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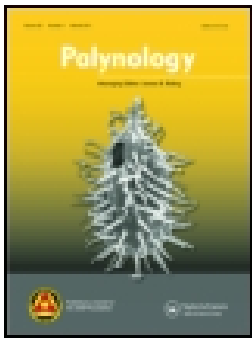
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Determining if honey bees (*Apis mellifera*) collect pollen from anemophilous plants in the UK

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ABSTRACT

Whether insect pollinators use wind-pollinated plants have implications for insect monitoring and conservation strategies in a wide range of environments. Habitats, such as coniferous plantations and arable crops of the Poaceae family are not typically considered priority for the monitoring of insect pollinators or habitat enhancement. Further many pollinator monitoring techniques focus on flowers and do not count insect interactions with wind-pollinated plants. Using two honey bee colonies from distinct environments (urban and rural) in north east England, we investigate the use of wind-pollinated plants over the summer of 2021. We combine honey bee pollen pellet analysis with airborne pollen sampling to investigate whether honey bees use three common wind-pollinated plant groups (*Pinus* sp., *Plantago* sp. and Poaceae) that have previously been considered sources of forage. Our results show that honey bees do forage on *Plantago* and Poaceae pollen, in line with previous studies. However, we show statistically that *Pinus* pollen is contamination from the atmosphere and not actively collected. It is important to consider airborne contamination before making interpretations based on small amounts of pollen in samples of bee products. The use of members of the Poaceae has implications for insect pollinator monitoring in urban environments, which has not always been considered in past studies.

KEYWORDS

Insect pollinators; urban pollen collection
contamination Poaceae
Pinus *Plantago*


1. Introduction

Global insect pollinator decline is a consequence of a wide range of factors, including land management decisions, climate change, pesticides, pathogens and species introductions (Potts et al. 2016; Baldock 2020). Headline figures regarding insect pollinator importance are commonly associated with agricultural land where pollinators improve the quantity, or quality, of the yield (Potts et al. 2016; Garibaldi et al. 2021). These agricultural systems, associated with insect pollinators, are typically dominated by entomophilous (insect-pollinated) plants. However, there is a growing awareness that insect pollinators use anemophilous (wind-pollinated) plants (Jones 2014; Saunders 2018). This has led to calls for the promotion of more sustainable practices and conservation management strategies in agricultural and forestry communities that were not previously considered priority for insect pollinators (Saunders 2018). Use of anemophilous plants has been documented through direct and indirect observations. Direct observations of insect pollinators foraging on anemophilous plants include many members of the Poaceae family and species of *Plantago* (Jones 2014; Saunders 2018). However, far more observations are

indirect, coming mainly from pollen analysis of honey, corbiculae pollen loads, brood cells or nests (Severson and Parry 1981; Keller et al. 2005; Baum et al. 2011; Saunders 2018; El-Sofany et al. 2020). Such indirect observations show a wide range of anemophilous plants apparently being used by pollinators (Saunders 2018). For honey bees (*Apis mellifera* L.), the use of some anemophilous plants is well established (Keller et al. 2005; Saunders 2018). Observations and pollen analysis show the use of *Zea mays* crops and a variety of tree species as a widespread phenomenon (Severson and Parry 1981; Keller et al. 2005; Di Pasquale et al. 2016; El-Sofany et al. 2020). However, indirect observations alone present something of a paradox, especially for anemophilous plant pollen present in a sample in small quantities – was it collected?

Honey bees collect pollen as a source of amino acids, fats, minerals, proteins, starch, sterol and vitamins (Brodschneider et al. 2018). A diverse selection of floral sources is required for a colony to get all their nutritional needs (Roulston and Cane 2000). Whilst the general rule still persists that honey bees forage on one plant per foraging trip, multiple studies have shown that around 40% of pollen pellets contain two

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forage sources (Betts 1935; Brodschneider et al. 2018; Hornby et al. 2022). Within all pellets, are typically small quantities of other pollen. Betts (1935) termed these as 'doubtfuls' and suspected they were not actively collected by the honey bee. Brodschneider et al. (2018) proposed a value of >10% pollen in a pellet for a plant to have been actively foraged on. Whereas, anything below 10% was considered contamination. Suggestions for these sources of contamination range from previous pollinator activity on flowers, residual pollen left on hairs from previous foraging trips, bee to bee contact, or contact with another contaminated surface (Betts 1935; Brodschneider et al. 2018). One source that has not been widely considered is atmospheric contamination.

