The background features a large, semi-transparent watermark of the University of Delaware seal. The seal is circular and contains the text 'UNIVERSITY OF DELAWARE' around the perimeter, '1743' at the bottom, and a central shield with the words 'GRAMM', 'METAPH', 'PHILOSOPHY', 'RHETOR', 'MATHEM', 'ETHICA', and 'PHYSICA'.

**Novel techniques for evaluating the potential host range
of candidate biological control agent *Trissolcus japonicus*
(Hymenoptera: Platygasteridae)**

Sean Boyle

Dr. Kim Hoelmer

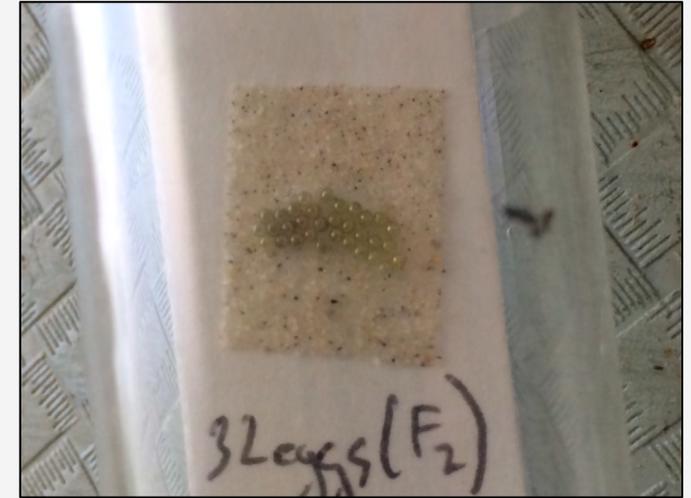
Dr. Judith Hough-Goldstein





Traditional Host Range Evaluations

- **Choice & No-choice** testing = physiological host range
 - Constricted to the parasitoid-host interaction
- Some native species are physiologically-suitable hosts
 - *Podisus maculiventris*
 - Possible over-estimation of NT effects
- How do we improve and/or enhance these host range assessments?



(1) Integrate egg parasitoid host foraging strategies

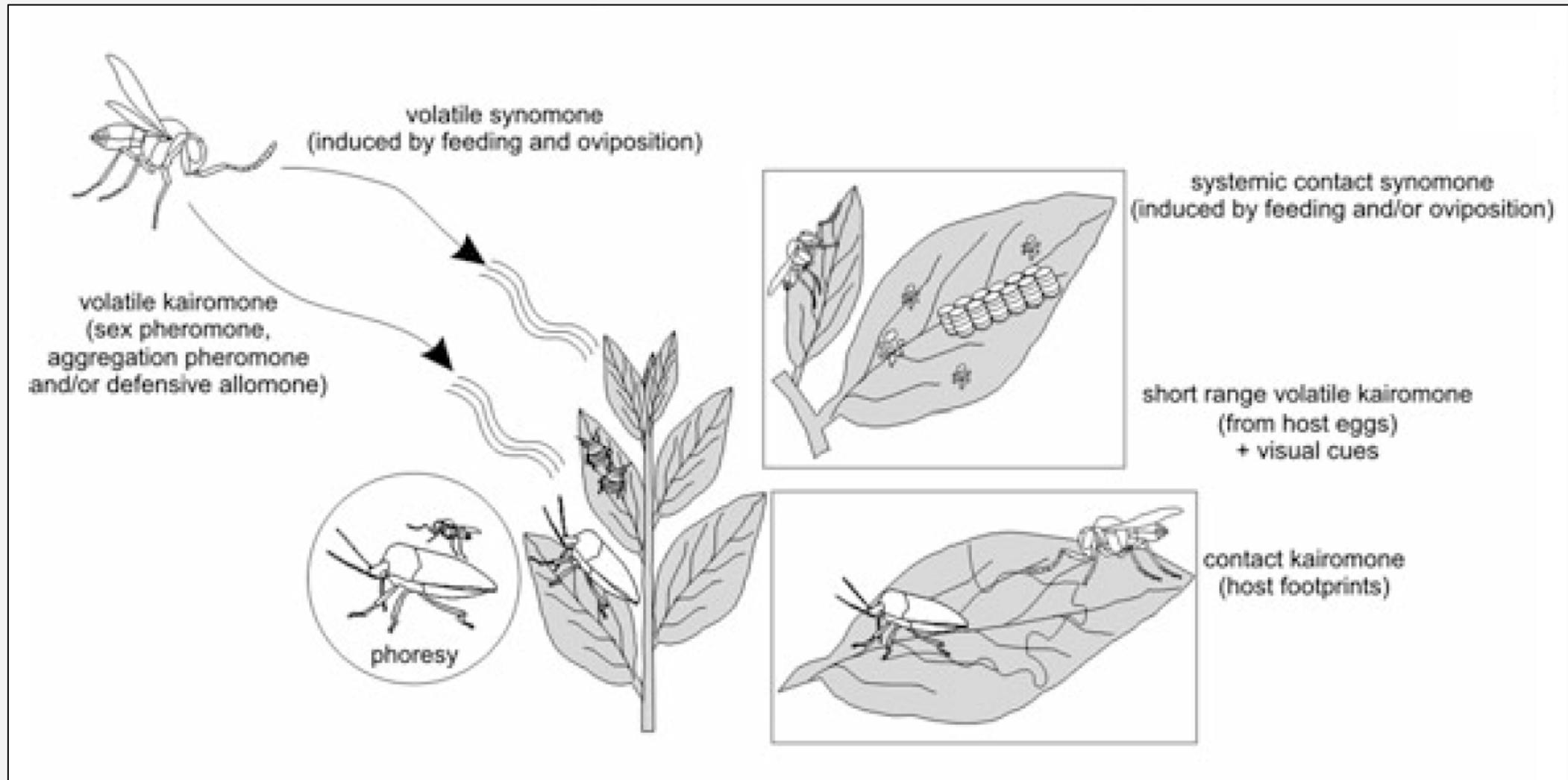
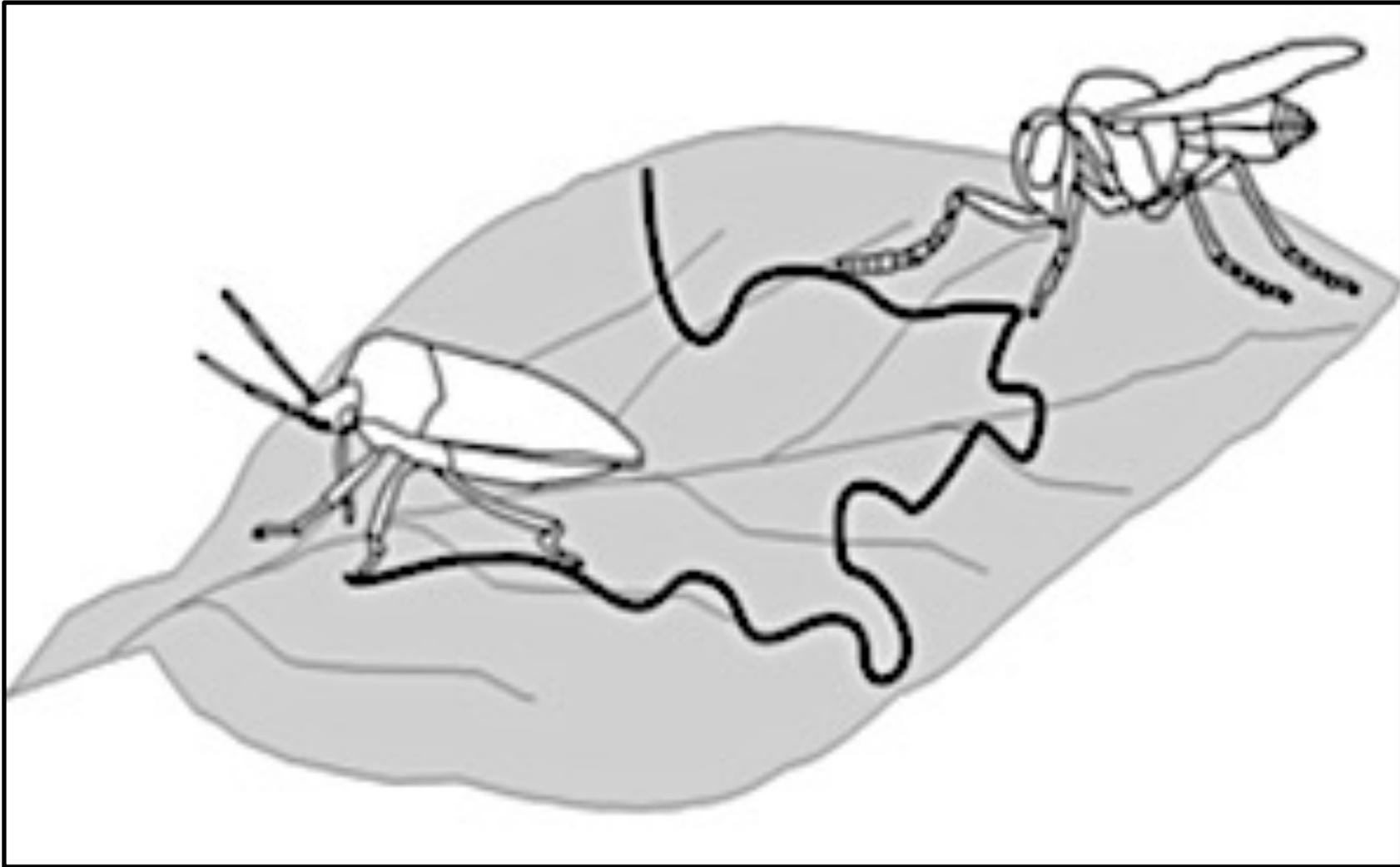


