

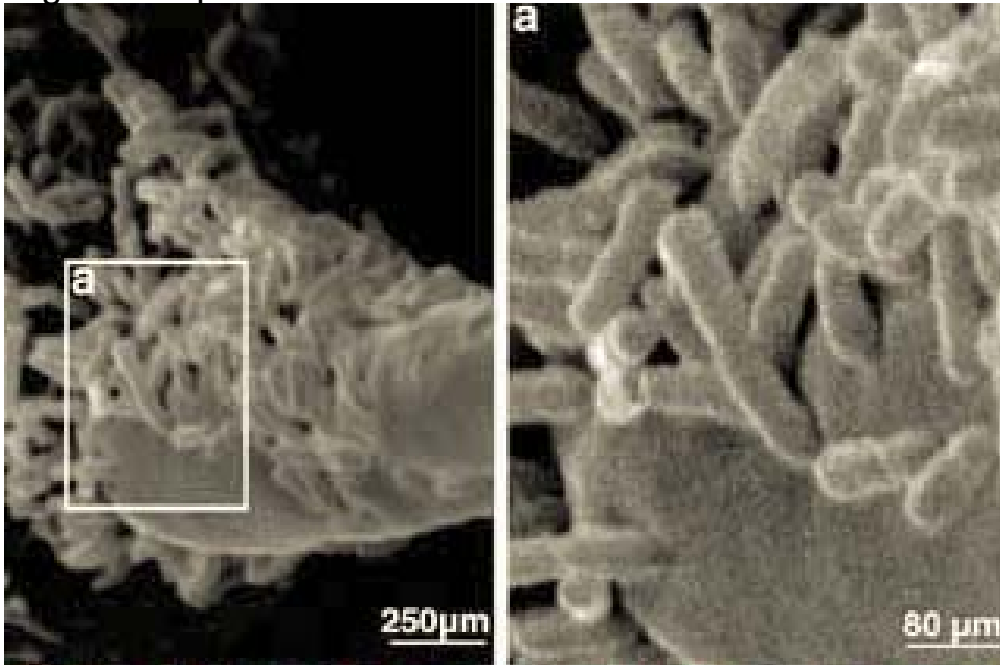
Crown Gall: Current and Future Management Methods



The crown gall pathogen

Agrobacterium tumefaciens

Agro on aspen roots



<http://www.mindfully.org/GE/Vitaly-Citovsky-Projects.htm>

Agro on tobacco

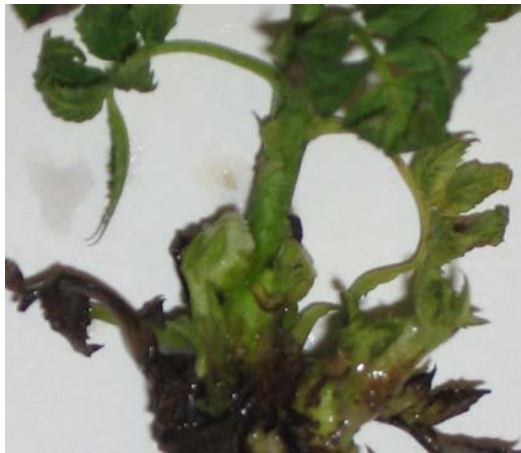


SCIMAT/SCIENCE PHOTO LIBRARY
B2420004-Agrobacterium_tumefaciens-SPL.jpg
<http://www.sciencephoto.com/media/13176/enlarge>

- Gram negative soil-borne bacterium
- free living or plant associated

Walnut susceptibility

Some *Juglans* spp. are susceptible especially Paradox



'Chandler' in tissue culture



NCB potted seedling



J. hindsii potted seedling



field seedlings



subterranean galls

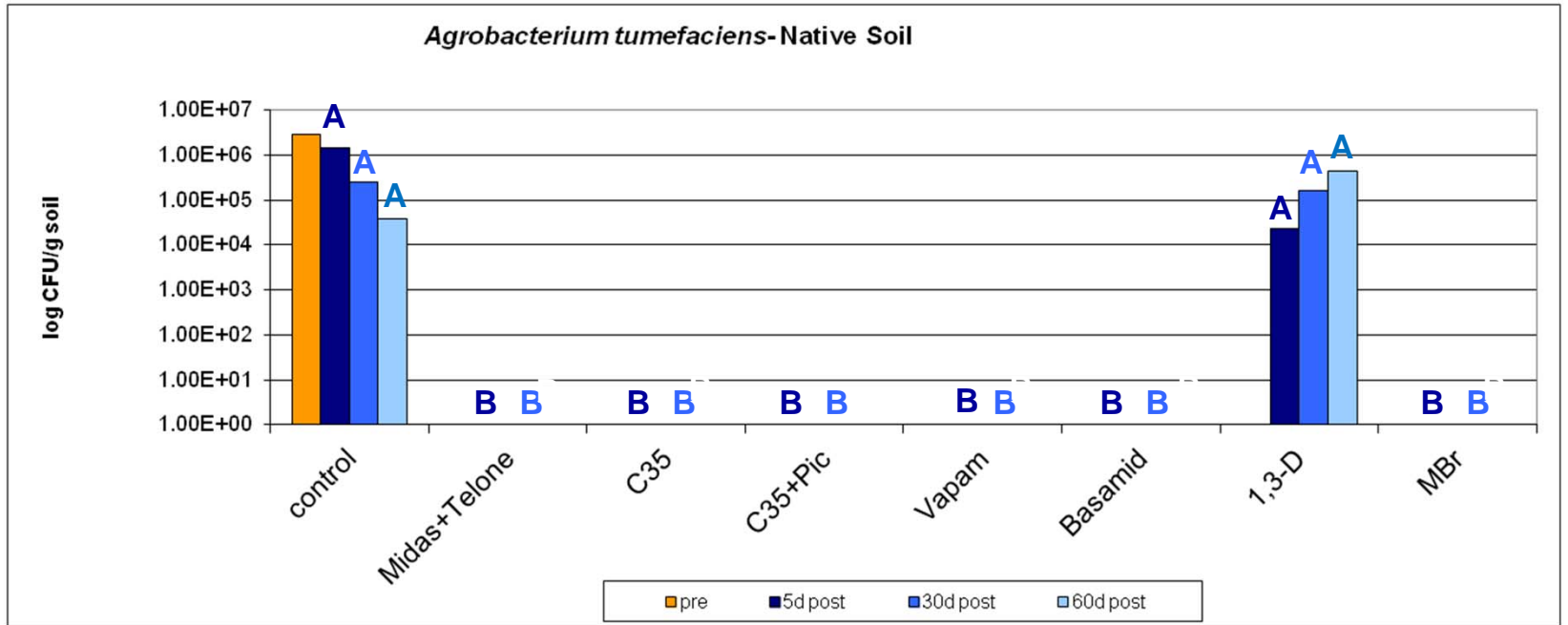
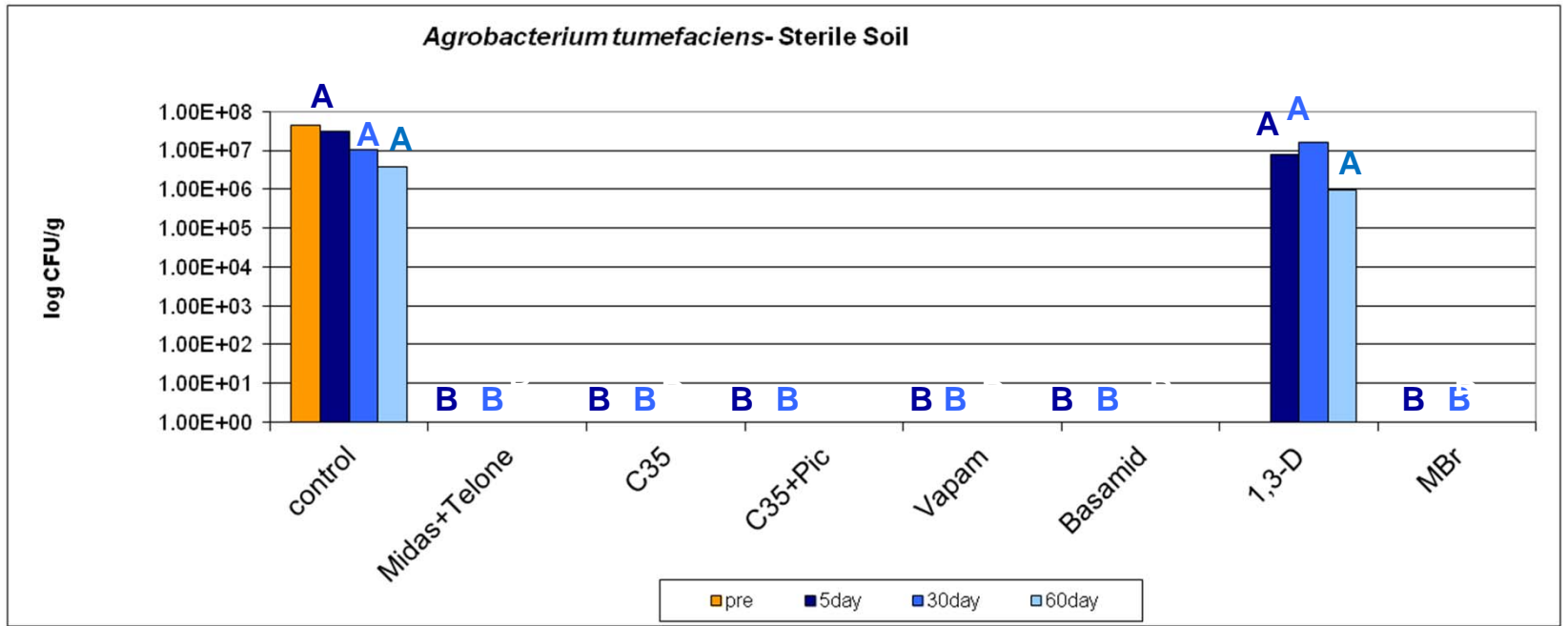


