TRANSLOCATION PROPOSAL
Round Leaf Honeysuckle,
Lambertia orbifolia C.A. Gardner (PROTEACEAE) - Narrikup Form.

1. SUMMARY

Lambertia orbifolia was first collected at Scott River in January 1945, and named in 1964 by Charles Gardner. It is a shrub that grows to 3m in height. The branches are erect and spreading, or arching with young branches brown and covered in soft hairs. Leaves are opposite, sessile rounded in shape, with a 1.5 - 2 cm diameter. Flowering occurs all year round with peaks from December to January (Hnatiuk 1995) and from May to July (Blackall and Grieve 1988). Between four and six orange-red coloured flowers form the inflorescence. The perianth is 4 - 5 cm long, tubular with hairs on the outside. The New Holland Honeyeater is believed to be the major pollinator for this species (Whitaker and Collins 1997). The fruit is between 0.7 - 1 cm in diameter, with a short beak and contains up to two seed. Initial percentage viability of the seed ranges from 77 - 100%. Percentage viability after one year storage at -18°C ranged from 53 - 100% (A. Cochrane pers. comm).

Lambertia orbifolia is an obligate seeder. It has been recorded as being killed by fire (Sage 1994), with a major recruitment event following fire and low levels of establishment occurring in the interfire period (Sage 1994, D. Coates *pers. comm*).

L. orbifolia is listed as Declared Rare Flora and is ranked as endangered. It is known from seven populations in two widely disjunct locations. Five populations occur on the sandy ironstone soils, grey sand over ironstone soils or shallow sands associated with ironstone soils near winter wet areas around the Scott River Plains. These populations have a combined total of about 24, 000 individuals. Two populations occur some 200 km to the east near Narrikup with a combined total of just 169 individuals. The Narrikup form occurs on grey sands over laterite.

The Narrikup and Scott River Plains populations have been shown to have a level of genetic divergence that can equate to species differences. The Narrikup form of *L. orbifolia* is critically endangered and in need of translocation to a secure site. *Lambertia orbifolia* is considered to be very susceptible to *Phytophthora cinnamomi* (Obbens and Coates 1997). Both of the Narrikup populations have had *P. cinnamomi* and Canker (spp.) confirmed as being present at the sites and deaths of numerous adult plants have been attributed to *P. cinnamomi*. Both Narrikup populations occur on narrow degraded Shire Road verges where damage from road maintenance activities has already resulted in the deaths of ten adult plants, and there is a possibility of this occurring again. Due to the number of threats to the genetically distinct Narrikup populations of *L. orbifolia* the translocation of this form should now be considered to be of extreme urgency.

This translocation proposal outlines the need for translocation of the critically endangered Narrikup form of *L. orbifolia*, the site selection process, the design of the translocation site and the provisions for monitoring. In addition it outlines the criteria for success or failure of this proposed translocation.

2. PROPONENTS

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TABLE OF CONTENTS

3. BACKGROUND

3.1 History, Taxonomy and Status

Lambertia orbifolia C.A. Gardner (Proteaceae) was first collected at Scott River in January 1945 by R. D Royce. It was named in 1964 by Charles Gardner from a collection made by A. J. Gray at King River. Gardner named it *orbifolia*, derived from the Latin words *orbis* and *folia* referring to the rounded shape of the leaves.

It is a shrub that grows to 3m in height, apparently lacking a lignotuber (Hnatiuk 1995). The branches are erect and spreading, or arching with young branches brown and covered in soft hairs. Leaves are opposite (or rarely whorled in groups of three), sessile or with a short petiole, orbicular to cordate (rounded) in shape, with a 1.5 - 2cm diameter.

Flowering occurs all year round with peaks from December to January (Hnatiuk 1995) and from May to July (Blackall and Grieve 1988). Between four and six orange-red coloured flowers form the inflorescence in the upper leaf axils. The perianth is 4 - 5 cm long, tubular in shape with a slight bulge in the middle and hairs on the outside. A small whorl of bracts subtend the flowers, allowing the flowers to spread widely so that they can be easily probed by honeyeaters (Hnatiuk 1995). The New Holland Honeyeater is believed to be the major pollinator for this species (Whitaker and Collins 1997).

