

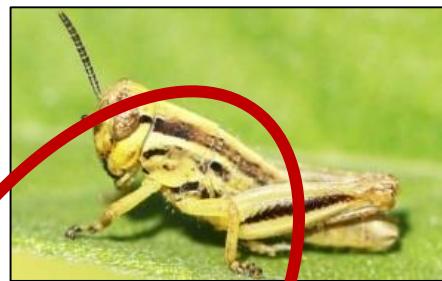
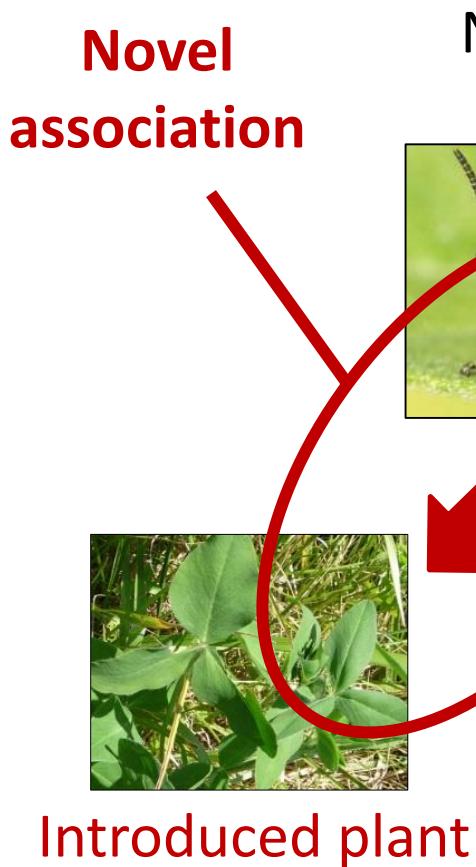
# Novel Plant-Insect Associations: Implications of the Lack of Coevolution



Alina Avanesyan

Department of Entomology, University of Maryland  
November 30, 2018

# Novel Plant-Insect Associations



Native plants

# Novel Plant-Insect Associations

- a combination of resident (native) and non-resident (exotic) plant or insect species “in which at least one species has little or no experience with relevant ecological traits of its interaction counterpart” (Saul and Jeschke, 2015).



Introduced plant's  
native range



Introduced plant

Lack of coevolution



Native insect

Native community

# In the introduced range...



Establishment

Spread

Impact

Species invasion

Introduced species

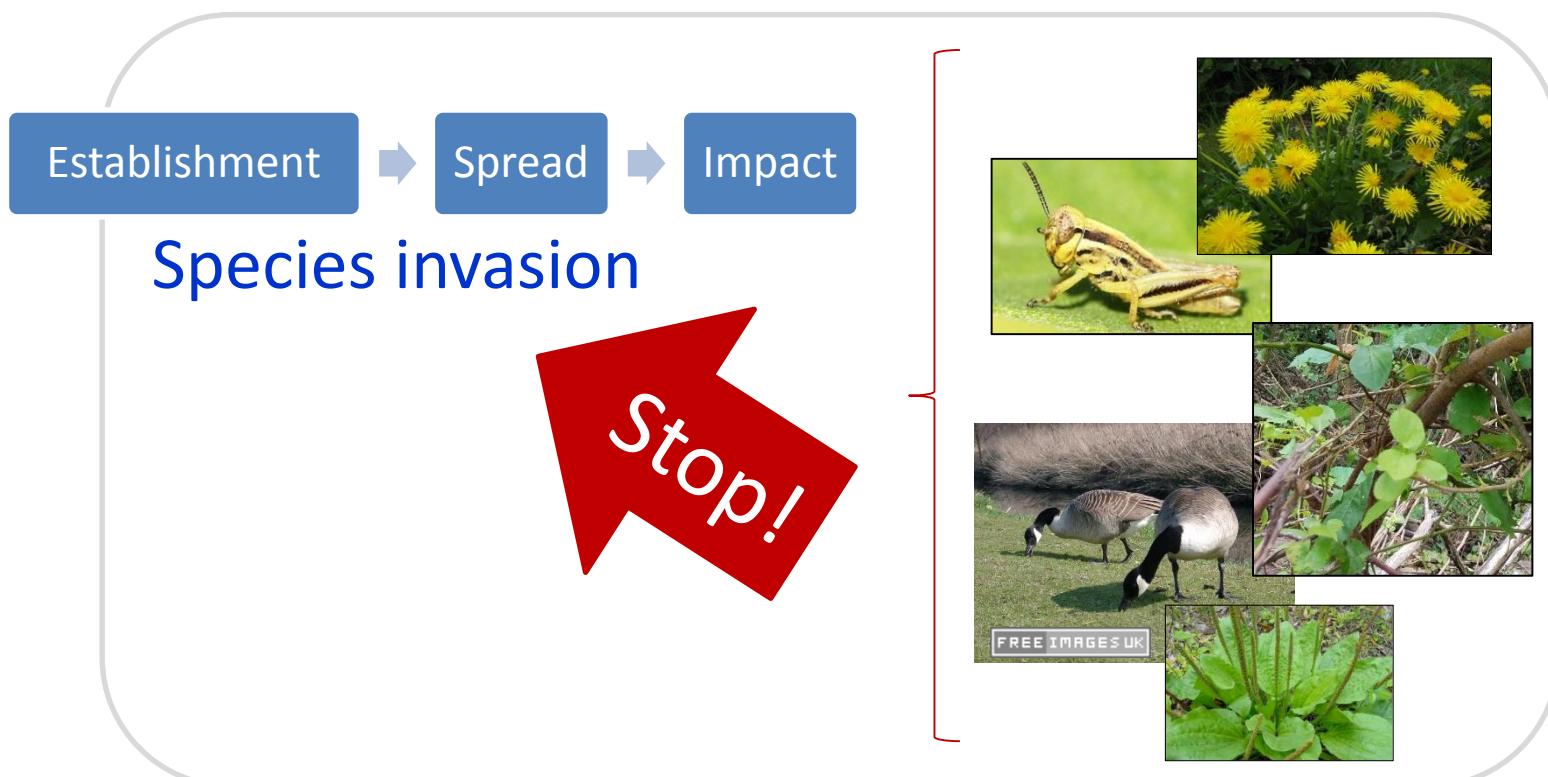
Fail to establish

Native community

Why do introduced species fail to establish in a new range?

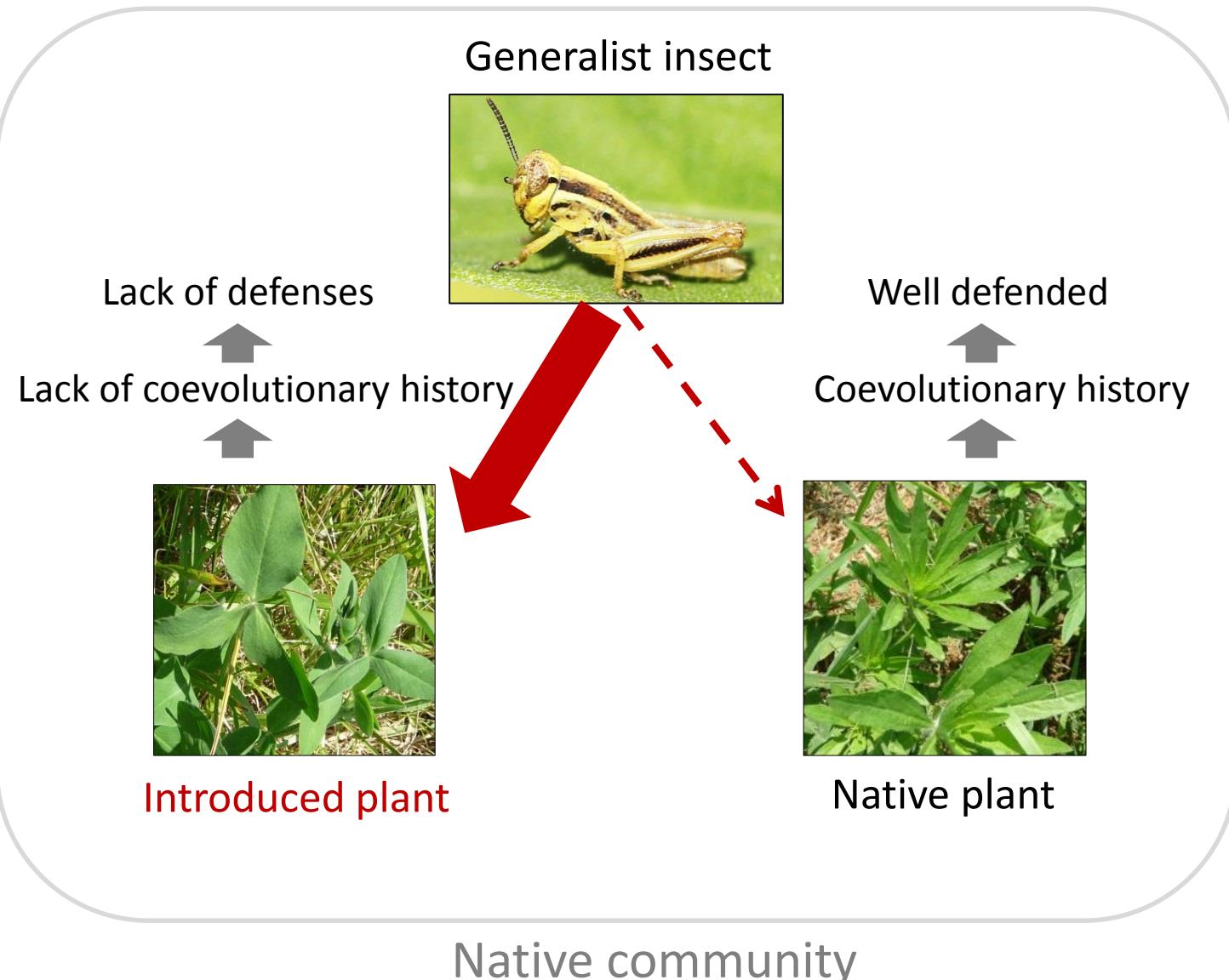
# Biotic resistance

- "the ability of resident species in a community **to reduce the success of exotic invasions**" (Levine et al., 2004) – i.e. competition, parasitism, herbivory, or predation, etc.



Native community

# Biotic Resistance Hypothesis



# Why do introduced species fail to establish in a new range?

## Novel species interactions



➤ How do insect herbivores respond to their novel host plants?



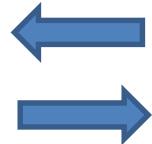
➤ How do plants respond to their novel insect herbivores?

# Study system



*Melanoplus* grasshoppers  
(Orthoptera: Acrididae)

Native



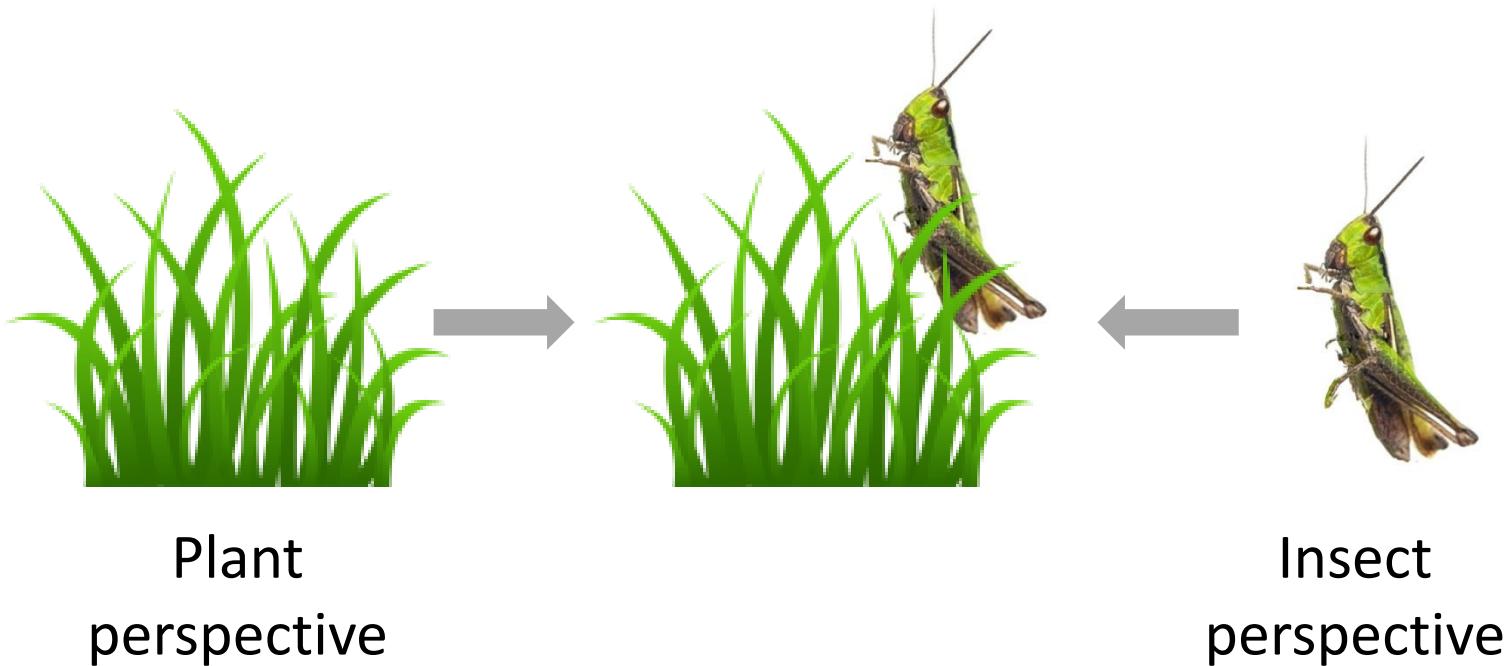
Grasses (Poaceae)

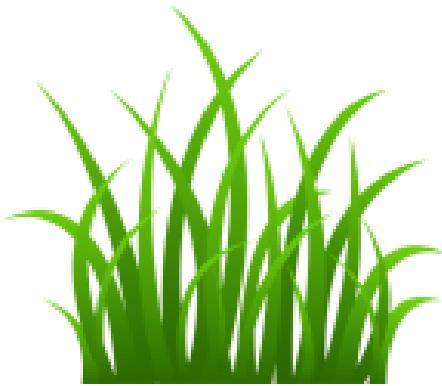
Native and Exotic

# Outline

- **Ph.D. research:** *Melanoplus* grasshoppers on native vs. exotic grasses
- **Review:** Acridid grasshoppers and their novel host plants
- **Current research:** *Melanoplus* grasshoppers and *Miscanthus sinensis* cultivars

# Native versus Exotic Grasses: The Interaction between Generalist Insect Herbivores and Their Host Plants





- Do native and exotic grasses differ in their resistance to herbivory by *Melanoplus* grasshoppers?
- Do native and exotic grasses differ in their tolerance to herbivory by *Melanoplus* grasshoppers?



