Considerations for Risk Assessment Procedures of RNAi-based Crops

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Scientists are Interested in Using RNAi as an ~~Insecticide~~

Industry has created GM corn that will kill *Diabrotica* larvae using RNAi

Baum et al. 2007. Nature Biotechnology 25(11): 1322-1326
Bolognesi et al. 2012. PLoS ONE 7: e47534

RNAi-based insecticidal sprays are in development

Gordon et al. 2007. Nature Biotechnol. 25: 1231.
Price and Gatehouse. 2008. Trends in Biotechnol. 26: 393.
Huvenne and Smagghe 2010. J. Insect Physiol. 56: 227.
Burand and Hunter 2013. J Invert. Pathol. 112: S68-S74

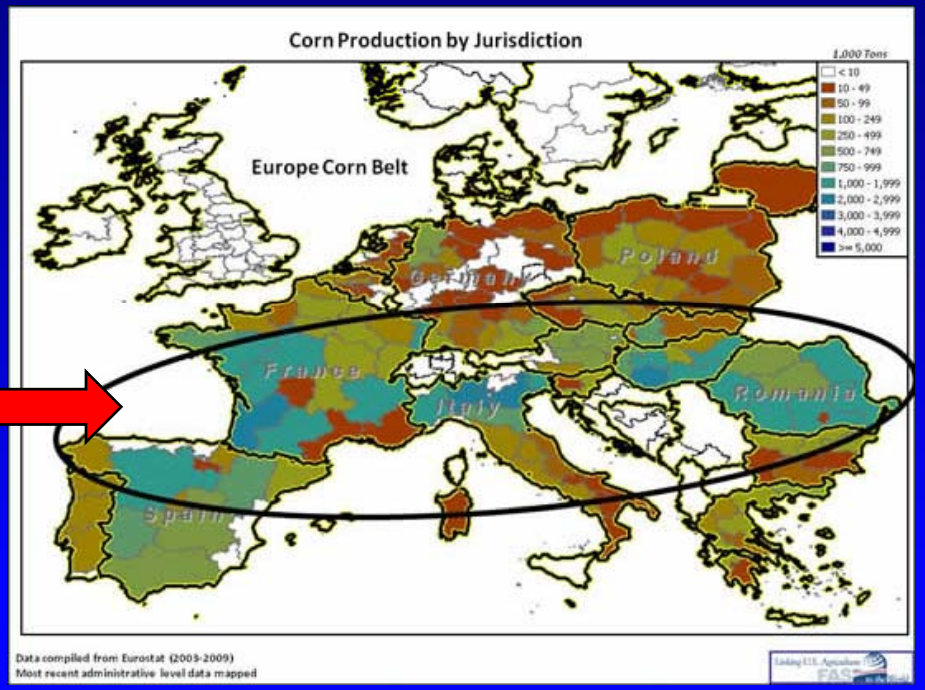
Experience with RNAi

Functional Genomics

Jackson and Linsley. 2010. Nature Reviews: Drug Discovery 9: 57-67

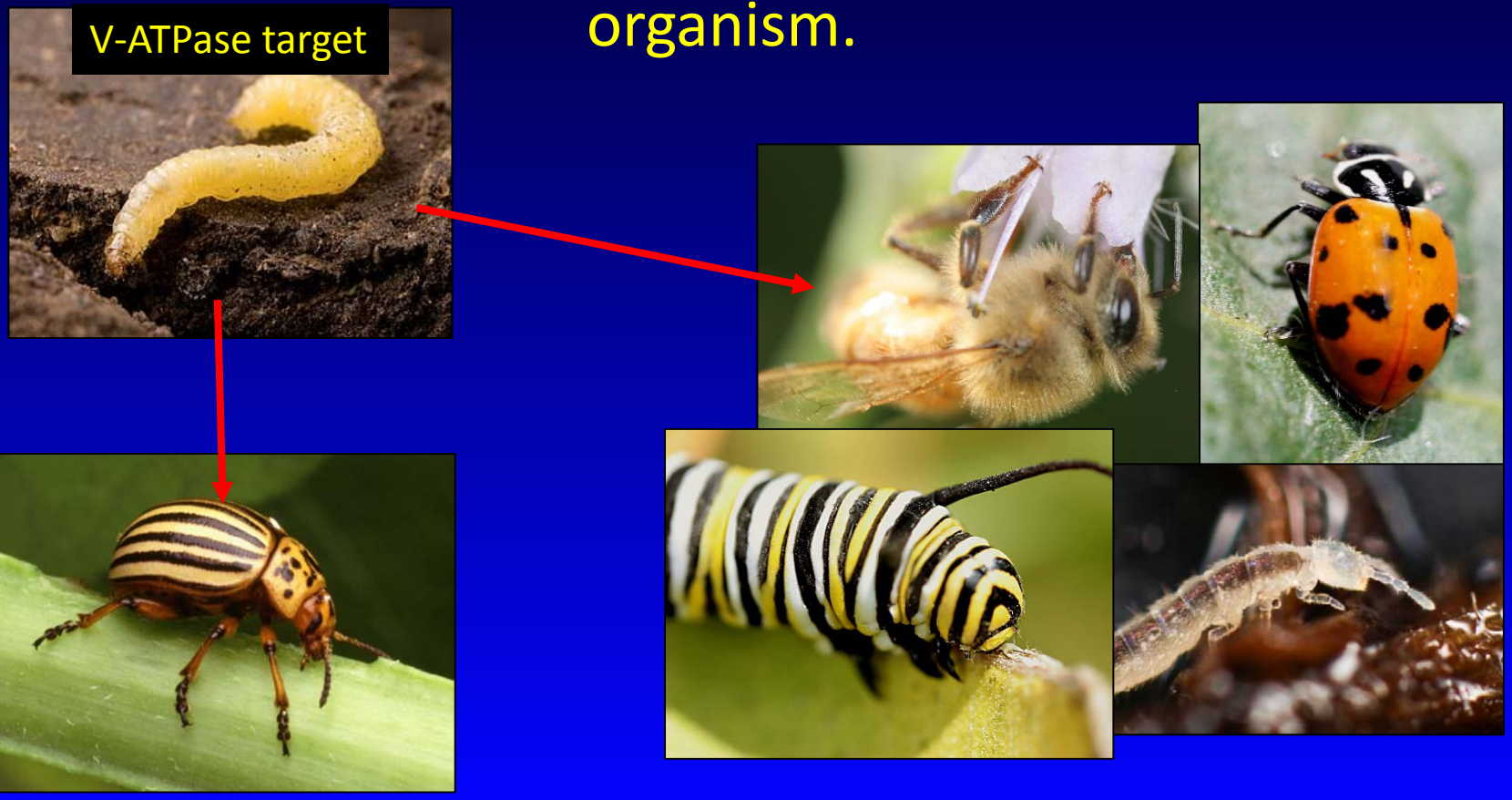
Potential Hazards Posed by RNAi-based Pesticides

Pesticides are deployed on a much broader scale than current uses of RNAi



Unintended Gene Silencing

RNAi sometimes silences the correct gene in the wrong organism.

