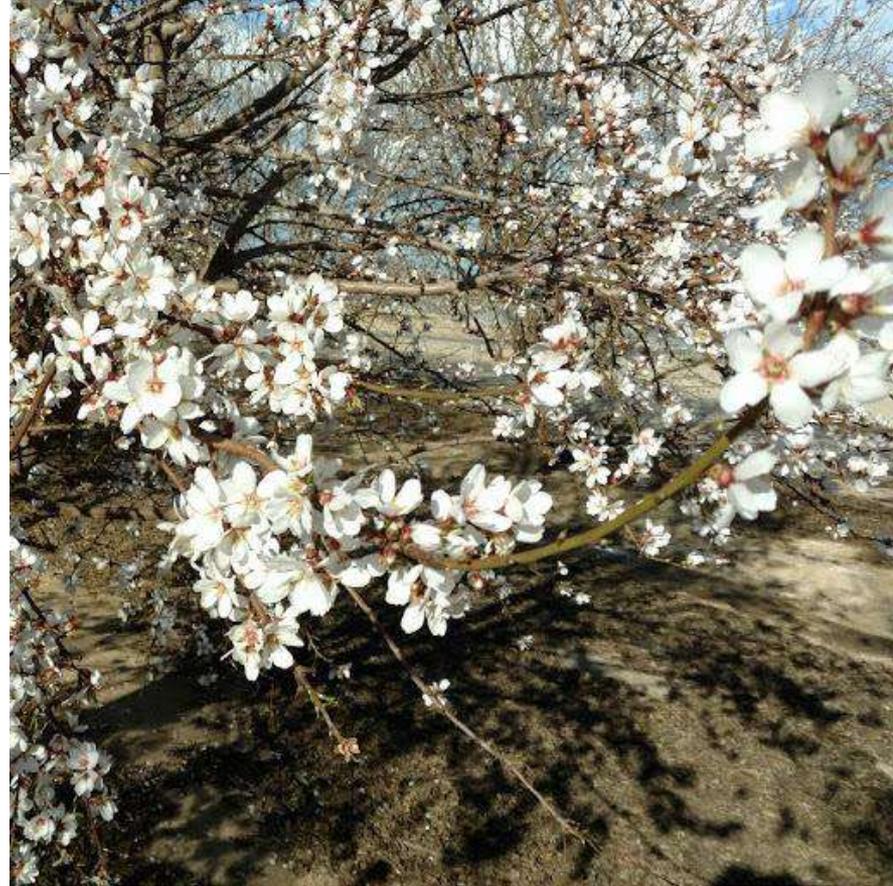


# Can Application Time Limit Fungicide Exposure to Honey Bees in Almonds?

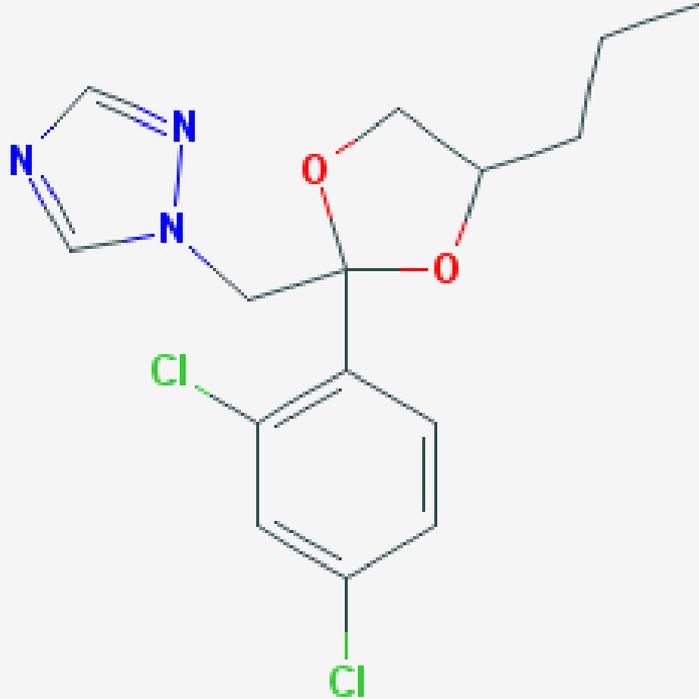
J. Johnson, Cullaborate, LLC, Baltimore MD  
G. Wardell, Wonderful Orchards, Lost Hills, CA,  
D. Lopez, USDA Bee Res. Lab, Beltsville, MD,  
P. Snyder – Stevenson Univ., Owings Mills, MD  
H. Boncristiani, USDA Bee Res.Lab, Beltsville, MD  
J. Pettis, Pettis and Associates, LLC, Salisbury, MD

