Endophytes and Plant/Microbe Interactions

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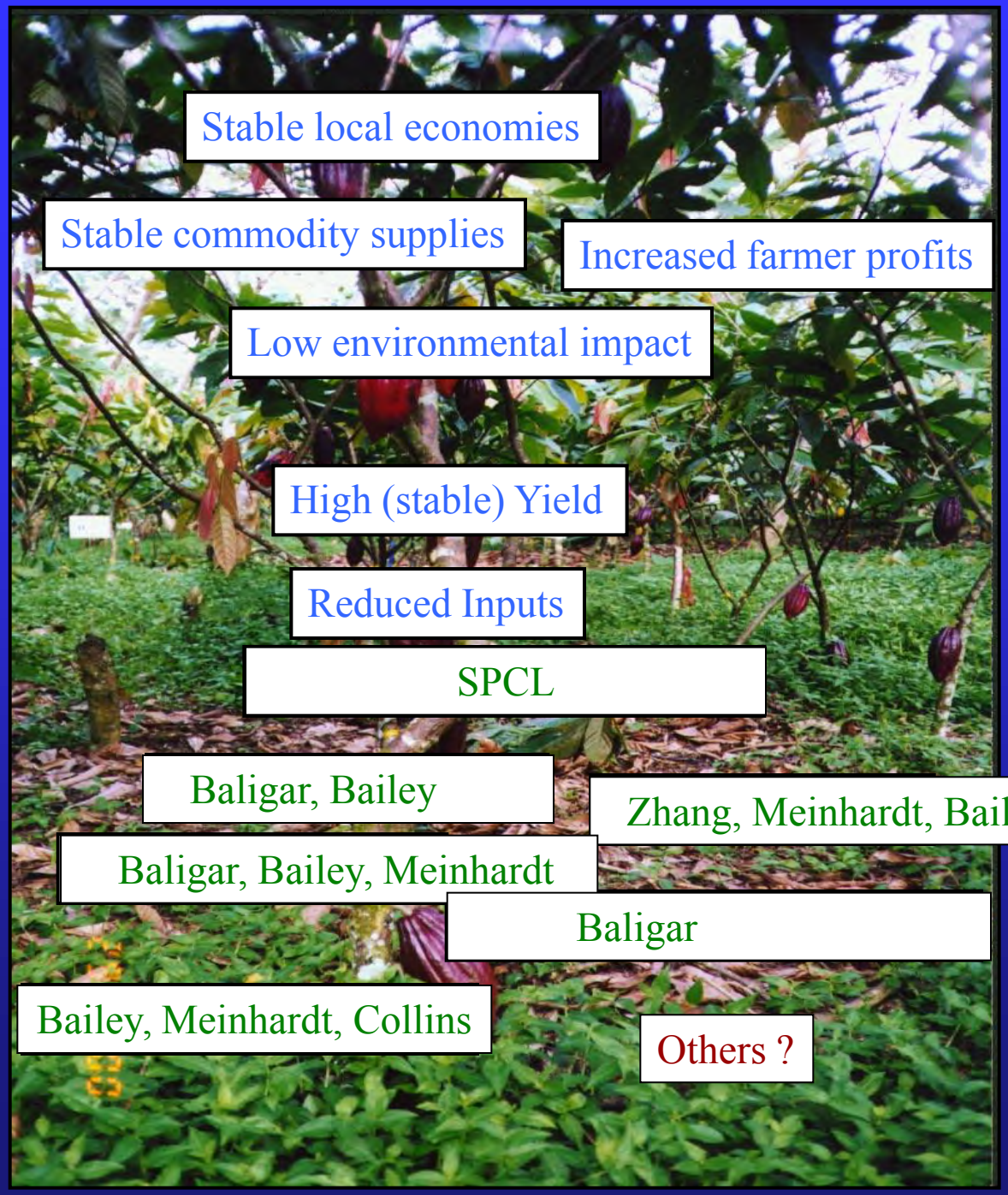
Beltsville Area Research Center

Agriculture Research Service

United States Department of Agriculture

- For the FY 2005 SPCL became its own entity.

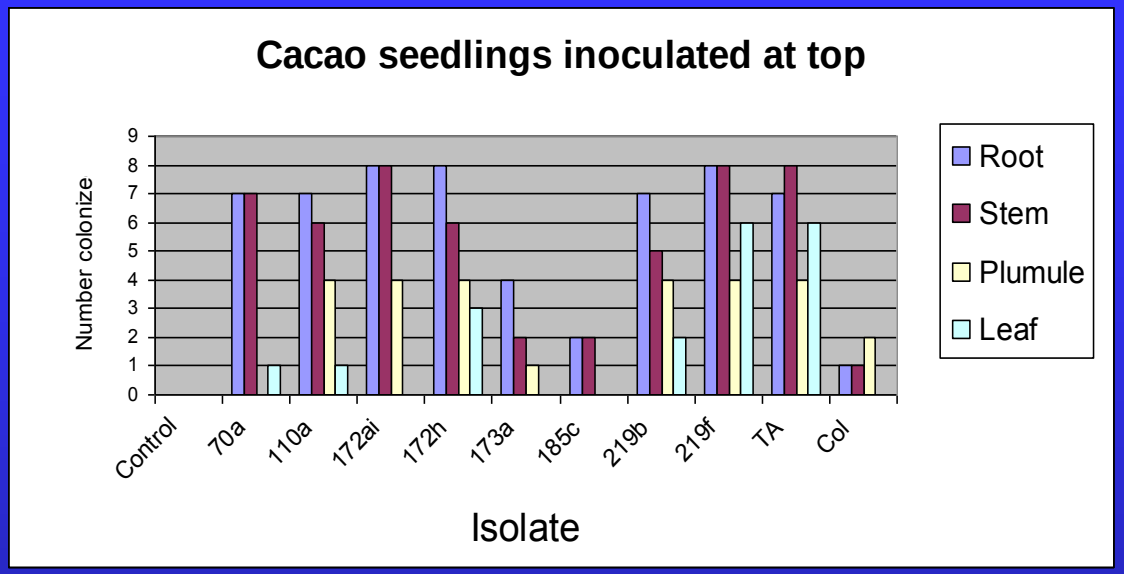

Stable local economies
Stable commodity supplies
Increased farmer profits
Low environmental impact
High (stable) Yield
Reduced Inputs
SPCL
Baligar, Bailey
Zhang, Meinhardt, Bailey
Baligar, Bailey, Meinhardt
Baligar
Bailey, Meinhardt, Collins
Others ?

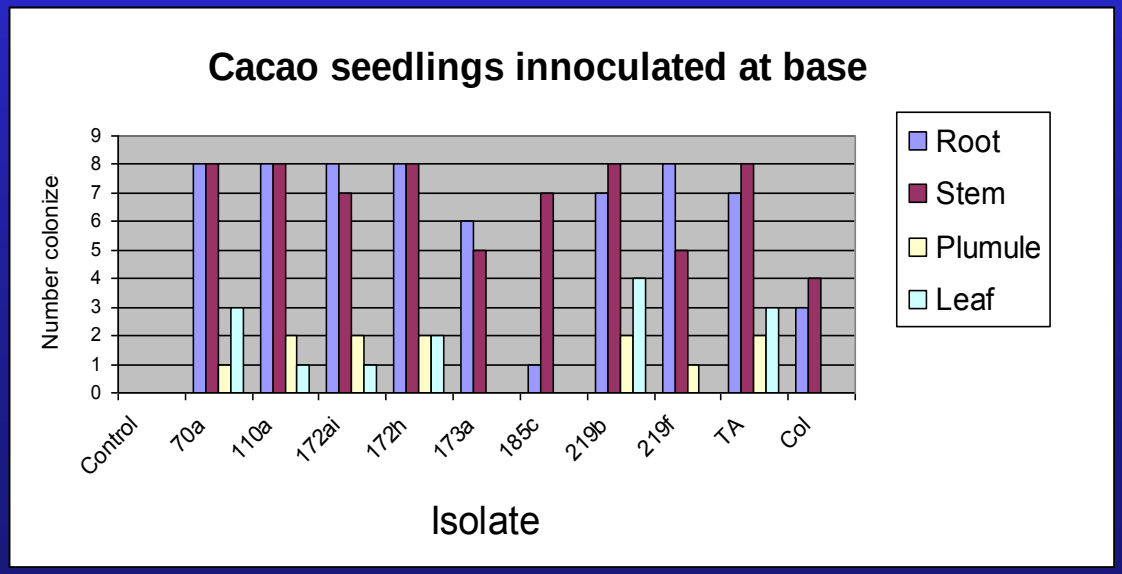
Major Diseases of cacao

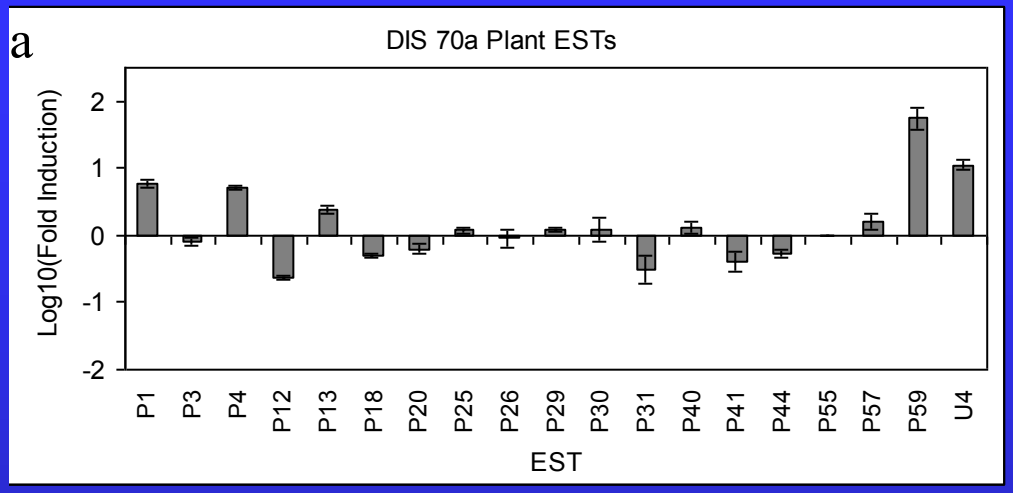
Table 1. List of *Trichoderma* spp. studied for their biocontrol potential against *Phytophthora capsici* on hot pepper.

