Hybridisation within *Chrysanthemoides monilifera* and implications for the eradication of bitou bush from Victoria, Australia

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Summary Two subspecies of the South African shrub *Chrysanthemoides monilifera* (L.) T.Norl. are naturalised in Australia and hybridise at Daveys Bay, Victoria. Comparisons described here showed that hybrids are more variable in morphological characteristics than parent subspecies. One subspecies (bitou bush, *C. monilifera* subsp. *rotundata* (DC.) T.Norl.) is subject to eradication in Victoria, but hybridisation confuses recognition of target plants and complicates eradication planning and implementation.

Keywords Boneseed, bitou bush, eradication, *Chrysanthemoides monilifera.*

INTRODUCTION

The southern African shrub *Chrysanthemoides monilifera* (L.) T.Norl. (Asteraceae) has two subspecies naturalised in Australia: boneseed (*C. m.* subsp. *monilifera* (L.) T.Norl.) and bitou bush (*C.m.* subsp. *rotundata* (DC.) T.Norl.). Both subspecies cause ecological harm in natural ecosystems and are weeds of national significance in Australia. In Victoria, boneseed is widespread and can form dense and extensive infestations. In contrast, bitou bush is only known from four localised populations (Kew, Frankston, Mallacoota and Daveys Bay), the largest occurring at Daveys Bay, SE of Melbourne (Adair 2009). In New South Wales, bitou bush forms extensive infestations in coastal habitats north of Jervis Bay (Cherry *et al.* 2008).

At Daveys Bay, a small infestation (<20 ha) of bitou bush occurs on steep coastal cliffs with highly erodible clay soils adjoining urban development fringing Port Phillip Bay. Boneseed occurs extensively throughout the area.

Eradication of weeds in early stages of invasion is recognised in Victoria's weed policy as a highly cost-effective way to reduce future weed problems (The state of Victoria 2008). Bitou bush is subject to eradication in Victoria, but at Daveys Bay suspected hybridisation with boneseed may complicate the identification of bitou bush individuals. This would necessitate a tactical position be taken on the extent, within a bitou bush-boneseed continuum, that eradication effort should be directed.

This paper provides the first quantitative evidence for hybridisation between *C. monilifera* taxa in Australia, and outlines an assessment process used to determine the feasibility of eradication of bitou bush and its hybrids from Victoria.

MATERIALS AND METHODS

Hybridisation At Daveys Bay, 45 *C. monilifera* plants were sampled for morphological characterisation. Of the plants sampled, nine were boneseed collected at Pelican Point, near the hybrid zone, and another 36 plants were within the hybrid zone that exhibited bitou bush or intermediate characteristics. Ten mature leaves, buds, flowers and fruits were collected from each plant and the following diagnostic features (Harden 1992) measured: leaf length, leaf width, petiole length, leaf apex, leaf tooth number, leaf tooth length, ray floret number, ray length, ray width, seed (putamen) length and seed width. Reference populations of boneseed from Crib Point (Vic) (n = 4) and bitou bush from La Perouse (NSW) (n = 10) and Ballina (NSW) (n = 7) were also sampled.

Statistical methods For each measurement, the mean value of all samples from a plant was calculated and these means were used as the unit of analysis. A residual maximum likelihood (REML) analysis was used to model the data to have a separate mean, and a separate variance, for each of the five plant sources (Crib Point, Daveys Bay (hybrid swarm), Pelican Point, Ballina and La Perouse). The means for tooth number and tooth length were square root transformed prior to the REML analysis, to reduce the skewness in the data. Standard errors for the mean and standard deviation of each plant source were calculated using an asymptotic normal approximation.

Wald F tests for comparing treatment means, and change in deviance chi-square tests for comparing treatment variances were carried out for the comparisons shown in Tables 1 and 2. Tests were chosen to maintain marginality between the models being compared.

A principal components analysis, using the seven key diagnostic characteristics (square root transformed

for tooth number and length) after they had been standardised by dividing by sample standard deviations, was carried out on the samples from La Perouse.