Study duration Feb 6 -12 2018

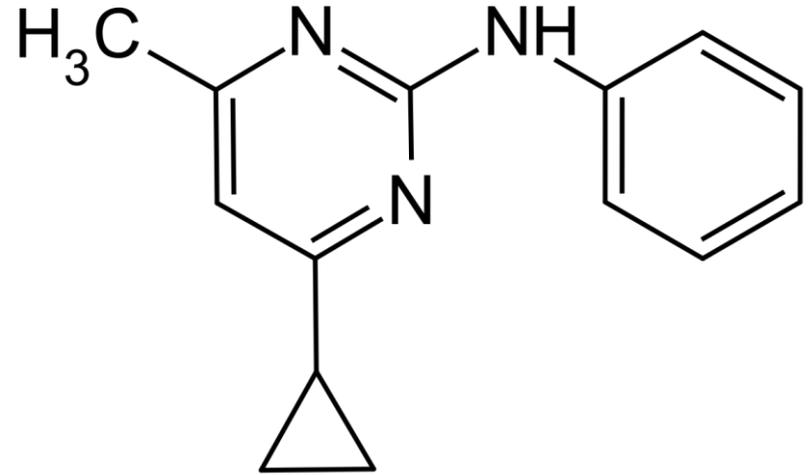


# Chemical structures of fungicides used in this study

**Propiconazole-** Tilt (CA) (100-617-ZG,  
41.80% prop)



**Cyprodinil-** Vanguard WG (CA) (100-828-ZB,  
75.00% cyp)

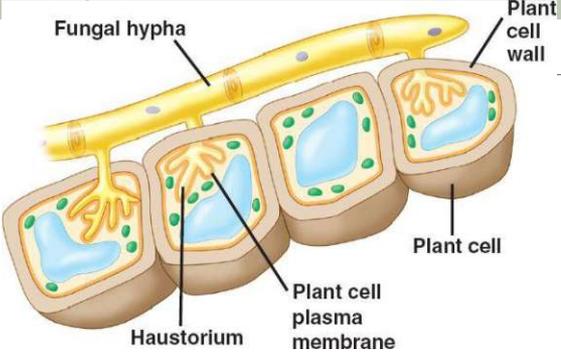


# Propiconazole = a.i.

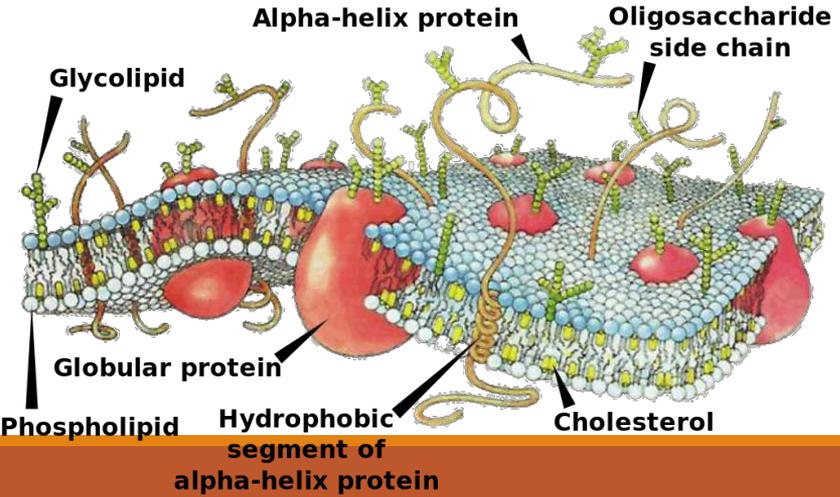
Formula = Tilt  
demethylation inhibitor



*Puccinia graminis* (stem rust)  
haustorium penetrating wheat  
cell



Brown rot on almond



UC Statewide IPM Project  
© 2000 Regents, University of California

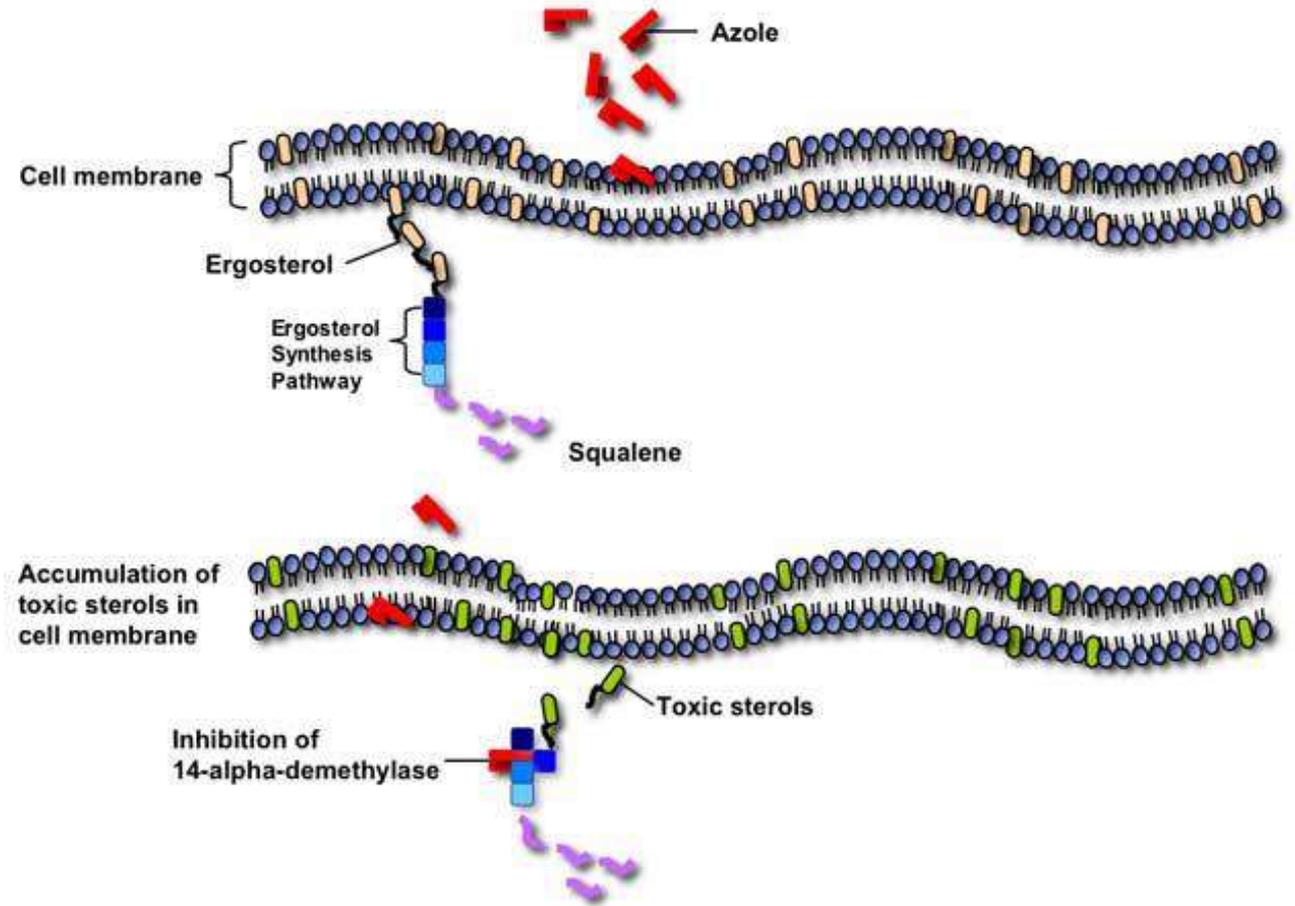
# Propiconazole Demethylation

Demethylation required for ergosterol synthesis and functional fit in membrane.

