



# PALYNOLOGY OF JARRAH AND MARRI HONEY: SITE SURVEYS AND ANALYSIS

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## Introduction

"Pollen analysis is still the most important method for the determination of the botanical origin of honey" (Ruoff and Bogdanov 2003), but the standard percent occurrence of the nominal pollen type differs for different plant species. For example - >90% for chestnut and >10% for citrus. We aim to establish (a) the standard pollen content of Jarrah (*Eucalyptus marginata*) and Marri (*Corymbia calophylla*) honey to verify it's monofloral status, (b) determine the peak period within a season and, (c) compare our results with the DNA analysis.

## Scope

The extraction of pollen from Jarrah and Marri honey and trap pollen samples for DNA and palynology, and chemical processing and analysis of Jarrah and Marri honey.

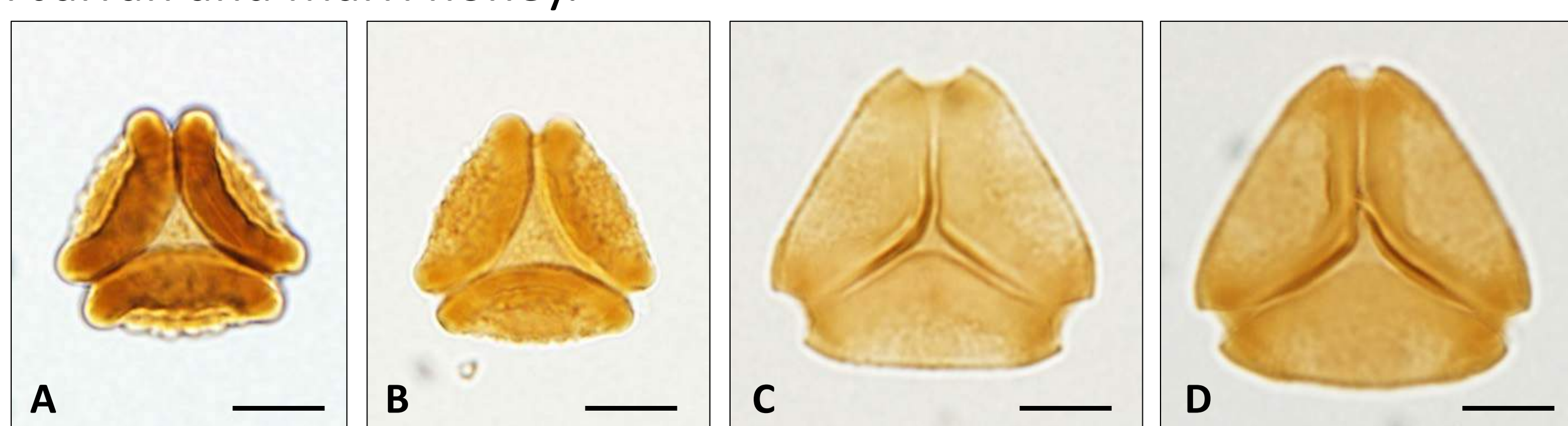


FIGURE 1: A, B *Corymbia calophylla* (Marri) pollen C, D. *Eucalyptus marginata* (Jarrah). Scale bars 10  $\mu$ m.

## Materials and Methods

**Botanical surveys** of collection sites were conducted to identify other species flowering on site and to collect pollen and leaf samples from these as well as Jarrah and Marri. Pollen from each sample taken was chemically processed (acetolysed), microscopically characterised, photographed and databased.

**Pollen extraction from honey.** (40 mL) of honey was diluted with 40 mL of warm distilled H<sub>2</sub>O, sieved with a 125  $\mu$ m mesh to remove bee parts and wax, diluted further with 360 mL of distilled H<sub>2</sub>O, spun in 50 mL centrifuge tubes at 3,000 rpm for three minutes to concentrate the pollen residue, and further warm-rinsed to remove residual honey.

**Trap pollen.** approximately 5g was suspended in 20 mL of warm distilled water, sieved, centrifuged and rinsed.

**Analysis.** Half of the pollen recovered from each procedure was sent for DNA analysis, the other half was acetolysed to remove pollen contents, mounted on glass microscope slides, and palynologically characterised.



FIGURE 2: Examples of other pollen types present in honey samples. A, *Ocimum basilicum* (Basil). B. *Grevillea* sp 1. C. *Ptilotus manglesii*. Scales bars 10  $\mu$ m.