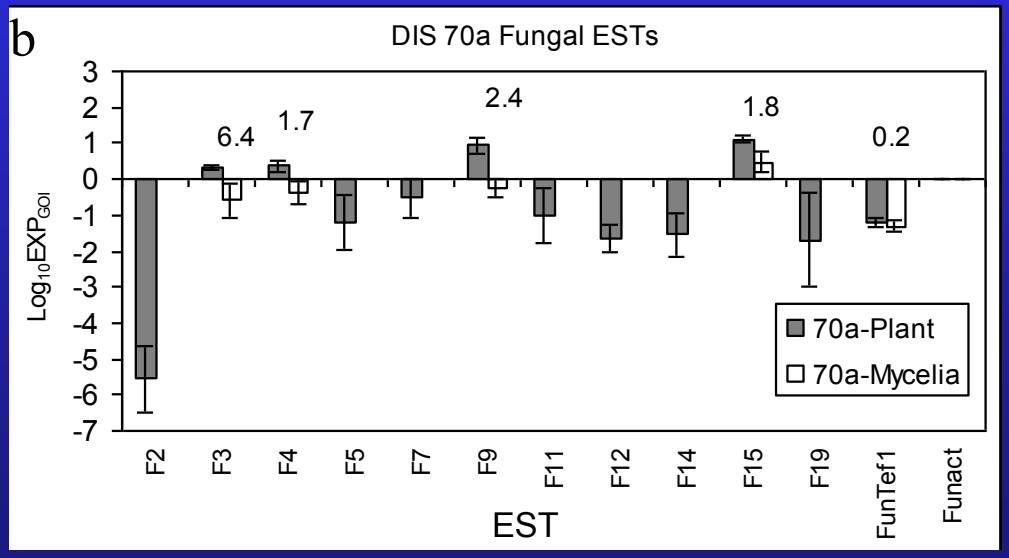
No.	Isolate	Species	Host	Country
1	DIS 65v	*T. harzianum clade*	*T. cacao*	Ecuador
2	DIS 70a	*T. ovalisporum*	Tropical forest	Ecuador
3	DIS 85f	*T. theobromicola*	*T. cacao*	Peru
4	DIS 172ai	*T. koningiopsis*	*T. grandiflorum*	Brazil
5	DIS 218f	endophyte	*T. gileri*	Ecuador
6	DIS 219b	*T. hamatum*	*T. gileri*	Ecuador
7	DIS 219f	*T. harzianum*	*T. gileri*	Ecuador
8	DIS 246d	endophyte	*T. cacao*	Ecuador
9	DIS 259j	*T. stilbohypoxyli*	*T. cacao*	Ecuador
10	DIS 314b	*T. harzianum clade*	*Cola*	Cameroon
11	DIS 320c	*T. caribbaeum var. aequatoriale*	*T. gileri*	Ecuador
12	DIS 326h	*T. koningiopsis*	*T. gileri*	Ecuador
13	DIS 376f	*T. theobromicola*	*Cola*	Cameroon
14	DIS 378f	*T. koningiopsis*	*Sterculia*	Cameroon
15	DIS 384d	*T. caribbaeum var. aequatoriale*	*Ancistrocladus*	Cameroon
16	GJS 00-08	endophyte	*soil*	Mexico
17	GJS 01-07	*T. koningiopsis*	*T. cacao*	Ecuador
18	GJS 03-66	endophyte	*Scalesia*	Galapagos
19	GJS 03-103	*T. stilbohypoxyli*	*Wood*	Ghana

Isolates of *Trichoderma* spp. vary in their aggressiveness in colonizing cacao seedlings

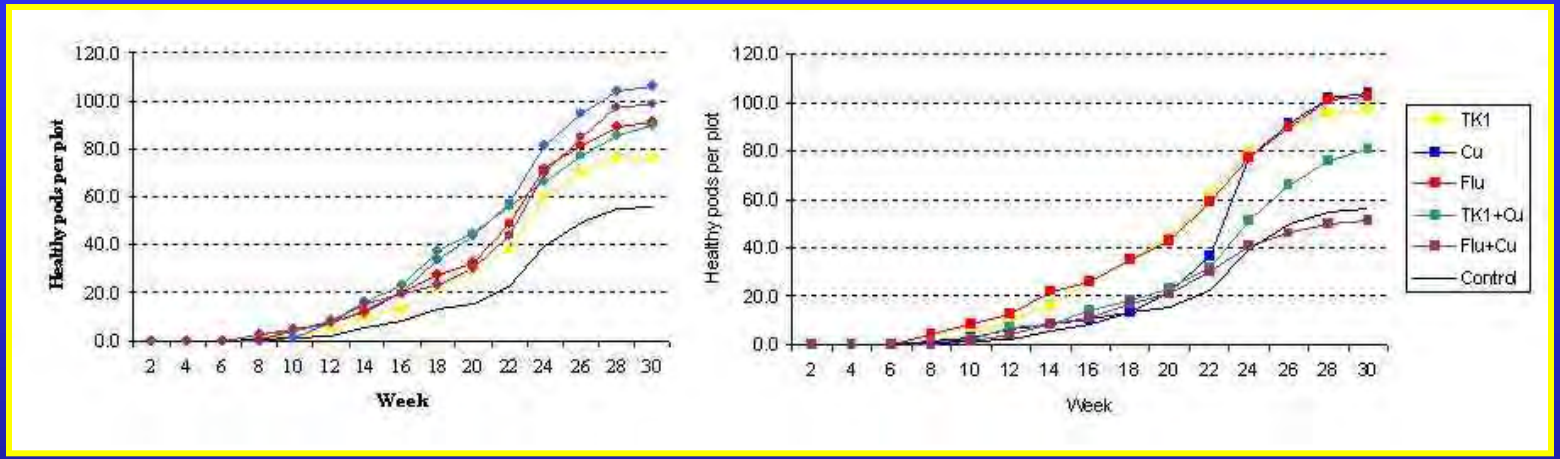





a
DIS 70a Plant ESTs
Log10(Fold Induction)
2
1
0
-1
-2
P1
P3
P4
P12
P13
P18
P20
P25
P26
P29
P30
P31
P40
P41
P44
P55
P57
P59
U4
EST


b
DIS 70a Fungal ESTs
Log10EXPGOI
3
2
1
0
-1
-2
-3
-4
-5
-6
-7
6.4
1.7
2.4
1.8
0.2
70a-Plant
70a-Mycelia
F2
F3
F4
F5
F7
F9
F11
F12
F14
F15
F19
FunTef1
Funact
EST

Almost all field studies carried out to date used a surfactant, a complex starch, or water alone



Hot pepper infected with *P. capsici* after 9 days. Seedlings were inoculated with or without *Trichoderma* isolate 376f at planting and grown for 39 days before being transplanted into *P. capsici* infested soil.

