Domestication for Conservation of an Endangered Species: The Case of the Wollemi Pine

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ABSTRACT

A small population of tall slender conifers was discovered in 1994 in a deep rainforest canyon of the Wollemi National Park, New South Wales, Australia. The living trees closely resembled fossils that were more than 65 million years old, and this 'living fossil' was recognised as a third extant genus in the Araucariaceae (*Araucaria*, *Agathis* and now *Wollemia*). The species was named the Wollemi pine (*W. nobilis*). Extensive searches uncovered very few populations, with the total number of adult trees being less than 100. *Ex situ* collections were quickly established in Sydney as part of the Wollemi Pine Recovery Plan. The majority of the *ex situ* population was later transferred to our custom-built facility in Queensland for commercial multiplication. Domestication has relied very heavily on the species' amenability to vegetative propagation because seed collection from the natural populations is dangerous, expensive, and undesirable for conservation reasons. Early propagation success was poor, with only about 25% of cuttings producing roots. However, small increases in propagation success have a very large impact on a domestication program because plant production can be modelled on an exponential curve where each rooted cutting develops into a mother plant that, in turn, provides more rooted cuttings. An extensive research program elevated rooting percentages to greater than 80% and also provided *in vitro* methods for plant multiplication. These successes have enabled international release of the Wollemi pine as a new and attractive species for ornamental horticulture.

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INTRODUCTION

During a bushwalking and canyon expedition in September 1994, an off-duty field officer from the National Parks and Wildlife Service of New South Wales, Australia, encountered an unfamiliar population of tall slender conifers. The trees were growing in the base of a deep rainforest ravine in the Wollemi National Park, about 150 km north-west of Sydney (Woodford 2002). Branches, leaves, and male and female strobili were collected from the trees in October and November 1994 (Jones *et al*. 1995; Woodford 2002), and it was soon realised that the previously-unseen living specimens closely resembled Araucariaceae fossils from the Cre-

taceous period (Hill 1996; Chambers *et al*. 1998; Hill and Brodribb 1999). This southern conifer family was widespread in both northern and southern hemispheres during the Jurassic (205-145 million years ago) and early Cretaceous periods but it contracted in distribution during the Cretaceous (145-65 million years ago) and Tertiary (65-2 million years ago) periods, and was thought to include only two surviving genera, *Agathis* and *Araucaria*, each comprising about 20 species (Enright *et al*. 1999; Kershaw and Wagstaff 2001; Dettmann and Clifford 2005). Instead, the number of extant genera in the Araucariaceae was three. The new genus and species was named the Wollemi pine, *Wollemia nobilis* (Jones *et al*. 1995), with the specific epi-

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thet referring both to the trees' stature and to their discoverer, David Noble.

Fossil records indicate that the oldest and most widespread species in the Araucariaceae belong to *Araucaria*. Analyses of *rbcL* gene sequences suggest that *Wollemia* may have derived prior to *Araucaria* and *Agathis* (Setoguchi *et al.* 1998), although other cladistic analyses place *Wollemia* and *Agathis* in the same clade, descended from a common ancestor (Gilmore and Hill 1997; Stefanović *et al.* 1998; Wagstaff 2004; Dettmann and Clifford 2005). The genus *Wollemia* was once widely distributed in Australia, Antarctica, and possibly New Zealand (Chambers *et al.* 1998; Hill and Brodribb 1999; Kershaw and Wagstaff 2001). Searches of the Wollemi National Park have uncovered very few surviving populations. Their coppicing and multi-stemming habit (Hill 1997; Offord *et al.* 1999; Burrows *et al.* 2003) makes it difficult to precisely determine the number of living Wollemi pines, but the total number of adult trees is considered to be less than 100 (Offord and Meagher 2001; Benson 2002; Woodford 2002).

The species has been dubbed a pine dinosaur (Anderson 1994), a lonesome pine (Anderson 1995, 1997; Jamieson 2005), a living fossil (Hill 1996; Raven *et al.* 1999; Woodford 2002) and a dinosaur tree (Woodford 2005). The discovery is regarded as one of the most significant botanical finds of the twentieth century, akin to rediscoveries of other ancient species like the gingko tree (*Gingko biloba*) and the dawn redwood (*Metasequoia glyptostroboides*) (Woodford 2002; Offord and Meagher 2006). The Wollemi pine has attracted enormous international media interest, featuring in thousands of newspaper articles as well as film and television documentaries. As such, it is a flagship species for plant science, ornamental horticulture, and biological conservation.

EX SITU COLLECTIONS

The New South Wales National Parks and Wildlife Service recognised that the widespread publicity surrounding the Wollemi pine's discovery created a new and significant series of threats to the species' survival, including from unauthorised seed collection, introduction of pathogens or weeds, trampling of seedlings, compaction of soil, and increased likelihood of fire. It prepared a recovery plan (Anon. 1998) with specific objectives that included: (a) protection of natural populations and habitat from human-induced threatening processes; (b) greater understanding of the species' ecology; (c) assessment of genetic variability of natural populations; and (d) establishment of representative *ex situ* populations in botanic gardens.

