Evaluation of wild *Juglans* species for crown gall resistance

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Crown Gall Disease of Walnuts

Causative agent:
Agrobacterium tumefaciens

- > ubiquitous soil-borne bacterium
- > long term persistence
- > natural genetic engineer
- > wide host range; (”all” dicots)
- > economic impact on walnut
- > essentially “girdles” trees
- > **Paradox root stock highly susceptible**

Outline

- Selection of CG resistant walnut germplasm
- Fumigation; alternatives to MeBr for Agrobacterium control
- Enhanced collection/propagation procedures for Paradox seed

Screening of NCGR *Juglans* germplasm for Crown Gall Resistance

- Collect open-pollinated nuts from "mother trees" in germplasm collection
- Germinate nuts and grow seedlings under greenhouse conditions
- Inoculated with suspension of *A. tumefaciens* strain EC-1:
- evaluated tumor development:


Osmocote PLUS
15-9-12




Office DEPOT

Stem inoculations

- A “T-cut” is made through the cambium layer of the seedling stem

Stem inoculations

- **Outer bark/phloem is gently peeled back**

Stem inoculations

- 0.5 ml of log-phase *Agrobacterium* inoculum is pipeted into wound

Stem inoculations

- **Wound is wrapped with parafilm and grown in the greenhouse for 1-3 months**

Germplasm Screening

Agrobacterium inoculated

Water Control

Germplasm Screen: 2005-2008 Summary

- Todate we have screened over 1900 individual germplasm seedlings under greenhouse conditions for Crown Gall Resistance.
- This work has examined:
    - 2 genera;
    - 12 species;
    - 328 accessions.

Germplasm Screened 05-08						Root Cuttings		
Genus	**Species**	**Accessions**		**No. of Trees Tested**	**Trees Retained @ 60 Days**		**Retested**	**Retained**
Juglans	hindsii	105		645	53		6	0
Juglans	**major**	**80**		**505**	**63**		**8**	**4**
Juglans	**microcarpa**	**22**		**85**	**13**		**7**	**6**
Juglans	nigra	8		56	8		0	0
Juglans	regia	15		34	15		2	0
Juglans	ailantifolia	64		291	89		5	0
Juglans	cathayensis	2		12	1		0	0
Juglans	**mandshurica**	**11**		**74**	**18**		**1**	**1**
Juglans	sinensis	2		7	0		0	0
Pteracarya	**sp**	**5**		**29**	**13**		**15**	**9**
Totals	**12**	**328**		**1803**	**282**		**44**	**20**

Where will we go from here?

- Material showing high levels of resistance will be will be clonally propagated.
- Graft compatibility determination.
- Re-examine original mother tree to determine frequency of CG resistant offspring. Perform directed crosses…generate “new” CG resistant Paradox.

Outline

- Selection of CG resistant walnut germplasm
- Fumigation; alternatives to MeBr for Agrobacterium control

Fumigation Outline

1. Efficacy of MeBr alternatives
2. Fumigation of gall tissue
3. Effects of fumigants on soil aerobic bacteria
4. Post-fumigation soil recolonization by *Agrobacterium tumefaciens*

