Development of Gene Editing Methods to Retain Access to Foreign Markets for American Hops

Presented by: Chris Willig
Oregon State University

Project to be funded through USDA TASC program

- Existing hop biotechnology research group at OSU worked with HRC to develop a research proposal submission to the USDA-FAS Technical Assistance for Specialty Crops (TASC) program—spring 2023
- We were informed this year that the proposal will be funded, >$2,000,000 over 5 years
- With this funding, we will:
  - Develop advanced biotechnology tools to support hop genetic research and production
  - Investigate a strategy for a long-term solution to overcome trade barriers due to HPM fungicide MRLs

Our research group and prior work

- A hop biotech research collaboration began at OSU in 2021 supported by a two-year grant from USDA-NIFA awarded to PIs Steve Strauss, Dave Gent, and John Henning
- The project focused on establishing methods for gene transfer and CRISPR gene editing in public US hops with the goal of studying genes associate with powdery mildew disease
- The Strauss lab has decades of experience in plant biotech (with a focus on forest trees), while the Gent and Henning groups bring expertise in hop pathology and breeding, respectively

USDA NIFA

Strauss Lab

Chris Willig
Postdoctoral Researcher

Steve Strauss
PI

Tanner Whiting
Undergraduate Technician

Gent Lab

Michele Wiseman
PhD candidate

Dave Gent
PI

Carly Cooperider
Undergraduate Technician

Consulting

Greg Goralogia
Postdoctoral Researcher
(Strauss Lab)

Cathleen Ma
Senior Research Technician
(Strauss Lab)

Henning Lab

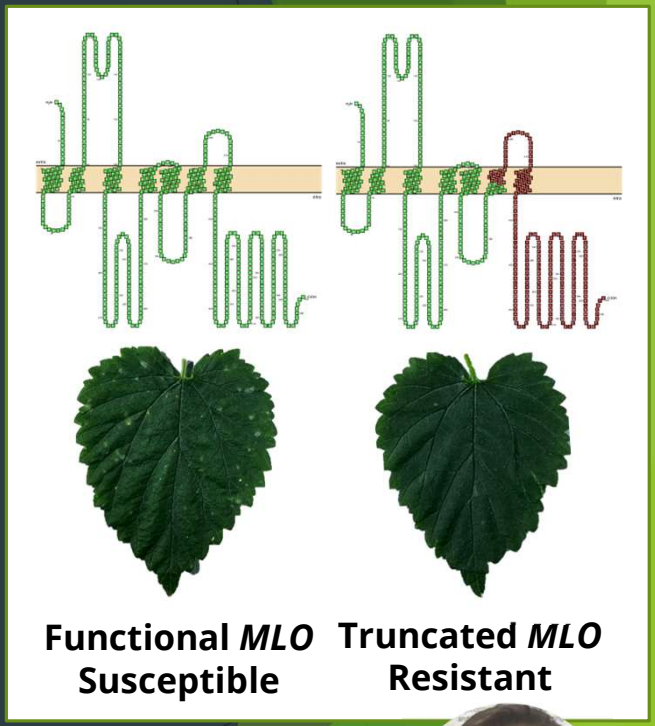
John Henning
PI

Rationale used in TASC proposal

- MRLs are a potential barrier for US hop exports. Applications of fungicides effective for controlling hop powdery mildew (HPM) are limited by MRLs set for major markets.
- Restrictive MRLs could constrain variety of fungicide chemistries used to control HPM, making pathogen more likely to develop tolerances
- Durable genetic resistance to powdery mildew could reduce dependence on fungicide application
- Some markets with strict MRL standards are open to gene-edited products—others moving in that direction
  - This project aims to lay groundwork ahead of anticipated changes in global regulatory environment

Rationale used in TASC proposal

- We propose to address fungicide MRLs by testing a strategy to endow hop plants with *genetic* resistance to HPM
  - Idea is that this could reduce need for fungicide application
- Variants of genes in the *Mildew Locus O* (*MLO*) family have provided durable resistance to PM in several crop species
  - However, in some instances (not always) there can be yield trade-offs
- PhD candidate Michele Wiseman's doctoral research focuses on identifying *MLO* genes associated with susceptibility to HPM
- Gene editing with CRISPR could allow us to:
  - Establish a genetic link between hop *MLO* candidate genes and HPM susceptibility
  - Create plants with edited variants of *MLO* genes that can be tested in the field for yield viability



Michele Wiseman

Gene editing vs. genetic engineering (GMO)

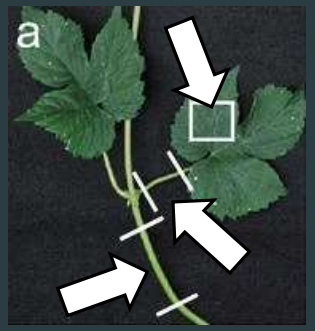
- Genetic engineering / transformation – method for delivering a "package" of genetic material into a plant to alter a trait

- Gene editing is using the package to deliver "machinery" that then changes genes *already inside the plant* to alter a trait

Hop tissue culture, transformation and regeneration

Roadmap to establishing a tissue culture-based gene transfer system

- Regeneration
  - Which cultivars will regenerate?
  - Media composition
    - Hormones
    - Sugars
    - Macronutrients
  - Starting plant tissue type
  - Lighting conditions

