.E.	Z-Value	p-Value
Intercept	−1.84	0.45	−4.05	<0.001
Girdling level 1	1.25	0.78	1.61	0.109
Girdling level 2	2.39	0.71	3.39	<0.001
Girdling level 3	3.09	1.12	2.77	0.006
rPPFD	1.73	0.79	2.20	0.028

In darker environments, such as where rPPFD = 0.2, fewer than 20% of trees in the control group (i.e., intact stems) produced female cones (Figure 2). On the other hand, in the girdling level 3 group, more than 70% of trees produced female cones, even at similar light intensities.

Figure 3 shows the genotype-specific reproductive response to light conditions. Gray lines indicate response curves estimated for each of the 44 clones. The among-clone variation in the probability of female cone production was more apparent in darker areas, and less apparent under more brightly lit conditions ( $rPPFD > 0.8$ ).



**Figure 2.** Relationship between fruiting probability of trees and light intensity (relative photosynthetic photon flux density) for different girdling treatment levels. Blue: fruiting index 1, trees not producing female cones. Green: fruiting index 2, trees producing female cones sparsely within their crown. Orange: fruiting index 3, trees producing abundant female cones within their crown.



**Figure 3.** Genotype-specific relationship between fruiting probability and light intensity (relative photosynthetic photon flux density) across different girdling treatment levels. Each gray line indicates each *L. kaempferi* genotype (clone).

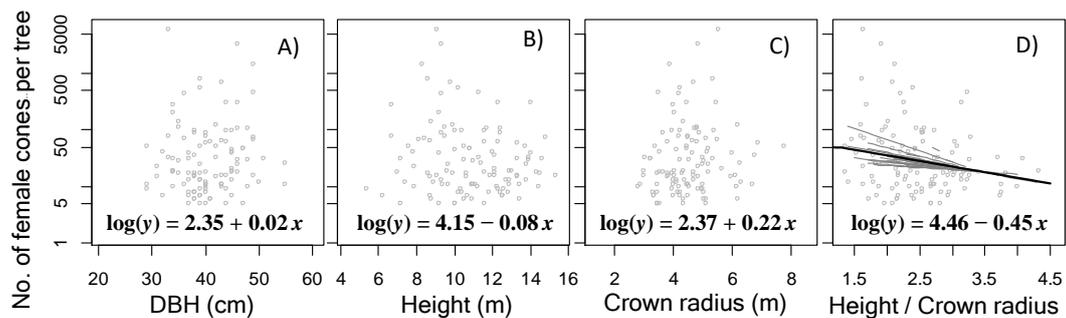
Based on the coefficient of variances (Table 2), among-clone variances in the probability of producing cones were much evident under conditions of lower light intensities and girdling levels, while the variation among clones decreased with increasing light intensities.

### 3.3. Relationship between Female Cone Production and Tree Size

When examining the relationships between female cone production and traits of the trees (size and shape), a significant negative relationship was observed between female cone production and the height/crown radius ratio ( $p < 0.05$ ; bold black line in Figure 4D), and smaller trees with a relatively wider crown radius tended to produce more female cones. No significant relationships were observed between female cone production and the other traits (Figure 4A–C).

**Table 2.** The coefficient of variance (CV) for among-clone differences in the probability of producing female cones.

	rPPFD: 0.2	rPPFD: 0.5	rPPFD: 0.8
Girdling level 0	26.4%	13.1%	2.9%
Girdling level 1	16.8%	7.4%	1.4%
Girdling level 2	8.3%	3.3%	0.6%
Girdling level 3	4.8%	1.8%	0.3%

**Figure 4.** Relationship between number of female cones per tree and (A) tree diameter, (B) tree height, (C) crown radius and (D) height/crown radius ratio. In panel D, the thick black line indicates a significant regression relationship across all genotypes ( $p < 0.05$ ), while gray lines indicate relationships for each genotype. There were no significant relationships between female cone production and the other traits (A–C).

#### 4. Discussion

In northern conifer species including Japanese larch, mast seeding, i.e., low frequent abundant fruiting events, is a limiting factor for sustainable seed production, and numerous efforts have been made to overcome this limitation [12,13]. Several trials for increasing cone production have been conducted on larch species, such as *L. kaempferi*, *L. decidua* and *L. laricina*, and some success has been reported for treatments involving girdling [18–23], gibberellins [40], nitrogen fertilizer [19], drought [24] and branch bending [41]. However, the results obtained from yet other studies using similar treatments have often been inconsistent, such as ineffective stimulation by gibberellins [42–44], nitrogen fertilizer [15] and root pruning [24]. These disparities could be attributed to differences in the age and growing conditions (natural stands, seed orchards or pots in greenhouses, etc.). In old seed orchards containing trees that are not well managed, restoring the reproductive capacity could be considered to be relatively difficult. However, the findings of our study showed that female cone production of old *L. kaempferi* trees can be enhanced by girdling and increasing the light intensity.

