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Clopyralid applied to winter oilseed rape (*Brassica napus* L.) contaminates the food products nectar, honey and pollen

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ABSTRACT

Clopyralid is a systemic herbicide used in oilseed rape and other crops. It was found in Danish honey from 2016 in concentrations exceeding the maximum residue level (MRL) of 0.05 mg kg^{-1}. About 50% of the Danish honey is based on nectar from winter oilseed rape. In 2019 and 2020, winter oilseed rape fields were sprayed with clopyralid just before the assigned spraying deadline. At flowering, nectar and pollen samples were collected and the content of clopyralid was measured. Honey and pollen samples were also collected from beehives next to ten conventional winter oilseed rape fields sprayed with clopyralid. Clopyralid was found in nectar and pollen from the experimental fields, and in honey and pollen from beehives next to the conventional fields. For most samples the content in nectar and honey exceeded the MRL. The concentrations found, may not pose any health risk for consumers, as the MRL is based on the original detection limit and not on toxicological tests. However, it can have a significant economical consequence for the beekeepers, who are not allowed to sell the honey if the concentration of clopyralid exceeds 0.1 mg kg^{-1}. Reducing the acceptable applicable rate of clopyralid or implementing an earlier deadline for spraying of clopyralid may reduce the risk of contaminating bee food products. However, if it is not possible to obtain a satisfactory effect of clopyralid on the weed flora under these conditions, spraying with pesticides containing clopyralid should be restricted in winter oilseed rape. Determination of an MRL value based on toxicological tests might result in a higher value and make it acceptable selling the honey containing higher levels of clopyralid.

1. Introduction

Pesticides are often applied to crops in conventional agriculture (e.g., against weeds, insects, and diseases) to keep a high production and quality of the harvested products. However, pesticide application constitutes a risk exposing non-target organisms to the chemicals. Agricultural crops are often the primary source for honey production, and a large amount of honey is harvested from fields of oilseed rape (*Brassica napus* L.). Pesticide residues have been detected in honey from several countries at varying levels, and sometimes the pesticide concentrations have exceeded the MRL (Souza Tette et al., 2016).

In the last decades, a concern about a global decline of pollinators has resulted in an increasing focus on the effect of pesticides on honeybees (*Apis mellifera* sp.) and wild bees (Faita et al., 2018; Lundin et al., 2015; Mulvey & Cresswell, 2020; Stanley et al., 2015). Also, pesticide residues in nectar and pollen in flowers (Bonmatin et al., 2015; Botias et al., 2015; Gierer et al., 2019), and in the pollen and honey collected by the bees have attracted more attention (El-Nahhal, 2020; Mitchell et al., 2017; Mullin et al., 2010; Raimets et al., 2020; Simon-Delso et al., 2017). In 2016, the Intergovernmental Science-Policy Platform on Ecosystem Services and Biodiversity (IPBES) (2016) reported that pesticide use, reduction in habitats, and changing in landscapes were the most significant factors responsible for the decline of pollinators.

Pesticide residues in honey and pollen in beehives originate from flowers (nectar and pollen) and other sources the honeybees collect (e.g., water) (Bonmatin et al., 2003; 2015). The concentration in honey from beehives can be both lower or higher than in nectar from flowers, and lower in pollen collected by bees compared to pollen collected directly from flowers (Bonmatin et al., 2015; Byrne et al., 2014; Schmuck et al., 2001). The reasons for these differences are not well understood, but a lower concentration could be caused by a dilution effect obtained in the beehives when the honeybees mix contaminated

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honey and pollen with uncontaminated honey and pollen, respectively (Bonmatin et al., 2015).

Clopyralid, which is a systemic herbicide and the active ingredient in several products (e.g., Galera and Matrigon 72SG manufactured by Corteva Agriscience, Denmark), was found in Danish honey samples collected from all over Denmark in 2016 (Landbrugsinfo.dk, 2017). Residues of clopyralid have also been found in honey in Estonia (Karise et al., 2017; Raimets et al., 2020) and Israel (Bonmuraj et al., 2019). According to the Danish Ministry of the Environment, 14.285 tons, 13.536 tons and 10.229 tons of the active ingredient clopyralid were sold in Denmark in 2013, 2014 and 2015, respectively. The maximum residue level for clopyralid in honey is set to 0.05 mg kg^{-1} (EU Pesticides Database), which was the original detection limit and considered harmless for humans. Now the detection limit is as low as 0.001 mg kg^{-1} (Crocoll, University of Copenhagen). However, it is not legal to sell honey with a content of 0.1 mg kg^{-1} clopyralid (Landbrugsinfo.dk, 2017).

Clopyralid was found in 81% of 55 Danish honey samples from 2016. For 60% of the samples the concentration of clopyralid exceeded 0.05 mg kg^{-1}, and for 31% of the samples the content was higher than 0.1 mg kg^{-1} resulting in prohibition of sale (Landbrugsinfo.dk, 2017). Data from 14 samples collected in the spring 2017 by the Danish Beekeepers Association showed that clopyralid was found in 93% of the samples, the concentration exceeded 0.05 mg kg^{-1} for 64% of the samples and the content was higher than 0.1 mg kg^{-1} for 50% of the samples (Rune Havgaard Sørensen, Danish Beekeepers Association, personal communication).

In 2017, winter and spring oilseed rape was grown on 176,754 ha and 786 ha in Denmark, respectively (Danmarks Statistik, 2017). Oilseed rape sprayed with clopyralid may constitute a serious economic problem for beekeepers as approximately 50% of honey comes from nectar collected from oilseed rape in Denmark (Kryger et al., 2011), and in 2017 several batches were refused due to the content of clopyralid (Rune Havgaard Sørensen, Danish Beekeepers Association, personal communication).

According to the European Food Safety Authority (EFSA), the routes of exposure of bees to pesticides have been categorized from 0 (no route of exposure) to four (very relevant route of exposure). Both nectar and pollen belong to category four (EFSA, 2012). Several pesticides have been identified in nectar and pollen from various plant species (Bonmatin et al., 2015; EFSA, 2012). Investigation of the occurrence of clopyralid in nectar and pollen in flowers of oilseed rape, and in honey and pollen collected by honeybees during the flowering period can be a foundation for an evaluation of the restrictions for clopyralid application decided by the Danish Environmental Protection Agency. Such investigation may contribute to explain why clopyralid sometimes ends up in the beehives polluting the honey.

