Trichoderma atroviride for control soil-borne pathogens

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IPM Innovation in Europe, 14th January 2015

FEM, San Michele all'Adige, Italy

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Soil-borne diseases:

- **Banning** of methyl-bromide; **exclusion** of other chemical fumigants in IPM programs
- High value crops or greenhouse and tunnels: **often monoculture**
- **Crop rotation useless** in case of pathogens with wide host-range (Rhizoctonia, Armillaria, Rosellinia, Pythium, etc.)
- **Long-lasting** inoculum in soil
- Solarization or anaerobic soil disinfestation often **unfeasible or ineffective**

Armillaria spp.

- World wide presence (forest and agriculture)
- Polyphagous (>300 hosts known)
- Long lasting inoculum in soil
- Emerging disease in several perennial crops (grape, apple, prunus, berries, etc.)
- Increasing problem for growers

Armillaria mellea

Trichoderma atroviride SC1

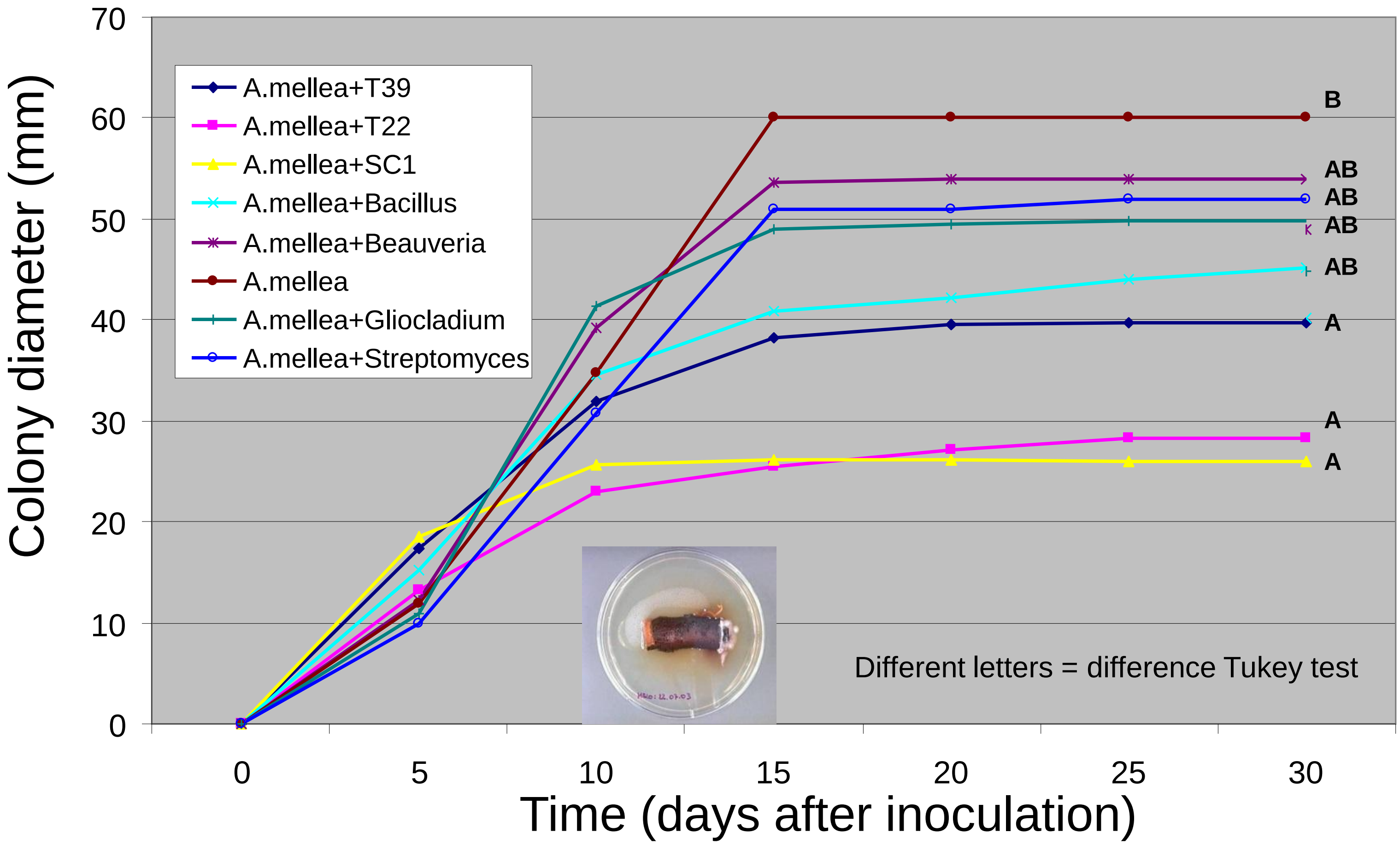
- SC1 has biocontrol properties against several soil-borne plant pathogens
  - *Armillaria mellea, A. gallica, Rhizoctonia solani, Fusarium, spp., Verticillium spp., Pythium spp. Phytophthora spp.*
- Very good colonizer of dead wood and vegetable material
  - Registration against wood pathogens (expected in 2016)

