

# The use of imidacloprid affects two parasitoids of the tobacco budworm differently

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# Imidacloprid

Has low mammalian toxicity but is under intense scrutiny for environmental effects

Is the industry standard in tobacco for control of aphids, thrips and flea beetles

But...

Has no activity against the tobacco hornworm or tobacco budworm.

# Imidacloprid and the Hornworm parasitoid *Cotesia congregata*

In previous work, we determined...

Imidacloprid is toxic to adult *C. congregata*

Imidacloprid can move from caterpillar to wasp larvae feeding in them

Foliar applications of the insecticide reduce parasitism of hornworms (but transplant-timed applications don't)

# The budworm endoparasitoids *Campoletis sonorensis* and *Toxoneuron nigriceps*...

...occur in overlapping regions of the southeastern United States.

...females prefer to lay eggs in 3<sup>rd</sup> instar *H. virescens* larvae.

*Campoletis sonorensis*



*Toxoneuron nigriceps*



# Routes of insecticide exposure

1. Topical exposure of adults to sprays and/or residues
2. Oral exposure of adults to nectar and/or pollen
3. Developmental exposure – topical and/or oral exposure of larvae inside the body of the host





# Field study design

Treatments

1. Untreated control
2. Admire Pro 0.8 oz./1,000 plants Greenhouse spray-in
3. Admire Pro 0.8 oz./1,000 plants Transplant water drench

Plots

401 2	402 1	403 3
301 1	302 2	303 3
201 3	202 1	203 2
101 2	102 3	103 1

3 years, 2 locations, 4 replications,  
rbc design

Collection/  
analysis



1. Infestations monitored until flowering.
2. Budworms  $\geq 3^{\text{rd}}$  instar collected.
3. Budworms observed in laboratory for successful parasitism and adult wasps observed for longevity.
4. Means compared using GLIMMIX procedure in SAS version 9.3; means separated using Tukey-Kramer.

# ELISA Design



- A subset of field-collected larvae and parasitoids were tested for imidacloprid and metabolite concentrations.
  1. Insects were rinsed 3 times in distilled water and dried with clean sterile Kim Wipe® lab tissues.
  2. Insects were homogenized in a 1:1 or 1:2 ratio by weight with distilled water and centrifuged at 12,000 x g (Eppendorf® minispin® Hamburg, Germany) for 2 minutes at room temperature.
  3. An aliquot of 40 µl of the resulting supernatant was added to 100 µl of distilled water.
  4. The prepared samples were measured for imidacloprid concentrations by enzyme-linked immunosorbent assay (ELISA) using the QuantiPlate™ Kit for Imidacloprid (ENVIROLOGIX, Portland, Me) following manufacturer's instructions.





# Results: Imidacloprid residues



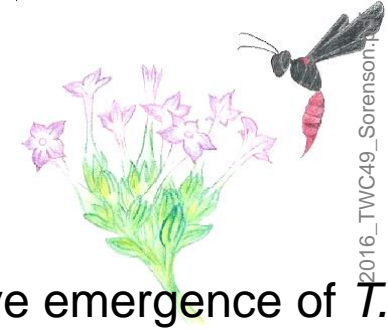
Species (life stage)	Concentration ( $\pm$ SEM)		
	Untreated	Imidacloprid – greenhouse (23.65ml/1000 plants)	Imidacloprid – transplant water (23.65ml/1000 plants)
<i>H. virescens</i> (larvae)	0.69 (0.07)*	7.66 (0.02)	7.91 (0.01)
<i>T. nigriceps</i> (larvae)	0.09 <sup>†</sup> (0.02)*	0.55 (0.07)	0.70 (0.06)
<i>T. nigriceps</i> (adults)	0.22 <sup>†</sup> (0.01)*	0.31 (0.03)	0.37 (0.03)
<i>C. sonorensis</i> (larvae)	-	-	-
<i>C. sonorensis</i> (adults)	-	-	-