Ex situ plants were established between 1995 and 1999 at the Royal Botanic Gardens at Mount Annan in Sydney from both seeds and cuttings collected from the Wollemi National Park (Fensom and Offord 1997; Anon. 1998; Offord *et al.* 1999; Offord and Meagher 2001). Emphasis was placed on minimising collection of plant material from wild populations and maximising propagation from stock plants. Seed collection involved the dangerous practice of suspending a national parks ranger from a helicopter cable, so that mature cones could be clutched from their terminal positions on very high branches before seeds were scattered naturally by wind or birds (Offord *et al.* 1999; see photograph in Woodford 2002). Some seeds were gathered from nets suspended beneath the canopy (Offord *et al.* 1999). Given optimal temperature, light, and stratification treatments, up to 80% of seeds germinated (Offord and Meagher 2001). Plants were then propagated as rooted cuttings by collecting orthotropic shoots from the juvenile seedlings. Vegetative propagation of conifers is much easier when cuttings are taken from juvenile stock plants (Thorpe and Harry 1990; Greenwood and Hutchison 1993; Mitchell *et al.* 2004), but a core objective of the Wollemi Pine Recovery Plan was to ensure that as many as possible of the wild adult trees were represented in the *ex situ* collections. Therefore, rooted cuttings were also raised from the more-

Table 1 Some international public gardens, museums and theme parks featuring living specimens of the Wollemi pine.

Country	Location
Australia	Royal Botanic Gardens, Sydney
	Taronga Park Zoo, Sydney
	Adelaide Botanic Garden, Adelaide
	Australian National Botanic Gardens, Canberra
	Royal Tasmanian Botanic Gardens, Hobart
	Kings Park and Botanic Garden, Perth
Austria	Schönbrunn Palace, Vienna
Belgium	National Botanic Garden of Belgium, Brussels
Canada	Kingsbrae Garden, St Andrews
France	National Museum of Natural History, Paris
	Botanic Gardens of Bordeaux, Lille and Nancy
Germany	Botanic Gardens of Berlin-Dahlem, Bonn, Hamburg, and Munich
	Palmengärten, Frankfurt
	Zoological and Botanic Garden, Stuttgart
Ireland	National Botanic Gardens of Ireland, Glasnevin
Italy	Botanic Gardens of Florence, Palermo, Cagliari and Pisa
	Trauttmansdorff Castle, Merano
Japan	Disneyland, Tokyo
Monaco	Saint Martin Gardens
Netherlands	Hortus Botanicus, Amsterdam, Haren and Leiden
	Arboretum Trompenburg, Rotterdam
	Botanic Gardens of Delft, Utrecht and Wageningen
Taiwan	National Museum of Taiwan, Taipei
United Kingdom	Royal Botanic Gardens, Kew
	Royal Botanic Gardens, Edinburgh
	The Eden Project, Cornwall
	Cambridge University Botanic Garden, Cambridge
United States of America	United States Botanic Gardens, Washington DC

A listing of other locations is available at the official Wollemi pine web site (www.wollemipine.com)

mature plants that had been raised from wild cuttings.

During establishment of the conservation collection in Sydney, several feature trees were transferred to major botanic gardens in other Australian states and to the Royal Botanic Gardens at Kew, United Kingdom (Hanson 2001; Woodford 2002). These transfers acted as part of a risk management strategy to ensure that not all *ex situ* specimens were housed in the one location, and they allowed preliminary assessment of the range of climatic conditions under which the species could be grown successfully. Further plantings and displays have been installed in major international botanic gardens, museums and theme parks since 2004 (**Table 1**). These feature displays provide an important educational experience for the public, giving visitors the chance to read about, view, photograph and touch the 'living fossils', albeit sometimes through the bars of a large protective cage (**Fig. 1A**).

Growing interest in the Wollemi pine's horticultural potential, coupled with concerns for protection of the natural populations from illegal plant and seed collection, provided strong impetus for development of a commercial propagation program. Several species of Araucariaceae have a long history as ornamental and amenity trees, particularly the Pehuén or Monkey Puzzle tree from Chile and Argentina (*Araucaria araucana*) and the Norfolk Island pine (*Araucaria heterophylla*) and Bunya pine (*Araucaria bidwillii*) from Australia (Matschke *et al.* 1993; Bekessy *et al.* 2002; Haebich 2005; Huth and Holzworth 2005). Many also have great value as forestry species, including the kauri pines (e.g. *Agathis australis* from New Zealand), the Paraná pine (*Araucaria angustifolia*) from Brazil and Argentina, and the hoop pine (*Araucaria cunninghamii*) from Australia, Papua New Guinea and Indonesia (Guerra *et al.* 2000; Duarte *et al.* 2002; Sousa and Hattemer 2003; Boswijk 2005; Huth and Holzworth 2005; Scott *et al.* 2005; Stewart and McKinley 2005).

A large proportion of the *ex situ* Wollemi pine collection

Fig. 1 (**A**) Feature specimen of Wollemi pine at the Royal Botanic Gardens, Mt Annan, Sydney. (**B**) Hedged stock plants in a 50%-shadehouse. (**C**) Cuttings under glasshouse mist irrigation. (**D**) Soft, pruned cuttings, used initially by the commercial program. (**E**) Firm, unpruned cuttings, used as part of the refined propagation method. (**F**) New orthotropic shoots developing after collection of cuttings. (**G**) *In vitro* shoots after subculture (left) and at the end of an 8-week passage (right). (**H**) *Ex vitro* plants.

in Sydney was transferred to the Queensland Department of Primary Industries and Fisheries in 1999 and 2000, with the aim of propagating commercial quantities of the species by 2006. Only limited information was available about conditions required for optimal multiplication of Wollemi pine but this organisation was selected to undertake the commercial program, in part, because of its long-standing expertise in large-scale propagation of the related hoop pine (*Araucaria cunninghamii*) for forestry plantations (Woodford 2002; Huth and Holzworth 2005).

