# Identification Of Disease Resistant Genotypes For Use In Walnut Rootstock Development

## USDA-NIFA-SCRI Project

# Disease-Resistant Walnut Rootstocks

**Long-term goal**: Develop, evaluate, and deploy walnut rootstocks with resistance to the major soil-borne pathogens.

**Target Pathogens:**

*Agrobacterium tumefaciens* (crown gall)

*Phytophthora* spp. (Phytophthora root/crown rot)

*Pratylenchus vulnus* (lesion nematode)

# Disease-Resistant Walnut Rootstocks

## Objectives:

1. Generate/exploit a genetically diverse *Juglans* germplasm collection

2. Identify *Juglans* germplasm resistant to key soil-borne pathogens

3. Generate and clonally propagate hybrid and elite disease-resistant genotypes for validation in field trials

4. Develop genomic tools to facilitate rootstock breeding

5. Deliver disease resistant rootstocks to growers

# Evaluation of wild *Juglans* species for disease resistance

## Exploiting the USDA-ARS Walnut Germplasm Collection

*J. ailantifolia*

*J. californica*

*J. cathyensis*

*J. hindsii*

*J. major*

*J. hopeiensis*

*J. mandshurica*

*J. microcarpa**

*J. nigra*

*J. regia*

*Pterocarya*

*J. sinensis*

Malli Aradhya
Chuck Leslie

# Germplasm Generation / Propagation for disease resistance testing



Chuck Leslie
Malli Aradhya
Wes Hackett

# Generation of Interspecific Full-sib Hybrids from Selected "Mother Trees"



*J. microcarpa* X

*J. regia* 'Serr' pollen

C. Leslie,
D. Kluepfel,
M. Aradhya

# In vitro Production of Clonal Plants from Hybrid Seed for Replicated Pathogen Resistance Testing

**Plant Propagation Goal:**

50 plants each of 600 hybrid genotypes
for pathogen resistance screening
(300 each from 31.01 and 31.09) = 30,000 plants

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**Produced to date:**

- Total of 43,800 fully acclimated hybrid clonal plants for
pathology resistance screening trials.

- >37,000 of these were 31.01 and 31.09 genotypes
> 15,000 compliments of Duarte Nursery

# Disease-Resistant Walnut Rootstocks

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   (OP and interspecific hybrids)

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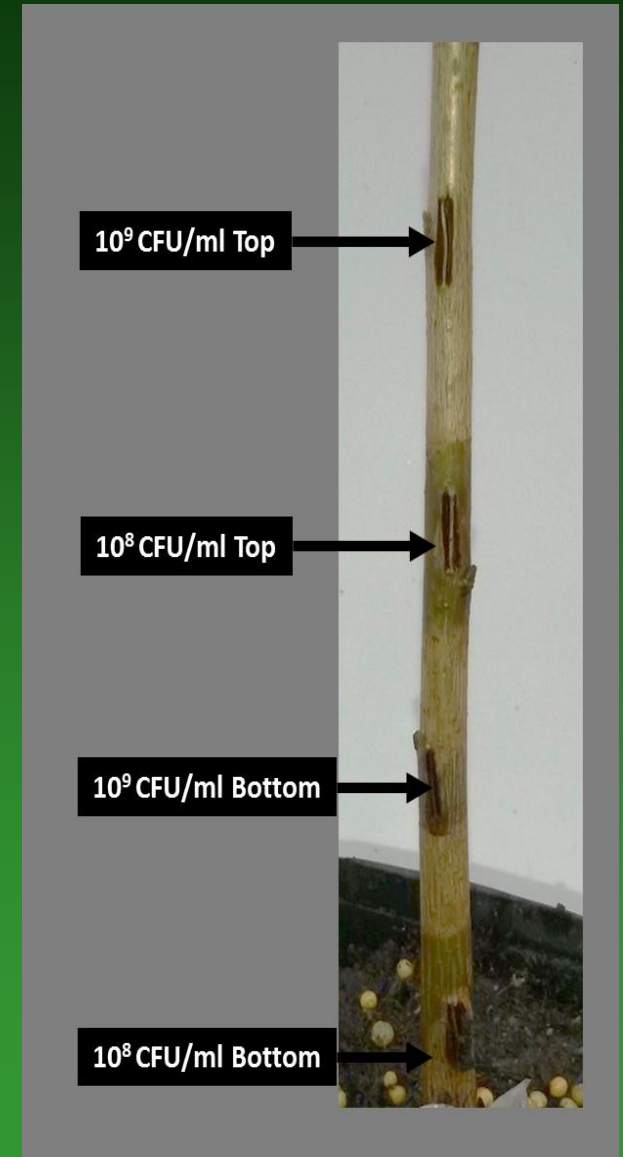
# Pathogen Resistance Screening



