WEED RESEARCH An International Journal of Weed Biology, Ecology and Vegetation Management

DOI: 10.1111/wre.12029

Genetic variation and structure of *Solanum elaeagnifolium* in Australia analysed by amplified fragment length polymorphism markers

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Received 3 November 2012
Revised 16 January 2013
Subject Editor: Pietro Iannetta, James Hutton Institute, UK

Summary

Solanum elaeagnifolium is a weed of national significance in Australia. However, the genetic diversity of *S. elaeagnifolium* is poorly understood. Four amplified fragment length polymorphism primer combinations were utilised to investigate the genetic variation and structure of 187 *S. elaeagnifolium* individuals collected from 94 locations in Australia. High genetic diversity was found, with an average Jaccard's genetic similarity at 0.26. Individuals were assigned to two genetic clusters or considered as admixed according to their membership coefficient value (q) calculated by Bayesian model-based genetic structure analysis. This suggested that Australian *S. elaeagnifolium* may have originated from two distinct gene pools. These results were further supported by principal co-ordinates analysis. Large spatial groups of individuals assigning to these two gene pools were found in western Victoria and south-western New South Wales (NSW) and northern NSW, which correlated well with the early records of *S. elaeagnifolium* in both regions. The high genetic diversity found here could add difficulties to effective control of *S. elaeagnifolium* across regions.

Keywords: silverleaf nightshade, invasive weed, AFLP, genetic diversity, genetic structure.

ZHU XC, WU HW, RAMAN H, LEMERLE D, STANTON R & BURROWS GE (2013). Genetic variation and structure of *Solanum elaeagnifolium* in Australia analysed by amplified fragment length polymorphism markers. *Weed Research* **53**, 337–343.

Introduction

Solanum elaeagnifolium Cav. (silverleaf nightshade) belongs to the *Leptostemonum* subgenus in the *Solanum* genus (Levin *et al.*, 2006) and is believed to be a native of Central America (Stanton *et al.*, 2009). It was first recorded in Australia in 1901 at Bingara, New South Wales (NSW). Subsequent infestations were reported in Victoria (VIC) in 1909 and South Australia (SA) in 1914, suggesting the possibility of multiple introductions (Cuthbertson *et al.*, 1976). Isolated infestations also occur in Western Australia (WA) and Queensland (QLD). As a weed of national significance, *S. elaeagnifolium* infests at least 0.35 million hectares in Australia and has the potential to infest 398 million hectares (Feuerherdt, 2009).

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Solanum elaeagnifolium is a deep-rooted, summer-growing perennial that reproduces both sexually as a self-incompatible outcrosser and vegetatively from adventitious buds in the root system (Hardin *et al.*, 1972; Petanidou *et al.*, 2012). Sexual reproduction is important for long-distance seed dispersal. By contrast, root systems can generate multiple adventitious shoots in a single season and contribute to rapid population increases or new infestations through movement of viable root fragments (Stanton *et al.*, 2011).

Effective weed management strategies are limited for *S. elaeagnifolium*, especially for large and dense infestations. Chemical control is often expensive and may have a residual effect that can damage sensitive crop or pasture species sown in subsequent years (Stanton *et al.*, 2009). Biocontrol proved to be unreliable in Australia due to harsh climatic conditions and the strong plant regenerative ability of the root system (Stanton *et al.*, 2009). Comprehensive assessment of the genetic diversity in *S. elaeagnifolium* is required for effective management, particularly in developing appropriate control strategies (Dekker, 1997).

Molecular markers have been widely used to assess genetic diversity in many weed species, such as wild oats (*Avena fatua*; Li *et al.*, 2007) and weedy red rice (*Oryza sativa*; Shivrain *et al.*, 2010). Dominant random amplified polymorphic DNA (RAPD) markers detected a high level of genetic diversity in *S. elaeagnifolium* in SA (Hawker *et al.*, 2006). In addition, high level of genetic diversity was also detected in 10 locations in Australia using 36 simple sequence repeat SSR markers (Zhu *et al.*, 2012, 2013). However, information is scarce on the genetic diversity of *S. elaeagnifolium* populations growing across Australia.