➔Good candidate to be used in IPM solutions *(no impact on health and environment, renewable, biological)*

Trichoderma spp.

- Several **species and strains**
- *T. atroviride* good colonizer of wood or plant residues
- Very effective against **many soil-borne pathogens**
- Hyperparasite, production of lytic enzymes and toxins, competition for space and nutrients, (induced resistance limited contribution to the efficacy)

Dual culture against *Armillaria mellea*

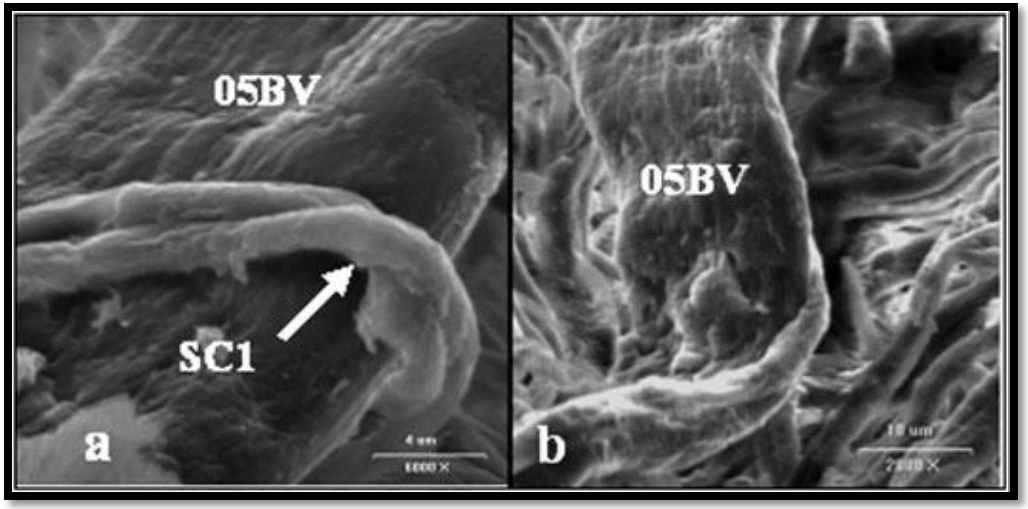


The most efficient biocontrol agents were *T. atroviride* SC1, *T. harzianum* T22 (commercial), *T. harzianum* T39 (experimental)

Mechanisms

- It can mycoparasitize fungal pathogens

Coiling of BCP511B (SC1) round a hypha of *Armillaria mellea* 05BV (a) compared with untreated hypha of *A. mellea* 05BV (b)



- It produces lytic enzymes

	Chitobiase activity* (mU ml^{-1}±SE)[a]	Protease activity* (Abs ml^{-1}±SE)[b]	Glycolytic activity* (µmol mg^{-1} h^{-1} ±SE)[c]
SC1	13.84 ± 0.38	10.22 ± 0.09	199.27 ± 4.07

