Pollen characterization of English honey from Worcestershire, West Midlands (UK).

Beverley Adams-Groom[1], Peter Martin[2], Ana Luisa Sierra Bañón[1]*

[1]National Pollen and Aerobiology Research Unit, School of Science and Environment, University of Worcester, Worcester, WR2 6AJ

[2] 81 Top Road, Kingsley, WA6 8BU

Corresponding author: Beverley Adams-Groom, ORCiD: 0000-0002-1097-8876

Introduction

Beekeeping in England, United Kingdom, is very popular since much of the country has favourable habitats for honey production. Worcestershire county, in the West Midlands region, has habitats typical of much of the lowland regions of England, comprising a patchwork of pasture with small woodlands, orchards, gardens, meadows, hedgerows, riverbanks and arable crops including oilseed rape and field bean, amongst others. While most beekeepers keep bees as a hobby, commercial honey companies do derive some of their honey from this region, from both smaller and large-scale producers.

Pollen surveys of honey are useful for several reasons: to help determine honeybee forage activities for honey producers; to provide information for honey analysts and to compare with earlier surveys to see if habitat and climate change are altering foraging habits, which may also help with bee conservation. In the United Kingdom, peer-reviewed surveys of bee forage and honeys have been quite limited (Balfour, Fensome, Samuelson, & Ratnieks, 2015) but there are good sources of information available to the beekeeper about the likely sources of pollen, such as Kirk and Howes (2012) or Hodges (1984). Although observing bee activity can indicate which plants the bees are using for their various requirements, it may not always be clear which plants are contributing directly to the honey. Furthermore, an empirical, peer-

reviewed study of the pollen content of honey in England has not been conducted since 1952 when Deans (1957) analysed 854 honey samples from across the British Isles, including 14 samples from Worcester (110 from the Midlands). Two recent DNA studies have also provided data on the floral composition of eight Welsh honeys and one from the Isle of Wight (Hawkins et al. 2015) and one for the National Botanic Garden of Wales (de Vere et al. 2017).

The aim of this research was to characterize the typical pollen content of honey from Worcestershire in the West Midlands region of England.

Methods

Forty-five of the Worcestershire Beekeepers kindly provided 83 honey samples for pollen analysis from the 2016 season. The weather in 2016 comprised a mild, dry Winter, followed by a cool, wet April, a warm, wet May, then an average early Summer and a largely dry late Summer and Autumn. Beekeepers were supplied with labelled sample tubes and a return envelope. Participants supplied approximately 10g of honey along with the harvest date, location and an indication as to whether or not the honey had been filtered. The samples came from extracted honey from single hives. The locations from which the samples came were spread quite well across the sampling area (Fig 1).

Samples were then processed using an amended version of the method recommended by Lutier and Vaissierre (1993). This involves placing c. 10g of honey in a solution of distilled water and 0.5% sulphuric acid which is then heated to remove sugars. The sample is then filtered through a 5 micron filter to catch the pollen grains. This is washed in acetic acid and then centrifuged. The excess liquid is decanted and a pollen pellet remains. The pellet is then subjected to acetolysis to remove other extraneous material and the pollen cell contents, which aids identification of the pollen grains. Acetolysis involves mixing 9 parts acetic

anhydride with 1 part sulphuric acid and then heating at 90ºC for 25 minutes. There is a final wash in acetic acid and the remaining pollen pellet is mounted with glycerogelatine on a microscope slide and counted.

