Trichoderma atroviride SC1 for biocontrol of fungal diseases in plants

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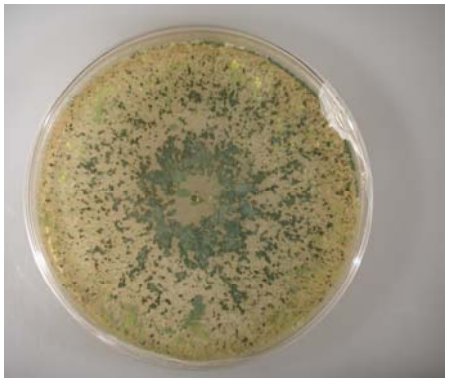
ISTITUTO AGRARIO DI SAN MICHELE ALL'ADIGE

***Trichoderma atroviride* SC1 (CBS122089) isolated from hazelnut in northern Italy (2000)**



Initially developed against *Armillaria* spp.
Active against 10 plant pathogens (more?)

Patent deposit
(PCT/IT2008/000196)

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T. atroviride SC1 grows on mycelium and rhizomorphs of Armillaria and kills it

Treatments

Grown on rice and applied in/on the soil

Grown on liquid media and applied to the soil

Pre-treatment of organic substrates or mulching barks

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Efficacy trials

Armillaria mellea
A. gallica

ROOT
Armillaria spp.

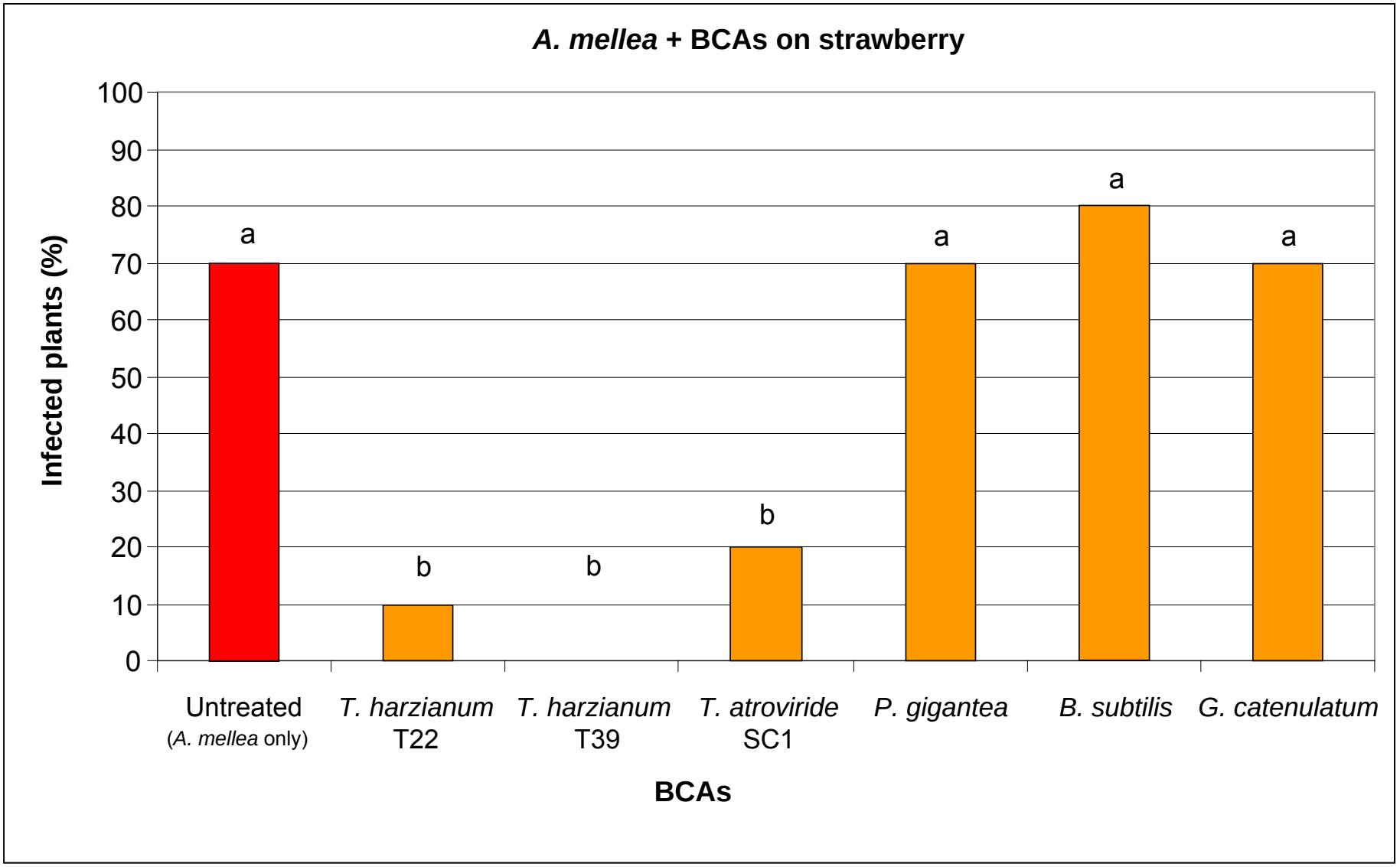
Phaeomoniella chlamydospora
Phaeoacremonium aleophilum
Fomitiporia mediterranea

TRUNK
ESCA disease

Podosphaera xanthii

LEAF
Powdery mildew

Efficacy trial on strawberry/blueberry plants: inoculation of *A. gallica* and *A. mellea* by infected wood pieces and BCAs



Different letters indicate significant differences ($p \leq 0.05$) according to the Kruskal-Wallis test.

Assessments done 150 days after inoculation with *A. mellea*

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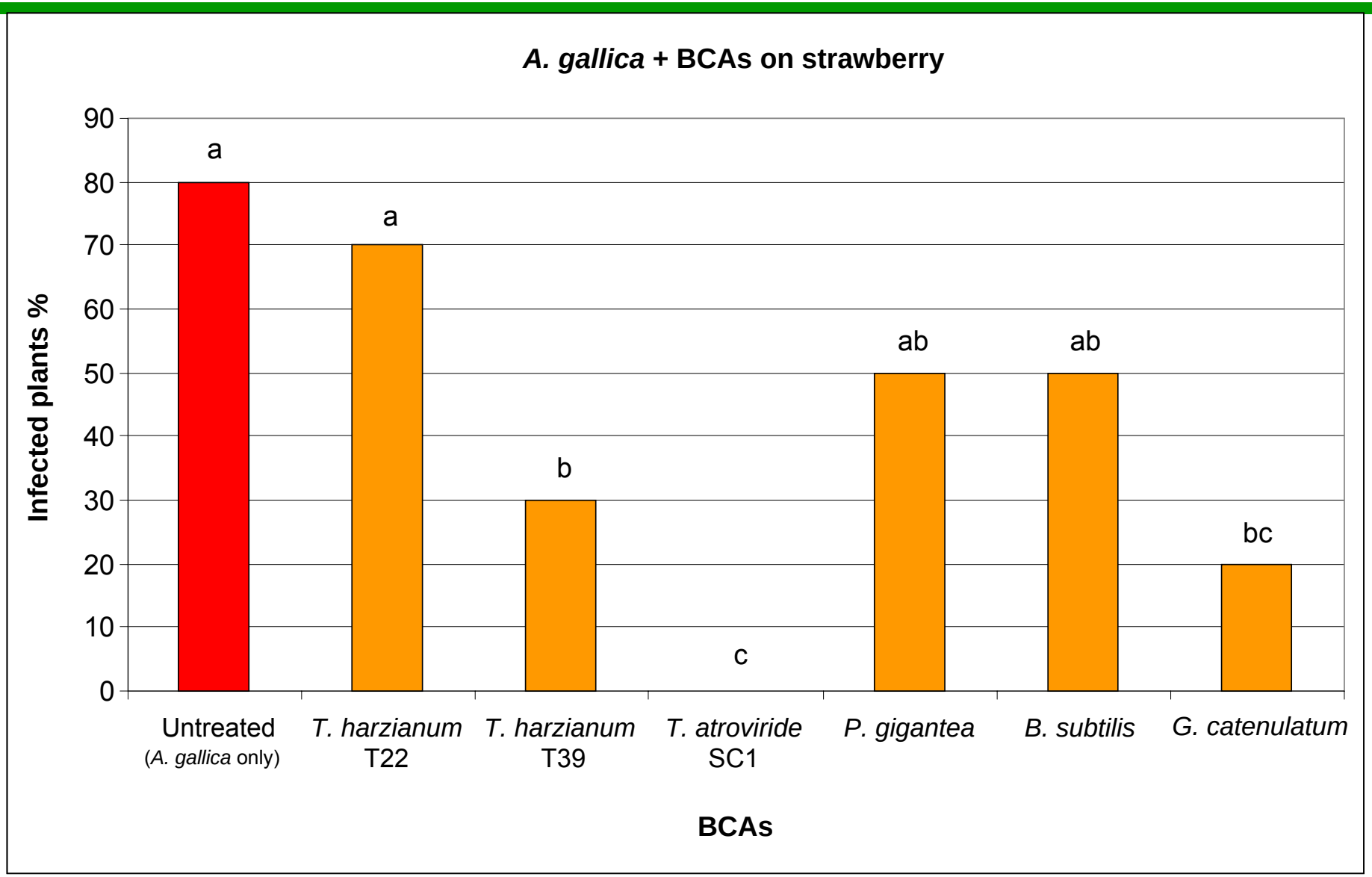

Different letters indicate significant differences ($p \leq 0.05$) according to the Kruskal-Wallis test.

