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Assessment of the potential connection between *Tilia* trees and bumblebee death

Opinion of the Panel on Alien organisms and trade in endangered species (CITES) of the Norwegian Scientific Committee for Food Safety

Report from the Norwegian Scientific Committee for Food Safety (VKM) 2017: 15
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Assessed and approved

The opinion has been assessed and approved by Panel on Panel on Alien Organisms and trade in Endangered Species (CITES). Members of the panel are: Vigdis Vandvik (chair), Hugo de Boer, Jan Ove Gjershaug, Kjetil Hindar, Lawrence R. Kirkendall, Nina Elisabeth Nagy, Anders Nielsen, Eli K. Rueness, Odd Terje Sandlund, Kjersti Sjøtun, Hans Kristen Stenøien, Gaute Velle.

(Panel members in alphabetical order after chair of the panel)

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The Norwegian Scientific Committee for Food Safety (Vitenskapskomiteen for mattrygghet, VKM) has appointed a working group consisting of both VKM members and external experts to answer the request from the Norwegian Food Safety Authority/Norwegian Environment Agency. Project leader from the VKM secretariat has been Micael Wendell. The members of the working group, Anders Nielsen, Jan Ove Gjershaug, Gaute Velle (Panel on Alien Organisms and Trade in Endangered Species (CITES), Micael Wendell (VKM staff), Torgils Fossen (UiB) and Frode Ødegaard (NINA) are acknowledged for their valuable work on this opinion. The Panel on Alien Organisms and Trade in Endangered Species (CITES) are acknowledged for comments and views on this opinion.

Competence of VKM experts

Persons working for VKM, either as appointed members of the Committee or as external experts, do this by virtue of their scientific expertise, not as representatives for their employers or third party interests. The Civil Services Act instructions on legal competence apply for all work prepared by VKM.

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Summary

Key words: VKM, (benefit and) risk assessment, Norwegian Scientific Committee for Food Safety, Norwegian Environment Agency, bumblebees, lime tree, linden trees, landscape trees, urban trees, pollinators, insects, *Tilia*, *Bombus,* toxic nectar, mannose, pesticides.

The Norwegian Environmental Agency (NEA) commissioned the Norwegian Scientific Committee for Food Safety (VKM) to assess the relationship between flowering *Tilia* trees and dead bumblebees observed underneath them. NEA asked for a thorough evaluation of relevant literature that describes the phenomenon, with particular attention to the species of *Tilia* and the species and castes of bumblebees involved. Furthermore, VKM was asked to evaluate the extent of the phenomenon, i.e. the number of dead bumblebees observed under *Tilia* trees in Norway, and how the phenomenon varies in time and space. NEA also asked whether the phenomena is known from other countries. Finally, NEA called for a summary of the hypotheses put forward to explain the phenomenon and whether it may have negative consequences for the populations of bumblebees in Norway.

In Germany workers, drones and queens, representing many different species of bumblebees have been found dead under several species of *Tilia*. There is however, no scientific study, in Norway or abroad, that has quantified the extent of the phenomena, including the number of dead bumblebees found, variation among the species of *Tilia* involved and the geographic and temporal variation of the phenomenon.

A number of hypotheses have been put forward to explain how *Tilia* may cause bumblebees to die. We have chosen to divide the hypotheses into four groups:

Natural death. *Tilia* are late flowering trees and represent a floral resource hotspot attracting numerous foraging bumblebees at a time when alternative floral resources are scarce. As individual bumblebees have short life expectancy and die after only a couple of weeks, dead specimens will tend to accumulate by chance under the trees where they forage. No data exist on the age of the bumblebees dying under *Tilia* trees or on the availability other floral resources at the time the bumblebees are found dead. Our conclusion is that we lack information to assess this hypothesis.

The bumblebees starve to death because *Tilia* do not contain sufficient nectar. This hypothesis claim that *Tilia* flowers do not contain sufficient nectar to feed the bumblebees. Studies have shown that the nectar content in *Tilia* flowers is highly variable in time with a decrease in nectar volume throughout the day and towards the end of the season. This hypothesis cannot be out-ruled, but there is a lack of information on the diurnal temporal dynamics of bumblebee deaths and nectar availability, and on the stomach content of the bumblebees that are found dead under *Tilia* trees*.* We have concluded that we lack information to assess this hypothesis.

The flowers or other parts of *Tilia* are poisonous. Several studies have claimed that the death of the bumblebees underneath *Tilia* trees is due to poisonous nectar from its flowers. One hypothesis that has received much attention claims that nectar from *Tilia* contain mannose, a carbohydrate that can be toxic to bumblebees. Unpublished data from Dr Torgils Fossen, professor at Institute of Chemistry, University of Bergen, revealed that mannose was not present in the nectar of flowers from several *Tilia* trees in Bergen. Studies from abroad have reached the same conclusion. We therefore regard this hypothesis as very unlikely. Secondary metabolites in the nectar and poisonous pollen have also been hypothesised as causing bumblebee deaths in relation to *Tilia* trees. Based on available information we regarded these hypotheses as unlikely. We finally evaluated the hypotheses that the bumblebees were subject to alcohol intoxication or toxic honeydew. These hypotheses could not be assessed due to lack information.

Human impact. There are several reports of dead bumblebees under *Tilia* trees in the USA subsequent to application of pesticides (against aphids). It is prohibited to apply pesticides in parks in Norway and we therefore regard this hypothesis as very unlikely in a Norwegian setting.

Based on our evaluation of relevant literature we are unable to assess whether the dead bumblebees found under flowering *Tilia* have any negative effects on Norwegian bumblebee populations. The available literature cannot out rule that *Tilia* trees may in fact be a valuable resource during a time of the season when few alternative nectar sources are available.

To get a better understanding of the spatio-temporal dynamics of the death of bumblebees in relation to *Tilia* trees we suggest establishing a monitoring program. The monitoring should assess the species, castes and age distribution of the bumblebees involved. It should also assess whether other insects are dying under *Tilia* trees and whether alternative nectar sources are available at the time the bumblebees are dying. We also suggest that the stomach content of bumblebees found dead under *Tilia* trees are analysed, to test the validity of the "starvation hypothesis". Extensive assessment of the amount and constituents of *Tilia* nectar would also add valuable knowledge informing the assessments of the hypotheses related to the *Tilia* trees being the ultimate cause of the phenomenon. Finally, there is a need to establish whether dead bumblebees also occur in connection with other nectar sources, if available, at the time when dead bumblebees are observed under *Tilia* trees. It is still not known whether bumblebee deaths occur in relation to the same trees every year or whether local weather conditions may play a role. A monitoring program should therefore cover several regions and span over several years.

Sammendrag på norsk

Vitenskapskomiteen for mattrygghet (VKM) fikk tidlig 2017 i oppdrag av Miljødirektoratet å vurdere om det er en sammenheng mellom blomstrende lindetrær og døde humler som er observert under dem. Miljødirektoratet ønsket en gjennomgang av relevant litteratur som beskriver sammenhengen mellom blomstrende lindetrær og humledød, med særlig blikk på hvilke arter av lind og hvilke arter og kaster av humler som er involvert. Videre ønsket det en vurdering av omfanget av fenomenet, det vil si antall døde humler observert under lindetrær i Norge og om omfanget varierer med hensyn til sted og tid. Direktoratet ønsket også å vite om fenomenet er kjent fra andre land. Videre ønsket det en gjennomgang av hypotesene som er fremsatt for å forklare fenomenet, og hvorvidt fenomenet kan ha negative konsekvenser for bestanden av norske humler.

Døde humler har blitt observert under en rekke arter av lindetrær. I Tyskland er både arbeidere, droner og dronninger, fra mange arter, blitt identifisert blant døde humler under blomstrende lind. Det finnes ingen vitenskapelige studier som har kvantifisert omfanget av fenomenet, hverken med hensyn på antallet humler som dør, hvilke arter av lindetrær det skjer under eller hvordan fenomenet varierer geografisk og tidsmessig, hverken i Norge eller i andre land.

Det er blitt fremsatt en rekke hypoteser, som forsøker å forklare hvordan lindetrær forårsaker humledød. Vi har valgt å dele hypotesene i fire grupper:

Naturlig død: Parklind blomstrer sent på sommeren og kan, i alle fall i et bymiljø, være en av få planter som fremdeles er i blomst. Humlene vil derfor samles på lindetrærne, som kan være store og ha mange blomster. Humler lever ikke mer enn noen få uker, og da dette er sent i sesongen vil mange av humlene uansett dø i denne perioden. At de dør der de sanker mat er derfor ikke uventet. Denne hypotesen har aldri blitt studert, og vi har vurdert at vi mangler informasjon for å kunne vurdere om den stemmer.

Humlene sulter ihjel fordi lindeblomstene er tomme for nektar: Denne hypotesen hevder at lindeblomstene ikke inneholder nok nektar til at humlene får nok næring. Det er ikke funnet studier som på en troverdig måte har konkludert med at så er tilfelle, for eksempel studier som har analysert mageinnholdet til humler som er funnet døde under lindetrær. Vi har vurdert at vi mangler informasjon for å kunne vurdere om hypotesen stemmer.

