Opportunities and Challenges in Augmenting Honey Bee Forage Resources with Pasture Legumes in Southern Australia

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Opportunities and challenges in augmenting honey bee forage resources with pasture legumes in southern Australia

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Abstract
More than 80% of Australian honey is produced from native tree and shrub species. However, with increasing demands on public lands along with production risks posed by drought, floods and wildfires, there is a need to identify alternative forage resources to augment Australian honey production. With over 30 pasture legumes now available for agriculture in southern Australia, opportunity exists to increase the utilisation of some species with co-benefits to multiple production industries. However, there is little understanding of the potential value of most pasture legumes for honey production, and side by side comparisons are complicated by factors such as differences in phenology, flower morphology and low nectar quantities. This paper describes a preliminary investigation presently underway in Australia comparing the floral attributes of 22 annual and short-lived perennial pasture legumes. The objective of the project is to prioritise species for their potential value to the local honey bee industry based on floral attributes, as well as existing and potential zones of adaptation. Methods being used to compare species in the Clover4Bees Pilot Study are described.

Introduction
Forage legumes play a unique role in the functioning and productivity of grasslands through nitrogen fixation and improved forage quality. With the increasing imperative for sustainable intensification of agriculture, legumes provide an opportuinity to increase output from a given area of land through alternative products such as honey. The Australian beekeeping industry has traditionally relied little on pasture legumes for honey production. This has been due to nomadic beekeeping practices where the majority of honey is produced from sporadic flowering of native tree and shrub species. The dominance of self-pollinating, self-regenerating legumes that are thought to be of little value to honey bees (*Apis mellifera*), such as subterranean clover (*Trifolium subterraneum*) or annual medics (*Medicago* spp.), is also a contributing factor (Somerville 2019). Perennial species, such as white clover (*T. repens*) or lucerne (alfalfa; *M. sativa*), are recognised for their honey production potential (Johnson 1946; Pedersen 1961), although generally confined to niche environments or, in the case of lucerne, rotationally grazed and not managed for flower production (Lodge 1991). There is substantial opportunity to harness the potential of a large suite of alternative legume species now available in Australia (Foster et al. 2021, Nichols et al. 2012) but for most, little is known of their value for honey production.

Agrifutures Australia, the industry body responsible for investing in research and development in the Australian honey bee industry, sought to invest in legume research to diversify the floral resourses available to honey bees. However, few previous studies had directly compared floral attributes of different legume species and it soon became clear that a pilot study was required to establish appropriate methodology for such a comparison. This paper details some of the challenges that impede side-by side comparison of contrasting legume species for attributes important to honey production, and how the 'Clover4Bees' pilot study is addressing those challenges to provide one of the first evaluations of legume species for the honey bee industry.

Challenges in methodology
Measuring honey production
The only way to directly measure honey production is to have bees forage the target crop. However, this approach is often not feasible to compare a large number of species, due to the large plantings required to support a hive isolated from other nectar sources. It was therefore deemed necessary to conduct an initial screening of species in the absence of direct measures of honey production, which instead focused on key component traits. The following sections describe the traits being assessed in the Clover4Bees pilot study.

Environmental conditions and experimental design

Environmental conditions are a governing factor in the performance of any organism. An inherent risk with a screening activity such as was proposed with the Clover4Bees project was that the results of individual species could be biased by the growing conditions. This can include seasonal conditions, such as rainfall, temperature, relative humidity, or radiation; soil conditions, such as soil fertility, pH or texture; or site conditions, such as the presence of pests or diseases. To mitigate these risks, field sites were established at four contrasting locations in New South Wales (NSW) Australia, at Glen Innes (average annual rainfall; AAR 840 mm), Tocal (AAR 938 mm), Cowra (AAR 619 mm) and Wagga Wagga (AAR 551 mm).

There are over 30 different species of winter-growing pasture legume species now available in Australia (Nichols et al. 2012), developed to improve the range of legumes available to the grazing and cropping industries. A subset of twenty-three annual or short-lived perennial species was selected for the initial evaluation (Table 1). Experiments were replicated three times and sown in autumn 2022. Each site was prepared similarly with weed mat (0.91 m width) laid in parallel lengths about 1.1 m apart. Each plot was established by burning 20 holes (0.05 m diam.) into the weed mat in two parallel rows (0.1 m apart), with holes offset and spaced at 0.2 m along each row. Plots were spaced 1.0 m apart along the length of the weed mat. At the appropriate sowing time, about four germinable seeds were sown into each hole with the objective of establishing 20 plants in each plot (Fig. 1).