Assessments done 150 days after inoculation with *A. gallica*

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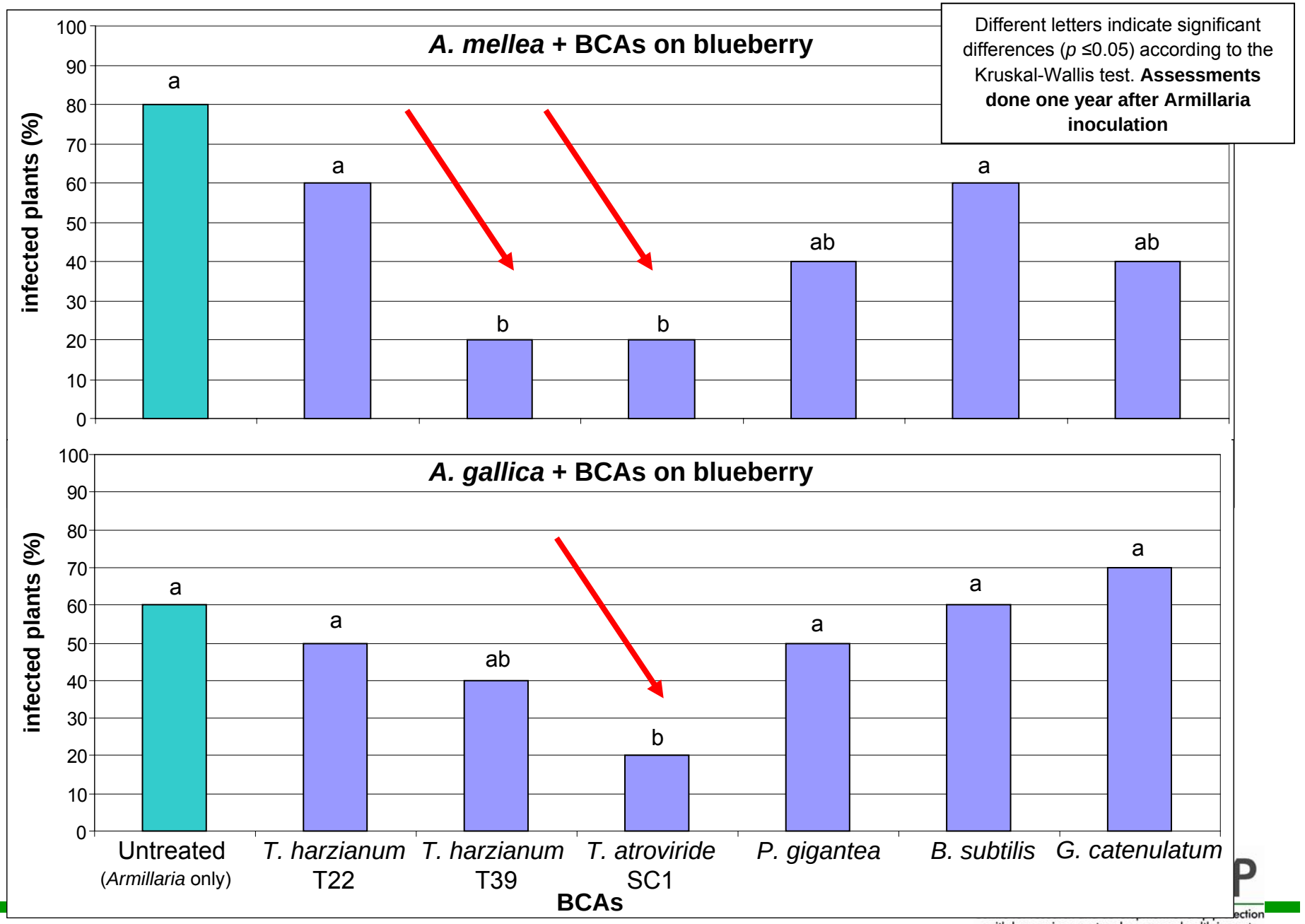
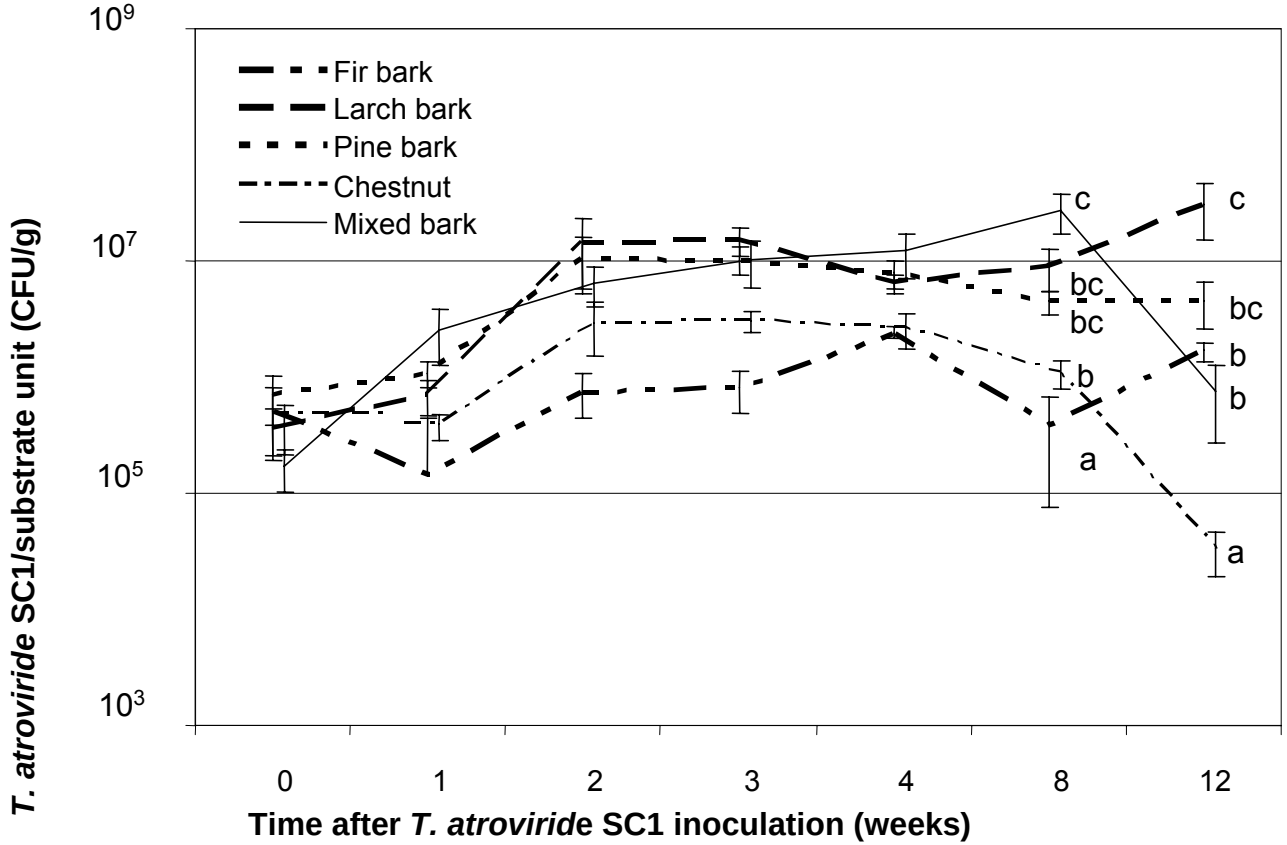

A. mellea + BCAs on blueberry
infected plants (%)
100
90
80
70
60
50
40
30
20
10
0
a
a
b
b
ab
a
ab
Different letters indicate significant differences (p ≤0.05) according to the Kruskal-Wallis test. Assessments done one year after Armillaria inoculation
A. gallica + BCAs on blueberry
infected plants (%)
a
a
ab
b
a
a
a
Untreated (Armillaria only)
T. harzianum T22
T. harzianum T39
T. atroviride SC1
P. gigantea
B. subtilis
G. catenulatum
BCAs