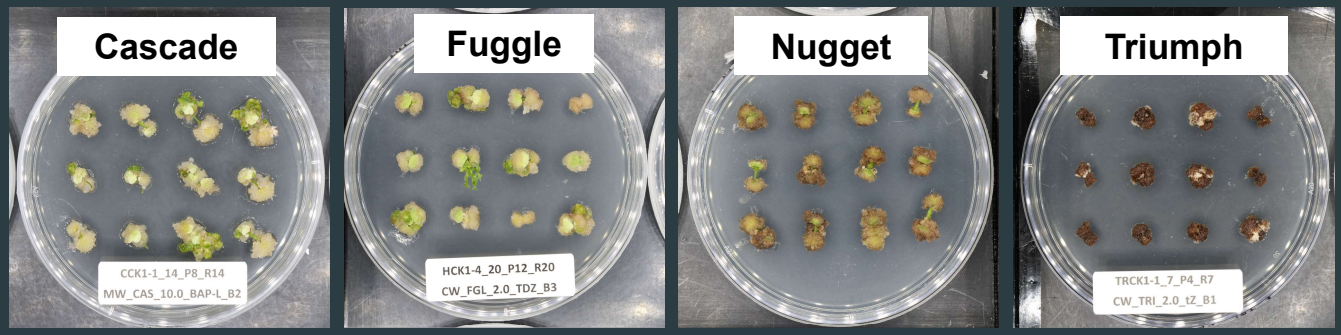


Horlemann et al., 2003

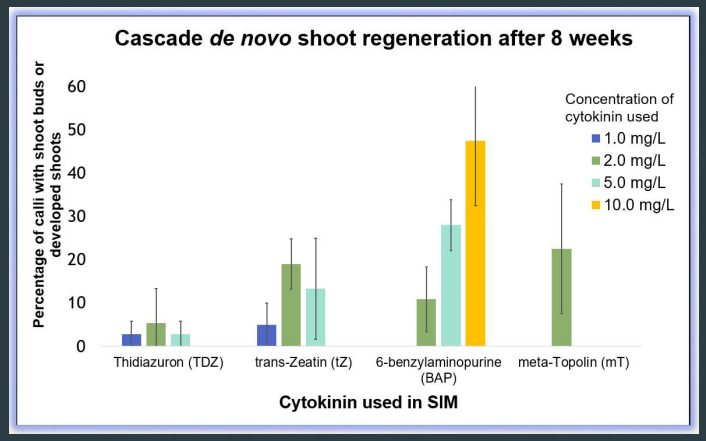
- Transformation
  - Which strains of *Agrobacterium* to use
  - How much *Agrobacterium* inoculum to use
  - Which marker genes to use
  - Starting plant tissue type
  - Techniques to help *Agrobacterium* deliver DNA to more cells

Experiments testing regeneration

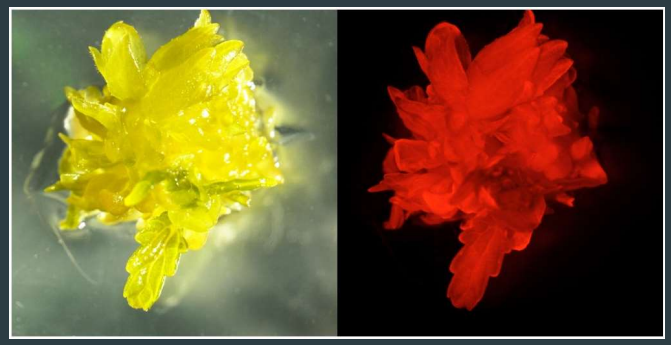
- Screened for shoot regeneration capacity in several public hop cultivars



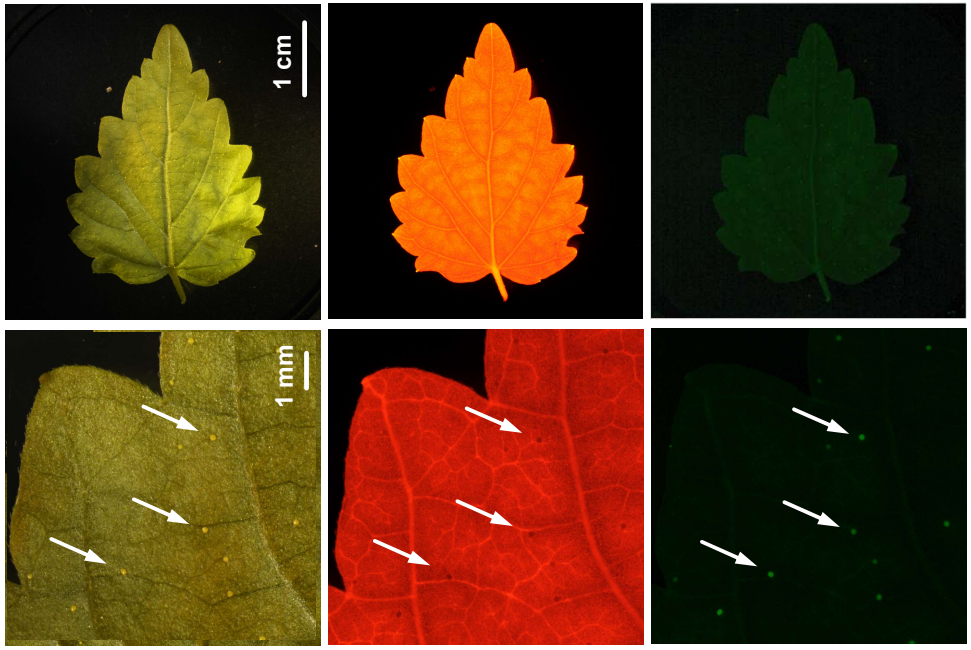
- Optimization for media hormone content in individual cultivars