Table 1. Legume cultivars being evaluated in the initial pilot study for potential use in honey production

No.	Common name	Genus	Species	Cultivar	Growth habit
1	Biserrula	*Biserrula*	*pelecinus*	Casbah	annual
2	Burr medic	*Medicago*	*polymorpha*	Scimitar	annual
3	Messina	*Melilotus*	*siculus*	Saltleader	annual
4	Yellow serradella	*Ornithopus*	*compressus*	Avila	annual
5	French serradella	*Ornithopus*	*sativus*	Erica	annual
6	Berseem clover	*Trifolium*	*alexandrinum*	Elite II	annual
7	Eastern star clover	*Trifolium*	*dasyurum*	Sothis	annual
8	Gland clover	*Trifolium*	*glanduliferum*	Prima	annual
9	Rose clover	*Trifolium*	*Hirtum*	Hykon	annual
10	Crimson clover	*Trifolium*	*incarnatum*	Dixie	annual
11	Balansa clover	*Trifolium*	*michelianum*	Bolta	annual
12	Purple clover	*Trifolium*	*purpureum*	Electra	annual
13	Persian clover	*Trifolium*	*resupinatum*	Lusa	annual
14	Bladder clover	*Trifolium*	*spumosum*	Bartolo	annual
15	Subterranean clover	*Trifolium*	*subterraneum*	Goulburn	annual
16	Arrowleaf clover	*Trifolium*	*vesiculosum*	Zulu II	annual
17	Common vetch	*Vicia*	*sativa*	Timok	annual
18	Woolly pod vetch	*Vicia*	*villosa* sp. *dasycarpa*	Hay maker	annual
19	Purple vetch	*Vicia*	*benghalensis*	Popany	annual
20	Sulla	*Hedysarum*	*coronarium*	Wilpena	Short-lived perennial
21	Red clover	*Trifolium*	*pratense*	Relish	Short-lived perennial
22	White clover	*Trifolium*	*repens*	Haifa	Short-lived perennial
23	Sainfoin	*Onobrychis*	*viciifolia*	Othello	Perennial

Flower morphology

Although all from the Fabaceae family, the flower morphology among the species in question differs markedly, even just among the candidates from the *Trifolium* genus. Flower morphology can be an important determinant of the pollinators that visit flowers (Aygoren Uluer 2021). The Clover4Bees pilot study is characterising key attributes of flower morphology by detailed sampling of flowers from the field site at Tocal. Through microscopy, inflorescence, sepal, petal, keel, banner and wing dimensions are being documented (Fig.1).

Legume phenology and floral abundance

Legume phenology here defines the flowering window, which is an important consideration for apiarists looking to augment existing floral resources. Contrasting phenology between species makes side-by-side comparisons difficult because of different environmental conditions (especially temperature and relative humidity) at flowering that can impact traits such as nectar secretion and honey bee activity (Somerville 2019).

Legume phenology is being assessed at the four field sites by assessing plants every 2-4 days to monitor the commencement and cessation of flowering. Once 50% of plants within a given plot had developed at least one flower, the number of inflorescences was counted every 2-4 days for the duration of flowering, within a fixed quadrat (0.2m x 0.91m) laid perpendicular to the weed mat .

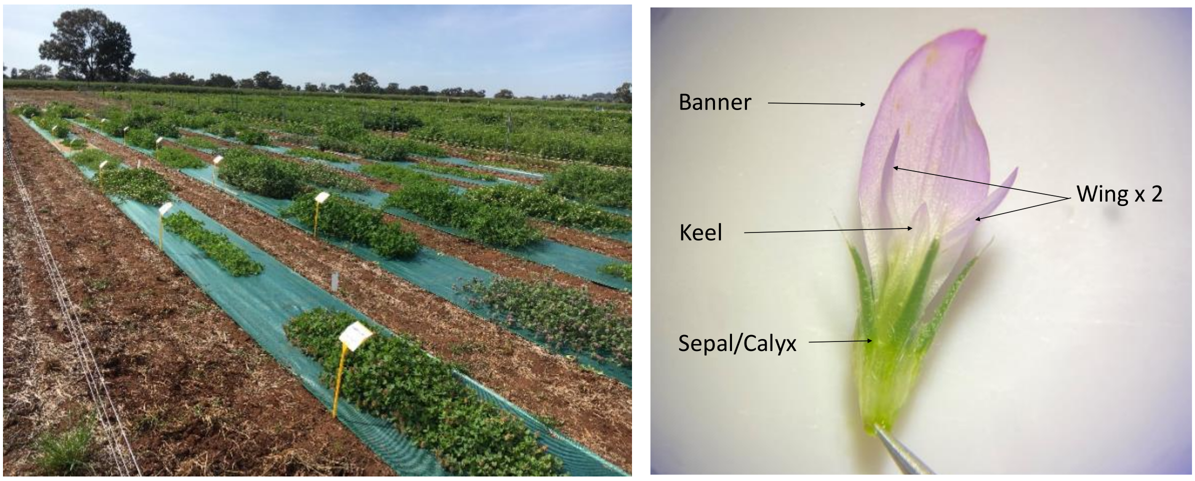

Fig. 1. (Left) The Clover4Bees field experiment established at Wagga Wagga, NSW, showing legume species grown on weed mat representing 20 individual plants in plots 2 m long. (Right) Illustration of the basic structure of a legume (gland clover) flower.

