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The flowering phenology of a natural population of *Acacia dealbata* (Fabaceae) in southeast Tasmania, Australia

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Abstract

Information on flowering phenology is necessary for full understanding of a plant's pollination ecology and breeding system. This paper reports such a study for a natural population of *Acacia dealbata* Link (family Fabaceae) in southeast Tasmania, Australia. Over a 4-year period, field observations were made on up to 11 mature trees, recording phenological events at the population, tree, raceme, and individual flower head levels. There is a single annual flowering event with the population in flower for an average of 53 days, peaking in late August and varying by <17 days between years. The rank order of flowering of trees is conserved. On average a raceme bears reproductively functional flower heads for a period of 25 days, substantially longer than reported for other *Acacia* species. Styles within individual flowers extend in advance of anther filaments but there is substantial overlap between male and female phase within heads implying that protogyny is an inefficient barrier to self-pollination.

We conclude that flowering phenology is strongly repeatable from year-to-year and broadly seasonally conserved between Tasmania and other countries where the species grows as an alien. It is argued that the relatively lengthy functional period for flower heads, together with mass-flowering, are adaptations promoting outcrossing in a pollinator-scarce late winter environment.

This baseline study under natural conditions contributes to understanding of the reproductive biology of this internationally important species and to the more general unresolved question of why so many supposedly insect-pollinated temperate *Acacia* taxa flower so early in the season.

Keywords: *Acacia dealbata*; reproductive biology; phenology; receptivity; anthesis

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Introduction

Phenology, or the seasonal timing of life cycle events, can be critical to the survival and reproduction of plants (Rathke and Lacey 1985). Description of the floral phenology, at structural levels from the whole plant to the individual flower, is an important step in elucidating the reproductive strategy of a species. In this paper we present results of such a study of a natural population of *Acacia dealbata* Link (Silver Wattle) in southeast Tasmania. This species is native to south-eastern Australia, with a range extending from Tasmania and western Victoria to northern New South Wales. It is common in forest and woodland communities in Tasmania from sea level to 900 m and dominates many transitional forests on disturbed sites (Kitchener and Harris, 2013, Nghiem *et al* 2018), varying in size from a low shrub on dry sites to a tall tree over 25 m in height on deep soils on wetter sites (Boland *et al*., 2006). It has also been widely planted in countries outside Australia for ornamental purposes, perfumery and fuelwood (Griffin *et al*., 2023) and has a reputation for invasiveness via both seed and root suckering (Gibson *et al*., 2011; Fuentes-Ramirez *et al*., 2011; Montesinos *et al*., 2016). It has been introduced to over 40 countries, more than any other species of *Acacia*, and become naturalised in about 30% of these (Botella *et al*. 2023). For more details of introduction and invasion history in Mediterranean Europe, Chile and New Zealand consult papers in Richardson *et al*. (eds.) 2023.

Genetic analyses by Broadhurst *et al*. (2008) showed that open-pollinated seed from six natural populations of *Acacia dealbata* in NSW was highly outcrossed, though (Gibson *et al* 2011) classified the species as having a mixed mating system on the basis of controlled self-pollination experiments in South Africa (Rodger and Johnson 2013) and Portugal (Correia *et al*. (2014). In Tasmania, regular heavy crops of seed are produced in winter/early spring when insect vectors, considered largely responsible for pollination of the genus (Bernhardt 1989, Stone *et al* 2003, Wandrag *et al* 2023), are not abundant (Griffin *et al* (2020). The possibility that wind pollination may also be important, as suggested for *Acacia longifolia* (Giovanetti *et al* 2018), Griffin *et al* (2025 in press) is under investigation and would provide an explanation for the temporal stability of the flowering phenology of this and many other species of *Acacia* in SE Australia, which also flower at that time of year (Costermans 2009).

The current study documents flowering phenology of a natural stand of *Acacia dealbata* at structural levels from the population to individual flowerheads, over a four year period.. The full reproductive cycle from early development of inflorescences to pod maturation is illustrated in Figure 1 but in this paper, we focus only on the flowering period *per se*.