\* denotes means significantly different within a row at  $\alpha=.05$

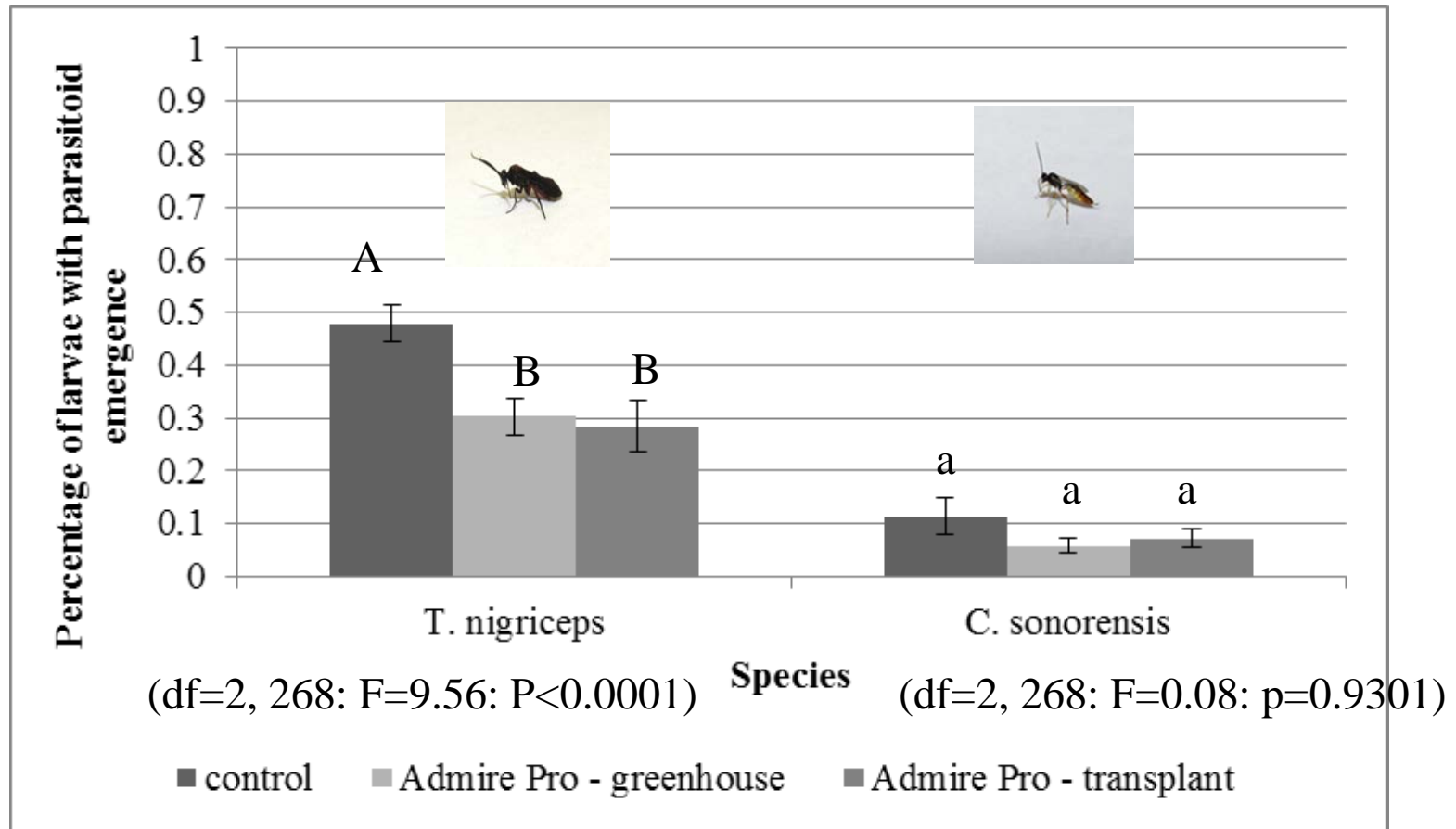
<sup>†</sup> denotes mean concentration below the limit of quantification (0.3 ppb)

- denotes mean concentration below the limit of detection (0.07 ppb)

# Results: Parasitism rates



ANOVA results: Mean percentage of field-collected *H. virescens* with live emergence of *T. nigriceps* and *C. sonorensis*. Vertical bars denote SEM.

