

## Phenology of the threatened *Diuris praecox* (Orchidaceae), a range-restricted terrestrial orchid from central eastern New South Wales

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**Abstract:** Phenological studies are important to gain insights into the ecology of plant species, particularly those that are threatened and require specific management actions such as regular population monitoring. For many species of terrestrial orchids, limited fundamental knowledge on peak flowering, pollination and seed production restricts effective monitoring outcomes. In this single-season study, phenology data from one population of the vulnerable *Diuris praecox* were collected, with the aim of informing future management relating to monitoring surveys and to assist in conservation of this species.

To this end, six sub-populations (three each in forest habitat and along maintained powerline easements) were visited weekly from the onset of flowering until seed release, with observations made on 134 tagged individuals within 10 x 10 m plots. During the 2019 flowering season, 37% of all plants developed capsules, and 35% released seed. However, success varied between locations, with greater floral displays along powerline easements resulting in stronger pollination rates, while sparse sub-populations in forested locations showed lower pollination. Significantly more flowers per inflorescence (range 1-7) were evident in forest than easement sites, but there was no significant difference in inflorescence height across these habitats. For most sub-populations at least one orchid set seed, even when occurring in low densities (<10 plants). Overall, substantial floral displays did not necessarily result in abundant fruiting, and impacts from desiccation, predation and grazing likely prevented more successful capsule production in any given sub-population.

The synchronously flowering shrubs *Daviesia ulicifolia* and *Pultenaea villosa* co-occurred across all sub-populations, suggesting that the nectar-less *Diuris praecox* may mimic these species to attract pollinators.

Peak flowering was determined to be approximately 20 days from the onset of flowering, with 83% of all plants in flower at that time. For ongoing monitoring, the timing of surveys to occur approximately three weeks after the first observed flowering, will likely maximize return-for-effort, particularly when survey resources are limited, although it is acknowledged that different seasons and populations may vary from this timeframe.

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## Introduction

The Orchidaceae family is one of the most diverse groups of all flowering plants (Fay 2018). This diversity allows orchids to grow in a wide variety of environments, and in some cases their specificity to particular habitats makes them excellent indicators of ecosystem health (Newman *et al.* 2007; Swarts & Dixon 2009). Within the Orchidaceae there is a high proportion of threatened species, a situation evident in Australia (Backhouse 2007) and replicated globally (Swarts & Dixon 2009; Fay 2018). Estimates suggest that in Australia one third of all orchids are terrestrial, the balance being epiphytic or lithophytic on a range of plant and rock hosts (Weston *et al.* 2005; Swarts & Dixon 2009), and threatening processes are widely acknowledged as being greater in terrestrial than in other orchid groups (Duncan *et al.* 2005; Backhouse 2007; Swarts & Dixon 2009). The majority of threats imposed on terrestrial orchids are human-based, and include land clearing, fragmentation, weed and pest introductions, tourism and recreation, and illegal plant collecting (Weston *et al.* 2005; Swarts & Dixon 2009; Wraith & Pickering 2017). However, natural rarity and restricted geographical distributions in some species often mean that impacts from threats are exacerbated. Orchids with reproductive strategies offering a nectariferous reward to visiting pollinators are generally more successful in setting fruit, and consequently more widespread, than are nectarless species (Neiland & Wilcock 1998).

Terrestrial orchids possess two main life-traits that contribute to their intrinsic rarity; their mycorrhizal association with specific fungal endophytes, and their often specific pollinator requirements, involving pollinator attraction by visual deception or scent (promises of food or sex) to enact pollination (Adams & Lawson 1993; Weston *et al.* 2005; Swarts & Dixon 2009; McCormick & Jacquemyn 2014). As a group, all orchids are primarily pollination limited, the severity of which is tempered by the availability of resources (Tremblay *et al.* 2005) which may vary temporally and geographically. Terrestrial orchids depend on specific fungal associations for the germination of seed and the exploitation or reciprocation of nutrients; the distribution of these fungi across the landscape are influential in where orchid populations flourish (Batty *et al.* 2002; Swarts & Dixon 2009). Mycorrhizal fungi are generally widespread in the landscape, and occur independently of orchid distribution: orchids need fungi, but the reverse is not also true (McCormick & Jacquemyn 2014). High taxonomic diversity in this family is consequently attributed to specialization of either pollinator or mycorrhizal fungi, inherently increasing the risk of extinction in highly specialized species (Tremblay *et al.* 2005; Brundrett 2016; Fay 2018).