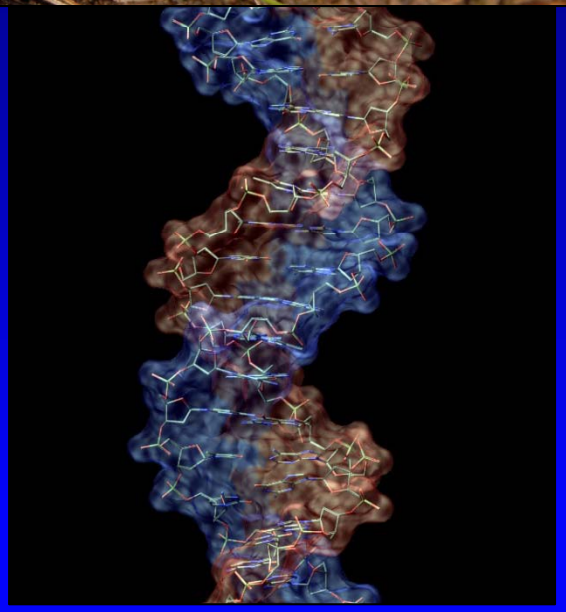


Baum et al. 2007. Nature Biotechnology 25(11): 1322-1326
DvSnf7: Bachman et al. 2013. Transgenic Res 22: 1207-1222

Zhou and Siegfried's NIFA-funded work

Off-target Binding

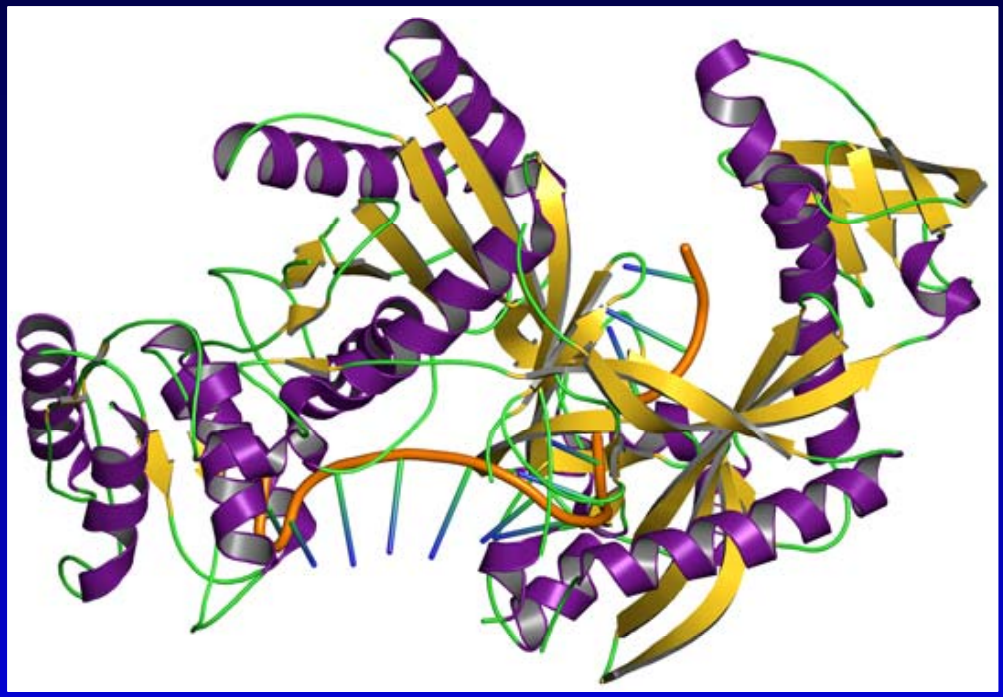
RNAi sometimes silences the wrong gene entirely



Saxena et al. 2003. J Biol. Chem. 278: 44312.
Qiu et al. 2005. Nucleic Acids Res. 33: 1834.
Federov et al. 2006. RNA 12: 1188.
Jackson et al. 2006. RNA 12: 1179.
Kulkarni et al. 2006. Nature Methods 3: 833.
Aleman et al. 2007. RNA 13: 385.
Davidson and McCray. 2011. Nature Rev. 12: 329

