# An Adaptive Framework for Non-target Risk Assessment of RNAi-based, Insect Resistant GM Crops

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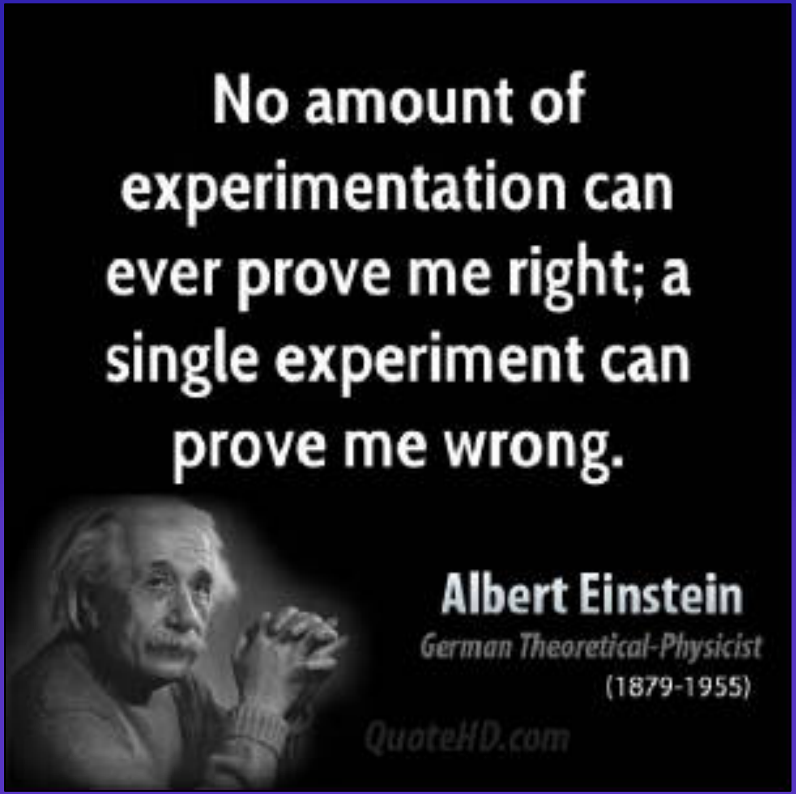
# Environmental Risk Assessment

Proving a negative




*"GM crops do not pose an extraordinary risk to the environment"*

# RNAi

*RNAi is a post-transcriptional technique for sequence-selective silencing of genes*

A concern is unintended gene silencing in non-target organisms

Lundgren and Duan. 2013. Bioscience 63: 657

For an animation on RNAi and associated risks, search youtube for "Cable Hardin RNAi"

Cable Hardin, SD State

# How Do We Select Species for Risk Assessment?

You can’t measure all of the species in a habitat.