(Fungistatic vs fungicidal)

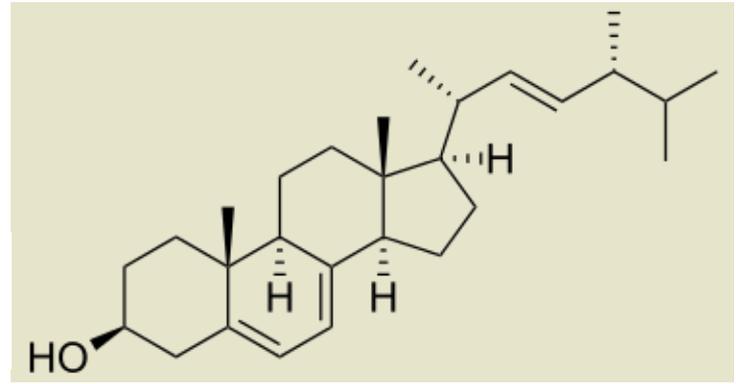
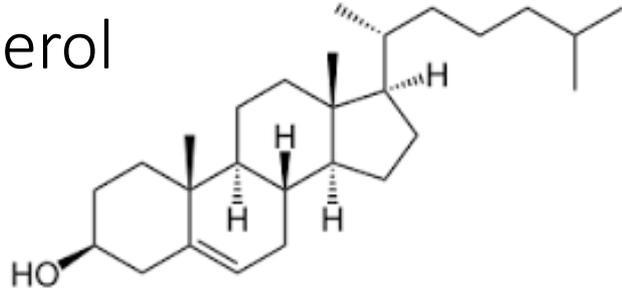
Cholesterol found in animal cell, ergosterol found in fungal cells

Membranes = physical barrier, regulate material exchange



Propiconazole effect:

cholesterol



ergosterol

During the biosynthesis of cholesterol, three demethylations occur, one on the C14 named 14 $\alpha$ -demethylation and two on the C4 named 4 $\alpha$ -demethylations (Cabrera-Vivas et al 2003)

P450's

Propiconazole inhibits microsomal cytochrome P450s from midguts of *Spodoptera eridania* sixth-instar caterpillars. (Brattsen et al. 1994).

# Cyprodinil

Interrupts methionine (amino acid) synthesis

may activate aryl hydrocarbon receptors (possible endocrine disruptor) and may induce nuclear translocation of AHR

may act as an endocrine disruptor

may disrupt the ERK pathway

source: Fang et al. 2013 Toxicology 304, 32-40

# Concerns:

---

- 1) There are reported effects in HBs (Johnson et al.2013, Pettis et al 2013, Brattsten et al.1994)
- 2) The fungicide's mode of action may share common biochemistry with the animal kingdom. (Radice et al.1998)
- 3) The fungicide may interfere in an unanticipated way with the animal's biochemistry (Iwasa et al.2004, Brattsten et al.1994)
- 4) Beneficial fungi utilized by HB to make bee bread may be negatively impacted (Yoder et al. 2013)