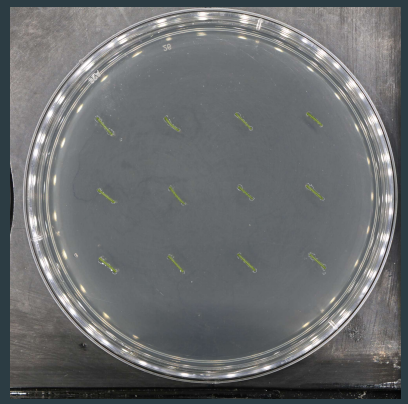
Transgenic Cascade plants produced – promising result showing gene editing should be achievable



Credit: Michele Wiseman

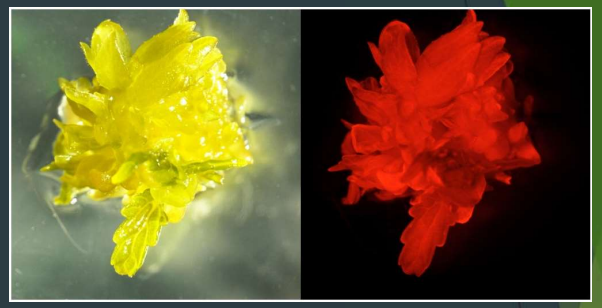


Next steps—optimizing our protocol



Rate of transgenic shoot production?

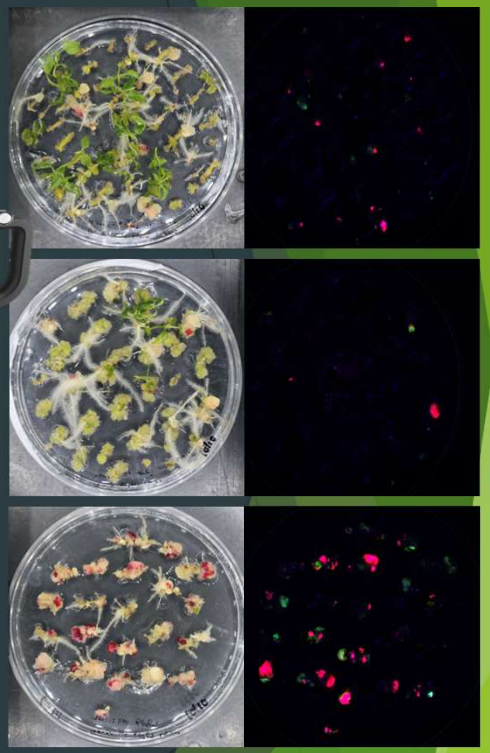
Estim. ~0.5%



- Transformation efficiency – how much original plant material and labor does it take to get one transgenic shoot cluster?
- We are and will continue testing whether any of a variety of additional tweaks to our procedures can reliably boost the efficiency of three factors:
  - Transformation (gene delivery)
  - Regeneration
  - Target gene editing efficiency

Work to date supporting success of this project

- Testing 6 different hop cultivars for regeneration capacity (in tissue culture)
- ~30 experiments to develop suitable regeneration/ transformation parameters in Cascade alone
  - ~1,500 petri plates
  - ~8,000 hop tissue segments