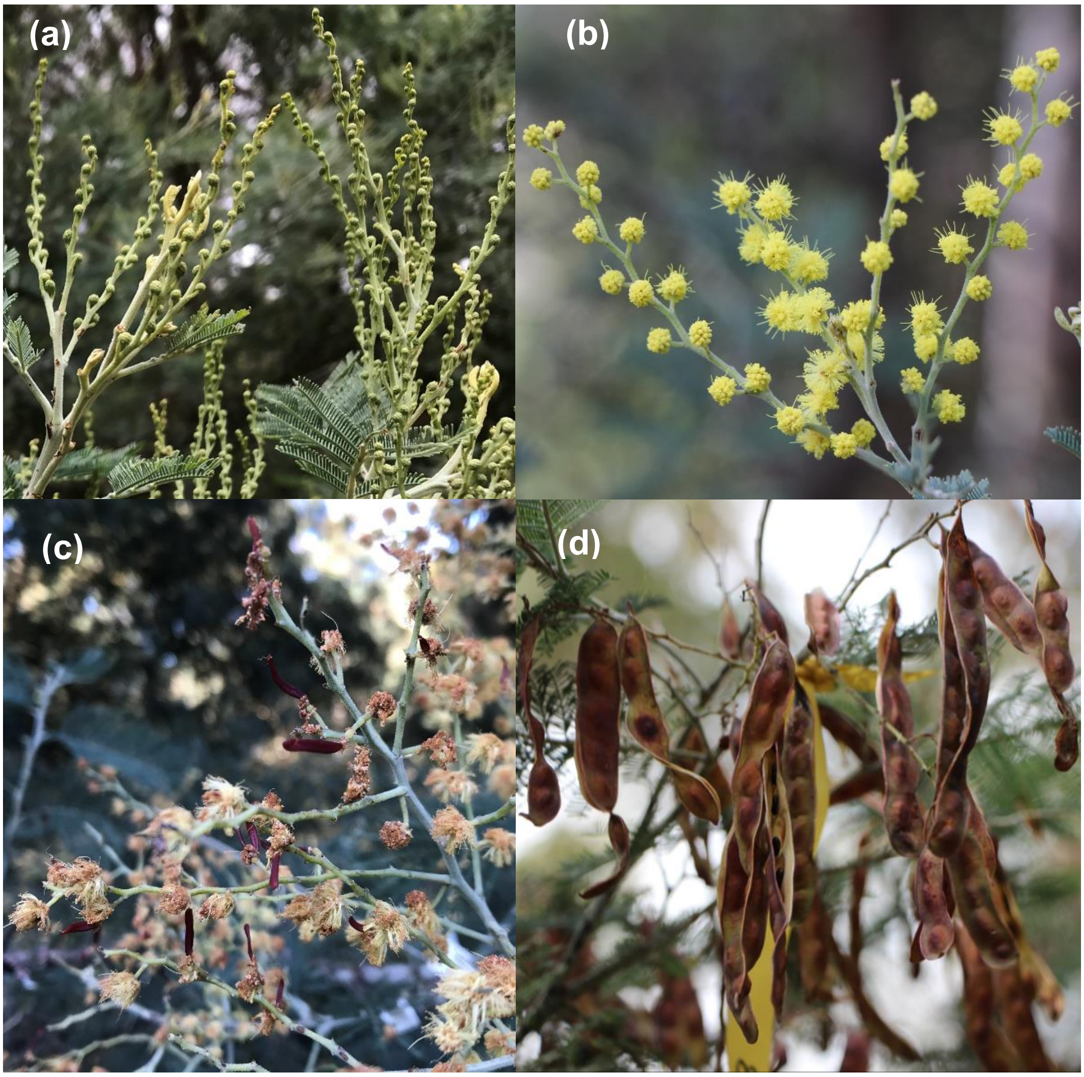

Figure 1. Stages in the flowering/fruiting cycle. a) extending raceme rachises; b) early-stage flowering; c) initial development of pod clusters and d) mature pods beginning to dehisce.

Methods

The annual flowering period of a sample of trees was documented from 2020-2023, to assess year-to-year repeatability in phenology of individual trees and the whole population. Additional studies in 2018-2019 documented temporal development of inflorescences and individual flower heads (fh).

The globose flower heads of *Acacia dealbata* are arranged in racemes, which are often paniculate (Blazek *et al* 2001) and we refer to the multiple racemes produced on a vegetative branch as an inflorescence. These are initiated on the new vegetative shoots produced in late spring/early summer. Each rachis extends until about mid-April (Figure 1a). Individual heads continue to increase in size and, unless browsed by insects or subject to fungal infection, the number remains constant to the time of flowering. Following Kendrick (2003) we consider the fh as the floral unit, rather than individual flowers within heads. Indeed, a case could be made to extend this definition to the whole raceme level. Biologically meaningful stages of development of a flower head are defined (see Figure 2). Each stage is reasonably easy to identify when making repeated on-tree observations and has meaning in terms of the progressive expression of male and female function within the head. The time course for progression through these stages provided a basis for assessment of phenology at higher structural levels within inflorescences and for the tree as a whole. Stages 1-5 correspond to Stages 6, 6+, 7, 7+ and 8 of the classification used by Knox *et al*. (1989) for *Acacia retinodes,* which also has globose flower heads.

When the filaments are fully expanded, each globose fh measures about 9 mm wide by 8 mm long (Griffin unpubl. data). The number of individual flowers per head ranges from about 20-40 (Roger & Johnson 2013, Correia *et al*. 2014), a varying proportion of which may have undeveloped gynoecia and are functionally male. Each flower has an average of 33 stamens (Correia *et al*. 2014). Temporal development during flowering is described using the stage classification shown in Figure 2.

Figure 2. Developmental stages of *Acacia dealbata* flower heads. From left: 0: yellow but not open; 1: at least 1 style unfurling; 2: styles all unfurled; 3: anther filaments extended; 4: style and filaments still erect but some pollen shed and head paler yellow; 5: some flowers beginning to wither and brown but may still include some un-dehisced anthers and receptive stigmas.