Indicator species

- Represent a functional or phylogenetic “guild”
- Are easy to rear and assay

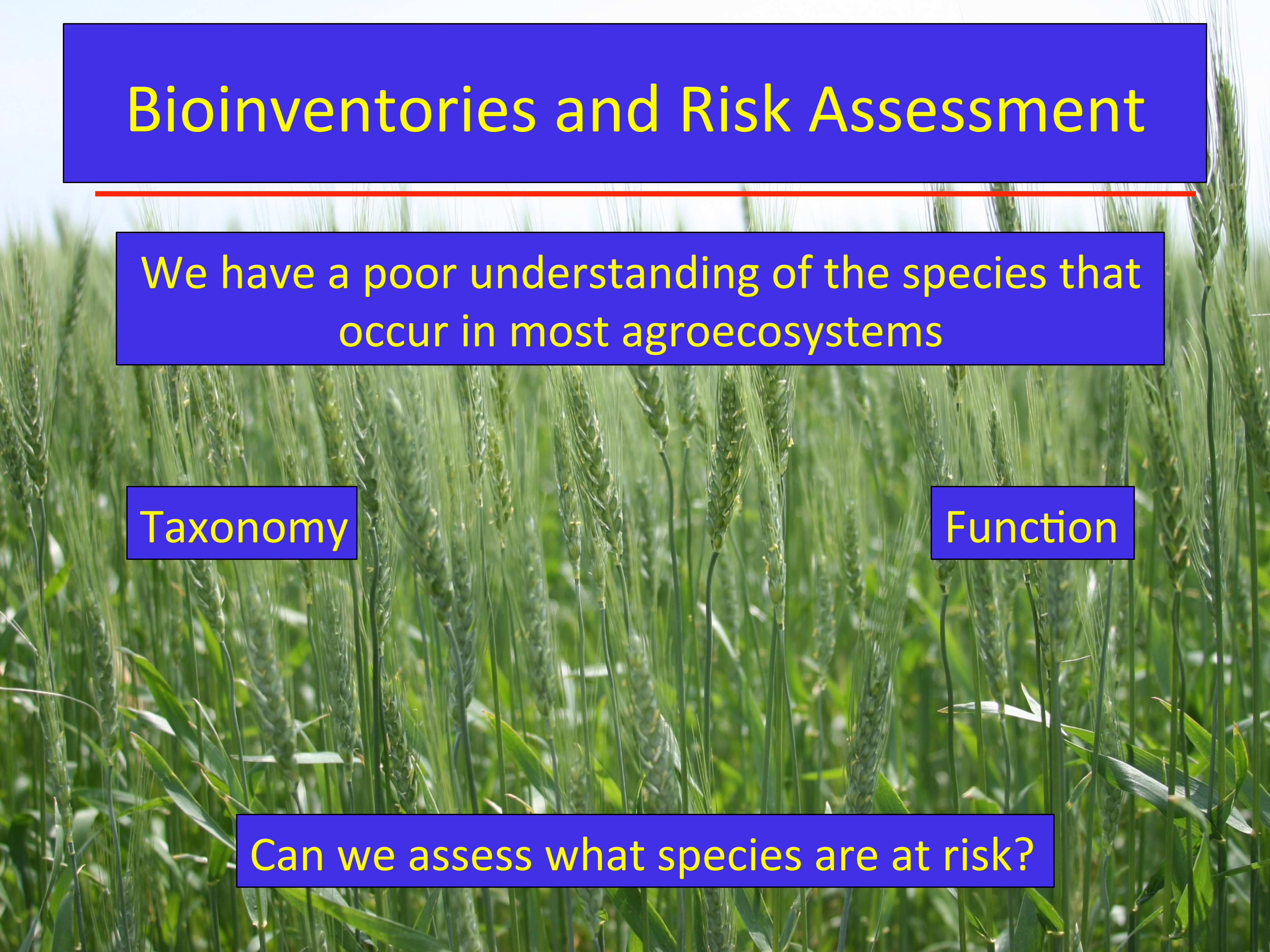
# Bioinventories and Risk Assessment

We have a poor understanding of the species that occur in most agroecosystems

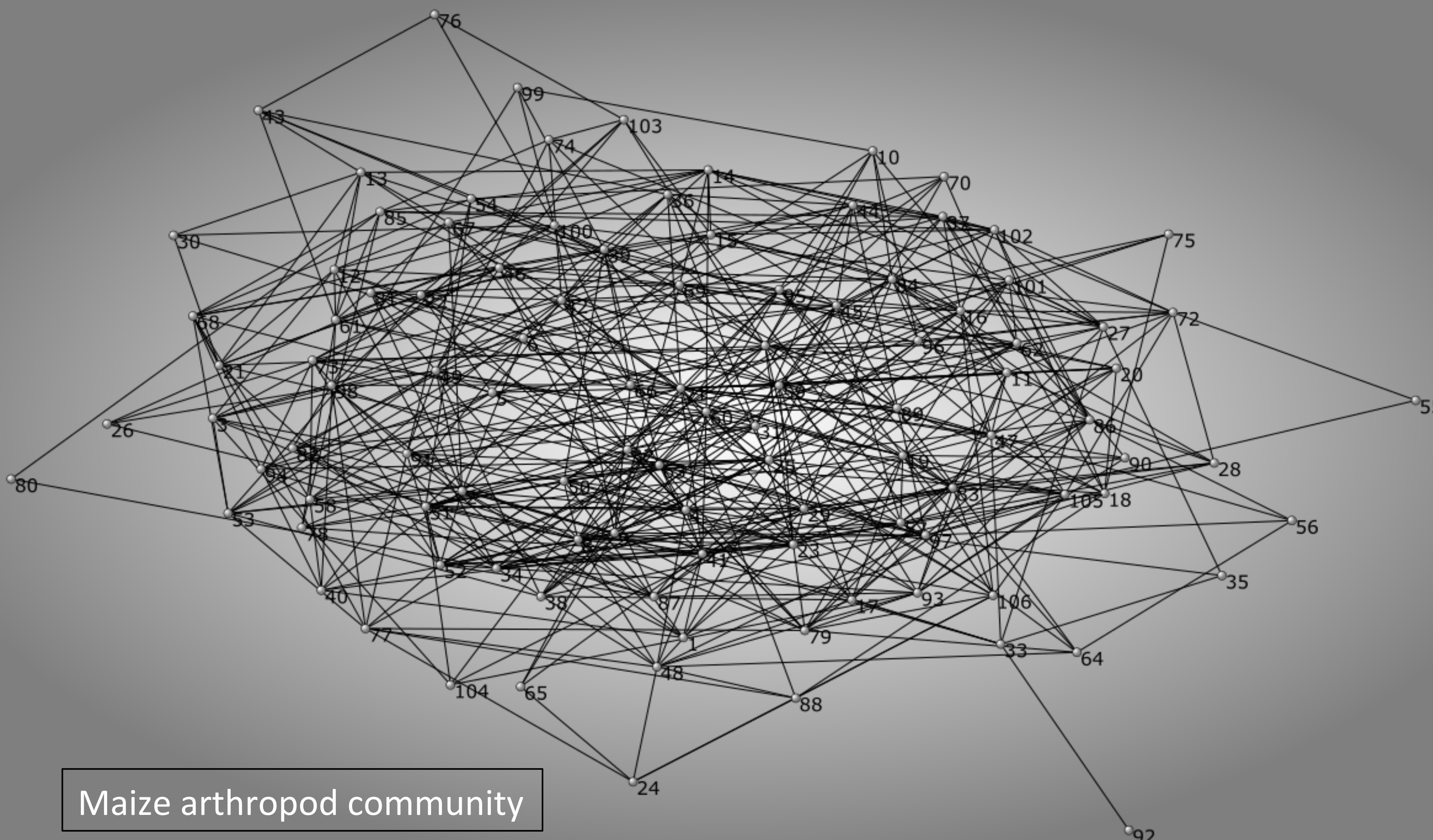
Taxonomy

Function

Can we assess what species are at risk?

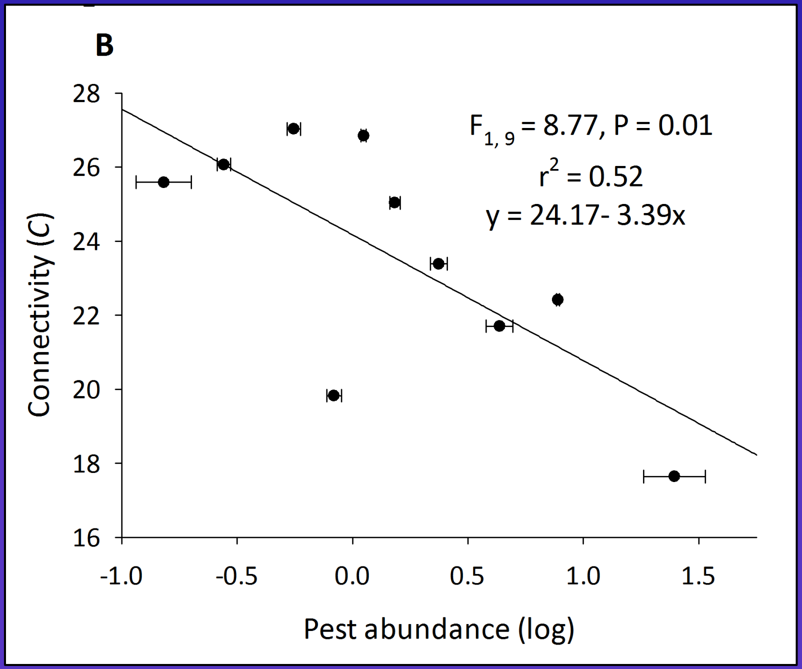


# Community Network in Agroecosystems




Maize arthropod community

# Network Strength and Pest Populations




More linked communities have fewer pests

Lundgren and Fausti. 2015. Trading biodiversity for pest problems. Science Advances, in press

# Using Community Ecology to Select Exposed Species

Bioinventory

Abundance and Incidence

Top corn consumers ⟷ Network connection to corn feeders


Bioinventory of Corn Arthropods
Soil column, soil surface, plant foliage
18 farms across eastern SD
Mid-vegetative and anthesis
382 taxa collected
11,939 specimens
Lundgren et al. 2015. Spatial and numerical relationships of arthropod communities associated with key pests of maize. J Appl Entomol, in press
Welch and Lundgren. An exposure-based, ecologically driven framework for selection of indicator species for insecticide risk assessments. Food Webs, in review

# Most Abundant Taxa

Taxa present in at least 50% of fields

Herbivore

*Rhopalosiphum padi*
*Frankliniella* sp.

Predator

*Solenopsis* (Thief ant)

Detritivore

Oribatid mite

# Corn Consumers

21 taxa were found to eat corn

Herbivore

*Lygus lineolaris*

Predator

*Chrysoperla* sp.

Detritivore

*Lepidocyrtus* sp. (Collembola: Entomobryidae)

# Most Connected Species

Number of network connections to corn consumers

Herbivore

*Frankliniella* sp.
*Malloewia* sp. (Chloropidae)

Predator

*Chrysoperla* sp.

Pollinator

*Toxomerus* sp. (Syrphidae)

Detritivore

Oribatid mite

# Summary

A diverse community resides in corn, with numerous species that are trophically exposed to PIPs.