COMMERCIAL PROPAGATION

Genetic diversity

The Royal Botanic Gardens in Sydney ensured that plants transferred to the commercial collection in Queensland comprised a wide range of the potential genetic diversity of the species, to contribute to the aim of establishing representative *ex situ* collections in other gardens. The commercial collection was made up of approximately 1000 stock plants, with about 300 plants raised from cuttings of wild adult trees, 500 seedlings raised from wild seed, and 200 rooted cuttings that had been collected from these seedlings. At the time of establishing the commercial collection, it was considered that at least 26 of about 40 wild trees were represented among the 300 plants raised from wild cuttings, including 18 trees from the population discovered in 1994 and eight trees from a second population discovered in 1995. The number of commercial stock plants from each wild tree

ranged from 1 to 47. The exact number of wild trees represented in the commercial collection is not entirely clear because their multi-stemming and coppicing habit makes identification of individual wild trees difficult, and because a survey of trees and stems has revised higher the number of wild trees since the commercial collection was established (Anon. 1998; Benson 2002).

In addition to the problem of identifying individual trees from among the large number of wild stems, surveys encompassing 13 allozyme, 20 SSR and more than 800 AFLP loci (Hogbin *et al.* 2000; Peakall *et al.* 2003) have failed to detect genetic polymorphism in Wollemi pine, leading to some suggestions that the adult natural population is actually a single clone that has only been reproducing asexually via coppice stems (Anderson 1997; Banks 2003). If this were the case, then all of the 300 commercial stock plants raised from cuttings of wild adult trees would be just one clone (i.e. ramets of the same genet). Extensive clonality within remnant or endangered populations has been found for several woody plant species (e.g. Warburton *et al.* 2000; Bartolome *et al.* 2002; Weekley *et al.* 2002; Jusaitis and Adams 2005). However, an important point to note is that Hogbin *et al.* (2000) and Peakall *et al.* (2003) failed to detect genetic variation not only among 26 wild trees but also among plants raised *ex situ* from seeds. They concluded that there is 'exceptionally low' rather than no genetic variation within Wollemi pine.

Even if the natural Wollemi pine populations were just a single clone, genetic variation should be expected among the hundreds of wild and *ex situ* seedlings unless either (a)

the clone was producing seeds asexually (i.e. by apomixis) or (b) the clone was completely homozygous at all loci across its genome. Apomixis is common among many families of flowering plants (Hanna and Bashaw 1987; Carneiro *et al*. 2006) but it has not been identified in conifers (Dogra 1966; Sedgley and Griffin 1989; Mogie 1992; Bicknell and Koltunow 2004). It is likely that Wollemi pine seedlings have arisen from sexual reproduction and will therefore exhibit genetic variation, even if they are progeny of a single clone, provided their parent possesses some level of heterozygosity. However, genetic theory predicts that heterozygosity can be lost from small populations if they have a low mutation rate and little or no selection against homozygosity (Peakall *et al*. 2003; Scott *et al*. 2005). Low, but nonzero, levels of heterozygosity have been detected in *Agathis robusta* and *Araucaria cunninghamii* (Peakall *et al*. 2003; Scott *et al*. 2005), but high levels have been found in *Araucaria araucana* and *Araucaria bidwillii* (Bekessy *et al*. 2002; Pye and Gadek 2004). The Wollemi pine could, just possibly, be an extreme case of a single clone completely lacking heterozygosity, with all plants in the *ex situ* collections being, effectively, ramets of the same clone. More likely, there is some genetic variation among the seedling populations both *in situ* and *ex situ*, whether or not there is clonality in the adult natural populations. We have observed considerable variation in proliferation rates among seedlings that have been germinated and multiplied *in vitro*, where environmental differences between plants have been negligible. These phenotypic differences *in vitro* suggest strongly that there are genotypic differences among Wollemi pine seedlings.

Germplasm multiplication

Domestication of the Wollemi pine has relied heavily on its amenability to vegetative propagation, because ongoing collection of seed from the species' natural populations would be very expensive, dangerous, and undesirable for conservation reasons. Methods used initially for cuttings propagation were based on those employed for commercial hoop pine. Briefly, newly developed orthotropic shoots of Wollemi pine were collected from hedged stock plants grown in a 50%-shadehouse (**Fig. 1B**). Shoots were dissected into 8-cm cuttings, and the position of each segment on the shoot (i.e. tip, second, third or fourth) was recorded. Cuttings were pruned to remove about 60% of their branch lengths.

They were dipped in a commercial rooting gel containing 3 g IBA per litre, and set 2-cm deep in 220-cm^3 propagation tubes containing 50% shredded pine bark and 50% perlite. Tubes were placed under automatic misting in an adjacent glasshouse, custom-built for Wollemi pine propagation (**Fig. 1C**). Mist intervals during the night were 120 mins. Daytime mist intervals were progressively decreased from 15-30 mins (June 2000 collection) to 10 mins (December 2000 and subsequent collections). Rooting was assessed after 6 months, and rooted cuttings were then hardened for several weeks under mist irrigation in the shadehouse before being transplanted to larger pots with drip, rather than mist, irrigation.

Rooting percentages were higher for cuttings collected from seedling stock plants (**Fig. 2**) than for those collected from stock plants raised from wild cuttings (**Fig. 3**). Tip cuttings, in most instances, were more likely than lower segment cuttings to form roots, and so cuttings were collected frequently to increase the proportion of tip cuttings in each setting. The use of tip cuttings is particularly advisable when rooted cuttings are to be raised directly for sale, because retention of the apical shoot helps to produce a symmetrical plant without visible wounding on the main stem. Plant form *per se* is not a major consideration when raising rooted cuttings as stock plants for further collection of cuttings.