Amplified fragment length polymorphism (AFLP) markers are more reproducible and informative than RAPD markers (Jones *et al.*, 1997; McGregor *et al.*, 2000). Compared with single locus specific SSR markers, AFLPs are multilocus markers generated by digestion and specific amplification of fragments representing the entire genome (Vos *et al.*, 1995) and reveal the greatest amount of genetic diversity in several crops including potato (*Solanum tuberosum* L.; McGregor *et al.*, 2000). AFLPs provide higher resolution at the individual level and are suitable for determining genetic structure (Van der Wurff *et al.*, 2003). In this study, AFLP markers were used to investigate genetic diversity and structure of 187 individuals of *S. elaeagnifolium* and to provide a comprehensive analysis of the distribution of genetic variation in this weed in Australia.

Materials and methods

Plant material

A total of 187 individuals were collected from 94 locations across NSW, SA, VIC, WA and QLD (Fig. 1 and Appendix 1). One to three individuals were randomly selected at each location, depending on the levels of infestation. Each individual was spaced at least 50 m apart from each other to reduce the probability of sampling identical clones. Leaf materials were collected and stored as previously described (Zhu *et al.*, 2012).

DNA isolation

Genomic DNA was isolated individually from leaf materials using the standard phenol/chloroform method with three extractions (Sambrook *et al.*, 1989). DNA quality was determined by electrophoresis (MI-DEAR, SYS-MD 120, USA) in a 1.0% agarose gel at 200 V for 10 min. The concentration of DNA was then adjusted to 20 ng μL^{-1} for further analysis.

AFLP analysis

DNA digestion, ligation and pre-selective amplification were performed as described in the manual of AFLP ® Analysis System I (Invitrogen, Australia). Four primer combinations (E-ACC/M-CAA, E-ACC/M-CTT, E-AGC/M-CAA and E-AGC/M-CTT) which showed high polymorphism in preliminary screening (data not shown) were chosen for selective amplification. PCR amplification (12 μL) was carried out in a Gene Amp PCR System 2700 (Applied Biosystems, Singapore), containing 0.4 U Taq (Promega, Australia), 1.2 μL 10 × buffer, 6 mM $MgCl_2$, 240 mM of each dNTP's, 0.3 μM WellRED D4-PA labelled forward primer (Sigma Aldrich, Australia), 0.3 μM reverse primer (Sigma Aldrich) and 2 μL of a 1/20 dilution of pre-amplified template.

PCR products were separated using a CEQ8000 (Beckman Coulter Inc.). Data collection and fragment analysis were performed using CEQ™ 8000, version 8.0. Peak criteria were 5% slope threshold, 5% relative peak height and 95% size estimation confidence. The 600 internal size standard (Beckman Coulter Inc.) was used to calibrate allele sizes.

Fragments were scored according to Stodart *et al.* (2005). Fragments that ranged from 60 to 600 bp were recorded and binned into two nucleotide differences. A binary matrix was obtained using the CEQ 8000 software. Only fragments with more than 10% frequency were used for further analysis to ensure that fragment

