

# Crown Gall Update:



# Control of Crown Gall Disease

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- Fumigation/Chemical Control
- Plant Clean Paradox Seeds
- Systemic movement of *Agrobacterium*
- Resistant *Juglans* Genotypes
- Cultural Practices
  - Post Cold Storage Handling
  - Surface disinfestation
  - limit wounding
- Surgical/chemical removal of tumors
- Biological Control, i.e. K84

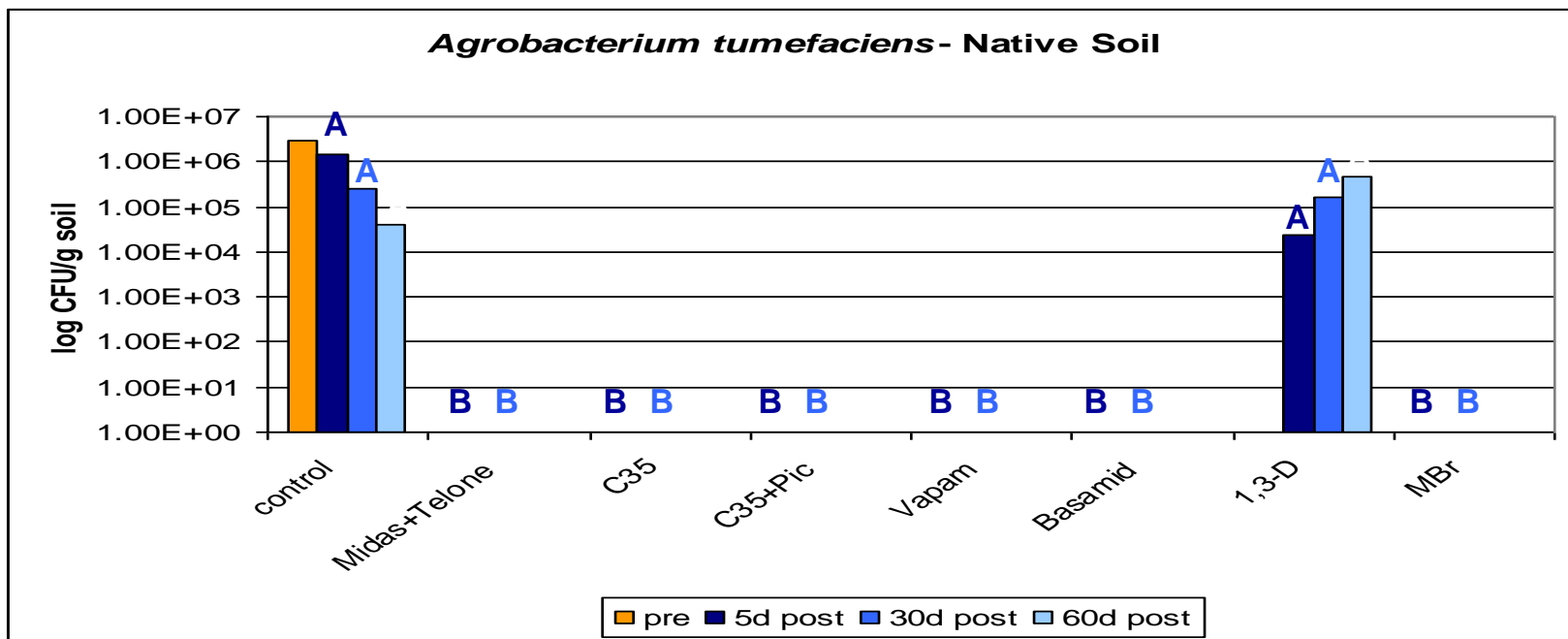
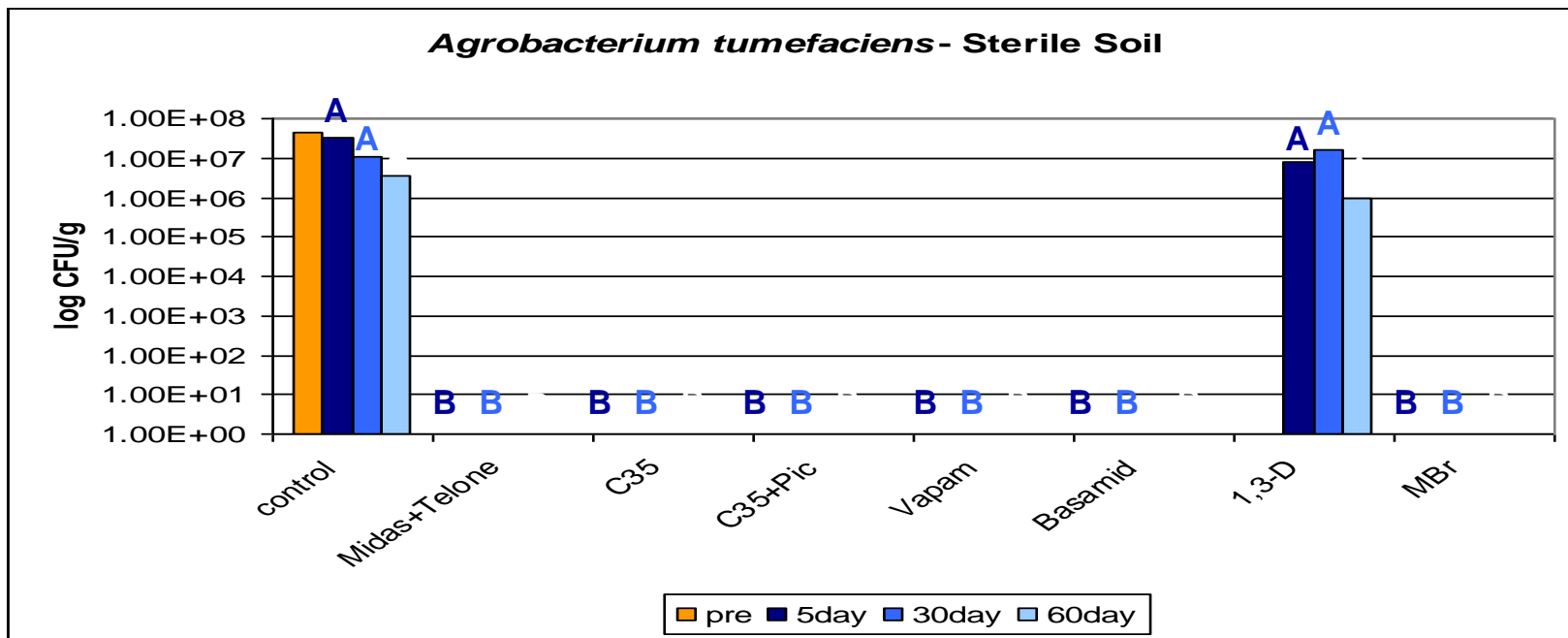
# MeBr Alternatives

Treatment	rate
methyl bromide	400 lbs/acre
Telone II	33.7 gal/acre
Telone C35	49 gal/acre
Telone C35 + chloropicrin	49 gal/acre + 250 lb/acre
iodomethane + Telone II	400 lb/acre + 150 lbs/acre
Vapam	75 gal/acre
Basamid	200 lb/acre



- Efficacy
  - *Phytophthora cactorum*
  - *Agrobacterium tumefaciens*
    - in soil
    - inside galls
  - Aerobic bacteria
- *A. tumefaciens* recolonization

