Melissopalynology of honey from Ponteland, UK shows the role of *Brassica napus* in supporting honey production in a suburban to rural setting

Matthew Pound[a*], Alice Dalgleish[a], Jessica McCoy[b] and Jessica Partington[b]

[a]*Department of Geography and Environmental Science, Northumbria University, Newcastle upon Tyne, NE1 8ST, UK;* [b]*Newcastle Sixth Form College, Newcastle upon Tyne, NE4 7SA, UK*

*Corresponding author: Email: matthew.pound@northumbria.ac.uk

Whether honeybees utilize oilseed rape, and thus come into contact with neonicotinoid pesticides, has been questioned in the UK. Here we report the melissopalynology of honey samples taken from hives in the northeast of the UK from 2014-2015. The results show that *Brassica* pollen is predominant in honey extractions from June, following the mass bloom of oilseed rape. Honey extractions from July and September show more diverse sources of nectar from entomophilous crops, weeds and garden plants. Our results clearly show that honeybees will extensively utilise oilseed rape mass blooms in Spring and any change in the current European Union moratorium on neonicotinoids should be carefully considered. We also confirm the importance of gardens (when planted with “bee friendly flowers”) in sustaining pollinators within suburban to rural environments.

Keywords: honey; oilseed rape; honeybee; northeast UK; pollination

1. Introduction

Insect pollination has been estimated to account for 75-80% of crop pollination globally, which has been converted into economical terms as a value of €153 billion (Gallai et al. 2009). Of these pollination services, the greatest value is added to vegetable, fruit and oil crops (Gallai et al. 2009). The global decline in pollinators has led to fears of a pollination crisis (Holden 2006). One of the most salient declines with widespread public attention has been the decline of honeybees in Europe and

North America (Becher et al. 2013; Goulson et al. 2015; Meixner & Le Conte 2016). Multiple causes have been identified for the witnessed decline in honeybees, including: parasites, pathogens, environmental impacts, beekeeping practices and pesticides (Becher et al. 2013; Goulson et al. 2015; Meixner & Le Conte 2016). Pesticides in particular have captured the public's opinion, with strong accusations placed against neonicotinoids, leading to restrictions, or bans in their usage (Barbosa et al. 2015). A growing body of literature has demonstrated the negative impacts that neonicotinoids can have on honeybee colonies through chronic exposure combined with parasites, pathogens and insufficient flowers for foraging (Sandrock et al. 2014; Doublet et al. 2015; Goulson et al. 2015).

Of particular importance to many honey producers in the UK is oilseed rape (OSR; *Brassica napus*), which is the most abundant oil crop in Europe (Carre & Pouzet 2014). Spring flowering OSR blankets fields in densely packed yellow flowers that provide a pollen and nectar source to a variety of pollinators (Westphal et al. 2003; Budge et al. 2015). Being susceptible to numerous pests, OSR crops were, until the European Union moratorium in 2013, treated with neonicotinoids (Gross 2013; Dewar, 2017). Since this ban, there have been crop losses and reductions in yields in eastern England from cabbage stem flea beetles (Dewar 2017); in some instances emergency use of neonicotinoids on OSR crops has been granted (Case 2015). With a decision yet to be made on whether the European Union moratorium will continue and with the UK voting to leave the European Union (and hence having to determine its own policy on neonicotinoid pesticides), evidence is needed on how much honeybees and wild bees utilise OSR crops (Woodcock et al. 2016). Evidence from western France demonstrated that honeybees will exploit mass flowering crops such as OSR for their nectar (Requier et al. 2015). However, decoded waggle dance data and pollen pellet analysis from southern England showed limited honeybee foraging on OSR (Garbuzov et al. 2015). The analysis of pollen pellets for OSR is unlikely to yield *Brassica* pollen, as bees only use OSR as a source of nectar (Requier et al., 2015). The aim of this paper is to present melissopalynological data for two honey extraction seasons (July 2014 and June to September 2015) from a small honey producer in the northeast of England to determine what the sources of nectar are for the production of these honeys and to what extent honeybees utilise OSR.