Photo by Conti et al. 2006

Adult contact kairomones (footprints)



Behavioral Responses to adult footprints

- Other *Trissolcus* spp display preference for specific chemical cues

- Gravid female vs. non-gravid & male cues

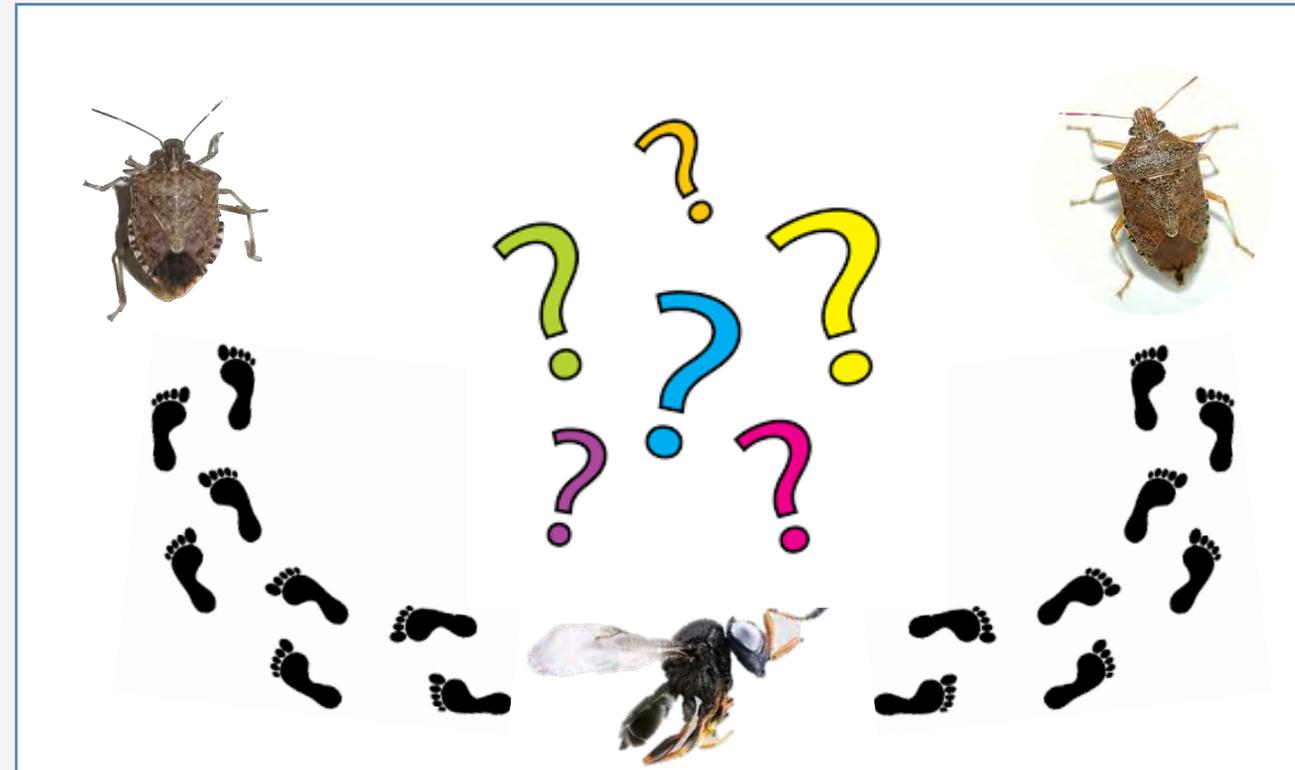
(Colazza et al., 1999, Conti et al., 2003)

- Coevolved hosts vs. non-coevolved host

(Salerno et al., 2006, Conti et al., 2004)

- Characterizing preference

1. Delayed flight response
2. Prolonged arrestment
3. Slowed walking
4. Increased turning while walking



- *T. japonicus* responses to host-related cues are largely unknown!

(2.) Simulate a non-target parasitism event

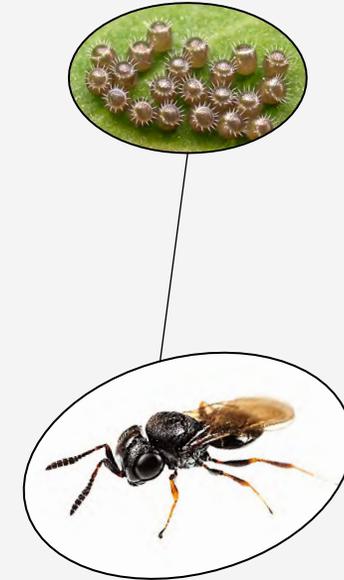
- “**Devil’s advocate**” approach
 - If *T. japonicus* emerges from (suitable non-target species), ...