Pollen released into the atmosphere can travel long distances, achieve great altitude and be in sufficient quantities to cause allergic reactions (Ziello et al. 2012; Szczepanek et al. 2017; Williams and Barnéoud 2021). Both *Pinus* and Poaceae pollen are ubiquitous in the atmosphere across Europe during their pollination season, including long-range transport of pollen to sampling stations when local plants are not releasing pollen (Kasprzyk 2006; Szczepanek et al. 2017). The potential for contamination in the beehive comes from the interaction with airborne particles during flight (Negri et al. 2015), during beekeeper inspections and hive management practices (Molan 1998), and by the proximity/size of the brood chamber to honeycomb (Fernandez and Ortiz 1994). In this paper, we aim to test whether honey bees collect pollen of anemophilous plants, with a focus on Poaceae, *Plantago* and *Pinus* pollen, or if airborne contamination can better explain observations. We include Poaceae and *Plantago* as extensive melissopalynological work and direct observational data confirm their usage (Severson and Parry 1981; Keller et al. 2005; Di Pasquale et al. 2016; El-Sofany et al. 2020). *Pinus* pollen is included because it has recently been identified as being used by honey bees through indirect observations of very small numbers of pollen grains (Saunders 2018 and references therein).

2. Materials and methods

Two hives were sampled from June to October 2021 in North East England. One hive (urban) was located in Newcastle-upon-Tyne city centre on an enclosed terrace (ground level was 45 m above sea level and the terrace is one floor up from ground level), with urban trees, amenity grasslands, parks and residential gardens within 3 km of the hive (Figure 1). The second hive (rural) was located ~18 kms to the west of Newcastle-upon-Tyne on a partially reforested disused airfield, located at 145 m above sea level and was mainly surrounded by farmland (Figure 1). An urban and rural hive were selected, to try and incorporate opposing surrounding environments in a landscape controlled by human intervention. This can be summarised by comparing the percentage of the two major land-uses form a 3-km radius circle around each hive (Figure 1). For the urban hive this is unsurprisingly Urban (52.3%) and Sub-Urban (27.8%). The rural hive is surrounded by Arable and Horticultural (58.1%) and Improved Grassland (31.7%).

In agreement with the beekeeper, pollen traps (Abelo Universal Pollen Trap) were attached for one-hour a week during fair weather and corbicular pollen samples (hereon 'pellets') were collected. A regular, but short collection period was opted to avoid placing the colonies under stress that might potentially modify their foraging behaviour (Baum et al. 2004) The collecting screen and collection draw were removed cleaned and stored indoors in between each sampling period. Airborne pollen was sampled by leaving a Tauber trap near each hive for the duration of the week, at the time of hive pollen sampling the Tauber trap was sampled and cleaned, before being placed back in the same spot. In total 19 weeks were sampled at the urban hive and 10 at the rural hive.

Pellets were sorted and colour was determined digitally following Hornby et al. (2022). The total number of pellets of each colour type was counted and a subset of these (3–5 pellets) were chemically treated to facilitate pollen identification. Chemical treatment on both airborne and pellet samples followed a modified version of the method presented in Jones and Bryant (2014). This involves the disaggregation (pellets only) in hot water (9 ml) and 95% Isopropyl Alcohol (1 ml), which is then centrifuged for 3.5 min at 3500 RPM and the supernatant decanted, before acetolysis treatment. Acetolysis treatment began with dehydration in 5 ml of acetic acid, before samples were heated to 90 °C for 3 min in a 9:1 ratio of acetic anhydride and sulfuric acid. Samples were then washed with acetic acid and centrifuged for 3.5 min at 3500 RPM, before being stored in distilled water and 10% copper sulphate solution. Whilst acetolysis treatment is beneficial for the identification of pollen grains, it can be detrimental to thinner walled specimens and fungal spores (Pound et al. 2021; Riding 2021). However, damage to pollen types we are interested in for this study is only observed after treatment periods in excess of 10 min (Jardine et al. 2015). Airborne pollen slides were mounted in dilute PVA (polyvinyl acetate) glue (Riding 2021), whereas pellet samples were analysed by placing one drop on a temporary slide and covering with a cover slip. Pollen counting followed Lau et al. (2018) using Leica DM500 microscopes. A minimum of 500 pollen grains were counted for pellets, and all pollen was quantified in airborne samples. Percentage values were then calculated from the count. Count data is presented in the [supplementary information](#).