Do *Melanoplus* grasshoppers have feeding preferences for native and exotic grasses?

→ behavioral approach (feeding activity, consumption, assimilation)

→ molecular approach (DNA barcoding of ingested plant material)

# Experimental Design

Plant responses,  
grasshopper feeding

Intact  
plants

Field



Leaf  
segments



Greenhouse

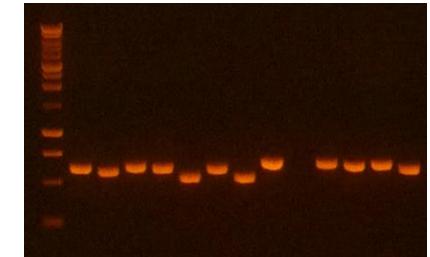


Molecular confirmation  
of diet

Protocol



Ingested  
plants

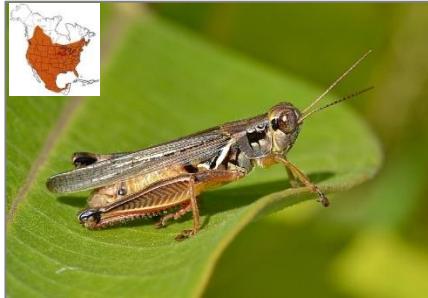


# Study Organisms



*Melanoplus differentialis*  
Differential Grasshopper

*Melanoplus femur-rubrum*  
Red-legged grasshopper



*Melanoplus spp.*  
(Orthoptera: Acrididae)  
Grasshopper nymph



*Andropogon  
gerardii*  
Big Bluestem



*Miscanthus  
sinensis*  
Chinese Silver  
Grass



*Bouteloua  
curtipendula*  
Side oats Grama



*Bothriochloa  
ischaemum*  
Yellow Bluestem

Native  
grasses

Exotic  
grasses

# Study Sites



University of Cincinnati  
Center for Field Studies  
(UCCFS)

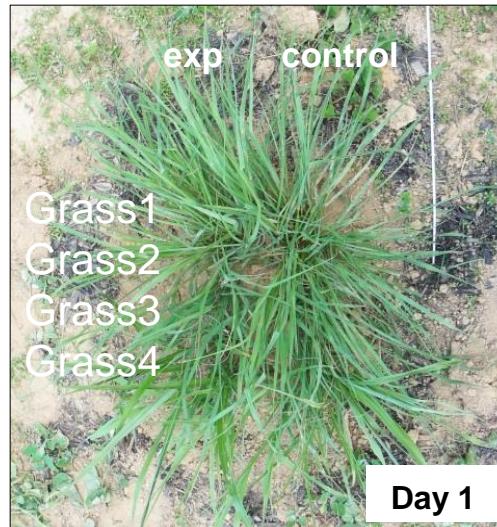


Western Maryland  
Research and Education Center  
(WMREC)



University of Cincinnati Greenhouse

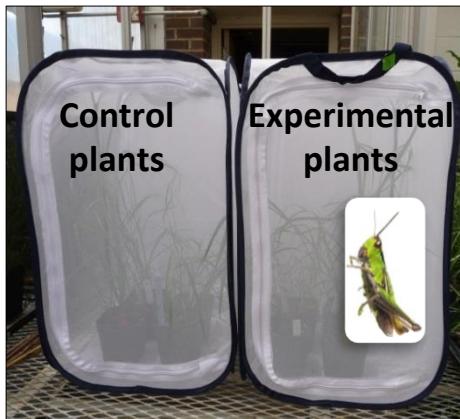
# Feeding Trials: Field



Plant growth / Grasshopper feeding

Plant regrowth  
Avanesyan and Culley (2017), J. Torrey Soc.

# Feeding Trials: UC Greenhouse





# Plant Resistance

- **The ability of a plant to decrease herbivore damage**

Price et al., 2011

- “A resistance trait is any plant character that influences the amount of damage a plant suffers”

Rausher, 1992



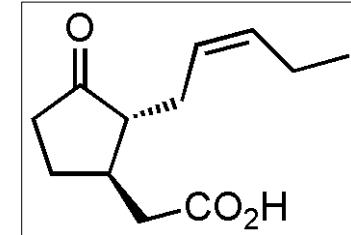
Wax



Spines



Trichomes



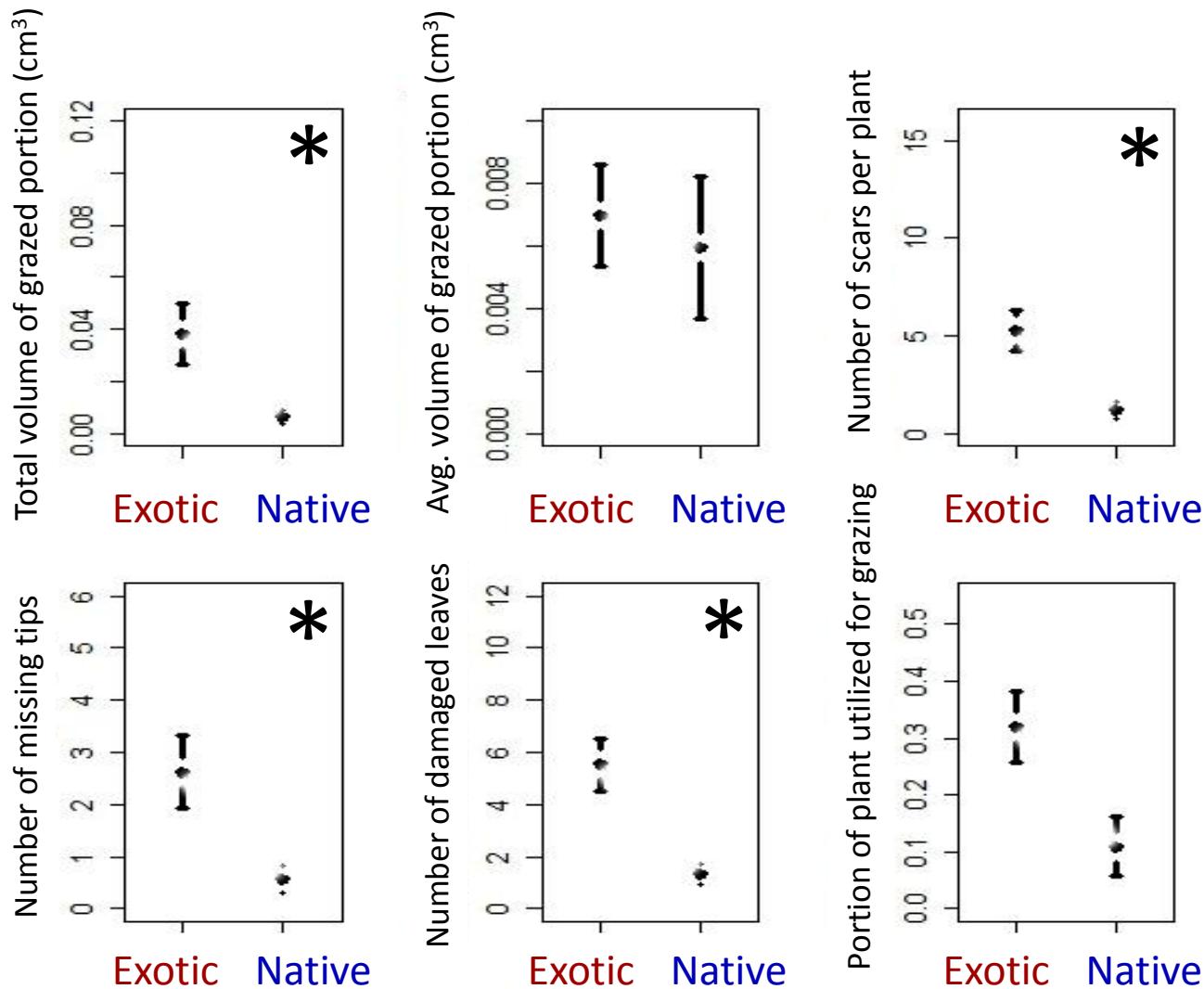
Jasmonic acid

- **Leaf damage** is one of the commonly used measurements for plant resistance
- Plants with more damage from herbivores are generally considered to have a lower level of resistance to herbivory

Mauricio 2000, Zou et al. 2008



# Results: Field

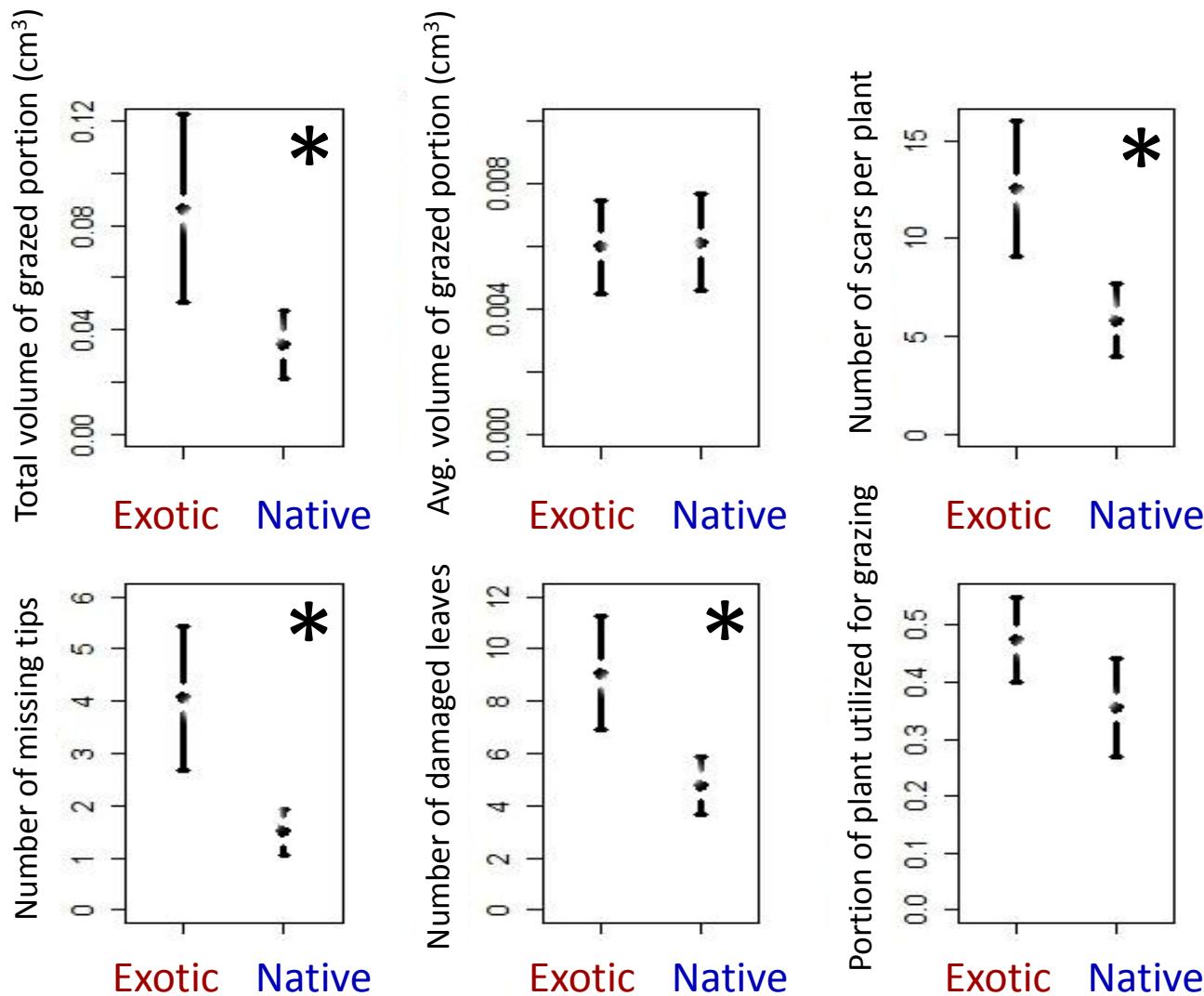


- Most measures of leaf damage were greater in exotic grasses at both field sites (MD and OH); mean  $\pm$  CI ; \*  $p_{\text{adj}} < 0.001$

Avanesyan and Culley (2015), *Plant Ecology*



# Results: Greenhouse



- Most measures of leaf damage were greater in exotic grasses; mean  $\pm$  CI ; \*  $p_{\text{adj}} < 0.001$



# Plant Tolerance

- **The ability of a plant to maintain fitness while sustaining herbivore damage**

Price et al., 2011

- Physiological components of plant tolerance:  
growth rate, storage capacity, photosynthetic rates, nutrient uptake etc.

