Propagation of *Pomaderris clivicola* and *Bertya pedicellata*

FINAL REPORT

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By

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Summary

This project attempted to propagate *Pomaderris clivicola* and *Bertya pedicellata* plants from a site assigned for remediation of the Humphery-Binjour Road by the North Burnett Regional Council.

The project aimed to produce a minimum of 34 *P. clivicola* potted plants by propagating all of the 34 plants within the site population. The project also attempted to produce as many *B. pedicellata* potted plants as possible by propagating from impacted plants and adjacent plants within the site population. This project complemented a separate project that excavated and translocated whole plants from the road remediation site to an offset management site.

Both *P. clivicola* and *B. pedicellata* were extremely difficult to propagate. However, cutting propagation and tissue culture were used successfully to raise potted plants of both species. Propagation of *P. clivicola* was mainly from cuttings, supplemented with tissue culture and the previous whole-plant translocations. Propagation of *B. pedicellata* was from a mixture of cuttings and tissue culture, augmented greatly by the previous whole-plant translocations.

All 34 of the 34 *P. clivicola* wild plants have been propagated or established successfully at the offset management site. 33 of these wild plants were propagated during this project, while the remaining wild plant was established successfully at the offset management site during the previous translocation project.

66 *B. pedicellata* plants have been propagated or established successfully at the offset management site. 39 potted plants were propagated during this project, while 27 wild plants were established successfully at the offset management site during the previous translocation project.

54 potted *P. clivicola* plants, representing 16 of the wild plants, were established at the offset management site in 2015. The remaining potted plants of *P. clivicola* and *B. pedicellata* are available now for planting at the offset management site.

Introduction

Populations of *Pomaderris clivicola* and *Bertya pedicellata* were identified within and adjacent to a site assigned for remediation of the Humphery-Binjour Road by the North Burnett Regional Council. This project attempted to produce a minimum of 34 *P. clivicola* potted plants by propagating all of the 34 plants within the site population. The project also attempted to produce *B. pedicellata* potted plants by propagating from impacted plants and adjacent plants within the site population. Plant production was attempted from both cuttings and tissue culture.

Methods

Field methods

Shoots of *P. clivicola* and *B. pedicellata* were collected from within and below the site that was to be impacted by remediation of the Humphery-Binjour Road (25°33'S, 151°27'E) following a major land-slip in January 2013. The site was on a steep hillside, mean altitude approximately 320 m, with red ferrosol soil.

Shoots of *P. clivicola* were collected from all four plants within the impact site and from 30 plants below the impact site on 5 Sep 2013, 29 Oct 2013, 3 Dec 2013 and 4 Mar 2014 (Table 1). Shoots were also collected from 18 *P. clivicola* plants below the impact site on five subsequent occasions, 8 Oct 2014, 14 Jan 2015, 26 Mar 2015, 22 Oct 2015 and 26 Nov 2015 (Table 1). The plants were labelled Pc1 – Pc34, with the four plants within the impact site being Pc21 – Pc24.

Shoots of *B. pedicellata* were collected from 63 plants within the impact site and from 19 plants below the impact site on 8 Oct 2013 (Table 1). Shoots were also collected from 27 *B. pedicellata* plants within the impact site and from 16 plants below the impact site on 3 Dec 2013. Shoots were again collected from 10 *B. pedicellata* plants below the impact site on 8 Oct 2014, from 3 plants below the impact site and 11 plants above the impact site on 14 Jan 2015, and from 38 plants above the impact site on 26 Mar 2015 (Table 1). The plants were labelled Bp1 – Bp120, with the plants within the impact site being Bp20 – Bp82. Shoots were also collected on 22 Oct 2015 and 26 Nov 2015 (Table 1) from 23 plants established at the offset site 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 *P. clivicola* and *B. pedicellata* 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 the first, fifth, sixth, seventh, ninth and tenth occasions, and 20 cuttings per plant, when available, were dissected on the second, third, fourth and eighth occasions (Table 1). Forty cuttings per plant, when available, were dissected from potted plants during routine pruning.