Based on abundance, corn consumption, and network connectivity, some ecologically relevant species that are:

## Herbivore

Thrips
Lygus

## Predator

Green lacewing
Ants
(*Orius* also scored highly)

## Detritivore

Oribatid mite
Entomobryid Collembola

# Bioinformatics as a Tool for RNAi Risk Assessment

Given the diversity of non-target species, can we

Reduce the number of species to test
Optimize hazard assessments to detect phenotypic changes

Unintended gene silencing in non-target organisms

Mogren and Lundgren. *In silico* identification of off-target pesticidal dsRNA binding in honey bees. Nature Biotechnology, in review

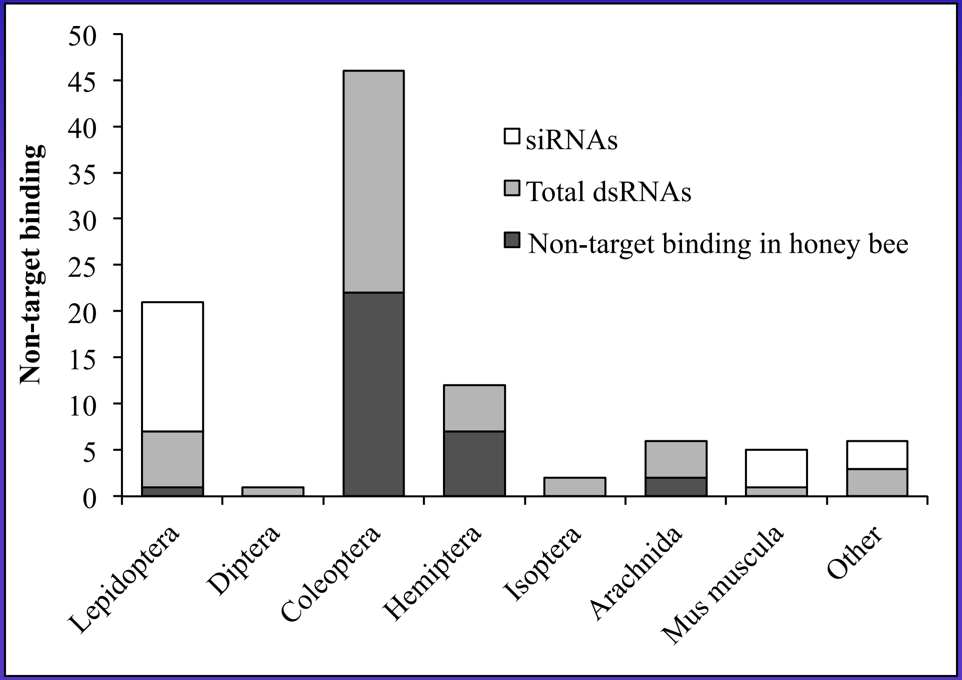
# The Database

Toxins
101 pesticidal dsRNAs (and siRNAs)
57 gene targets
23 species of taxonomic targets

Searched for sequence homologies (19/21, 20/21, and 21/21 nt) in honey bee genome

# Off-target Gene Homologies

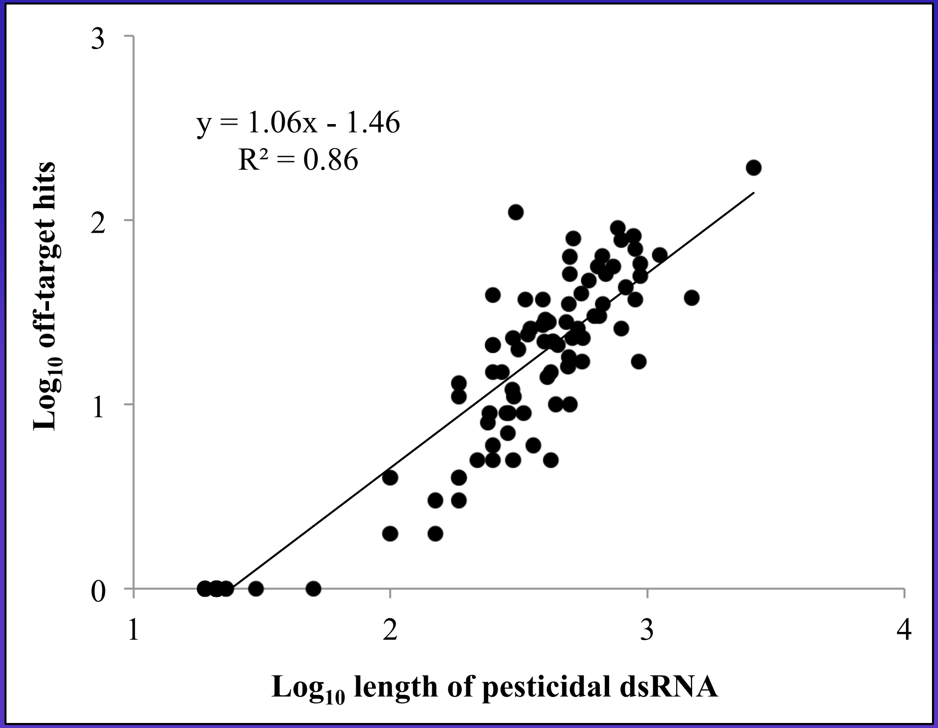
***All*** of the the dsRNAs had at least one perfect sequence homology in honey bees




siRNAs had no gene homologies in bees

Taxonomy of target does not affect the likelihood of silencing the targeted gene in the honey bee.