# Efficacy – *A. tumefaciens*



# Agro in Galls

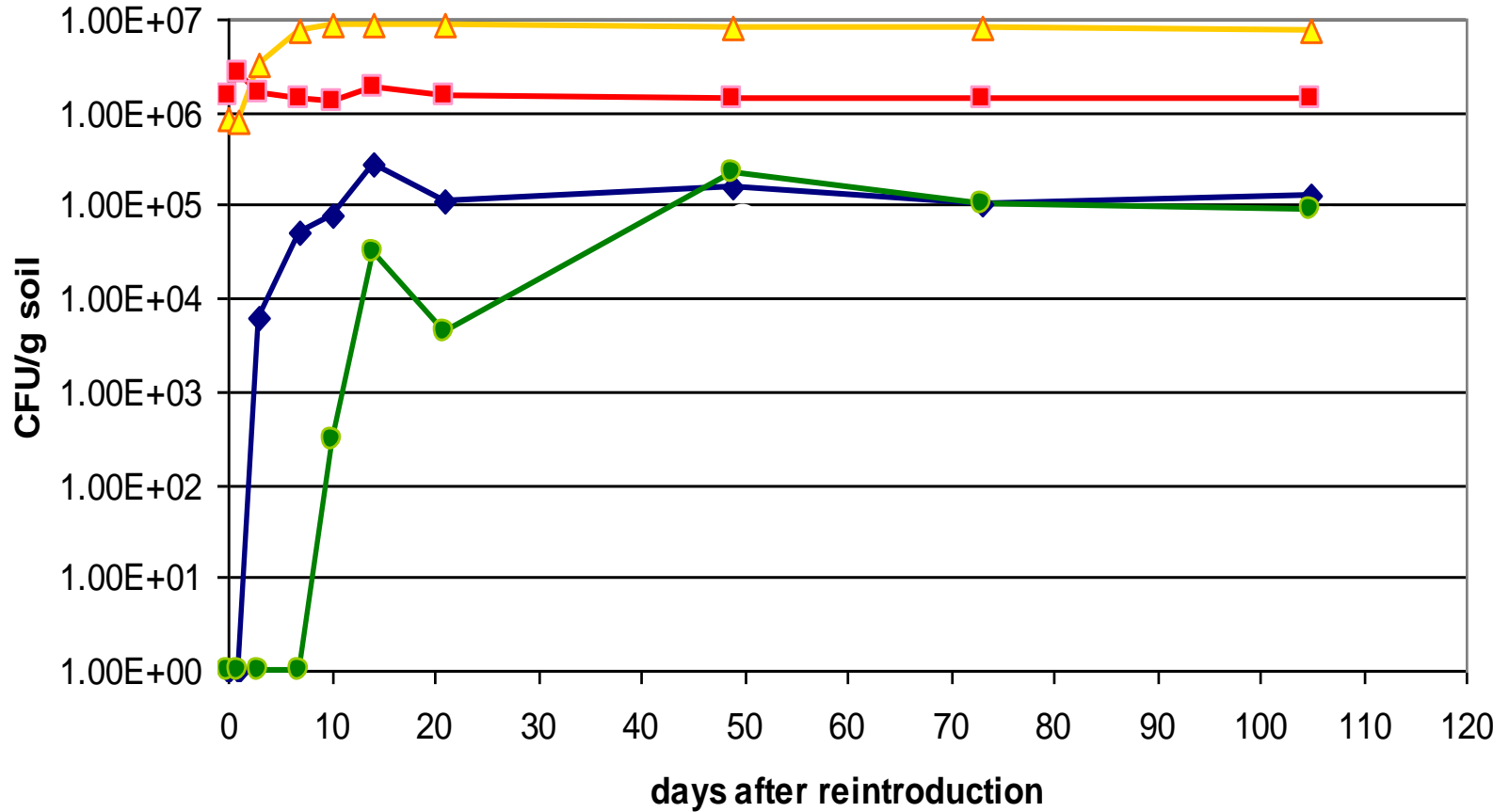
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- 2"-4" galls buried in sterile soil
  - Treatments: MeBr 400lb/acre  
Telone C35 49 gal/acre
  - Assay 120 days after fumigation
    - in soil
    - inside galls

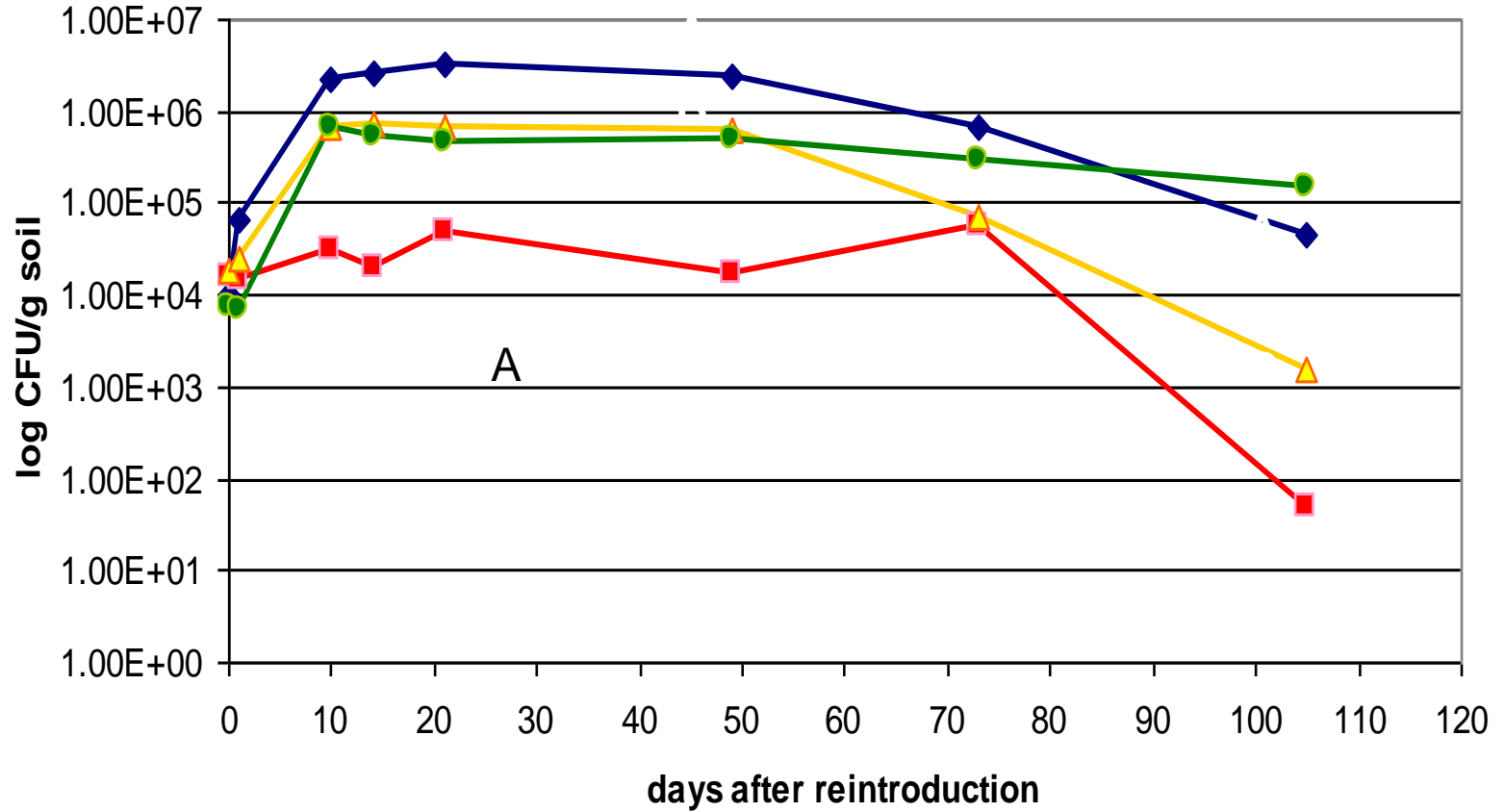
Treatment	<u><i>A. tumefaciens</i> Detected</u>	
	<u>In galls</u>	<u>In soil</u>
MeBr	0/6*	0/6*
TC35	3/6	5/6
TC35 + pic	0/8*	0/8*
Non-fumigated control	16/16	14/16