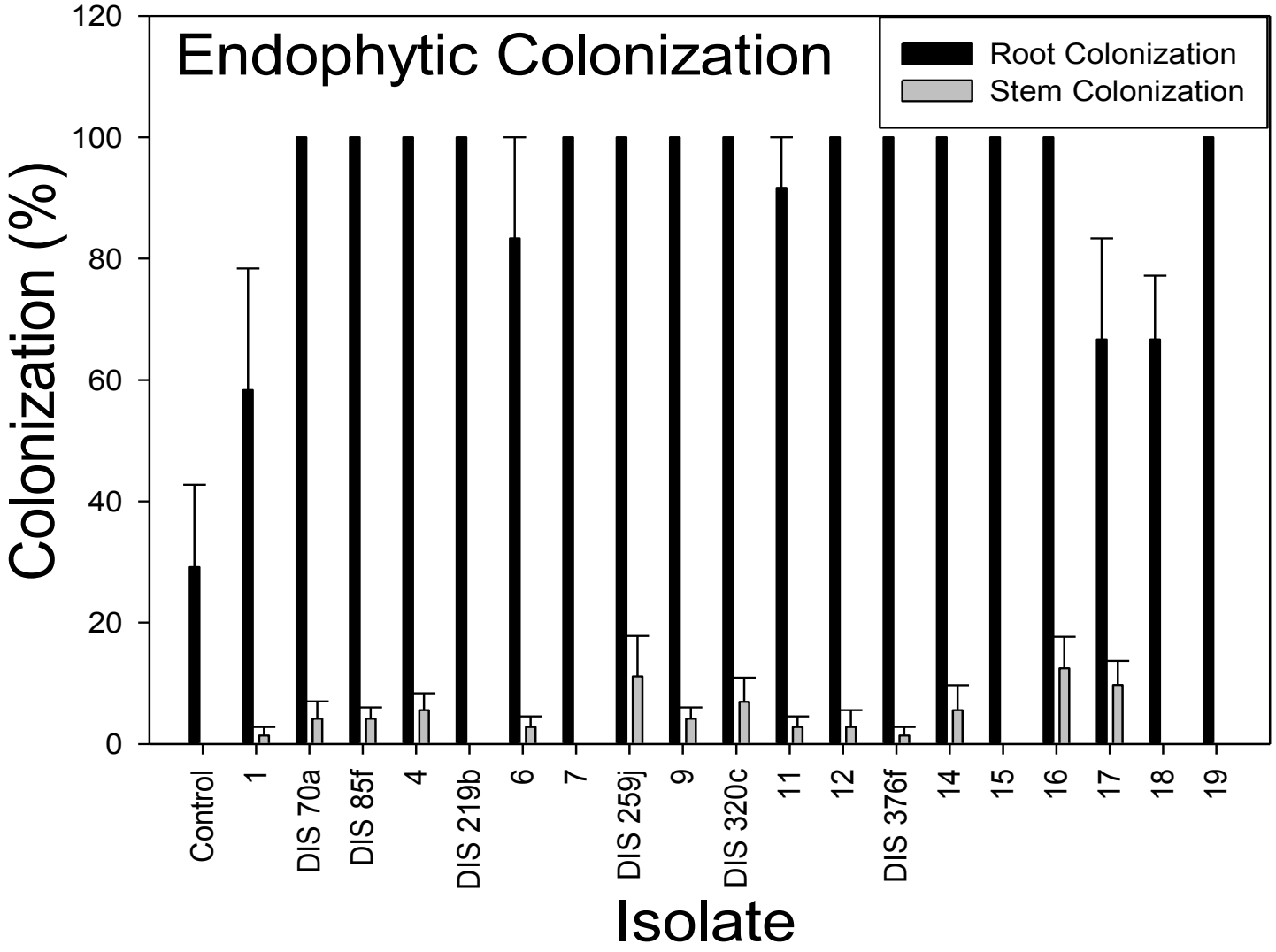
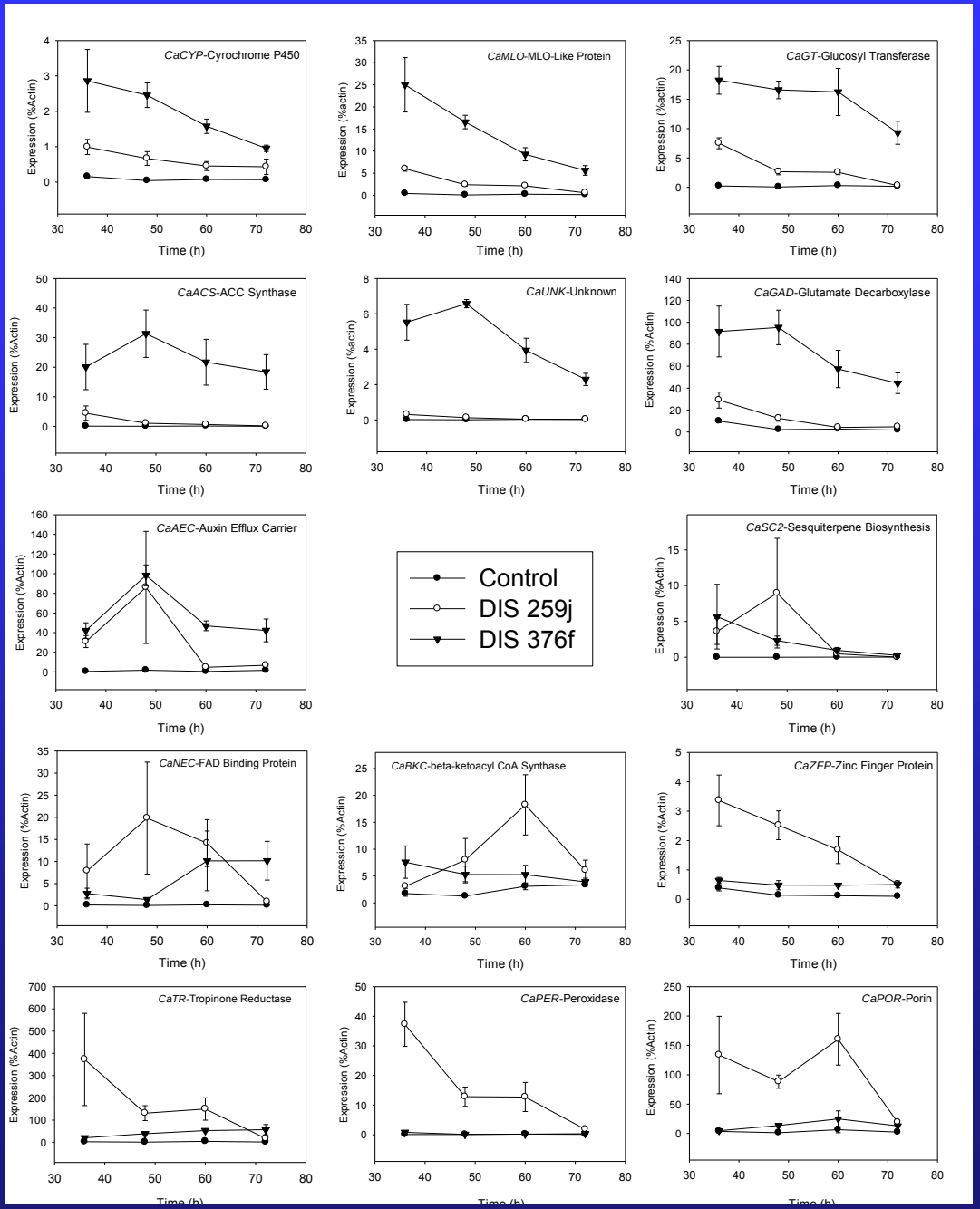
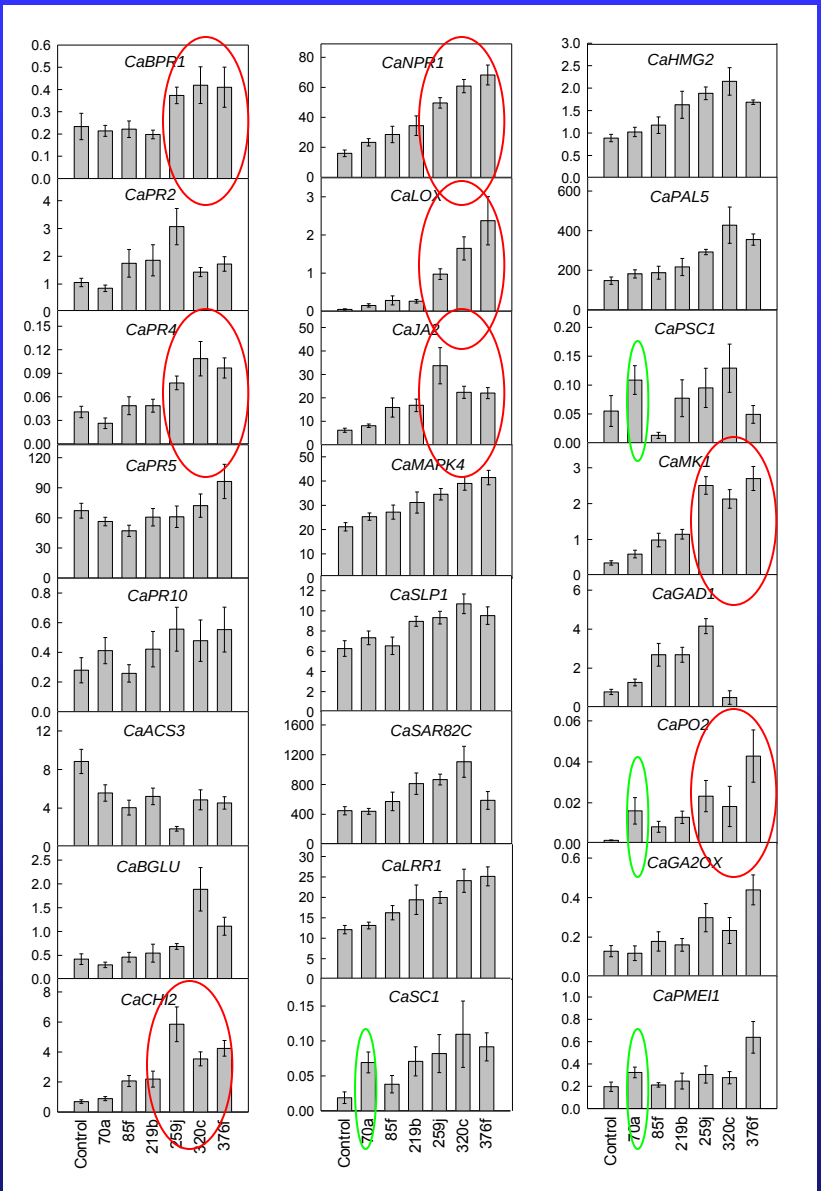

Endophytic Colonization
Root Colonization
Stem Colonization
Colonization (%)
120
100
80
60
40
20
0
Control
1
DIS 70a
DIS 85f
4
DIS 219b
6
7
DIS 259j
9
DIS 320c
11
12
DIS 376f
14
15
16
17
18
19
Isolate

Figure 8. ESTs encoding stress related proteins are differentially induced by *Trichoderma* isolates DIS 259j and DIS 376. QPCR was carried out using primers for 12 ESTS indicated highly induced (Table V) by microarray analysis of RNA samples collected 48 h after *Trichoderma* inoculation. Pepper seedlings were grown 14 days and inoculated with *Trichoderma* on agar plates. Pepper roots were harvested 36, 48, 60, and 72 hours after inoculation and RNA was extracted for QPCR analysis. ESTs include *CaCYP*, *CaMLO*, *CaGT*, *CaACS*, *CaUNK*, *CaGAD*, *CaAEC*, *CaSC2*, *CaNEC*, *CaBKC*, *CaZFP*, *CaTR*, *CaPER*, and *CaPOR*.