Lindeblomster er giftige: Flere studier har hevdet at humledøden skyldes giftig nektar i lindeblomstene. En hypotese som har fått mye oppmerksomhet hevder at lindenektar inneholder mannose, et sukker som kan være giftig for humler. Vi har fått tilgang til upubliserte data fra Torgils Fossen, professor ved Kjemisk institutt, UiB, som ikke viser mannose i nektar i lindeblomster i Bergen. Andre studier fra utlandet har også kommet til samme konklusjon. Denne hypotesen anser vi som svært usannsynlig. Andre faktorer vi har vurdert er sekundære metabolitter, som vi anser som usannsynlig, og giftig pollen, som vi anser som usannsynlig. Vi har også vurdert alkoholforgiftning og giftig honningdugg. Vi mangler informasjon for å kunne gjøre en vurdering av hypotesene om at alkohol eller giftig honningdugg forårsaker humledød.

Menneskelig aktivitet: Fra USA er det rapportert flere episoder hvor humler har blitt funnet døde under lindetrær etter at trærne har blitt sprøytet med insektmidler (mot bladlus). Det er ikke tillatt å sprøyte parktrær på denne måten i Norge og vi anser denne hypotesen som svært usannsynlig.

På bakgrunn av vår gjennomgang av relevant litteratur har vi ikke funnet det mulig å vurdere hvorvidt humledød under blomstrende lindetrær påvirker norske humlepopulasjoner. Vi vurderer det slik at det er en mulighet for at lindetrær bidrar med en ekstra nektarressurs i en tid i sesongen der det finnes få alternativer. I så måte kan det faktisk hende at lindetrærne bidrar til at humlene lever noe lenger enn de ville gjort hvis ikke en gang lindeblomstene var tilgjengelige.

Det er store kunnskapshull når det gjelder sammenhengen mellom lindetrær og humledød. Etablering av overvåkingsprogram vil kunne bidra til å kvantifisere romlig og temporært omfang av fenomenet, hvilke arter og kaster som er rammet i Norge, aldersfordelingen på humlene som dør og hvorvidt andre insekter dør under lindetrær. Storskala studier av variasjonen i mengde og innhold i nektar i alle lindearter som har blitt assosiert med humledød vil også kunne gi mer kunnskap. Studiene bør dekke store områder og gå over flere år. Det bør også studeres hvorvidt humledød forekommer i forbindelse med andre større nektarkilder og hvorvidt magesekkene til humlene er tomme.

Background as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

Bumblebees and other pollinators provide an important ecosystem service. Lately, several observations of large amounts of dead bumblebees and other pollinators under flowering lime trees (Tilia spp.) have been recorded. Most observations seem to be of dead bumblebees (Bombus spp.) found under the alien species European lime (Tilia x europea). Because the plant is not observed reproductive in the wild the common lime is not risk assessed by the Norwegian Biodiversity Information Centre in Alien species in Norway- with Norwegian Black list 2012.

Due to several findings of dead bumblebees and other pollinators (bumblebees henceforth) under the lime trees, many have questioned whether there are aspects of species in genus Tilia that leads to increased death of bumblebees or whether the death is caused by factors other than the presence of lime trees in an area. Several conflicting hypotheses have been proposed about the causality between the bumblebee death and lime trees.

In order to provide more knowledge about the risk of damage to biological diversity the linden trees poses the Norwegian Environment Agency finds it necessary to obtain more knowledge on the issue through a compilation and evaluation of the available literature on the topic.

Terms of reference as provided by the Norwegian Food Safety Authority/ Norwegian Environment Agency

Norwegian Environment Agency requests the Norwegian Scientific Committee for Food Safety (VKM) to undertake the following:

I. A review of the literature on the possible correlation between the death of bumblebees and occurrence of lime trees, including:

- under what tree species, include species within the linden genus and other genera, has there been found dead bumblebees in Norway,
- which species of bumblebee and castes in the caste system have been found dead under lime trees in Norway,
- how many bumblebees (rough estimate) is found dead under lime trees in Norway,
- annual variation in the number of dead bumblebees under lime trees in Norway, geographical variation in the number of dead bumblebees under lime trees in Norway and
- whether dead bumblebees are observed under lime trees abroad.

II. A review of the various hypotheses on why bumblebees are found dead under linden trees, as well as an assessment of the validity of the different explanatory models.

III. Based on the findings in parts I and II, an assessment of whether the amount of dead bumblebees lime trees have a negative impact on the population development of the relevant bumblebee species, and, if present, a presentation of the knowledge gaps regarding the causality between lime trees and early bumblebee death.

IV. Propose future measures to improve knowledge of the issue, focusing on clarifying to what extent lime trees may have a negative impact on the bumblebees' population trend.

Assessment

1 Introduction

1.1 Purpose and scope

This opinion was prepared by the VKM Panel on Alien Organisms and trade in Endangered Species (CITES) (hereafter referred to as the Panel), in response to a request from the Norwegian Environmental Agency. The opinion is an assessment of the perceived widespread phenomenon where large numbers of bumblebees are found dead under *Tilia* trees. Knowledge on the spatial and temporal dynamics of the phenomenon is reviewed based on a wide variety of sources. The potential impact the phenomenon might have on native bumblebee populations is then discussed. Furthermore, the opinion identifies and evaluates hypotheses proposed to explain causative mechanisms.

This Opinion focus on bumblebees and *Tilia* trees. Other pollinators and other species of trees are only briefly discussed. This in accordance to the background information and ToR provided by the Norwegian Environmental Agency.

2 Methodology and data

2.1 Information collection

2.1.1 Previous environmental risk assessments

No previous environmental risk assessments were identified in the search in WEB of Science (described in section 2.1.2), or in Google combining the search words "*Bombus*" or "Bumblebees" or "pollinators" and "*Tilia*" or "*lime* tree" or "basswood" or "lime" and "ERA" or "environmental risk assessment" or "risk evaluation" or "risk".

Tilia x *europaea* do not reproduce sexually in the wild in Norway and the Norwegian Biodiversity Information Centre therefore did not evaluate the ecological impacts of *Tilia* in their 2012 black list of alien species in Norway (Gederaas et al., 2012). However, the Norwegian Biodiversity Information Centre is planning to include *T.* x *europaea* in the ongoing risk assessment because of the assumed "long-range impact of bumblebee populations" (Sandvik et al., 2017).

2.1.2 Literature search

Information on the possible link between *Tilia* and bumblebee deaths was found through on-line databases and libraries, such as the ISI Web of Science, PubMed, Scifinder and Library of Congress. Information was also obtained through Google searches, including information from the grey literature. Search terms included «tilia» and/ or «lime» and/ or «linden» and «nectar» and/ or «honeydew» and/ or «pollen» and «tree» and «bumblebee» and/ or «bombus» and/ or «bees» and/or «insects» and «death» and/ or «dead» and «toxic» and/ or «intoxication» and/ or «poison». The searches were performed with the use of wildcard (*). In addition, the cited references in the retrieved papers were searched for relevant studies, as well as citing articles.

2.1.3 Data collection

During the flowering season of 2015 and 2016, the research groups of Torgils Fossen and Dag Olav Øvstedal at University of Bergen (UiB) conducted a research survey on the occurrence of dead bumblebees under trees of *T.* x *europaea* in Bergen city centre (Fossen and Øvstedal, *in prep*). The study, which is the only research work on the subject performed in Norway, also included thorough analyses of the chemical constituents of the nectars from *Tilia* under which dead bumblebees were harvested over the two consecutive flowering seasons. The research data was made available to VKM.

3 Dead insects under *Tilia* trees

3.1 *Tilia* trees

Lime trees (*Tilia* spp.), also referred to as linden trees (Table 1), are widely distributed and form locally important parts of northern temperate deciduous forests (Pigott, 2012). The genus comprises 23 species and 14 subspecies growing in the temperate zone of the northern hemisphere (Pigott, 2012). The trees reach a height of 20 to 40 m and live for 300-500 years.

Table 1. Names of the *Tilia* trees mentioned in this report, and whether dead bumblebees have been observed beneath the trees in Norway and abroad (based on (Illies and Mühlen, 2007; Madel, 1977; Mühlen et al., 1994; Pawlikowski, 2010) and observations by authors). Multiple common names exist for many of the species, some of which are mentioned in the table. Latin names are used in this report. The ratio between observed bumblebees foraging and dead bumblebees found under the tree is computed based on data from Illies and Mühlen (2007). The ratio is only indicative since it is methodological demanding to count foraging bumblebees.

Latin name	English name	Norwegian name	Dead bumblebees
Tilia	Lime (UK) Linden (US)	Lind	
Tilia x *europaea* (synonyms: *T. cordata* x *platyphyllos*, *T. vulgaris* *T. intermedia,* *T. officinarum*)	Common lime, European lime	Parklind	Observed in Norway and abroad
T. cordata	Small-leaved lime, Little-leaf lime Greenspire lime Winter lime	Vanlig lind	Observed abroad. Ratio dead/ foraging = 1.4
T. platyphyllos ssp. *cordifolia*	Large-leaved lime, Summer lime	Storbladlind, Storlind	Observed abroad. Ratio dead/ foraging = 0.1
T. tomentosa	Silver lime	Sølvlind	Observed abroad. Ratio dead/ foraging = 3.7*
T. americana	American linden Basswood	Amerikansk lind	Observed in the USA, caused by insecticides?

