Vivipary in the Marlborough rock daisy, *Pachystegia insignis* (Asteraceae)

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Vivipary involves the germination of seeds prior to their dispersal from the parent plant. It requires seed dormancy to be absent and has only been recorded in a very small number of phylogenetically diverse higher plants of varying life forms from a wide range of habitats. This study confirms a lack of seed dormancy in the New Zealand native Marlborough rock daisy, *Pachystegia insignis* (Hook. f.) Cheeseman (Asteraceae). High frequencies of germination in *P. insignis* immediately following seed maturation are documented, as well as an instance of prolific vivipary. We provide experimental validation that, when achenes are retained in capitula, vivipary is induced *in planta* by wet conditions.

Keywords: achenes, Asteraceae, Pachystegia insignis*, seed germination, vivipary*

Introduction

Vivipary in flowering plants involves the development of embryos in sexually produced seed through to germination while still attached to the parent plant (Farnsworth 2000). This seed germination phenomenon occurs prior to seed dispersal and is observed in species with no inherent seed dormancy. In true vivipary the seedling penetrates through the fruit pericarp and becomes well developed prior to seedling dispersal. Cryptovivipary involves situations where the embryo significantly develops but does not penetrate the pericarp before dispersal (Tomlinson 1986). In contrast, pseudovivipary describes the apomictic development of asexual plants or bulbils instead of embryos derived from sexual fertilisation (Emlqvist & Cox 1996). True vivipary has been recorded in at least 65 species belonging to over 20 families and is usually observed in tropical species, especially in mangroves (Farnsworth 2000).

Pachystegia insignis (Hook. f.) Cheeseman, the Marlborough rock daisy (Asteraceae), is a stout spreading shrub endemic to New Zealand. It grows naturally in rocky outcrops from coastal to montane areas (Allan 1961) from the Wairau River in southern Marlborough to Lowry Peaks and Hawkswood Ranges in northern Canterbury (Heenan & Molloy 2022). It is commonly grown in New Zealand as a garden plant (Fisher et al. 1988; Metcalf 1987). This study confirms a lack of seed dormancy in *Pachystegia insignis* with high frequencies of germination immediately following seed maturation. It also documents an instance of prolific vivipary and provides experimental validation of vivipary being induced by wet conditions when achenes are retained in capitula.

Methods

Seed germination in Pachystegia insignis

The plants under study involved a small population of five plants growing on the Canterbury Agricultural and Science Centre campus at Lincoln, New Zealand. They were well established plants of cultivated origin, growing for over 35 years in a 'courtyard' protected from adverse weather by surrounding buildings. Achenes harvested from individual capitula were kept separate as independent replicates, with five replicates per treatment. Achenes were placed on Whatman filter paper (#113, 11 cm diameter circles) pushed into the base of clear plastic pottles with lids (98 mm diameter x 60 mm high; supplied by Alto Packaging, Hamilton). The filter paper was moistened with 20 ml of sterile water and 30 achenes were sown into each pottle. Pottles were placed under cool white fluorescent lights (80-100 μmol/m^2/sec; 16 h light: 8 h photoperiod) at a temperature of 22°C during the day and ambient at night. For the dark treatment, pottles were placed in a light-proof container and not opened until germination was scored. Achenes with emerging radicles were considered germinated, with their total number recorded after ten days.

Vivipary in Pachystegia insignis

Following an observation of vivipary *in planta*, the status of all capitula on the small population of plants was recorded with respect to achene dispersal and presence of vivipary. Ten intact capitula with no achene dispersal were harvested and the achenes dissected out under a binocular microscope (10x magnification) and the number of germinated and non-germinated achenes counted.

Vivipary was observed within the moist areas of the dissected capitula. Two experiments were therefore designed to examine the induction of vivipary by wet conditions. To induce vivipary *in situ*, twenty intact capitula in a sheltered position against a building, with no sign of vivipary, were tagged. Ten of these remained untreated and ten were inverted into a container of water for 30 seconds, then 20 ml of water was injected into the pappus of each capitulum using a syringe. This latter water treatment was repeated every second day until the achenes were dissected and scored for seed germination and development as described above. A further twenty intact capitula from a sheltered position under an overhang against a building, with no sign of vivipary, were excised and individually placed in the germination pottles described above. Ten of these were treated with water in the manner as described above and all were incubated under the same conditions as for seed germination.