Survival of *T. atroviride* SC1 in barks



Barks treated with *T. atroviride* SC1

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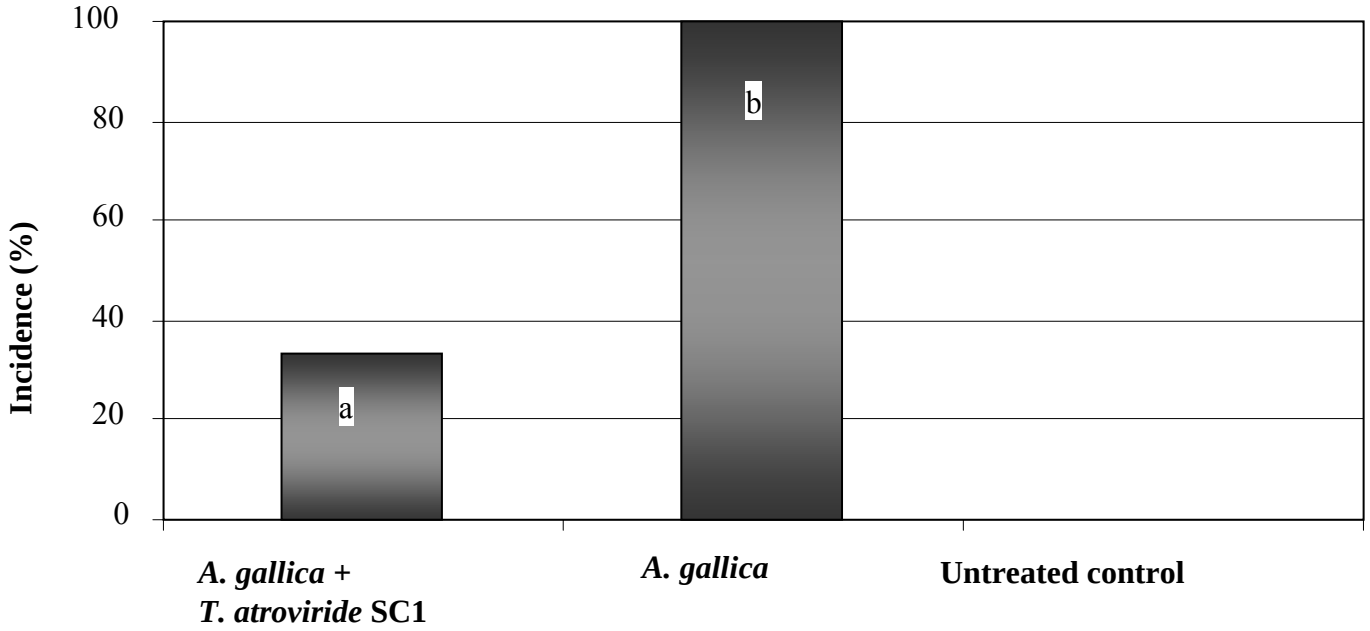
Biocontrol activity of *Trichoderma atroviride* SC1 against *Armillaria gallica* in a bark mixture



Wood pieces infected by *A. gallica* placed in barks and treated with *T. atroviride* SC1
Assessment (percentage of infected wood pieces): after one year

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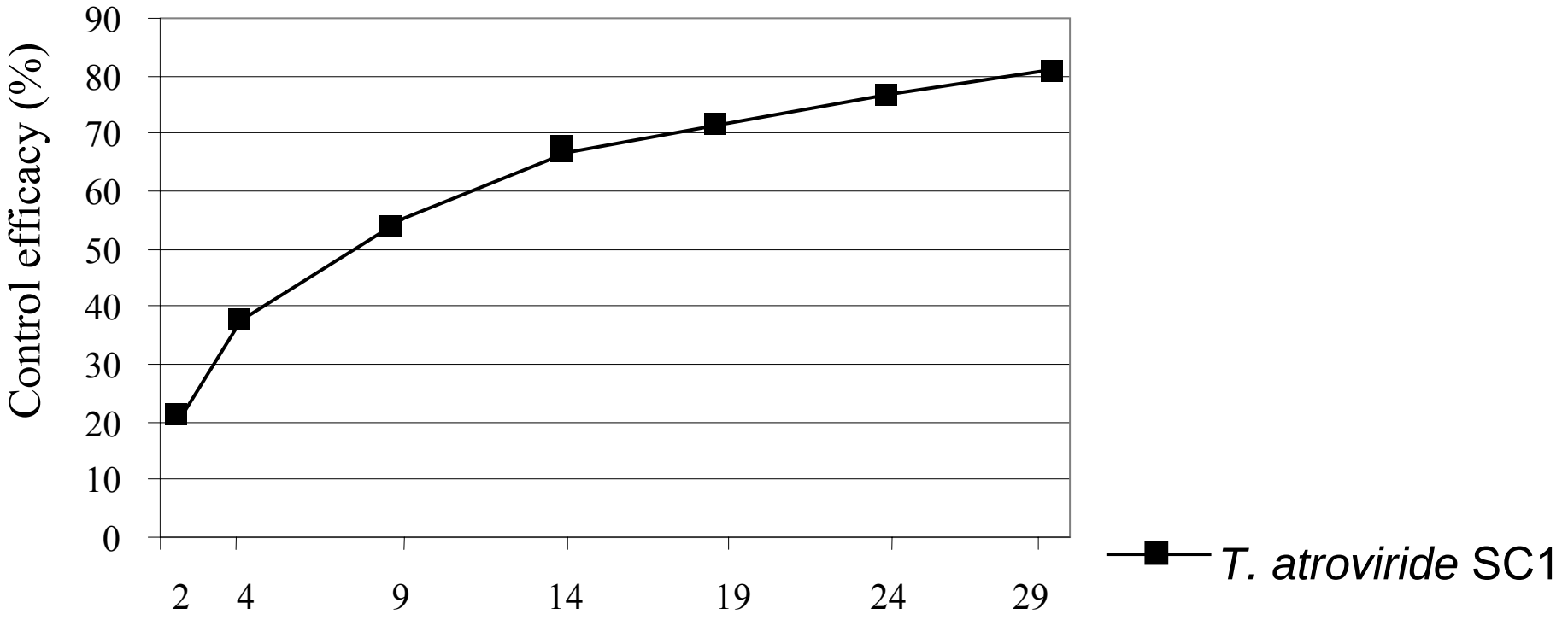
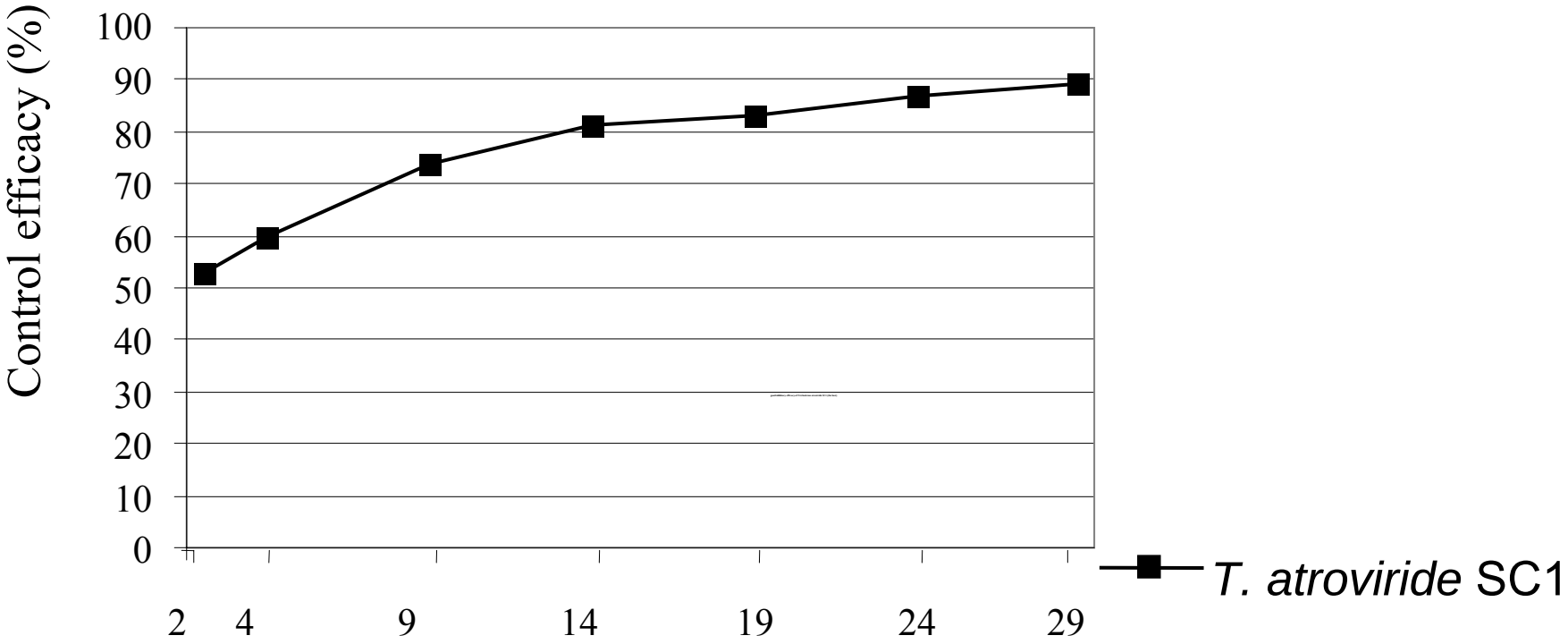
Phaeomoniella chlamydospora