Crown gall disease – Kluepfel Lab

# Walnut Germplasm Screen

## Inoculation method
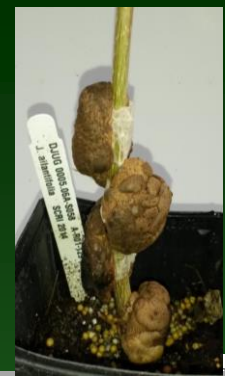


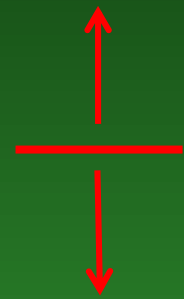Stab technique using
*A. tumefaciens*
infested blade





$10^9$ CFU/ml Top

$10^8$ CFU/ml Top

$10^9$ CFU/ml Bottom

$10^8$ CFU/ml Bottom

# Rating System



Rating = 1     Rating = 2     Rating = 3     Rating = 4

1 = no disease symptoms

2 = gall symptoms with stem girdling less than 25%

3 = gall symptoms and stem girdling between 25% and 50%
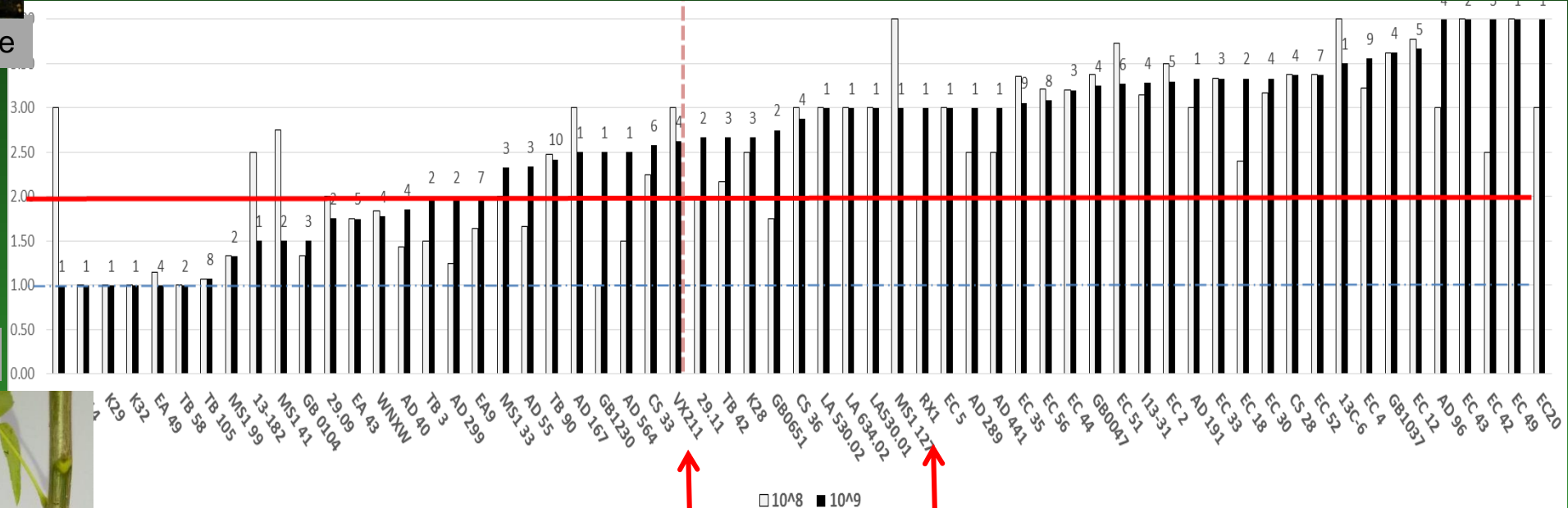
4 = galls symptoms with stem girdling >50%

