Do Linden trees kill bees? Reviewing the causes of bee deaths on Silver Linden (Tilia tomentosa)

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Do Linden trees kill bees? Reviewing the causes of bee deaths on Silver Linden (*Tilia tomentosa*)

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Abstract

For decades, linden trees (basswoods or lime trees), and particularly Silver Linden (*Tilia tomentosa*), have been linked to mass bee deaths. This phenomenon is often attributed to the purported occurrence of the carbohydrate mannose, which is toxic to bees, in *Tilia* nectar. In this review, however, we conclude that from existing literature there is no experimental evidence for toxicity to bees in linden nectar. Bee deaths on *Tilia* likely result from starvation, due to insufficient nectar resources late in the tree's flowering period. We recommend ensuring sufficient alternative food sources in cities during late summer to reduce bee deaths on Silver Linden. Silver Linden metabolites such as floral volatiles, pollen chemistry, and nectar secondary compounds remain unexplored, including their toxic or behavioural effects on bees. Some evidence including the presence of caffeine in linden nectar suggests linden trees may chemically deceive foraging bees to make sub-optimal foraging decisions, in some cases leading to their starvation.

Keywords: Bumblebee, ecotoxicology, pollinator decline, urban ecology

Introduction

Pollinators face increasing pressure from anthropogenic environmental impacts including land use intensification, climate change, and pesticides [1]. Concurrently, agricultural and urban environments can support abundant and species-rich pollinator communities if suitable floral resources are available [2-4]. Accurate knowledge about how plant species benefit or harm pollinators is therefore of central importance for creating pollinator-friendly environments. For example, non-native plants interact with native pollinators and the whole ecosystem, with direct or indirect effects that benefit or hinder pollinators and ecosystem services they provide [5]. Non-native plant species can have negative consequences for local non-adapted pollinators where toxins occur in nectar, as shown for the invasive *Rhododendron ponticum* in the British Isles [6].

Linden or lime trees (*Tilia* sp., Malvaceae) have at times been regarded as either beneficial food sources, or deadly traps for bees. In antiquity, linden trees were regarded as bountiful food plants for honeybees [7]. Linden trees have been planted in Europe to support honeybees since medieval times [8] and are productive nectar sources [3,9]. Conversely, since at least the 16th century, other authors have suggested linden can harm bees [10,11]. The potential dual nature of linden is most apparent by reoccurring mass deaths on flowering linden trees with sometimes thousands of dead bees (Table 1). Silver Linden (*Tilia tomentosa* Moench) are most often associated with bee deaths and have been asserted in numerous accounts to produce toxic nectar [12-19].

Silver Linden (Fig. 1) originates from South-Eastern Europe, but is planted widely outside its native range across Europe and North America [8,12]. Linden are among the most common urban trees throughout Europe and North America [20], and so have the greatest potential to affect urban pollinators. Their high drought and pest tolerance qualifies Silver Linden as excellent urban trees [21,22]. Given the importance of urban habitats and trees for pollinator populations [2,3], it is necessary to review whether linden trees have detrimental effects on bees, and how these may arise.

Dead bees under flowering linden have been reported from the UK [12], Switzerland [23], Germany [13,24-26], Norway [27], Poland [17], Austria [28,29] and the USA [19,30] (Table 1). The Crimean Linden (*Tilia* x *euchlora*), a putative hybrid between *T. cordata* and *T. dasystyla* [8], is also associated with bee deaths (Table 1). Small-leaved Linden (*Tilia cordata*), Large-leaved Linden (*Tilia platyphyllos*), and their hybrid Common Linden (*Tilia* x *europaea*) are generally not linked to this phenomenon, with the exception of a recent bumblebee kill under *T. cordata* in Oregon (USA) (Table 1).

Bumblebees are most affected, accounting for over 75% of dead bees [13,31] (Table 1). Short-tongued bumblebee species like *Bombus terrestris* dominate (Table 1; Fig. 1). Fewer honeybees (*Apis mellifera*) die, even though they forage as abundantly on the tree as bumblebees [17,26].

While dead bees under *T. tomentosa* and other linden trees are still recorded in many countries, uncertainty and confusion prevails over the causes. Here, we categorize and assess the published explanations under 5 hypotheses, examine their plausibility considering existing research, and identify key research gaps (see Table 2).