Leaves (32 days, Pepper)



Disease Development

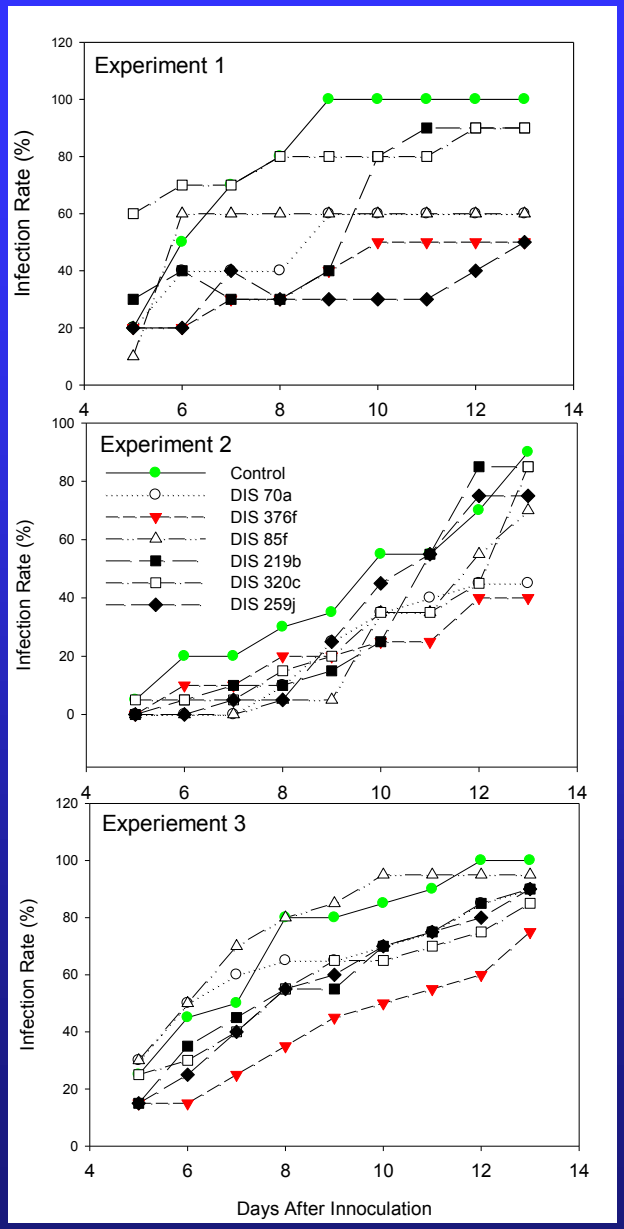


Table 3. Disease suppression as measured by the average number of days seedlings within treatments showed symptoms of disease before the experiment was terminated (SE/DBT, Symptom Expression/ Days Before Termination). Pre-germinated pepper seed were planted in *Trichoderma* colonized soil in seed trays and grown in growth chambers for 42 days before being transplanted into *P. capsici* infested soil. The experiments were monitored for disease symptoms for 14 days after transplanting into *P. capsici* infested soil before termination.

	SE/DBT	MS[1]
Experiment 1		
Control	8.2	A
DIS 70a	5.5	B
DIS 376f	3.9	B
DIS 85f	5.5	AB
DIS 219b	6.0	AB
DIS 320c	7.9	A
DIS 259j	3.3	B
Experiment 2		
Control	4.7	A
DIS 70a	2.6	C
DIS 376f	2.4	C
DIS 85f	2.8	C
DIS 219b	3.8	B
DIS 320c	3.1	BC
DIS 259j	3.7	B
Experiment 3		
Control	7.5	AB
DIS 70a	6.7	BC
DIS 376f	4.3	E
DIS 85f	8.0	A
DIS 219b	6.2	CD
DIS 320c	5.7	D
DIS 259j	6.0	CD

[1]Means not followed by the same letters are significantly different (P≤0.05).

Are there basic differences in how perennials (cacao) and annuals (pepper) plant species interact with non-pathogenic microbes that internally colonize their tissues?

Bacterial endophytes as biological control agents for cacao diseases

Dr. Rachel L. Melnick

The Pennsylvania State University

What about bacterial endophytes?

- Are there bacterial endophytes associated with cacao?
- Can endospore-forming bacteria from cacao be used to manage disease?

Bacterial Endophytes

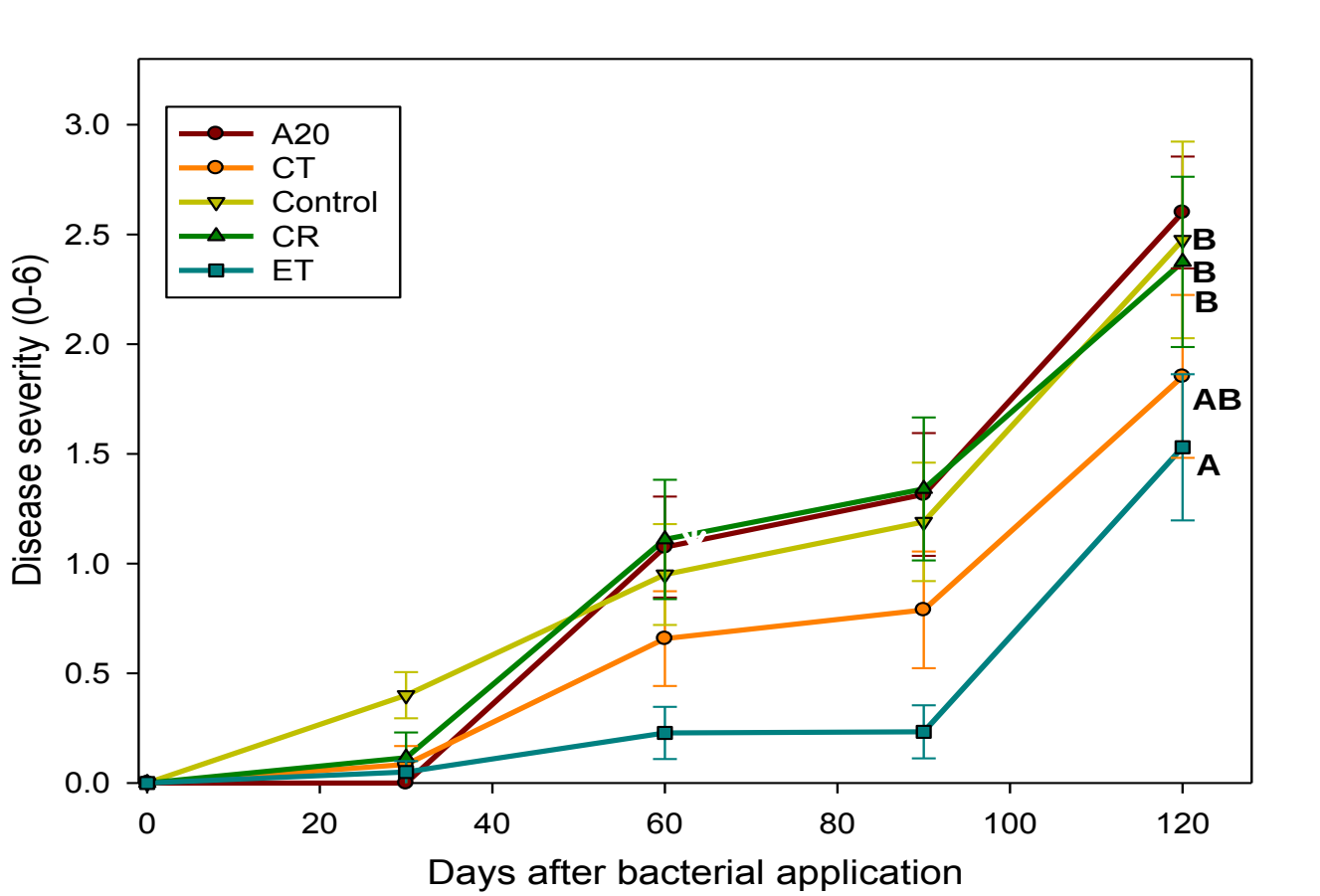
- INIAP tropical research station in Pichilingue, Ecuador
- Endospore-forming bacteria were isolated from pods, leaves, branches, floral cushions
- Screened for elite qualities for biocontrol agent

Bacterial Endophytes

- Endospore-formation
- Chitinase production
- *Bacillus cereus* agar
- Colonization
- *In planta* disease challenge
- Antibiosis plate assay
- Sequencing