Study Site

The study population grows on fertile soil in the margin of eucalypt forest at 300 m asl near Turnip Fields Rd (TFR) (42°54′53″S 147°16′23″E) on the lower slopes of Kunanyi/Mt Wellington in SE Tasmania and had regenerated from seed following fire in 1998. Open-grown individuals with large crowns accessible from the ground and 11-24 m in height, were used for this and related studies (see Griffin *et al* 2020 Plate 1).

Whole tree flowering phenology

In an earlier study of a nearby population (Griffin *et al* 2020), we characterised phenology of whole trees using a subjective assessment of flower colour, since this was observed to change with developmental stage. Flower colour has been successfully used in aerial mapping of the distribution of invasive *Acacia dealbata* populations in Spain (Vazquez de la Cueva *et al* 2022). Individual flower heads change in colour from green to yellow as they mature, with stamens progressing from pale to brighter yellow and then paler again past the peak anther dehiscence, which we designate as Stage 4 (Figure 2).

Six trees in the TFR population were observed in four successive years (2020-23) with an additional five added for 2021-23. Weekly observations commenced when the first open flowers appeared on any tree. The % of Stage 4 heads on the whole tree were estimated on a scale from 0 < 100% (peak flowering) at 10 percentile intervals, reducing from this peak date, again in 10 percentile steps, as the proportion of open heads declined over time. The same observer was responsible for all records. To avoid placing undue weight on small numbers of early or late opening flowers we considered the flowering period for each tree to start when 10% were scored as open, and end when less than 10% remaining open. Dates were scaled for analysis by designating June 1st as Day 1 in each year.

To address the lack of balance in the data set, we conducted a mixed model analysis of the phenology variates start day, peak day, end day and period of flowering, with treatment factor year set as fixed and tree as random in the model. The significance of differences among years was evaluated using Wald tests and that of differences among trees by the reduction in the Aikake Information Criterion (AIC) when tree was included in the model. For presentation of results, we revert to calendar dates.

Phenology of flower heads within racemes

Three ground-accessible branches were selected on two early and two late flowering trees in 2018. On each branch, two racemes bearing an average of 15 fh mainly at Stage 1, were tagged. At intervals from marking (designated Day 1 for this study), the number of fh at each developmental stage was recorded. Day 1 for trees 1 and 2 was July 25th and for trees 3 and 4, August 17th. Flowering was deemed over when all heads within a raceme had reached Stage 5. Data for racemes within trees were pooled and the percentage of fh at each stage on each date was tabulated.

These data also permitted us to determine whether there was any consistent baso- or acro-petal pattern of development. For each individual raceme we selected the earliest date at which at least one fh was at Stage 4; divided the number of fh in the raceme at that time into four quartiles from base to apex, and calculated the mean Stage score for those in each quartile. Analysis of variance was used to determine significance of position effect.

Variation in development within flower heads

Although difficult to document because of the small size of individual flowers and the head configuration, the extent to which development is synchronized within fh must greatly affect the probability of self-pollination. This is particularly important in *Acacia* because the stigma frequently only accommodates one polyad (Kendrick and Knox, 1982, Kendrick 2003)

Stigmatic Receptivity: The most direct assessment of receptivity is via controlled pollination of individual flowers of known different ages, but with the small globose flower heads of *Acacia dealbata* this was impracticable. Receptivity of *Acacia* stigmas has also been determined by various chemical assays (Kendrick 2003, Vicente *et al*. 2022), combined with microscopic observation of stigma exudates (Kendrick & Knox 1981) and pollen tube growth (Sornsathapornkul and Owens 1998), but none of these tests clearly define start or end point on a flower-head scale observable in the field. We used time to full extension of all styles within a head as a measure of full receptivity although it is likely that even unfurled stigmas are receptive as suggested for the *Acacia mangium x auriculiformis* hybrid (Sornsathapornkul & Owens 1998) and *Acacia longifolia* (Vicente *et al* 2022). Three racemes with between 5 to 8 fh at early Stage 1 (at least one style beginning to extend) were marked on each of two trees in 2020 and the number of fully extended styles per head counted daily until all were unfurled. We similarly recorded the time taken for all anther filaments to extend (transition to Stage 3), thus giving an indication of the degree of protogyny of heads as a whole. Knox *et al* (1989) noted browning of the stigma at the onset of male phase in flowers of *Acacia retinodes* and we chose whether individual stigmas on turgid styles were discoloured rather than clear, as the best available indication that an end point to receptivity had been reached. In 2021, a total of 33 fh at Stage 4 and 22 at Stage 5 from 3 trees were dissected and the percentage of discoloured stigmas per fh determined.

Anthesis: A preliminary study determined that viable pollen could be recovered from flower heads until at least 22 days following Stage 1 (J Harbard unpubl. data). We quantified the amount of pollen recoverable from flower heads at different stages, using the methodology developed by Stone *et al* (1998) and Prescott (2005). On four dry days in 2019 with varying temperature and humidity conditions, we collected three flower heads at each of Stage 3, 4 and 5 from each of three trees. Each head was gently rolled onto a piece of sticky tape which was then inverted and placed on a microscope slide for later examination of pollen removed, using a Zeiss Axiovert 25 4x objective and 16x eyepiece. Number of polyads was counted in each of 5 fields of view per tape and summed to give a relative estimate of pollen present on dehisced anthers. Analysis of variance of log transformed polyad counts was used to demonstrate the main effects of trees, dates, developmental stages and their respective interactions.