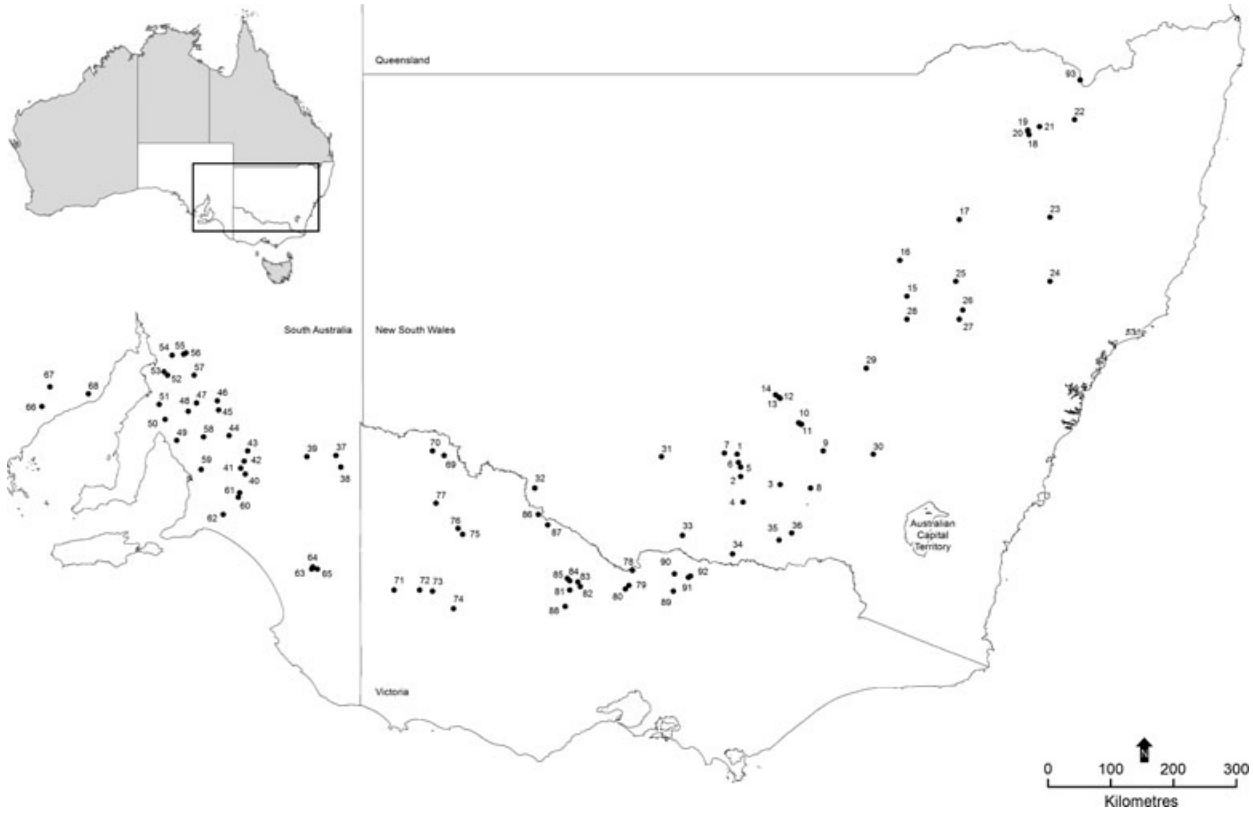

Fig. 1 Distribution of sampled locations across south-eastern Australia (individual from WA not shown).

artefacts were not considered as polymorphism (Stodart *et al*., 2005).

Genetic diversity and structure analysis

The binary matrix was used to calculate the Jaccard's genetic similarity matrix using PAleontological STatistics software package (PAST), version 2.02 (Hammer *et al*., 2001).The Bayesian model-based structure analysis was obtained by STRUCTURE, version 2.3 to infer the genetic structure of *S. elaeagnifolium* in Australia, using the admixture model (Falush *et al*., 2007). In this model, K clusters are assumed and characterised by allele frequencies. A burn-in period of 30 000 was applied, followed by 300 000 steps of Markov Chain Monte Carlo simulations. K was set to vary from 1 to 20 with five iterations. A ΔK value was calculated and used to identify the number of clusters that best explained the data (Evanno *et al*., 2005). Individuals were assigned to clusters according to their membership coefficient (q) value. The threshold $q \geq 0.8$ was applied as described by Menchari *et al*. (2007). The outputs of the estimated K were plotted using Distruct 1.1 (Rosenberg, 2004). In addition, principal co-ordinates analysis (PCoA) was calculated based on the Jaccard's genetic similarity matrix using the same software to investigate the relationship between individuals.