MeBr ALTERNATIVES and WALNUTS

Efficacy

Phytophthora cactorum

Agrobacterium tumefaciens

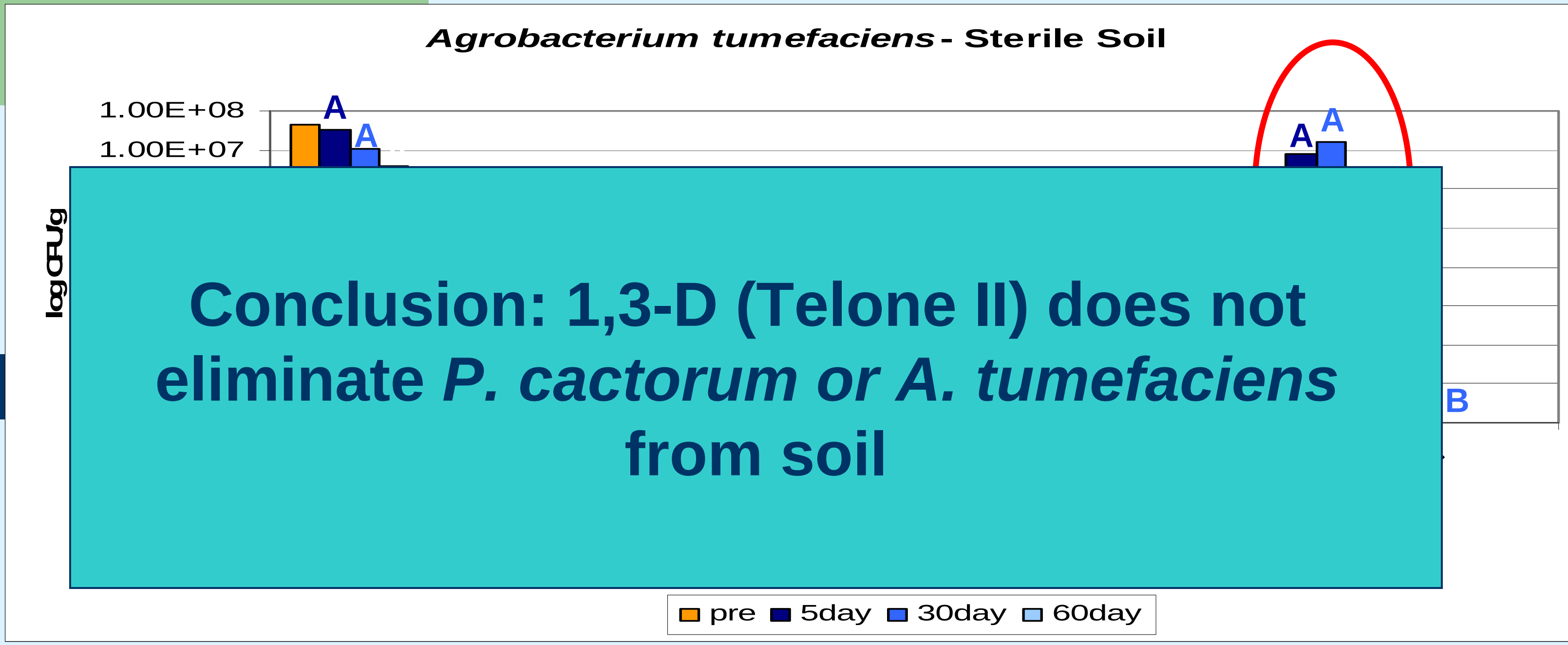
in soil

inside galls

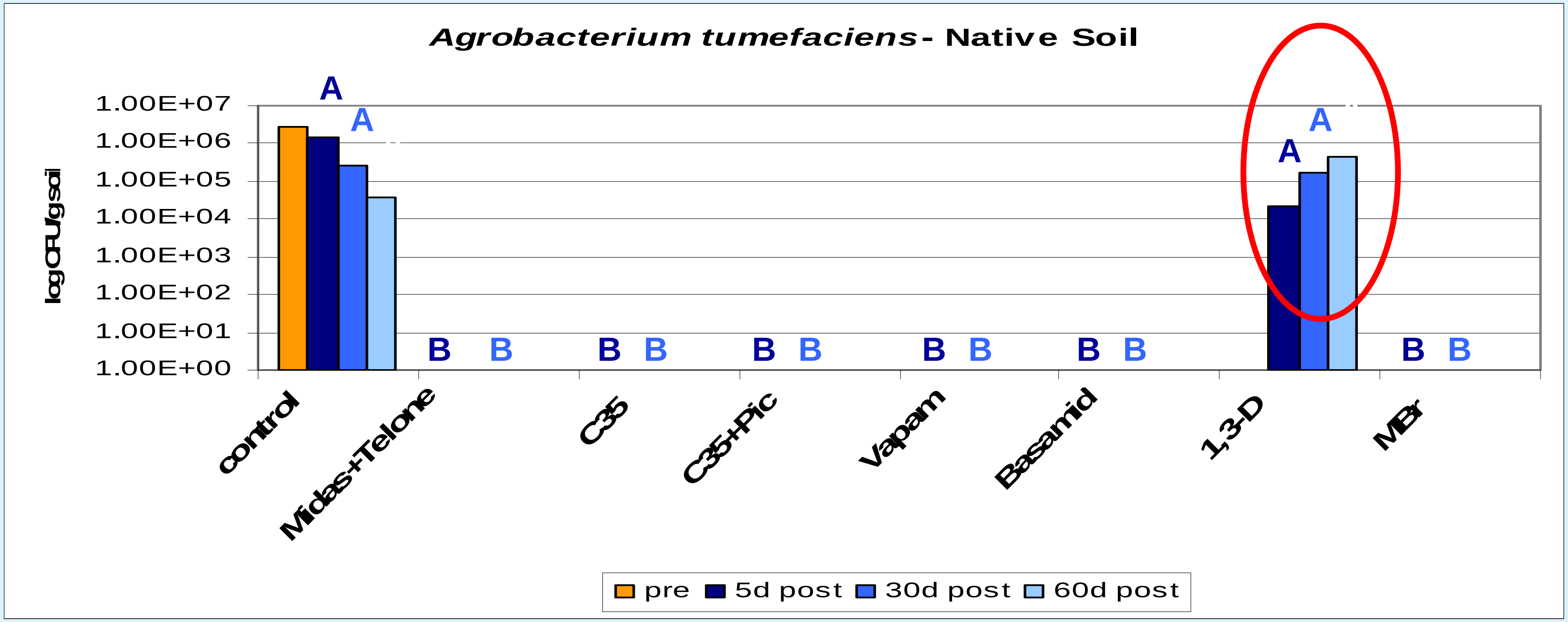
Aerobic bacteria

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

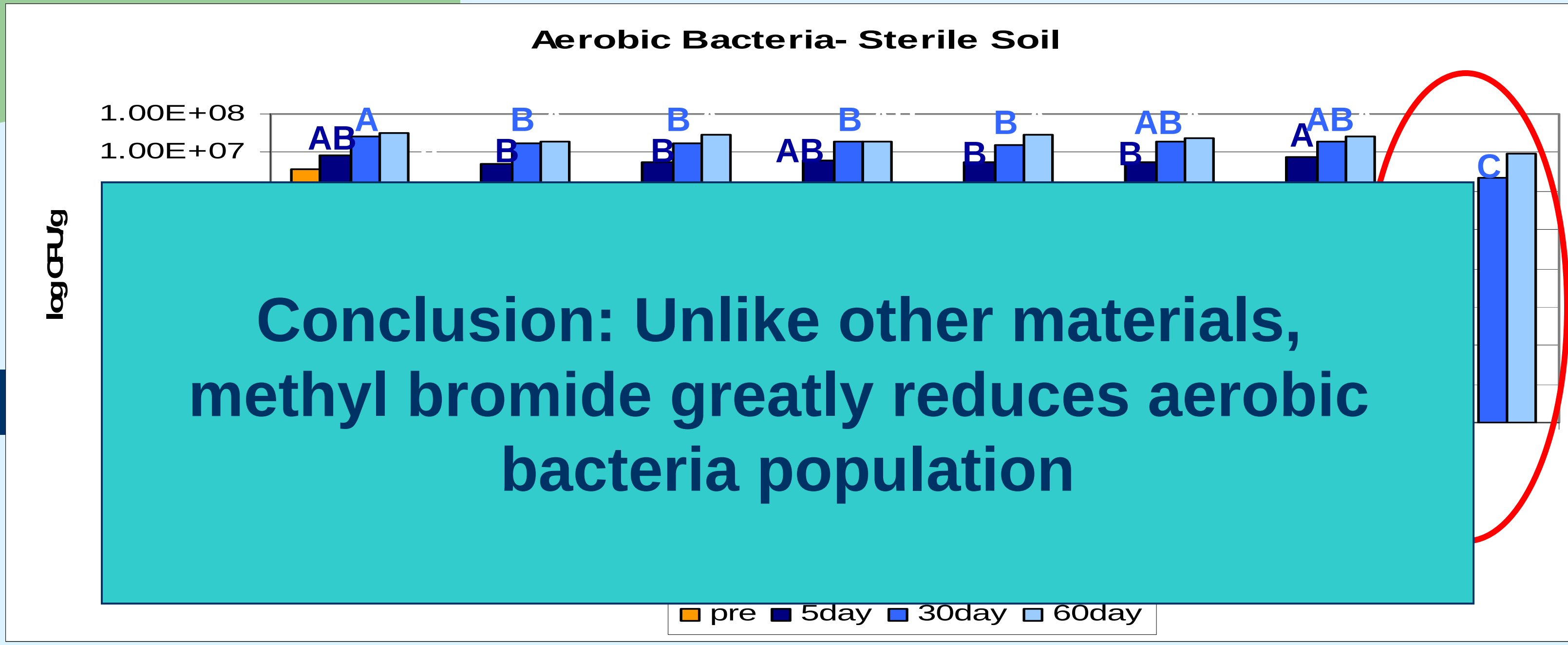
Efficacy – *A. tumefaciens*



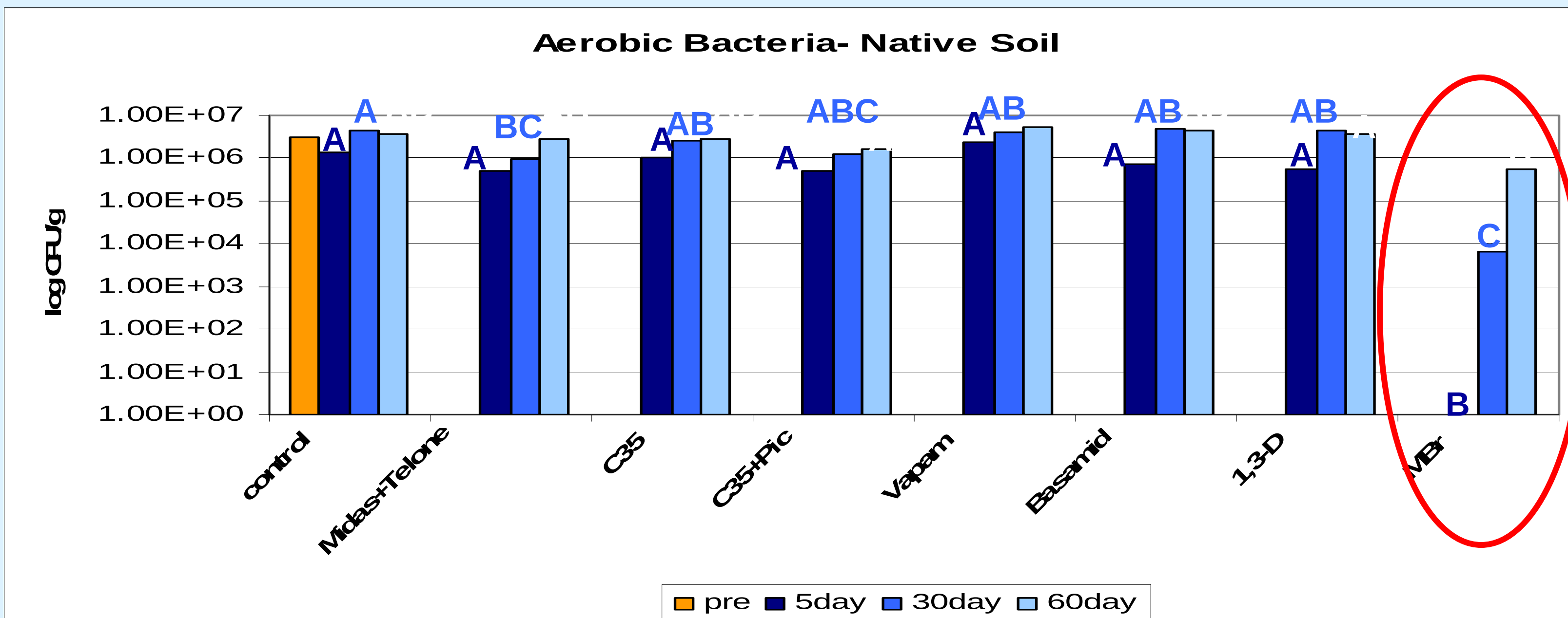
Conclusion: 1,3-D (Telone II) does not eliminate *P. cactorum or A. tumefaciens* from soil



Efficacy – Aerobic Bacteria



Conclusion: Unlike other materials, methyl bromide greatly reduces aerobic bacteria population



Efficacy – *A. tumefaciens*

A. tumefaciens in Galls

- 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

+

120 days → Assay the gall and soil

900 grams soil + 2-4” galls

Efficacy – *A. tumefaciens*

Conclusion: Telone C35 does not eliminate *A. tumefaciens* from gall tissue. Methyl bromide does.

TREATMENT	REP	A. tumefaciens DETECTION	
		IN GALLS	**IN SOIL**
MeBr	6	0	0
Telone C35	6	3*	5
Non-fumigated Control	8	8	6

* Galls were degraded. *A. tumefaciens* was detected in soil of samples w/o detection in gall remnants

Recolonization- Aerobic bacteria

Effect of fumigation on recolonization rate of soil

400 grams
of soil

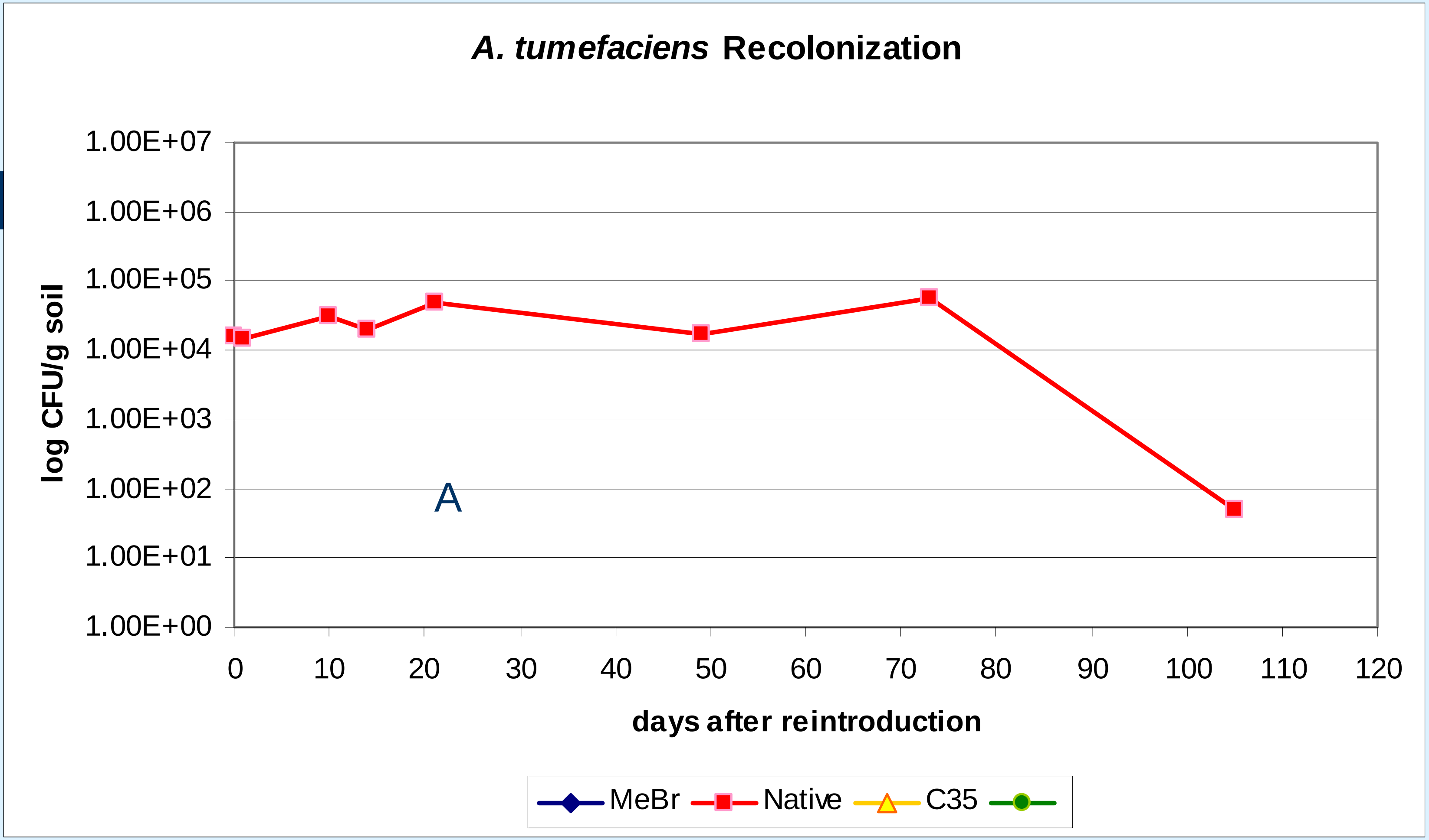
+ 1 gram of
Infested soil
5d post
fumigation

Sample soil from
1 to 105 days
after reintroduction

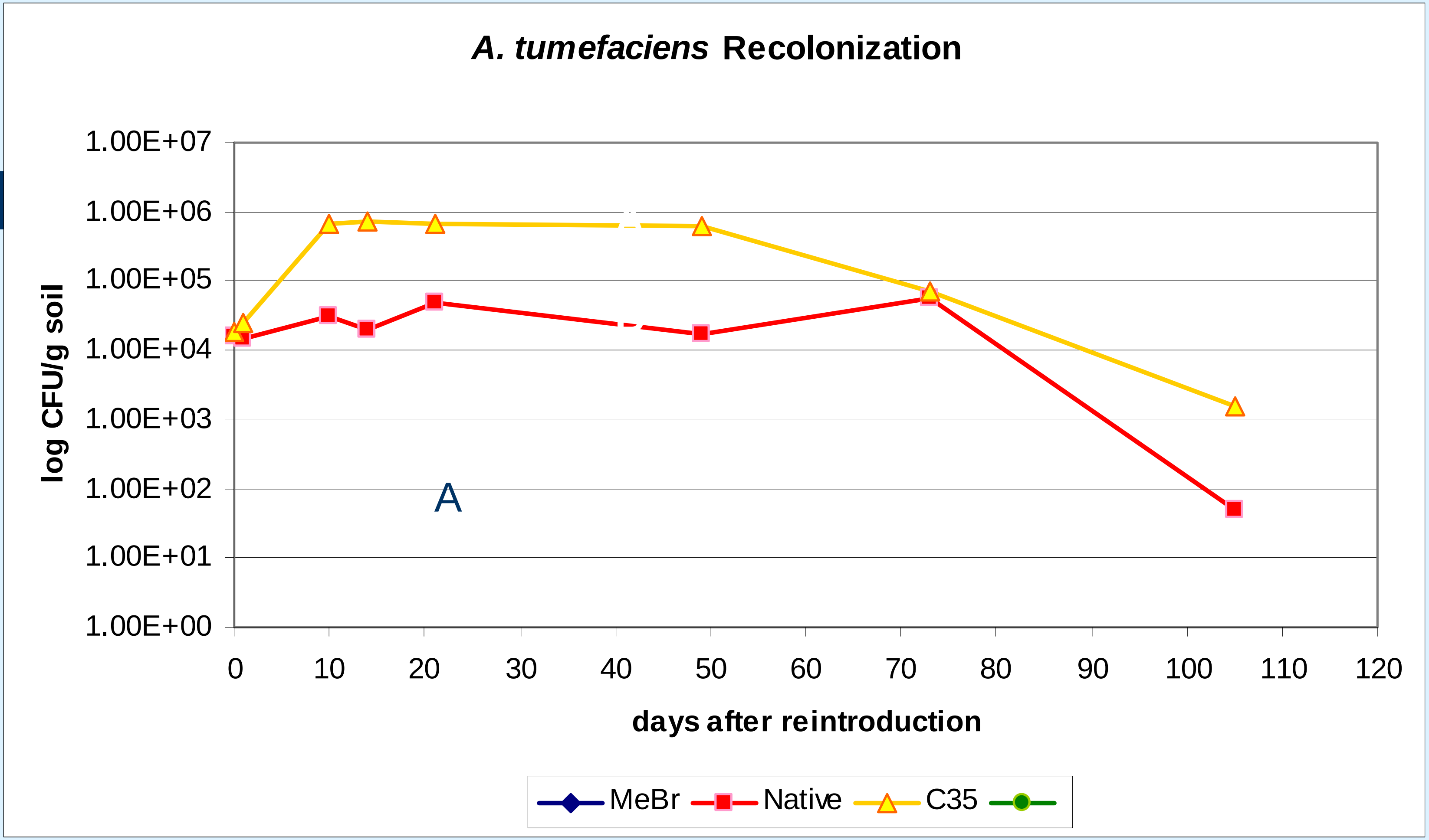
Treatments:

nontreated soil
sterilized soil
Telone C35
MeBr

Recolonization- *A. tumefaciens*

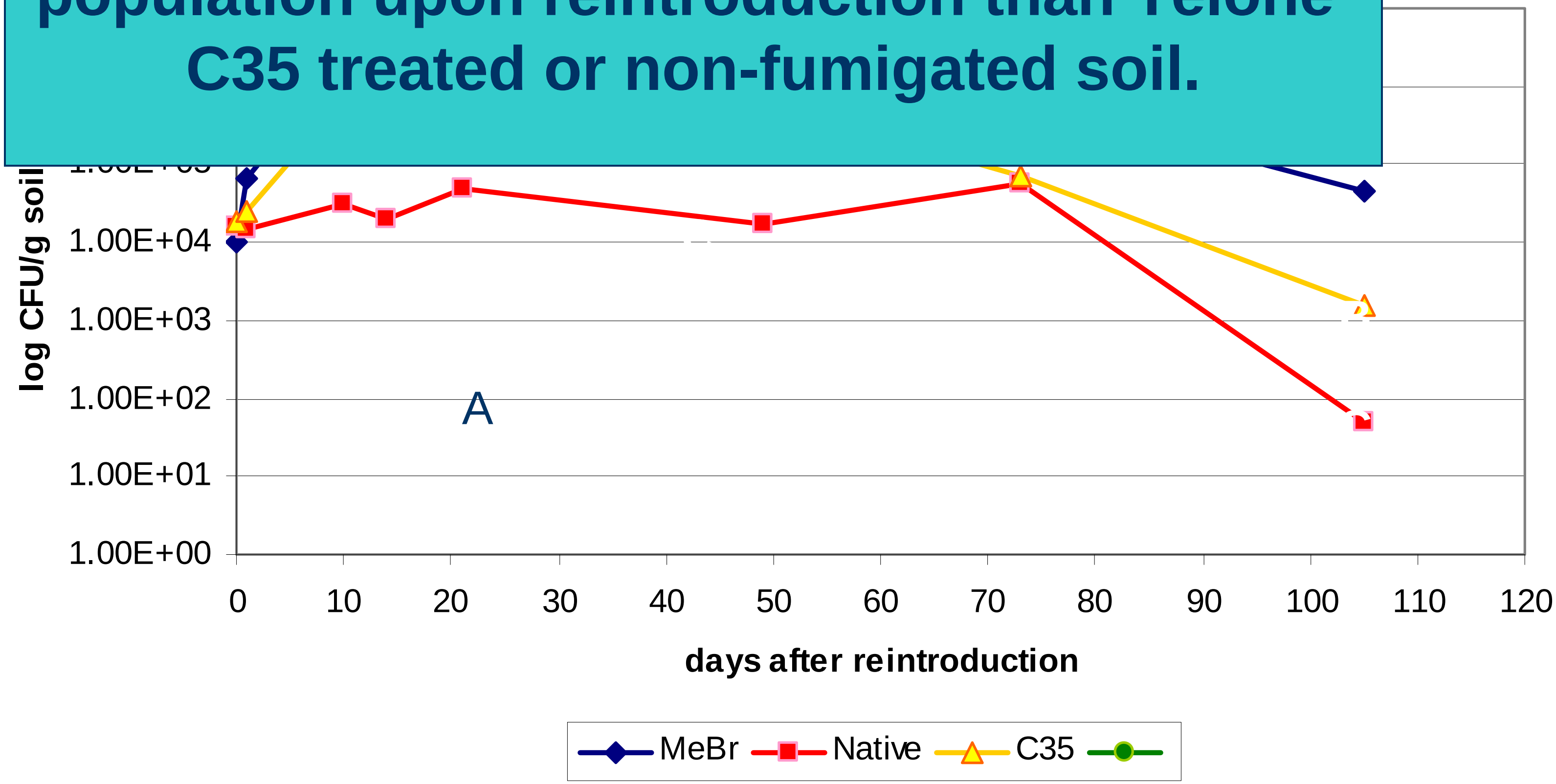


Recolonization- *A. tumefaciens*



Recolonization- *A. tumefaciens*

Conclusion: MeBr treated soil produces and sustains a greater *A. tumefaciens* population upon reintroduction than Telone C35 treated or non-fumigated soil.



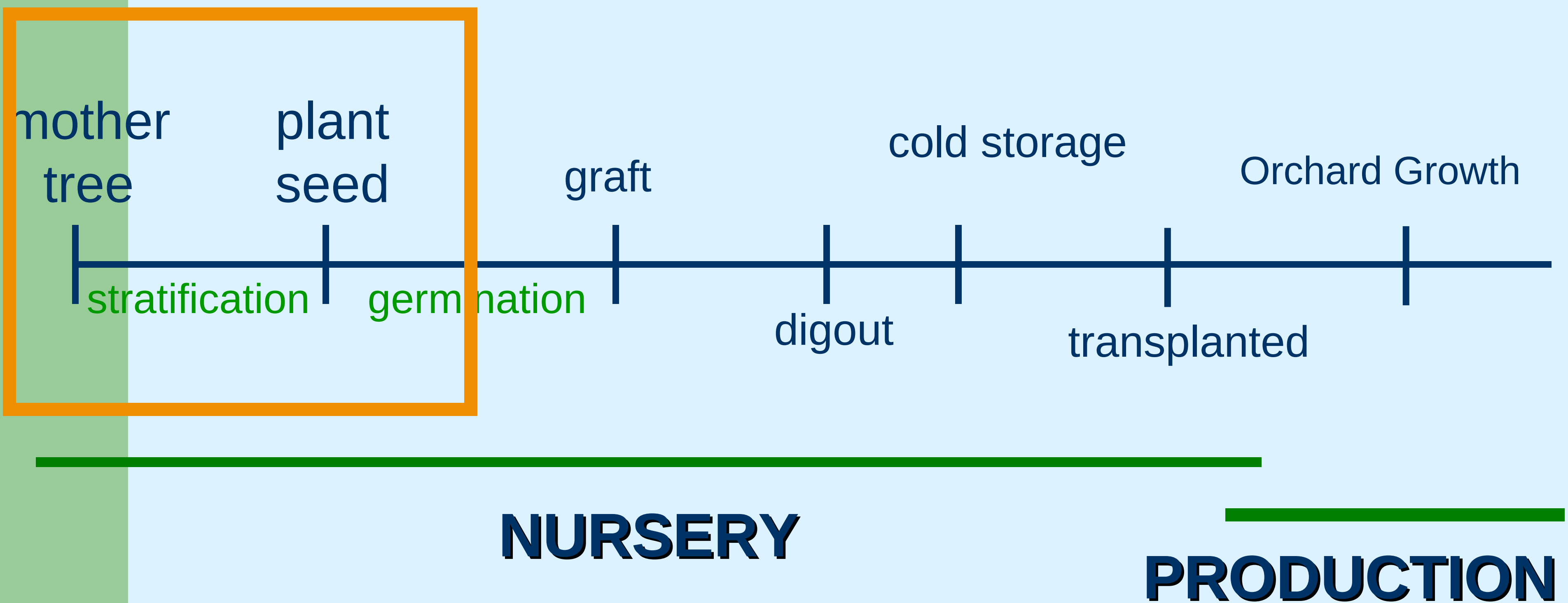
Conclusions

1. MeBr and most alternatives are effective on both pathogens
   - Telone II reduces but does not eliminate *Phytophthora* and *A. tumefaciens* populations
   - Telone C35 is the best MeBr alternative for walnut industry
2. Telone C35 does not eliminate *A. tumefaciens* from gall tissue; MeBr does
3. Fumigation significantly alters soil community
4. *A. tumefaciens* can re-colonize soil to higher levels in MeBr treated than non-fumigated or C35 soil

Outline

- Selection of CG resistant walnut germplasm
- Fumigation; alternatives to MeBr for Agrobacterium control
- Enhanced collection/propagation procedures for Paradox seed: Implications for crown gall management