Results

Phenology at whole tree level

The multi-year flowering periods for individual trees are shown in Appendix Table 1, with population mean values (presented as calendar dates) in Table 1. The mean start date over all trees and years was July 28th and the corresponding mean dates for peak and end of flowering August 30th and Sept 20th respectively. Between years the range in peak flowering date was 17 days with the earliest (2020) on Aug 23rd and the latest (2022) on Sept 9th. Despite the variation in starting dates the total period of flowering each year was very uniform, averaging 53 days (range 50 to 55). Year effects were highly significant ($P<0.001$) for start, peak and end dates but not for period of flowering. Based on the reduction in AIC, differences among trees were judged significant ($P<0.05$) for all four phenology variates. Trees 1 and 5 were the earliest flowering in all years and tree 3 the latest (Appendix Table 1).

Table 1. Weighted mean phenology parameters for the TFR *Acacia dealbata* population over four years (for individual tree data see Appendix Table S1)

	2020	2021	2022	2023	Range earliest to latest years (days)	Mean Dates
Start	Jul-17	Jul-31	Aug-13	Jul-24	27	Jul-28
Peak	Aug-23	Aug-31	Sep-09	Aug-25	17	Aug-30
End	Sep-10	Sep-20	Oct-05	Sep-12	25	Sep-20
Total Period (days)	55	51	53	50	5	53

Phenology of racemes

Interpretation of the pattern of development within racemes is complicated because, despite an attempt to select racemes with uniform development, not every fh was at Stage 1 on Day 1 (Figure 3). In part this variability was due to a significant tendency for slower development of those in the distal quartile of a raceme (Supp. Table 2). It was impracticable to keep track of the stage of each individual fh over time but, assuming each develops at a similar rate, this initial variation must have influenced the stages recorded on subsequent dates. Nevertheless, some general patterns are evident: Averaged over all trees the highest numbers of fh at Stages 0 and 1 were on Day 1; Stage 2 and Stage 3 fh were most abundant at Days 4 and 8 respectively; and Stage 4 peaked on Day 21. The earliest fh on any tree reaching Stage 5 was Day 25. On trees 1 and 2 most were at that stage by Day 28 though comparable data for trees 3 and 4 are missing.