1. Toxic *Tilia* metabolites

A widely held belief and historic explanation of bee deaths under *Tilia* is that components in nectar poison bees, first suggested by Elwes & Henry [12]. Geissler & Steche [32] and Madel [13] proposed that the presence of the monosaccharide mannose (Fig. 1) in *T. tomentosa* nectar was responsible, after von Frisch [33] and Staudenmayer [34] had discovered toxicity of mannose to honeybees and bumblebees. This toxic effect results from a metabolic disease, in which an intermediate product, mannose 6-phosphate, accumulates and adenosine triphosphate is depleted, resulting in paralysis and death [35]. However, Madel's assertion [13] that this explained *T. tomentosa* toxicity was supported by scant detail about the detection of mannose beyond stating that he had conducted preliminary paper-chromatographic investigations. Biological evidence was limited to a feeding trial with 8 bumblebees caged with 7 *T. tomentosa* flowers without control [13]. All bumblebees tested died within 12 hours, leading Madel to conclude *T. tomentosa* nectar was toxic. However, Baal *et al*. [31] showed nectar of 7 flowers was inadequate for 8 caged bumblebees, meeting less than 2% of their energetic demand, and suggested starvation explained Madel's results [31]. Geissler & Steche [32] analysed sugars with paper chromatography and did not detect mannose in linden (*T. platyphyllos*) nectar. A hydrolyzed linden nectar sample revealed a sugar bound as a glycoside that was tentatively identified as mannose based on relative retention time, but was not clearly distinguishable from galactose. Via a colorimetric test, Geissler & Steche [32] also detected a sugar in dead bees collected under linden they concluded to be galactose or mannose. Notably, Geissler & Steche [32] pointed out their identifications were tentative, as they could not isolate sufficient sugar quantities for more refined analytical procedures. Subsequent chemical analyses, described below, discount these earlier proposed identifications. Despite this, Crane [14] later popularized the idea that mannose was responsible, erroneously presenting the riddle of bee deaths on linden as solved (Fig. 2).

Baal *et al*. [31] and Krasenbrink *et al.* [36] re-examined the nectar sugar chemistry of *T. tomentosa* and other *Tilia* species using gas chromatography of derivatised sugars, following standard methods by Sweely *et al.* [37]. Chromatograms published in Baal *et al.* [31] and Krasenbrink *et al.* [36] demonstrate their methods distinguished mannose from other nectar sugars. Glucose, fructose, sucrose, and mannose were furthermore enzymatically quantified [31]. These analyses showed unequivocally that mannose was absent in nectar of *T. tomentosa* (n = 36 trees), *T. platyphyllos* (n = 20), *T. cordata* (n = 12) and *T.* x *euchlora* (n = 14). Only the non-toxic sugars sucrose, glucose, and fructose were detected. Since mannose might be produced as a nectar metabolite by bees [32], Baal *et al*. [31] analyzed guts, abdomina and heads/thoraxes of 80 dying bumblebees from flowering *T. tomentosa* and *T*. x *euchlora*, but recorded no mannose in the bumblebees. Finally, Baal *et al*. [31] fed *T. tomentosa* nectar to 30 caged *B. terrestris*, and again mannose was absent from guts and haemolymph. Bumblebees fed on *T. tomentosa* nectar for 5 days showed no adverse effects. Baal *et al*. [31] thus disproved the hypothesis of mannose poisoning by *T. tomentosa*. Nevertheless, non-nutritive sugars in *Tilia* nectar, including sugar moieties in glycosides (see [32]), deserve further study. We suggest carbohydrate chemistry of linden nectar and pollen will become clearer through more accurate and sensitive methods including nuclear magnetic resonance spectroscopy.

Despite the lack of evidence, the received wisdom of mannose poisoning by *T. tomentosa* nectar continues to prevail as fact in much scientific and technical literature (Fig. 2), including reviews [14,18,38-44], original research papers [17,45,46], horticultural and botanical guides [15,16], pest control [47], and governmental advisories [48].

The non-sugar chemistry of *T. tomentosa* nectar and pollen remains largely unstudied. Naef *et al*. [49] and Frérot *et al*. [50] described the volatile nectar constituents from the related *T. cordata* and found secondary compounds including terpenoids, flavonoids, and a novel cyclohexa-1,3-diene-1-carboxylic acid and its *β*-gentiobiosyl ester. The disaccharide gentiobiose occurs in crops of honeybees foraging on *T. tomentosa* [51], and in linden honey [52]. Gentiobiose is most likely the product of enzymatic cleavage of the *β*-gentiobiosyl moiety of the above-mentioned glycoside in *Tilia* nectar [50]. Effects of gentiobiose on bees are unknown, but the feeding trials by Baal *et al*. [31] (see above) suggest no adverse effects should be expected.

Bumblebees collect pollen on linden [53] (Fig. 1), but the importance of *T. tomentosa* pollen remains unknown. Melville [54] observed that only bumblebees and not honeybees collected pollen from *T. tomentosa*, and speculated a toxic compound in the pollen could explain why the majority of dying bees are bumblebees. However, no published pollen chemistry analysis beyond amino acids and sterols in Somme *et al*. [3] exists. It remains unknown if foraging bees directly consume *Tilia* pollen on the tree, or rather carry pollen back externally to the nest as larval food.

We conclude the available evidence shows mannose does not occur in *Tilia* nectar and therefore cannot explain mass bee deaths on *Tilia*. There is no convincing experimental evidence for toxicity of *T. tomentosa* nectar or pollen to bees. However, the exposure of bees foraging on *T. tomentosa* flowers to toxic compounds other than mannose cannot be completely excluded, given the incomplete knowledge of *Tilia* pollen and nectar metabolites, and the limited experimental tests of *T. tomentosa* forage on bumblebee individual or colony health. Plant metabolites in *T. tomentosa* nectar and pollen therefore need to be analyzed further, and their potentially lethal or sub-lethal effects on bees should be tested experimentally.