# Orchard orientation

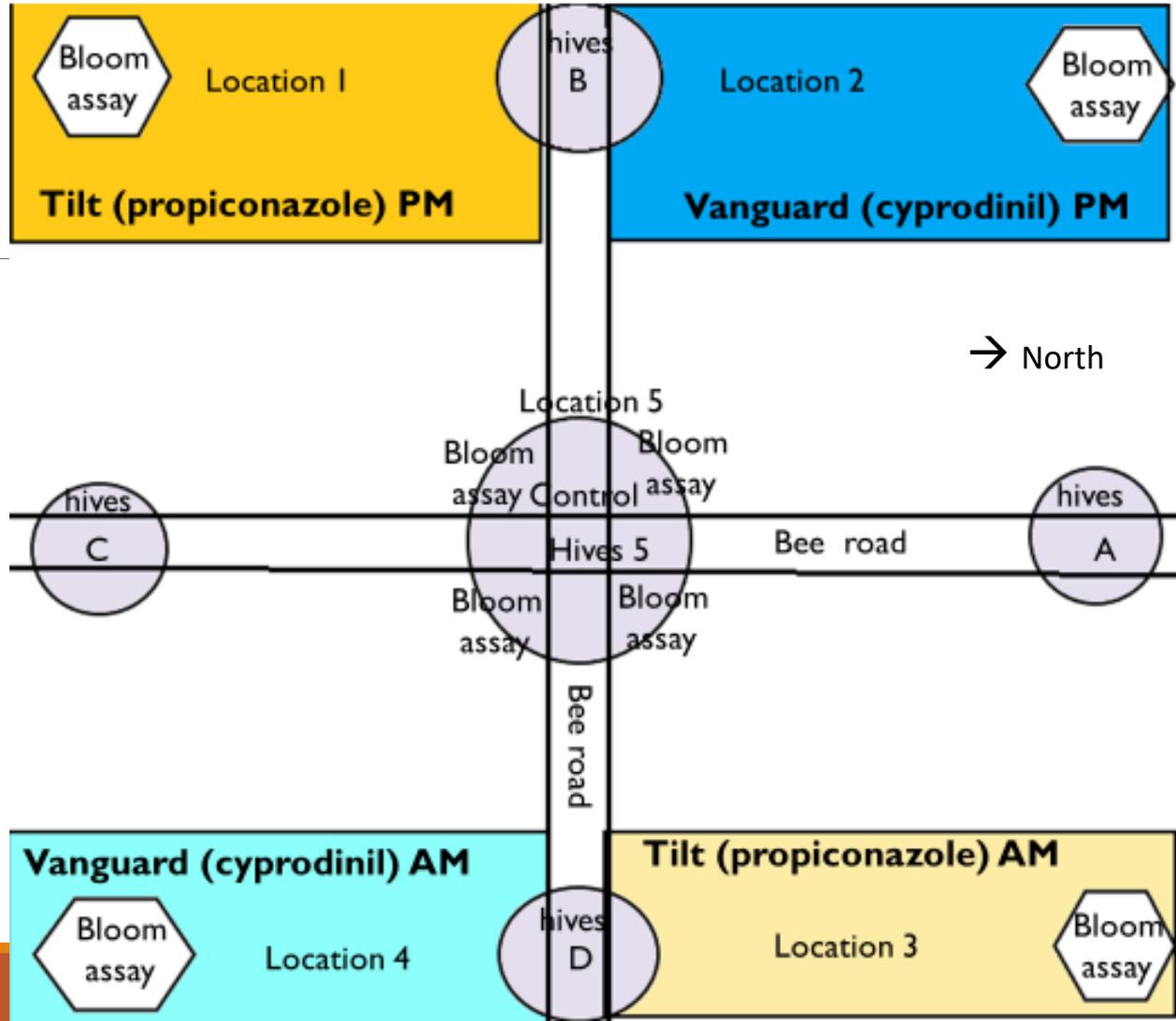
300 acres, ~ 5151ft x 2,542ft

33,950 almond trees with each tree 18 ft. apart in rows , 22-24 ft. apart

600 hives along the bee roads

Prevailing winds from the northwest.

This orchard is completely surrounded by other orchards and is edged by asphalt and hard packed dirt roads



# Measurements

Five metrics before and after AM and PM applications:

Forager counts: flower foragers within specific areas

all returning foragers to hive

pollen-bearing bees returning to hive

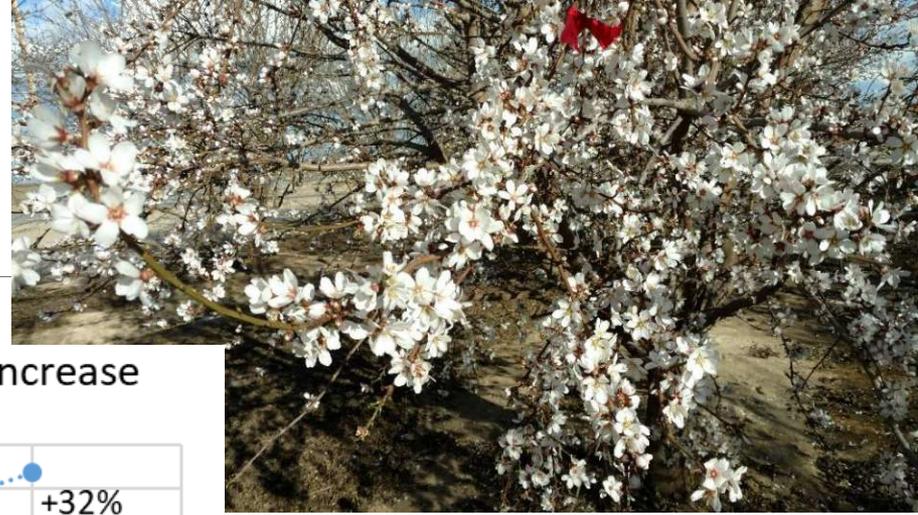
Fungicide conc.: pollen sampled from anthers

pollen collected at hive (traps)

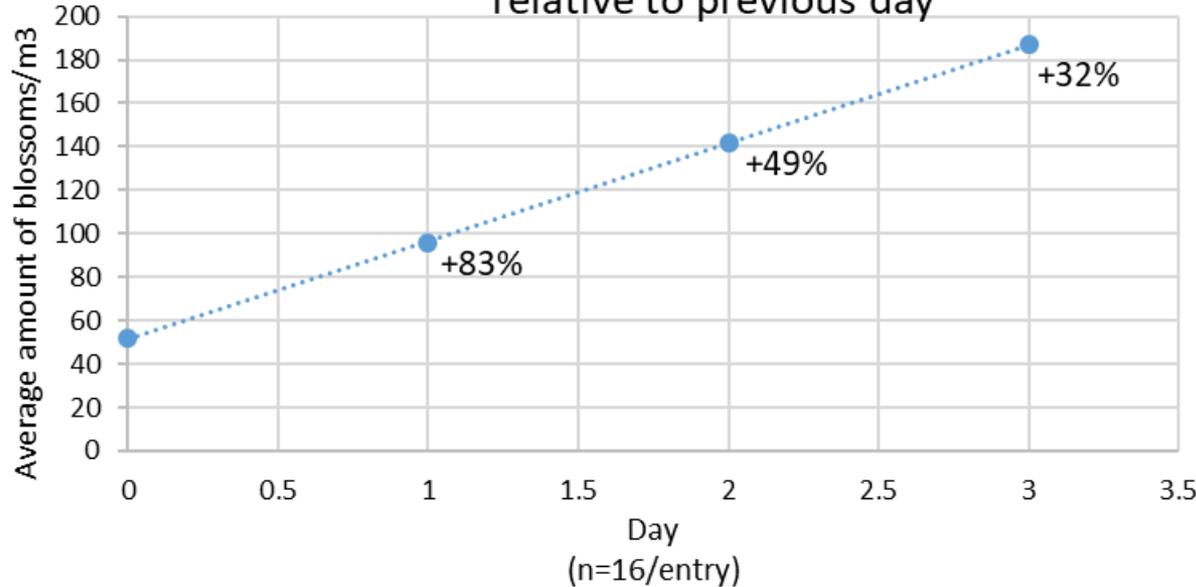


Bloom progress and weather monitored throughout study.

# Bloom density increased during study



Bloom density expressed as percent increase relative to previous day



When counting foragers in blooms, each team member marked a branch and counted bees visiting blooms for 3 mins in m<sup>3</sup> (am and pm counts/daily).