Pesticide residues in nectar and pollen from flowers are usually detected in very low concentrations. Nectar is commonly collected with calibrated micropipettes from flowers with more than 0.5 μl nectar (Human et al., 2013), while nectar from flowers with lower amounts of nectar can be extracted by centrifugation from flowers collected in the field (Bertazzini & Forlani, 2016; Enkegaard et al., 2016). However, there is a risk of diluting the nectar because the centrifugation may result in more liquid than the bees are able to collect from the flowers. The content of nectar in the rapeseed flowers varies between varieties, time of the day, and the time after flowering has begun (Mohr & KJay, 1990; Pernal & Currie, 1998). For some rapeseed varieties, the content is less than 0.5 μl per flower (Bertazzini & Forlani, 2016; Enkegaard, Kryeger & Boldt, 2016; Pierre et al., 1999). Calibrated micro-pipettes have previously been used to collect rapeseed nectar with the purpose of detecting pesticides in nectar and is considered the most reliable method to avoid cross contamination (Botias et al., 2015; Mesquida et al., 1988).

The assigned deadline for spraying clopyralid in winter oilseed rape is when the first individual flowers are visible and still closed (BBCH scale: growth stage 55) (Canola Council of Canada, 2017).

This project aimed to investigate if clopyralid sprayed just before the legal spraying deadline can be detected in nectar and pollen in winter oilseed rape flowers and in honey and pollen from beehives placed next to the fields. We tested the following hypotheses: 1) nectar and pollen, collected from flowers of winter oilseed rape sprayed with clopyralid before flowering and no later than at growth stage 55 may contain clopyralid. 2) honey and pollen collected from beehives next to winter oilseed rape fields sprayed with clopyralid no later than stage 55 may contain clopyralid.

2. Materials and methods

2.1. Field experiments at University of Copenhagen

Two independent field experiments (experiment 1 and 2) with winter oilseed rape (cultivar: Butterfly) were conducted at the University of Copenhagen (UCPH), Hoejbakkegaard (55°38′ N, 12°17′ E), Taastrup, Denmark in an area of 100 m × 48 m. The experiments were sown in August 2018 and August 2019, respectively. In each field, eight plots of 25 m × 12 m were marked. Four of the plots were sprayed and four plots were left untreated. Sprayed and untreated (control) plots were placed randomly and separated with plots of 25 m × 12 m to avoid pesticide drift from sprayed plots to the control plots. After spraying, oilseed rape was removed with a combine harvester around each plot and around the experimental area creating 3 m wide trails. Oilseed rape plants were also removed in the middle of each experimental plot, creating a 1.6 m wide path along the length of the plots to create easy access to collect nectar and pollen in the plots (Fig. 1). Samples were not taken from the outer 2 m of the plots.

2.1.1. Application of clopyralid in the field

The plots were sprayed once with 0.11 kg ha^{-1} matrigon 72SG (water soluble granules) containing 720 g kg^{-1} clopyralid (Corteva Agriscience, Copenhagen, Denmark) mixed in 200 l ha^{-1} water with a pressure of 1 bar using Lechler IDKT 120-03 POM nozzles (Lechler GmbH, Metzingen, Germany). In 2019, the oilseed rape field was sprayed at BBCH growth stage 53 (flower buds raised above the youngest leaves) to 55 (individual flower buds (main inflorescence) visible

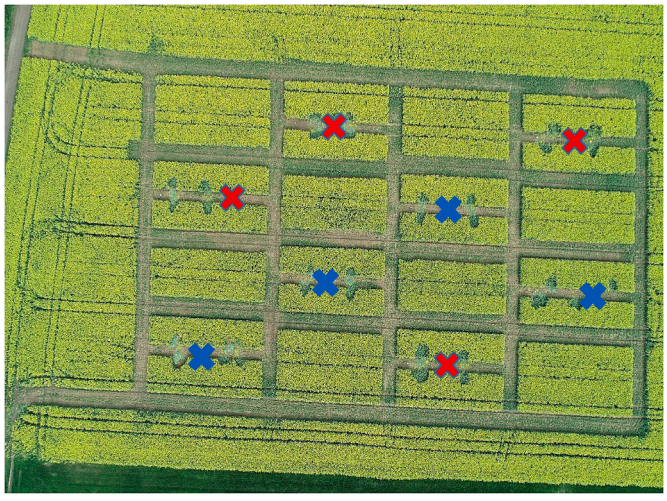

Fig. 1. Drone photo of the winter oilseed rape field at the field station in Taastrup, University of Copenhagen in 2019 showing the sprayed (red **x**) and unsprayed plots (blue **x**) from where the pollen and nectar samples were collected. A path was made in the middle of the plots to allow for access for sampling. The traces close to the red and blue crosses in the fields are areas where the plants have been cut and used for pollen collection (Image: Morten Dürr Resen, University of Copenhagen).

but closed) April 5 at 10 am (Canola Council of Canada, 2017; Lancashire et al., 1991). In 2020, the oilseed rape field was sprayed at growth stage 53–55, March 20, 9 am.

2.1.2. Collection of samples from the fields at University of Copenhagen

In each of the eight plots, four samples of 50 μl nectar and four samples of 200 mg pollen were collected in 2019 and 2020. The samples were collected from plants in BBCH growth stage 63 (30% of flowers on main raceme were open) to 65 (full flowering: 50% flowers on the main raceme open, older petals falling). There was no rainfall during the collection periods and it was relatively windless. In 2019, the collection took place between 25 April and 2 May, and in 2020, between 17 and 22 April.

The nectar was collected from one random flower on the main stem of each plant from flowers that started blooming the same morning. The nectar was sucked from the flowers using 5 or 10 μl micro pipettes (Fig. 2). The flower of oilseed rape has six stamens, four long and two short ones. At the bottom of the flower, there are four nectary's, of which two produce large nectar droplets and the others produce small droplets. The large droplets were collected with the micro-pipette and transferred to 50 μl tubes. Approximately 50 μl nectar per sample were required for chemical analysis. The content of nectar varied a lot between the flowers. In 2019, nectar from 108 to 923 flowers was collected to obtain 50 μl, and 109–851 flowers were needed in 2020. In both years, some flowers did not contain any collectable nectar. Plants needed to dry after the night to avoid collecting too thin liquid nectar, which often is the case in the morning. Therefore, nectar collection was started between 9:30 a.m. and 11:00 a.m., when the dew had evaporated, and continued until approximately 50 μl was collected from each plot, which usually was achieved before or around 3:45 p.m.