Results

Four AFLP primer combinations amplified 532 polymorphic fragments among the 187 *S. elaeagnifolium* individuals. High genetic diversity was detected, with the average Jaccard's genetic similarity at 0.26, ranging from 0.07 to 0.69.

The genetic structure of *S. elaeagnifolium* was inferred using STRUCTURE (Fig. 2). The LnProb (D) value showed an incremental increase, which is common in STRUCTURE analysis (Evanno *et al*., 2005). However, the ΔK value clearly suggested $K = 2$ (Fig. 3). At the threshold of $q \geq 0.8$, 53 (28.3%) and 51 (27.3%), individuals were assigned to clusters while the other 83 (44.4%) individuals could not be assigned to any clusters and were considered admixed.

The first two PCoA axes explained 15.4% of the total variation (Fig. 4). Individuals are widely spread along the axes, reflecting the high level of genetic diversity. The PCoA achieved similar result with STRUCTURE analysis. Individuals that were assigned to Cluster 1 or Cluster 2 in the STRUCTURE analysis were clearly separated from each other and those that were admixed were placed in the middle of Cluster 1 and Cluster 2 individuals (Fig. 4).

Several spatial groups were defined (Fig. 5). Within each spatial group, more than 70% of individuals

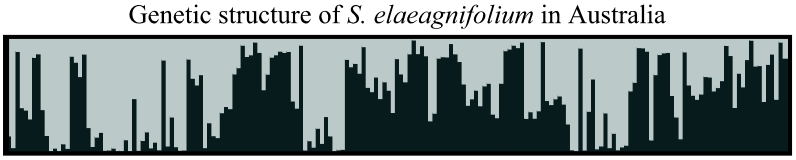

Fig. 2 Genetic structure of *Solanum elaeagnifolium* in Australia inferred by STRUCTURE according to AFLP data.

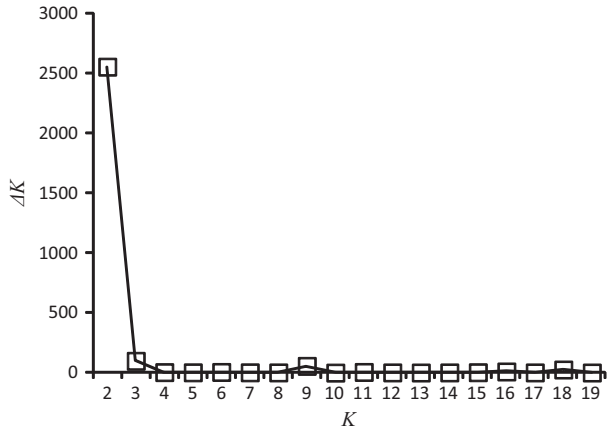

Fig. 3 Plot of the second order rate of change of lnProb (D), ΔK, as a function of the number of genetic clusters or gene pools, K, based on STRUCTURE analysis of AFLP data, showing that ΔK value peaked at $K = 2$.

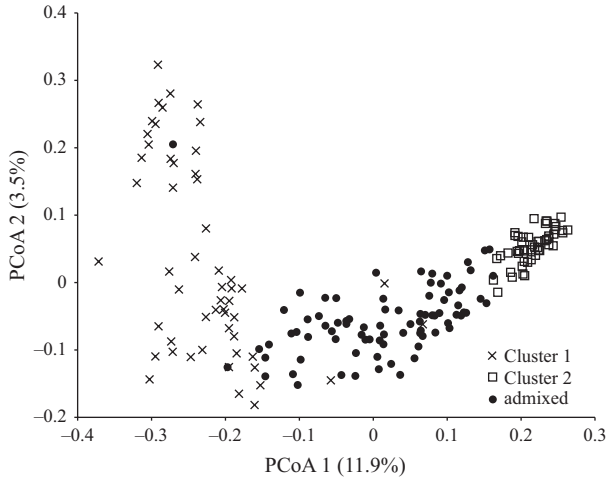

Fig. 4 Principal co-ordinates analysis (PCoA) of 187 *Solanum elaeagnifolium* individuals using four AFLP primer combinations based on Jaccard's genetic similarity, showing that individuals that were assigned to Cluster 1 and Cluster 2 or were admixed were well separated.