# Homeobox and developmental genes had a disproportionately high level of sequence homology with pesticidal dsRNAs




Longer dsRNAs produced more off-target hits

# Conclusions

Pesticidal dsRNAs likely will find off-target binding sites in non-target organisms

*In silico* gene homologies does not imply phenotypic suppression

Predicted gene suppression can help to hone hazard assessments

# Conclusions

Risks of pesticidal RNAi should be predictable and may be avoidable.

Filling the numerous knowledge gaps surrounding these risks will improve this predictability.

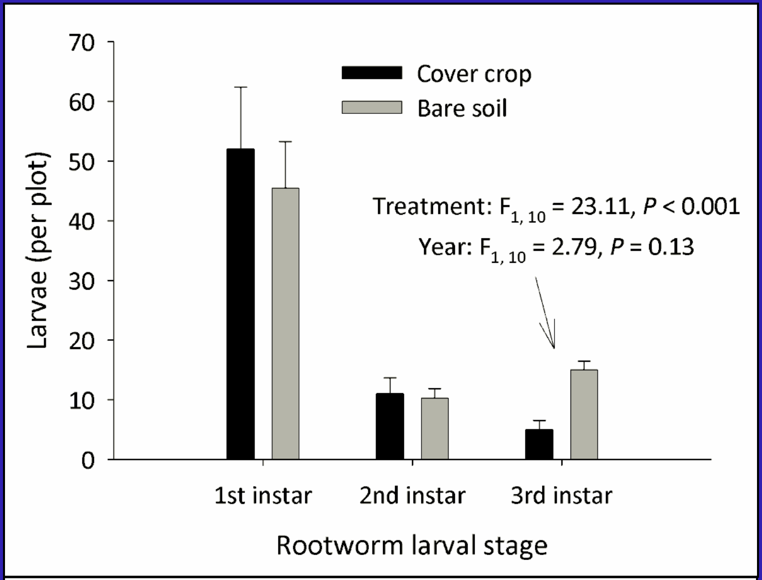
Now is the time to be asking these questions

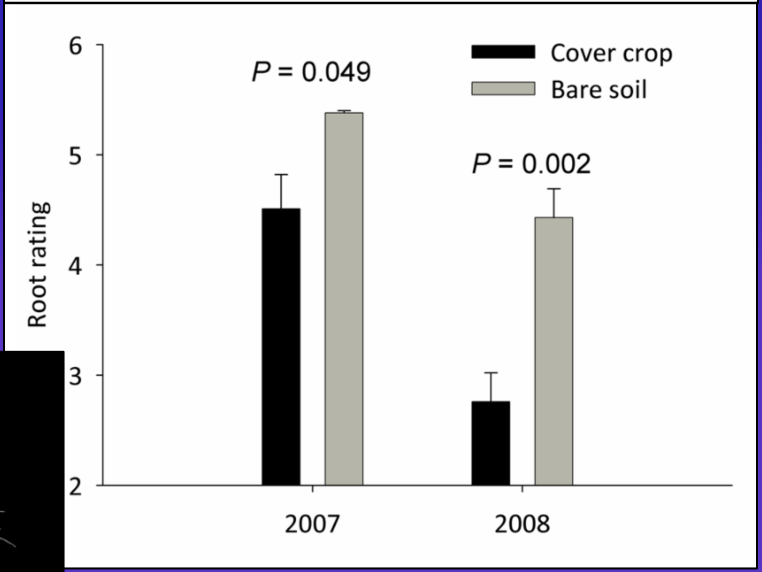

Pests are a Symptom, NOT the Problem!
What causes pest problems?

# High Disturbance Causes Pest Problems

Tillage

Brust et al. 1985. J Econ Entomol 78: 1389
Lehman et al. 2015. Sustainability 7: 988