Analysis and plotting was conducted in R-Studio software (R Development Core Team 2021). To test the hypothesis that *Pinus*, *Plantago* and Poaceae pollen in pellets were caused by high amounts of these pollen in the atmosphere (contamination), Pearson Correlation and Granger Causality tests were performed. Pearson correlation shows how two datasets change and correlate, it offers no insight into cause and effect. The Pearsons Correlation simply identifies if high pollen content in the pellets is correlates with that in the airborne samples. Whilst the Granger Causality test does not provide a true measure of causality (and indications of causality will be presented in italic font to indicate this), it does offer *a priori* rather than *post-hoc* assumptions of causality (Dorestani and Aliabadi 2017). Using the Granger Causality

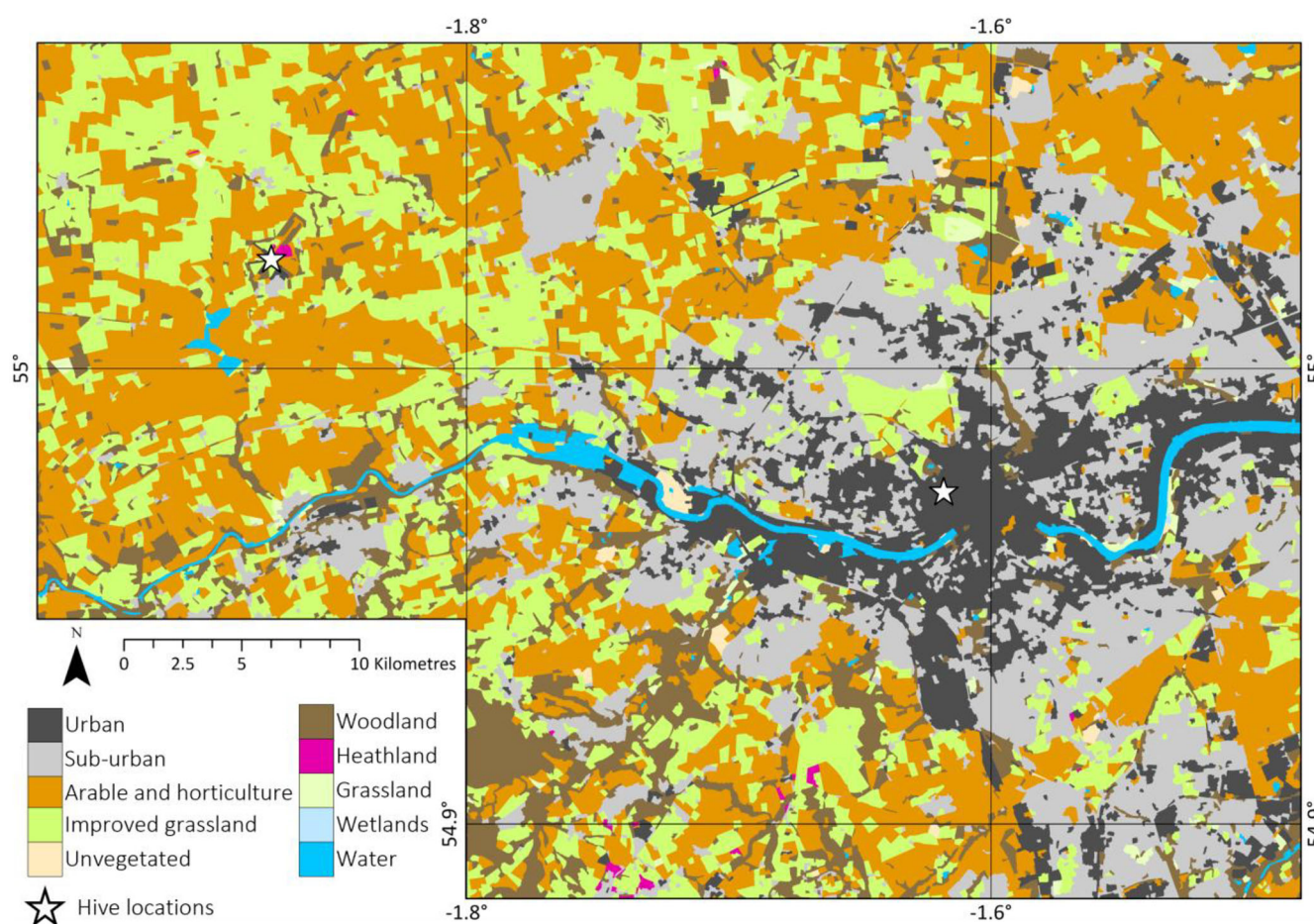


Figure 1. Location of the two hives in the study area and photographs of the hive sites. The location map shows land use classification based upon LCM2007 © NERC (CEH) 2011. Contains Ordnance Survey data © Crown Copyright 2007. © third party licensors (for full details see Morton et al. 2011) and was projected in ArcGIS Pro. Newcastle-upon-Tyne and Gateshead are the large urban and sub-urban areas on the east of the map. The river running east to west in the centre of the map is the River Tyne.

test allows us to test the hypothesis that high airborne pollen is *causing* high pollen in the pellets (contamination). The Granger Causality test was run using a one-week time-lag.

3. Results

Over the six-month period a total of 2424 pellets were collected and analysed, 1454 came from the urban hive and

970 from the rural hive. Of these, 296 pellets from the urban hive (20.4%) contained Poaceae pollen and 172 pellets from the rural hive (17.7%) contained Poaceae pollen. Only one pellet contained more than 10% Poaceae pollen (from the urban hive). Poaceae pollen is present in the pellet samples for the entire study period and shows peaks in July and September, which is coincident with peaks in the airborne samples (Figure 2). *Pinus* pollen was present in 347 pellets (23.9%) from the urban hive and 64 pellets (6.6%) from the