2. Insecticides

Whereas *T. tomentosa* does not poison bees, insecticide application to the tree can. *Tilia* trees are occasionally treated with insecticides against aphids. Several instances of bumblebee deaths under *T. cordata* have recently occurred in Oregon, USA. In one outstanding case, over 50,000 bumblebees died under *T. cordata* trees in Wilsonville, Oregon [19]. Due to the widespread misconception about the presence of toxic sugars in linden nectar (see above), some sources erroneously suggested naturally occurring nectar toxins causing these bee kills (e.g. [47]). The Oregon Department of

Agriculture judged the neonicotinoid dinotefuran (Fig. 1), that had been applied to the trees prior to the event, as the cause [48]. Neonicotinoids are potent neurotoxins for honeybees and bumblebees [55]. Even when applied outside the flowering period, neonicotinoids can persist in plant tissues and subsequently occur at concentrations detrimental to bees in pollen and nectar [55]. The neonicotinoid use on flowering trees like *Tilia* spp. should therefore be prohibited.

Bee deaths under linden trees predate the introduction of neonicotinoid insecticides in the 1990s [12,13,24]. Neonicotinoids therefore cannot explain this phenomenon more broadly, but can account for isolated recent cases. The widespread confusion over the erroneously presumed presence of toxic mannose in *Tilia* nectar (see above) could however misguide policy makers and pest control professionals (e.g. [47,48]).

3. Death by natural causes: Predators and old age

T. tomentosa flowers later than other linden species, between mid-July and early August for Europe [25,26,54]. Large trees can accommodate thousands of foraging bees [26]. Many bumblebee species approach the end of their colony cycle at this point in the season. The high bee population on a mass flowering tree like *T. tomentosa* may see significant numbers of older bumblebees dying of natural causes, giving an impression of toxicity. However, Mühlen *et al*. [25] classified only 6% of 4116 dead bumblebees collected under *T. tomentosa* as old, based on characters like loss of pile and wing wear. The vast majority of dead individuals consisted of younger age classes, including young bumblebee queens. These findings led Mühlen *et al*. [25] to discount old age as major cause of bee deaths.

Predators including great tits and wasps attack bees on flowering linden trees [25]. Mühlen *et al*. [25] found 76.1% of 10984 dead bees from *T. tomentosa* had damage indicating predator feeding. Mühlen *et al*. [25] found high variability between trees and seasons for predator damage, with some trees having high death counts but few signs of predation. This suggested predators mostly attacked dying or dead bees, and predation was only a secondary factor.

In conclusion, natural deaths due to old age or predators account for some of the observed bee deaths, but appear insufficient to fully explain the many thousands of deaths recorded by Mühlen *et al*. [25] and others.

4. Starvation

The late flowering period of *T. tomentosa* can coincide with a scarcity of nectar resources in the wider landscape [31]. After the often more abundant linden species *T. platyphyllos*, *T.* x *europaea* and *T. cordata* (generally not linked to bee deaths) have stopped flowering, bees concentrate foraging on the rarer *T. tomentosa* due to missing alternative nectar sources. The large honeybee and bumblebee populations at the flowering time of *T. tomentosa* then face intense competition for remaining nectar [31].

In a detailed temporal study of nectar production, foraging bee species, and dead bees covering the flowering period of *T. tomentosa*, Illies [26] observed an increase of dead bumblebees towards the end of the flowering period. During this time, flowers secrete less nectar, but bumblebees continue visiting [26,56]. This drop in available nectar may lead to large scale starvation [56]. Similarly, Surholt & Baal [57] monitored foragers of a *B. terrestris* colony close to a *T. tomentosa* tree throughout its eleven day flowering period, and found that, coinciding with the cessation of nectar production by the tree at day eight, bumblebee foragers returned from foraging trips without collected nectar. Individually marked workers from the colony were at this point found dead or dying beneath the tree, and the colony died of starvation [57]. Support for the “starvation hypothesis” also comes from the analysis of sugar reserves in bumblebees’ bodies [31]. Foragers dying under *T. tomentosa* had less than a third of the energy reserves left compared to foragers on *T. cordata* or *T. platyphyllos* [31]. Surholt *et al*. [58] reported that paralyzed bumblebees under *T. tomentosa* recover when provided with *T. tomentosa* nectar. Bumblebees feeding on this nectar recovered fully after 30-40 minutes [58]. Honeybees may be better able to deal with late summer nectar shortage because of available honey stores in the colony, possibly explaining fewer dead honeybees under *T. tomentosa* compared to bumblebees [26].

Baal *et al*. [31], Surholt & Baal [57] and Illies [26] thus presented a compelling case for the bee mass deaths on *T. tomentosa* resulting from starvation, and considering current evidence this seems the most likely explanation. However, why this happens is still unknown. The best management decision to avert dead bees under *Tilia* should be to increase late season floral resources in urban environments. This would reduce competition between honeybees and bumblebees. In contrast, felling of *T. tomentosa* would be counterproductive by further reducing available nectar resources and leading to increased bee losses [26]. Linden including Silver Linden are valuable nectar sources for bees [3,9].