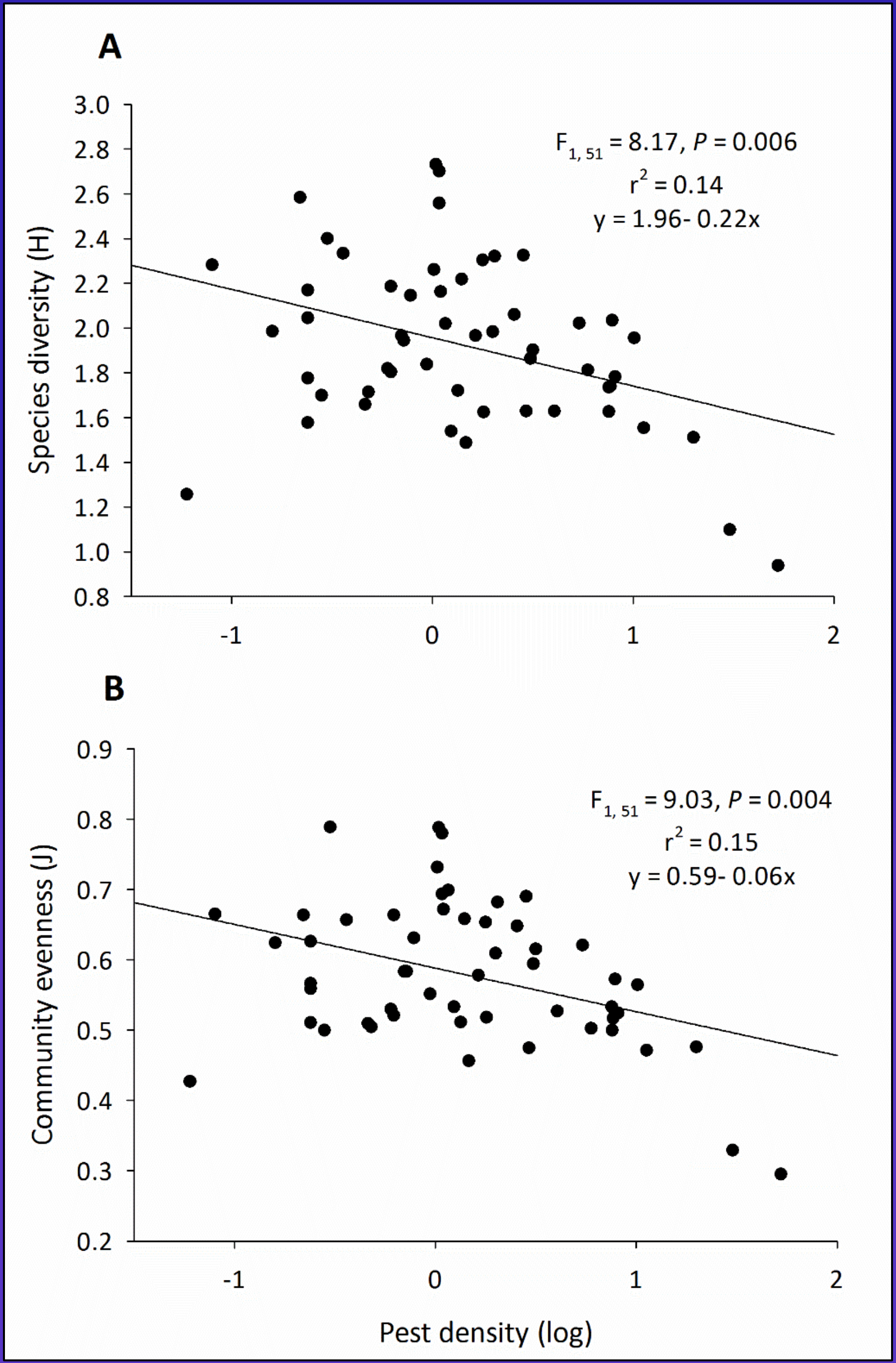



Bare soil

Lundgren and Fergen 2010. Environ Entomol 39: 1816
Lundgren and Fergen 2011. Appl Soil Ecol 51: 9

