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Case Study
Plant Propagation for Environmental Offset Planting: A Case Study of Endangered *Pomaderris clivicola* and Near-threatened *Bertya pedicellata*

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Abstract

Background: Environmental offset programs for threatened plant species can be limited by the capacity to propagate sufficient plants for establishing offset plantations. This study describes the use of clonal propagation techniques for an offset program that propagated two species impacted by a landslide and road works. **Materials and Methods:** Cuttings were collected from *Pomaderris clivicola* (Rhamnaceae) and *Bertya pedicellata* (Euphorbiaceae) on 9 and 7 occasions, respectively. Shoots were also initiated into tissue culture. **Results:** Both species proved extremely difficult to propagate from cuttings and tissue culture. Rooting frequencies for *P. clivicola* cuttings were 4.3%. Repeated harvests of cuttings from the impacted plant population over more than 2 years eventually provided most of the *P. clivicola* plants required for the offset planting. However, the offset population of *P. clivicola* also had to be supplemented with some plants produced by tissue culture and with one plant that was excavated from the impacted population. Rooting frequencies for *B. pedicellata* cuttings were only 1.3%. A combination of cutting propagation and tissue culture did not produce sufficient *B. pedicellata* plants for the offset planting, but the offset population was supplemented with 27 plants that were excavated during road works. **Conclusion:** Success in producing the offset plants depended ultimately on a combination of (a) Cutting propagation, (b) Tissue culture propagation and (c) Whole plant excavation and translocation. This case study highlights challenges and successes in propagating poorly-known species for an environmental offset program within a short timeframe and with little prior knowledge of suitable propagation methods.

Key words: Conservation, cuttings, environmental offset, rooting, tissue culture

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Environmental offset programs for human-impacted and threatened plant populations depend upon the capacity to propagate the species, often within short timeframes and with little prior knowledge of suitable propagation methods. The choice of propagation method can be limited by the availability of plant material, such as seeds or actively growing shoots[1,2] . It can also be determined by permit conditions that stipulate the plant parts to be collected from the impacted population and the number and genetic composition of plants that are required in the offset population[3-6].

Propagation of seedlings can provide an offset population that is comprised entirely of genetically unique individuals, but seedling propagation requires a reproductively-mature population with a seed crop as well as reliable techniques to germinate the seed[7,8]. An offset population produced from seeds will not replicate exactly the genetic composition of the impacted population and it will be composed entirely of juvenile plants that may take many years to reproduce, especially for woody species[1,9-12]. Propagation from cuttings or tissue culture can provide a reproductively more-mature offset population that is of the same genetic composition as the impacted population, although this plant production strategy can be challenging if shoots from the impacted population have low amenability to clonal propagation[2,9,13,14].

This study describes a cutting propagation and tissue culture program for two plant species, *Pomaderris clivicola* (Rhamnaceae) and *Bertya pedicellata* (Rhamnaceae) that were impacted by a landslide during a tropical cyclone and by subsequent road remediation works. Vegetative propagation from cuttings is often considered a more convenient and less expensive technique than tissue culture propagation, but tissue culture has the potential to produce many plants rapidly from a limited source of plant material[13-16]. However, as is often the case with environmental offset programs for poorly-known plants, no previous information was available on clonal propagation techniques for the 2 impacted species. This study provides a case study in the use of cutting propagation and tissue culture approaches for an environmental offset program and it will assist in developing propagation methods for other *Pomaderris* and *Bertya* species.

MATERIALS AND METHODS

Study species: *Pomaderris clivicola* and *Bertya pedicellata* are small trees that are listed as 'Endangered' and 'Near-threatened', respectively under the Queensland (Australia) Nature Conservation Act 1992 and Nature Conservation (Wildlife) Regulation 2006. The only known wild populations of *P. clivicola* are the impacted population of approximately 50 plants, a 2nd population of about 1600 plants approximately 45 km East and a 3rd newly-discovered population of several hundred plants approximately 70 km South of the impacted population in Southern Queensland, Australia. Most *P. clivicola* plants are on private land except that 34 plants in the impacted population grew within a road reserve that included the road remediation site. The other species, *B. pedicellata* comprises less than 50 known populations along approximately 750 km of central and Southern Queensland. The *Bertya pedicellata* population at the impacted site comprised several hundred plants. Federal and State permits that were issued to allow road remediation works at the landslide site required that each of 34 *P. clivicola* plants be propagated clonally to establish a genetically-matching population of this endangered species at an environmental offset site. The permits also required propagation of *B. pedicellata* plants from the impacted site but did not require that the offset population of this near-threatened species replicate the genetic composition of the impacted population. The propagation program described in this study was supplemented with a whole-plant translocation program for those individual plants that were impacted directly by the road remediation works[17].