Doubts remain, however, whether simple starvation due to insufficient alternative food sources completely explains the phenomenon. Zucchi [59] suggested bee deaths occur under *T. tomentosa* in areas with alternative flowering forage plants based on observations in a flower rich park in Osnabrück and observations by Breinl [60] in a Botanical Garden in Gera (both in Germany). Similarly, we observed 403 dead bumblebees over the flowering period of a single *T. tomentosa* tree at Royal Botanic Gardens, Kew (Richmond, UK) in July 2016, when many other nectar providing plants were still flowering in the surrounding garden (Table 1; Fig. 1).

Bees have been shown to adopt an ideal free distribution across resources [61,62]. This would suggest that if bees are starving on *T. tomentosa*, they should be starving to an equal extent on other flowering plants simultaneously. Bee deaths on *T. tomentosa* would thus only be a “canary in a coal mine”, highlighting a general lack of nectar resources in a particular area. To test the “starvation hypothesis”, bumble bee mortality on *T. tomentosa* and surrounding flowering plants should be compared, and bumble bee colonies foraging in comparable landscapes with and without *T. tomentosa* should be monitored for their food intake and starvation. If bumble bee deaths on *T. tomentosa* are due to simple starvation, similar levels should be observed on *T. tomentosa* and other plants, or in colonies foraging in comparable landscapes with or without *T. tomentosa*. If, however, elevated rates of individual or colony mortality are observed in the presence of *T. tomentosa*, starvation alone cannot account for the observed phenomenon, and alternative hypotheses outlined in this review need to be considered.

5. Chemical deception

Plants can chemically manipulate pollinator behaviour against the pollinators’ best interests, to optimize pollination services at minimal cost. Bee orchids (*Ophrys* spp.) offer bees no nectar reward, but instead mimic female bee sex pheromones to trick corresponding male bees into visiting and transferring pollen [63]. Other plant species may still offer nectar rewards, but chemically induce pollinators to over-value these rewards and visit with greater frequency than would be optimal for pollinators [64].

Despite the continued interest in the bee deaths on *T. tomentosa,* the floral chemistry including nectar, pollen and floral volatiles remains understudied. Bumblebees, and to a lesser extent honeybees, are attracted to linden even at the end of the flowering period, when little nectar is produced [56]. The potent scent of *T. tomentosa* has long been noted [12]. Illies [26] speculated *T. tomentosa* scent may mimic unknown bumblebee pheromones, causing bees to visit without receiving nectar rewards and thus act as a “scent trap”. Returning bumblebee (*B. terrestris*) foragers emit three pheromones within the colonies that recruit idle workers to start foraging: eucalyptol, farnesol and ocimene [65]. All three compounds occur in flower volatiles or nectar of *Tilia* species [49,66,67]. Exposure to these volatiles either on the tree or in the colony through returning foragers with *Tilia* scent could exploit the bumblebees’ sensory bias and increase foraging intensity even at times of low nectar production. However, all three volatiles are common amongst European flowering plants [68]. This suggests that, while the volatiles could have been selected in plants to act as innate stimuli attracting foraging bumblebees, any behavioural effects would not necessarily be unique to *Tilia*. The specific volatiles emitted by *T. tomentosa* flowers should be investigated and compared to species of *Tilia* that are not associated with bee deaths. Their effects on bumblebee foraging behavior and persistence to return to empty flowers should furthermore be tested experimentally with artificial flowers.

Intriguingly, Naef *et al*. [49] reported caffeine (Fig. 1) in *T. cordata* nectar, and Mathon *et al*. [69] detected caffeine in *Tilia* sp. flower tea. Additional studies should verify if, and at what concentrations foraging bees are exposed to caffeine or related alkaloids across different *Tilia* species. We propose that recent experimental studies investigating the effect of caffeine on bees could help explain the mystery behind bee deaths. Wright *et al*. [70] demonstrated caffeine enhances odour memory associated with food rewards in honeybees, predicting this induced greater floral fidelity. This was later demonstrated in free-flying honeybees by Couvillon *et al*. [64] who showed caffeine-laced sugar water increased foraging intensity and recruitment behaviour. Notably, caffeine increased persistence of honeybee foragers to return to previously rewarding but subsequently empty feeders, and increased site specificity, i.e., reducing searching behaviour for other food rewards around the caffeine-laced feeder. Caffeine may allow plants to reduce their nectar investments by misleading bees into making sub-optimal foraging decisions, depleting honey stores despite increased foraging activity [64]. Thomson *et al.* [71] demonstrated nectar caffeine also affects bumblebee foraging behaviour, with ecologically relevant caffeine levels (10^{-5} M) leading to increased deposition of a pollen substitute on artificial flowers.