* The number of dead bumblebees may be underestimated since it was not possible to count all bumblebees beneath one half of the two trees included in the study (Illies and Mühlen, 2007).

Small-leaved lime (*T. cordata*) is the only native *Tilia* in Norway. It spread to Norway during the mid-Holocene thermal maximum at about 6000 years ago and constituted as much as

25-30% of all trees in southern Scandinavia at about 5000 years ago (Huntley and Birks, 1983). It is now much less common, and *Tilia* occurs as stands on warm and dry habitats north to Sunnmøre in western Norway and in isolated stands even further north. It can reach about 30 m in height and have a trunk up to about 1 m in diameter. In addition to *T. cordata*, some species of *Tilia* are cultivated and planted in Norwegian gardens, parks and along streets, including large-leaved lime (*T. platyphyllos* ssp. *cordifolia)*, silver lime (*T. tomentosa*), American linden (*T. americana*) and the intraspecific hybrid European lime (*T.* x *europaea*) Grundt et al. (2015) (Table 1). *T. cordata* and *T. platyphyllos* naturally forms the hybrid *T.* x *europaea* when individuals of the parental species exist in close proximity (Mauer and Tabel, 1995). *T.* x *europaea*, and possibly also other hybrids, comprised about 70% of all cultivated *Tilia* in Norway in the year 2000 (Sæbø and Pedersen, 2002). Both *T. platyphyllos* and *T.* x *europaea* have spread by vegetative clones in a few places in Norway. *T. tomentosa* grows naturally only in the Balkans, especially in the former Yugoslavia, Bulgaria, Romania and Greece (Radoglou et al., 2008). The natural northern limit of *T. platyphyllos* is found in Denmark and south Scandinavia (Radoglou et al., 2008).

Tilia produce small yellow-green flowers in July to early August, with exact timing varying depending on the site and local climate. The flowers have a rich and heavy scent and grow in clusters (inflorescence) of four to forty (Anderson, 1976). The flowering period for each species at a particular site is at most two weeks, and the flowers are predominantly pollinated by insects. Once flowers begin to open, all stages of floral development are present on a single tree as well as in a single inflorescence. Each tree produces large quantities of flowers and abundant and easily available rewards (pollen, nectar) that attracts large quantities of insect of different species (Anderson, 1976), including bees. There are not many other flowering plants this late in the summer, highlighting that *Tilia* may be an attractive resource for bumblebees during late summer. Production of nectar is depending on temperature, which must be above 15 ºC for *T. platyphyllos* and *T.* x *europaea* (Pigott, 2012). The nectar starts to form when the flower opens, and the formation rate increases up to the end of the second day and declines during the subsequent 2 to 3 days. Three to 4 mg of nectar can be sucked from a single flower during maximum production, and this amount will be replaced (Anderson, 1976). On average, the nectar composition is close to that of pure sucrose. However, the concentration of solutes and sugars vary highly even within a single flower (Pigott, 2012). The concentration of sucrose also depends on the relative humidity of the air (Corbet et al., 1979), suggesting that the sucrose concentration is at least partly depending on the rate of evaporation in the open flowers.

Cold temperatures during winter are likely not a limiting factor for *Tilia* growth in Norway, and *T. cordata* can tolerate daily minima down to -20 to -22ºC (Pigott, 2012). The temperature limit for a successful sexual reproduction for *T. cordata* is about 20 ºC during the flowering season, and subsequent development of the fertilized ovules and seed-development is also temperature-dependent (Pigott, 1981). *T. cordata* can still grow in colder conditions than the requirements for sexual reproduction, and the distribution of *T. cordata* is correlated with the north Europe annual isotherm of + 2 ºC and July isotherm of + 17 ºC (Pigott and Huntley, 1978; Pigott and Huntley, 1980). This suggests that most trees in

Norway today mainly produce vegetative clones from the root collar, and only (very) rarely sexual offspring (Mong, 2005). Cloning also implies a limited ability for natural long-distance dispersal and spread.

3.2 Bumblebees

Bumblebees are conspicuous insects that have received increased attention in recent years due to their appealing appearance and the general appreciation of the ecosystem service they provide, through pollination. The increased attention is manifested through a number of papers in the scientific literature, books, reports, numerous articles in the daily press and other media coverage, and an increased awareness of bumblebees in the general public, including designated NGOs such as the British "Bumblebee trust" and the Norwegian "La humla suse".

Bumblebees are important pollinators in northern latitudes and forage in flowers of numerous wild plant species and entomophilous (*viz.* insect pollinated) crops. Bumblebees live from nectar and pollen and can appear in large numbers on floral resource hotspots. Although plants produce nectar solely for attracting pollinators, e.g., bumblebees, several plants display nectar that appears toxic to certain pollinators. In particular, nectar can contain secondary metabolites, such as alkaloids, terpenoids and (poly)phenolic compounds that are often associated with defence against foliar herbivory (Adler, 2000). A search in ISI Web of Science (accessed February 20th 2017) using the term "toxic nectar" resulted in 183 hits. Refining the search with the phrase "bumble*" retrieved a list of 30 studies showing that the issue of nectar toxicity is highly relevant also for bumblebees. For example, Jones and Agrawal (2016) found that bumblebees showed deterrence from milkweed flowers with cardenolides at the highest natural concentrations. Tiedeken et al. (2014) found that generalist bumblebees (*Bombus terrestris*) are unable to detect relevant doses of several naturally occurring nectar toxins, suggesting that "... it is difficult for them to learn to associate floral traits with the presence of toxins". Several theories have been posed for the beneficial effects of toxic nectar. However, a review by Adler (2000) highlights the potential for toxic nectar to be solely an artefact of anti-herbivore toxic compounds present in the phloem, which have subsequently been transferred into the nectar.

No published studies exist on Bumblebee death under *Tilia* trees in Norway. According to scattered information available in the grey literature, most reported bumblebee deaths occur beneath *T.* x *europaea* (Table 2). However, deaths in other countries have also been reported for *T. cordata*, *T. tomentosa*, *T. platyphyllos* (Illies and Mühlen, 2007; Madel, 1977; Mühlen et al., 1994; Pawlikowski, 2010) and horse chestnuts *(Aesculus hippocastanum*) (Crane, 1977; Somme et al., 2016). There is also a non-conformed account on bumblebee death under flowering sitka spruce (*Picea sitchensis*) and Norway maple (*Acer platanoídes*) during the spring (http://humleskolen.no/nyheter/dodelig-lind) and an unpublished observation of several dead bumblebees under *Echinops ritro* in Norway (observed by Frode Ødegaard). In addition, some flowers found in Norway, such as poppies (Papaveroideae), dodder (*Cuscuta* spp.) and *Anemone nemorosa*, may have a toxic effect on bees (Eckert, 1946; Howes, 1945; Løken, 1991; Mussen, 1979). Despite the great media attention of the, it has only been anecdotally reported in popular science articles and scientific reports and books (e.g., (Grundt et al., 2015; Løken, 1991; Ødegaard et al., 2015) and often with reference only to the daily press (Table 2).

Table 2. Examples of the many accounts of bumblebee death in Norway. Note that scientific studies are lacking.

Media type	Link
Blog	www.beetlebee.me/archives/stort-kunnskapsbehov-om-massiv-og-mystisk-humledod
Blog	teronor.wordpress.com/tag/humledod
Education	humleskolen.no/nyheter/dodelig-lind
Politics	oslo.mdg.no/nyhet/ber-om-svar-om-humledod-i-oslo
Politics	suzy.no/ta-humledod-pa-alvor-ogsa-i-tonsberg
Daily press	www.aftenposten.no/norge/Ett-av-Norges-vanligste-bytrar-dreper-humler-84188b.html
Daily press	www.tb.no/nyheter/vestfold/nyheter/flere-teorier-om-mystisk-humledod/s/2-2.516-1.8621069
Daily press	www.bt.no/nyheter/lokalt/Mystisk-humledod-ved-insekthotell-271258b.html?r=C
Popular science	forskning.no/insekter/2014/11/hvorfor-dor-humlene
Popular science	Løken, A. 1991. Planter som forgifter humler og bier. Insekt-Nytt 16: 17–19
Popular science	Ødegaard et al. 2009. Status for humler i Norge 2010. Fauna 62(4): 94–104.
Book	Ødegaard et al. 2015. Humler i Norge. NINA Trondheim 231 p.