Field methods: Shoots of *P. clivicola* and *B. pedicellata* were collected from within and around the site at Humphery-Binjour road (25°33'S, 151°27'E) that experienced a landslide during cyclone Oswald in January, 2013. The site was on a steep hillside, mean altitude 320 m a.s.l., with red ferrosol soil (Fig. 1).

Shoots of *P. clivicola* were collected from all 4 plants within the impacted site and from 30 plants below the impacted site on 5 September, 2013, 29 October, 2013, 3 December, 2013 and 4 March, 2014 (Table 1). Shoots were also collected from 18 *P. clivicola* plants below the impacted site on 5 subsequent occasions, 8 October, 2014, 14 January, 2015, 26 March, 2015, 22 October, 2015 and 26 November, 2015 (Table 1). The plants were labelled Pc1-Pc34, with the 4 plants within the impacted site being Pc21-Pc24. Shoots were collected from low on the stem, wherever possible in an attempt to obtain vigorous juvenile cuttings[11,12].

Shoots of *B. pedicellata* were collected from 63 plants within the impacted site and from 19 plants below the impacted site on 8 October, 2013 (Table 1). Shoots were also collected from 27 *B. pedicellata* plants within the impacted site and from 16 plants below the impacted site on

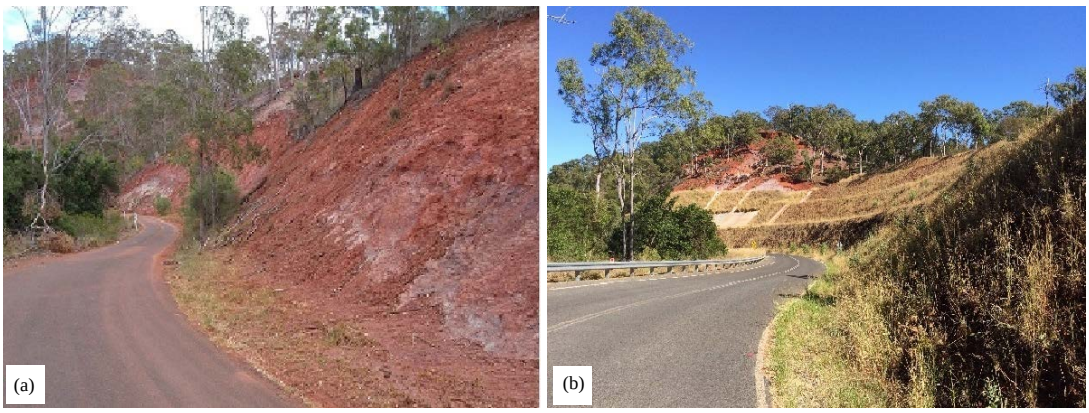

Fig. 1(a-b): Impacted site (a) Before and (b) After earthworks to remedy a landslide across a road. The main land slippage occurred in a gully below the summit in the background of (a) and (b). A deep layer of soil was removed from the road prior to the photograph in (a). Thirty of the 34 *Pomaderris clivicola* plants were in the forest below the road (left of each photograph) while *Bertya pedicellata* plants were scattered below within and above the earthwork site

Table 1: Collection dates, No. of plants sampled, No. of cuttings set per plant and percentage of cuttings that formed roots from *Pomaderris clivicola* and *Bertya pedicellata* at the natural population at Binjour (B) and the environmental offset planting at Gurgeena (G)

Collection date	No. of *P. clivicola* plants	Maximum No. of cuttings per *P. clivicola* plant	*Pomaderris clivicola* cuttings with roots (%)	No. of *B. pedicellata* plants	Maximum No. of cuttings per *B. pedicellata* plant	*Bertya pedicellata* cuttings with roots (%)
25 September, 2013	34 (B)	40	3.3±2.2	-	-	-
8 October, 2013	-	-	-	84 (B)	20	0.1±0.1
29 October, 2013	34 (B)	20	3.0±1.4	-	-	-
3 December, 2013	34 (B)	20	3.8±1.4	43 (B)	20	0.2±0.2
4 March, 2014	34 (B)	40	0.1±0.1	-	-	-
8 October, 2014	18 (B)	40	0	10 (B)	40	0
14 January, 2015	18 (B)	40	15.7±4.3	14 (B)	80	7.2±2.7
26 March, 2015	18 (B)	20	4.3±2.2	38 (B)	20	0.8±0.8
22 October, 2015	18 (B)	40	0	23 (G)	40	0
26 November, 2015	18 (B)	40	8.6±3.2	23 (G)	40	0.6±0.3
Mean rooting (%)			4.3±0.5			1.3±1.0