Most terrestrial orchids are cryptic geophytes, remaining dormant in the soil for at least six months of the year in the form of an underground tuber, and may remain in that state for up to three years, if environmental conditions are unfavourable (Weston *et al.* 2005). This presents challenges for surveying and monitoring threatened orchids, as many populations flower irregularly while they await suitable environmental conditions (e.g. adequate rainfall at the right

time of year); some authors refer to orchids displaying this habit as ‘time travelers’ (e.g. Brundrett 2016). Further, the variability in magnitude of orchid emergence extends across both time and space (Gillman & Dodd 1998; Kindlmann & Balounová 2001), with populations observed at some locations budding or flowering freely while nearby others in seemingly identical habitat are yet to appear. A wide suite of biological and ecological factors may govern this, including the successful production of seed and its dispersal, the availability of mycorrhizal fungi within the soil, the provision of adequate and timely populations of appropriate pollinators, and suitable environmental conditions to encourage and maintain emergence and flowering (Swarts & Dixon 2009; McCormick & Jacquemyn 2014). Such a range of influential factors ensures that surveys designed to detect terrestrial orchids are often hit-and-miss affairs, beholden to the interaction of climatic and ecological variables which are difficult to control and predict.

*Diuris praecox* (Rough Double-tail) is a terrestrial nectarless orchid endemic to a restricted area of central eastern New South Wales (NSW). It is listed as Vulnerable under both the NSW *Biodiversity Conservation Act 2016* (BC Act 2016) and the Commonwealth *Environmental Protection and Biodiversity Conservation Act 1999*. *Diuris praecox* is a site-managed species under the *Saving our Species* (SoS) program being implemented by the NSW Department of Planning, Industry and Environment. Under this initiative, management actions are required to secure this species in the wild for the next 100 years and maintain or improve its conservation status under the BC Act 2016 (OEH 2019). As with many orchid species included in this management stream, annual surveys of known populations are undertaken to monitor their persistence and magnitude, and to identify appropriate actions that may be required to lessen the impact of threats (Emergent Ecology 2018). Previous monitoring actions within this strategy have included the surveying of parallel transects during the flowering season in known and suspected habitat to identify occupancy area, the establishment of permanent monitoring ‘macro-plots’ to further refine occupancy area, and installation of smaller 10 x 10 m plots within macro-plots to map individuals and investigate reproductive success (Emergent Ecology 2017, 2018; OEH 2019). To date, surveys for this species have occurred at or near perceived peak flowering periods, but there have been no longitudinal survey data across a single season to determine the optimal time to detect this species during surveys.

Given the often unpredictability of flowering in terrestrial orchids, the frequency of visitation and timing of surveys will inevitably affect the outcomes of actions requiring the reporting of population size. Currently there is little knowledge on the best time to conduct surveys for *Diuris praecox*. This paper examines the phenology of one population of *Diuris praecox* over a single season, to identify peak flowering at this location, and investigates the influence of habitat type on flowering and fruiting success.

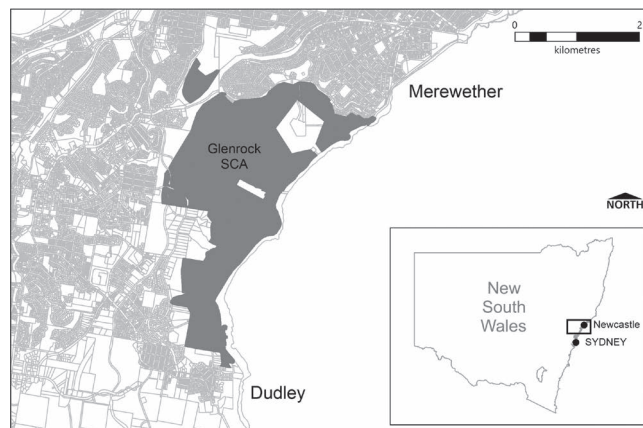
## Study species and population

*Diuris praecox* occurs in sclerophyll forests with a grassy or dense understorey, on sandy to sandy-clay soils in coastal and near coastal areas of central eastern NSW, from Ourimbah to Nelson Bay (Jones 1991; Emergent Ecology 2017; PlantNET 2019). Larger populations occur in disturbed habitats, along tracks and maintained power line easements, with fewer individuals in adjacent forest habitat. Individuals can occur as isolated plants or in clumps (Jones 2006), and Emergent Ecology (2017) have noted its absence when leaf litter reaches 10 cm depth or greater. There are currently four SoS management sites for this species across its distributional range. The present study was undertaken in one of these, Glenrock State Conservation Area (SCA), which comprises 550 hectares of coastal forest and heath immediately south of Newcastle, between the suburbs of Merewether and Dudley (Figure 1). Urban development surrounds the reserve on three sides, the fourth being the Pacific Ocean. Glenrock SCA is managed by the NSW National Parks and Wildlife Service, and its flora and vegetation have been previously documented in Bell (1998). *Diuris praecox* has been known from Glenrock SCA for several decades, along with other threatened plants including *Cynanchum elegans*, *Tetratheca juncea* and *Syzygium paniculatum*. Other conserved *Diuris praecox* populations are in Wyrabalong National Park, Munmorah State Conservation Area, Tomaree National Park and the Worimi Conservation Lands (Bell 1997, 2002; Burton 2008; Bell & Driscoll 2010). No other species of *Diuris* were recorded flowering within our study population at the time of this study.

