New approaches for rapid response to plant disease threats

Anna Whitfield, Marcé Lorenzen, Dorith Rotenberg, Peg Redinbaugh, Guo-Liang Wang, Lucy Stewart, Astri Wayadande, Peter Balint-Kurti, Max Scott, Tim Sit, Surapathrudu Kanakala, Cesar Augusto Diniz Xavier, Ordom Huot, Kathleen Martin, Kirsten Lahre, Colin Murphree, Hong Hanh Tran, Anandrao Patil, William Klobasa, and Nathaniel Grubbs

The Ohio State University

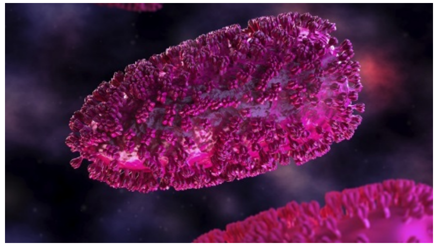
NC State University

Oklahoma State University

USDA ARS

Insect Allies: How the Enemies of Corn May Someday Save It

Plant-infecting virus



Herbivorous insect vector

Maize plant

- Virus engineered to deliver plant-protective traits
- Virus delivery by insect vectors genetically modified to transmit and die
- Protection of mature plants from drought, pathogens, and/or pests

Team Maize Hopper

Virus Team

Lucy Stewart, USDA-ARS

Tim Sit, NCSU

Anna Whitfield, NCSU
Peg Redinbaugh, OSU

Vector Team

Anna Whitfield, NCSU

Dorith Rotenberg, NCSU

Astri Waydande, OSU

Marcé Lorenzen, NCSU

Max Scott, NCSU

Lucy Stewart, USDA-ARS

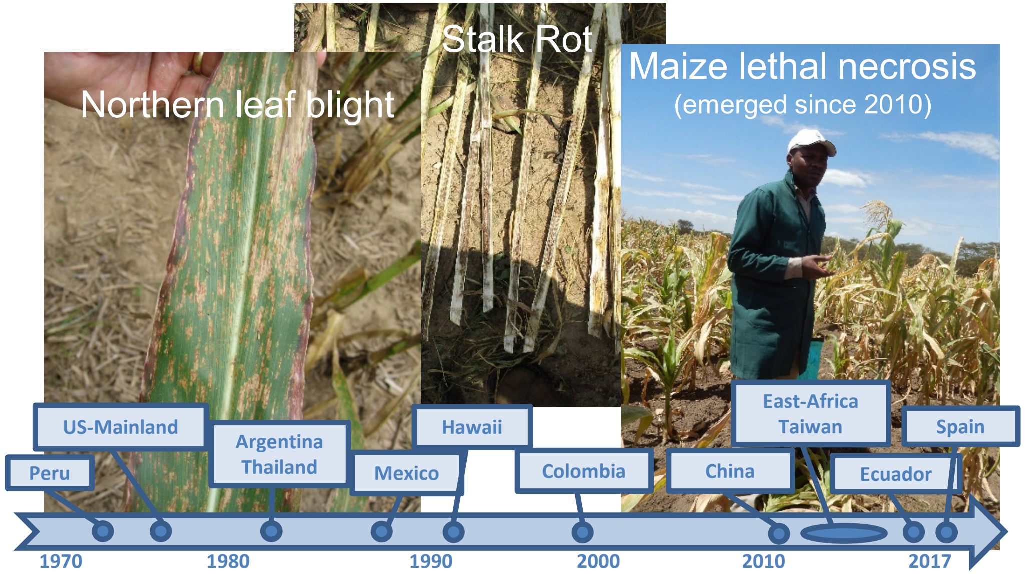
Plant Team

Peg Redinbaugh, OSU
Guo-liang Wang, OSU

Peter Balint-Kurti, USDA-ARS

The U.S. corn crop

- Maize, wheat and rice are the most important staple crops worldwide.
- The U.S. is the largest maize (corn) producer, accounting for 40% of worldwide production.
- **>90 million acres** of corn are planted and **>15 billion bushels worth >$50 billion** are harvested per year.
- About 14% of the crop ($7 billion) is exported.
- **Drought, floods, pests and diseases threaten the crop and food security.**



Team Maize Hopper: The Big Picture

1. Identify and develop virus systems allowing stable expression of multiple heterologous sequences in maize.

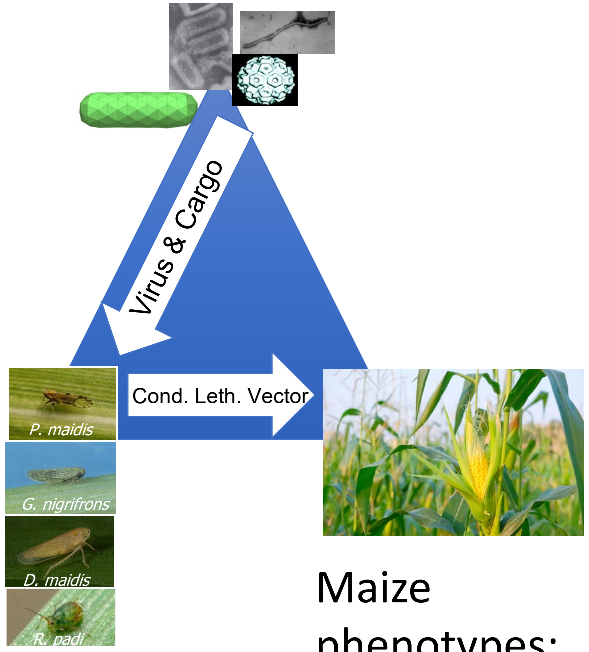
2. Develop systems for specific, controlled and efficient insect delivery of the modified viruses to maize.

