Flowering by selected serradella and subterranean clover lines in response to vernalisation and photoperiod treatments

Goward L[1], Haling R[1], Smith R[2], Penrose B[3] and Simpson R[1]

[1] CSIRO Agriculture & Food, GPO Box 1700, Canberra, ACT 2601, www.csiro.au, Email: laura.goward@csiro.au
[2] University of Tasmania, Mt. Pleasant Laboratories 165 Westbury Road, Launceston, TAS 7250
[3] University of Tasmania, Sandy Bay Campus, Hobart, TAS 7001

Abstract
Serradellas are promising alternative annual legumes for permanent, temperate pastures. However, many current cultivars exhibit unstable flowering dates relative to subterranean clovers. This is a potential risk for seed production and persistence. Early and late maturing cultivars of yellow serradella, French serradella and subterranean clover were subjected to six vernalisation (VRN) treatments (0, 1, 3, 5, 7 or 9 weeks at 5°C) and four photoperiods (PPD) (8, 12, 16 or 20 hours) to determine how these stimuli affected flowering time, as measured by nodes to first flower. The cultivars differed in their responses to VRN and PPD treatments. The flowering time of early maturing cultivars was insensitive (cv. King) or weakly responsive (cvv. Eliza, Izmir) to photoperiod and required 0-1 week of VRN to minimise the time to flowering. In contrast, late season types (cvv. Avila, Serratas, Goulburn) had stronger photoperiod and vernalisation responses with longer photoperiods required to hasten flowering when vernalisation was <3 weeks. These results inform selection of cultivars with appropriate flowering times and improved flowering date stability.

Keywords
Pasture, legumes, persistence, stability, phenology.

Introduction
The permanent pasture zone of south-eastern Australia would benefit from the release of modern cultivars of legume species adapted to temperate climates with enhanced nutrient-efficiency such as serradellas (*Ornithopus* spp.). Yellow serradellas (*Ornithopus compressus* L.) have been used in light, acid soils where their production can exceed that of benchmark pasture legumes such as subterranean clover (*Trifolium subterraneum* L.) and to date have been most widely used in phase farming systems (southern WA, northern NSW). Recent development of hardseeded cultivars of French serradellas (*Ornithopus sativus* Brot.) (Loi et al., 2005) has seen increasing use of French serradellas in southern WA and southern NSW.

More recently, serradellas have also been identified as potentially having a role in the permanent pasture systems of south-eastern Australia (Sandral et al., 2019). The species are highly phosphorus-efficient, and it is anticipated that serradella-based grazing systems could require up to 30% less phosphorus fertiliser than subterranean clover-based pastures (Sandral et al., 2019). This has both national and global significance given Australia's dependence on phosphorus fertiliser and the importance of the world's phosphate rock reserves for food security.

Further expansion of serradellas use into the permanent pasture zone depends on the suitability of cultivars for high yields and persistence. Persistence is dependent on having hardseeded cultivars that flower reliably at a suitable time for adequate seed set. For an annual plant in temperate environments this usually means flowering at a time that balances the risk of frost, heat, and drought stress. In contrast to cropping systems, germination date in permanent pastures depends on the time of opening rainfall and cannot be managed beyond the establishment year. Adapted pasture cultivars rely on internal control of flowering to ensure a similar (stable) flowering date occurs each year, irrespective of the germination date. Coordinated field experiments in contrasting environments have demonstrated useful differences in flowering date (maturity type), but relatively poor flowering date stability among serradella cultivars relative to subterranean clover controls (Boschma et al., 2019). Late maturing serradella cultivars tended to display better flowering date stability.

Flowering date is determined by a cultivar's intrinsic tendency to flower combined with its response to VRN and PPD stimuli. Flowering is delayed when the plant's VRN and/or PPD requirements are not satisfied (Aitken, 1955). We hypothesized that quantification of these influences on flowering in serradellas will improve our understanding of cultivar maturity type and how flowering date stability may be improved. A controlled-environment study was conducted to examine the range in flowering responses to daylength and temperature cues likely to be displayed among current Australian serradella cultivars.