Table 1. Collection dates, number of plants sampled, and number of cuttings set per plant from* Pomaderris clivicola *and* Bertya pedicellata *at the natural population at Binjour (B) and the offset planting at Gurgeena (G)

Collection date	Number and location of *P. clivicola* plants	Maximum number of cuttings per *P. clivicola* plant	Number and location of *B. pedicellata* plants	Maximum number of cuttings per *B. pedicellata* plant
1. 25 Sep 2013	34 (B)	40	—	—
2. 8 Oct 2013	—	—	84 (B)	20
3. 29 Oct 2013	34 (B)	20	—	—
4. 3 Dec 2013	34 (B)	20	43 (B)	20
5. 4 Mar 2014	34 (B)	40	—	—
6. 8 Oct 2014	18 (B)	40	10 (B)	40
7. 14 Jan 2015	18 (B)	40	14 (B)	40
8. 26 Mar 2015	18 (B)	20	38 (B)	20
9. 22 Oct 2015	18 (B)	40	23 (G)	40
10. 26 Nov 2015	18 (B)	40	23 (G)	40

Each cutting was dipped 0.5 cm into powder containing 3 g kg^{-1} IBA for 1 s (Kilkenny et al. 2012; Trueman and Adkins 2013) and placed 1 cm deep into a 70-mL Hyco propagation tube containing propagation mix. The propagation mix consisted of a 75/25 (v/v) mixture of perlite and shredded pine bark, with 3 kg of 8-9 month Osmocote™ fertiliser (Scotts International, Heerlen, the Netherlands) and 1 kg gypsum (Queensland Organics, Narangba, QLD) incorporated per m^3 (Trueman *et al.* 2013a, b).

The propagation trays were placed under mist irrigation in a translucent white polyethylene chamber (Fig. 1) or glasshouse, with misting provided for varying durations from 45 s every 10 min to 60 s 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 had died.

Cuttings with roots were transferred to 1.6-L pots containing the eucalypt seedling mixture described by Trueman et al. (2013a, b). 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, four 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 varying durations from 10 min to 30 min either twice or three times per day, depending on the season. Plants were transplanted into 9-L or 16-L pots if they were being maintained in the nursery for longer than 6 months.

