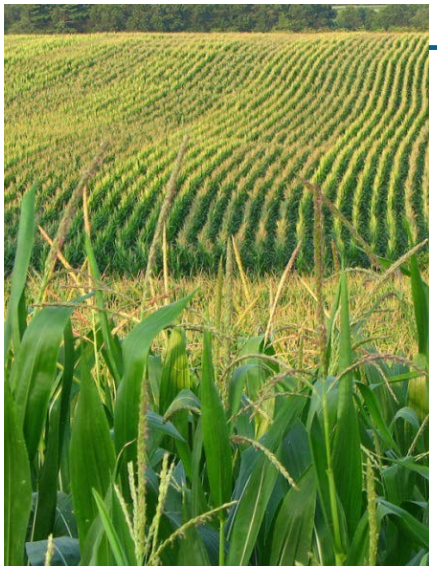
Georg Jander, Boyce Thompson Institute for Plant Research, Team VIPER

Funded



Project Overview: Viruses and Insects as Plant Enhancement Resources

- Annual maize crop in the United States = *90 million acres*, *14 billion bushels*, *$50 billion*
- Productivity is limited by cold, drought, pests, pathogens, and other environmental challenges.

DARPA Insect Allies technical areas

1. develop a plant virus that delivers a transgene to a target plant species
2. produce insect vectors for the modified virus to be transmitted to mature plants
3. introduce functional traits to mature plants

Technical approaches to implement

- Virus-induced gene silencing in whole maize plants
- Transient gene overexpression in maize plants
- Genome manipulation in mature maize
- Identification of suitable maize aphid vectors

Challenges to overcome

- Uniform gene silencing in all maize tissues
- Limited gene capacity of the virus vectors
- Engineering of maize DNA viruses
- Transmission without vector spread

Teaming Overview and Objectives

Dan Voytas, University of Minnesota
Site-directed mutagenesis in mature maize

S.P. Dinesh-Kumar, U.C. Davis
Virus-induced gene silencing in maize

Steve Whitham, Iowa State University
Virus constructs for maize gene expression

Georg Jander, Boyce Thompson Institute
Optimization of maize aphid vectors

Impact

Anticipated Impact

Manipulation of gene expression in mature maize plants will allow rapid response to environmental threats.

Potential Applications

Introduction of new pathogen resistance genes
Expression of toxic proteins for pest control
Targeted modification of endogenous gene expression

Milestones

Phase 1: development of a efficient maize virus vector
Phase 2: engineering of maize aphids for virus transmission
Phase 3: targeted trait introduction into mature maize plants

Transition of the Technology

Publication of methods in academic journals
Viruses and vectors made available for further research
Contact with established industry partners

Contact Information – gj32@cornell.edu – (607) 254-1365

Rhabdo-Graminella-Maize (RGM) Team

Lucy Stewart, Ohio State Univ./USDA-ARS et al.

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Project Overview

Goal–Develop genome editing systems for maize based expression of CRISPR-Cas from RNA virus vectors.

Advantages – infectious clone, larger inserts, non-persistent transmission by multiple species
Challenges – insert stability, resistance

Advantages –recombination suppressed, large inserts, persistent transmission, specific vector
Challenges – infectious clone

Teaming Overview and Objectives

Team Members:

- Lucy Stewart, USDA, ARS/Ohio State Univ
- Anna Whitfield, Kansas State Univ/NCSU
- Feng Qu, Ohio State Univ
- Peg Redinbaugh, USDA, ARS/Ohio State Univ

Strengths

- Scientifically strong individuals; successful at team research; previous collaborations.
- Experience working with viruses, vectors and hosts; virus transmission to maize; virus-based vectors. MDMV clone.
- BSL2P, BSL3P, insect rearing and plant transformation facilities; most virus and vector permits; IBC protocols in place.

Additional expertise needed:

- TA1: Developing and infecting leafhopper cell cultures.
- TA2: Target gene selection.
- TA3: Containment for large scale experiments.

Impact and applications

- Development of maize virus vectors for expressing and silencing genes; CRISPR/Cas system for maize.
- In field phenotype manipulation; plant, virus, and insect gene function studies; virus-vector-maize interaction studies.

Metrics

TA1: alter maize phenotype by heterologous expression of bacterial gene(s); infectious rhabdovirus clone.

TA2: Vector transmission of virus expressing bacterial gene, Cas9 and guide RNAs.

TA3: Large-scale gene editing in maize.