Flower preference

With present levels of understanding, it is not possible to predict flower preference by bees from any one or combination of traits described above. A direct measure of bee activity is required on the species in question. However, such a measure can be compromised by site conditions at the time of sampling. The level of bee activity and the availability of pollen or nectar will vary throughout the day, making the result dependant upon the time at which it was sampled. The presence of other pollen and nectar sources in the vicinity may also impact the level of visitation by bees, and this will vary through time, further confounding direct comparisons of legume species with contrasting phenologies. Nevertheless, the Clover4Bees pilot project is assessing bee preference at each site every 2-3 days by counting the number of bees that visit a plot in a two-minute period.

Nectar production

Nectar provides bees with their prime source of energy in the form of sugars, mostly consisting of sucrose and smaller amounts of glucose and fructose. The sugar concentration of nectar and the amount produced is impacted by environmental conditions, such as temperature and humidity (Corbet 2003). Honey bees show some preference of nectar with sugar concentrations between 30 – 50 % (Wright et al. 2018).

Quantifying nectar can be problematic in small flowers, due to challenges extracting low volumes (Corbet 2003; Morrant et al. 2009). Even the use of microcapillaries, for example, is unlikely to extract all of the nectar in a flower (Morrant et al. 2009). In addition, there always remains uncertainty of how much nectar a honey bee would have extracted, which is a limitation of interpretation of results from such sampling. Previous research (Swanson and Shuel 1950) has demonstrated successful use of a centrifuge in extracting nectar from red clover flowers. This would seem the most promising methodology for the range of species in question but further testing is required to validate this technique, especially for very small flowers such as biserrula.

Pollen production and composition

Pollen provides honey bees with their primary source of protein (amino acids), fats, minerals and vitamins and is essential during brood rearing and reproduction (Wright et al. 2018). Despite large variation in the nutritional composition or pollen, there is evidence that pollen from the same genus show similarities in nutrtitional profile, specificially in macronutrient content such as total protein content (Pamminger et al., 2019). For such comparisons, it is important to consider the method of pollen collection as, for example, bee-collected pollen can contain up to 50 % carbohydrates added by bees upon collection (Wright et al., 2018), reducing the absolute concentration of protein and lipid nutrients per weight. Bees also collect different quantities of pollen from different plant species (Somerville 2019). The challenge again lies in comparing the amount of pollen that bees can collect from legumes, which is only possible from large plantings by the use of pollen traps mounted to hives. In this study we are, in the first instance, observing which legume species honey bees are foraging for pollen.

Discussion

At the time of writing, few results were available from the field experiments. Nevertheless, the novel approach used in this project to overcome the many challenges that constrain comparison of floral attributes of diverse species is worth recording. A key outcome of the Clover4Bees pilot study will be to enhance knowledge among pasture scientists to increase the emphasis on pollinator activity in mainstream grassland research.

Another outcome of the project will be to short-list priority legume species for more detailed assessment of their value to honey bees. It is acknowledged that the 'short-list', using the methods described above, cannot be definitive given the many challenges in making such an assessment. However, it is essential that the range of legumes being tested be reduced in order to be able to directly assess the benefits for honey production. Specifically, it is essential that larger plantings are used and metrics defining hive health and productivity are measured. Therefore, species that are omitted from the short-list from this initial project may still have potential for further development in the future.

There are a range of criteria important when assessing flora for their value to bees. In his comprehensive catalogue of flora for honey bees in south-eastern Australia, Somerville (2019) assessed not only the attributes of the flower but also the geographic adaptation, reasoning that species confined to niche environments are likely to have only limited impact at an industry level. The same thinking applies to the evaluation of legume species, noting that many of the 'newer' legume species are yet to achieve their potential reach.

The species in the initial testing fall into one of two categories. Either, they are perennial or self-regenerating annual species that can persist in permanent pasture settings, or they are more like forage crops that will not persist reliably beyond the year of sowing. The adaptation of each species will be context-specific, for example, some species are better suited to crop/pasture rotations and others to longer-lived grasslands (Nichols et al. 2012). However, a constraint to the potential geographic footprint of many of the species in question is the lack of cultivars, meaning that a constrained range of traits are available for many species (Hayes et al. 2023).

Conclusions and/or Implications

Honey production offers grasslands great opportunity to increase agricultural productivity from a given unit of land, due to the many synergies with existing livestock and crop production systems. Moreover, a greater emphasis on pollinators promises other benefits to grasslands, notably in seed production of some species. However, a greater awareness of how to measure key attributes of grassland species is required for this line of research to be embraced more broadly.

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