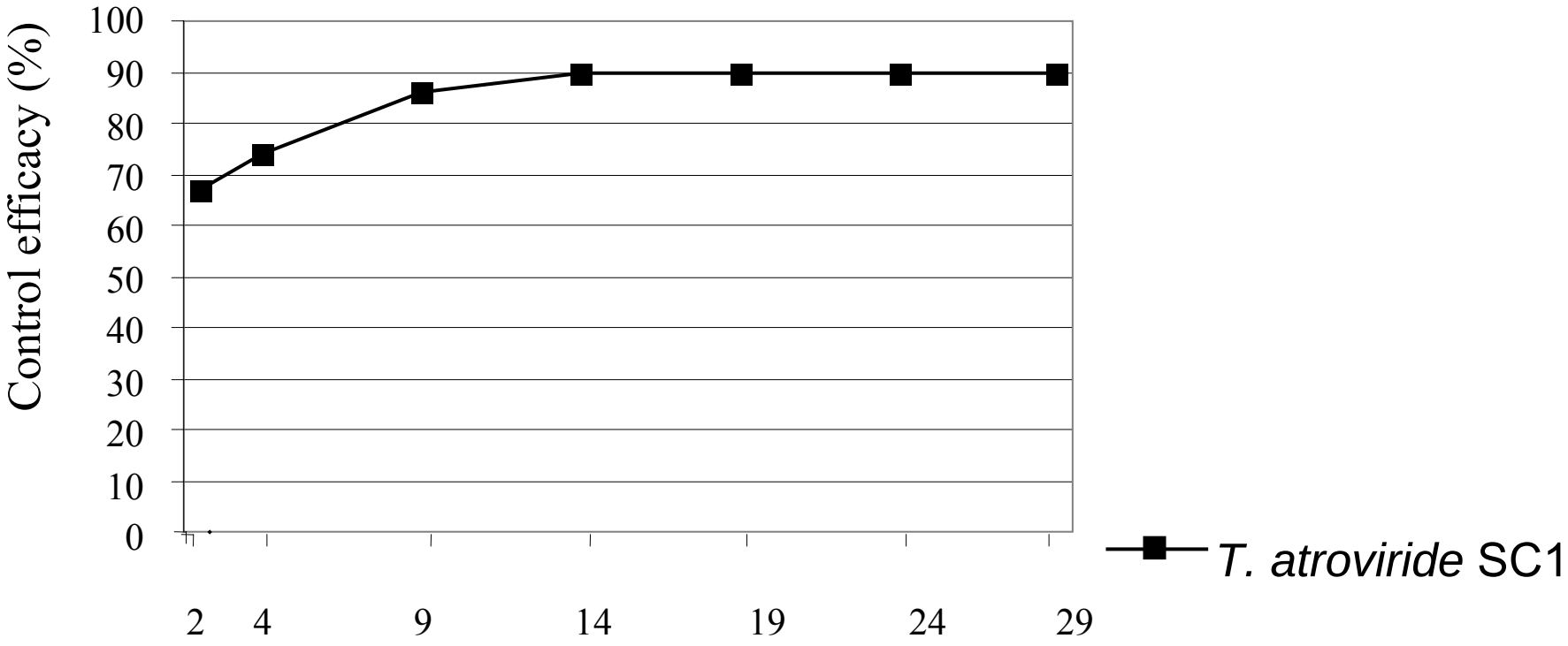
Growth inhibition by *T. atroviride* SC1

Phaeoacremonium aleophilum



Growth inhibition by *T. atroviride* SC1

Fomitiporia mediterranea



Growth inhibition by *T. atroviride* SC1 and by *B. subtilis* F77

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Esca: efficacy trials on plants

Plants were cut and **wounds treated** with *T. atroviride* (10^6 conidia/cm^2)
Inoculated with Pal or Pch (10^5 conidia/cm^2) 24 h after
Untreated controls (no *T. atroviride* SC1 treatment)
10 plants/treatment

5 months after treatment, slices below the cut, PDA. Repeated (two independent exp.)

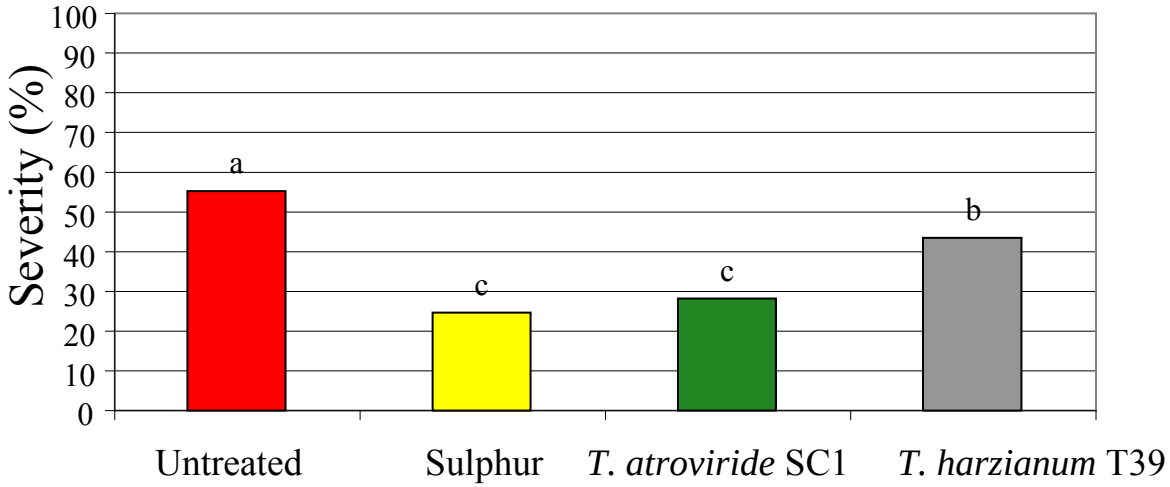
No Pal or Pch infection occurred on treated wounds
Pal and Pch presence in the wood (5 cm below the cut) in untreated control
• SC1 a tool to prevent Esca infections

Powdery mildew: efficacy trials on plants

Plants: **cucumber and zucchini** (4 replicates of 5 plants per treatment)
Treatments: water suspensions of ***T. atroviride* SC1** (10^4 conidia/ml^2)
Untreated control and standard (Sulphur; Thiovit, Syngenta)
Inoculation: *Podosphaera xanthii* (10^5 conidia/ml^2) 2 h after

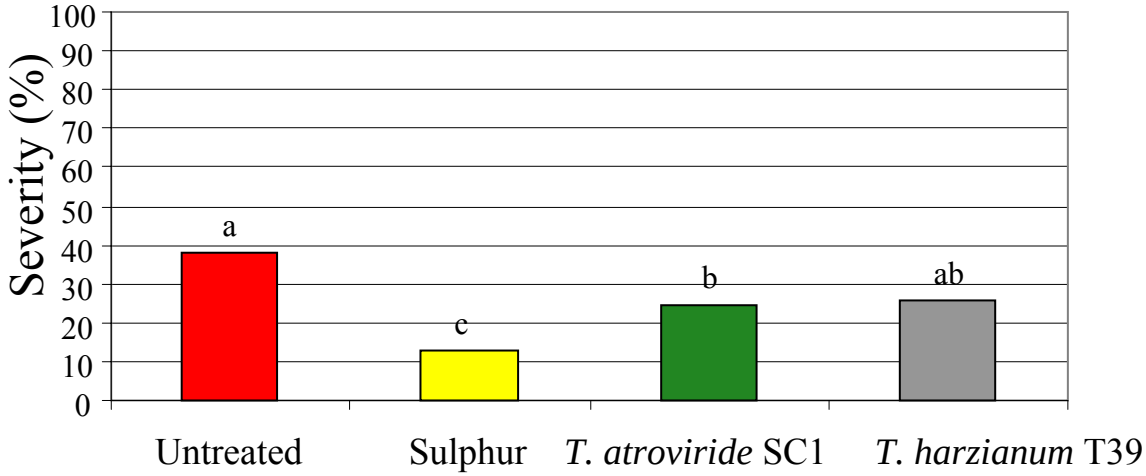
2 weeks after treatment, assessment of **severity** (% of symptomatic leaf surface)