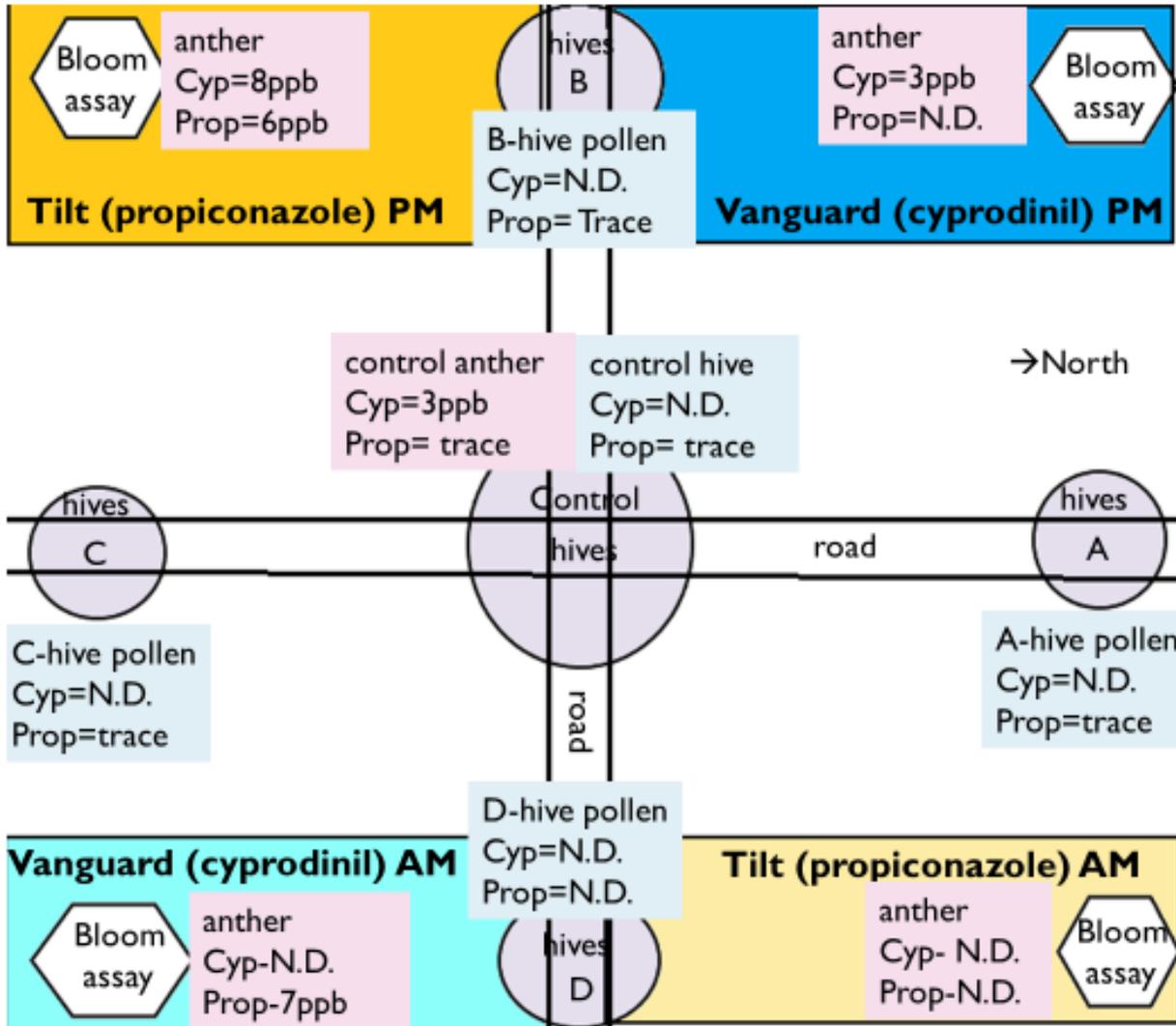
# Weather (time designated in military time)

<b>D</b>	<b>Highest temp °C/time</b>	<b>Lowest Temp °C/time</b>	<b>Rel. Humid. High/low</b>	<b>Avg Wind Speed mph</b>	<b>Highest wind speed mph</b>	<b>% clear day</b>	<b>Atm Press High/low in Hg</b>
<b>1</b>	<b>23.9/13:54</b>	<b>10/23:54</b>	<b>69/32</b>	<b>6</b>	<b>14</b>	<b>100</b>	<b>29.43/29.30</b>
<b>2</b>	<b>22.2/14:54</b>	<b>5.6/6:54</b>	<b>64/17</b>	<b>4.9</b>	<b>16</b>	<b>100</b>	<b>29.49/29.21</b>
<b>3</b>	<b>15.6/14:54</b>	<b>5.6/3:54</b>	<b>73/33</b>	<b>5.3</b>	<b>11</b>	<b>54</b>	<b>29.44/29.29</b>

# Air blast rig

R-11<sup>®</sup> Spreader-Activator (2935-50142) spreader with an 8000 Ga tank and a nozzle size 16. The spray rate of the spreader was 2.5 mph. Fungicides were sprayed at maximum allowable rates.