Witches' broom: Rainy Season 2008

- *Bacillus pumilis* ET reduced disease



Melnick et al. 2009. PDMR 3:V146

Cacao Trichomes

Figure 1

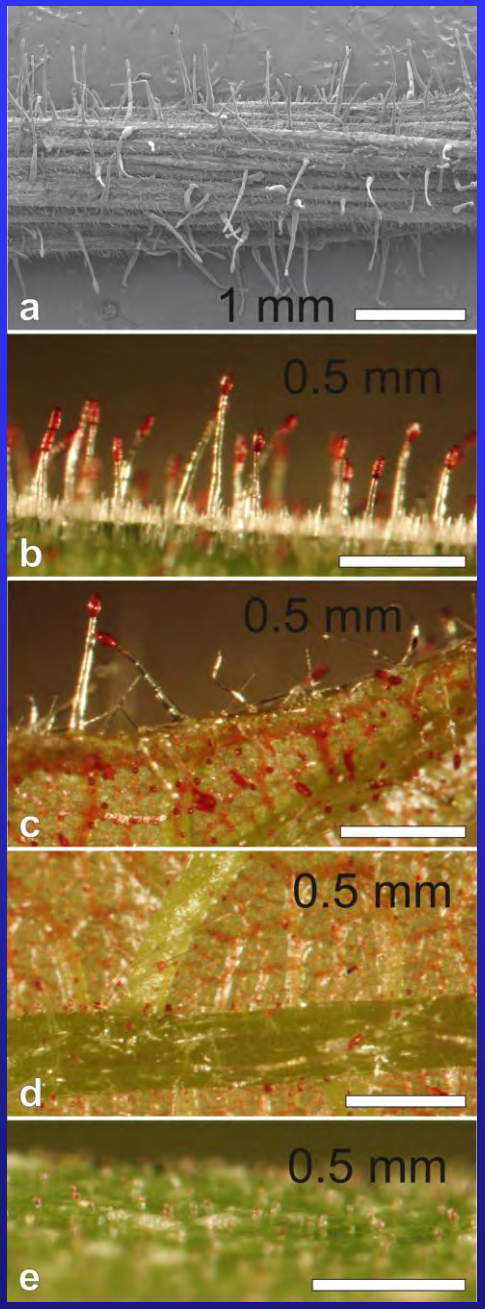
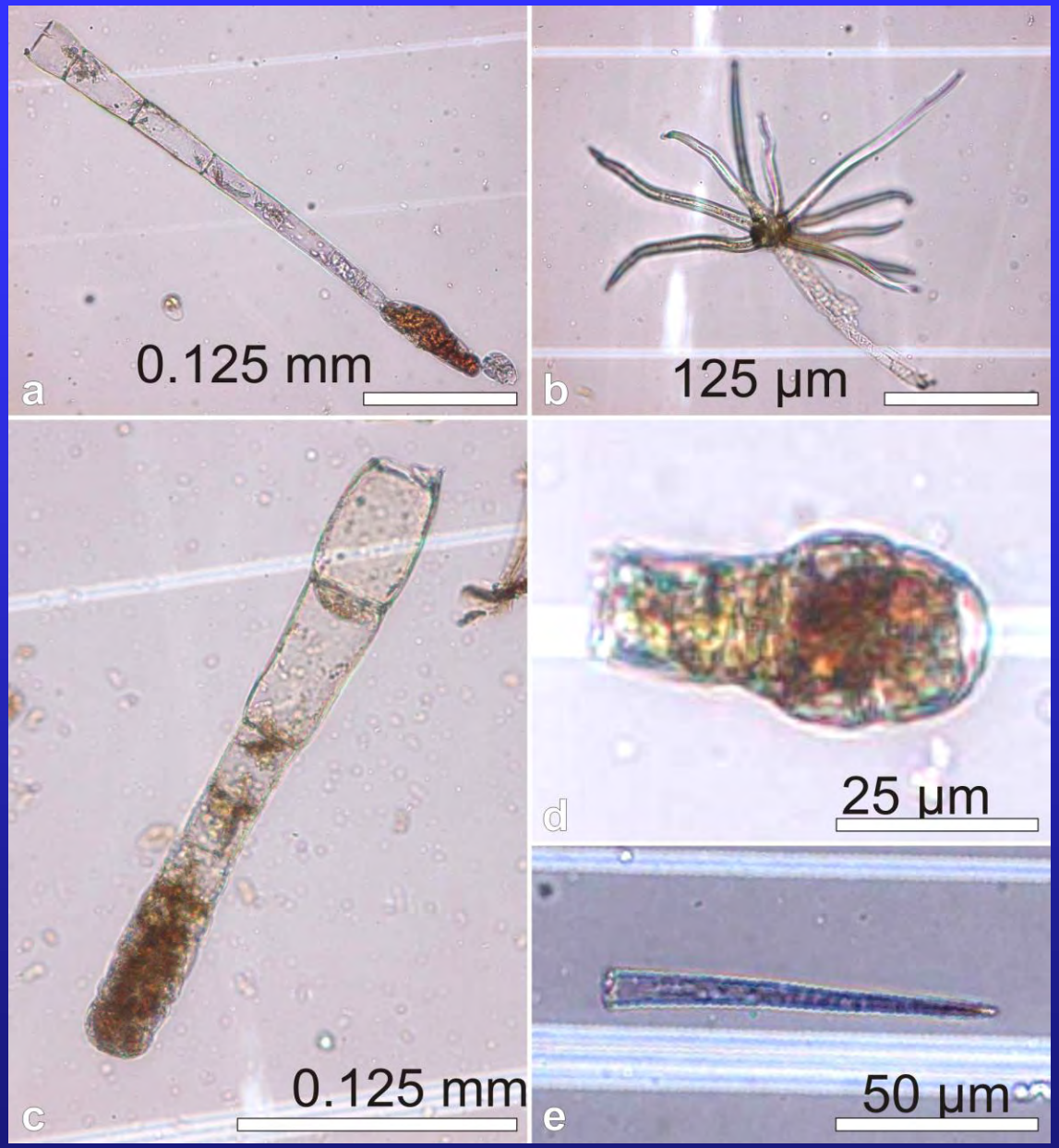
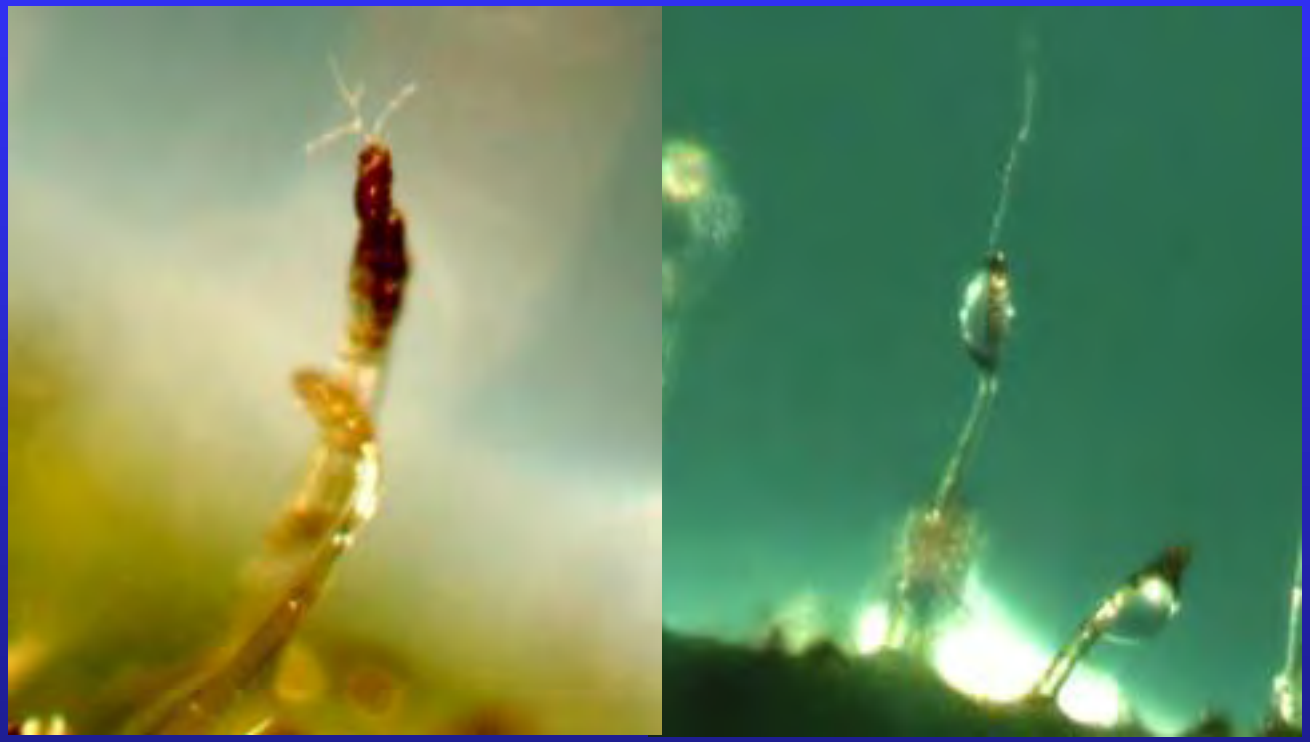