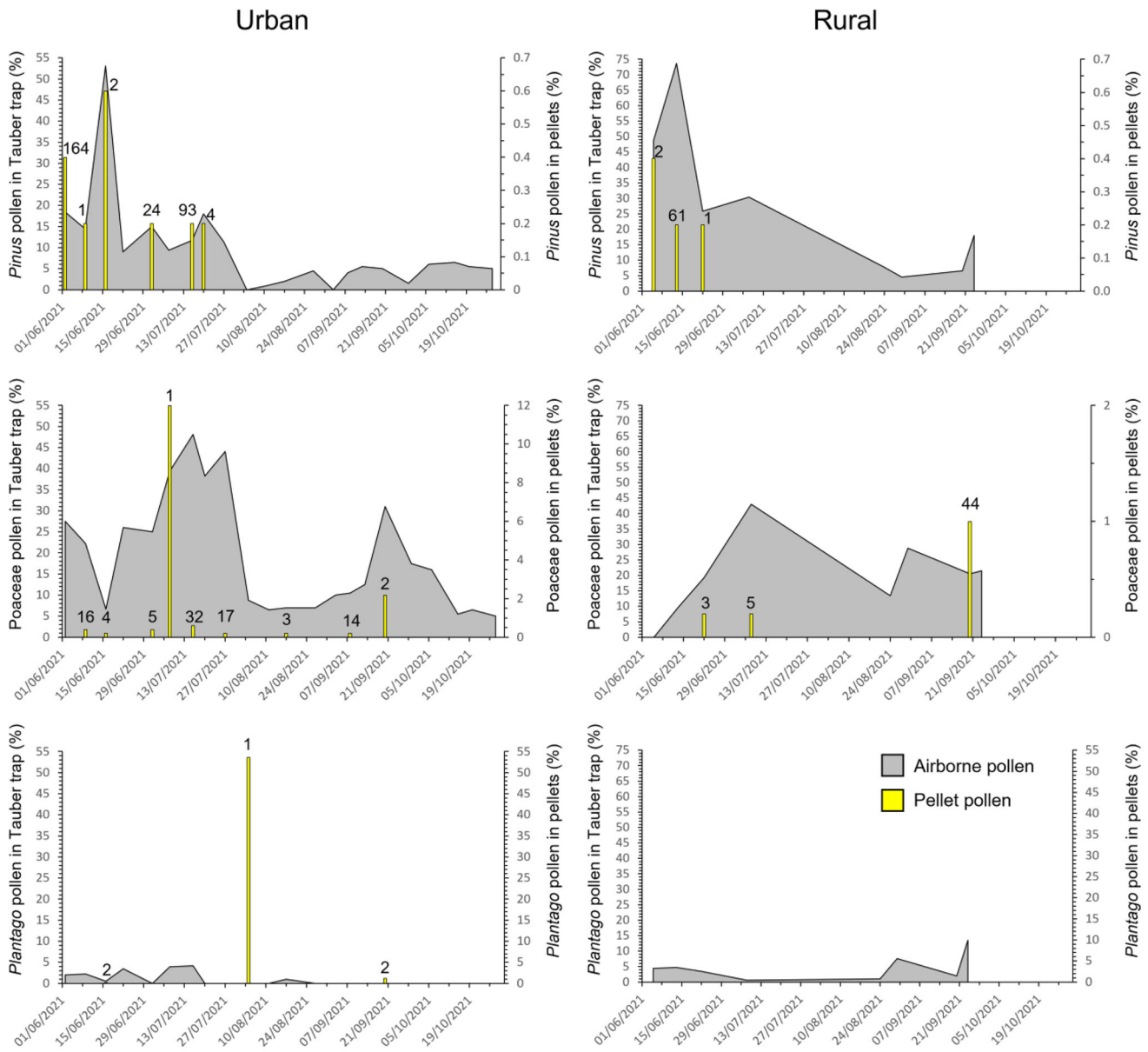


Figure 2. Comparison of the percentage of pollen (*Poaceae*, *Pinus* and *Plantago*) recorded in airborne samples (grey curves) and pellet samples (yellow bars) for the urban and rural hive. Numbers above the yellow bars show how many of that pellet type (based on colour – see methods) were recorded in each sample. Primary and secondary y-axes are scaled differently to show key features in the data. The sampling interval finished earlier at the rural site due to weather conditions and an earlier finish to the beekeeping season.

rural hive during June to July. These coincide with airborne levels greater than 10% in the urban setting and 25% in the rural. In the pellets *Pinus* pollen never exceeds 1% of the counted sample. *Plantago* pollen was present in 52 pellets (3.6%) from the urban hive from June to September, but not reported from the rural hive (Figure 2). In one pellet during August, it comprises 54% of the assemblage, but for the other samples rarely exceeds 1%. It is present in the rural airborne samples throughout the study interval, but more sporadically in the urban airborne samples (Figure 2).

Pearson correlation shows no correlation between the airborne samples and the pollen in pellets for *Poaceae* and *Plantago* (Table 1). The Granger Causality tests allows us to reject the hypothesis that high atmospheric pollen causes high amounts of *Poaceae* pollen in pellets, and the same is true for *Plantago* (Table

Table 1. Granger Causality tests of whether atmospheric pollen causes high pollen content in pellets and Pearson correlation between the datasets.

	Urban				Rural			
	Granger causality test			Pearson	Granger causality test			Pearson
	F	PR	Significance	Pearson	F	PR	Significance	Pearson
<i>Poaceae</i>	0.1711	0.684	n/a	0.389	0.498	0.519	n/a	0.182
<i>Pinus</i>	1.8293	0.193	n/a	0.911	5.086	0.087	0.05	0.702
<i>Plantago</i>	0.3734	0.549	n/a	-0.128	n/a	n/a	n/a	n/a

