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Title: Bees Associated with Linden (*Tilia* spp.) Trees and their Susceptibility to Toxic Sugars in Nectar

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Bees provide critical pollination services to diverse agricultural crops, native plants and trees. Globally, there are reports of bee declines which have been attributed to diseases, exposure to pesticides and changes in land use that are believed to have led to a reduction in foraging resources and nesting habitats for native bees. Other factors such as low nectar production caused by water stress in plants or toxins present in nectar may also be responsible but have received little attention. Risks associated with foraging behaviors are particularly critical as bees spend considerable time seeking food resources. Linden, a common ornamental tree in urban areas, produces an abundance of nectar and pollen, and thus benefits bees. However, in the late 1970's, dead bees were observed under linden in Europe when environmental conditions were dry. European researchers speculated that the causal factor was the presence of the sugar mannose in linden nectar under drought stress. Mannose is similar in structure to glucose which is used by bees as a carbohydrate source. The toxicity of mannose was believed to be due to disruption of glucose metabolism resulting from competition between mannose and glucose for the enzyme hexokinase during the glycolysis cycle that provides energy for bees. In laboratory studies mannose and galactose were shown to be toxic to honey bees. Their impacts on bumble bees were, however, not determined. There is little information available about the associations of bees with linden in the USA. Occasionally dead

bumble bees have been observed under linden trees in western Oregon in the west coast of the USA. The current study was conducted to: 1) Examine bloom and nectar production in linden, correlate nectar production with environmental conditions, and with diversity and abundance of foragers; and 2) Determine the impacts of mannose and galactose on honey bees and bumble bees.

The study was conducted in 2014 and 2015 in the city of Corvallis in western Oregon. Honey bees, five species of bumble bees, solitary bees (*Halictus* spp.), yellow jackets and dipterans (primarily syrphids), visited four species of linden surveyed during bloom. Honey bees were the dominant foragers, and accounted for 69% of foragers in 2014 and 84% in 2015. Nectar production in linden flowers was highest in the morning, and was positively correlated with relative humidity and negatively with temperature. However, there was no correlation between nectar production and the abundance of foragers over both years. A preliminary HPLC analysis of linden nectar samples collected from three linden trees showed a peak with the same retention time as a mannose standard. Further analyses are needed for confirmation of the presence of mannose in the nectar of linden. In a laboratory bioassay, mannose and galactose were toxic to both honey bees and bumble bees. However, when the toxic sugars were presented to honey bees and bumble bees in combination with the non-toxic glucose, the toxic impact was significantly lower ($p < 0.05$) if the proportion of glucose was high (90%) compared to combinations with lower proportions (10% or 50%) of glucose. These results provide support for the hypothesis that mortality of bees when exposed to mannose is due to competition with glucose for the hexokinase enzyme during glycolysis. However, it is still not known why higher bumble bees have been reported to die after foraging on linden when honey bees are the dominant foragers on linden, and are susceptible to the toxicity of mannose. It is possible that honey bees and bumble bees differ in their ability to assess the presence of toxins in nectar or that other factors are involved. Further research is needed for determining differences, if any, in the foraging behaviors of honey bees and bumble bees on linden trees, and for detecting other nectar compounds in linden that may differ in their impacts on different species of bees.

Bees Associated with Linden (*Tilia* spp.) Trees and their Susceptibility to Toxic Sugars in Nectar

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Chapter I
General Introduction

Bees provide critical pollination services to diverse agricultural crops, native plants and trees, and thus help sustain crop production and our natural resources. However, while the demand for bee pollinated crops increases each year with human population growth, there are global reports of bee declines (Kremen et al., 2007; Goulson et al., 2008; Grixti et al., 2009). Many factors are believed to be responsible including diseases, exposure to pesticides and, in the case of honey bees, the colony collapse syndrome. For native bees, changes in land use are believed to have led to a reduction in foraging resources and nesting habitats. Other factors may also be responsible for bumble bee mortality but have received little attention to date.

Risks associated with foraging behaviors are particularly critical as bees spend considerable time seeking food resources. Bees forage on multiple plants, and hence species that pollinate crops are affected by diverse factors across the landscape. If high numbers of bees die suddenly, plants/crops with blooming periods later in the same year as well as in the following year will be affected.

Linden (*Tilia* spp.; Malvaceae), also known as lime or basswood, is a common ornamental tree in urban landscapes. It produces an abundance of fragrant flowers that draws diverse foragers (Anderson, 1976). Nectar and pollen produced by linden benefits bees. However, in the late 1970's, many dead bumble bees and a few honey bees were observed under linden in Europe when environmental conditions were dry. European researchers speculated that the causal factor was the presence of the sugar mannose in linden nectar under drought conditions (Crane, 1977). Mannose is a monosaccharide that is very similar in structure to glucose which is used by bees as a primary carbohydrate source. Its toxicity to bees was speculated to be due to disruption of glucose metabolism resulting from competition between mannose and glucose for the enzyme hexokinase during the glycolysis cycle that provides energy for bees (Sols et al., 1960). Studies by Saunders et al. (1969) and van Handel (1971) refuted the competitive inhibition of glycolysis hypothesis but a later study by de la Fuente (1986) provided support. Mannose

and galactose, which is also a monosaccharide, has been shown to be toxic to honey bees (von Frisch, 1950; Sols et al., 1960; Saunders et al., 1969; van Handel, 1971; Barker and Lehner, 1974; de la Fuente, 1986). Impacts of mannose and galactose on bumble bees are, however, not known. There is also little information available about the associations of bees with linden in the USA. Occasionally dead bumble bees have been observed under linden trees in western Oregon in the west coast of the USA (Rao, 2016).

In this dissertation, I explore the association of pollinators with linden trees in western Oregon. In Chapter 2, I describe a survey that I conducted for assessing the diversity and abundance of pollinators foraging on four *Tilia* species. I made observations on: 1) periods of bloom in each species, 2) nectar production throughout bloom, and 3) the diversity and abundances of foragers on each *Tilia* species. In addition, correlations were determined between nectar production and: 1) environmental variables (temperature and humidity), and 2) the abundance of foragers on linden.

In Chapter 3, I compared the impacts of galactose and mannose with those of fructose, glucose and sucrose on honey bees and bumble bees. Laboratory bioassays were conducted to compare mortality of honey bees and bumble bees when presented with toxic (mannose and galactose) and non-toxic sugars. In a second experiment, the dose responses of mannose and galactose to honey bees and bumble bees were determined. A third bioassay was conducted to determine the impacts of these two toxic sugars in the presence of varying levels of the non-toxic glucose.

Chapter 4 synthesizes the results of all studies, provides an assessment of the linden-bee mortality phenomenon, and offers suggestions for future research for understanding the differential impacts of linden trees on honey bees and bumble bees.

Chapter II

Diversity and abundance of foragers on trees of linden (*Tilia* spp.) in Oregon

Introduction

Linden (*Tilia* spp.; Malvaceae), also known as lime or basswood, is a common ornamental tree in urban landscapes. Worldwide, there are 30 species of *Tilia.* A single species, *T. americana* is native to the US but several European species and cultivated hybrids are planted nationwide. Linden has economic importance as linden honey is valued in countries like Poland (Weryszko-Chmielewska and Sadowska, 2010; Waś et al., 2011). In addition, linden has medical importance as flower infusions have diuretic and anti-inflammatory properties (Konarska, 2013).

Linden trees produce an enormous amount of nectar and pollen in summer. There can be 4-40 flowers/inflorescence which produce strong odors that persist throughout the blooming season. Flowers of the same umbel do not open at the same time and all stages can be found in the same inflorescence. Flowers of *Tilia* open for the first time late in the afternoon release a significant amount of pollen for 24 hours and start producing nectar on the second day when the flower is fully opened and the stigma has matured (Anderson 1976). According to Weryszko et al. (2010) one flower of *Tilia cordata* is able to produce 43,000 pollen grains, while an inflorescence produces 200,000 pollen grains.

Linden trees draw a great diversity of foragers during bloom. A study on the pollination biology of linden conducted in eastern (Connecticut) and midwestern (Nebraska) USA by Anderson (1976) indicated that 66 insects belonging to 29 families visited *T. americana*, *T. cordata*, and *T. platyphyllos*. These included honey bees, bumble bees and solitary bees. However, sampling details and the abundance of each forager species were not provided. Subsequent studies (Illies and Muhlen, 2007; Pawlikowski, 2010) on foragers of linden conducted in Europe were focused on bees. In the study by Pawlikowski (2010) conducted in Poland, honey bees were the dominant (~ 90%) foragers on *T. cordata* and *T. tomentosa* while in the study conducted in Germany by Illies and Muhlen (2007), honey bees were dominant (55%) but to a lesser extent.

While linden trees are beneficial to bees, on occasion bees have been observed to die after foraging on linden flowers. In the 1970's, many bumble bees and a few honey bees were observed lying dead, paralyzed or drunk under linden trees in Europe in regions where the soil drained quickly, or in years when the summer was exceptionally dry (Crane, 1977). A few dead bumble bees were observed under linden trees in a study conducted in Poland by Pawlikowski (2010) though drought stress was not mentioned. Similar observations of bumble bee mortality associated with linden trees under non-drought conditions were also reported from Oregon on the west coast of the USA (Rao 2016) but no information was provided on other foragers visiting linden trees.

The objectives of this study were to compare the following across *Tilia* species: 1) periods of bloom; 2) nectar production; 3) diversity and abundance of foragers. In addition, we were interested in determining if there were correlations between nectar production and 1) environmental conditions; and 2) forager abundance.

Materials and Methods

Study Site

The study was conducted in 2014 and 2015 on linden trees in urban areas in the city of Corvallis (44.567843, -123.282166) in western Oregon on the west coast of USA.

Periods of Bloom

Six trees belonging to each of three *Tilia* species, *T. americana, T. cordata*, and *T. tomentosa* were surveyed in 2014. One additional species, *T. platyphyllos*, was included in 2015. All trees were observed for determining the start and end of bloom in each species.

Nectar Production

A preliminary survey was conducted to determine the daily pattern of nectar production in linden flowers. Nectar was collected (as described below) from 20 individual linden flowers on trees every two hours from 7AM until 7PM. Nectar was found to be produced only once a day, prior to 7 AM. For this study, nectar was collected

in the morning to minimize losses due to evaporation as the temperature rose during the day.

The day before each forager survey, two to three umbels were enclosed in organza bags (11.43 x 8.89cm) to prevent foragers from feeding on the nectar (Fig 2.1). Five organza bags were attached to each survey tree. The following day, the organza bags were removed, and nectar was collected in the morning (7 AM). From the five bags, 10 flowers/bag were selected randomly for collection of nectar present from each flower. More flowers than needed were collected as only fully opened flowers were selected for estimation of nectar. In addition, in 2014, on a single day during peak bloom, nectar production per flower was determined by estimating the amount of nectar present in each of 50 flowers per *Tilia* species.

Nectar was collected using 15 µl glass microcapillary tubes (Drummond Scientific) and measured in mm by placing the capillary tube adjacent to a ruler (Fig 2.2). All nectar droplets/flower were carefully collected with minimal damage to the petals. Nectar was stored at -20°C.

The following formula was used to transform values from mm to µl.

$$\frac{L_{Nectar}\ (mm) * V_{Microcap}\ (\mu L)}{L_{Microcap}(mm)}$$

L_{Nectar}= Quantity of nectar collected measured in mm

$V_{Microcap}$= 15 µl (Total volume of the microcapillary tube)

$L_{Microcap}$= 54 mm (Total length of the microcapillary tube)

For determining if mannose is present in linden nectar, nectar samples (collection of 50 flowers) from the three *Tilia* trees (2 *T. tomentosa*, and 1 *T. americana*) surveyed in 2014 were analyzed for sugars using High-performance liquid chromatography (HPLC) by Robert Durst at the Linus Pauling Science Center at Oregon State University.

Environmental Conditions

Mean daily temperature (°C) and relative humidity (%) were obtained from the AgriMet System Station CRVO (Corvallis Oregon Weather Station 5N 3E).

Diversity and Abundance of Foragers

The survey of foragers on linden trees was conducted using protocols adapted from those used by Illies and Muhlen (2007) and Pawlikowski (2010). The six trees belonging to each *Tilia* species were monitored for foragers three times a week from the start to the end of bloom. Observations were made in the morning (7 AM to 9 AM) when nectar quantities were expected to be the highest during the day.

Linden trees included in the survey varied in tree height and crown diameter, and hence forager observations were made by standing 2 m away from the edge of the crown of the tree, and recording foragers on flowers within a 3 m x 3 m region of the crown. Observations were made over 2 minutes each while standing on four sides (north, south, east and west) of each tree. Thus, foragers were counted for a total time interval of 8 minutes per tree. The numbers of foragers belonging to each of five forager groups (honey bees, bumble bees, halictids, dipterans, and wasps) were recorded. Samples of each bee groups were collected for confirmation of identification and deposition of voucher specimens at Oregon State University. Bumble bees are easily recognized due to variation in body colors (Koch et al. 2012), and hence these were further identified to the species level.

Data Analysis

For the study on nectar production, the data were subjected to a one way ANOVA with nectar quantity as the dependent variable and *Tilia* species as the independent variable. A two-way ANOVA was used for analysis of the forager data with the abundance of foragers as the dependent variable, and forager group and *Tilia* species as two independent variables. ANOVA analyses were performed at a significance level of $\alpha = 0.05$ using SPSS.

Pearson correlations were performed to determine if there were any correlations between nectar production and the two environmental conditions (temperature and relative humidity), and with the abundance of foragers. Pearson correlation coefficients were analyzed at the five percent level using SPSS.

When significant differences were observed, pairwise comparisons were made using the Tukey's HSD (honest significant difference) test using R 3.2.2. Tests were performed at a significance level of $\alpha = 0.05$.