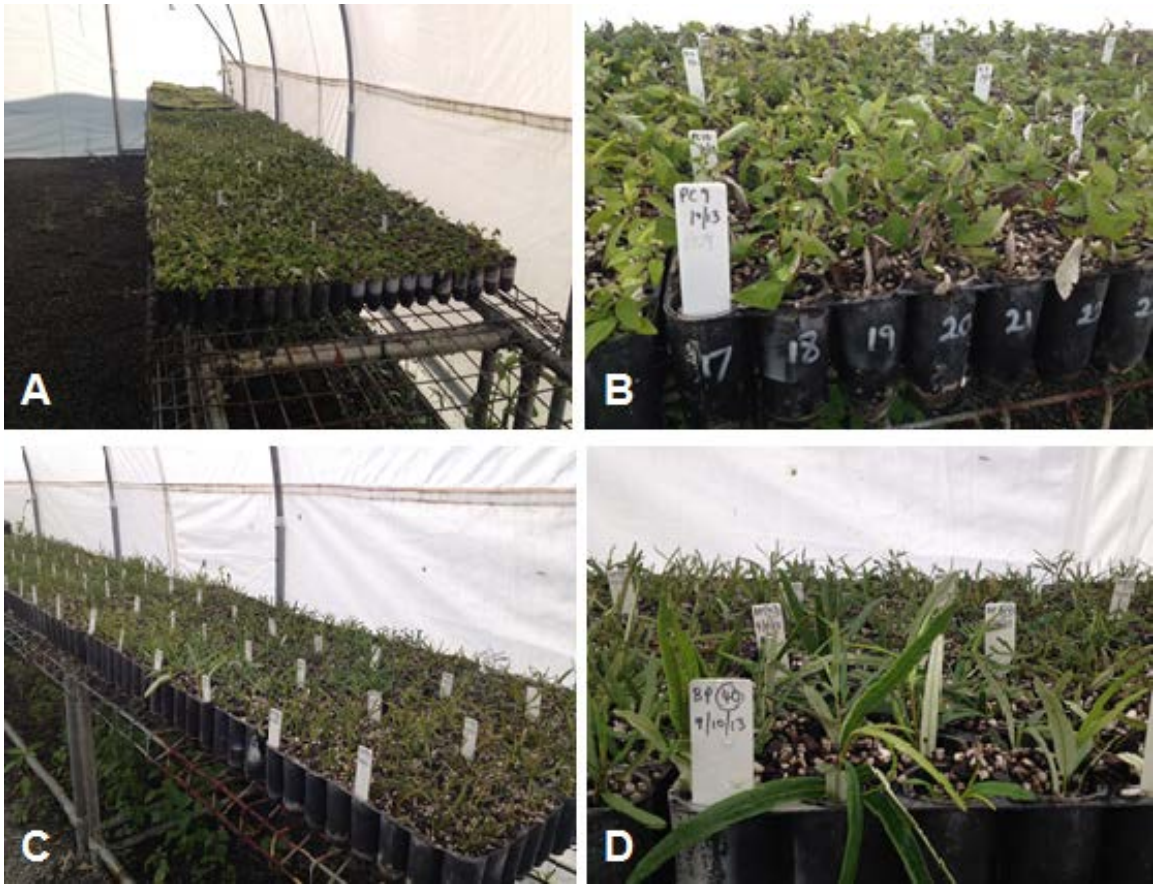

Fig. 1. Propagation of* (A, B) Pomaderris clivicola *and* (C, D) Bertya pedicellata *cuttings in a misting chamber.

Tissue culture

Excess shoots from the first, second and fourth collections (Table 1) were placed in a cold room at 4ºC (Kowalski and van Staden 1998) on 26 Sep 2013, 9 Oct 2013 and 4 Dec 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 Sep 2013 and 10–11 Oct 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 Dec 2013 to initiate additional 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 Dec 2013) with one drop of Tween 20 (Trueman and Richardson 2007). 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 (Hung and Trueman 2011, 2012). 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 (121ºC, 20 min). The shoots were maintained at 25ºC for 4 weeks under a 16-h photoperiod (~50 µmol m^{-2} s^{-1} with fluorescent tubes).

Uncontaminated shoots from each donor plant were then transferred to 90-mm Petri dishes containing shoot proliferation medium (Hung and Trueman 2011, 2012) and maintained for one 4-week passage at 25ºC under a 16-h photoperiod (approx. 100 µmol m^{-2} s^{-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-week passages at 25ºC under a 16-h photoperiod (approx. 100 µmol m^{-2} s^{-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 (approx. 50 µmol m^{-2} s^{-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 (Dwan and Trueman 2014). The shoots in this root induction medium were placed in darkness for 7 d at 25ºC to allow formation of root primordia. They were then transferred to shoot induction medium and maintained at 25ºC under a 16-h photoperiod (approx. 100 µmol m^{-2} s^{-1}).

Plantlets or somatic embryos were transplanted into punnets containing sterile potting mix, based on the *in vitro* soil-less (IVS) method of Newell et al. (2003, 2005) and Dwan and Trueman (2014). The punnets, each containing fifteen 12-mL tubes, were placed in sterile 1-L plastic containers that were then covered with another plastic container to provide a volume of 2 L (see Dwan and Trueman 2014). The sealed punnets were maintained at 25ºC under a 16-h photoperiod (approx. 50 µmol m^{-2} s^{-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. Plantlets received overhead watering in the glasshouse for 3 min, four times per day. They were then transferred to 1.6-L pots containing the eucalypt seedling mixture described by Trueman et al. (2013a, b), and maintained in the same manner as the plants produced from cuttings (above).