Morphological identification was done using binocular microscopes and 300 grains were counted for each sample. The raw data was then transformed into percentages. When all the results were collated it was clear that sweet chestnut (*Castanea sativa*) had occurred in high amounts in many of the samples and, since this pollen type is heavily over-represented in honey, it was decided to use pollen coefficents on this data set (Sawyer, 1988) to give a better picture of the nectar sources contributing to the honey of Worcestershire Beekeepers. Quantities of pollen in honey do not directly correlate to the quantities of nectar in honey from those same plant species. Pollens can be over or under-represented due to the size of the pollen grains or quantities of pollen produced by plants. For example, some pollen grains are filtered out of the nectar and consumed by the foraging bee before she returns to the hive. Honeybees have a filter between their stomach and honey stomach which removes a lot of pollen and unwanted debris. However, not all pollen is filtered out and some types are removed more efficiently than others, often due to shape and size of the grain (Bryant & Jones, 2001). Pollen that is not removed therefore occurs in the honey. Repeated experiments by researchers into the absolute pollen concentration (APC) in honey of various common pollen types eventually resulted in pollen coefficients, where the average amount of pollen in 1g of honey is expressed numerically (Bryant & Jones, 2001). For example, the pollen coefficient for lime (*Tilia* spp) is 10/g while for sweet chestnut it is 1000/g (Sawyer, 1988). Therefore, lime is under-represented compared to the highly over-represented sweet chestnut. Not all pollen coefficients have been worked out so some types may still be somewhat over- or under-represented (Bryant & Jones, 2001). Those types that do not have a pollen coefficient worked out, may have a standard one of 50 applied (Sawyer, 1988, p. 115).

Finally, the frequency of occurrence and dominance of each pollen type were analysed (after the application of pollen coefficients). Pollen types that occurred only once were not included in these results (unless they occurred at a percentage greater than 3%) as there were a lot of them and they may be incidental. The pollen classes were determined according to Louveaux, Maurizio and Vorwhol (1970), as a percentage of the sample as follows: Dominant: greater than 45%; Secondary: 16-45%; Important minor: 3-15%; Present: less than 3%.

After the pollen analysis was done, the honey that had been taken off on or before the 1st July was classified as 'Spring' and honey taken off from 2nd July onwards was classified as 'Summer', as there seemed to be the most differences before and after these dates. It was therefore determined that 13 samples from the Spring collection and 70 samples from the Summer collection had been tested.

Results

The frequency of occurrence and abundance of the pollen types in the honey samples can be seen in Table 2. Clearly, blackberry/raspberry (*Rubus* spp) was the most frequent and dominant pollen type in the samples. Oil-seed rape/mustards (*Brassica* spp) were also important, along with lime trees (*Tilia* spp), vetch/field bean (*Vicia* spp), sweet chestnut (*Castanea sativa*), hawthorn (*Crataegus* spp), white clover (*Trifolium repens*), Himalayan balsam (*Impatiens glandulifera*), cherry/plum (*Prunus* spp), willow (*Salix* spp), butterfly bush (*Buddleia* spp) and onions (*Allium* spp). The top ten most abundant Spring and Summer pollen types by total percentage are given in Figure 2. Oilseed rape/mustards dominated in the Spring while blackberry/raspberry dominated in the Summer.

Discussion

After applying pollen coefficients, this study found more than one third (37 of the 83 samples) to be monofloral/unifloral blackberry/raspberry honey, which is a normally represented pollen type. Butterfly bush may be over-represented as it has a small pollen grain but, since a pollen coefficient has not been worked out for this type, we cannot be sure of its relative importance in the honeys. Butterfly bush is popular with honey bees, often available when there are fewer other plants in flower. Honey bees cannot collect nectar from the species with a long flower-tube, although they sometimes collect pollen, while both pollen and nectar can be gathered from *Buddleia globosa,* which has a short flower tube (Kirk & Howes, 2012).

Sweet chestnut trees are quite prevalent in Worcestershire and although not a dominant pollen type (once the pollen coefficients are applied), it was the second most frequently occurring pollen type, found in 68 of the samples. When Deans (1957) was doing the honey research 60 years ago, the most important pollen type in Worcestershire honey was white clover, dominant in 36% of the samples and secondary in a further 36%. In the current research, white clover was present in just over half the samples (47 samples) but did not occur as a dominant type and only occurred as a secondary type in six samples. Due to changes in agricultural and garden lawn practices, white clover is no longer such an available source of nectar and pollen in the UK (Frame, 1987).