Rosenthal & Kotanen 1994

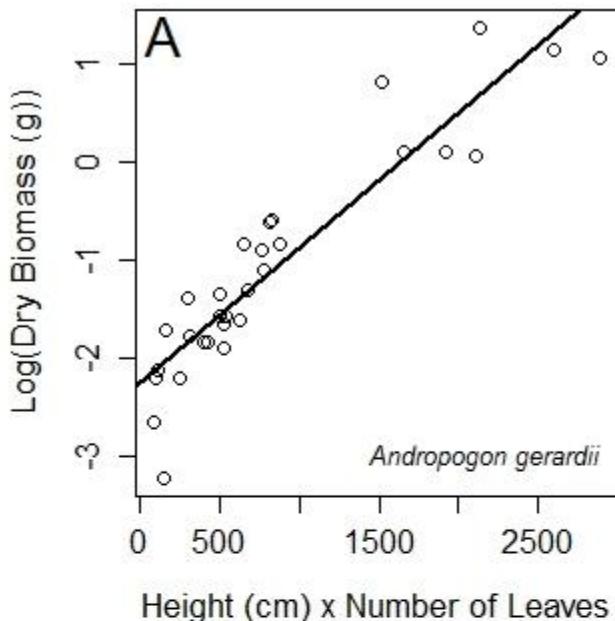
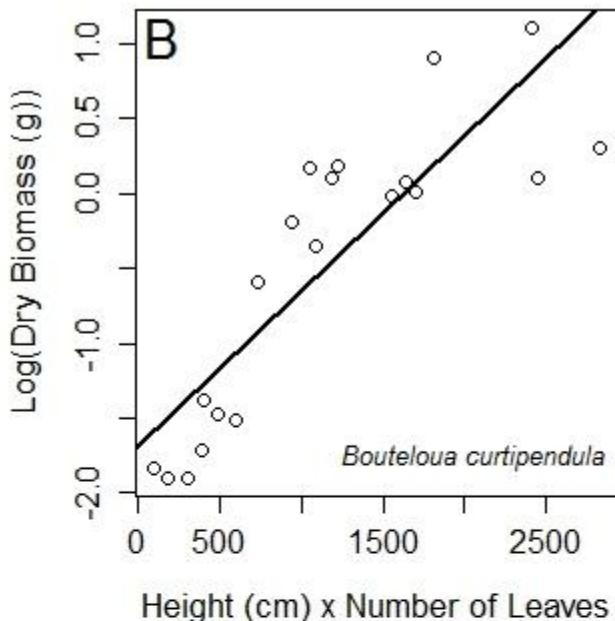
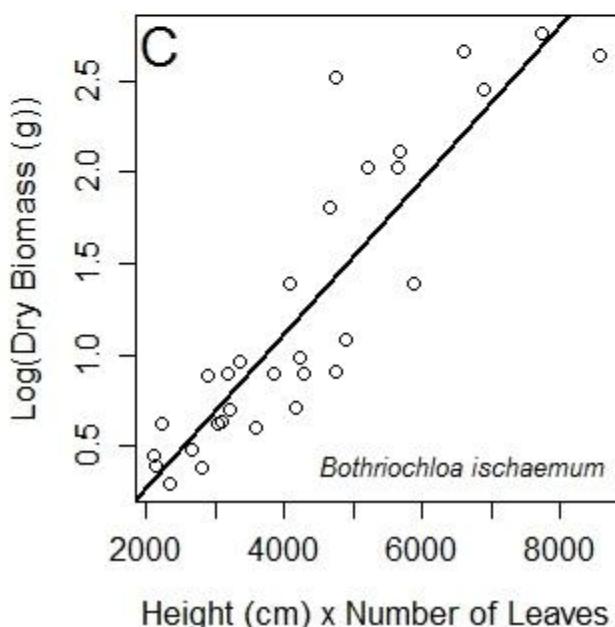
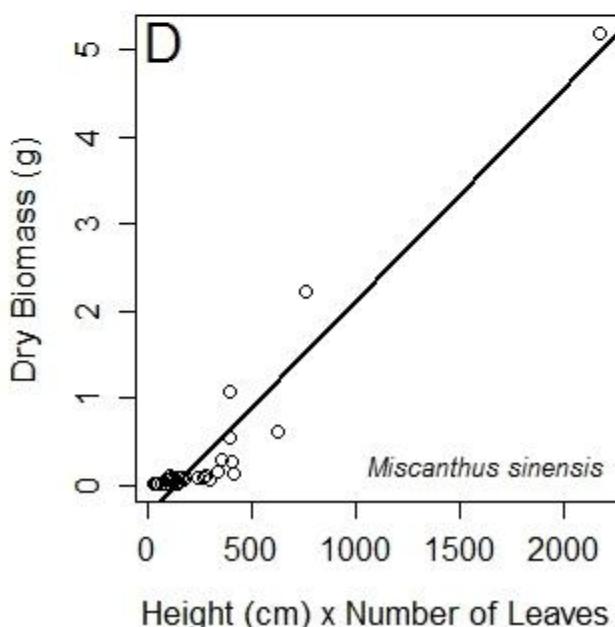
- **Plant compensatory growth in terms of aboveground plant biomass**  
is one of the fundamental and commonly used measurements for  
plant tolerance to herbivory, especially in grasslands

Rosenthal & Kotanen 1994; Atwood & Meyerson 2011;  
Leis & Morrison 2011



- Estimating biomass should be **non-destructive**, accurate, and easy to implement

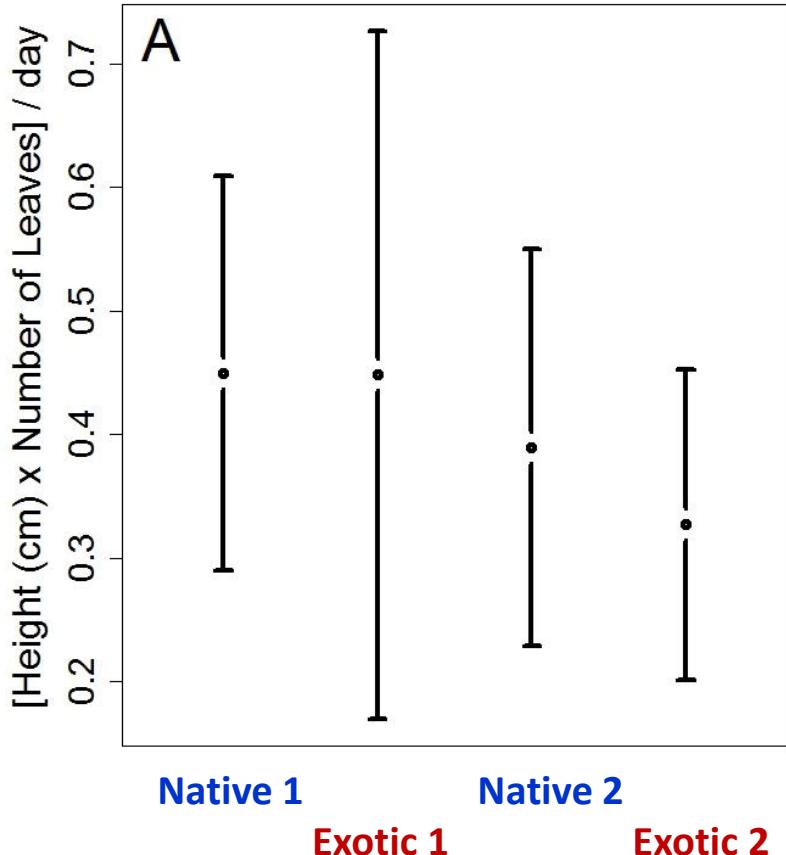
Redjadj et al. 2012

 $R^2=0.84$  $R^2=0.72$  $R^2=0.80$  $R^2=0.92$ 

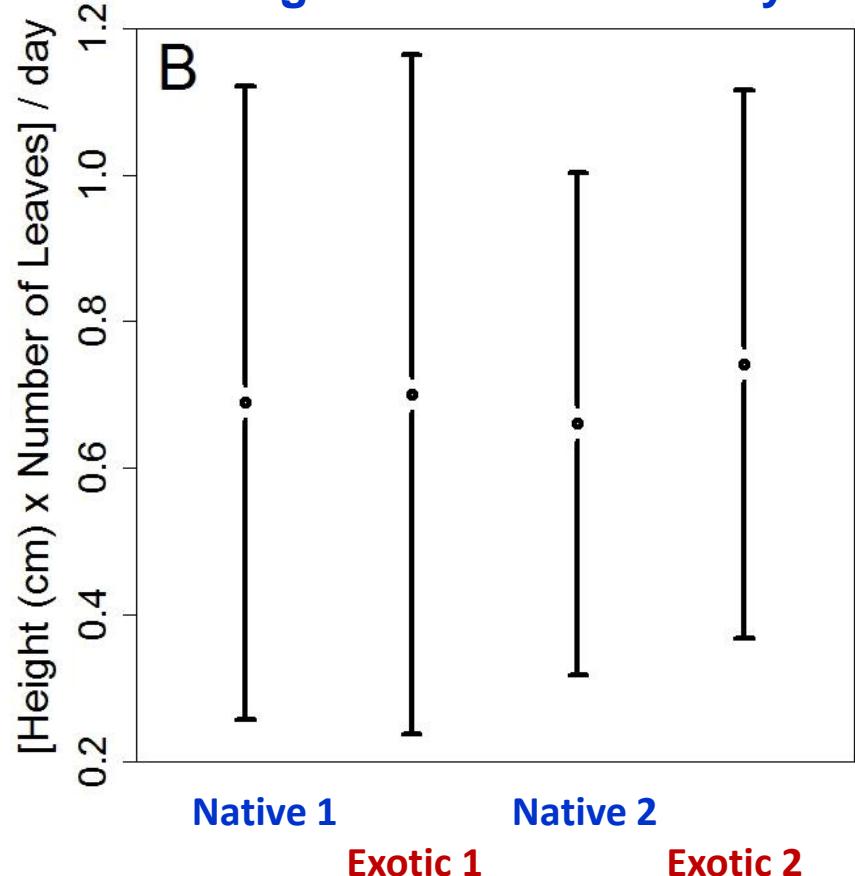


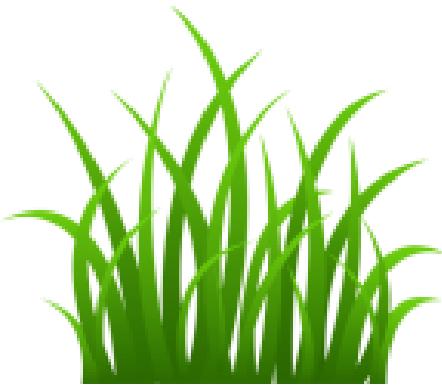
# Plant Tolerance

Growth during herbivory



Regrowth after herbivory





Do native and exotic grasses differ in their resistance to herbivory by *Melanoplus* grasshoppers?

Exotic < Native



Do native and exotic grasses differ in their tolerance to herbivory by *Melanoplus* grasshoppers?

Exotic = Native



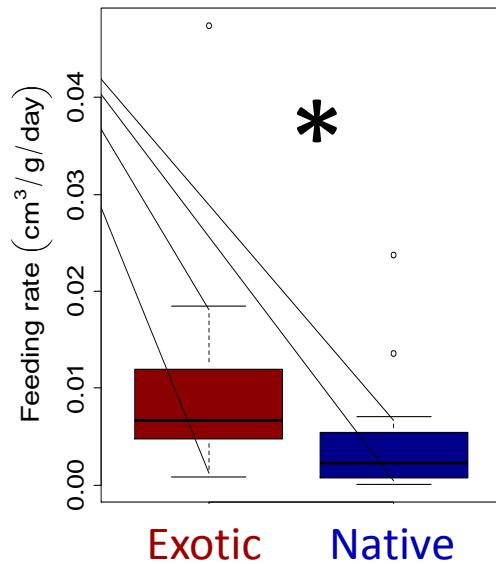
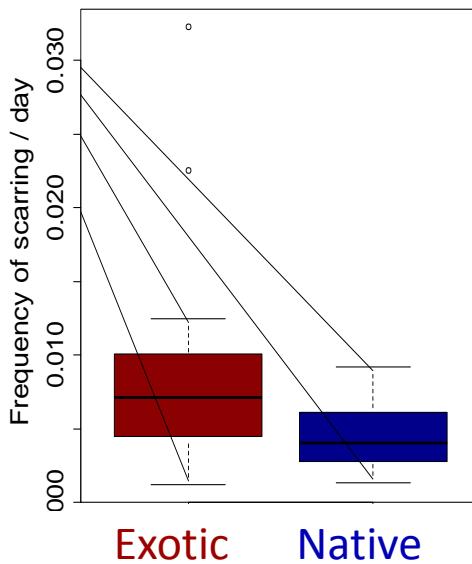
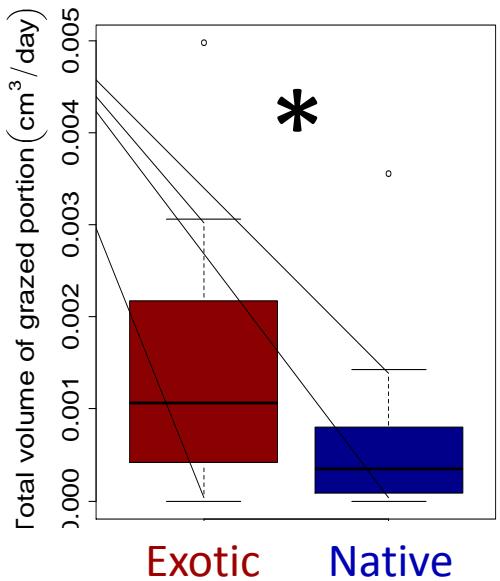
Do *Melanoplus* grasshoppers have feeding preferences for native and exotic grasses?



