Nematostatic N_2-fixing *Crotalaria* for greenhouse vegetable production (GVP) in Mediterranean conditions

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www.legumefutures.de

Nematostatic N_2-fixing *Crotalaria* for greenhouse vegetable production

Legume Futures

Tunnel in South France

Canarian shelter in Spain

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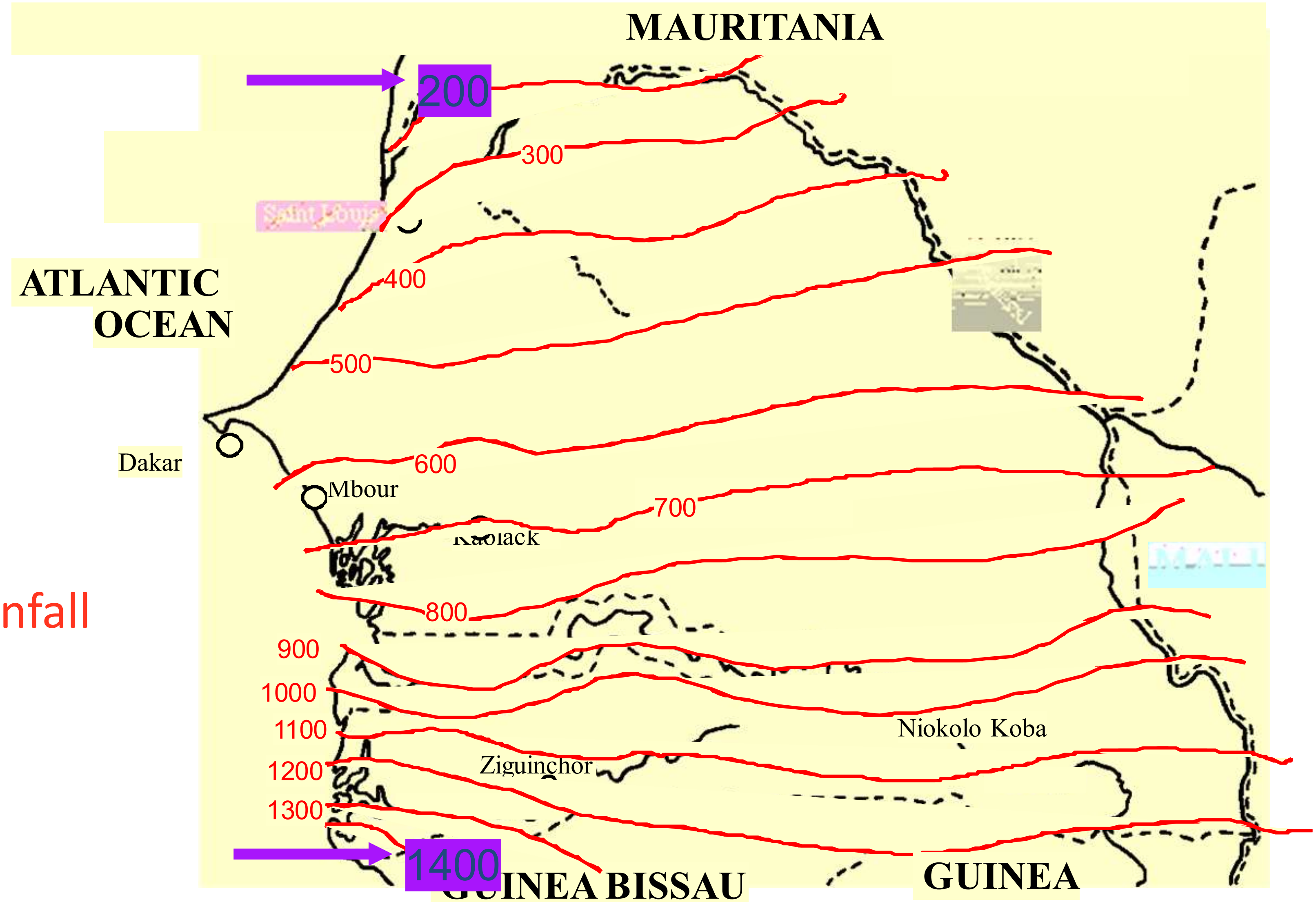
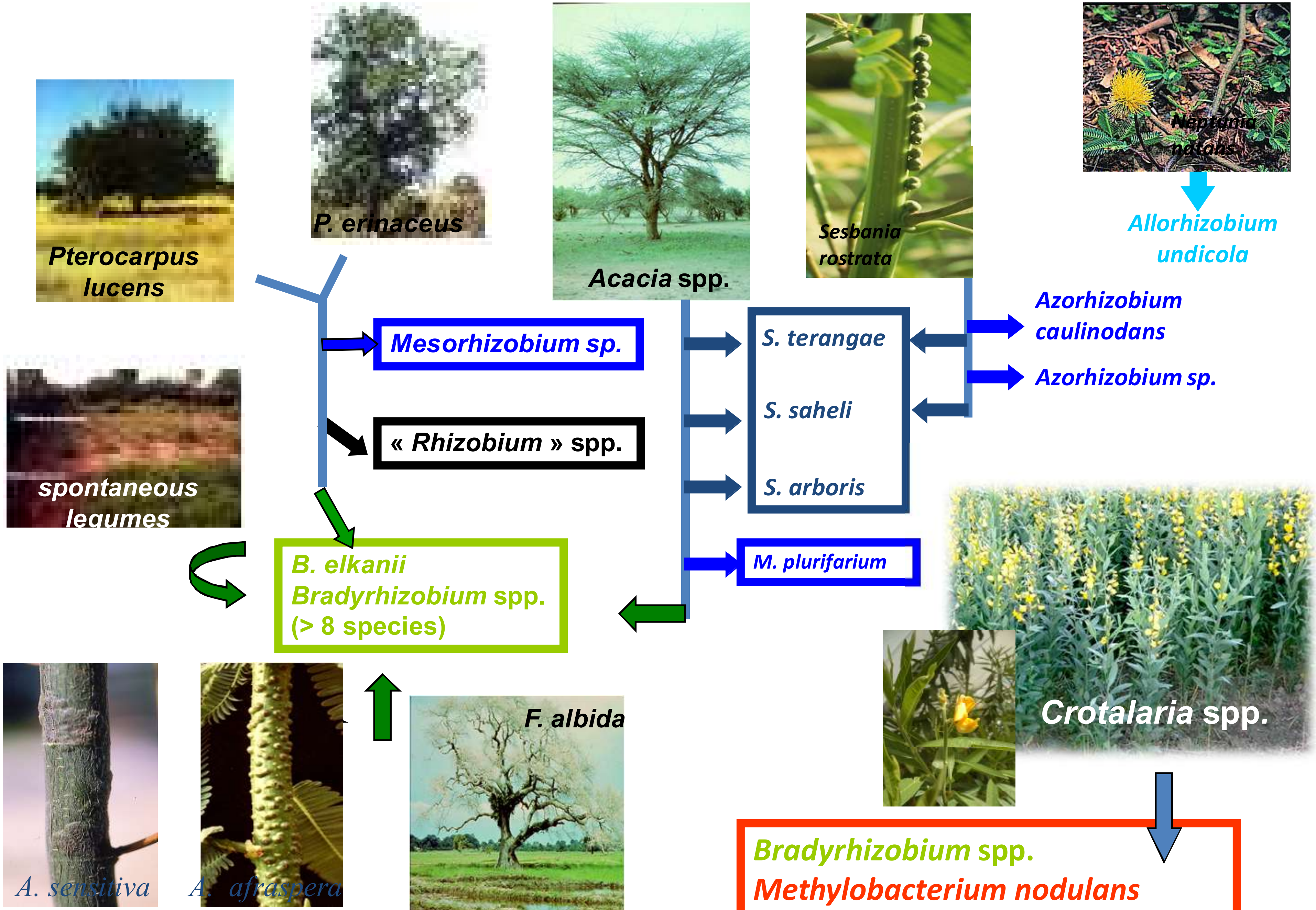
Investigating and promoting local legume symbioses for development in Senegal

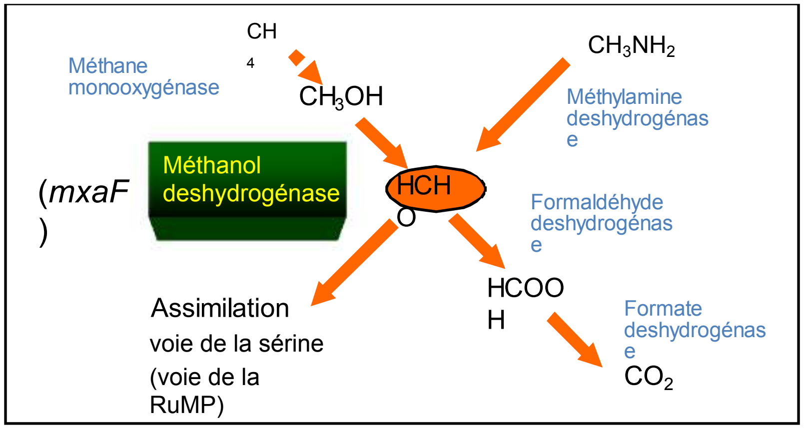
Rainfall





de Lajudie et al., 1994;1998ab;
Diouf et al., 1999 ; 2007; Doignon-Bourcier et al., 1999;2000; Sylla et al., 2002; Sy et al., 2001; Jourand et al., 2004

Investigating local legume symbioses → Unexpected properties in LNBs



New models

Potential of Legume symbioses

- Plant nutrition (BNF, P)
- Metabolite production
- Land reclamation
- Crop protection (parasites)
- …

Méthylotrophy

Carotenoïds

Photosynthesis

Stem nodulation

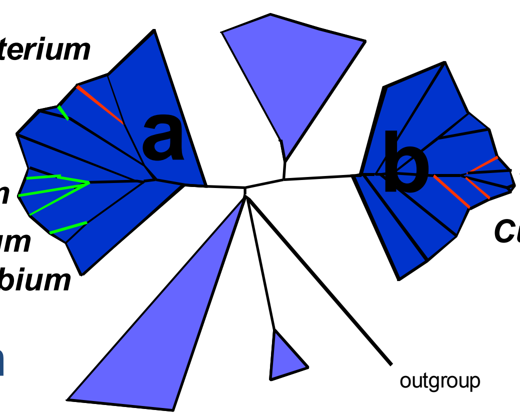
Free-living N_2 fixation

nod gene independant nodulation

(endo)colonization of non-legumes: rice, maize

P solubilisation (FABATROPIMED)

Biodegradation



Dreyfus et al., 1981
Lorquin et al., 1997
Molouba et al., 1999
Chaintreuil et al., 2000
Giraud et al., 2000, 2002
Sy et al., 2001
Moulin et al., 2001
Chen et al., 2001
Jourand et al., 2004, 2005
N'Zoué, 2008
Merabet et al., 2010