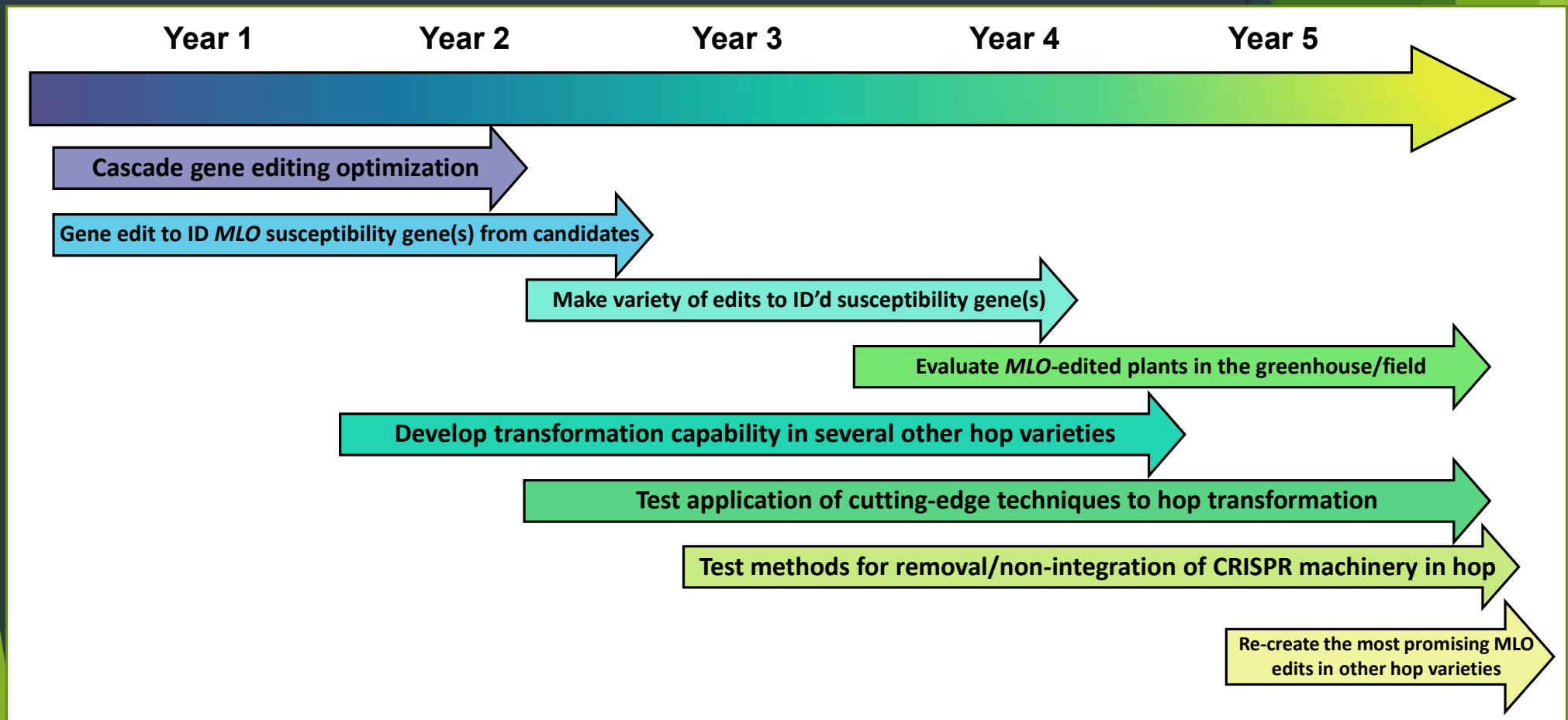
- Michele's work identifying candidate *MLO* genes and attempting to validate by methods independent of hop transformation
- Experiments started/ongoing to attempt editing of an *MLO* candidate gene

Bottom line: developing a transformation procedure in hop has been tough, labor intensive (compared to many other plants)

What new TASC funding will allow us to do

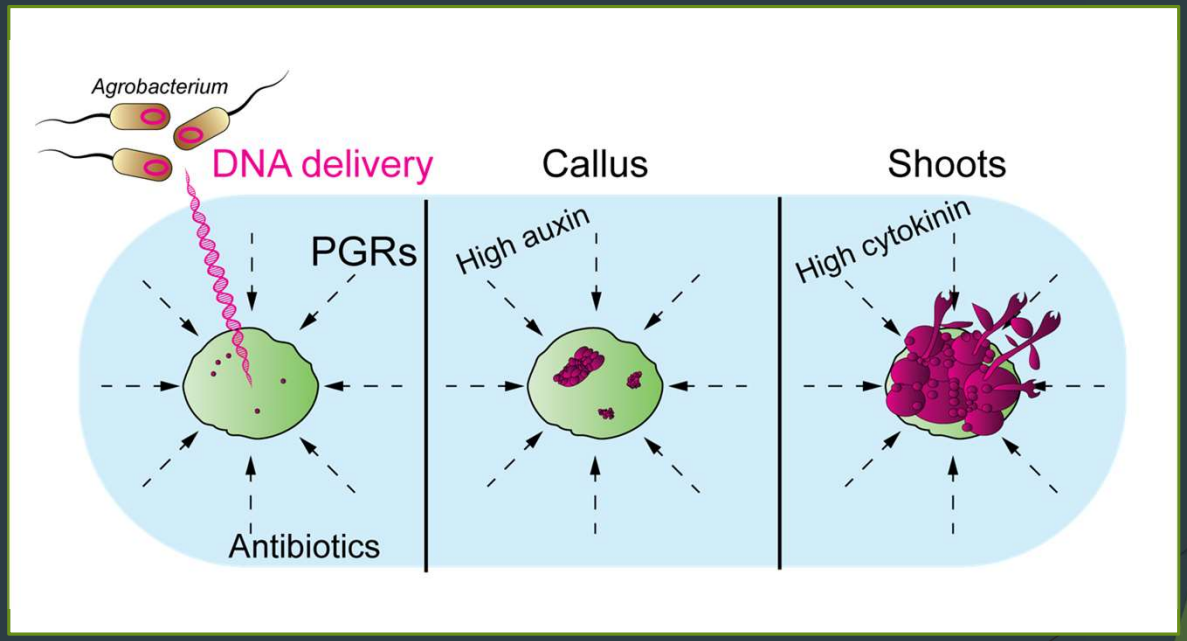
- Dedicate more time and attention to R&D work for transformation optimization
  - Strauss lab will hire a technician to focus on hop research full-time
- Longer funding period enables monitoring our *MLO* target trait from lab to field
- Develop transformation methods in multiple varieties rather than focusing on only one (Cascade)
- Advanced methods that enable more regulatory, consumer-friendly gene edited hops
- Consult intensively with stakeholders on best ways to apply gene editing in hop