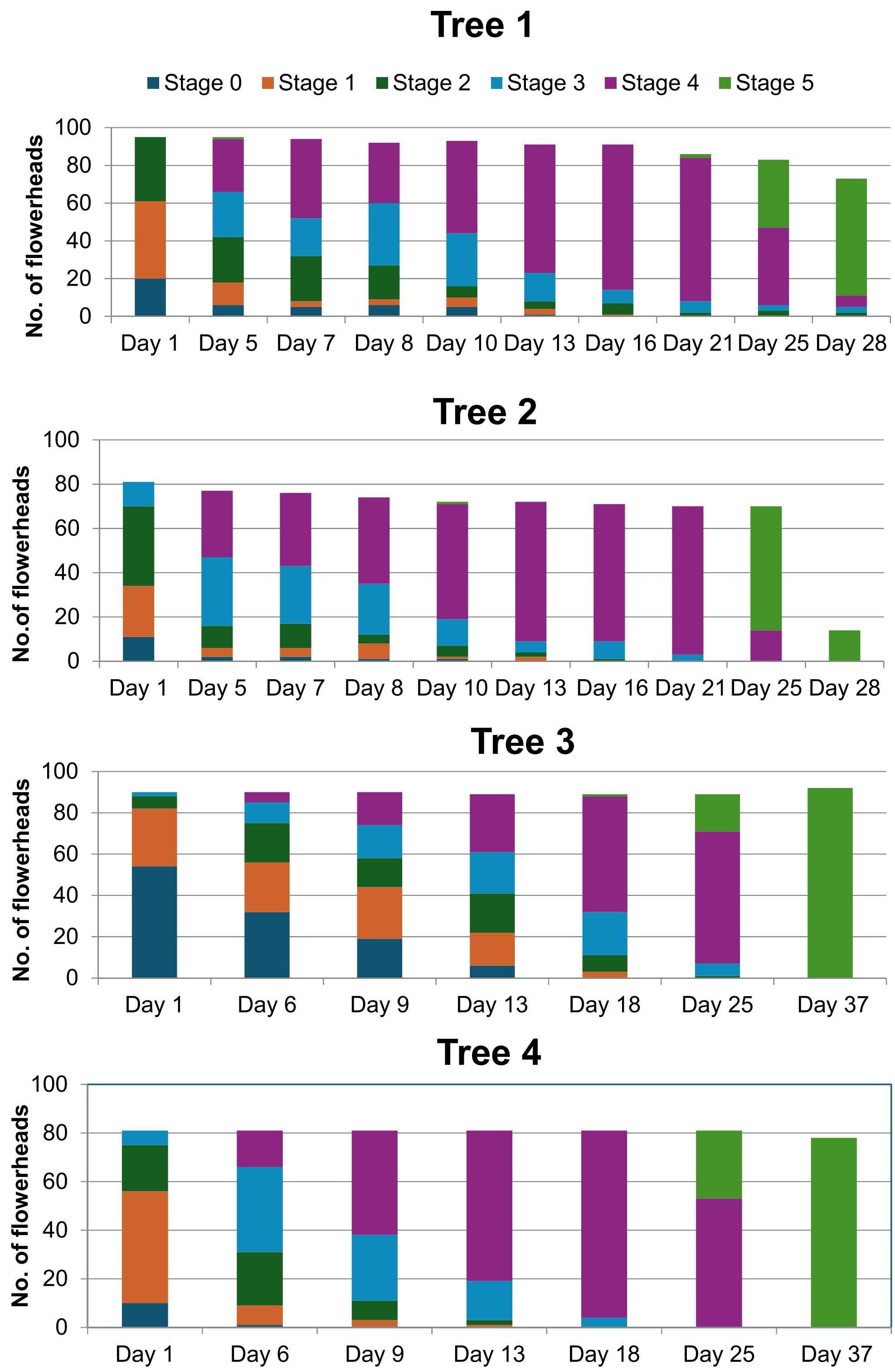

Figure 3. *Acacia dealbata* - Percentage of flowerheads at each developmental stage over time for four TFR trees in 2018. Pooled data for 6 racemes per tree.

Phenology within flower heads

The Stage classification (Fig 2) indicates the protogyny commonly reported for individual acacia flowers, but in terms of functional significance in limiting self-pollination, the degree of synchronicity of development of individual flowers within heads is more important. All the styles on the 55 individual fh observed in 2020 had progressed from Stage 1 to full extension by Day 4 (Table 2). Anther filaments of the same set of fh took between 5 and 12 days to fully extend with a weighted mean period of 9 days.

A total of 33 fh from three trees were dissected and condition of 407 stigmas observed. 67% of stigmas of Stage 4 flower heads were clear and presumed still receptive, falling to 23% for those at Stage 5. However, 19 of the 22 Stage 5 heads dissected also included at least one stigma which was still clear (Table 3).

The potential of flower heads of different developmental stages to shed pollen was assessed for three trees on four different days in 2019 (Table 4). By analysis of variance tree, stage and date main effects were all highly significant. The count from Stage 4 flower heads was greater than that from Stage 3 or 5, with that from those two Stages being comparable. Counts varied significantly between days, being highest on the day with highest temperature and lowest Relative Humidity. The significance of such weather effects in terms of the species' pollination ecology is discussed in another paper (Griffin *et al* in prep).

Table 2. *Acacia dealbata*-Time taken for all styles within Stage 1 flower heads to fully unfurl (Stage 2) and for anther filaments to fully extend (Stage 3), on each of two trees observed in 2020. Pooled data from 4 racemes per tree.

					Cumulative % of fh with fully extended styles							
Tree No.	Racemes	Flower heads	Total styles	styles/ head	Day 1	Day 2	Day 3	Day 4*	Day 5	Day 6	Day 8	Day 12
5	4	15	120	8	67	82	93	100				
6	4	40	84	2	76	93	96	100				

*4 days to full style extension (weighted mean)

					Cumulative % of fh with fully extended anthers							
Tree No.	Racemes	Flower heads	Total styles	styles/ head	Day 1	Day 2	Day 3	Day 4*	Day 5	Day 6	Day 8*	Day 12*
5	4	15			0	0	0	0	40		100	
6	4	40			0	0	0	0	30		68	100
Total fh		**55**										

*9 days to full filament extension (weighted mean)

Table 3. *Acacia dealbata*- Proportion of discoloured stigmas (assumed past receptivity) for open pollinated Stage 4 and 5 heads from three trees in 2021

	Stage 4			Stage 5		
Tree No	# fh dissected	# stigmas observed	% red	# fh dissected	# stigmas	% red
1	8	90	20	6	83	87
5	16	209	38	6	144	71
6	9	108	41	10	215	74
total	33	407		22	442	
mean			33			77

Table 4. *Acacia dealbata* - Mean polyad counts from pollen rolls of 3 flower heads at three developmental stages (see Figure 2) from three trees on four dry days with varying Temperature and Relative Humidity conditions. Meteorological data from BOM Station 094029 records. Analysis conducted on log transformed values.

Date	Tree	Stage 3	Stage 4	Stage 5	No. polyads Date Mean	3pm Temp °C	3pm RH%
26/08/2019	6	26	34	103			
	7	8	23	82	**51**	13.5	47
	8	39	77	91			
29/08/2019	6	46	177	23			
	7	18	135	21	**70**	12.4	57
	8	58	85	64			
2/09/2019	6	179	94	85			
	7	14	159	39	**123**	18.3	35
	8	155	191	133			
9/09/2019	6	23	83	13			
	7	3	33	8	**39**	10.5	46
	8	66	78	41			
No. polyads Stage Mean		**53**	**97**	**59**	**71**		

Fixed term	n.d.f[1]	d.d.f[1]	F statistic	F probability
tree	2	67	17.73	***
stage	2	67	18.8	***
date	3	67	11.19	***
tree.stage	4	67	4.19	**
stage.date	6	67	4.69	***
tree.date	6	67	1.25	n.s.
tree.stage.date	12	67	1.16	n.s.