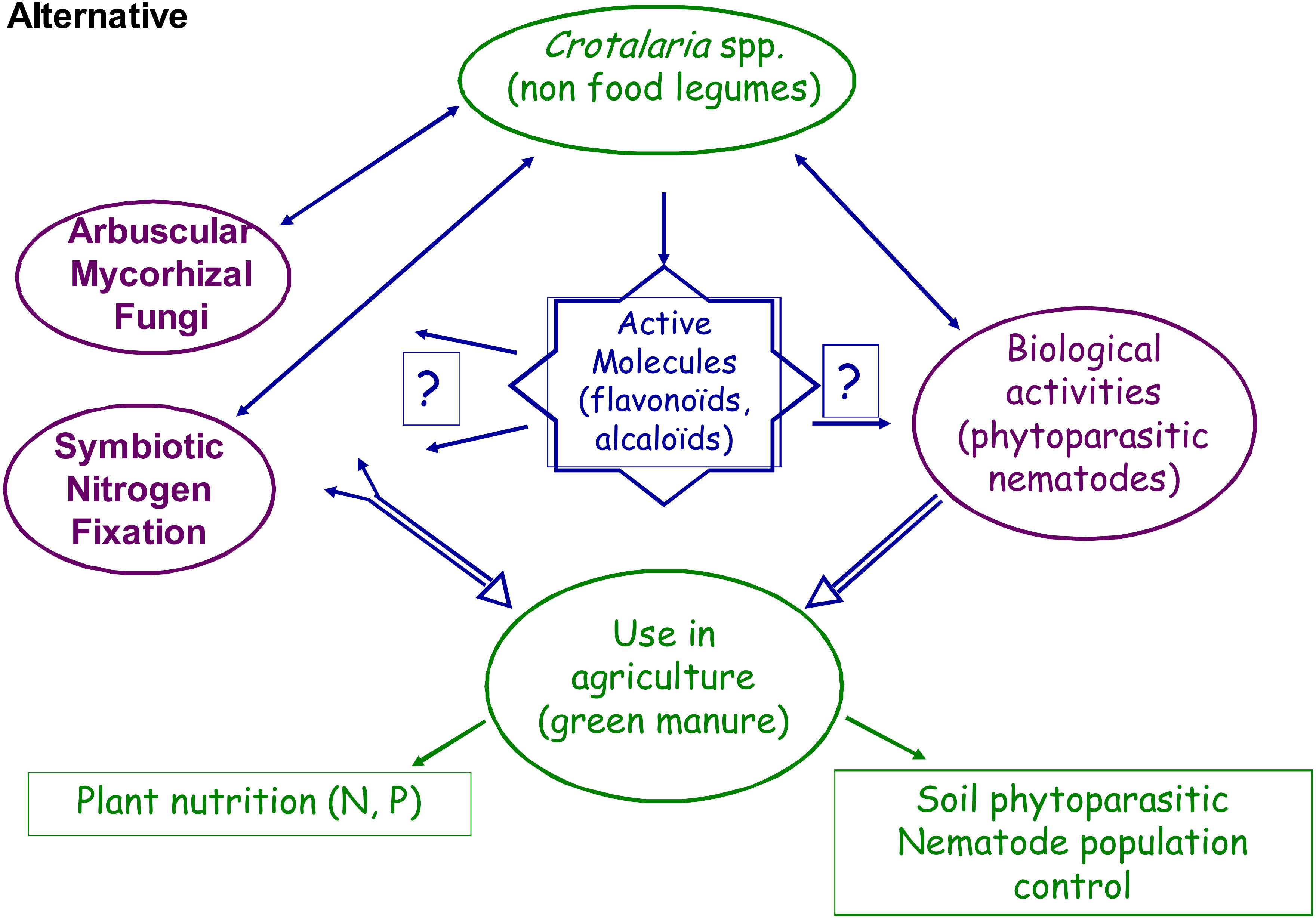
Background

No efficient treatment against nematodes.
Tourteau of castor-oil plant reputed active
Common practice: 3 month sanitary period (fallow)
Solarization / xolone treatment

Chemicals against nematodes are:
- toxic to the users
- soon banned in Europe

Alternative
Non-food legume *Crotalaria*

Alternative



Crotalaria Nematostatic Activity

Early data

Tomatoe Plants
+ *Crotalaria grantiana*
foliar extracts

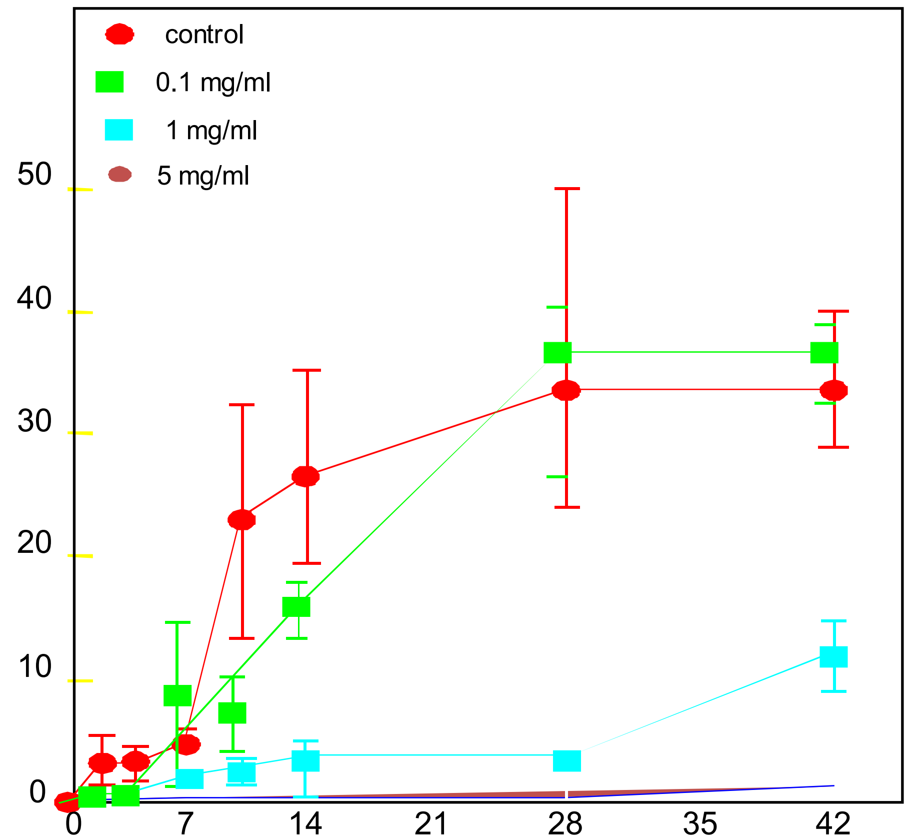
↓

Inoculated with 100 *M. incognita* juveniles

↓

% root infestation
fuschine coloration

% juveniles infested roots



Days after inoculation

Phytoprotection effect : delay and infestation inhibition

Jourand et al., Nematology, 2004, Vol. 6(5), 765-771

Nematostatic effect of *Crotalaria*
Early data in Senegal

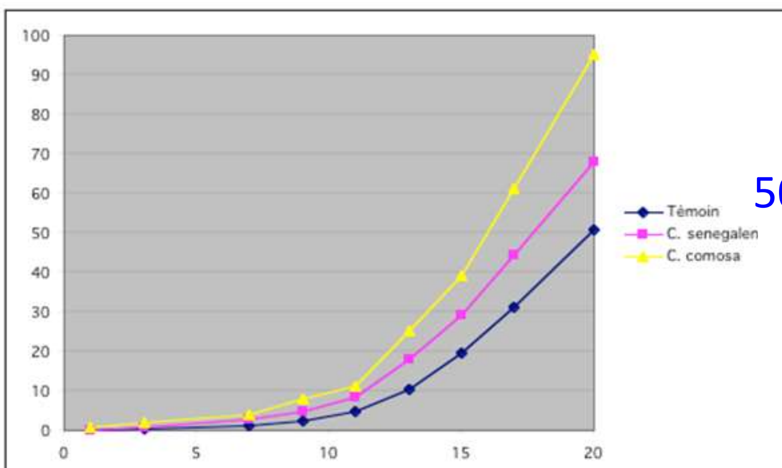
	Meloïdogyne incognita		*Meloïdogyne javanica*	
	Penetration rate	Dev to adult	Penetration rate	Dev to Adult
Crotalaria (15 species)	0,2 - 7% 0%	0,6 - 5% 0%		
Tomato	97%	74% 77%	13%	

Jourand, 2004

Nematostatic effect of *Crotalaria*
Early data in LCM, Dakar, Senegal

95

50



Tomato yield (**kg** / 20 plants)

Temps (j)

Control

Jourand et al., 2004, 2005

Nematostatic *Crotalaria* for vegetable production in France, Spain, Morocco

LSTM Objectives

To test *Crotalaria* spp. for **plant protection** against nematodes as intercrop/rotational **green manure in industrial greenhouse vegetable production** on the commercial scale for a cost-effective, environment-friendly and **sustainable agriculture in 3 Mediterranean countries** with the private company Delbon

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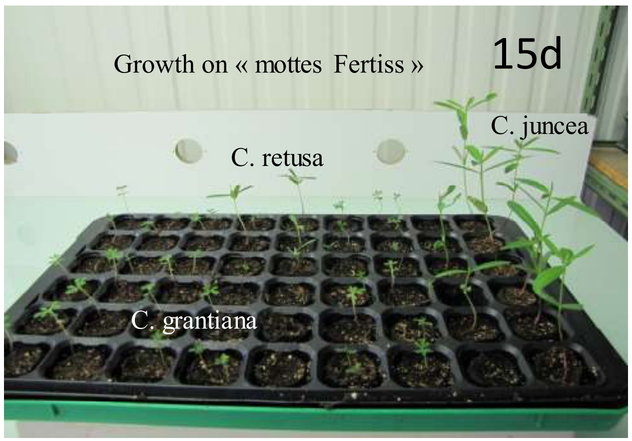
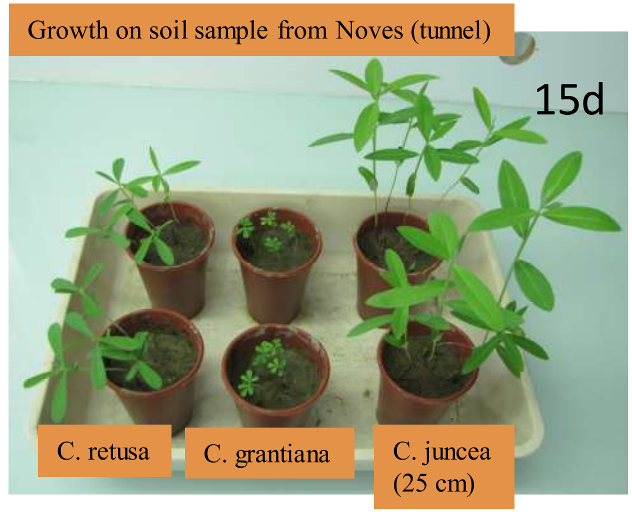
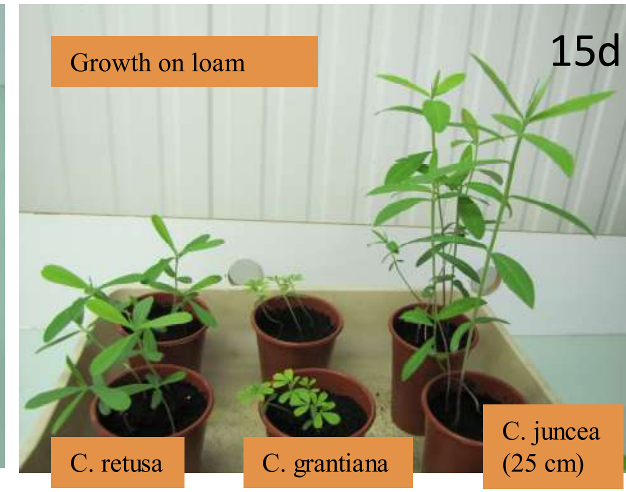
Plant growth test in lab

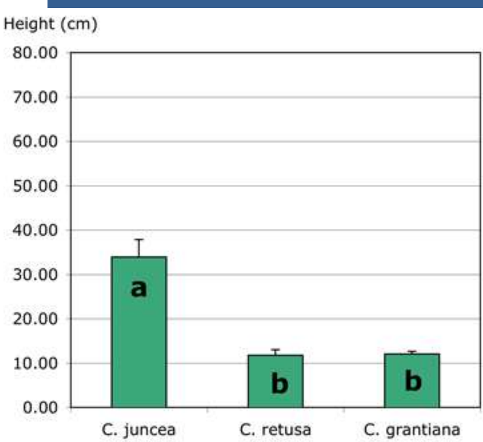
Comparative growth of 3 *Crotalaria* species on 3 substrates

