VIPER: Viruses and Insects as Plant Enhancement Resources

Dan Voytas, University of Minnesota • S.P. Dinesh-Kumar, University of California, Davis
Steve Whitham, Iowa State University • Georg Jander, Boyce Thompson Institute for Plant Research

Project Overview

- Annual maize crop in the United States = *90 million acres*; *14 billion bushels*; *$50 billion*
- Maize productivity is limited by cold, drought, pests, pathogens, and other environmental challenges.
- Aphid-vectored viruses will be designed to rapidly alter gene expression in mature maize plants.
- Three DARPA Insect Allies technical areas will be addressed: (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, and (3) introduce functional traits to mature plants.

Technical approaches that will be implemented

- Virus-induced gene silencing will be developed for whole maize plants.
- Virus vectors will be designed for transient gene overexpression in maize plants.
- The genome of mature maize plants will be manipulated by site-directed mutagenesis.
- Suitable maize aphid vectors for virus transmission will be identified or engineered.

Specific challenges that will be addressed

- Uniform gene silencing in all maize tissues will be required.
- Currently available virus vectors have limited gene capacity for overexpression.
- Engineering of maize DNA viruses will be required for site-directed mutagenesis.
- Insect vectors need to be designed for efficient virus transmission without vector spread.

Teaming Overview and Objectives

Existing team members and their relevant experience

Dan Voytas, College of Biological Sciences, University of Minnesota
Expertise in site directed mutagenesis and genome editing
Carroll D, Van Eenennaam AL, Taylor JF, Seger J, and Voytas DF (2016) *Nature Biotechnology* 34:477-479.
Tang X, Zheng X, Qi Y, Zhang D, Cheng Y, *et al.* (2016) *Molecular Plant* 9:1088-1091.

S.P. Dinesh-Kumar, Department of Plant Biology, University of California Davis
A leader in vector design and virus-induced gene silencing
Hayward A, Padmanabhan M, and Dinesh-Kumar SP (2011) *Methods in Molecular Biology* 678:55-63.
Dong Y, Burch-Smith TM, Liu Y, Mamillapalli P, et al. (2007) Plant Physiology 145:1161-1170.

Steve Whitham, Department of Plant Pathology and Microbiology, Iowa State University
Prior experience cloning and modifying the genomes of maize-infecting viruses
Mei Y, Zhang C, Kernodle BM, Hill JH, and Whitham SA (2016) *Plant Physiology* 171:760-772.
Zhang C, Whitham SA, and Hill JH (2013) *Methods in Molecular Biology* 975:149-156.

Georg Jander, Boyce Thompson Institute for Plant Research
Investigates maize-aphid interactions and aphid gene expression silencing
Tzin V, Yang X, Jing X, Zhang K, Jander G, *et al.* (2015) *Journal of Insect Physiology* 79:105-112.
Meihls LN, Handrick V, Glauser G, Barbier H, Kaur H, *et al.* (2013) *The Plant Cell* 25:2341-2355.

Institutional assets

- Extensive experience with maize, viruses, and insect vectors
- Prior collaborative interactions among the project team members
- Plant tissue culture and transformation facility
- Field sites for growing maize
- Transgenic plant and insect containment facilities
- Established maize industry contacts

Impact

Anticipated Impact

- Completion of the proposed project will allow manipulation of gene expression in mature maize plants as a rapid response to new biotic and abiotic threats.
- Government, industry, and academic scientists will benefit from making use of the viral vectors that will be developed through this project.
- Methods that will be developed can be implemented not only in maize but also other crop species.

Potential Applications

- Introduction of new pathogen resistance genes
- Expression of toxic proteins for insect pest control
- Targeted modification of endogenous gene expression to increase abiotic stress tolerance
- Maize germline modification for lasting resistance

Milestones

- Phase 1: development of a virus that delivers genes and silencing constructs to whole maize plants
- Phase 2: engineering of maize aphid vectors for efficient and safe virus transmission
- Phase 3: targeted introduction of functional traits into mature maize plants