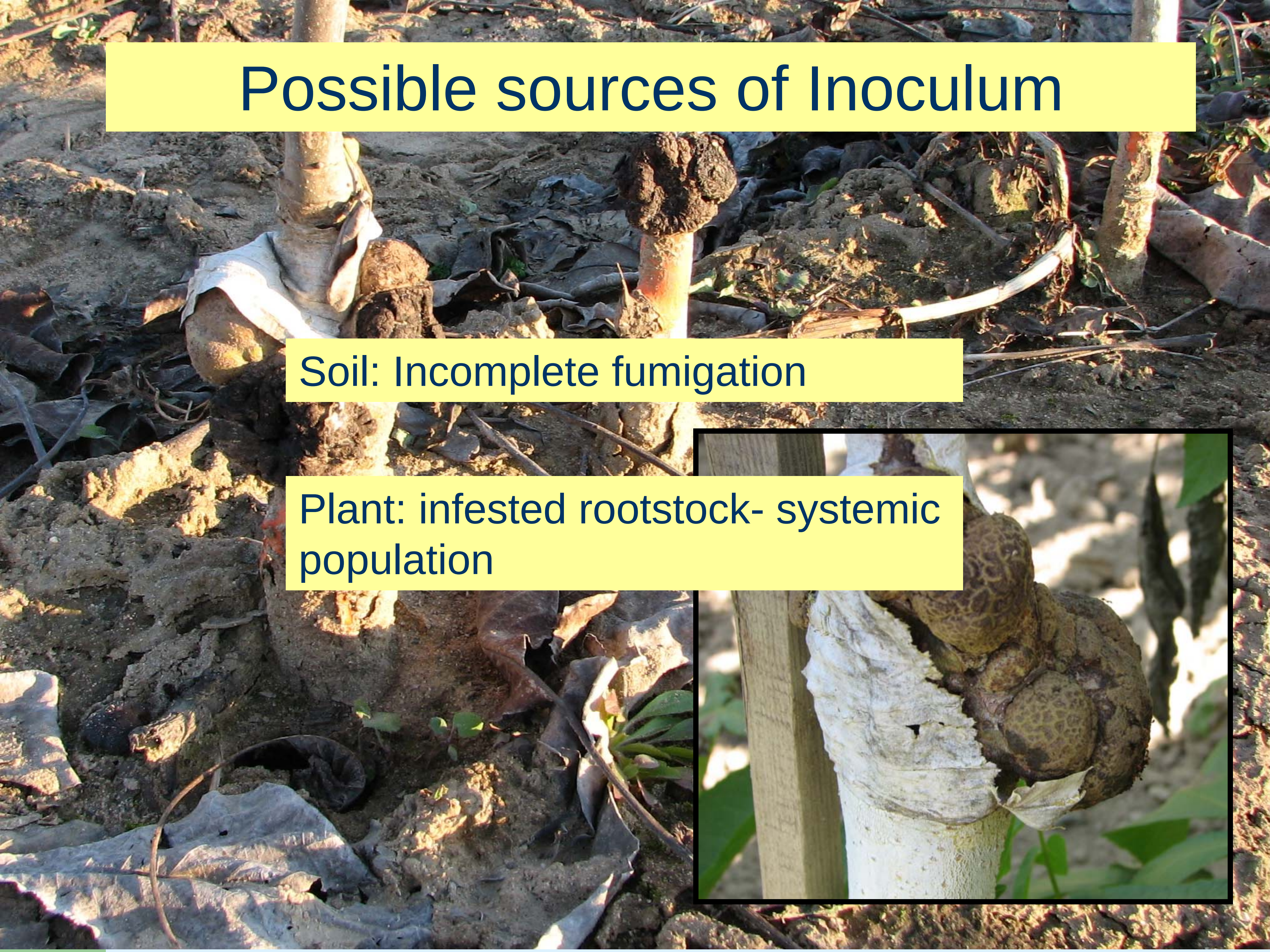
Walnut Production Time Line

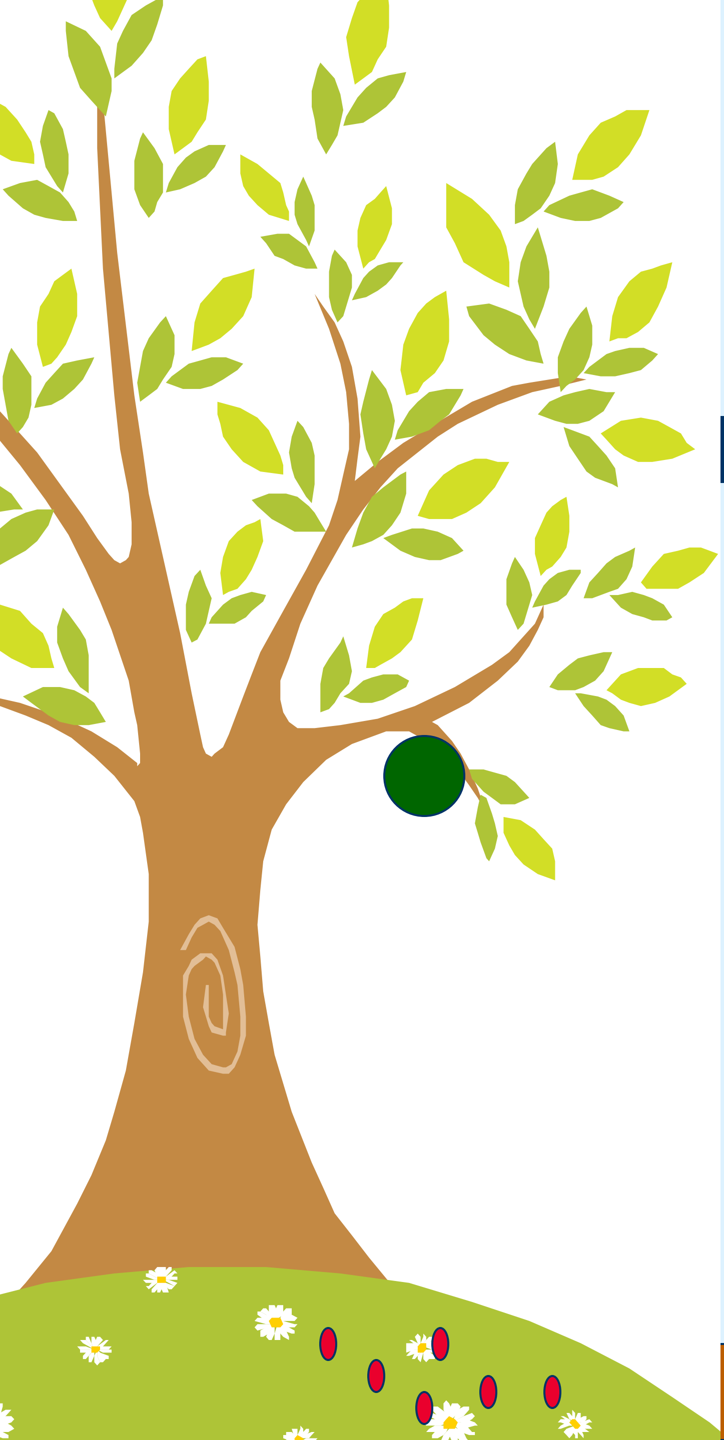


Possible sources of Inoculum

Soil: Incomplete fumigation

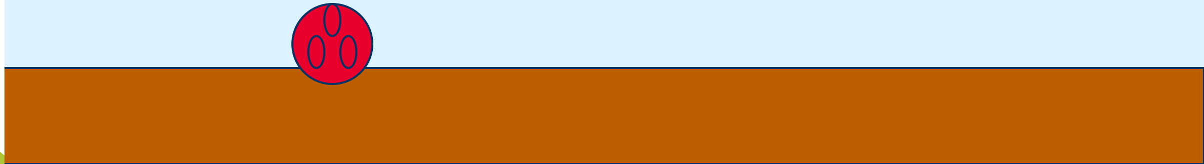
Plant: infested rootstock- systemic population

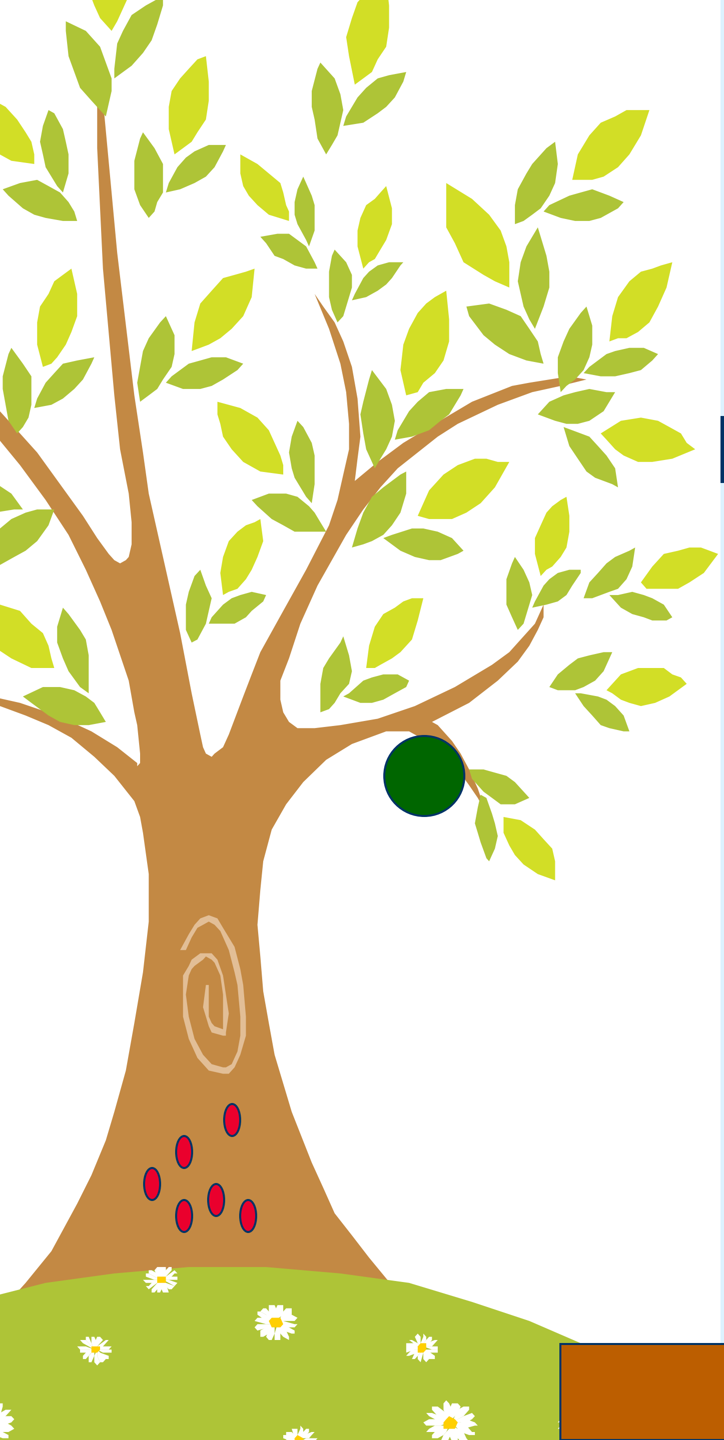




Scenario 1.