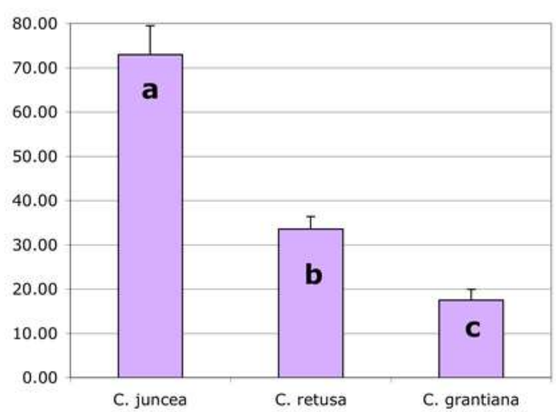


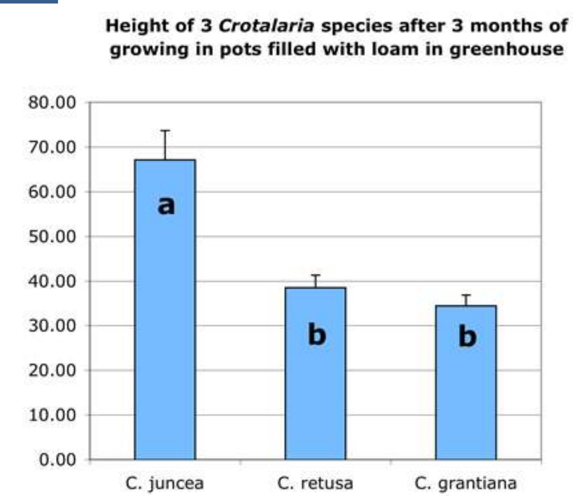




Crotalaria juncea produces significant more biomass







Crotalaria juncea

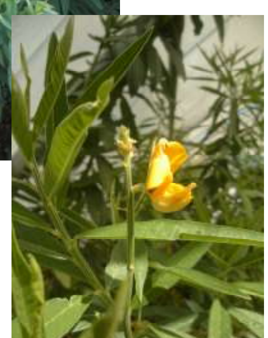
Tropical **Shrub** Legume (1-4m ; *Fabaceae, Papilionoidae, Crotalariae*)

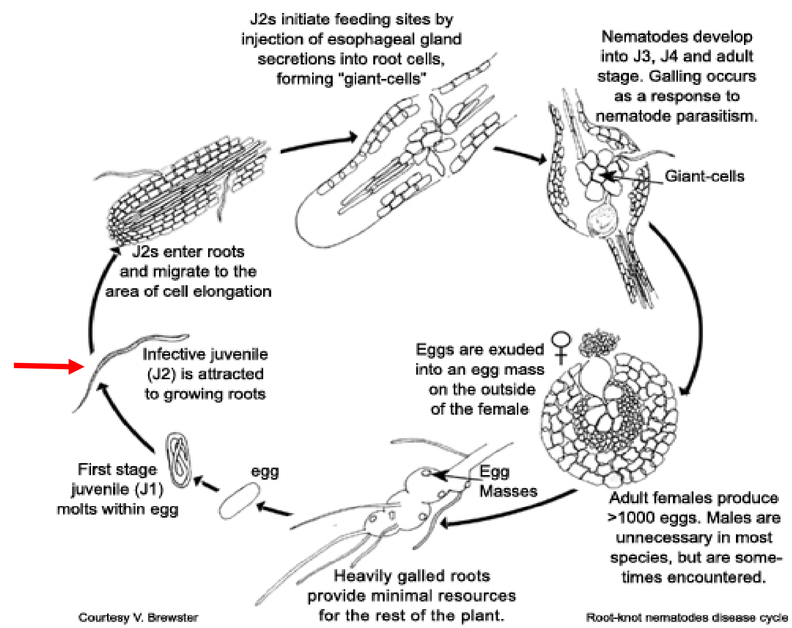
Not eatable. Cultivated in India for **fibers and rope** production

Nitrogen Fixing symbiosis

Highly **mycotrophic** (symbiosis with endomycorhizal fungi)

Pyrrolizidinic alcaloids content in whole plant parts (nematostatic effect)





Research questions

⇨ **Growth, Biomass & BNF** (^{15}N) of *C. juncea* in vegetable producing tunnels in Mediterranean conditions (France & Spain)

⇨ Incidence of *C. juncea* on following crop: **yield, nitrogen supply, nematode protection**

⇨ **Effect on soil biological activity**: microbial activity, mycorhizogenous potential, microbial populations

⇨ Symbiosis : **nodulation & survival** of rhizobia in Mediterranean conditions

⇨ **Soil** biology, fertility & sanitary improved after 3 years ?

Nematostatic *Crotalaria* for vegetable production in South France

Assay on nematostatic and green manuring effects of *Crotalaria juncea* In tunnel greenhouse vegetable production (lettuce)

Current rotation cropping
Jan-Mar lettuce
Mar-Jul Melon
Jul-Sep Sorghum (intercrop)
Oct-Dec Lettuce

Mr Ginoux (farmer in Noves, Provence)
Mr Deleuze (Delbon)
Dr Prin (CIRAD)

Severe nematode problem

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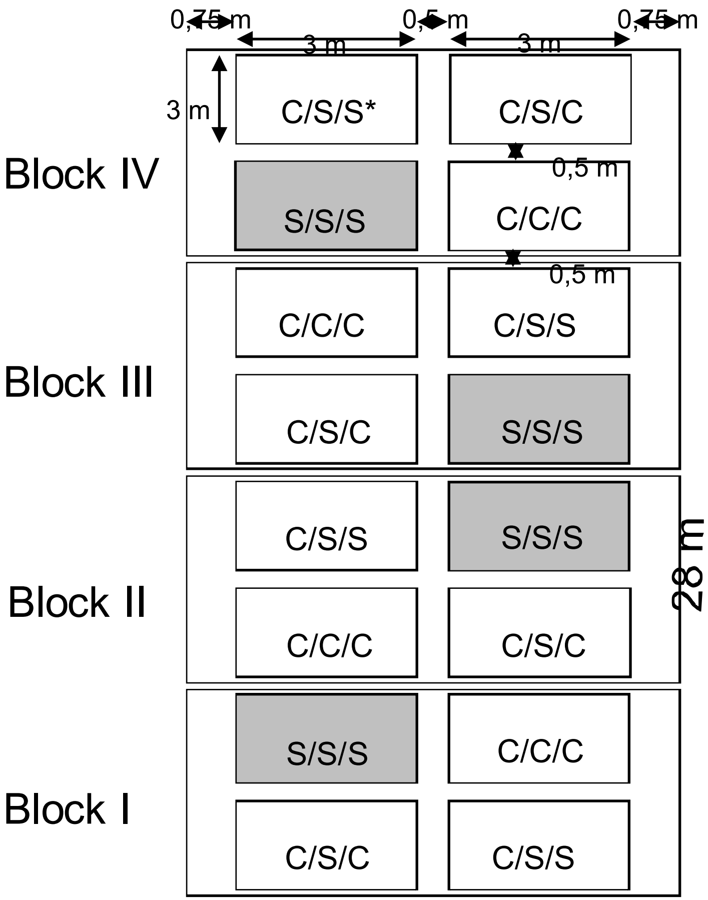
Experimental design over 3 years
4 blocks × 4 treatments



Crotalaria/**S**orgho/**C**rotalaria

Crotalaria/**C**rotalaria/**C**rotalaria

Crotalaria/**S**orgho/**S**orgho

Sorgho/**S**orgho/**S**orgho

Inoculation
5 strains

3 experimental sites:
Noves, Aimargues (France)
Archena (Spain)

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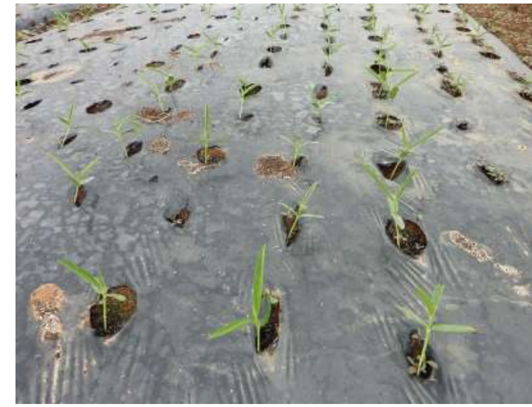
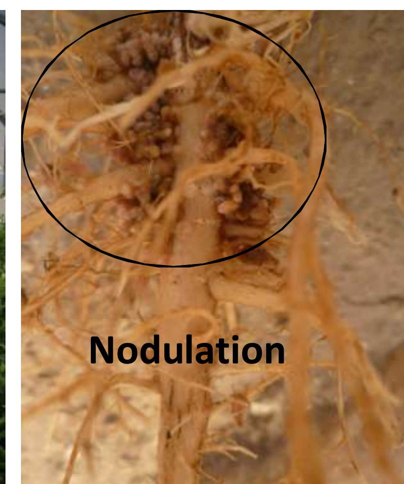
Crotalaria juncea