[1] n.d.f. = numerator degrees of freedom, d.d.f. = denominator degrees of freedom

Discussion

The aim of the study was to document the floral phenology of the species in its natural habitat. This is a pre-requisite for full understanding of the pollination ecology and breeding system (Wesselingh 2007).

Time of flowering at whole tree/population level

The trees flowered at approximately the same time in each of four successive years (Table 1). The population mean peak date was August 30th, ranging from Aug 21st in 2020 to Sept 7th in 2022. *Acacia dealbata* was also the earliest flowering in a community of seven sympatric *Acacia* species studied by Prescott (2005) in Victoria, peaking in mid-August, and Broadhurst et al. (2008) noted that it flowered in winter-spring in New South Wales. In South Africa at latitude 29° 13′ 48″S, flowering was also in July-Sept (Roger and Johnson 2013) while in Portugal (about 40°N latitude) Correia *et al* (2014) and Montesinos *et al* (2016) reported seasonal equivalence, with flowering beginning in January. The reliable repeatability of flowering time of *Acacia dealbata* is the basis of a significant tourist attraction in southern France. To quote "*The Mimosa route is at its best from late January to early March, with mid-February the most popular time with visitors*" (France this Way 2024). It appears that the natural season of flowering is highly conserved at least when growing at temperate latitudes. Wandrag *et al* (2015) broadened this conclusion to include a wider measure of relative reproductive success of the species growing in Australia and as an alien species in New Zealand.

The total period of flowering was strongly repeatable over the four years, averaging 53 days (range 50 to 55) and comparable to the 51 days previously reported for a nearby population (Griffin *et al* (2020). Rank order of flowering of trees was also repeatable with trees TFR1 and TFR5 the earliest in all years and TFR 3 the latest. Since there was only one observation per genotype it is not possible to separate genetic from micro-environmental causation. However, since the earliest flowering tree (TFR 5) and the second last (TFR 4) were growing closely together (see Plate 1 Griffin *et al* 2020), genetic variation seems most likely. In a study of *Acacia mearnsii,* another temperate tree-form species from Acacia Section Botrycephalae (Murphy and Maslin 2023), Moncur *et al*. (1991) also reported that trees flowered in a similar order each year but there was annual variation in the time flowering commenced.

In many temperate plant taxa, variation in vegetative and flowering phenology can be related to seasonal temperature/rainfall conditions (Rathke & Lacey (1985), Kozlowski & Pallardy 1997) with heat sums and various other computational approaches used to quantify such effects (Chuine *et al* 1999). We reviewed monthly max. and min. temperature records from a nearby BOM station but were unable to find a pattern which was convincingly associated with the modest observed between-year variation on time of peak flowering (Table 1), so results are not presented. Perhaps focus should be on environmental control of the vegetative growth cycle rather than flowering *per se*. Complex interactions between temperature, light intensity and water availability have been shown to affect floral initiation and development in two other temperate taxa *Acacia pycnantha* and *Acacia baileyana* (Sedgley (1985, 1987), Morgan & Sedgley (2002). The latter species is closely related to *Acacia dealbata* in *Acacia* Section Botrycephalae and, in Tasmania, flowers even earlier in the winter (R. Griffin unpubl data).

Flowering phenology in the tropical taxa *Acacia mangium* and *Acacia auriculiformis* in Atherton Queensland (17° 15′ 27″S) is much less seasonally constrained (Sedgley *et al* 1992), with flowering occurring between February and August. Flowering was not continuous but rather flowering and non-flowering periods occurred in cycles. Subsequent observations in Sumatra, Indonesia (Latitude: 0°31′00″ N) showed that this pattern was related to variation in vegetative growth in response to rainfall and could be annually bimodal in that environment (R.Griffin unpubl. data). In Vietnam (lat 11° 15′ N) Nghiem *et al* (2010) also reported that the main flowering of these two species followed the start of the rainy season and that, depending on the year, there may be one or two flowering periods. Irregular flowering related to rainfall events is also a feature of many arid zone *Acacia* species (Costermans 2009).