3. Limit the spread of modified viruses.

4. Modify maize resistance phenotypes at relevant developmental stages.

5. All work performed under APHIS permit and designed to prevent off-target effects and release of organisms.

Nine viruses, four prioritized



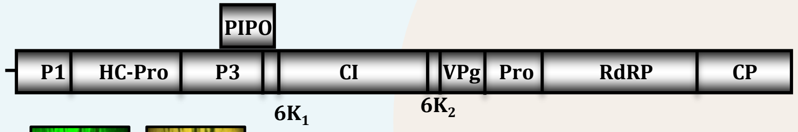
Seven insect vectors, narrowed to three

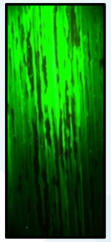
Maize phenotypes: Disease and drought resistance

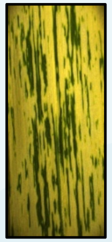
Top Virus-Vector Systems

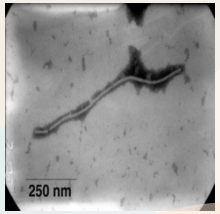
System 1: Potyvirus/Aphid/Maize

MDMV-*R. padi*



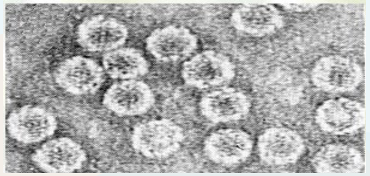




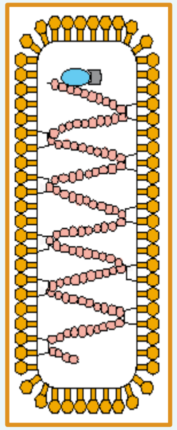


System 2: Marafivirus/Leafhopper/Maize

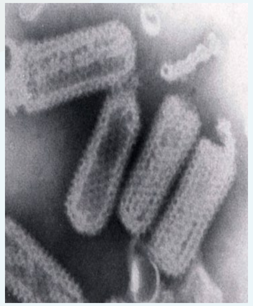
MRFV-*G. nigrifrons*



MMV-*P. maidis*

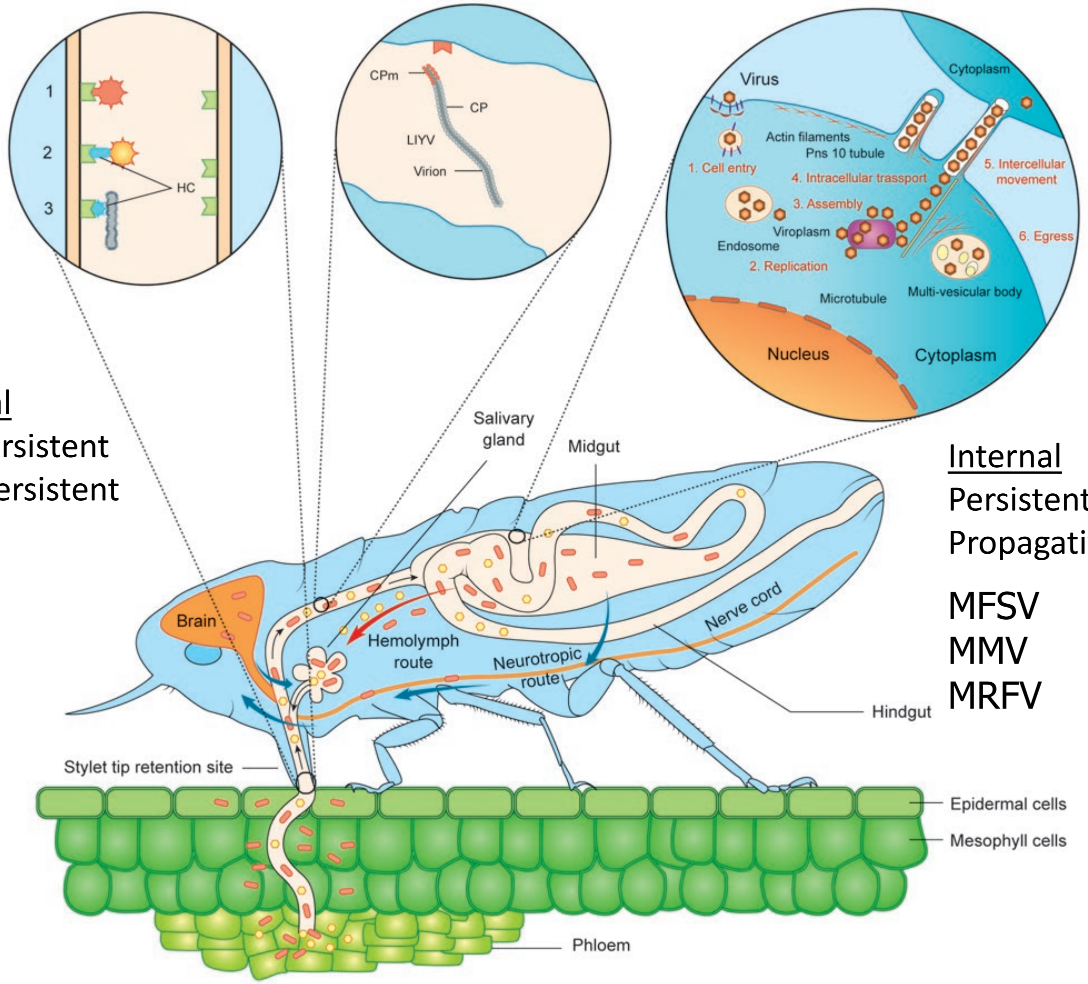


MFSV-*G. nigrifrons*



System 3: Rhabdovirus/Hopper/Maize

Viruses with diverse transmission strategies were chosen



<u>External</u>
Non-persistent
Semi-persistent

MDMV

<u>Internal</u>
Persistent/circulative
Propagative

MFSV
MMV
MRFV

Whitfield, Falk, & Rotenberg, Virology, 2015

Tiered phenotype manipulation approach

- Virus-induced gene silencing (VIGS) sequences (50-300 nt, smallest insertions required)