***Crotalaria* seedling**

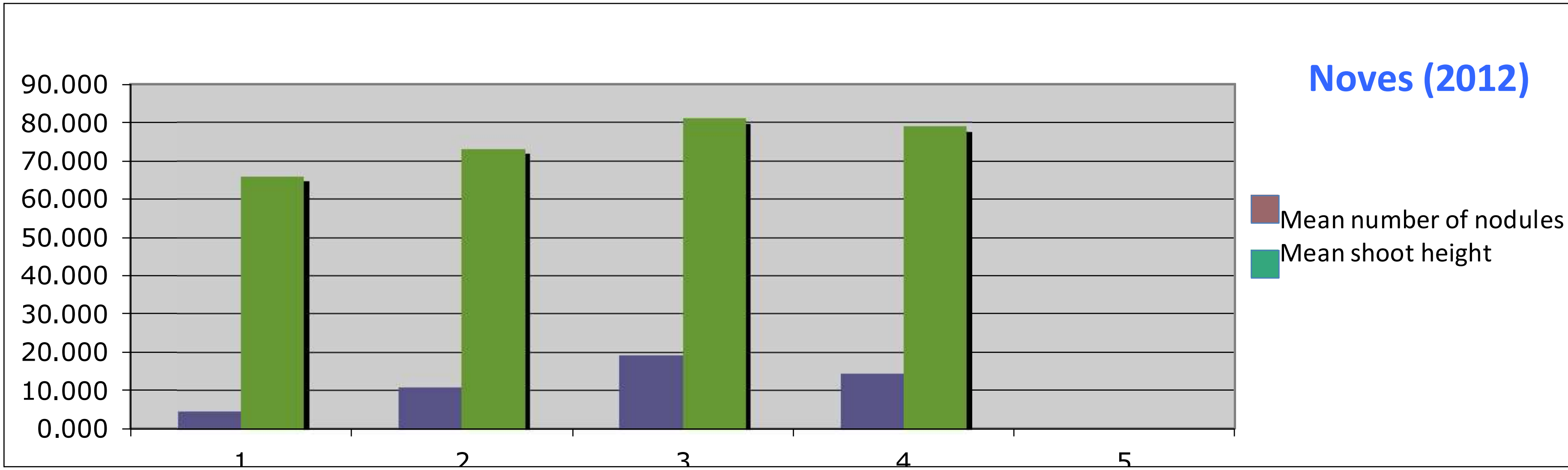
Inoculation

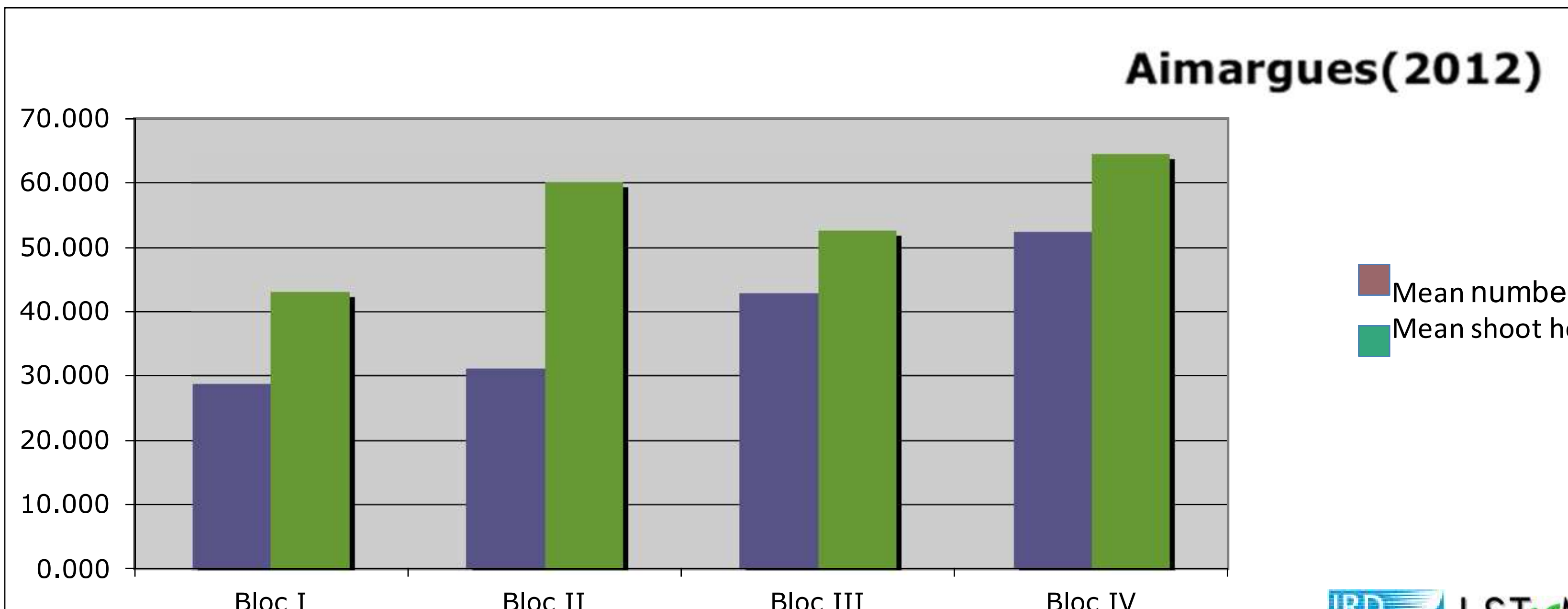




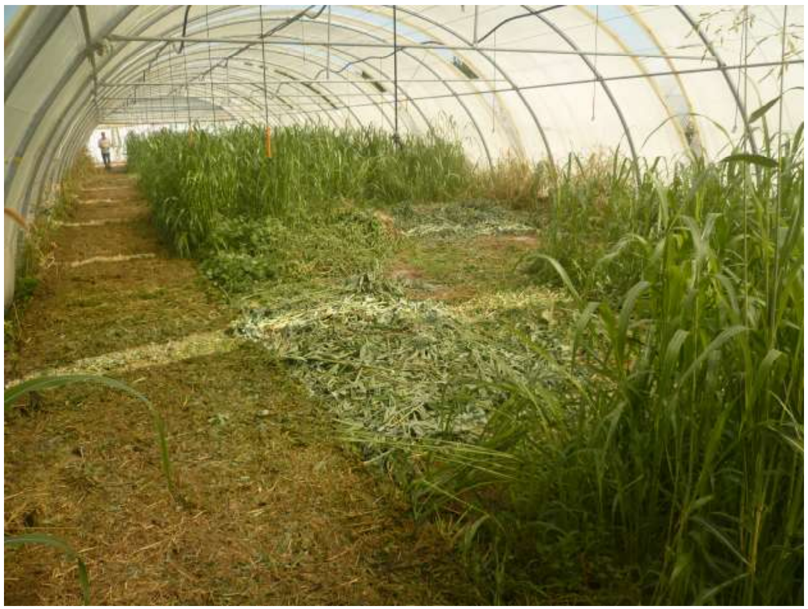
Crotalaria juncea in South France Growth, biomass, nodulation

Plant growth and nodulation of C. juncea after 6 weeks growing in the 4 blocks of the experimental designs





Cut, shear, weigh, bury



Lettuce crop

Nematostatic *Crotalaria* for vegetable production in greenhouse in South France

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Observation of nematode galls on lettuce roots at harvest (75 days)

After *Sorghum* or *Crotalaria*

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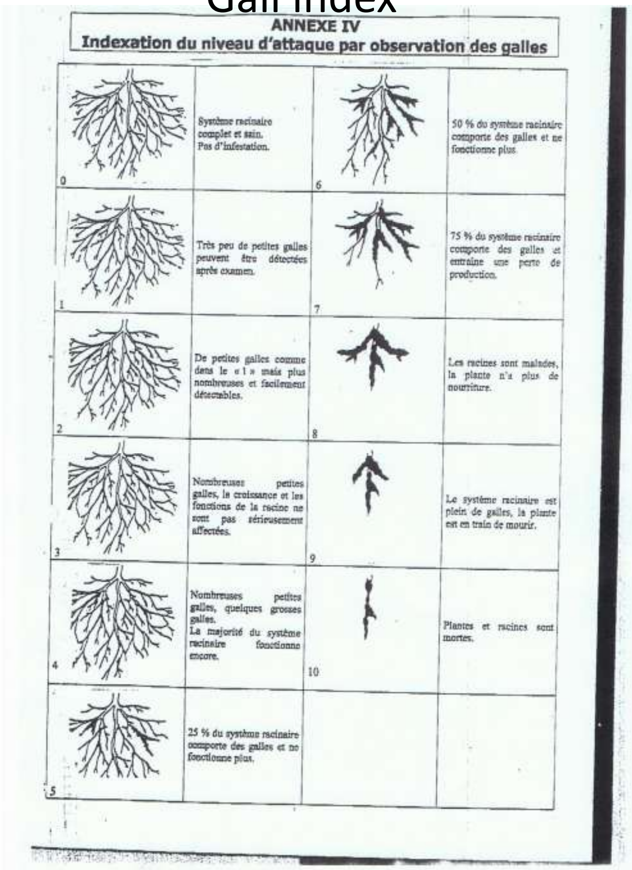
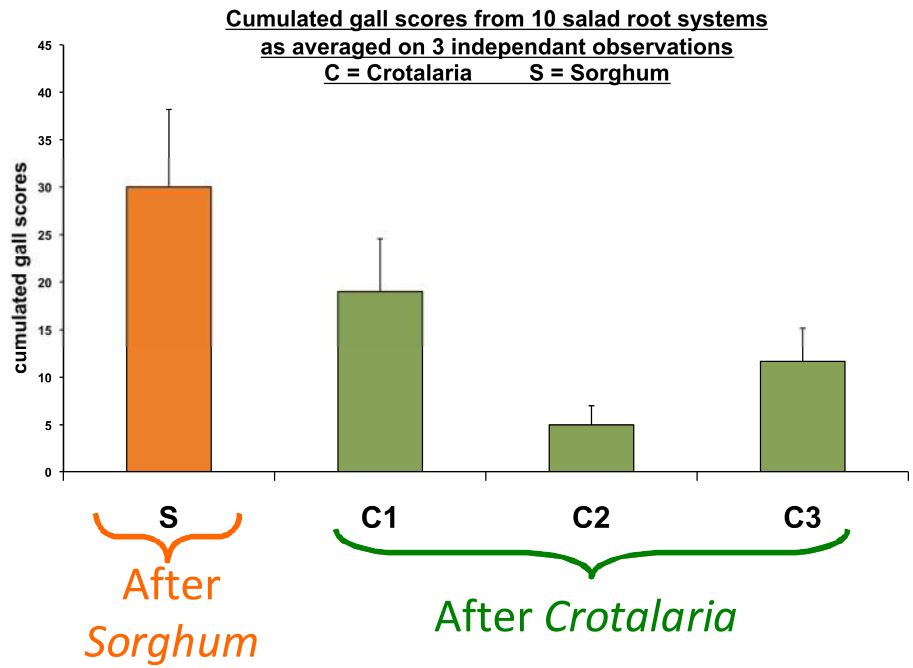
Evaluation of nematode infection on lettuce roots at harvest

Gall index



ANNEXE IV
Indexation du niveau d'attaque par observation des galles

0	Système racinaire complet et sain. Pas d'infestation.	6	50 % du système racinaire comporte des galles et ne fonctionne plus.
1	Très peu de petites galles peuvent être détectées après examen.	7	75 % du système racinaire comporte des galles et entraîne une perte de production.
2	De petites galles comme dans le « 1 » mais plus nombreuses et facilement détectables.	8	Les racines sont malades, la plante n'a plus de nourriture.
3	Nombreuses petites galles, la croissance et les fonctions de la racine ne sont pas sérieusement affectées.	9	Le système racinaire est plein de galles, la plante est en train de mourir.
4	Nombreuses petites galles, quelques grosses galles. La majorité du système racinaire fonctionne encore.	10	Plantes et racines sont mortes.
5	25 % du système racinaire comporte des galles et ne fonctionne plus.		



→ **Significant differences between plots and treatments**

Lettuce harvest (fresh weight) Year 1

France Site 1 Year 1

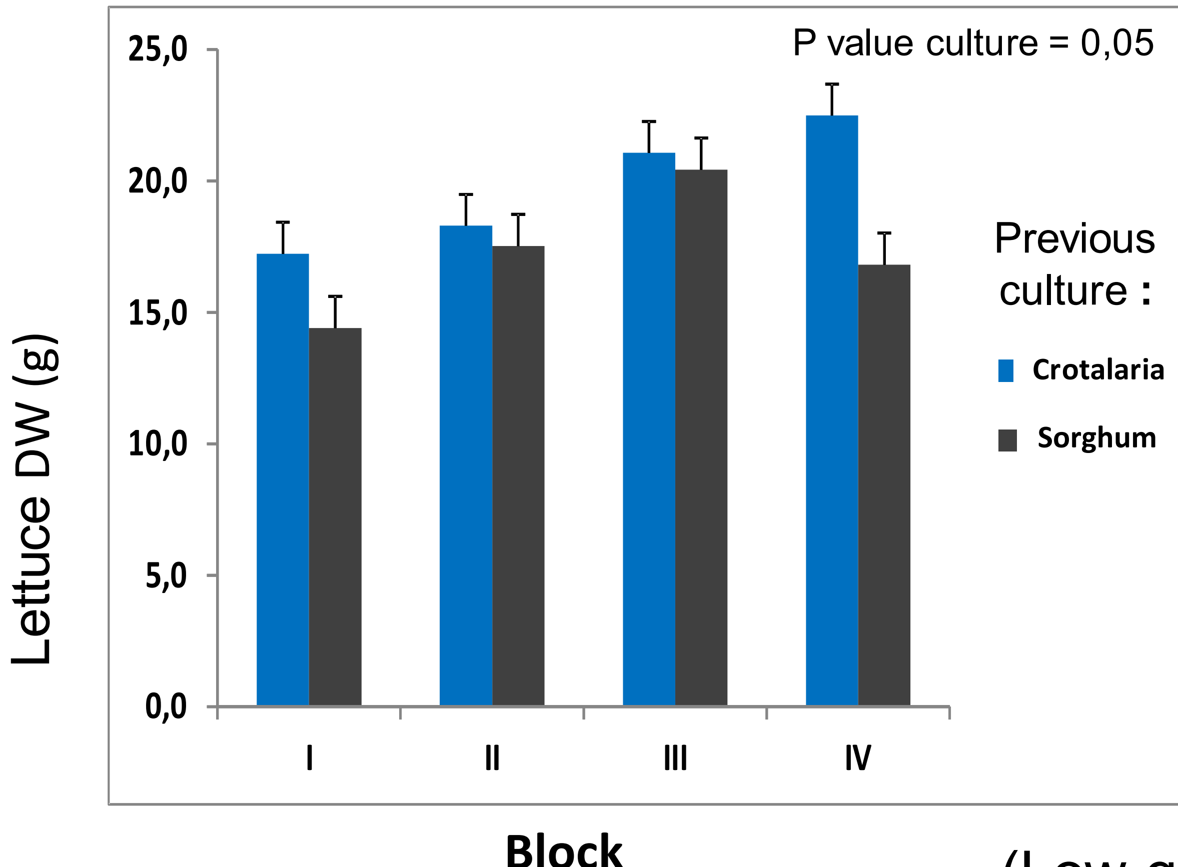
Newman-Keuls (SNK)		P < 0.001
Treatment	Average (g)	Groups
Crotalaria	364	A
Crotalaria	352	A
Crotalaria	352	A
Sorghum	300	B