orchard tree

Control of Crown Gall Disease

- Fumigation/Chemical Control
- Plant Clean Paradox Seeds
- Implications of contaminated graft wood
- “other approaches”
 - K84 biocontrol
 - BioSI
 - Popcorn Sulfur

Efficacy – *A. tumefaciens*

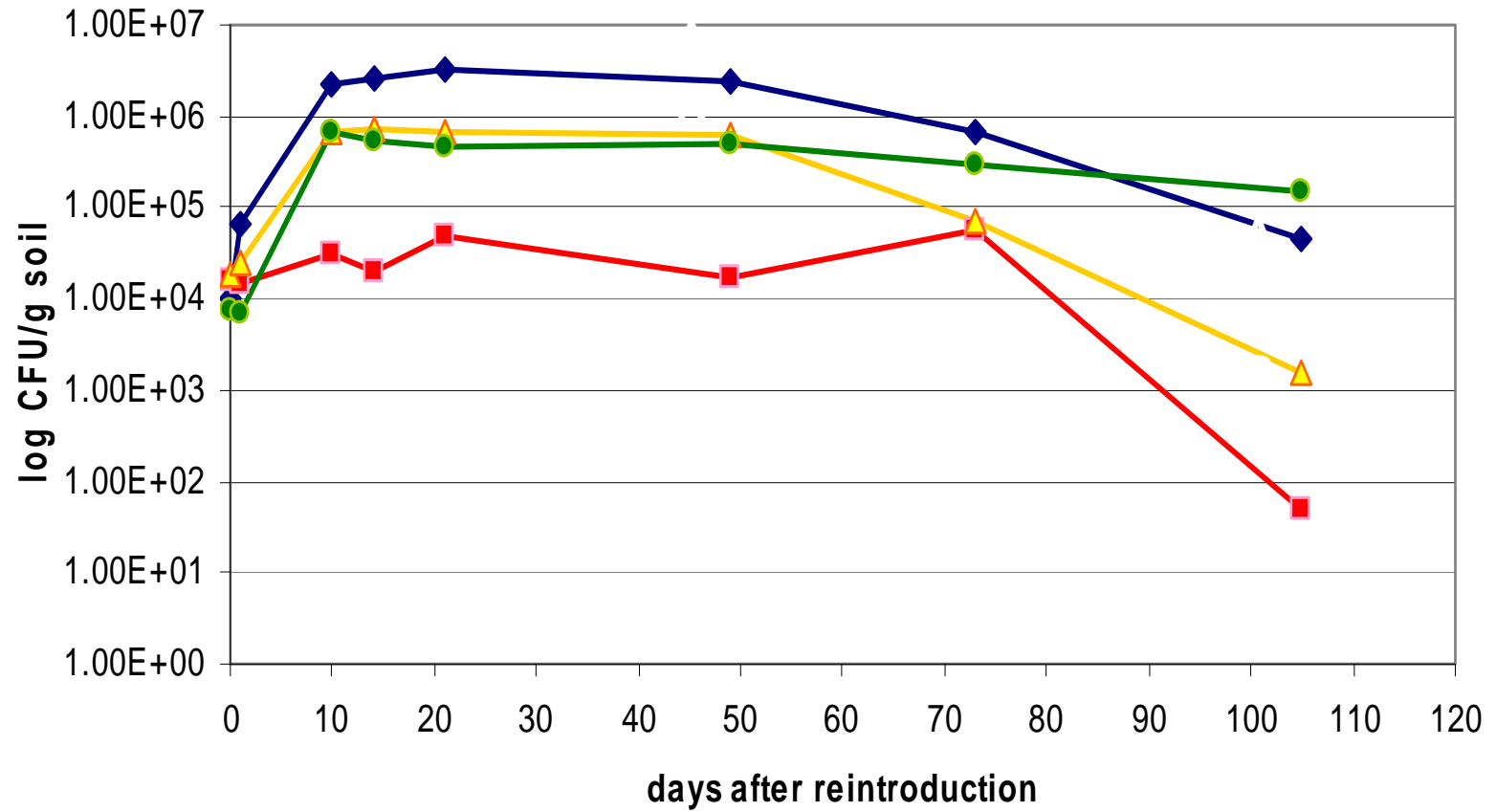


Agro in Galls

- 2"-4" galls buried in sterile soil

Treatment	<u><i>A. tumefaciens</i> Detected (120d)</u>	
	<u>In galls</u>	<u>In soil</u>
MeBr (400lb/acre)	0/6*	0/6*
TC35 (49 gal/acre)	3/6	5/6
TC35 + pic	0/8*	0/8*
Non-fumigated control	16/16	14/16