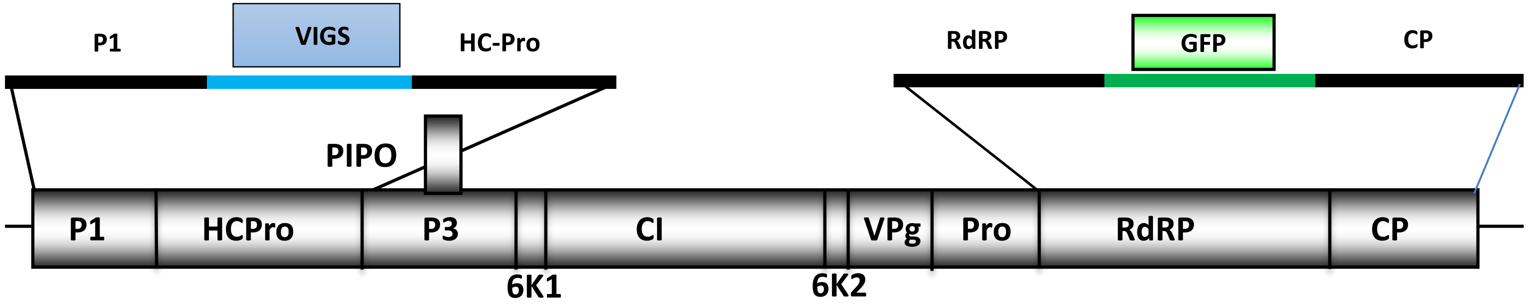
- Gene & multigene expression (500nt – 9+ kb full gene cassettes required)

- Gene editing (CRISPR/Cas9 genes and guide RNA required)

Building a virus that modifies multiple plant traits: Maize dwarf mosaic virus (MDMV)

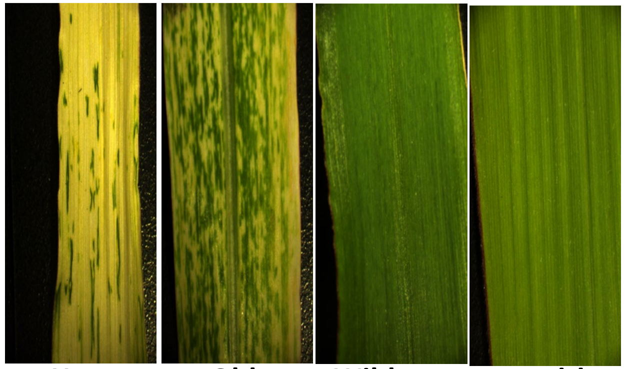
MDMV-OH-VIGS-GFP (pWX56)

Lucy Stewart Lab
USDA-ARS



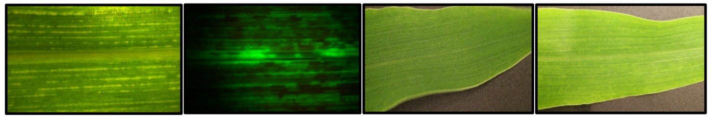
- *Maize dwarf mosaic virus* (MDMV) is a potyvirus, non-persistently transmitted by cereal aphids (short acquisition and inoculation periods)
- Engineered MDMV-OH to report GFP and to carry three maize-expressing traits (triple virus-induced gene silencing = VIGS)
  - VIGS: Three partial fragments of maize (*Zea mays*) genes inserted in-frame with viral polyprotein
    - magnesium chelatase subunit I precursor (ZmChlI, chlorophyll biosynthesis),
    - lemon white1 (ZmlspH, isopentenyl diphosphate biosynthesis), and
    - phytoene desaturase (ZmPDS, essential plant carotenoid biosynthetic enzyme)
- Confirmed expected traits with rub-inoculated virus (VIGS phenotypes, and GFP reporter expression in maize)

VIGS-photobleaching phenotype in maize leaves expressed by MDMV-OH-VIGS-GFP (pWX56) infection