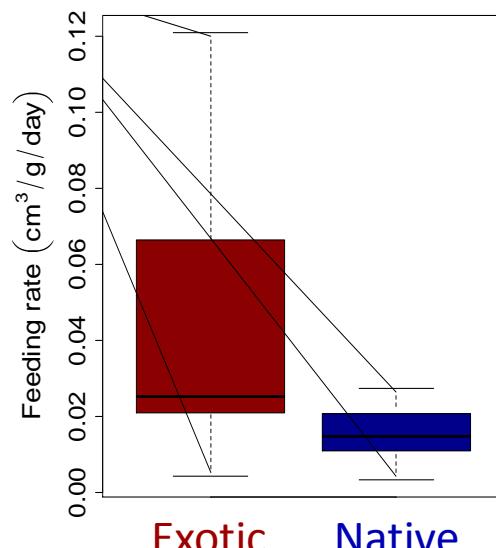
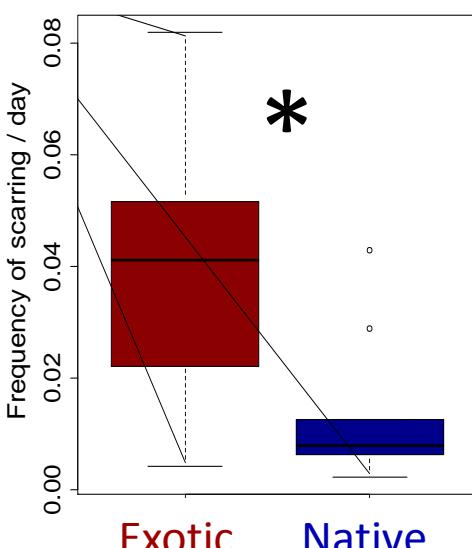
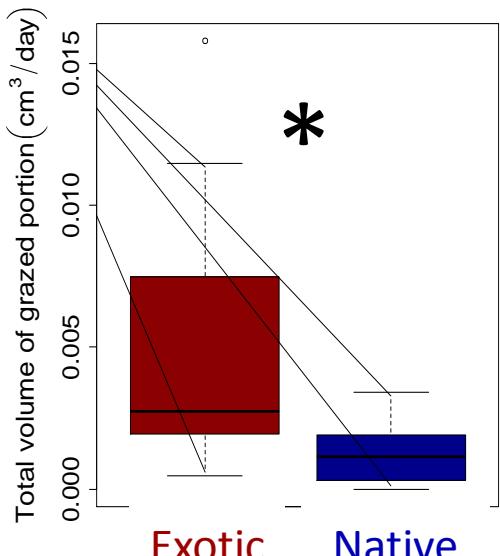
behavioral approach



**Field**



**Greenhouse**

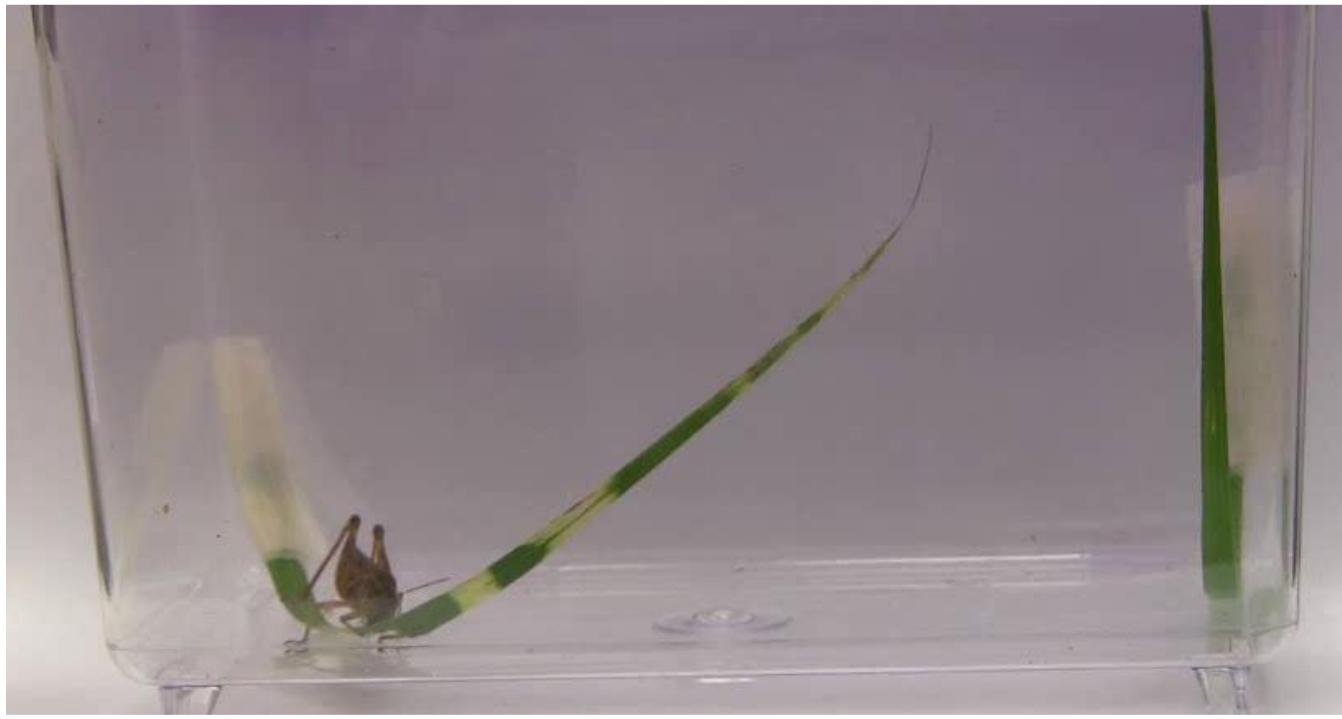


- Grasshopper food consumption and feeding activity were greater on exotic grasses;

\*  $p < 0.05$

Avanesyan and Culley (2015), *Entom. Exp. Appl.*

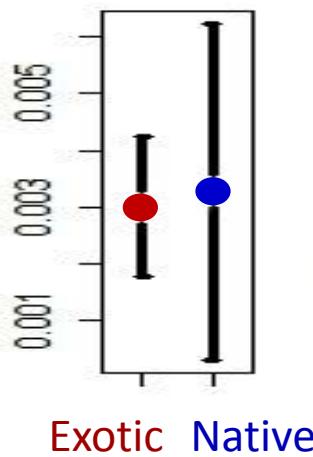
# Lab Assays (Leaves)



Hours 1-3

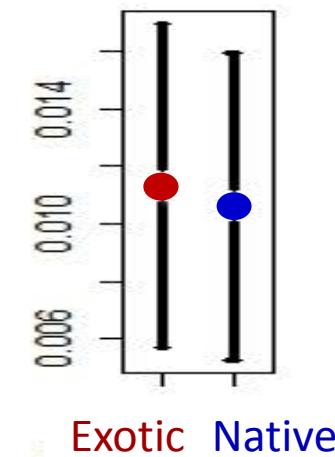


# Lab Assays: Food Consumption

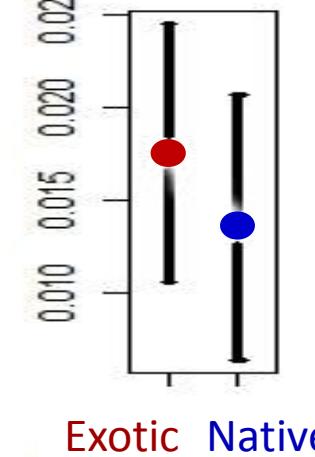
Total volume of grazed portion ( $\text{cm}^3$ )

Exotic Native

Food intake (g)

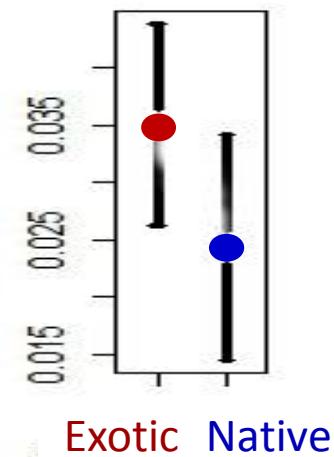


Exotic Native

Feeding rate ( $\text{g/g/hour}$ )

Exotic Native

Consumption index

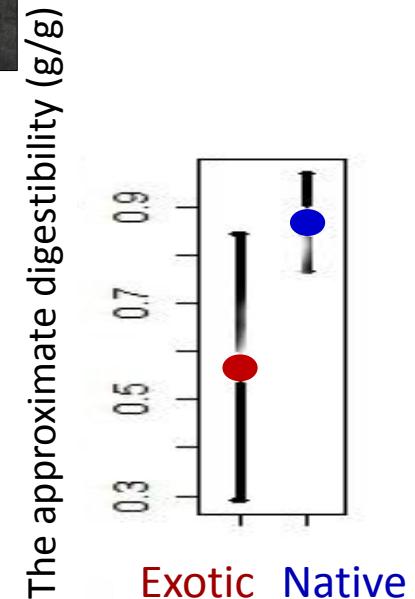
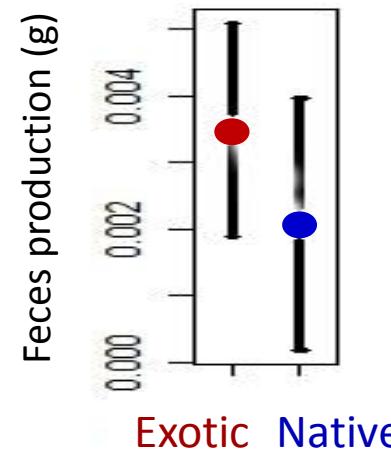
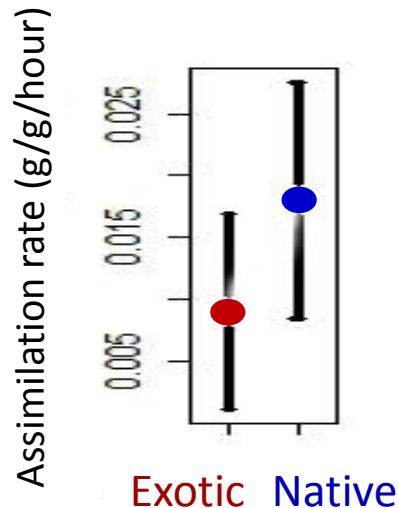
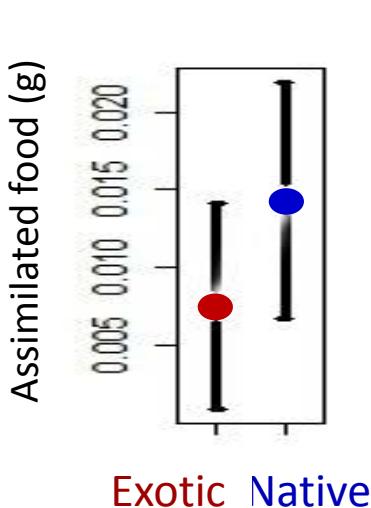
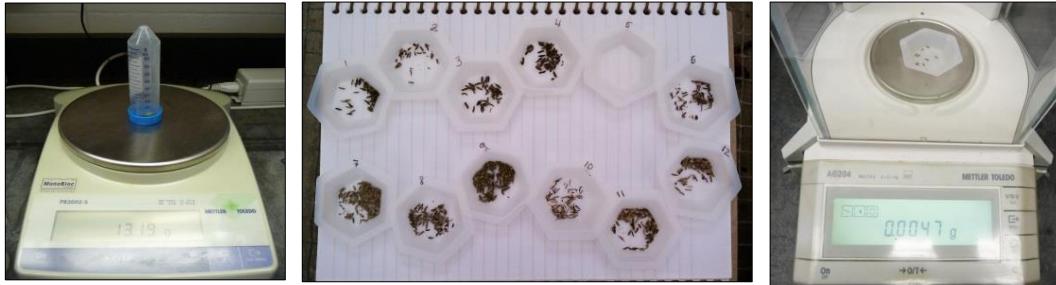


Exotic Native

- Grasshopper food consumption did not differ on the leaves clipped from native and exotic grasses ( $p > 0.05$ )



# Lab Assays: Food Assimilation



- Grasshopper food assimilation did not differ on the leaves clipped from native and exotic grasses ( $p > 0.05$ )