The fruit is asymmetrical, between 0.7 - 1 cm in diameter, flattened, smooth with a short oblique beak. Each fruit may contain up to two seed. Initial percentage viability of the seed ranges from 77 - 100%. Percentage viability after one year storage at -18°C ranges from 53 - 100% (A. Cochrane pers. comm).

Hnatiuk (1995) considers *L. orbifolia* to be most closely related to *L. ericifolia* and *L. inermis*. This is due to these species having outward facing flowers, lacking bracts at the base of the inflorescence (which allows easy access for the Honeyeater pollinators which perch on the stems below the flowers), having asymmetric, cuneate seeds and obtuse leaves.

L. orbifolia is an obligate seeder. It has been recorded as being killed by fire (Sage 1994), with a major recruitment event following fire and low levels of establishment occurring in the interfire period (Sage 1994, D. Coates *pers. comm*). The mean age of plants in population 2 is 15 years, with the maximum age observed to be 20 years (Sage 1994). It is not known at what age this species sets its first flowers or seeds, however, a six month old seedling grown for this translocation has set its first flower whilst being raised at the Kings Park and Botanic Gardens.

L. orbifolia is considered to be very susceptible to *Phytophthora cinnamomi* (Obbens and Coates 1997). At this stage only one Scott River population (1E) is considered to be at risk of being infected with the disease. However, both Narrikup populations have tested positive to the presence of *P. cinnamomi*, and Canker (spp.) A total of 20 individuals died in the largest of the two Narrikup populations during a two month period in 1995 due to *P. cinnamomi*. As a result Phosphite was applied at a rate of 0.2% on two occasions (42 days apart) at both Narrikup sites in autumn/winter 1995 (Obbens and Coates 1997). This spray program appears to have halted the high death rate, however sick looking adult plants have still been observed and this is considered to be due to *P. cinnamomi* (E. Hickman *pers. comm*).

The species is listed as Declared Rare Flora under the Western Australian Wildlife Conservation Act and ranked as endangered. At present there is no recovery plan for this species and it is anticipated one will be written for this species soon. However, the Narrikup form is critically endangered and there is an urgency for translocation that necessitates translocation prior to a full recovery plan being written.

3.2 Evolutionary Status of the Narrikup Populations

Population genetic structure and patterns of differentiation among populations have been investigated using isozyme markers. (Coates and Hamley, in prep). Single locus genetic diversity measures *A* (mean number of alleles per locus), *P* (mean percentage polymorphic loci), *He* (expected panmictic heterozygosity), and *Ho* (the average observed heterozygosity) are presented in Table 1. These data indicate that *L. orbifolia* has relatively high levels of genetic diversity for an endemic species although there are marked differences between

populations (see Hamrick and Godt, 1989). These population differences are particularly evident when comparing the Scott River Plains populations with the Narrikup populations.

Both Narrikup populations have significantly higher genetic diversity levels, based on all single locus diversity. These results are unexpected given the small fragmented nature of the Narrikup populations compared with the three larger populations in the Scott River Plains area. They suggest that quite different evolutionary mechanisms may be operating in the two population systems. These results also indicate that there is a suitable broad range of genetic diversity in the each of the Narrikup populations to support successful translocation and population enhancement programs.

The large discrepancy between the observed heterozygosity (*Ho*) and the expected panmictic heterozygosity (*He*) also suggest significant levels of inbreeding within all populations. The apparent level of inbreeding was unexpected when compared to other bird pollinated Proteaceae although heterozygote deficits are not unusual in plants with mixed mating systems and significant population structuring (Sampson *et al*., 1996).