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# Aerobic Bacteria Recolonization



### *A. tumefaciens* Recolonization



◆ MeBr    ■ Native    ▲ C35    ● Sterile

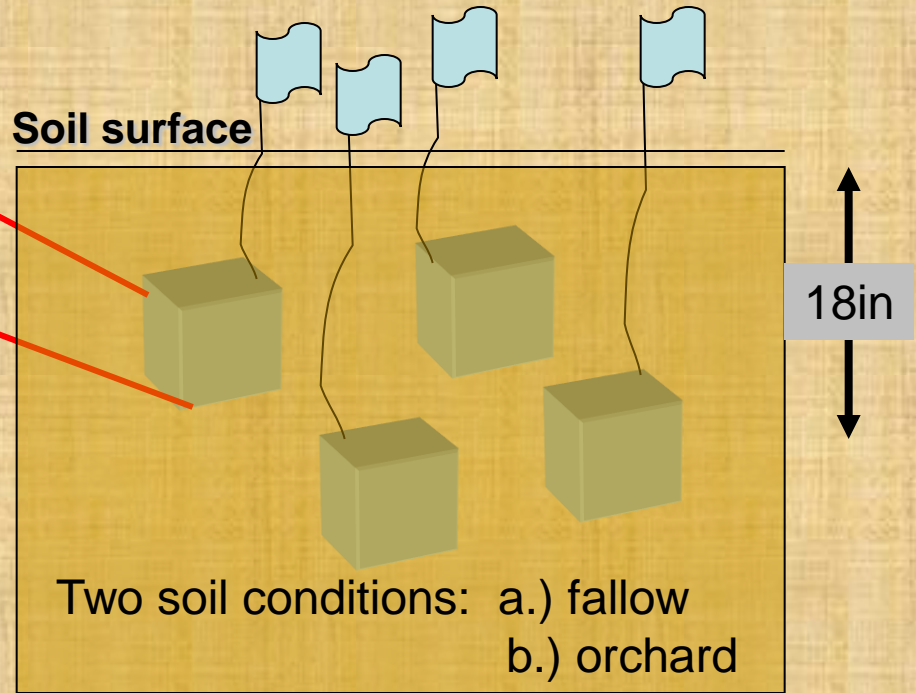
# Conclusions

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- MeBr and most alternatives control *A. tumefaciens*
  - 1,3-D only “reduces” *A. tumefaciens* populations
  - A combination of 1,3-D and chloropicrin (Telone C35) best alternative for control of *Agrobacterium tumefaciens*/crown gall
- Fumigation alters the soil microbial community; result, pathogens can re-colonize to higher levels than in non-fumigated soil

# Long term soil survival

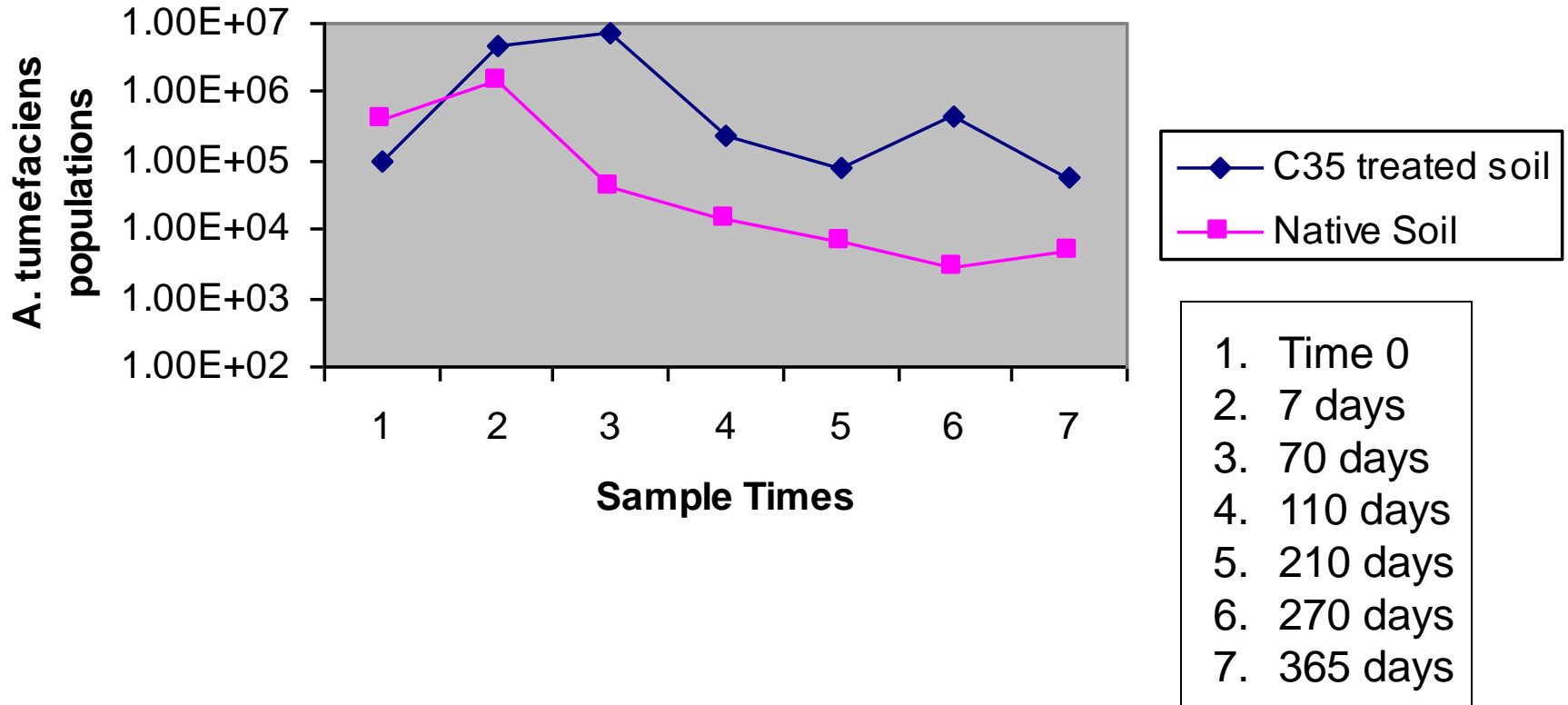
- a) C35 trt soil
  - b) Native soil
- +
- A. tumefaciens*



Dig up pouch after  
given time interval

Determine *A. tumefaciens*  
populations in soil pouches

# Soil Survival of *A. tumefaciens* Orchard Row



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Tracking the origin/importance of *A. tumefaciens*  
infection in/on Paradox seeds