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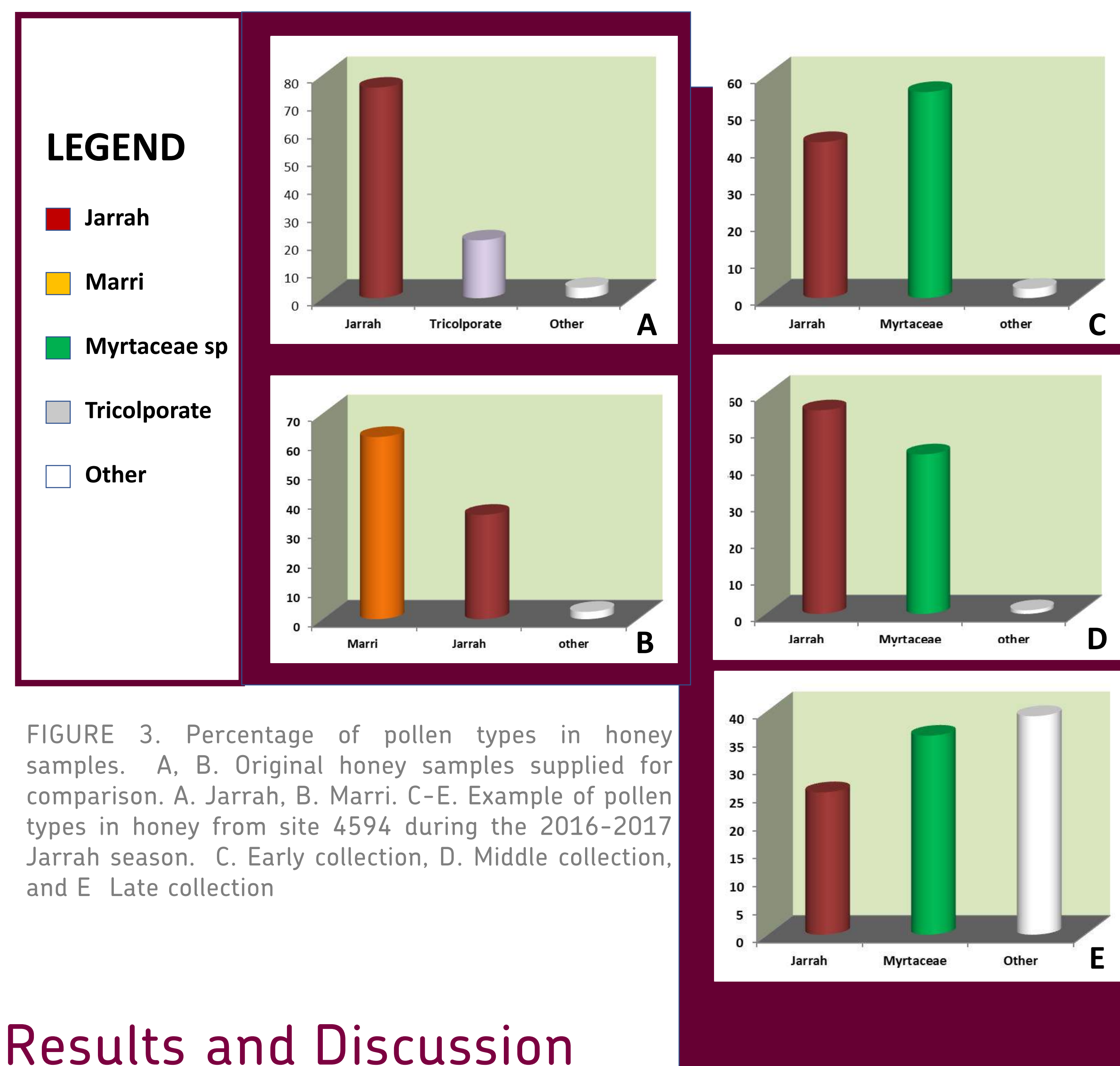


FIGURE 3. Percentage of pollen types in honey samples. A, B. Original honey samples supplied for comparison. A. Jarrah, B. Marri. C-E. Example of pollen types in honey from site 4594 during the 2016-2017 Jarrah season. C. Early collection, D. Middle collection, and E. Late collection

## Results and Discussion

Pollen assemblages from the processed Jarrah and Marri honey provided as a standard for the development of methods yielded approximately 75% and 60% of the nominated species respectively (Fig. 3A, B). The limited number of Jarrah honey samples palynologically processed and analysed to date show a trend of approximately 40% Jarrah pollen in early season collections, 55% in mid-season collections and 25% in late-season collections. The 2016-2017 Jarrah season was quite poor, and at some localities there was a variety of other myrtaceous species flowering at the same time - this is reflected in the pollen counts (Fig. 3, C-D). As more samples are studied it is expected that both greater and smaller percentages of the nominated species will occur at different sites and during different seasons before a standard percent occurrence for each honey type is reached.

## Summary

- Methods for the extraction of pollen from honey have been established.
- Jarrah and Marri pollen and pollen from other species at collection sites has been palynologically characterised and databased.
- Pollen has been extracted from the 2016-2017 Jarrah honey samples and the Jarrah and Marri trap pollen samples.
- Some of each of the pollen samples, and plant material from sites, have been supplied for DNA analysis.
- Some of the Jarrah honey pollen samples have been chemically processed and analysed.

### References

Ruoff K. and Bogdanov S. 2003. Authenticity of honey and other bee products. *Apiact* 4.