Table 1. Single locus genetic diversity measures: *A* (mean number of alleles per locus), *P* (mean percentage polymorphic loci), *He* (expected panmictic heterozygosity), and *Ho* (the average observed heterozygosity) for each population of *L. orbifolia.*

Population		N_e	A	P	H_e	H_o
Scott River Plains	1E	≈100	1.2	15.0	0.066(0.038)	0.031(0.022)
	4	60	1.4	30.0	0.080(0.038)	0.038(0.016)
	5	68	1.4	35.0	0.119(0.046)	0.060(0.023)
	6	6810	1.4	35.0	0.081(0.036)	0.052(0.028)
	7	≈10,000	1.4	35.0	0.095(0.038)	0.048(0.023)
Mean			1.36	30.0	0.088	0.046
Narrikup	2	139	1.5	45.0	0.149(0.047)	0.076(0.025)
	3	30	1.6	65.0	0.116(0.034)	0.063(0.027)
Mean			1.55	0.55	0.133	0.067
Mean			1.41	37.1	0.129	0.053

A UPGMA analysis of genetic differentiation between populations reveals striking genetic differences between the Narrikup and Scott River Plains areas (See Figure 1). The level of genetic divergence between these two population groups (> 0.20) is indicative of species differences in some plant groups (Gottleib, 1981). This result clearly supports the view that these two population groups are distinct evolutionary units or evolutionary significant units (ESUs) as discussed by Moritz (1995). This is despite the fact that there are no detectable morphological differences between the two groups (Keighery *pers comm*).

These two population groups appear to have been isolated historically from each other for a long period of time and are relic populations from a previously more continuous population system which linked the two groups. These conclusions are further supported by recent molecular studies (Byrne in prep) which show that the Narrikup populations are characterised by six unique mutations in the chloroplast DNA. The critically

endangered Narrikup populations should therefore be considered a distinct conservation unit for the purposes of management, particularly translocation.

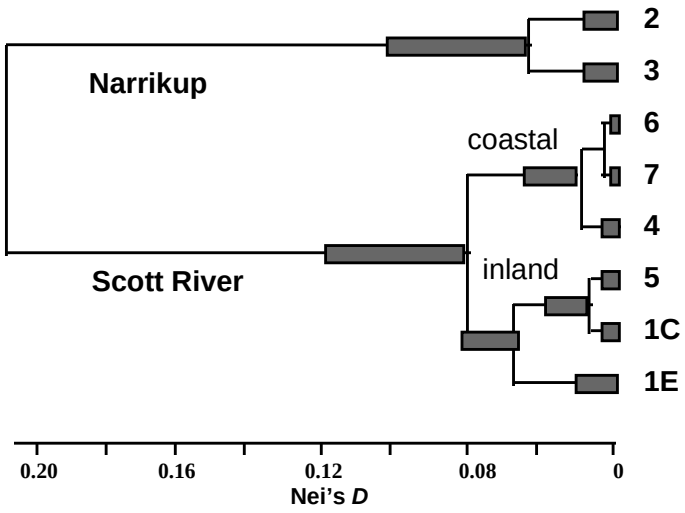

Figure 1. UPGMA clustering (based on Nei's genetic distance, *D*) of *Lambertia orbifolia* populations. A cluster in the UPGMA phenogram is significant if the shaded standard error bar is less than half the branch length.

3.3 Distribution and Habitat

L. orbifolia is known from seven populations in two widely disjunct locations. Five populations occur on the sandy ironstone soils, grey sand over ironstone soils or shallow sands associated with ironstone soils near winter wet areas around the Scott River Plains. Two populations occur some 200 km to the east near Narrikup, on grey sands over laterite.

At the Scott River Plains *L. orbifolia* is found in two habitat types. The coastal form is found adjacent to low *Agonis flexuosa* and *Eucalyptus marginata* woodlands, forming dense thickets with *Banksia littoralis, B. grandis* and *B. ilicifolia*. The inland form occurs in seasonally inundated dense shrub heath associated with *Calothamnus* aff. *crassus*, *Agonis flexuosa* and several *Banksia* species. The Narrikup form occurs in an open low woodland of *Eucalyptus marginata* and *E. calophylla*, with *Banksia grandis*, *Agonis parviceps*, *Hakea ferruginea* and *Xanthorrhoea preissii*.