Like most terrestrial orchids, *Diuris praecox* remains dormant for most of the year, leaves emerging in May and flowering occurring from late July to early September (Jones 2006). Leaves are linear, 150–350 mm long and up to 5 mm wide, and individuals are often associated with habitats supporting a grassy understory, ensuring difficulty in detection prior to flowering. Flowers resemble members of the Fabaceae family ('peas') in shape and colour, and some studies have shown that certain *Diuris* taxa rely on co-occurring pea flowers to attract pollinators to the area through false nectar pathways (Beardsell et al. 1986; Indsto et al. 2006, 2007). For example, *Diuris maculata* was found to have similar ultra-violet nectar guides to those in the Fabaceae, including red and yellow pea flowers of the genera *Pultenaea* and *Daviesia* (Indsto et al. 2006).

Relative to the past three years, Glenrock SCA received below average rainfall in 2019 for all months except June, August and September (BOM 2019). Falls in June at the Burwood Beach weather station (station #061391, located within Glenrock SCA) occurred largely over two six day periods, within the first week and last ten days of the month, and were 106 mm above the average. July rainfall was less than half of the average, but spread over two periods early and late in the month. In August (28 mm above average), 89 mm of rain fell over the last two days, while in September (40 mm above average) 101 mm fell in the first two days of the month. For the late winter flowering *Diuris praecox*, these falls were

sufficient to saturate the soil prior to flowering, and then to maintain soil moisture over the full flowering period.

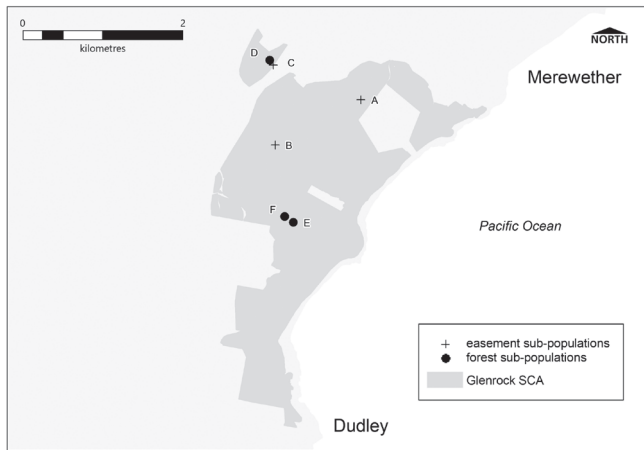


**Figure 1:** Location of Glenrock State Conservation Area (SCA) within central eastern New South Wales.

## Methods

Six sub-populations of *Diuris praecox* in Glenrock SCA form the basis of this study (Figure 2); three along regularly slashed powerline easements ('easement': sub-populations A to C), and three in undisturbed forest habitat ('forest': sub-populations D to F). Monitoring plots 10 x 10 m in size were established in each sub-population, and a 1-metre grid was used to progressively map and tag all individuals with a unique number. Tags were placed in the ground c. 10 cm from the flower stem to avoid damaging tubers. In cases where several stems occurred in close proximity, only a single individual was tagged; a minimum separation distance of 30 cm was used to demarcate the next nearest tagged plant.

This study focused on monitoring orchids at weekly intervals (from August to September 2019). For each tagged plant, information on flowering status and micro-habitat was recorded, including: inflorescence stem height (in cm); presence and number of buds; flowering number and stage (closed, fully open, wilting); evidence of pollination (ovary swelling, capsule development); capsule number and stage (dehisced with seed release, fail); apparent abortion or cessation of flowering (not pollinated, trampled, predated, other/unknown); and micro-habitat (grass, leaf litter, moss etc.). As *Diuris* inflorescences open acropetally, flowering stage of the least advanced flowers on each inflorescence only were recorded, and represented flowering progress of that individual at each inspection. For example, an individual was categorized as in flower only when no developing buds were present, but was scored as in bud if all but one flower was yet to open. Additional habitat information, such as associated plant species occurring within each plot, vegetation height (within easements only), observable threatening processes and/or disturbance events, was also recorded. Co-occurring peas observed to be flowering synchronously with *Diuris* were also noted.