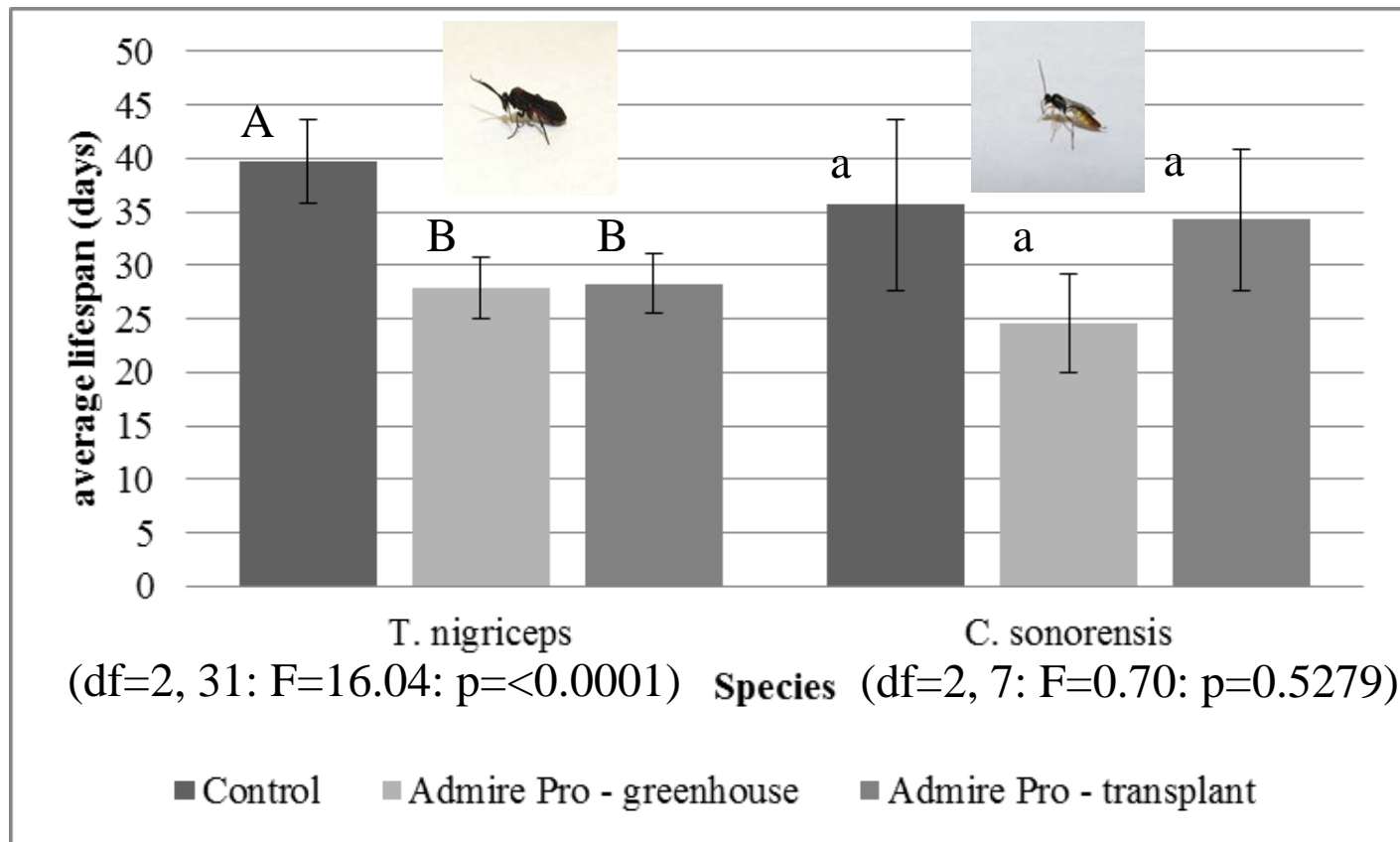


Treatments followed by the same letter are not significantly different according to Tukey-Kramer. *T. nigriceps* is denoted with capital letters and *C. sonorensis* with lower case letters.

# Results: Adult lifespan



ANOVA Results: Average lifespan (in days) of adult female *T. nigriceps* and *C. sonorensis* from field-collected *H. virescens*. Vertical bars denote SEM.



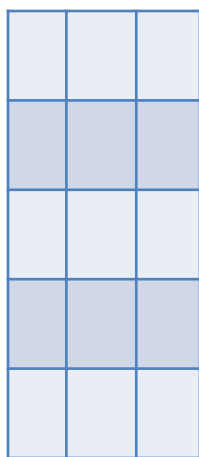
Treatments followed by the same letter are not significantly different according to Tukey-Kramer. *T. nigriceps* is denoted with capital letters and *C. sonorensis* with lower case letters.