Means are provided with standard errors

3 December, 2013. Shoots were again collected from 10 *B. pedicellata* plants below the impacted site on 8 October, 2014, from 3 plants below the impacted site and 11 plants above the impacted site on 14 January, 2015 and from 38 plants above the impacted site on 26 March, 2015 (Table 1). The plants were labelled Bp1-Bp120 with the plants within the impacted site being Bp20-Bp82. Shoots were also collected on 22 October, 2015 and 26 November, 2015 (Table 1) from 23 plants established by whole-plant translocation[17] at the environmental offset planting at Gurgeena (25°27'S, 151°23'E).

The shoots from each plant were placed in a plastic clip-lock bag with a light spray of water and the bags were kept cool in tubs containing ice-bricks. The shoots were transported overnight on each occasion to the University of the Sunshine Coast (26°43'S, 153°04'E). Shoots were also collected during routine pruning of potted plants that were produced from cuttings or tissue culture. These shoots were also placed in plastic clip-lock bags with a light spray of water, but were used on the same day rather than being transported overnight.

Propagation of cuttings: The shoots were dissected into apical cuttings, ~5 cm length without pruning of leaves. Forty cuttings per plant, when available were dissected on the1st, 5th, 6th, 7th, 9th and 10th occasions and 20 cuttings per plant, when available were dissected on the 2nd, 3rd, 4th and 8th occasions (Table 1). An additional 40 cuttings were dissected from two *B. pedicellata* plants, Bp104 and Bp105 on the 7th occasion (Table 1) because they were suspected to possess a more-juvenile type of foliage. Forty cuttings per plant, when available were dissected from potted plants during routine pruning.