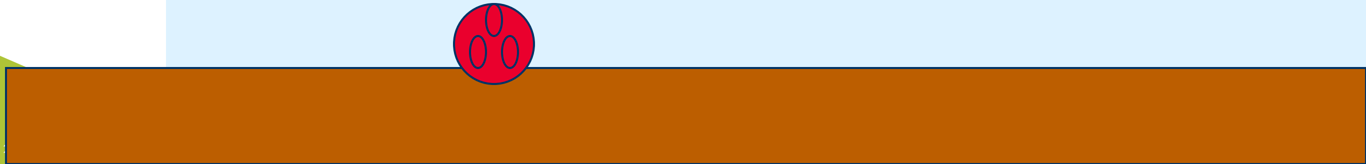
A. tumefaciens in soil

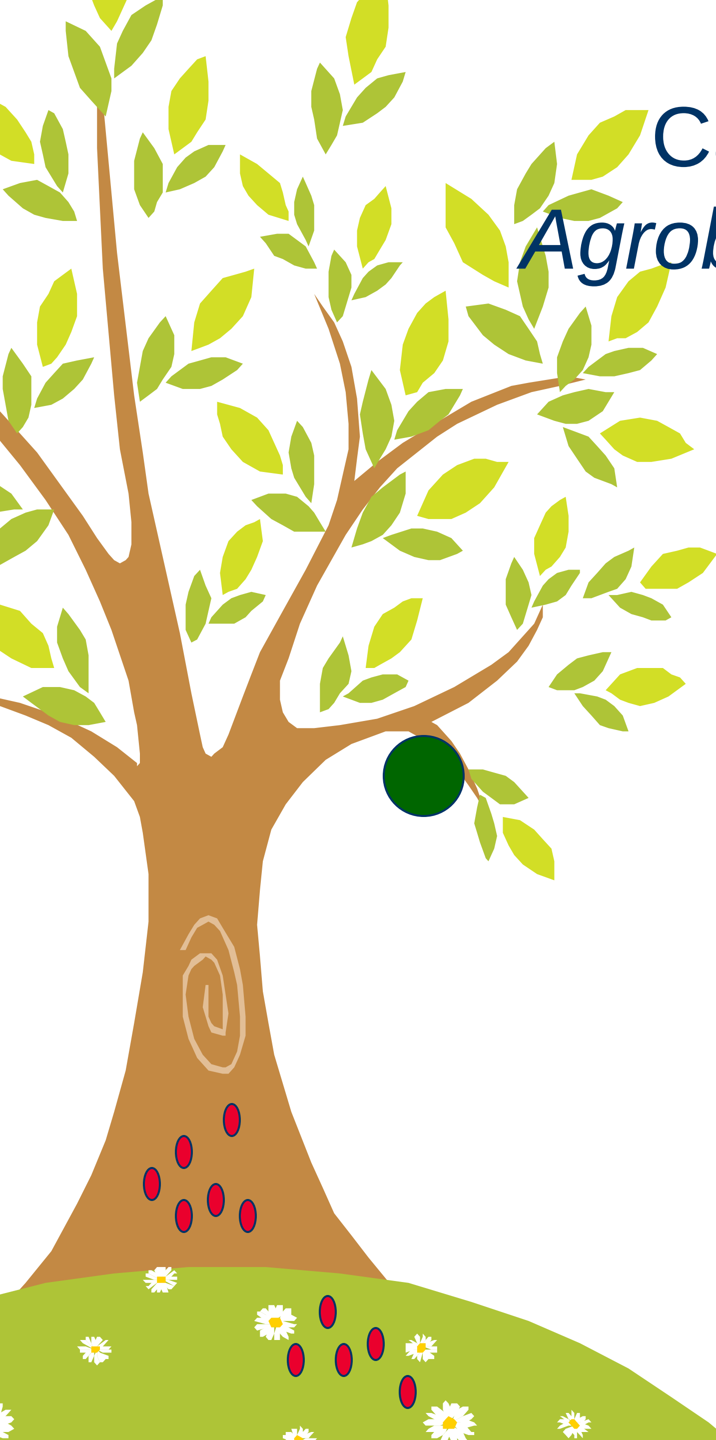




Scenario 2.

A. tumefaciens in tree



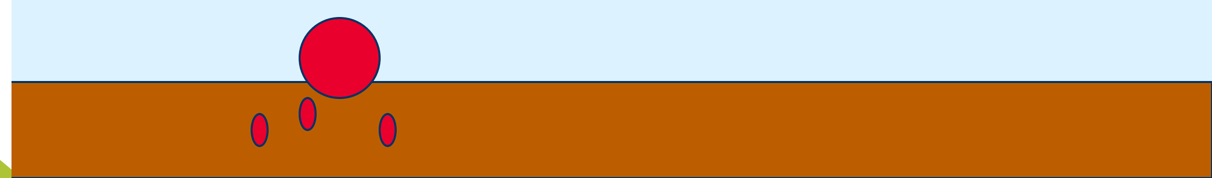


Can systemic populations of *Agrobacterium tumefaciens* exist in walnut seed or trees?

Where does the inoculum originate?

What is the incidence of systemic infections in the industry?

How does this impact disease management?

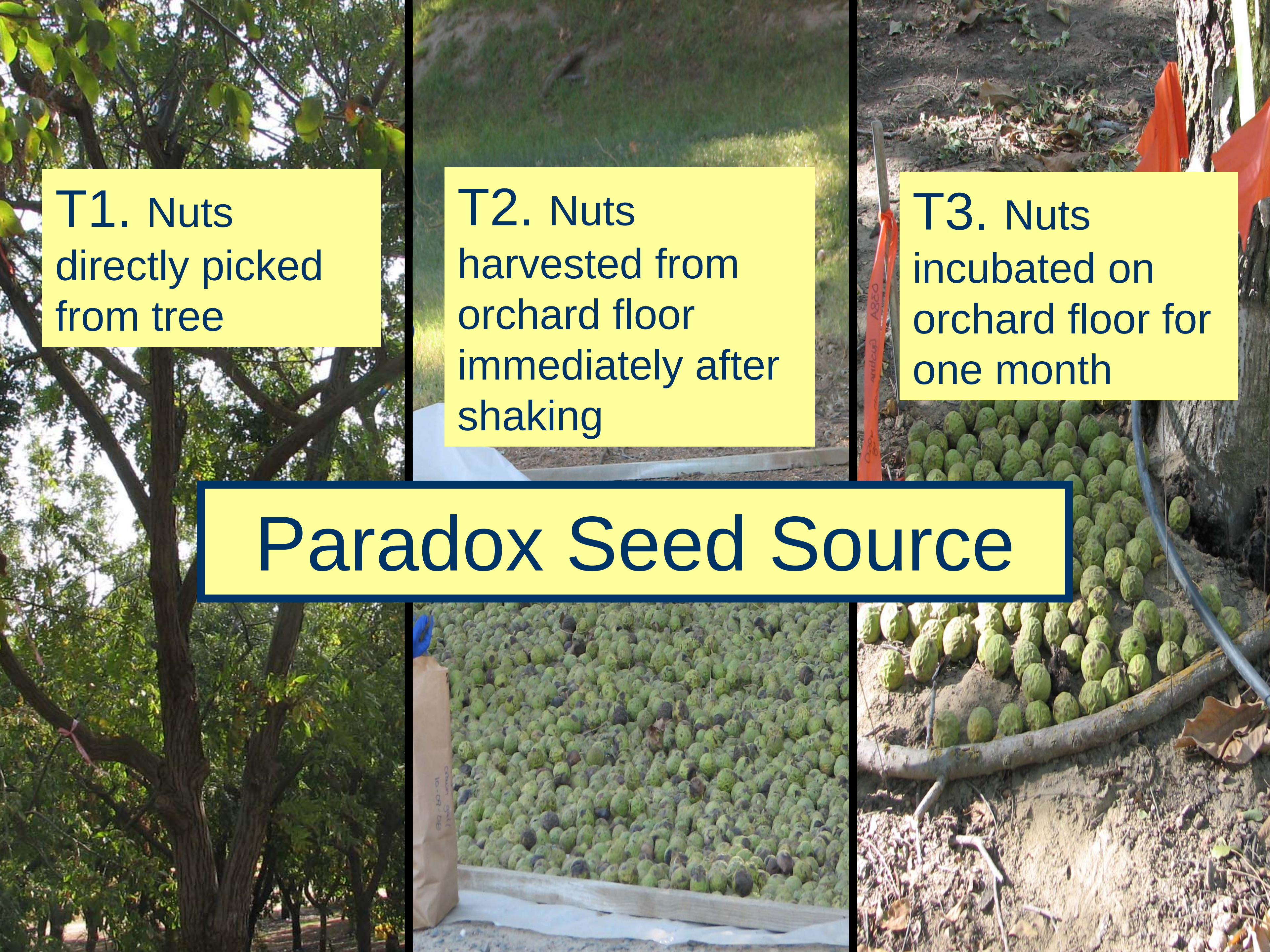


outline

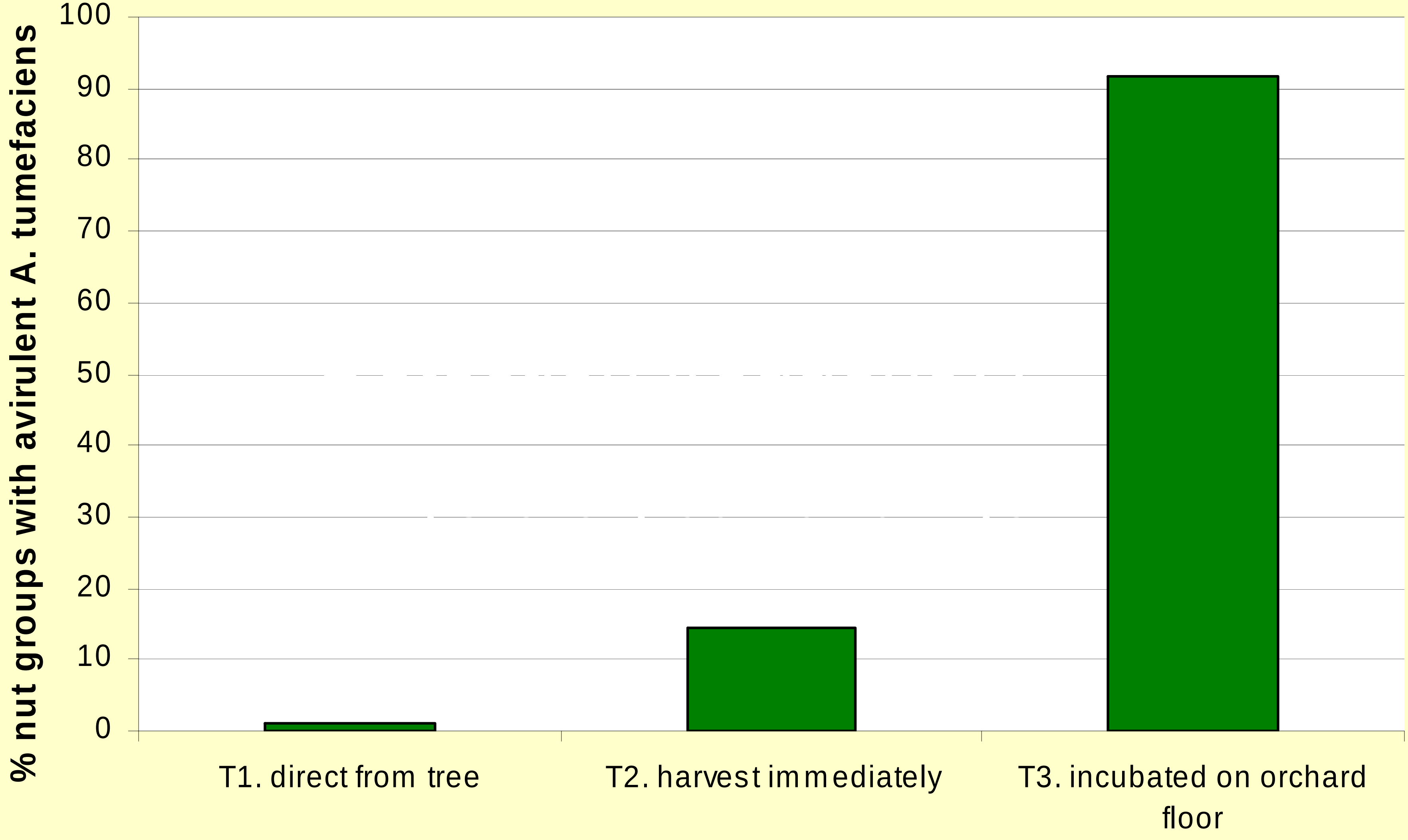
- Paradox seed source
  - Is *A. tumefaciens* in/on seeds?
  - Where was the seed inoculated?
    - mother tree?
    - orchard floor?

- Preemergent-systemic study
  - Do systemic populations establish from seed infestation?
  - Does CG develop from infested seeds?