- **Parental host effects**

1. Phenotypic traits (Abrams et al., 2015)
2. Associative learning (Peri et al., 2006)
3. Fitness traits (Allahyari et al., 2004; Keller, 1987)
4. Host preferences (Henry et al., 2010)



VS



- *T. japonicus* host range tests have only used BMSB as the rearing host

Research Objective #1

Distinguish the host foraging preferences of *T. japonicus* through its exposure to different host contact kairomones on various plant substrates



Research Objective #2

Identify if parental host species affects *T. japonicus* kairomone-induced behavioral responses

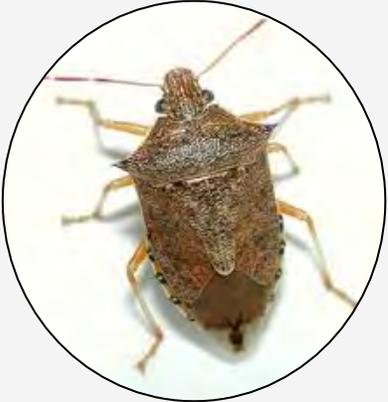
Research Objective #3

Determine if parental host species influences *T. japonicus* performance in no-choice tests using large, more complex arenas



Objective #1

Experimental Set-Up: Behavioral Assays



Stink bug species

H. halys
P. maculiventris

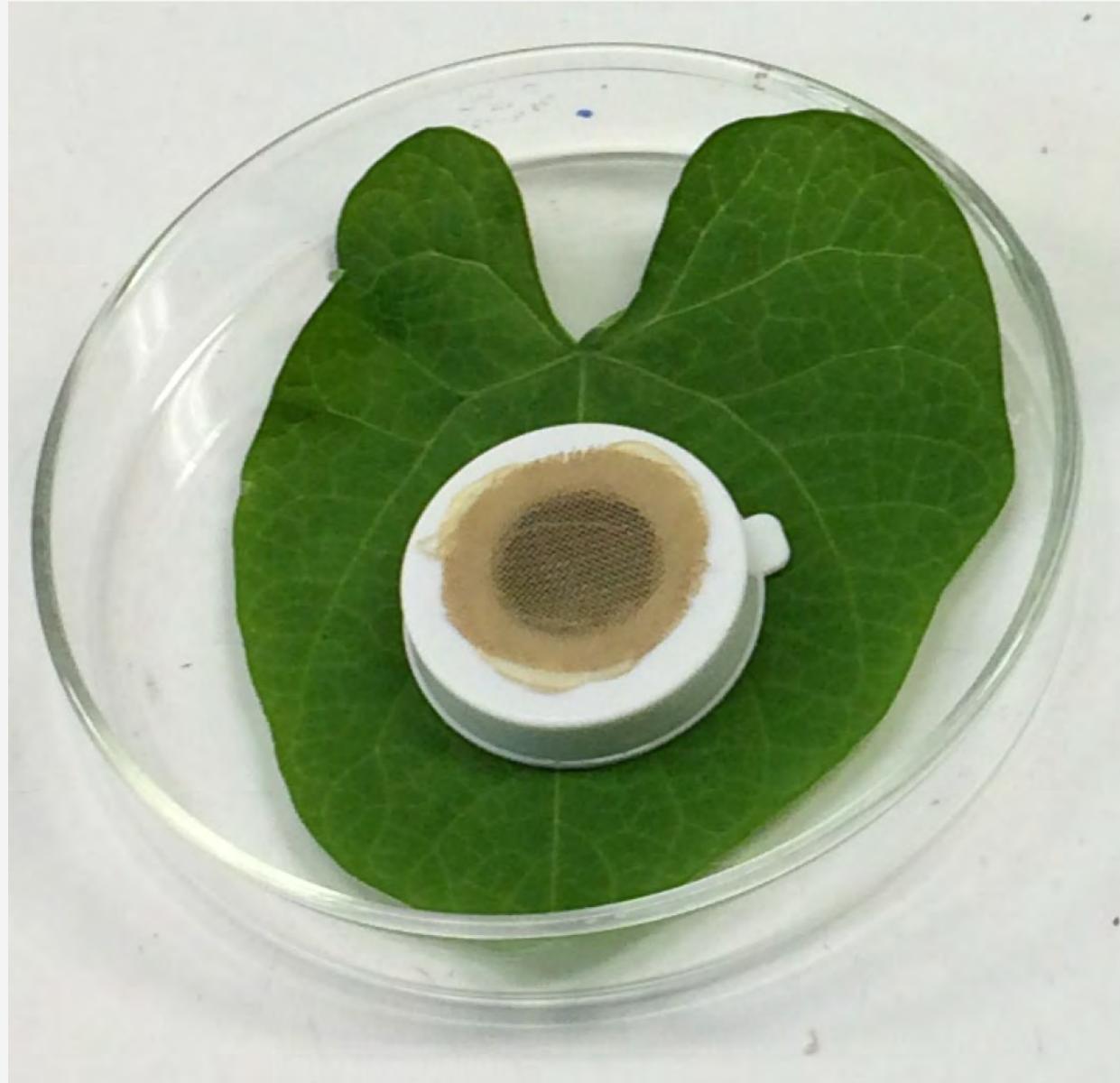
gravid ♀'s

Plant species

Acer rubrum
Malus domestica
Glycine max

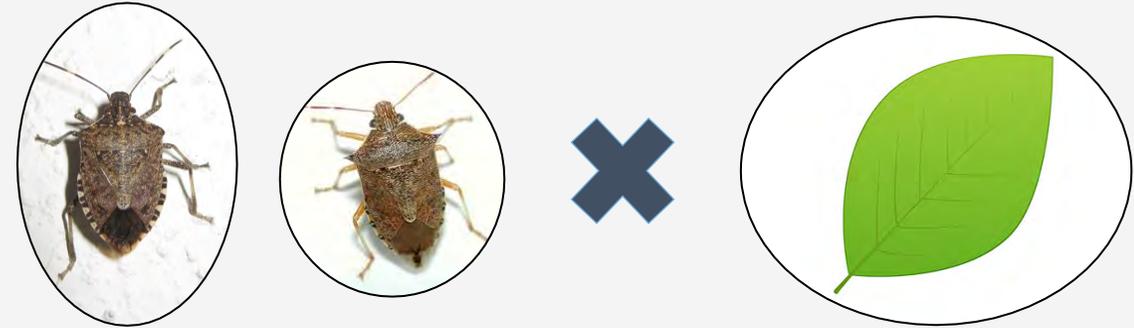
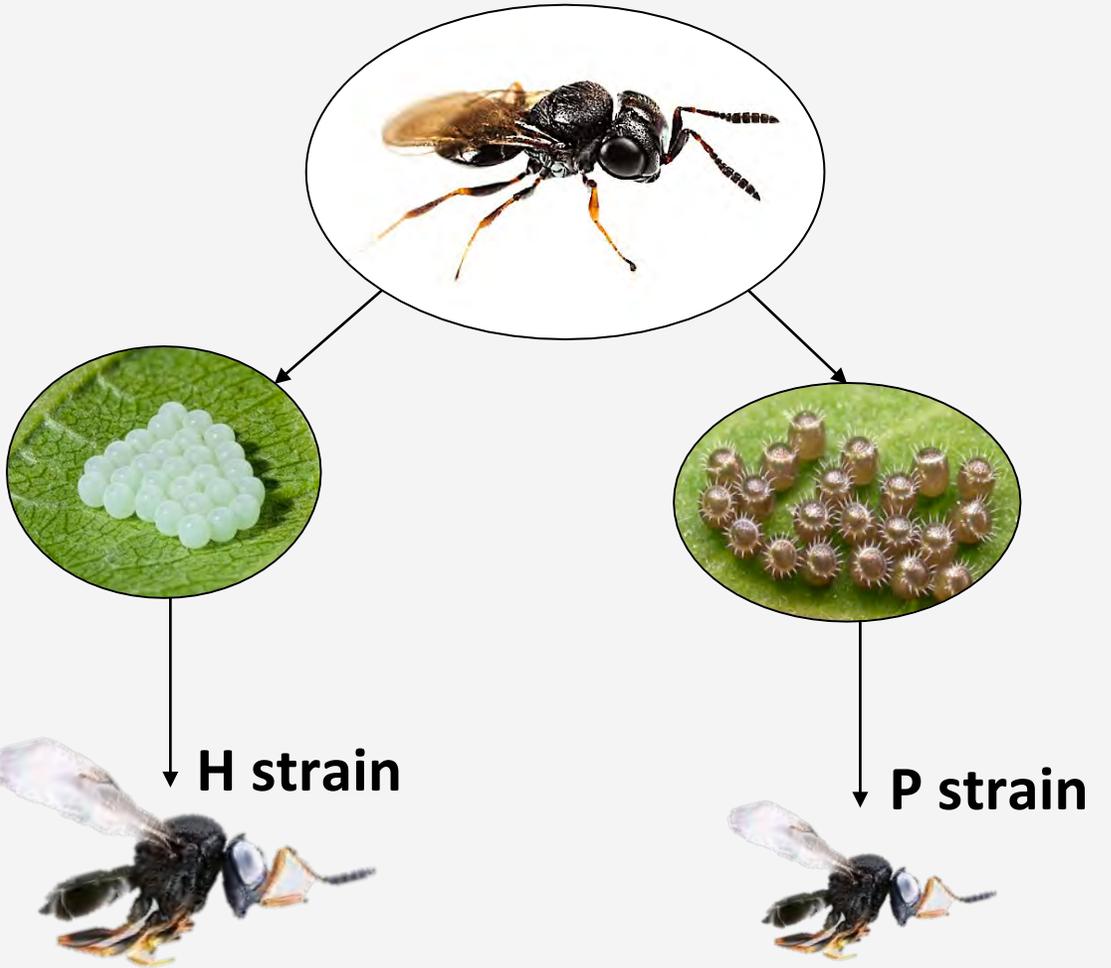
SA ≈ 16 cm²

Leaf surface kairomone contamination



Objective #2

Experimental Set-Up: Behavioral Assays

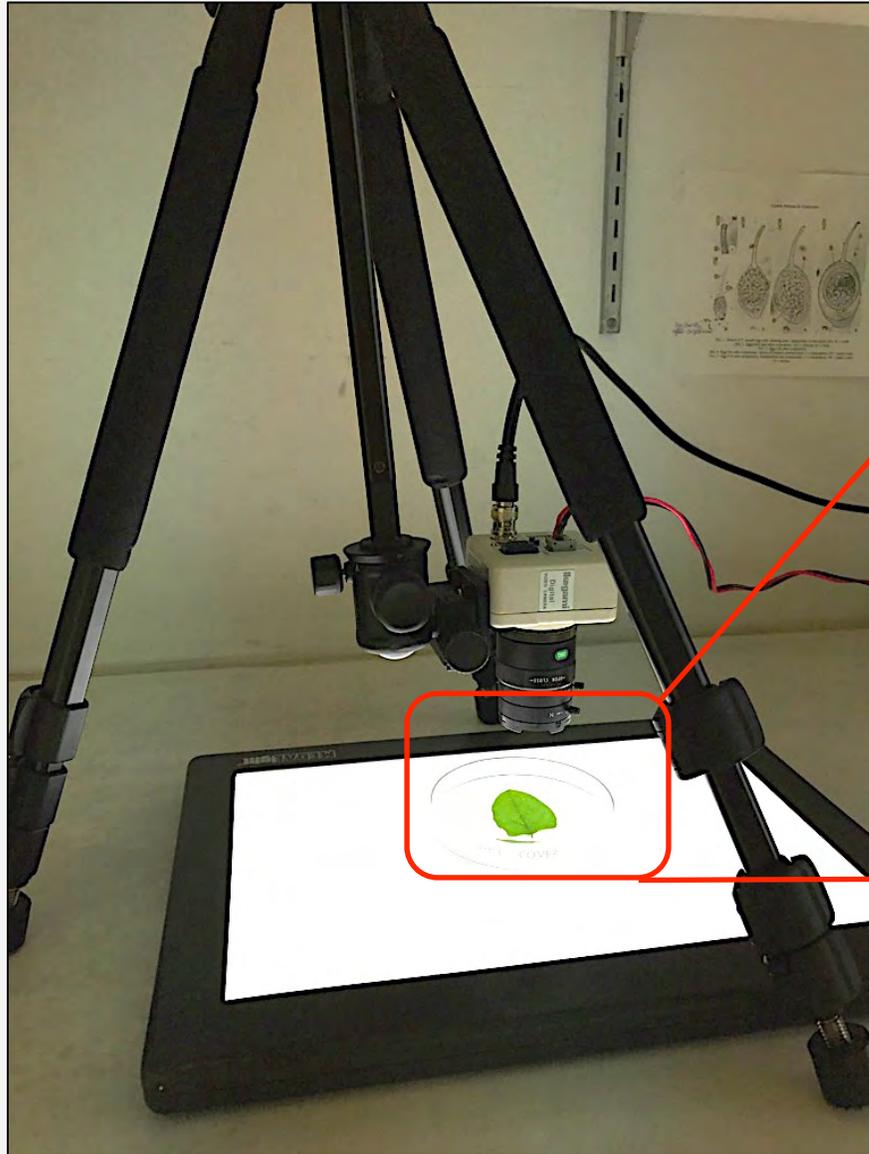


H. halys or *P. maculiventris*
gravid females

Soybean leaf
(~ 16 cm²)