The work by Deans (1957) also showed that fruit tree pollen of the Rosaceae family (cherries, pears, plums etc.) used to be much more available to bees. Many other forage plants have seen a decline in their abundance in the British landscape, including red clover (*Trifolium pratense*) (Carvell et al. 2006) and heather (*Erica* spp), both of which were also more important in honey 60 years ago. Conversely, blackberry/raspberry, oilseed rape/mustards, lime, Himalayan balsam and sweet chestnut did not occur as either dominant or secondary types at all in Worcestershire in Deans's (1957) study. Therefore, either honeybees have had

to focus on other sources for their pollen and nectar or maybe newly-available plants now interest them more. Brassicas, particularly oilseed rape, are now a major source of nectar for bees since the expansion of these crops over the last 30-40 years. There is an important mutualistic interaction between brassicas and bees, with the yield of oilseed rape and mustard increasing where there are high numbers of pollinators to set the seed (Abrol, 2007). The invasive species Himalayan balsam has also become much more prolific in recent decades, allowing it to become an important late season forage plant for some bee colonies (Titze 2000).

Conclusion

Pollen types occurring most prevalently in the honeys of Worcestershire have been determined in this study. Worcestershire's honeybees are favouring a variety of pollen from various habitats such as: crops, e.g. oil-seed rape and field bean; hedgerows and riverbanks, e.g. blackberry, hawthorn and Himalayan balsam; gardens, e.g. alliums and white clover and from trees, e.g. sweet chestnut and limes.

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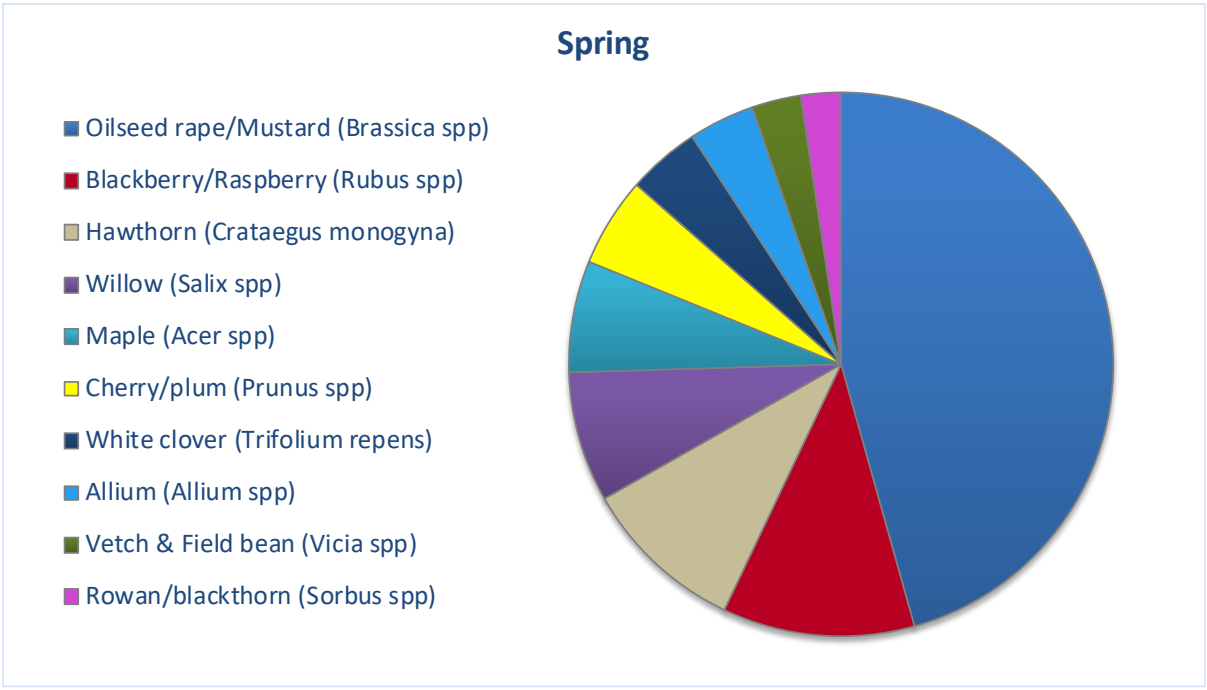
*New affiliation: Polytechnic University of Madrid, General Pardiñas 107 4ª A, 28006, Madrid, Spain.