assigned to a single cluster (Cluster 1, Cluster 2) or were admixed. A total of 127 individuals from 63 locations formed such spatial groups, which left 60 individuals from 31 locations that failed to form any spatial structure, either because of mixed genotypes in the locations or the spatial isolation from other locations with similar genotypes (such as the location 93 in QLD and 94 in WA). There was a large group of individuals located in western VIC and south-western NSW that was closely associated with Cluster 1, and a concentrated group in northern NSW and SA that was closely associated with Cluster 2. In addition, groups of admixed individuals were usually found spatially close to groups of Cluster 1 and 2 individuals (Fig. 5).

Discussion

Genetic diversity of Solanum elaeagnifolium

Four AFLP primer combinations were successfully used to assess the genetic variation among 187 individuals of *S. elaeagnifolium* collected from across Australia. High level of genetic diversity was detected in *S. elaeagnifolium* from Australia, with a mean Jaccard's genetic similarity of all individuals at 0.26. Combined with a previous study (Hawker *et al.*, 2006), a high genetic diversity in *S. elaeagnifolium* was determined in Australia at both state and national levels. The high level of genetic variation in Australia might be attributed to multiple introductions (Cuthbertson *et al.*, 1976), heterogeneous and/or heterozygous nature of the initial introduction(s) and/or inter- and intra- species hybridisation events. *Solanum elaeagnifolium* is a xenogamous species with the potential to hybridise with other Solanaceae species (Hardin *et al.*, 1972). *Solanum esuriale*, a native species often occupying the same habitat as *S. elaeagnifolium*, could potentially hybridise with *S. elaeagnifolium*. This native species also belongs to the *Leptostemonum* subgenus and shares the same chromosome number ($n = 12$) with *S. elaeagnifolium* (Randell & Symon, 1976) and is noted to be morphologically very similar to *S. elaeagnifolium* (Bean, 2004). Many intermediate forms between *S. elaeagnifolium* and *S. esuriale* were found during our sampling trips (data not shown). Artificial hybridisation between these two *Solanum* species is underway to determine whether cross-species hybridisation occurs in the field. Preliminary results showed that crosses between the two species can form berries. The viability and identity of the suspected F1 seeds are to be further investigated. Once confirmed,

Fig. 5 Spatial groups of individuals assigning to the two genetic clusters inferred by STRUCTURE analysis of AFLP data. Within each spatial group, more than 70% individuals were assigned to a single cluster (Cluster 1: dotted line ellipse, Cluster 2: solid line ellipse) or were admixed (broken line ellipse). A total of 60 individuals from 31 locations failed to form any spatial group either because of the mixed genotypes in locations or the spatial isolation from other locations (such as the location 93 in QLD).

this potential interspecies hybridisation could increase the genetic diversity in local populations of *S. elaeagnifolium*.