Figure 2

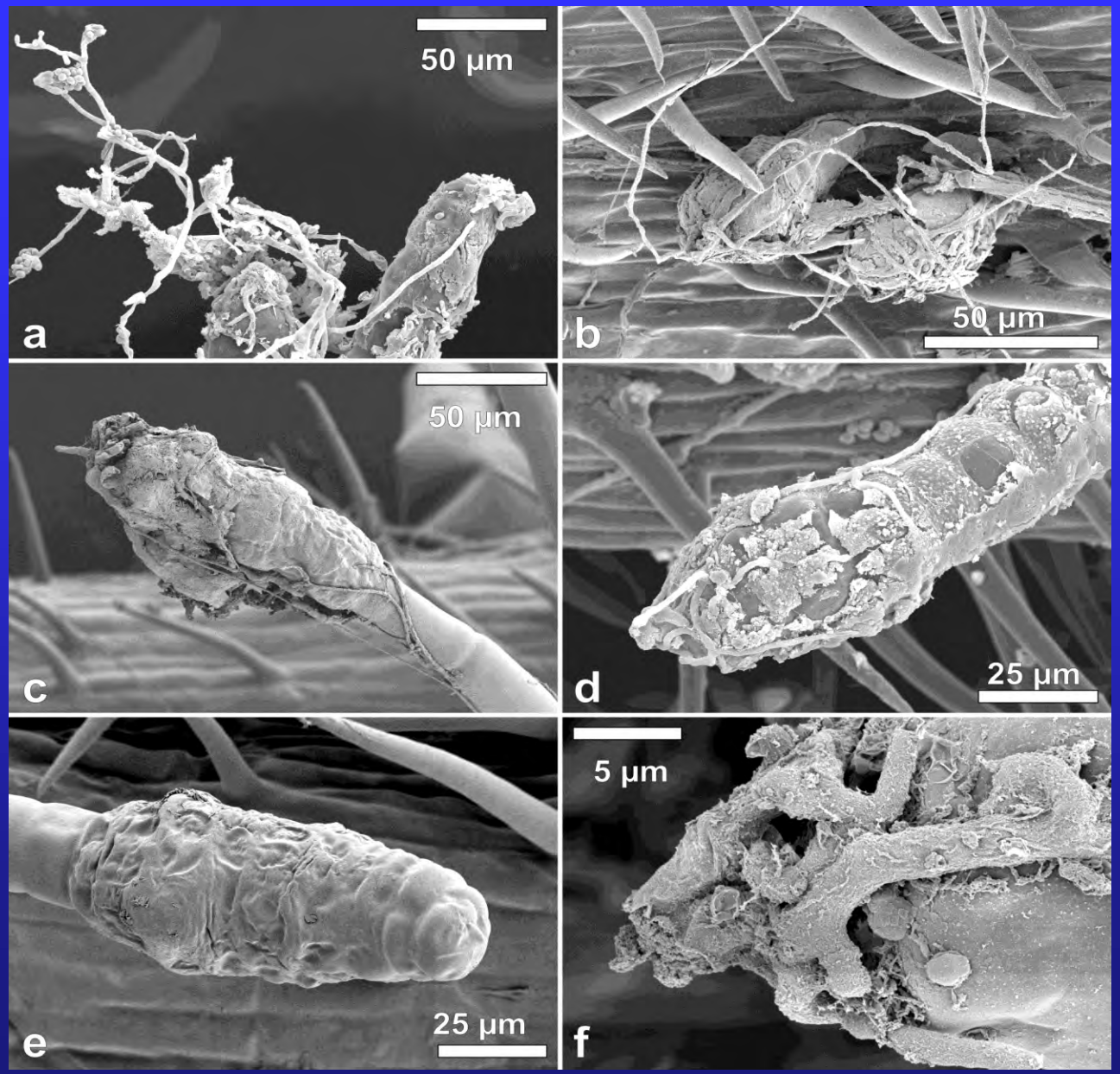


Trichoderma growing from tips of cacao stem trichomes



Trichoderma **growing from surface sterilized cacao stem trichomes.**

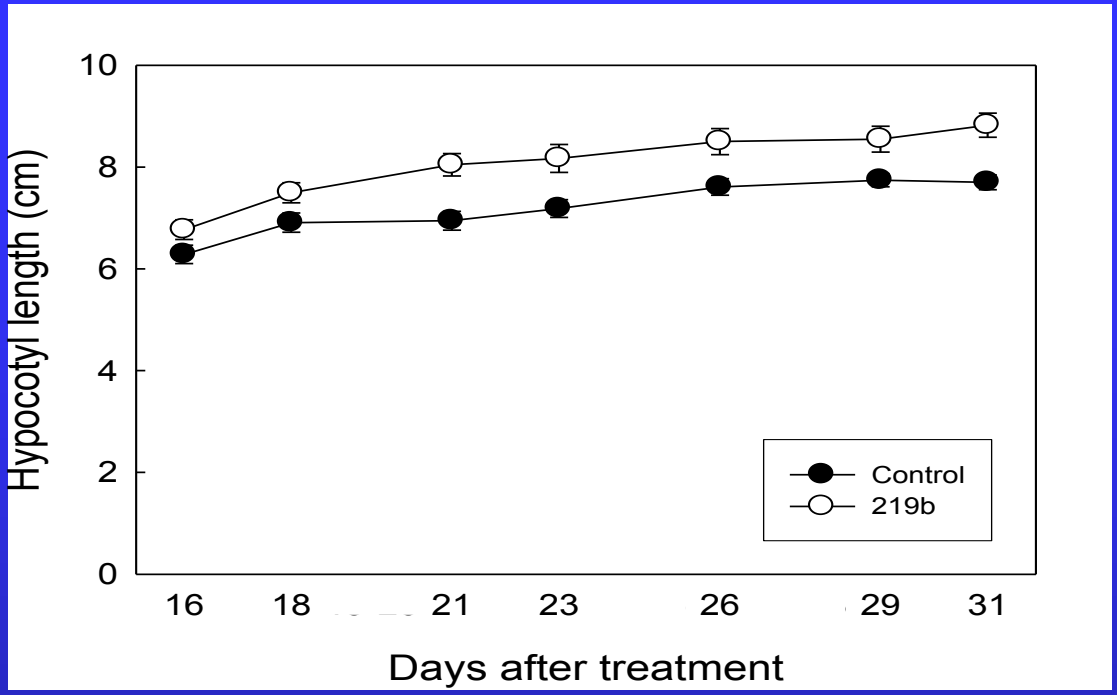
Figure 3



Drought Tolerance in Cacao

Figure 1.

A



B

219b control

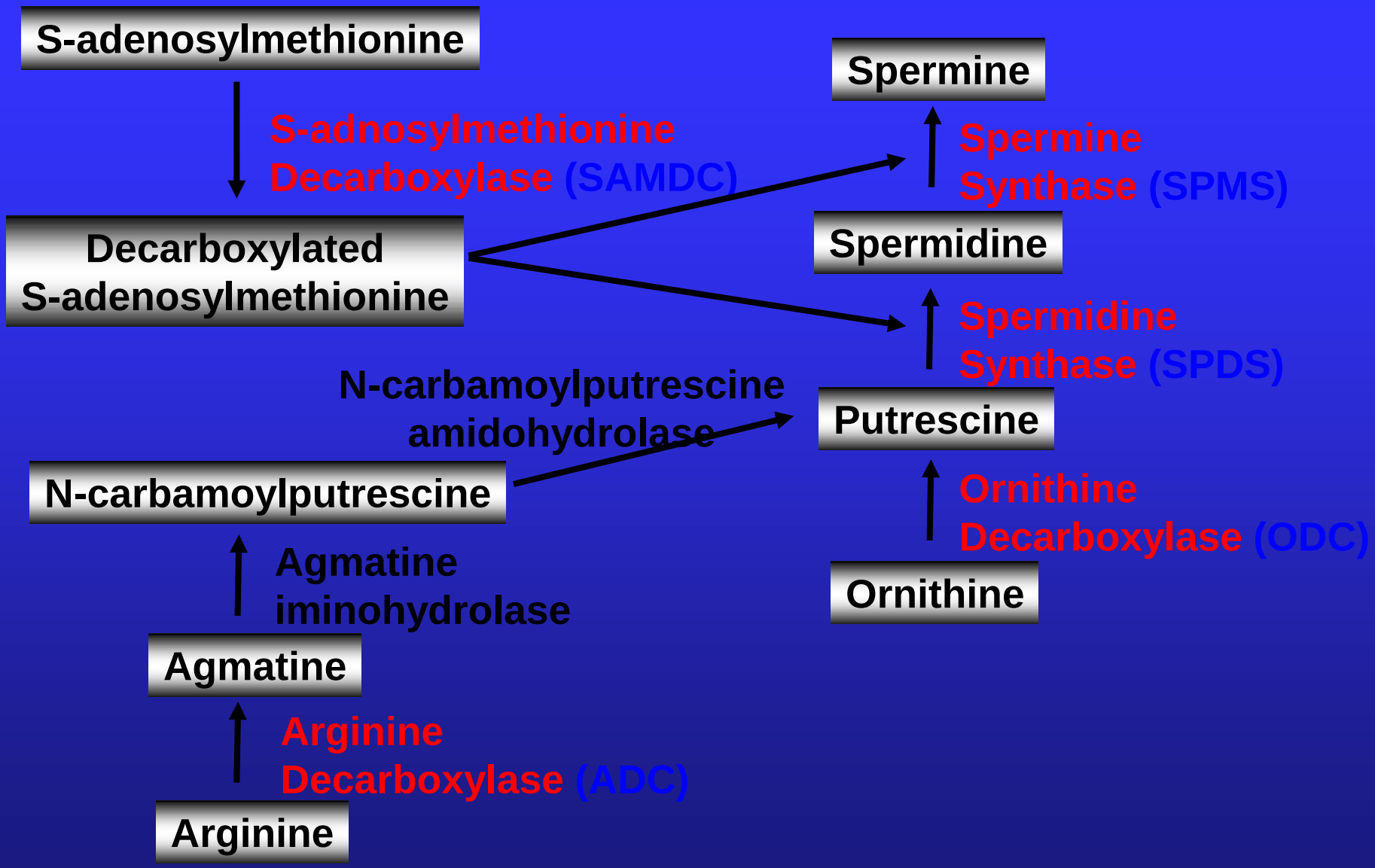


Figure 1. The plant polyamine biosynthesis pathway.

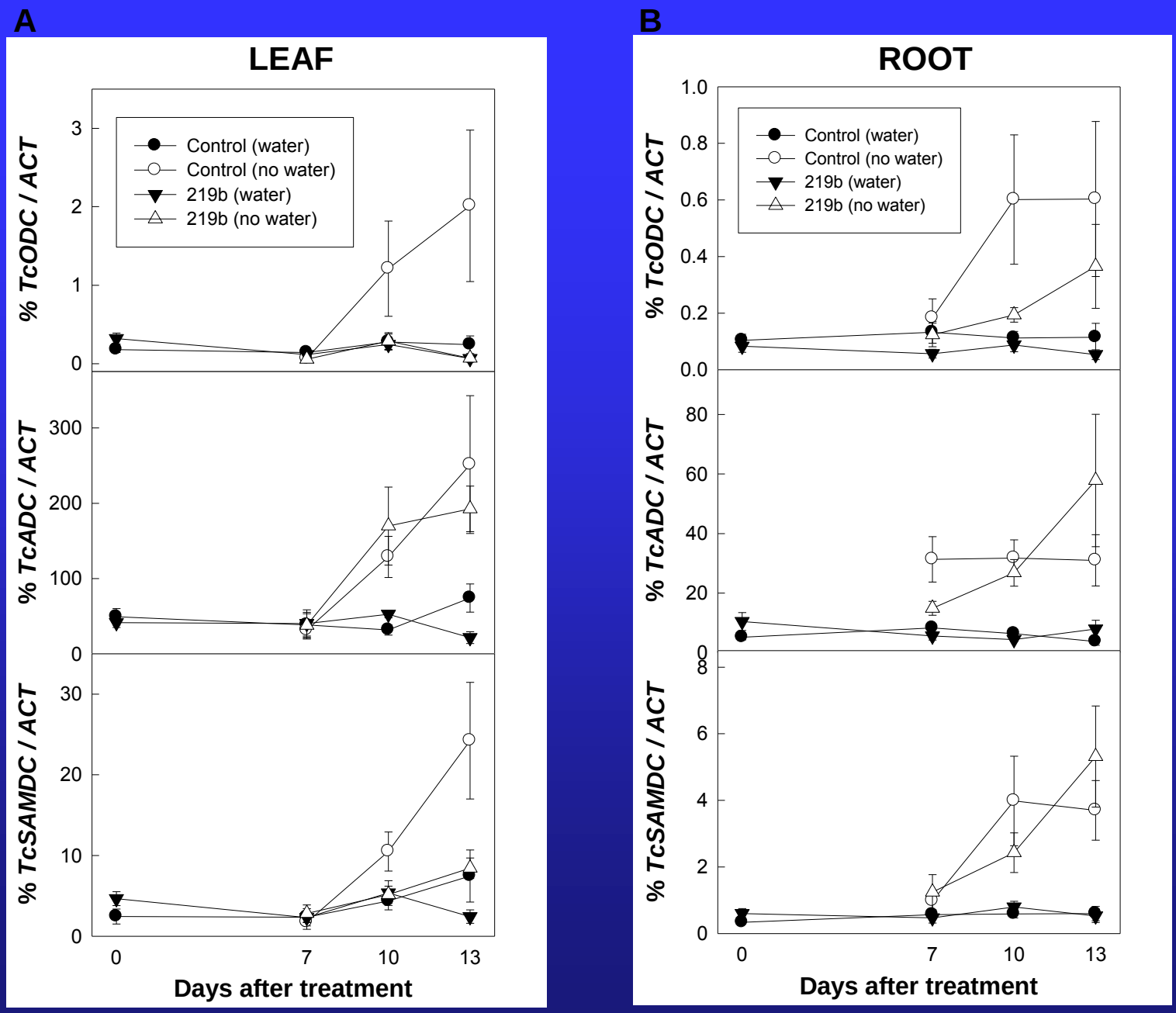

A
LEAF
% TcODC / ACT
% TcADC / ACT
% TcSAMDC / ACT
Control (water)
Control (no water)
219b (water)
219b (no water)
0
1
2
3
0
100
200
300
0
10
20
30
0
7
10
13
Days after treatment
B
ROOT
% TcODC / ACT
% TcADC / ACT
% TcSAMDC / ACT
Control (water)
Control (no water)
219b (water)
219b (no water)
0.0
0.2
0.4
0.6
0.8
1.0
0
20
40
60
80
0
2
4
6
8
0
7
10
13
Days after treatment

Table 4. Changes in root biomass (dry weight) and water content in cacao seedlings due to drought treatment in cacao seedlings treated with or without *Trichoderma hamatum* isolate DIS 219b. Treatments were: non-colonized seedlings (Control) watered every 1, 3, 7, or 10 days; colonized seedlings (DIS 219b) watered every 1, 3, 7, or 10 days; Means for 14 individual seedlings are presented. The seedlings were grown at 25°C and an average of 80% humidity. The watering cycle was initiated after 32 days and continued for 21 days.