Timeline for TASC project



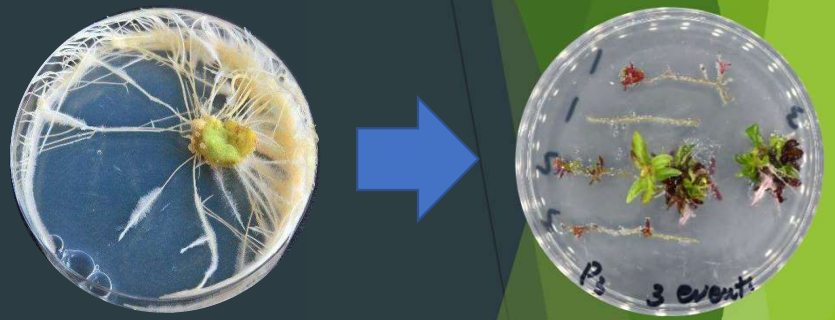
Examples of cutting-edge techniques we will test

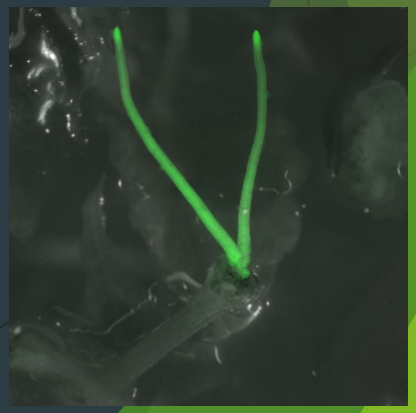
- Development ("DEV") genes to facilitate transformation



Examples of cutting-edge techniques we will test

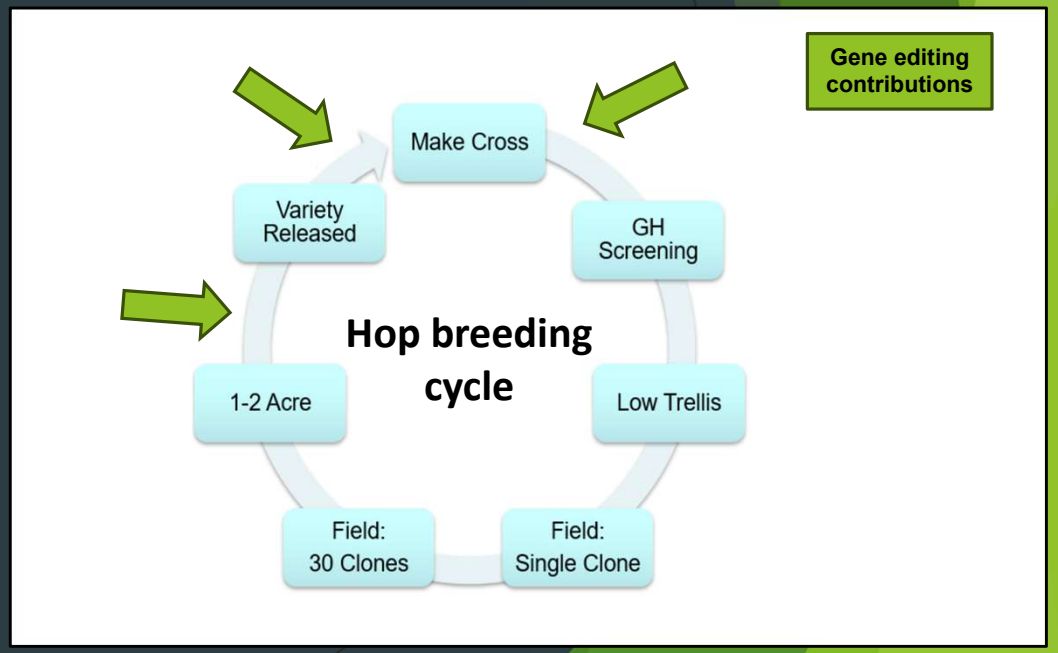
- Testing an alternative transgenic “hairy root”-to-shoot transformation approach
- Approach has been reported worked in other crops recently
- We have shown that we are able to get transgenic hairy roots in 4 hop varieties