Genetic structure of Solanum elaeagnifolium

Bayesian model-based STRUCTURE analysis and PCoA analysis achieved similar results. Individuals that were assigned to different clusters or were admixed in the STRUCTURE analysis were separated by PCoA (Fig. 4). These results suggested that there were probably two main distinct genetic clusters in Australia. Spatially concentrated individuals from the same genetic demes were found (Fig. 5). AFLP analysis indicated that Cluster 2 was the main genetic deme in northern NSW, while a large spatial group of Cluster 1 individuals was found in western VIC and south-western NSW. The spatial distribution of these two genetic clusters correlated with the early record of *S. elaeagnifolium* in Australia. *Solanum elaeagnifolium* was first detected in Bingara, NSW, where most individuals were assigned to Cluster 2 (location ID: 18, 19, 20; six individuals studied in this area, with five grouped into Cluster 2 and one identified as admixed by STRUCTURE). In VIC, one of the earliest records of *S. elaeagnifolium* was in Hopetoun, where 75% individuals were assigned to Cluster 1 (location ID: 75 and 76). This result indicated the possible sites of first establishment of *S. elaeagnifolium* in Australia. In addition, small spatially isolated groups of these two genetic demes were also found in south-eastern SA and southern NSW, indicating that multiple introductions (Cuthbertson *et al*., 1976) were likely in those areas. *Solanum elaeagnifolium* fruits can be eaten by livestock (Heap & Honan, 1993). Subsequent long-distance transport and trade of these livestock could have led to the spread of contaminated faeces, thus causing the spatially isolated groups of genetic demes. Sexual crossing between these two genetic clusters may also have happened as groups of admixed individuals were usually spatially close to groups of Cluster 1 and 2 individuals (Fig. 5).

The high level of genetic diversity of *S. elaeagnifolium* identified in this study suggests that successful management of this weed may be a challenging task. Weeds of higher genetic diversity were considered to be more difficult to manage than those of lower genetic diversity (Dekker, 1997). Control strategies suitable for one

weed population in a given area might not be effective for a genetically distinct population in other areas. In addition, survival rate of biocontrol agents may be different between different weed genotypes. For example, larvae survival rate of stem-mining midge, a biocontrol agent for *Hydrilla verticillata* (L.f.) Royle, was significantly different among the genotypes of this weed (Schmid *et al*., 2010). Therefore, management strategies might need to be modified between genetically distinct populations. Genetic structure analysis indicated that *S. elaeagnifolium* from different gene pools was distributed across south-eastern Australia. Thus, it is important to prevent seed contamination from various sources, as seed is the main contributor to long-distance dispersal.

Acknowledgements

We would like to acknowledge Charles Sturt University, Australia, for funding this research. We thank a number of state and local organisations across Australia for their assistance in field sampling.

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Appendix 1 The Location ID, sample size and locations of *Solanum elaeagnifolium* collected from different states of Australia: New South Wales (NSW), South Australia (SA), Victoria (VIC), Queensland (QLD) and Western Australia (WA)