A. tumefaciens Recolonization



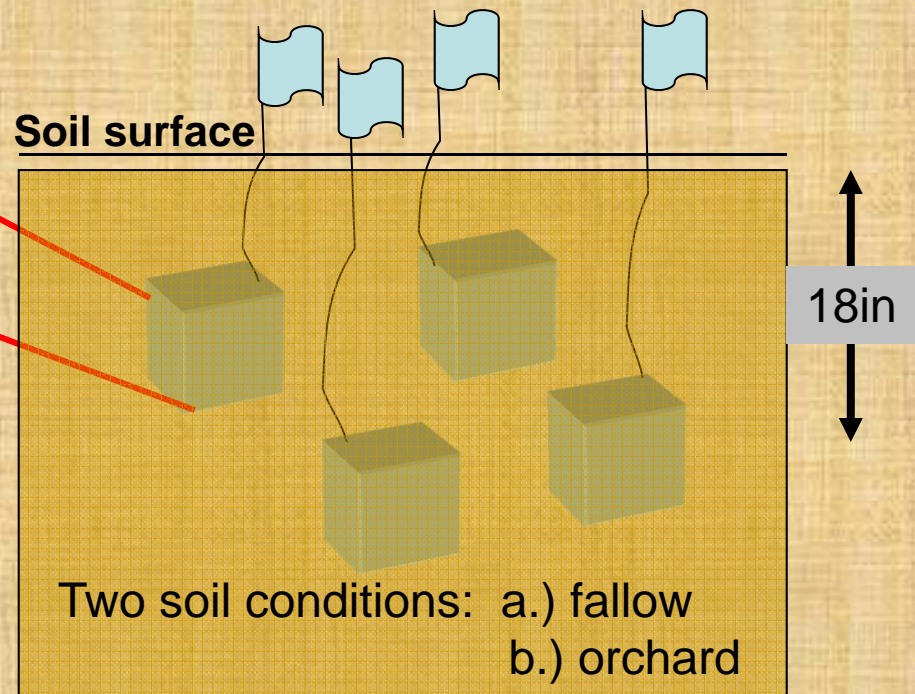
◆ MeBr ■ Native ▲ C35 ● Sterile

Conclusions

- MeBr and many alternatives control *Agrobacterium 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

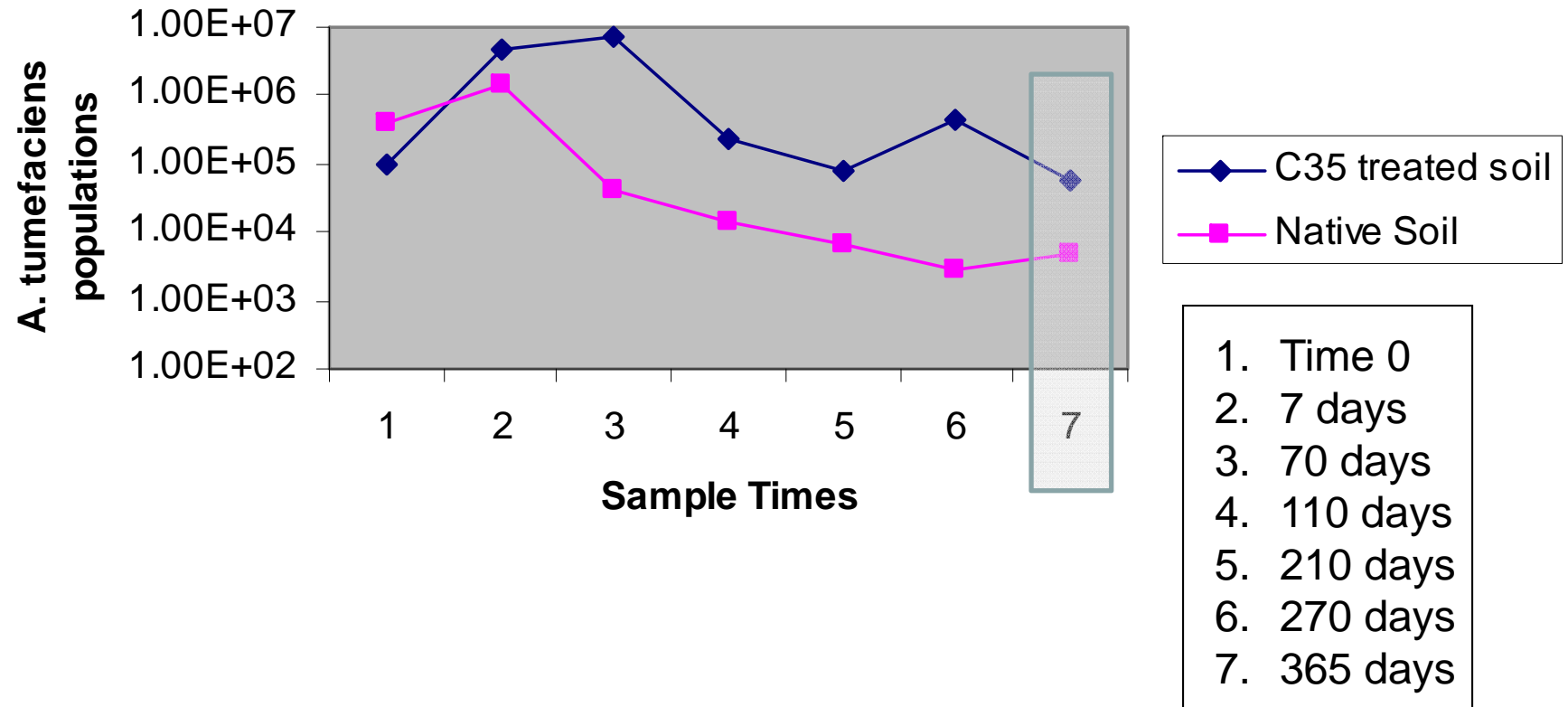
- a) C35 trt soil
 - b) Native soil
- +
- A. tumefaciens*