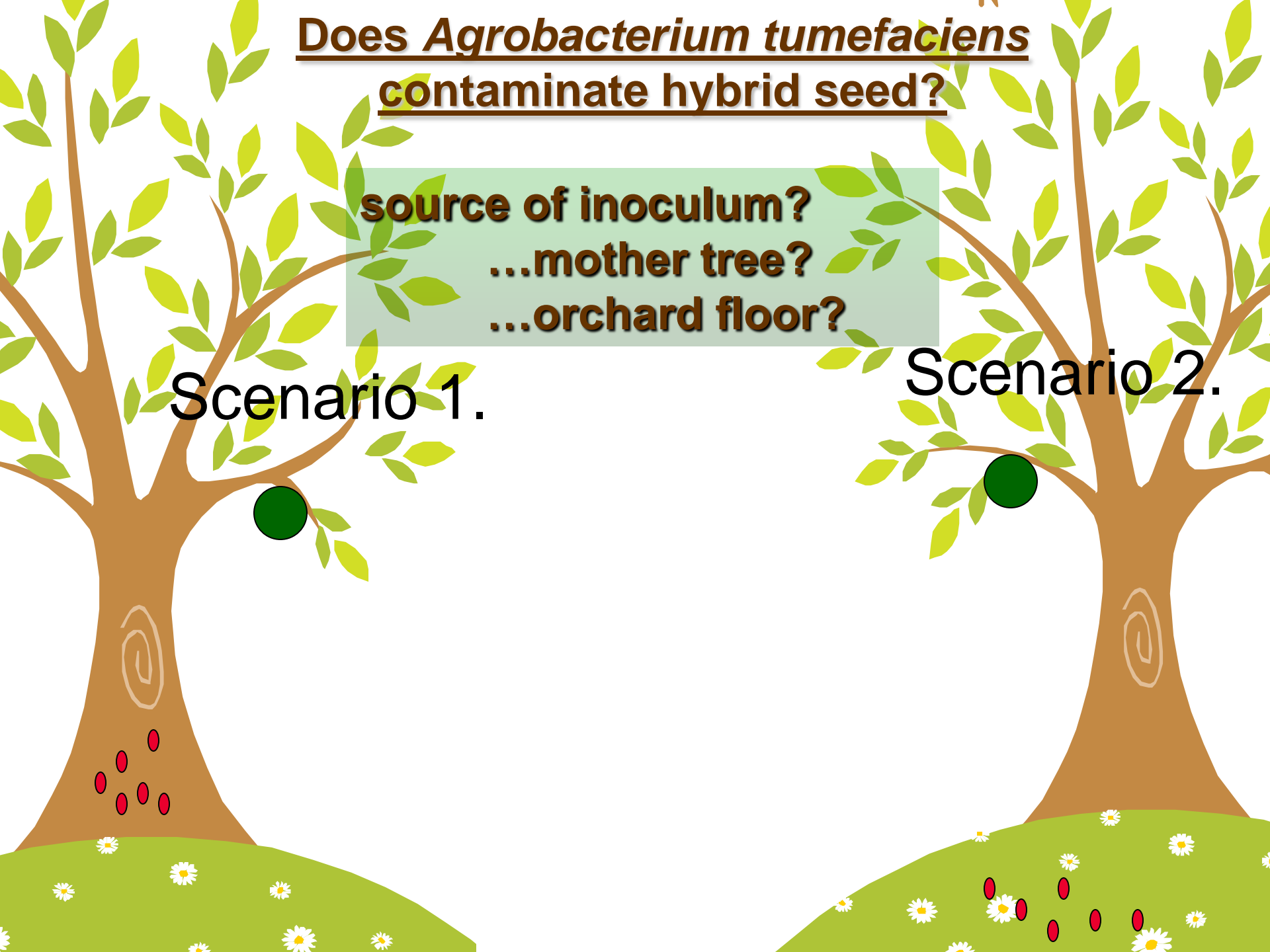
USDA-ARS CPGRU Davis, CA

Does *Agrobacterium tumefaciens* contaminate hybrid seed?

source of inoculum?  
...mother tree?  
...orchard floor?

Scenario 1.

Scenario 2.



# outline

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- **Mother Block Survey**

- Where does the *A. tumefaciens* inoculum come from?
  - ...mother tree?
  - ...orchard floor?



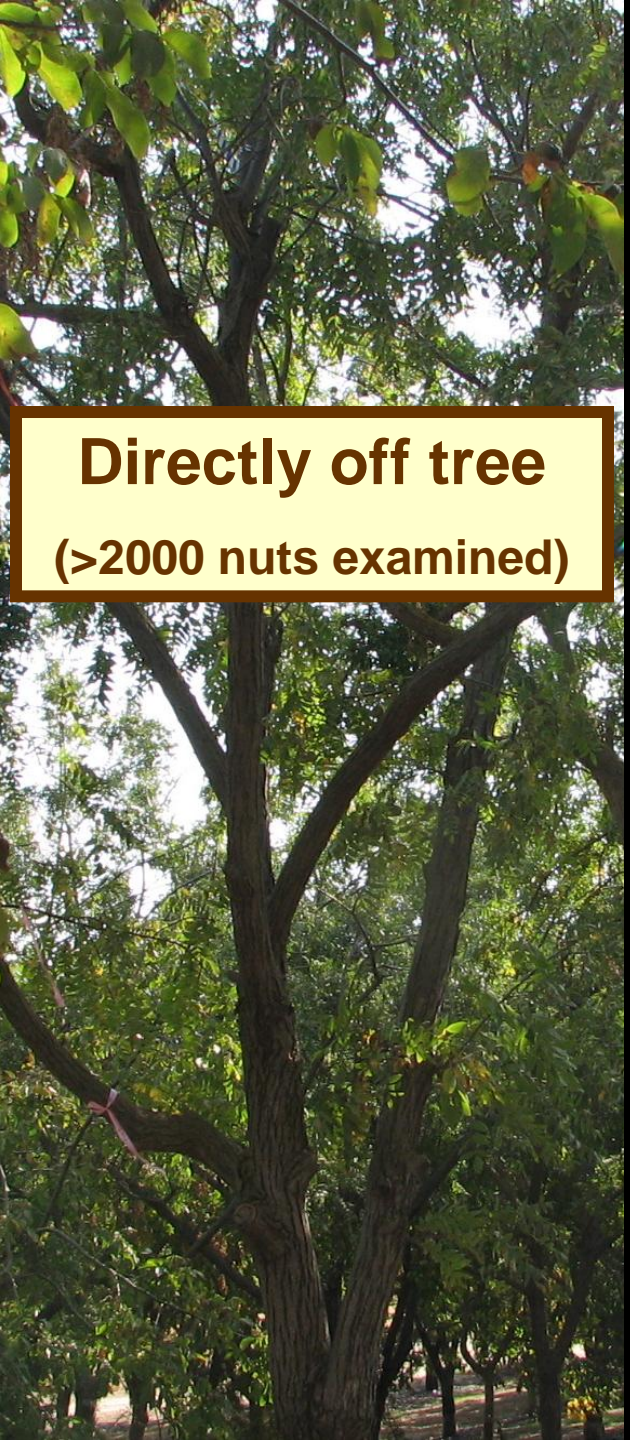
**Directly off tree**

**Off orchard floor**

**(1,3,7,14,28 days on soil)**

# Mother Block Survey





shell/ embryo

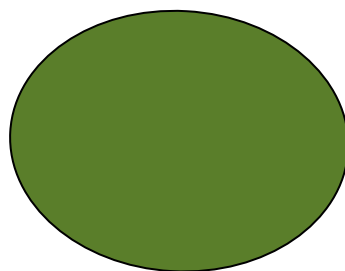
No Agro Detected



husk

No Agro Detected

-inhibitors



exterior

No Agro Detected

**Directly off tree**

(>2000 nuts examined)

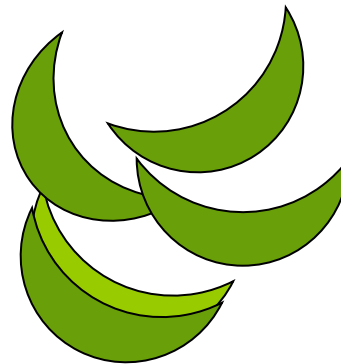
No Agro detected...yet



shell/ embryo

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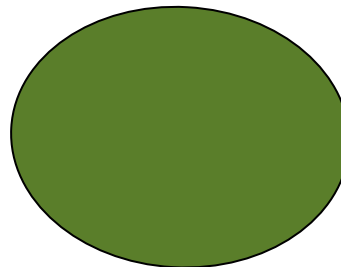
**Virulent/Avirulent**  
**Agro. Detected**