Location ID	Location	State	Sample size	Longitude/ Latitude
1	Leeton	NSW	2	−34°27/146°22
2	Narrandera	NSW	2	−34°46/146°25
3	Ganmain	NSW	2	−34°53/146°59
4	Boree Creek	NSW	2	−35°08/146°27
5	Yanco 1	NSW	2	−34°38/146°25
6	Yanco 2	NSW	2	−34°34/146°23
7	Griffith	NSW	1	−34°26/146°11
8	Cartwrights Hill	NSW	2	−34°56/147°25
9	Temora	NSW	2	−34°24/147°36
10	West Wyalong 1	NSW	1	−34°00/147°15
11	West Wyalong 2	NSW	2	
12	Ungarie 1	NSW	2	−33°39/146°59
13	Ungarie 2	NSW	1	−33°38/146°58
14	Ungarie 3	NSW	3	−33°36/146°55
15	Dubbo	NSW	2	−32°11/148°48
16	Gilgandra	NSW	2	−31°40/148°42
17	Coonabarabran	NSW	2	−31°05/149°33
18	Bingara 1	NSW	2	−29°52/150°33
19	Bingara 2	NSW	2	−29°48/150°32
20	Bingara 3	NSW	2	−29°49/150°32
21	Delungra	NSW	2	−29°45/150°42
22	Inverell	NSW	2	−29°39/151°12
23	Tamworth	NSW	2	−31°03/150°51
24	Scone	NSW	2	−31°58/150°51
25	Dunedoo	NSW	2	−31°58/149°30
26	Gulgong	NSW	2	−32°23/149°36
27	Mudgee	NSW	2	−32°31/149°33
28	Wellington	NSW	2	−32°31/148°48
29	Parkes	NSW	2	−33°13/148°13
30	Young	NSW	2	−34°27/148°19
31	Hay	NSW	1	−34°29/145°17
32	Balranald	NSW	2	−34°56/143°28
33	Finley	NSW	2	−35°37/145°35
34	Corowa	NSW	2	−35°53/146°18
35	Culcairn	NSW	2	−35°41/146°58
36	Morven	NSW	2	−35°35/147°09
37	Loxton 1	SA	3	−34°28/140°37
38	Loxton 2	SA	2	−34°38/140°41
39	Wunkar	SA	3	−34°29/140°12
40	Angas Valley	SA	3	−34°44/139°19
41	Cambrai	SA	1	−34°39/139°15
42	Sedan	SA	1	−34°33/139°18
43	Annadale	SA	1	−34°24/139°21
44	Eudunda	SA	1	−34°11/139°05
45	Koonoona	SA	3	−33°49/138°56
46	Burra	SA	3	−33°41/138°55
47	Clare	SA	3	−33°43/138°37
48	Blyth	SA	1	−33°50/138°30
49	Avon	SA	3	−34°15/138°20
50	Lochiel	SA	1	−33°57/138°10
51	Snowtown	SA	3	−33°44/138°05
52	Crystal Brook	SA	3	−33°19/138°12
53	Port Pirie	SA	2	−33°16/138°09
54	Wirrabara	SA	2	−33°02/138°16
55	Appila 1	SA	1	−33°01/138°26
56	Appila 2	SA	1	−33°00/138°28
57	Spalding	SA	1	−33°19/138°35
58	Tarlee	SA	1	−34°12/138°43
59	Adelaide	SA	3	−34°40/138°41
60	Murray Bridge	SA	2	−35°04/139°13
61	Mannum	SA	3	−35°00/139°14
62	Langhorne Creek	SA	3	−35°19/139°00
63	Keith 1	SA	2	−36°06/140°16
64	Keith 2	SA	2	−36°04/140°17
65	Keith 3	SA	2	−36°06/140°21
66	Mount Priscilla	SA	2	−33°46/136°24
67	Mangalo	SA	2	−33°29/136°31
68	Mitchellville	SA	2	−33°35/137°04
69	Carwarp	VIC	2	−34°28/142°10
70	Red Cliffs	VIC	2	−34°24/142°00
71	Nhill 1	VIC	1	−36°24/141°27
72	Nhill 2	VIC	3	−36°24/141°49
73	Dimboola	VIC	2	−36°25/142°00
74	Longerenong	VIC	2	−36°40/142°18
75	Hopetoun 1	VIC	2	−35°36/142°26
76	Hopetoun 2	VIC	1	−35°31/142°22
77	Walpeup	VIC	2	−35°09/142°03
78	Echuca	VIC	2	−36°07/144°52
79	Nanneella	VIC	2	−36°20/144°49
80	Rochester	VIC	2	−36°23/144°46
81	Serpentine	VIC	2	−36°24/143°58
82	Calivil 1	VIC	2	−36°21/144°07
83	Calivil 2	VIC	2	−36°17/144°05
84	Jarklin 1	VIC	1	−36°16/143°58
85	Jarklin 2	VIC	2	−36°14/143°56
86	Swan Hill	VIC	2	−35°19/143°31
87	Lake Boga	VIC	2	−35°28/143°39
88	Bridgewater	VIC	2	−36°38/143°54
89	Shepparton	VIC	3	−36°25/145°27
90	Wunghnu	VIC	3	−36°10/145°28
91	Dookie 1	VIC	1	−36°13/145°40
92	Dookie 2	VIC	3	−36°12/145°42
93	Inglewood	QLD	3	−29°05/151°17
94	Katanning	WA	1	−33°39/117°44
	Total		187	

Appendix 1 (Continued)