Phenology of flowers within racemes and flower heads

The time course of floral development at these structural levels must influence the probability that a receptive stigma will be pollinated and the proportion of such pollinations which are effective outcrosses rather than selfs. The most distinctive attribute of *Acacia dealbata* compared to reports for other species of *Acacia*, is the 25 days or more taken for flower heads within racemes to progress through development Stages 1-5 (Figure 3), with temporal variation both within and between fh within racemes. Kendrick (2003) considered that flowering is generally synchronous within globose heads but this is clearly not the case for this species or indeed for other temperate taxa e.g. *Acacia paradoxa, Acacia pycnantha* and *Acacia melanoxylon* described by Prescott (2005) or *Acacia ligulata* (Gilpin *et al*. 2014). Variation in phenology of individuals flowers extends the functional longevity of the fh as a whole. Our methodology did not allow direct determination of the time an individual unpollinated stigma remains receptive, however it is clearly substantially longer that the less than one day reported for species with spicate heads as geographically diverse as the tropical *Acacia mangium x auriculiformis* (Sedgley *et al* 1992, Sornsathapornkul & Owens 1998) and the temperate *Acacia longifolia* (Vicente *et al* 2022). By Day 4 after marking as Stage 1, all styles within a head were fully extended to Stage 2. By Day 21 a high proportion of heads had progressed to Stage 4 and 67% of such heads still had some clear and putatively receptive stigmas (Table 4). Taking the developmental variation within heads into account, data presented in Figure 3 indicate that some stigmas within a fh will be receptive for at least 21 days or longer if it can be proved that stigmas are receptive prior to full extension. Likewise, pollen can still be shed by some flowers in heads judged to be at Stage 5 (Table 4).

The style and filament extension data (Table 2) suggests that individual flowers are protogynous as reported for many other species of *Acacia*, with styles within heads extending between Days 1 and 4 and anther filaments by Day 8. However, this cannot be an effective mechanism for avoiding self-pollination within heads (Gilpin *et al* 2014). The finding that heads within the distal quartile of the raceme developed more slowly (Table S2), further enhances the probability of geitonogamous pollination, as does the variation within crowns inferred from the average period of 53 days over which whole trees flowered (Table 1) –

approximately twice the period for individual racemes. There was no obvious difference in rate of development between the two trees which flowered early (1 and 2) and those which were later (3 and 4), all having progressed to the beginning of Stage 5 by day 25.

Adaptive advantages of the floral phenology of Acacia dealbata

The genus *Acacia* is considered to be of tropical origin, with an explosive evolutionary radiation across Australia since the mid-Pliocene (Raven & Polhill 1981). *Acacia dealbata* grows at the southern latitudinal limit of this radiation. Rathke & Lacey (1985) noted that flowering of plants from the tropics may be more constrained by rainfall than temperature and may occur more than once in a year. This is certainly true of the *Acacia* taxa *Acacia mangium*, *Acacia auriculiformis* and their hybrid, (Sedgley *et al* 1992). In contrast the flowering of *Acacia dealbata* and other temperate zone taxa is much more seasonally constrained (Costermans 2009, Bernhardt 1989, Prescott 2005). We did not investigate the conditions for floral induction and subsequent initiation of *Acacia dealbata* but the high repeatability of flowering time over years (Table 1) is strong evidence of an environmental trigger occurring sometime in conjunction with commencement of vegetative growth in spring. At this latitude both photoperiod and temperature vary to a very predictable pattern and so could have applied an adaptive selection pressure. Prescott (2005) reported that the relative order of flowering of seven sympatric species of *Acacia* in Victoria was repeatable, implying genetic variation in response to common environmental conditions.

Although ambient temperatures are low during the flowering period of *Acacia dealbata* it seems unlikely that this is solely responsible for the observed functional longevity of flower heads. A more likely postulate is that, in combination with mass-flowering and synchronisation of flowering time within a population, this is an adaptative response to a low probability of effective pollination by seasonally scarce biotic vectors (Griffin *et al*. 2020). Ashman & Schoen (1994) developed a model for evolution of optimal floral longevity treated as a resource allocation strategy, which was predictive of such a response. In the case of *Acacia dealbata,* pollen is most readily shed on warm dry days (Table 4) which would also favour insect activity, but such conditions are unpredictable, and chances of pollination must be enhanced if many heads remain in a functional state on many trees for many days. As noted by Gilpin *et al* (2014), mass flowering of a population means that pollinators are not biased to individual plants and outcrossing is therefore enhanced. Based on observations of *Acacia longifolia,* which is also winter flowering (Fernandes *et al*. 2015, Giovanetti *et al*. 2018), Vicente *et al* (2022) propose an alternative strategy for that species which, unlike *Acacia dealbata*, has sequentially produced spicate axillary inflorescences, whereby individual flowers are short-lived, but the plant as a whole can carry some flowers for as long as 4-5 months.

It is noteworthy that there is no equivalent variation in developmental time from flowering to seed maturation which takes about 18 weeks in both *Acacia dealbata* (Griffin unpubl. data) and the hybrid *Acacia mangium x auriculiformis* in Vietnam (Nghiem *et al* (2016), in spite of the fact that mean daily temperatures during the September-December period of development were 13.3 °C in Hobart (Bom.gov.au. (2023)) and 27.8 °C in Vietnam (Nghiem CQ pers. comm.), for the comparable pod maturation phase.