(ETA- Spring 2009)

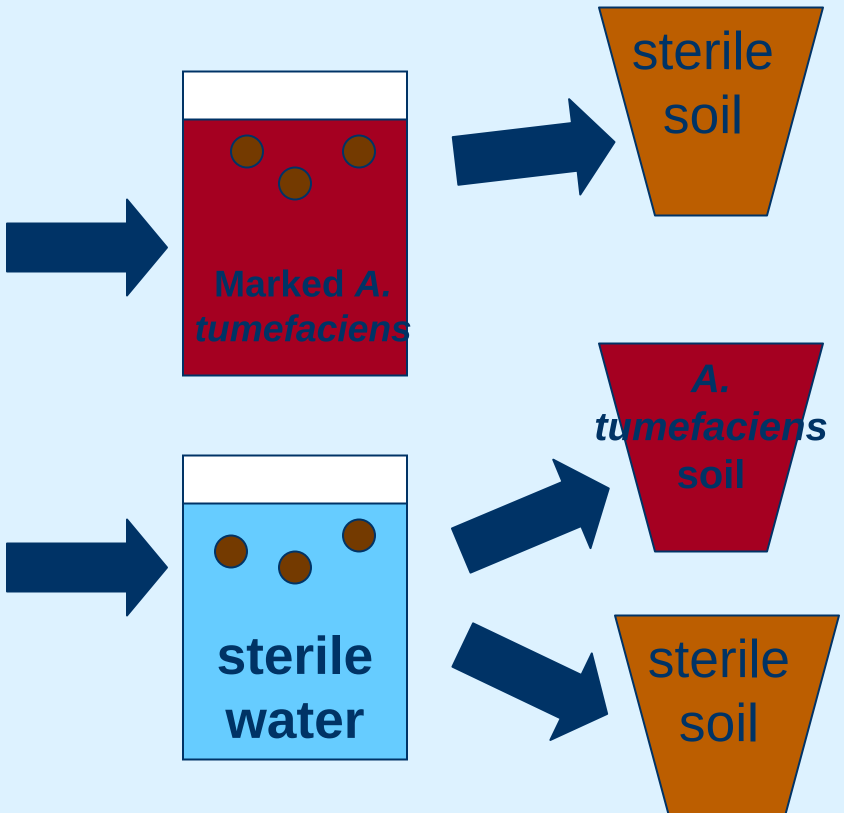

T1. Nuts directly picked from tree
T2. Nuts harvested from orchard floor immediately after shaking
T3. Nuts incubated on orchard floor for one month
Paradox Seed Source

Agrobacterium infested Paradox seeds



Preemergent-Systemic Study

- Will disease occur?
- Will a systemic population result?




Section and plate:
to determine
A. tumefaciens
presence
Wound on stem:
to determine if disease
can occur


Non Inoculated
Seed Inoculated


Seed Inoc.


Soil Inoc.

Systemic Nature of *A. tumefaciens*.

Conclusions

- *A. tumefaciens* populations were extremely low on nuts directly picked from trees
  - *A. tumefaciens* incidence increased with soil contact
  - Nuts colonized by *Agrobacterium* can develop crown gall on roots and support systemic populations of *A. tumefaciens*

Conclusions cont'd

- We will identify CG resistant germplasm for root stock development
- Several MeBr alternatives are effective against Agrobacterium (CG) and Phytophthora
- Improved handling of Paradox seed source will reduce CG incidence.

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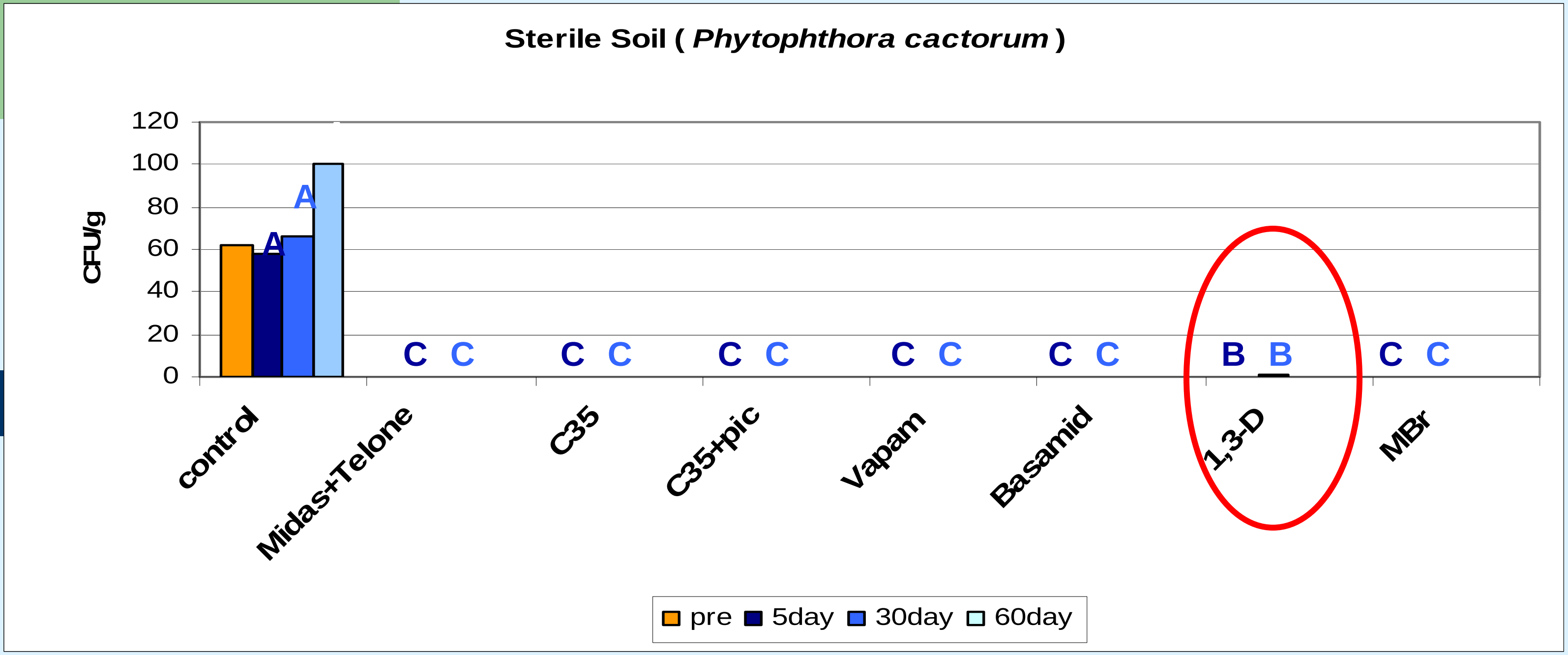
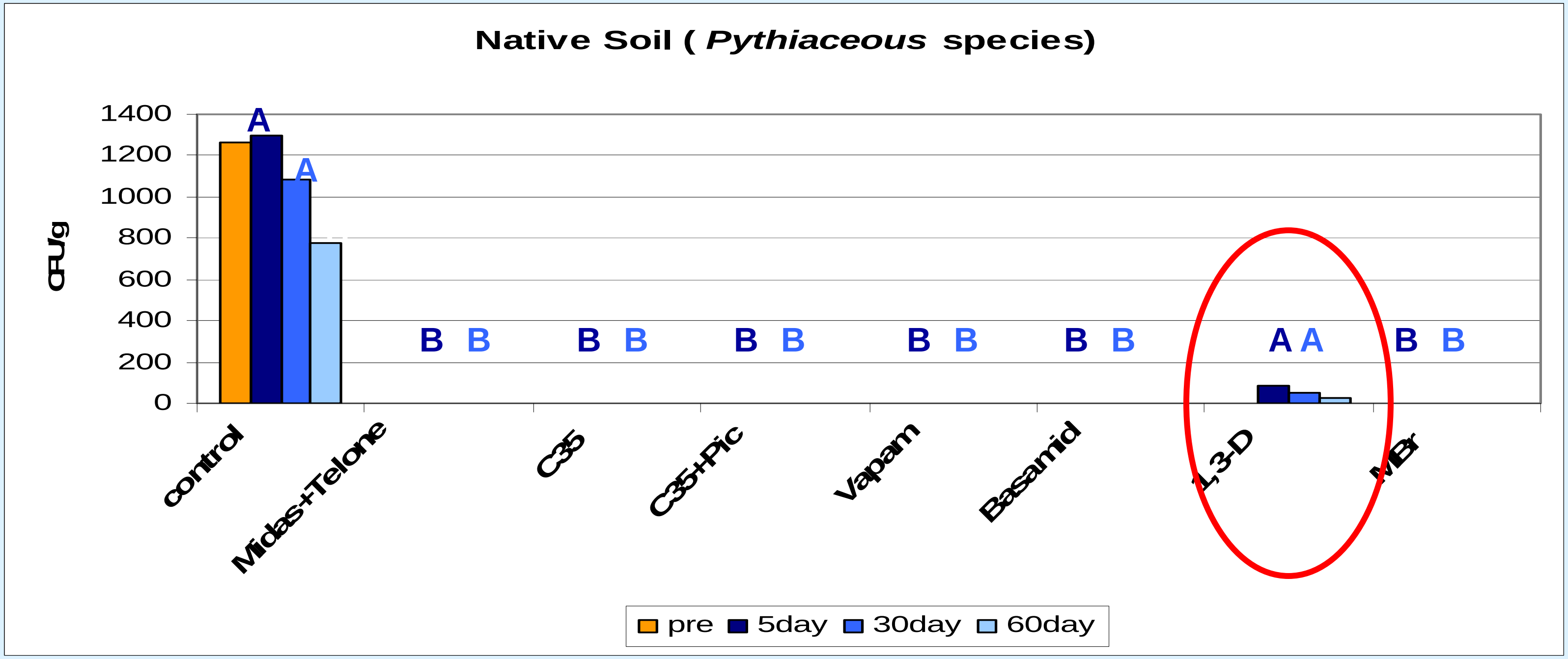
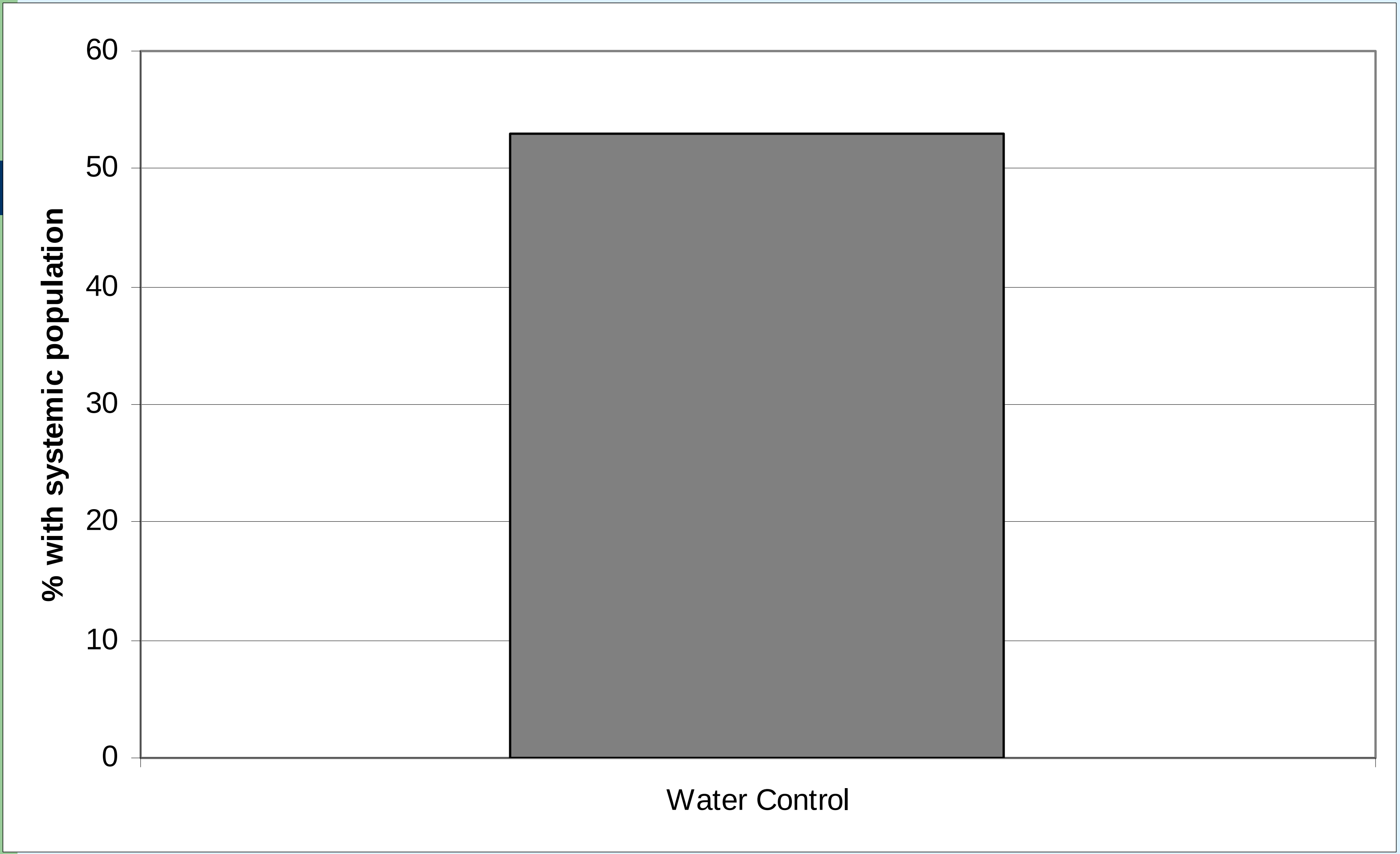
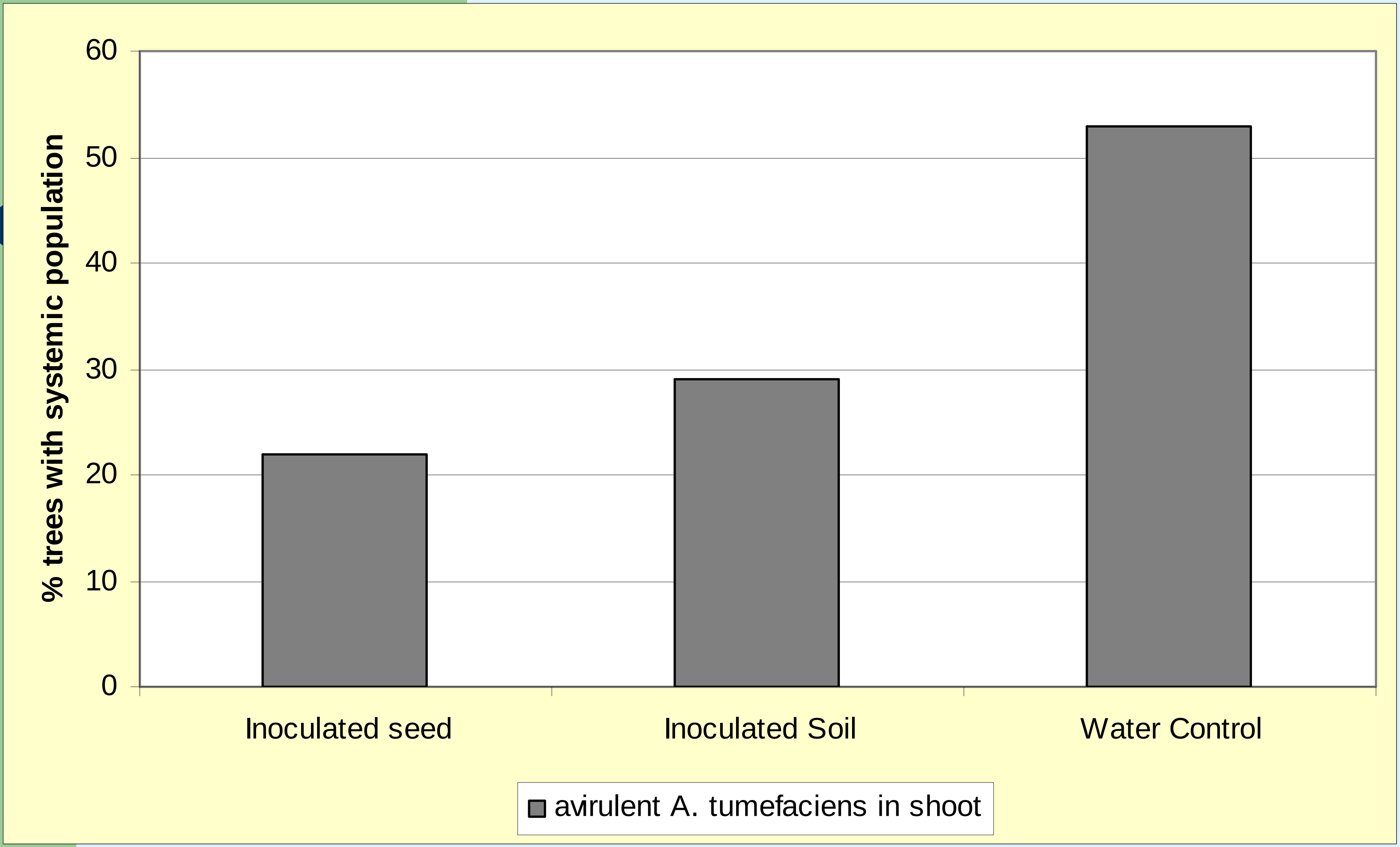
Efficacy - Phytophthora