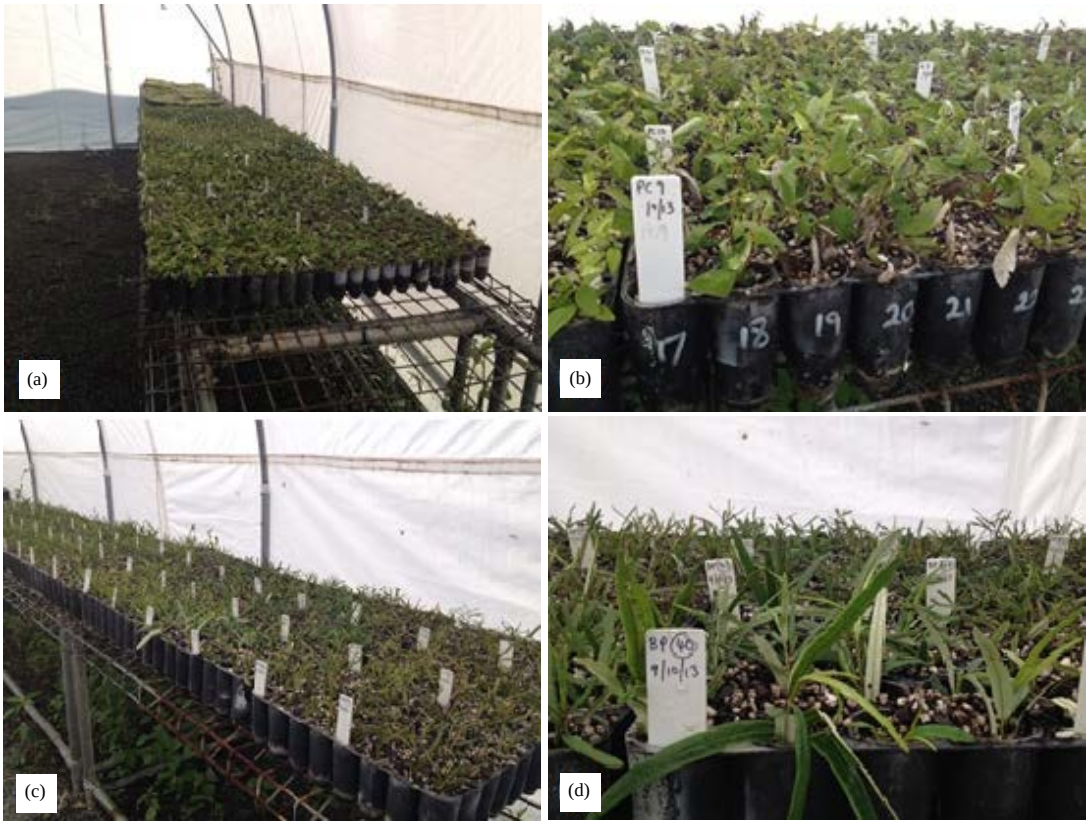

Fig. 2(a-d): Propagation of (a, b) *Pomaderris clivicola* and (c, d) *Bertya pedicellata* cuttings in a polyethylene misting chamber

Each cutting was dipped 0.5 cm into powder containing 3 g kg^{-1} IBA for 1 sec[18-20] and placed 1 cm deep into a 70 mL hyco-propagation tube containing potting mix. The potting mix consisted of a 75/25 (v/v) mixture of perlite and shredded pine bark with 3 kg of 8-9 month Osmocote Natives™ fertiliser (Scotts International, Heerlen, the Netherlands) and 1 kg gypsum (Queensland organics, Narangba, Australia) incorporated per meter cube[21,22]. The propagation trays were placed under mist irrigation in a translucent white polyethylene chamber (Fig. 2) or glasshouse with misting provided for varying durations from 45 sec every 10 min to 60 sec every 30 min, depending on the season. Each cutting remained under mist irrigation until roots had protruded through the base of the propagation tube or the cutting died.

Cuttings with roots were transferred to 1.6 L pots containing the same potting mix. The pots were initially kept in the misting chamber or glasshouse before they were moved to a glasshouse cell with additional 50% shade cloth. There, they received overhead watering for 3 min, 4 times per day. The plants were moved outdoors under 50% shade cloth at least 6 weeks before transfer to the offset site and the shade-cloth was removed at least 2 weeks before transfer to the offset site. During this period, the plants received overhead watering for 10-30 min, either twice or 3 times per day, depending on the season. Plants were transplanted into 9 or 16 L pots if they were being maintained in the nursery for longer than 6 months. Propagation from individual *P. clivicola* genotypes (i.e., original wild plants) was discontinued when at least 20 plants from that genotype were established in the nursery.

Tissue culture: Excess shoots from the 1st, 2nd and 4th collections (Table 1) were placed in a cold room at 4°C[23] on 26 September, 2013, 9 October, 2013 and 4 December, 2013, respectively. Shoot tips and nodes from each of the 34 *P. clivicola* plants and 41 of the *B. pedicellata* plants were dissected to ~3 mm length on 27-28 September, 2013 and 10-11 October, 2013, respectively to initiate *in vitro* cultures. Fresh shoot tips and nodes were also dissected from each of 18 *P. clivicola* plants and 18 *B. pedicellata* plants on 5-6 December, 2013 to initiate cultures.

All macroscopic leaves were removed from each dissected shoot and the shoots were washed in 70% ethanol (v/v) for 1 min in 70 mL vials containing one drop of tween 20. They were then rinsed in sterile distilled water for 1 min and

transferred into new vials containing 3% sodium hypochlorite (most explants) or 1% sodium hypochlorite (*B. pedicellata* in December, 2013) with one drop of tween[24] 20. The vials were swirled for 20 min on an orbital shaker at 160 rpm and the shoots were rinsed in sterile distilled water. Shoots were placed on sterile paper to remove excess liquid between solutions. Shoots were then plated (five shoots per 90 mm petri dish with two dishes per donor plant) onto shoot induction medium[16,25]. This medium consisted of half-strength Murashige and Skoog (MS) medium with 30 g L^{-1} sucrose, solidified with 8 g L^{-1} agar and with pH adjusted to 5.8 prior to autoclaving (121°C, 20 min). The shoots were maintained at 25°C for 4 weeks under a 16 h photoperiod (~50 μmol m^{-2} sec^{-1} with fluorescent tubes).