Results

Seed germination in Pachystegia insignis

Radicles were observed emerging from achenes of *P. insignis* five days after achene harvest and sowing during April-May 2021. A high frequency of germination (82-88% across all experiments) was recorded after ten days. No significant differences were determined in germination frequency of achenes derived from ray florets versus disc florets, light versus dark conditions, or achenes with or without pappus (Table 1).

Natural vivipary in Pachystegia insignis

A high incidence of vivipary was observed on 16 June 2021 among the small population of five *P. insignis* plants from which the achenes for the prior germination experiments were obtained. At this time, these plants had a combined total of 371 capitula. Of these, 164 capitula (44%) were 'empty' i.e. all achenes had dispersed, while 207 capitula (54%) still retained some achenes. Of the capitula with achenes still attached, 149 (72%) exhibited vivipary, with precocious germination of achenes still attached to the capitula (Figure 1). The capitula with vivipary were generally more exposed and wet from severe rain late May-early June 2021. The remaining 58

Table 1. Immediate germination of *Pachystegia insignis* seeds. Achenes were harvested and sown on the same day.

Date of experiment initiation	Treatment	Germination after 10 days (%)	t-test^
4 April 2021	Ray achenes	86	
	Disc achenes	88	$t_s = 0.3873$, P = 0.71
14 April 2021	Light	85	
	Dark	82	$t_s = 0.6489$, P = 0.53
1 May 2021	With pappus	87	
	Without pappus	85	$t_s = 0.3203$, P = 0.76

^t-test performed on the number of seeds germinated (n= 5 replicates, each with 30 achenes).

capitula with achenes (28%), with no apparent vivipary, were dry and generally in a sheltered position under the eve of a building.

The wet pappus of capitula exhibiting vivipary became embedded together and retained moisture. As the seedling grew within the capitula, the accumulated weight eventually caused these capitula to detach from the plants and fall to the ground by mid to late July 2021. While these seedlings started to establish beneath the parent plants, they eventually succumbed to the activity of birds (mostly blackbirds, *Turdus merula*) foraging in the plant litter.

Ten capitula exhibiting vivipary from which no achenes had dispersed were recovered. These were carefully dissected and the frequency of vivipary determined. A mean of 229 achenes were recovered per capitulum (range 106 to 340; n = 10).

Figure 1. Examples of vivipary in *Pachystegia insignis* from the Canterbury Agricultural and Science Centre campus at Lincoln, New Zealand. Photographs taken 16 June 2021.

Seeds had not germinated from 38% of the achenes (range 16% to 57%; n = 10), with these achenes generally situated in dry sectors of the capitula. The remaining achenes, all from damp areas of the capitula, exhibited germination; 17% with emerging radicles (range 6% to 23%; n = 10) and 45% with well-developed green cotyledons (range 24% to 75%; n = 10).

Induced vivipary in Pachystegia insignis

Since vivipary was observed within the moist areas of the dissected capitula, experiments were designed to examine the induction of vivipary by wet conditions. Firstly, water was applied *in planta* to capitula that had developed in a sheltered position against a building and therefore less exposed to rainfall. These capitula were kept moist for three weeks, resulting in prolific vivipary (Figure 2). Dissection of the capitula revealed 11% ungerminated achenes, 23% having radicle emergence, and 66% exhibiting full cotyledon development (Table 2). Without the water treatment, no achenes germinated (Table 2). Secondly, capitula originating from a similar sheltered position were excised from plants, treated with water in the same manner and incubated in a growth room. Abundant vivipary was observed after only ten days (Figure 2). Upon dissection of capitula, 10% of achenes were ungerminated, 19% exhibited radicle emergence, and 71% had full cotyledon development (Table 3). Consistent with the earlier experiment, no achenes germinated without the water treatment (Table 3).

Figure 2. Induced vivipary in *Pachystegia insignis* by treatment of capitula with water; three weeks after first applying water *in planta* (left) and ten days after first applying water to an excised capitulum in growth room (right).

Discussion

Vivipary occurs in a very small number of higher plants that are phylogenetically diverse, of varying life forms and from a wide range of habitats (Farnsworth 2000). This seed germination prior to dispersal from the parent plant requires the absence of seed dormancy. High frequencies of germination immediately following seed maturation were observed for *P. insignis* in this study (Table 1), which establishes an absence of seed dormancy. This confirms a previous report by Simpson & Molloy (1978) who observed up to 82% germination within 2 weeks for freshly harvested seed. It is also consistent with the observation of numerous self-sown seeds appearing beneath cultivated *P. insignis* plants in the autumn after seed production (Fisher et al. 1988, Metcalf 1987).

Among the plants used to establish the absence of seed dormancy in *P. insignis,* prolific vivipary was observed (Figure 1). An instance of vivipary has also been reported on a garden plant of *Pachystegia minor* during an especially wet autumn (Simpson 1979). Vivipary has been recorded in several other Asteraceae species, including: *Abrotanella linearis* var. *apiculata* (Simpson 1979), *Ageratina adenophora* (Karmakar & Hazra 2016), *Bidens pilosa* (Karmakar et al. 2018), *Grindella squarrosa* (Pliszko & Górecki 2021), *Saussurea lappa* (Chauhan et al. 2018), and *Tagetes erecta* (Anand & Mathur 2012). In all these examples of Asteraceae species, vivipary has been associated with seed germination occurring in wet capitula following prolonged periods of rainfall or flooded habitats. Furthermore, in the Asteraceae species *Eclipta alba*, vivipary was observed in excised capitula cultured *in vitro* on a liquid medium (Bimal et al. 2014).

The key advance in this paper is the experimental validation that vivipary in the Asteraceae is induced when capitula with mature achenes become wet. This was clearly established *in planta* and for excised capitula. Applying water *in planta* to dry capitula and maintaining the moisture for 3 weeks resulted in prolific vivipary, while without the water treatment no achenes germinated in capitula (Figure 2, Table 2). Similar results were observed following the treatment of

Table 2. Induced vivipary in situ on capitula of *Pachystegia insignis*. Water was first applied to capitula on 23 June 2021 and moisture maintained for three weeks before data were recorded.

Parameter (n=10 capitula)	No water applied to capitula	Water applied *in situ* to capitula
Number of achenes/capitulum		
Mean	223	234
Standard deviation	27	32
Achenes not germinated (%)		
Mean	100	11
Range	-	8-15
Achenes with radicle emergence (%)		
Mean	0	23
Range	-	15-49
Achenes with cotyledon emergence (%)		
Mean	0	66
Range	-	43-77

Table 3. Induced vivipary on excised capitula of *Pachystegia insignis*. Water was first applied to excised capitula on 9 July 2021 and moisture maintained for ten days in a growth room before data were recorded.

Parameter (n=10 capitula)	No water applied to capitula	Water applied *in situ* to capitula
Number of achenes/capitulum		
Mean	229	225
Standard deviation	31	35
Achenes not germinated (%)		
Mean	100	10
Range	-	7-14
Achenes with radicle emergence (%)		
Mean	0	19
Range	-	14-25
Achenes with cotyledon emergence (%)		
Mean	0	71
Range	-	66-76

excised capitula with water (Figure 2, Table 3). Vivipary was apparent after three weeks for the *in planta* experiment, whereas it was very evident after only ten days in excised capitula. This can be accounted for by difference in temperature, with the *in planta* experiment involving ambient outdoor winter temperatures, whereas the excised capitula were incubated in a growth room at 22°C.

Vivipary is a rare reproductive strategy in flowering plants, especially in non-mangrove species; it has been reported in less than 0.1% of angiosperm species (Cota-Sánchez 2004). It has been suggested that the development of vivipary in flowering plants has had independent evolutionary origins because of its prevalence in a wide range of families (Elmqvist & Cox 1996). Can vivipary be an advantage in Asteraceae species when capitula with mature achenes become wet, or is it just a consequence of the lack of seed dormancy? The pappus of Asteraceae achenes is an adaptation for wind dispersal of propagules. *Pachystegia* species are naturally found in xerophytic, exposed habitats providing opportunity for quick dispersal of seeds upon maturity. Without inherent dormancy *Pachystegia* seeds may have adapted to use the first available exposure to moisture to promote germination. However, if protected from the wind and the pappus becomes soaked with water from rainfall prior to dissemination, the individual achenes become embedded together. The added weight from a large mass of wet pappus is less conducive for wind dispersal. Vivipary then offers an opportunity for immediate germination, with young seedlings falling to the ground due to the accumulated weight from the growth of seedlings in the capitula.

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