The collection of pollen took place in the field after the flowers had dried in the morning to avoid that pollen was mixed with water and nectar. Pollen was collected from all flowers in a raceme. The raceme was cut from the stalk and gently beaten against a sieve with a mesh size of 0.50 mm. Then the sieved pollen was sieved one more time (mesh size of 0.25 mm). Eventual impurities were removed before the pollen was frozen to −18 °C. In 2019, 43–151 plants were used for each sample and in 2020, 80–200 plants were used except for one sample, where 300 plants were used. Each sample contained more than 200 mg pollen.

2.2. Samples from farmer's winter oilseed rape fields

In 2019 and 2020, ten commercial fields of winter oilseed rape distributed across the country were included in the investigation. Table 1 shows the spraying dates and stage of development of the winter oilseed rape at the spraying dates.

A beehive with a honeybee colony was placed next to each field. A honey and a pollen sample from each beehive were taken in 2019 and 2020. In each beehive, a new frame with wax foundation was placed just above the queen excluder. Honey was only harvested from this frame. The honey was taken just when the first torn flower (*Crataegus* sp.) appeared in the landscape no matter whether the frame was sealed or not, because until that time it would be most likely that the honey was only collected from the oilseed rape field. The honey was scraped into two honey glasses. Remains of wax and eventual impurities floating on the honey were removed. Individual samples weighed at least 500 g in 2019. In 2020, a few samples weighed less than 500 g, but it was more than sufficient for the chemical analyses.

A pollen trap was placed in front of the entrance of each of the ten beehives each year. Pollen was collected from the traps the day after the traps were mounted during the flowering period of the oilseed rape. The samples contained pollen from two or three days.

Table 1
Clopyralid spraying dates in the conventional winter oilseed rape fields, and the plants stage of development at the time of spraying according to the BBCH scale (Lancashire et al., 1991) in 2019 and 2020, respectively. Stage 50: Flower buds present, still enclosed by leaves. Stage 55: individual flower buds (main inflorescence) visible but closed.

2019			2020		
Field number	Date of spraying	Stage of development	Field number	Date of spraying	Stage of development
1	3 April	52	11	16 March	53–55
2	7 April	52	12	26 March	55
3	3 April	53–54	13	26 March	55
4	[a]	55	14	5 April	55
5	28 March	55	15	27 March	55
6	6 April	52–55	16	6 April	52–55
7	30 Mach	55	17	[a]	55
8	4 April	51	18	26 March	50
9	28 March	53–55	19	10 April	55
10	3 April	54	20	4 April	55

[a] The farmer did not note the date.

2.3. Collection of bees with pollen from oilseed rape fields

Ten honeybees were caught while they were collecting pollen from oilseed rape plants in 2019. The pollen was used as a reference for identification of pollen from oilseed rape because honeybees only collect pollen from one plant species at each trip of foraging (Grüter et al., 2011). The pollen was taken from the bees and stored at −18 °C. The pollen was later studied visually in natural size and under the microscope and compared with the pollen collected from the commercial fields to separate pollen from other plant species. From each of the commercial fields, four randomly chosen lumps of pollen were studied microscopically and compared with the controls. Pollen lumps deviating slightly in color from the controls were discarded.

A container with glycerin jelly with fuchsin (GJF) (Beeequipment.glopalstore.com) was heated in a microwave oven in 10 s to make it liquid. A droplet of water was placed on a glass slide and a small piece of pollen from a pollen lump was placed in the droplet, which was stirred to separate the pollen grains from each other and distribute the grains in the droplet. The glass slide was placed on a heating plate (50 °C) until the water had evaporated. A cover glass was placed on the heating plate and a droplet of GJF was placed on each dried pollen sample. Afterward, the warm cover glass was placed on the pollen sample. The glass slide was placed on the heating plate for 10 min and thereafter moved to a flat surface for 2 h. Finally, the cover glass was sealed with transparent nail polish, stored and used for identification of pollen. Based on the lumps of verified pollen from oilseed rape, random samples were taken for chemical analyses.

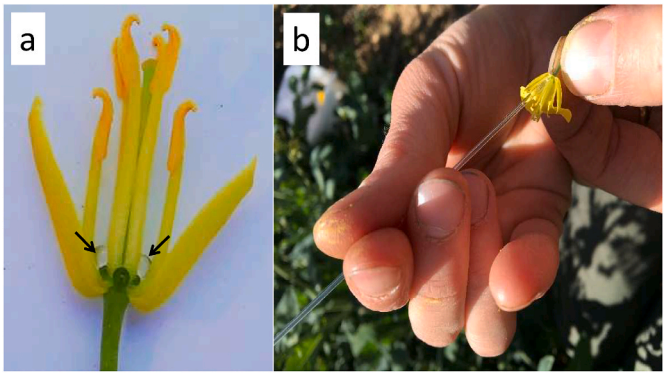

Fig. 2. a) The two large droplets of nectar at the bottom of the flower at the two short stamens are indicated by arrows (image: Lise Hansted). **b)** Sampling of nectar droplets by micro capillaries (image: Marie-Louise Olsen).