% with systemic population
60
50
40
30
20
10
0
Water Control

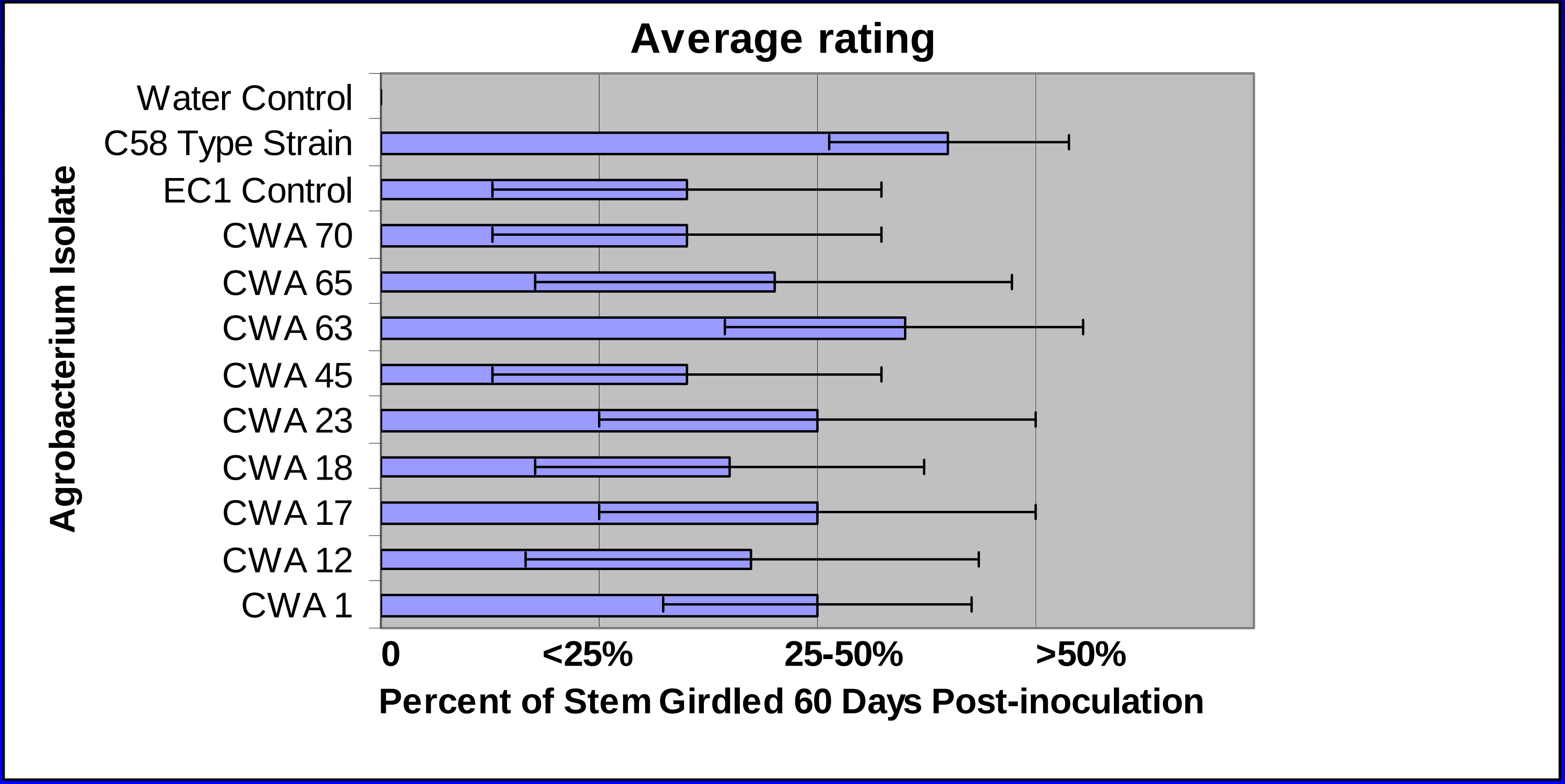


60
50
40
30
20
10
0
% trees with systemic population
Inoculated seed
Inoculated Soil
Water Control
avirulent A. tumefaciens in shoot

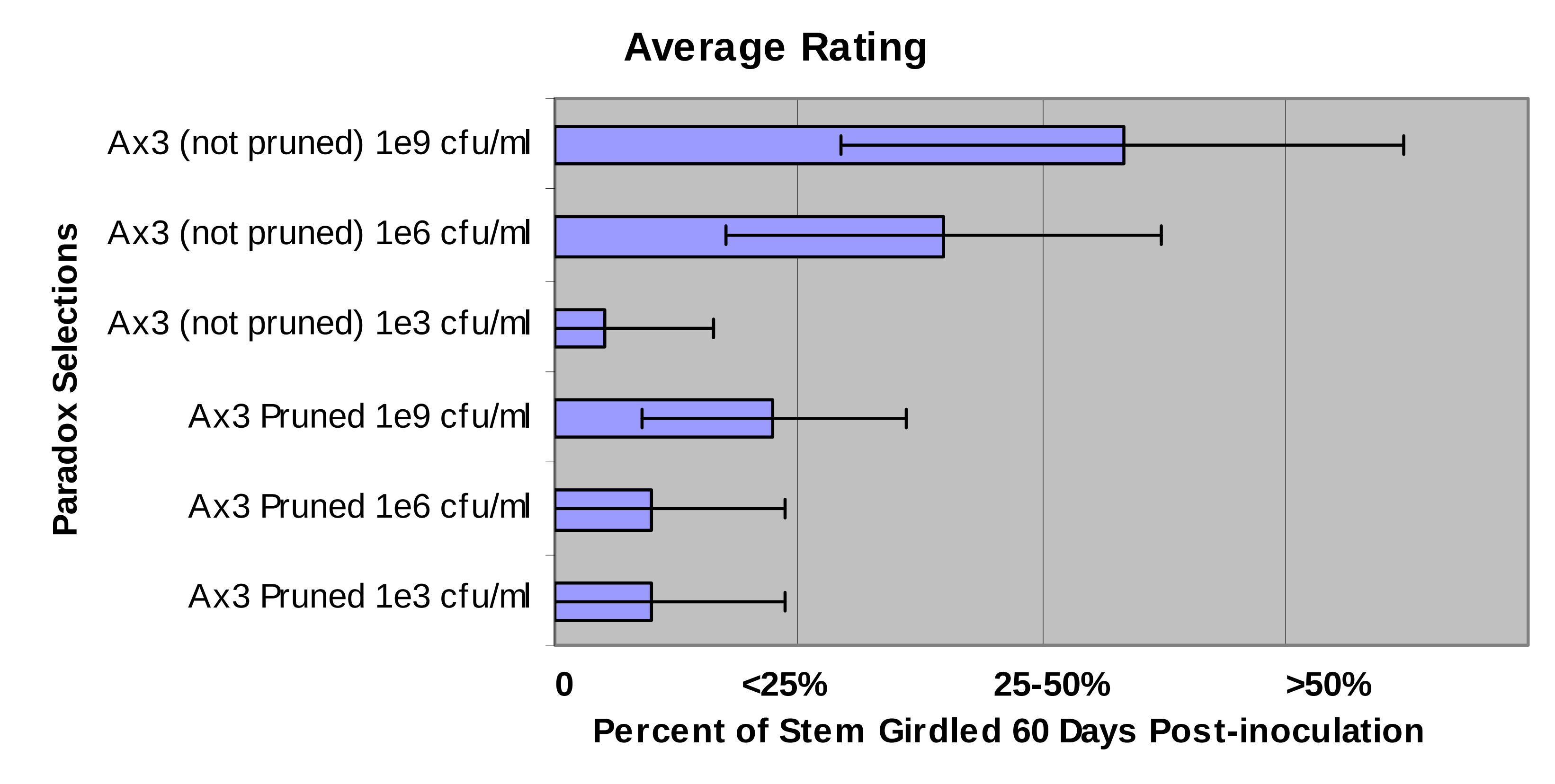
Anticipated Outcome

- New sources of resistance to crown gall will be identified and clonally propagated
- This resistance will be incorporated into new hybrids/potential rootstocks with greatly reduced crown gall susceptibility
- This material will be available for broader testing for horticultural characteristics and resistance to other diseases and pests