Many of the stock plants raised from wild cuttings possessed adult foliage but all of the seedling stock plants had juvenile foliage, indicating that the lower rooting percentages from wild-cutting plants could have been a consequence of donor maturation commonly encountered in conifer propagation programs (Greenwood and Hutchison 1993; Menzies *et al*. 2004; Mitchell *et al*. 2004). Plant production via vegetative propagation can be modelled on an exponential curve where each rooted cutting develops into a stock plant that, in turn, provides more rooted cuttings and more stock plants. In this situation, lower rooting percentages associated with mature stock plants can have a very large effect on the final number of domesticated plants. Although this could be viewed as a major limitation to a domestication program, it provided an opportunity to segregate the commercial collection into two product groups:

(1) Many of the rooted cuttings from mature stock plants were not used as mother plants, but were grown as a premium collection of large specimen trees bearing the highly unusual adult foliage. Approximately 300 of these
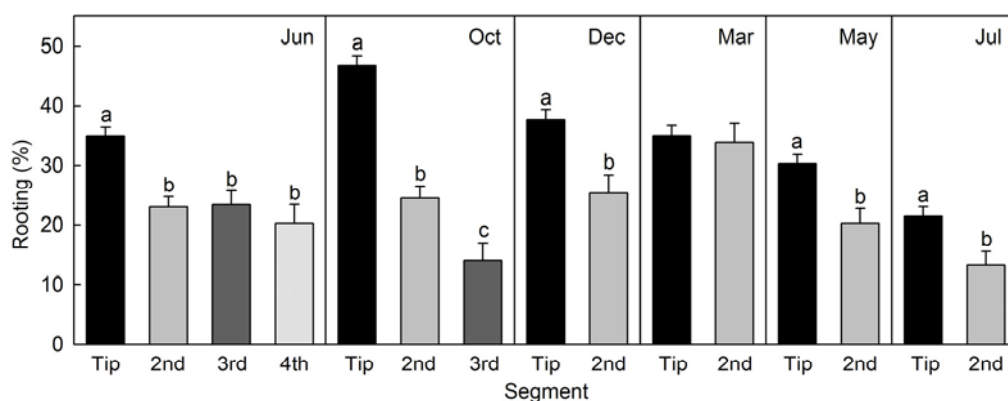

Fig. 2 Rooting of tip cuttings and lower segment cuttings from seedling stock plants of Wollemi pine. Results are from the first six commercial settings (June 2000 - July 2001). Means (+ s.e.) with different letters within a setting are significantly different ($P < 0.05$, ANOVA or t-test).
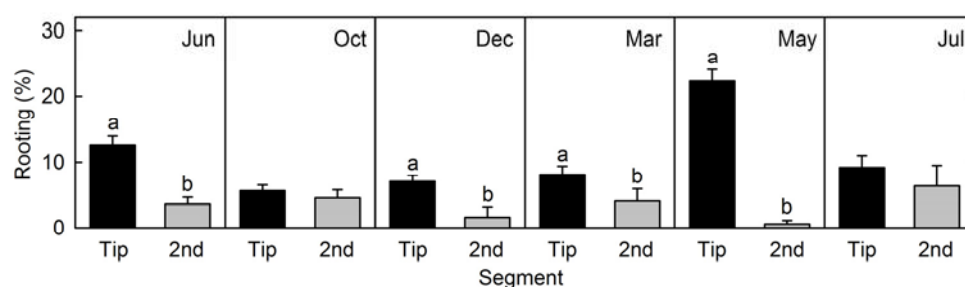

Fig. 3 Rooting of tip cuttings and second segment cuttings from stock plants raised from wild cuttings of Wollemi pine. Results are from the first six commercial settings (June 2000 - July 2001). Means (+ s.e.) with different letters within a setting are significantly different ($P < 0.05$, *t*-test).

feature trees, between 70-cm and 225-cm height, were auctioned internationally by Sotheby's in October 2005 as 'The First Generation Wollemi Pines: Collectors Edition'. Each of the auction trees could be traced to one of 15 individual wild trees, with an authentication certificate providing provenance information on each wild tree's height, girth, number of major coppice stems, and approximate location within the natural population. The average price per auction tree was approximately $US 2700.

(2) Rooted cuttings from all juvenile and some mature stock plants were used to raise more stock plants, so that plant numbers in this system rose exponentially until a selection was prepared for the first release of routine potted plants in April 2006. Most of these plants were raised from seed, and they were presumed to be genetically distinct from the wild trees. Their foliage is usually juvenile, and any available pedigree information (e.g. maternity of seed) has not been retained to point-of-sale. Prices have depended on geographic location and associated transport costs; e.g. 40-cm potted Wollemi pines have retailed for $US 45 in Australia and 25-cm potted plants for $US 100 in North America.

The initial rooting percentages from juvenile stock plants (**Fig. 2**) were considered poor in comparison with hoop pine, and so a research and development program was implemented to raise rooting percentages to at least 70%. Fifty experiments were conducted over a three-year period, concentrating on four factors considered to affect rooting ability (Leakey *et al.* 1994; Leakey 2004): (a) the stock plant environment (e.g. irradiance, potting media, substrate pH, stock plant nutrition, and plant growth regulator applications); (b) the origin of the cutting within the stock plant (e.g. cutting length, shoot diameter and stem anatomy, and cutting position); (c) post-severance treatments (e.g. rooting hormones, foliage pruning, foliar fertilisers, and fungicides); and (d) the propagation environment (e.g. irradiance, temperature, propagation tubes, propagation media, and non-mist propagators).

No factor was found singularly responsible for the initially poor rooting of Wollemi pine cuttings, but incremental gains of about 10% from each of several experiments led to refined methods with greater than 80% rooting. A major change to the original hoop pine method has been a move from soft, pruned cuttings (**Fig. 1D**) to firm, unpruned cuttings (**Fig. 1E**). By allowing branch expansion and development of secondary xylem in orthotropic shoots, and then not pruning foliage from the cuttings, root formation and root growth rate are increased in our mist-propagation system (Pohio *et al.* 2005). Use of unpruned cuttings also saves time in preparing cuttings, and allows cuttings to retain their full complement of branch apical meristems (Cameron *et al.* 2001).