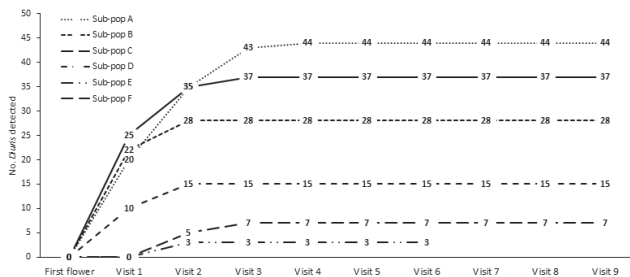
**Figure 2:** Study sub-populations within Glenrock State Conservation Area.

Data were analysed with JMP software (SAS Institute 2019) to investigate differences in flowering and capsule development, peak flowering and inflorescence stem height between the two habitat types (forest, easement). Independent-samples t-tests were used to test for significance in the number of flowers per inflorescence and inflorescence height across easement and forest sites. Analysis of variance and bivariate linear regression were used to determine whether there was a relationship between flower display and fruit set. A graph of flower numbers against visitation was constructed to determine peak flowering.

**Results**

*Diuris praecox* detection

The maximum number of individuals was quickly reached in all plots (Figure 3) particularly for subpopulations in easements (sub-populations A to C), where 28-55% of individuals were detected after the initial visit. For sub-population E (forest), no additional *Diuris* were located after the first detection at Visit 2, and unexplained removal of tags at Visit 6 curtailed further monitoring of this sub-population. Most plants had been detected within each sub-population by Visit 3. A total of 134 *Diuris* were located across all sub-populations; 24.6% of these were detected while still in bud, 74.6% were in flower, and 0.8% when flowers were wilting (Table 1).



**Figure 3:** Cumulative total of *Diuris* detected at each sub-population. Sub-populations A to C = Easement, D to F = Forest.

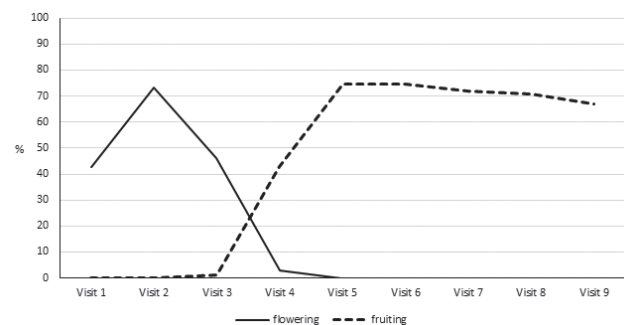
**Table 1: Flowering stage of *Diuris praecox* at first detection, Glenrock SCA.**

Habitat	Sub-population	Flowering stage			Total plants
		Buds	Open flowers	Wilted flowers	
Easement	A	14	30	-	44
	B	6	22	-	28
	C	8	28	1	37
Forest	D	3	12	-	15
	E	-	3	-	3
	F	2	5	-	7
Total		33	100	1	134
Proportion (%)		25	75	<1	100

*Flowering Period*

First flowering in *Diuris* was detected on the 29th of July 2019. Weekly inspections subsequently commenced on 7 August and continued until no further flowering was observed within plots, on 17 September 2019. The total flowering period spanned 42 days, and incorporated six visits to all plots over this period (Visit 1, 7-12 August; Visit 2, 17-19 August; Visit 3, 24 August; Visit 4, 2 September; Visit 5, 8 September; Visit 6, 17 September). Three additional visits subsequent to this to monitor capsule development did not record any further flowering.

Flowering was at its peak on Visit 2, with most flowering occurring during the initial three weeks of observations (Figure 4). Expressed as a proportion of the total tagged population (n = 134), flowering peaked at Visit 2 (73.1%), while fruiting peaked at Visit 5 (74.6%). Most flowers had either been grazed, failed to be fertilized or were developing capsules by Visit 4, however the occasional flower was observed up until Visit 5. Some buds did not progress to flowering or fruiting, and a small number of new individuals evaded detection when in flower and were discovered only when in fruit. No flowering was recorded on Visit 6 or subsequently. Buds were last observed at Visit 3, with flowering finishing up by the following inspection one week later. Pollination was first detected on Visit 2, and the first capsules were observed on Visit 4. Seed set was first recorded at the beginning of October and continued into mid-October and beyond.