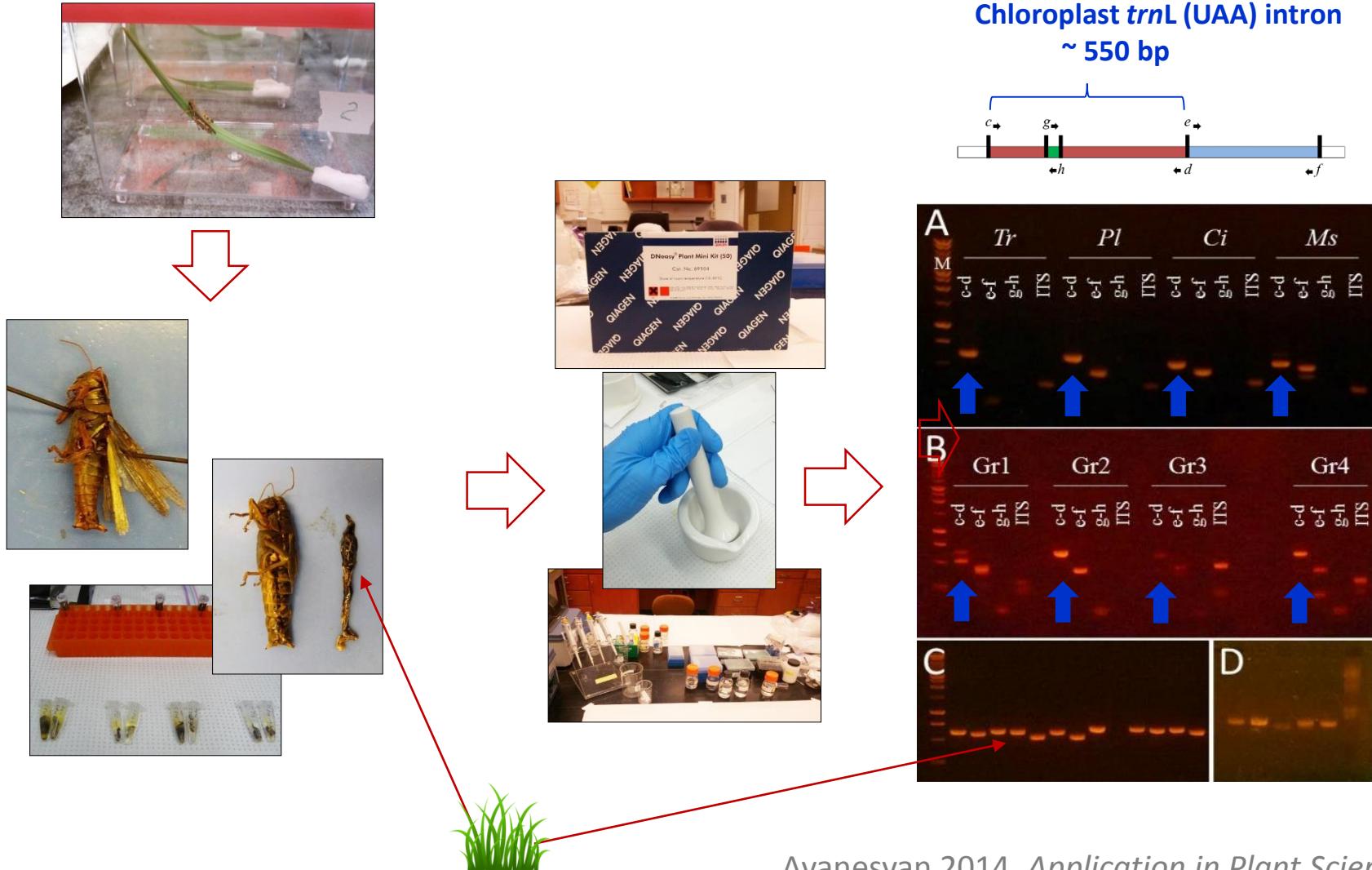
Do *Melanoplus* grasshoppers have feeding preferences for native and exotic grasses?



behavioral approach (feeding activity, consumption, assimilation)

Exotic ≥ Native

# Molecular Confirmation of Diet



# Testing the Protocol

Grasshoppers of different sizes



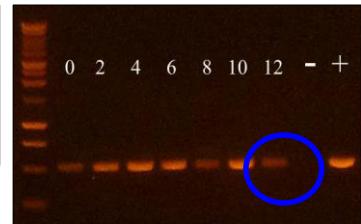
*Melanoplus spp.*  
nymph



12 h PI: choice, two plants



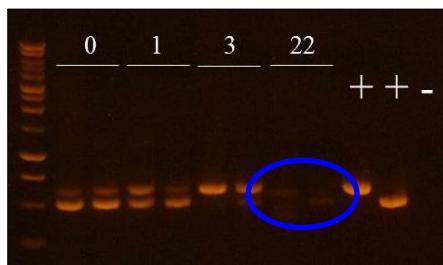
*M. femur-rubrum*



12 h PI: no choice, single plant

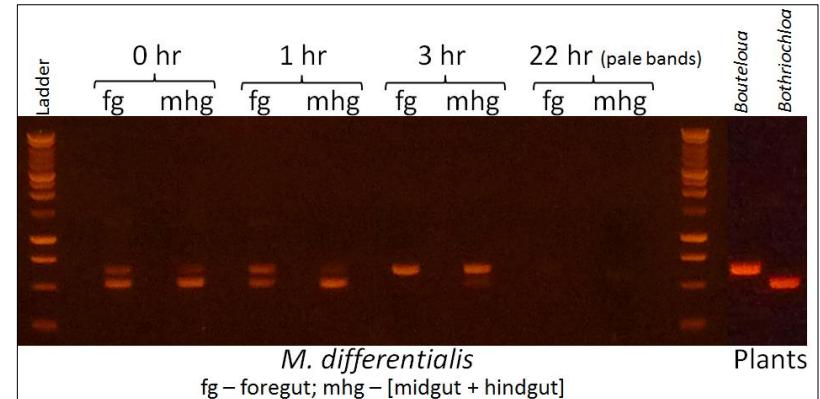
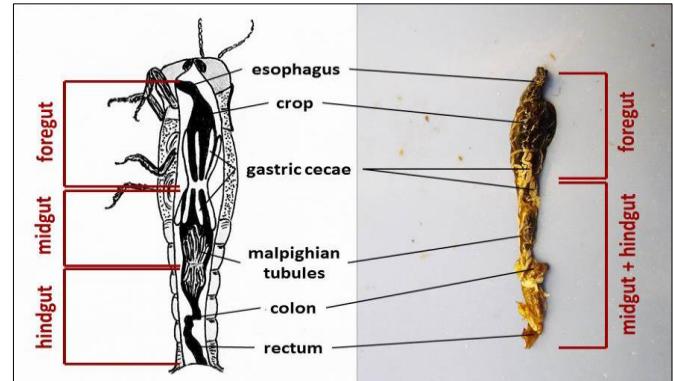


*M. differentialis*



22 h PI: choice, two plants

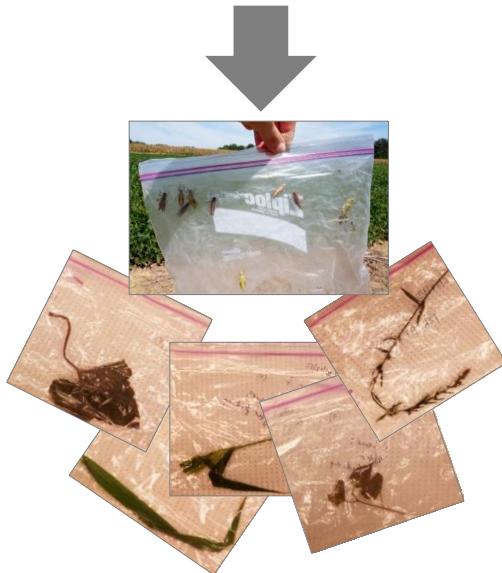
Different parts of grasshopper digestive system



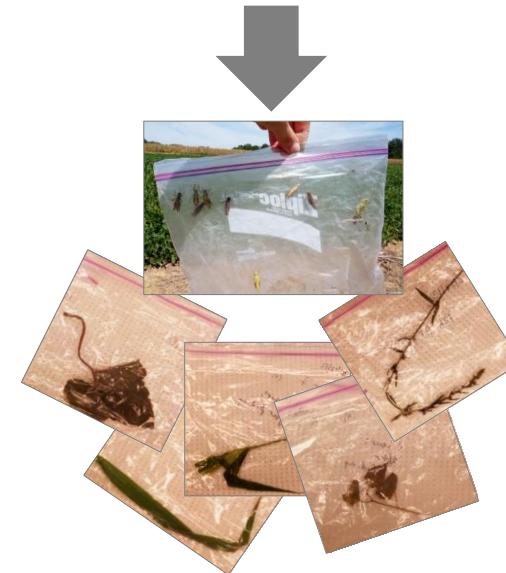
# Applying the Protocol



Cincinnati Center  
for Field Studies (OH)

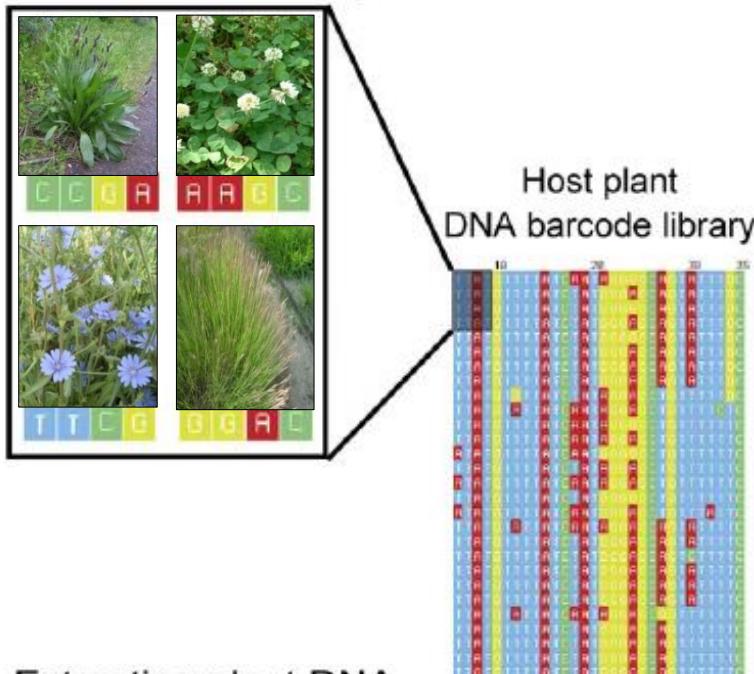


Western Maryland  
Research and Education Center (MD)



# Host Plant Identification

## A. Assembling a host plant DNA barcode library

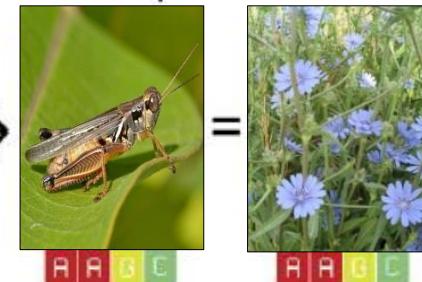


## B. Extracting plant DNA from insect herbivores



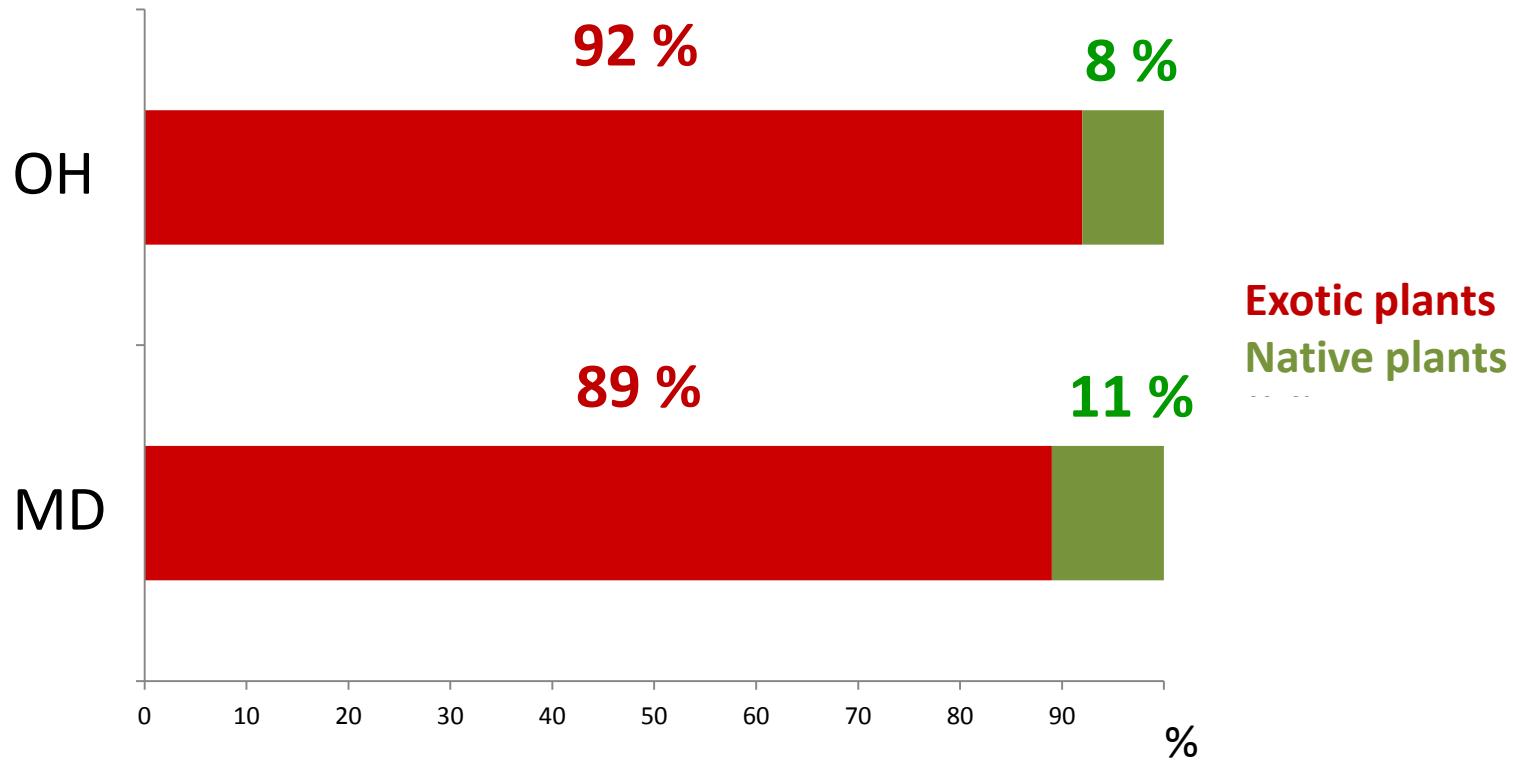
## C. Comparing extracted DNA with sequences in the DNA barcode library

## D. Matching DNA sequences and host plant identification



- Plant ID
- Plant Origin

# Proportions of Ingested Plants



- Grasshopper gut contents contained greater numbers of exotic plant species at both field sites ( $p < 0.0001$ , Binomial test)



Do *Melanoplus* grasshoppers have feeding preferences for native and exotic grasses?