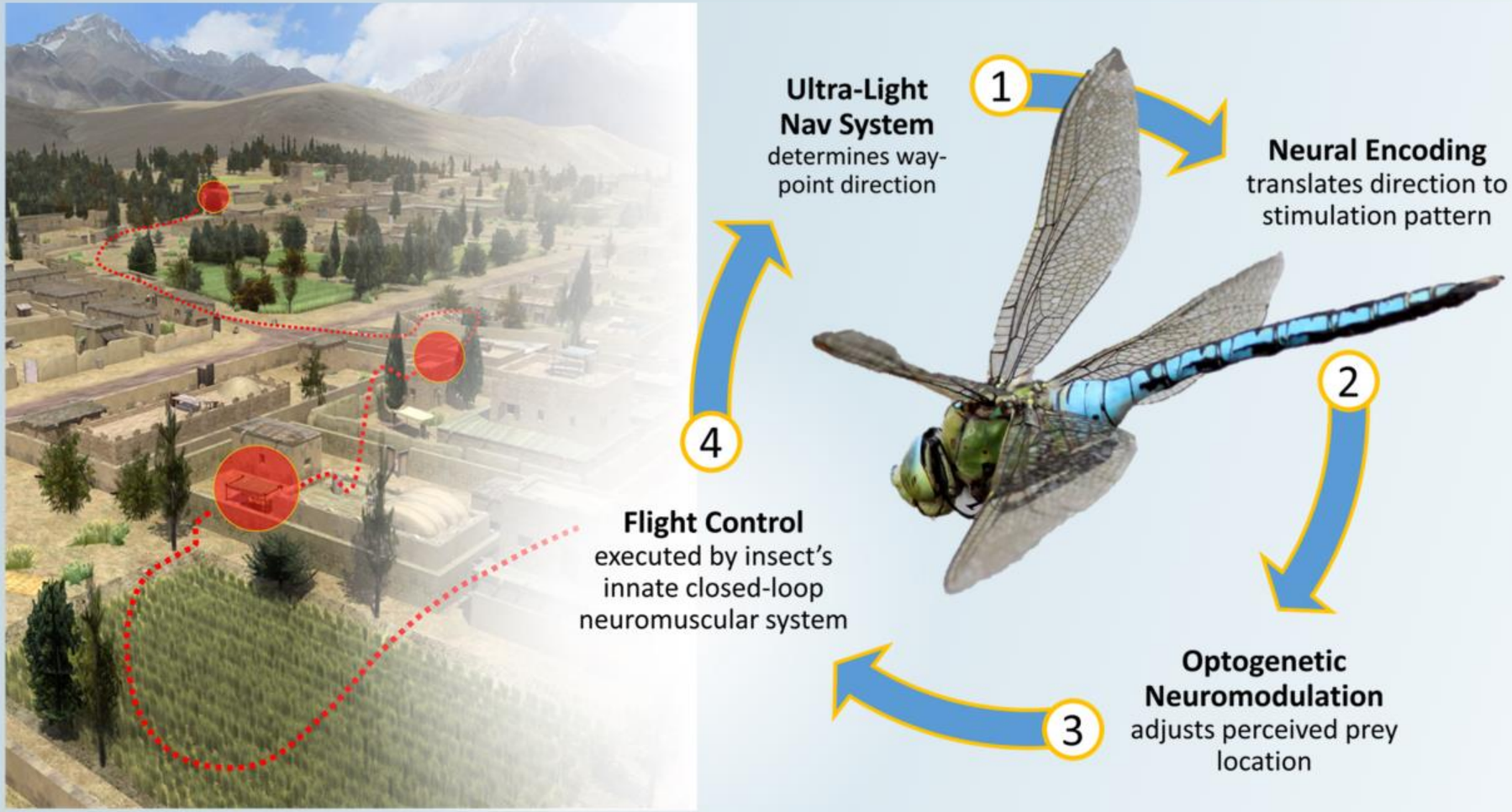
Transition of the Technology

- Methods will be published in academic journals.
- Virus and insect vectors will be made available for further research.
- Contact with established industry partners will allow implementation in the field.

Contact Information: gj32@cornell.edu – (607) 254-1385 – Boyce Thompson Institute for Plant Research, 533 Tower Road, Ithaca, NY 14853

DRAGONFLeye Steering Insect Flight with Optogenetic Neuromodulation

Jesse Wheeler, Andrew Magyar, Draper Laboratory



NEW FRONTIERS IN CO-DESIGN OF BIOLOGY, MATERIALS, AND ELECTRONICS

Teaming Overview and Objectives

- Expertise in MEMS fabrication, sensors, and synthetic biology

Impact

- Control insect flight, enabling directed navigation to targets of interest.