Another method that greatly increases root growth, and hence decreases the time that cuttings remain under mist irrigation, is the incorporation of slow-release fertiliser into propagation medium (Pohio *et al.* 2005). Methods that improve root growth have a major impact on the exponential plant production model because they accelerate the phase from root formation to collection of cuttings from the resultant stock plant, even if they do not affect the percentage of cuttings that forms roots. Other experimental results provided time and cost savings to the commercial program. For example, application of rooting hormones was found unnecessary when more than 70% of cuttings were forming roots without exogenous hormones, and most cuttings were being transferred from the propagation environment with an extensive root system within four months (Trueman and Peters 2006).

Some issues relating to health and vigour of potted stock plants were addressed outside the experimental program. Extensive growth of weeds (especially liverworts) became a major long-term problem in irrigated and fertilised potting medium, but this was easily controlled using weed matting. Stock plants were also found to retain their vigour when a small portion of the shoot (including two or three
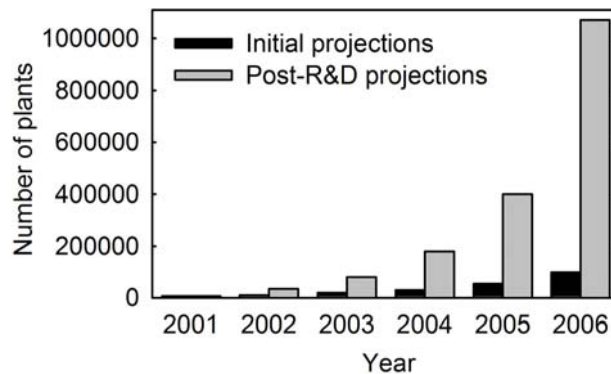

Fig. 4 Projections for plant production of Wollemi pine before and after research-based refinements in the propagation method. Initial projections were based on 25% rooting and post-R&D projections were based on 80% rooting.

branches) was retained on the mother plant when each cutting was collected. Several new orthotropic shoots often form on this remaining portion of shoot (**Fig. 1F**), without the usual appearance of dieback at the cut stump. Retention of these shoot portions leads to a gradual increase in the size of stock plants. Although this might be expected to counteract the maturation-delaying effect of stock plant hedging (Aimers-Halliday *et al.* 2003; Mitchell *et al.* 2004; Trueman 2006), rooting percentages appear to have remained high after six years of harvesting cuttings.

A modest investment in research and development elevated average rooting percentages of Wollemi pine from about 25% to 80%. As a result, the number of plants expected to be produced by the initial public release (including stock plants) was revised in 2003 from 99,000 to more than 1,000,000 (**Fig. 4**). Since then, the research program has focused on developing somatic embryogenesis and shoot culture methods for *in vitro* propagation (Grace *et al.* 2005). Somatic embryogenesis allows long-term cryopreservation of embryogenic cell lines and it can provide exceptionally high proliferation rates (Cyr and Klimaszewska 2002; Jones 2002). However, despite success in initiating embryogenic cultures, it has proven difficult to convert embryogenic tissue into germinated plantlets (i.e. emblings) for Araucariaceae species (Aitken-Christie and Platt 1992; Steiner *et al.* 2005; Silveira *et al.* 2006), including Wollemi pine.

Attempts to propagate Wollemi pine by shoot culture have been more fruitful (Grace *et al.* 2005, Woodford 2005). Methods are based on those developed for *Pinus radiata* in New Zealand (Horgan 1987; Aitken-Christie *et al.* 1988), using orthotropic shoots from Wollemi pine seedlings that have been raised *in vitro* (**Fig. 1G**). Shoots are subcultured at intervals of approximately 8 weeks (at 25°C with constant irradiance of 70 μmol photons m^{-2} s^{-1}). Some seedlings provide a two-fold multiplication each passage, but proliferation rates have consistently varied greatly among individual seedlings. Rooting occurs readily *ex vitro* and plantlets are progressively conditioned for use as nursery stock plants (**Fig. 1H**). Plantlets are initially smaller than rooted cuttings, but high proliferation rates during *in vitro* culture provide greater flexibility for the commercial program in managing plant production to meet expected horticultural demand. *In vitro* plant material also has potential advantages for overcoming some of the export quarantine restrictions associated with *ex vitro* plants or rooted cuttings.

Pests and diseases

The commercial propagation program in Queensland implemented a regular program of pest and disease inspections as soon as plants arrived from the Royal Botanic Gardens in Sydney. Refined methods for Wollemi pine propagation, and the resulting rapid increase in stock plant numbers, created a new series of challenges for the domestication program. A dense canopy of wet leaves above a high-nutrient

substrate in a warm glasshouse or shadehouse can provide a suitable incubation environment for pathogens and pests.

Minor pathogens

A large number of potential pathogens was identified from the commercial program. However, many of the fungi detected were considered as secondary colonisers of dead leaf and stem tissue and not primary pathogens. These included species of *Pestalotiopsis*, *Fusarium*, *Glomerella*, *Phyllosticta*, *Phoma*, *Epicoccum* and *Alternaria*.