4. THE TRANSLOCATION

4.1 The Need to Translocate

Genetic studies show that there is a level of genetic divergence between the Narrikup and Scott River Plains populations that is indicative of species level differences and substantial historical isolation. These data show that the Narrikup populations are a distinct conservation unit and need to be considered separately for management, particularly translocation.

The Scott River Plains populations have a combined total of around 24,000 individuals in five populations. The genetically distinct Narrikup populations have a combined total of just 169 individuals in only two populations. The presence of *Phytophthora cinnamomi* and Canker (spp.) has been confirmed at both of these populations and, as mentioned previously, the deaths of 20 adult plants have been confirmed as being due to *P. cinnamomi*. Both Narrikup populations occur on narrow degraded Shire Road verges, with population three extending into remnant vegetation on private property. Damage to the Spencer Road population from road maintenance activities occurred in 1995, with ten adult plants being killed. With the high turn over of Shire staff and the use of contract shire employees it is difficult to ensure that every person undertaking maintenance activities adjacent to the *L. orbifolia* populations received information vital to the conservation of this taxon. Further damage is likely from routine road maintenance activities.

Due to the number of threats to the genetically distinct Narrikup populations of *L. orbifolia* the translocation of this form to a secure, disease free area should now be considered to be of extreme urgency.

4.2 Translocation Site Selection

A search was carried out on the 10th March 1998 to locate a suitable disease free area in the vicinity of the known Narrikup populations. An area on the south western corner of Reserve (#) was selected. As this species has not previously been recorded from this reserve this translocation can be considered an introduction under the definitions provided by Policy Statement 29 and the Guidelines for Translocation of Threatened Plants in Australia. A map of the proposed translocation site in relation to the known populations is shown in Appendix 1.

This area was chosen because it is only a short distance (in a direct line) from the Narrikup populations (3.1 km from population 2 and 3.6 km from population 3) and is *P. cinnamomi* free. It is the opinion of the District Dieback Interpreter (M. Grant pers. comm) that *P. cinnamomi* is highly unlikely to infect the translocation site. Strict hygiene procedures will be followed to prevent infection of the site during translocation planting and monitoring.

The proposed translocation site has a soil type of grey sand over laterite, with an underlying geology of white, grey or brown sand overlying laterite and commonly containing iron pisoliths (conglomerates of spherical bodies) (Muhling and Brakel 1985). The known Narrikup populations have a similar soil type of sand to the depth of 18 m, with laterite beginning at a depth of 15 - 30 cm. They have an underlying geology of alluvially (soils deposited from river systems) and colluvially (soils found at the foot of a slope) deposited pebbles, sand, silt and clay, as well as soils of white, grey or brown sand overlying laterite and commonly containing iron pisoliths (Muhling and Brakel 1985).

Both the translocation site and the Narrikup *L. orbifolia* populations have similar vegetation structure of Open Low Woodland over Mid-Dense Heath A (using Muir's (1977) classification). The translocation site has many associated species in common with the known populations, none of these are listed as rare or threatened. These are shown below in Table 1.

The pollinators of this species, New Holland Honeyeaters (Whitaker and Collins 1997), have been confirmed as being present at the proposed translocation site. The presence of pollinators is necessary for perpetuation of the translocated population through outcrossed seed and therefore the creation of a viable self sustaining population.

Table 1. Main associated vegetation at the proposed translocation site within Reserve # compared to the associated vegetation at the known Narrikup populations of *Lambertia orbifolia*.

Main associated species of the proposed translocation site within Reserve #	Main associated species of the original populations of *Lambertia orbifolia* at Narrikup
	Agonis hypercifolia
Agonis parviceps	*Agonis parviceps*
Banksia grandis	*Banksia grandis*
Beaufortia anisandra	
	Bossiaea ornata
Eucalyptus calophylla	*Eucalyptus calophylla*
Eucalyptus marginata	*Eucalyptus marginata*
Eucalyptus staeri	
Hakea ceratophylla	
Hakea ferruginea	*Hakea ferruginea*
	Hakea ruscifolia
	Isopogon formosus
Leucopogon glabellus	
	Leucopogon verticillata
	Xanthorrhoea platyphylla
Xanthorrhoea preissii	*Xanthorrhoea preissii*

The proposed translocation site was therefore chosen because the environmental attributes of climate, soil type, vegetation structure and associated vegetation are almost identical to the known Narrikup populations of this species.