2. Materials and Methods

Five samples of honey, extracted from hives in July 2014, June 2015 (two extractions), July 2015 and September 2015, were taken from a small-scale honey producer based in Ponteland, northeast England (Fig. 1). A 10 gram sample of each honey was processed following the methods presented in Jones and Bryant (2004). Two *Lycopodium clavatum* spore tablets (Northumbria University Batch 3862; 9666 spores per tablet) were added to each sample to facilitate the calculation of pollen concentrations. Following acetolysis, the sample was transferred to glass vials using isopropyl alcohol and silicon oil was added.. Slides were made and the pollen counting was undertaken on a Leica DM2000 microscope. Total pollen concentration per 10 grams was calculated using the formula presented in Jones and Bryant (2014).

3. Results

A total of 1293 pollen grains were counted across the five samples and 35 pollen taxa from 26 plant families were identified (Table 1). A brief description of the pollen content of the five samples is presented below and pollen taxa are referred to as "predominant" when present at >45%, "secondary" at frequencies of 16-45%, "important minor" when present at 3-16% and minor when they make up <3% of the total pollen percentage.

3.1. July 2014 honey extraction

Brassica is the predominant pollen taxa in this honey and there are no pollen types present that qualify as secondary (Fig. 2; Table 1; 2). Important minor elements include *Borago* (probably *B. officinalis*), *Trifolium*, *Vicia faba* and *Solanum* (Fig. 2; Table 1; 2). Minor pollen types account for <2% of the sample and include (in alphabetical order) *Anthyllis*-type, Apiaceae, *Papaver* and *Primula* (Fig. 2; Table 1). The anemophilous pollen of Cupressaceae and Poaceae are present (Table 1). This honey was classified as category II (intermediate) based on the pollen concentration (Table 1).

3.2. June 2015 1st honey extraction

This honey was extracted from the hive in early June and is dominated by *Brassica* pollen (Fig. 2; Table 1; 2). There are no pollen classified as secondary and the only important minor pollen is *Vicia faba* (Table 1; 2). Minor pollen taxa present are *Bistorta*-type, *Fraxinus*, Rosaceae, *Ruta* and *Thalictrum*-type (Table 1). Poaceae and *Quercus* are likely anemophilous contaminants. This honey was classified as category II (intermediate), based on the pollen concentration (Table 1).

3.3. June 2015 2nd honey extraction

This honey was extracted from the end of June and is again dominated by *Brassica* pollen with no elements classified as secondary (Fig. 2; Table 1; 2). *Solanum* and Rosaceae are the only important minor elements (Table 1; 2). Minor taxa include Apiaceae, *Borago*, Liliaceae, *Papaver*, *Plantago* and *Vicia faba* (Table 1). Anemophilous pollen are represented by *Pinus* and Poaceae (Table 1). This honey sample had the highest pollen concentration and was classified as category III (rich) (Table 1).

3.4. July 2015 honey extraction

There are no predominant pollen taxa in this honey extraction (Table 2). Instead, the honey contains two secondary taxa: *Brassica* and *Solanum* that are the most common pollen types (Fig. 2; Table 1; 2). *Vicia faba*, Apiaceae, *Borago*, Ranunculaceae and *Papaver* are the important minor components of the assemblage (Table 1; 2). Nine minor pollen types are present *Geranium*-type, *Micropus*-type, *Plantago*, *Primula*, *Rhamnus*, Rosaceae, *Ruta*, *Trifolium* and *Valeriana*-type (Table 1). Anemophilous pollen types include *Alnus*, Cupressaceae, Poaceae and *Quercus*. This honey sample was classified as category II (intermediate).

3.5. September 2015 honey extraction

There were no predominant pollen taxa present in this honey extraction (Table 2). The secondary taxa *Vicia faba* and *Brassica* are the most common pollen types present (Fig. 2; Table 1; 2). There are five important minor pollen taxa present, including: Apiaceae, *Solanum*, *Borago*, *Androsace*-type and Liliaceae (Table 1; 2). Eleven minor elements present in this honey are *Anthyllis*-type, *Castanea*, *Fabaceae*, *Globularia*-type, *Micropus*-type, *Papaver*, *Ribes*, Rosaceae, *Rubus*, *Taraxacum*-type and *Tilia* (Table 1). Anemophilous pollen taxa recorded were Poaceae and *Quercus* (Table 1). This honey was classified as category III (rich), based on its pollen concentration (Table 1).