Given sub-optimal honeybee foraging under the influence of caffeine [64], could *T. tomentosa* similarly manipulate bumblebees to visit after cessation of nectar secretion, until they starve? Certainly, caffeine exposure of bees foraging on *Tilia*, and its resulting effects should be investigated. Studying *T. tomentosa* volatiles and their effects on bees, alongside interactive effects with caffeine on scent-reward association learning [70] using artificial flowers [cf. 71], could help bring two of the more plausible explanations together to understand this extraordinary natural phenomenon.

6. Interactive effects

The interaction of stressors such as pesticides and nutritional deficits is more damaging to pollinators than each stressor in isolation [1]. Similarly, interactions of factors in the preceding five hypotheses could increase bee mortality on *T. tomentosa*. For example, if compounds in *T. tomentosa* paralyze bees, they would be more vulnerable to predation. Nutritionally stressed bees may be more susceptible to effects of toxic metabolites in nectar or pollen. *T. tomentosa* metabolites could interact with insecticides causing additive or synergistic toxic effects. Chemical deception of *T. tomentosa* may be more effective if fewer alternative flowering resources are available in the contiguous landscape. These interactive effects should be considered and tested experimentally.

Conclusion

There is no convincing evidence for direct toxicity of *T. tomentosa* nectar or pollen to bees. Mannose does not occur in *T. tomentosa* nectar, and the hypothesis of mannose poisoning by foraging bees on this tree has been refuted. In isolated cases, neonicotinoid treatment against aphids can explain some mass bee death events, and insecticide treatment of *Tilia* trees should be prohibited. In general, starvation of bees due to insufficient nectar availability is the most likely cause of bee deaths on *T.*

tomentosa. Yet, as the event occurs in the presence of alternative food sources in gardens, starvation alone may not explain the deaths. Starvation rates of individual bees and bee colonies in landscapes with and without *T. tomentosa* trees associated with bee deaths should be investigated. Ensuring alternative floral resources in late summer during *T. tomentosa* flowering could be the best way of avoiding associated bee deaths. *T. tomentosa* flower chemistry (including nectar, pollen and volatiles) remains incompletely known, and should be analysed and experimentally tested for bumble bee toxicity. Further research should determine if *T. tomentosa* can chemically manipulate bee foraging behaviour. A combination of caffeine and *Tilia* volatiles could lead to sub-optimal foraging in bees, in some cases leading ultimately to starvation.

Author contributions

HK drafted the manuscript. HK and PCS revised the manuscript, gave their final approval, and are accountable for its content.

Competing interests

We have no competing interests.

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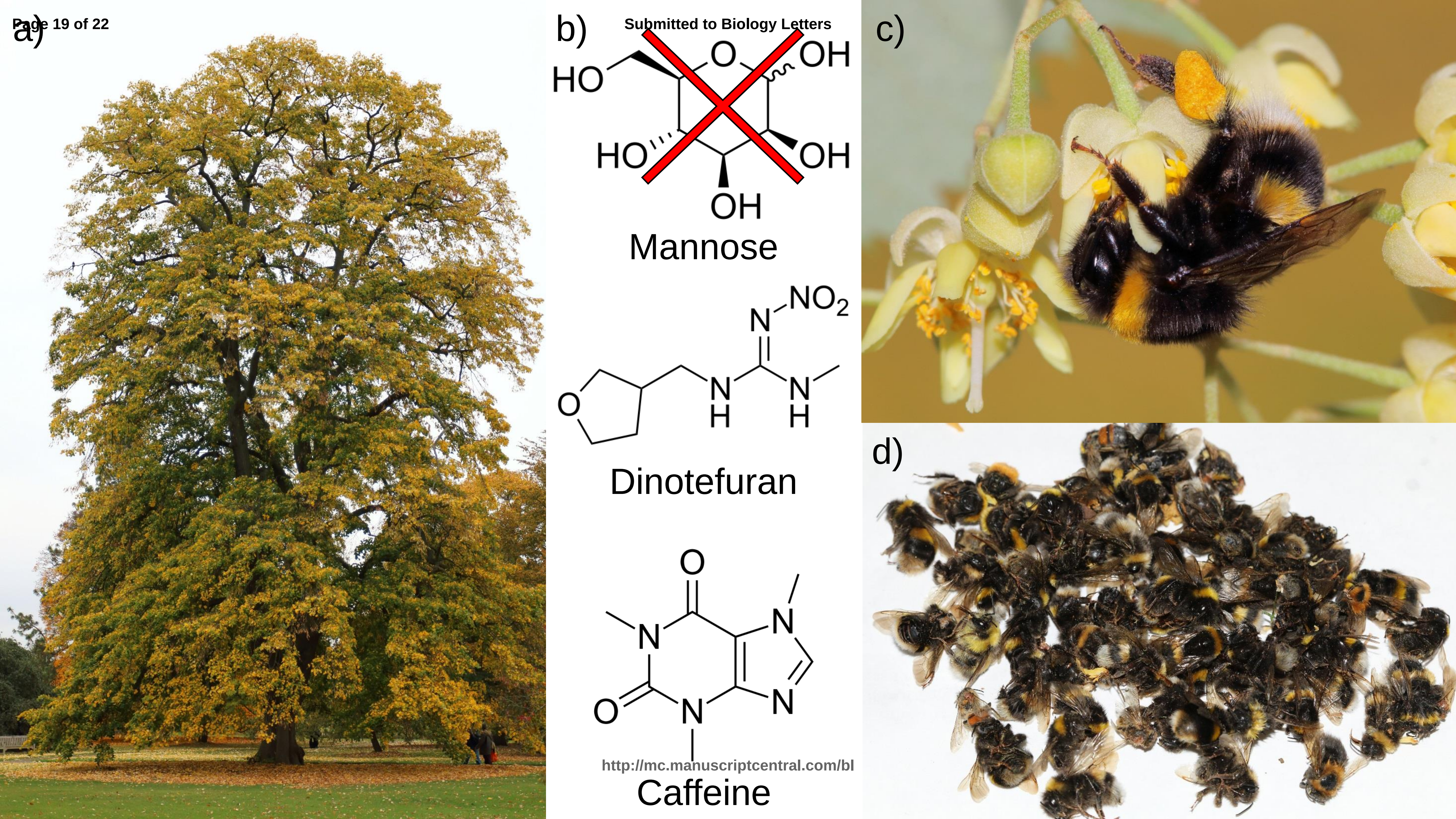
Figures:

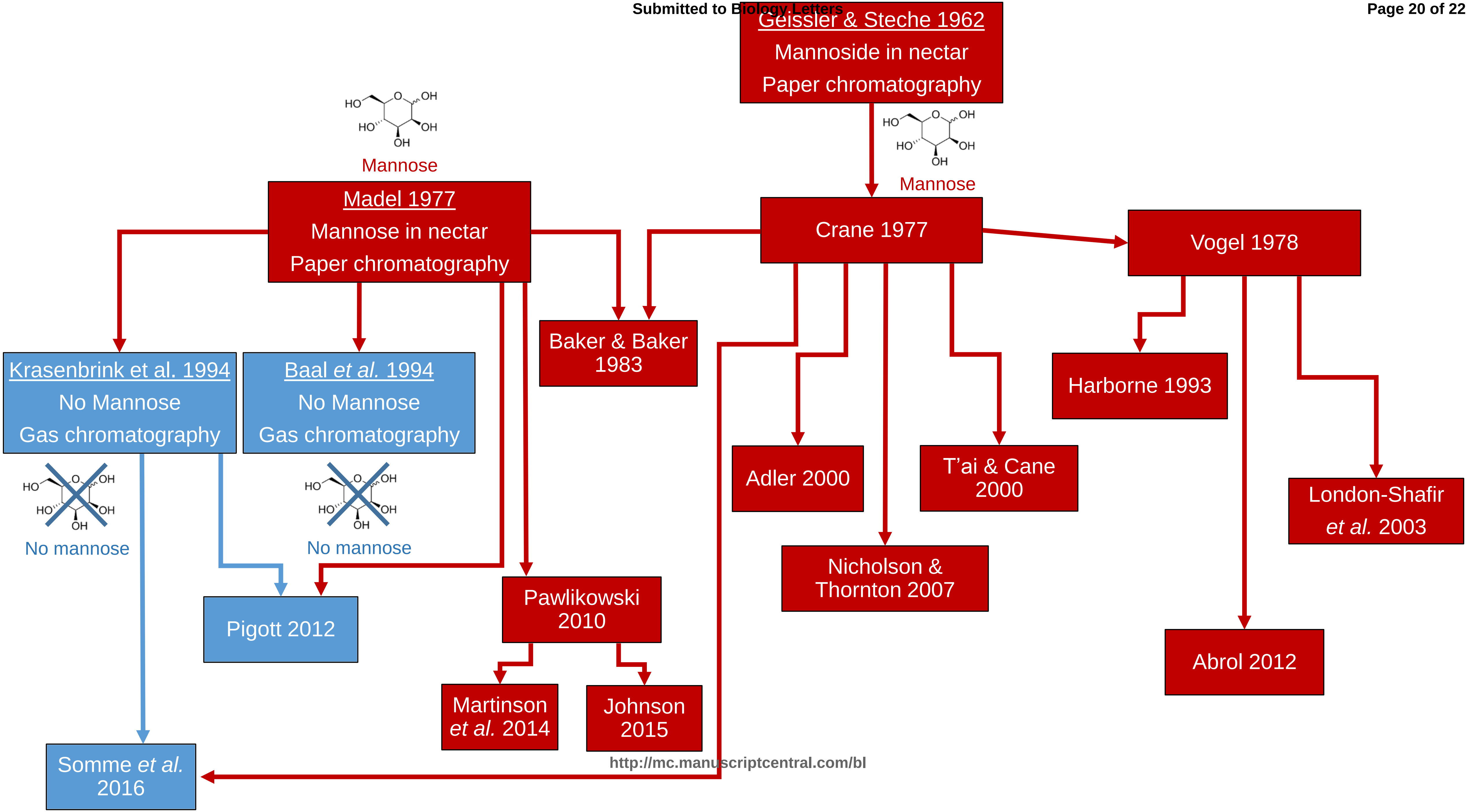
Figure 1: a) Silver Linden (*T. tomentosa* "Petiolaris") at Kew Gardens, UK; b) Chemicals implied in bee deaths c) Buff-tailed bumblebee (*B. terrestris*) worker foraging on *T. tomentosa*; d) Dead bees (*B. terrestris*, *B. hypnorum*, *B. lucorum*, *Apis mellifera*) collected during one day (07/29/2016) under flowering *T. tomentosa*.

