New Forests **28:** 1–9, 2004.

Selection of *Prunus cerasoides* D. Don seed trees for forest restoration

GREUK PAKKAD[1], STEPHEN ELLIOTT[1]
and DAVID BLAKESLEY[2,]*
[1]*Forest Restoration Research Unit, Biology Department, Science Faculty, Chiang Mai University, Thailand 50200;* [2]*Horticulture Research International, East Malling, West Malling, Kent ME19 6BJ, UK; *Author for correspondence (e-mail: david.blakesley@hri.ac.uk; phone: +44 1732 843833; fax: +44 1732 849067)*

Received 28 August 2002; accepted in revised form 5 May 2003

Key words: Germination, *Prunus cerasoides*, Seedling development, Tropical forest restoration

Abstract. *Prunus cerasoides* D. Don has been identified as a 'framework species' for restoring evergreen forest in seasonally dry climates. The aim of this study was to develop criteria to select parent trees for supplying seed to forest restoration projects based on seedling performance in the nursery and in the field. Seed progenies were collected from 50 individual parent trees; 13 in Doi Inthanon National Park; 14 in Doi Ang Khang; and 23 in Doi Suthep-Pui National Park. Criteria were developed for the selection of superior parent seed trees based on nursery and field performance, and a provisional selection of parent seed trees for forest restoration projects has been made based on these criteria. Four standards for selection of superior seed trees were recognised: (i) 70% or greater seedling survival in the field, (ii) a seedling height of 100 cm or taller after the first growing season in the field, (iii) 40% or greater germination in the nursery and (iv) 70% or higher seedling survival in the nursery. Twenty one seed trees met these standards.

Introduction

Forest loss is a very serious environmental problem in developing tropical countries such as Thailand. Watershed degradation is a primary consequence of deforestation, and soil erosion leads to droughts, whilst siltation of water courses worsens flood damage. To retain any significant natural forest cover in the long-term, stricter forest protection must be complemented with natural forest restoration to degraded sites, especially within protected areas (FORRU 1998). The Forest Restoration Research Unit (FORRU) of Chiang Mai University has found the so-called 'framework species method' to be successful in the highlands of northern Thailand (Blakesley et al. 2000; Elliott et al. 2002). First developed in Queensland, Australia (Goosem and Tucker 1995; Lamb et al. 1997; Tucker and Murphy 1997), the method involves planting 20–30 indigenous tree species to rapidly restore basic forest structure and function, whilst relying on wildlife to bring about biodiversity recovery. The selection criteria for framework tree species include high rates of survival and growth when planted out in deforested sites; dense, spreading crowns to shade out weeds and production of resources attractive to wildlife (e.g. fruit, nectar, nesting sites) at an early age (Elliott et al. 2002). Ease of propagation is also an important consideration (Blakesley et al. 2000). If the framework species method is widely adopted, framework species identified in Thailand could be planted across their natural range.

High germination percentages and growth rates that lead to the production of suitably sized seedlings, 50–60 cm tall at the optimal planting time (June in northern Thailand) are required to produce a high quality crop of seedlings with the highest chances of survival in the hot, dry and sunny conditions that prevail in deforested sites (FORRU 1998). Initial planting trials in deforested sites within Doi Suthep-Pui National Park identified *Prunus cerasoides* D. Don (Rosaceae) as meeting all the criteria of a framework species (Elliott and Anusarnsunthorn 2001). *P. cerasoides* is distributed throughout the mountains of the Himalayas, Yunnan, Myanmar, northern Thailand and northern Indo-China, where it occurs in evergreen and deciduous forest, evergreen forest, and evergreen and pine forest, particularly in disturbed areas, at elevations of 1040–1700 m (FORRU 2000). In the future, *P. cerasoides* could be widely planted across the region.

The concept of *in situ* Seed Production Plus Trees was first proposed by Steiner (1986) for the improvement of high-value hardwoods. However, no studies have been carried out to select superior seed trees for natural forest restoration. The main aim of this work was to study the variability in nursery and field performance of seed progenies collected from parent trees growing in three national parks and to develop criteria for the selection of superior parent seed trees for natural forest restoration projects based on these criteria.