4. Discussion

Mellisopalynological data shows a dominance of *Brassica* pollen grains in honeys extracted from bee hives in June and July (Fig. 2). In the first two extractions of 2015 (both in June) *Brassica* pollen accounted for >75% of all pollen encountered, strongly suggesting that OSR was the dominant botanical source for the honey and the principle foraging target for the colonies during late Spring – early Summer. Our interpretive step from *Brassica* pollen to OSR is based on field observations of honeybees from these hives flying towards OSR crops and returning from these fields coated in bright yellow pollen in April – June (L. Elliot pers. comm.). In July, the percentage of *Brassica* pollen decreases to 39-57%, but is still the most frequent pollen grain encountered (Fig. 2). This result is in agreement with a number of other studies that found Spring OSR to be an important nectar and pollen source for pollinators (Westphal et al. 2003; Budge et al. 2015; Requier et al., 2015). Our findings are however contradictory to the pollen pellet analysis and waggle dance data of Garbuzov et al. (2015) who found evidence for limited foraging on OSR. This discrepancy might arise from the sampling of different bee resources: Garbuzov et al. (2015) sampled pollen pellets, which are a source of proteins, minerals and fats for bees, whereas we sampled honey - a food source for the colony. When both pollen pellets and honey have been co-sampled during spring it has been shown that *Brassica* pollen types will be dominate in honey (when OSR mass blooms are present), but that woody species are the main foraging source for pollen pellets (Requier et al. 2015).

Following the OSR mass blooming, the honey samples extracted in July and September show an increase in the number of different pollen types, including entomophilous crops, garden plants and weeds (Table 2). The importance of entomophilous crops (e.g. *Solanum* and *Vicia faba*) and weeds (such as *Papaver*) in the period after mass-blooms for sustaining bee colonies has been previously demonstrated (Requier et al. 2015; Rollin et al. 2013). Crops of Fabaceae in particular are extensively visited by bees (Rollin et al. 2013). The importance of weeds has been identified for providing a diverse diet throughout the flowering season (Garbuzov et al. 2015; Goulson et al. 2015; Requier et al. 2015). This is epitomized by *Papaver*, which is essentially a nectar-less plant (Louveaux et al. 1978), but the pollen is an important part of the honeybee's annual diet (Requier et al. 2015). The amount of *Borago* pollen in honeys extracted in July and September clearly demonstrates the role of gardens in supporting honeybee diets within a suburban to rural area (Table 2). The planting of appropriate flowers in gardens has already been suggested as a means to help pollinator biodiversity (Blackmore & Goulson 2014), but many garden plants widely advertised as "bee friendly", are often based purely on anecdotal evidence (Garbuzov & Ratnieks 2014). This study shows that *Borago* (Borage) is an important nectar source for bee colonies with gardens in their foraging range (Fig. 1) and should therefore be promoted as "bee friendly".

5. Conclusions

Honeybee colonies in a suburban to rural environment extensively utilise OSR mass blooms as a source of nectar in Spring. This finding is in disagreement with previous research from the UK, which suggested honeybees do not use OSR mass blooms. Differences in sampling strategies is the likely reason for this disconnect and a methodology that samples honey and pollen pellets would likely find results in agreement. As multiple studies have shown that honeybees will forage extensively on OSR any change in the policies concerning neonicotinoid pesticides should be carefully considered. Following the mass bloom of OSR the nectar source for the Ponteland honey becomes more diverse and shows the importance of entomophilous crops, weeds and gardens for sustaining honeybee colonies in suburban to rural environments.

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Disclosure statement

The authors declare that they have no conflict of interest.

Author Biographies

MATTHEW POUND is a senior lecturer in physical geography at Northumbria University. His main research is on Cenozoic palaeoenvironments and palaeoclimates, but he is also interested in palynomorphs from honey and faeces – you have to have a hobby.