Establishment of 2 separate *T. japonicus* lines

Experimental Set-Up: Behavioral Assays



60 mm petri dish arena

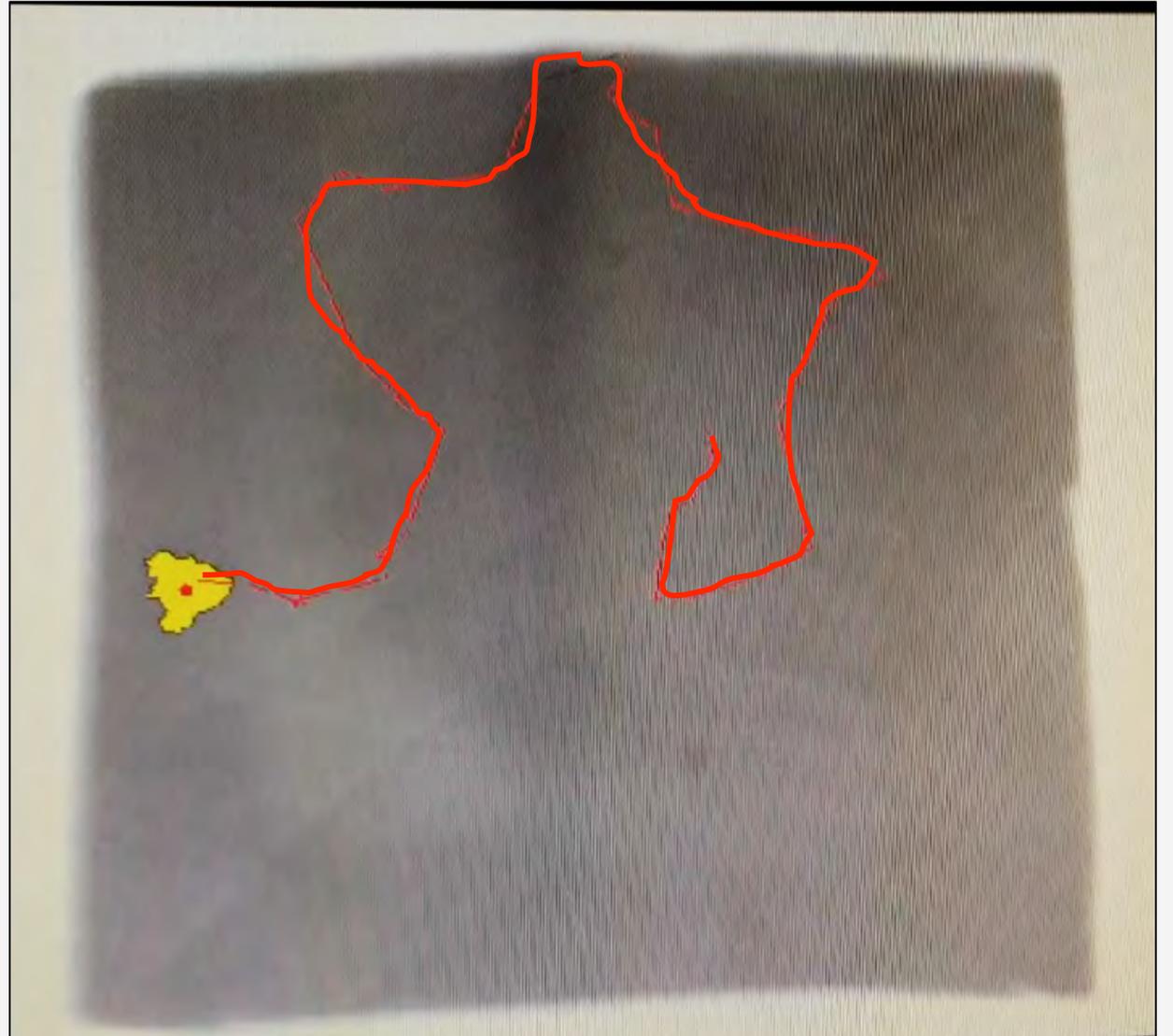


Ethovision XT 8.0 (Noldus Technologies)

Ethovision Tracking Software

Recorded Behaviors:

1. Residence time (s)
2. Linear Walking Velocity (mm/s)
3. Angular Walking Velocity ($^{\circ}$ /s)

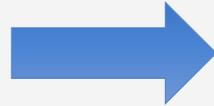


Objective #3

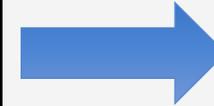
Experimental Set-Up: No-choice tests



Kairomone contamination of *P. vulgaris* leaf surfaces

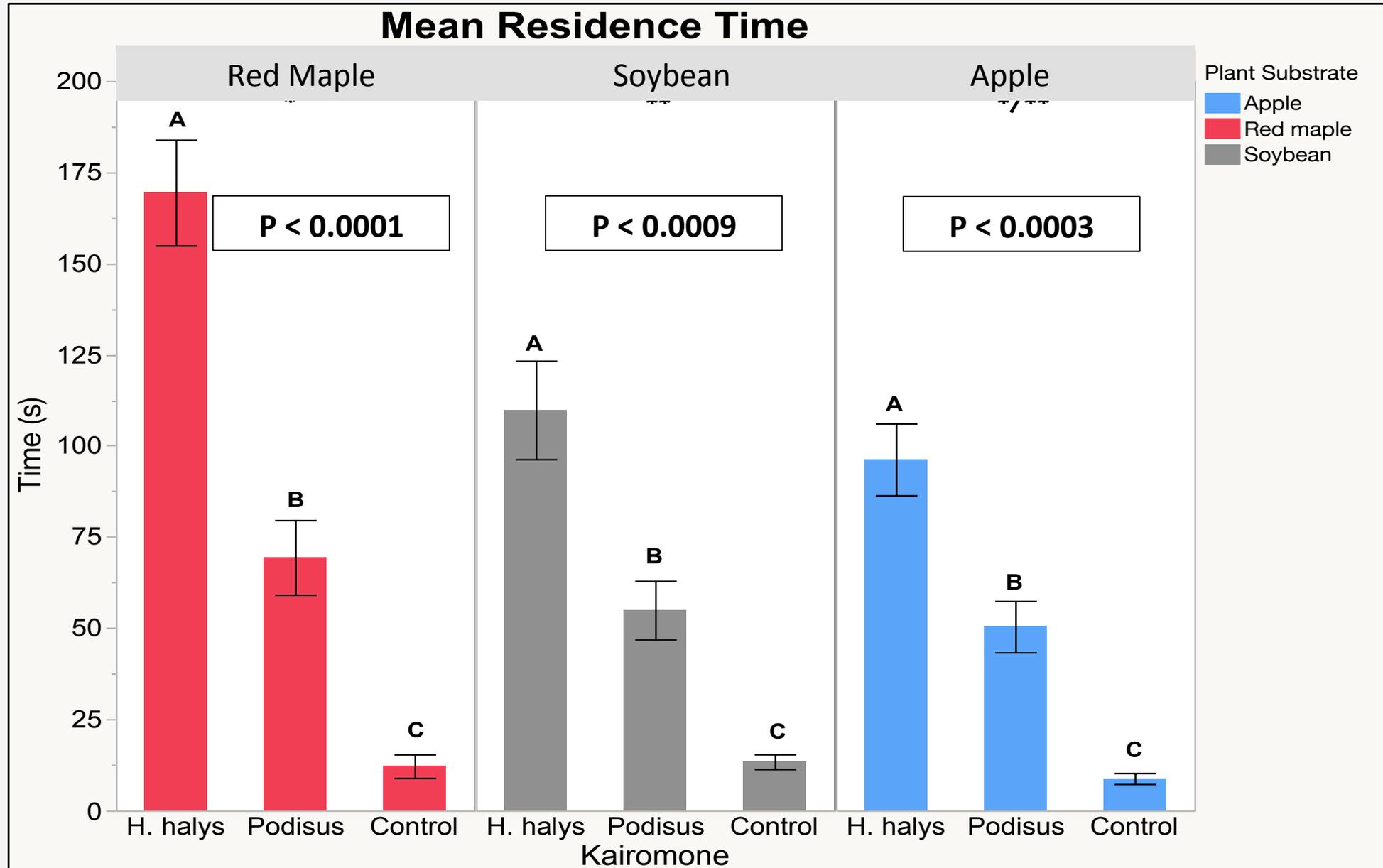


Attach *H. halys* or *P. maculiventris* egg mass to contaminated plant



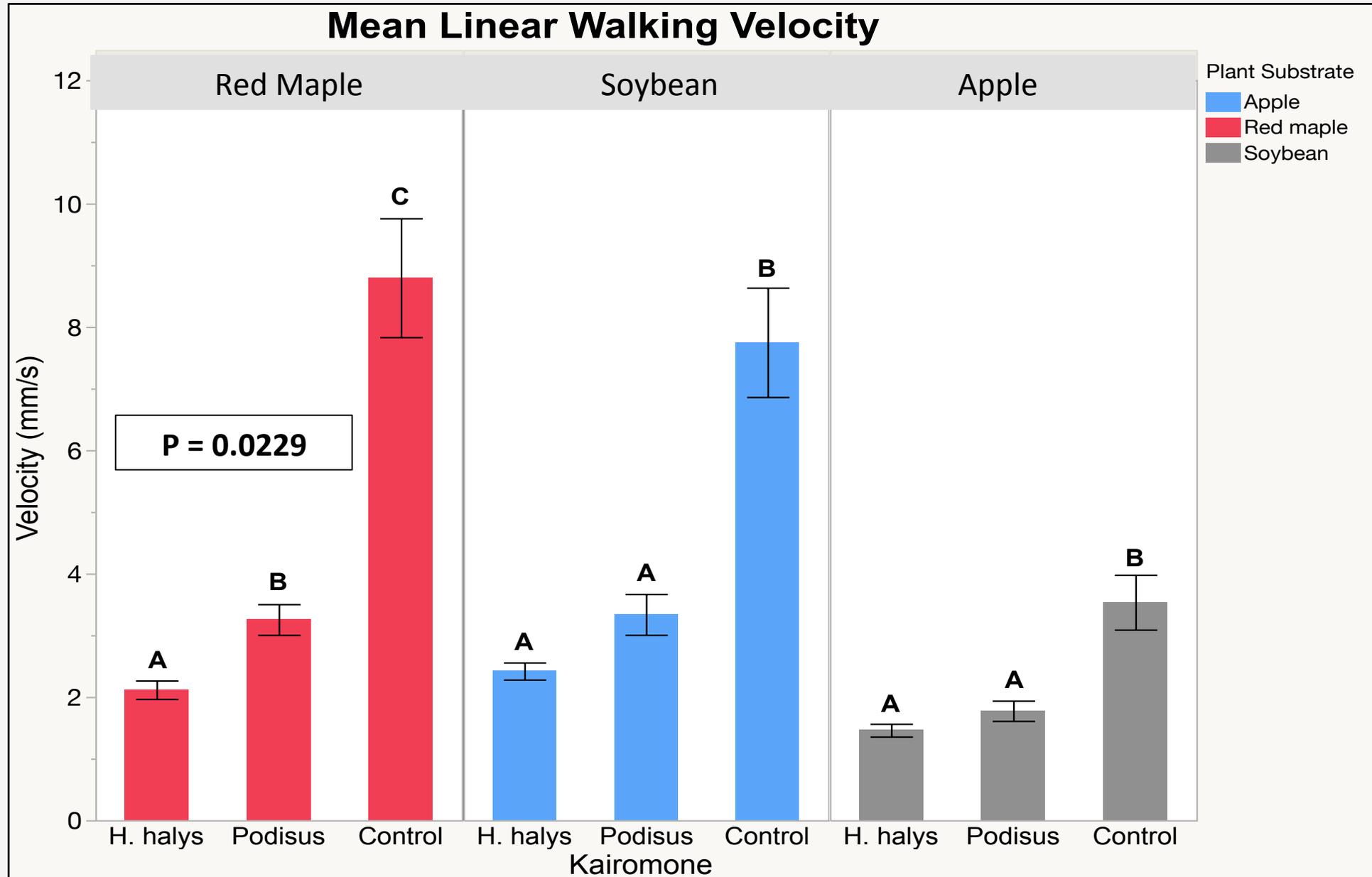
24 h exposure of **H-** or **P-strain** *T. japonicus* females (mated, naïve, 3-5 days old)