# Greenhouse study design

Treatments

1. Untreated control
2. Admire Pro drench 0.8 oz./1,000 plants Transplant

Layout



30 plants per treatment per species, rbc design.  
3 replications

Collection/  
analysis

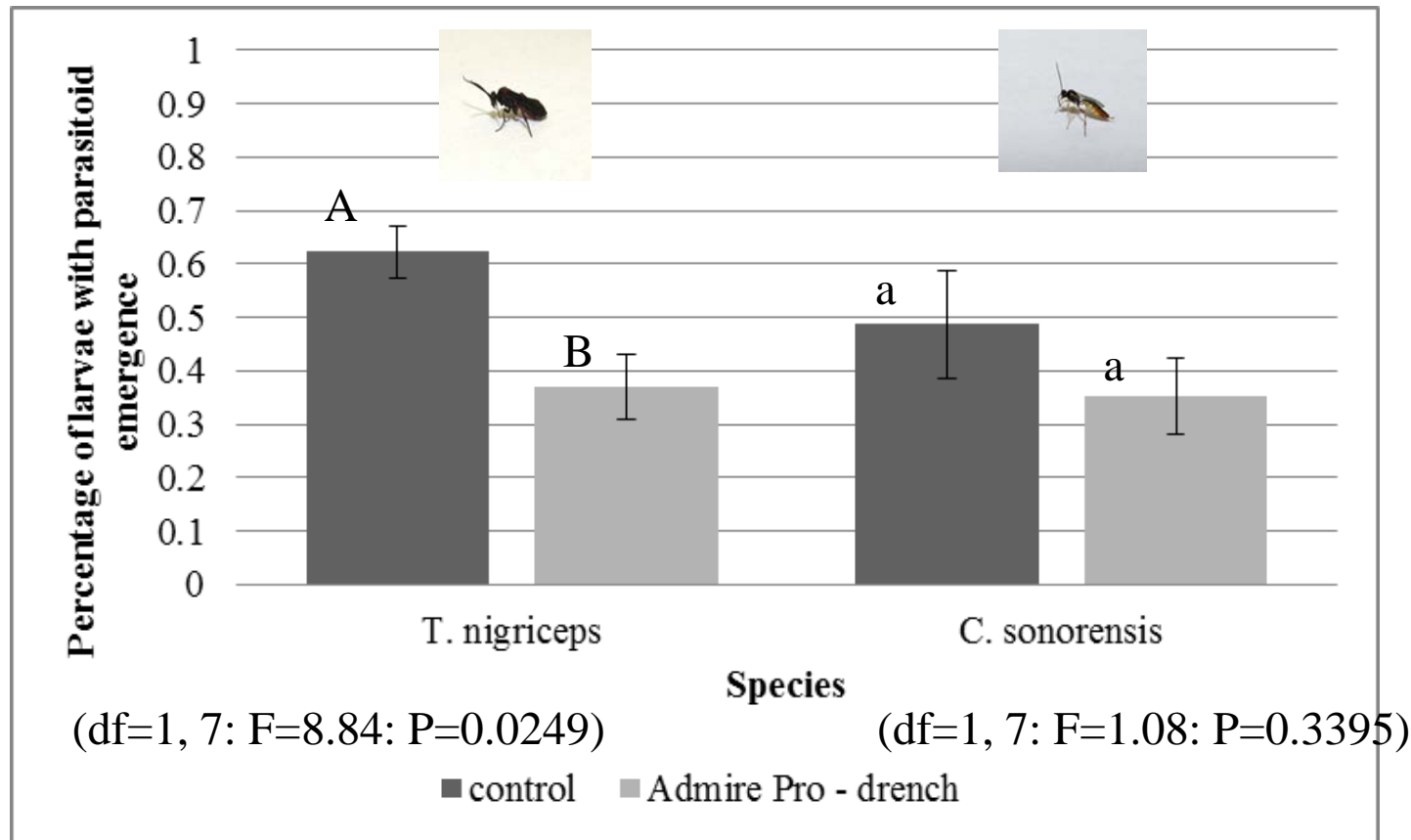


1. Budworms reared on plants and parasitized by wasps in the lab.
2. Plants bagged to prevent movement.
3. Units checked after ~2 weeks for parasitism.
4. Means compared using MIXED procedure in SAS version 9.3.