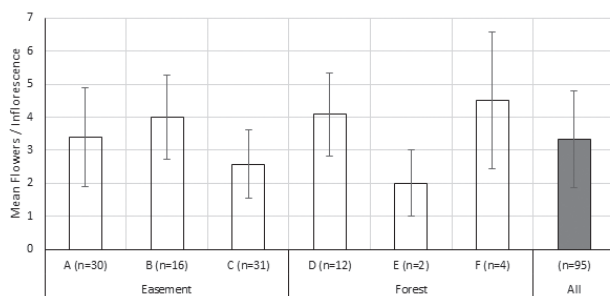
**Figure 4:** Percentage of *Diuris* flowering (n = 100) and fruiting (n = 59) across all sub-populations at Glenrock SCA over the full monitoring period.

*No. of flowers per stem*

For individually tagged orchids (n = 95), the minimum number of flowers per inflorescence was one, the maximum was seven, and the overall mean was 3.34 +/- 1.47 (Figure 5). An independent-samples t-test showed there to be a significant difference between the number of flowers in easement ( $\bar{x} = 3.19$ , SD = 1.40) and forest ( $\bar{x} = 3.94$ , SD = 1.61) sites:  $t(93) = 1.990$ ,  $p = 0.0495$ . This suggests that more flowers are borne on inflorescences occurring in forest than in easements, and perhaps reflects the observation that plants in forest habitat tend to be taller than those that have colonized easements.

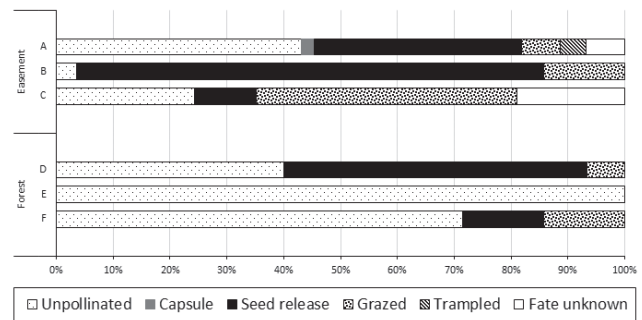
*Successful capsule development*

First development of capsules was observed on Visit 3 at sub-population A (easement) on 24 August, and most capsules were evident across the majority of sub-populations on Visit 5 and Visit 6 (Figure 4). No capsules were observed at sub-population E (forest), and only one at sub-population F (forest). Of 53 capsules developing, all but one (in sub-population A) progressed to seed release. Sub-population B (easement) had the highest level of capsule development, with 82% of individuals progressing to seed (Figure 6). This is significantly higher than other sub-populations examined and comprises approximately half of all other orchids successfully reaching this stage. Sub-populations E and F (both forest) returned particularly low rates of capsule development, only one orchid progressing to seed. Cessation of flowering/fruitletting due to grazing, as determined by missing flowers/fruit and chewed stems, was significantly higher at sub-population C (46%) than all other areas. This site was seen to be infested with a large number of phasmid insects not present elsewhere, and although no direct observations of grazing were made it is posited that these insects grazed on flowers and/or capsules.

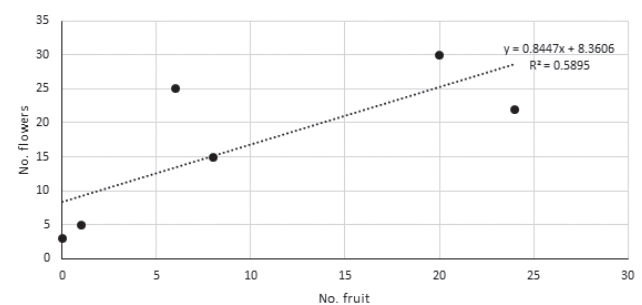


**Figure 5:** Mean (with standard deviations) of the number of *Diuris* flowers per inflorescence across all sub-populations in easement (A-C) and forest (D-F) sites at Glenrock SCA.

Overall, flower display and fruit development gave a strong positive relationship across all sub-populations, with a correlation of  $r = 0.77$ . However, regression analysis (Figure 7) returned an  $R^2$  value of 0.59, indicating only a moderate fit for all samples and this was not significant ( $p = 0.075$ ). This suggests that substantial floral display in this species does not necessarily result in abundant fruit production, likely due to factors such as desiccation and grazing.



**Figure 6:** Fate of 134 *Diuris praecox* individuals within Easement (sub-populations A to C) or Forest (sub-populations D to F) habitats, showing relative proportion remaining un-pollinated and those attaining or failing to reach capsule or seed release stage.



**Figure 7:** Regression analysis of flower display and fruit development in *Diuris praecox* across six study sub-populations.