4.3 Translocation Design

Five 14 metre by 5 metre plots will be selected and then measured out at the translocation site. Plots will not be cleared of vegetation, instead seedlings will be planted in gaps in the vegetation, adhering as close as possible to the grid pattern presented in this proposal. In this way there will be minimal disturbance to the natural vegetation. There appears to be no reason that there would be adverse effects on the conservation values of the reserve from this translocation.

Within each plot a grid of 13 m by 4 m will be measured so that a 1 metre border will be left between the outer of the grid and the inner of the plot. Four treatments will be chosen: control, mulched, shaded or gro-cone (see Table 2). A total of 65 seedlings will be randomly assigned to each treatment. Each group of 65 seedlings will be randomly divided in to 5 smaller groups of 13 and then assigned to a plot, such that each plot has 52 seedlings: 13 controls, 13 mulched, 13 shaded and 13 gro-cones.

Each of the four treatments will be randomly assigned to one of the four lines in the grid. The plot will be planted with the seedlings spaced at a distance of 1m apart within the grid (see Appendix 2). Each plot will be fenced with rabbit netting to prevent predation of the plants by large herbivores.

Seedlings have been raised at the accredited nursery at Kings Park and Botanic Gardens and therefore are considered disease free. All equipment used during seedling planting will be maintained under strict disease hygiene.

Table 2. Description of experimental treatments.

Treatment	Description of Treatment
Control	Plants not given any treatment
Mulched	A layer of mulch is placed around the plant to see whether it enhances survival by increasing water retention.
Shaded	A circle of wire netting, approximately 1m in diameter covered in shade cloth is placed around the plant after planting to see whether survival is enhanced by the creation of a shaded environment around the plant.
Gro-cone	A Gro-cone is placed around the plant after planting to see whether it enhances survival by creating a sheltered environment around the plant

Monitoring of the translocated population will be undertaken every second month over the length of the project. Monitoring will include counting the number of surviving seedlings, height of the surviving seedlings, width of the crown of the surviving seedlings in two directions (so that crown volume can be calculated), reproductive state, number of flowers and fruit, whether second generation plants are present and general health of the plants.

Monitoring of the original populations of *L. orbifolia* (population 2 and 3) will also occur every second month. Monitoring will include counting the number of individuals, height and crown width of the individuals, reproductive state, number of flowers and fruit and general health of the plants.

At the end of this project monitoring will continue on a yearly basis until such a time as the long term criteria for success is considered to have been met. Yearly monitoring will include the above information.

4.4 Source of Plants

Seed was collected under guidelines outlined in Appendix 3. Seed has been sourced from a bulk collection from 40 plants from population two, and from a bulk of 10 plants from population 3 for planting at the translocation site in 1998. Seeds were germinated at the Threatened Flora Seed Centre. Seeds were surface sterilised with a 10% solution of 4g/L sodium hypochlorite for five minutes prior to being placed on agar plates. Agar plates were placed in germination cabinets at 15°C with a photoperiod of 12 hours of light and 12 hours of darkness.

A total of 268 seedlings were raised from seed sourced from population 2 and 30 seedlings were raised from seed sourced from population 3. Any subsequent plantings will also be grown from seed collected from these sources. Seedlings from the two populations were mixed together at the translocation site. Seedlings have been raised at Kings Park and Botanic Gardens accredited nursery.