2.4. Chemical analyses at University of Copenhagen

Due to the small volume of the samples and the expected low concentration of clopyralid, the analyses were performed by LCMS-QqQ (Agilent UHPLC 1290 Infinity II and MS 6465). Separation was achieved on a C18 column (Zorbax Eclipse Plus C18, 50 × 2.1 mm, 1.8 μm) which polarity is well suitable to polar chemical compounds as clopyralid. The gradient was optimized to achieve the best possible separation from other chemicals. MilliQ water with 0.05% formic acid and acetonitril with 0.05% formic acid were used as solvents A and B, respectively. The gradient was 0–0.2 min 5% B, 0.2–1.9 min 5−40% B, 1.9–2.0 min 40−100% B, 2.0–2.8 min 100% B, 2.8–2.85 min 100−5% B and 2.85–4.0 min 5% B. Column temperature was 40 °C. The analysis on the mass spectrometer was done in multi reaction monitoring (MRM) mode to increase sensitivity. MRM parameters were optimized to obtain increased sensitivity and precise quantification of clopyralid. Analysis was run in combined positive and negative mode as clopyralid can be detected in both modes: positive mode precursor ion *m/z* 192.0 → product ions *m/z* 146.0 (Collision energy, CE = 20 V), 173.8 (CE = 8 V), 109.9 (CE = 40 V) and 74.9 (CE = 72 V); negative mode precursor ion *m/z* 189.9 → product ions *m/z* 146.0 (CE = 4 V) and 34.9 (CE = 12 V). Product ion 146.0 was used as quantifier ion in both modes. Further parameters for ionization were as follows: fragmentor voltage 48 V, dwell time 74.47 ms, gas temperature 325 °C, gas flow 10 L min^{-1}, nebulizer 30 psi, sheath gas temperature 250 °C or sheath gas flow 12 L min^{-1}. Capillary voltage was 4500 V in positive mode and 2000 V in negative mode. In positive mode the nozzle voltage was set to 1000 V and 0 V in negative mode. Detection limit (LLOD) was measured as 0.1 ng ml^{-1}, while the quantification limit (LLOQ) was 1 ng ml^{-1}. Linearity was confirmed from 0.2 ng ml^{-1} to 2 μg ml^{-1}. Quantification was done by an external standard solution series ranging from 0.01 ng ml^{-1} to 10 μg ml^{-1} clopyralid. All samples were analysed in triplicates. All pollen and honey samples were diluted 5 times with MilliQ-grade water before analysis to avoid matrix effects. Nectar samples were diluted 10 times with MilliQ-grade water prior to analysis.

The nectar samples were prepared by mixing 5 μl nectar with 45 μl 20% methanol directly in analytical vials. Pollen and honey were extracted in 85% methanol. Approximately 100 mg honey and 100 mg pollen were extracted in 1 ml 85% methanol, while approximately 30 mg pollen from beehives were extracted with 350 μl 85% methanol. All samples were vortexed and incubated at 20 °C for 1.5 h. Afterward, samples were vortexed again and centrifuged for 3 min at 20,000×*g*. The supernatant was transferred to new tubes and frozen at −18 °C until analysis. Prior to MS analysis, the supernatant was thawed and diluted 5 times with MilliQ-grade water.

Corresponding analyses for honey were done by Quality Services International GmbH, Bremen, Germany (QSI).

2.5. Estimation of clopyralid content in honey based on nectar from fields at University of Copenhagen

The analysis results for clopyralid content in nectar are given in μg L^{-1}. When converting to mg kg^{-1}, the sugar content of the nectar must be considered, as it affects the density.

Once the bees have brought the nectar to the beehive, they process it into honey, and as part of the process, they evaporate it to a water content of 17–20% to avoid fermenting (Crane, 1980). The water content corresponded to the average value for water content found by Ohe and Ohe (1998) (16.7%) for 399 oilseed rape honeys. According to the EU Honey Directive (Council of the European Union, 2002), honey, with a few exceptions, must contain a maximum of 20% water. The rest is mainly different sugars, meaning that honey contains around 80% sugar (Crane, 1980).

Due to the small sample size, it was not possible to measure the sugar content in the nectar. As the sugar concentration in nectar varies, we calculated the potential content of clopyralid in a honey the bees could have produced from the nectar samples if the sugar concentration in the samples had been 30, 50 or 80%. For 80%, the measured data was used as it corresponds to the sugar content in honey according to the EU Honey Directive (Council of the European Union, 2002).

In Table 3, the clopyralid content of the collected nectar is converted to the content in the honey the bees could have produced from the nectar if there had been 30%, 50% or 80% sugar in the nectar.

Example: For plot 2, one of the samples had a clopyralid content of 80.760 μg l^{-1} in 2019. If the nectar contained 50% sugar, the conversion factor according to Vinolab (2021) is 1.189 meaning that there is 80.760 × 1.189/1000 mg kg^{-1} = 0.096 mg clopyralid kg^{-1} nectar with 50% sugar. If the nectar is evaporated into a honey with 80% sugar this corresponds to (0.096 mg clopyralid kg^{-1})/(50% × 80%) = 1.54 mg clopyralid kg^{-1}–1.5 mg clopyralid kg^{-1}

2.6. Statistical analyses

A paired *t*-test was used to analyze differences between the content of clopyralid in honey samples analysed by KU and QSI. An unpaired *t*-test was used to analyze differences between the content of clopyralid in pollen samples collected by bees and directly from flowers. Both were done in Excel.

3. Results and discussion

3.1. Percentage of oilseed rape pollen in honey samples from conventional fields

There were 35–96% and 87–98% pollen from oilseed rape in the honey samples from farmers' fields in 2019 and 2020, respectively (Supplementary file 1) showing that the bees had visited the oilseed rape fields in varying degree in 2019, while the bees mainly visited the oilseed rape fields in 2020. Hence, the bees had primarily collected honey from oilseed rape in 2020.

3.2. The concentration of clopyralid in nectar and honey samples

Table 2 shows the concentration of clopyralid in honey samples collected from beehives next to conventional fields. Table 3 shows the amount of clopyralid in honey, the bees could have produced from the nectar collected from flowers at the experimental field at UCPH. Rapeseed nectar contains 26–84% sugar (Knopper et al., 2016; Westcott & Nelson, 2001; Enkegaard et al., 2016; Hansted & Jørgensen, 2019), and bees appear to prefer nectar with a sugar content of 50–65% (Kim, Gilt & Bush, 2011; Knopper et al., 2016). Therefore, we have converted the

Table 2
Clopyralid content in honey samples collected from beehives next to conventional winter oilseed rape fields sprayed with clopyralid. SD = standard deviation.

	2019		2020	
	Analysed at UCPH[a]	Analysed at QSI[b]	Analysed at UCPH	Analysed at QSI
Number of samples (N)	10	10	10	10
N > 0.05 (mg kg-1)	9	10	10	8
N > 0.1 (mg kg-1)	4	4	7	4
Interval (mg kg-1)	0.05–0.27	0.05–0.32	0.07–6.83	0.04–4.20
Mean ± SD (mg kg-1)	0.11 ± 0.07	0.11 ± 0.08	0.80 ± 2.12	0.51 ± 1.30
Median (mg kg-1)	0.09	0.09	0.12	0.09

[a] University of Copenhagen.
[b] Quality Services International GmbH, Bremen, Germany.

Table 3
Clopyralid content in nectar samples collected from the experimental field at University of Copenhagen. The amount of clopyralid in nectar was converted to the relevant amount in honey the bees can produce from the nectar depending on the sugar percentage. SD = standard deviation.