Methods

Study sites

Plant material was collected from three sites in northern Thailand. Doi Suthep-Pui National Park (18°43′–19°08′N, 98°48′–98°58′E) covers an area of 262.5 km^2 and rises from an elevation of 330–1685 m. Doi Inthanon National Park (18°24′–18°41′N, 98°21′–98°38′E), includes the highest mountain in Thailand, rising to 2565 m elevation. It covers an area of 272 km^2 and is part of the Thanon Thongchai Range located south of Doi Suthep-Pui National Park. Doi Ang Khang (19°50′–19°57′N, 99°01′–99°06′E) covers an area of 91.4 km^2 and rises to an elevation of 1800 m. Ripe fruits of *P. cerasoides* were collected in March 2000 from 13 trees in Doi Inthanon National Park, 14 trees in Doi Ang Khang and 23 trees in Doi Suthep-Pui National Park. Many trees sampled were close to the roadside forest edge, suggesting that some may have been dispersed locally (collected and germinated or transplanted) by Forestry Department Officials.

Propagation

Trees were propagated in a nursery at 1000 m elevation, near the headquarters of Doi Suthep-Pui National Park, north-west of Chiang Mai, Northern

Thailand (l8°51′N, 98°54′E). The fruit pulp was removed and the pyrenes were soaked in water, and then exposed to air until their surface was dry. Seventy-two pyrenes were randomly selected from each seedlot, and sown within 2–3 days of collection on the surface of a medium of two parts forest soil to one part coconut husk to one part peanut husk in modular plastic trays. The seeds were divided into three replicate batches of 24, which were randomly assigned to different benches and watered daily. Each replicate consisted of 24 adjacent compartments (3.5 × 3.0 × 7.0 cm) in one seed tray. Trays were placed on top of concrete benches, partially shaded under a transparent plastic roof (approximately 40% full sunlight, similar to the light intensity in partially regenerating gaps). Germination was monitored throughout the germination period and was defined as emergence of any part of the shoot. The dates of the first and last seeds to germinate were recorded, and the median length of dormancy (MLD) calculated from the germination times of all seeds which germinated. Once the first pair of leaves had fully expanded, all seedlings were pricked out and transplanted into black plastic bags (6.5 × 23 cm), filled with the potting medium described above. Seedlings were shaded inside the nursery under a plastic roof (approximately 20% full sunlight) for 2 weeks. After this time, the seedlings were placed outside, under black shade netting (slan, approximately 50% of full sunlight). Seedlings were randomised within one block, with the border rows not included in the measurements. Ten granules of Osmocote slow-release fertiliser (15-15-15) were applied every 3 months. Seedling height and root collar diameter (RCD) were measured regularly. Monitoring ceased when the seedlings exceeded the ideal planting size of 60 cm tall. All seedlings were hardened off in full sunlight 4 weeks before being dispatched for planting in early June of the year following sowing.

The experimental plots were located in a degraded watershed in Doi Suthep-Pui National Park (18°52′N, 98°51′E), 1207–1310 m ASL. Originally, the plots would have been covered in evergreen forest, which was cleared between 1960–1970. Subsequently the area was cultivated for vegetables and further degraded by frequent fires. Although a few scattered mature trees remain, the area is now dominated by weedy herbaceous vegetation. One month before planting, weeds were cuts back with hand tools and a non-residual herbicide, glyphosate, was applied. Holes about twice the size of the container were dug, with 100 g of NPK 15:15:15 mixed in with loose soil at the bottom of each planting hole. Where possible, 20 seedlings were randomly selected for planting from every batch of seeds. Seedlings were planted randomly at a density of 3125 ha^{-1} in three 0.16 ha plots. They were planted together with 30 other species, with a 1.8 m spacing. Seedling height and RCD were measured 6 months after planting, in the dry season.

Analysis of variance was carried out within and between populations on all parameters measured. The percent relative growth rate per year of each seedling in the nursery and in the experimental plots was calculated using the following equation:

$$RGR = \frac{[\ln(X_2) - \ln(X_1)]}{(T_2 - T_1)} \times 100 \times 365$$

where X_2 = RCD or height at time T_2 (at the end of measurement), X_1 = RCD or height at time T_1 (at the beginning of measurement), $T_2 - T_1$ = Number of days between the beginning (T_1) and the end (T_2) time of measurement

Results

Germination and nursery studies

In the nursery, seed germination and seedling performance were recorded for seed batches collected from 13 trees in Doi Inthanon, 14 trees in Doi Ang Khang and 23 trees in Doi Suthep-Pui. Ripe fruit was collected late in the dry season. The germination percentage ranged from 12.5 to 87.5% for trees from Doi Suthep-Pui, 7.5 to 70.8% for trees from Doi Inthanon and 18.1 to 80.6% for trees from Doi Ang Khang. The mean germination percentage, averaged across all trees, was 55 ± 20.5% (SD) (Table 1). Thirty-nine trees had a germination percentage of 40% or greater, which we believe is the minimum acceptable for efficient nursery production. Germination did not differ amongst the three study sites ($P > 0.05$; ANOVA).