Results and Discussion

Pomaderris clivicola

All of the 34 *P. clivicola* plants have been propagated or translocated successfully (Table 2).

Thirty-one of the wild plants were propagated successfully by cuttings, and three of the wild plants were propagated successfully by tissue culture (Table 2). One of these plants, Pc8, was propagated successfully by both cuttings and tissue culture. Therefore, one wild plant, Pc22, was not propagated successfully by either cuttings or tissue culture but this plant was excavated and established successfully at the offset site at Gurgeena (Table 2). The one wild plant that died after excavation from the Humphery-Binjour Road remediation site, Pc21, was already propagated successfully from cuttings.

Two of the *P. clivicola* plants, Pc8 and Pc31, that were propagated in tissue culture were produced using conventional shoot cultures (Fig. 2A). The other plant that was propagated in tissue culture, Pc23, produced somatic embryos (Fig. 2B). Both types of culture provided fully-acclimatised plants in the nursery (Fig. 3).

Fifty-four potted plants, representing 16 of the wild *P. clivicola* plants, were transferred to the offset site in January and May 2015 (Table 2). The remaining *P. clivicola* plants are available now for planting at the offset management site at Gurgeena.

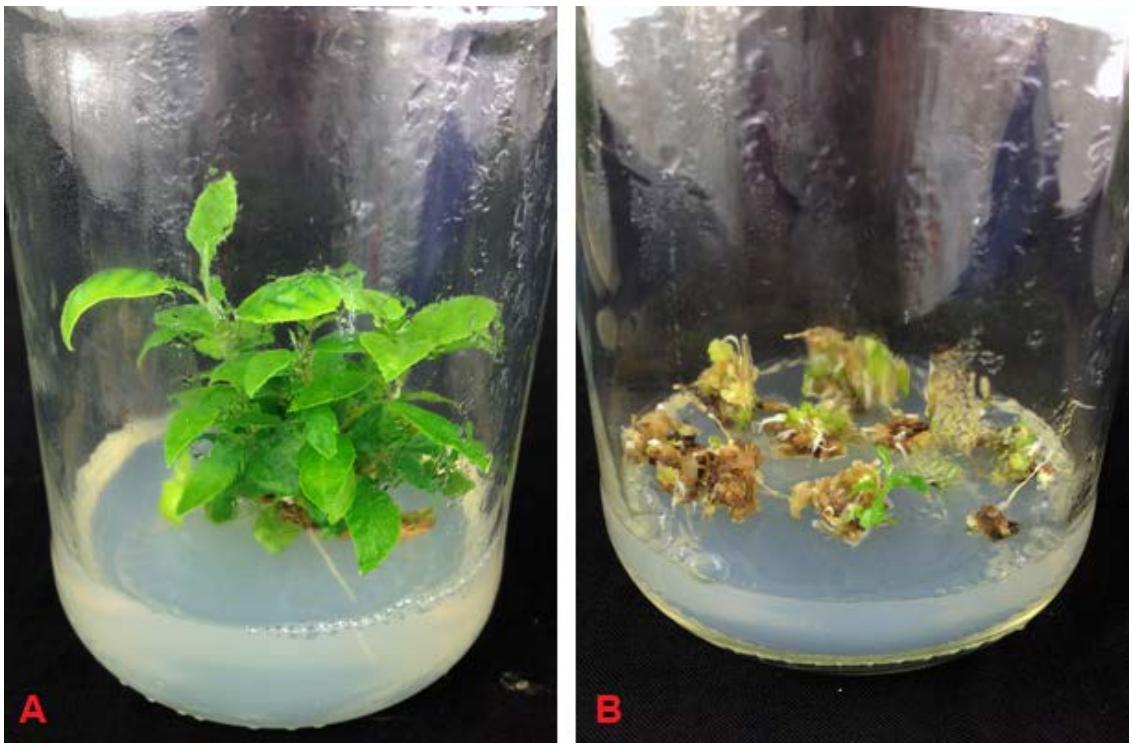

Fig. 2.* Pomaderris clivicola *in tissue culture: (A) shoots (with one root visible) and (B) somatic embryos.