Dig up pouch after
given time interval

Determine *A. tumefaciens*
populations in soil pouches

Soil Survival of *A. tumefaciens* Orchard Row



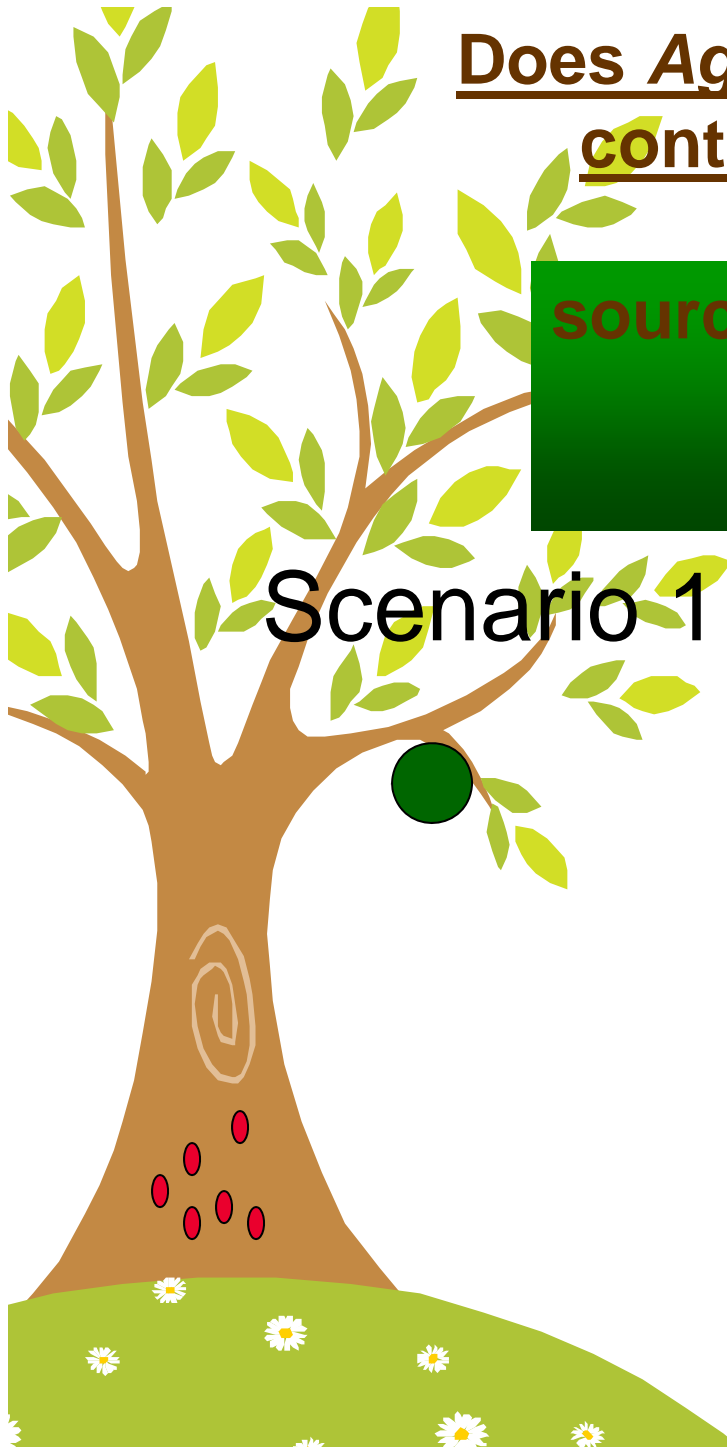
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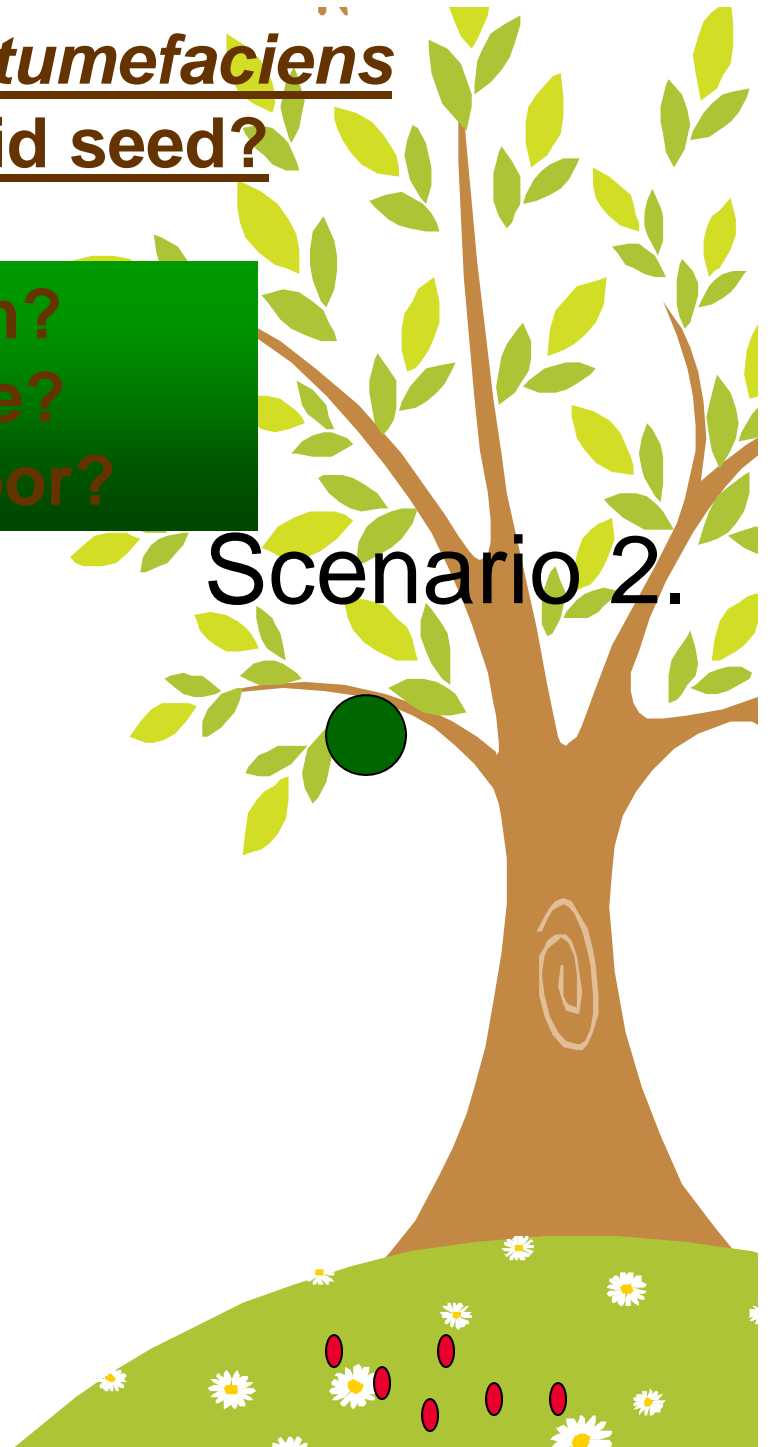
Does *Agrobacterium tumefaciens* contaminate hybrid seed?

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

Scenario 1.



Scenario 2.





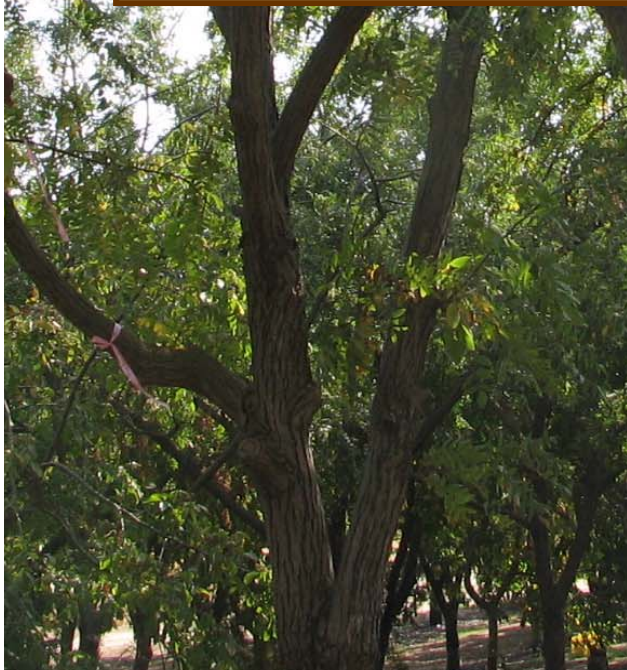
Directly off tree



Off orchard floor

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

Mother Block Survey



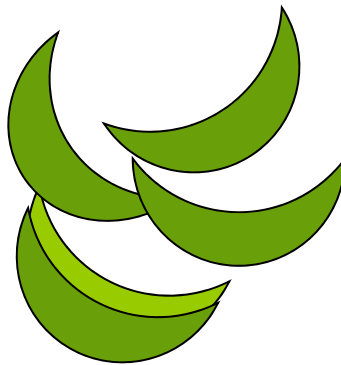


Directly off tree
(>2000 nuts examined)