*Growth habit & associated species*

Across all plots, 25% of tagged individuals occurred in a clumped arrangement (several stems <30 cm apart) rather than as widely spaced individuals. These clumps may possibly be clonal, although a more likely explanation is leptokurtic dispersal (seed dispersal close to founding individuals). Overall mean stem height of flower inflorescences was 22.8 cm (n = 107) for easement and 24.4 cm (n = 25) for forest sub-populations. Although sample sizes were unequal, a Levene test returned a non-significant result ( $f = 1.718$ ,  $p = 0.192$ ), suggesting that variances were homogeneous. An independent-samples t-test subsequently showed there to be no significant difference in inflorescence height between easement ( $\bar{x} = 2.80$ , SD = 7.13) and forest ( $\bar{x} = 24.42$ , SD = 6.00) sites:  $t(133) = 1.050$ ,  $p = 0.2955$ .

At the micro-habitat scale, most *Diuris* were found growing amongst grass, most commonly *Themeda triandra* (20%) or other grass species (39%), while some sub-populations were in bare ground (10%), or within clumps of *Lomandra longifolia* (7%) or in leaf litter (7%). Some *Diuris* occurred under shrubs (10%), but this was a rarity. Collectively, *Diuris* occurred within habitat dominated by a canopy of *Angophora costata*, *Eucalyptus acmenoides*, *E. paniculata*, *E. punctata* and *Corymbia maculata*, although such species were absent along easement sites. Native shrubs present in this habitat comprised *Acacia ulicifolia*, *A. irrorata*, *A. stricta*, *A. longifolia*, *A. falcata*, *Breynia oblongifolia*, *Daviesia ulicifolia*, *Dodonaea triquetra*, *Leucopogon juniperinus*,

*Pittosporum undulatum* and/or *Pultenaea villosa*. Common ground layer species included the grasses *Entolasia stricta*, *Imperata cylindrica* and *Themeda triandra*, the graminoids *Lomandra longifolia*, *L. multiflora* and *Dianella caerulea*, the fern *Cheilanthes sieberi*, and the forb *Lobelia purpurascens*. Weed species, such as *Andropogon virginicus*, *Asparagus aethiopicus*, *Briza subaristata*, *Lantana camara* and *Lilium formosanum* were also present to varying degrees in some sub-populations.

Within the immediate vicinity of study plots, the shrubs *Daviesia ulicifolia* and *Pultenaea villosa* (both Fabaceae) flowered synchronously with *Diuris praecox* and were present at all sub-populations. *Pultenaea villosa* came into flower during Visit 2 (mid-August), and flowering in this species appeared to be heaviest from mid-August to the beginning of September. *Daviesia ulicifolia* flowered earlier than *Pultenaea villosa*, and remained in bloom for the duration of the survey period. The abundance of both of these species appeared to be typically greater at the easement locations, although this attribute was not measured quantitatively. Other species also in flower at this time were *Hibbertia dentata* (Dilleniaceae) and *Hardenbergia violacea* (Fabaceae). No observations on visitation by potential pollinators to either *Diuris praecox* or these co-occurring species were made at the time of surveys.

## Discussion

### *Diuris* detection and flowering trends

As with most terrestrial orchids, *Diuris* is visible predominantly when in flower and hence there is clearly a greater chance that individuals will be detected in plant surveys during this life-stage. This is particularly so for those species with bright and showy flowers, like most *Diuris*, that are easily detected by the human eye. However, it should never be assumed that all individuals of an orchid population will be detected in targeted surveys (Sanger & Waite 1998; Kéry & Gregg 2003; Bell 2019). There is a range of factors influencing detectability on any given day, such as available soil moisture, ambient temperatures, pressures from grazing and trampling on flowering stems, asynchronous flower opening of individuals and populations, experience of field surveyors, and over-shading or shielding by co-occurring vegetation (Duncan *et al.* 2005; Backhouse 2007; Light & MacConaill 2007; Milberg *et al.* 2008; Swarts & Dixon 2009; Duncan & Moloney 2018). One way to reduce uncertainty of knowing when to best survey a population is to identify the peak flowering period. An identified peak may not necessarily be consistent from year to year (Kindlmann & Balounová 2001), but it will give some guidance when planning monitoring surveys. Importantly, the implicit assumption made by many field workers that all plants in a search area will always be found may often prove incorrect (Kéry & Gregg 2003; Milberg *et al.* 2008).