Young leaf | **Old leaf** | **Wild-type MDMV** | **Healthy**

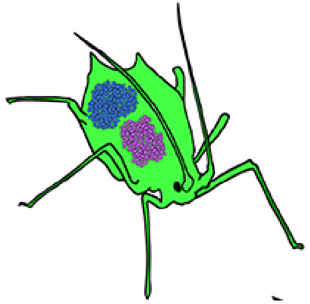
GFP reporting by MDMV-OH-VIGS-GFP (pWX56) expression in maize leaves (fluorescence)



pWX56, bright field | **pWX56, fluorescence** | **Healthy** | **MDMV WT**

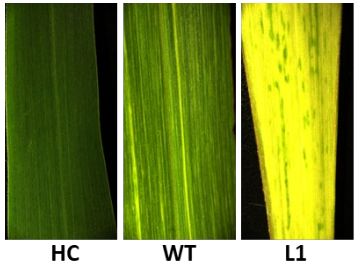
Insect transmission of engineered virus to multiple plants

Dorith Rotenberg
Kirsten Lahre, Cesar A. D. Xavier, Ithai A. Lopez, Anandrao Patil, NCSU



Clear aphids of obligate endosymbiont

Aphids transmit recombinant virus during initial probes on plants



Virus infects plant and alters phenotype

- Aphid vectors were treated with antibiotic to clear endosymbionts
  - Cleared aphids can reach plants to inoculate the virus before rapid decline
- Reduced fitness and rapid decline in endosymbiont-cleared aphid populations after delivery of recombinant virus to maize
- Multi-trait-expressing virus dispersed to and infected <u>**62.5% of maize plants**</u> within cages by endosymbiont-cleared (rifampicin) aphids. [45% plants infected for water-treated aphids]
- Multi-trait expression = VIGS mediated by MDMV-OH-VIGS-GFP **significantly and consistently reduced** the transcript levels of **three** maize genes

Top Virus-Vector Systems

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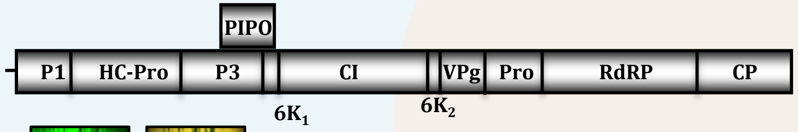
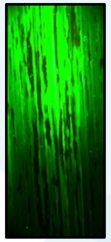
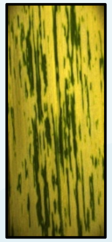
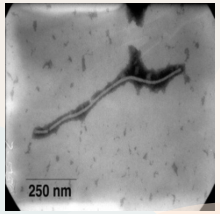
System 2: Marafivirus/Leafhopper/Maize

MDMV-*R. padi*



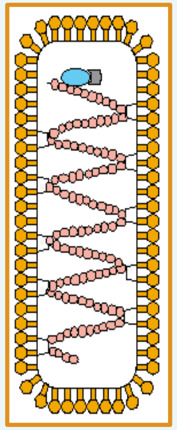




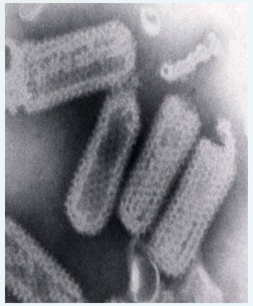


MRFV-*G. nigrifrons*

MMV-*P. maidis*



MFSV-*G. nigrifrons*



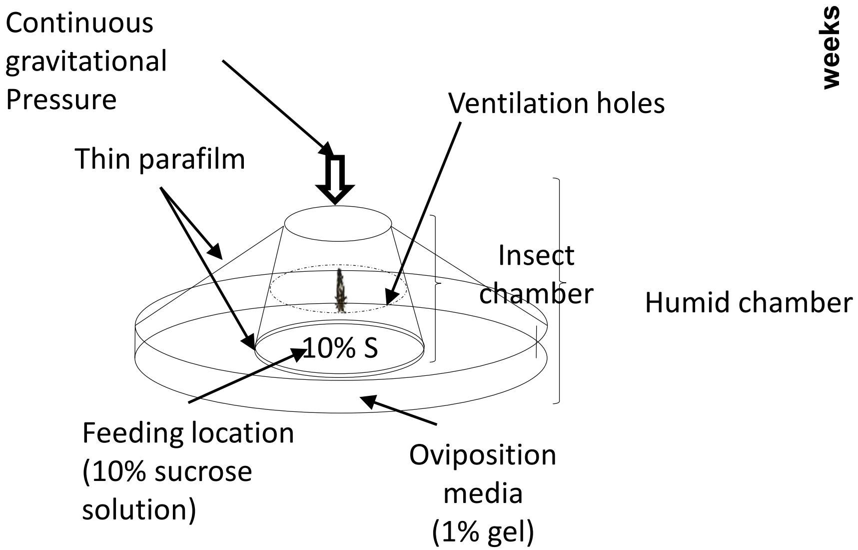
System 3: Rhabdovirus/Hopper/Maize

Peregrinus maidis is an efficient vector of maize mosaic virus (MMV) and tractable system for generation of transgenic insects