Objective #1 Results



ANOVA + Tukey's HSD

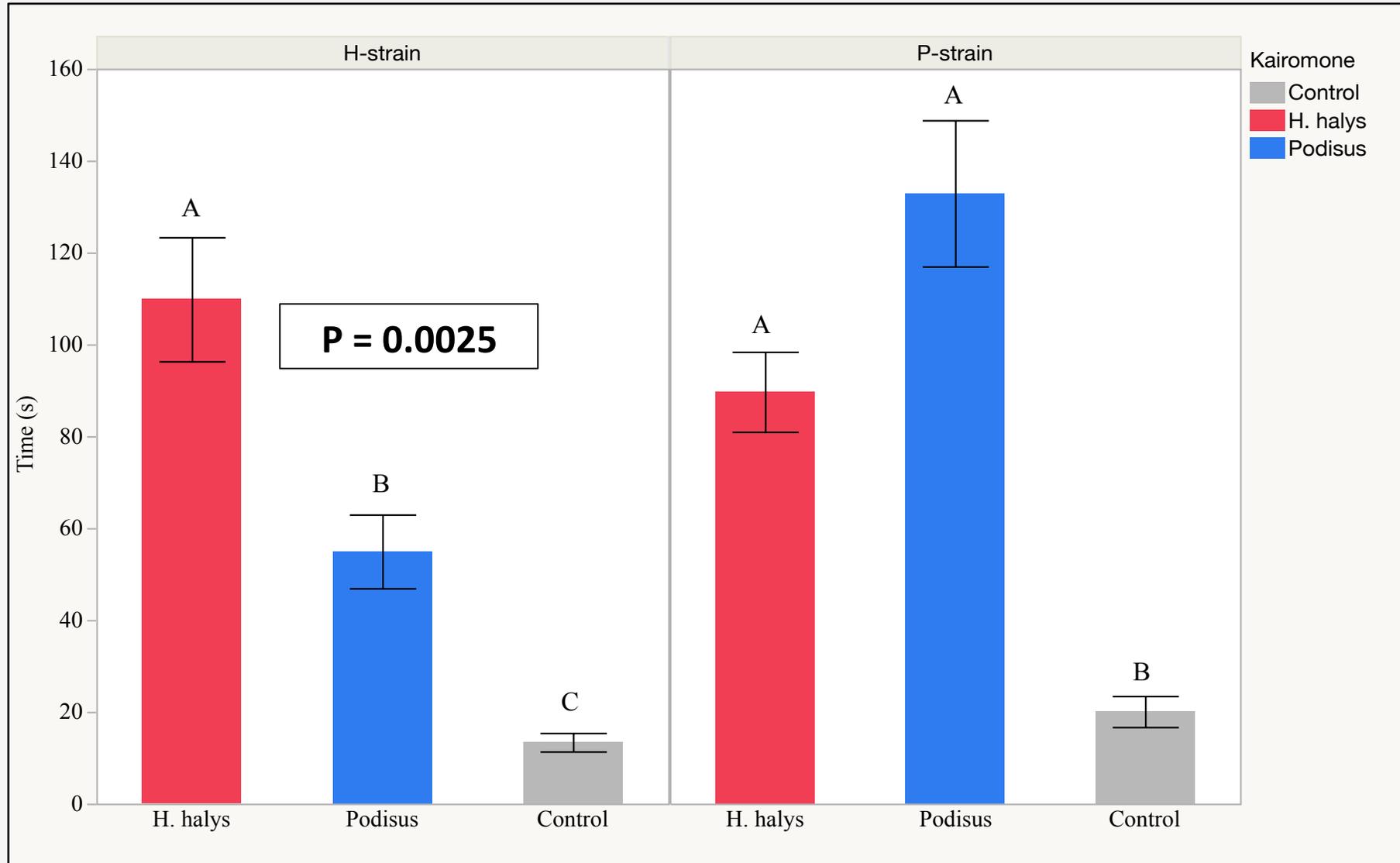
Objective #1 Results



ANOVA + Tukey's HSD

Objective #2 Results: Residence Time

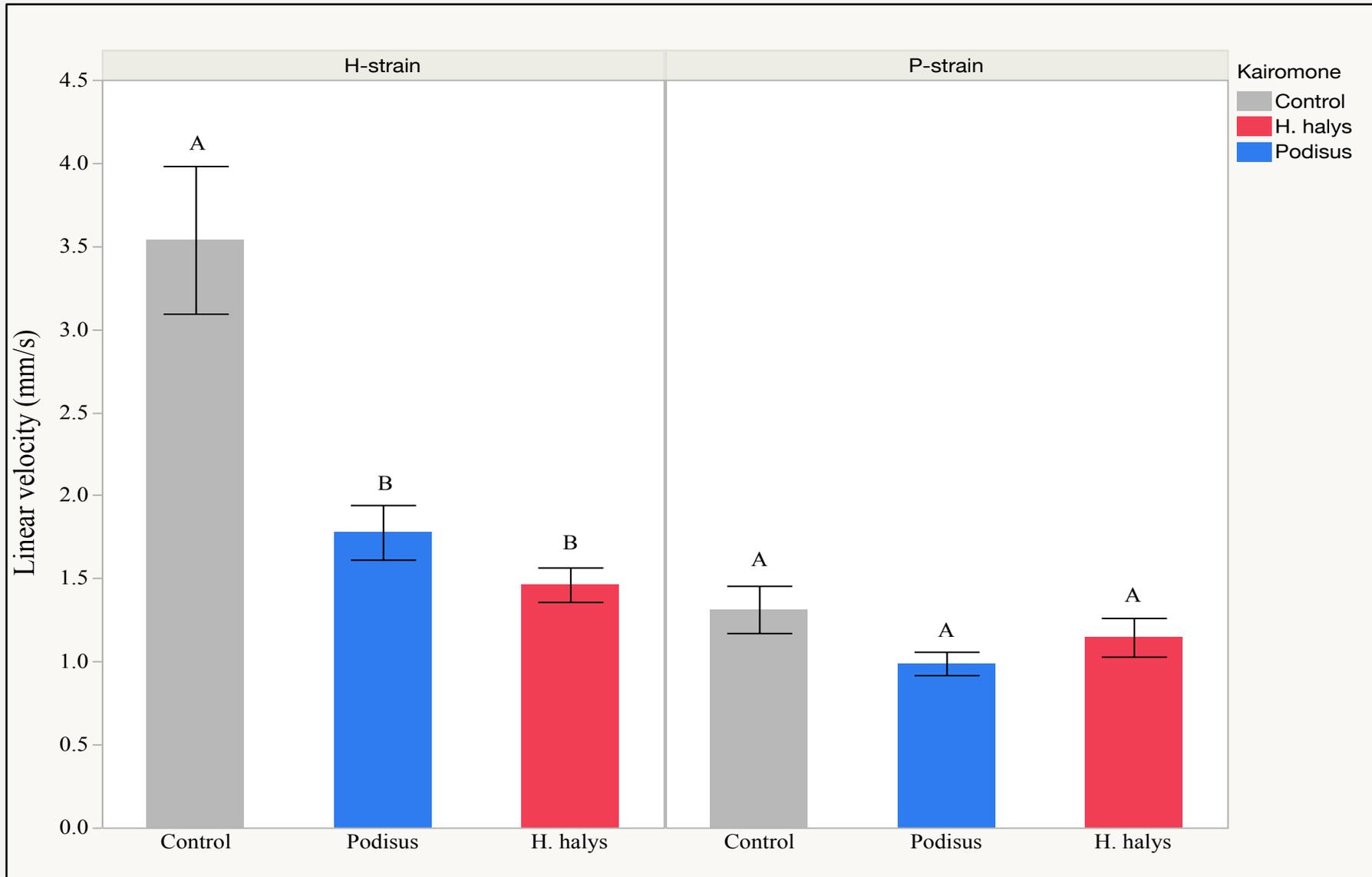
A significant interaction was observed between wasp strain and kairomone type ($P = 0.0013$)