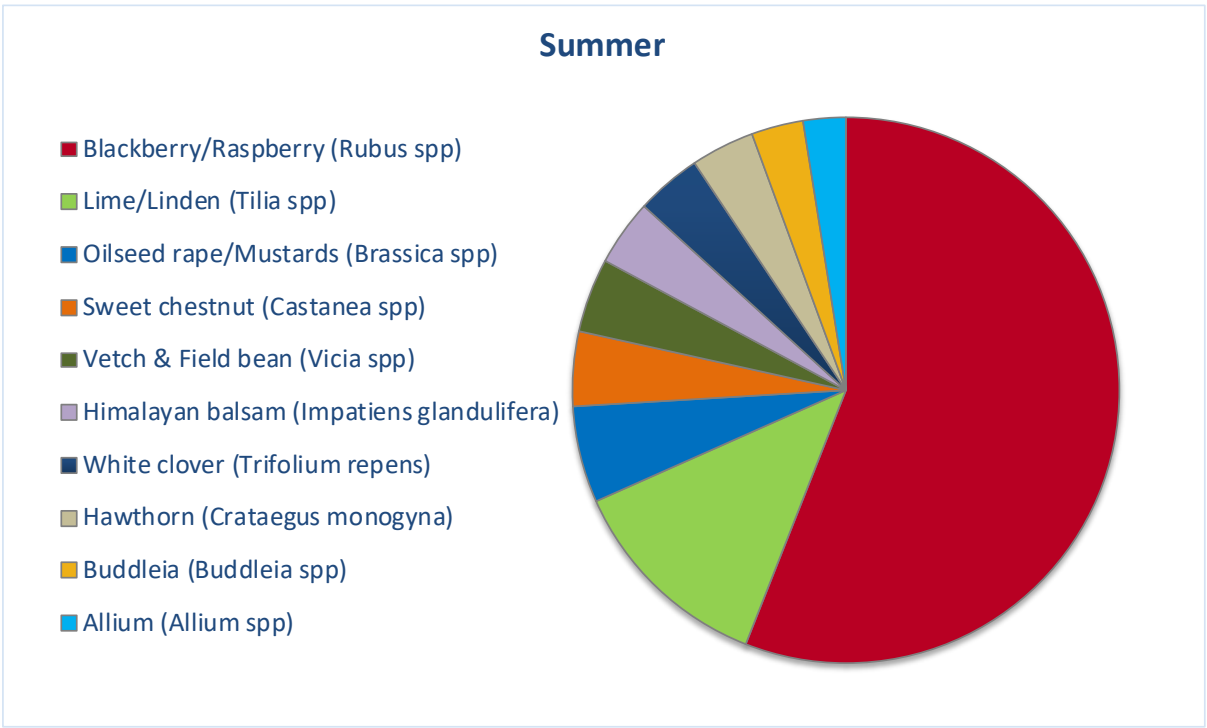


Figure 2. The ten most abundant pollen types in Spring and Summer honey extractions from 83 Worcestershire hives (2016) (total percentages per type, after pollen coefficients have been applied).