For the study population of *Diuris praecox* at Glenrock SCA, peak flowering occurred on Visit 2, twenty days after the first open flower was observed. At this time, a large proportion

(83%) of all plants were flowering, and by Visit 4 (c. 35 days post first-flower) the bulk of orchids had ceased flowering. Limited rainfall at this time may have shortened the overall flowering period, although this is not expected to have been a significant constraint. It required four visits to sub-population A (easement, 44 *Diuris*) to detect all individuals, which was considerably longer than all other sub-populations, and this trend has been noted previously (pers. obs.; Emergent Ecology 2018). This may have been due to the difficulty in detecting orchids amongst the dense grass/shrub ground layer at this location, or may suggest that this sub-population has a longer flowering span. Initial detection and tagging of some *Diuris* individuals was enacted while in bud (25%), but the majority were in full flower. One individual was not located until flowering had ceased, implying that others may also have been overlooked in the dense ground layer vegetation.

### Pollination & pea flower presence

*Diuris* is one of several terrestrial orchid genera that commonly rely on visual deception (mimicry) to attract pollinators to their predominantly nectar-less flowers (Adams & Lawson 1993; Brundrett 2019). In these situations, successful pollination will only occur if nectar-producing, synchronously flowering non-orchid plants displaying similar floral features occur within and around *Diuris* populations. With few exceptions in this genus, pollinators are mistakenly attracted to *Diuris* flowers in the belief that a reward of nectar awaits them. Some previous studies have drawn a link between successful fruit development in *Diuris* with co-occurring pea species from the Fabaceae family. Beardsell *et al.* (1986), for example, documented how *Diuris maculata* fruiting success was tied to the presence of *Daviesia virgata*, *D. mimosoides* and *Pultenaea scabra*, all three of which flowered synchronously with *Diuris* during their study. Vizer (2013) suggested that the shrubs *Templetonia stenophylla* and *Daviesia genistifolia* may be important species to attract pollinators to areas where the vulnerable *Diuris tricolor* occurs in the upper Hunter Valley, and Indsto *et al.* (2007) targeted *Daviesia ulicifolia* in an effort to capture potential pollinators in their study of *Diuris maculata*.

Although not assessed quantitatively, a greater proportion of synchronously flowering pea plants (*Daviesia ulicifolia*, *Pultenaea villosa*) were observed within easements compared to forests at Glenrock SCA, suggesting that high pollination rates at these sub-populations may have been assisted by pea co-occurrence. This trend was variable, however, with sub-population B (showing the highest rate of pollination success at 82% from 28 *Diuris*) occurring in an easement dominated by exotic grasses with minimal shrubs. No flowering peas were present within the study plot here, although they were evident 5-10 m away in the adjacent forest and were dominant in the easement approximately 20-50 m from the plot. In contrast, sub-population A (also an easement, with 44 *Diuris*) supported many individuals of *Daviesia ulicifolia* and *Pultenaea villosa* but returned a success rate of only 39%. Sub-population C (easement, 37 *Diuris*) similarly had a high presence of *Daviesia* and *Pultenaea* but a low pollination success rate (11%). The most likely cause for this poor result

is presumed grazing by a species of phasmid (stick insect, c. 8 cm long), which were observed in large numbers within the study plot on Visit 4. To our knowledge, there is no mention elsewhere in the literature of phasmids grazing on *Diuris* or other terrestrial orchids, although a range of other insects (including beetles, weevils, flies, bugs, wasps, butterflies, moths, thrips) have been observed and implicated elsewhere (Light & MacConaill 2011). Phasmids have been briefly noted as feeding on epiphytic orchids (including *Dendrobium*, possibly in cultivation) in Singapore (Ridley 1894), but this remains the only reported evidence of such herbivory.

At the Glenrock population, *Hibbertia dentata* (Dilleniaceae) and *Hardenbergia violacea* (Fabaceae) were also flowering synchronously with *Diuris praecox*, although to a considerably lesser extent. These two species may also play a role in attracting pollinators, given the suggestion by Brundrett (2019) that other representatives in these genera attract pollinators to *Diuris magnifica* colonies in Western Australia. A review of all Fabaceae recorded for Glenrock SCA in Bell (1998) suggests several other possible mimic models (e.g. *Phyllota phyllicoides*, *Podolobium ilicifolium*, *Pultenaea daphnoides*, *P. euchila*, *P. paleacea* and *P. retusa*), however few of these were observed in the vicinity of the *Diuris* study sub-populations.