France Site 2 Year 1

Newman-Keuls (SNK)		P < 0.05
Treatment	Average (g)	Groups
Crotalaria	425	A
Crotalaria	409	AB
Crotalaria	403	AB
Sorghum	376	B

- a block effect
- **previous crotalaria improves salad biomass compared to control sorghum**

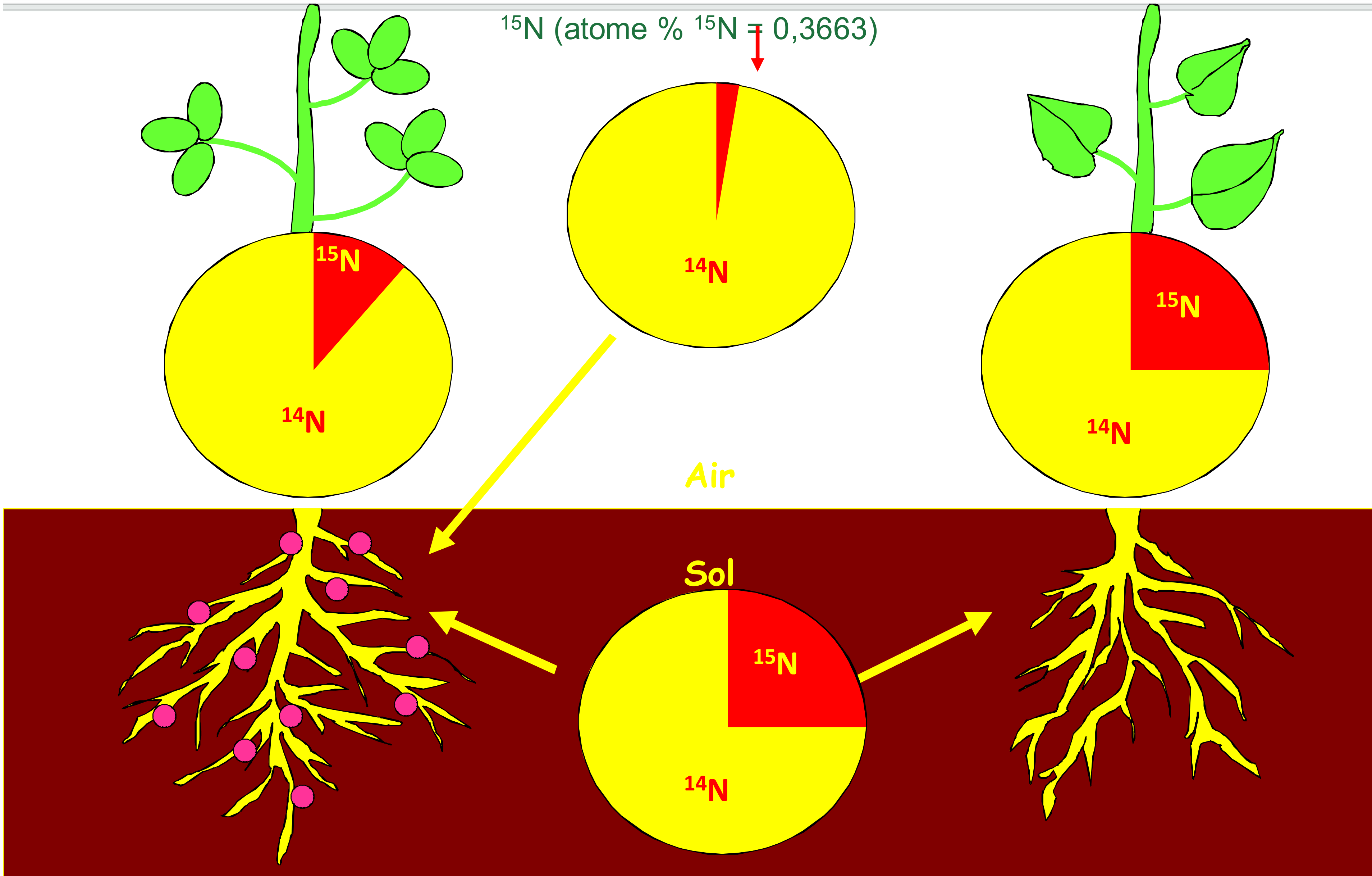
Effect of previous culture *Crotalaria* vs. Sorghum on lettuce biomass (DW) Noves year 2



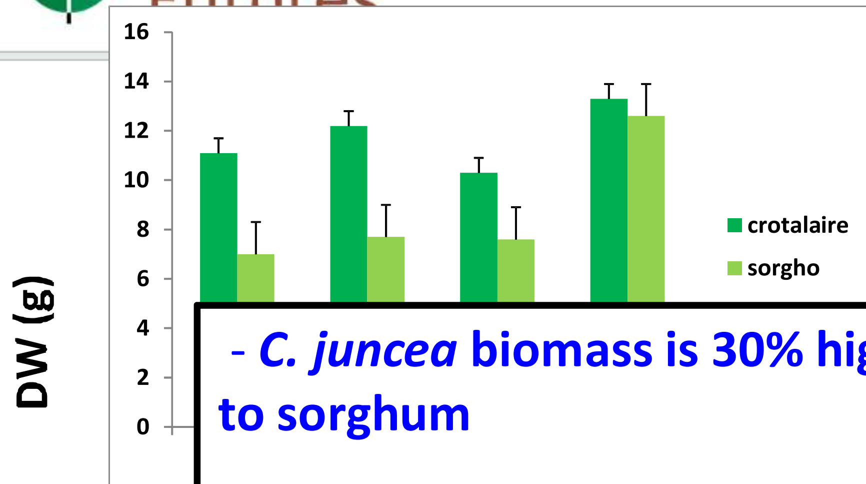
(Low gall attack)

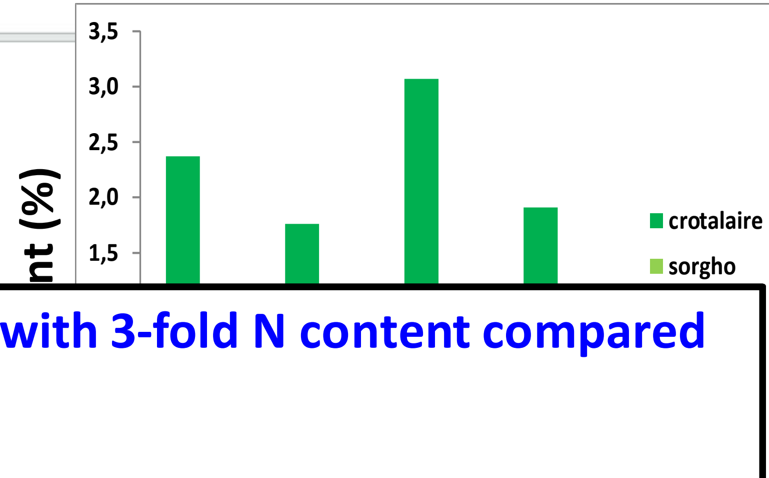
Nitrogen fixation
Isotopic approach

Estimation of nitrogen fixation by ^{15}N natural abundance measurement



^{15}N natural abundance, N content & $\%N_2$ fixed in *C. juncea* after 2 month-growth (experimental designs / 4 blocks)

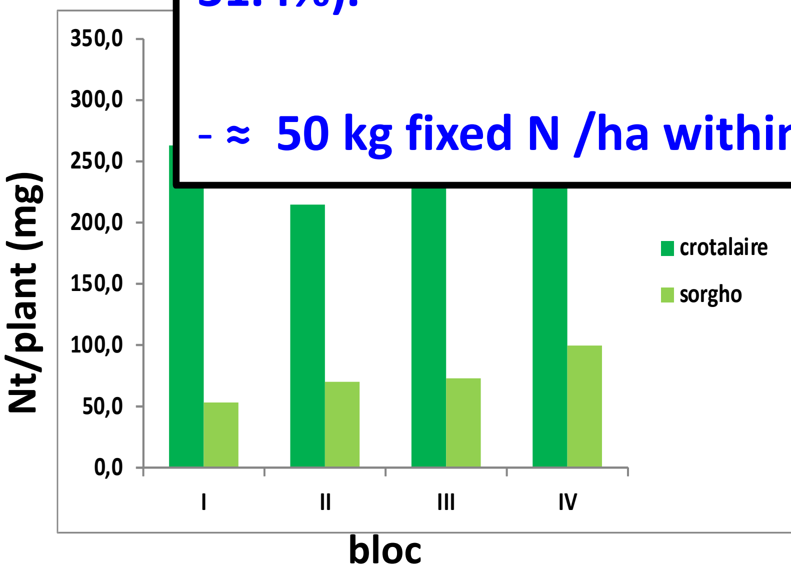


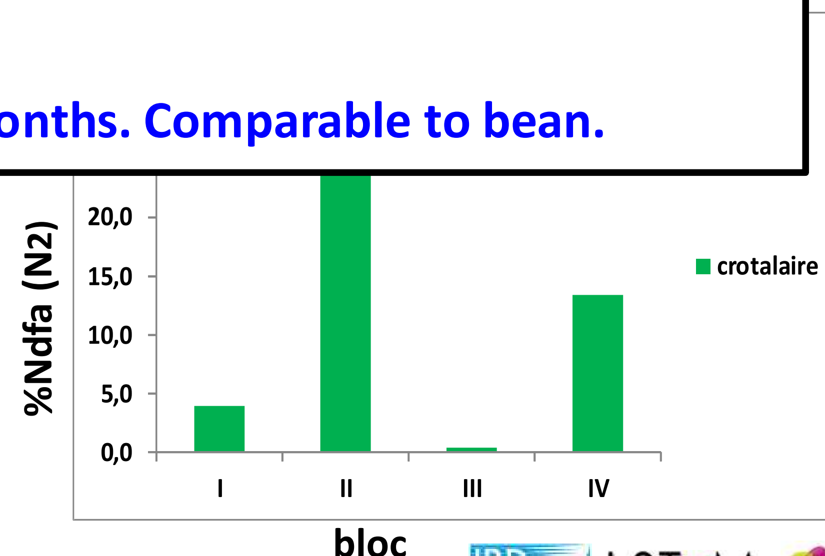


- *C. juncea* biomass is 30% higher with 3-fold N content compared to sorghum

-The part of N_2 fixation ($\Delta^{15}N$) in *C. juncea* varies in blocks (0.4-31.4%).

- ≈ 50 kg fixed N /ha within 3 months. Comparable to bean.