Year		Control	Sprayed with clopyralid		
			Honey with 30% sugar in nectar	Honey with 50% sugar in nectar	Honey with 80% sugar in nectar
2019	Number of samples (N)	16	16	16	16
	N > 0.05 (mg kg^{-1})	0	16	16	15
	N > 0.1 (mg kg^{-1})	0	15	12	6
	Interval (mg kg^{-1})	0.00–0.00	0.08–0.42	0.05–0.27	0.04–0.18
	Mean ± SD (mg kg^{-1})	0 ± 0	0.22 ± 0.09	0.14 ± 0.06	0.09 ± 0.04
	Median (mg kg^{-1})	0	0.19	0.12	0.08
2020	Number of samples (N)	16	16	16	16
	N > 0.05 (mg kg^{-1})	0	16	16	16
	N > 0.1 (mg kg^{-1})	0	16	16	16
	Interval (mg kg^{-1})	0.00–0.00	0.26–0.63	0.16–0.40	0.11–0.27
	Mean ± SD (mg kg^{-1})	0 ± 0	0.42 ± 0.10	0.27 ± 0.06	0.18 ± 0.04
	Median (mg kg^{-1})	0	0.39	0.25	0.17

content to 30% and 50% sugar in the nectar. No conversion was done for 80% sugar in the nectar. Nectar with 80% sugar would have been too thick to collect with micro-pipettes, whereas 50% sugar is slightly less than the average sugar content found by Hansted and Jørgensen (2019) in Denmark using the same method.

There were only minor differences between the measured content of clopyralid in honey done by the two institutions (UCPH and QSI), indicating that the analysis method was credible. According to both laboratories, 10 out of 10 samples from farmers' fields had a content of clopyralid exceeding or equal to 0.05 mg kg^{-1} in 2019, and 4 (UCPH) or 5 (QSI) had a content exceeding or equal to 0.1 mg kg^{-1}.

In the samples from fields 2 and 9, the amount of clopyralid in the honey was less than 0.1 mg kg^{-1} in 2019, but the proportion of rapeseed pollen was low (35% and 77%, respectively). Pollen from different plants occurs in varying amounts in the honey. The structure of the flower affects how much pollen the bees bring home to the hive when they collect nectar. Oilseed rape pollen is generally slightly over-represented (Ohe & Ohe, 2000). When the pollen content in honey is estimated, one will find a larger proportion of the rapeseed pollen than the actual proportion of honey collected from oilseed rape. For the two fields mentioned, the amount of honey collected from oilseed rape was lower than 35% and 77%, respectively. If the bees had collected the honey exclusively from oilseed rape, we would expect that the proportion of clopyralid would have been at least 0.1 mg kg^{-1} for the honey with 35% oilseed rape pollen and perhaps also for the one with 77%. In 2020, one of the honey samples had an extremely high content of clopyralid (UCPH: 6.83 mg kg^{-1}; QSI: 4.2 mg kg^{-1}). If we exclude this outlier, 9 (UCPH) or 7 (QSI) samples had a content of clopyralid exceeding or equal to 0.05 mg kg^{-1}, and 6 (UCPH) or 3 (QSI) samples had a content exceeding or equal to 0.1 mg kg^{-1}. Raimets et al. (2020) found clopyralid in 27.3% of the honey samples collected from 23 commercial apiary sites located in southeastern Estonia. The median concentration for samples exceeding the detection limit was 0.03 mg, and the maximum concentration over all samples was 0.27 mg kg^{-1}. Bommuraj et al. (2019) examined the content of clopyralid and other chemicals in honey and beeswax samples collected from apiaries in Israel. They found an average range of 8.6 μg kg^{-1} clopyralid (SD = ± 9.2) in honey samples. All of the honey samples analysed complied with the Israeli and European MRL's. They detected ten different pesticides/pesticide metabolites in the honey samples.

The corresponding numbers for the 16 nectar samples from the field at UCPH in 2019 were 15 and 6. If the values for nectar are converted to honey with a water content of 20%, based on presumed sugar content in the nectar of either 30% or 50%, then 16 samples had a content of clopyralid exceeding or equal to 0.05 mg kg^{-1} and 15 and 12 samples, respectively, had a content exceeding or equal to 0.1 mg kg^{-1}. In 2020, all similar samples had a content of clopyralid exceeding or equal to 0.1 mg kg^{-1}. All mean values were larger than the medians, indicating that one or more large values significantly affected the means. We did not find any content of clopyralid in nectar samples from control plots.

3.3. The concentration of clopyralid in pollen

All pollen samples collected from the farmers' sprayed fields (Table 4) and the university plots (Table 5) had a content of clopyralid exceeding or equal to 0.1 mg kg^{-1}. We found no clopyralid in pollen samples from control plots. All mean values were larger than the medians for all pollen samples. In both years, the content of clopyralid was significantly larger in pollen manually collected from flowers in Taastrup than in pollen collected by the bees from farmers' fields (t critical two-tail = 2.10; df 21; $p < 0.05$). Raimets et al. (2020) found clopyralid in 3.4% of the pollen samples collected from 23 commercial apiary sites. The median concentration for samples exceeding the detection limit was 0.056 mg kg^{-1}, and the maximum concentration over all samples was 0.056 mg kg^{-1}.

3.4. Consequences of a high clopyralid content

Residuals of clopyralid were found in all the samples of nectar, pollen and honey from the sprayed experimental plots and beehives next to farmers' fields. In contrast, no residuals of clopyralid were found in unsprayed control plot samples. In a large proportion of the samples, the content exceeded 0.05 mg kg^{-1} and 0.1 mg kg^{-1}. If the Danish national authorities measure the content of clopyralid in honey exceeding 0.05 mg kg^{-1}, samples are toxicologically evaluated at the National Food Institute, at the Technical University of Denmark (DTU). If the pesticide content in the honey constitutes a human health risk, the honey must be revoked from the market. If the National Food Institute does not consider the content to constitute a health risk, the honey should only be revoked if the residual concentration of clopyralid exceeds 0.1 mg kg^{-1}. According to the EU's guidance document about analytic control (SANTE/12682/2019) the confidence interval for pesticide analysis is, in general, ± 50%, which means, in practice, that the residual concentration of clopyralid in honey below 0.1 mg kg^{-1} will not be considered as a significant exceedance of MRL. In case of a considerable exceedance of MRL, the product cannot be marketed. For *Apis mellifera* the LD50 value for oral uptake >100 μg bee^{-1} for 48 h (Hartley and Kidd, 1978).