Some fungi were identified as minor pathogens and impacted on tree quality, particularly from an aesthetic perspective, and had the potential to affect domestic and international plant sales. These included *Colletotrichum acutatum*, which was identified from leaf blight symptoms on the foliage of cuttings and young *ex vitro* plants growing within climate-controlled polythene chambers and glasshouses. High humidity and extended periods of leaf wetness created ideal conditions for *C. acutatum* to become established, along with high levels of algae, on young Wollemi pine foliage. The disease was not detected as readily on older trees located in the shadehouse environment. A species of *Nectria* was found to be associated with death of cuttings, causing blackened and decayed stems, especially during the warmer summer months. The disease was typified by the presence of orange-red coloured, superficial fruiting bodies (perithecia) on dead and decaying stems of cuttings. The fungus was associated with cuttings that had failed to produce sufficient root systems in polythene chambers, where moisture and humidity levels remained high for extended periods of time. It was not clear whether *Nectria* caused the lack of root formation or whether it occurred as a secondary agent accelerating the decay of cuttings. A new species of *Mycosphaerella*, *M. wollemiae*, was identified as the causal agent of leaf spotting and blighting on older Wollemi pine foliage (Sivanesan and Shivas 2002). Its impact on tree health was limited and it appeared to be restricted to mature foliage in the lower branches.

Two fungal agents, *Fusicoccum* (anamorph of *Botryosphaeria*) and *Phytophthora cinnamomi*, were identified as having a significant impact on the successful establishment of Wollemi pine cuttings and their health and survival during commercial propagation.

Fusicoccum dieback - Fusicoccum spp. (Botryosphaeria spp.)

Fusicoccum dieback was the most severe disease within the commercial propagation program in Queensland. Species of *Fusicoccum* were found to be associated with stem and branch dieback, cankers, wilting and death of older potted trees and stem cankers and decay of cuttings (**Fig. 5A-C**). Pathogenicity tests, using isolates of *Fusicoccum* taken from disease samples, confirmed that *Fusicoccum* was the causal agent. The disease could be recognised by the visible mass of black fruiting bodies (pycnidia) on the upper and lower leaf surfaces and young green stems as well as dying and dead foliage of older potted trees. These fruiting structures were less common on woody stems and symptoms were usually confined to internal tissue discolouration or decay, which on occasions resulted in girdling of the stem, wilting, and tree death.

Fusicoccum dieback was generally associated with plants under stress, with symptoms more readily detected during the warmer wetter months. Water logging appeared to be one of the main triggers for the development of the disease in established plants. The disease was also commonly detected on senescing branches and foliage in the lower canopy particularly in areas where stocking rates were high, leading to elevated humidity levels and increased water logging of the potting mix. Branches affected by other stress events, such as sunburn, also became symptomatic and covered with masses of pycnidia.

Cuttings were also found to be susceptible to infection,

with necrotic areas appearing along the stem rapidly becoming covered in pycnidia. This was generally followed by rapid decay of the entire stem and death of the cutting. These symptoms were also reported by Bullock *et al*. (2000), who artificially inoculated Wollemi pine with *Botryosphaeria*. The frequency of disease was highest during the summer months with minimal deaths apparent in the cooler winter period.

Slippers *et al*. (2005) determined that two species of *Botryosphaeria* were associated with Wollemi pine. Using ITS and β-tubulin sequence data, Slippers *et al*. (2005) showed that both species were previously undescribed, with one closely related to *B. ribis* but sharing unique sequence polymorphisms with *B. parva*. The other grouped with *B. australis* but varied slightly in the gene regions sequenced. The two new species are yet to be described and it is not known whether they occur on other hosts, such as *Agathis* and *Araucaria*. More research is required to determine the origin of these pathogens, host range, and general biology in relation to the infection process of Wollemi pine.

Maintaining tree health and avoiding water logging appeared to aid in the reduction of Fusicoccum dieback and death. Hygiene strategies, including alcohol swabbing of pruning equipment and the use of copper sulphate paste on plant wounds, were implemented to limit the spread of *Fusicoccum* spp. within the propagation facilities. Controlled inoculation experiments demonstrated that tebuconazole (Folicur®), applied as a foliar spray prior to infection, was effective in reducing the size of *Fusicoccum*-induced lesions. It has now been adopted as part of a standard treatment regime within the program. This regime also includes removal of infected material from glasshouse and shadehouse areas to reduce the levels of fungal inoculum.

Phytophthora root rot - Phytophthora cinnamomi

Bullock *et al*. (2000) found that *Phytophthora cinnamomi* causes significant root rot disease on Wollemi pine. They reported that symptoms become obvious 3 weeks after inoculation, with wilting of new tip growth and browning of stems and leaf bases occurring after only four weeks.

Phytophthora cinnamomi was detected in the early stages of the commercial program in Queensland. It was identified as the causal agent of wilting and dieback of some of the original trees transferred from Sydney. Strict hygiene strategies were implemented to eliminate its spread to new cuttings, including disposal of the infected plants and sterilisation of all material used in collecting cuttings. The disease was also detected in association with wilting and death of trial stock plants that were planted in the ground. Patches of trees were dead or dying, with advanced root decay and cankers forming at the root collar (**Fig. 5D**). Trees in the infected area were treated with metalaxyl (Ridomil®), and the area was cordoned off to limit vehicle and pedestrian traffic and prevent spread to other areas of the nursery. Growing of stock plants in the ground was discontinued, and all other stock plants were maintained in pots to avoid infection and spread of *P. cinnamomi* to plants destined for horticultural release. However, given the apparent susceptibility of Wollemi pine to *P. cinnamomi*, this pathogen poses a threat to trees that are planted in gardens, potentially influencing the range of environments where the trees can be grown successfully.