→ behavioral approach (feeding activity, consumption, assimilation)

Exotic ≥ Native

→ molecular approach (DNA barcoding of ingested plant material)

Exotic > Native

# Main Conclusions



Overall, exotic grasses demonstrated lower resistance to grasshopper herbivory than native grasses in most experiments, while they tolerated the herbivory similar to native grasses

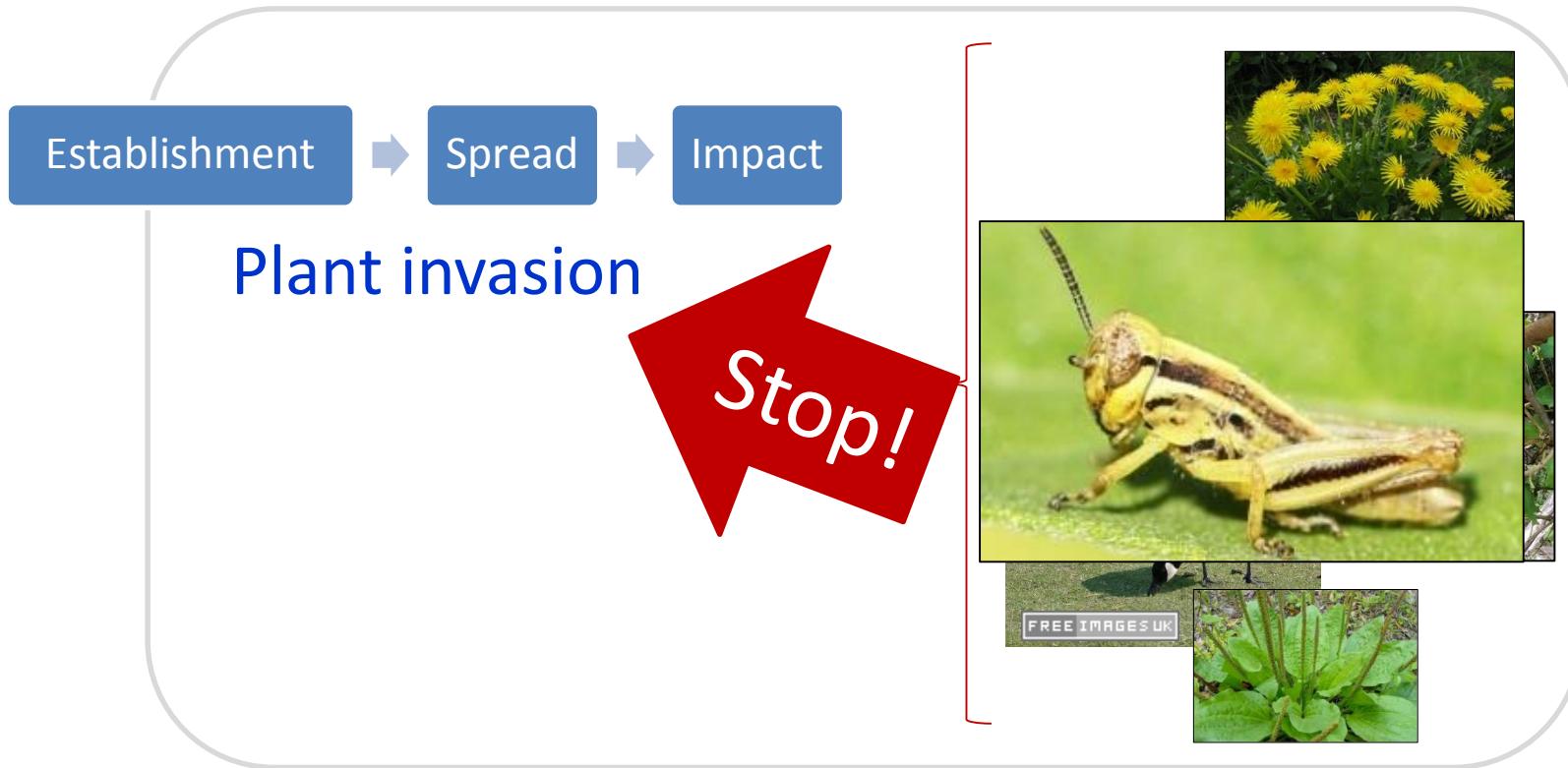
**Exotic  $\leq$  Native**



Grasshoppers did not avoid feeding on exotic grasses and even preferred them to native plants in most experiments.

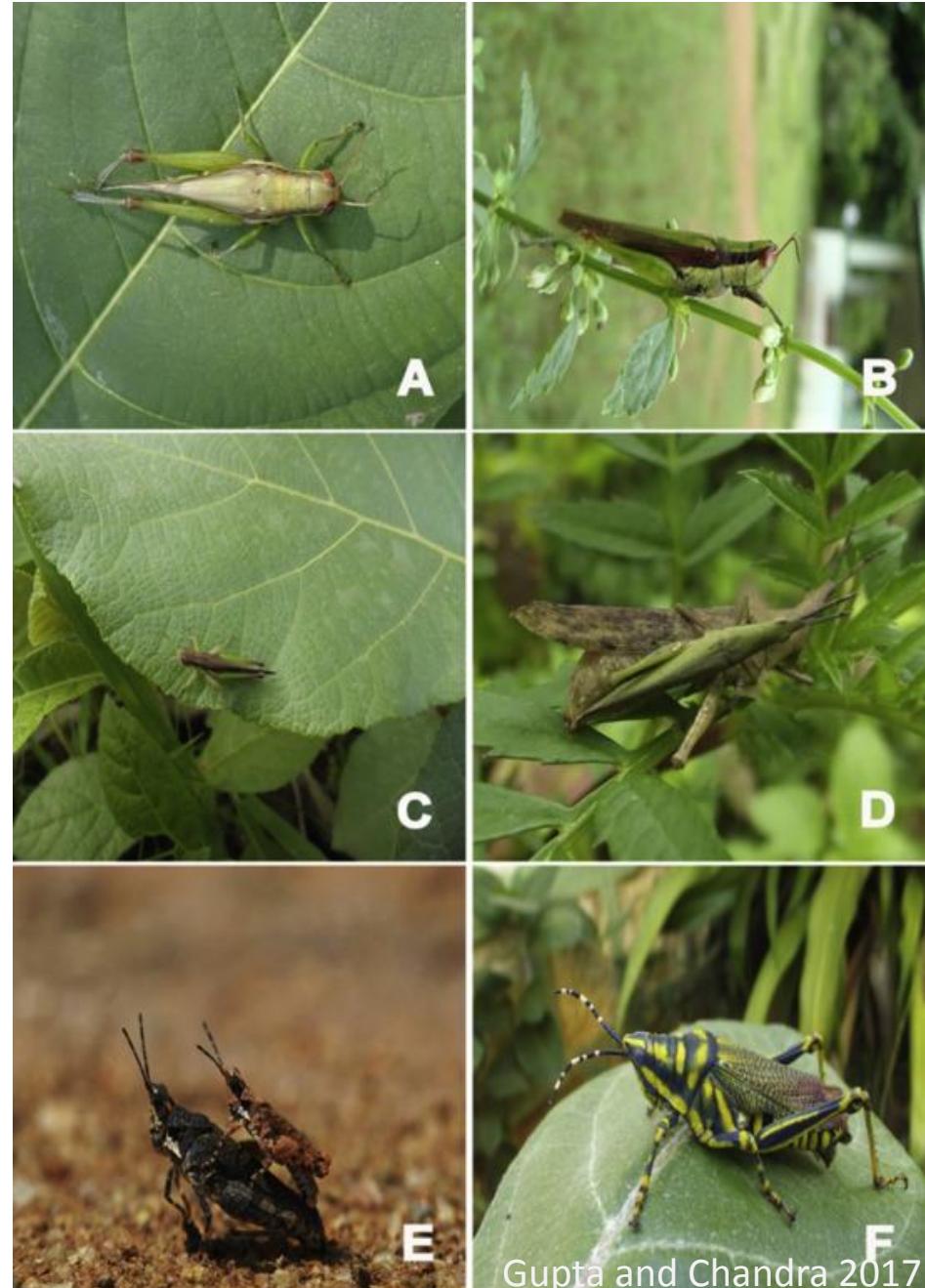
**Exotic  $\geq$  Native**

# Application to Biotic Resistance



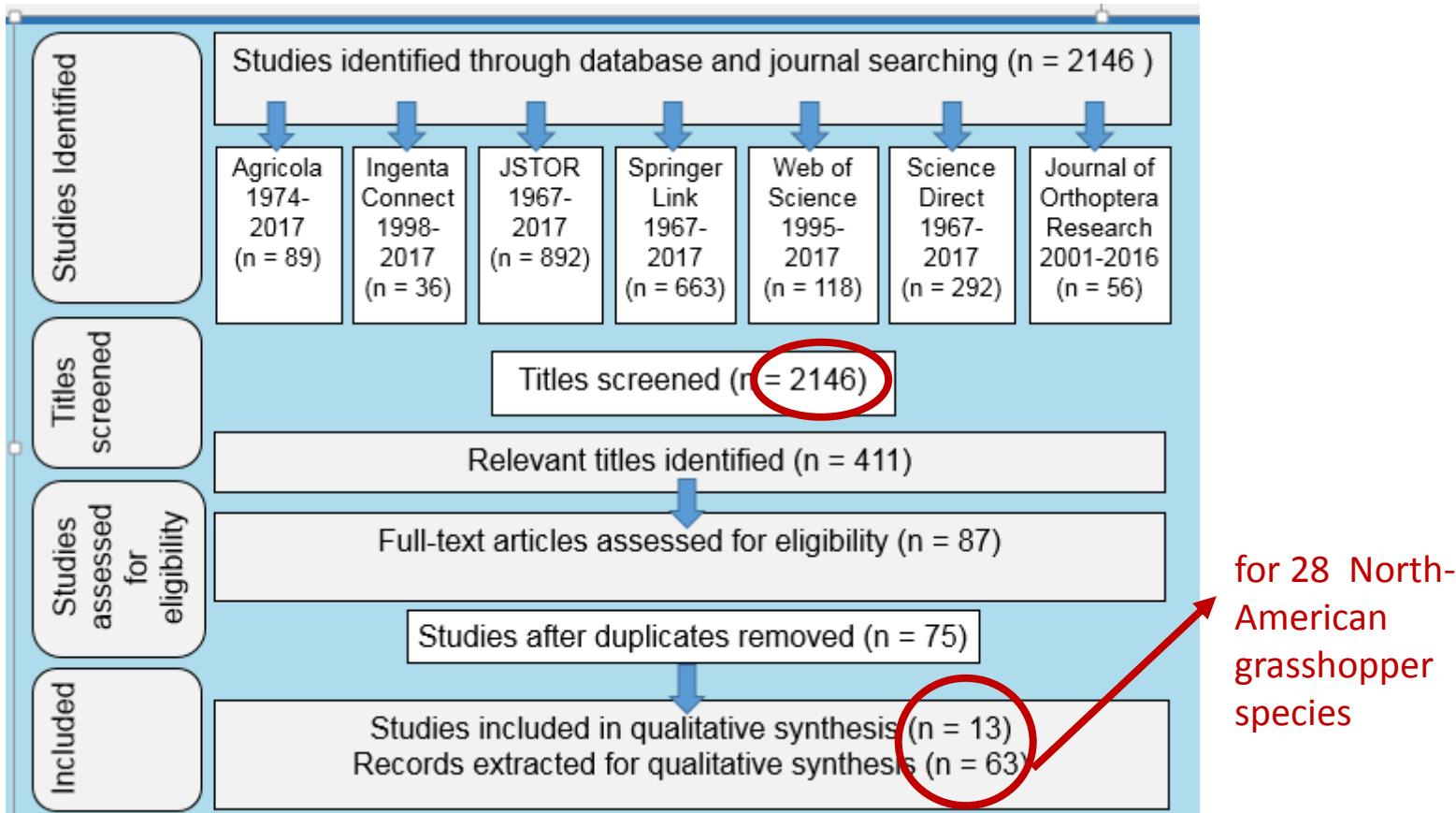
Native community

Do all the  
grasshoppers  
prefer to feed  
on exotic  
plants?