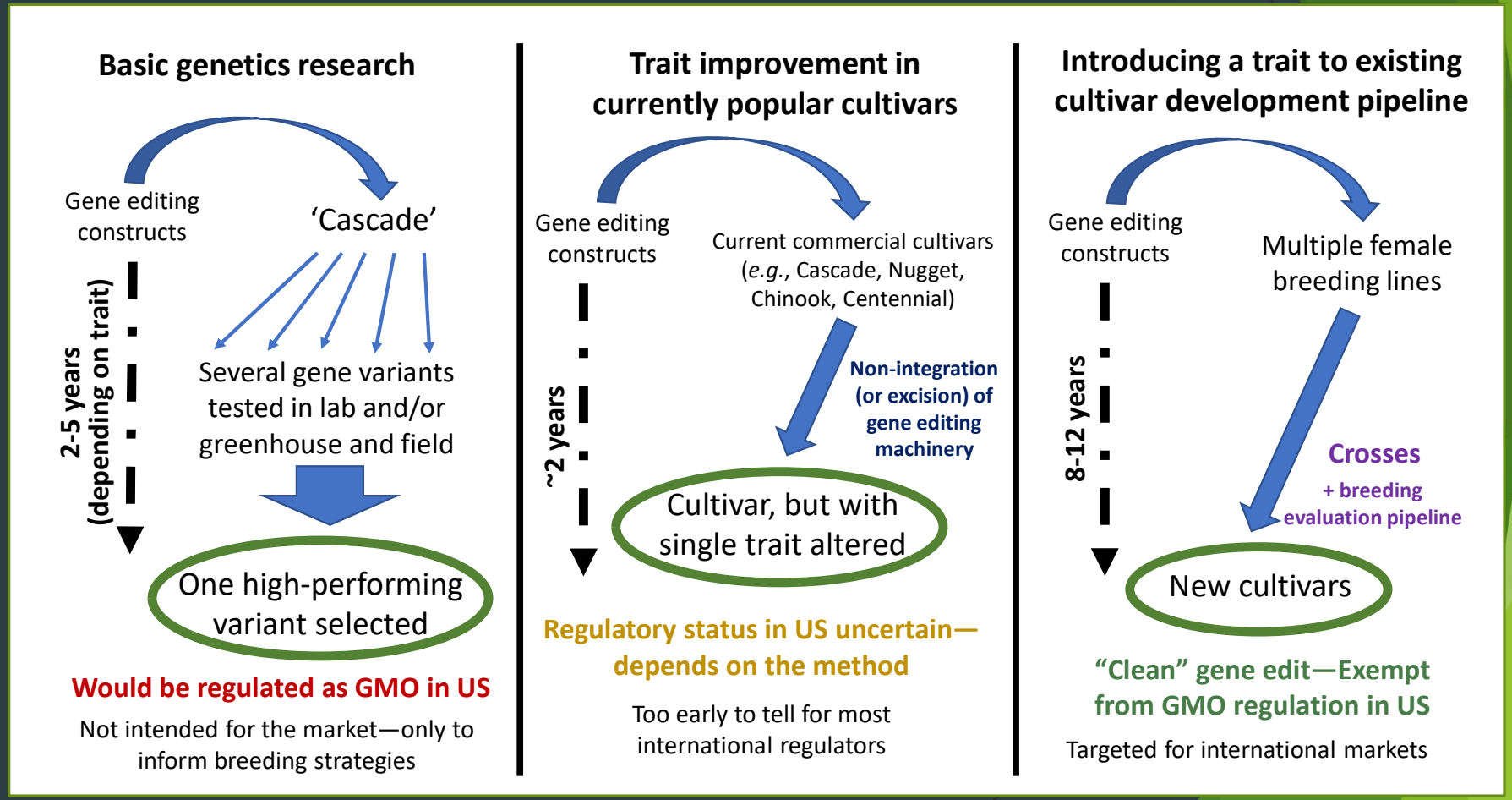


Gene editing can work hand-in-hand with breeding

- Support for breeding
  - GE can be used as a tool for genetic research to uncover gene functional information to assist breeders in tracking desired traits
- Complement breeding capabilities
  - GE can be used to fill in gaps with respect to specific traits that are difficult to alter through breeding
  - It can also speed up the timeframe for addressing these traits

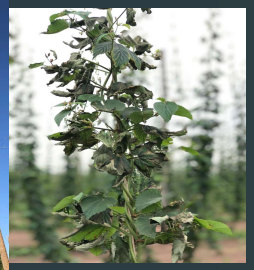


Multiple paths are open for applying gene editing to hop research/agriculture



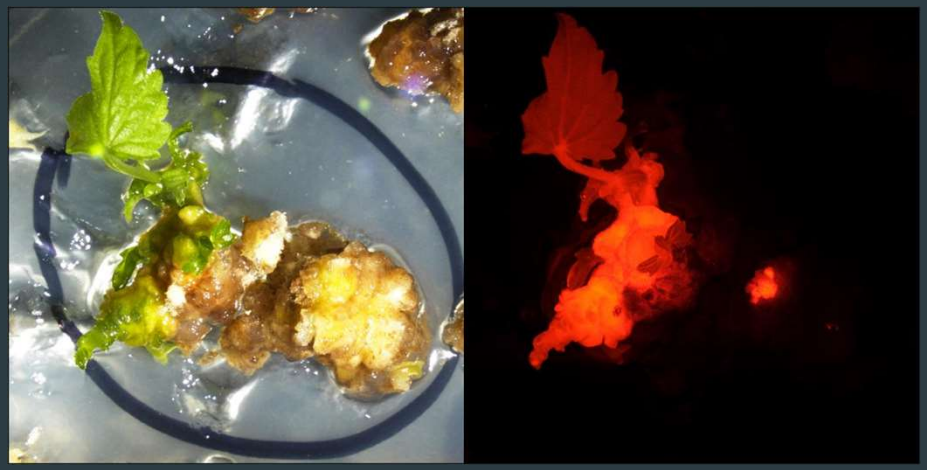
A long-term investment into future hop genetics research

- Hop agriculture is facing threats due to a changing global climate
  - Extreme temperature waves
  - Periodic drought
  - Disease and pest outbreak