shell/ embryo

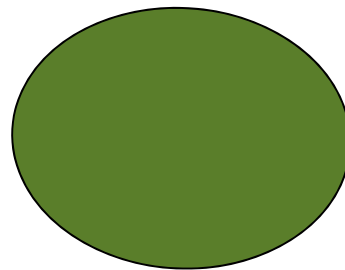
No Agro Detected



husk

No Agro Detected

-inhibitors



exterior

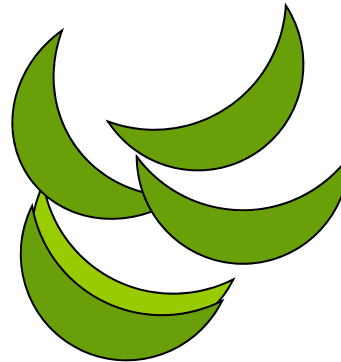
No Agro Detected

No Agro detected...yet



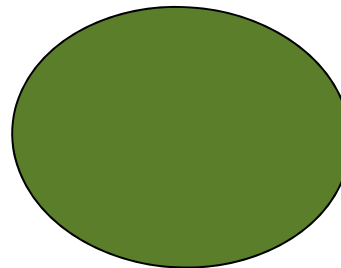
shell/ embryo

Virulent/Avirulent
Agro. Detected



husk

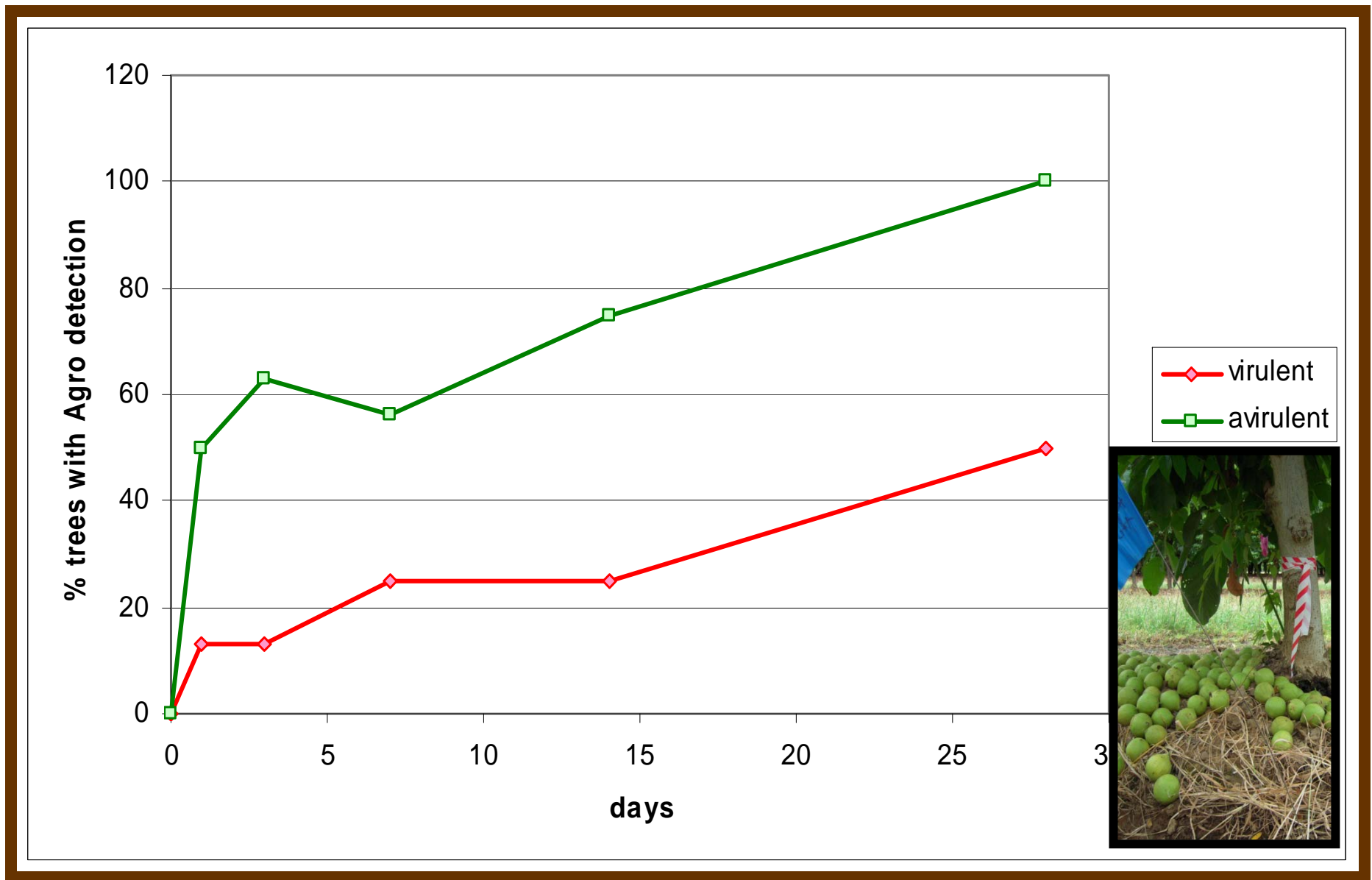
Virulent/Avirulent
Agro. Detected



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 on/in seeds collected directly from mother trees
- *A. tumefaciens* contaminates seeds which hit the orchard floor

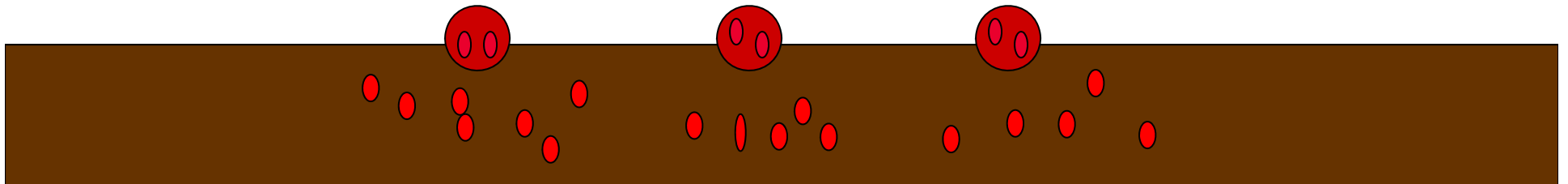


SO WHAT ??

