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### Introduction

Neonicotinoid insecticides are commonly used in landscapes and agriculture in the US, but levels of residue in ornamental plants are rarely measured. Neonicotinoid research showed lethal and sublethal effects on bee behavior and colony health (Scholer & Krischik 2014, Baron et al. 2017, Arce et al. 2017) and on butterfly survival, development, and behavior (Pecenka & Lundgren 2015, James 2019, Peterson et al. 2019, Krishnan et al. 2020).

### Objective 1: Materials & Methods

#### Objective 1: Comparing neonicotinoid residues in ornamentals and agriculture.

Plants were treated with label rates of imidacloprid, harvested at 5-10 wks, and residue was quantified by HPLC GC at the USDA lab in Gastonia, NC

### Objective 1: Results

**Objective 1.** Imidacloprid is common in ornamentals and residues are significantly higher compared to clothianidin residues near ag fields. The LD<sub>50</sub> is around 4 ng/bee for both and was shown to have similar colony effects on bumblebees (Scholer and Krischik 2014).

**Table 1. Neonicotinoid residues in urban and ag plants.**

Species/ label application	Flowers imid ppb	Leaves imid ppb	Many cloth ppb	Ref
<b>Neonicotinoid residues in agricultural fields (4mg/agft)</b>				
<i>Asclepias syriaca</i>			1.14 0.71	1,2
<i>Brassica napus</i> pollen		0.09 0.10		3
<i>Brassica napus</i> Wildflowers pollen	1.4 0.16			4
Bee Urban Bee Rural pollen	20		5 35	3
<i>Taraxacum</i> pollen	2.9		6.3	6,7
<b>Neonicotinoid residue in ornamentals (pot 300 mg/agft)</b>				
<i>Tilia cordata</i> 25 cm DBH trunk inj.	1,340 45 yr 2	36,283 680 yr 2		8
<i>Tilia cordata</i> 25 cm DBH soil dr.	34 yr 1 38 yr 2	290 yr 1 680 yr 2		8
<i>Tilia cordata</i> 70 cm DBH soil dr.	30 yr1 88 yr2	554 yr1 737 yr2		8
<i>Cornus racemosa</i> 4 cm DBH soil dr.	762	21,062		8
Rosa soil dr.	812	0a		8
<i>Asclepias incarnata</i> soil dr.	86	132		8
<i>Agastache</i> soil dr.	94	561		8
<i>Callitriche hybrid</i> Pot dr. 1L pot	333	25, 333		8
<i>Ruellia humilis</i> Pot dr. 1L pot	502	2,086		8
<i>Asclepias incarnata</i> pot dr. 6L pot	1568			8
<i>Agastache</i> Pot dr. 6 L pot	1973			8

1. Pecenka and Lundgren 2015, 2. Olaya-Arenas and Kaplan 2017, 3. Blacouiere et al. 2012, 4. Boffa et al. 2015, 5. David et al. 2016, 6. Krupke et al. 2012, 7. Krupke et al. 2017, 8. Kritechik unpublished

### Objective 2: Materials & Methods

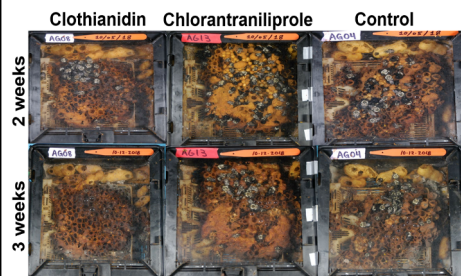
#### Objective 2: Flight cage studies on lethal and sublethal doses on bumblebees.

Bumblebees, *Bombus impatiens* (Koppert Biological Systems, Howell, MI), colonies were fed untreated Koppert Bee Happy syrup (35%) for 3 weeks after which clothianidin (20 ppb) or chlorantraniliprole (4 ppm) were dissolved in the syrup and fed ad libitum for 5 weeks (10 colonies/trt). Colonies were measured weekly for weight (g), syrup consumption (ml), and movement (sec). Photos were taken bi-weekly to measure brood numbers, brood cell age (1-3), worker numbers, and disease. Movement is the time for a worker to cross the brood.