ALICE DALGLEISH is a recent graduate in BSc Geography (2016) from Northumbria University with her main focus in Palaeoecology. She has enjoyed being able to continue her interests in pollen through this paper.

JESSICA MCCOY is an aspiring research scientist, aiming to study BSc Physical Geography at Northumbria University. She has a keen interest in melissopalynology and ecology.

JESSICA PARTINGTON is a student at Newcastle Sixth Form College who plans to study Physical Anthropology at Higher Education. She has a keen interest in human biology and the skeletal morphology of early hominids.

References

Barbosa WF, Smagghe G, Guedes RNC. 2015. Pesticides and reduced-risk insecticides, native bees and pantropical stingless bees: pitfalls and perspectives. Pest Management Science 71:1049-1053.

Becher MA, Osborne JL, Thorbek P, Kennedy PJ, Grimm V. 2013. Towards a systems approach for understanding honeybee decline: a stocktaking and synthesis of existing models. Journal of Applied Ecology 50:868-880.

Blackmore L M, Goulson D. 2014. Evaluating the effectiveness of wildflower seed mixes for boosting floral diversity and bumblebee and hoverfly abundance in urban areas. Insect Conservation and Diversity 7:480–484.

Carre P, Pouzet A. 2014. Rapeseed market, worldwide and in Europe. OCL - Oilseeds and Fats, Crops and Lipids 21:D102–D102.

Case P. 2015. Neonicotionoid emergency use approved for 5% of OSR area. Farmers Weekly 22 July (2015).

Dewar AM. 2017. The adverse impact of the neonicotinoid seed treatment ban on crop protection in oilseed rape in the United Kingdom. Pest Management Science In press DOI: 10.1002/ps.4511

Doublet V, Labarussias M, de Miranda JR, Moritz RFA, Paxton RJ. 2015. Bees under stress: sublethal doses of a neonicotinoid pesticide and pathogens interact to elevate honey bee mortality across the life cycle. Environmental Microbiology 17:969-983.

Gallai N, Salles J-M, Settele J, Vaissière BE. 2009. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. Ecological Economics 68:810-821.

Garbuzov M, Ratnieks FLW. 2014. Quantifying variation among garden plants in attractiveness to bees and other flower-visiting insects. Functional Ecology 28:364–374.

Garbuzov M, Couvillon MJ, Schürch R, Ratnieks FLW. 2015. Honey bee dance decoding and pollen-load analysis show limited foraging on spring-flowering oilseed rape, a potential source of neonicotinoid contamination. Agriculture, Ecosystems and Environment 203:62-68.

Goulson D, Nicholls B, Botias C, Rotheray E. 2015. Combined stress from parasites, pesticides and lack of flowers drives bee declines. Science 347:1432-1435.

Holden C. 2006. Report warns of looming pollination crisis in North America. Science 314:397.

Jones GD, Bryant VM. 2014. Pollen Studies of East Texas Honey. Palynology 38:242-258.

Louveaux J, Maurizio A, Vorwohl G. 1978. Methods of melissopalynology. Bee World. 59:139–157.

Meixner MD, Le Conte Y. 2016. A current perspective on honey bee health. Apidologie 47:273-275.

Morton D, Rowland C, Wood C, Meek L, Marston C, Smith G, Simpson IC. 2011. Final report for LCM2007 – the new UK land cover map. CS Technical Report No 11/07 NERC/Centre

Requier F, Odoux J-F, Tamic T, Moreau N, Henry M, Decourtye A, Bretagnolle V. 2015. Honey bee diet in intensive farmland habitats reveals an unexpectedly high flower richness and a major role of weeds. Ecological Applications 25:881-890.

Sandrock C, Tanadini M, Tanadini LG, Fauser-Misslin A, Potts SG, Neumann P. 2014. Impact of Chronic Neonicotinoid Exposure on Honeybee Colony Performance and Queen Supersedure. PLoS ONE 9:e103592.

Westphal C, Steffan-Dewenter I, Tscharntke T. 2003. Mass flowering crops enhance pollinator densities at a landscape scale. Ecology Letters 6:961–965.