1). For *Pinus*, Pearson correlation shows positive correlation, and the Granger Causality test suggests it is causation: high *Pinus* pollen in the airborne samples is causing *Pinus* pollen to be present in the pellets. *Pinus* pollen is only present in the pellets around the time of peak atmospheric amounts (Figure 2).

4. Discussion

Pollen analysis of two hives in North East England shows that honey bees do use some anemophilous plants (Figure 2). However, comparison with trapped airborne pollen shows this is more nuanced than recent reviews of the topic have proposed. Our results confirm the use of both Poaceae and *Plantago* pollen by *Apis mellifera* (Saunders 2018). *Plantago* pollen is intentionally collected by honey bees, as demonstrated by one urban pellet in our study where it constituted 54% of the pollen. Honey bees (and other flower-visiting insects) have been shown to sometimes actively forage on *Plantago* inflorescences (Stelleman 1984; Abrahamczyk et al. 2020) and previous pollen analysis routinely shows *Plantago* as a source of pollen (e.g. Percival 1947; Baum et al. 2004). Occurrences of these anemophilous plants being foraged on is during the challenging summer period (also referred to as the 'hungry gap'), seen in greater foraging distances and lower sugar content in foraged nectar (Couvillon et al. 2014; Timberlake et al. 2019). This could mean that these plants are being used as a result of more preferential foraging not being available. Both occurrences of Poaceae and *Plantago* pollen being collected are also from the urban hive (Figure 2). It is known that urban habitats can be beneficial for insect pollinators (Baldock 2020). However, hive densities and floral availability can negatively affect pollinator success in urban environments (Ropars et al. 2019; Egerer and Kowarik 2020). How the multi-factor pressures facing honey bees in urban areas is resulting in foraging on anemophilous plants, that may be sub-optimal, is beyond the scope of this study.