Uncontaminated shoots from each donor plant were then transferred to 90 mm petri dishes containing shoot proliferation medium[16,25] and maintained for one 4 weeks passage at 25°C under a 16 h photoperiod (approximately 100 μmol m^{-2} sec^{-1}). Shoot proliferation medium consisted of full-strength MS medium with 30 g L^{-1} sucrose and 4.4 μM Benzyl Adenine (BA), solidified with 8 g L^{-1} agar and with pH 5.8. All shoots were then transferred to 375 mL glass jars containing 50 mL of shoot proliferation medium and proliferated in these jars during 4 weeks passages at 25°C under a 16 h photoperiod (approximately 100 μmol m^{-2} sec^{-1}). Cultures from most donor plants continued to produce shoots in shoot proliferation medium. However, cultures from some *P. clivicola* genotypes produced embryogenic callus from which somatic embryos emerged. Embryogenic cultures were maintained at lower irradiance (approximately 50 μmol m^{-2} sec^{-1}).

Subsamples of shoots of at least 15 mm length were selected periodically for root induction. The medium for root induction was the same as the shoot induction medium except that it also contained 314.9 μM IBA[14]. The shoots in this root induction medium were placed in darkness for 7 days at 25°C to allow formation of root primordia. They were then transferred to shoot induction medium and maintained at 25°C under a 16 h photoperiod (approximately 100 μmol m^{-2} sec^{-1}).

Plantlets or somatic emblings were transplanted into punnets containing sterile potting mix, based on the *in vitro* soil-less (IVS) method of Newell *et al.*[26,27] and Dwan and Trueman[14]. The punnets, each containing 15 tubes of 12 mL were placed in sterile 1 L plastic containers that were then covered with another plastic container to provide a volume of 2 L[14]. The sealed punnets were maintained at 25°C under a 16 h photoperiod (approximately 50 μmol m^{-2} sec^{-1}). The lids were removed from the punnets and the plantlets or emblings were moved to a glasshouse with additional 50% shade cloth when newly emerged leaves had expanded fully. The plants received overhead watering in the glasshouse for 3 min, 4 times per day. They were then transferred to 1.6 L pots containing potting mix and maintained in the same manner as the plants produced from cuttings.

Offset planting: Twenty and 34 potted plants of *P. clivicola* (30-70 cm tall) were transferred to the environmental offset site at Gurgeena for planting on 14 January, 2015 and 22 May, 2015, respectively. A further 143 *P. clivicola* plants (20-140 cm tall) and 40 *B. pedicellata* plants (20-110 cm tall) were transferred to the offset site for planting on 4 April, 2016. The site described by Haskard[17] was prepared and planted by a commercial contractor. Survival and height of the offset plants were measured on 24 May, 2016 prior to light rain between 27 May and 3 June, 2016 and heavy rain (40 mm) on 4 June, 2016 that helped to firmly establish the final batch of plants. Tree height and rooting means are presented with standard errors.

RESULTS AND DISCUSSION

The endangered species, *P. clivicola* had low amenability to propagation from cuttings. Few or no cuttings from 3 of the 9 shoot collections produced roots; however, between 3.0 and 15.7% of *P. clivicola* cuttings from the other 6 collections produced roots, resulting in an overall rooting frequency of 4.3% (Table 1). Rooting capacity differed between shoots from different trees, so that for example, 20 potted plants had been produced from each of trees Pc16 and Pc19 (Table 2) after just two shoot collections. In contrast, no cuttings of tree Pc32 produced roots until the final shoot collection, more than 2 years later and trees Pc22, Pc23 and Pc31 were not propagated successfully from cuttings (Table 2). Trees Pc16 and Pc19 possessed atypical shoots that arose from low positions on the tree trunk and which had very narrow stems and long internodes. Their high rooting capacity, basal location and unusual morphology suggest that these shoots were more juvenile than other *P. clivicola* shoots, possibly having arisen from cells laid down early in the life of the tree[11,12,28].

A total of 124 potted plants of *P. clivicola* were produced from the cuttings obtained from wild plants. These represented 31 of 34 plants at the impacted site. Routine pruning of the potted plants in the nursery yielded more cuttings and these had a higher rooting capacity (13.8±3.6%)