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

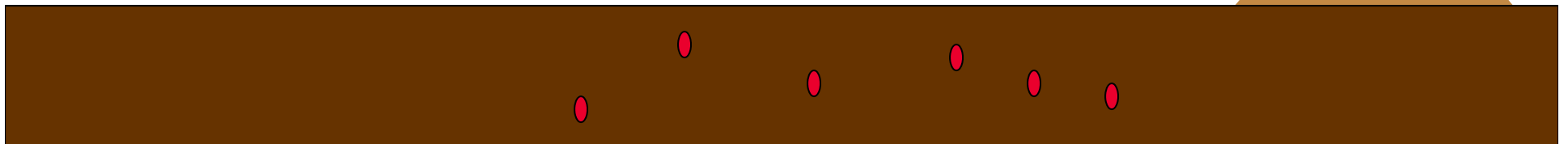


Control of Crown Gall Disease

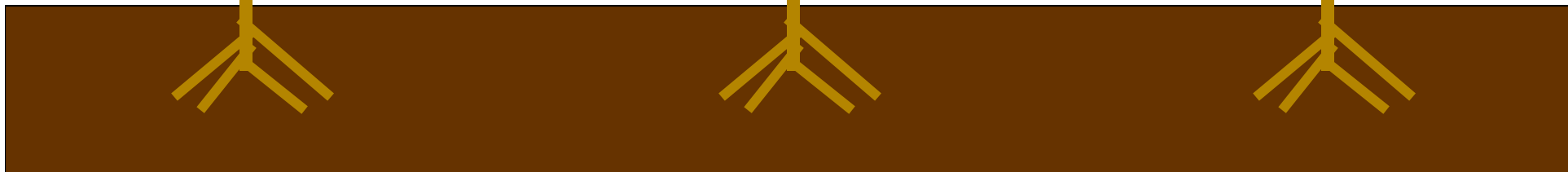
- Fumigation/Chemical Control
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Determine source of inoculum and role of surface contaminated cuttings in crown gall development



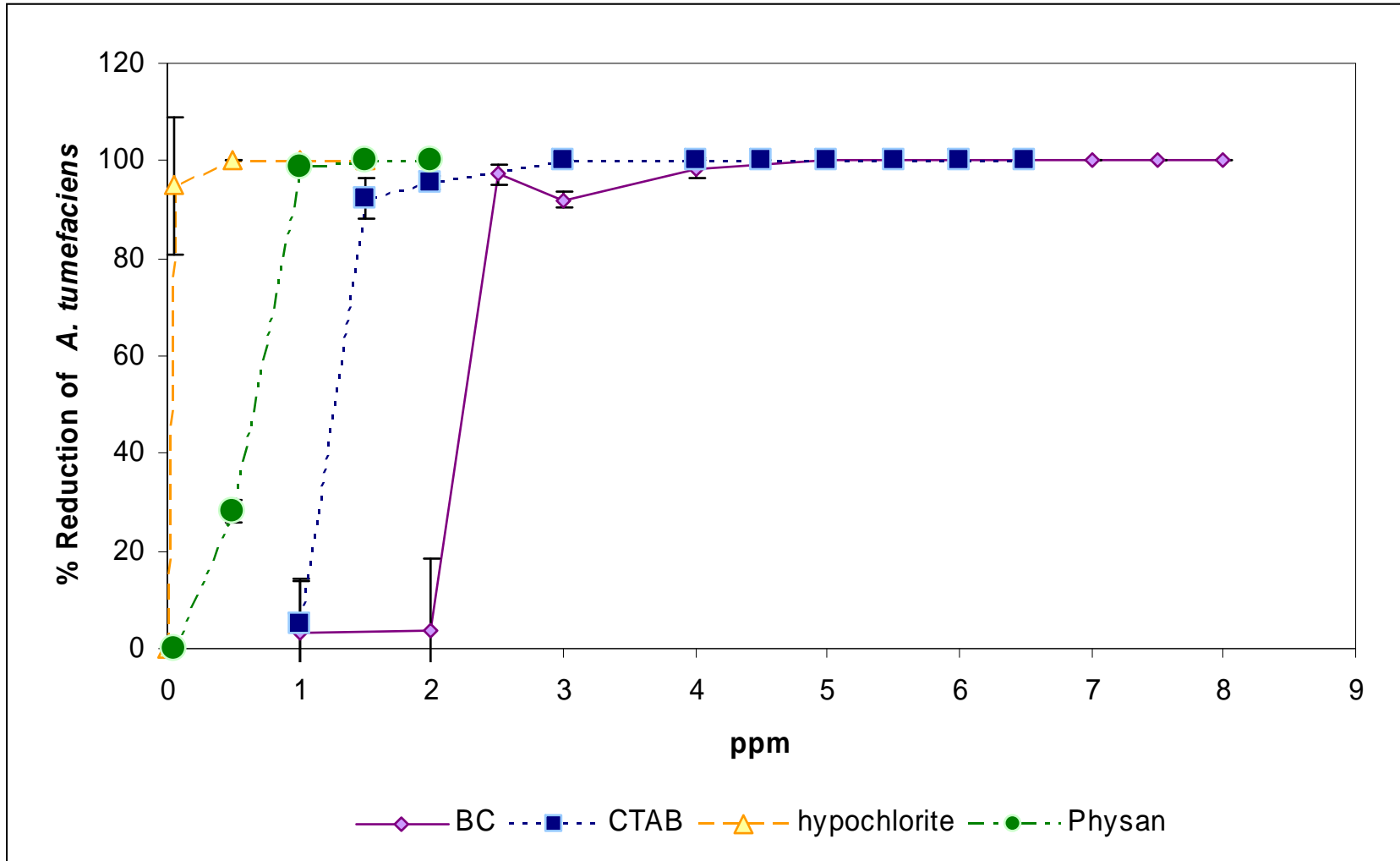
Is there a solution?