Results

Periods of bloom

The sequence of bloom across the *Tilia* spp. surveyed was the same over the two years of the study. *Tilia platyphyllos* was the first species to bloom followed by the native species *T. americana* which was in turn followed by *T. cordata,* while *T. tomentosa* was the last to bloom. The duration of bloom per *Tilia* species lasted for 20-21 days in 2014 and 8 to 14 days in 2015 (Table 2.1).

Nectar Production

Nectar production varied across the *Tilia* species during 2014 ($F_{2,20} = 16.04$, $p<0.001$) and 2015 ($F_{3,16} = 19.43$, $p<0.001$) (Table 2.1). Based on the Tukey HSD test, the native *T. americana* had significantly ($p< 0.05$) higher nectar production during the two years while other *Tilia* species did not differ in nectar production in either year (Table 2.2, 2.3, Fig 2.3). The mean nectar production per individual flower of *T. americana* was 7.3 µl (n=50), which was more than double the amount of nectar produced per flower in the other species (Table 2.1).

In general, all *Tilia* species produced more (1.3 to 2.5 times) nectar during 2015 despite the shorter blooming period compared with 2014.

Based on the HPLC analysis, all three nectar samples contained fructose, glucose, raffinose and sucrose (Fig.2.4). In addition, a peak with the same retention time as a

mannose standard was also recorded in two samples (Fig 2.4, 2.5). The remaining peaks could not be identified.

Environmental Conditions

In 2014, there was a significant correlation between nectar production and both environmental variables (temperature Pearson r= -0.47, p=0.022; relative humidity Pearson r= 0.58, p=0.003). Nectar production decreased with rising temperature and increased with higher humidity. In 2015, the correlation was not significant for both environmental variables (temperature Pearson r= -0.11, p=0.65; relative humidity Pearson r= 0.22, p=0.37) but the trend was similar to that observed in 2014 (Fig 2.6).

Insect foragers

Over both years of the study, a total of 11,731 foragers were recorded on all species of *Tilia* surveyed. These included honey bees, bumble bees, solitary bees (halictids), yellow jackets and dipterans (primarily syrphids) (Table 2.4).

Foragers did not show any preference for a particular species of tree (p>1.0) but the numbers of foragers and the diversity differed across the two years of the study (p<0.01) (Table 2.5, 2.6, Fig 2.7). In 2014, of the 2121 foragers observed, 69% were honey bees and 8% were bumble bees (Table 2.4). In 2015, of the 9610 foragers, 84% were honey bees and 13% bumble bees. Halictids, yellow jackets and dipterans accounted for 23% of foragers in 2014 and only 3% in 2015 (Table 2.4).

Over both years, five species of bumble bee foragers were observed: *B. griseocollis, B. melanopygus, B. mixtus, B. nevadensis* and *B. vosnesenskii.* In both years, *B. vosnesenskii* was the most abundant (Fig 2.8).

The forager abundance was not correlated with nectar production in either year of the study (2014 Pearson p= 0.78, 2015 Pearson p=0.94) (Fig 2.9). Figure 2.10 shows the daily temporal variation in abundances of honey bees and bumble bees relative to nectar availability.

Discussion

This is the first study that has examined diversity and abundance of foragers on linden trees in western Oregon. All four species of linden included in the study attracted honey bees, bumble bees, solitary bees (*Halictus* spp.), yellow jackets and syrphids. As indicated by Anderson (1976), *Tilia* is a generalist in regard to pollinators. In the current study, and in the study by Pawlikowski (2010) conducted in Poland, there were higher numbers of honey bees compared to any other bee across all *Tilia* spp. In the study conducted in Germany by Illies and Muhlen (2007), higher abundance of honey bees compared to bumble bees were recorded on *T. cordata* and *T. tomentosa* but bumble bees were more abundant on *T. platyphyllos.* Based on all these studies, it is surprising that in reports of dead bees from Europe and the US associated with linden, high numbers of bumble bees and few honey bees, if any, were mentioned (Crane, 1977; Rao, 2016).

In the current study, five species of bumble bees were observed foraging on linden though *B. vosnesenskii* was the most dominant. Studies on pollinators in western Oregon have recorded the presence of the same five species. A sixth species, *B. appositus* is also present in regions around Corvallis (Rao and Stephen, 2010). It was not observed in the current study possibly because it develops later in the summer (Koch et al. 2012). Multiple species of bumble bees foraging on linden were also reported in the USA study by Anderson (1976), and in the studies conducted in Europe by Illies and Muhlen (2007), and Pawlikowski (2010).

The linden species surveyed in the current study are the main species planted in western Oregon. Overall bloom across the four *Tilia* species lasted 49 days in 2014 and 29 days in 2015. In a study by Anderson (1976) conducted in Nebraska and Connecticut, bloom across *T. americana, T. cordata* and *T. platyphyllos* species lasted ~ 38 days. *Tilia tomentosa* was not included in that study. Thus, linden trees can provide foraging resources for bees for several weeks in the summer when only few other plant species are in bloom. They are valued as a landscape tree, but are also beneficial to bees due to the abundance of food resources that they provide.

The linden species included in the study differed in nectar production. The native *T. americana* has large flowers and produced more nectar than the introduced European species while *T. cordata*, *T. platyphyllos* and *T. tomentosa* did not differ in nectar production. However, in a study conducted in Europe by Illies and Muhlen (2007), *T. tomentosa* had the highest production of nectar, followed by *T. platyphyllos*, while *T. cordata* produced the least amount of nectar. Even though nectar production in *T. platyphyllos* was lower than *T. americana* in the current study, it has benefits for bees as it is the first to bloom. Similarly, *T. tomentosa* may be particularly beneficial to bees as it is the last to bloom, and could thus be a valuable food source towards the end of summer.

The lack of correlation between foragers and nectar production in linden recorded in the current study is surprising. It is possible that higher numbers visited the trees beyond the two hour sampling period of our study. In a study by Illies and Muhlen (2007), no correlation between nectar availability and foraging of bumble bees was observed over the course of the day but when the entire blooming period was considered, the numbers of honey bees were positively correlated with nectar production in *T. cordata* and *T. tomentosa* but not *T. platyphyllos.*

In the current study, we noted that nectar is produced once in the early morning and no additional nectar is produced later. Illies and Muhlen (2007) also reported that nectar production in linden was high in the morning. However, Anderson (1976) noted that nectar production increased in the afternoon in a study conducted in eastern and midwestern regions in the US where humidity is high in the summer unlike Oregon where the summers are dry.

When plants are under water stress, investment of energy in flower and nectar production is high and costly to the plant, and hence, if temperature increases, the plant invests in production of fewer and smaller flowers (Nicholson and Thornburg, 2007). This may be the reason why the flowering period was shorter in 2015 when the temperature was higher compared to 2014. We observed that flowers of *T. cordata,* in particular, senesced rapidly in 2015. In addition, we noticed that, during extremely warm days, flowers were wilted and shriveled, and the nectar produced was sticky and difficult

to collect with the capillary tube. Flower shape, corolla opening stage and size, temperature and humidity influence rates of evaporation of nectar (Nicolson et al., 2007). The morphology of linden flowers is such that the nectar is fully exposed which could lead to rapid evaporation and result in the nectar becoming sticky during warm days. The negative correlation recorded between nectar production and temperature in the current study is thus not surprising. The study does not support the speculation by Konarska (2013) that nectar production of linden trees increases with warm and sunny weather.

In the analyses of sugars in nectar samples conducted by Illies and Muhlen (2007), no mannose was detected. Based on the current study, mannose may be present in the nectar of linden. For confirmation of the presence of mannose additional analysis with mass-spectrometry are needed.

In the current study, a few (< 10) bumble bees were noted to be crawling and unable to fly under three of the six *T. tomentosa* trees surveyed. No dead bumble bees were recorded under any of the other *Tilia* spp. Honey bees were not observed dead under any linden tree surveyed. Similar results were reported from the study in Poland by Pawlikowski (2010). In the study by Illies and Muhlen (2007), a few bumble bees were found dead below *T. cordata* and *T. platyphyllos* while highest numbers were observed under *T. tomentosa*. Dead honey bees were also observed under *T. tomentosa* though in much lower abundance compared with bumble bees.

In summary, linden trees in western Oregon attract diverse foragers including honey bees, bumble bees and halictids bees. The four linden species surveyed in Oregon bloom at slightly different periods and thus serve as valuable food resources for bees over several weeks in summer. While nectar production was influenced by temperature and relative humidity, it did not appear to impact the abundance of foragers. Mannose may be present in linden nectar but this needs confirmation. Further research is needed to determine why higher bumble bees have been reported to die after foraging on linden when honey bees are the dominant foragers on linden, and are susceptible to the toxicity of mannose.

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Figure 2.1. Inflorescence of linden covered with organza bag in the evening to prevent foragers from collecting nectar prior to nectar estimation the following morning.

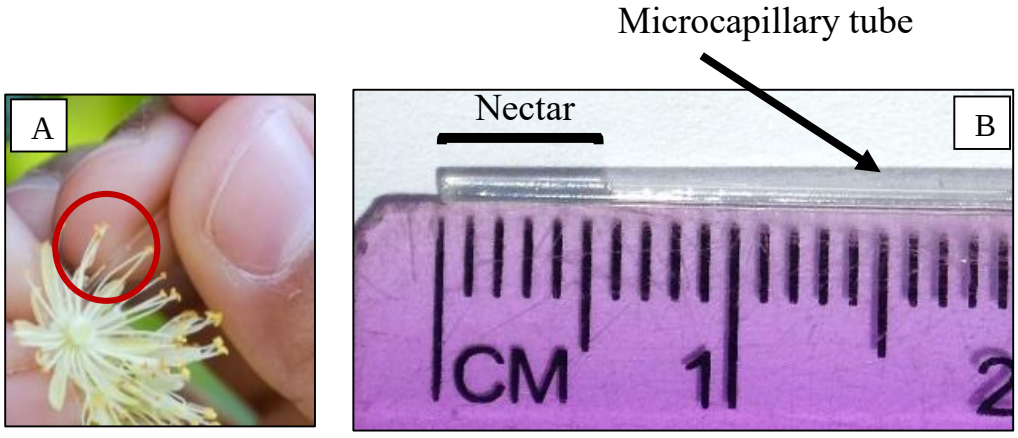

Figure 2.2. A. Nectar collection from linden flowers using a microcapillary tube. **B**. Estimation of the quantity of nectar collected from a single linden flower (in mm) using a ruler.

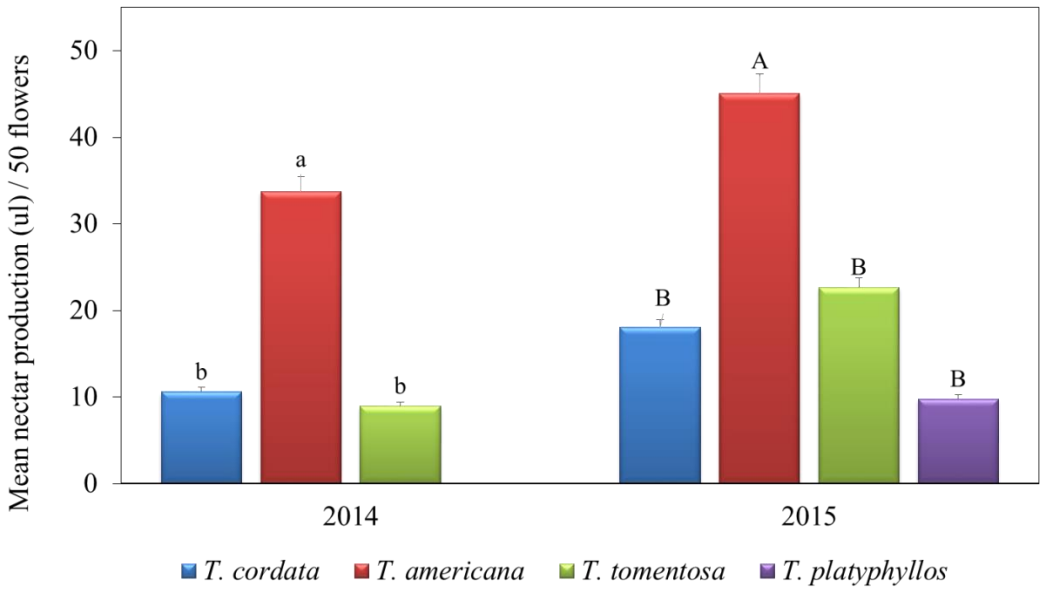

Figure 2.3 Mean (± SE) nectar in flowers of linden trees surveyed in 2014 and 2015. Bars with different letters in each year are significantly different at $\alpha = 0.05$ (Tukey HSD multiple means comparison).

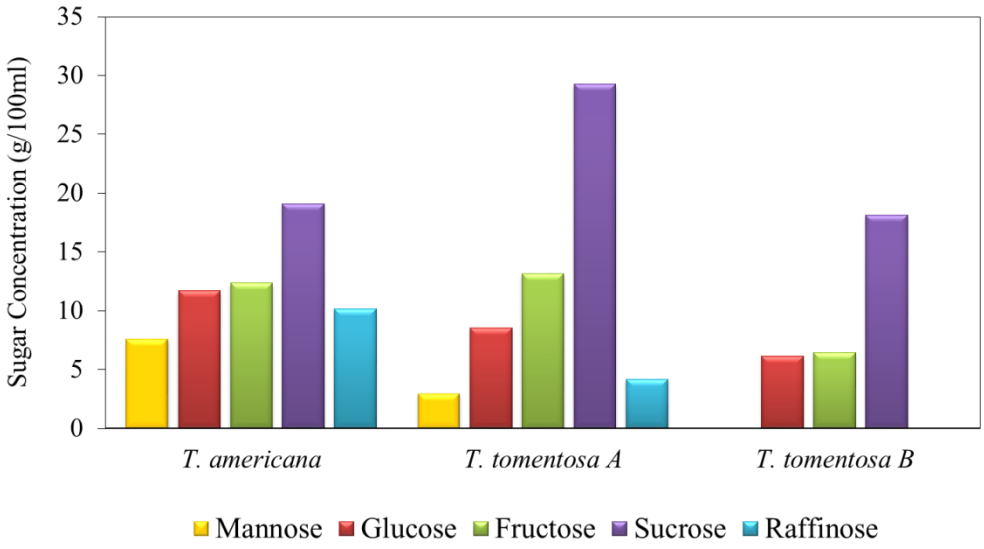

Figure 2.4. Composition of sugars in linden nectar samples (dilution = 10,000) analyzed by HPLC.