Table 4
Content of clopyralid measured in pollen samples from beehives next to the conventional winter oilseed rape fields sprayed with clopyralid. SD = standard deviation.

	2019	2020
Number of samples (N)	10	10
N ≥ 0.05 (mg kg^{-1})	10	10
N ≥ 0.1 (mg kg^{-1})	10	10
Interval (mg kg^{-1})	0.51–1.63	0.33–1.03
Mean ± SD (mg kg^{-1})	0.94 ± 0.41	0.70 ± 0.23
Median (mg kg^{-1})	0.83	0.62

Table 5
Content of clopyralid measured in pollen samples from the experimental field at the UCPH. SD = standard deviation.

	2019		2020	
	Control	Sprayed with clopyralid	Control	Sprayed with clopyralid
Number of samples	16	16	16	16
N ≥ 0.05 (mg kg^{-1})	0	16	0	16
N ≥ 0.1 (mg kg^{-1})	0	16	0	16
Interval (mg kg^{-1})	0.00–0.00	1.85–4.19	0.00–0.00	8.68–14.78
Mean ± SD (mg kg^{-1})	0 ± 0	3.21 ± 0.63	0 ± 0	11.16 ± 1.62
Median (mg kg^{-}1)	0	3.19	0	11.27

The concentrations in the honey samples are unlikely to cause any acute toxicity to honeybees.

3.5. Effect of application time and dose

The uptake of pesticides in nectar and pollen depends on the dose and timing of the pesticide application (Gierer et al., 2019). The smaller dosage the less pesticide will occur in the honey and nectar. The longer time between spraying and crop flowering, the less pesticide can be expected in the honey and nectar. The effect of application time, dosages, and the growth stage of winter oilseed rape at spraying on the residual clopyralid content in honey and pollen has not been investigated. Therefore, there is a need to study how lower dosages and earlier spraying will affect the weed control and the content of residual concentration in nectar and honey to ensure that it does not exceed the MRL. It may not be possible spraying the winter oilseed rape fields earlier than when the first individual flowers become visible but closed (BBCH 55). At that time, the temperature would rarely be high enough to get a good effect on the weed flora.

Another way to reduce the residual concentration of clopyralid in honey and pollen could be to spray only a part of the field, for example, by using site-specific weed management (Asaduzzaman et al., 2020). If the bees collect nectar and pollen from the whole field, site-specific spraying may reduce the content of clopyralid in honey and pollen because of the dilution effect. It is impossible to control in which part of the field the bees collect nectar and pollen, and we cannot exclude a scenario where the bees mainly collect pollen and nectar in the sprayed part of the field. Therefore, site-specific weed management, in some cases, may not reduce the residual concentrations of clopyralid in honey and nectar.

Increasing MRL to 3 mg kg^{-1}, approved for cabbage and cauliflower (EU Pesticides Database), would make most honey salable. The content in pollen from the sprayed plots in UCPH was significantly higher than 3 mg kg^{-1} in 56% and 100% of the pollen samples in 2019 and 2020, respectively. No MRL for pollen has been specified and listed in the EU Pesticides Database for apiculture products (Code number 1040000).

Determination of an MRL based on toxicological residual concentration may result in a considerable higher value and could legalize selling honey with higher clopyralid content. Consequently, some consumers may stop buying honey and pollen because they want natural products without pesticide residuals. Another consequence of a reduced sale could be that beekeepers terminate their business resulting in lacking bee families to pollinate the crops. In 2020, an increasing number of professional beekeepers, who are mainly responsible for the bee pollination of crops, have left their provisions because of increasing difficulties in selling honey, resulting in an abundant storage of honey produced in 2018–2020. At the same time, the wholesale prices for honey have declined from 30 to 36 DKK kg^{-1} (4.04–4.84 Euro kg^{-1}) in 2017/2018 to 15–18 DKK kg^{-1} (2.02 − 2.42 Euro kg^{-1}) in 2019/2020 samples (Dahlager, 2020a; Anon., 2021; Rune Havgaard Sørensen, Danish Beekeepers Association, personal communication). If the honey sale is further aggravating due to an accepted higher MRL for clopyralid in honey, it may have significant consequences for the pollination of crops (Dahlager, 2020b).

Reducing the acceptable applicable dose of clopyralid or implementing an earlier deadline for clopyralid spraying in winter oilseed rape could be ways to reduce the risk of contaminating bee food products. Suppose it is not possible to obtain a satisfactory effect of clopyralid on the weed flora under these conditions, then we suggest banning spraying with pesticides containing clopyralid in winter oilseed rape.

4. Conclusion

This work shows that clopyralid in honey can originate from nectar from oilseed rape fields. Clopyralid was found in nectar and pollen from the experimental fields, and in honey and pollen from beehives next to farmer's fields. The content exceeded the MRL in nectar and honey in varying levels between the years. The concentrations found may not pose any health risk for consumers, as the MRL is based on the original detection limit and not on toxicological tests. The concentrations found in honey are unlikely to cause any acute toxicity to honeybees. However, it can have a significant economically consequence for the beekeepers, who cannot sell the honey legally if the concentration of clopyralid exceeds 0.1 mg kg^{-1}. Reducing the acceptable applicable dose of clopyralid or implementing an earlier deadline for clopyralid spraying in winter oilseed rape may be ways to reduce the risk of contaminating bee food products but need to be studied. However, we propose to ban the use of pesticides containing clopyralid in winter oilseed rape if it is impossible to obtain a satisfactory weed control under such new restrictions. Determination of an MRL based on toxicological residual concentration might result in a considerable higher value and could legalize selling honey containing higher levels of clopyralid, but a consequence may be that many consumers no longer want to buy the honey and pollen.

Availability of data and material

Data was not shared.

CRediT authorship contribution statement

Lise Hansted: Conceptualization, data curation, formal analysis, funding acquisition, investigation, experimental design and methodology, supervision, validation, visualization, writing - original draft, review and editing. **Christoph Crocoll:** Data curation, formal analysis, methodology, validation, writing a part of the original draft, review and editing. **Zahra Bitarafan:** investigation, writing – review and editing. **Christian Andreasen:** Conceptualization, funding acquisition, experimental design and methodology, project administration, writing - original draft, review and editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. The funders had no role in the design of the study, in the collection, analysis, or interpretation of data, in the writing of the manuscript, or in the decision to publish the results.