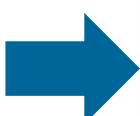


# Should I Eat or Should I Go?

## Acridid Grasshoppers and Their Novel Host Plants: Implications for Biotic Resistance

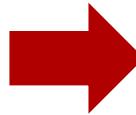


# Systematic Review and Meta-analysis

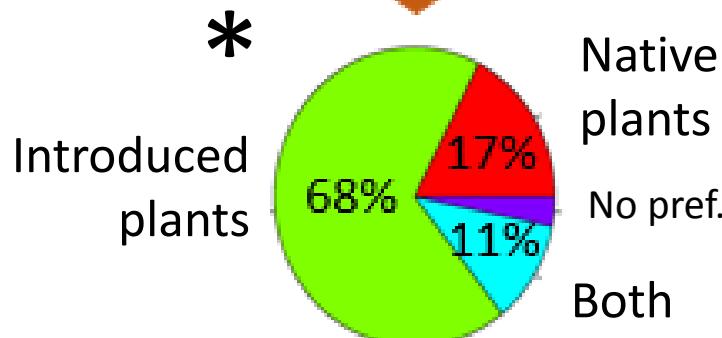


The authors used a very wide range of experimental conditions and measurements to assess grasshopper preferences

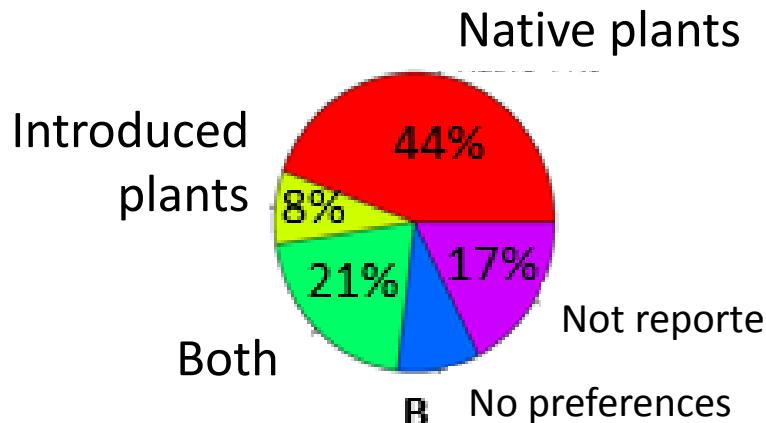
- **4 experimental environments:** common garden, greenhouse, lab (leaves), **lab (stems)**
- **3 types of feeding trials:** no-choice, choice (2 plants), **choice (plant mixture)**
- **2 types of plant material:** intact plants, **clipped plant parts**
- **Different stages:** **adults**, nymphs, mix
- **35 measurements** of feeding preferences!



Acridid grasshoppers prefer to feed on introduced plants



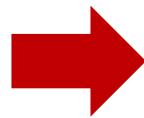
A



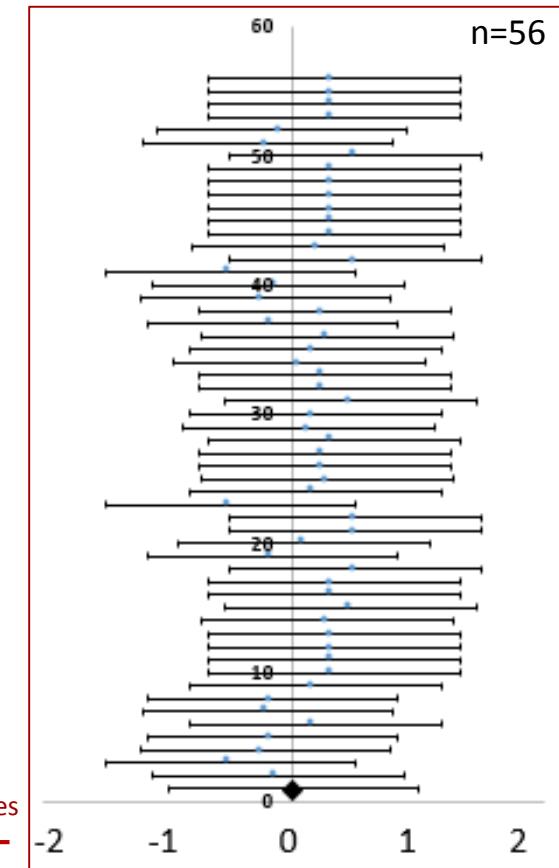
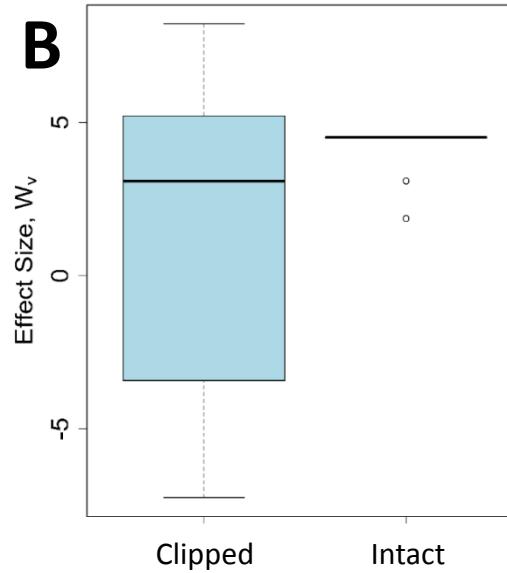
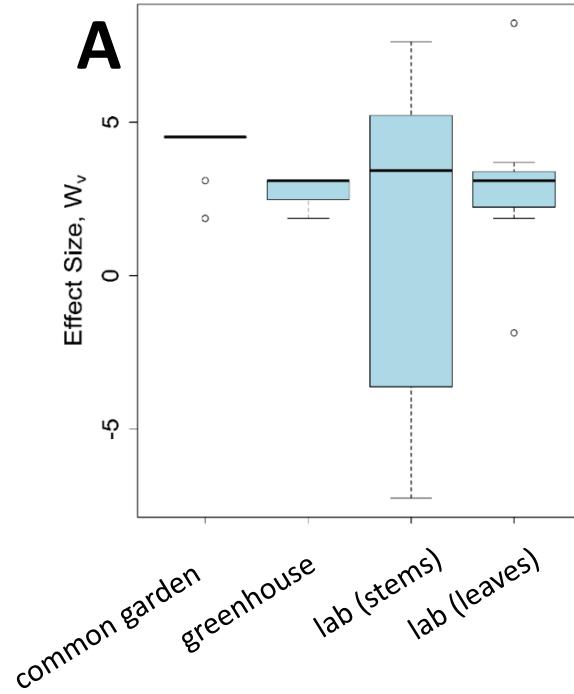
B

➤ Most preferred plants

➤ Least preferred plants



Acridid grasshoppers prefer to feed on introduced plants regardless the experimental conditions or plant material offered



**Preference Metric** = 
$$\frac{n_{\text{most preferred exotic plant species}} - n_{\text{most preferred native plant species}}}{n_{\text{total plant species offered}}}$$



Most of the preferred plants are highly invasive

- ❖ 20 introduced plant species (out of 22) were reported as “the most preferred”
- ❖ 12 species showed high or middle invasive rank
- ❖ *Bromus inermis* (smooth brome) and *Schedonorus arundinaceus* (tall fescue) are among the most preferred (for 50% grasshopper species)

# Application to Biotic Resistance

Establishment → Spread → Impact

Plant invasion

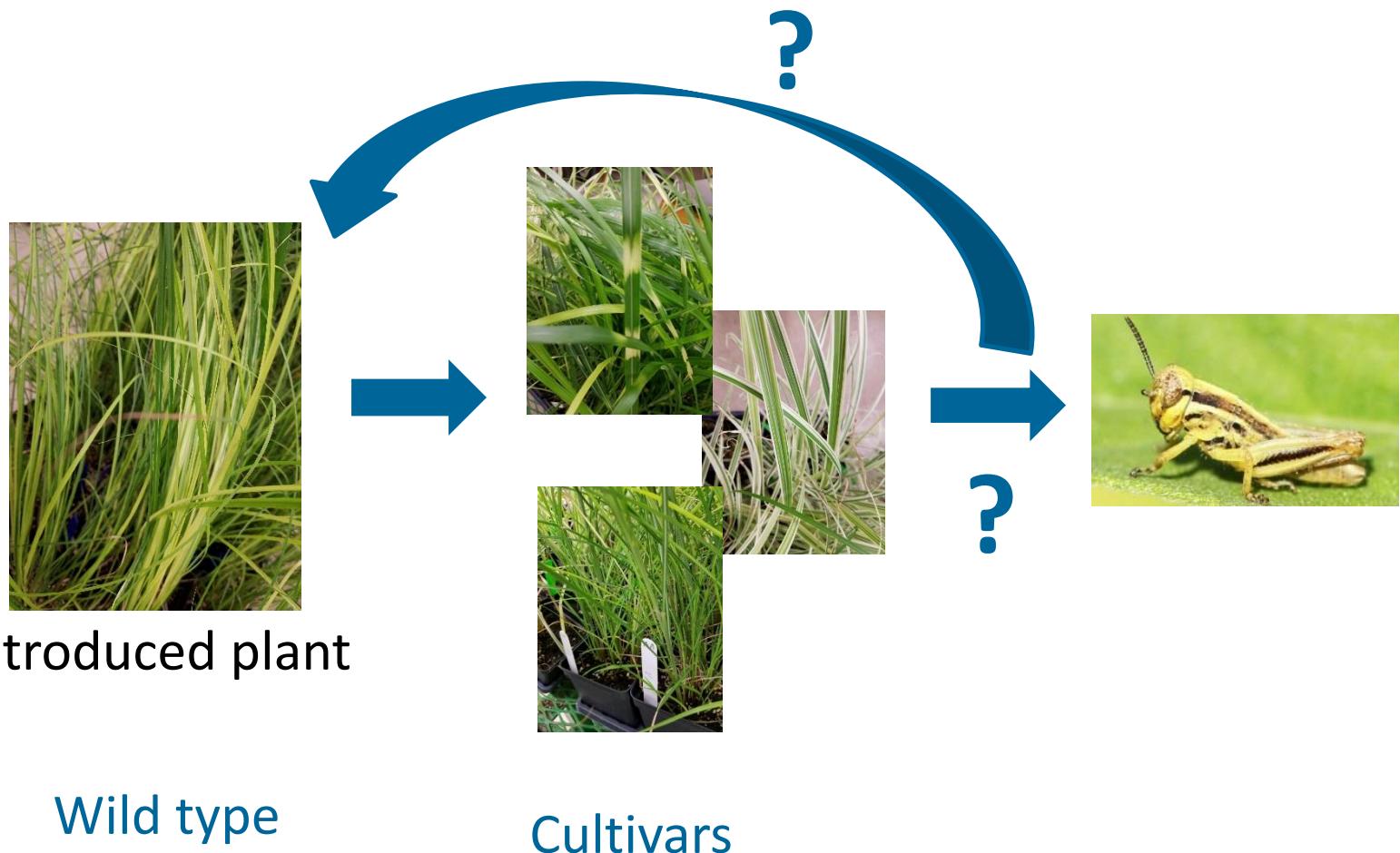


FREE IMAGES UK



Native community

# Grasshoppers and Introduced Plants

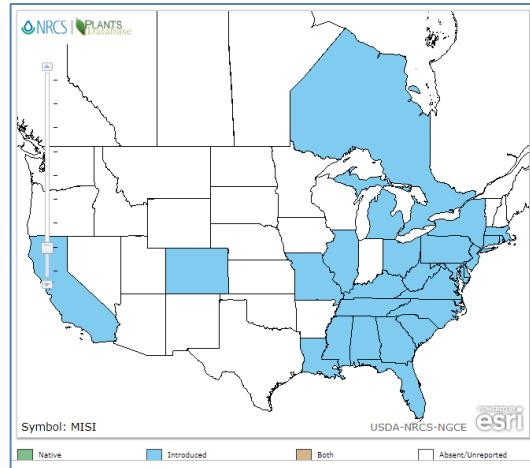


# Interactions between *Melanoplus* grasshoppers and *Miscanthus sinensis* varieties



# *Miscanthus sinensis* Andersson

## Chinese silvergrass



- Native to Japan
- 1893: introduced to Asheville NC; 1894: Washington DC
- 1940: naturalized populations in New York, Washington DC, Florida, West Virginia
- 2018: reported in 27 states
- disturbed areas, open fields, forest understories (in Maryland)

# *Miscanthus sinensis* varieties



- one of the most popular ornamental plants
- > 100 cultivated varieties



- Striped pattern
- Less vigorous, less invasive

- ‘all-green’ plants
- More aggressive

# *Miscanthus sinensis* varieties



*M. sinensis* 'Zebrinus' (ZE)



*M. sinensis* 'Autumn Anthem' (AA)



*M. sinensis* 'Gracillimus' (GR)



*M. sinensis* 'Morning Light' (ML)

# Research Questions

- Do *Miscanthus sinensis* cultivars differ in their resistance and tolerance to grasshopper herbivory?
- Do the plant responses to herbivory in *M. sinensis* cultivars differ from the plant responses in *M. sinensis* wild type?