*In vitro

Mechanisms

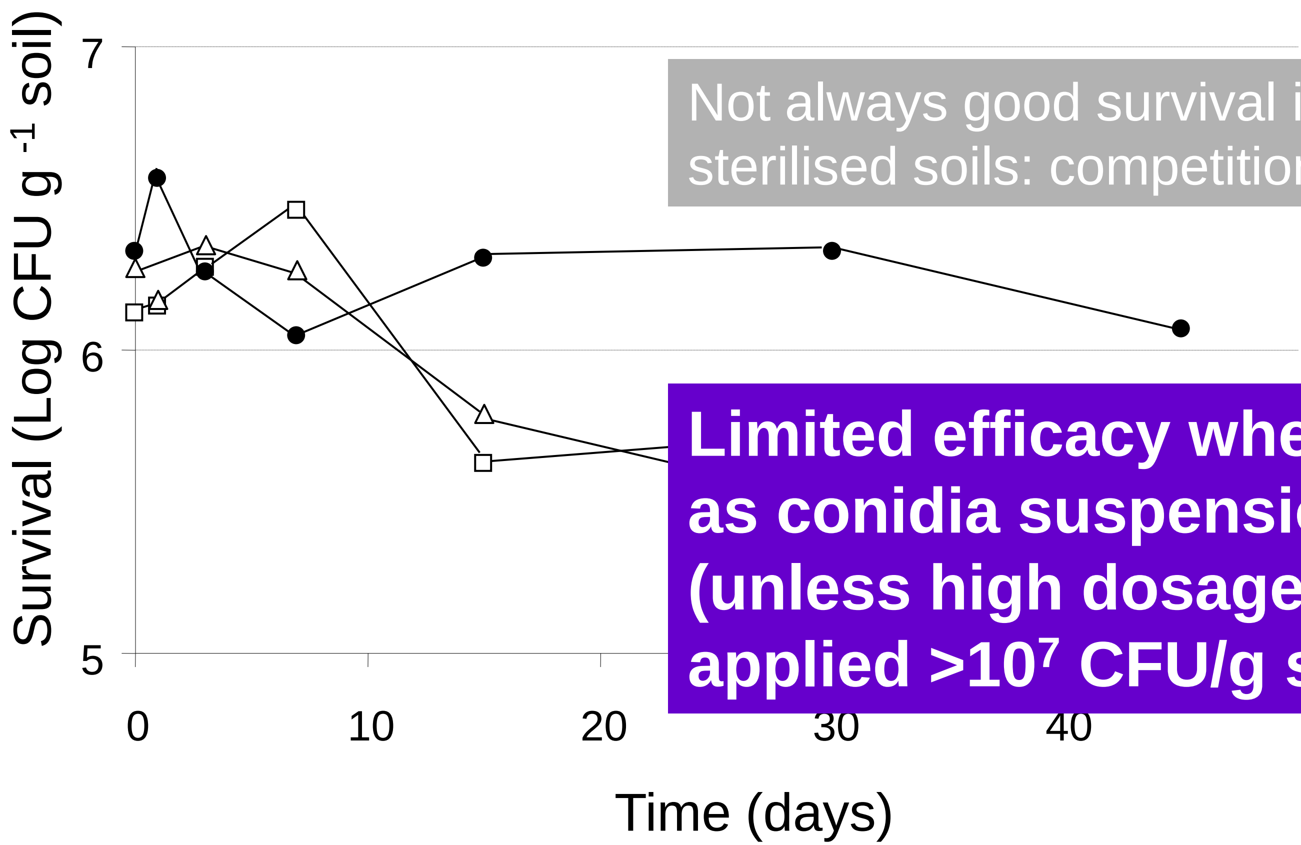
- Colonization of wood and exclusion of pathogens

example of *Phaeoacremonium aleophilum* (Pal) and *Phaeomoniella chlamydospora* (Pch) on grape

Wounds colonized by SC1 (%)	Presence of Pal (%) in colonized wounds	Presence of Pch (%) in colonized wounds
33.3 - 66.3 (min-max)	0	0