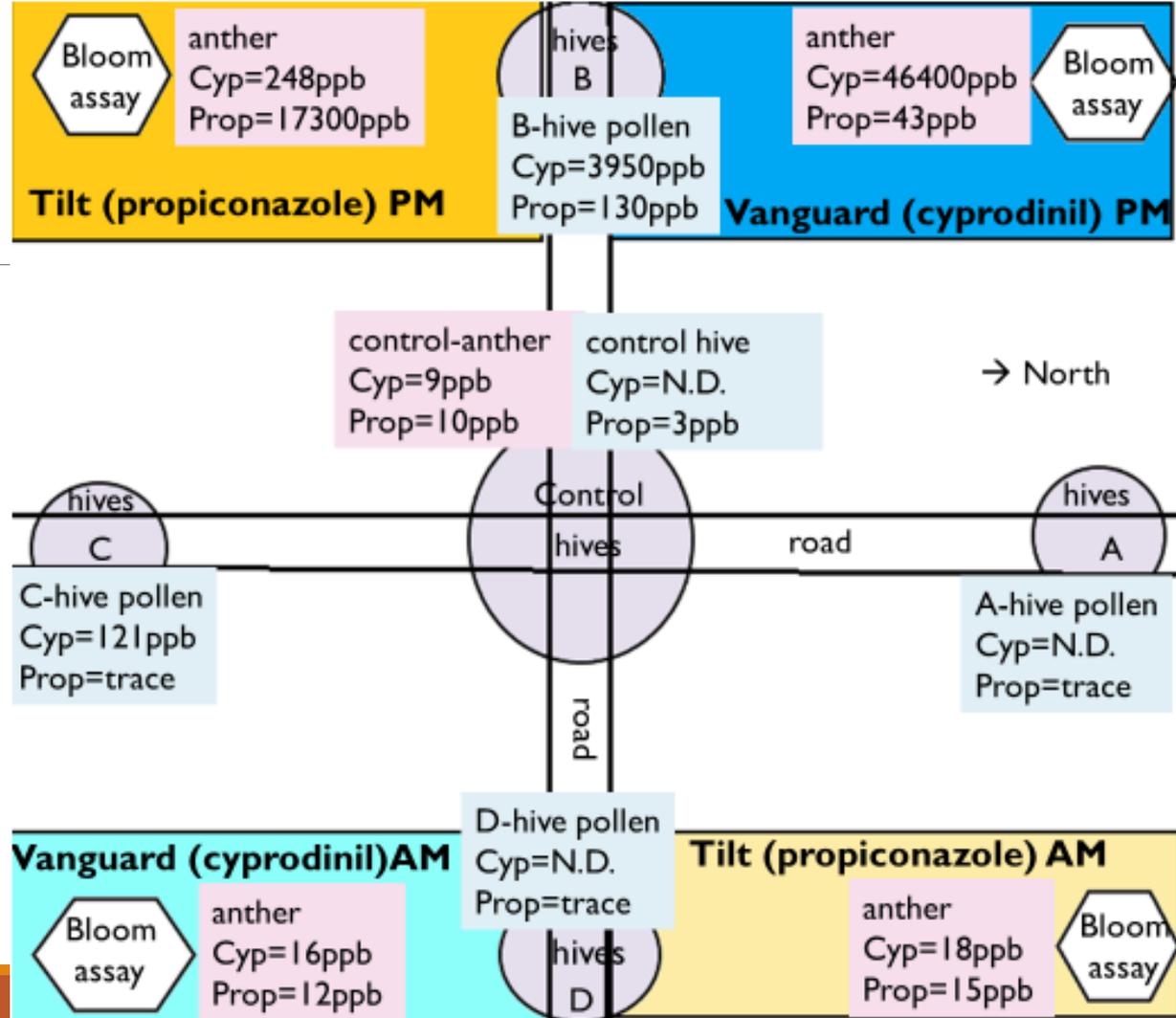
# Control Day, Day 1

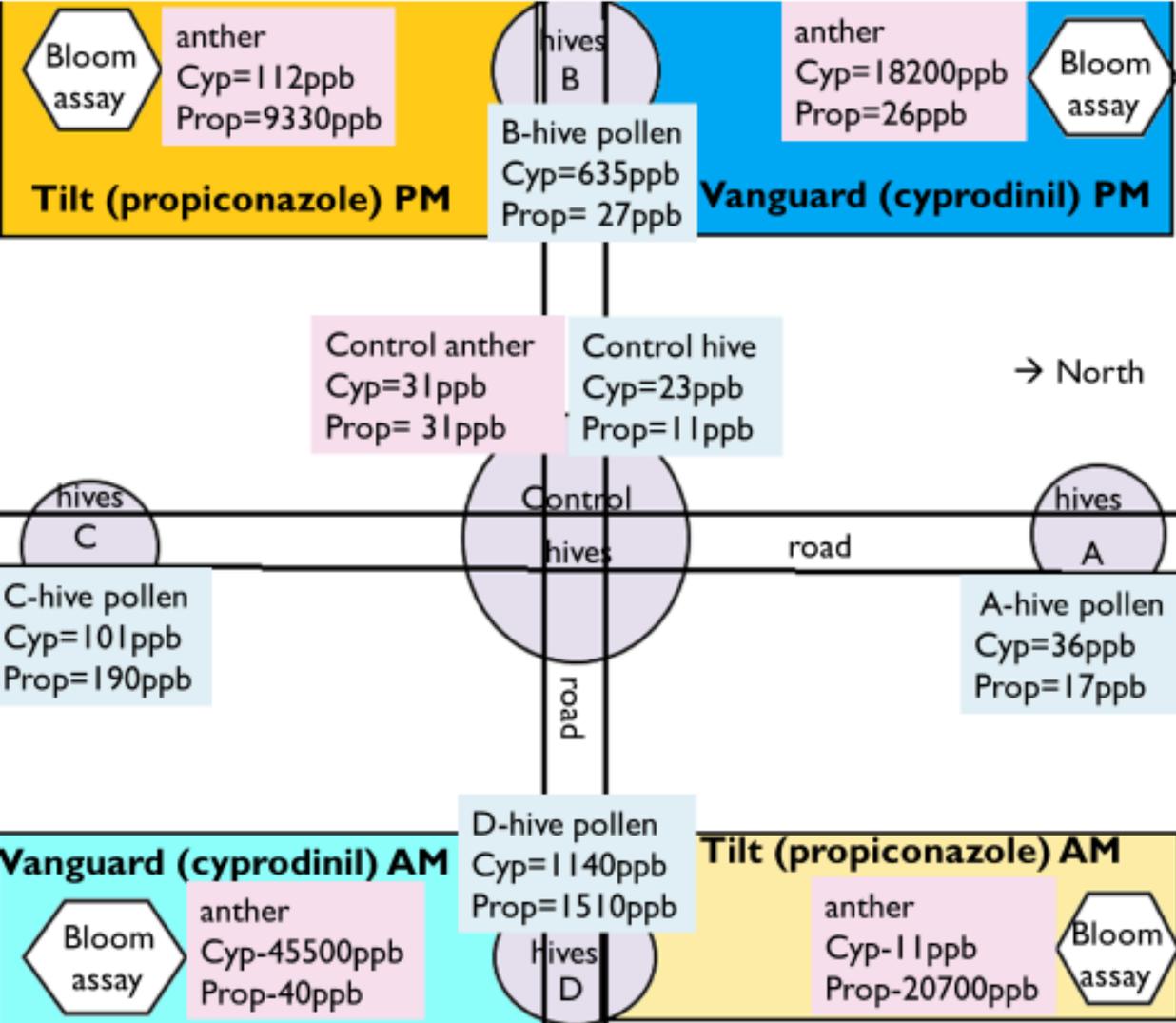
ppb= parts per billion  
 Cyp= Cyprodinil  
 Prop= Propiconazole  
 Anther= anther pollen  
 N.D.= not detected  
 LCMSMS = method to detect fungicide concentrations