Methods

A controlled-environment experiment (3 species x 2 cultivars of each species x 6 VRN treatments x 4 PPD treatments x 6 replicates) was established to test flowering responses to daylength and cold temperature cues. Early and late maturing cultivars of yellow serradella (cvv. King and Avila), French serradella (cvv. Eliza and Serratas) and subterranean clover (cvv. Izmir and Goulburn), reflecting the flowering time range among current cultivars, were sown in pots containing 2 kg of a nutrient-rich, pasteurised, sandy-loam soil. Hardseeded cultivars were scarified. Moistened pots were covered with dark plastic and kept at 4°C for 48 h to break embryo dormancy and to ensure even germination. The plants were then transferred to a growth cabinet set to 21/17°C (day/night) and 8 h daylength (non-PPD inducing conditions) to allow establishment for 14 days before being subjected to one of six VRN treatments (0, 1, 3, 5, 7 or 9 weeks at 5°C (Fig. 1), with 8 h daylength). Pots were inoculated with species-specific rhizobia after seedling emergence. The planting of each VRN treatment was timed so that all 6 VRN treatments entered the PPD treatments on the same day. Four PPDs (8, 12, 16 or 20 hours; Fig. 1) were established using cabinets fitted with fluorescent and incandescent lighting (480 µmol photons/m^2/s, visible range) and set to 8 h (21°C)/16 h (17°C). Extended daylengths (12, 16 and 20 h) were achieved by only using the incandescent lighting (18 µmol photons/m^2/s) for time periods that were centred on the daily 8-h period of full light.

Floral initiation was estimated by recording node of first flower (Aitken, 1955); changes in node of first flower demonstrated how vernalisation and photoperiod stimuli affected flowering time. In this paper, we use nodes to first flower as a surrogate measure for the time to flower. The minimum node to first flower (i.e., the intrinsic tendency of the cultivar to flower) was recorded when plants were grown with the longest VRN and PPD treatments. A 3-way factorial ANOVA analysis was conducted using log-transformed data to detect statistical differences ($P<0.05$) among the treatments.

Results and Discussion

The flowering time responses of the cultivars to VRN and PPD treatments and their interaction, had a much greater influence on the distinction between early and late flowering within a species than the intrinsic tendency of the cultivars to flower. Minimum node of first flower ranged from 7 to 11 among the cultivars: Eliza (9), King (7), Izmir (7); Serratas (11), Avila (9) and Goulburn (7) (Fig. 2). The early and late subterranean clover cultivars were the same. Within each serradella species, the early cultivar had a lower minimum node number than the late cultivar (but only 2 nodes less, $P<0.05$).