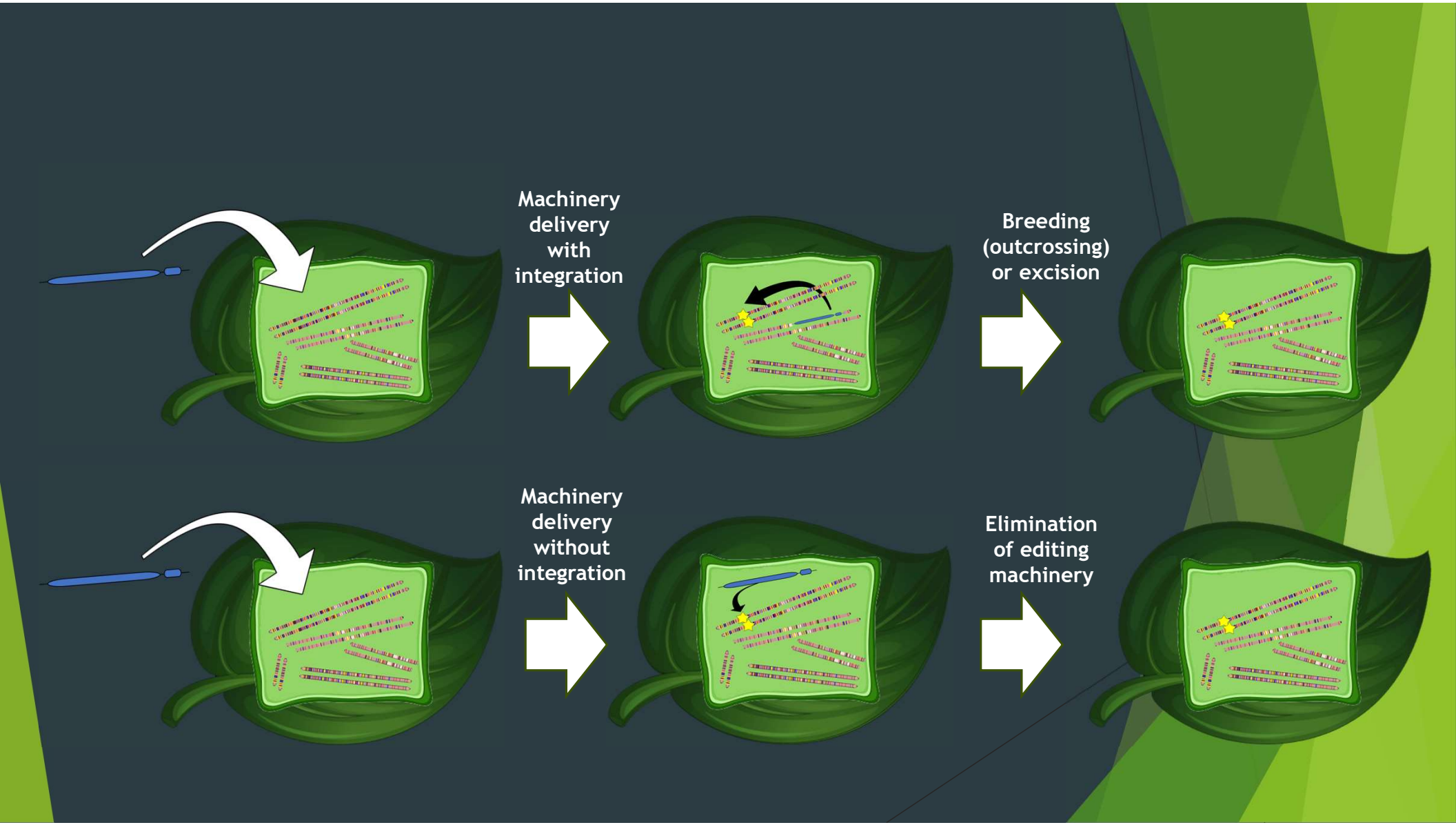
- Will be addressed by accelerating genetic research and breeding hop varieties with improved traits that offer some protection from these pressures

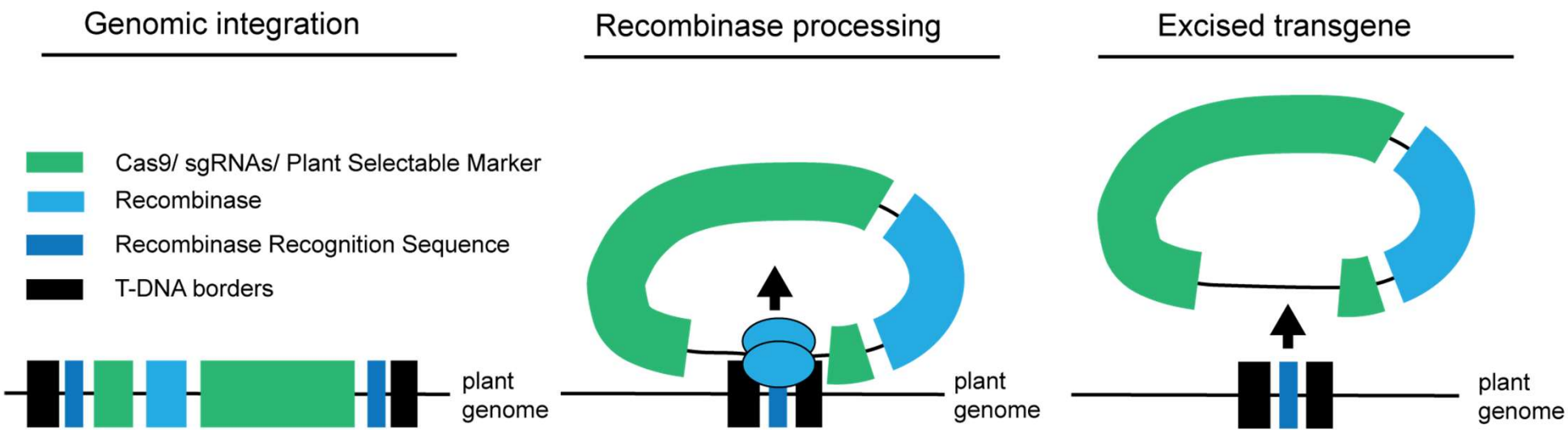
Thanks / Questions?