- Induction of resistance

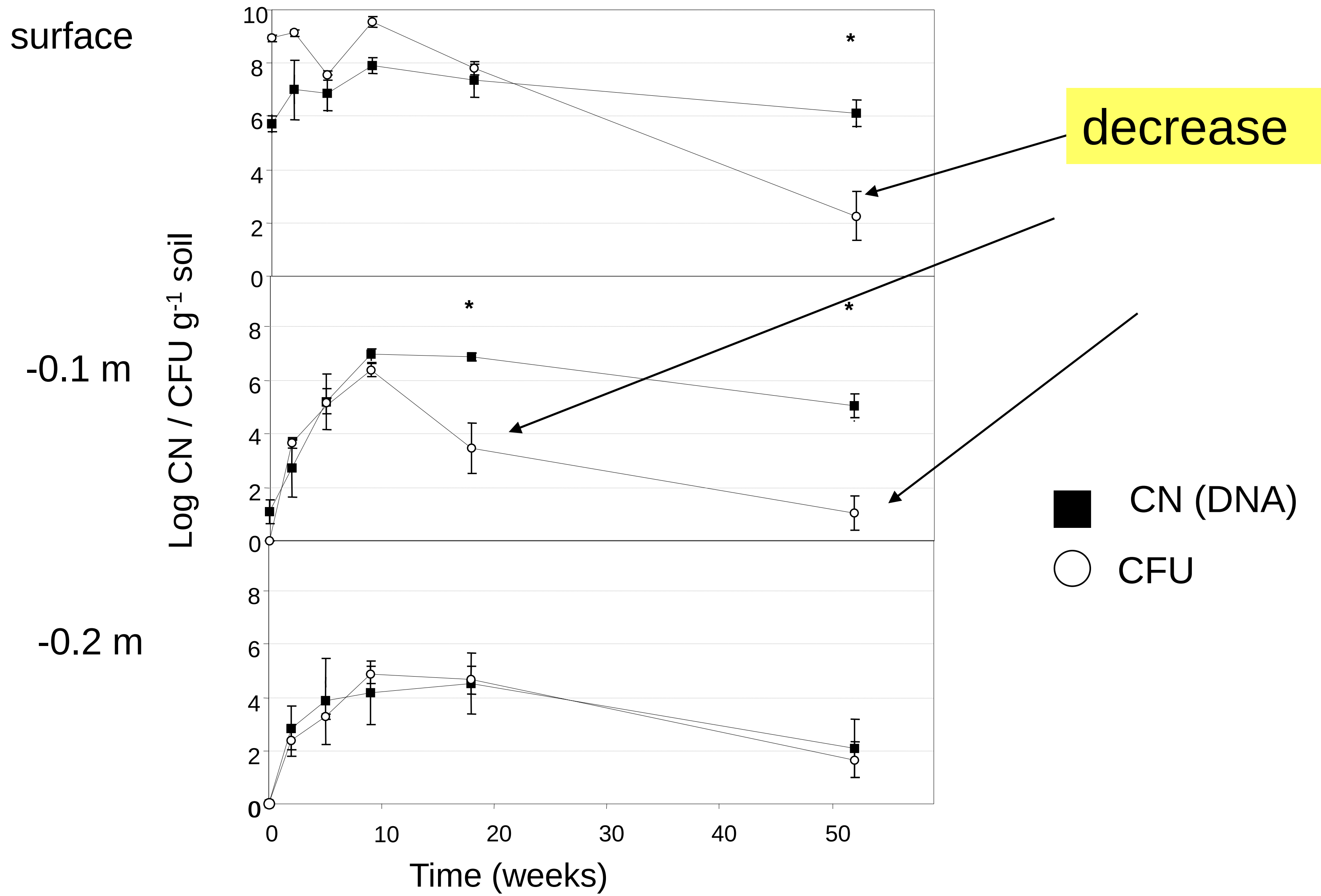
Survival in soil



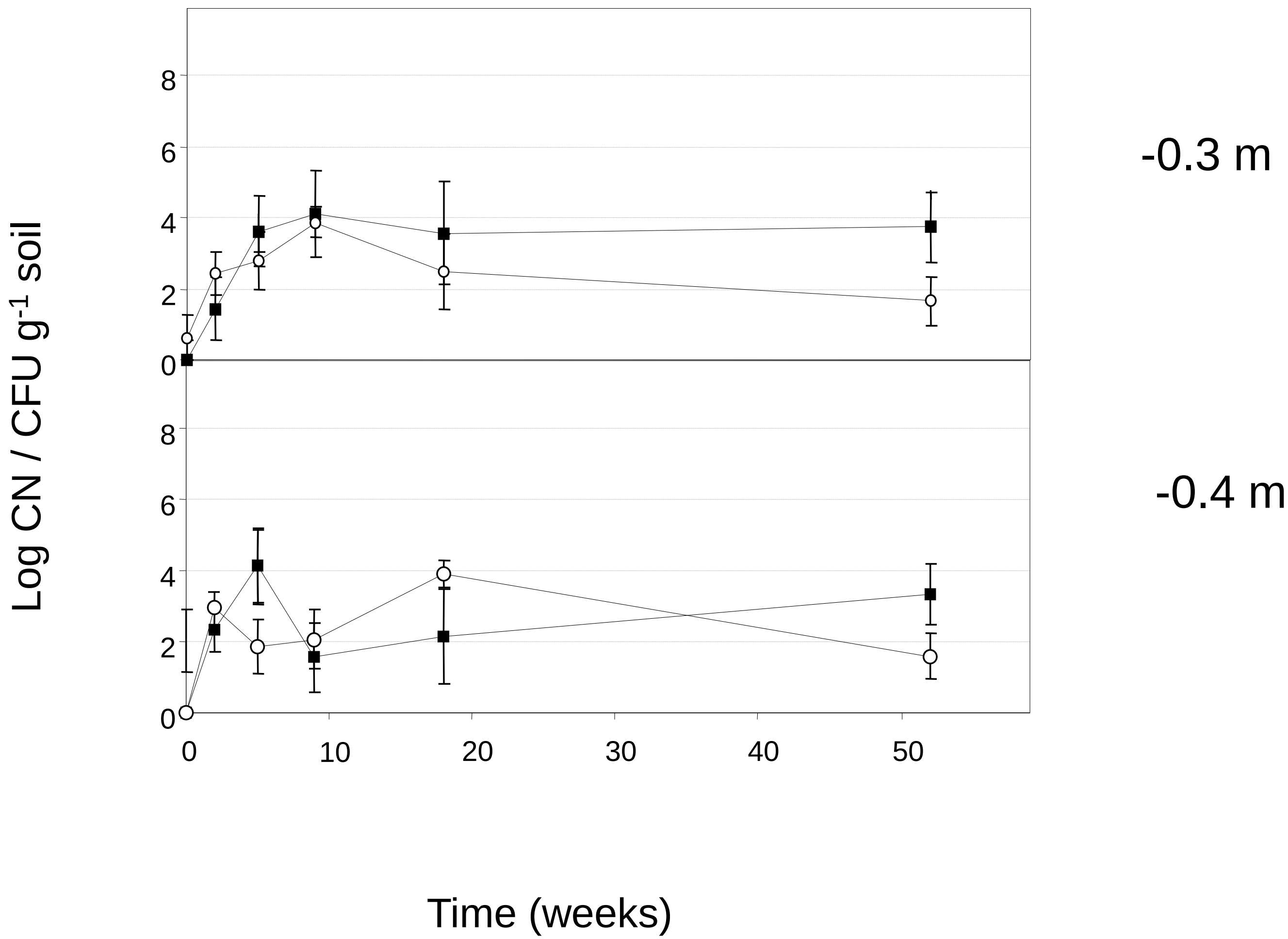
Not always good survival in non sterilised soils: competition

Limited efficacy when applied as conidia suspension in soil (unless high dosage are applied >10^7 CFU/g soil)

Non sterilized soils were inoculated at day 0 by a conidia-water suspension (1.5 x 10^6 CFU·ml^{-1}) with no formulation



Real time PCR and colony forming units on semi-selective medium



After 1 year *T. atroviride* SC1 at the constitutive level of Trichodermas in soil (10-10^2/g soil)

Armillaria mellea on grapevine

- plants stunted
- leaves that redden prematurely in autumn
- more sensitive to water stress and cold
- poor wine quality

- rotted roots
- wilted branches
- dead plants

Armillaria mellea or *A. gallica* on blueberry

- plants stunted
- small leaves that redden prematurely in autumn
- more sensitive to water stress and cold

- rotted roots
- wilted branches
- dead plants

On roots:

- white mycelium
- rhizomorphs

Blueberries are mulched with a layer of bark (coniferous bark)

and covered with plastic net

- *Armillaria* is a pathogen of trees, but can survive as saprophyte on wood or root residues
- We found white mycelium and rhizomorphs on bark heaps and on bark near symptomatic plants

Bark:

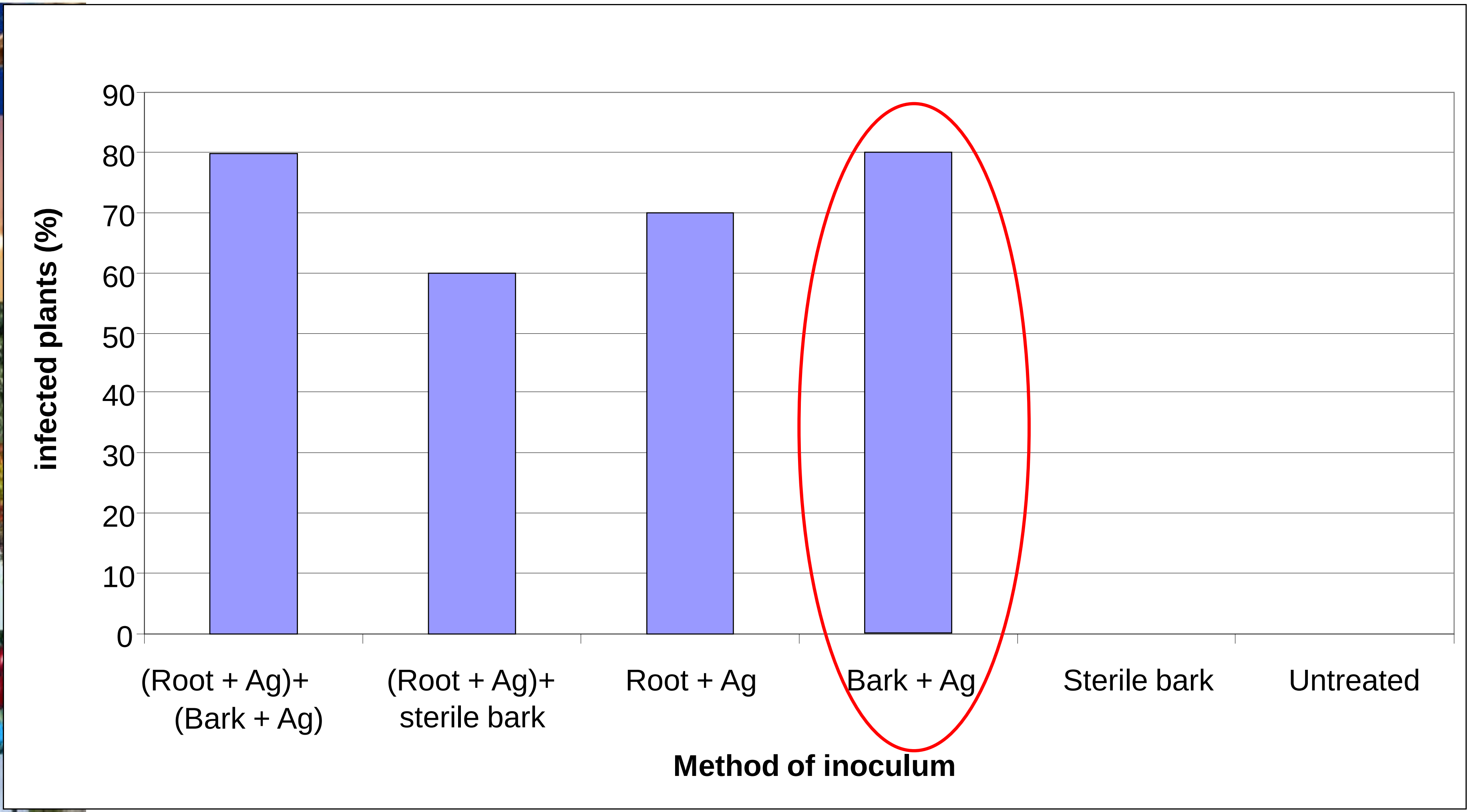
potential source of inoculum?

Role of barks and root debris as inoculum source

- young potted blueberry plants were inoculated with infected coniferous bark and/or infected wood pieces inserted between roots