# Walnut Germplasm Screen for Crown Gall resistance
## (hybrids and open pollinated genotypes)

# Pathogen Resistance Screening



Phytophthora crown and root rots– Browne Lab

# Assessing resistance to *Phytophthora*
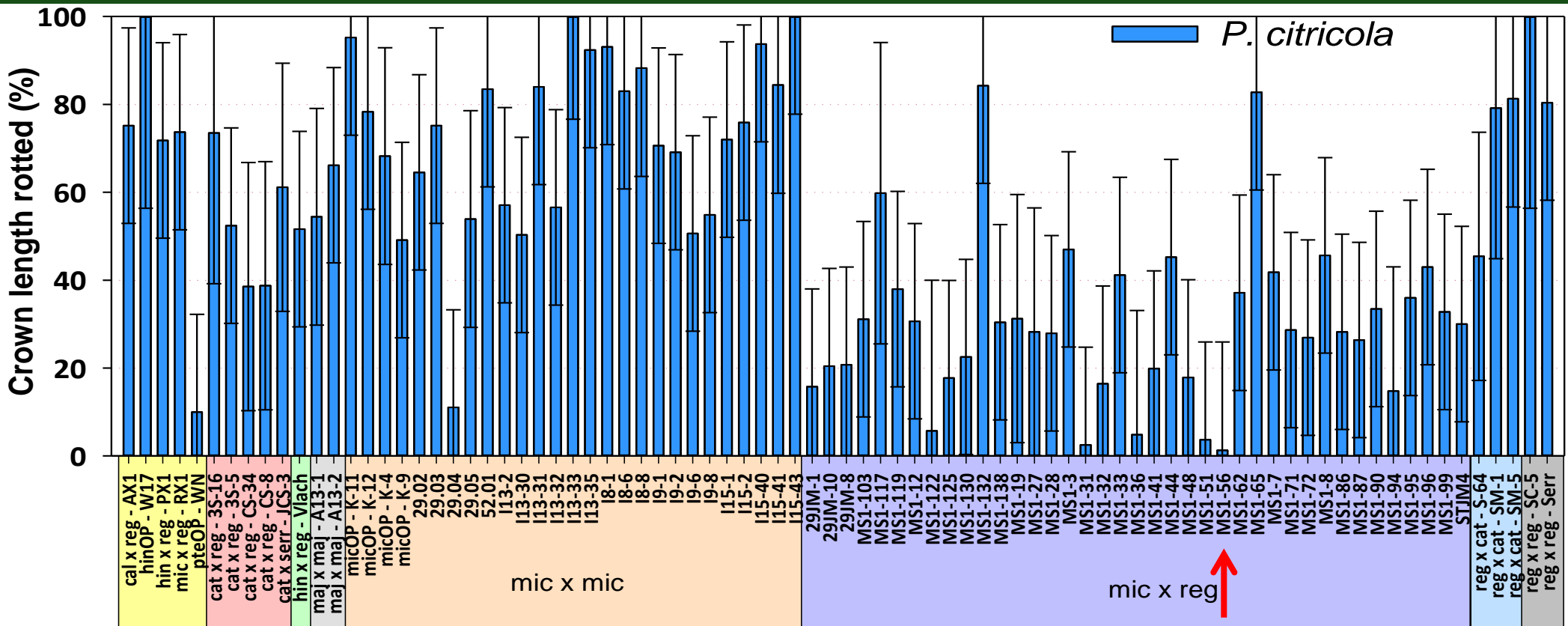


**2-3 month exposure to *P. cinnamomi, P. citricola***

**Resistance assessed according to:**
- **Survival duration; (ratings, 0 to 5)**
- **Crown length rotted (%) (measured)**
- **Root length rotted (%) (visual)**
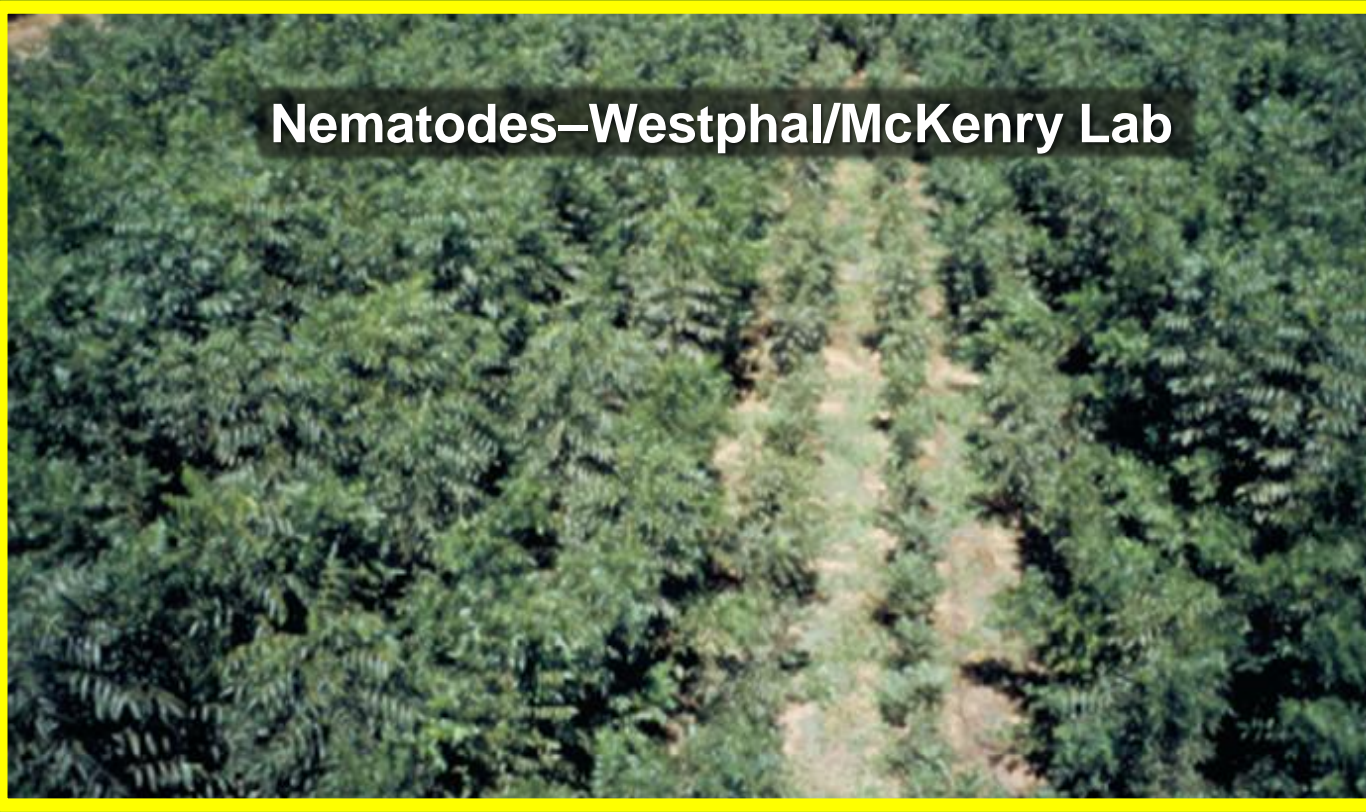
Assessing resistance to *Phytophthora*

# Pathogen Resistance Screening



Nematodes–Westphal/McKenry Lab

# Assessing Resistance to Nematodes

**Goal:** Select for resistance/tolerance to root-lesion and root-knot nematode (Westphal, McKenry)

**Activities:**

- Field screen of clones; inoculated with RKN and RLN.

- Selected candidates planted into a replicated nursery for continued evaluation.

- New potential candidates with high levels of resistance in seedling derivatives.