husk

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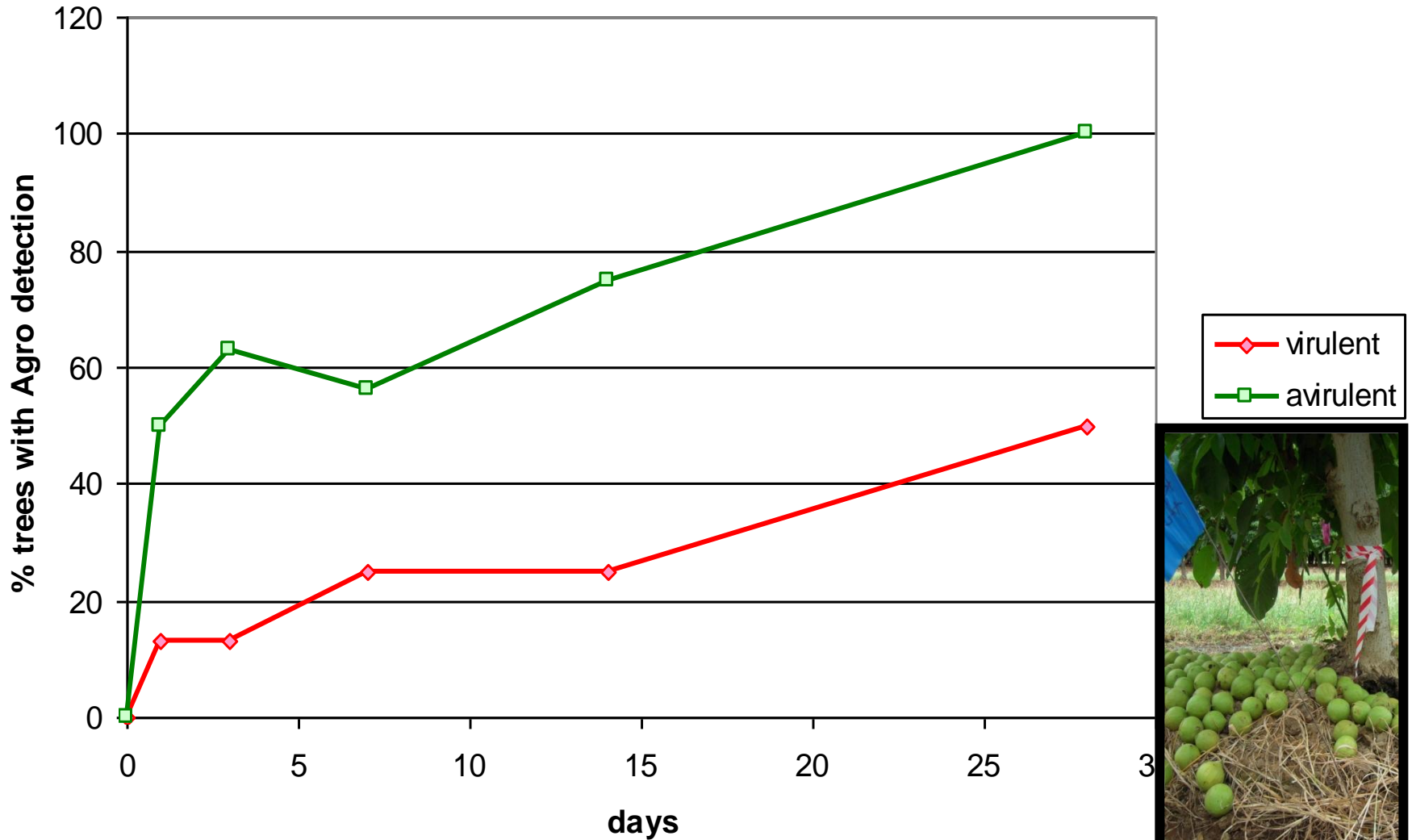
**Virulent/Avirulent**  
**Agro. Detected**



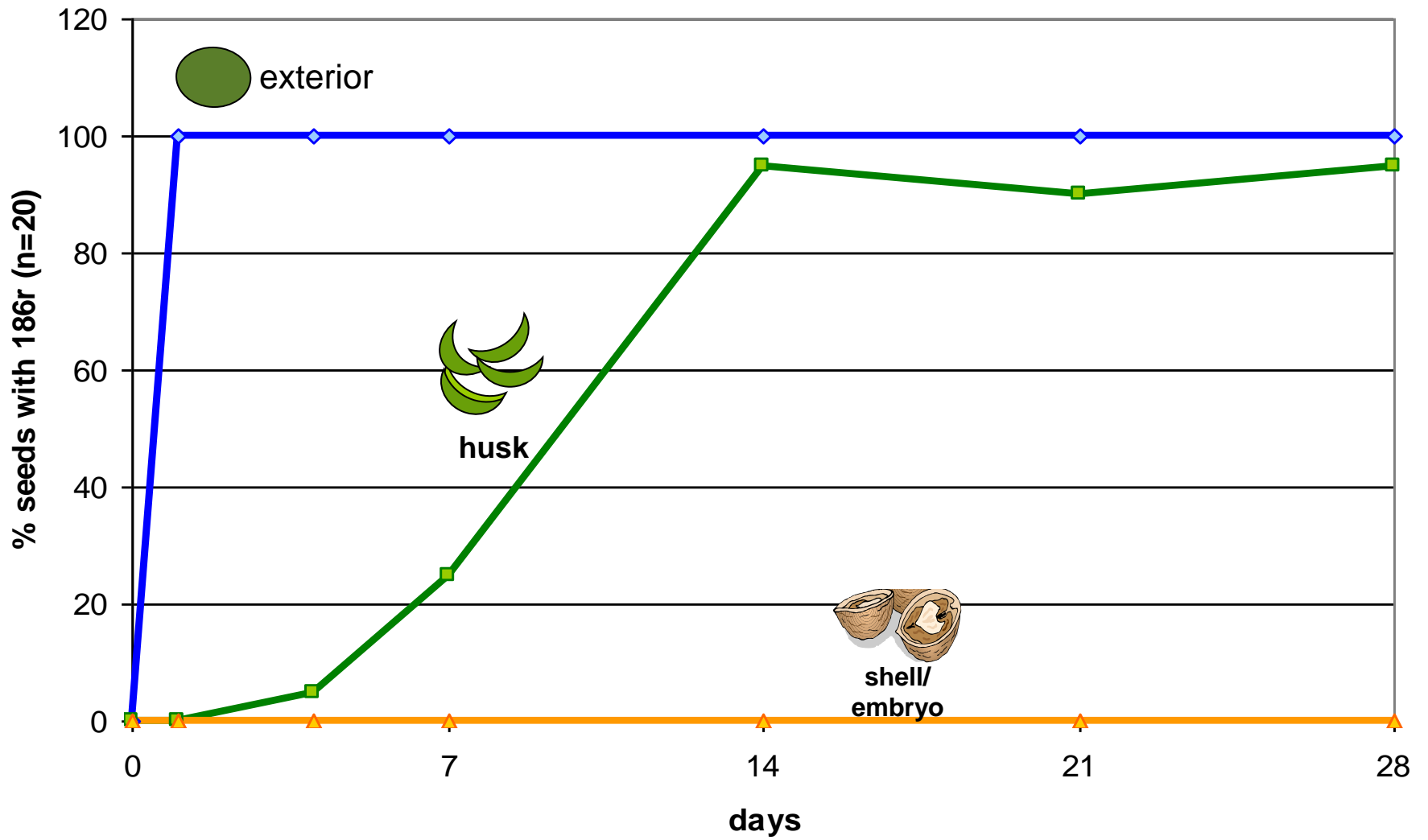
exterior



**Off orchard floor  
(1,3,7,14,28 days on  
soil)**



**Contact with the orchard floor increases the probability of picking up *A. tumefaciens*.**



# seed inoculum summary

- *A. tumefaciens* not detected from seeds collected directly from mother trees
- *A. tumefaciens* picked up from orchard floor