# Results: Parasitism rates



ANOVA Results: Percentage of *H. virescens* larvae parasitized by *T. nigriceps* and *C. sonorensis* with parasitoid emergence in greenhouse trials. Vertical bars denote SEM.



Treatments followed by the same letter are not significantly different according to Tukey-Kramer. *T. nigriceps* is denoted with capital letters and *C. sonorensis* with lower case letters.

1. Size = ~3.4 mg
2. Generalist
3. Length of time in host = 7-9 days
4. Host feeding stops immediately

*Campoletis sonorensis*



1. Size = ~17mg
2. Specialist
3. Length of time in host = 11-13 days
4. Host feeding stops in ~5 days

*Toxoneuron nigriceps*

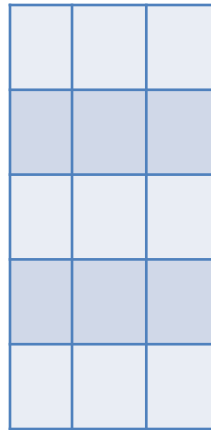


# ELISA for imidacloprid concentration study design

Treatments

1. Untreated control
2. Imidacloprid 0.8 oz./1,000 plants Transplant water drench
3. Imidacloprid 1.6 oz./1,000 plants Transplant water drench

Layout



30 plants per species, 3 replications, rbc design

Collection/  
analysis



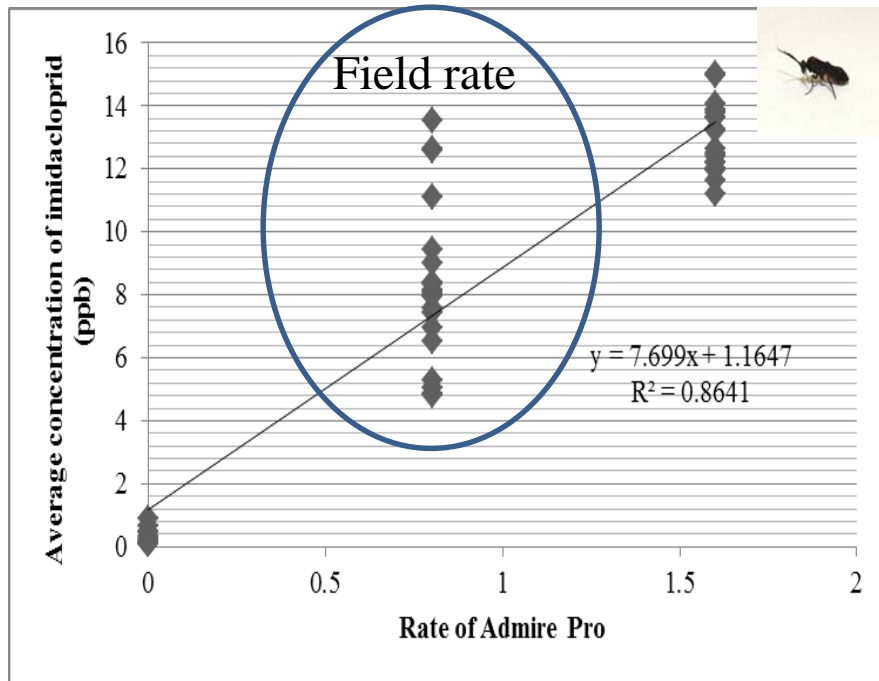
1. Budworms reared on plants and parasitized by wasps in the lab.
2. Plants bagged to prevent movement.
3. Budworms removed after 1 week.
4. Imidacloprid concentration in larvae tested using ELISA.
5. Means compared using MIXED procedure in SAS version 9.3.