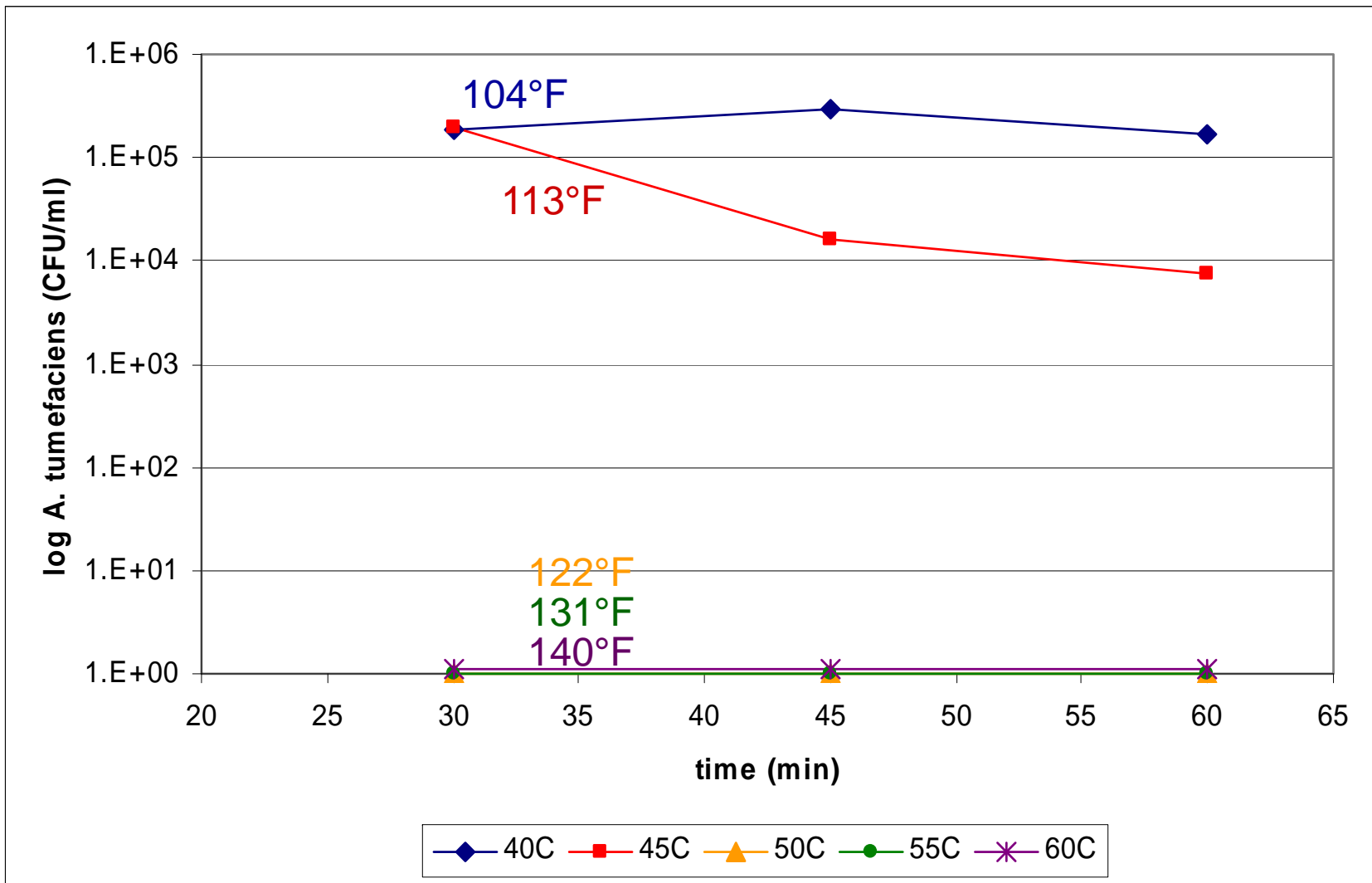
Objectives

- Develop treatment for graft wood
 - heat : 55°C for 60 min
 - 55°C for 45 min
 - 53°C for 60 min
 - surfactant : BC/CTAB 5000 ppm for 20 min

Surfactants – external contamination



Heat – external/internal contamination



Control of Crown Gall Disease

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Evaluation of wild *Juglans* species for crown gall resistance

Daniel Kluepfel¹, Malli Aradhya¹, Jeff Moersfelder¹,
Ali McClean¹, Diane Velasco¹,
and Wes Hackett²

¹USDA-ARS, ²University of California Davis, CA

Walnut Germplasm Screen



seedlings in greenhouse conditions



Walnut species screened previously

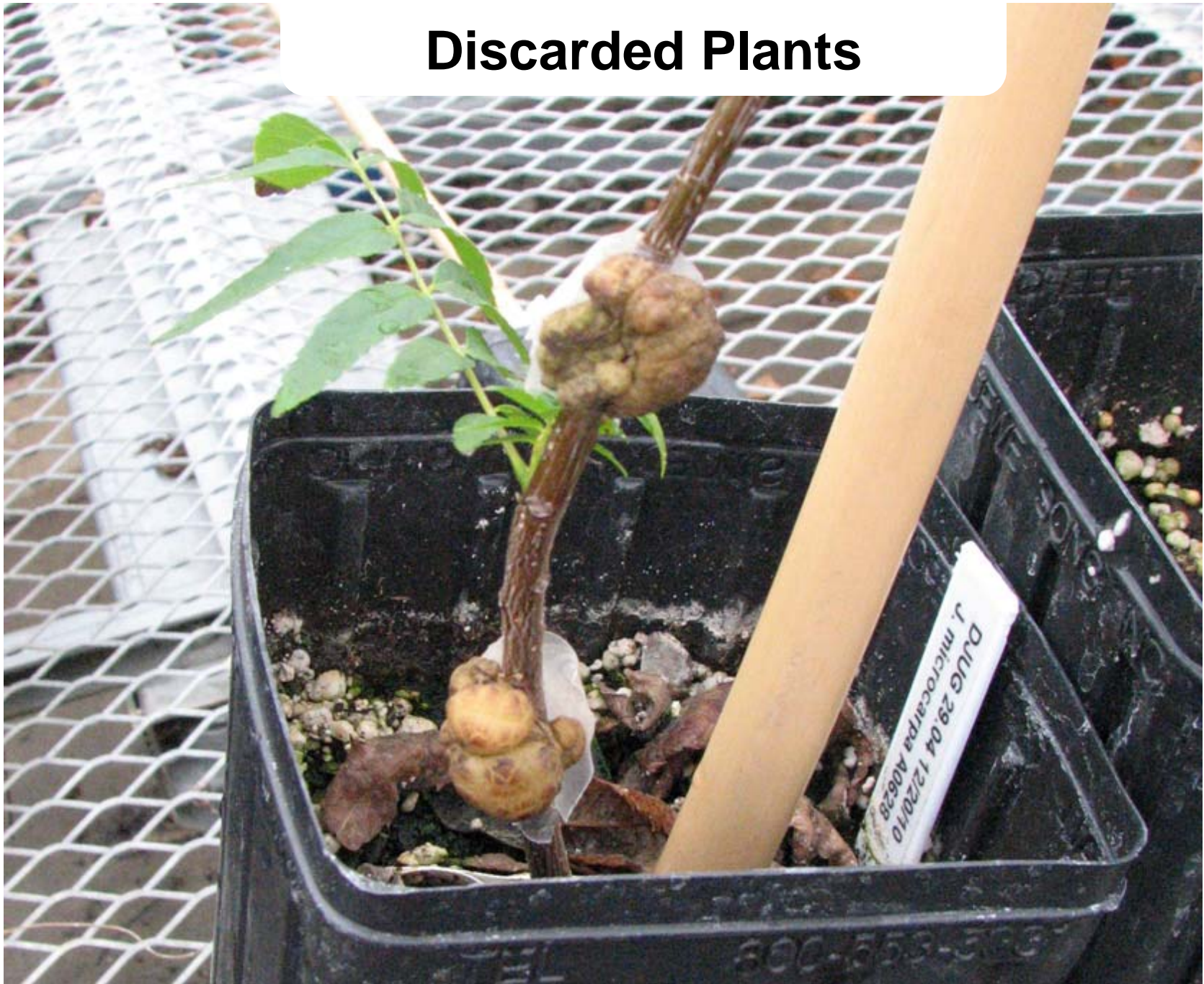
J. ailantifolia
J. californica
J. cathyensis
J. hindsii
J. major
J. hopeinsis
J. mandshurica
J. microcarpa
J. nigra
J. regia
J. sinensis
Pterocarya sp. (wingnut)