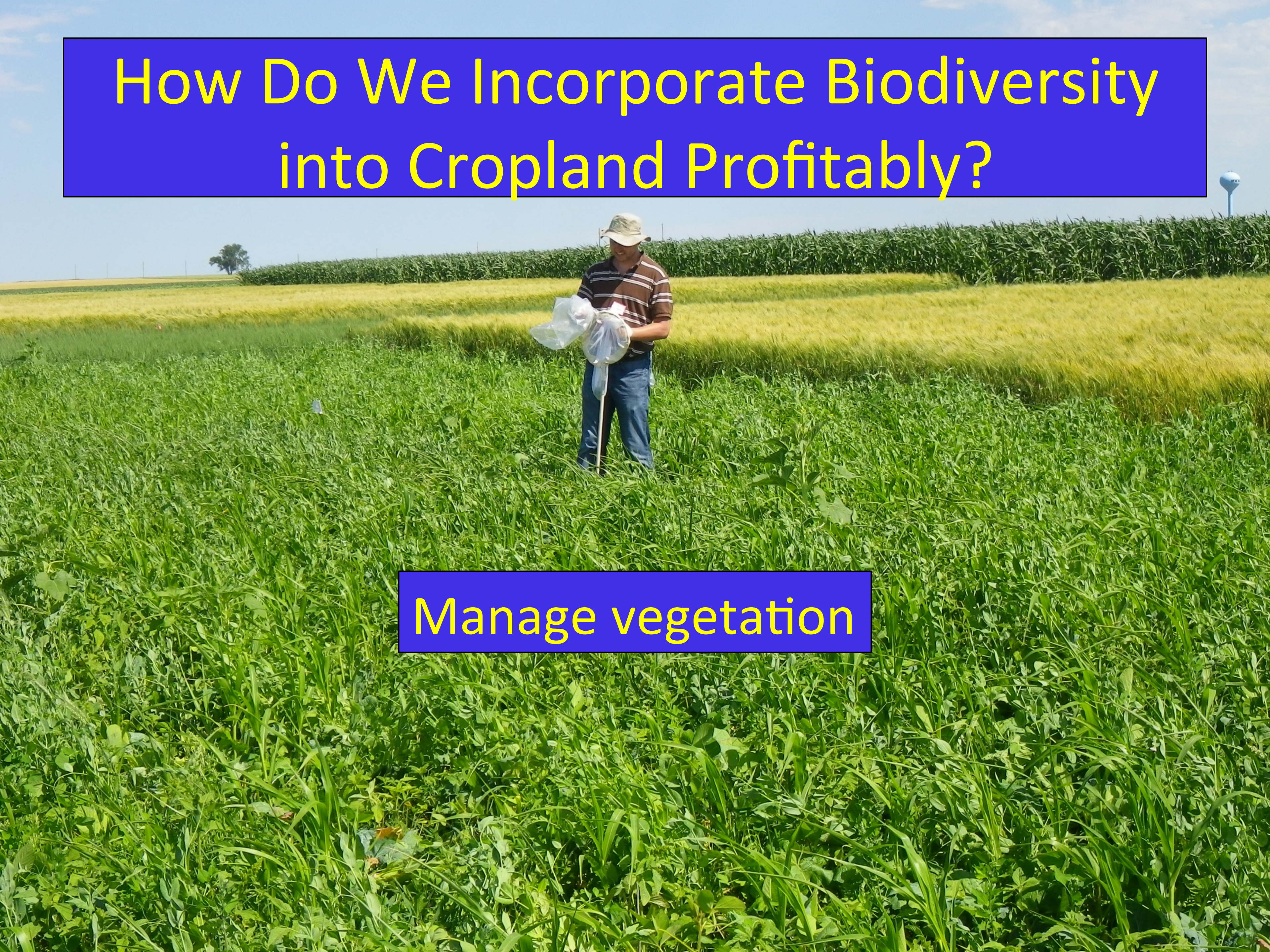
# Low Diversity Causes Pest Problems




Not just species richness, but the balance of species within that community and the interactions that they have

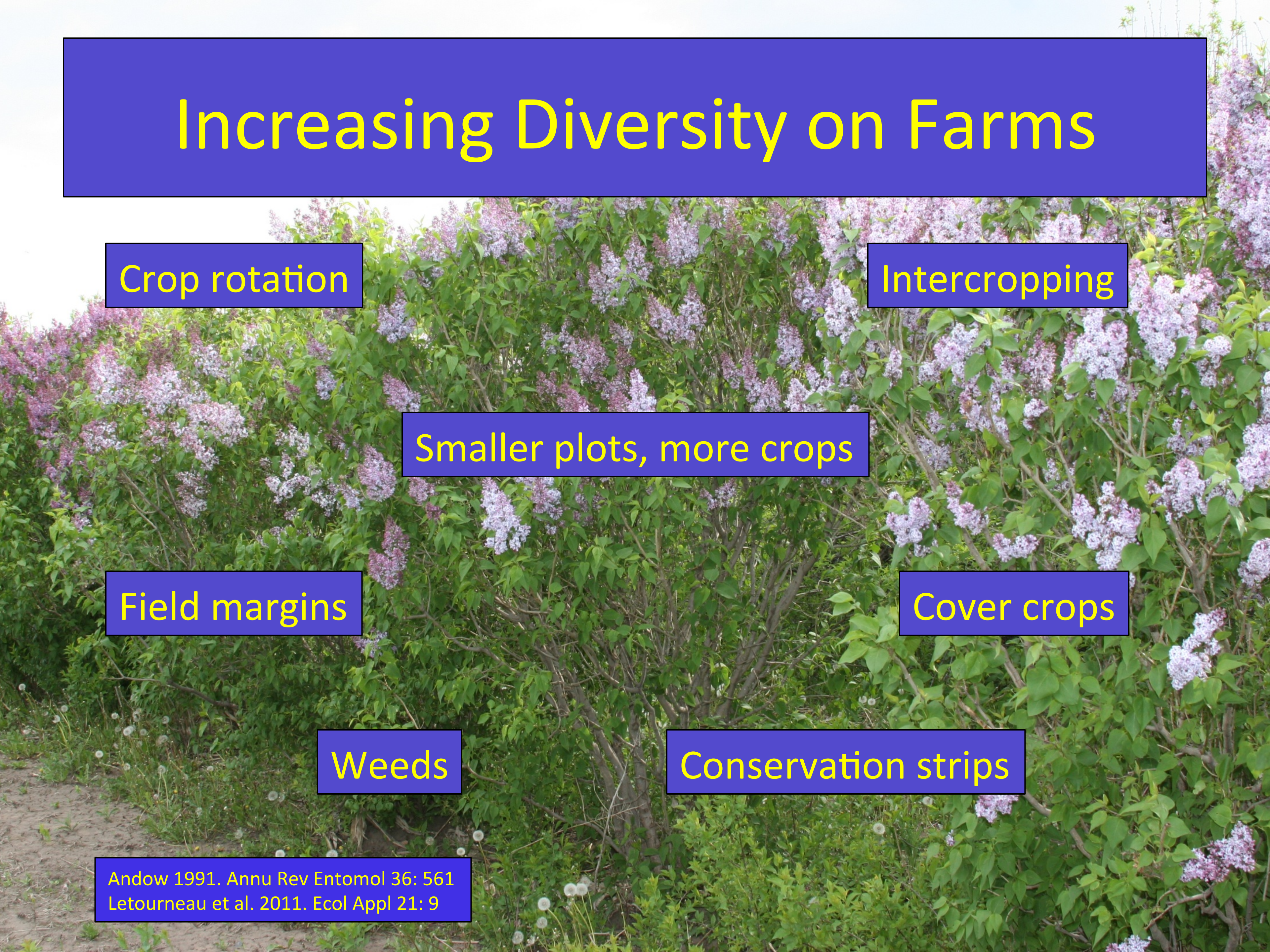
Lundgren and Fausti. Trading biodiversity for pest problems. Nature, *in review*