4.5 Criteria for Success or Failure

Criteria for Success

- Short Term: after one generation (juvenile transplant to seed producing adult plant) the number of individuals is sustained by natural recruitment
- Long Term: after two or more generations the number of individuals is sustained by natural recruitment

Criteria for Failure

- There is a significant decline in population size due to lack of natural recruitment

5. TIMETABLE

Time	Action
October 1997	Germination of seed started.
March 1998	Translocation site selected.
April 1998	Translocation proposal submitted for review and approval.
June - July 1998	Translocation of seedlings into reserve #.
July - August 1998	Follow up monitoring and maintenance of translocation site.
September 1998 - May 1999	Monitoring and maintenance of translocation site.
October 1998	Translocation proposal for 1999 translocation submitted for review.
November 1998	Further seed collection if necessary
November 1998	Second batch of seeds put down for germination. Resulting seedlings raised at Kings Park and Botanic Gardens.
April 1999	Progress report.
May - June 1999	Further translocation of seedlings into Reserve #.
June - July 1999	Follow up monitoring and maintenance of translocation site.
August 1999 - May 2001	Monitoring and maintenance of translocation site.
May 2001	Final Report

6. FUNDING

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7. ACKNOWLEDGMENTS

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8. REFERENCES

Blackall W.E. and Grieve B.J. (1988) *How To Know Western Australian Wildflowers, Part 1*. University of Western Australia Press, Perth W.A.

Hamrick, J. L. and Godt, M. J. (1989). Allozyme diversity in plant species. In Brown, A., Clegg, M. T., Kahler, A. L. and Weir, B. S. (eds) *Plant Population Genetics, Breeding and Genetic Resources*. Sinauer Associates, Sunderland, pp 43-63.

Hnatiuk R.J., (1995) *Lambertia, Flora of Australia* 16: 425-435

Gottleib, L. D. (1981) Electrophoretic evidence and plant populations. *Progress in Phytochemistry*. **7**. 54-64

Guidelines for the Translocation of Threatened Australian Plants. (1997) Produced by The Australian Network for Plant Conservation Translocation Working Group. Canberra, Australia.

Moritz, C. (1994) Defining "Evolutionary Significant Units" for conservation. *Trends in Ecology and Evolution*, 9, 373-375.

Muhling P.C. and Brackel A.T. (1985) Mount Barker - Albany Western Australia 1:250 000 Geological Series - Explanatory Notes. Geological Survey of Western Australia. Perth Western Australia.

Muir B.G. (1977) Vegetation and habitat of Bendering Reserve. Biological survey of the Western Australian Wheatbelt. Pt 2. Records of the Western Australian Museum Supplement 3.

Obbens F. J. and Coates D. J. (1997) Conservation biology and management of endangered Lambertia species. Final Report Submitted to the Commonwealth Threatened Species and Communities Section, Biodiversity Group, Environment Australia. Department of Conservation and Land Management, Perth W.A.

Sage L. (1994) The conservation requirements of the rare species *Lambertia orbifolia*. BSc. Honours Dissertation. School of Environmental Biology, Curtin University of Technology, Bentley W.A.

Sampson, J. F., Coates, D. J. and Van Leeuwen, S. J. (1986). Mating system variation in animal-pollinated rare and endangered populations in Western Australia. In: "*Gondwanan Heritage: Past, Present and Future of the Western Australian Biota*". Eds S. Hopper, J. Chappill, M. Harvey and A. George. Surrey Beatty, Sydney.

Whitaker P.K. and Collins B.G. (1997) Pollen vectors for the rare plant species *Lambertia orbifolia*. Report to the Department of Conservation and Land Management. School of Environmental Biology, Curtin University of Technology, Bentley W.A.

Appendices One, Three and Four may be available on contacting the authors.

Appendix Two.

Site Diagram for Proposed Translocation of *Lambertia orbifolia*

There is a total of 270 seedlings of *Lambertia orbifolia* available.
These will be planted as shown in the diagram below, with one seedling at each point marked with an asterix (*).
The four treatments of control, mulched, shaded or Gro-cone will be assigned as per the diagram below.