Plant Family	Pollen type: Latin / English names	Dominant (>45%)	Secondary (16-45%)	Important minor (3-15%)	Present (<3%)
Aceraceae	*Acer spp* / Maple		1	8	19
Adoxaceae	*Sambucus* spp / Elderflower			3	13
Amaryllidaceae	*Allium* spp / Allium		6	5	8
Aquifoliaceae	*Ilex aquifolium* / Holly			1	8
Apiaceae	Apiaceae / Carrot family			1	11
Araliaceae	*Hedera helix* / Ivy			2	9
Asparagaceae	*Asparagus officinalis* / Asparagus			1	3
Asteraceae	Asteraceae: liguliferous / Dandelion t.				9
	Asteraceae: tubuliferous / Daisy/aster t.				10
	Centaurea cyanus / Cornflower				4
	Centaurea nigra / Knapweed				3
Balsaminaceae	*Impatiens glandulifera* / Himalayan balsam		8	5	13
Berberidaceae	*Berberis* spp / Berberis				2
Boraginaceae	*Borago officinalis* / Borage				3
	Cynoglossum sp / Chinese forget-me-not			1	11
					2
	Echium spp / Viper's bugloss				3
	Lithospermum sp / Lithospermum		1		3
	Myosotis spp / Forget-me-not			1	5
	Phacelia tanacetifolia / Phacelia				
Brassicaceae	*Brassica* spp / Oilseed rape/Mustard	8	10	4	43
	Other Brassicaceae			7	22
Campanulaceae	*Campanula* spp / Bellflower				2
Caryophyllaceae	*Dianthus t.* / Carnation t.				2
Cistaceae	*Cistus* spp / Rock rose				3
	Helianthemum spp / Rock rose				8
Cucurbitaceae	*Bryonia dioica* / Bryony				3
Ericaceae	*Erica* spp / Heather				3
Fabaceae	*Lotus corniculatus* / Bird's foot trefoil			1	5
	Medicago sp / Alfalfa		1		
	Melilotus spp / Melilot			1	3
	Trifolium pratense / Red clover			1	10
	Trifolium repens / White clover		6	17	24
	Trifolium spp / Clover				2
	Vicia spp / Vetch & Field bean	1	3	17	19
Fagaceae	*Castanea sativa* / Sweet chestnut		5	16	47
	Quercus spp / Oak			1	11
Hypericaceae	*Hypericum* spp / St John's wort				4
Liliaceae	*Liliaceae* / Lily family				6
Lythraceae	*Lythrum salicaria* / Purple loosestrife				4
Myrtaceae	*Eucalyptus* spp / Eucalyptus			3	12
Oleaceae	*Ligustrum ovalifolium* / Garden privet		1	2	9
	Ligustrum vulgare / Common privet		2	1	8
Onagraceae	*Chamerion* spp / Willowherb	1			
Papaveraceae	*Papaver* spp / Poppy			1	8
Plantaginaceae	*Plantago* spp / Plantain			1	5
	Linaria spp / Toadflax				2
Poaceae	Poaceae / Grass family			5	15
Polygonaceae	*Polygonum* spp / Knotweed				3
Ranunculaceae	*Aquilegia* sp / Aquilegia				3
	Clematis spp / Clematis			1	6
	Ranunculus spp / Buttercup			1	4
	Thalictrum sp / Meadow rue				2
Rhamnaceae	*Frangula alnus* / Alder buckthorn				3
	Rhamnus spp / Buckthorn			3	11

Plant Family	Pollen type: Latin / English names	Dominant (>45%)	Secondary (16-45%)	Important minor (3-15%)	Present (<3%)
Rosaceae	*Amelanchier* sp / Amelanchier				2
	Crataegus monogyna / Hawthorn		9	12	22
	Filipendula ulmaria / Meadowsweet		1	9	21
					2
	Potentilla sp / Cinquefoil		4	11	16
	Prunus spp / Cherry/plum			2	
	Pyrus spp / Pear		1	3	14
	Rosa spp / Rose	37	29	5	5
	Rubus spp / Blackberry/Raspberry			3	8
	Spirea spp / Spirea			7	7
	Sorbus spp / Rowan				11
	Other Rosaceae				
Rutaceae	*Skimmia* spp / Skimmia				4
Salicaceae	*Salix* spp / Willow		2	14	23
Sapindaceae	*Aesculus hippocastanum* / Horse chestnut			3	11
Saxifragaceae	*Saxifraga* sp / Saxifrage				3
Scrophulariaceae	*Buddleia* spp / Butterfly bush	1	2	6	18
Simaroubaceae	*Ailanthus* sp / Tree of heaven			2	7
Tamaricaceae	*Tamarix* sp / Tamarisk				2
Tiliaceae	*Tilia* spp / Lime/linden	3	13	26	10
Urticaceae	Urticaceae / Nettle family			1	1