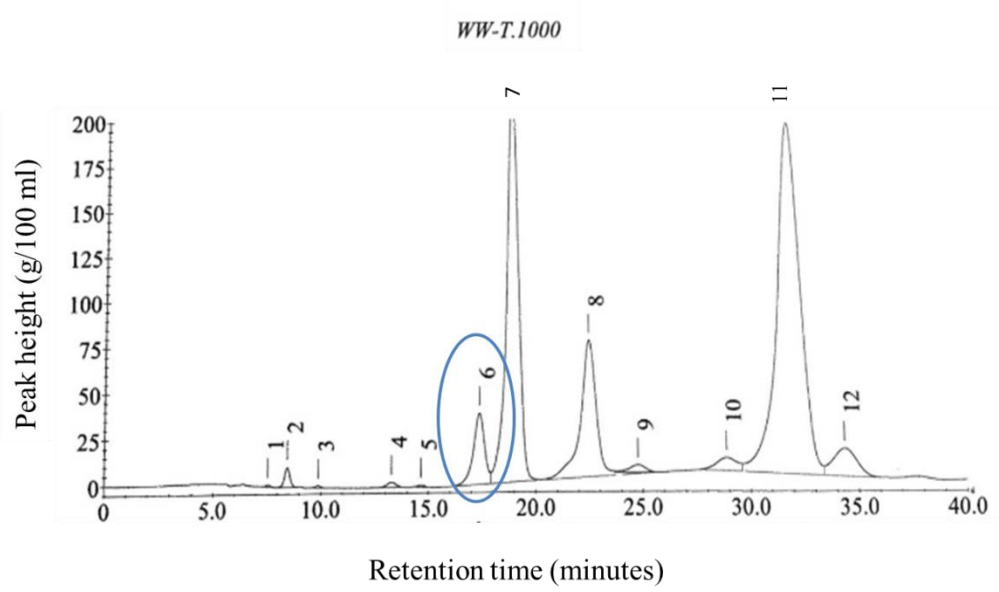

Figure 2.5. HPLC chromatogram of sugars in nectar sample from *Tilia tomentosa A.* Numbers correspond to different sugars identified based on the retention time of standards run on the same HPLC column: 6-Mannose, 7-Glucose, 8-Fructose, 11-Sucrose, 12-Raffinose. *Courtesy*: Bob Durst.

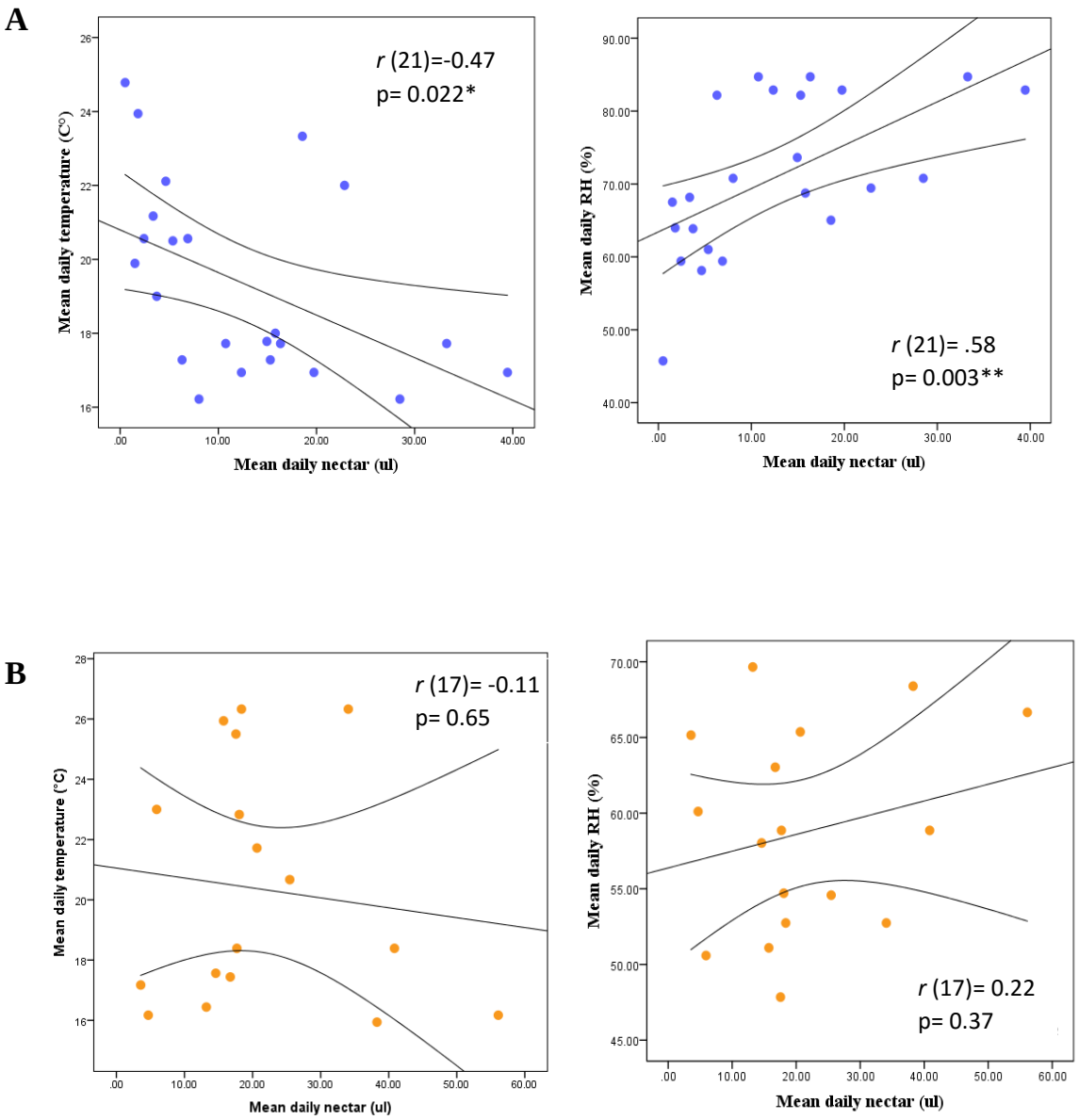

Figure 2.6. Correlation between environmental conditions and mean daily nectar production in 2014 (**A**) and 2015 (**B**). n=10. Mean daily values presented for temperature (on left) and Relative Humidity (on right). * Significant at the 0.05 level (2-tailed). ** Significant at the 0.01 level (2-tailed).

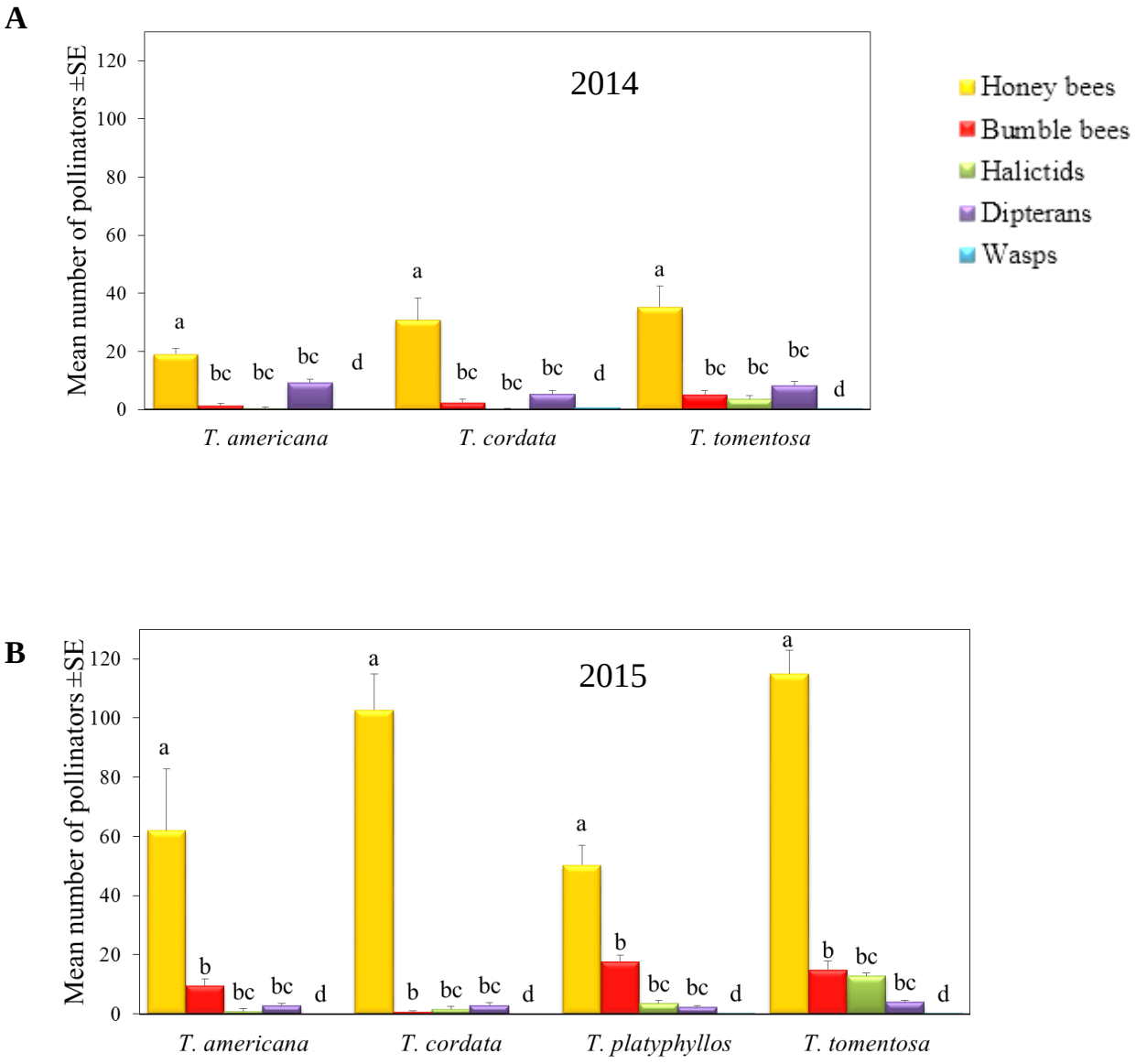

Figure 2.7. Abundance (Mean + SE) of five main forager groups observed on various *Tilia* species in 2014 (**A**) and 2015 (**B**). Bars with different letters are significantly different at $\alpha = 0.05$ (Tukey HSD multiple means comparison).

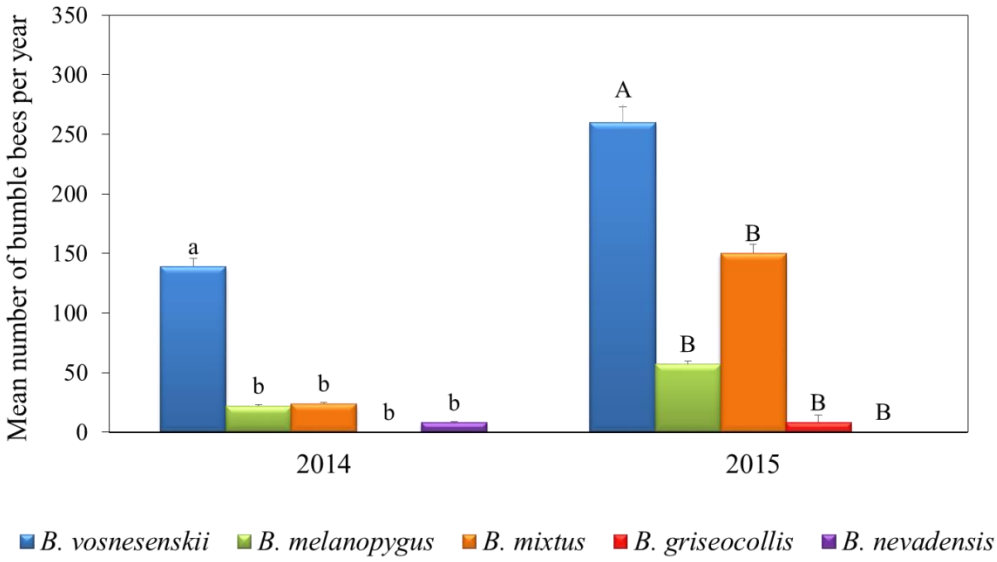

Figure 2.8. Abundance (Mean (± SE) of bumble bees observed foraging on linden trees in 2014 and 2015. Bars with different letters in the same year are significantly different at $\alpha = 0.05$ (Tukey HSD multiple means comparison).