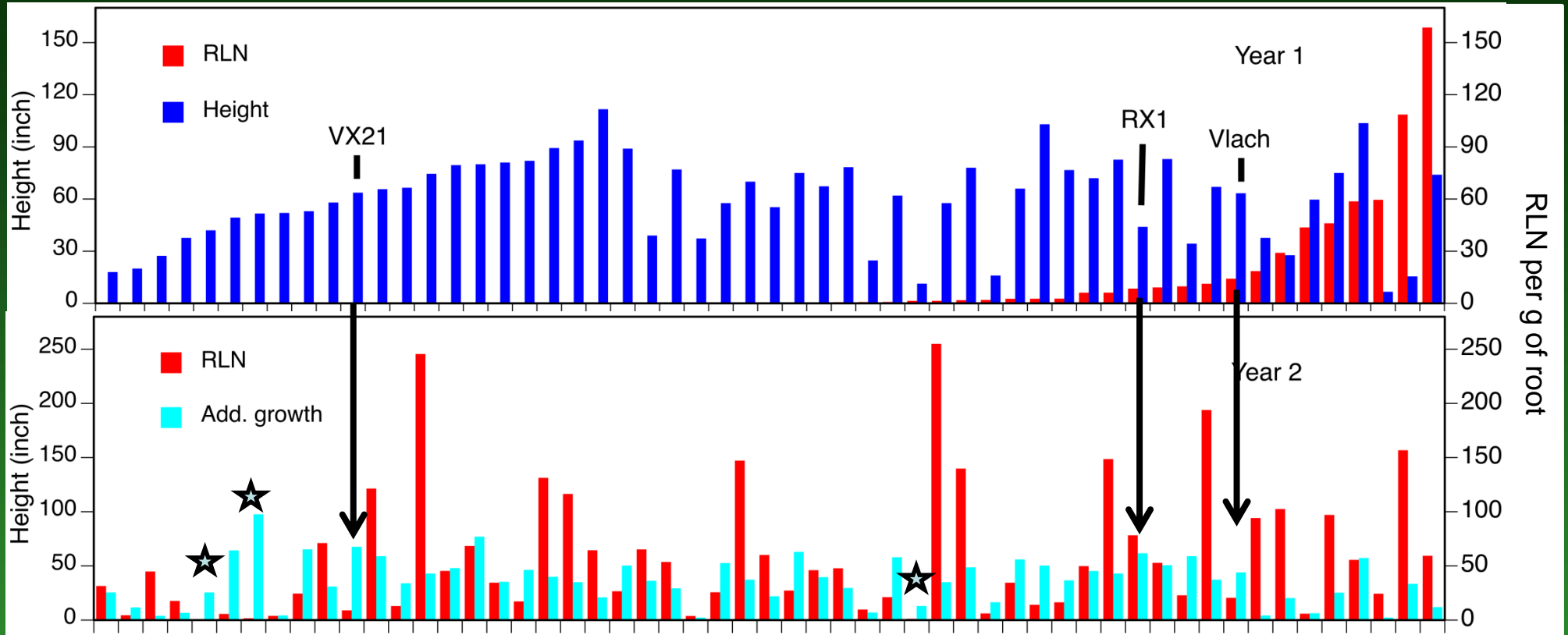**Initial field testing**



**Selection and transplant**



**Selection and transplant**



**Validation**

# Results: Evaluating MS1 – hybrid clones



**Clones of the MS1 cross**

## Outcome/ Deliverables

- Two years of testing provides the most meaningful nematode data.
- Experimental genotypes show greater nematode resistance than levels observed in standard industry clones.

**Future effort:** Validate nematode resistance/tolerance of top candidates.
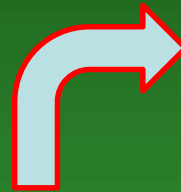
# Disease-Resistant Walnut Rootstocks

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# Field evaluation of "Elite" clonal rootstock germplasm

5 Elite genotypes are in field trials.
5 new clones are ready to enter field trials

**In vitro propagation under commercial conditions.**
*(compliments of Sierra Gold)*

Hardening off

Shoot/root development

Field trials

# Field evaluation of "Elite" clonal rootstock germplasm

Glenn Co. (Carriere) 11/3/16

Solano Co. (Armstrong)