RNAi Saturation

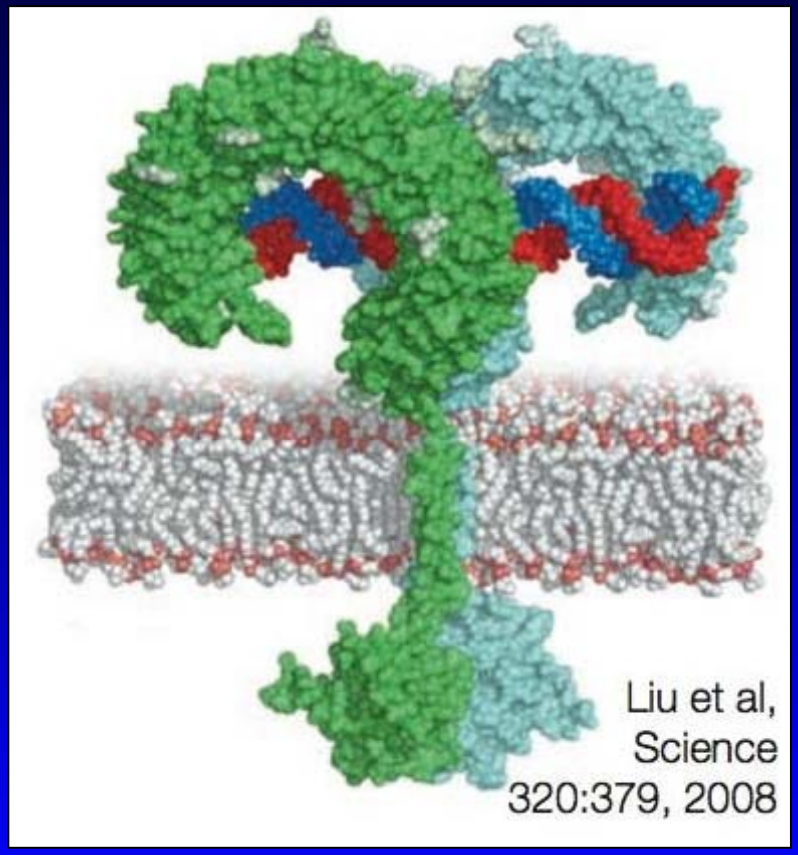
Cellular machinery involved with RNAi can become saturated. This temporarily inhibits cellular use of RNAi.



Argonaute protein

Kahn et al. 2009. Nature Biotechnol. 27: 549
Jackson and Linsley 2010. Nature Reviews 9: 57