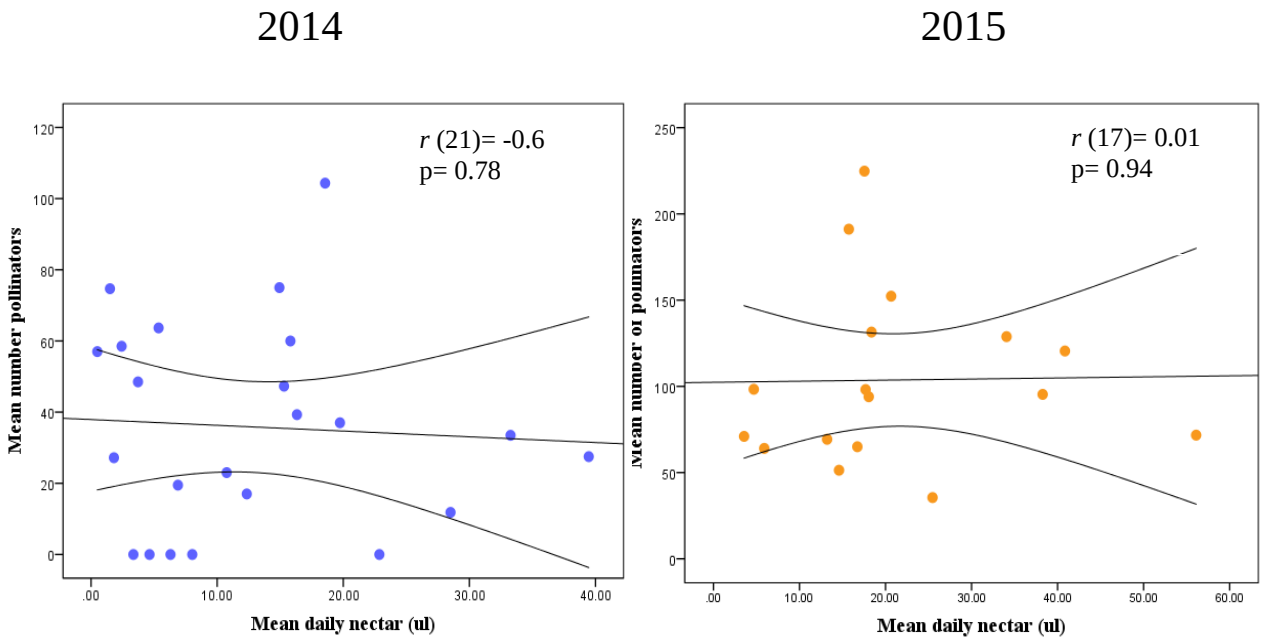

Figure 2.9. Correlation between daily mean nectar production and daily mean numbers of foragers (n=10 flowers). For both years correlation was not significant.

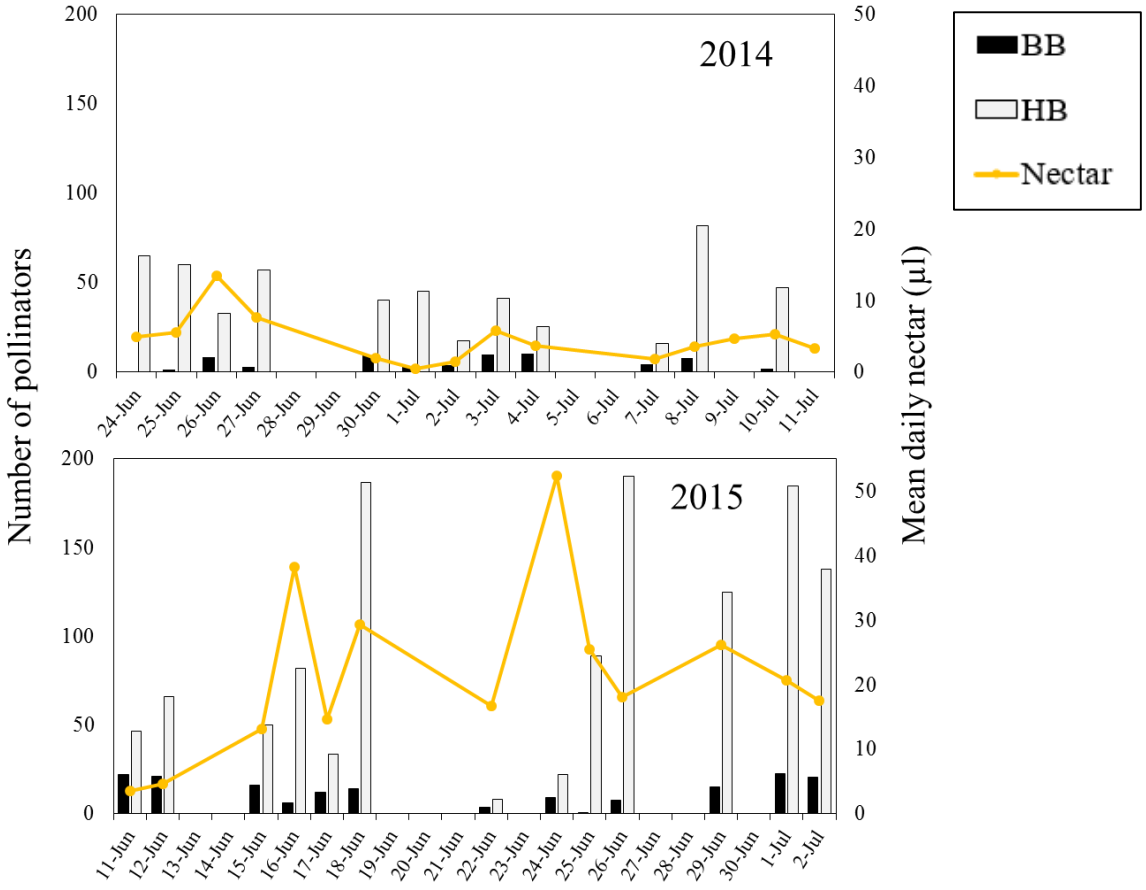

Figure 2.10. Temporal (daily) variation in nectar availability and abundance of honey bees and bumble bees observed foraging on linden trees in 2014 and 2015.

Table 2.1. Periods of bloom, nectar production and abundance of foragers observed on various *Tilia* species surveyed in 2014 and 2015.

	T. platyphyllos		***T. americana***		***T. cordata***		***T. tomentosa***	
	2014	**2015**	**2014**	**2015**	**2014**	**2015**	**2014**	**2015**
First day of bloom	26 May	5 Jun	Jun 7	Jun 11	Jun 10	Jun 17	Jun 24	Jun 23
Last day of bloom	16 Jun	19 Jun	Jun 27	Jun 22	Jul 2	Jun 25	Jul 14	Jul 3
Number of days of bloom	21	14	20	11	22	8	20	10
Mean (±SE) nectar production (µl)		9.78 ± 2.69	33.74 ± 3.99	45.07 ± 3.80	10.62 ± 2.6	18.03 ± 3.8	8.98 ± 1.9	22.69 ± 2.94
Nectar production per flower (µl) [a]	1.56		7.3		1.43		2.97	
Forager Bumble bees (mean/day)		17.55	1.50	9.33	2.54	0.72	5.29	14.80
Forager Honey bees (mean/day)		50.35	19.25	62.00	30.85	102.83	35.32	114.97
Forager Dipterans (mean/day)		2.30	9.25	2.71	5.31	2.89	8.18	3.97
B. vosnesenskii		345	15	195	26	12	107	251
B. melanopygus					2			
B. mixtus		6		5	5	1	17	45
B. griseocollis				24			24	146
B. nevadensis								2

[a] Mean value (N=50 flowers)

Table 2.2. Tukey HSD Post hoc test showing pairwise comparison of mean nectar collected from different *Tilia* species in 2014.

Tukey HSD						
(I) tree	(J) tree	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Americana	Cordata	23.1200*	4.777	0.000	11.035	35.205
	Tomentosa	24.7531*	4.434	0.000	13.536	35.971
Cordata	Americana	-23.1200*	4.777	0.000	-35.205	-11.035
	Tomentosa	1.633	3.245	0.871	-6.577	9.843
Tomentosa	Americana	-24.7531*	4.434	0.000	-35.971	-13.536
	Cordata	-1.633	3.245	0.871	-9.843	6.577

* The mean difference is significant at $\alpha = 0.05$.

Table 2.3. Tukey HSD Post hoc test showing pairwise comparison of mean nectar collected from different *Tilia* species in 2015.

Tukey HSD						
(I) tree	(J) tree	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Americana	Cordata	27.0367*	5.382	0.001	11.239	42.835
	Platyphyllos	35.2950*	4.661	0.000	21.613	48.977
	Tomentosa	22.3793*	4.814	0.002	8.249	36.510
Cordata	Americana	-27.0367*	5.382	0.001	-42.835	-11.239
	Platyphyllos	8.2583	4.661	0.329	-5.423	21.940
	Tomentosa	-4.6573	4.814	0.770	-18.788	9.473
Platyphyllos	Americana	-35.2950*	4.661	0.000	-48.977	-21.613
	Cordata	-8.2583	4.661	0.329	-21.940	5.423
	Tomentosa	-12.9157*	3.992	0.029	-24.632	-1.199
Tomentosa	Americana	-22.3793*	4.814	0.002	-36.510	-8.249
	Cordata	4.6573	4.814	0.770	-9.473	18.788
	Platyphyllos	12.9157*	3.992	0.029	1.199	24.632

* The mean difference is significant at $\alpha = 0.05$.

Table 2.4. Foragers observed during survey of *Tilia* trees surveyed in western Oregon in 2014 and 2015.

Forager group		**2014**[1]	**2015**[2]	**Total**
Apidae	*Apis mellifera*	1467	7795	**9262**
	B. grisecollis	24	170	**194**
	B. melanopygus	2	0	**2**
	B. mixtus	22	57	**79**
	B. nevadensis	0	2	**2**
	B. vosnesenskii	139	803	**942**
Diptera	Syrphids	335	282	**617**
Halictidae	*Halictus* spp.	108	496	**604**
Vespidae	*Polistes dominula*	24	4	**28**
TOTAL		**2121**	**9610**	**11731**

[1] Six trees each of : *T. americana, T. cordata, T. tomentosa* surveyed
[2] Six trees each of: *T. americana, T. cordata, T. platyphyllos, T. tomentosa* surveyed

Table 2.5. Two way ANOVA of the abundance of insect forager groups across *Tilia* species surveyed in 2014.

Dependent Variable: Abundance of foragers

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	32642.197[a]	14	2331.585	17.002	.000
Intercept	8365.132	1	8365.132	60.998	.000
Tree_sp	555.693	2	277.847	2.026	.134
Pollinators	13477.887	4	3369.472	24.570	.000
Tree_sp * Pollinators	713.286	8	89.161	.650	.735
Error	28798.843	210	137.137		
Total	81435.000	225			
Corrected Total	61441.040	224			

a. R Squared = .531 (Adjusted R Squared = .500)

Table 2.6. Two way ANOVA of the abundance of insect forager groups across *Tilia* species surveyed in 2015.

Dependent Variable: Abundance of foragers

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	550013.954[a]	19	28948.103	62.288	.000
Intercept	179911.600	1	179911.600	387.120	.000
Tree_sp	18648.465	3	6216.155	13.375	.153
Pollinators	436391.829	4	109097.957	234.748	.000
Tree_sp * Pollinators	56517.350	12	4709.779	10.134	.215
Error	204487.392	440	464.744		
Total	955225.000	460			
Corrected Total	754501.346	459			

a. R Squared = .729 (Adjusted R Squared = .717)

Chapter III

Impacts of galactose and mannose on the European honey bee (*Apis mellifera*) and the yellow faced bumble bee (*Bombus vosnesenskii*)[1]

Introduction

Bees provide critical pollination services to diverse agricultural crops, native plants and trees, and thus help sustain crop production and our natural resources. However, while the demand for bee pollinated crops increases each year with human population growth, there are global reports of bee declines (Kremen et al., 2007; Goulson et al., 2008; Grixti et al., 2009). Many factors are believed to be responsible including diseases, exposure to pesticides and, in the case of honey bees, the colony collapse syndrome. For native bees, changes in land use are believed to have led to a reduction in foraging resources and nesting habitats. Other factors such as low nectar production caused by water stress in plants, or toxins present in nectar may also be responsible for bee mortality but these have received little attention. Risks associated with foraging behaviors are particularly critical as bees spend considerable time seeking food resources.

Linden (*Tilia* spp.; Malvaceae), also known as lime or basswood, is a common ornamental tree in urban areas. It produces an abundance of fragrant flowers that draws diverse pollinators (Anderson, 1976). Nectar and pollen produced by linden benefits bees. However, in the late 1970's, many dead bumble bees and a few honey bees were observed lying dead, paralyzed or drunk under linden trees in localities where the soil drained quickly, and in 1976 when the summer was exceptionally dry (Crane, 1977). Researchers speculated that the casual factor was the presence of mannose in the nectar of linden during dry years (von Frisch, 1950; Sols et al., 1960; Saunders et al., 1969; van Handel, 1971; de la Fuente, 1986). Sugars that are typically present in the nectar of linden include fructose, glucose, sucrose and melibiose (Wykes, 1952). Mannose is a monosaccharide that is very similar in structure to glucose which is used by bees as a carbohydrate source. The toxicity of mannose to honey bees was speculated to be due disruption of glucose metabolism resulting from competition between mannose and glucose for

[1] In preparation for submission to Apidologie

the enzyme hexokinase during the glycolysis cycle that provides energy for bees (Sols et al., 1960). Studies by Saunders et al. (1969) and van Handel (1971) refuted the competitive inhibition of glycolysis hypothesis but a later study by de la Fuente (1986). The study showed that the presence of mannose led to large accumulation of mannose-6-phosphate and a marked depletion of ATP due to low levels of the enzyme mannosephosphate isomerase. Besides mannose, galactose, which was documented to be present in flowers of Darwin tulips, *Tulipa gregii* Regelhas, was also shown to be toxic to honey bees (Barker and Lehner, 1976. Galactose was also found to be present in the nectar of linden (Illies and Muhlen, 2007). In laboratory studies mannose and galactose were shown to be toxic to honey bees. Impacts of these sugars on bumble bees have, however, not been determined. The objectives of this study were to: 1) Compare the impacts of mannose and other sugars on honey bees and bumble bees; 2) Determine the dose-responses of galactose and mannose to honey bees and bumble bees; and 3) Determine the impacts of galactose and mannose in the presence of glucose to honey bees and bumble bees.

Materials and Methods

Three laboratory bioassays were conducted in 2014 and 2015 at Oregon State University (OSU) for determining the impacts of sugars on honey bees and bumble bees.

General Experiment Procedures:

Honey bee and bumble bee sources: Honey bee workers were randomly collected from ~15 colonies at the apiary at the Oak Creek Center for Urban Horticulture at Oregon State University. For Objective 1, bumble bee workers were collected from a red clover field in western Oregon during bloom in August 2014. For Objectives 2 and 3, bumble bee workers were collected from ~15 colonies of *B. vosnesenskii* purchased from a regional bumble bee propagator (Bee Man Exterminators LLC, Olympia, WA), and reared in the laboratory prior to use in the experiments.

Sugar Treatments: D-Glucose (G8270), D-fructose (F0127), D-Mannose (M6020), D-Galactose (G0750) and D-Sucrose (84097) were obtained from Sigma Aldrich. Stock solutions of 1M concentration were prepared in distilled water and refrigerated at 10°C until use in the experiments. Concentrations were adjusted based on the experiment (described below). Naturally

occurring concentrations of sugars in nectars are highly variable across plant species, and even across individual flowers, ranging from 0.3 M to 2.5 M (Nicolson and Thornburg, 2007). In the current study, 0.5 M to 2 M concentrations were used following studies by Sols et al. (1960), Barker and Lehner (1976) and de la Fuente (1986).

Bioassay: Cylindrical cages were fabricated with Phifer aluminium mesh (47 cm long x 12 cm wide) and plastic petri dishes (150mm x 15mm VWR), placed at the top and bottom as described by Johansen et al. (1983). Square openings were cut with a sharp blade in the petri dish at the top for placement of feeders. Fisher clear glass vials (7.2 ml) used as feeders were filled with 5 ml of sugar solution (concentration depending on the experiments described below) and covered with fabric nylon mesh attached with rubber bands. The system allowed feeding by bees while preventing the sugars from spilling when placed upside down in the cage (Fig 3.1).

Experiments with honey bees and bumble bees were conducted separately. For each test, 10 worker bees were introduced into the mesh cages after they were starved for 8 hours. After addition of the feeders, the cages were placed in an incubator set to 28°±2 °C and 50-55% of relative humidity (Figure 3.1). Each experiment was set up as a randomized block design with six replicates.

For the experiments with honey bees, observations on bee mortality were made during the following periods: 30 mins, 45 mins, 1 hour, 2 hours, 3 hours, 4 hours and 24 hours after bee exposure to the sugar treatments in the feeders. Bumble bees survived for longer periods, and hence additional observations were made hourly after 4 hours until 12 hours.

The following three studies were conducted sequentially using the bioassay set up described above. The experiments with honey bees and bumble bees were conducted separately.

Objective 1. Comparison of impacts of mannose and galactose on survivorship of honey bees and bumble bees.

Honey bee and bumble bee workers were exposed to 1 M concentrations of the following sugars: glucose, fructose, sucrose, mannose, galactose, and water (negative control resembling starvation).

Objective 2. Determination of the dose-response of galactose and mannose on honey bees and bumble bees

The toxic sugars, galactose and mannose were evaluated at different concentrations to determine the dose-response. The toxic sugars and glucose (positive control) were tested at 0.5, 1 and 2 M concentrations.

Objective 3. Determination of the impact on honey bees and bumble bees of galactose and mannose presented in combination with glucose.

The following ratios of toxic sugars (galactose and mannose) with glucose (all at 1M concentration) were presented to bees: 10 % toxic sugar: 90% glucose, 50 % toxic sugar: 50% glucose and 90 % toxic sugar: 10% glucose.

Data Analysis:

Data on mortality of honey bees and bumble bees were analyzed separately for all Objectives. Data from all three experiments were analyzed in SPSS Statistics 20 using a general linear model (GLM) with proportion of dead bees to total bees exposed at 24 hours as response variable. Explanatory variables depended on the Objective – see below. Since the experimental unit was at the cage level, and each cage contained ten bees, we assumed that the bees were not independent.

Explanatory variables:

Objective 1: sugars (fructose, galactose, glucose, mannose, sucrose, water).

Objective 2: sugars (galactose, glucose, mannose); concentrations (0.5M, 1M, 2 M).

Objective 3: sugars (galactose, mannose); combinations with glucose (90:10, 50:50, 10:90).

When significant differences in variables were observed in the GLM analysis, pairwise comparisons were made using Tukey's HSD (honest significance difference) post hoc analysis using R 3.2.2. All tests were performed at a significance level of $\alpha = 0.05$. Mortality across time was also analyzed using GLM with cumulative numbers of dead bees at each observation time

interval as response variable and time intervals of observation as explanatory variables. Honey bees and bumble bees were analyzed separately.

Results

Objective 1. Comparison of impacts of mannose and galactose on survivorship of honey bees and bumble bees.

Honey bees: Significant differences ($p < 0.001$) in mortality were observed when workers were exposed to the different sugars (Table 3.1). Mortality of bees when presented with galactose, mannose and water (negative control, starvation) was significantly greater than mortality when presented with fructose, glucose and sucrose (Table 3.2; Fig 3.2, 3.3).

Bumble bees: Significant differences ($p < 0.001$) in mortality were observed when workers were exposed to the different sugars (Table 3.3). Mortality of bees when presented with galactose, mannose and water (negative control, starvation) was significantly greater than mortality when presented with fructose, glucose and sucrose (Table 3.4, 3.7; Fig 3.2, 3.3).

Objective 2: Determination of the dose-response of galactose and mannose on honey bees and bumble bees

Honey bees: There were significant differences ($p<0.001$) in bee mortality across sugars and concentrations but the interaction was not significant ($p >0.05$) (Table 3.5). At all concentrations, bee mortality was higher with galactose and mannose compared with glucose (Table 3.6, 3.7; Fig 3.4). Bee mortality between galactose and mannose, however, did not differ (Table 3.6, 3.7). The cumulative numbers of dead bees at various observation times were similar for galactose and mannose treatments: 50% mean mortality was reached at 1 hour for both sugars (galactose 5.3±3.9; mannose 4.8±3.7) and 90% was reached at 4 hours (galactose 8.6±1.5; mannose 8.7±2.2) (Fig 3.5).

Bumble bees: There were significant differences in mortality across sugars ($p<0.001$) and concentrations ($p<0.05$) but the interaction was not significant ($p >0.05$) (Table 3.8). At all concentrations, mortality was higher with galactose and mannose compared with glucose (Fig

3.4, Table 3.9. 3.10). Mortality with galactose and mannose, however, did not differ (Table 3.9, 3.10). The cumulative numbers of dead bees at various observations times were not similar for the galactose and mannose treatments: 50% mean mortality with galactose was reached at 5 hours (mean 4.22±2.6) and at 2 hours for mannose (mean 4.83±3.0); 90% mean mortality for galactose was reached at 12 hours (mean 7.41±2.1) and at 8 hours with mannose (mean 8.00±1.9) (Fig 3.5).

Objective 3. Determination of the impacts on honey bees and bumble bees of galactose and mannose presented in combination with glucose.

Honey bees: There were significant differences in worker mortality across ratios of sugars that were tested ($p<0.001$) but not across sugars ($p=0.9$). The interaction of sugars and combinations was not significant ($p=0.2$) (Table 3.11). The mortality of bees exposed to the four combinations of 10:90 and 50:50 combinations of Glucose:Mannose and Glucose:Galactose did not differ from each other but differed significantly from the two combinations of 90:10 of Glucose:Mannose and Glucose:Galactose which did not differ from each other (Table 3.12, Fig 3.6, 3.7).

Bumble bees: There were significant differences in worker mortality across sugars ($p<0.001$), ratios of sugars that were tested ($p<0.001$) and the interaction of sugars and combinations ($p< 0.05$) (Table 3.13). The mortality of bees exposed to the four combinations of 10:90 and 50:50 combinations of Glucose:Mannose and Glucose:Galactose did not differ from each other but differed significantly from the two combinations of 90:10 and Glucose:Mannose and Glucose:Galactose which did not differ from each other (Table 3.14, Fig 3.6, 3.7).

Discussion

This is the first study to document the toxic impacts of mannose and galactose on bumble bees. Both sugars caused over 70% mortality at the lowest dose (0.5 M) in the dose response experiment. The current study corroborates earlier studies which documented the toxicity of mannose on honey bees (von Frisch, 1950; Sols et al., 1960; Saunders et al., 1969; van Handel, 1971; de la Fuente, 1986). These studies were conducted to determine if mannose could be used

as an alternative to sucrose when sugar was rationed during the World War II (Staudenmayer, 1939). There were also concerns of impacts on humans of honey produced in hives in which sugars such as mannose were used instead of sucrose (Howes, 1949). Impacts on bumble bees were, however, not determined.

The current study showed that when exposed to mannose (1M), 50% of the honey bees died within 1.5 hours while 90% died within 4 hours. These results are similar to those reported from the study by Sols et al. (1960), which showed that 50% of honey bees fed with mannose (1M) died at 1.5 hours and 90% died at 3 hours. In the current study mortality of bumble bee occurred less rapidly - 50% mortality occurred at 2 hours and 90% at 8 hours. In a study by de la Fuente (1986), 90% of honey bees fed mannose (1 M) died within 4 hours. In contrast, other insects, *Drosophila melanogaster* and *Ceratitis capitata*, survived for 24 hours highlighting the difference in impacts of mannose across insect groups (de la Fuente et al., 1986).

While mannose is toxic to bumble bees, its presence in the nectar of linden has not been documented. Illies and Mühlen (2007) analyzed linden nectar and determined that mannose was absent. However, Crane (1977) speculated that mannose is present in linden nectar only during dry conditions. Thus, dry conditions need to be simulated for documenting whether mannose is present in linden nectar and whether it is the basis for the bee mortality associated with linden.

This is the first laboratory study to document direct toxic impacts of galactose on honey bees and bumble bees. Galactose has been documented to be present in the stigma exudates from tulips (Barker and Lehner, 1976) and nectar of linden (Illies and Muhlen, 2007). As in the case of mannose, when honey bees were exposed to galactose (1M) 50% of workers died within 1 hour and 90% died in 4 hours. In the case of bumble bees 50% died at 5 hours and 90% at 12 hours.

In the current study, when sugar combinations contained 90% or 50% galactose or mannose, with 10% or 50%, of glucose, respectively, mortality was much higher than when honey bees and bumble bees were presented with 10% of toxic sugars with 90% glucose. This showed that while mannose and galactose were toxic to honey bees and bumble bees, the toxic impacts were dependent on the amount of glucose presented at the same time. In a sugar combination study by Barker and Lehner (1974), when mannose, galactose and arabinose were presented at three concentrations (0.125 M, 0.5 M, and 2 M) in combination with 0.5 M of

sucrose, honey bee survival was no different than the water treatment for all three concentrations tested. These results appear to provide support for the hypothesis that toxicity of mannose to honey bees is due to disruption of glucose metabolism resulting competition between mannose and glucose for the enzyme hexokinase during the glycolysis cycle that provides energy for bees (Sols et al., 1960). Studies by Saunders et al. (1969) and van Handel (1971) appeared to disprove the competitive inhibition of glycolysis hypothesis but a later study by de la Fuente (1986) provided support. The study by de la Fuente (1986) showed that the presence of mannose led to large accumulation of mannose-6-phosphate and a marked depletion of ATP due to low levels of the enzyme mannosephosphate isomerase. In contrast, mannosephosphate isomerase levels were 5-10 times higher in *D. melanogaster* and *C. capitata* that were tested in the same study. Based on the results of the current study, perhaps the presence of high amounts of glucose prevents the accumulation of mannose-6-phosphate thus enabling the bees to survive. There is no information on the basis of the toxicity of galactose to bees. Biochemical studies are needed to document the basis of the toxicity of mannose and galactose to honey bees and bumble bees.

Unlike mannose, the presence of galactose has been reported from Darwin tulips (Barker and Lehner, 1976), and linden (Illes and Mühlen, 2007). The current study corroborates the observation by Barker and Lehner (1976) that galactose is toxic to honey bees. Analysis of extracts from the thoraces of honey bees that died under flowers of Darwin tulips, *Tulipa gregii* Regel suggested the presence of mannose. However analysis of flower exudates using thin layer chromatography documented the presence of galactose and not mannose, and thus the former was speculated to be the cause for the bee deaths.

Our expectation was that bumble bees would be more sensitive than honey bees to mannose given that bumble bees and not honey bees were reported dead under linden trees. However, the results of the current study contradicted that expectation. Honey bee mortality reached 50% within 1.5 hours while only 15% of bumble bees died during the same period when presented with 1 M solutions of mannose and galactose. According to Tiedeken et al. (2014), honey bees are better at detecting toxic compounds in nectar. Hence, we speculate that the fewer honey bee deaths associated with linden is due to other factors such as differences in foraging behaviors of the two species of bees (Illes and Mühlen, 2007).

In summary, sugars such as mannose and galactose are toxic to honey bees and bumble bees, but the toxicity depends on the amount of glucose present at the same time. These toxic sugars could be the reason for bee deaths associated with linden. Further research is needed for confirming the presence of galactose and mannose in the nectar of linden under drought and non-drought conditions. In addition, research is needed for determining differences, if any, in the foraging behaviors of honey bees and bumble bees on linden trees, and for detecting other nectar compounds in linden that may differ in their impacts on different species of bees.

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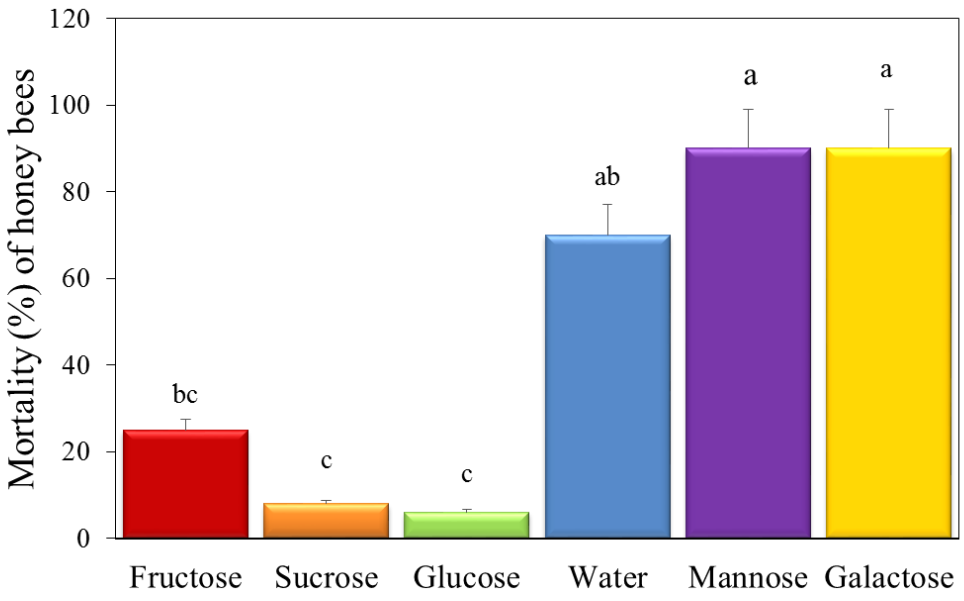
Fig 3.1. Cylindrical wire cages with feeders used for bioassays.

A



B

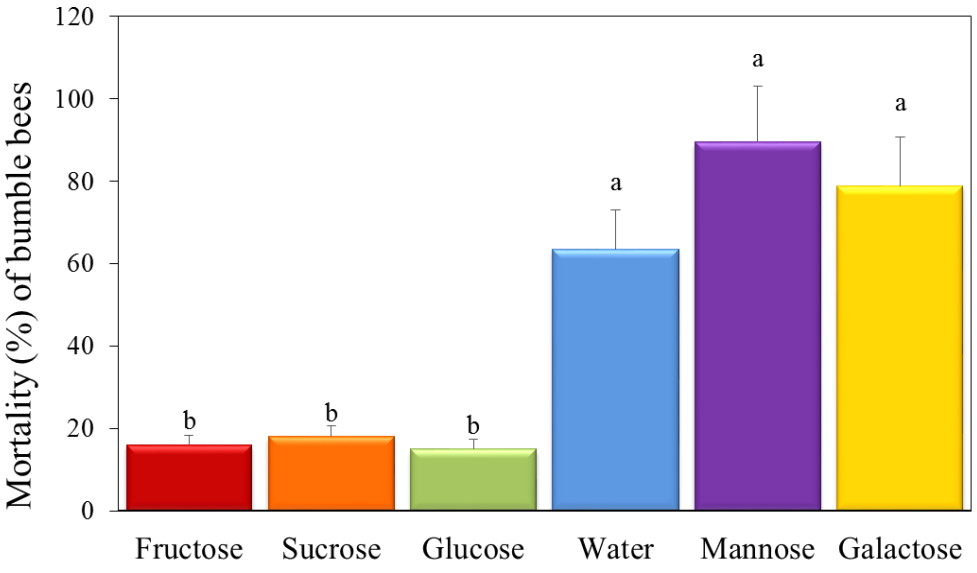


Fig 3.2. Mortality (%) (Mean +SE) of bees presented with sugars (1M in water) in a laboratory bioassay. **A.** Honey bees **B.** Bumble bees. Bars with different letters are significantly different ($p < 0.05$) (Tukey HSD multiple means comparison).

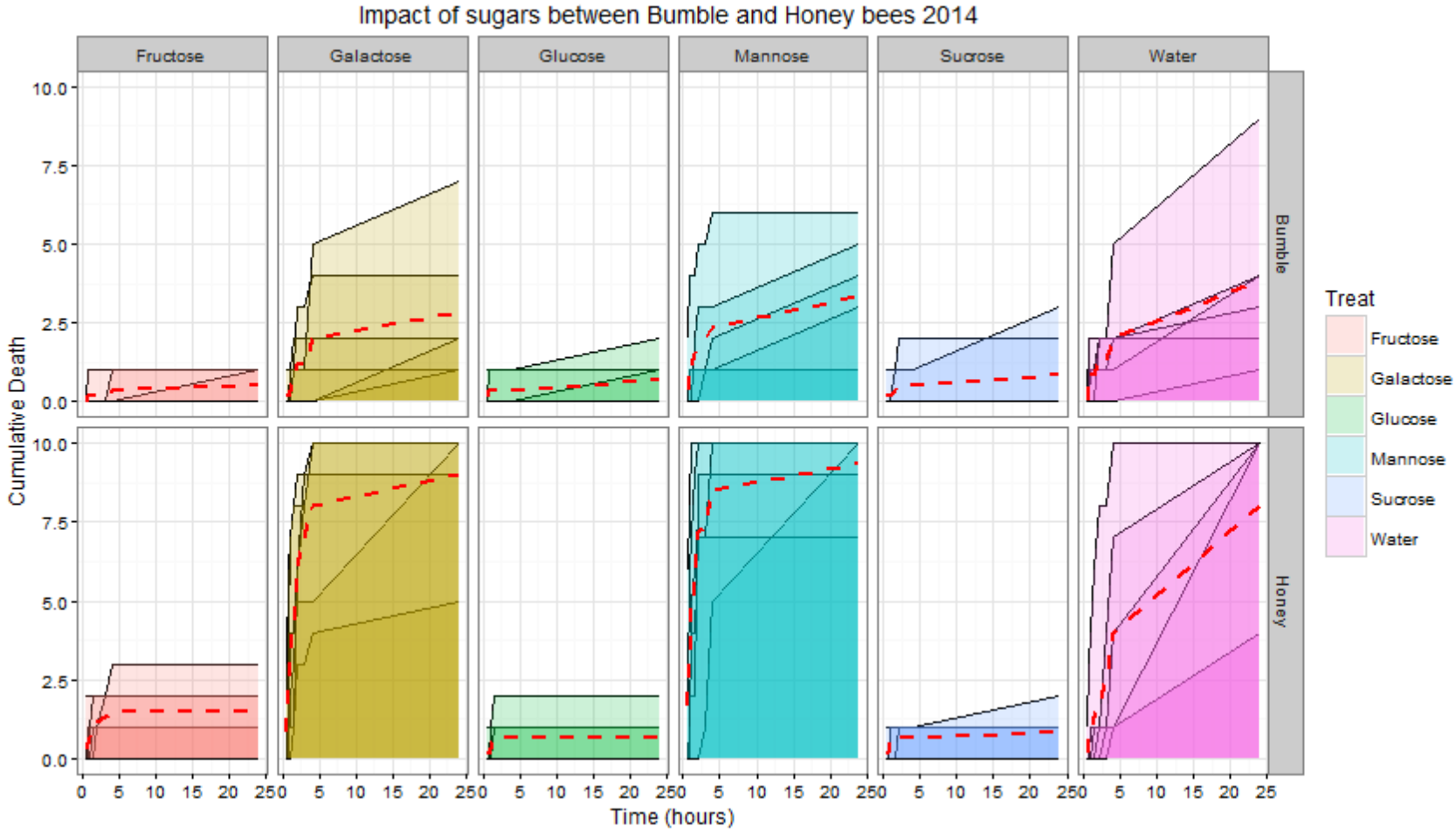


Fig 3.3. Cumulative deaths by bee species and sugar treatments across time. Each line within each graph represents one replicate. (n=6). Dotted line represents the mean cumulative death.

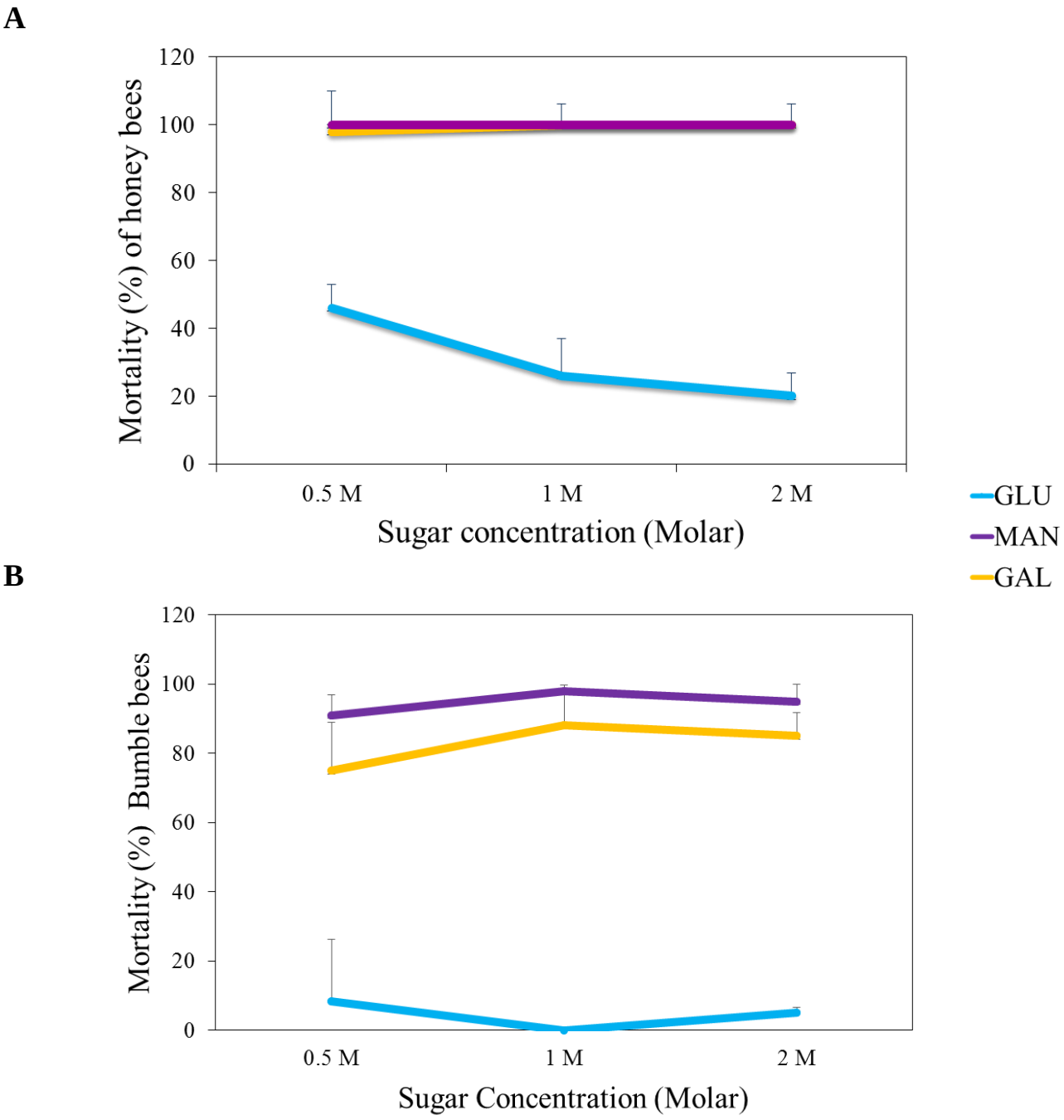


Fig 3.4. Mean number of dead bees presented with sugars at different concentrations in a laboratory bioassay. **A.** Honey bees. **B.** Bumble bees.

A

B

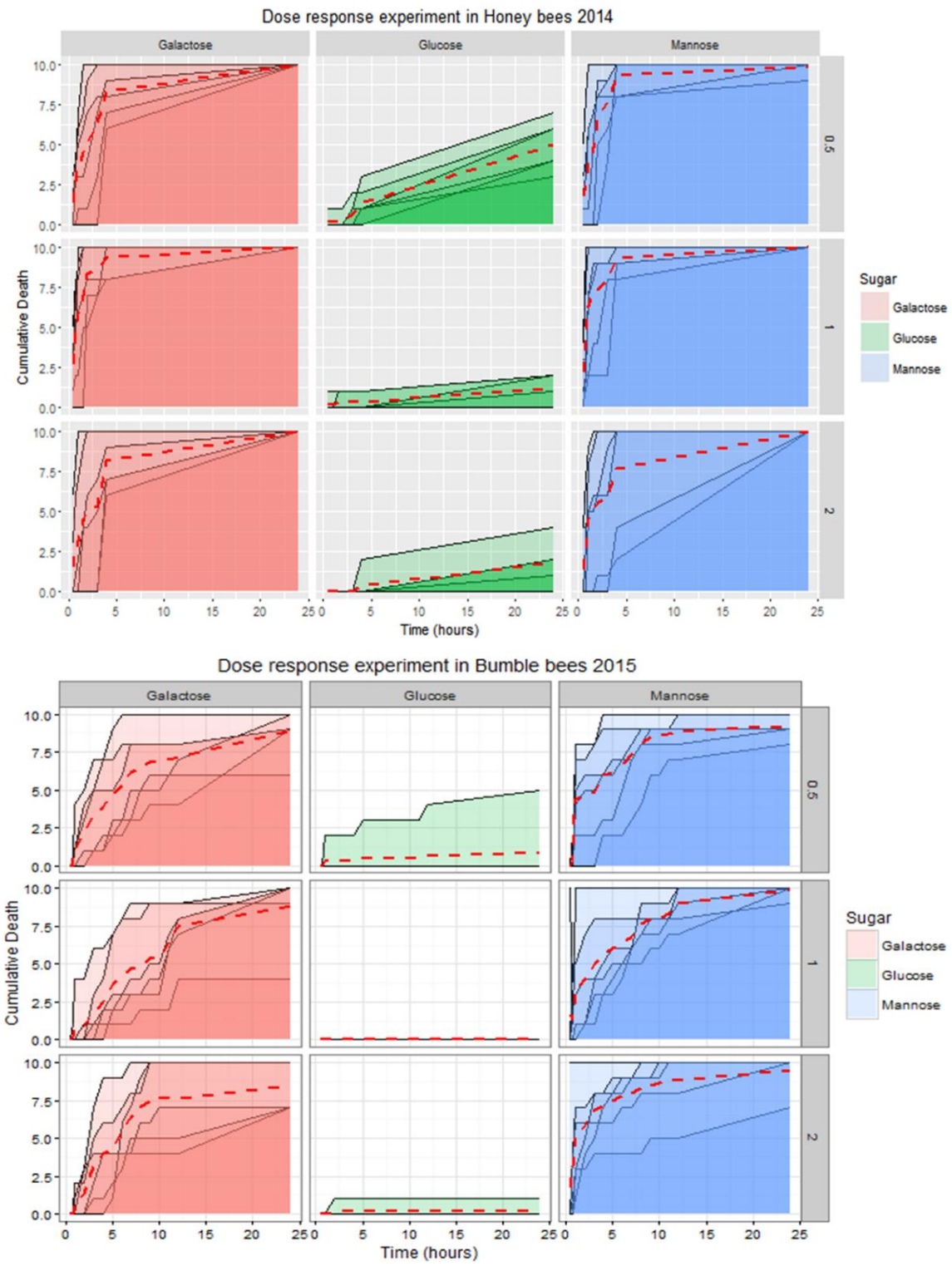
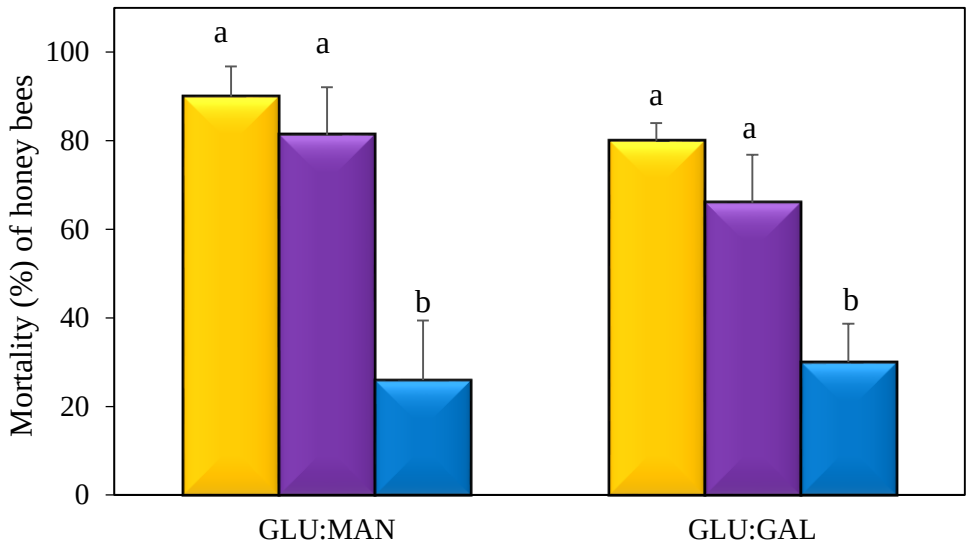


Fig 3.5. Cumulative deaths of bees exposed to various concentrations of sugars over time. **A**. Honey bees. **B.** Bumble bees. Lines in each small plot represent number of repetitions (N=6) and red dotted line represents the mean cumulative death.

A



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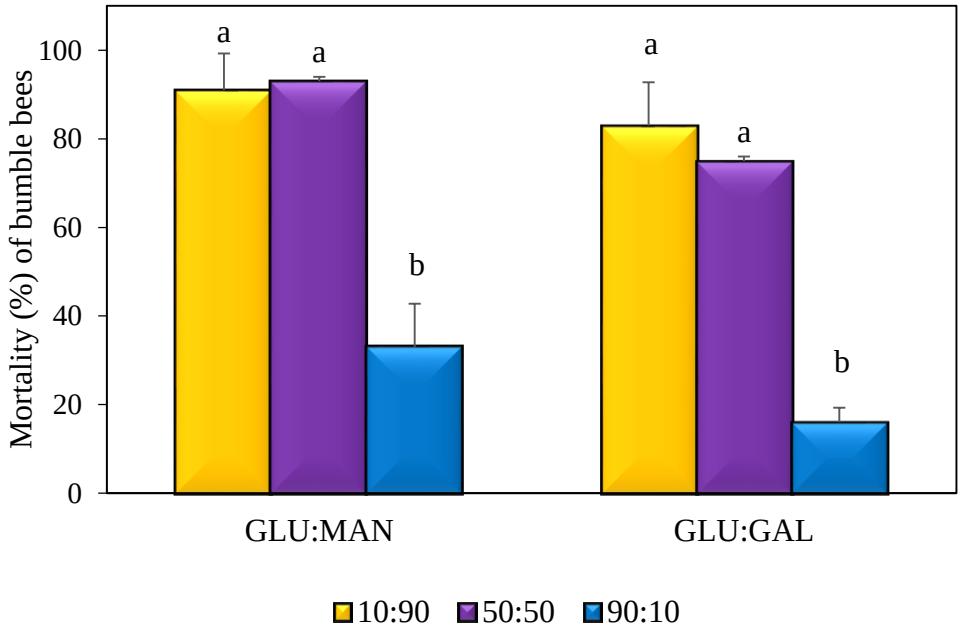
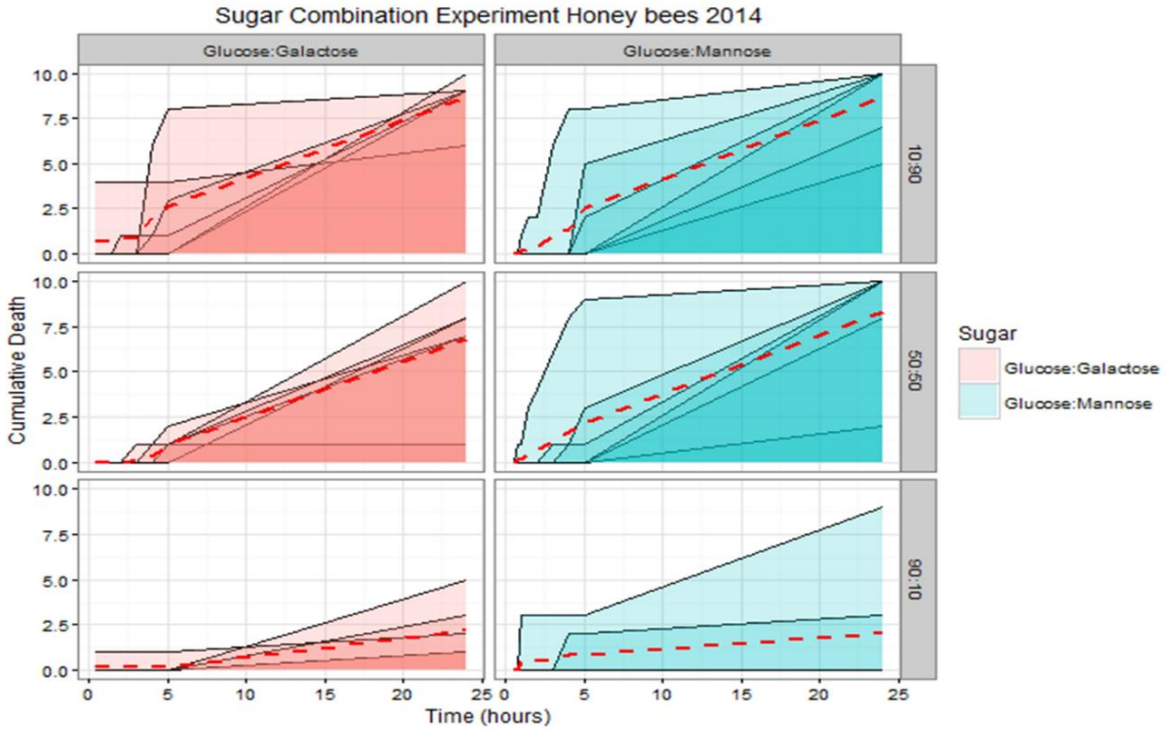


Fig 3.6. Mortality (Mean SE) of bees presented with combinations of sugars. **A**. Honey bees. **B**. Bumble bees. GLU-glucose; MAN=mannose; GAL = galactose. Bars with different letters denote significant differences at $\alpha = 0.05$. (Tukey HSD multiple means comparison).

A



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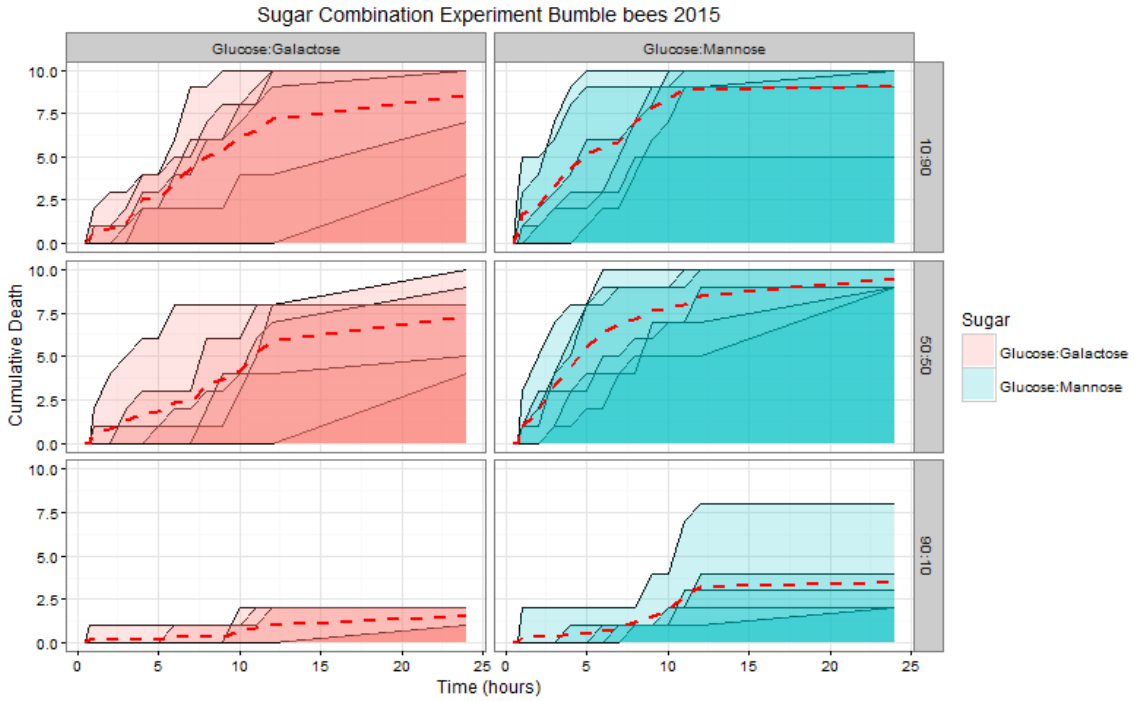

Fig 3.7. Cumulative deaths of bees exposed to combinations of sugars over time. **A**. Honey bees. **B**. Bumble bees. Lines in each small plot represent number of repetitions (N=6) and red dotted line represents the mean cumulative death.

Table 3.1. General linear model for mortality of honey bees exposed to different sugars

Dependent Variable: Total proportion of death					
Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	1346.392[a]	5	269.278	39.912	.000
Intercept	1886.003	1	1886.003	279.539	.000
Treat	1346.392	5	269.278	39.912	.000
Error	1902.604	282	6.747		
Total	5135.000	288			
Corrected Total	3248.997	287			

a. R Squared = .414 (Adjusted R Squared = .404)

$\alpha = 0.05$.

Table 3.2. Tukey HSD pairwise comparison of mortality of honey bees exposed to different sugars.

Multiple Comparisons

Dependent Variable: Total proportion of Death (24h)

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Fructose	Galactose	-4.10*	.530	.000	-5.63	-2.58
	Glucose	.50	.530	.935	-1.02	2.02
	Mannose	-4.83*	.530	.000	-6.35	-3.31
	Sucrose	.48	.530	.945	-1.04	2.00
	Water	-1.40	.530	.093	-2.92	.13
Galactose	Fructose	4.10*	.530	.000	2.58	5.63
	Glucose	4.60*	.530	.000	3.08	6.13
	Mannose	-.73	.530	.742	-2.25	.79
	Sucrose	4.58*	.530	.000	3.06	6.10
	Water	2.71*	.530	.000	1.19	4.23
Glucose	Fructose	-.50	.530	.935	-2.02	1.02
	Galactose	-4.60*	.530	.000	-6.13	-3.08
	Mannose	-5.33*	.530	.000	-6.85	-3.81
	Sucrose	-.02	.530	1.000	-1.54	1.50
	Water	-1.90*	.530	.005	-3.42	-.37
Mannose	Fructose	4.83*	.530	.000	3.31	6.35
	Galactose	.73	.530	.742	-.79	2.25
	Glucose	5.33*	.530	.000	3.81	6.85
	Sucrose	5.31*	.530	.000	3.79	6.83
	Water	3.44*	.530	.000	1.92	4.96
Sucrose	Fructose	-.48	.530	.945	-2.00	1.04
	Galactose	-4.58*	.530	.000	-6.10	-3.06
	Glucose	.02	.530	1.000	-1.50	1.54
	Mannose	-5.31*	.530	.000	-6.83	-3.79
	Water	-1.88*	.530	.006	-3.40	-.35
Water	Fructose	1.40	.530	.093	-.13	2.92
	Galactose	-2.71*	.530	.000	-4.23	-1.19
	Glucose	1.90*	.530	.005	.37	3.42
	Mannose	-3.44*	.530	.000	-4.96	-1.92
	Sucrose	1.88*	.530	.006	.35	3.40

Based on observed means.
The error term is Mean Square(Error) = 6.747.

* Significant at α = 0 .05

Table 3.3. General linear model for mortality of bumble bees exposed to different sugars.

Tests of Between-Subjects Effects

Dependent Variable: Total proportion of death

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	90.653[a]	5	18.131	12.213	.000
Intercept	206.722	1	206.722	139.255	.000
Treat	90.653	5	18.131	12.213	.000
Error	418.625	282	1.484		
Total	716.000	288			
Corrected Total	509.278	287			

a. R Squared = .178 (Adjusted R Squared = .163)

$\alpha = 0.05$.

Table 3.4. Tukey HSD pairwise comparison of mortality of bumble bees exposed to different sugars.

Multiple Comparisons

Dependent Variable: Total proportion of death (24h)

(I) Treat	(J) Treat	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Fructose	Galactose	-.88*	.249	.007	-1.59	-.16
	Glucose	-.15	.249	.992	-.86	.57
	Mannose	-1.50*	.249	.000	-2.21	-.79
	Sucrose	-.19	.249	.975	-.90	.53
	Water	-1.13*	.249	.000	-1.84	-.41
Galactose	Fructose	.88*	.249	.007	.16	1.59
	Glucose	.73*	.249	.042	.02	1.44
	Mannose	-.63	.249	.124	-1.34	.09
	Sucrose	.69	.249	.066	-.03	1.40
	Water	-.25	.249	.916	-.96	.46
Glucose	Fructose	.15	.249	.992	-.57	.86
	Galactose	-.73*	.249	.042	-1.44	-.02
	Mannose	-1.35*	.249	.000	-2.07	-.64
	Sucrose	-.04	.249	1.000	-.76	.67
	Water	-.98*	.249	.001	-1.69	-.27
Mannose	Fructose	1.50*	.249	.000	.79	2.21
	Galactose	.63	.249	.124	-.09	1.34
	Glucose	1.35*	.249	.000	.64	2.07
	Sucrose	1.31*	.249	.000	.60	2.03
	Water	.38	.249	.660	-.34	1.09
Sucrose	Fructose	.19	.249	.975	-.53	.90
	Galactose	-.69	.249	.066	-1.40	.03
	Glucose	.04	.249	1.000	-.67	.76
	Mannose	-1.31*	.249	.000	-2.03	-.60
	Water	-.94*	.249	.003	-1.65	-.22
Water	Fructose	1.13*	.249	.000	.41	1.84
	Galactose	.25	.249	.916	-.46	.96
	Glucose	.98*	.249	.001	.27	1.69
	Mannose	-.38	.249	.660	-1.09	.34
	Sucrose	.94*	.249	.003	.22	1.65

Based on observed means.
The error term is Mean Square (Error) = 1.484.

* Significant at $\alpha = 0.05$.

Table 3.5. General linear model of mortality of honey bees exposed to different concentrations of sugars.

Tests of Between-Subjects Effects

Dependent Variable: Total Proportion of Death (24 h)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	2917.463[a]	8	364.683	35.293	.000	
Intercept	7235.704	1	7235.704	700.256	.000	
Sugar	2727.574	2	1363.787	131.984	.000	***
Concentration	107.921	2	53.961	5.222	.006	***
Sugar * Concentration	81.968	4	20.492	1.983	.096	
Error	4370.833	423	10.333			
Total	14524.000	432				
Corrected Total	7288.296	431				

a. R Squared = .400 (Adjusted R Squared = .389)

*** = P<0.001

Table 3.6. Tukey HSD Post hoc test showing means for mortality of honey bees exposed to different concentrations of sugars in homogeneous subsets

Tukey HSD **Total Proportion of Death (24 h)**			
Sugar	N	Subset	
		1	2
Glucose	144	0.542	
Galactose	144		5.750
Mannose	144		5.986
Sig.		1.000	0.807

Means for groups in homogeneous subsets are displayed.

The error term is Mean Square(Error) = 10.333.

Uses Harmonic Mean Sample Size = 144.000.

α =0.05.

Table 3.7. Tukey HSD Post hoc test showing pairwise comparison of means related to morality of honey bees exposed to different concentrations of sugars.

Tukey HSD multiple comparisons of means **95% family-wise confidence level**						
Across Sugars and Concentrations		diff	lwr	upr	p adj	
0.5 M	Glucose - Galactose	-0.500	-0.644	-0.356	0.000	***
	Mannose - Galactose	-0.017	-0.161	0.128	1.000	
	Mannose - Glucose	0.483	0.339	0.628	0.000	***
1 M	Glucose - Galactose	-0.883	-1.028	-0.739	0.000	***
	Mannose - Galactose	0.000	-0.144	0.144	1.000	
	Mannose - Glucose	0.883	0.739	1.028	0.000	***
2 M	Glucose - Galactose	-0.817	-0.961	-0.672	0.000	***
	Mannose - Galactose	0.000	-0.144	0.144	1.000	
	Mannose - Glucose	0.817	0.672	0.961	0.000	***
Within Sugar concentrations						
Mannose	0.5 M – 2M	0.017	-0.128	0.161	1.000	
	0.5 M – 2M	0.017	-0.128	0.161	1.000	
	1 M - 2 M	0.000	-0.144	0.144	1.000	
Galactose	0.5 M – 1M	0.000	-0.144	0.144	1.000	
	0.5 M – 2M	0.000	-0.144	0.144	1.000	
	1 M - 2 M	0.000	-0.144	0.144	1.000	

$\alpha = 0.05$.

Table 3.8. General linear model of mortality of bumble bees exposed to different concentrations of sugars.

Tests of Between-Subjects Effects

Dependent Variable: Total Proportion of death (24 h)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	5560.956[a]	8	695.119	86.944	.000	
Intercept	10868.011	1	10868.011	1359.343	.000	
Sugar	5448.496	2	2724.248	340.742	.000	***
Concentration	66.763	2	33.381	4.175	.016	**
Sugar * Concentration	45.696	4	11.424	1.429	.223	
Error	6404.033	801	7.995			
Total	22833.000	810				
Corrected Total	11964.989	809				

a. R Squared = .465 (Adjusted R Squared = .459)

******* = p<0.001

Table 3.9. Tukey HSD Post hoc test showing means for mortality of bumble bees exposed to different concentrations of sugars in homogeneous subsets

Tukey HSD **Total Proportion of death (24 h)**				
Sugar	N	Subset		
		1	2	3
Glucose	270	0.185		
Galactose	270		4.393	
Mannose	270			6.411
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.

Based on observed means.

The error term is Mean Square(Error) = 7.995.

Uses Harmonic Mean Sample Size = 270.000.

α = 0.05

Table 3.10. Tukey HSD Post hoc test showing pairwise comparison of means related to morality of bumble bees exposed to different concentrations of sugars

Tukey HSD multiple comparisons of means **95% family-wise confidence level**						
Across Sugars and Concentrations		diff	lwr	upr	p adj	
0.5 M	Glucose - Galactose	-0.8	-1.060	-0.540	0.000	***
	Mannose - Galactose	0.033	-0.227	0.293	1.000	
	Mannose - Glucose	0.833	0.573	1.093	0.000	***
1 M	Glucose - Galactose	-8.83E-01	-1.143	-0.623	0.000	***
	Mannose - Galactose	1.00E-01	-0.160	0.360	0.939	
	Mannose - Glucose	0.983	0.723	1.243	0.000	***
2 M	Glucose - Galactose	-8.33E-01	-1.093	-0.573	0.000	***
	Mannose - Galactose	1.00E-01	-0.160	0.360	0.939	
	Mannose - Glucose	9.33E-01	0.673	1.193	0.000	***
Within sugar concentrations						
Mannose	0.5 M - 1M	6.67E-02	-0.193	0.327	0.995	
	0.5 M - 2M	3.33E-02	-0.227	0.293	1.000	
	1 M - 2 M	-3.33E-02	-0.293	0.227	1.000	
Galactose	0.5 M - 1M	1.67E-02	-0.260	0.260	1.000	
	0.5 M - 2M	-0.033	-0.293	0.227	1.000	
	1 M - 2 M	-0.033	-0.293	0.227	1.000	

$\alpha = 0.05$

Table 3.11. General lineal model of honey bee deaths when exposed to sugar combinations.

Tests of Between-Subjects Effects

Dependent Variable: Total Proportion of Death (24 h)

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.	
Corrected Model	145.460[a]	5	29.092	4.805	.000	
Intercept	402.225	1	402.225	66.435	.000	
Sugar	.151	1	.151	.025	.875	
Ratio	126.340	2	63.170	10.434	.000	***
Sugar * Ratio	18.969	2	9.485	1.567	.210	
Error	1925.315	318	6.054			
Total	2473.000	324				
Corrected Total	2070.775	323				

R Squared = .070 (Adjusted R Squared = .056)

*** = P<0.001

Table 3.12. Tukey HSD Post hoc test showing pairwise comparison of means for honey bee deaths when exposed to sugar combinations.

Tukey HSD multiple comparisons of means **95% family-wise confidence level**						
Across Sugars			diff	lwr	upr	p adj
Glucose:Mannose	10:90	10:90	-3.33E-16	-0.394	0.394	1.000
vs	50:50	50:50	1.50E-01	-0.244	0.544	0.853
Glucose:Galactose	90:10	90:10	-1.50E-01	-0.544	0.244	0.853
Between Sugars						
	50:50	10:90	-1.83E-01	-0.577	0.211	0.718
Glucose:Galactose	90:10	10:90	-6.50E-01	-1.044	-0.256	0.000 ***
	90:10	50:50	-4.67E-01	-0.861	-0.073	0.013 **
	50:50	10:90	-3.33E-02	-0.427	0.361	1.000
Glucose:Mannose	90:10	10:90	-8.00E-01	-1.194	-0.406	1.19E-05 ***
	90:10	50:50	-7.67E-01	-1.161	-0.373	2.42E-05 ***

α= 0.05

Table 3.13. General lineal model of bumble bee deaths when exposed to sugar combinations.

Tests of Between-Subjects Effects

Dependent Variable: Total Proportion of Death (24 h)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	
Corrected Model	1827.587[a]	5	365.517	41.601	.000	
Intercept	5035.557	1	5035.557	573.118	.000	
Sugar	350.417	1	350.417	39.882	.000	***
Ratio	1406.937	2	703.469	80.065	.000	***
Sugar * Ratio	70.233	2	35.117	3.997	.019	**
Error	4691.856	534	8.786			
Total	11555.000	540				
Corrected Total	6519.443	539				

R Squared = .280 (Adjusted R Squared = .274)

*** = P<0.001

Table 3.14. Tukey HSD Post hoc test showing pairwise comparison of means for bumble bee deaths when exposed to sugar combinations.

Tukey HSD multiple comparisons of means **95% family-wise confidence level**						
Across Sugars		diff	lwr	upr	p adj	
Glucose:Mannose	10:90 10:90	5.00E-02	-2.85E-01	0.385	0.997	
vs	50:50 50:50	0.217	-0.119	0.552	0.385	
Glucose:Galactose	90:10 90:10	0.2	-0.135	0.535	0.472	
Between Sugars						
	50:50 10:90	-1.17E-01	-4.52E-01	0.219	0.894	
Glucose:Galactose	90:10 10:90	-0.7	-1.035	-0.365	7.4E-06	***
	90:10 50:50	-0.583	-0.919	-0.248	0.000	***
	50:50 10:90	0.05	-0.285	0.385	0.997	
Glucose:Mannose	90:10 10:90	-0.55	-0.885	-0.215	0.000	***
	90:10 50:50	-0.6	-0.935	-0.265	9.06E-05	***

α= 0.05

Chapter IV

Summary

Review of findings

Linden, a common ornamental tree in urban landscapes, produces an abundance of fragrant flowers that provide nectar and pollen for bees during a period when few other plants are in bloom. Researchers have examined the diversity and abundance of bee pollinators on linden in Europe where many linden species are native, and in eastern and midwestern USA. This is the first study that examined foragers on linden in western USA. Over the two year study, a total of 11,731 foragers were recorded on four species of *Tilia* surveyed in western Oregon. These included honey bees, bumble bees, halictids, yellow jackets and dipterans (mostly sryphids). Honey bees were the dominant forager on all *Tilia* species.

Earlier research has shown that honey bees forage on *Tilia* species only at times of high nectar availability. In the current study, nectar production was, correlated with temperature and humidity. However there was no correlation between the amount of nectar and bee abundance. Interestingly, in 2014, when temperatures were lower, bloom occurred over a longer period (20-21 days) compared to 2015 (8-14 days) but there were fewer numbers of foragers during the cooler year.

In the late 1970's, many dead bumble bees and a few honey bees were observed under linden in Europe when environmental conditions were dry. Researchers speculated that the causal factor was the presence of the sugar mannose in linden nectar under drought stress. Mannose, a sugar that is similar in structure to glucose which is a carbohydrate source for bees. The toxicity of mannose to honey bees was speculated to be due disruption of glucose metabolism resulting from competition between mannose and glucose for the enzyme hexokinase during the glycolysis cycle that provides energy for bees. Preliminary HPLC analyses of three nectar samples in the current study suggested the presence of mannose in two

samples. A laboratory bioassay in the current study corroborated the toxicity of mannose and galactose to honey bees documented by earlier researchers, and also showed that both sugars are toxic to bumble bees. Interestingly, when the toxic sugars were combined with the non-toxic glucose and presented to the bees, the toxic impact was reduced if the proportion of glucose was high (90%). If the environmental drought stress indeed causes fluxes in levels of mannose in the flowers, bee mortality could be due to disruption of glucose metabolism when concentrations of toxic sugars are high. However, it is it is still not known why higher bumble bees have been reported to die after foraging on linden when honey bees are the dominant foragers on linden, and are susceptible to the toxicity of mannose. It is possible that honey bees and bumble bees differ in their ability to assess the presence of toxins in nectar or that other factors are involved

Future Research

Further research is needed for confirming the presence of galactose and mannose in the nectar of linden under drought and non-drought conditions. In addition, research is needed for determining differences, if any, in the foraging behaviors of honey bees and bumble bees on linden trees, and for detecting other nectar compounds in linden that may differ in their impacts on different species of bees.

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