Treatment Measurement	P value	Treatment	Weight (g)	Means Separation
Drought				
Root Fresh Weight	0.0006	1 day	1.91	A[1]
		3 day	2.07	A
		7 day	1.95	A
		10 day	1.61	B
Root Dry Weight	0.0007	1 day	0.35	B
		3 day	0.40	AB
		7 day	0.44	A
		10 day	0.38	AB
Root Water Weight	<0.0001	1 day	1.56	A
		3 day	1.67	A
		7 day	1.51	A
		10 day	1.22	B
DIS 219b				
Root Fresh Weight	<0.0001	Cont	1.74	B[1]
		219b	2.06	A
Root Dry Weight	<0.0001	Cont	0.36	B
		219b	0.43	A
Root Water Weight	<0.0001	Cont	1.38	B
		219b	1.63	A

[1]Measurement means within columns for the individual treatments of drought or DIS 219b followed by the same letter are not significantly different at the $P < 0.05$ level. The interaction between Drought and Endophyte was not significant.

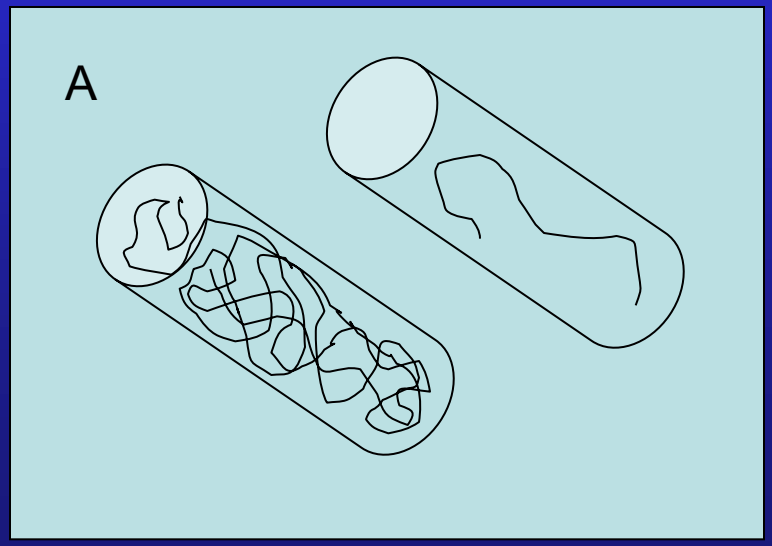
Formulation

What are the primary tissues targeted by biocontrol applications?

What tissues are the primary targets of disease?

We must also consider the impact of colonization on the effectiveness of biocontrol.

It cannot be assumed that the better colonizer provides the best disease control. Nor can it be assumed that the larger the population of a specific isolate the better its biocontrol efficacy.



Understanding factors that influence colonization are critical to making decisions on biocontrol isolates to be used, the methods of their application, the targets they are applied to, and the timing of their applications.

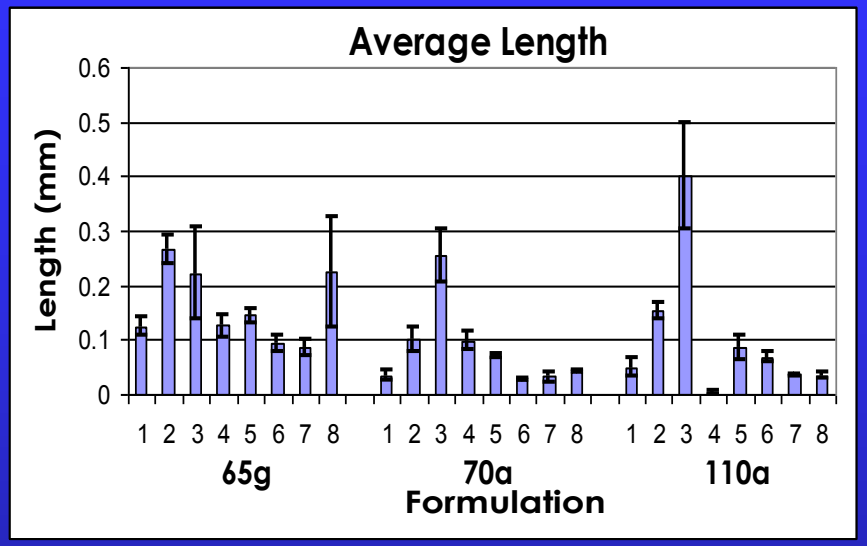

Green leaf (top)
Red Leaf (top)
Red Leaf (bottom)
Plumule
Flower Cushion
Pod Surface
Pod Surface (Cherelle)
Young Stem
Root
Cotyledon
Mature Stem
Flower Cushion

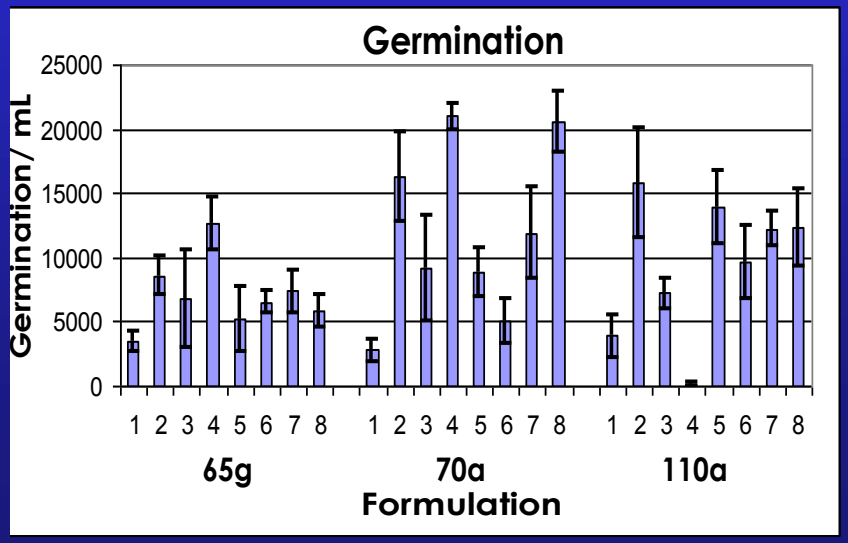
Formulation Results

Experiment 1:

Carbohydrate and Cacao Plant Component Treatments

1:control	5:chitin
2:mucilage	6:cellulose
3:cacao leaves	7:carboxymethyl cellulose
4:pectin	8:gelatin