Insect pests

Several insect species were collected while they were feeding on or damaging Wollemi pine plants in the glasshouse, shadehouse or polythene propagation chambers in Queensland. Their effects ranged from minor damage and loss of aesthetic value to defoliation, stem damage, and death. These insects were pests in protected situations, and little information is yet available about insect damage to Wollemi pines in home gardens and amenity plantings.
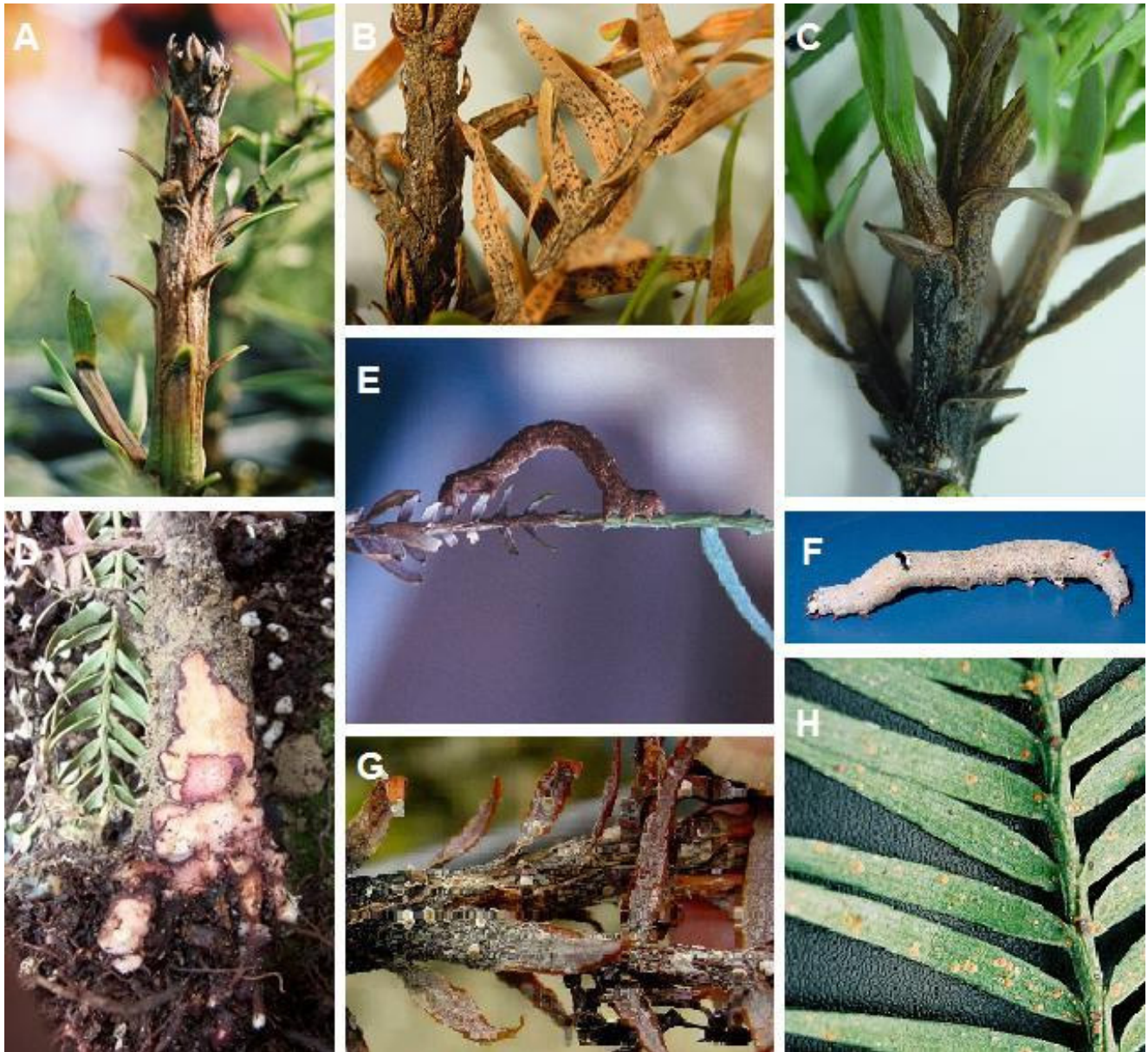
Pests were active throughout the year, but more in sum-

Fig. 5 (**A, B**) Fusicoccum dieback on stems and foliage of dead and dying Wollemi pine. (**C**) Cuttings affected by *Fusicoccum*. (**D**) Root collar lesion associated with infection by *Phytophthora cinnamomi*. (**E**) Early instar brown looper caterpillar. (**F**) Castor oil looper caterpillar. (**G**) Latania scale on dying cutting. (**H**) California red scale on the ventral surface of a Wollemi pine branch.

mer than in winter. Most pests were collected from plants in glasshouse or shadehouse situations where pesticides were applied only as necessary. Very few insects were detected where a routine program of regular insecticide application was in place, with the exception of two species of scale insects that show significant pesticide resistance (Smith *et al*. 1997). All the species collected are known polyphagous insects, some with a wide variety of hosts. Most belonged to two insect orders, Lepidoptera (moths and butterflies) and Hemiptera (bugs), and a single species belonged to order Thysanoptera (thrips).

Lepidoptera

In most Lepidoptera only the larvae (caterpillars) feed on and damage plant tissues. Moths laid eggs on Wollemi pines. The larvae chewed young foliage and soft stems, and larvae of larger moths also chewed mature foliage. Larvae of very small species webbed needles at the growing tips together with silk and fed inside the shelter. The severity of damage depended on the moth species and ranged from minor distortion of the growing tips to defoliation. The loss of tissues stressed and weakened the plants and interfered with growth and form.

Brown looper or Sinister moth (*Pholodes sinistraria*,

Geometridae) (**Fig. 5E**) was the most common and most damaging of the lepidopteran pests. Brown loopers were a constant problem in summer in one of the shadehouses. Their larvae caused extensive loss of foliage, they were voracious feeders, and mature larvae stripped entire stock plants. Castor oil looper (*Achaea janata*, Noctuidae) (**Fig. 5F**) and banana fruit caterpillar (*Tiracola plagiata*, Noctuidae) were sporadic pests but, when present, the larvae caused extensive defoliation, similar to the brown looper.