Role of barks and root debris as inoculum source

1 year after ***A. gallica* (Ag)** inoculation

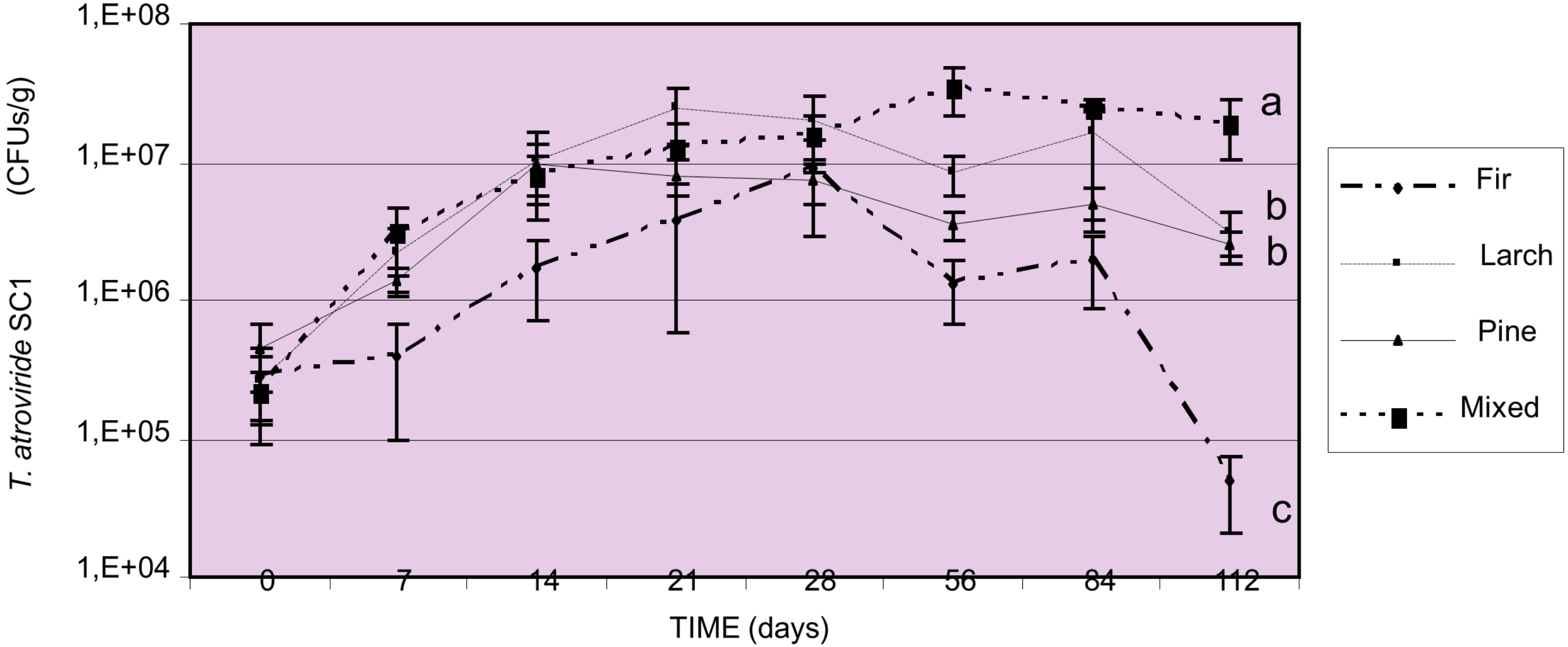



Sterile barks
A. gallica infected barks

Barks as carriers of *T. atroviride* SC1

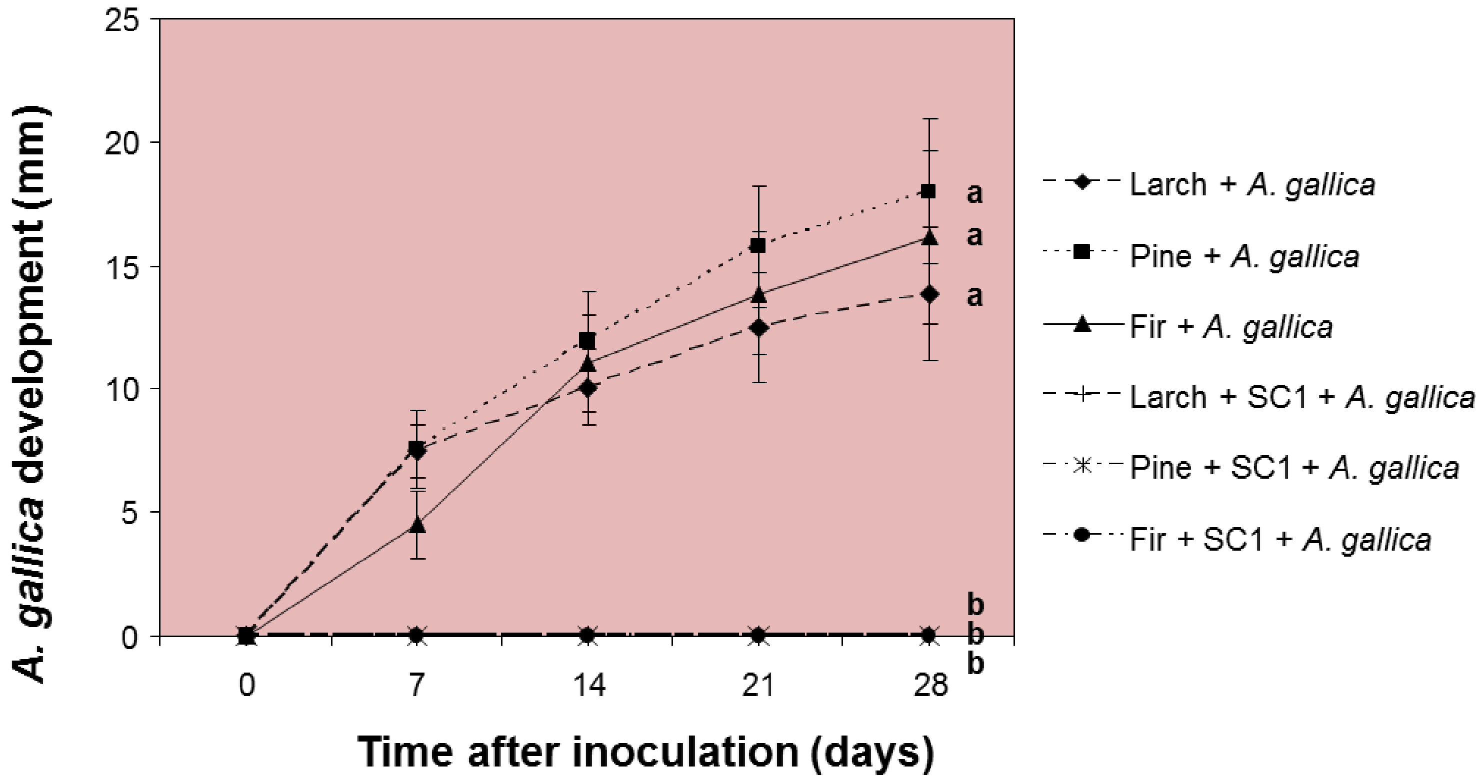
- Fir, larch and pine barks and mixture
- Half - inoculated with SC1 (1×10^{7}conidia/ml; 0.7 ml/g barks) and half - sterile water
- Colonization of SC1 on barks: CFUs at 0, 1, 2, 3, 4, 8, 12 and 16 weeks after inoculum