The phenomenon has been reported repeatedly outside Norway and there have been attempts to disentangle causal mechanisms in Germany and Poland (e.g., (Geissler and Steche, 1962; Maurizio, 1950; Pawlikowski, 2010). Some of these studies date back almost 70 years, indicating that this is not a recent phenomenon. Dead bumblebees under *Tilia* have also been reported in the USA (http://www.reuters.com/article/us-usa-bees-oregon-idUSKCN0PA04G20150630), where pesticide use has been suggested as the cause of death, but as far as we know, with a lack of scientific studies. No systematic information exists on dead bumblebees beneath trees of *Tilia* in Norway. There is a lack of monitoring schemes and large-scale studies, implying that the extent to which bumblebee death under *Tilia* affects bumblebee populations is mostly unknown. A study by Pawlikowski (2010) from Poland states that "The percentage of intoxicated bumblebees constituted only a few percent of all bumblebees visiting the flowers", suggesting that even though a large number of bumblebees were observed under *Tilia*, the number of bumblebees still foraging on the *Tilia* flowers were still much higher. They also reported that the number of dead bumblebees were higher in warmer periods, suggesting that weather conditions are an important explanatory variable.

Nectar from *T. tomantosa* has been fed to bumblebees without any harm (Baal, 1993). According to video recordings made by Surholt et al. (1992), bumblebees die about 45 minutes after fallout from *T. tomantosa.* However, they will recover if fed nectar from the same *T. tomantosa* tree shortly after fallout. Surholt et al. (1992) noted that "Before starting such an experiment, however, and exposing the bee to the silver lime tree flowers, it is necessary to protect the flowers for 24 hours against nectar collecting insects by enclosing them in a gauze bag." They did not provide further reasoning why this enclosing procedure is needed. Other anecdotal accounts have also suggested that bumblebees found on the ground recover if fed with *Tilia* nectar. Such reports may suggest that it is not the composition of the nectar that is causing the bumblebee death.

3.3 Other insects

Tilia is a generalist in regard to pollination and attracts more than bees. In a study from New England, 66 species of insects, representing 29 families, were found visiting *Tilia* flowers. Flies were the most common diurnal visitors and moths the most common nocturnal insect (Anderson 1976).

Illies and Mühlen Illies and Mühlen (2007) reported a significant number of dead honeybees under *T. tomentosa*, but in much lower numbers than bumblebees. In the same study, they found only very few dead honeybees under *T. platyphyllos* and *T. cordata*. In Norway, dead honeybees (Apis melifera), social wasps (Vespidae), flies (Muscidae, Calliophoridae and Sarcophagidae) and Bibio pomonae (Bibionidae) have been found in large amounts under and other insects are found under trees of T. x europaea during July and August 2016 (observation by authors, pers. comm. Frode Ødegaard). It may be assumed that the significance of other dead insects may be underestimated in some studies due to small size, and poor focus in registrations.

It is unknown the extent to which dead solitary bees (*Halictus*, *Lasioglossum*, *Andrena*, *Osmia*, *Hylaeus* etc.) occur under *Tilia*. Their local occurrence, low density and small size make them more difficult to detect. Solitary bees of the genera *Andrena*, *Halictus* and *Lasioglossum* were recorded on *Tilia* in an urban environment of Torun in Poland, however no dead bees were found under the trees (Pawlikowski, 2010). In Norway, very few species of solitary bees are active at the time of year when lime trees are in flower, and none of the bee species in our region is particularly associated with lime trees.

4 Identification and validation of hypotheses

Based on the literature review, information from media and ecological knowledge, we have identified several hypotheses that may explain findings of dead bumblebees beneath *Tilia* trees. The hypotheses are categorized into four groups, some of which includes sun-hypotheses:

1. Random processes not related to *Tilia*
2. Behavioural aspects of the bumblebees
3. Negative effects of *Tilia* on bumblebees
4. Human impact

The likelihood of the hypothesis being true is categorized into five:

1. **Very unlikely:** Published data exists that rejects the hypothesis
2. **Unlikely:** Unpublished data exists that rejects the hypothesis, but published data is lacking
3. **Likely:** Unpublished data exists that confirms the hypothesis, but published data is lacking
4. **Very Likely:** Published data exists that confirms the hypothesis and no studies have rejected the hypothesis
5. **Lack of information:** The hypothesis can potentially be true or not true, but its validity cannot be assessed because of insufficient data

The hypotheses are described and discussed in the section below. For each hypothesis, we conclude on its likelihood according to the categories described above.

4.1 Random processes

4.1.1 **"The natural death hypothesis"**

Tilia is mainly flowering at a time during late summer when there are limited availability of alternative floral resources and most other park trees have ceased flowering. The flowering coincides with a peak in the number of bumblebees and the floral resource of *Tilia* therefore represents a hotspot that attracts numerous foraging bumblebees. However, we have found no studies or anecdotal reports that assess the availability of alternative food sources near *Tilia* trees under which dead bumblebees are found. As individual bumblebee workers have a short life expectancy with a life span of only a few weeks, the natural bumblebee mortality is high during this period. Dead bumblebees are commonly observed in gardens and elsewhere, and since they tend to accumulate to forage on *Tilia*, the deaths observed under the trees might be a result of the patchy distribution of bumblebee workers. *Tilia* is often planted in parks and even on paved sidewalks with short, if any, ground vegetation. Dead bumblebees on the ground are therefore more easily visible under *Tilia* trees than under other floral resource hotspots, such as trees in the wild or flowerbeds. Therefore, if comparable floral resource hotspots exist, they should be associated with deaths to a similar extent. However, we are not aware of studies that have compared concurrent numbers of dead bumblebees under *Tilia* and under alternative floral resources. This hypothesis predicts that the dead bumblebees are of an age in proximity to their life expectancy. Another prediction would be that the wings of the dead bumblebees are torn or damages in the wing margins due to long lifetime. We have not found if this prediction has been tested. Mühlen et al. (1994) found that 34.4% of the dead bumblebees were males and 3.5% were queens under their focal *Tilia* trees in Germany. This minority of males and queens (approximately 40% of the total) is comprised by reproductive and presumably young individuals, which may contradict the hypothesis. However, the longevity of bumblebee males is unknown. Whether the males are old relative to their life expectancy can therefore not be assessed. Bumblebee males, at least in some species, establish perching sites in or close to visually conspicuous trees (Alcock and Alcock, 1983). However, the more common species of bumblebees in Norway (i.e. *Bombus hortorum*, *B. sylvestris*, *B. bohemicus*, *B. pascuorum*, *B. lapidarius*, *B. terrestris* and *B. lucorum*) does not seem to display such a perching behaviour, but instead conduct patrolling flights along regular circuits of a few hundred meters in length (Goulson et al., 2011and references therein). Irrespective of the exact mating behaviour, the males tend to accumulate in confined areas. Dead reproductive individuals may then accumulate under *Tilia* trees that by chance are located along the patrolling circuit. However, it should be noted that observations of dead insects other than bumblebees under *Tilia* trees as well as observations made by Frode Ødegaard (pers. comm. Frode Ødegaard may indicate that the hypothesis is unlikely. may indicate that the hypothesis is unlikely.

Concluding remarks: One should expect that *Tilia* trees may provide an additional nectar resource and prolong the bumblebee life for a week or so. However, we have not found studies that debate on the possibility that *Tilia* is not responsible for the accumulation of dead of bumblebees that may occur under the trees.

We assess the natural death hypothesis as having "**Lack of information**". The uncertainty of this judgement is rated as **high**.

4.2 Behavioural aspects of the bumblebees

4.2.1 **"The starvation hypothesis"**

Bumblebees are prone to starvation if they fail to find sufficient nectar resources during a foraging bout. Several studies have proposed that dead bumblebees under late flowering *Tilia* trees have starved (Beutler and Whal, 1936; Illies and Mühlen, 2007; Mühlen et al., 1992; Surholt and Baal, 1995). They suggest that bumblebees and other pollinators empty the flowers of nectar and that available alternative food in the surroundings is scarce at this time of the summer (*op. cit.*). However, these studies have not quantified the availability of additional floral resources. Illies and Mühlen (2007) studied nectar availability, flower visits and bumblebee deaths beneath trees of *T. platyphyllos*, *T. cordata* and *T. tomentosa* and found a noticeable number of dead insects (mainly bumblebees) only under the latter species. They found that the number of dead bumblebees increased throughout the season and reached the maximum at the end of the flowering period of *T. tomentosa*. The persisting bumblebee visits to *T. tomentosa* flowers late in summer may be due to a local lack of alternative nectar sources, as discussed by Surholt and Baal (1995). *Tilia* flowers contain highly variable amounts of nectar throughout the day and season (Pigott, 2012). According to Anderson (1976), as much as 3 to 4 mg of nectar can be extracted from single flowers and will be replaced. The sugar composition and density in *Tilia* nectar trees also varies among species (Krasenbrink et al., 1994). However, there is still a lack of information regarding the sugar content in the nectar at different times of day and over the flowering season. The amount of nectar has so far not been measured at the time directly preceding death of bumblebees.

Concluding remarks: We are not aware of studies that assessed the nectar content in the stomachs of dead bumblebees found under *Tilia* trees. Depletion of the metabolic nutrient trehalose in the dying bumblebees may support that the bumblebees starve (www.beetlebee.me/archives/stort-kunnskapsbehov-om-massiv-og-mystisk-humledod). Based on available information this hypothesis has some indirect support, at least for *T. tomentosa*, but further investigation is needed.