Table 1. Frequency and dominance of the various pollen types found across 83 honey samples from Worcestershire hives (2016), after pollen coefficients had been applied. Data shows the number of times a pollen type appeared in each pollen class.

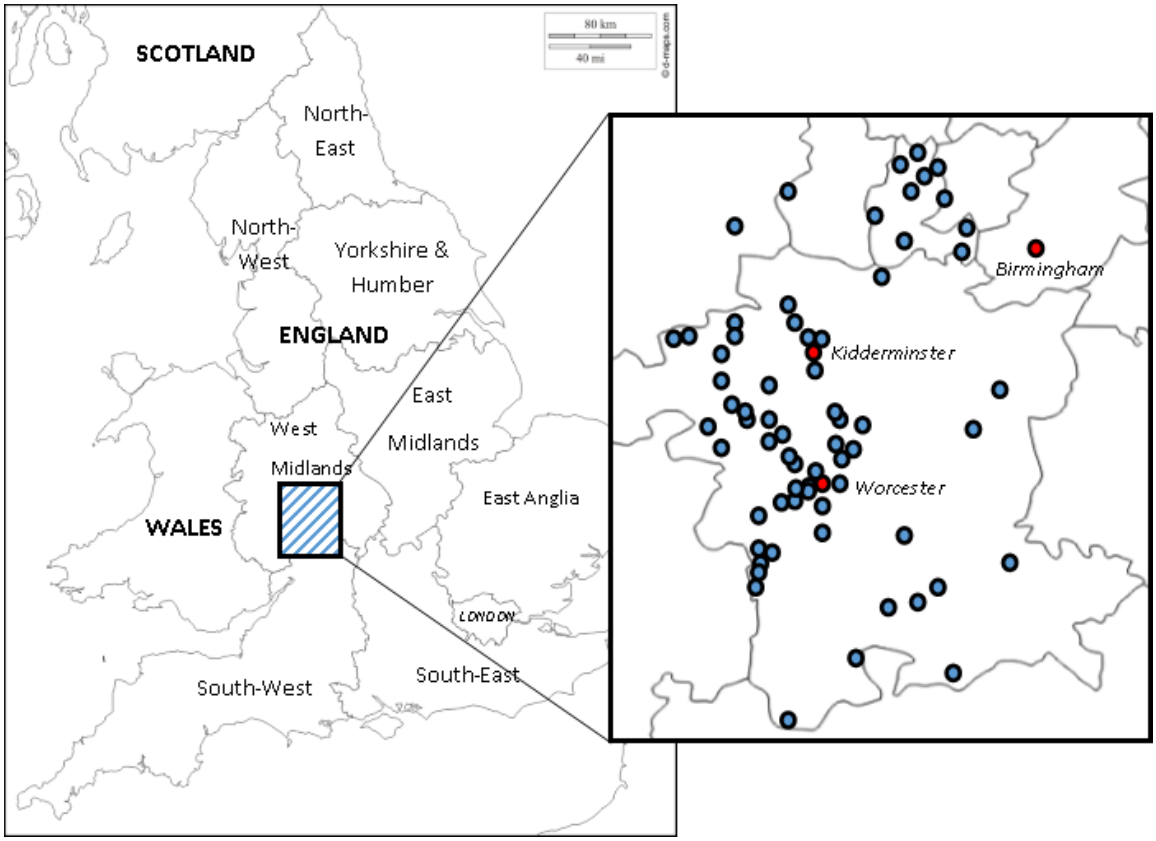


Figure 1. Locations of the bee hives used in the study.