Powdery mildew *(Podosphaera xanthii)*



Cucumber

Zucchini



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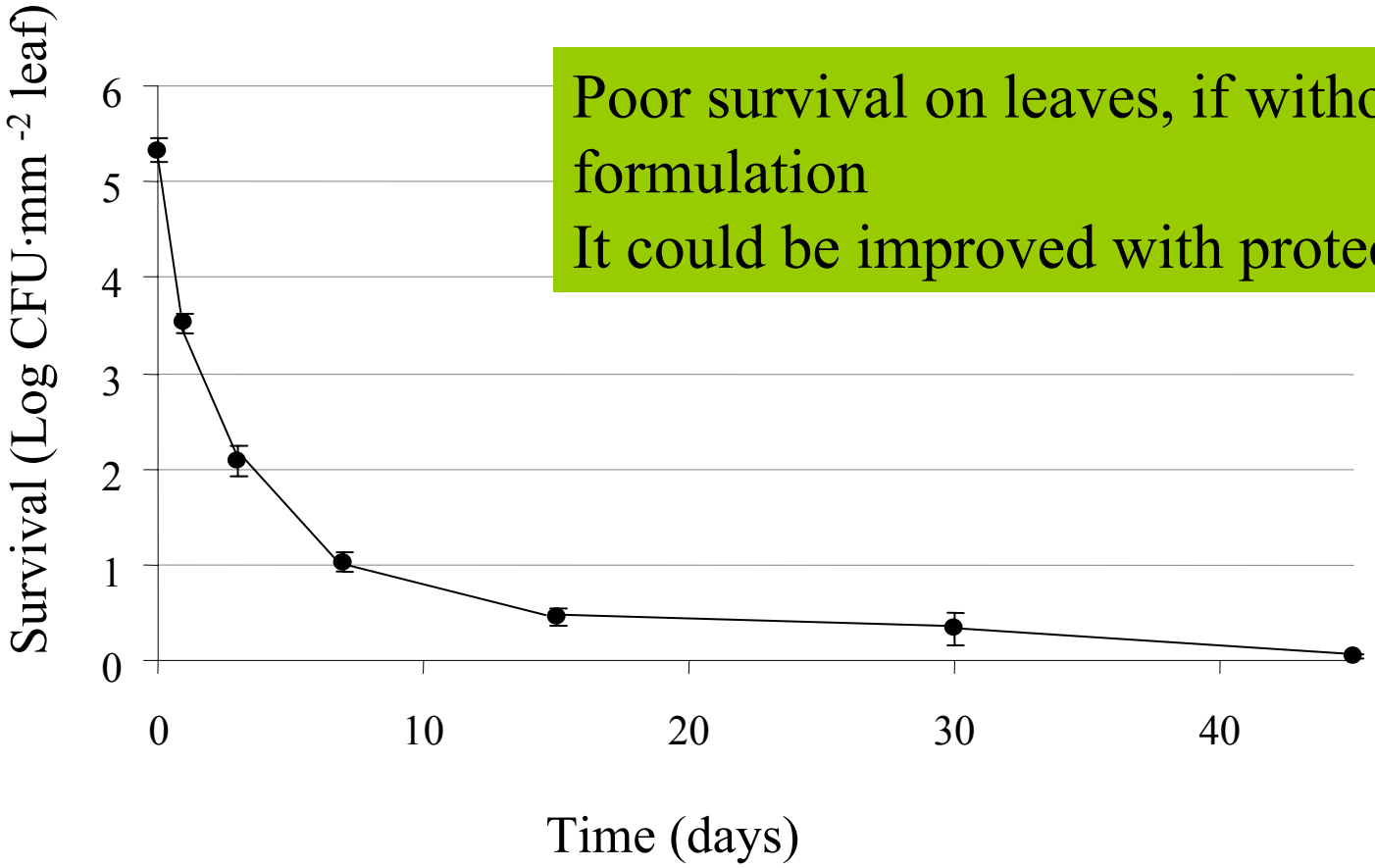
Survival on leaves



Poor survival on leaves, if without formulation
It could be improved with protectors

Leaves were inoculated at day 0 by spraying a conidia-water suspension (10^6 CFU·ml^{-1}) with no formulation

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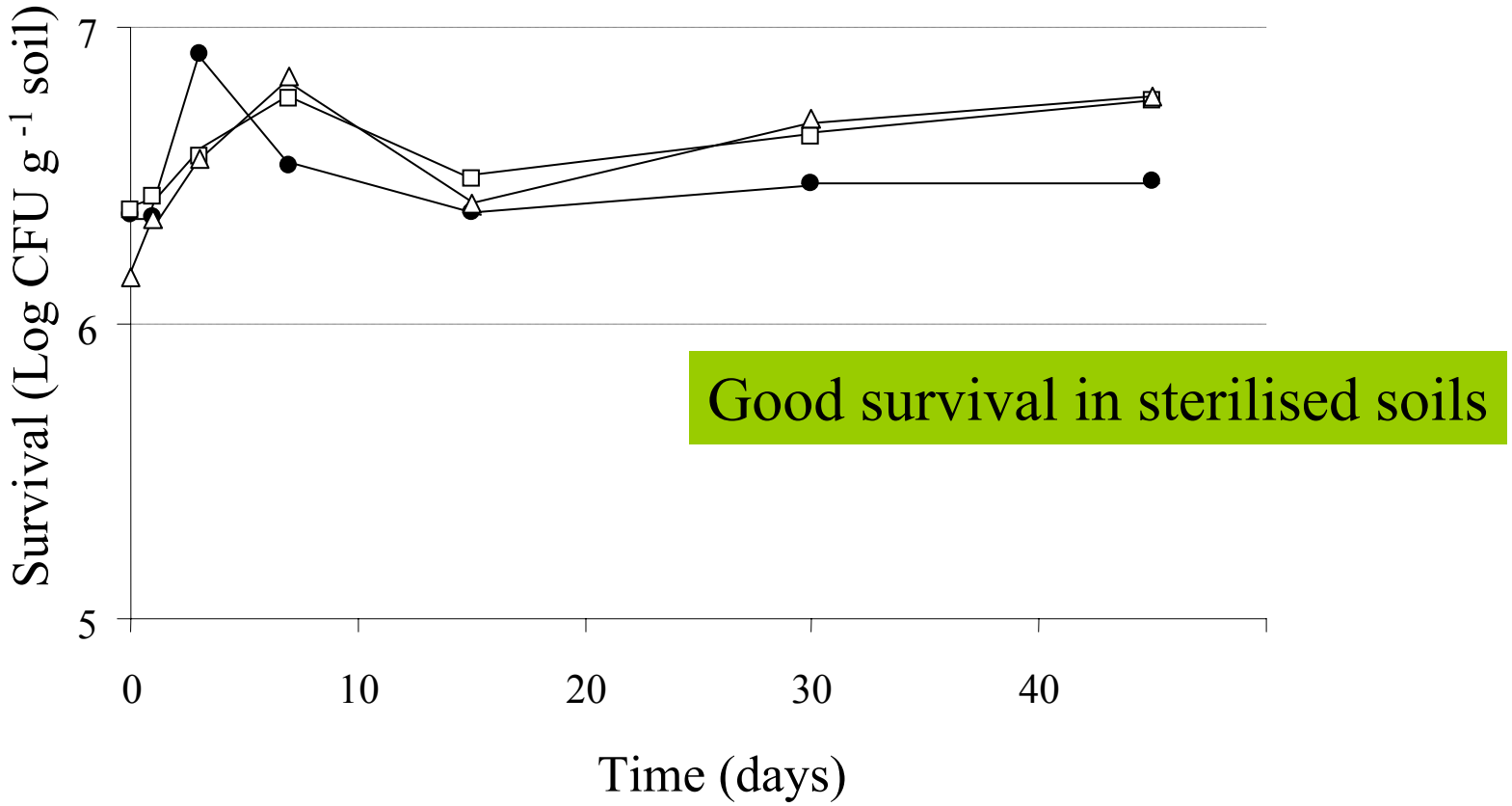
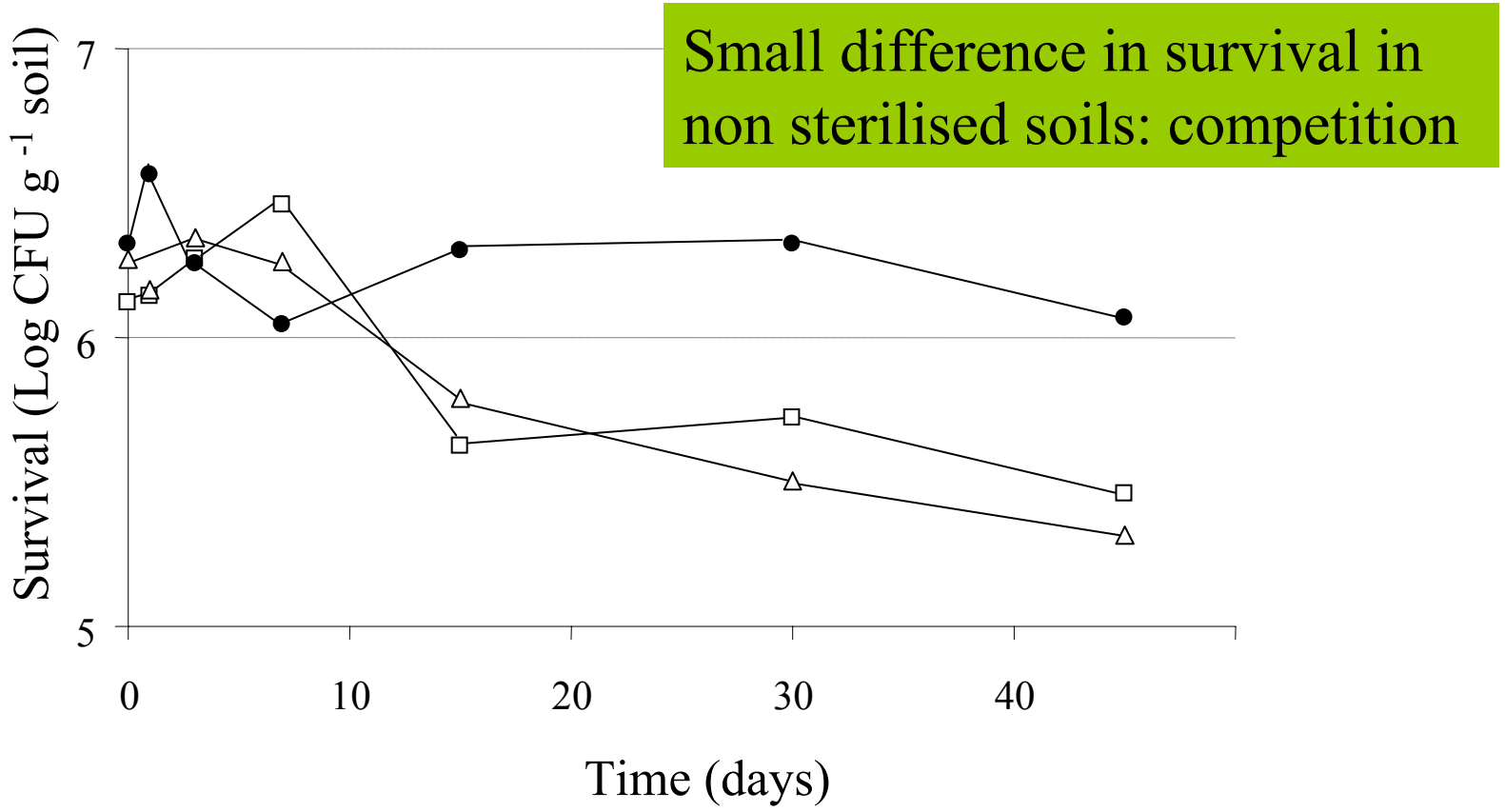
Survival in sterilised soils