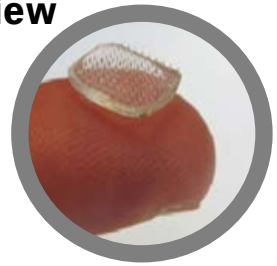
Peg Redinbaugh – peg.redinbaugh@ars.usda.gov - 330.263.3965

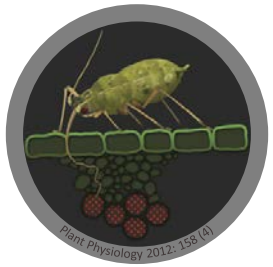
Universal Delivery System – M.stylet

Project Overview

The development and fabrication of insect-inspired universal delivery system of various biological agents to a wide range of crop species based on MEMS and newest CRISPR gene editing technology.

Rapid mature plant transformation (“Plant”) and development of species-specific insect vectors





Poplar
100% CRISPR editing

Teaming Overview and Objectives

Milad Navaei, **Gary McMurray** Research Faculty, Georgia Tech Research Institute
Chung-Jui Tsai Professor of Genetic, University of Georgia
Rajagopalbabu Srinivasan Associate Professor of Entomology, University of Georgia

The newest CRISPR genome editing technology was demonstrated to a tree species by Tsai lab at UGA for the first time. This was shown by mutating a lignin pathway gene in reddish wood by100% editing efficiency. Demonstration of patented patch microneedle system for delivering antimicrobial

Impact

Delivery of complex biological agents such as DNA , RNA (including viral vector), protein and chemical compounds agents to a wide range of crop species.

Spatiotemporal monitoring of the gene effect, specificity and stability.

Potential applications (1) native gene silencing or mutation, (2) introduction of foreign genes, (3) altering spatiotemporal expression of native genes, (4) delivery of chemicals, such as hormones, herbicides, antibiotics, or some other signaling compounds.

UNIVERSITY OF GEORGIA

Milad Navaei– milad.navaei@gtri.gatech.edu – (404) 407-6206

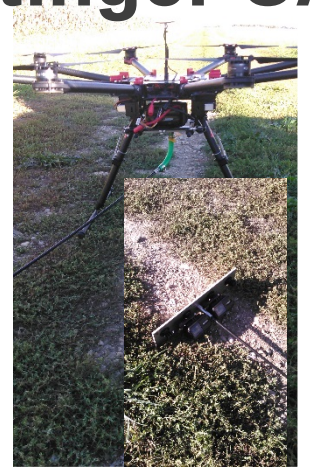
Georgia Tech Research Institute

Amul D. Tevar, Ag. Engineering Team, Ohio State Univ.

Project Overview

- Robotic video and non-destructive, in-ground sensor for plant canopy and root imaging for in field characterization and verification
- Technical Areas: In field verification of #1 (Trait Design) and #3 (Selective Gene Therapy in Mature Plants)
- Limits: Resolution vs. $/Acre

Stinger UAV



3D Point Cloud

Teaming Overview and Objectives

- Team: OSU Ag. Engineering & Mech. Engineering, Electroscience Lab
- Experience: In-field deployment and design of sensor and analytics suites
- Institutional assets: Stinger UAV, Trailer with 3D imaging, Below ground sensor system, Cloud-based computation
- Collaborator Areas: #1 (Trait Design) and #3 (Selective Gene Therapy in Mature Plants)

Impact

- Impact: Rapid, full phenotype verification (canopy to roots) through growing season
- Applications Enabled: Early, in-field determination of different cultivars/insect vectors/gene therapies
- Metrics: <$15/acre testing, mid-season trait verification/validation
- Transition: Collaboration and IP transfer to start-up or large agriculture partner through licensing

Wayne R. Curtis, The Pennsylvania State University, Penn State / PNNL

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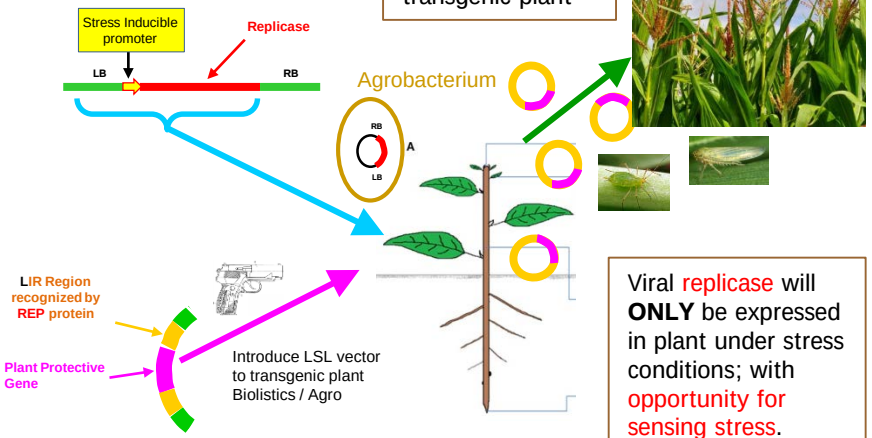
Crop Protection using non-Replicating Viral Vectors with Complementary Transgenic Plants