We assess the starvation hypothesis as having "**Lack of information**". The uncertainty of this judgement is rated as **high.**

4.3 Negative effects of *Tilia* on bumblebees

This group of hypotheses suggest that *Tilia* excrete a substance that is toxic to bumblebees and cause the bumblebee deaths.

4.3.1 "The mannose hypothesis"

Tilia nectar has been suggested to contain mannose (Geissler and Steche, 1962), a sugar that is toxic to bumblebees since it may negatively influence their carbohydrate metabolism. Even though this theory has been adopted by more recent authors (e.g., (Løken, 1991; Madel, 1977; Pawlikowski, 2010)) mannose has hitherto not been adequately characterized from *Tilia* nectar by modern spectroscopic methods. Indeed, several studies have stated that mannose is toxic and present in the nectar, without chemically identifying its presence in *Tilia* nectar (e.g., Argoti and Rao, 2015; Johnson, 2015; Løken, 1991; Sols et al., 1960; Wykes, 1952).

Using paper chromatography, Madel (1977) found mannose in the nectar of *T. tomentosa*. However, based on more precise techniques, i.e. coupled Gas Chromatography and Mass spectrometry (GC-MS) analysis, Mühlen et al. (1992) and Krasenbrink et al. (1994) failed to detect mannose in *Tilia* nectar. Krasenbrink et al. (1994) also analysed other parts of the flower, including stamens and flowers, for mannose. They detected other sugars and the sugar alcohol inositol, but not mannose. Very recently, Fossen and Øvstedal (*in prep.*) found that *Tilia* nectar collected from trees in Bergen (Norway) did not contain mannose. A significant number of dead bumblebees were found under the same trees. The latter analyses were performed by application of a combination of several modern multidimensional high resolution Nuclear Magnetic Resonance (NMR) spectroscopic techniques.

Concluding remarks: The assumption that *Tilia* nectar contain mannose originate from a time when present modern methods for accurate structure determination of natural products, such as multidimensional high resolution NMR spectroscopy and hyphenated GC and HPLC combined with high resolution MS, had not yet been developed. It has been verified that a significant number of natural products from the epoch predating the widespread use of these techniques have been incorrectly identified. This is highlighted by the fact that more recent analyses of the chemical composition of *Tilia* nectar based on modern methodology (Fossen and Øvstedal, *in prep;* Krasenbrink et al., 1994; Mühlen et al., 1992) agree that mannose is not present in *Tilia* nectar.

We assess the mannose hypothesis as being "**Very unlikely**". The uncertainty of this judgement is rated as **low**.

4.3.2 **"Toxic secondary metabolite hypothesis"**

Several plant species have been shown to display nectar that contains compounds which may be toxic to certain pollinators. Such compounds may include secondary metabolites, such as alkaloids, certain terpenoids and (poly)phenolic compounds, some of which are associated with defence against foliar herbivory (Adler, 2000). It has therefore been hypothesized that *Tilia* nectar contains nicotine and/or other potentially toxic natural products including certain volatile compounds.

According to Naef et al. (2004) *Tilia* nectar contains a complex mixture of volatile constituents. Using GC-MS-methods they identified products of fatty acid degradation (nonanal, decanal and tetradec-1-ene), phenylpropanoids (3-(4-methoxyphenyl)propan-1-ol, 3-(4-methoxyphenyl)propanal, 3-(4-methoxyphenyl)prop-2-enal, isoprenoids (vomifolione, vomifoliol and 3,5,5-trimethyl-4-(3-oxobutyl)cyclohex-2-ene-1-one), alkaloids (caffeine, theophylline and trace amounts of nicotine), and a complex mixture of monoterpenes, among them *Tilia* ether and 1,8-cineole, in addition to several unknown compounds having molecular weights of 148, 150, 152, 166, 168 and 170, respectively (Naef et al., 2004).

Trace amounts of nicotine have also been indicated to occur in *Tilia* nectar (Naef et al., 2004). Baracchi et al. (2015) suggested that bumblebees might use nicotine-containing nectar for self-medication towards microbial infections, although this is controversial. The fact that to date, no alkaloid has been identified from *Tilia* questions the reliability of the tentative identification of nicotine in nectar from *Tilia*. Moreover, initial toxicological studies of the non-volatile constituents of *Tilia* nectar showed that these compounds are not toxic for *Artemia salina* at concentrations as high as 1 mg/ml (Fossen and Øvstedal, *in prep*.).

Concluding remarks: *Tilia* nectar contains a myriad of natural products, as is common for plant nectar. Whether or not some of these compounds may have a function as chemical defence against foliar herbivory remain elusive. Nectar from *T. tomantosa* has been fed to bumblebees without any harm (Baal, 1993; Fries, 2009), and the bumblebees may even recover if fed nectar from the same *T. tomantosa* tree shortly after falling to the ground (Surholt et al., 1992). These studies question the toxicity for bumblebees of secondary metabolites in *Tilia* nectar. However, toxicity tests of the volatile secondary metabolites found in the nectar are lacking.

We assess the secondary metabolite hypothesis as being "**Unlikely**". The uncertainty of this judgement is rated as **medium**.

4.3.3 **"The alcohol hypothesis"**

4.3.3.1 Ethanol

Microorganisms are commonly present in nectar and can modify a variety of traits important for pollinator attraction, such as sugar content, alcohol, pH, scent, amino acids and temperature (Ehlers and Olesen, 1997; Schaeffer et al., 2016). Insects can exhibit symptoms of ethanol intoxication (Heberlein et al., 2004) and the negative effects of alcohol on bees and bumblebees have long been recognized (Cumming, 1864; Kevan et al., 1988). Unpublished observations suggests that bumblebees under *Tilia* trees show symptoms of such ethanol intoxication, including spiralling flight towards the ground, unsteady walking and dizziness before they die or return to a healthy state (pers. comm. Frode Ødegaard). A higher occurrence of dead bumblebees under *Tilia* during warmer periods (Pawlikowski, 2010) with increased activity of microorganisms may support the hypothesis. Also, observations of other dead insects under *Tilia* along with bumblebees (Illies and Mühlen, 2007) may suggest a common source of intoxication, such as alcohol. Still, flowers containing ethanol may remain attractive due to the caloric value of ethanol, which is nearly twice that of carbohydrates (Dudley, 2000).

Ehlers and Olesen (1997) identified microorganisms from orchid flower nectar which were able to produce ethanol *in vitro* in the presence of sugars. The nectar from these orchids is known to be toxic to wasps. The authors did not examine the nectar for alcohol. However, the ability of identified microorganisms to produce ethanol in the presence of sugars *in vitro* led to the suggestion that they may produce ethanol from sugars when they are residing in the nectar. However, ethanol has not been found in the nectar of *Tilia*. For example, Naef et al. (2004) screened for volatile molecules with molecular weights in the range 27-350 and did not detect ethanol.

4.3.3.2 Inositol

Krasenbrink et al. (1994) analysed nectar, stamens and flowers of *T. tomentosa* and found several sugars and the sugar alcohol inositol, but not ethanol. The potential effect of inositol, which is a common natural product, was not reported.

Concluding remarks: The circumstantial evidence presented above gives support to the hypothesis. However, we have found no studies that report the presence of ethanol in *Tilia* nectar or reports suggesting toxicity of inositol. Also, *Tilia* nectar has been fed to bumblebees without any harm (Baal, 1993; Fries, 2009), including nectar from the same tree shortly after fallout (Surholt et al., 1992). Such information gives this hypothesis limited support.

We assess the alcohol hypothesis as having "**Lack of information**". The uncertainty of this judgement is rated as **high**.

4.3.4 "The toxic pollen hypothesis"

Some plants produce toxic pollen, for example *Stryphnodendron polyphyllum* (Fabaceae: Mimosoidea) releasing sacbrood-like symptoms in honeybee larvae (Pimentel de Carvalho and Message, 2004). From the Norwegian flora, *Anemone nemorosa* is known for producing toxic pollen that can kill bumblebees in the spring when there are few other blooming plants (Løken, 1991). We find it unlikely that pollen of *Tilia* is causing bumblebee death, because the observed bumblebee mortality increases towards the end of the blooming period, when the trees produce only small quantities of pollen (Illies and Mühlen, 2007).

Concluding remarks: We have found no study that have showed toxic substances to be present in *Tilia* pollen, which gives this hypothesis limited support.

We assess the toxic pollen hypothesis as being "**Unlikely**". The uncertainty of this judgement is rated as **low**.

4.3.5 "The toxic honeydew hypothesis"

Honeydew is a sugar-rich sticky liquid secreted by aphids and some scale insects (Hemiptera: Sternorrhyncha) as they feed on plant sap. Nectar-feeding insects, such as bumblebees, have been observed feeding on the honeydew. If infected by fungi, the honeydew may include some toxic substances. In New Zealand, the vine hopper (*Scolypopa australis*) produce honeydew from the poisonous plant tutu bush (*Coriaria arborea*) (Robertson et al., 2010). This honeydew is toxic to mammals, including humans, but not for the honeybee or other insects (Palmer-Jones, 1947). Reports from the US and personal observations from Norway suggest that honeydew can be produced in large quantities on trees of *Tilia*.