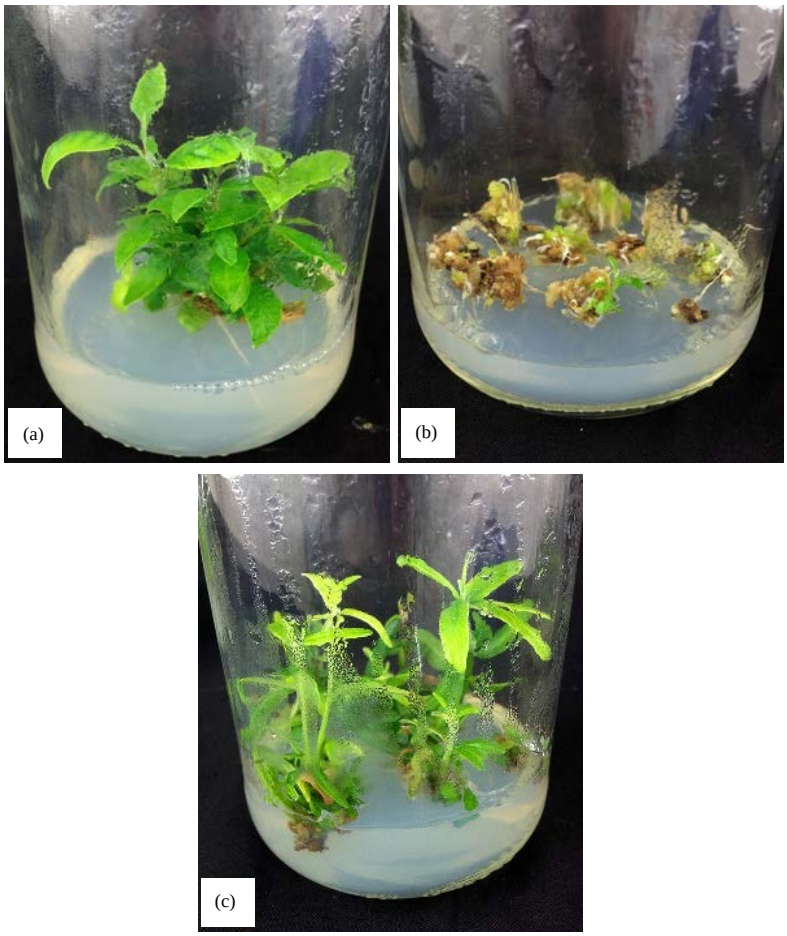

Fig. 3(a-c): Propagation of *Pomaderris clivicola* and *Bertya pedicellata* in tissue culture, (a) *P. clivicola* shoots with one root visible, (b) *P. clivicola* emblings and (c) *B. pedicellata* shoots

Table 2: Final numbers of *Pomaderris clivicola* potted plants produced by cuttings or tissue culture for establishment at the environmental offset planting

Original plant number	No. from cuttings	No. from tissue culture	Original plant number	No. from cuttings	No. from tissue culture
Pc1	5	-	Pc18	1	-
Pc2	2	-	Pc19	20	-
Pc3	2	-	Pc20	14	-
Pc4	10	-	Pc21	1	-
Pc5	2	-	Pc22*	-*	-*
Pc6	1	-	Pc23*	-*	4
Pc7	1	-	Pc24*	8	-*
Pc8	2	19	Pc25	6	-
Pc9	5	-	Pc26	2	-
Pc10	3	-	Pc27	7	-
Pc11	4	-	Pc28	1	-
Pc12	2	-	Pc29	1	-
Pc13	4	-	Pc30	3	-
Pc14	2	-	Pc31	-	4
Pc15	6	-	Pc32	4	-
Pc16	20	-	Pc33	17	-
Pc17	1	-	Pc34	13	-

Total number of potted plants: 197, *Pc22, Pc23 and Pc24 were established at the environmental offset planting by whole-plant translocation[17]

than wild cuttings, presumably reflecting more-optimal plant water and nutrient status in the nursery[29-31]. These nursery-harvested cuttings provided an additional 46 potted plants for the environmental offset planting. These 46 plants