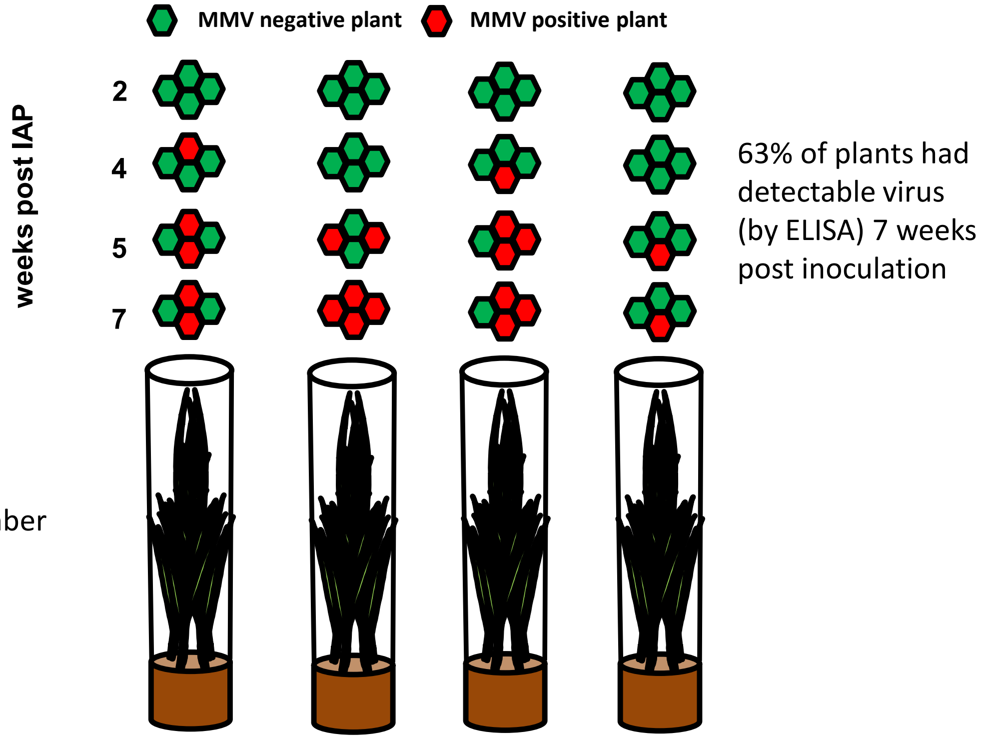
Whitfield and Lorenzen lab groups, NCSU

- *P. maidis* is a pest of corn and transmits MMV
- Methods developed for insect oviposition in agar (facilitates egg harvest for transgenic work)
- Transcriptomes generated for all stages embryo-adult

<u>Egg production chamber</u>

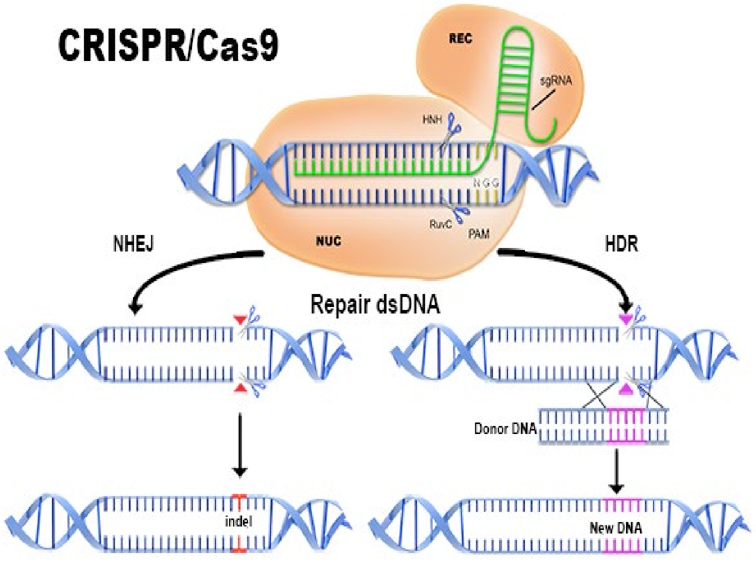


MMV detection in corn at varying times post inoculation access period (IAP)



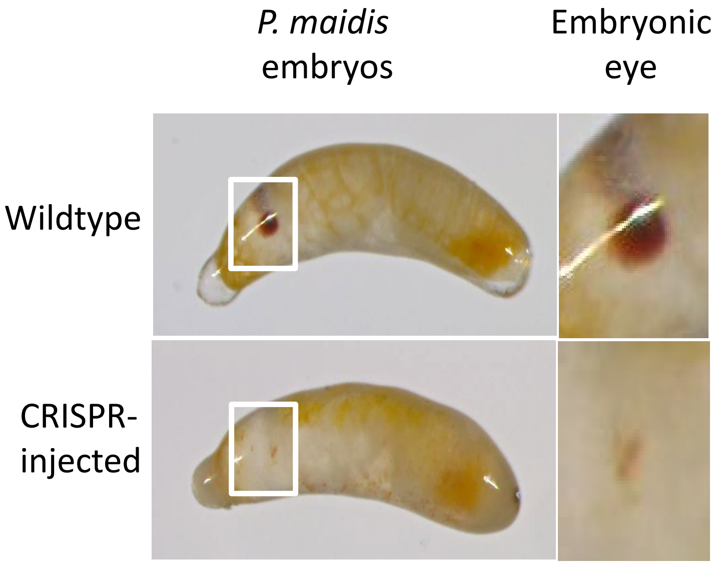
Progress towards genetic modification of vector insects