# Field Experiments



WMREC, June 2018

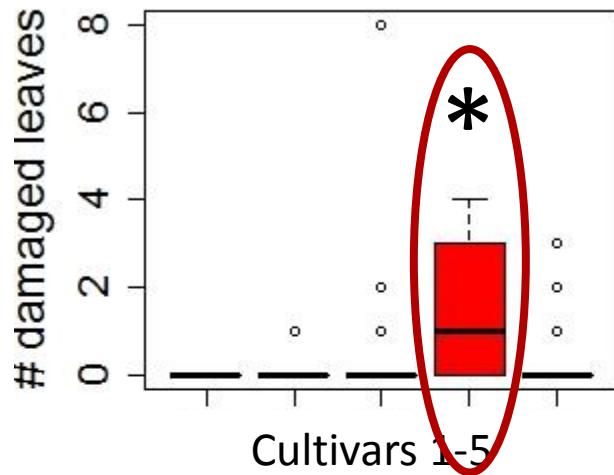
- 5 cultivars
- 30 plants/cultivar
- measured plant growth and leaf damage at 4 time points



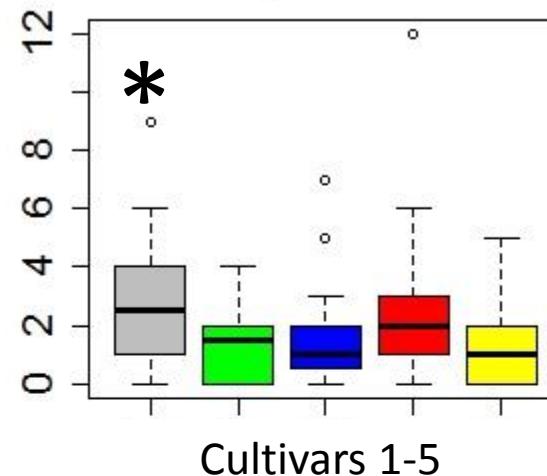
WMREC, August 2018



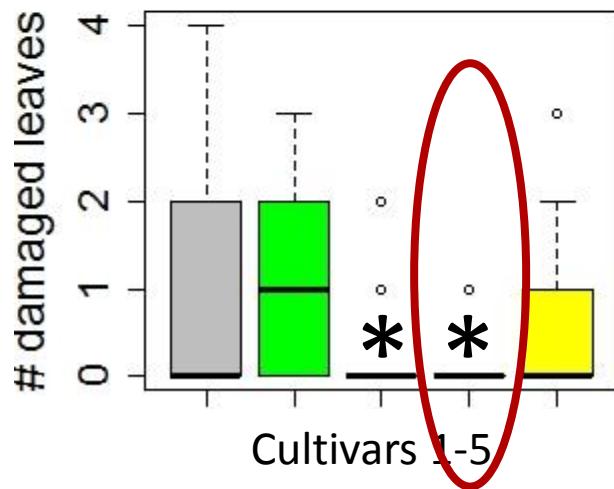
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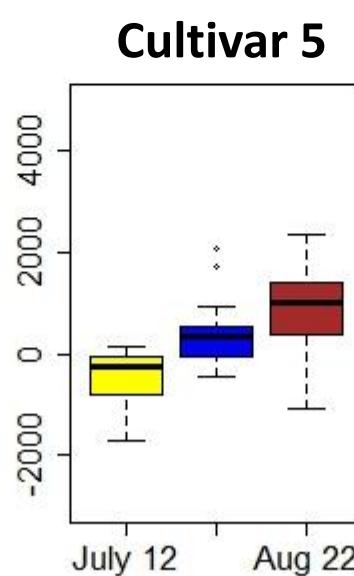
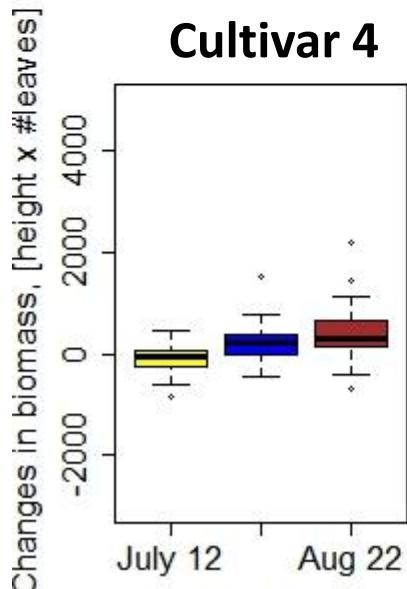
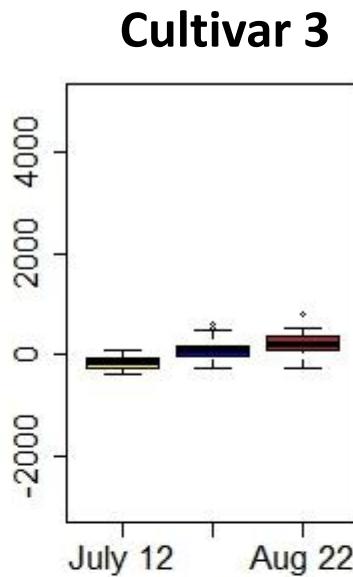
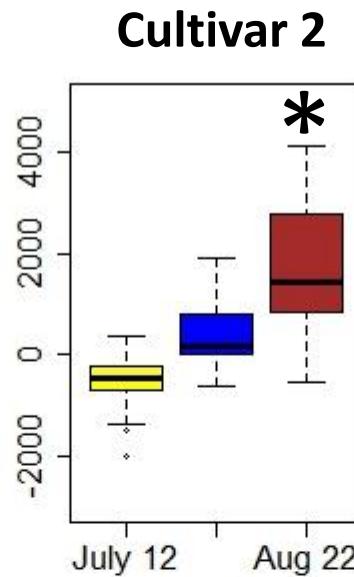
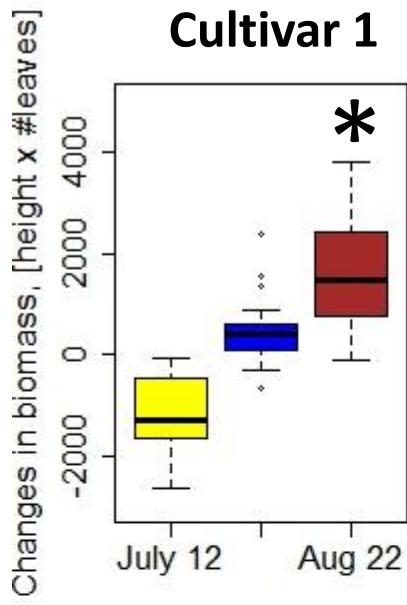
Aug 3, 2018



Aug 22, 2018



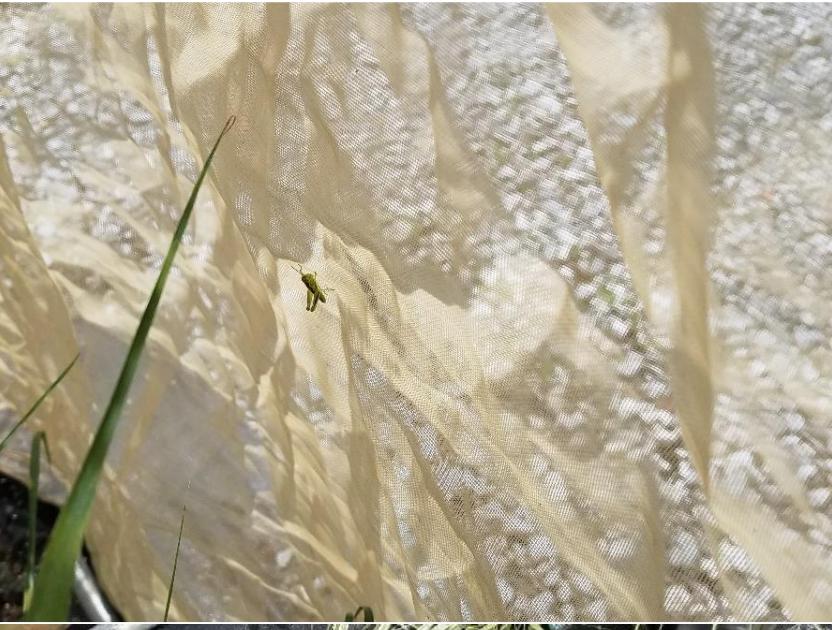
Plant resistance to  
herbivory: field



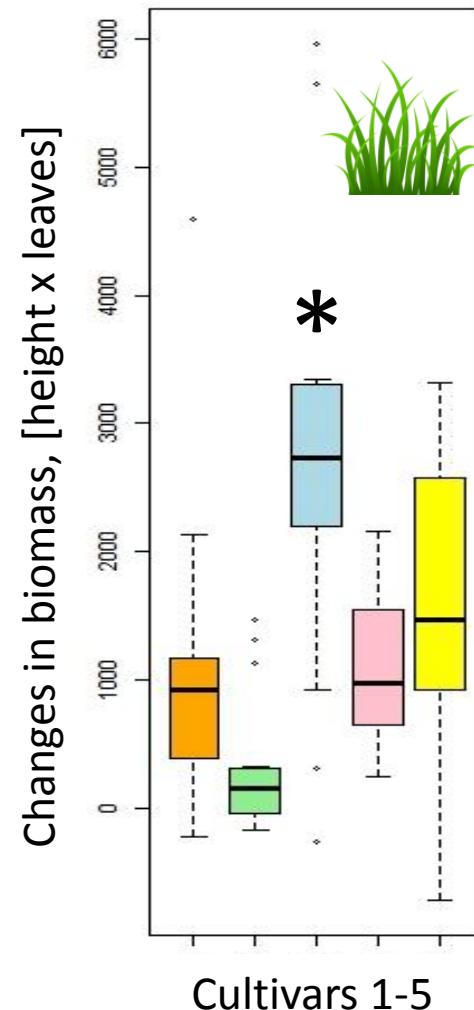
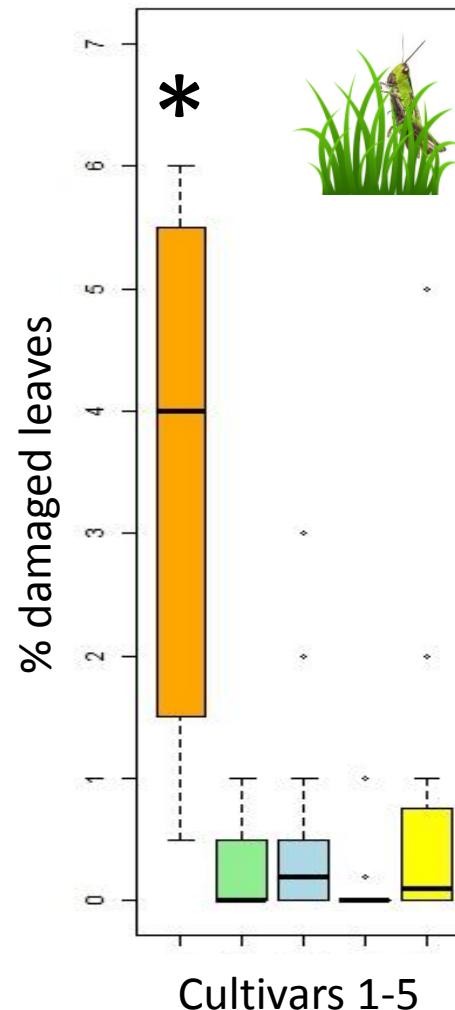
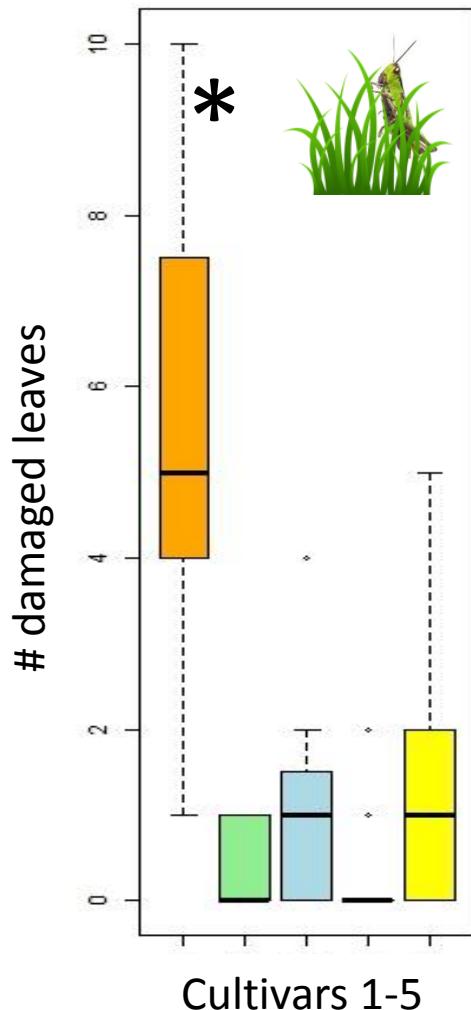
Plant tolerance to  
herbivory: field

# Greenhouse experiments





# Plant Resistance and Plant Tolerance to Herbivory: Greenhouse



# Preliminary Conclusions

- Grasshoppers feed on all the cultivars
- Plant responses differ among the cultivars



## Field:

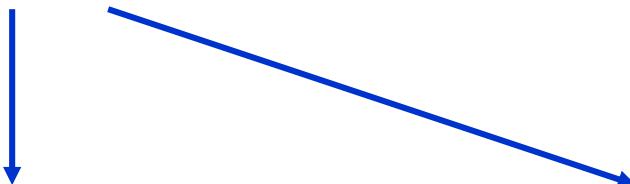
- plant resistance to herbivory in '[Gracillimus](#)' and '[Morning Light](#)' ('all-green' cultivars) is significantly lower than that in other cultivars in the beginning of the season, but it is significantly higher at the end of the season
- plant tolerance in '[Gracillimus](#)' and '[Autumn Anthem](#)' ('all-green' cultivars) is significantly higher than that in other cultivars

## Greenhouse:

- plant resistance in '[Autumn Anthem](#)' ('all-green' cultivar) is significantly lower than that in other cultivars
- plant tolerance in '[Gracillimus](#)' ('all-green' cultivar) is significantly higher than that in other cultivars

# Next step..

- Do Miscanthus sinensis cultivars differ in their resistance and tolerance to grasshopper herbivory?
  
- Do the plant responses to herbivory in *M. sinensis* cultivars differ from the plant responses in *M. sinensis* wild type?



Plant morphology

Plant population genetics

# Summary

## Why do introduced species fail to establish in a new range?

Native insect  
herbivore



- Behavioral mechanisms
- Morphological adaptations

- Plant chemistry
- Insect seasonal phenology



- Phylogenetic relatedness

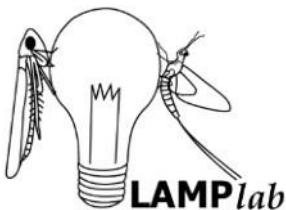


Native plant

Introduced plant

- ....many other mechanisms

Native community



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