ANOVA + Tukey's HSD

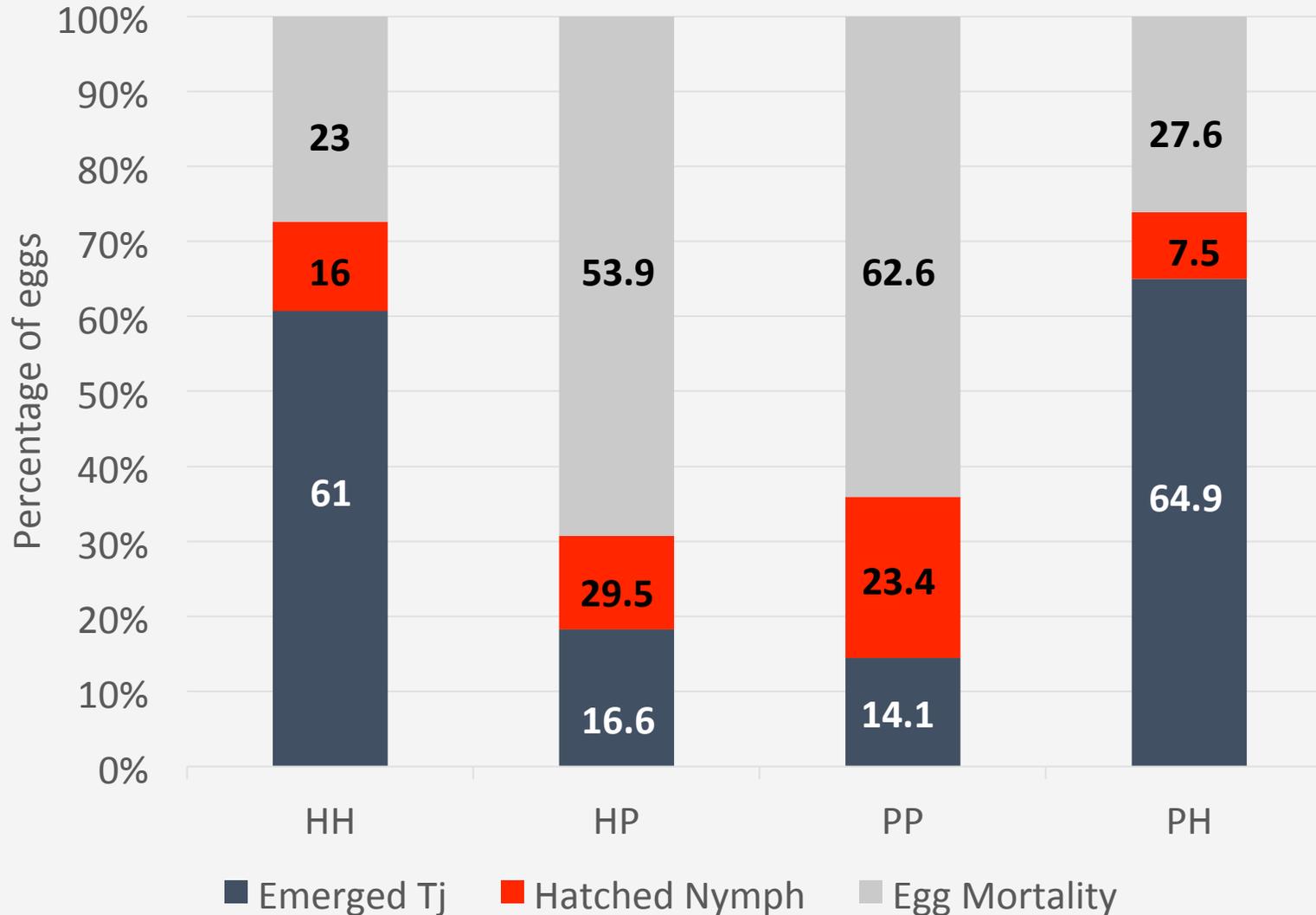
Objective #2 Results: Linear Walking Velocity

A significant interaction was observed between wasp strain and kairomone type ($P = 0.0022$)



Objective #3 Results

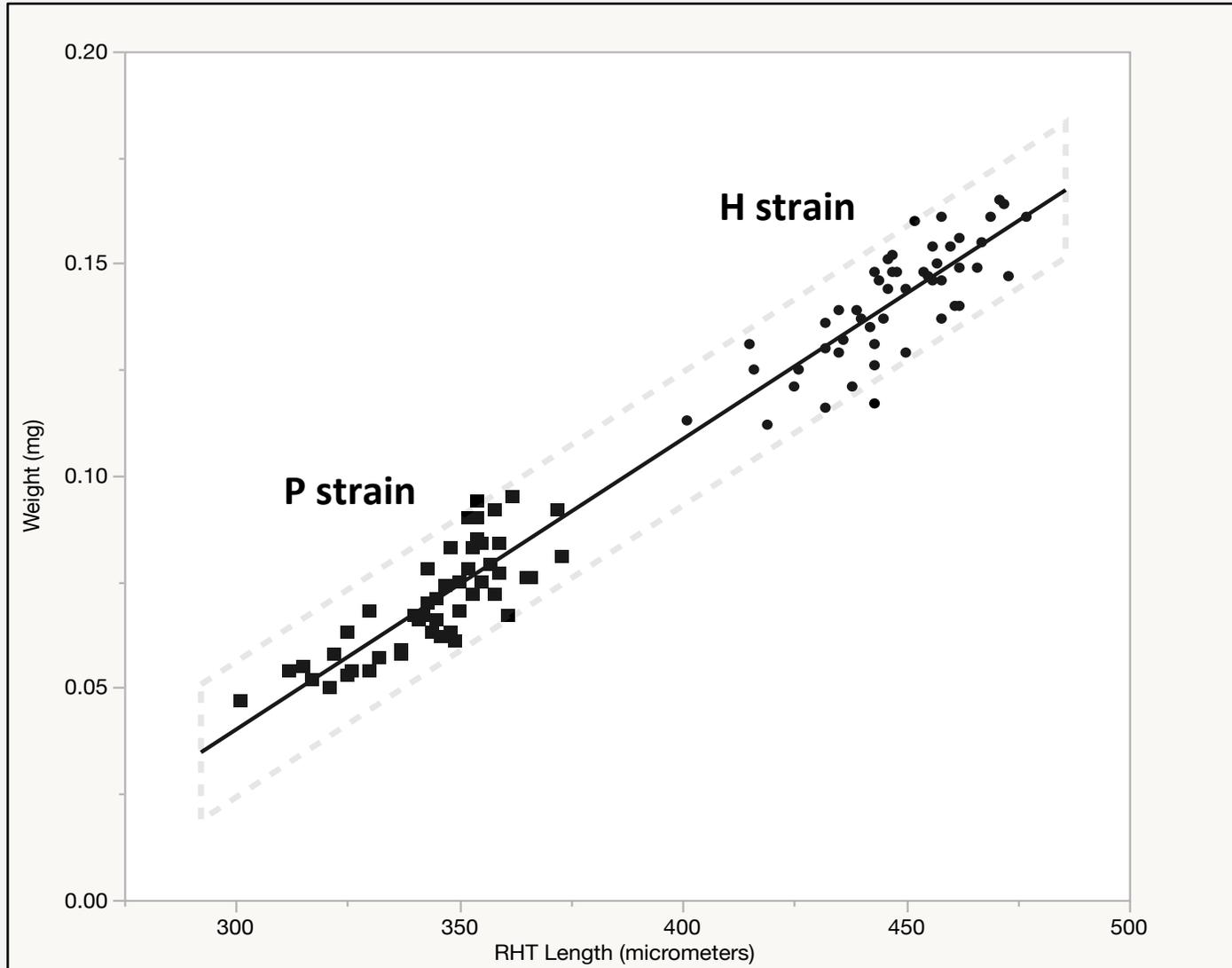
Fate of host eggs in No-Choice tests



- H and P strain wasps parasitized equal percentages of eggs from the same host species
- Among parasitized egg masses, *H. halys* eggs were more suitable than *Podisus* eggs for both strains ($P < 0.0001$)

ANOVA + Tukey's HSD

Parasitoid Size



- H strain wasps possessed 30% longer RHT ($P < 0.0001$; ANOVA)
- H strain wasps weighed roughly twice as much as P strain wasps ($P < 0.0001$)
- Strong positive correlation between *T. japonicus* RHT length and weight ($R^2=0.957$; $P < 0.0001$)



H Strain

P Strain

Conclusions

- Our study was the first to display *T. japonicus* preferences for *H. halys* adult contact kairomones on leaf substrates.
- Signs of host fidelity were exhibited in kairomone behavioral assays
 - A function of early adult experience with parental host egg ?
- Reduced walking velocity of P-strain *T. japonicus* may indicate reduced foraging abilities
 - Soybean pubescence clearly hindered smaller wasps' movement
- Parental host species alters phenotypic traits of *T. japonicus*, but does not change its preference for *H. halys* in laboratory no-choice tests
 - Successive rearing generations on *P. maculiventris* would validate these results

Questions???



Help us, *Trissolcus japonicus*.
You're our only hope.