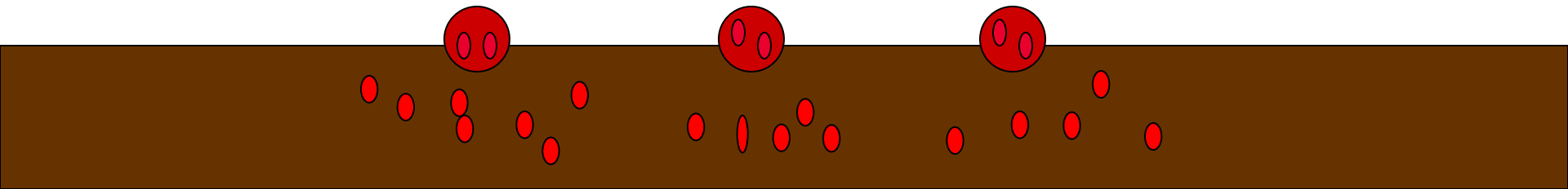


**SO WHAT ??**

## MeBr fumigated soil

- 1) Biological vacuum generated by MeBr fumigated soil results in... no/limited microbial antagonists

New direction: modifying soil-borne microbial communities in fumigated soils



# Conclusions

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- If left on orchard floor, Paradox seeds can become contaminated with *A. tumefaciens*
- Nuts harvested directly from mother trees did not contain *A. tumefaciens*

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# Evaluation of wild *Juglans* species for crown gall resistance

Daniel Kluepfel<sup>1</sup>, Malli Aradhya<sup>1</sup>, Malendia Maccree,  
Jeff Moersfelder<sup>1</sup>, Ali McClean<sup>1</sup>,  
and Wes Hackett<sup>2</sup>

<sup>1</sup>USDA-ARS, <sup>2</sup>University of California Davis, CA

# Goals

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1. Identify sources of crown gall (CG) resistance in the *Juglans* germplasm collection. (USDA/ARS National Clonal Germplasm Repository in Davis, CA)
2. Generate new hybrids between *J. regia* and select *J. microcarpa* (TX black walnut) mother trees with demonstrated CG resistance.  
“i.e. generate new “Paradox-like” rootstocks”
3. Clonally propagated cuttings from genotypes exhibiting crown gall resistance.

# Walnut Germplasm

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**Open pollinated seedlings grown in greenhouse conditions**

# Walnut Germplasm Screen

Inoculation method



**Stab technique**

# Walnut species screened previously

*J. ailantifolia*  
*J. californica*  
*J. cathyensis*  
*J. hindsii*  
*J. major*  
*J. hopeinsis*  
*J. mandshurica*  
*J. microcarpa*  
*J. nigra*  
*J. regia*  
*J. sinensis*  
*Pterocarya sp.*

*J. microcarpa* and *Pterocarya* species exhibited highest levels of gall resistance (>3,500 seedlings examined)

# Walnut germplasm screened in 2011

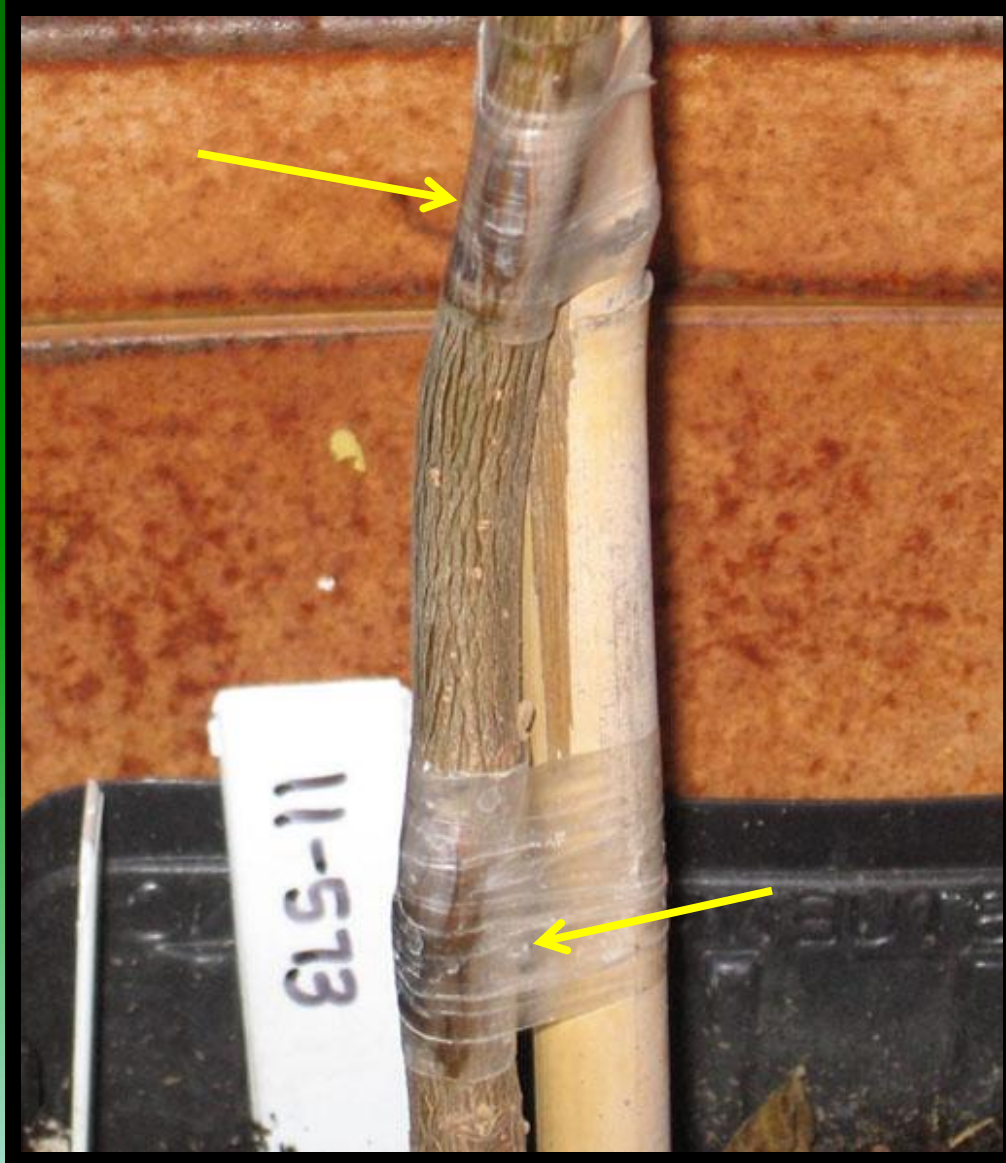
<b>Tree classes</b>	<b>Tested</b>	<b>Retained</b>	<b>% Retained</b>
Individual trees	273	95	36
<i>Juglans</i> species	6	3	50

<b><i>Juglans</i> species</b>	<b>Tested</b>	<b>Retained</b>	<b>% Retained</b>
<i>J. ailantifolia</i> var. <i>cardiforum</i>	2	0	0
<i>J. californica</i>	3	3	100
<i>J. cathayensis</i>	16	3	19
<i>J. major</i>	2	0	0
<i>J. mandshurica</i>	1	0	0
<i>J. microcarpa</i> (26 MT's)	249	89	37