Microbiological approach

Soil microbial activity

Genetic structure of soil bacterial and fungal communities

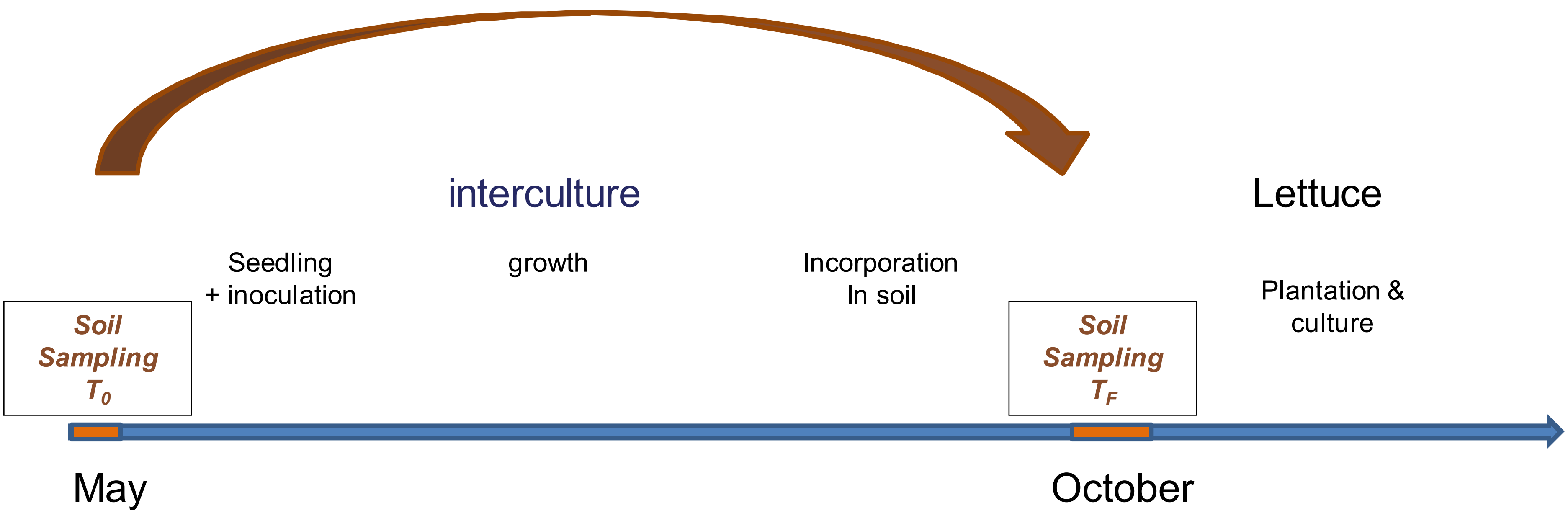
Soil mycorhizogenous potential

Inoculum survival in soil (MPN)

Inoculum strain efficiency

Noves (France) 2011, 2012

Interculture *Crotalaria juncea versus Sorghum bicolor*



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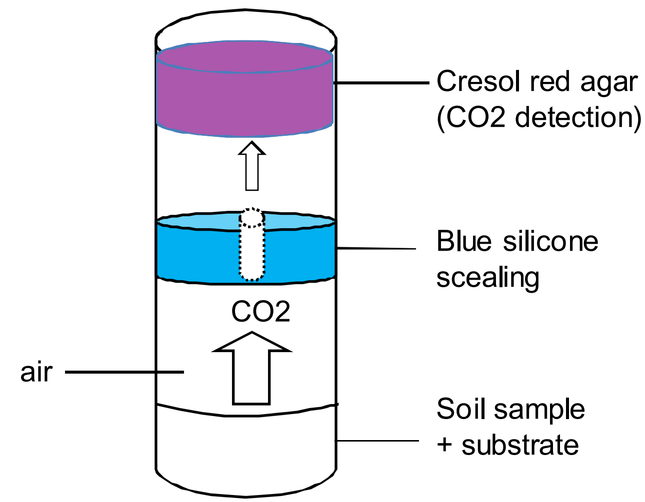
Soil microbial catabolic diversity
Respirometry



sugars

Organic acids

Amino acids

	1	2	3	4	5	6	7	8	9	10	11	12
A	mannose	mannose	mannose	mannitol	mannitol	mannitol	trehalose	trehalose	trehalose	arabinose	arabinose	arabinose
B	H2O	H2O	H2O	xylose	xylose	xylose	sucrose	sucrose	sucrose	galactose	galactose	galactose
C	inositol	inositol	inositol	sorbitol	sorbitol	sorbitol	rhamnose	rhamnose	rhamnose	arabitol	arabitol	arabitol
D	glutamate	glutamate	glutamate	citrate	citrate	citrate	maleate	maleate	maleate	ac.malique	ac.malique	ac.malique
E	gluconate	gluconate	gluconate	ascorbate	ascorbate	ascorbate	etoglutarate	etoglutarate	etoglutarate	D-oxalate	D-oxalate	D-oxalate
F	asparagine	asparagine	asparagine	valine	valine	valine	méthionine	méthionine	méthionine	glutamine	glutamine	glutamine
G	N-acétylglu	N-acétylglu	N-acétylglu	alanine	alanine	alanine	sérine	sérine	sérine	histidine	histidine	histidine
H	proline	proline	proline	leucine	leucine	leucine	lysine	lysine	lysine	arginine	arginine	arginine

32 substrates
(triplicates)

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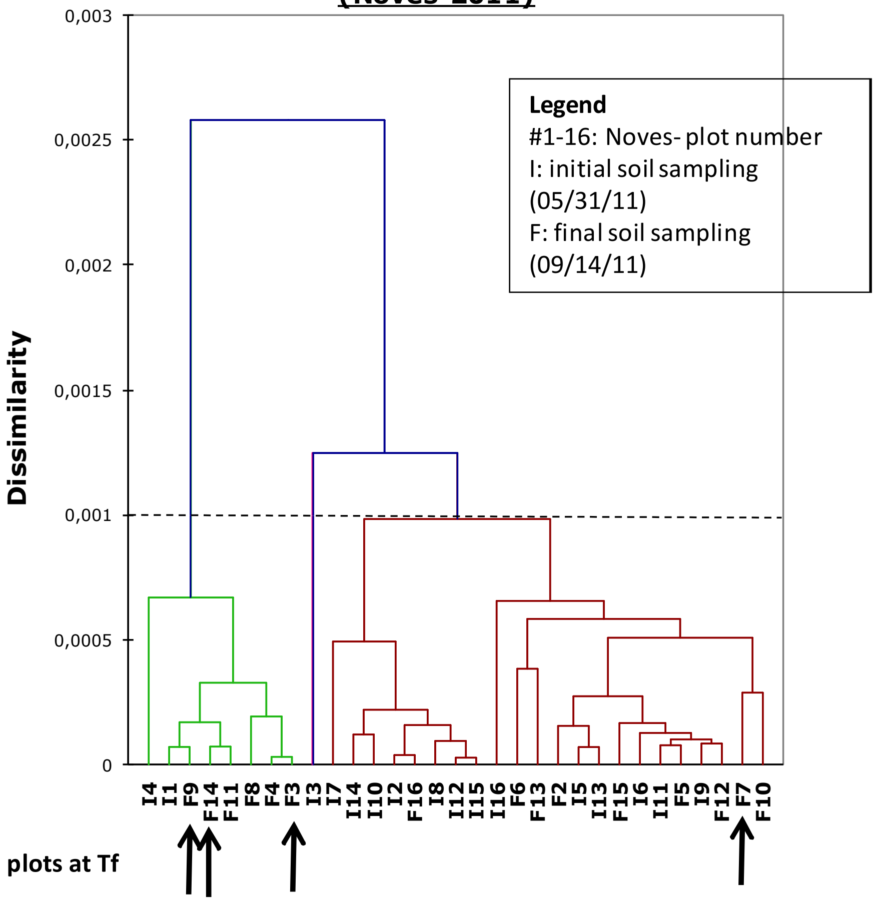
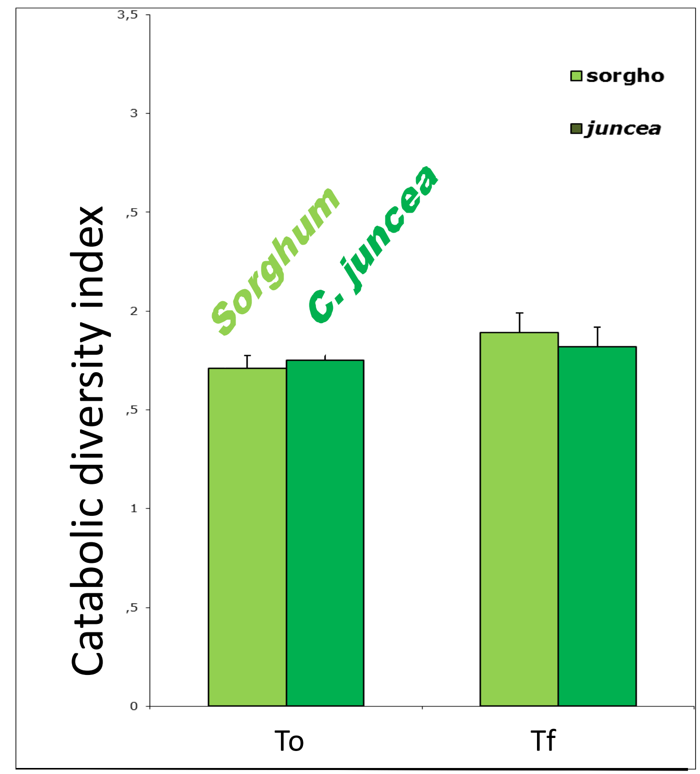
Soil catabolic profiles To vs. Tf

(Noves-2011)



Cluster analysis of the catabolic profiles caracterizing soils sampled before the *C. juncea* seeding and after its soil incorporation and mineralisation.



H = - Σ Pi ln Pi

Pi : relative oxydation intensity of a substrate i reference to the total oxydation level of all substrates

Microbiological approach

Soil microbial activity

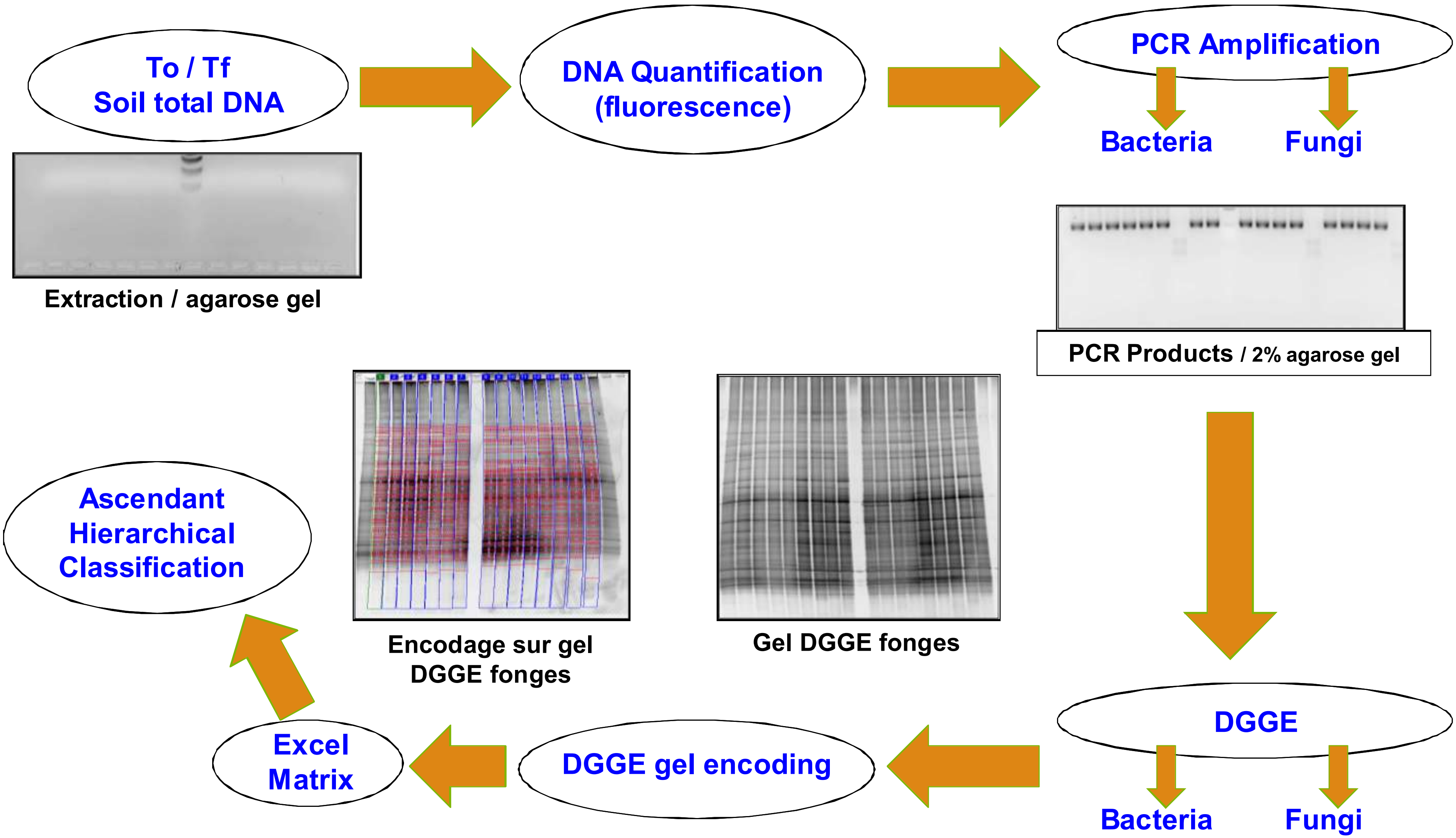
Genetic structure of soil bacterial and fungal communities