Irrespective of synchronously flowering peas, successful pollination and capsule development within the study population of *Diuris praecox* was generally higher in areas where greater densities of the orchid were evident, such as in easement sites. Sub-population D (forest, 15 *Diuris*) at Glenrock also showed a high pollination/capsule development rate, which supported more *Diuris* than all other forest sub-populations (range 3 to 10). Good rainfall received in August and early September, corresponding with the fruiting phase, likely contributed to successful capsule development across this and all studied sub-populations. Overall, regression analysis showed that substantial floral displays do not necessarily result in abundant fruiting, and impacts from grazing likely prevent more successful capsule production in any *Diuris* population. As in this study, other research with *Diuris* has shown greater presence and flowering in open habitats rather than in forest (e.g. Burton 2008; Vizer 2013; Tierney et al. 2017), although proximity to habitats where sufficient nectar-producing species occur will always be important for long-term persistence.

#### Growth habit

One in four plants of *Diuris praecox* occurred within a clump (<30 cm from neighbours), however the bulk of detected orchids presented as individuals. As no further investigation was undertaken within clumps, it is unknown if these plants are clonal or represent several individuals from a previous successful germination event. Orchids emerging in close proximity to one another may be distinct individuals, having germinated in an area where the necessary mycorrhizal fungi were particularly abundant, however most cases of aggregated distributions in orchids are thought to be a result of clonal growth (Bates 1986; Brundrett et al. 2003). Vizer (2013) considered laboratory-raised specimens of *Diuris*

*tricolor* bearing three leaves (rather than the usual 1-2) to be clonal, after finding leaf emergent points on axillary branches of tubers.

Although not significant, inflorescence stem height differed between easement and forest sub-populations, with the former having shorter average stems than the latter. This may be due to a number of factors, and one possibility is that the higher densities of orchids along easements reflect a cohort of younger plants that have colonized these habitats since easement creation. Taller orchids, such as are present in forested habitats, are more likely to be older and have larger storage reserves, enabling greater vegetative growth including a larger number of flowers with taller stems (Gregg & Kéry 2006). They may also be a developmental consequence of the need to grow taller to outcompete surrounding vegetation. Significantly more flowers per inflorescence were found in forest than easement habitat, supporting suggestions that older, taller individuals bear more flowers than younger plants.

#### Implications for management

The results of this study can now be applied to the management of *Diuris praecox* at Glenrock SCA and at other populations. For this late-winter flowering species, the greatest number of open flowers occurred approximately three weeks (20 days) after the first detection of a flowering individual in the population. This would suggest that monitoring surveys designed to quantify the size of a population, when survey resources are limited, are best undertaken approximately twenty days after first flowering. However, if resourcing allows for more regular surveys repeated inspections of an area either side of this optimum are more likely to capture a greater proportion of individuals in a population.

Although dense colonies of *Diuris praecox* appear to attract sufficient pollinators (such as occurred at easement sub-populations), this study has also implied that successful pollination of sparse colonies and isolated individuals will likely be greatly assisted by the presence of the synchronously flowering *Daviesia ulicifolia* and *Pultenaea villosa*. Management of the habitats supporting *Diuris* should therefore ensure that populations of these peas are maintained in the near vicinity of known orchid sub-populations, preferably in a mosaic pattern of age classes to ensure ongoing supply of reward for pollinating insects. Both of these peas require regular fire to replenish their populations (Benson 1985; Benson & McDougall 1996; Clarke et al. 2009), however fires of high frequency may potentially render them locally extinct. Many species of *Daviesia* (but not *D. ulicifolia*; Benson & McDougall 1996) also reportedly resprout following fire, although this will likely depend on fire intensity. Flowering in both *Daviesia* and *Pultenaea* generally does not occur for the first three years after fire (Benson & McDougall 1996), and for *Diuris maculata* Brown et al. (2007) suggested that the limited availability of these nectar-rewarding species immediately post-fire may restrict pollination of this species of *Diuris*. Additionally, they raise the possibility that pollinator activity may be influenced by moisture availability (rainfall) in and

around *Diuris* and pea populations, ultimately reducing the extent of capsule development. While for *Diuris praecox* this remains untested, the interplay of rainfall and fire at Glenrock SCA may prove important in the maintenance of pollinating insect populations.

Terrestrial orchids are prone to a wide range of threats, both natural and man-made (Duncan *et al.* 2005; Backhouse 2007; Swarts & Dixon 2009; Wraith & Pickering 2017). Grazing by vertebrates and invertebrates is perhaps one of the most pressing natural stressors operating on orchid populations, yet it is relatively poorly understood. This is particularly the case for invertebrate grazing; observations made in this study of the potential predation on *Diuris* by phasmids, and its impact on the production of fruiting capsules, has not been previously reported in the literature (see Light & MacConaill 2011). Further research into how these insects affect population dynamics would be beneficial, and managers may need to monitor and act upon these insects during *Diuris* flowering, perhaps through the use of grazing exclusion cages.

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