APPROACH:

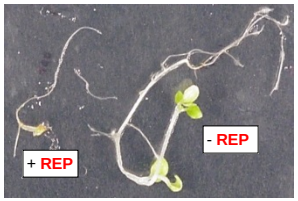
Sub-genomic virus can 'only' replicate in its complementary transgenic plant

Stable Plant Transformation

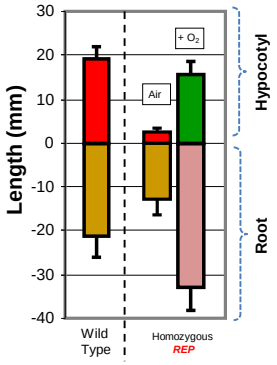


Viral replicase will **ONLY** be expressed in plant under stress conditions; with opportunity for sensing stress.

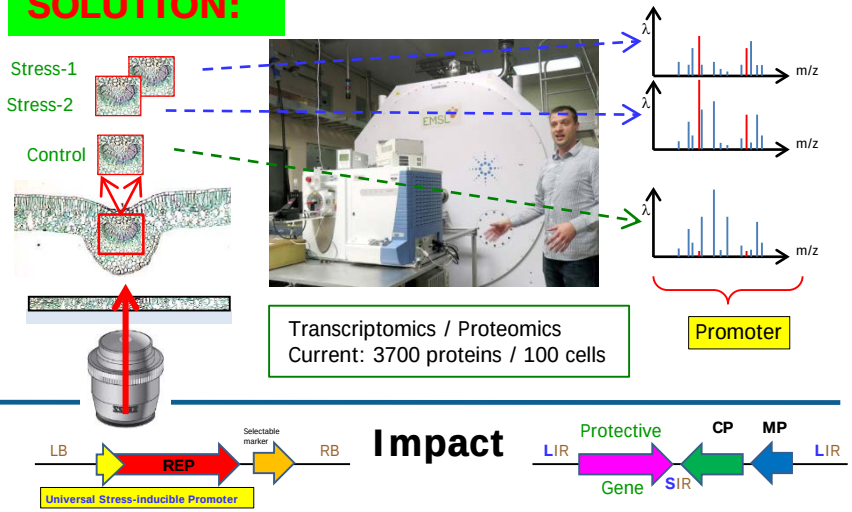
KEY BOTTLENECK:



Replicase prevents hypocotyl cell expansion (and other developmental abnormalities)



SOLUTION:



Transcriptomics / Proteomics
Current: 3700 proteins / 100 cells

Impact

- Generalizable technology to deliver genes to cultivated plants.
- The goal is proof-of-principle for delivery of heterologous gene; with an appropriate teaming partner, we would hope to demonstrate plant protection in greenhouse implementation.
- Translation as a stackable trait component of integrated pest management via traditional seed / agricultural chemicals companies.

Teaming Overview and Objectives

PI: Wayne Curtis, Professor of Chemical Engineering, Penn State University
Co-PI: Ryan Kelly, Sr. Research Scientist & Capability Lead, EMSL/PNNL.

Agrobacterium-mediated viral vector-amplified transient gene expression in Nicotiana glutinosa plant tissue culture. DOI: 10.1021/bp060342u

RNA viral vectors for improved *Agrobacterium*-mediated transient expression of heterologous proteins in *Nicotiana benthamiana* cell suspensions and hairy roots. DOI: 10.1186/1472-6750-12-21

Enhancing bottom-up and top-down proteomic measurements with ion mobility separations; DOI: 10.1002/pmic.201500048

- PSU: > 50,000 sq.ft. greenhouse; BL3 facility. DOE EMSL/PNNL > 150 analytical / OMICS capability.
- Seeking teaming in viral entomology, crop stress physiology