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Appendix A. Supplementary data

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References

Anon. (2021). *EU beekeeping sector, national apiculture programmes 2020–2022. 2021*. Retrieved from https://ec.europa.eu/info/sites/default/files/food-farming-fisheries/animals_and_animal_products/documents/honey-apiculture-programmes-overview-2020-2022pdf.

Asaduzzaman, M. D., Pratley, J. E., Luckett, D., Lemerle, D., & Wu, H. (2020). Weed management in canola (*Brassica napus* L.): A review of current constraints and future strategies for Australia. *Archives of Agronomy and Soil Science, 66*, 427–444. https://doi.org/10.1080/03650340.2019.1624726

Bertazzini, M., & Forlani, G. (2016). Intraspecific variability of floral nectar volume and composition in rapeseed (*Brassica napus* L. var. *oleifera*). *Frontiers of Plant Science, 7*, 288. https://doi.org/10.3389/fpls.2016.00288.

Bonmatin, J. M., Giorio, C., Girolami, V., Goulson, D., Kreutzweiser, D. P., Krupke, C., Liess, M., Long, E., Marzaro, M., Mitchell, E. A., & Noome, D. A. (2015). Environmental fate and exposure; neonicotinoids and fipronil. *Environmental Pollution Research, 22*, 35–67. https://doi.org/10.1007/s11356-014-3332-7

Bonmatin, J. M., Moineau, I., Charvet, R., Fleche, C., Colin, M. E., & Bengsch, E. R. (2003). A LC/APCI MS/MS method for analysis of imidacloprid in soils, in plants, and in pollens. *Annals of Chemistry, 75*, 2027–2033. https://doi.org/10.1021/ac020600b

Bonmuraj, V., Chen, Y., Klein, H., Sperling, R., Barel, S., & Shimshoni, J. A. (2019). Pesticide and trace element residues in honey and beeswax combs from Israel in association with human risk assessment and honey adulteration. *Food Chemistry, 299*. https://doi.org/10.1016/j.foodchem.2019.125123. article 125123.

Botías, B., David, A., Horwood, J., Abdul-Sada, A., Nicholls, E., & Goulson, D. (2015). Neonicotinoid residues in wildflowers, a potential route of chronic exposure for bees. *Environmental Science and Technology, 49*, 12731–12740. https://doi.org/10.1021/acs.est.5b03459

Byrne, F. J., Visscher, P. K., Leimkuehler, B., Fischer, D., Grafton-Cardwell, E. E., & Morse, J. G. (2014). Determination of exposure levels of honeybees foraging on flowers of mature citrus trees previously treated with imidacloprid. *Pest Management Science, 70*, 470–482. https://doi.org/10.1002/ps.3596

Canola Council of Canada. (2017). *Growths stages of the canola plant*. Retrieved from https://www.canolacouncil.org/canola-encyclopedia/growth-stages/. (Accessed 1 March 2022).

Council of the European Union. (2002). *COUNCIL DIRECTIVE 2001/110/EC of 20 December 2001 relating to honey*. Retrieved from https://www.fsai.ie/uploadedfiles/Dir%202001.110%20EC.pdf.

Crane, E. (1980). *A book of honey* (p. 193). UK: Oxford University press.

Dahlager, L. (2020a). *Biavlere trækker stikket på stribe* (pp. 4–6). Lørdagsliv: Politiken.

Dahlager, L. (2020b). *Land, mænd: Stort problem, hvis bierne mangler* (Vol. 6). Lørdagsliv: Politiken.

Danmarks Statistik. (2017). http://www.statistikbanken.dk/PEST1. (Accessed November 2017).

EFSA. (2012). EFSA panel on plant protection products and their residues (PPR); scientific opinion on the science behind the development of a risk assessment of plant protection products on bees (*Apis mellifera, Bombus* spp. and solitary bees). *EFSA Journal, 10*(2668), 275. https://doi.org/10.2903/j.efsa.2012.2668. Available online: www.efsa.europa.eu/efsajournal.

El-Nahhal, Y. (2020). *Pesticide residues in honey and their potential reproductive toxicity*. Science of the Total Environment, 139953.

Enkegaard, A., Kryger, P., & Boelt, B. (2016). Determinants of nectar production in oilseed rape. *Journal of Apicultural Research, 1*, 89–99. https://doi.org/10.1080/00218839.2016.1192341

EU Pesticides Database. (2021). Retrieved from https://ec.europa.eu/food/plant/pesticides/eu-pesticides-database/products/?event=search.pr. (Accessed 7 June 2021).

Faita, M. R., de Medeiros, E. O., Júnior, V. V. A., Orth, A. I., & Nodari, R. O. (2018). Changes in hypopharyngeal glands of nurse bees (*Apis mellifera*) induced by pollen-containing sublethal doses of the herbicide Roundup. *Chemosphere, 211*, 566–572. https://doi.org/10.1016/j.chemosphere.2018.07.189

Gierer, F., Vaughan, S., Slater, M., Thompson, H. M., Elmore, J. S., & Girling, R. D. (2019). A review of the factors that influence pesticide residues in pollen and nectar: Future research requirements for optimising the estimation of pollinator exposure. *Environmental Pollution, 249*, 236–247. https://doi.org/10.1016/j.envpol.2019.03.025

Grüter, C., Moore, H., Firmin, N., Helanterä, H., & Ratnieks, F. L. (2011). Flower constancy in honey bee workers (*Apis mellifera*) depends on ecologically realistic rewards. *Journal of Experimental Biology, 214*, 1397–1402.

Hansted, L., & Jørgensen, A. S. (2019). Vinterraps – en attraktiv trækkilde for bier? *Tidsskrift for Biavl, 5*.

Hartley, D., & Kidd, H. (Eds.). (1987). *The agrochemicals handbook* (2nd ed.). Lechworth, Herts, England: The Royal Society of Chemistry. A433/AUG 87.