Woodcock BA, Isaac NJB, Bullock JM, Roy DB, Garthwaite DG, Crowe A, Pywell RF. 2016. Impacts of neonicotinoid use on long-term population changes in wild bees in England. Nature Communications 7:12459.

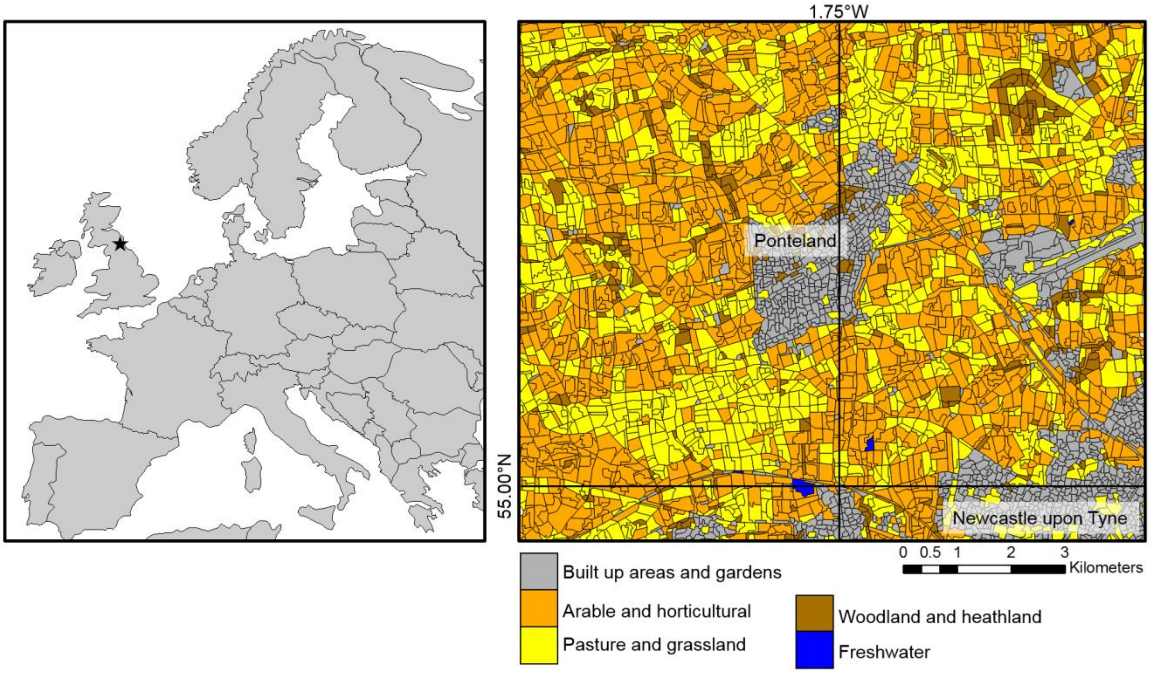

Figure 1. The location of Ponteland - a small settlement to the northwest of Newcastle upon Tyne. The beehives are located in the southern part of the settlement. The land cover classification shows the dominance of arable and horticultural fields (50.75 km2) in the surrounding area. Pasture and grassland is the next most abundant land cover class (37.15 km2), followed by built-up areas and gardens (16.66 km2) and the woodland and heathland land cover class (5.36 km2). This map is based upon LCM2007 © NERC (CEH) 2011. Contains Ordnance Survey data © Crown Copyright 2007. © third party licensors (for full details see: Morton et al., 2011) and was projected in ArcGIS 10.