Concluding remarks: We are not aware of any study suggesting honeydew to be toxic to insects in general or bumblebees in particular, which gives this hypothesis limited support.

We assess the toxic honeydew hypothesis as having "**Lack of information**". The uncertainty of this judgement is rated as **low**.

4.4 Human impact

4.4.1 "The pesticide hypothesis"

A variety of insecticides have been shown to negatively impact bumblebees by either killing individuals directly upon exposure or harming colonies indirectly via sub-lethal effects (e.g., (Franklin et al., 2004; Morandin et al., 2005; Rundlof et al., 2015)). Bumblebees are particularly vulnerable to sprays that are applied early in the morning (when they are more active) and in the spring (when queens are the lone foragers) (Colla, 2016; Thompson and Hunt, 1999).

Insecticides are widely used to kill harmful insects, often having negative effects also on non-target species (Pisa et al., 2015). Neonicotinoid pesticides in particular have been shown to have sub-lethal effects on bumblebees in subtle ways. Neonicotinoids target nicotinic acetylcholine receptors and neurons in the bumblebee brains (Moffat et al., 2016; Palmer et al., 2013) causing slower learning and reduced flower handling efficacy (Stanley et al., 2015). These altered behaviours may cause reduced foraging capacity (Feltham et al., 2014; Stanley and Raine, 2016) and slower colony growth (Rundlof et al., 2015; Whitehorn et al., 2012). Neonicotinoids may also negatively impact the immune system of bees (Di Prisco et al., 2013). Neonicotinoid exposure can originate from direct spraying while the bumblebees are foraging on flowers or indirectly as the bumblebees forage on contaminated nectar or pollen.

Newspaper articles and professional biologists from the US have suggested that pesticide use on *Tilia* is to blame for massive death of bumblebees under *T. Americana* and *T.* x *europaea* (e.g., www.treehugger.com/natural-sciences/25000-bumble-bees-found-dead-target-parking-lot.html). The problem seems to be particularly apparent in Oregon, where the largest known incident of bumblebee deaths in the United States occurred in 2013 when an estimate of 25 000 bumblebees were found dead under 55 *Tilia* trees (oregonlive.com/environment/index.ssf/2013/06/25000_bubblebees_killed_droppi.html). The Oregon Department of Agriculture suspects they were killed by improper application of pesticide, which has resulted in a permanent rule banning the use of four particular types of neonicotinoids on *Tilia*. However, no scientific studies have supported this theory or the underlying mechanisms.

Concluding remarks: Neonicotinoid pesticides are harmful to bumblebees, however it is illegal to spray trees in parks in this fashion in Norway and we regard the hypothesis as very unlikely in a Norwegian setting.

We assess the pesticide hypothesis as being “**Very unlikely**”. The uncertainty of this judgement is rated as **low**.

5 Extent and significance for bumblebee populations

Mühlen et al. (1994) collected dead insects under *Tilia* in Germany in 1990-1992. Of the 5117 insects, 4339 were bumblebees of the *Bombus terrestris/lucorum* complex and 218 were from eight other species within *Bombus.* In addition, they found 532 honeybees (*Apis*), 14 wasps (Vespidae) and 14 other insect species. Of the bumblebees, 62.1% were female workers, 34.4% were males and 3.5% were queens.

It is unknown what impact the mortality observed in proximity to *Tilia* has on bumblebee populations. *Tilia* is flowering in late summer coinciding with a peak in the number of bumblebees and at a time with high natural mortality of workers. Nevertheless, if many workers die earlier than normal, at a time when new queens and males need feeding in the nest, the negative impact may be substantial. Furthermore, if the young queens are attracted by *Tilia* after they have left the nest, they can potentially also die. Mühlen et al. (1994) found that 3.5% of the dead bumblebees were queens. However, Pawlikowski (2010) stated that "The percentage of intoxicated bumblebees constituted only a few percent of all bumblebees visiting the flowers", suggesting that even though a large number of bumblebees were observed under *Tilia*, the number of bumblebees still foraging on the tree flowers were much higher.

The result from Germany (Mühlen et al., 1994) indicate that the most common species of bumblebees are found dead under *Tilia*. This finding corresponds with results from Bergen and findings of dead *B. hortorum, B. lucorum* and *B. pascuorum* (Fossen and Øvstedal *in prep.*). In Trondheim, *B. terrestris*, *B. lucorum* and *B. pascuorum* are the most abundant species of bumblebees, and the species most often found dead under *T.* x *europaea* (observations by Gjershaug).

Short-tongued bumblebee species are generally attracted by *Tilia* flowers, but also the long-tongued species *Bombus hortorum* and *B. pascuorum* have been found dead under *Tilia* trees in Bergen (Fossen and Øvstedal, *in prep*). It is therefore possible that the long-tongued species *B. distinguendus* and *B. subterraneus*, that are endangered (EN) and vulnerable (VU) on the Norwegian Red List (Henriksen and Hilmo, 2015), may use *Tilia* flowers. These two species of bumblebees have been found in some urban areas in Southern Norway (Gjershaug et al., 2013; Røsok et al., 2016), and their small populations may be more affected by *Tilia* trees than the more common bumblebee species. However, we do not know if there are any species-specific responses to *Tilia* trees. There are no information whether red listed species belonging to other insect groups may be affected positively or negatively by *Tilia* trees.

So far, no systematic monitoring has been performed in Norway on the extent of dead bumblebees or on the species of trees involved. Such monitoring should ideally also include information on the total population size of bumblebees in the area, on the bumblebee species found that are found dead under the trees, including sex and caste, and on other dead insects. Such information is needed in order to assess the impact of *Tilia* on the bumblebee populations.

6 Temperature considerations

Climate change may potentially influence the number of bumblebees that die given that the distribution and abundance of *Tilia* is temperature-dependent and that *Tilia* also causes bumblebee deaths. For example, in a study from Germany more dead bumblebees were found under *T. tomentosa* than under other species of *Tilia* (Table 1). *T. tomentosa* is native to central Europe and does not occur naturally in Norway today. It can be expected to thrive in Norway given sufficient future warming. If it does, dead bumblebees can expectedly be found under such trees.

The distribution of *Tilia* seems to be restricted by summer temperatures (Pigott and Huntley, 1978; Pigott and Huntley, 1980; Pigott, 2012). Currently, the warmest summer temperatures in Norway (period 1971-2000) are in the southern part of Østlandet and the coastal areas of Sørlandet, with an average of about 17°C. Given the mid-range CO_2 emission scenario RCP4.5, these warm areas can expect an annual temperature increase of 2.0°C during the next 50 years, with the highest increase (2.4°C) occurring during winters (Table 1). The increase in temperature will be more pronounced given the emission scenario RCP8.5 (Table 3). The number of growing season days is expected to increase under both climate scenarios (Table 3).

Table 3. Modelled increase in temperature and growing season days from the period 1971-2000 and towards 2067 under the greenhouse gas concentration trajectories RCP4.5 (emission peak 2040-2050, then decline) and RCP 8.5 (business as usual). These two scenarios are recommended by the ICPP. The projections are based on an ensemble of ten different climate models. Source, including uncertainties in the projections: klimaservicesenter.no

	Annual °C	Summer °C	Winter °C	Growing season days
Norway RCP 4.5	2.2	2.0	2.5	0-60
South-eastern Norway RCP 4.5	2.0	1.9	2.4	0-60*
Norway RCP 8.5	3.3	2.9	3.5	0-60
South-eastern Norway RCP 8.5	3.0	2.6	3.2	30-60

Summer= June, July, August; winter= December, January, February. *Small areas in southernmost Norway may experience up to 60 days of increase.

Given an increase in summer temperatures of 2.6°C for south-eastern Norway, the average summer temperature will reach a maximum right below 20°C in the warmest areas of Norway in 2067. However, the modelled future temperature increase (Table 3) represents averages, suggesting that the increase may be larger for some areas. The temperature requirement for natural reproduction of *T. cordata* is 20°C during the flowering season, and this temperature may perhaps be reached for a few warm sites in southern Norway during the next 50 years. Reproduction of *T. cordata* in Norway may be possible in such sites under a warmer climate, whereas the temperature will likely be too low in most Norwegian areas, suggesting very limited if any sexual reproduction. *T. platyphyllos* is currently found in Denmark and south Sweden (Radoglou et al., 2008) and may spread to the warmest areas of Norway given warmer summers and successful colonization. Hybrids (i.e., *T.* x *europaea*) may form naturally in the warmest areas of Norway in the case of co-existence between *T. cordata* and *T. platyphyllos* (see section on *Tilia* trees above) *T. tomentosa* will likely not be able to thrive and reproduce in Norway within the next 50 years. Overall, the potential harmful effects on bumblebees caused by *Tilia* may increase as a consequence of climate change in Norway during the next 50 years, but only to a limited extent in a few warm areas.