For trees from Doi Suthep-Pui, the first seed germinated after 14 days, and the last seed after 82 days. The germination period ranged from 26 to 82 days, with a mean of 43.39 ± 9.92 days. The MLD ranged from 20 to 44 days with a mean of 32.65 ± 7.25 days. For trees from Doi Inthanon, the first seed germinated after 13 days and the last seed after 78 days. The germination period ranged from 32 to 60 days, with a mean of 44.54 ± 8.04 days. The MLD ranged from 20 to 51 days with a mean of 31.92 ± 10.1 days. For trees from Doi Ang Khang, the first seed germinated after 11 days and the last seed after 72 days. The germination period ranged from 28 to 62 days, with a mean of 41.64 ± 10.16 days. The MLD ranged from 17 to 41 days with a mean of 28.21 ± 6.73 days. Overall, the germination period and MLD showed no significant differences among the three study sites ($P > 0.05$; ANOVA) (Figure 1).

RCD and height of seedlings were measured 6 months after pricking out. Although there was considerable variation in growth both within and among study sites, the majority of seedlings reached a height suitable for planting (50–60 cm tall well before the optimal planting time (June in northern Thailand) (FORRU 1998). For trees from Doi Suthep-Pui, RCD ranged from 2.40 ± 1.12 to 4.39 ± 1.24 mm, with a mean of 3.08 ± 0.62 mm, whilst seedling height ranged from 59.82 ± 25.16 to 103.23 ± 23.67 mm with a mean of 74.97 ± 11.85 mm. For trees from Doi Inthanon, RCD ranged from 1.66 ± 0.87 to 3.75 ± 1.37 mm, with a mean of 2.96 ± 0.57 mm, whilst seedling height ranged from 40.65 ± 21.12 to 99.00 ± 27.70 mm with a mean

Table 1. Germination, survival and growth of *P. cerasoides* seed progenies collected from 50 parent trees from three locations. Growth measurements taken 6 months after pricking out in the nursery, and 6 months after planting out in the field. Mean number of seedlings from each parent tree planted out was 17.5

Parameter	Nursery			Field		
	Mean	SD	Range	Mean	SD	Range
Percent germination	55.0	20.5	7.5–87.5	–	–	–
Percent seedling survival	74.7	11.7	7.1–93.8	81.6	11.5	37.5–100
Mean size: RCD (mm)	3.1	0.6	1.7–4.8	8.6	1.1	6.3–11.7
Mean size: Height (cm)	74.6	14.0	40.7–133.1	110.0	12.8	87.1–156.3
Relative growth rate (% $year^{-1}$): RCD	–	–	–	212.6	48.7	94.6–343.9
Relative growth rate (% $year^{-1}$): Height	–	–	–	179.0	44.3	77.6–292.3

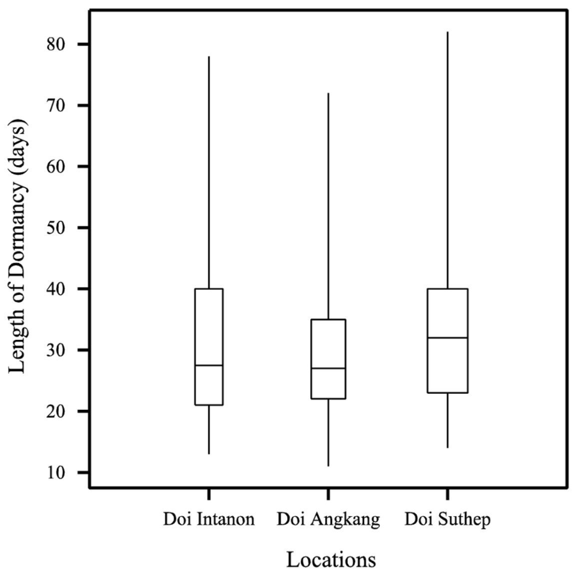

Figure 1. The range of germination period and mean MLD of all trees collected in Doi Inthanon National Park, Doi Suthep-Pui National Park and Doi Ang Khang. Each box contains 50% of the seed germinated, and the line in the box shows the mean MLD. Vertical lines below and above the boxes represent 0–25%, and 75–100% of seeds which germinated, respectively. Extreme values are at both bounds.

of 74.05 ± 15.58 mm. For trees from Doi Ang Khang, RCD ranged from 2.41 ± 1.09 to 4.84 ± 1.56 mm, with a mean of 3.19 ± 0.66 mm, whilst seedling height ranged from 57.17 ± 22.05 to 113.14 ± 27.24 mm with a mean of 74.50 ± 15.48 mm. Overall, growth rates in the nursery showed no significant differences among the three study sites ($P > 0.05$; ANOVA).