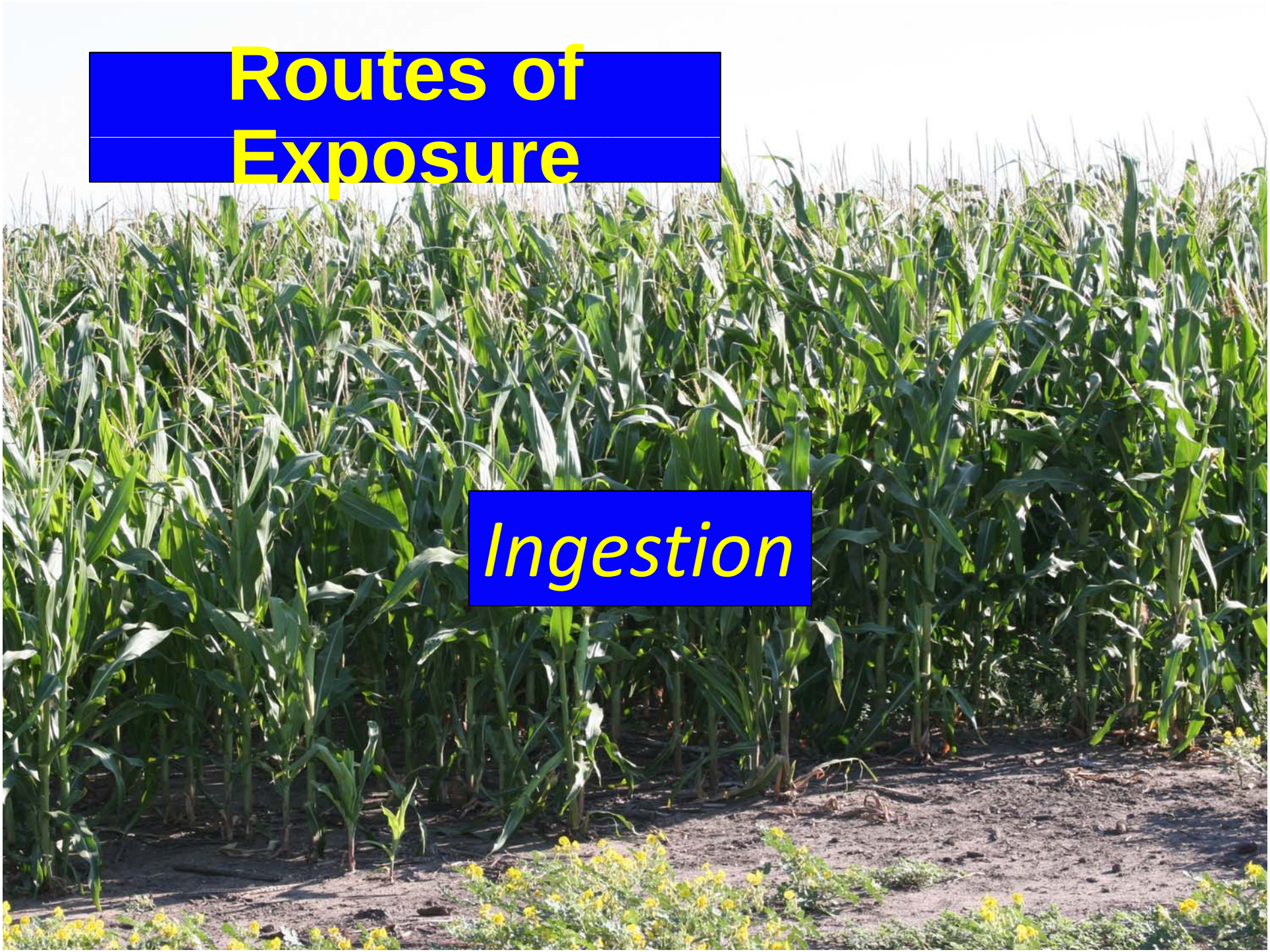
Immune Stimulation



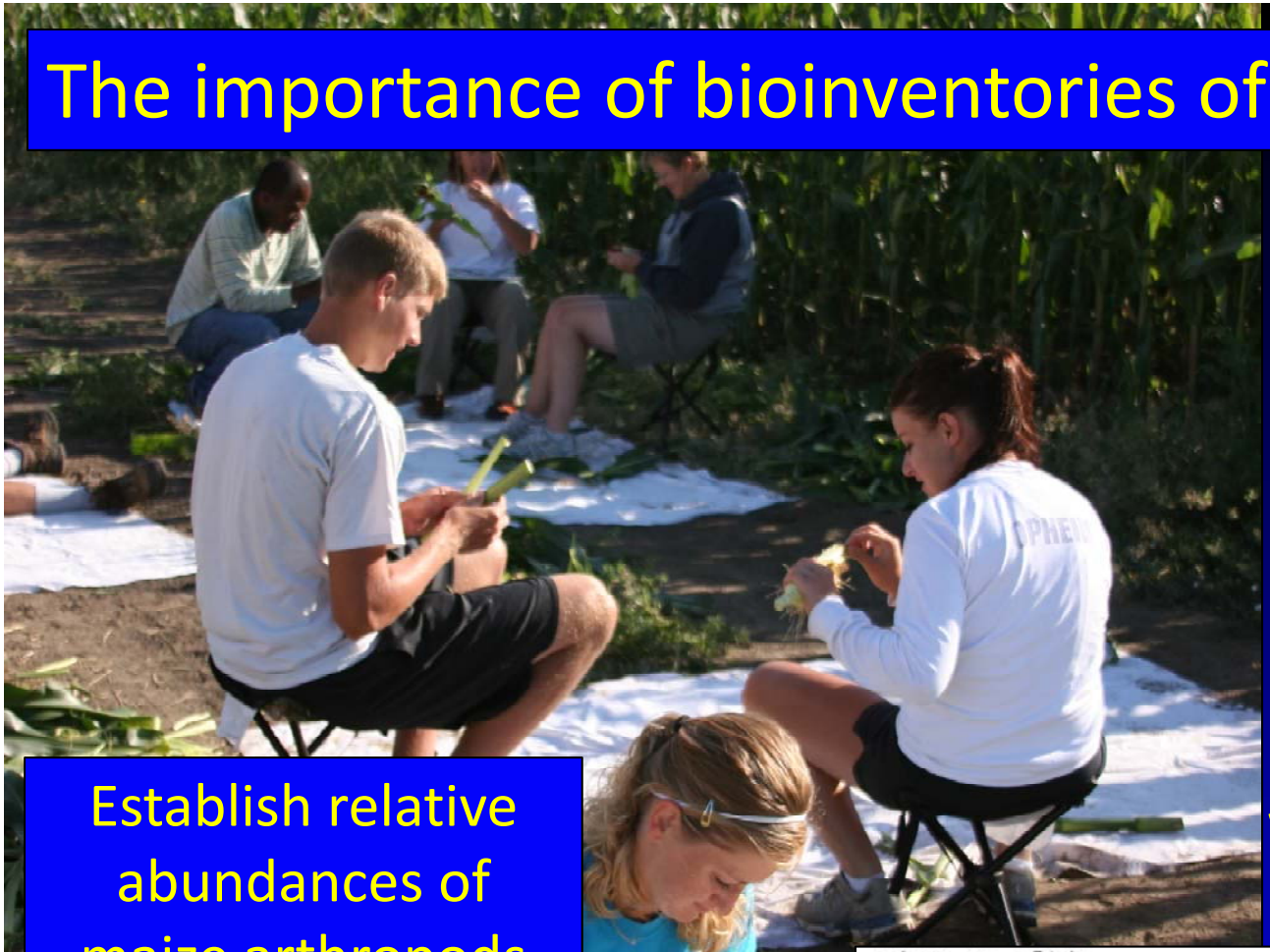
Nucleic acids can stimulate the innate immune system of animals

Whitehead et al. 2011. Annual Review of Chemical and Biomolecular Engineering 2: 77-96
Flenniken and Andino 2013. PLoS ONE 8(10): e77263