Marcé Lorenzen lab group, NCSU



CRISPR/Cas9 pathways. https://www.quora.com/How-does-CRISPR-Cas9-work

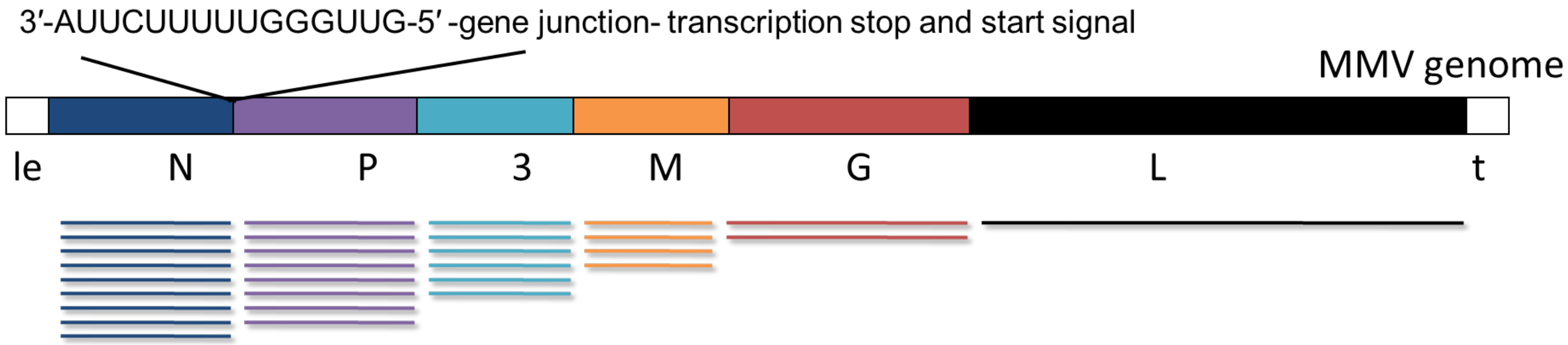
- Cas9 protein complexed with guide RNAs targeting the *Peregrinus maidis white* gene were injected into precellular *P. maidis* embryos
- Expected result: insertion and/or deletions in *white* eye-color gene and change in eye color

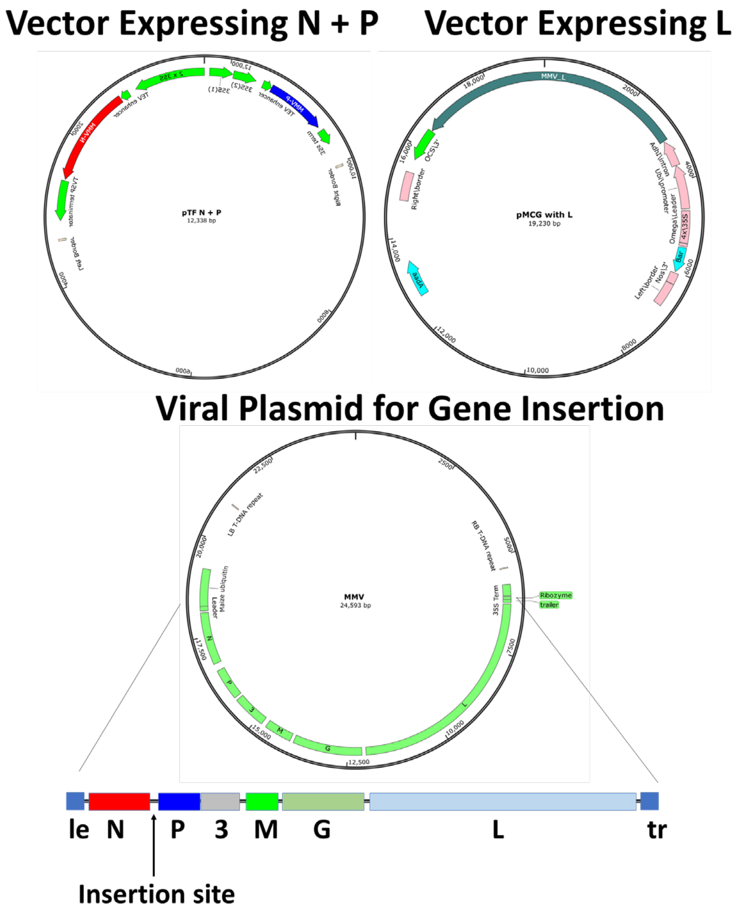


Embryonic eye-spot phenotypes.

Rhabdoviruses for delivery of large cargo

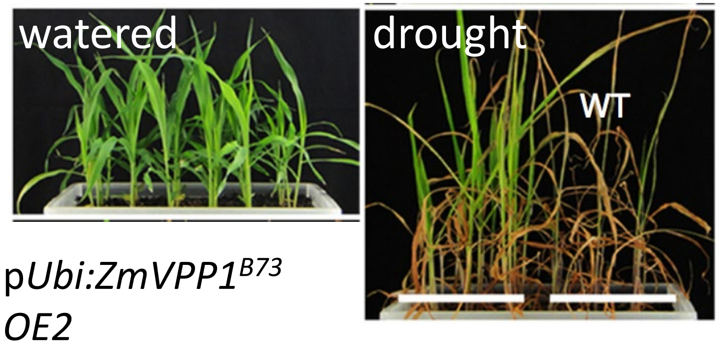
Surapathrudu Kanakala, Cesar Augusto Diniz Xavier, Kate Martin





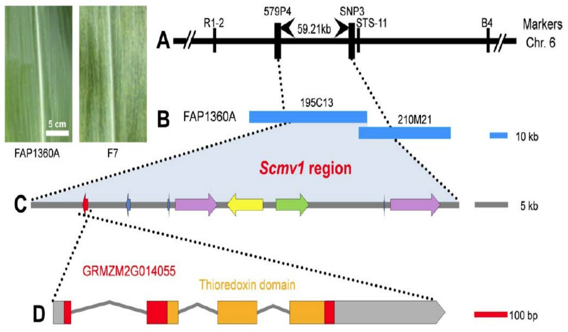
- Polar transcription of genes from the leader sequence (le) enables regulation of gene expression
- mRNAs have 5′ caps and poly A tails
- Particle shape (rod/bullet) allows addition of large genes (Cas9) and multiple genes (gRNAs)