Contact Information – wrc2@psu.edu – cell: 814.360.3069

PI: W. Allen Miller, Iowa State University
Team (so far): Bryce Falk, UC Davis

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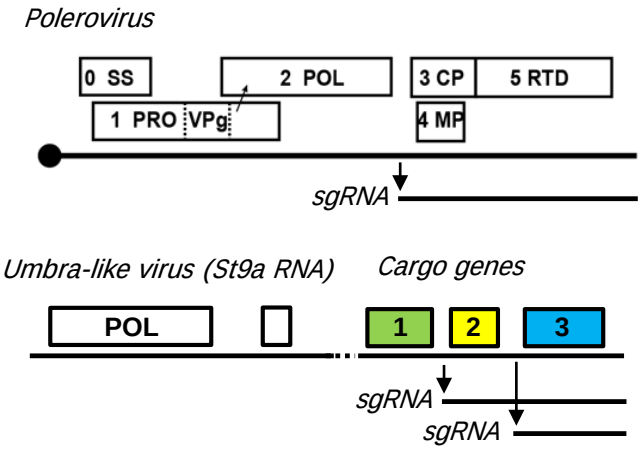
Project Overview

- Engineer RNA replicons that partner with poleroviruses to replicate and express multiple genes in plants. These RNAs are naturally encapsidated in polerovirus virions, and thus are transmitted by aphids.
- Insect Allies Technical Area **TA1:** Engineer plant virus. Hurdles: Identify sequences required for: RNA replication, subgenomic mRNA synthesis and translation, packaging in polerovirus virion. These should be straightforward, and in our area of expertise.
- **TA2:** Viral delivery by insect vector (aphid): Engineered RNA transmission by aphid relies on unaltered, natural polerovirus as helper. Hurdle: Ways of controlling aphid in the field (seeking collaborator).



Teaming Overview and Objectives

- Existing team members and partners:
  - W. Allen Miller
  - Bryce Falk
- Combined >60 years experience in engineering diverse insect-transmitted plant viruses:
  - RNA replication and subgenomic mRNA synthesis
  - Translation enhancing mechanisms
  - Virus-aphid vector interactions
  - Satellite RNAs
- Institutional assets: UC Davis has high-containment greenhouse.
- For which Technical Areas are you seeking collaborators?
  - Aphid biology, Plant genes

Impact

- What is the anticipated impact of the team's success (in terms of technique AND capability)?
  - New and more efficient ways to rapidly express cargo genes in plants without altering plant genome.
- Potential applications enabled by this technology.
  - Any technology that requires rapid expression of foreign genes without plant breeding.
- What metrics and milestones will the team aim to achieve?
  - Those outlined in the DARPA proposal.
- How will the team pursue transition of this technology?
  - University resources to interact with industry.
  - e.g. NSF IUCRC CamTech at ISU.

wamiller@iastate.edu, bwfalk@ucdavis.edu

John O'Neil Oak Ridge National Laboratory Soybean Defense

(for Xiaohan Yang)

Project Overview

Soybean is one of the major crops in the U.S. and world, with a production value of ~$40 billion in USA in 2014. Soybean is a major source of protein for humans. It is also one of the largest sources of vegetable oil and of animal protein feed in the world. Severe disease epidemics are a major threat to soybean production worldwide.

Our team seeks to develop a synthetic tri-partite antimicrobial system for improving biotic stress resistance in soybean crops. We intend to pursue the following three Technical Area(s): TA1: Engineered plant virus ("Virus"); TA2: Viral delivery by insect vector ("Insect"); and TA3: Rapid mature plant transformation ("Plant"). Specifically, we will engineer novel genetic circuitry in a virus and deliver it to soybean plants by insect for synthesis of antimicrobial molecules.

One technical challenge is to develop conditional lethal safeguard for insect.

Teaming Overview and Objectives

- Dr. Xiaohan Yang is a staff scientist, with experience in plant genomics, plant-microbe interaction, and synthetic biology. The Yang lab is well equipped for gene construct design and construction, plant transformation, gene stacking, and plant phenotyping in greenhouse. His major contribution will be to TA3.
- Dr. Gerald A. Tuskan is a Corporate Fellow at ORNL. He focuses on the genetics of renewable feedstock for bioenergy. His major contribution will be to TA3.
- Dr. Tessa Burch-Smith is an Assistant Professor at the University of Tennessee, Knoxville. She has a strong background in plant viruses and extensive experience with virus-induced gene silencing. Her lab is well equipped for plant virology and is currently permitted as a BSL2 lab. Her major contribution will be to TA1.
- We are seeking collaborators in TA2: Viral delivery by insect vector ("Insect").

Impact

- The success of our research will create a novel synthetic plant–virus–vector system as an alternative strategy to defend soybean crops against multiple biotic stresses.
- It will benefit soybean growers worldwide.
- The milestones to be achieved include construction of genetic circuits, genetically modified viruses compatible with plant host, improvement of insect vector for efficient viral delivery.
- The new technology will be transferred to agricultural industry through the institution's licensing program.

Contact: Xiaohan Yang – yangx@ornl.gov – 865-241-6895