Soil mycorhizogenous potential

Inoculum survival in soil (MPN)

Inoculum strain efficiency

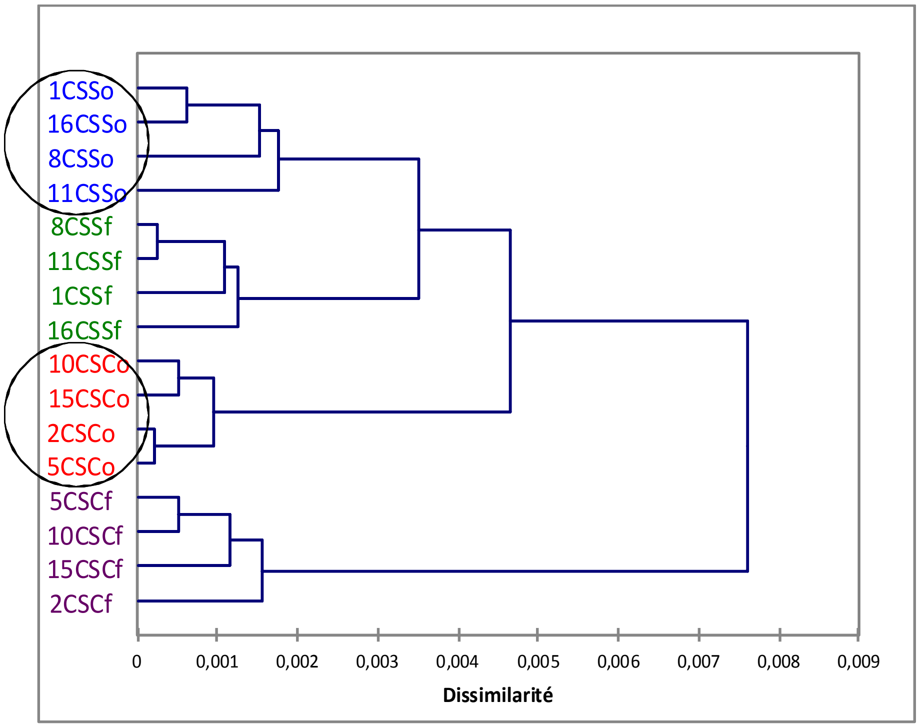
- Total bacteria : 16S rRNA coding gene (200 bp)
- Total fungi : 18S rRNA coding gene (400 bp)



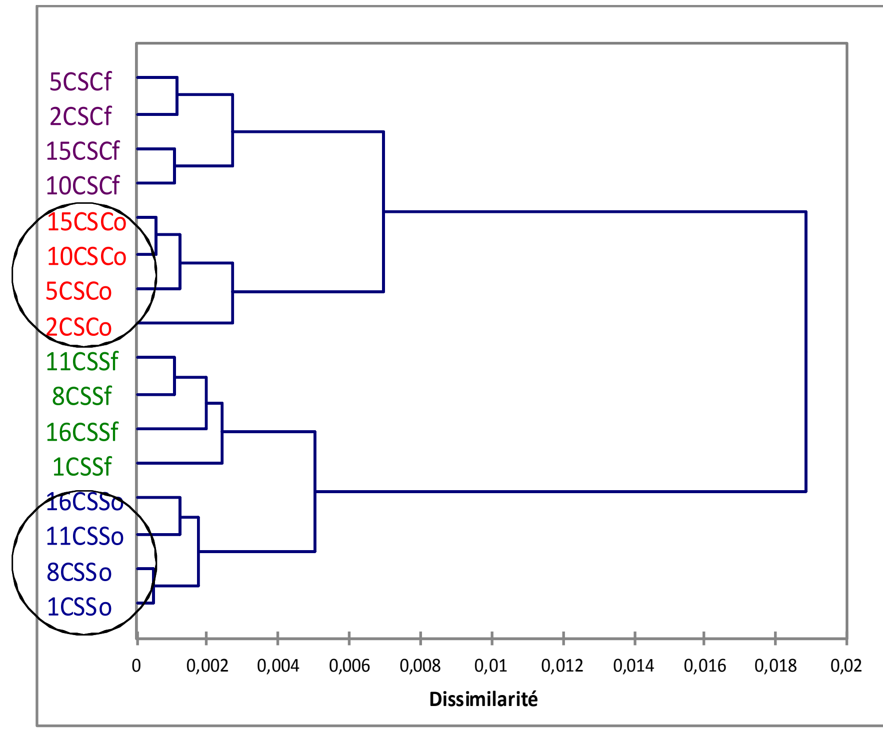
Noves 2011 (1st year)

Crotalaria Plots To

AHC **Bacterial community**



AHC fungal community

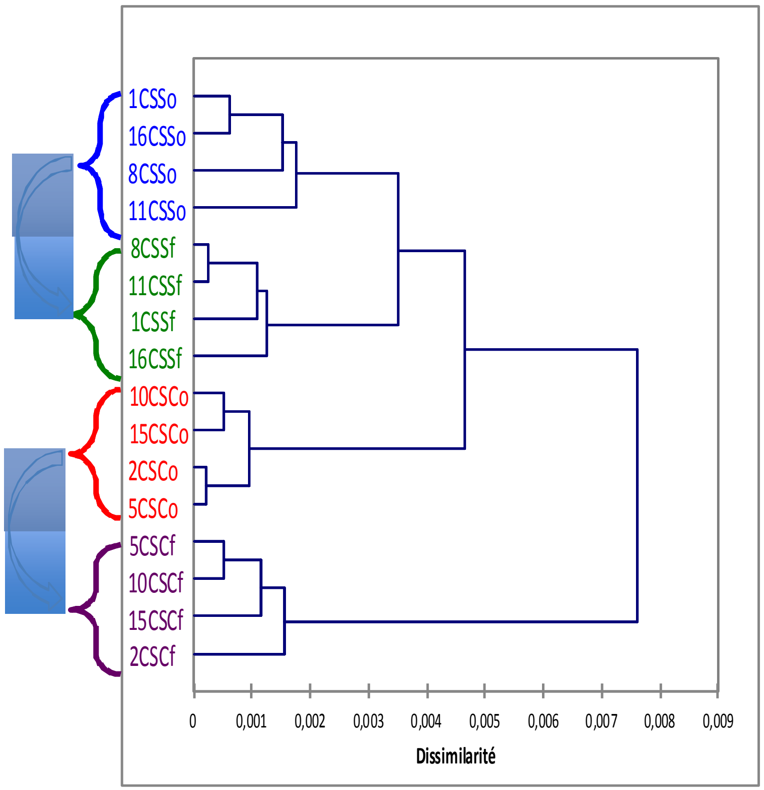


T_0 : initial heterogeneity of plots

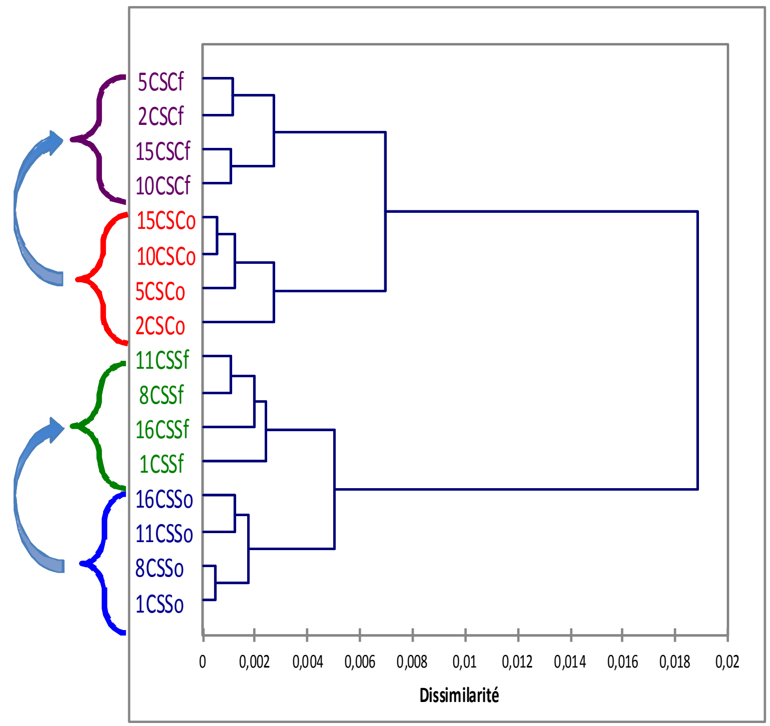
Noves 2011 (1st year)

***Crotalaria* Plots To / Tf**

AHC **Bacterial community**



AHC fungal community



***Crotalaria* Effect**

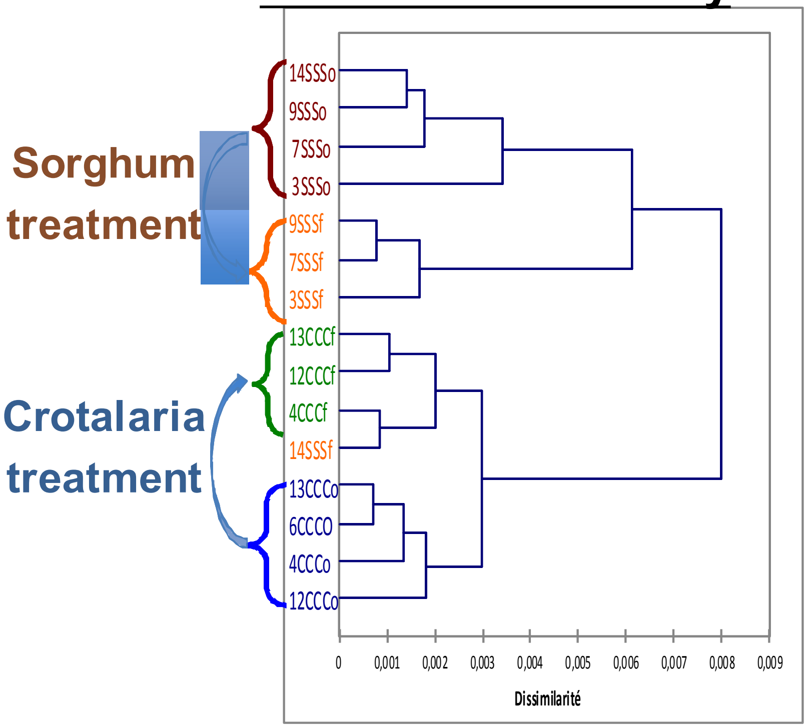
Tf ≠ To

2011 (1st year)