As in previous studies [18–23], our study confirmed that girdling was effective for improving female cone production, even in the old *L. kaempferi* orchard. In an orchard of 42-year-old *L. kaempferi*, more than 90% (19/20) of the girdled trees totally produced about 8000 cones, while only 25% (5/20) of the intact trees yielded 42 cones [22]. Similarly, female cone production in girdled trees increased more than ten times in natural stands of 70- [45] and 90-year-old [19] western larch, compared to intact trees. However, when girdling was conducted on young trees (17 years old), the effects of girdling on the proportion of trees which produced cones and the cone production per tree were less apparent [40], suggesting that age-dependent sexual maturity may affect the efficiency of girdling. Although the efficiency of girdling at different ages has not yet been clarified, our study found that girdling is a low-cost method that can be used to efficiently restore the cone production capacity of old *L. kaempferi* seed orchards.

Girdling has been regarded as a cost-efficient and useful technique for disrupting phloem transport while limiting detrimental effects [46]. However, it has also been reported that the positive effects of girdling are relatively limited in duration, and repeated girdling might adversely affect tree vigor and

decrease total reproductive output [47]. In our study, there was considerable variation in the magnitude of the girdling effect among the different girdling treatment levels (see the coefficients in Table 1: larger coefficient values indicate a larger positive effect); the probability of producing cones was significantly increased as the treatment level was higher (Figure 2). However, 2 out of 12 girdled trees in the level 3 group died, while none of the girdled trees in the level 1 and 2 groups died. These results suggest that level 2 girdling might be better for balancing tree vigor and reproduction, while level 3 might be too severe. Although the sample size of severe girdling levels in the present study may be slightly small and the long-term effects of girdling on cone production are still unclear, the short-term effect on increasing female cone production of *L. kaempferi* was confirmed in the old seed orchard.

In the present study, improvements in light intensity also had a positive influence on the probability of producing cones. Previous studies similarly reported that trees on southern slopes that received higher levels of insolation tended to produce abundant cones, and the branches in a crown that received full sunlight achieved the highest cone production [48–50]. In our estimates, even in intact (ungirdled) trees, more than half of the trees tended to produce cones when the light conditions reached rPPFD > 50%. In a study on *L. gmelinii* var. *japonica*, Uchiyama et al. [14] recommended that light intensity at over 50% rPPFD in an orchard is optimal. Since there was a large spatial variation in light intensity in our studied orchard, thinning the darker areas to reduce the stem density appears to be an efficient means of improving the reproductive status of *L. kaempferi* trees and for improving the seed production capacity of the seed orchards.

Moreover, our quantitative estimates demonstrated the existence of a genotype-specific response to increases in light intensity. When light intensity was sufficient, all of the clones had similar reproductive potentials. However, among-clone differences in reproductive potential were much evident under conditions of insufficient light intensity, and less-reproductive *L. kaempferi* clones were susceptible to light conditions. Uchiyama et al. [14] also reported a significant  $G \times E$  interaction among eight *L. gmelinii* var. *japonica* clones. Large among-clone variation in fecundity often makes it difficult to achieve panmixia, as seed orchards are designed on the premise of an equal reproductive contribution of each constitutive clone [51]. In some cases, less than half of the mother trees were responsible for more than half of the parentage in the seed orchard, resulting in an uneven genetic contribution among seedlings [52,53]. It has been reported that treatments often have a greater effect on less-reproductive trees, improving their genetic contribution across an orchard [52]. Improving light conditions by thinning can therefore be an effective method for ensuring that mating in a seed orchard more closely approximates panmixia through increasing the participation of clones in reproduction. In this context, improving the light conditions and collecting information on genotype-specific responses to light intensity may be useful for optimizing management regimes for improving seed production capacity of old seed orchards, not only in terms of seed quantity but also in quality.

In an experiment on young *L. kaempferi* trees [41], bending the upright branches so they were oriented downward had the effect of increasing cone bud initiation, especially on the lower surfaces of the horizontal shoots. In the early stages of orchard management, top pruning (cutting the main trunk and upright leader shoots) is typically conducted at a height of 3–4 m. However, in the less-intensively managed old seed orchards of *L. kaempferi*, it has been found that upright branches often re-sprouted dense adventitious shoots from the cut-off position, and the tree height was recovered. Based on our findings, ensuring that trees have a wide crown radius and relatively low height could be a better management strategy for improving female cone production in old orchards.

## 5. Conclusions

This study quantitatively evaluated the effects of girdling and increasing light intensity on female cone production in an *L. kaempferi* seed orchard. The findings showed that female cone production was augmented by both girdling and increasing light intensity. The probability of producing cones was markedly increased when higher girdling levels were applied. When the light intensity reached rPPFD > 50%, more than half of the mother trees tended to produce cones, even intact (ungirdled) trees,

and the genotype-specific response to light intensity was more apparent in less-reproductive clones. A significant negative relationship was observed between female cone production and the height/crown radius ratio. Taken together, these findings suggested that a management procedure that combines girdling less-reproductive trees and improving light intensity could be used to optimize and improve the cone production capacity of old *L. kaempferi* seed orchards.

**Author Contributions:** M.M., H.N., A.T. and M.T. conceived and designed the research. M.M., H.N. and A.T. performed the field survey. H.N. managed the study site. M.M. conducted data analyses and wrote the original draft. A.T. and M.T. conducted supervision and project administration. M.M. and M.T.; funding acquisition. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest:** The authors declare no conflict of interest.

## Appendix A



**Figure A1.** Example photos for girdling levels 1–3.

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