Plant traits selected for crop protection



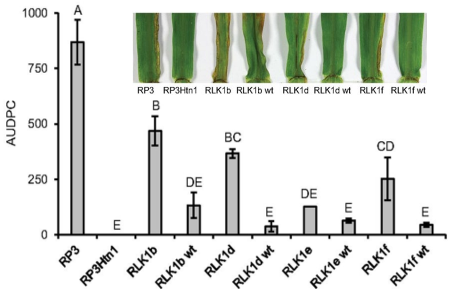
Drought tolerance *ZmVPP1*

WANG ET AL. 2016 Nat Genet 48: 1233-1241



Virus (SCMV) resistance *ZmTrxh*

LIU ET AL. 2017 MOL PLANT 10: 483-497



Fungal (SCLB) resistance *LHT1*

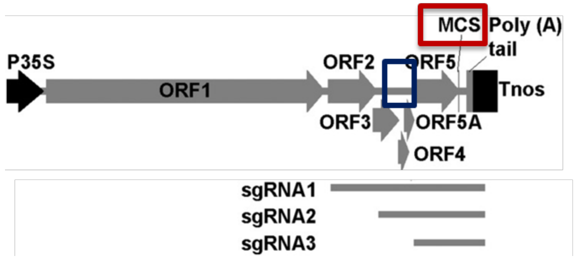
ZHAO ET AL. J INTEGR PLANT BIOL. 2012. 54: 321-329.

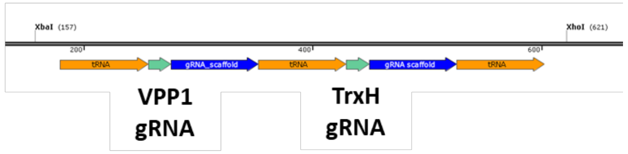
Modification using viral delivery of:

- dsRNA/siRNA
- Cas9 and guide RNAs
  - Editing/knock-in
  - Activation/repression

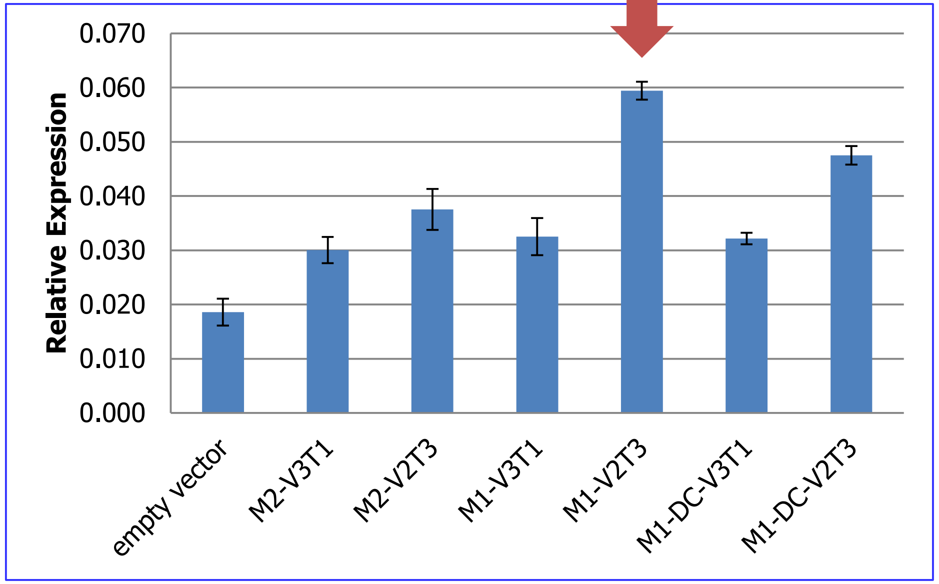
Gene activation/suppression with virus-delivered gRNAs

Guo-Liang Wang, Peg Redinbaugh, and Peter Balint-Kurti Teams

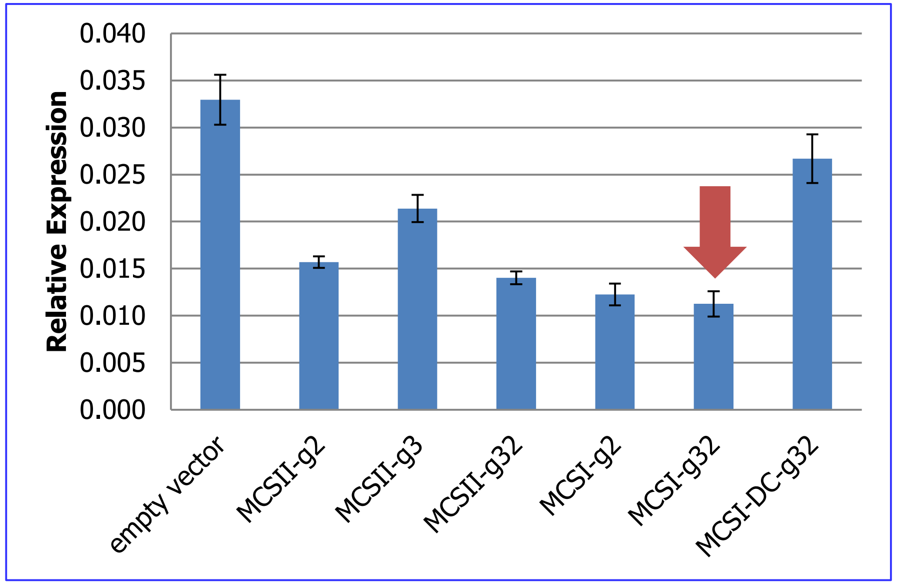




FoMV-based constructs* for gRNA expression testing: activation and suppression of *VPP1* and *PDS1*