Sampling issues

The study highlighted a number of practical issues which need to be considered, with trade-offs required between replication at each structural level; time spent per observation; appropriate biologically meaningful intervals between repeat observations; and ease of access to laboratory facilities. Our study would not have been possible had not the senior author lived on-site with ready access to a good microscope. Accessibility is a non-trivial benefit which needs to be considered at the earliest stage of planning. Strengths and weaknesses of the sampling regime at each structural level are:

1) *Whole plant phenology*. Repeat observations at least at weekly intervals are required for estimation of within and between population variation. Size of individual plants will affect time required per observation for different species. The smaller the stature, the more plants can be assessed. For species with no or little prior knowledge, population replication is correspondingly important.
2) *Raceme level phenology*. Our approach was a compromise between time required per observation, replication within plants, and number of different genets assessed. The selection of both early and late flowering trees was an attempt to cover possible temporal variation. More plants and more racemes per plant would strengthen the generality of conclusion. Ideally developmental stages of at least some individual fh within racemes would have been recorded.
3) *Receptivity*. Our method for recording temporal development of style and anther filament extension would benefit from greater replication of both trees and fh within trees. However, the demonstration of protogynous development of flowers was quite clear-cut. The project would have benefitted from preliminary investigation of the various published chemical tests for onset of receptivity, however none of those define an end point *per se*. Confidence in the use of browning of the stigma as indicative of "beyond receptivity" could have been improved if we had taken daily fh samples for dissection and observation under the microscope once the first brown stigma was observed.

   New camera technology is being used to more accurately determine the time course of development (https://cavicams.com/) but only 1 fh per camera can be observed, so again we run into the sampling issue and generalisation of conclusions. Hopefully such detailed observations will confirm the validity of the quicker visual assessment, which can then be used with more confidence.
4) *Statistical design considerations*. Analysis is simpler if balanced data sets are acquired at any stage in the investigation. Replication of observations in more than one year would strengthen conclusions, however environmental variables cannot be controlled and are likely to lead to imbalance. Where observations were required at all structural levels, it proved difficult to complete in one flowering season. The implicit assumption that year effects at the fh level are minor is acknowledged.
5) *Environmental correlates*: Multi-year observation studies provide an opportunity to explore the environmental drivers of variation in flowering phenology. Our failure to

find any simple relationship between onset of flowering and key meteorological variables emphasises that this is a statistically challenging question and will probably require acquisition of data on vegetative as well as flowering phenology *per se* (Chuine et al.1999).

Some unresolved questions:

1) What is the relationship between vegetative and reproductive phenology, both during inflorescence development and during the October-December period when new shoots are developing in parallel with pod maturation and resource allocation may be an issue affecting seed quality?
2) If the lengthy period of receptivity/pollen availability in *Acacia dealbata* is an adaptive response to scarce pollinator populations in winter, then a testable hypothesis would be that temperate bipinnate *Acacia* species flowering later in the spring/summer would have more rapid floral development.
3) If insects are the predominant pollinators of Acacia (Stone *et al*. 2003) and these are less abundant and active in the winter/early spring, why do we not see evidence of selection favouring later flowering of *Acacia dealbata* populations?
4) Studies of development at the individual flower level are required to reinforce conclusions regarding the period of stigma receptivity and the environmental conditions conducive to anther dehiscence.
5) Our floral phenology data suggest that a high level of self-pollination is inevitable. If the species is partially self-fertile (Gibson *et al* 2011) then how is it that the mating system, as judged at the mature seed stage of the life cycle, indicates a very high level of outcrossing (Broadhurst *et al*. 2008)?

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Appendix

Table S1. *Acacia dealbata* - Individual Phenology data for 11 trees over 4 year period 2020-23

	start day				Peak				end day			
Tree No.	**2020**	**2021**	**2022**	**2023**	**2020**	**2021**	**2022**	**2023**	**2020**	**2021**	**2022**	**2023**
1	18	29	47	39	69	82	87	76	87	106	110	97
3	78	82	90	74	101	102	107	96	120	115	136	110
4	69	66	83	63	96	100	107	94	110	113	143	110
5	22	29	50	30	61	75	81	66	80	102	107	88
6	28	61	68	43	73	97	107	81	105	122	143	112
9		66	86	60	97	97	110	90	117	110	125	108
10		77	84	60	91	102	105	94	117	120		112
11		70	71	55	85	92	103	90	102	113		105
12		64	79	60	91	92	97	85	104	109		100
13		61	72	50	85	89	106	81	102	113		105
20		64	82	63		86	103	90		109		102
Mean	**43**	**56**	**71**	**54**	**83**	**92**	**100**	**86**	**103**	**111**	**127**	**104**

Table S2. *Acacia dealbata* - Mean Stage of development of flower heads by quartiles from base of racemes, assessed at the time when each raceme carried at least one flower head at Stage 4

Tree No	#racemes	total fh	Q1	Q2	Q3	Q4
1	5	86	3.41	3.18	3.08	1.39
2	5	70	3.58	3.56	3.31	2.15
3	6	89	2.03	2.23	1.53	0.91
4	6	81	2.37	2.9	3.17	2.5
Total	22	326				
Mean			**2.85**	**2.97**	**2.77**	**1.74**

There are significant differences between trees (P<0.01) and between quartiles (P<0.01)

Analysis of variance

Variate: meanstage

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
tree	3	4.8391	1.613	9.52	0.004
quartile	3	3.868	1.2893	7.61	0.008
Residual	9	1.525	0.1694		
Total	15	10.2321			