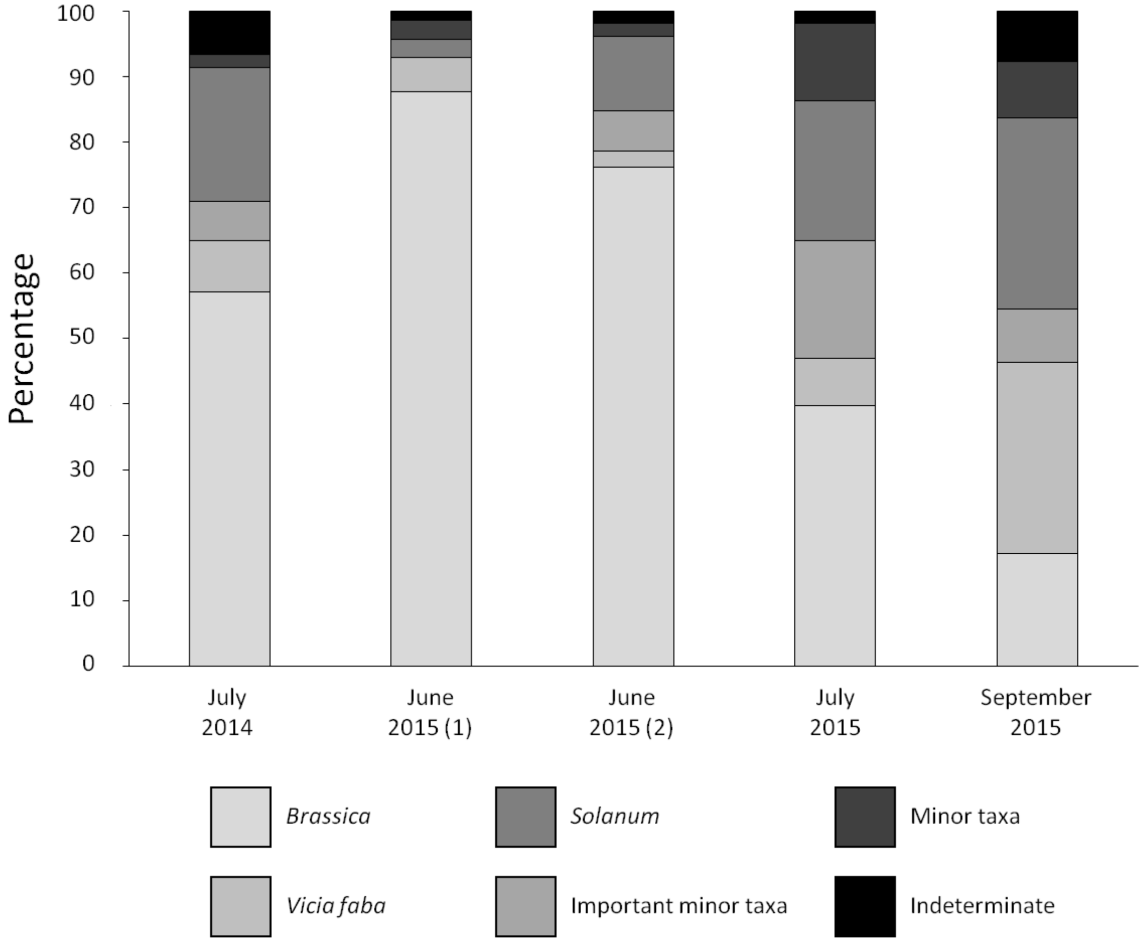

Figure 2. Stacked bar charts to show the changing dominance of pollen types found in the Ponteland honey. The two June 2015 extractions come from the beginning (1) and end (2) of the month. The trend shows a high-reliance on *Brassica* (OSR) during the early part of the production season, with a gradual shift to entomophilous crops and garden plants towards the end. All data presented in table 1.