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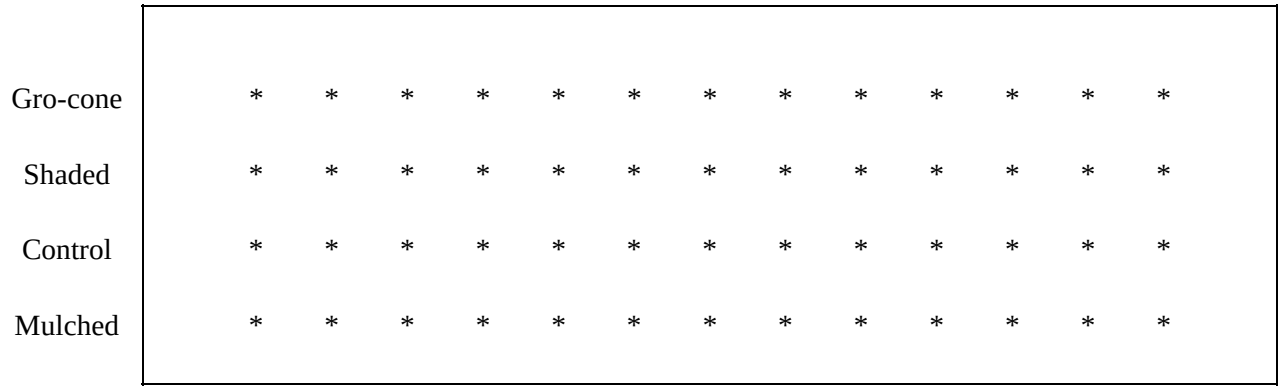

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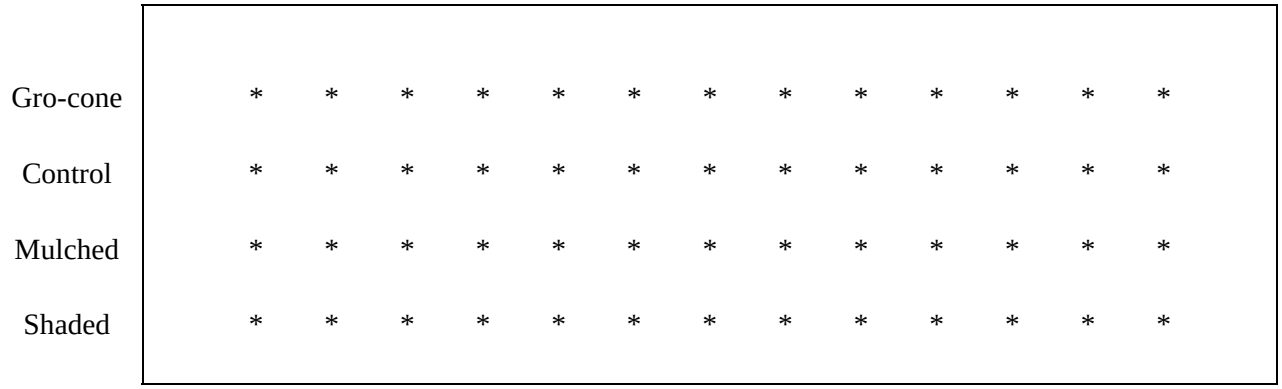

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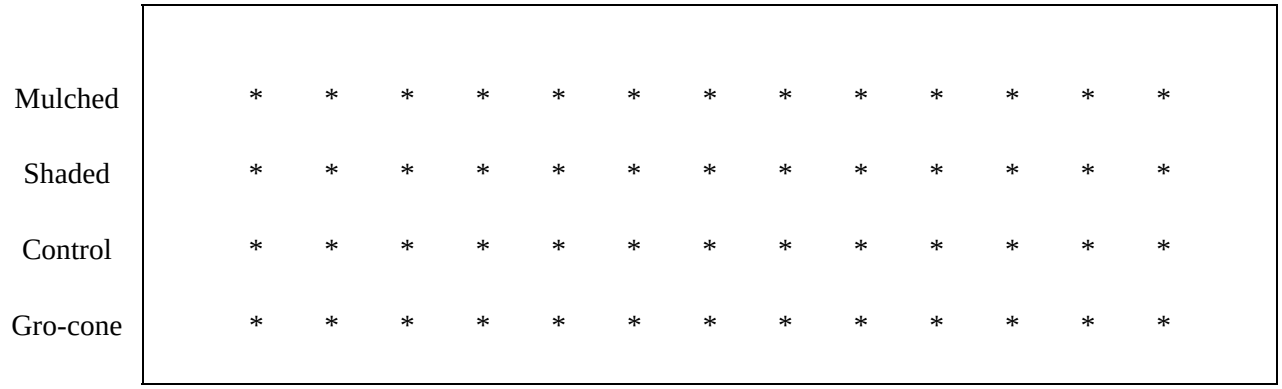