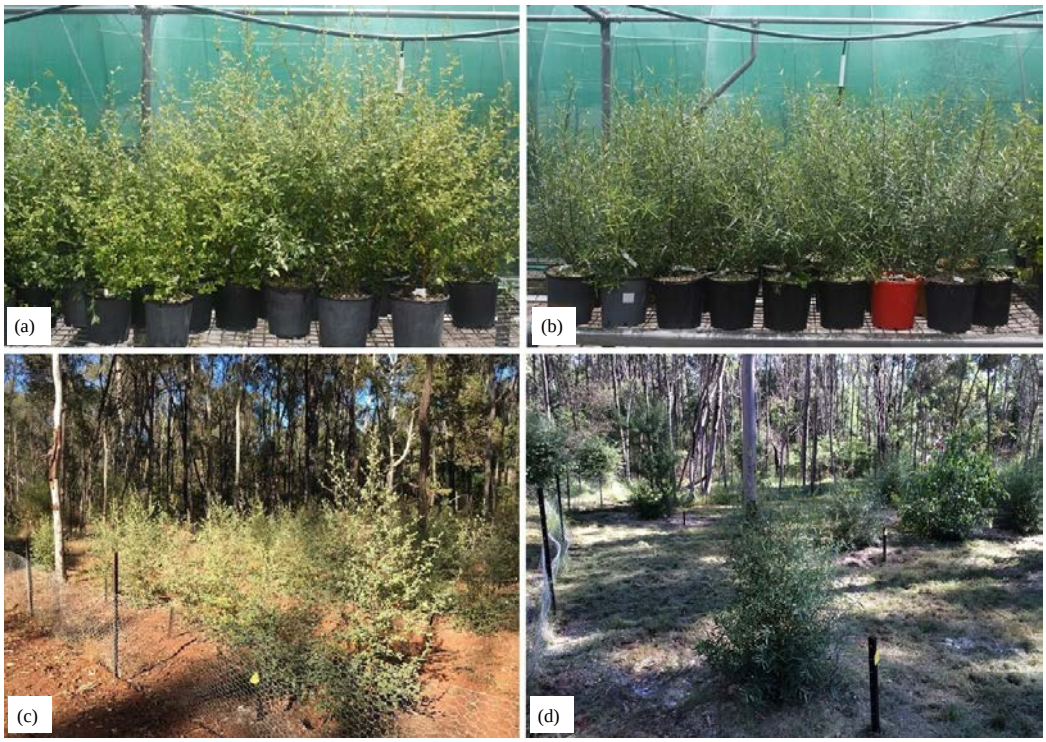

Fig. 4(a-d): Potted plants of (a) *Pomaderris clivicola*, (b) *Bertya pedicellata* in the nursery and established plants of (c) *Pomaderris clivicola* and (d) *Bertya pedicellata* at the environmental offset planting. Each plant at the offset site is marked with a steel peg bearing a yellow number tag

did not add to the genetic diversity captured by the offset program but they did provide additional plants in the event of plant deaths in the nursery or at the offset site.

Shoots from 4 of the 34 wild *P. clivicola* plants were established in tissue culture. The Pc8 and Pc31 produced conventional shoot cultures (Fig. 3a). The rooting frequencies of their shoots were 25.3 and 20.0%, respectively and plantlets from both trees were acclimatised to nursery conditions (Table 2). The shoot proliferation medium for *P. clivicola* contained the cytokinin, BA at 4.4 mM which is generally used in a conventional shoot culture system to stimulate the formation of new shoots from existing lateral buds or from *de novo* adventitious buds[14,16,25,32]. However, cultures from two other *P. clivicola* trees, Pc22 and Pc23 instead developed embryogenic callus on this medium. Embryogenesis is unusual on medium with this low level of cytokinin although it has been observed previously with *Abies fraseri*[33]. Somatic embryogenesis has the capacity to produce an extremely large number of cloned plants[15,34,35] but this was unnecessary for *P. clivicola* because only one plant was required to be established at the offset site from each of the 34 wild plants. Fully-developed somatic emblings (Fig. 3b) emerged from Pc23 but not from Pc22. The number of potted plants of *P. clivicola* (Fig. 4a) that arose from tissue culture was 27 and importantly, 2 of the 3 wild trees that had not been captured by cuttings were propagated successfully by tissue culture (Table 2).

The remaining wild *P. clivicola* tree that was not propagated successfully by cuttings or tissue culture, Pc22 was 1 of the 3 trees planted at the environmental offset site (Table 2) as part of the whole-plant translocation program[17]. All three of these translocated *P. clivicola* trees survived and became established at the offset site. Their latest recorded heights are 3.42±0.27 m and flowering has occurred on these trees. All 54 of the *P. clivicola* potted plants that were planted in 2015 have been established successfully (Fig. 4c) with latest tree heights being 1.62±0.08 and 1.68±0.04 m for the January, 2015 and May, 2015 plantings, respectively. In addition, 130 of the 143 *P. clivicola* potted plants that were planted in 2016 have been established. Their latest heights are 0.97±0.03 m. Thirteen plants that did not survive were generally short plants of 20-30 cm height at planting time. Importantly, all of the original wild *P. clivicola* plants are represented in the offset population. Therefore, the program has been successful in establishing each of 34 *P. clivicola* plants as a genetically-matching population at the environmental offset site.