Several outbreaks of Tussock moth or Painted pine moth (*Orgyia australis*, Lymantriidae) occurred throughout one of the shadehouses, resulting in defoliation of the trees. Newly hatched larvae disperse by 'ballooning'; i.e. they spin a long silken thread, which is picked up and carried by air currents. They can rapidly colonise plants in a confined space. Larvae of the closely related brown tussock moth (*Olene mendosa*, Lymantriidae) were collected once in the same shadehouse.

Some of the plants that arrived from Sydney were infested with light brown apple moth (*Epiphyas postvittana*, Tortricidae). At the growing tips the needles were webbed together and chewed, and the plants were effectively tip pruned. This small leaf-tier is not known to occur in coastal Queensland.

Minor lepidopteran pests, detected only once or very few times during six years of commercial propagation, were:

- Illustraria looper (*Cleora illustraria*, Geometridae);
- Repetita looper (*Cleora repetita*, Geometridae);
- Bark moth (*Ectropis* sp., Geometridae);
- Twig looper (*Ectropis excursaria*, Geometridae);
- Orange fruitborer (*Isotenes miserana*, Tortricidae); and
- Green leaf-tier (*Acropolitis canana*, Tortricidae).

Hemiptera and Thysanoptera

Both adults and nymphs (immatures) of bugs and thrips damaged the Wollemi pine. They fed by piercing or scraping leaf tissues and stems of the host plant and sucking the sap. They often fed together, developing large, localised populations on the host. The loss of water and nutrients stressed the plants, affecting growth. The associated tissue damage could interfere with nutrient uptake, cause distortion, allow entry of pathogens through wounds, and sometimes result in plant death.

Among the pests causing significant damage were scale (**Fig. 5G-H**), including the California red scale (*Aonidiella aurantii*, Diaspididae) and Spanish red scale (*Chrysomphalus dictyospermi*, Diaspididae). These two species are serious pests of other horticultural crops, such as citrus. They occurred together, with very high populations developing mainly on foliage but also stems. Some plants died due to a combination of water and nutrient loss and extensive tissue damage. Infestations were persistent and difficult to control because of the humid atmosphere and crowded canopy in the shadehouses. Citrus trees in the vicinity of the propagation facility were a probable source of the infestation, and were removed.

Long tailed mealy bugs (*Pseudococcus longispinus*, Pseudococcidae) were a particularly difficult problem on cuttings in the glasshouse, where they thrived in the warm moist conditions and caused significant stress and tissue injuries. There were also sporadic infestations in the shade house. These bugs are recognised as severe pests of a variety of horticultural crops and ornamentals (Smith *et al*. 1997). Populations build rapidly, and can be difficult to control chemically because of resistance to many insecticides. Latania scale (*Hemiberlesia lataniae*, Diaspididae) also infested cuttings in the glasshouse. Outbreaks were patchy, but heavily infested cuttings died. This pest, too, was recorded occasionally from potted stock plants in the shadehouse.

Minor hemipteran and thysanopteran pests were:
- Golden mealybug (*Nipaecoccus aurilanatus*, Pseudococcidae). Golden mealy bug is a pest of several *Araucaria* species including *A. bidwillii*, *A. cunninghamii* and *A. norfolkensis*. It was recorded several times on Wollemi pine in a shadehouse that was located close to mature *A. bidwillii* and *A. cunninghamii* trees.
- Spiraea aphid or apple aphid (*Aphis spiraecola*, Aphididae) and cotton aphid or melon aphid (*Aphis gossypii*, Aphididae). Low level infestations of spiraea aphids were occasionally recorded from the shade house. Cotton aphids were collected several times in the glasshouse and shadehouse, but they were heavily parasitised by wasps.
- Greenhouse thrips (*Heliothrips haemorrhoidalis*, Thripidae). These thrips were initially present in large numbers on some stock plants in a glasshouse, but they disappeared when the plants were moved into a shadehouse.

CONCLUSION

The domestication program for Wollemi pine represents a case of the environmental, economic, and social value of plant science. This conifer genus had survived as a small population of less than 100 adult trees in deep ravines of the rugged Wollemi National Park, but its discovery and subsequent international publicity created a new series of threats to its existence. A Wollemi pine recovery plan was formulated, which included plans for establishment of *ex situ* plantings in botanic gardens. The attractiveness of the species, and the extensive media interest, also encouraged its development as an ornamental plant.

Commercial plant production was initially difficult but, by assembling a diverse team of scientists with expertise in plant propagation, tissue culture, physiology, nutrition, pathology, and entomology, refined methods were developed that allowed stock numbers to be raised from 1,000 to several million in five years. However, a note of caution is warranted. Domestication of a poorly-understood species requires a commitment to investment in research, development and routine production, and it may also require major investment in infrastructure and marketing. One advantage of investing in plant propagation research is that small gains in rooting percentage or rooting rate can result in very large gains in final stock numbers because of the exponential nature of the cuttings – stock plant production curve. These gains have their own costs. Expenditure on labour, further infrastructure, and marketing also rise rapidly as the number of stock plants rises, but revenue must be postponed until plant numbers have reached a level sufficient to allow a coordinated market release. Nevertheless, the returns on investment can be more than just economic.

The Wollemi pine can now be viewed, experienced and appreciated in botanic gardens, books, television documentaries and films, making plant science, ornamental horticulture and biological conservation more accessible to many millions of people. The Wollemi pine is also available in many countries as an indoor potted plant or outdoor feature tree, reducing the risk of poaching from the wild population and, most importantly, alleviating the threat of absolute extinction of the species.

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