# How Do We Incorporate Biodiversity into Cropland Profitably?



Manage vegetation

# Increasing Diversity on Farms



Crop rotation

Intercropping

Smaller plots, more crops

Field margins

Cover crops

Weeds

Conservation strips

Andow 1991. Annu Rev Entomol 36: 561
Letourneau et al. 2011. Ecol Appl 21: 9

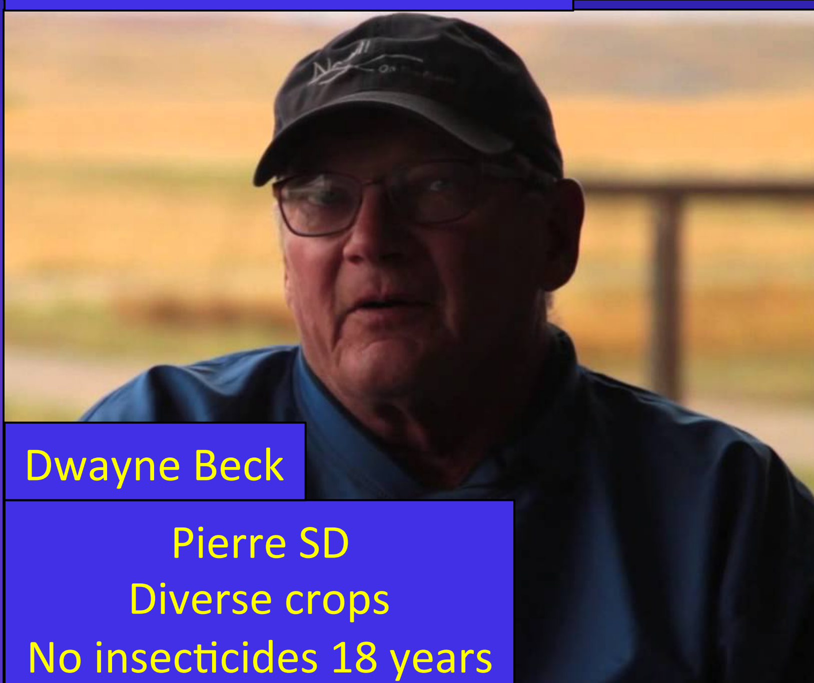
# The Farmers are leading the way

Gabe Brown

Bismarck ND
Diverse crops and livestock
No insecticides 28 years

Dave Brandt

Carroll, OH
Diverse crops
No insecticides 8 years



Dwayne Beck

Pierre SD
Diverse crops
No insecticides 18 years

Gail Fuller

Emporia KS
Diverse crops and livestock
No insecticides 8 years


Translating Research to Practice
Research supports these ideas
It is diffuse
The endpoints are often wrong
There are numerous knowledge gaps

# Why?


The Washington Post
Magazine
Was a USDA scientist muzzled because of his bee research?
By Steve Volk March 3
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Suspended USDA researcher alleges agency tried to block his research into harmful effects of pesticides on bees, butterflies
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# A New Way for Science to Help Bee Keepers and Farmers

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