Table 3: Final numbers of *Bertya pedicellata* potted plants produced by cuttings or tissue culture for establishment at the environmental offset planting

Original plant number	No. from cuttings	No. from tissue culture
Bp6	1	-
Bp11	-	5
Bp31*	-	5
Bp37*	1	-
Bp54*	-	2
Bp85	1	-
Bp101	1	-
Bp102	1	-
Bp104	21	-
Bp105	1	-
Bp108	1	-

Total number of potted plants: 40, *27 plants among Bp20-Bp82 were established at the environmental offset planting by whole-plant translocation[17]

The near-threatened species, *B. pedicellata* was much more difficult than *P. clivicola* to propagate from cuttings. The overall rooting frequency for *B. pedicellata* cuttings was 1.3% with rooting frequencies consistently lower than 1% except for shoots collected on 14 January, 2015 (Table 1). On this occasion, 28.7% of cuttings from one plant, Bp104 produced roots. A total of 25 potted plants of *B. pedicellata* were produced from the cuttings obtained from wild plants. These represented eight of the plants at the impacted site (Table 3). Routine pruning of the potted *B. pedicellata* plants in the nursery yielded only three more potted plants, all of which were obtained from Bp104. Therefore, Bp104 which was suspected of possessing juvenile shoots, contributed 21 of the 28 potted plants that were propagated from cuttings (Table 3).

Shoots from 6 of the wild *B. pedicellata* plants were established in tissue culture. These produced conventional shoot cultures (Fig. 3c). Rooting was not obtained on shoots from 3 of the 6 trees, but rooting frequencies of 27.7, 26.5 and 21.4% were obtained from *in vitro* shoots of Bp11, Bp31 and Bp54, respectively. Twelve of 22 plantlets were acclimatised successfully in the nursery (Table 3). These raised the number of potted plants of *B. pedicellata* (Fig. 4b) from 28-40 and increased the number of wild trees propagated successfully from 8-11. In addition, 27 *B. pedicellata* plants were excavated from the impacted site and planted at the offset site[17]. All 27 of these translocated plants survived and became established at the offset site (Fig. 4d), as have all 40 of the potted plants. Their latest recorded heights are 2.77±0.10 and 0.90±0.03 m, respectively. Extensive flowering has occurred on the translocated and propagated plants at the offset site with many honey bees (*Apis mellifera*) observed foraging at *B. pedicellata* flowers. In total, 67 *B. pedicellata* plants have been propagated or translocated successfully during the environmental offset program.

Pomaderris clivicola and *B. pedicellata* proved to be extremely difficult to propagate, both from cuttings collected in the wild and from cuttings collected in the nursery. Commercial nurseries generally prefer to propagate species or varieties with greater than 70% rooting[36,37]. Many species, particularly from dryland ecosystems, such as at the study sites have very low rooting capacity[38,39] possibly because adventitious rooting has often evolved as an adaptive response to flooding or waterlogging[40,41]. Rooting capacity can also vary across seasons, especially between warmer periods in spring/summer and cooler periods in autumn/winter[21,22,30,31]. Both species in the offset program had very low rooting from wild cuttings collected from early spring through summer to early autumn, as well as from nursery cuttings collected under controlled environmental conditions. Rooting capacity did vary among genotypes although this might have been related to different levels of maturation of the shoots from different trees. Cuttings are usually much easier to propagate from juvenile than from mature shoots[42-45] but it is often difficult to identify and source juvenile shoots among an established mature population of wild trees. Juvenile shoots are most likely to be obtained from a "Cone of juvenility" near the base of the tree[11]. The two most-easily propagated *P. clivicola* trees, Pc16 and Pc19 both possessed basal stems of atypical morphology that suggested the shoots were derived from juvenile tissue. The only easily-propagated *B. pedicellata* tree, Bp104 possessed stems with unusual foliage that suggested these shoots might also be juvenile.

CONCLUSION

The propagation and translocation program achieved its aims of establishing a genetically-matching population of *P. clivicola* and replacing impacted *B. pedicellata* plants with a new population at the environmental offset site. However, the program proved challenging because both species were extremely difficult to propagate from both cuttings and tissue culture. Success relied ultimately on a combination of: (a) Repeated collection of shoots from the impacted wild populations, (b) The combined use of both cuttings and tissue culture techniques and (c) The excavation and translocation of whole plants.

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