Seedling establishment in the field

The target number of 20 seedlings of each seed tree transplanted into the plots was not reached in all cases, due to variation in nursery mortality. At the end of the wet season, seedling survival amongst the progenies was high, ranging from 37.5 to 100%, with a mean of 81.6 ± 11.5 % (SD) (Table 1). Seedlings from 46 seed trees had a survival rate of 70% or greater. After 6 months in the plots, the mean height of all seedlings was 110.0 ± 12.8 cm (SD), approximately 50% taller than when planted. Seedling heights ranged from 87.09 ± 36.8 cm (SD) to 156.3 ± 6.4 cm (SD). The mean RCD of all seedlings was 8.6 ± 1.1 mm (SD), more than double that when planted, ranging from 6.3 ± 2.1 mm (SD) to 11.7 ± 3.7 mm (SD). Consequently, RGRs also showed considerable variation between trees, and significant variation between the three study sites ($P < 0.05$; ANOVA).

Selection of seed trees based on seedling performance

Criteria used to identify seed trees of *P. cerasoides* 'adapted' to the local environment were; survival and growth of seedlings in the field and germination percentage and seedling survival in the nursery. Survival in the field is the most important criterion. We considered that a standard of 70% survival over the first growing season was the minimum which would be acceptable for a restoration programme. The second criterion applied was growth in the field. An acceptable standard was considered to be a height of 100 cm or more by the end of the first growing season. Parent seed trees which met the 1st and 2nd criteria were then considered for their nursery performance. Standards of more than 40% germination and more than 70% survival in the nursery were applied. Twenty-one of the seed trees satisfied all four of the standards outlined above.

Discussion

Performance

Considerable variation was found among seed trees in the germination percentage of their progeny. Similar levels of variation were found at each study site. This is important as it indicates that the choice of seed trees may have profound effects on the efficiency of the nursery operation and the ability to achieve production targets. An acceptable germination percentage needs to be established and seed should be collected only from those trees that meet this target. In our case, 40% represents the lower limit (Blakesley et al. 2002), although a higher % would be preferable. The range of germination period and MLD showed no differences among the three study sites, but varied considerably among trees within study sites. Ideally, a nursery manager would chose seed which germinated synchronously, with a short MLD, for maximum efficiency and uniformity of plant material. Growth rate of the *P. cerasoides* seedlings in the nursery, although variable, was too rapid. The seedlings reached a plantable size well before the optimum planting time and required pruning before planting. This wastes labour and space in the nursery. Only one tree from Doi Inthanon, three trees from Doi Suthep-Pui and three trees from Doi Ang Khang did not require pruning. However, it is possible that these trees may also exhibit slower growth rates following planting in the field. This contrasts with other framework species such as *Lithocarpus craibianus* and *Castanopsis acuminatissima*, which may require in excess of 12 months to produce a seedling suitable for transplantation (Elliott et al. 2002).

A high survival rate of seedling survival in the field plots was obtained. A standard of 70% survival or higher, was considered the minimum acceptable for our restoration planting programme and seedlings from 46 seed trees met or exceeded this standard. Growth rates of seedlings in the field were also high, with a mean seedling height across seed trees was 110.0 $\pm$ 12.8 cm (SD). Seedlings from 39 seed trees had a mean seedling height of 100 cm or more, which met the standard criteria for selection of a superior parent tree.

Selection of seed trees

It is important that the planting stock for forest restoration is well adapted, and contains adequate genetic diversity. The ability to reproduce (fitness) is important both as an indice of adaptation and for practical nursery production. Survival and growth rates in the field show adaptation to the site, and are important for management and effectiveness of the restoration process. The primary aim of this study was to develop criteria to select *P. cerasoides* seed trees for forest restoration programmes which meet these objectives. For efficient site capture, selection would be made on the basis of nursery and field performance. Twenty-one 'superior' mother trees were identified whose seedlings appear to be 'well adapted' to the planting site, and hence effect efficient site capture. However, a simultaneous study of genetic diversity of *P. cersasoides* mother trees using microsatellites (Pakkad et al. in press) has shown that populations in the three National Parks are genetically distinct. Consequently, seed should be collected locally, and not moved between the three National Parks. Our selection criteria identified five trees from Doi Inthanon, seven trees from Doi Suthep-Pui and nine trees from Doi Ang Khang as suitable seed sources for our restoration programme. Further work is therefore necessary to identify more parent trees from each location. Diversity is also necessary, not only to ensure the present adaptability of a species, but also to allow the continued evolution of the species. A baseline figure of 50 individuals has been suggested (Brown and Marshall 1995; FAO, DFSC, IPGRI 2001), although this may be modified depending on the aims of the sampling strategy.