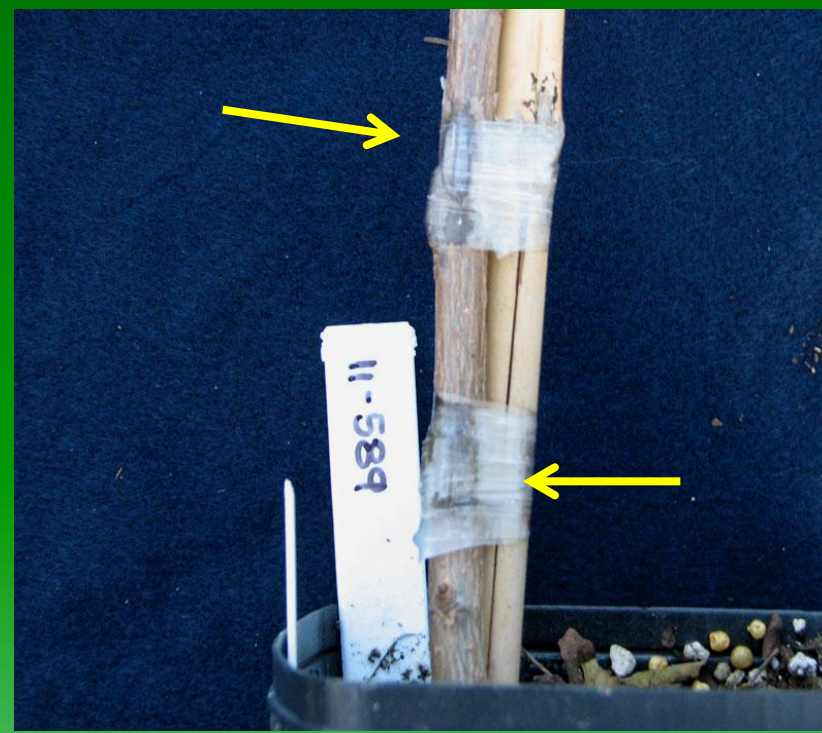
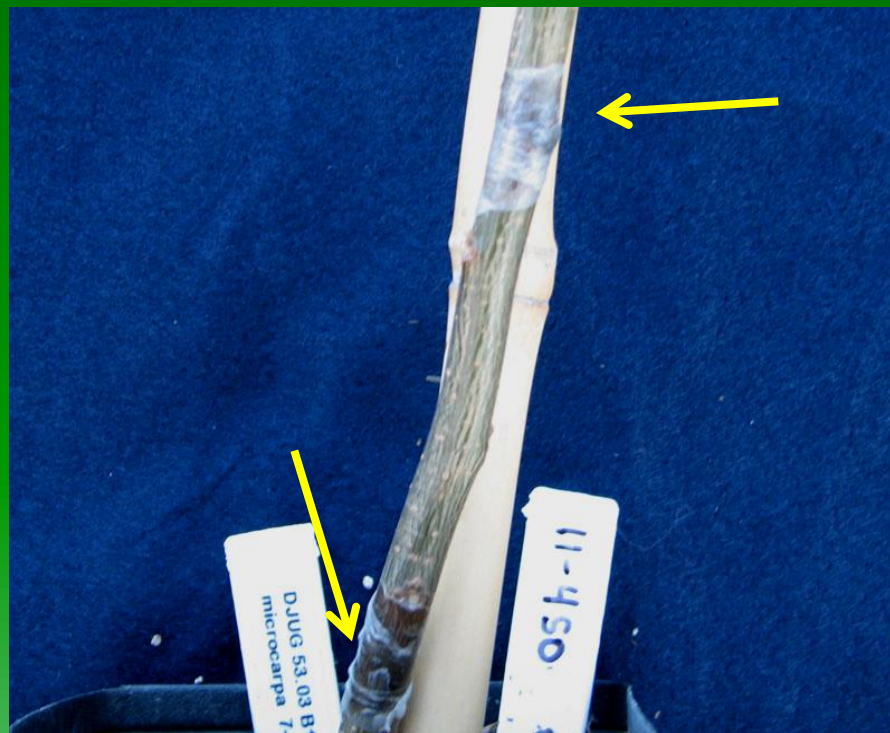
# Discarded Plants



# Resistant Plant



# More Resistant Plants



# How do we validate “resistant” plants?

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Rescreen all putative resistant trees after at least one dormancy period.

Test rooted cuttings derived from putative resistant plants for persistence crown gall resistance or tolerance.

## 2010 cuttings re-screened in 2011

Species	Total cuttings	Rooted cuttings	Rooting efficiency	2011 retest	
				No. cuttings	No. retained
<i>J. microcarpa</i>	333	25	7%	22	18
<i>Pterocarya sp.</i>	47	31	66%	31	21
<i>J. ailantifolia</i>	2	1	50%	1	1
<i>Juglans sp.</i>	11	0	0%	0	0
	<b>total</b>	<b>total</b>		<b>total</b>	<b>total</b>
	<b>393</b>	<b>57</b>	<b>14%</b>	<b>54</b>	<b>40</b>

# Conclusions

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Crown gall resistant *Juglans* and *Pteracarya* genotypes have been identified after screening over 3500 seedlings.

*J. microcarpa* is the most promising *Juglans* species tested and can be propagated clonally via rooted cuttings.

Efforts are ongoing to improve rooting efficiency.

This resistance will be incorporated into new hybrid rootstocks with reduced crown gall susceptibility.

# “Suggestions” for limiting Crown Gall

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Limit exposure of paradox seeds/graft wood to soil.

Surface sterilize grafting tools frequently.

Limit time between nursery pickup and planting.

Fumigate planting hole/avoid replanting in same hole

Treat bare roots with Galltrol K84(?) (no dip tanks).

Limit wounding.

Avoid planting too deep.

Avoid mounding soil up on newly planted trees.

Keep base/crown of tree dry.

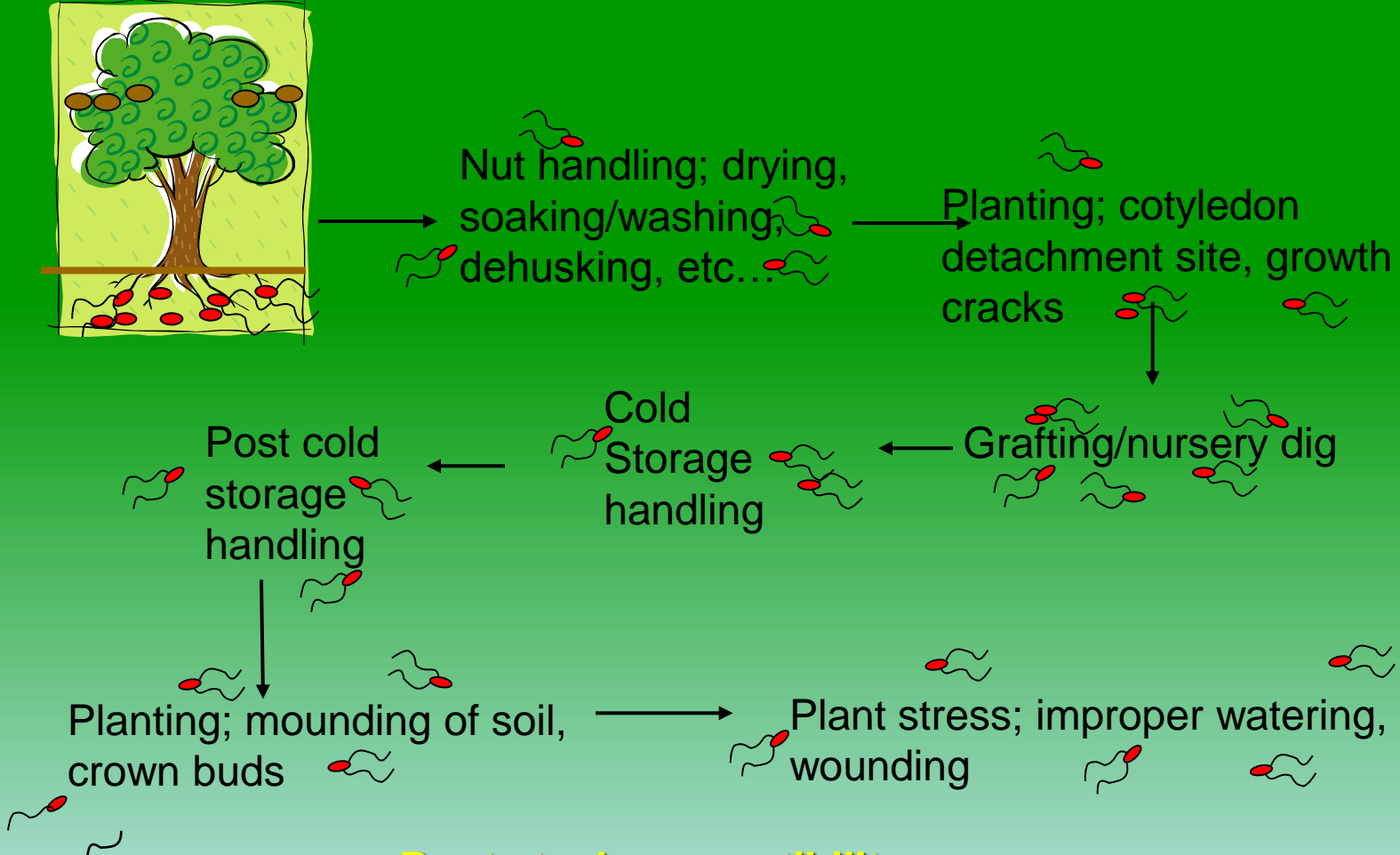
# Crown Gall Update:





# The "Big" Picture

## Paradox source tree

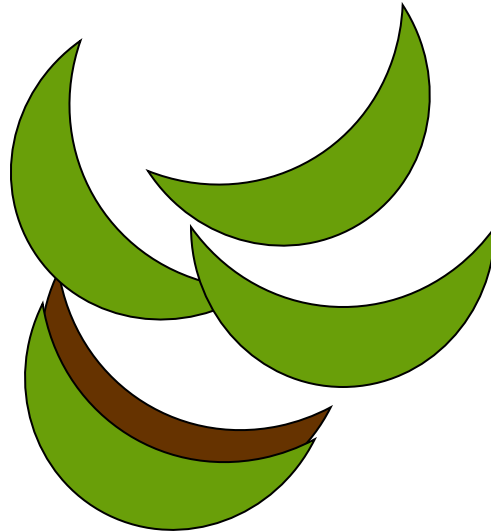


**Root stock susceptibility**

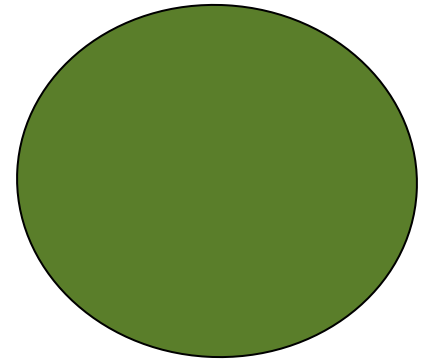
### 3) Is *A. tumefaciens* in/on the seed?



shell/ embryo

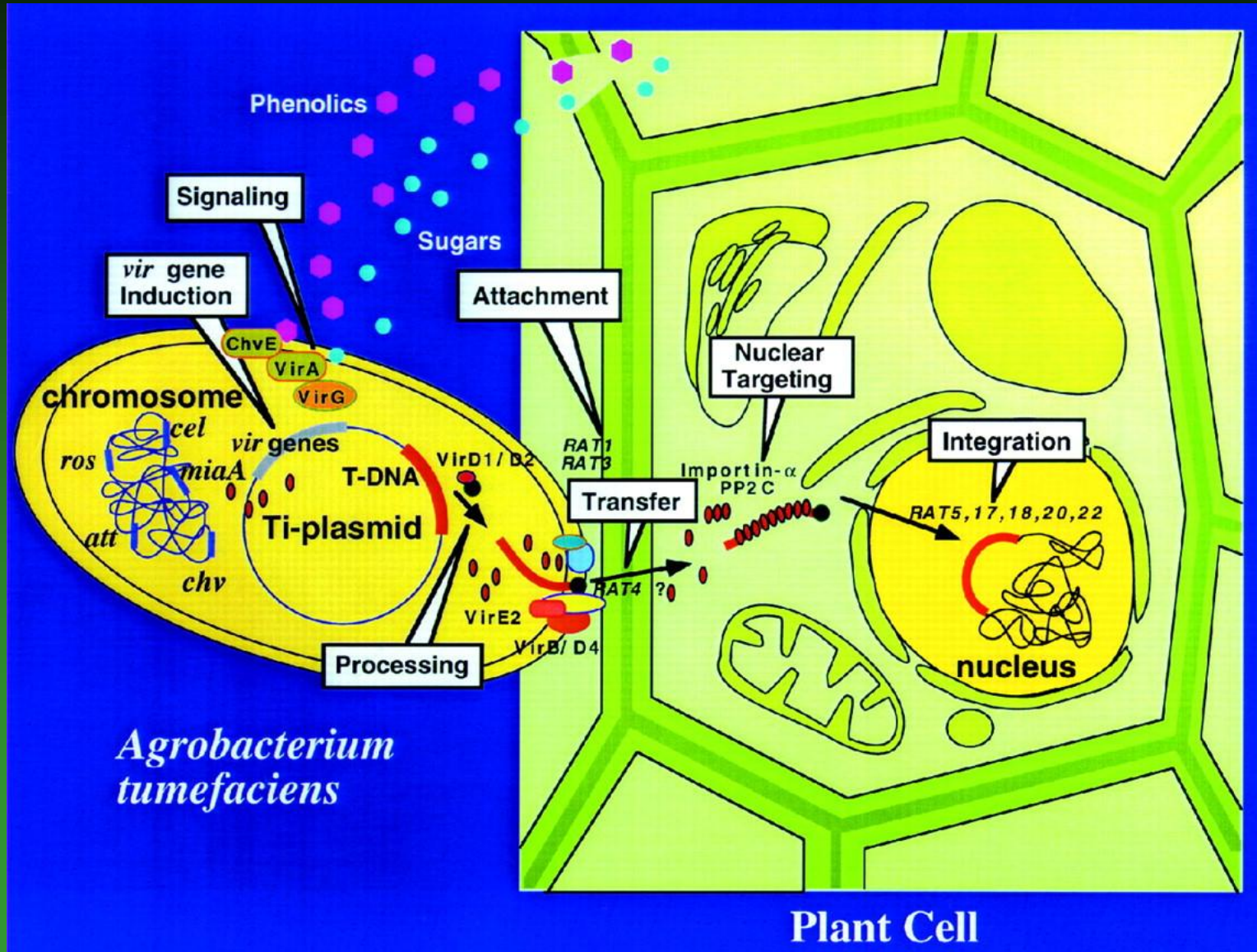


husk

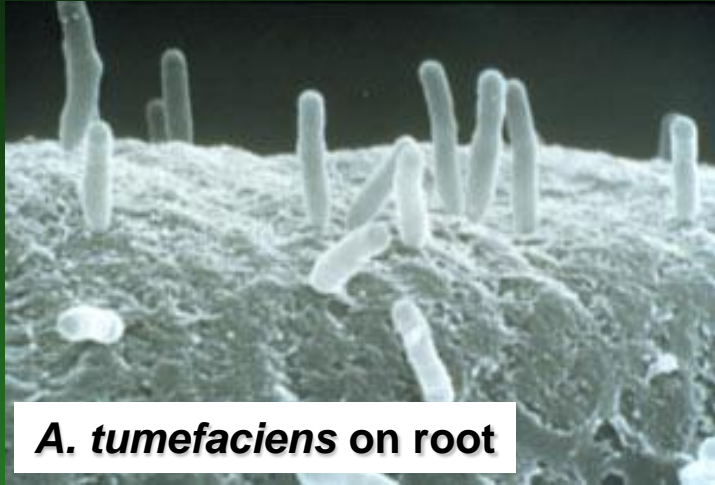


exterior

# Crown Gall Disease



# Crown Gall Disease



**A. tumefaciens on root**

## Agrobacterium tumefaciens

- soil-borne bacterium
- long term persistence
- natural genetic engineer
- widest host range of any plant pathogen (i.e. “all” dicots)

## Paradox is highly susceptible

- good rhizosphere colonist



# Soil Survival of *A. tumefaciens* Fallow Soil