# Results: ELISA

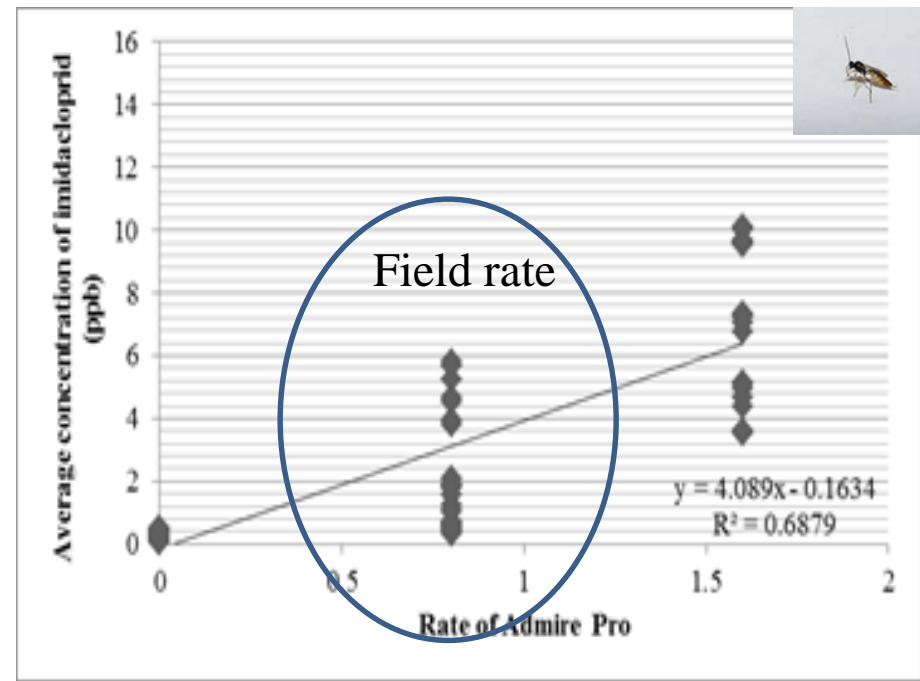


ANOVA Results: Concentration of imidacloprid (ppb) in *H. virescens* larvae parasitized by *T. nigriceps* (A) and *C. sonorensis* (B).

A)



B)



\*Concentration levels differed by imidacloprid rate (df=2,108: F=288.31: P<0.0001) and parasitoid species (df=2,108: F=162.04: P<0.0001). The interaction of the imidacloprid rate and the parasitoid species was also significant (df=2,108: F=35.32: P<0.0001).



# Routes of potential exposure

1. Topical exposure to sprays and/or residues
2. Oral exposure of adults to nectar and/or pollen
3. “Developmental” exposure – topically and/or orally through exposure to imidacloprid and/or its metabolites sequestered in the body of the host