The PM spray took place at 6PM on Day 1 after control data had been collected

# Post PM spray, Day 2

The PM spray had taken place 6pm on Day 1 (the previous evening)



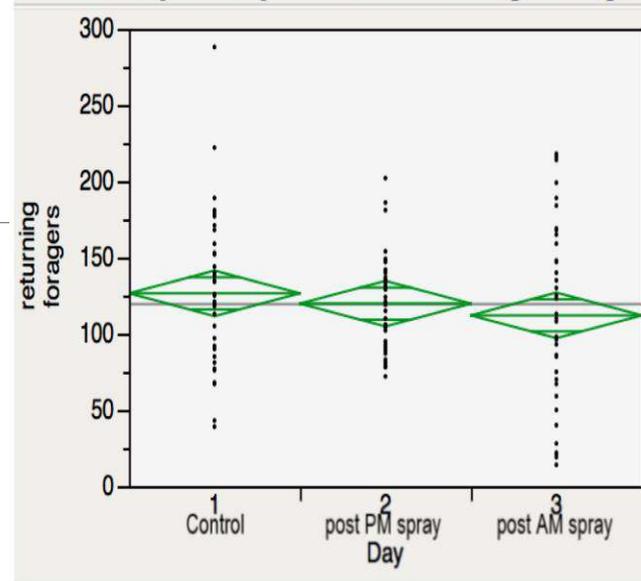


# Post AM spray, Day 3

The AM spray took place at 7 am on this day, Day 3

Analysis of all returning foragers was not significantly different across the three day study

## ▼ Oneway Analysis of returning foragers By Day



Missing Rows 120

## ▼ Oneway Anova

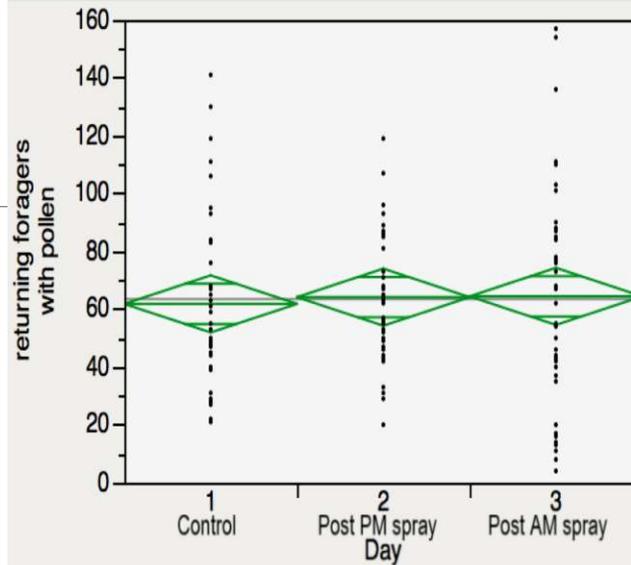
### ▼ Summary of Fit

Rsquare	0.015492
Adj Rsquare	-0.00134
Root Mean Square Error	47.66069
Mean of Response	119.3833
Observations (or Sum Wgts)	120

### ▼ Analysis of Variance

Analysis of all returning pollen-bearing foragers was not significantly different across the three-day study

### ▼ Oneway Analysis of returning foragers with pollen By Day



Missing Rows 120

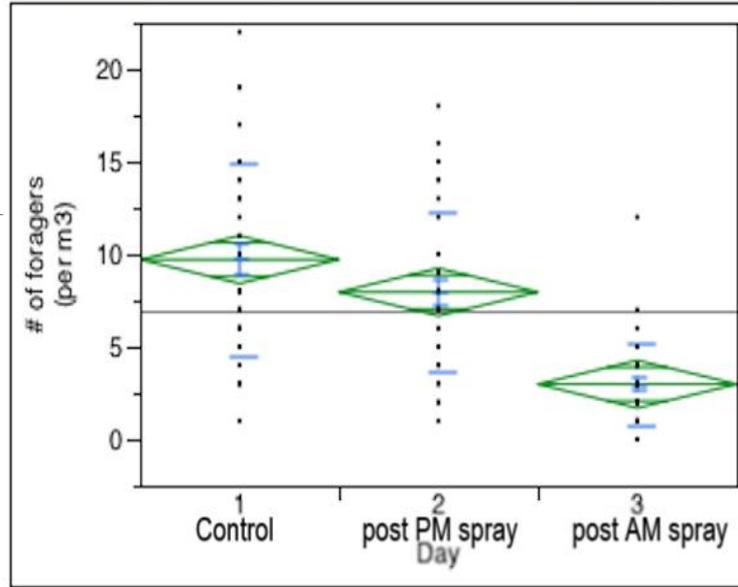
### ▼ Oneway Anova

#### ▼ Summary of Fit

Rsquare	0.0014
Adj Rsquare	-0.01567
Root Mean Square Error	31.54381
Mean of Response	63.44167
Observations (or Sum Wgts)	120

Analysis of foragers visiting blooms was significantly different across the three day study

## Oneway Analysis of # of foragers (per m3) By Day



Missing Rows 120

## Oneway Anova

### Summary of Fit

Rsquare	0.329101
Adj Rsquare	0.317632
Root Mean Square Error	4.119243
Mean of Response	6.875
Observations (or Sum Wgts)	120

## Foragers are not visiting trees- why?

Are they sensing a **physical change** in the bloom? ex. the fungicide formulations make the blooms sticky or slippery

Are they responding to the **chemistry** of the fungicides? ex. the fungicides burn their feet, smell badly, or taste badly

Are they responding **biologically**? ex. the fungicides make them dizzy or sick

# Does application time make a difference on exposure level to bees?

## Prop

Post PM Prop concentrations in blooms were 16% lower than post AM Prop concentrations.