T. atroviride SC1 during time



Different letters = differences at $p<0.05$, Kruskal-Wallis test.

Efficacy against *A. gallica* on barks



Different letters = differences at $p<0.05$, Kruskal-Wallis test.

Prevention of *A. gallica* by barks pre-inoculated with *T. atroviride* SC1

Bark mixture (best survival)

Strawberry, cv. Elsanta/*A. gallica* pathosystem (fast response)

- Bark mixture (carrier) inoculated with *T. atroviride* SC1 (50 ml/l of bark, 3 $\times 10^7$ conidia/ml) and incubated (14 days, room temperature)
- Applied to potted strawberry plants as mulch inoculation with apple wood that had been infected with *A. gallica*

*20 plants*replicate*treatment, repeated experiment*

Results

Treatment	**Incidence**
Bark pre-treated with *T. atroviride* SC1 + *A. gallica* inoculum	25 ± 2.3% b
Untreated bark + *A. gallica* inoculum	70 ± 1.4% a
Untreated bark + uninoculated (control)	0 ± 0% c
3 months; different letters= Kruskal-Wallis test P<0.5	

Barks used **as carrier** of *T. atroviride* SC1 controlled the disease originating from soil inoculum

T. atroviride SC1 to control *A. gallica* originating from infected barks

Plants and barks as previous experiment

- Infected bark mixture (*A. gallica* until full colonization of barks, 3 months)
- Half of these infected bark treated with a *T. atroviride* SC1 suspension and incubated for 14 days then used as mulch
- Half untreated
- Infected bark (treated and untreated) was used as mulch

*20 plants*replicate*treatment, repeated experiment*

Results

Treatment	Incidence
Bark infected by *A. gallica* and then treated with *T. atroviride* SC1	10 ± 1.4% b
Bark infected by *A. gallica*	45 ± 1.6% a
Healthy bark untreated (control)	0 ± 0% c
3 months; different letters= Kruskal-Wallis test P<0.5	

T. atroviride SC1 applied on infected barks (**disinfestation treatment**) controlled the disease originating from *A. gallica* infected bark

Conclusions

- Currently, preventive **agronomic practices** are the main way to manage Armillaria root rot in IPM (crop rotation, alternation, removal of infected roots)
- However: **rotation often useless** (polyphagous pathogen) and residue removal expensive and difficult

o Use of Trichoderma to **prevent** source of inoculum originating from infected bark

- Use of Trichoderma **as carrier** to prolong the survival and efficacy
- + Cheap, easy, increase in organic matter
- - Regulation: registration of this type of use may be difficult

Prospects

- Test on other pathosystems
  - Pre-treated barks as carrier of Trichoderma on other crops and pathogens
  - Composted material with Trichoderma
  - Check influence of barks on other pathogens (they can act as substrate)
  - Other carriers (chitin-rich substrates as shrimps shells)

- Regulation issues
  - Registration as bark disinfectant
  - Registration as new formulation
- Scaling up production and logistic issues

Thank you for your attention!

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The research leading to these results received funding from the European Union Seventh Framework Programme (FP7/ 2007-2013) under grant agreement no. 265865- PURE