Table 2. Propagation of* Pomaderris clivicola (Pc) *by cuttings and tissue culture as at 23 February 2016.
Green: successfully propagated. Blue: excavated wild plants.

Propagation by cuttings	Propagation by tissue culture
Pc1: 4 plants (2 at Gurgeena)	Pc1
Pc2: 2 plants	Pc2
Pc3: 2 plants (1 at Gurgeena)	Pc3
Pc4: 4 plants (already at Gurgeena)	Pc4
Pc5: 2 plants	Pc5
Pc6: 1 plant (already at Gurgeena)	Pc6
Pc7: 1 plant	Pc7
Pc8: 2 plants	Pc8: 19 plants (1 at Gurgeena)
Pc9: 5 plants	Pc9
Pc10: 3 plants	Pc10
Pc11: 4 plants	Pc11
Pc12: 2 plants (1 at Gurgeena)	Pc12
Pc13: 4 plants	Pc13
Pc14: 2 plants (1 at Gurgeena)	Pc14
Pc15: 6 plants	Pc15
Pc16: 20 plants (already at Gurgeena)	Pc16
Pc17: 1 plant	Pc17
Pc18: 1 plant (already at Gurgeena)	Pc18
Pc19: 20 plants (12 at Gurgeena)	Pc19
Pc20: 14 plants	Pc20
Pc21: 1 plant (already at Gurgeena)	Pc21
Pc22: 1 wild plant translocated*	Pc22
Pc23: 1 wild plant translocated*	Pc23: 4 plants (1 at Gurgeena)
Pc24: 8 plants (2 at Gurgeena) + 1 wild plant translocated*	Pc24
Pc25: 4 plants	Pc25
Pc26: 2 plants (1 at Gurgeena)	Pc26
Pc27: 7 plants	Pc27
Pc28: 1 plant	Pc28
Pc29: 1 plant (already at Gurgeena)	Pc29
Pc30: 3 plants	Pc30
Pc31	Pc31: 2 plants
Pc32: 3 plants	Pc32
Pc33: 17 plants (4 at Gurgeena)	Pc33
Pc34: 13 plants	Pc34
Number by cuttings: 31	**Number by tissue culture: 3**
Number of *Pomaderris* wild plants propagated: 34 (including Pc22)	

* These three wild plants were excavated and established successfully at Gurgeena (Haskard 2014)

Fig. 3. Potted plants of* Pomaderris clivicola *in the nursery.

Bertya pedicellata

B. pedicellata was extremely difficult to propagate but we have produced 39 potted plants using either cuttings or tissue culture (Table 3). In addition, 27 *B. pedicellata* plants have already been translocated successfully to Gurgeena (Haskard 2014).

This brings the total number of *B. pedicellata* plants to 66.

The number of potted *B. pedicellata* plants produced from cuttings was 27, and these represent seven of the wild plants (Table 3). The number of potted *B. pedicellata* plants produced from tissue culture was 12, and these represent three of the wild plants (Table 3). The tissue-cultured *B. pedicellata* plants all arose through conventional shoot culture (Fig. 4).

All of the *B. pedicellata* potted plants are available now for planting at the offset management site at Gurgeena (Fig. 5).

Fig. 4. **Bertya pedicellata** ***shoots in tissue culture.***

Fig. 5. Potted plants of **Bertya pedicellata** ***in the nursery.***

Table 3. Propagation of* Bertya pedicellata (Bp) *by cuttings and tissue culture as at 23 February 2016.
Green: successfully propagated.* *Blue: some of these wild plants were excavated.