# Topical LD50 study design



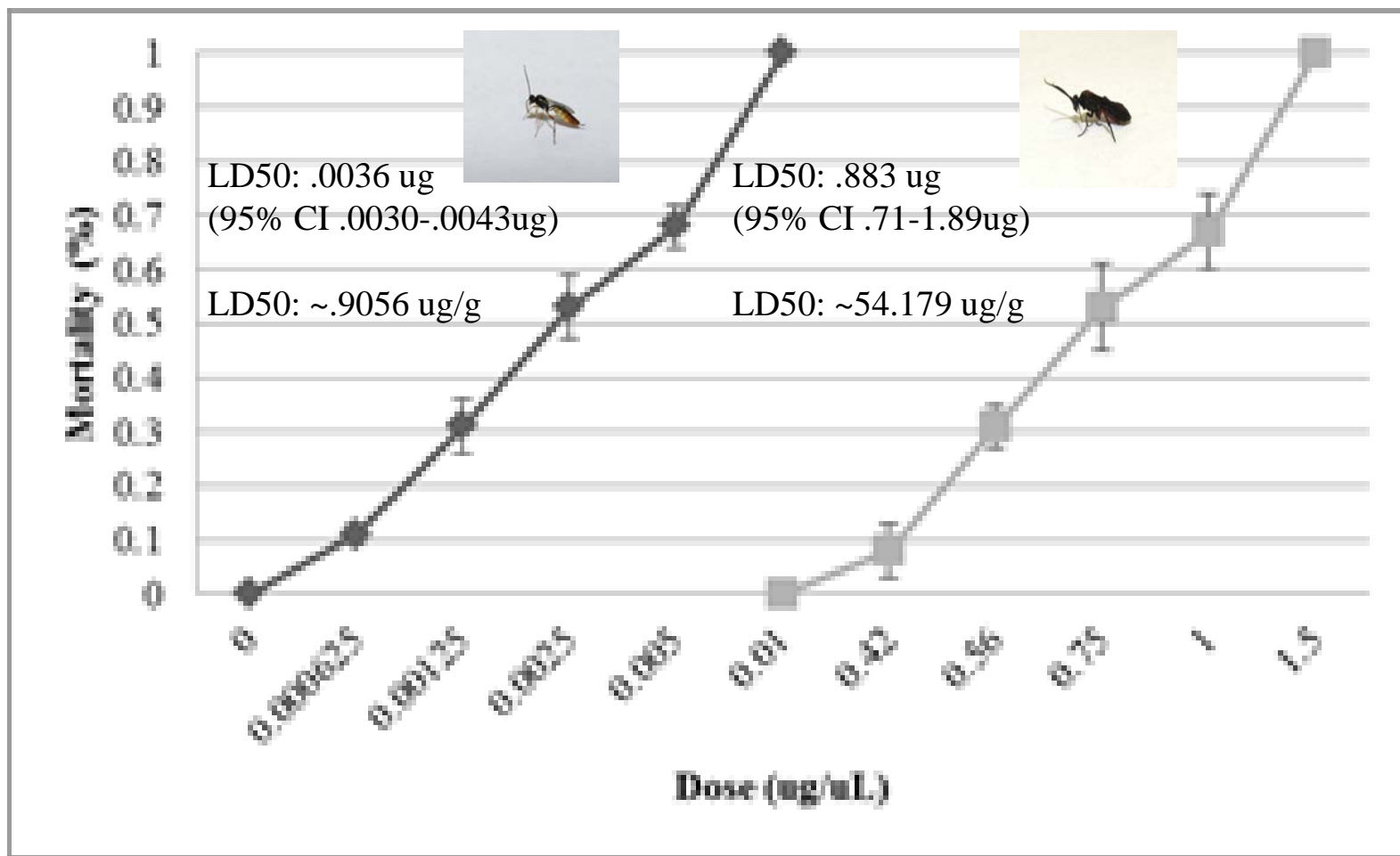
- 5 concentrations of imidacloprid in acetone base, 1 acetone-only control
- 10 adult wasps per dose per replicate; 3 replicates
- Dose applied to pronotum
- Observed at 12, 24 & 48 hours
- Treatments compared using GLIMMIX and PROBIT procedures in SAS version 9.3.



# Results: Topical LD50



The dose-response relationship resulting from contact exposure to imidacloprid. The lack of error bars represents SD=0.



	<b>Parasitism Rate</b>	<b>Adult Longevity</b>	<b>Adult Topical toxicity</b>
<i>C. sonorensis</i>	No change	No change	More susceptible
<i>T. nigriceps</i>	Decreases	Decreases	

*C. sonorensis* adults are more susceptible to topical imidacloprid exposure, but larvae are exposed to lower imidacloprid rates during development, and parasitism rates are unaffected.

*T. nigriceps* adults are more tolerant to topical imidacloprid exposure, but larvae are exposed to higher imidacloprid rates during development, and parasitism rates are reduced.

# Conclusions

Two parasitoid species that inhabit the same agricultural environments and utilize the same host respond differently to imidacloprid exposure.

There are differences in imidacloprid toxicity between adult and immature parasitoids and between parasitoid species.

Imidacloprid is capable of moving through trophic levels of the agroecosystem, and that this movement causes deleterious effects on some, but not all, beneficial species.

# Acknowledgements



2016\_TWC49\_Sorenson.P

## **Sorenson/Roe/Burrack Labs:**

Tanner Stanfield  
Anirudh Dhammi  
Heather Moyette  
Aurora Toennisson  
Damien Stone

## **Funding:**

Cotton Incorporated  
NIH/NCSU Molecular Biotechnology  
Training Program  
Thrips Tospovirus Education Network  
North Carolina Tobacco Commission  
E.G. Moss Fellowship  
Fred G. Bond Scholarship  
Philip Morris USA Fellowship  
Lorillard Tobacco Company Fellowship  
Altria Fellowship



***H. virescens***: Sandra Paa & Gould Lab

TWC2016(47) - Document not peer-reviewed

Questions?