Sutter Co. (SGN) 12/2/16

Lake Co. (Valadez) 11/17/16

Tulare Co. (Lindcove) 10/21/16

| Location | Advisor | Potential Site Problems | VX211, RX1, Vlach, Sdling PDX | Experimental Germplasm |
|---|---|---|---|---|
| Lindcove REC Tulare Co. | Fichtner | *Phytophthora*, lesion nematode | Yes | K3, JM4, JM8, 11-99 |
| Lake Co. | Elkins | *Armillaria* root rot, Nematodes, Crown Gall | Yes | K3, JM4, JM8, CC |
| Glenn Co. | Lightle | *Phytophthora*, lesion nematode, Crown Gall | Yes | K3, JM4, JM8 |
| Sutter Co. SGN | Hasey | Marginal Soil | Yes | K3, JM4, JM8, 11-99, 3S-17, CC |
| Solano Co. UCD Armstrong | Pope | Inoculated: CG *Phytophthora spp* | Yes (no sdlg PDX) | K3, JM4, JM8, 11-99, 3S-17 |

# Disease-Resistant Walnut Rootstocks
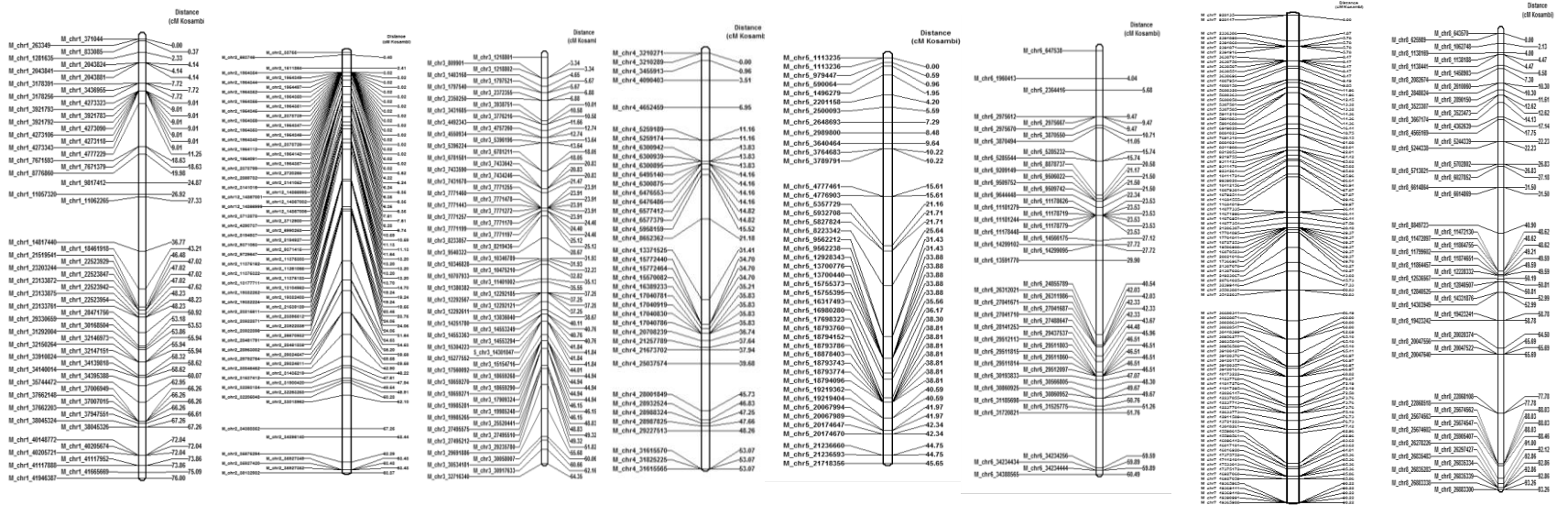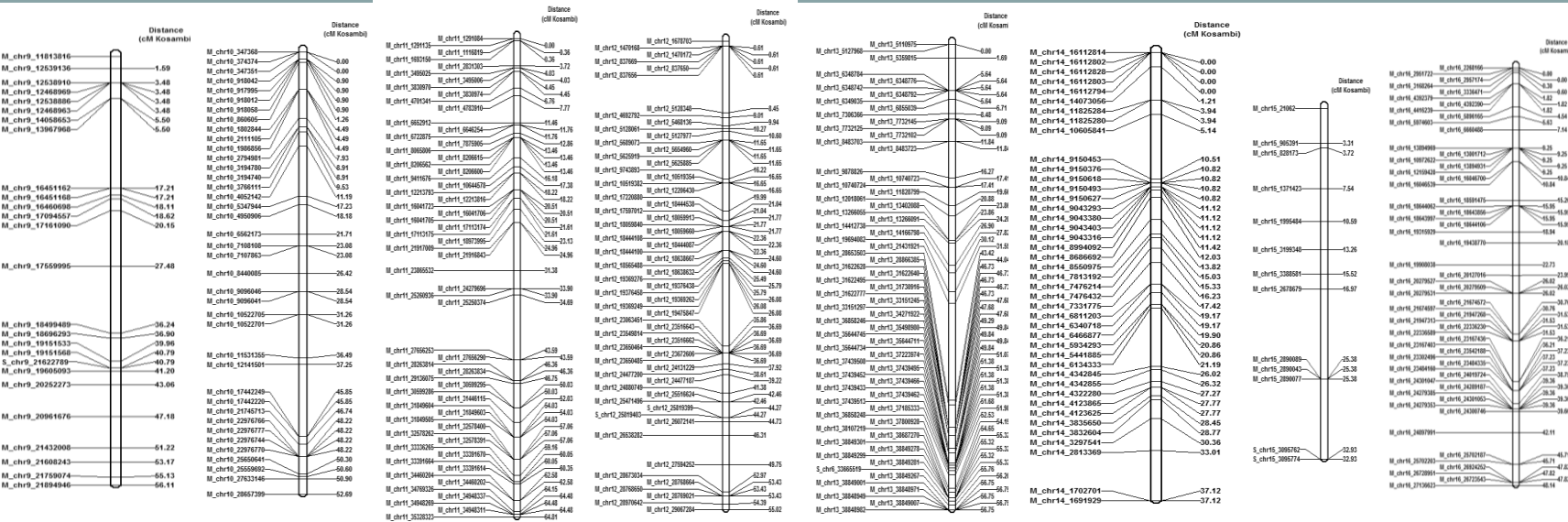
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# Genetic Linkage Map of DJUG 31.01 (*Juglans microcarpa*)