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

Survival in non sterilised soils



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

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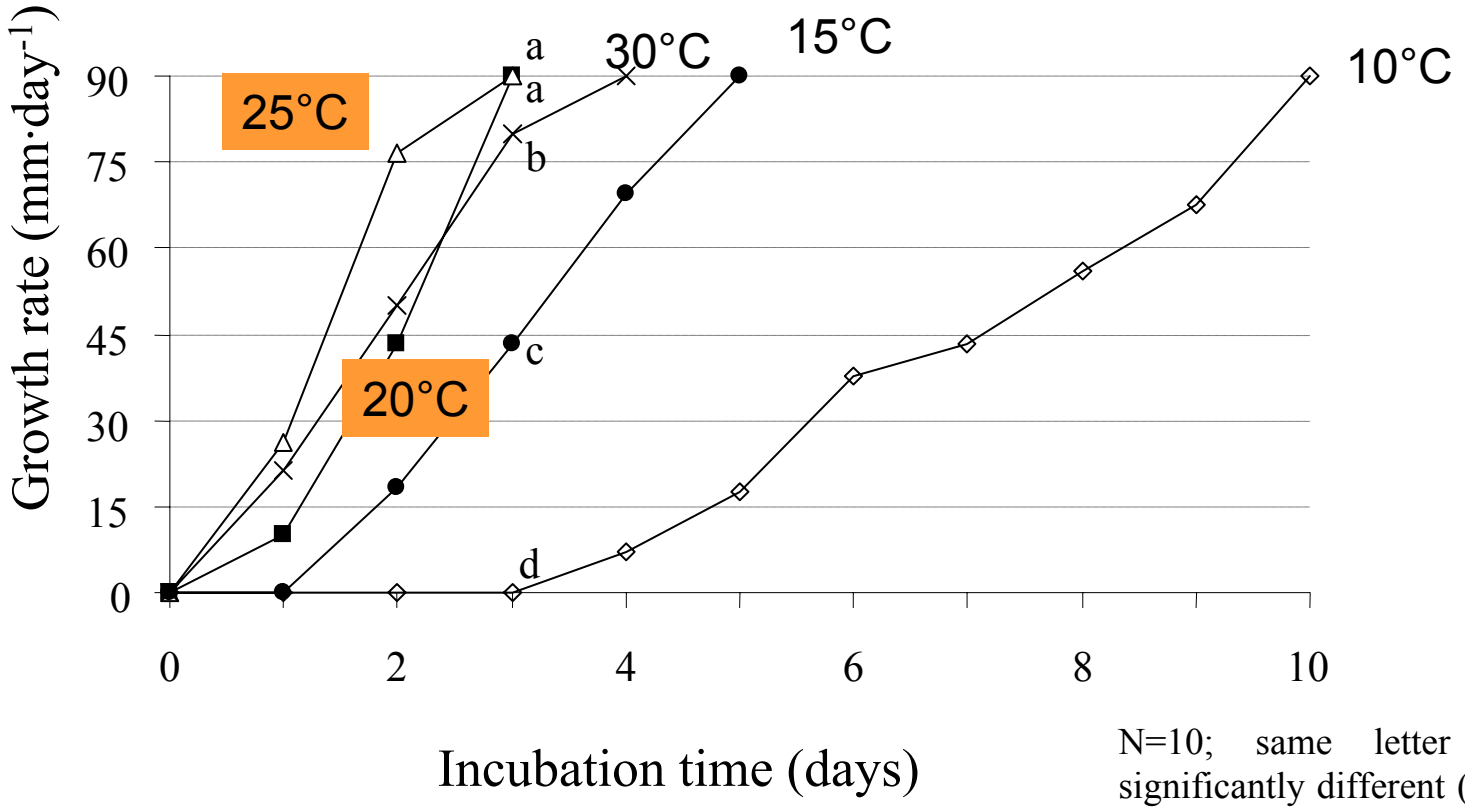
Growth parameters:
Useful information for industrial production, but also for registration

Effects of temperature and pH on *T. atroviride* SC1

T: -1, 5, 10, 15, 20, 25, 30, 37 and 40°C, with pH 5 and a_w 0.998.

pH: 3, 4, 5, 6, 7, 8, 9 and 10 with a_w 0.998 at 25°C.