Figure 2: Citation pattern of scientific papers discussing bee poisoning on *T. tomentosa* by mannose. Underlined citations are original research studies investigating nectar mannose presence. Red coloured publications suggest mannose as causative agent, blue coloured publications suggest alternative causes.

Table 1: Accounts of bee deaths on linden (*Tilia* sp.).

477 **Table 2**: Hypotheses explaining bee deaths on *Tilia.*


a)
b)
HO
O
OH
HO
OH
OH
Mannose
NO_2
N
N
H
N
H
O
Dinotefuran
O
N
N
O
N
N
Caffeine
c)
d)



Geissler & Steche 1962
Mannoside in nectar
Paper chromatography

Mannose

Madel 1977
Mannose in nectar
Paper chromatography

Mannose

Crane 1977

Vogel 1978

Baker & Baker 1983

Krasenbrink et al. 1994
No Mannose
Gas chromatography

Baal *et al.* 1994
No Mannose
Gas chromatography

Harborne 1993

Adler 2000

T’ai & Cane 2000

London-Shafir *et al.* 2003

No mannose

No mannose

Pigott 2012

Nicholson & Thornton 2007

Pawlikowski 2010

Abrol 2012

Martinson *et al.* 2014

Johnson 2015

Somme *et al.* 2016

Table 1: Accounts of bee deaths on Linden (*Tilia* sp.) trees.

Tilia species	# dead bees	# trees	† bees/tree	City	Country	Date	% *Bombus*	% *Apis*	main species	Notes	Reference
T. tomentosa	417	13	32	Bonn	Germany	29.07.1975	100%	0%	*B. terrestris*	*A. mellifera* not recorded	Madel 1977
T. tomentosa	1937	2	968	Münster	Germany	14.07.2000	83%	17%	*B. terrestris*		Illies 2005
T. tomentosa	1833	5	367	Eberswalde	East Germany	13.08.1987	79%	21%	*B. terrestris*		Donath 1989
T. tomentosa	716	9	80	Berlin	East Germany	07.1988	61%	39%	*B. lucorum*		Donath 1989
T. tomentosa	49	25	2	Casel	East Germany	08.08.1987	100%	0%	*B. lucorum*	Incomplete collection?	Donath 1989
T. tomentosa	1637	1	1637	Steinfurt-Borghorst	Germany	07.1990	99.5%	0.5%	*B. terrestris*		Mühlen et al. 1994
T. tomentosa	1702	1	1702	Steinfurt-Borghorst	Germany	07.1991	?	?	*B. terrestris*	same tree as above	Mühlen et al. 1994
T. tomentosa	1412	1	1412	Steinfurt-Borghorst	Germany	07.1992	?	?	*B. terrestris*	same tree as above	Mühlen et al. 1994
T. tomentosa	2210	1	2210	Münster	Germany	07.1992	?	?	*B. terrestris*		Mühlen et al. 1994
T. tomentosa	300	1	300	Torun	Poland	2003	83%	17%	*B. terrestris*		Pawlikowski 2010
T. tomentosa	1608	1	1608	Dülmen	Germany	07.1993	100%	0%	?	*A. mellifera* not recorded	Surholt & Baal 1995
T. tomentosa	1603	?	?	Osnabrück	Germany	1994	100%	0%	*B. terrestris*	*A. mellifera* not recorded	Zucchi 1996
T. tomentosa	141	1	141	Gera	East Germany	07.1989	89%	11%	*B. terrestris*	Incomplete collection	Breinl 1990
T. tomentosa "Petiolaris"	"hundreds"	1	"hundreds"	Innsbruck	Austria	07.1994	?	?	*Bombus* spp.		Schedl 2015
T. tomentosa "Petiolaris"	403	1	403	Richmond	UK	05.08.2016	99%	1%	*B. terrestris*	Kew Gardens	H. Koch pers. obs.
T. tomentosa "Petiolaris"	?	1	?	Tortworth	UK	1908	?	?	?	dead bees "manured ground"	Elwes & Henry 1913
T. tomentosa "Petiolaris"	660	1	660	Gera	East Germany	07.1989	67%	33%	*B. terrestris*	Botanical Garden	Breinl 1990
T. tomentosa "Petiolaris"	86	1	86	Gera	East Germany	07.1989	83%	17%	*B. terrestris*	Incomplete collection	Breinl 1990
T. x *euchlora*	247	30	8	Luckau	East Germany	02.08.1987	71%	29%	*B. hypnorum*		Donath 1989
T. x *euchlora*	82	11	7	Erfurt	East Germany	24.07.1988	93%	7%	*B. lapidarius*		Donath 1989
T. x *euchlora*	983	1	983	Steinfurt-Borghorst	Germany	07.1990	97%	3%	*B. terrestris*		Mühlen et al. 1994
T. x *euchlora*	816	70	12	Gera	East Germany	07.1989	72%	28%	*B. terrestris*	Incomplete collection	Breinl 1990
T. x *euchlora*	336	1	336	Gera	East Germany	07.1989	89%	11%	*B. terrestris*	Incomplete collection	Breinl 1990
T. x *euchlora*	372	50	7	Gera	East Germany	07.1989	67%	33%	*B. terrestris*	Incomplete collection	Breinl 1990
T. cordata	10	1	10	Münster	Germany	22.06.2000	80%	20%	*B. terrestris*		Illies 2005
T. cordata	534	4	134	Steinfurt-Borghorst	Germany	07.1990	77%	23%	?		Mühlen et al. 1994
T. cordata	12	1	12	Torun	Poland	2003	50%	50%	*B. terrestris*		Pawlikowski 2010
T. cordata	50000	55	909	Wilsonville	USA	13.06.2013	100%	0%	*B. vosnesenskii*	Killed by Dinotefuran?	Black & Vaughan 2013
T. platyphyllos	40	2	20	Münster	Germany	15.06.2000	71%	29%	*B. terrestris*		Illies 2005
T. platyphyllos	78	1	78	Berlin	East Germany	13.06.1988	5%	92%	*A. mellifera*		Donath 1989
T. platyphyllos	373	4	93	Steinfurt-Borghorst	Germany	07.1990	62%	38%	?		Mühlen et al. 1994
T. spp.	608	102	6	Linz	Austria	04.08.1978	41%	59%	*A. mellifera*	Incomplete collection	Pfitzner 1978