Prop might be degrading/evaporating overnight?

Over the next 24 hrs, the prop concentrations in the PM spray area dropped 46% within the PM spray area.

## Cyp

Post PM Cyp concentration levels in blooms were only 2% different after PM versus after AM spray.

Over the next 24 hrs, cyp concentrations in the PM spray area dropped 61% within the PM spray area.

Could the chemistries of the fungicides cause one to degrade faster in the presence of nighttime dew and the other to degrade faster in sunlight?

# Did the bees respond differently to application time? Perhaps

Of the hives closest to the spray areas,

bees collected 0.75% of the post PM prop concentration on Day 2.

bees collected 7.3% of the post AM prop concentration on Day 3

bees collected 8.5% of the post PM cyp concentration on Day 2

bees collected 2.5% of the post AM cyp concentration on Day 3

Hive pollen concentrations that were sampled closest to the PM spray areas decreased (84% Cyp, and 79% Prop) from Day 2 to Day 3, a result that may reflect forager avoidance learning.

Several factors may be contributing: application time, degradation rates of the fungicides, learning, changing bloom density

Thank you to my team!  
(Dawn, Phoebe, Humberto)



# Thank you especially to

ALMOND BOARD OF CALIFORNIA FOR SUPPORTING THIS  
STUDY!

DR. GORDON WARDELL FOR INVALUABLE HELP AND  
DIRECTION

ERIK WILKINS, MARK SZCZERBA, DOUG BLAIR, MIKE  
MENDES, WONDERFUL

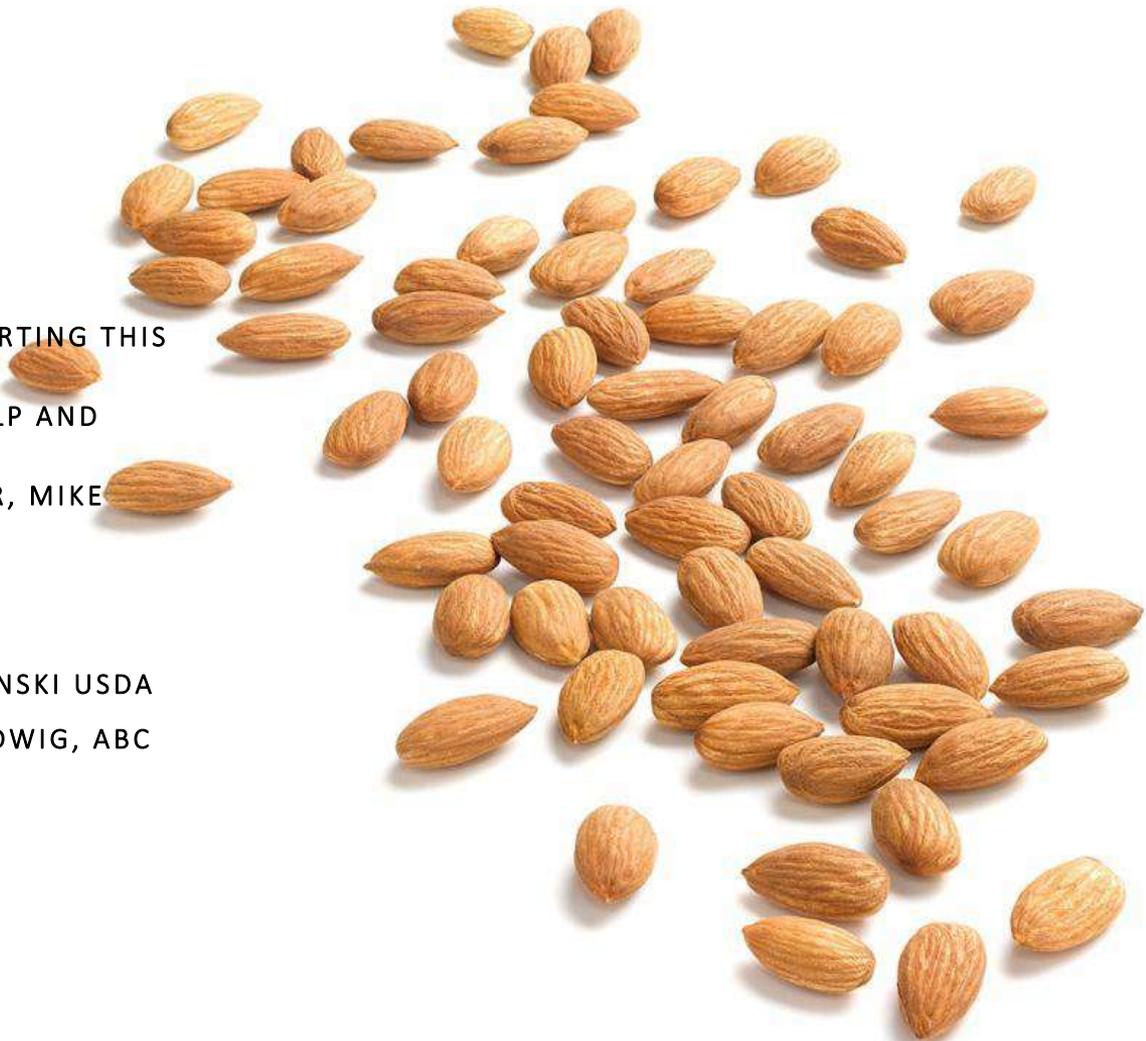
ELIAS, URIEL, EMMANUEL SPRAY TEAM

WILL NESSON

STEVE COOK, JONATHAN BARBER, MARIE DENSKI USDA

DEBYE HUNTER, BOB CURTIS, GABRIELLE LUDWIG, ABC

APIMONDIA FOR INVITING ME TO SPEAK



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