Radial growth of *T. atroviride* SC1 at different temperatures



N=10; same letter = not significantly different ($P \leq 0.05$) according to Tukey's test

No growth: at -1, 5, **37** or 40°C

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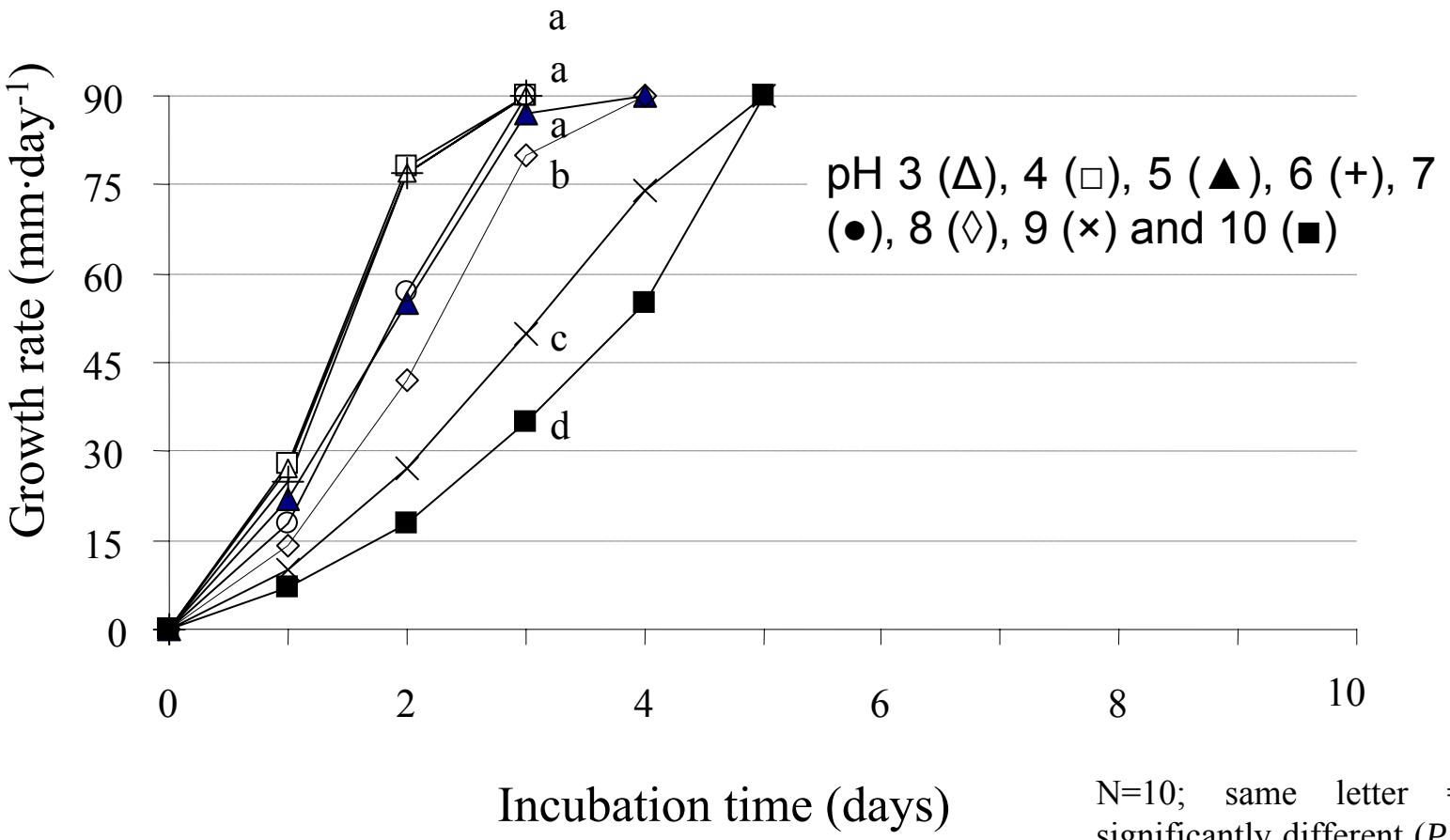
Radial growth of *T. atroviride* SC1 at different pH



N=10; same letter = not significantly different ($P \leq 0.05$) according to Tukey's test

Best growth between pH 3 and 5

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Specific real time PCR for detection and quantification
Necessary for precise identification and quantification

Real-time PCR primers and probe

The real-time PCR primers and the strain-specific TaqMan probe set were designed based on these two nucleotide mismatches on the 3' strand of the *ech42* gene of *T. atroviride* SC1

A **second primer set** and TaqMan **probe** were designed for the *tga3* gene, which encodes the G protein α subunit

Real time PCR: Specificity

tga3 probe acts as internal control of the reaction

Real-time PCR (*ech42* and *tga3* primers) resulted in amplification products from all DNA from *Trichoderma* spp. strains, other fungi and soil samples

Conversely, in the presence of the *ech42* TaqMan probe (two SC1-specific point mutations), **only *T. atroviride* SC1 produced a single signal** and **no probe hybridization occurred with amplification products** of other fungi, grapevine and soils samples

Real time PCR: Quantification

By interpolating the **threshold cycle (Ct) values of the sample** with the Ct values of **known concentrations of purified genomic *T. atroviride* SC1 DNA**

T. atroviride SC1 quantification: as haploid copy number (CN) of genomes, considering that the single copy *Trichoderma* genome size is 0.034 pg

Survival and vertical dispersion in soil (2006-2007)

Six plots of 0.6 × 0.6 m each, located between grapevine plants in the row **in a vineyard**

Three plots were inoculated with *T. atroviride* SC1 (500 g of the boiled-rice with the fungus grown on it)

The inoculum **was mixed into the soil surface** layer (approximately 3 cm deep)

The initial concentration of the fungal inoculum in this layer was estimated to be 10^8 CFU g dry soil^{-1}

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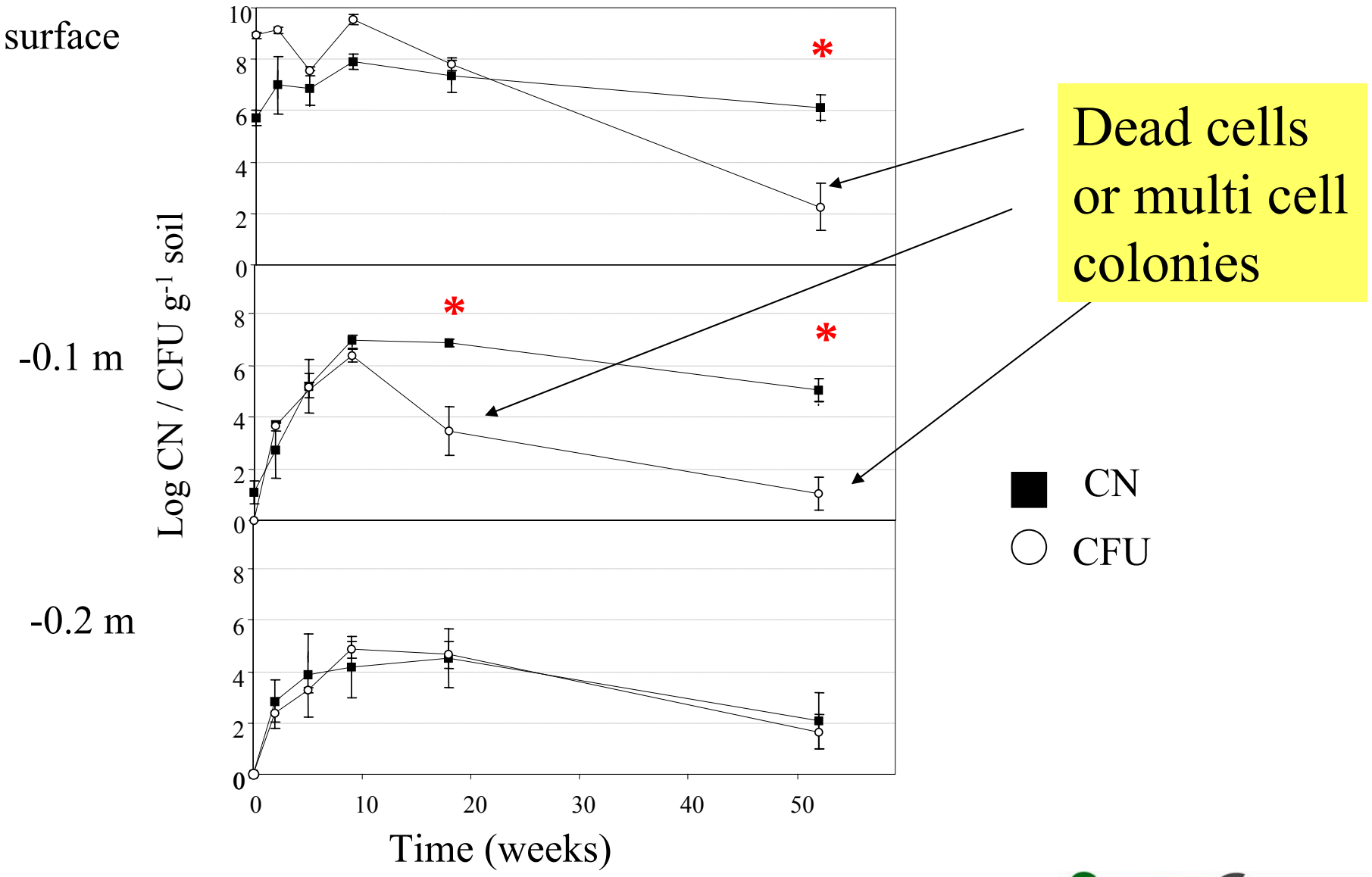
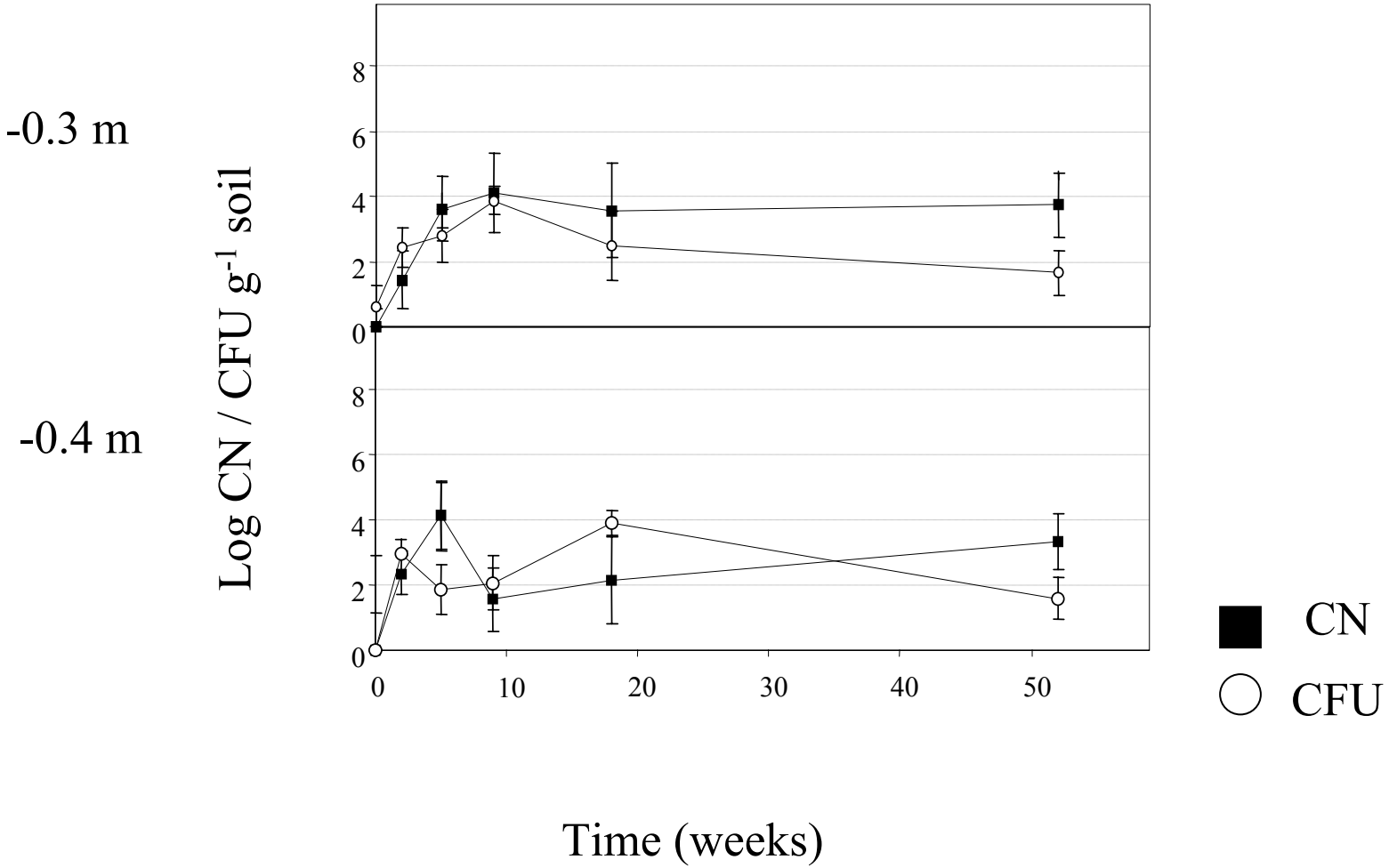
- **DNA extraction + Real time PCR**
- **Colony forming unit (CFU) counting on semi-selective medium**