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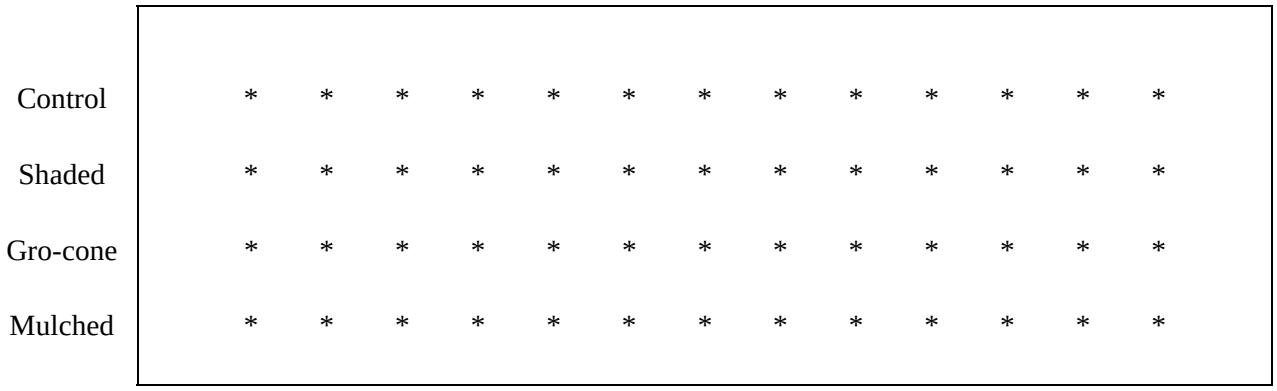

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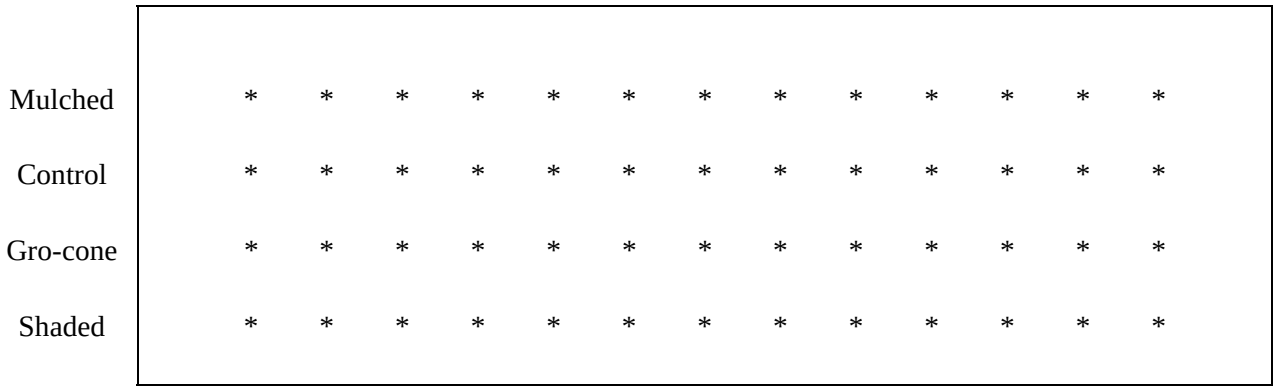

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