The presence of *Pinus* pollen in pellet samples is here shown to co-occur with periods of high *Pinus* pollen in the atmosphere and therefore is most likely contamination (Table 1; Figure 2). Simple correlation analysis and Granger Causality tests both support the idea that *Pinus* is present due to airborne contamination (Table 1). Given the quantity of *Pinus* pollen in the atmosphere it is not surprising that this could be the source of contamination (Kluska et al. 2020; Sicard et al. 2021). Pollen of *Pinus* has been commonly reported in small percentages in samples from wide range of bee features and products: rectums, honey, pellets and propolis (Warakomska and Maciejewicz 1992; Coffey and Breen 1997; Dimou and Thrasyvoulou 2009; Pound et al. 2018; Radaeski and Bauermann 2021). It is not always present in Pine honey, which is a honeydew type honey (Tsigouri et al. 2004). Even when stands of *Pinus* are proximal to hive locations and have abundant pollen they are not used (Percival, 1947). In controlled experiments, Pernal and Currie (2000) showed that worker honey bees do not readily consume pollen of *Pinus*. They also showed it was little better than no pollen for hypopharyngeal gland and ovary development (Pernal and Currie 2000). In a recent review on pollinator use of anemophilous plants, four studies were cited showing indirect evidence for *Apis mellifera* using species of *Pinus* (Saunders 2018). Three of these studies show the presence of *Pinus* pollen in individual samples at quantities <1% (Pearson and Braiden 1990; Aronne et al. 2012; Girard et al. 2012) and the other study, on propolis, has a maximum *Pinus* pollen content of 6.5% (Warakomska and Maciejewicz

1992). Based on our comparison of airborne pollen and pollen presence in pellets we would suggest that <1% does not represent active collection of *Pinus* pollen. This is in line with suggestions by Brodschneider et al. (2018) for pollen in a pellet by contamination.

Considering how pollen gets into a pellet, previous workers have shown that multiple plants can be foraged on for one pellet and that any pollen present in a value >10% should be considered actively collected (Brodschneider et al. 2018). For those present in smaller amounts a range of contamination pathways were previously proposed: previous pollinator activity on flowers, residual pollen left on hairs from previous foraging trips, bee to bee contact, or contact with another contaminated surface (Betts 1935; Brodschneider et al. 2018). To this list our results add airborne contamination, either by direct contact in flight or through contaminated surfaces. Although not part of the current study, it is also possible that very small amounts of entomophilous pollen (those in a pellet in <1%) could come from the regurgitated nectar used during pellet formation (Matherne et al., 2021).

Given anemophilous pollen types have been associated with honey-induced anaphylaxis (Di Costanzo et al. 2021), understanding the incorporation of these pollen types into hives and bee products is important. Especially as experimental and observational data have shown that pollen production increases with atmospheric CO₂ concentration (LaDeau and Clark 2006; Anderegg et al. 2021). Pollen of Pinaceae has been increasing annually in the atmosphere of Europe, whilst there may be a slight decline in the amount of Poaceae pollen (Ziello et al. 2012). Creating a scenario under 21st Century climate change were the contamination of hives and bee products by non-foraged anemophilous plants will increase.

5. Conclusions

Pinus pollen is only found in urban and rural honey bee pellets during periods of high atmospheric concentration. Conversely when *Pinus* pollen is not abundant in the atmosphere it did not contaminate the pellets. Whereas Poaceae and *Plantago* pollen were present in single pellets in values indicative of active collection by *Apis mellifera* in urban areas during the 'challenging summer period'. Low percentages (<1%) of anemophilous pollen in bee products is the result of airborne contamination. Statistically we show that during, and following, high periods of atmospheric pollen content, bee products become contaminated with airborne pollen. It is therefore important to consider a threshold value for assuming actively collected pollen, rather than simply assuming presence of a taxa indicates that it was foraged on.

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No potential conflict of interest was reported by the authors.

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