Routes of Exposure



The importance of bioinventories of agroecosystems



Establish relative abundances of maize arthropods

Genetic gut content analysis to determine which insects are eating maize

Quantitative food webs to rank most exposed species

Environmental Persistence of Small RNAs

Naked nucleic acids break down rapidly in soil

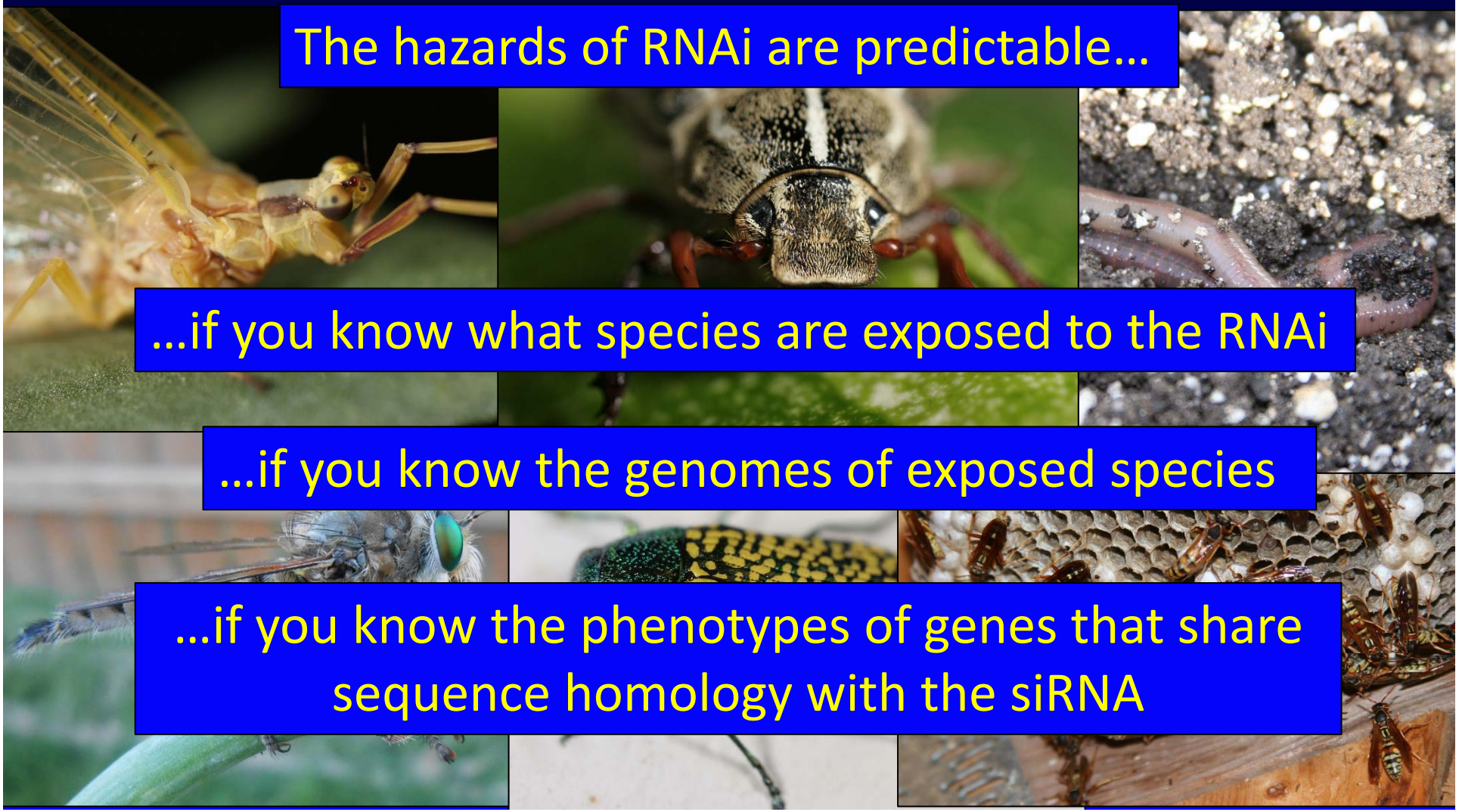
Dubelman et al. 2014. PLoS ONE 9(3): e93155

Are some forms of small RNAs more persistent?

Are small RNAs taken up by other organisms (plants, microbes)?

Hunter et al. 2010. Southwestern Entomologist 37(1): 85-87

Activity Spectrum



The hazards of RNAi are predictable…

…if you know what species are exposed to the RNAi

…if you know the genomes of exposed species

…if you know the phenotypes of genes that share sequence homology with the siRNA

Bioinformatics as a Risk Assessment Tool

Bioinformatics is an important tool for narrowing down the spectrum of activity

Additional tools are necessary to complete a risk assessment

RNAi vs Bt-based GM Plants

Similarities

- A similar suite of exposed species
- Similar initial target species
- Both are systemic and constitutive

Differences

- Potential activity spectrum is greater for RNAi
- Different assays may be necessary to measure sublethal effects of RNAi.
- Range of targets is greater for RNAi.

Conclusions

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

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

We are asking the right questions, and at the right time.

Acknowledgements

efsa
European Food Safety Authority

NIFA

USDA ARS

Dr. Chrissy Mogren, USDA-ARS

Dr. Jian Duan, USDA-ARS

Dr. Kelton Welch, USDA-ARS