Feasibility of eradication The occurrence, distribution and population boundaries of bitou bush and bitou-boneseed hybrids were determined by systematic searches on private and public land and mapped using GPS co-ordinates. At Daveys Bay, a letter drop survey delivered to all properties (n = 75) within and adjacent to the invasion zone collected additional information on distribution and helped assess local awareness of the invasion and attitudes to *C. monilifera* and possible future control programs.

RESULTS

While there are definitely differences in the key diagnostic characteristics between the two boneseed populations and the two bitou populations, these are small compared to the differences between boneseed populations and the Daveys Bay population and compared to the differences between bitou populations and the Daveys Bay population (Tables 1 and 2, Figure 1). In particular, the Daveys Bay population is much more variable, on most diagnostic characteristics, than the pure boneseed and pure bitou populations (Tables 1 and 2, Figure 1).

Nevertheless the Daveys Bay population overlapped the boneseed populations on all seven key diagnostic characteristics (Figure 1). In fact, 6% of plants (n = 2) at Daveys Bay closely matched the La Perouse bitou bush population on all seven characteristics, as well as on the scores derived from the principal components analysis of the bitou bush population at La Perouse (Figure 2). The first three principal components accounted for about 80% of the variation. For

Table 1. Mean values (columns A) and standard deviation (columns B) of traits at each site. Standard errors in parentheses.

	Boneseed				Hybrids		Bitou bush			
	Crib Point		Pelican Point		Daveys Bay		Ballina		La Perouse	
Trait	A	B	A	B	A	B	A	B	A	B
Ray number	4.9 (0.17)	0.34 (0.14)	5.5 (0.09)	0.27 (0.07)	10.9 (0.24)	1.44 (0.17)	10.7 (0.28)	0.73 (0.21)	11.7 (0.18)	0.58 (0.14)
Seed width	6.3 (0.22)	0.44 (0.18)	6.1 (0.06)	0.17 (0.04)	3.9 (0.09)	0.54 (0.07)	–	–	3.2 (0.05)	0.15 (0.04)
Seed length/ width ratio	1.05 (0.014)	0.028 (0.011)	1.07 (0.007)	0.022 (0.005)	1.43 (0.037)	0.214 (0.026)	–	–	1.73 (0.032)	0.101 (0.024)
Square root of tooth number	2.1 (0.07)	0.14 (0.06)	2.0 (0.05)	0.14 (0.04)	1.3 (0.17)	1.05 (0.12)	1.1 (0.40)	1.05 (0.30)	1.1 (0.16)	0.52 (0.12)
Square root of tooth length	1.34 (0.045)	0.09 (0.037)	1.79 (0.034)	0.101 (0.025)	0.57 (0.069)	0.421 (0.05)	0.56 (0.2)	0.527 (0.153)	0.46 (0.064)	0.201 (0.047)
Petiole length	11.5 (0.43)	0.9 (0.4)	10.1 (0.32)	1.0 (0.2)	16.9 (0.41)	3.5 (0.4)	20.6 (1.07)	2.8 (0.8)	18.9 (0.71)	2.2 (0.5)
Leaf length/ width ratio	2.45 (0.097)	0.19 (0.079)	2.31 (0.058)	0.17 (0.043)	1.98 (0.054)	0.33 (0.039)	2.10 (0.049)	0.13 (0.038)	2.02 (0.064)	0.20 (0.048)

Table 2. P values for testing equality of means (columns A) and P values for testing equality of standard deviation (columns B). Values in bold are significant.

	Hybrids vs. boneseed		Pelican Point vs. Crib Point		Hybrids vs. bitou bush		Ballina vs. La Perouse	
Trait	A	B	A	B	A	B	A	B
Ray number	**5.3×10^{-25}**	**1.5×10^{-6}**	**0.032**	0.60	0.054	**0.0012**	**0.016**	0.51
Seed width	**4.4×10^{-23}**	**0.016**	0.44	**0.033**	**2.9×10^{-8}**	**2.2×10^{-4}**	–	–
Seed length/width ratio	**1.2×10^{-11}**	**2.0×10^{-9}**	0.24	0.60	**7.1×10^{-7}**	**0.016**	–	–
Square root of tooth number	**4.2×10^{-4}**	**2.0×10^{-8}**	0.14	1.0	0.33	0.18	0.95	0.055
Square root of tooth length	**7.8×10^{-20}**	**6.7×10^{-6}**	**1.4×10^{-4}**	0.81	0.29	0.55	0.64	**0.010**
Petiole length	**3.3×10^{-13}**	**3.3×10^{-5}**	**0.037**	0.81	**0.0047**	0.16	0.22	0.53
Leaf length/width ratio	**1.7×10^{-5}**	**0.025**	0.28	0.81	0.17	**0.0099**	0.31	0.25

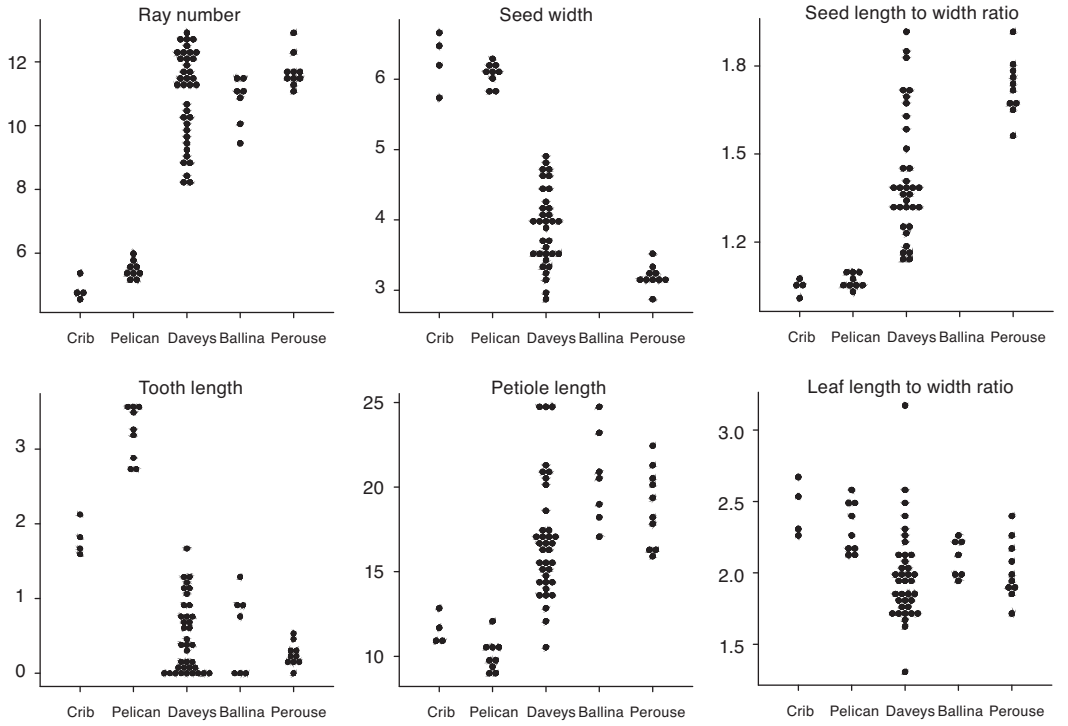

Figure 1. Dot histograms of six of the seven key diagnostic characters for each of the five populations.

each key characteristic, the distribution of the Daveys Bay population extended towards or into the values obtained in the boneseed populations (Figure 1).

Bitou bush and bitou bush-boneseed intermediates occur over approximately 20 ha at Daveys Bay. Most plants are located on public land between the high-tide mark and private residential allotments. Invasion on private land was detected on at least 25% (n = 14) of properties within the invasion zone. A 43% (n = 32) response rate was achieved from the letter drop survey. Ninety-one percent of respondents supported the eradication of bitou bush and hybrids and most were willing to contribute in-kind support. Two survey respondents (6%) opposed the control of *C. monilifera* from the area.

DISCUSSION

Eradication of weeds in the early stages of invasion is a key component of Victoria's approach to weed management and is also a feature of the Australian Weed Strategy. Boneseed is well established in Victoria, where it forms dense and often extensive infestations, and is clearly beyond eradication at a state scale. Bitou bush is restricted in Victoria to two extant populations (Daveys Bay and Mallacoota),

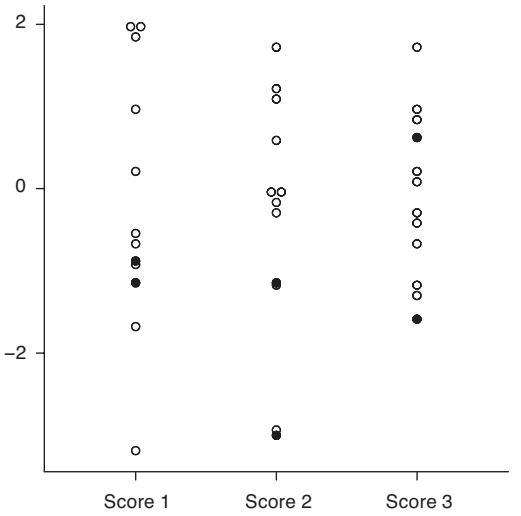

Figure 2. First three principal component scores for bitou bush plants (open circle) and two Daveys Bay plants (closed circle).

which are outliers of the southern Australian bitou bush containment zone (Cherry *et al*. 2008) and therefore

a desirable target for eradication. Two populations of bitou bush have been eradicated from Victoria (Kew, Frankston), while the Mallacoota infestation is well advanced in achieving eradication within several years.

At Daveys Bay, *C. monilifera* plants with key morphological features closely matching bitou bush are present, but form a small component of the population. Most plants were either boneseed or bitou-like hybrids, with intermediate characters between boneseed and bitou bush. Recent analysis of nuclear and chloroplast markers reaffirm that hybridisation has occurred between bitou bush and boneseed at Daveys Bay (R. Watts, CSIRO, pers. comm.). Variation in morphological characters was substantially greater in the hybrid plants than populations of boneseed and bitou bush, making identification of individuals difficult in many instances. Hybridisation between weed taxa can result in hybrid vigour and affect invasion patterns and susceptibility to control (Gaskin *et al*. 2009, Ward *et al*. 2008, Swarbrick *et al.* 1998, Roche and Roche 1991). At Daveys Bay, values of the seven morphological characteristics extended from those of NSW bitou bush towards, and sometimes into, those from boneseed, suggesting hybrid invigoration has not yet occurred in these traits. However, whether there are fitness advantages associated with intermediates values was not determined in this study.

The occurrence of bitou bush and hybrids on steep erodible cliffs at Daveys Bay necessitates a phased approach of management actions, commencing from the upper cliff region and followed up with intensive revegetation using local native species with good soil-binding properties. Soil-stored seed of bitou bush is possibly transient with viability of less than 5 years, although seed of boneseed may remain viable for considerably longer (Schoeman *et al*. 2010). The longevity of hybrid seed is presently unknown. An eradication program for bitou bush at Daveys Bay is proposed to run over 10 years, with the first 3 years dedicated to the removal of established plants (Adair 2009).

Residents within and adjoining the bitou bush infestation zone are largely supportive of a potential eradication program. Although bitou bush is clearly a desirable eradication target for Daveys Bay, hybridisation complicates the identification of *C. monilifera* subspecies. Therefore, an eradication program for bitou bush needs to encompass hybrid plants to ensure the depletion and eventual extinction of bitou bush genes from the Daveys Bay infestation. Simple and readily recognisable characters such as flowering time (April–May), bush architecture (sprawling cf. erect) and ray number would assist control contractors to distinguish bitou bush and hybrids from boneseed.

An eradication program for bitou bush at Daveys Bay is estimated to cost $323K over 10 years (Adair 2009). While bitou bush has not aggressively invaded Victoria, the subspecies has the potential to colonise all coastal habitats in the State (Adair 2009), a situation that would undoubtedly result in substantial declines in biodiversity values. Hybridisation between bitou bush and boneseed has the potential to occur wherever these subspecies are sympatric; therefore, management of *C. monilifera* in New South Wales, in particular, should take into account possible hybridisation risks. Although boneseed may invade areas following the eradication of bitou bush, suppression efforts will reduce the impact of this subspecies.

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