7 Uncertainties

A measure of the uncertainty is included with a 3-point scale: low-, medium- or high uncertainty (Table 4).

Table 4. Ratings used for describing the level of uncertainty

Rating	Descriptors
Low	Information on potential negative effects on bumblebees is available. No subjective judgement is introduced. No unpublished data are used.
Medium	Some information is missing on the potential negative effects on bumblebees. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used.
High	Most information is missing on the potential negative effects on bumblebees. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used.

7.1 Uncertainty related to the hypotheses

7.1.1 Random processes

*7.1.1.1 **"The natural death hypothesis"***

This hypothesis predicts that the dead bumblebees are of an age in proximity to their life time expectancy. Mühlen et al. (1994) found that 34.4% of the dead bumblebees were males and 3.5% were queens. This minority (approximately 40%) is comprised by presumably young individuals, which may contradict the hypothesis. However, the expected mortality of young individuals needs to be taken into consideration. Furthermore, the longevity of bumblebee males is unknown. Whether the males are old relative to their life expectancy can therefore not be assessed. Our conclusion is that there is a lack of information needed to assess the natural death hypothesis. Systematic studies on the life cycle, life expectancy and mortality of bumblebees are required for a more certain conclusion.

The uncertainty of this judgement is rated as **high**.

7.1.2 Behavioral aspects of the bumblebees

7.1.2.1 ***"The starvation hypothesis"***

The amount of nectar found in *Tilia* flowers vary throughout the day and throughout the season. Based on existing studies (see the section describing the hypothesis), it is reasonable to conclude that the persisting bumblebee visits on *T. tomentosa* flowering in late summer may be caused by a local lack of alternative nectar sources. The tendency of bumblebees to visit *Tilia* flowers increasingly throughout the day when the amount of nectar is decreasing, may result in starvation. Our conclusion is that the starvation hypothesis has some support for *T. tomentosa*, which was studied by Illies and Mühlen (2007). However, there is a lack of information concerning the hypothesis since no similar studies exists in Norway or for *T.* x *europaea*. More detailed monitoring of nectar availability related to bumblebee death beneath trees of *T.* x *europaea* is needed in order to assess the hypothesis.

The uncertainty of this judgement is rated as **high**.

7.1.3 Negative effects of *Tilia* on bumblebees

7.1.3.1 ***"The mannose hypothesis"***

Published and unpublished studies using modern spectroscopic and chromatographic methods have failed to detect mannose in *Tilia* tree nectar from which dead bumblebees were found. This suggest that the mannose hypothesis is very unlikely.

The uncertainty of this judgement is rated as **low**.

7.1.3.2 "Toxic secondary metabolite hypothesis"

Tilia nectar contains a multitude of chemical substances. However, whether they are toxic to bumblebees or occur in concentrations that are relevant for exerting potential toxic effects, are unclear. The hypothesis cannot be rejected but has limited support. Extensive chemical analysis of lime tree nectar from trees under which death of bumblebees are observed and trees under which no such observations occur, could provide arguments that strengthen or weaken this hypothesis. Our conclusion is that the toxic secondary metabolite hypothesis is unlikely but cannot be rejected.

The uncertainty of this judgement is rated as **medium**.

7.1.3.3 ***"The alcohol hypothesis"***

Ethanol has not been detected in *Tilia* nectar. According to (Pigott, 2012) and references therein, the sugar alcohol inositol has only been found in other parts of the flower, i.e.

stamens and/or pollen. Further analyses of ethanol- and inositol concentrations in nectar and its potential toxicity to bumblebees are needed. Whether alcohol or sugar alcohol concentrations in *Tilia* nectar can be at a level causing toxic effect in bumblebees is not confirmed. The alcohol hypothesis has limited support from the published literature, but cannot be rejected. Our conclusion is that there is a lack of information needed to assess the hypothesis. The uncertainty of this judgement is rated as **high**.

The uncertainty of this judgement is rated as **medium**.

7.1.3.4 ***"The toxic pollen hypothesis"***

Only a limited number of plant species have toxic pollen. We have found no study documenting toxic substance in pollen from *Tilia*. Also, the number of dead bumblebees and the pollen production of *Tilia* seems to be inversely correlated. The toxic pollen hypothesis is unlikely but cannot be rejected.

The uncertainty of this judgement is rated as **low**.

7.1.3.5 ***"The toxic honeydew hypothesis"***

Although honeydew can be produced in high quantities on *Tilia*, no study has reported that the honeydew contains toxic substances. However, the toxic honeydew hypothesis cannot be assessed further since there is a lack of information.

The uncertainty of this judgement is rated as **low**.

7.1.4 Human impact

7.1.4.1 "The pesticide hypothesis"

Landscape trees are not treated with insecticides in Norway. Pesticides might indeed be a cause of bumblebee death elsewhere (suggested to be causative in the USA), but in the case of Norway our conclusion is that the pesticide hypothesis is very unlikely.

The uncertainty of this judgement is rated as **low**.

7.2 Uncertainty related to other questions listed in the Terms of reference

I. The possible correlation between the death of bumblebees and occurrence of *Tilia*, including:

a) *Under what tree species, including species within the linden genus and other genera, have been found dead bumblebees in Norway?*

In Norway, bumblebee death has been reported beneath trees of *T.* x *europaea*. Elsewhere, deaths have also been reported to other species, including *T. cordata*, *T. tomentosa T. platyphyllos*, *T. americana* and the unrelated Horse-chestnut tree *Aesculus hippocastanum* (Crane, 1977; Somme et al., 2016). There is also a non-conformed account on bumblebee death under flowering sitka spruce (*Picea sitchensis*) and Norway maple (*Acer platanoídes*) during the spring (http://humleskolen.no/nyheter/dodelig-lind).

The uncertainty related to this information is rated as **medium**.

b) *which species of bumblebee and castes in the caste system have been found dead under trees of* Tilia *in Norway?*

The results from University of Bergen (Fossen and Øvstedal, *in prep*) found dead *Bombus hortorum*, *B. lucorum* and *B. pascorum* under *Tilia*. From Trondheim, *B. terrestris* and *B. lucorum* have been found dead under *T.* x *europaea* (personal observations by Gjershaug, J.O.). We have not been able to find data on the bumblebee caste involved in Norway. From Germany, Mühlen et al. (1994) found that workers, males and queens represented 62.1%, 34.4% and 3.5%, respectively. We conclude that it is likely that several species of bumblebees can be found dead under *Tilia* in Norway and that all castes are involved.

The uncertainty of this judgement is rated as **low**.

c) *how many bumblebees (rough estimate) are found dead under lime trees in Norway?*

There are no studies documenting the number of dead bumblebees found under Tilia trees in Norway. However, based on the number of dead bumblebees observed under *Tilia* trees in Norway (pers. obs. authors) and abroad (Illies and Mühlen, 2007; Pawlikowski, 2010), it is reasonable to assume that the number in Norway must be in the order from tens to hundreds per tree of *T.* x *europaea* per summer.

The uncertainty of this judgement is rated as **high**.

d) *what is the annual variation in the number of dead bumblebees under the lime trees in Norway, geographic variation in the number of dead bumblebees under lime trees in Norway?*

Fossen and Øvstedal (*in prep*) found comparable numbers of dead bumblebees under four trees for two consecutive years in Bergen. However, long-term monitoring that assess the inter-annual variation in the number of dead bumblebees is lacking from Norway and abroad. It is reasonable to assume that the incident is greatest in urban areas where most of *T.* x *europaea* are planted.

The uncertainty of this judgement is rated as **high**.

e) *whether dead bumblebees are observed under lime trees abroad?*

Substantial documentation of this phenomenon exists from abroad. Some of the studies dates back almost 70 years.

The uncertainty of this statement is judged as **low**.

II. Is the extent of bumblebee death under lime trees affecting bumblebee populations?

There is a lack of information on the spatial and temporal extent of bumblebee death under Tilia *trees. Hence, the importance of this phenomenon for bumblebee population dynamics cannot be assessed. Based on the lack of information, we cannot assess the effect of death related to* Tilia *relative to the importance of other causes of mortality (e.g., natural death, parasites and diseases, predation by birds or road kills). Bumblebees die naturally in large numbers during late-season and* Tilia *may potentially provide an additional nectar resource and prolong the bumblebee life for an extra week or so.*

III. Based on the findings in part I and II, an assessment of whether the number of dead bumblebees under lime trees have a negative impact on the population development of the relevant bumblebee species

No assessments are available on the potential impact that dead bumblebees beneath *Tilia* trees can have on the population development of bumblebees. In addition, it is presently not conceivable to conduct such an assessment for Norway because of lack of data. However, in Poland, Pawlikowski (2010) found that "The percentage of intoxicated bumblebees constituted only a few percent of all bumblebees visiting the flowers", suggesting that even though a large number of bumblebees were observed under *Tilia*, the number of bumblebees still foraging on *Tilia* flowers were still much higher.

The uncertainty of this judgement is rated as **high**.

8 Conclusions (with answers to the terms of reference)

Below we respond to each point highlighted in the terms of reference.