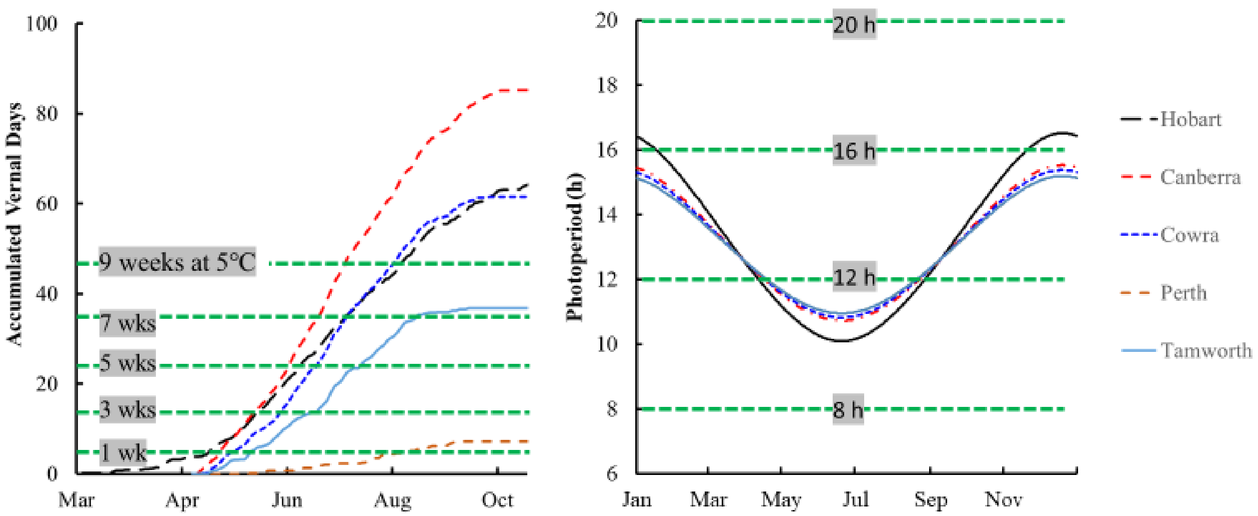

Figure 1. VRN and PPD in southern Australia. Green dashed lines represent imposed treatments.

The early maturing cultivars were essentially insensitive (cv. King) or weakly responsive (cvv. Eliza, Izmir) to PPD with low requirements for VRN (0-1 week) to hasten progress to flowering. This was evidenced by the appearance of the first flower at, or close to the minimum node to flower in treatments with short PPD and VRN >1 week. However, flowering in cvv. Eliza and Izmir was delayed by 3-8 nodes when VRN was <1 week and they were grown in short days (PPD <16 h). Cultivar King was delayed in flowering by up to 10 nodes when the VRN treatment was <1 week but only required 3 weeks of VRN treatment to minimise node of first flower.

Flowering date instability has been observed at Perth, Cowra and Canberra for most of these early-maturing cultivars. Flowering occurred up to 5 weeks (395 dd at Canberra) earlier when they were sown 6 weeks earlier in mid-March, as opposed to early May (i.e., 744 dd difference in sowing time, Bochma et al., 2019). The phenology data reported here, indicate that this is a consequence of flowering time in these cultivars not being delayed strongly by VRN or PPD. Cultivar King was an exception because it has a small VRN