Human, H., Brodschneider, R., Dietemann, V., Dively, G., Ellis, J. D., Forsgren, E., Fries, I., Hatjina, F., Hu, F. L., Jaffé, R., & Jensen, A. B. (2013). Miscellaneous standard methods for *Apis mellifera* research. *Journal of Apicultural Research, 1*, 1–53. https://doi.org/10.3896/IBRA.1.52.4.10

IPBES. (2016). The assessment report of the intergovernmental science-policy Platform on biodiversity and Ecosystem services on pollinators, pollination and food production. In S. G. Potts, V. L. Imperatriz-Fonseca, & H. T. Ngo (Eds.), *Secretariat of the intergovernmental science-policy Platform on biodiversity and Ecosystem Services, Bonn, Germany* (p. 552). Retrieved from https://ipbes.net/assessment-reports/pollinators.

at al. Karise, R., Raimets, R., Bartkevics, V., Pugajeva, I., & Pihlik, P. (2017). Are pesticide residues in honey related to oilseed rape treatments? *Chemosphere, 188*, 389–396. https://doi.org/10.1016/j.chemosphere.2017.09.013

Kim, W., Gilet, T., & Bush, J. W. (2011). Optimal concentrations in nectar feeding. *Proceedings of the National Academy of Sciences, 108*, 16618–16621. https://doi.org/10.1073/pnas.1108642108

Knopper, L. D., Dan, T., Reisig, D., Johnson, J. D., & Bowers, L. M (2016). Sugar concentration in nectar: A quantitative metric of crop attractiveness for refined pollinator risk assessments. *Pest Management Science, 72*, 1807–1812. https://doi.org/10.1002/ps.4321

Kryger, P., Enkegaard, A., Strandberg, B., & Axelsen, J. A. (2011). *Bier og blomster – honningbiens fødegrundlag i Danmark. DJF Rapport Markbrug 150 – Maj 2011* (p. 65). Denmark: Aarhus University. https://dcapub.au.dk/djfpublikation/djfpdf/djf_rapport_markbrug_150_55645_internet.pdf.

Lancashire, P. D., Bleiholder, H., Langelüddecke, P., Stauss, R., Van Den Boom, T., Weber, E., et al. (1991). An uniform decimal code for growth stages of crops and weeds. *Annals of Applied Biology, 119*, 561–601. https://doi.org/10.1111/j.1744-7348.1991.tb04895.x

Landbrugsinfo, dk (2017). Retrieved from https://www.landbrugsinfo.dk/basis/1/3/2/pl_17_2439_matrigon_ikke_anvendes_i_blomstrende_vinterraps. (Accessed 17 June 2021).

Lundin, O., Rundlöf, M., Smith, H. G., Fries, I., & Bommarco, R. (2015). Neonicotinoid insecticides and their impacts on nees: A systematic review of research approaches and identification of knowledge gaps. *PLoS One, 10*, Article e0136928. https://doi.org/10.1371/journal.pone.0136928

Mesquida, J., Marilleau, R., Pham-Delègue, M. H., & Renard, M. (1988). A study of rapeseed (*Brassica napus* L. var. *oleifera* Metzger) flower nectar secretions. *Apidologie, 19*, 307–318.

Mitchell, E. A., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G., & Aebi, A. (2017). A worldwide survey of neonicotinoids in honey. *Science, 6*, 6359. https://doi.org/10.1126/science.aan3684, 109–11.

Mohr, N. A., & KJay, S. C. (1990). Nectar production of selected cultivars of *Brassica campestris* L. and *Brassica napus* L. *Journal of Apicultural Research, 29*, 95–100. https://doi.org/10.1080/00218839.1990.11101203

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., & Pettis, J. S. (2010). High levels of miticides and agrochemicals in North American apiaries: Implications for honeybee health. *PLoS One, 5*. https://doi.org/10.1371/journal.pone.0009754. article 9754.

Mulvey, J., & Cresswell, J. E. (2020). Time-dependent effects on bumblebees of dietary exposures to farmland insecticides (imidacloprid, thiamethoxam and fipronil). *Pest Management Science, 76*, 2846–2853. https://doi.org/10.1002/ps.5838

Ohe, K. von der, & Ohe, W. von der (2000). *Celle's Melissopalynological collection*. Celle, Germany: Niedersächsisches Landesinstitut für Bienenkunde.

Pernal, S. F., & Currie, R. W. (1998). Nectar quality in open-pollinated pol CMS hybrid, and dominant SI hybrid oilseed summer rape. *Canadian Journal of Plant Science, 78*, 79–89. https://doi.org/10.4141/P97-071

Pierre, J., Mesquida, J., Marilleau, R., Pham-Delègue, M. H., & Renard, M. (1999). Nectar secretion in winter oilseed r,ape, *Brassica napus*— quantitative and qualitative variability among 71 genotypes. *Plant Breeding, 118*, 471–476. https://doi.org/10.1046/j.1439-0523.1999.00421.x

Raimets, R., Bontšutšnaja, A., Bartkevics, V., Pugajeva, I., Kaart, T., Puusepp, L., et al. (2020). Pesticide residues in beehive matrices are dependent on collection time and matrix type but independent of proportion of foraged oilseed rape and agricultural land in foraging territory. *Chemosphere, 238*, 124555. https://doi.org/10.1016/j.chemosphere.2019.124555

Schmuck, R., Schöning, R., Stork, A., & Schramel, O. (2001). Risk posed to honeybees (*Apis mellifera* L, *Hymenoptera*) by an imidacloprid seed dressing of sunflowers. *Pest Management Science, 57*, 225–238. https://doi.org/10.1002/ps.270

Simon-Delso, N., San Martin, G., Bruneau, E., Delcourt, C., & Hautier, L. (2017). The challenges of predicting pesticide exposure of honeybees at landscape level. *Scientific Reports, 19*, 3801.

Stanley, D. A., Garratt, M. P. D., Wickens, J. B., Wickens, V. J., Potts, S. G., & Raine, N. E. (2015). Neonicotinoid pesticide exposure impairs crop pollination services provided by bumblebees. *Nature, 528*(7583), 548–550. https://doi.org/10.1038/nature16167

Tette, P. A. S., Guidi, L. R., Glória, M. B. A., & Fernandes, C. (2016). Pesticides in honey: A review on chromatographic analytical methods. *Talanta, 149*, 124–141. https://doi.org/10.1016/j.talanta.2015.11.045

Vinolab. (2021). Retrieved from https://www.vinolab.hr/calculator/gravity-density-sugar-conversions-en19. (Accessed 17 June 2021).

Westcott, L., & Nelson, D. (2001). Canola pollination: An update. *Bee World, 82*, 115–129. https://doi.org/10.1080/0005772X.2001.11099514