VPP1 activation in B73 protoplasts co-transfected with dCas9-TV and FoMV-gRNA constructs

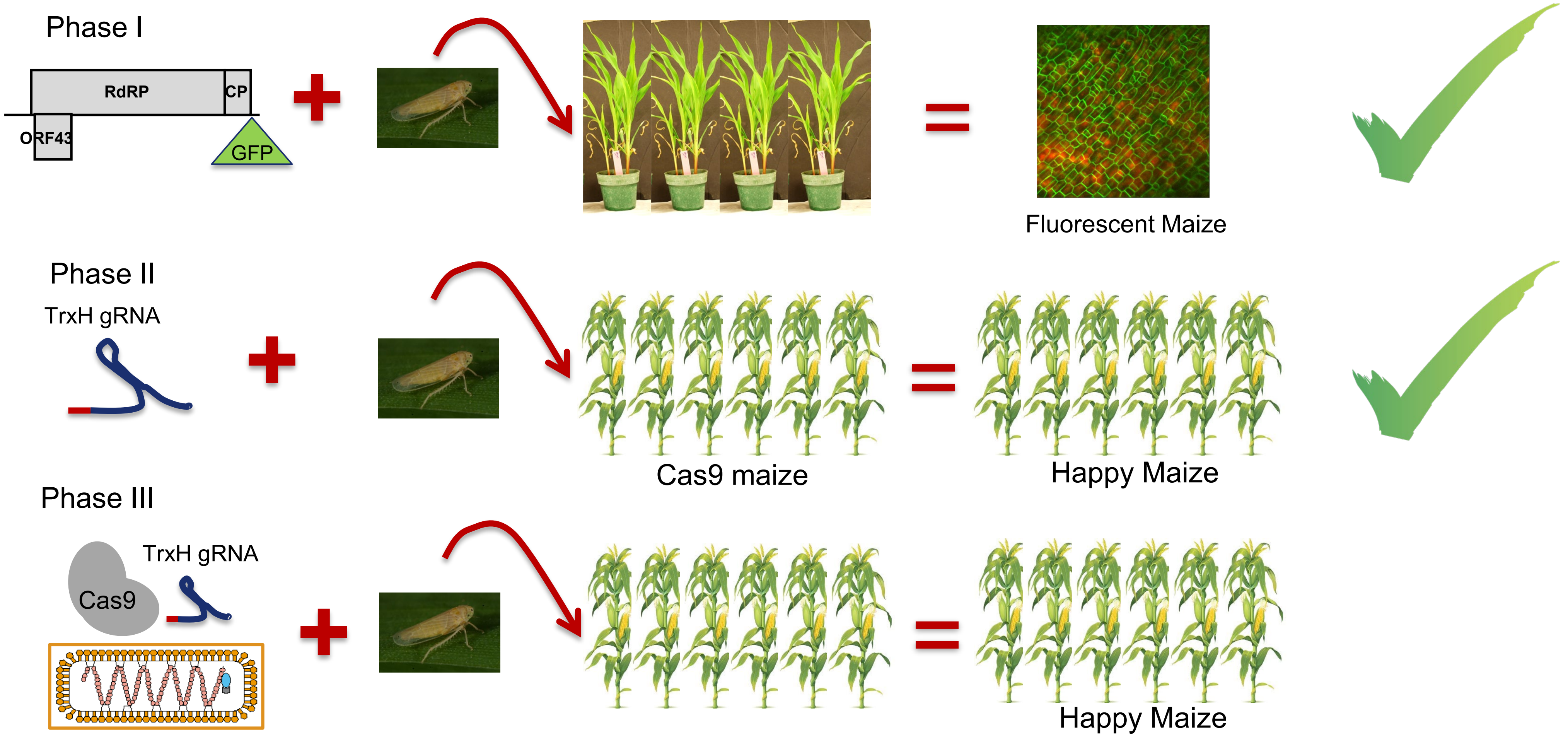


PDS1 suppression in B73 protoplasts co-transfected with dCas9-SDRX and FoMV-gRNA constructs

Potential to change expression of two traits using single gRNAs for each gene cloned into a virus delivery system (FoMV)

***Steve Whitham @ISU**

Putting It All Together



Viruses as allies in the fight against plant diseases

Citrus greening

- Recombinant citrus tristeza virus developed to carry foreign genes
- Expresses spinach defensin gene that confers resistance to greening
- Graft transmitted to plants

Emerging virus diseases

- Maize lethal necrosis
- Tomato brown rugose fruit virus

Southern Gardens Citrus Nursery, LLC Permit 17-044-101r to Release Genetically Engineered *Citrus tristeza virus*
Preliminary Pest Risk Assessment
https://www.aphis.usda.gov/brs/aphisdocs/17_044_101r_CTV_ppra.pdf

TEAM MAIZE HOPPER

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