Cryptic Plant Virus Discovery / Insecticidal Agent Team

Sijun Liu and Bryony C. Bonning, Iowa State University

Project overview

- Identify novel, cryptic, aphid-transmitted viruses that do not cause significant pathology in the host plant.
  - Identification of appropriate plant viruses for use as delivery vehicles is Step 1 for the Insect Allies Program.
  - The use of viruses that are relatively benign to the host plant will be essential to achieve the full benefit of vector use.
- Provide bioactive agents for delivery by plant viral vectors to target insect pests.
- Insect Allies Technical Area TA1: Engineer plant virus. Technical challenge that must be overcome –
  1) identification of plant viruses that do not cause significant pathology in the host plant;
  2) identification of bioactives appropriate for suppression of insect pests

Teaming Overview and Objectives

Sijun Liu, Associate Scientist
Bryony Bonning, Professor

- Extensive expertise with
- discovery from insect and plant virus-derived sequences from deep sequencing datasets (Liu)
- insect viruses, and insect transmitted plant viruses (Liu, Bonning)
- diverse insecticidal agents for plant virus delivery (Bonning)
- We are offering our services for projects requiring plant virus discovery and / or identification of appropriate insecticidal bioactives for use in other proposed projects.

Key Publications

Liu S., Chen, Y. Bonning BC, 2015 RNA virus discovery in Insects Current Opinion in Insect Science 8:54-61
Bonning, BC, Pal, N. Liu, S, Wang, Z, Sivakumar, S, Dixon PM, King, GF, Miller WA 2014 Toxin delivery by the coat protein of an aphid-vectored plant virus provides plant resistance to aphids . Nature Biotechnology 32, 102-105.

Impact

- Anticipated impact: Provision of
  1) candidate novel, cryptic plant viruses for vector development;
  2) toxins suitable for virus delivery to target specific insect pests
- These two areas will enable development of plant virus delivery vehicles, and delivery of insecticidal molecules to target insect pests
- Both objectives (identification of candidate viruses and identification of insecticidal bioactives) would be achieved in the first year of the project.

Sijun Liu, sliu@iastate.edu 515-294-1815

Bryony C. Bonning, bbonning@iastate.edu 515-294-1989

BCHEPP – Thrips and Tospoviruses for Responsive Crop Protection

Jeffrey Dean, Angus Catchot, Jonas King, Shien Lu, Fred Musser, Sorina Popescu, and Sead Sabanadzovic
Dept. of Biochemistry, Molecular Biology, Entomology and Plant Pathology, Mississippi State University

Project Overview

- This project will demonstrate the feasibility of using tospoviruses, a broad host-range plant pathogen, to deliver protective transgenes to environmentally challenged crop species at any growth stage post emergence using sterile tobacco thrips as the insect vector.
- The project will address all three Technical Areas of the Insect Allies call for proposals. Technical challenges to overcome include:
  - Adapt available techniques for transgene delivery by bunyaviruses to tospoviruses and demonstrate systemic expression in infected plants
  - Create artificial laboratory diet and demonstrate uptake and persistent infection of larval thrips with engineered tospoviruses
  - Develop protocols to bulk up mass-rearing of tobacco thrips from existing laboratory colonies and demonstrate efficient sterilization protocols
  - Identify optimal tospovirus combinations for different crops and investigate means to constrain transgene expression to targeted crop species

Teaming Overview and Objectives

- Personnel & Expertise
  - Dean – Plant and Insect Biochemistry and Genomics
  - Catchot – Field Crop Entomology
  - King – Insect Molecular Genetics
  - Lu – Plant Bacteriology and Biocontrol
  - Musser – Crop Entomology and Insect Husbandry
  - Popescu – Plant Molecular Genetics
  - Sabanadzovic – Plant Virology
- Experience – Project PIs are all highly published experienced investigators whose combined expertise covers all project needs
- Assets – Insect husbandry facility; CRISPR/Cas9 experience; susceptibility gene collection; crop pathogen collection

Impact

- Successful outcomes from the proposed work will provide the means to modify crop susceptibility to pathogens *in situ* for corn, soybean and cotton. Thrips/tospovirus combinations available for all major crop species
- Potential applications include knock-out of susceptibility genes and knock-in of resistance genes
- The project will demonstrate systemic expression of small (e.g. CRISPR guide expression) and large transgenes in seedling and mature plants after infected thrip exposure
- Technology transfer will proceed through private sector partners

Jeffrey F.D. Dean (Dept. Head) – jeffdean@bch.misstate.edu – 662-325-2640
BCHEPP, Mailstop 9655, Mississippi State, MS 39762