Propagation by cuttings	Propagation by cuttings	Propagation by tissue culture	Propagation by tissue culture
Bp1	Bp61*	Bp1	Bp61
Bp2	Bp62*	Bp2	Bp62
Bp3	Bp63*	Bp3	Bp63
Bp4	Bp64*	Bp4	Bp64
Bp5	Bp65*	Bp5	Bp65
Bp6: 1 plant	Bp66*	Bp6	Bp66
Bp7	Bp67*	Bp7	Bp67
Bp8	Bp68*	Bp8	Bp68
Bp9	Bp69*	Bp9	Bp69
Bp10	Bp70*	Bp10	Bp70
Bp11	Bp71*	Bp11: 5 plants	Bp71
Bp12	Bp72*	Bp12	Bp72
Bp13	Bp73*	Bp13	Bp73
Bp14	Bp74*	Bp14	Bp74
Bp15	Bp75*	Bp15	Bp75
Bp16	Bp76*	Bp16	Bp76
Bp17	Bp77*	Bp17	Bp77
Bp18	Bp78*	Bp18	Bp78
Bp19	Bp79*	Bp19	Bp79
Bp20*	Bp80*	Bp20	Bp80
Bp21*	Bp81*	Bp21	Bp81
Bp22*	Bp82*	Bp22	Bp82
Bp23*	Bp83	Bp23	Bp83
Bp24*	Bp84	Bp24	Bp84
Bp25*	Bp85: 1 plant	Bp25	Bp85
Bp26*	Bp86	Bp26	Bp86
Bp27*	Bp87	Bp27	Bp87
Bp28*	Bp88	Bp28	Bp88
Bp29*	Bp89	Bp29	Bp89
Bp30*	Bp90	Bp30	Bp90
Bp31*	Bp91	Bp31: 5 plants	Bp91
Bp32*	Bp92	Bp32	Bp92
Bp33*	Bp93	Bp33	Bp93
Bp34*	Bp94	Bp34	Bp94
Bp35*	Bp95	Bp35	Bp95
Bp36*	Bp96	Bp36	Bp96
Bp37*	Bp97	Bp37	Bp97
Bp38*	Bp98	Bp38	Bp98
Bp39*	Bp99	Bp39	Bp99
Bp40*	Bp100	Bp40	Bp100
Bp41*	Bp101: 1 plant	Bp41	Bp101
Bp42*	Bp102: 1 plant	Bp42	Bp102
Bp43*	Bp103	Bp43	Bp103
Bp44*	Bp104: 21 plants	Bp44	Bp104
Bp45*	Bp105: 1 plant	Bp45	Bp105
Bp46*	Bp106	Bp46	Bp106
Bp47*	Bp107	Bp47	Bp107
Bp48*	Bp108: 1 plant	Bp48	Bp108
Bp49*	Bp109	Bp49	Bp109
Bp50*	Bp110	Bp50	Bp110
Bp51*	Bp111	Bp51	Bp111
Bp52*	Bp112	Bp52	Bp112
Bp53*	Bp113	Bp53	Bp113
Bp54*	Bp114	Bp54: 2 plants	Bp114
Bp55*	Bp115	Bp55	Bp115
Bp56*	Bp116	Bp56	Bp116
Bp57*	Bp117	Bp57	Bp117
Bp58*	Bp118	Bp58	Bp118
Bp59*	Bp119	Bp59	Bp119
Bp60*	Bp120	Bp60	Bp120
Number of potted plants from cuttings: 27		**Number of potted plants from tissue culture: 12**	
Total number of *Bertya* potted plants: 39			
Total number of *Bertya* plants: 66 (including the 27 excavated translocated plants)			

* Approximately 55 of these wild plants were excavated and 27 were established successfully at Gurgeena (Haskard 2014)

Conclusions

The cuttings method was successful for propagating 31 of the 34 *P. clivicola* wild plants. This species was not especially amenable to tissue culture, but the additional use of tissue culture and whole-plant translocation has ensured that all 34 *P. clivicola* wild plants were propagated or established successfully at the offset management site.

Both cutting propagation and tissue culture were used to produce *B. pedicellata* plants. This species was extremely difficult to propagate but the combined use of cutting propagation, tissue culture and whole-plant translocation ensured that the offset management site will soon be stocked with more *B. pedicellata* plants than were directly impacted at the road remediation site.

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