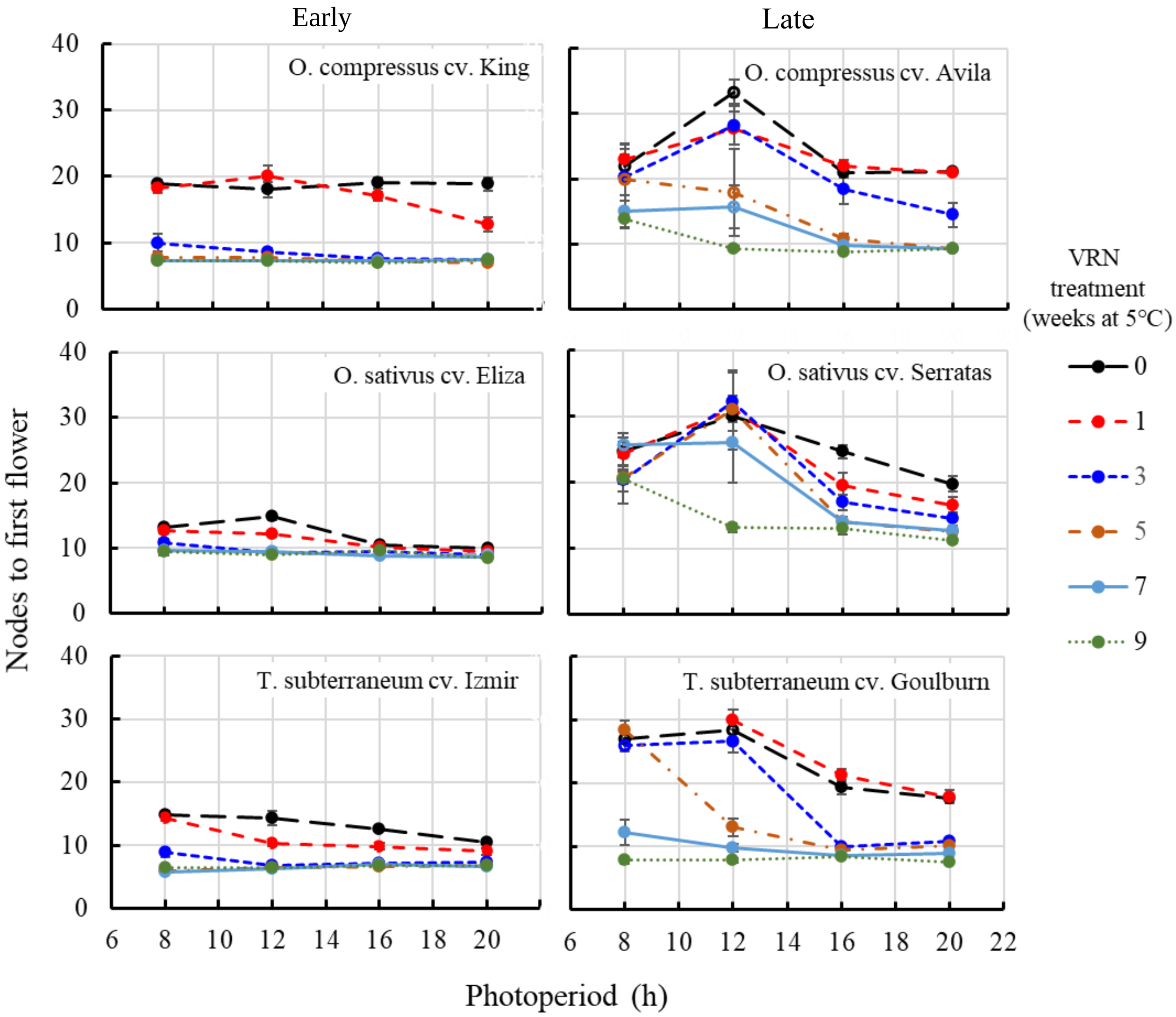

Figure 2. Early and late maturing yellow and French serradella and subterranean clover flowering time responses to vernalisation (VRN) and photoperiod (PPD) stimuli with standard error bars. Open symbols denote data where only 3 of 6 replicates were measured for flowering.

requirement which delayed flowering when not satisfied. This low VRN requirement was, nevertheless, likely to be satisfied during winter at most locations in southern Australia and potentially would even be satisfied during late autumn/early winter in colder regions (e.g. Southern, Central and Monaro tableland regions, NSW). Consequently, cv. King can also be expected to flower prematurely after an early germination event in many grassland regions.

All late season cultivars (cvv. Avila, Serratas, Goulburn) exhibited a higher requirement for PPD and VRN to stimulate earlier flowering. For example, longer daylengths were required to hasten flowering when VRN treatment was <3 weeks for cvv. Avila and Goulburn, and <9 weeks for cv. Serratas. These responses (expressed as a delay in flowering) deliver a late maturity phenotype. We argue that they are also required for the achievement of more stable flowering dates than exist among the early maturing, less VRN/PPD sensitive cultivars. In temperate Australia, PPD ranges between 10-16 hours (Hobart) and 11-15 h (mainland states), but the accrual of vernalisation is more location-specific (Fig. 1). Late cultivars with significant VRN requirements must pass at least part of the winter to satisfy their VRN response and early spring, to satisfy any PPD response that has not yet been diminished by interaction with VRN.

Conclusion

The early cultivars were essentially PPD insensitive with weak VRN requirements. Among the serradella and subterranean clover cultivars examined, requirements for VRN and sensitivity to PPD were the major factors that distinguished the early and late maturity type of the cultivars. VRN and PPD responses are anticipated to also be critical for flowering date stability.

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