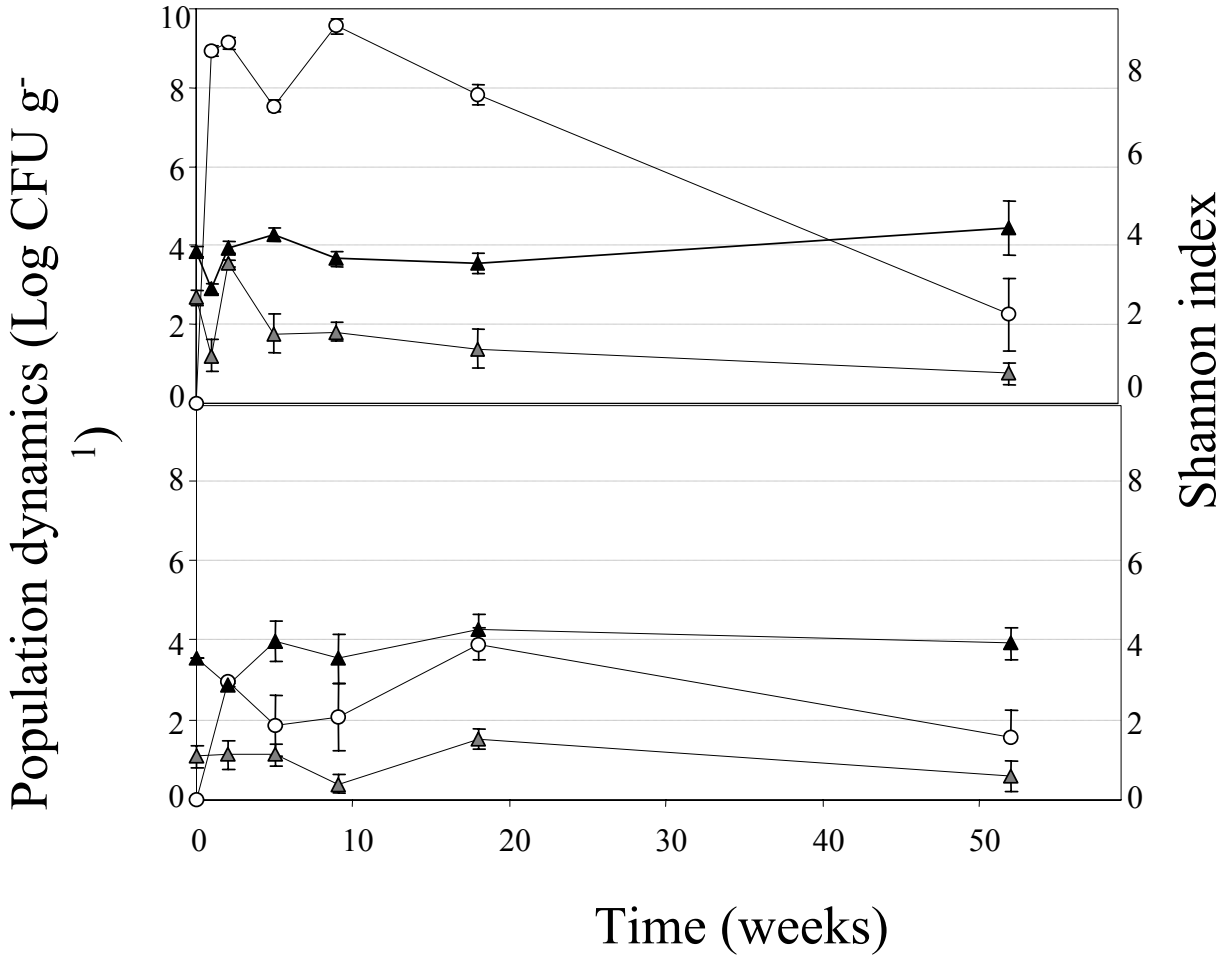
* Significantly different (ANOVA, P<0.05)


-0.3 m
-0.4 m
Log CN / CFU g-1 soil
8
6
4
2
0
8
6
4
2
0
0
10
20
30
40
50
Time (weeks)
CN
CFU

Impact of *T. atroviride* SC1 on microbial communities in soil

Native communities of bacteria and fungi in the soil were analyzed using **automated ribosomal intergenic spacer analysis (ARISA)**
PCR amplification of the **intergenic region between the small and the large subunit rRNA genes** in the rRNA operon with fluorescence-tagged oligonucleotide primers from the total bacterial or fungal community DNA and **automated detection of fluorescent DNA fragments** (Fisher & Triplett, 1999)

Impact of *T. atroviride* SC1 on microbial communities in soil: biodiversity



Surface

- 40 cm

▲ Fungi Shannon I.

▲ Bacteria Shannon I.

○ SC1 population

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Conclusions:

- *T. atroviride* SC1 is **effective against several pathogens**
- **Need to improve formulation** for leaf treatments
- Survives **for long time in soil** (1-2 years after application, levels ~ similar to native *Trichoderma* spp.)
- **Transient effect** on soil microflora

Thank you for your attention!

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