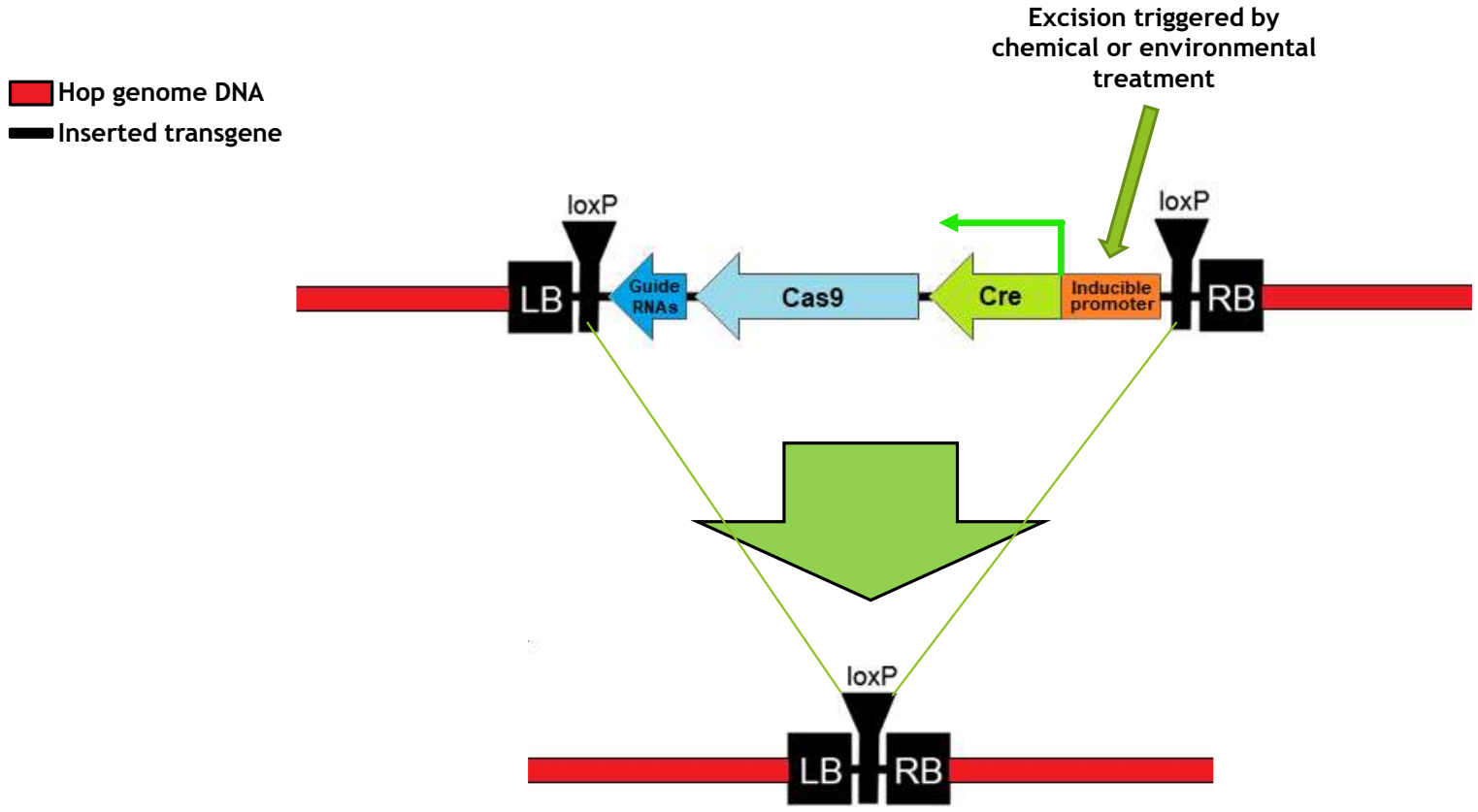
Connect with the Strauss lab

Lab website: https://biotechlab.forestry.oregonstate.edu/
Steve's email: Steve.Strauss@oregonstate.edu
My email: Chris.Willig@oregonstate.edu


Machinery delivery with integration
Breeding (outcrossing) or excision
Machinery delivery without integration
Elimination of editing machinery



We use the Cre-lox system, but other options exist


Hop genome DNA
Inserted transgene
Excision triggered by chemical or environmental treatment
loxP
loxP
LB
Guide RNAs
Cas9
Cre
Inducible promoter
RB
loxP
LB
RB

Examples of cutting-edge techniques we will test

- *In planta* transformation methods – still a very new technology, but could allow us to bypass tissue culture