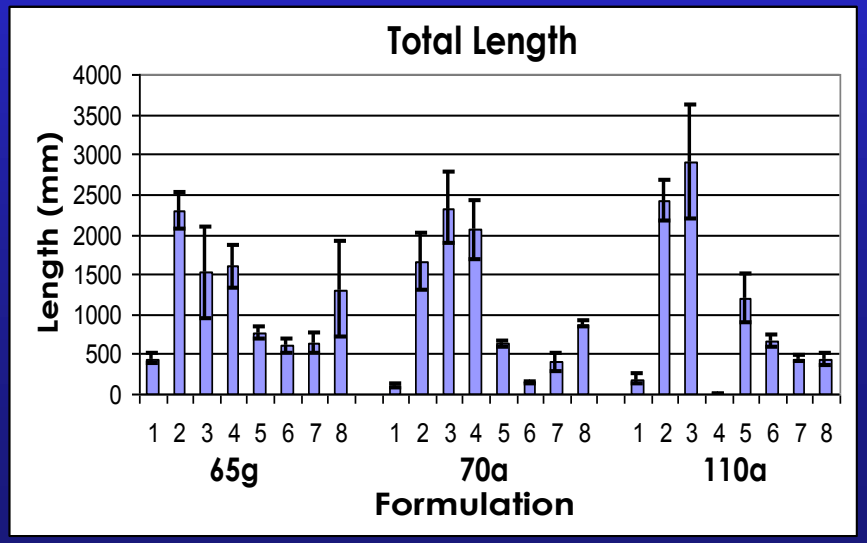


Figure 1

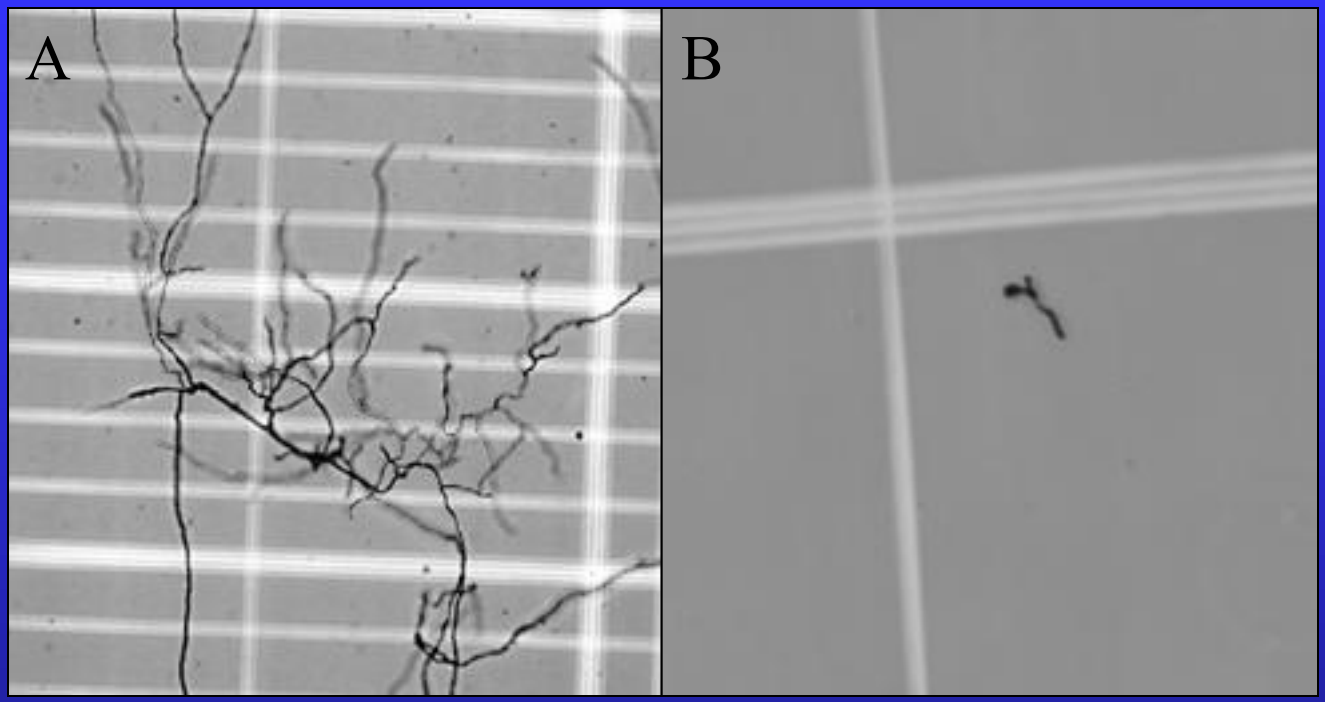
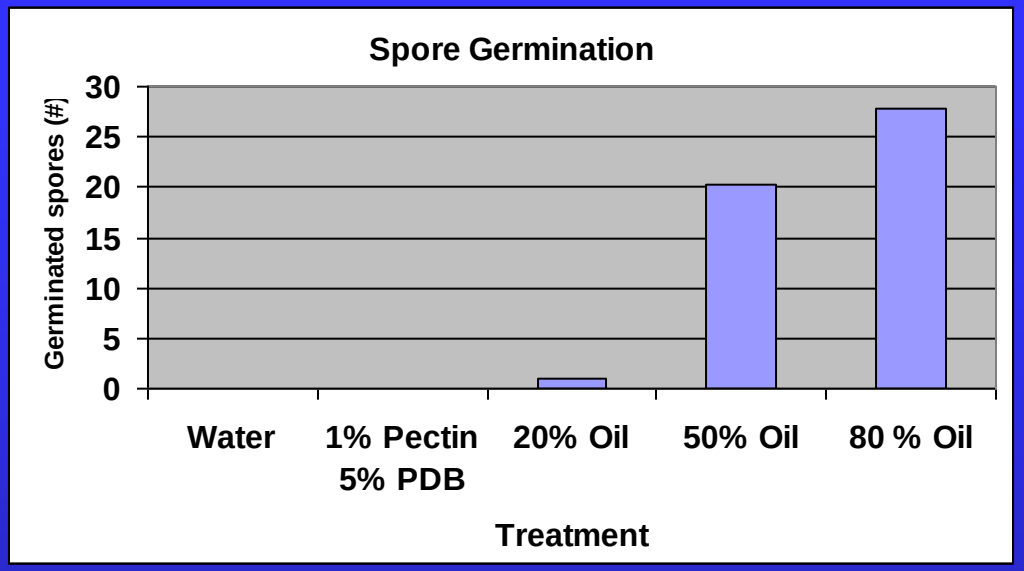
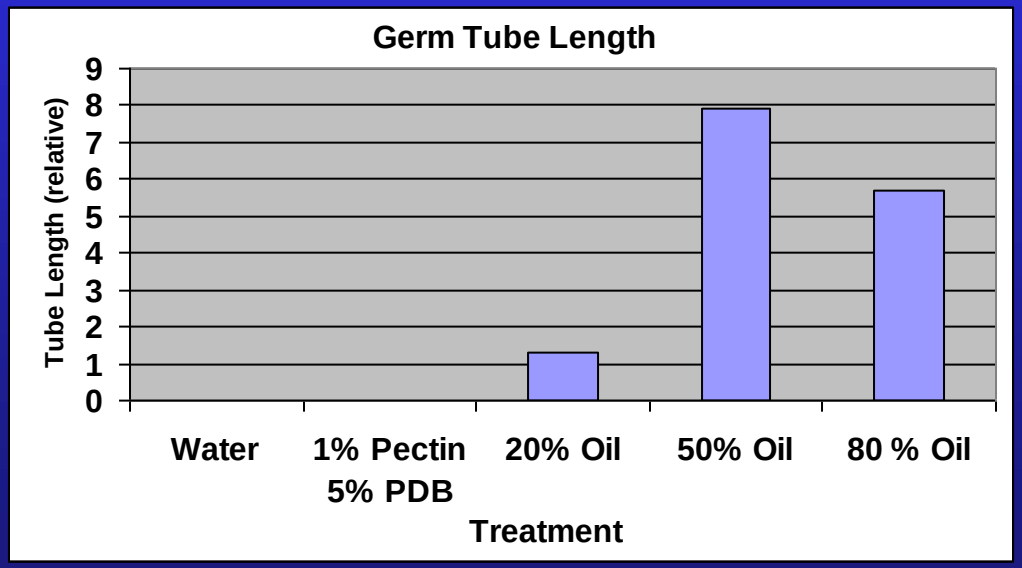


Figure 2. *Trichoderma hamatum* spore germination and growth after 24 h: (A) Growth in extract from cacao stem (0.2% musilage) and (B) Growth in water.

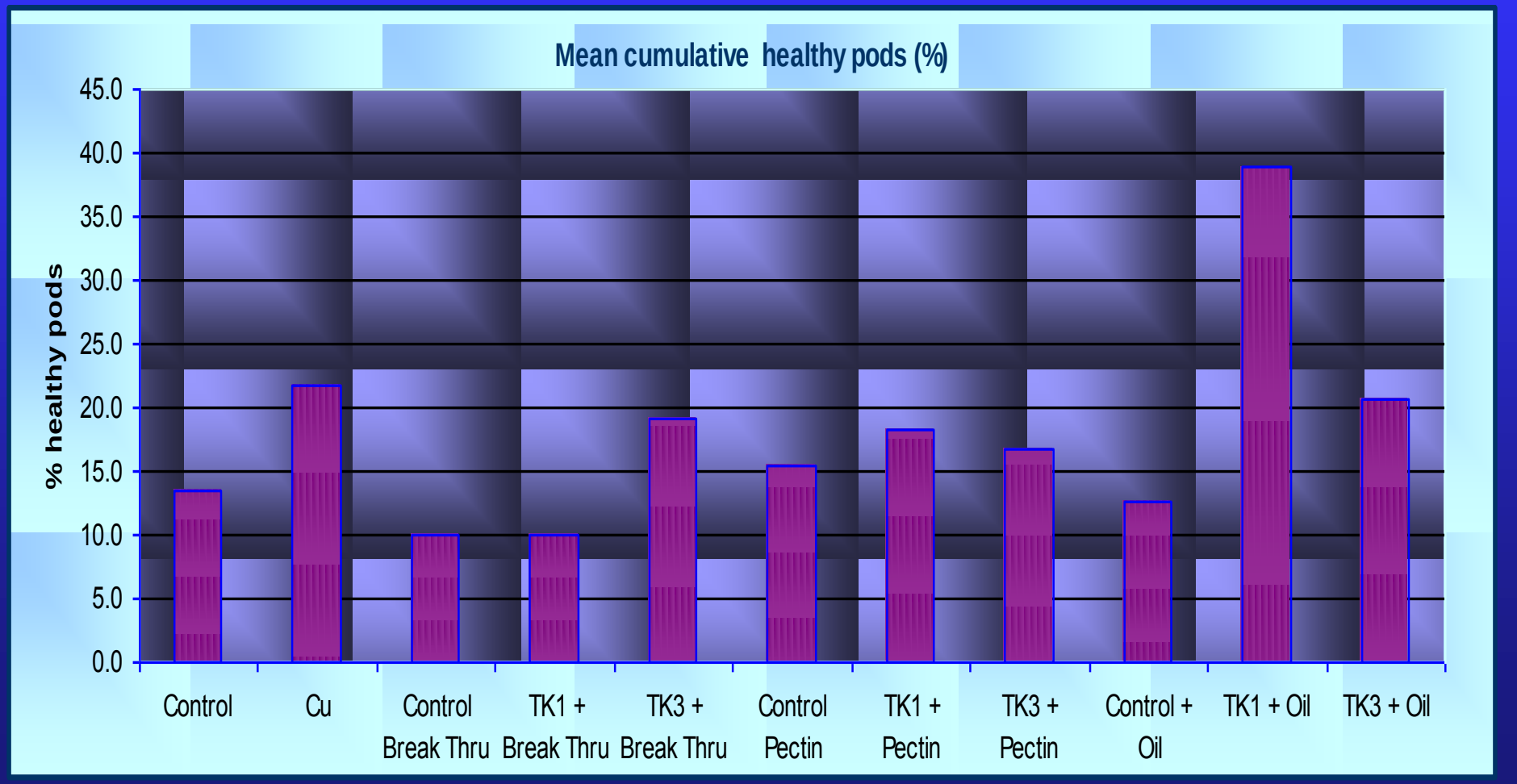
70a conidia from plates
10^{-6}spores/mL

16h
70%-90% humidity
32 C-24C





For the first time, invert oil formulations have been used successfully to enhance biocontrol of frosty pod in the field. Repetition of the experiment has begun (Bailey, Collins, Crozier, Philips).



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Thank You