# Conclusions/Results

Crossed the "best" *Juglans* species with disease resistance with *J. regia* and invitro propagated hybrid progeny from these crosses ("Paradox"-like seedlings)

Disease resistant OP and some interspecific hybrids (*J. microcarpa x J. regia)* have been identified and will be used to map the genetic loci which mediate disease resistance.

Elite putative disease resistant hybrids have been clonally propagated and are being examined in field tests at multiple locations in the CA central valley

Developing Genomic information for future use in breeding

## Current Conventional Breeding and Selection

- Effective but Time consuming
- Resource Intensive
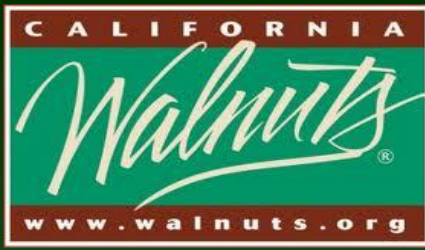- Complex Disease Testing

## Genomics & Genetics

- ID genes associated with disease resistance. i.e. Genetic Marker discovery
- Genetic and Physical maps

## Evaluate progeny from a Conventional Cross
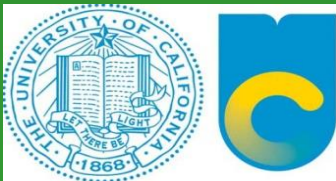
- Juvenile selection

## Walnut Rootstock Improvement

Pathogen resistance
Adaptability to varied soil conditions
Productivity/Longevity
Sustainability

# Exploiting walnut wild relatives to identify disease resistant genotypes for use in commercial rootstock development.

## USDA-NIFA-SCRI Project

# Phytophthora research overview, Browne Lab – H. Forbes, N. Ott, H. Gouran

## Goal:

- Rootstock clones resistant to *Phytophthora spp .,* markers for resistance to *Phytophthora*

## Activities:

- Screening (2 *Phytophthora* species with: 290 clones, 38 genetic backgrounds; 13,800 plants tested since 2010, 2986 plants in 2016)
- Orchard trials, diagnostics

## Products:

- Clones of *Juglans microcarpa x J. regia* and J. *cathayensis x reg* putatively low in susceptibility to *P. cinnamomi* and *P. citricola.*
- Orchard-validated resistance to *P. cinnamomi* in 'RX1', orchard trials established w/ 'STJM4', '29JM8', & '3s17'