J. microcarpa and *Pterocarya* species exhibited the most crown gall resistance after multiple annual screenings

Discarded Plants



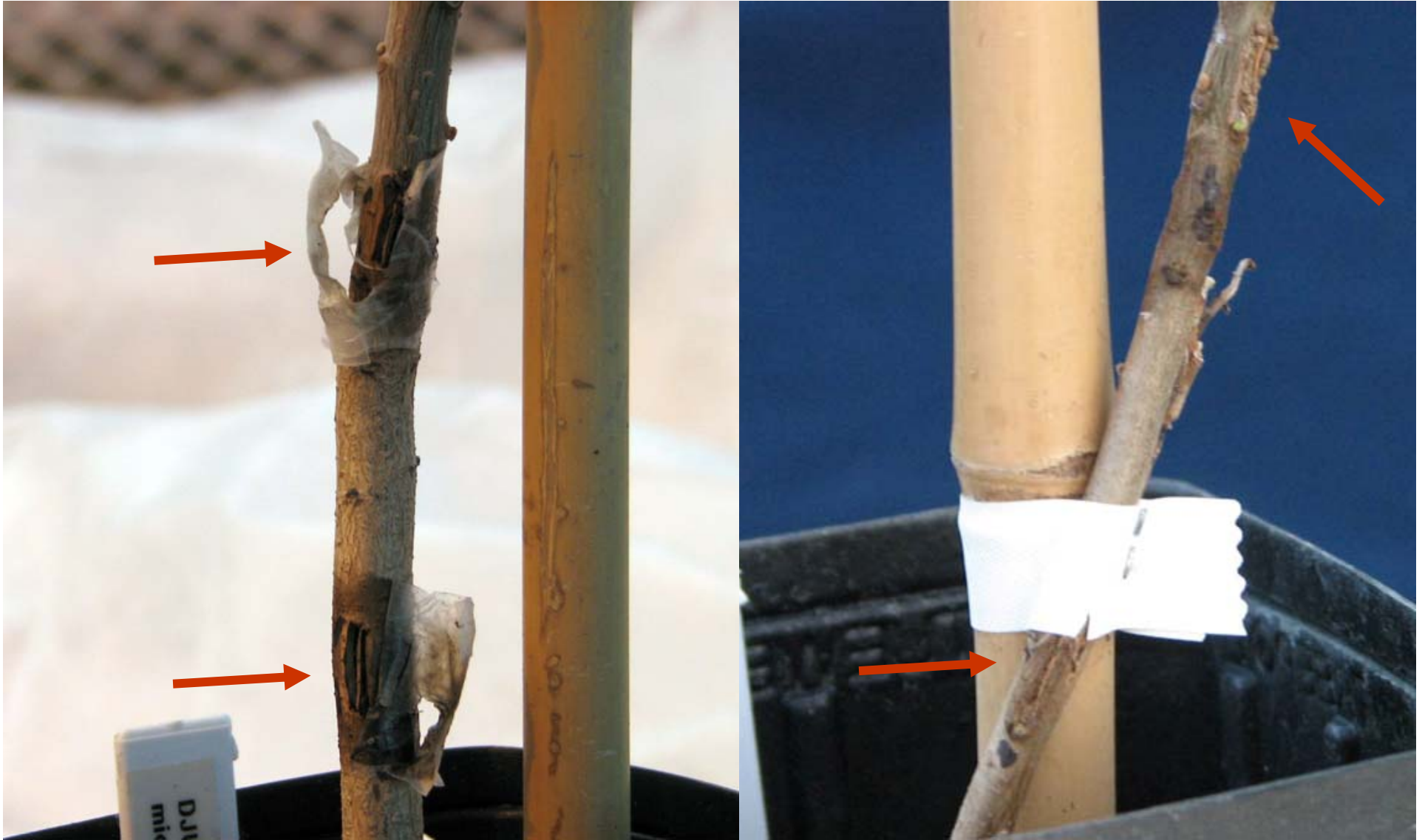
Discarded Plants



Resistant Plants



Resistant Plants



Progress in Genetic Mapping of Crown Gall Resistance

J. microcarpa (CG resistant) **X** *J. regia* 'Serr' (CG susceptible)
A "new" Paradox-like rootstock

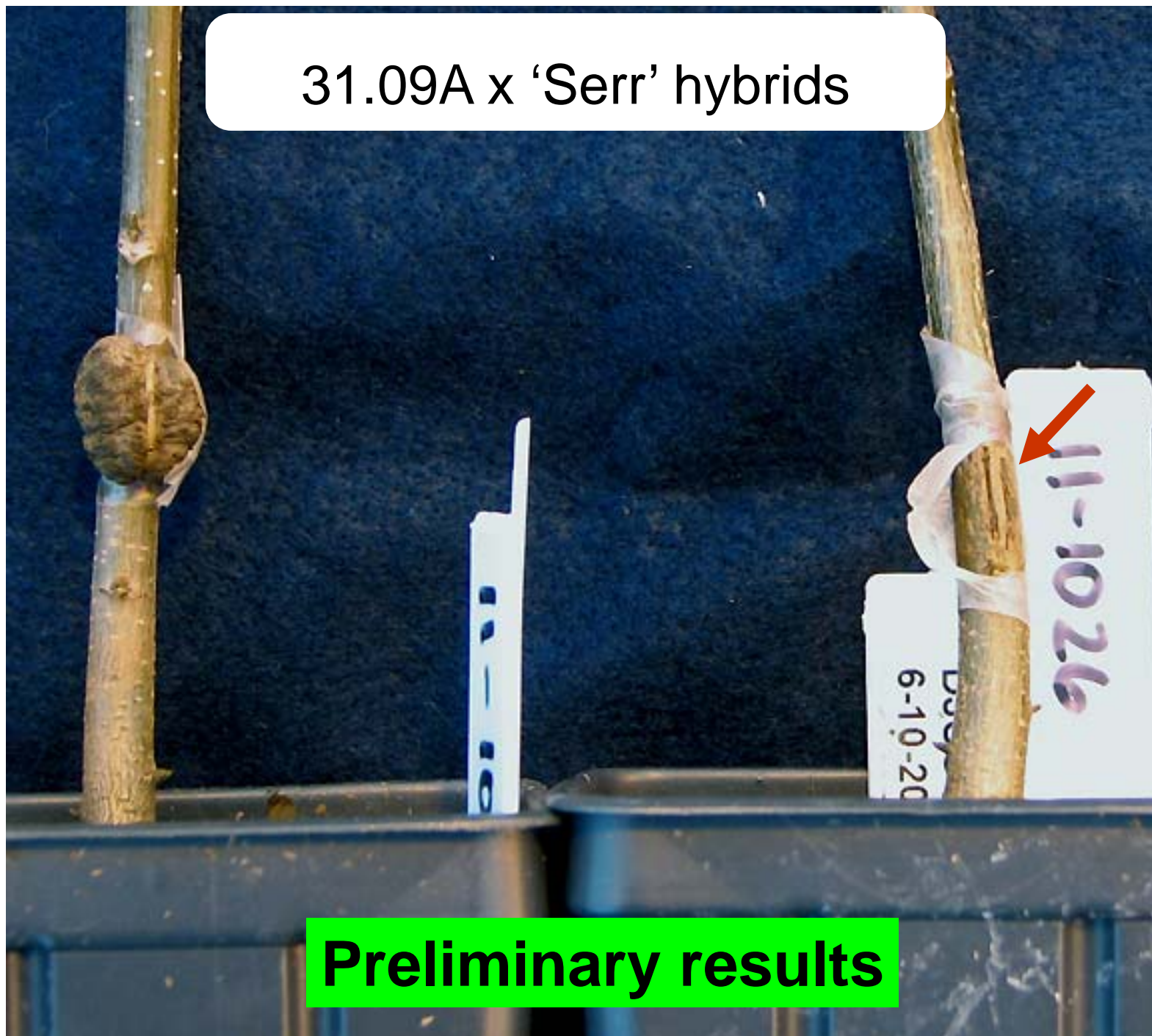
F1 hybrid progeny was propagated

54 seedlings were screened for CG resistance

Preliminary assessment: **35/54** with galls and **19/54** with no galls

Resistant individuals will be re-evaluated in the spring

31.09A x 'Serr' hybrids



Preliminary results

“Suggestions” for limiting Crown Gall

“The 9 point program”

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 crown of tree dry.



Crown Gall Update:

