10.1071/BT23029

Australian Journal of Botany

Supplementary Material

Seed biology can inform conservation actions: a case study on Geijera parviflora

Ganesha S. Liyanage^{A,*}, Amy-Marie Gilpin^{B,C}, Catherine A. Offord^A, and Amelia J. Martyn Yenson^{A,B}

^AThe Australian PlantBank, Botanic Gardens of Sydney, Mount Annan, NSW, Australia.

^BSchool of Science, Western Sydney University, Penrith, NSW, Australia.

^cHawkesbury Institute for the Environment, Western Sydney University, Penrith, NSW, Australia.

*Correspondence to: Ganesha S. Liyanage The Australian PlantBank, Botanic Gardens of Sydney, Mount Annan, NSW, Australia Email: Ganesha.boralaliyanage@botanicgardens.nsw.gov.au

Supplementary Material S1

Details of each of the pollination treatments are as follows:

- (i) Open pollination treatment Buds were left to open over three days and exposed to normal pollinator activity without any intervention.
- (ii) Manipulative self-pollination On each day of the experiment any recipient flowers that were open within the bagged manipulative self-pollination treatment had their anthers removed. From the same tree, two freshly opened flowers with pollen present (identified through visual assessment) were then rubbed over the stigma of each of the recipient flowers, with new donor flowers used for each recipient on each day. Recipient flowers were marked to indicate whether they had been hand pollinated on one, two or three occasions depending on the day each flower opened during the experiment.
- (iii) Autonomous self-pollination Bagged buds were left to develop into flowers throughout the experiment. Each day of the experiment bags on the autonomous self-pollination treatment branches were removed and immediately replaced, to maintain consistency of the bag removal procedure across all of the pollination treatments (ii-v).
- (iv) Manipulative outcross pollination – On each day of the experiment, approximately 100 newly opened flowers with pollen present were harvested early in the morning from three selected donor trees in the donor cluster. For the five trees at cluster 1, four donor trees at site 2 were selected and vice versa, with the same trees used each day of the experiment for the five trees at cluster 1 and two days for the four trees at cluster 2. The harvested flowers from each of the four donor trees were pooled, with two flowers randomly selected to pollinate each of the open recipient flowers. Before the hand pollination was performed, all anthers on the newly opened recipient flowers were removed with tweezers to prevent self-pollination each day of the experiment. Whilst holding the donor flower using tweezers, the pollen on the anthers were carefully rubbed all over the stigma of the recipient flower. Once a donor flower had been used to hand pollinate it was disposed of. Between each flower all equipment and hands were washed using 70% ethanol and wiped clean as a precautionary method to prevent cross contamination of pollen between treatments. Each day of the experiment the number of flowers open and subjected to this pollination treatment were documented. After all flowers had been pollinated the bag was re-fastened to prevent any pollinators gaining access to the flowers.

Bag control treatment – The same bags that were used in the three other pollination treatments (ii, iii, iv treatments) had three large holes cut out to allow for insect access to the flowers. The bags were then secured like the other treatments around a cluster of buds and left to develop. Each day of the experiment the cut bags were removed and replaced to simulate the same procedure used for the other treatments.

Supplementary Material S2

Table S1. Temperature data recorded by Thermochron® ibutton in 1-2cm depth at Jerrys Plains, NSW Australia for year 2020 and 2021. All temperature data represented in °C. Site 1, Site 2 and Site 3 indicates the location site of seed bags.

2020									
	Site1			Site 2			Site3		
	Average			Average			Average		
Month	of site	Average	Range	of site	Average	Range	of site	Average	Range
						11.5-			
December	24.9		13-57	25.8		61	22.6		12-41
			16-						
January	24.5		49.5	24.6		15-58	23.0		15.5-41.5
			15.5-			13.5-			
February	22.3	23.9	53	21.7	24.0	55	20.7	22.1	15-45.5
			15.5-						
March	21.1		29.5	19.9			18.3		12-31
			12-						
April	19.1		27.5	17.3			19.2		13-27
May	14.3	18.2	8-22	13.2	16.8	9-23	14.7	17.4	9.5-21
June	12.5		6.5-20	11.5			13.1		8.5-19.5
July	11.9		6.5-19	10.7			12.5		8-17.5
August	12.2	12.2	6-23	10.3	10.8	7-13.5	12.4	12.7	7.5-19.5
September									
October	Data limited due to capacity restrictions of the ibuttons								
			12.5-			9.5-			
November	22.6	22.6	50	24.5	24.5	62.5	21.3	21.3	11.5-45

	2021								
	Site1			Site 2			Site3		
	Averag			Averag			Averag		
	e of	Averag		e of	Averag		e of	Averag	
Month	site	е	Range	site	е	Range	site	е	Range
Decembe									
r									
January									
February									
						11.5-			13.5-
March	18.4		12-31	18.8		41.5	20.2		36.5
April	15.9		9-24	15.7		9-27	18.1		11-32.5
						7.5-			
May	9.8	14.72	8.5-12	10.5	15.02	18.5	13.2	17.17	9.5-25
June									

2021

Season	Average	SD	SE	Minimum	Maximum
Summer	23.3	1.4	0.8	14.1	51.3
Autumn	17.5	3.5	2.0	11.7	26.4
Winter	11.9	0.3	0.2	7.1	18.9
Spring	22.8			11.3	31.2

Table S2. Average, minimum and maximum temperature (°C) data calculated for each season