The review of available literature includes:

- What species of lime (subspecies/crosses/varieties) has been reported to cause bumblebee death?

Several species of Tilia have been reported to be associated with bumblebee death. These include T. cordata, T. platyphyllos, T. tomentosa and T. x europaea. However, bumblebee death has also been reported under other tree species which are not related to Tilia, e.g., horse chestnut. In Norway, reports of dead bumblebees are linked to T. x europaea.

- What species and castes of bumblebees are to be found under lime trees?

The only study reporting the species composition of dead bumblebees under Tilia trees in Norway (Fossen and Øvstedal, in prep) found workers of B. hortorum, B. lucorum and B. pascuorum. Mühlen et al. (1994) identified nine bumblebee species under their focal Tilia trees, of which ~95% of the specimens were of the Bombus lucorum/terrestris complex (the most common bumblebees in the area). ~35% of the bumblebees were males and ~3.5% were queens, suggesting that not only workers die under Tilia.

- How many bumblebees are found dead under lime trees in Norway?

There is a lack of information on the number of dead bumblebees found under Tilia trees in Norway. Anecdotal reports from the US indicated that as many as 25 000 bumblebees were found dead under 55 Tilia trees after an incident of pesticide application. Mühlen et al. (1994) reported ~4450 dead bumblebees in total over three consecutive years in Germany, while Fossen and Øvstedal (in prep) found 70-90 dead bumblebees under four trees over an entire flowering season in Bergen. Pawlikowski (2010) found dead bumblebees under Tilia trees and assumed that they constituted only a few percent of all bumblebees visiting flowers.

- Annual variation in the number of bumblebees under lime trees.

There is a lack of monitoring in Norway and abroad. However, Fossen and Øvstedal (in prep) studied four trees over two consecutive summers and found comparable numbers of dead bumblebees during both years. The focal trees were not the same both *years, however, the chemical composition of their nectar was identical. Inter-annual variation cannot be assessed from existing data.*

- Geographic variation in numbers of dead bumblebees under lime trees.

Large-scale monitoring over time and space has not been conducted in Norway or abroad. All available information originates from local and small-scale studies or anecdotal reports. An assessment of the spatial variation cannot be made.

- Are dead bumblebees under lime trees reported from other countries?

Yes, reports exist from Poland, Sweden, Denmark, Germany and the US, although their scientific quality is highly variable. They are either anecdotal reports or case studies investigating the phenomenon on a limited spatial and temporal scale. The earliest reports date back to the 1950s, suggesting that bumblebee death under Tilia trees is not a recent phenomenon. One of these studies concluded that neonicotinoid pesticides, rather than Tilia, possess a major threat to bumblebee populations. It is likely that dead bumblebees will be present under Tilia trees regardless of country.

I. A review of the available hypotheses trying to explain why large numbers of dead bumblebees are found under lime trees:

We defined four groups of hypotheses that have been proposed to explain bumblebee death under Tilia *trees:*

- ***Random processes, not related to the* Tilia *trees – natural death.*** Tilia *represent a floral resource hotspot attracting numerous foraging bumblebees. As individual bumblebees have short life expectancy and die after only a couple of weeks, dead specimens will tend to accumulate by chance under the trees where they forage. No data exist on the age of the bumblebees dying under* Tilia *trees or on other floral resource hotspots. Our conclusion is that there is a lack of information needed to assess the natural death hypothesis.*

- **Behavioural aspects of the bumblebees – starvation.** Tilia *trees are attractive to bumblebees due to their large number of flowers. However, in a period of limited floral resource availability numerous flower visits may empty the nectar, whereupon the bumblebees roam among depleted flowers and starve. Mühlen et al. (1994) assessed the nectar availability in* Tilia *flowers throughout the day and throughout the season and found that nectar volumes decreased towards the end of the season. Fossen and Øvstedal (in prep) found surplus of nectar in the flowers of the* Tilia *trees they studied in Bergen. However, the nectar was collected at noon, and no monitoring of the diurnal pattern of nectar availability was performed. This hypothesis cannot be out-ruled, but there is a lack of information on the diurnal temporal dynamics of* *nectar availability or on the stomach content of the bumblebees that are found under* Tilia*.*

- ***Negative effects of* Tilia *nectar constituents on bumblebees.*** *It has been suggested that* Tilia *can be toxic to bumblebees due to the chemical composition of nectar, pollen or honeydew. We have found no support for these hypotheses based on available information. Modern chemical analytical tools have revealed that* Tilia *nectar does not contain the toxic substance mannose. None of the identified secondary metabolites hitherto identified in* Tilia *nectar have been reported to exhibit toxic effects. Initial toxicological studies of the non-volatile constituents of* Tilia *nectar showed that these compounds are not toxic at concentrations as high as 1 mg/ml. Thus, there is a lack of information for assessing the hypothesis that the chemical constituents hitherto identified in* Tilia *nectar have toxic effects.*

- **Human impact – pesticides.** *This theory is of little relevance for Norway since the pesticides suggested to cause bumblebee death in the USA are not used on* Tilia *in Norway.*

II. Is the extent of bumblebee death under lime trees affecting bumblebee populations?

There is a lack of information on the spatial and temporal extent of bumblebee death under Tilia trees. Hence, the importance of this phenomenon for bumblebee population dynamics cannot be assessed. Based on the lack of information, we cannot assess the effect of death related to Tilia relative to the importance of other causes of mortality (e.g., natural death, parasites and diseases, predation by birds or road kills). Bumblebees die naturally in large numbers during late-season and Tilia may potentially provide an additional nectar resource and prolong the bumblebee life for an extra week or so.

III. Future measures to improve knowledge

- *Monitoring program to assess:*
  - *The spatial and temporal extent of the phenomenon*
  - *The bumblebee species (and castes) involved*
  - *Whether significant numbers of non-bumblebees also die under* Tilia *trees*
  - *Whether other tree species are associated with bumblebee death*
  - *The age distribution of dead bumblebees under* Tilia *trees*
- *More spatial and temporarily extensive studies on the chemical composition and available quantities of nectar from the assortment of* Tilia *species associated with bumblebee death*
- *Assess whether other floral resource hotspots are associated with bumblebee death*
- *Measure the stomach content of dead bumblebees under* Tilia *trees*

9 Data gaps

Bumblebee death under *Tilia* trees is not a recent phenomenon. The first international scientific publications appeared already in the 1950s and 1960s (Geissler and Steche, 1962; Maurizio, 1950). However, several shortcomings have been identified with respect to the current status of knowledge in the scientific literature. Especially, a monitoring of the number of bumblebees that are affected is needed and scientific testing of hypotheses relating death of bumblebees to *Tilia* is needed. The knowledge needs are interrelated and can be divided into three:

1. Knowledge needs related to the mortality of bumblebees in the presence and absence of *Tilia*

- Systematic monitoring of dead bumblebees found under *Tilia* trees, including the species of bumblebees affected and with special focus on red-listed species.
- Information on the time of day when they die.
- Information on the natural life cycle, life expectancy and mortality of different bumblebees (species and castes).
- Information on dead insects other than bumblebees under *Tilia* trees. Bumblebees are conspicuous insects and anecdotal reports might miss out on other insect groups, such as solitary bees, wasps, flies and beetles.
- Number of dead bumblebees under floral resource hotspots other than *Tilia*.

2. Knowledge needs related to causes for the observed phenomenon

- A characterisation on the chemical constituents of *Tilia* nectar and its secondary metabolites, inositol and ethanol, performed by modern spectroscopic methods. The first publications on the chemical constituents of *Tilia* nectar (Geissler and Steche, 1962; Madel, 1977) were based on inaccurate methods, such as thin layer or paper chromatography. Even though more recent work has rejected the existence of several constituents reported to occur in *Tilia* nectar (such as mannose), these early studies are still being cited uncritically as authoritative sources (e.g., Pawlikowski, 2010).
- A comparison of the chemical constituents of nectar among species and cultivars of *Tilia*. The influence of nectar on bumblebees differs among the species of *Tilia* (Mühlen et al., 1994).
- Feeding experiments on bumblebees including nectar, pollen and honeydew from different species and cultivars of *Tilia.*
- Information on the amount of nectar found in the stomachs of bumblebees that are found dead under *Tilia* trees and under other floral resource hotspots.
- Information on the number of dead bumblebees found under different species and cultivars of *Tilia*.

- Analyses of concurrent environmental factors, such as temperature and moisture, and dead bumblebees. Comments, such as "on warm days" or "increased death during warmer temperatures" occur, but has been subject to limited testing.
- Analyses of traces of pesticides in *Tilia* and in bumblebees.

2. Knowledge needs related to effects on the population dynamics of bumblebees

- Long-term monitoring to assess trends in the spatial and temporal extent of the phenomenon. The increase in reported incidents in recent years could be due to elevated public awareness.
- Information on the population dynamics of bumblebees and the density of *Tilia* trees.
- Information on the age, caste and sex of the bumblebees found under *Tilia* trees.
- Information on effects on red listed species of bumblebees and other insects.

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