Table 2: Hypotheses explaining bee deaths on *Tilia*

Hypothesis	Prediction	Supporting evidence	Opposing evidence	Research need
1. Toxic *Tilia* metabolites	Toxic metabolites in *Tilia* nectar or pollen with lethal or sub-lethal effects on bees	Affected bees appear paralyzed before dying [13]; suggestion of mannose (toxic to bees) in *Tilia* nectar based on limited paper-chromatographic investigations [13,32]	No detection of mannose by gas-chromatography in *T. tomentosa* nectar or dead bees; no experimental evidence for toxicity of *T. tomentosa* nectar [31,36]	Detailed chemical analysis of *Tilia* pollen and nectar metabolites, experimental tests of toxicity
2. Insecticides	Insecticide (e.g. neonicotid) application to *Tilia* trees killing bee foragers	Prior application of neonicotinoids to *Tilia* recorded in isolated cases [19,48]	Phenomenon existed before use of neonicotinoids [12,13,24], most cases without known previous insecticide application (see Table 1)	Persistence of neonicotinoids in *Tilia* and exposure of bees from *Tilia* pollen and nectar when neonicotinoids are applied outside flowering period
3. Natural causes: Predators / old age	Dead bees due to background mortality from e.g. predators and old age	*T. tomentosa* flowers during the end of the colony cycle of some bumblebee species; birds and wasps observed preying on bees on flowering *Tilia* [25]	Majority of dead bees are not old, bee deaths also occur without predator attacks [25]	Additional quantification of background mortality from predation or old age of bees foraging on *Tilia*
4. Starvation	Dead bees due to Insufficient nectar resources during *T. tomentosa* flowering period causing starvation	Most deaths occur at end of *Tilia* flowering period when nectar production is very limited [26,56,57], foragers on *T. tomentosa* have depleted body sugar reserves [31], dying bees can recover when fed *Tilia* nectar [58], scarcity of alternative nectar resources during *T. tomentosa* flowering suggested [31,57]	Bee deaths can occur when alternative food sources are available [59,60]	Comparison of bumblebee mortality on *T. tomentosa* and nearby plants flowering simultaneously; comparison of colony resource intake and mortality in comparable landscapes with and without *T. tomentosa*
5. Chemical deception	Chemical deception (e.g. by volatiles, caffeine) causes overvaluation of *Tilia* as resource and increased foraging persistence once nectar is depleted, leading to starvation	Presence of caffeine in *Tilia* honey [49], caffeine modulates bee foraging, increasing persistent return to depleted food sources and causing overvaluation of sugar rewards [64,70,71], presence of volatile compounds in *Tilia* flower scent that act as foraging recruitment pheromones in bumblebees [49,66,67]	Known *Tilia* flower volatiles are common in plants not associated with bee deaths [68]	Analysis of volatiles from *T. tomentosa* flowers; exposure of bees to caffeine on *Tilia* and effects on foraging behaviour; interaction of *T.tomentosa* volatiles and caffeine in reward association learning
6. Interactive effects	Bee deaths due to interaction of factors in hypotheses 1-5	Plausible, but not investigated	Not investigated	Interactions between factors in hypotheses 1-5 should be studied