Relative Virulence of *A. tumefaciens*



Host Plant – *Agrobacterium* interactions



Continuing Proposal: Objectives

1. Identify and characterize novel sources of CG resistance in the NCGR *Juglans* collection
   a) Examine additional progeny from germplasm mother trees producing CG resistant seedlings
2. Develop a range of hybrids using identified CG resistant material to further evaluate resistance and finally characteristics as rootstocks
3. Screen rooted cuttings made from accessions exhibiting crown gall resistance.

Methods: Objective 1, Identifying novel sources of CG-resistance, 2008

- Preparing for inoculation trials in 2009 and 2010. Nuts are being stratified, germinated and propagated under greenhouse conditions for inoculation spring/summer 2009
- Evaluate tumor development
- Infect “resistant” accessions with genetically diverse CA agro isolates

Methods: Objective 2, Develop hybrids & new Paradox using CG-resistance , 2006-8

- Controlled crosses have produced 80 hybrids using identified sources of resistance
  (Black walnut x regia crosses to synthesize “New” Paradox)

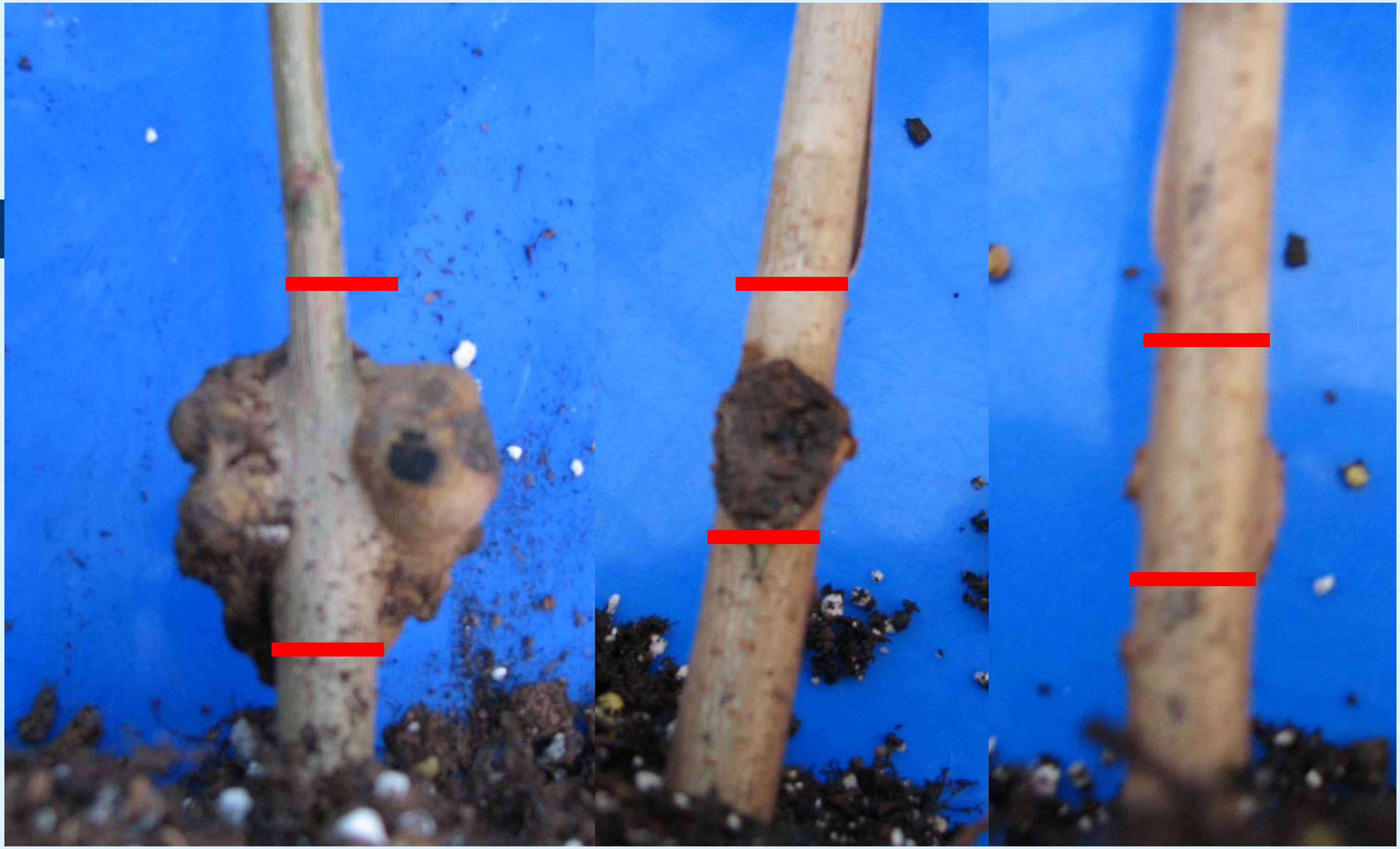
- Controlled crosses will be made during 2009 to generate combinations that may be adapted to California walnut growing conditions.

- Resulting hybrids will be included in the screening for CG resistance as described in the other objectives

Methods: Objective 3, Screen rooted cuttings made from accessions exhibiting crown gall resistance.

- Collect dormant cuttings from previously identified “resistant” accessions.
- Root dormant cuttings.
- Grow-out rooted cuttings under greenhouse conditions.
- Using genetically diverse *Agrobacterium* strains to screen rooted cuttings for crown gall crown gall resistance.

Girdled Stems



Rating = 4
>50% girdled

Rating = 3
25-50% girdled
Front view

Rating = 3
25-50% girdled
Back view

Summary of Germplasm Screened 05-08

Genus	Species	Accessions	Seedling Years Tested	No. of Trees Tested	Trees Retained @ 60 Days
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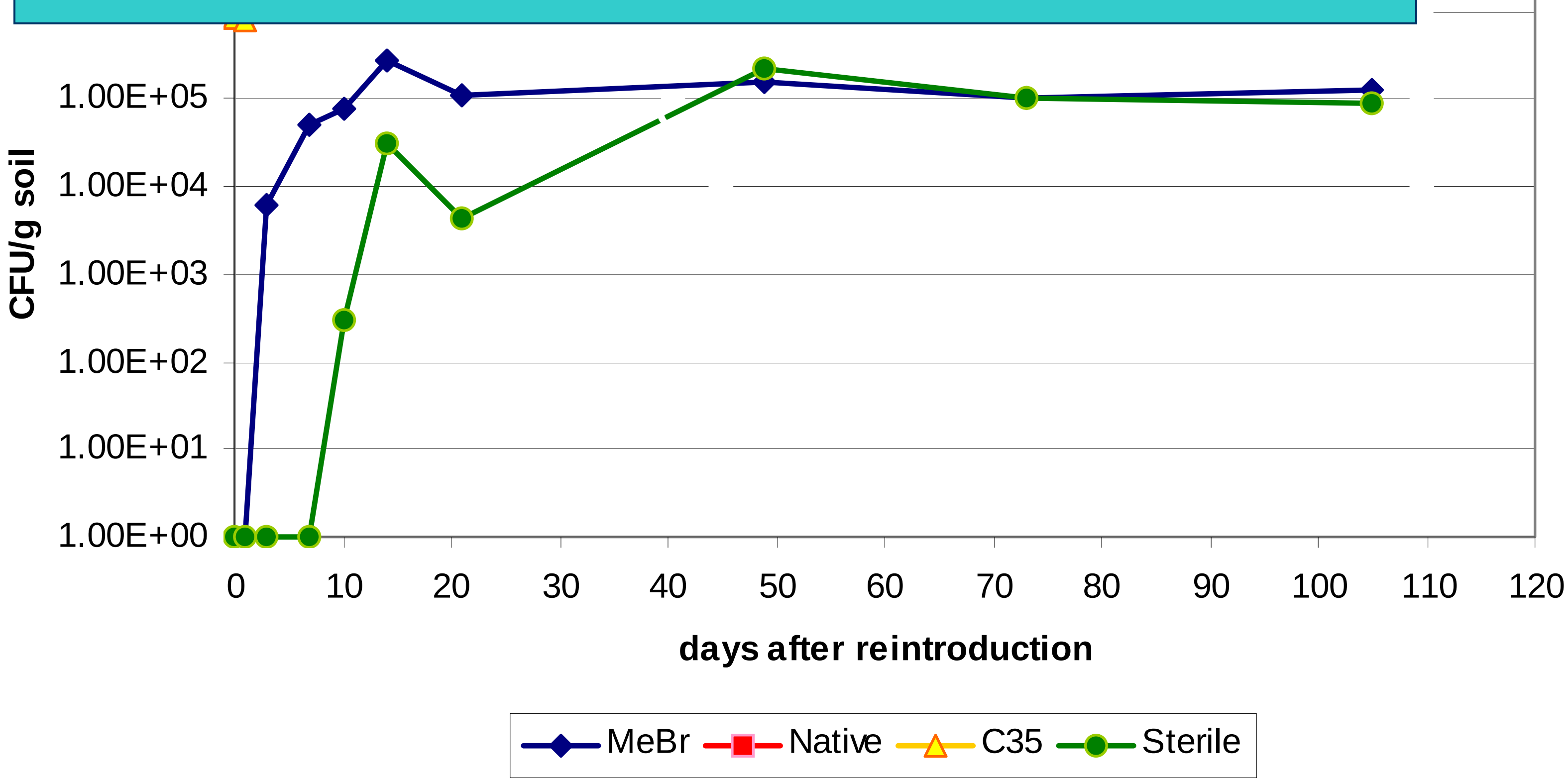
Juglans	cathayensis	2	3	12	1
Juglans	**mandshurica**	**11**	**3**	**74**	**18**
Juglans	sinensis	2	4	7	0
Pteracarya	**Spp**	**5**	**1**	**29**	**13**
Totals	**12**	**328**	**4**	**1803**	**282**

Recolonization- Aerobic bacteria

Conclusion: Telone C35 retains a bacteria community greater than native soil and methyl bromide



Potential efficacy on gall tissue

Treatment	**rate**
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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
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