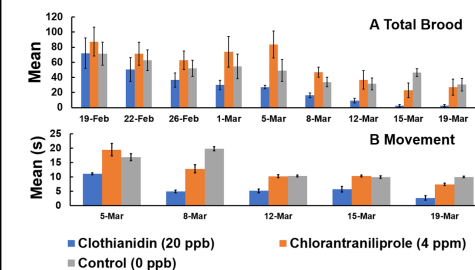
### Objective 2: Results

**Objective 2:** *Bombus impatiens* fed 20 ppb of clothianidin (LC<sub>50</sub> 100 ppb, Scholer and Krischik 2014) in syrup had reduced brood production and movement. At 4 ppm chlorantraniliprole (LC<sub>50</sub> 7 ppm, Smagghe et al. 2015) caused no effects

#### Figure 1. Clothianidin (20 ppb) had lower colony weight and brood.



**Figure 2. (A) Brood numbers (mean ± SE) and (B) movement (mean ± SE), both were lower for clothianidin (20 ppb).**



### Objective 3: Materials & Methods

#### Objective 3: Lab studies on lethal and sublethal doses on butterflies

For LC<sub>50</sub> bioassays, larvae were fed swamp milkweed, *Asclepias incarnata* (*Danaus plexippus*) or common mallow, *Malva sylvestris* (*Vanessa cardui*) dipped in insecticides. Adults in 3 m cages were fed Bee Happy syrup at eclosion. On day 1 adults (30/trt) were force fed syrup containing bifenthrin, (0.1 ppm), clothianidin (10 ppm), imidacloprid (0.05 ppm), or chlorantraniliprole (0.001 ppm). Butterflies were dropped and the ability to open wings was measured on 3 days (day 1, 8, 10).

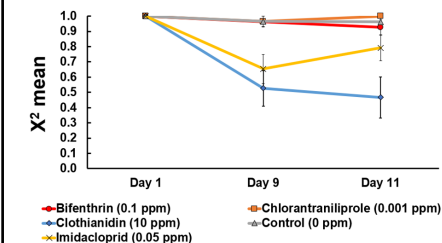
### Objective 3: Results

**Objective 3:** For clothianidin, larval *D. plexippus* had an LC<sub>50</sub> of 4 ppm and *V. cardui* 96 ppm and for chlorantraniliprole 0.20 ppm and 0.03 ppm. Sublethal effects of clothianidin (10 ppm) reduced flight (wing opening), but did not lower fecundity

**Table 2. LC<sub>50</sub> ppm values for *D. plexippus* and *V. cardui***

Insecticide	n	LC <sub>10</sub>	LC <sub>50</sub>	LC <sub>90</sub>	Slope ±SE	X <sup>2</sup>	P
<b>LC<sub>50</sub> fifth instar painted lady larvae, <i>Vanessa cardui</i></b>							
clothianidin	180	28.9	96.2	801	0.6 ± 0.1	2.8	0.6
imidacloprid	180	43.3	256	1309	0.8 ± 0.1	3	0.4
bifenthrin	180	7.1	9	70	0.6 ± 0.1	0.5	0.9
chlorantraniliprole	180	0.004	0.03	0.2	0.7 ± 0.1	0.06	0.9
<b>LC<sub>50</sub> fifth instar monarch larvae, <i>Danaus plexippus</i></b>							
clothianidin	262	0.15	3.7	89	0.4 ± 0.05	8.1	0.2
imidacloprid	99	0.19	1.1	6.24	0.7 ± 0.18	1.2	0.3
bifenthrin	191	0.18	1.5	12.49	0.6 ± 0.08	4.2	0.4
chlorantraniliprole	300	0.02	0.2	2.8	0.5 ± 0.07	2.0	0.8
<b>LC<sub>50</sub> adult painted lady adults, <i>Vanessa cardui</i></b>							
clothianidin	100	6.0	13.2	28.9	1.6 ± 0.4	1.5	0.5

**Figure 3. Proportion of *D. plexippus* adults that did not fly.**



### References

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### Discussion & Conclusion

Two butterfly species had higher LC<sub>50</sub> for the neonicotinoids clothianidin (4, 96 ppm) and imidacloprid (1, 256 ppm) than bumblebees (100 ppb imidacloprid, clothianidin). LC<sub>50</sub> for butterflies was lower for chlorantraniliprole (30, 200 ppb) than bumblebees (7 ppm). Clothianidin (10 ppm) reduced flight in monarch butterflies. The 20 year decline of endangered Rusty patched bumblebee, *B. affinis*, may be correlated to ubiquitous neonicotinoid residues and bumblebee sensitivity (20 ppb).