	Extraction date	July 2014			June 2015 (1)			June 2015 (2)			July 2015			September 2015		
Family	Pollen taxon	Count	%	Frequency	Count	%	Frequency	Count	%	Frequency	Count	%	Frequency	Count	%	Frequency
Apiaceae	Apiaceae	7	1.73%	L	0			4	1.90%	L	13	6.16%	M	32	12.45%	M
Asteraceae	Micropus-type	0			0			0			1	0.47%	L	1	0.39%	L
	Taraxacum-type	0			0			0			0			1	0.39%	L
Betulaceae	Alnus	0			0			0			1	0.47%	A	0		
Boraginaceae	Borago	42	10.37%	M	0			1	0.48%	L	11	5.21%	M	18	7.00%	M
Brassicaceae	Brassica	231	57.04%	D	184	87.62%	D	160	76.19%	D	84	39.81%	S	44	17.12%	S
Caprifoliaceae	Valeriana-type	0			0			0			3	1.42%	L	0		
Cupressaceae	Cupressaceae	1	0.25%	A	0			0			3	1.42%	A	0		
Fabaceae	?Anthyllis	3	0.74%	L	0			0			0			1	0.39%	L
	Fabaceae	0			0			0			0			2	0.78%	L
	Trifolium	33	8.15%	M	0			0			4	1.90%	L	0		
	Vicia faba	32	7.90%	M	11	5.24%	M	5	2.38%	L	15	7.11%	M	75	29.18%	S
Fagaceae	Castanea	0			0			0			0			1	0.39%	L
	Quercus	0			1	0.48%	A	0			1	0.47%	A	3	1.17%	A
Geraniaceae	Geranium-type	0			0			0			1	0.47%	L	0		
Grossulariaceae	Ribes	0			0			0			0			6	2.33%	L
Liliaceae	Liliaceae	0			0			6	2.86%	L	0			8	3.11%	M
Malvaceae	Tilia	0			0			0			0			3	1.17%	L
Oleaceae	Fraxinus	0			1	0.48%	L	0			0			0		
Papaveraceae	Papaver	1	0.25%	L	0			2	0.95%	L	7	3.32%	M	2	0.78%	L
Pinaceae	Pinus	0			0			1	0.48%	A	0			0		
Plantaginaceae	Globularia-type	0			0			0			0			1	0.39%	L
	Plantago	0			0			1	0.48%	L	4	1.90%	L	0		
Poaceae	Poaceae	1	0.25%	A	1	0.48%	A	2	0.95%	A	2	0.95%	A	1	0.39%	A
Polygonaceae	?Bistorta	0			1	0.48%	L	0			0			0		

Primulaceae	Androsace-type	0			0			0			0			10	3.89%	M
	Primula	3	0.74%	L	0			0			1	0.47%	L	0		
Ranunculaceae	?Thalictrum	0			1	0.48%	L	0			0			0		
	Ranunculaceae	0			0			0			9	4.27%	M	0		
Rhamnaceae	Rhamnus	0			0			0			1	0.47%	L	0		
Rosaceae	Rosaceae	0			6	2.86%	L	11	5.24%	M	1	0.47%	L	5	1.95%	L
	Rubus	0			0			0			0			2	0.78%	L
Rutaceae	Ruta	0			1	0.48%	L	0			1	0.47%	L	0		
Saxifragaceae	Saxifraga	0			0			0			6	2.84%	L	0		
Solanaceae	Solanum	24	5.93%	M	0			13	6.19%	M	38	18.01 %	S	21	8.17%	M
	Indeterminate	27	6.67%	M	3	1.43%	L	4	1.90%	L	4	1.90%	L	20	7.78%	M
	Sum	405			210			210			211			257		
	Pollen concentration	38952.54			90216.00			238807.06			33711.17			105709.02		
	Honey classification	Intermediate			Intermediate			Rich			Intermediate			Rich		
	International Honey Category	Category II			Category II			Category III			Category II			Category III		

Table 1. Pollen counts (count), percentages (%)and frequency classifications (frequency) for the Ponteland honey samples. Frequency classifications: D = predominant, S = secondary, M = important minor, L = less important minor and A = anemophilous. Pollen concentrations and honey classification based upon the methodologies of Louveaux et al. (1978) and Jones and Bryant (2014).

Pollen taxa frequency	July 2014	June 2015 (1)	June 2015 (2)	July 2015	September 2015
Predominant >45%	*Brassica*	*Brassica*	*Brassica*		
Secondary (16-45%)				*Brassica*, *Solanum*	*Vicia faba*, *Brassica*
Important minor (3-16%)	*Borago*, *Trifolium*, *Vicia faba*, *Solanum*	*Vicia faba*	*Solanum*, Rosaceae	*Vicia faba*, Apiaceae, *Borago*, Ranunculaceae, *Papaver*	Apiaceae, *Solanum*, *Borago*, *Androsace*-type, Liliaceae

Table 2. Pollen taxa frequency classifications for the five Ponteland honey samples. Classification of pollen taxa frequency is based upon Louveaux et al. (1978).