There are no clear published guidelines for forest restoration practitioners concerned with wildlife conservation. However, our work with microsatellite markers (Pakkad et al. in press) indicates a large amount of genetic diversity in *P. cerasoides*, highlighting the importance of genetic diversity conservation, and the need to collect seed from as many trees as possible, certainly within, or close to the FAO recommendation of 25–50 individuals per population (FAO Forest Resources Division 1995).

Acknowledgements

The authors are grateful for institutional support of this work provided by Chiang Mai University and especially the Herbarium and the FORRU attached to the Biology Department, Faculty of Science. We are deeply grateful to the staff of FORRU for helping to take care of seedlings in the nursery, especially Thonglaw Seethong, Jumpee Bunyadit, Loontiwa Pakaad, Cherdsak Kuarak and Puttipong Navakitbumrung. Financial support of the 1st author came from the Kanchanapisek Program of the Thailand Research Fund.

References

Blakesley D., Anusarnsunthorn V., Kerby J., Navakitbumrung P., Kuarak C., Zangkum S., Hardwick K. and Elliott S. 2000. Nursery technology and tree species selection for restoring forest biodiversity in northern Thailand. In: Elliott S., Kerby J., Blakesley D., Hardwick K., Woods K. and Anusarnsunthorn V. (eds) Forest Restoration for Wildlife Conservation. International Tropical Timber Organisation and The Forest Restoration Research Unit, Chiang Mai University, Thailand, pp. 207–220.

Blakesley D., Elliott S., Kuarak C., Navakitbumrung P., Zangkum S. and Anusarnsunthorn V. 2002. Propagating framework tree species to restore seasonally dry tropical forest: implications of seasonal seed dispersal and dormancy. Forest Ecol. Manag. 164: 31–38.

Brown A.H.D. and Marshall D.R. 1995. A basic sampling strategy: theory and practice. In: Guarino L., Ramanath Rao V. and Reid R. (eds) Collecting Plant Genetic Diversity (Technical Guidelines). CAB International. 748 p.

Elliott S. and Anusarnsunthorn V. 2001. Research to restore biodiversity to degraded land in Northern Thailand's conservation Areas. Final report on project 240002 to the Biodiversity Research and Training Program, Bangkok, Thailand.

Elliott S., Kuarak C., Navakitbumrung P., Zangkum S., Anusarnsunthorn V. and Blakesley D. 2002. Propagating framework trees to restore seasonally dry tropical forest in northern Thailand. New Forest. 23: 63–70.

FAO 1995. State of the World's Forests. FAO, Rome.

FAO, DFSC, IPGRI 2001. Forest genetic resources conservation and management. Vol. 2. In: Managed Natural Forests and Protected Areas (*in situ*). International Plant Genetic Resources Institute, Rome, Italy, 89 p.

Forest Restoration Research Unit 1998. Forests for the Future: Growing and Planting Native Trees for Restoring Forest Ecosystems. Elliott S., Blakesley D. and Anusarnsunthorn V. (eds), 60 p.

Forest Restoration Research Unit 2000. Tree Seeds and Seedlings for Restoring Forests in Northern Thailand. Kerby J., Elliott S., Maxwell J.F., Blakesley D. and Anusarnsunthorn V. (eds), 152 p.

Goosem S.P. and Tucker N.I.J. 1995. Repairing the Rainforest – Theory and Practice of Rainforest Re-establishment in North Queensland's Wet Tropics. Wet Tropics Management Authority, Cairns, 71 p.

Lamb D., Parrotta J., Keenan R. and Tucker N. 1997. Rejoining habitat fragments: restoring degraded rainforest lands. In: Laurance W.F. and Bierregaard R.O. (eds) Tropical Forest Remnants: Ecology, Management and Conservation of Fragmented Communities. University of Chicago Press, Chicago, pp. 366–385.

Pakkad G., James C., Torre F., Elliott S. and Blakesley D. 2003. Genetic variation of *Prunus cerasoides* D. Don, a framework tree species in northern Thailand. New Forest. in press.

Steiner K.C. 1986. Integrating tree improvement with hardwood seedling production. Proc. Northeastern Area Nurseryman's Conf. (State College, PA) 39: 24–30.

Tucker N.I.J. and Murphy T.M. 1997. The effects of ecological rehabilitation on vegetation recruitment: some observation from the wet tropics of north Queensland. Forest Ecol. Manag. 99: 133–152.