***Crotalaria* Plots** versus ***Sorghum* plots**

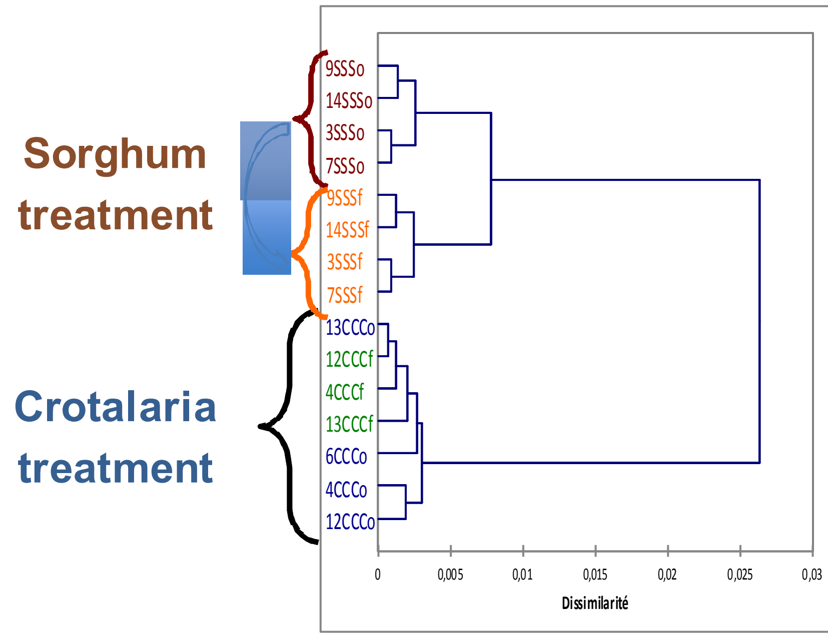
BACTERIA

AHC **Bacterial community**



FUNGI

AHC fungal community



Plant effect

Sorghum* > *Crotalaria

Partial conclusions

Interculture impacts genetic structures of both soil bacterial and fungal communities,

Sorghum ≠ Crotalaria

Intensity varying in campaings.

Initial soil heterogeneity persists after intercultures.

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Microbiological approach

Soil microbial activity

Soil mycorhizogenous potential

Inoculum survival in soil (MPN)

Inoculum strain efficiency

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Soil mycorrhizogenous infective potential evaluation (t=0) trap culture

Trap cultures of mycorrhizal fungi using *C. juncea* and *L. multiflorum* on soils sampled in the 4 sorghum plots (Noves-tunnel, 2011).

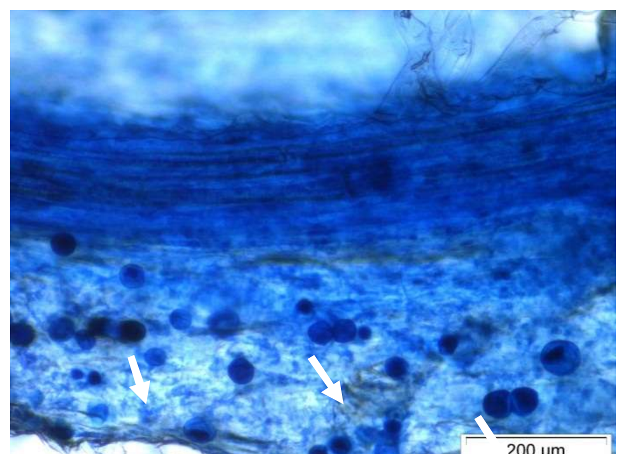
C. juncea

Ray grass

Root systems at harvest.

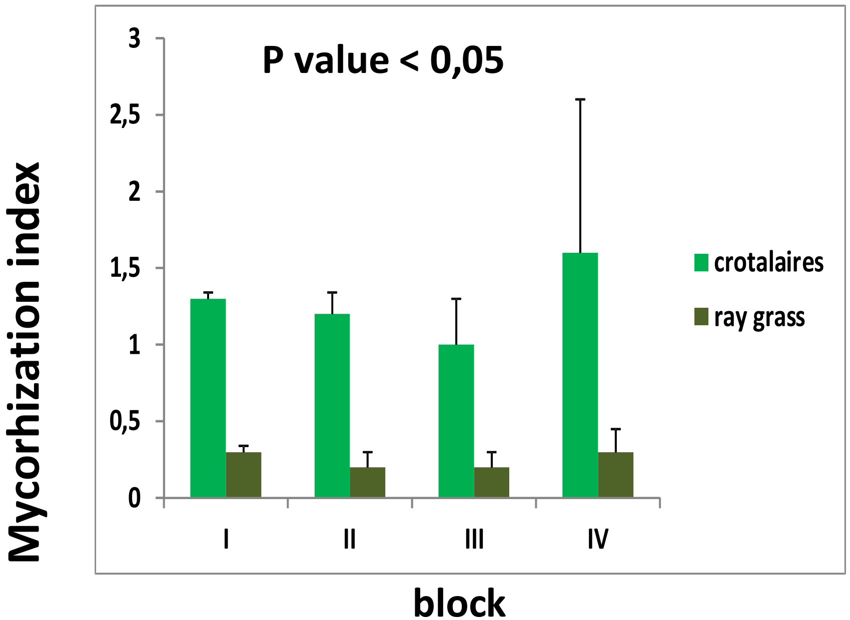
Soil mycorhizogenous infective potential evaluation (t=0) trap culture

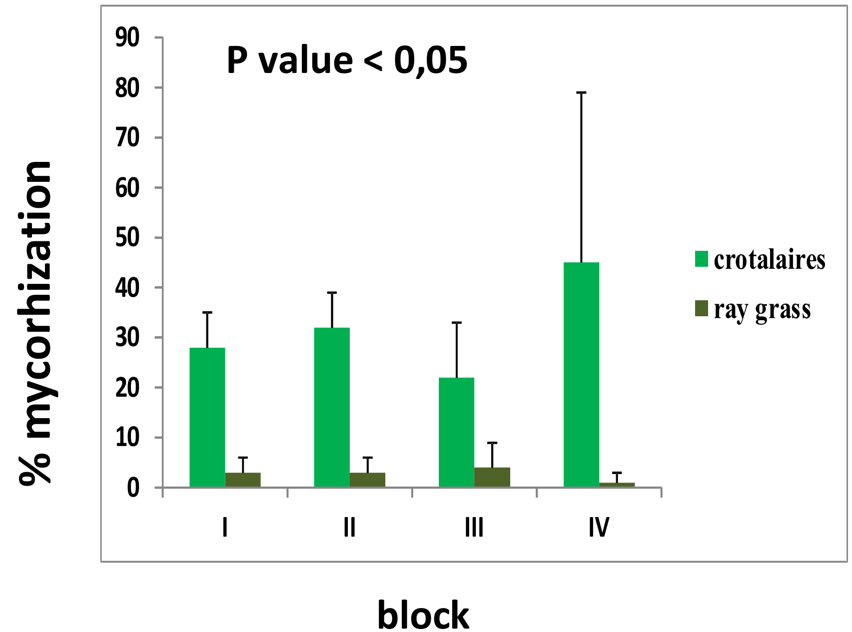
Noves soil +
{45% attapulgite, 45% sand, 10% loam}
subdued 2 mm, 36 day cultivation



Microscopic observation of inner root mycorrhizal structures from Trypan Blue stained roots

Soil mycorhizogenous infective potential evaluation (t=0) trap culture





Crotalaria >> Ray grass

C. juncea **may monitor soil mycorrhizal infectivity**

Microbiological approach

Soil microbial activity

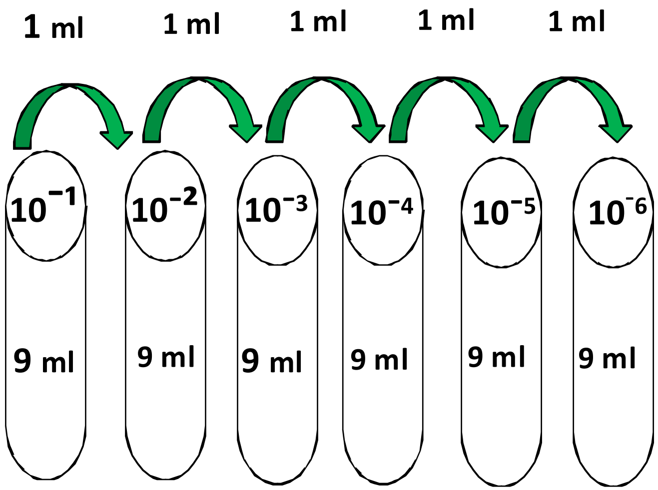
Soil mycorhizogenous potential

Inoculum survival in soil (MPN)

Inoculum strain efficiency

Rhizobial trapping after 1 year

Rhizobial inoculum persistence after *C. juncea* cultivation (MPN)



× **4 C/C/C soil samples × 4 repeats**

$$X = (m*d)/v$$

X – estimated number rhizobiums/g soil

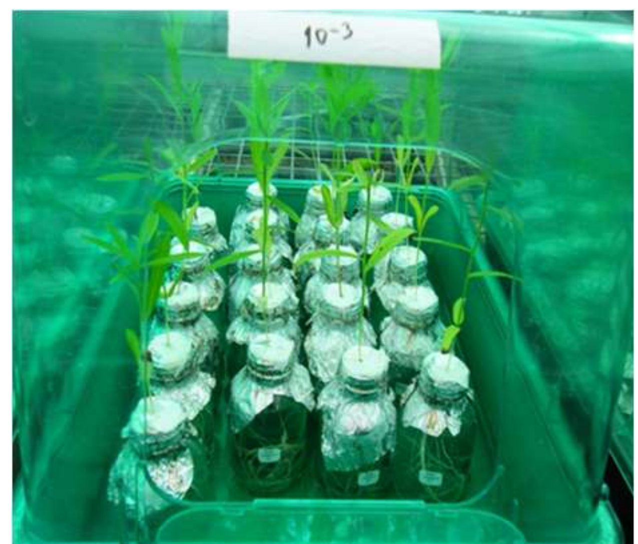
m –bacterial number at the lowest dilution

d – the lowest dilution

v –inoculum volume (= 1 ml)

bloc		I					II					III					IV			
répétition	1	2	3	4	total	1	2	3	4	total	1	2	3	4	total	1	2	3	4	total
dilution																				
10^{-1}	+	+	+	+	4	+	+	+	+	4	+	+	+	+	4	+	+	+	+	4
10^{-2}	+	+	-	+	3	-	+	+	-	2	+	+	+	+	4	+	+	+	-	3
10^{-3}	-	+	-	-	1	+	-	-	-	1	+	+	+	+	4	-	-	-	+	1
10^{-4}	+	+	-	-	3	-	-	+	+	2	+	+	+	+	4	+	-	+	+	3
10^{-5}	-	-	+	+	1	-	+	-	-	1	-	+	-	-	1	-	-	+	-	1
10^{-6}	-	-	-	-	0	+	+	-	-	2	+	+	+	-	3	-	-	-	-	0
total/bloc					12					13					20					12

$X = 1,7 * 10^3$ bact /g soil



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Microbiological approach

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West Africa Crotalaria

nitrogen fixing bacterial symbionts

Long Bacilli	Short Bacilli
Slow growth	Fast growth
Bradyrhizobium spp.	*Methylobacterium nodulans*
(Samba *et al.*, 1999)	

Methanol	+
Formate	+
Formaldehyde	+
Methylamine	-
nodA, nifH	+

(Sy *et al.*, 2001

Jourand *et al.*, 2004)

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Efficiency potential of the 5 *Bradyrhizobium inoculant* strains on *C. juncea*

Sterile substrate :
45% perlite, 45% sand,
10 % loam
+ N-free nutritive solution
Analysis 30 dai.

Bradyrhizobium

ORS 1816 (origin *C. hyssopifolia)*

ORS 1815 (origin *C. hyssopifolia)*

ORS 1810 (origin *C. lathyroides)*

ORS 1929 (origin *C. comosa)*

ORS 1935 (origin *C. goreensis)*

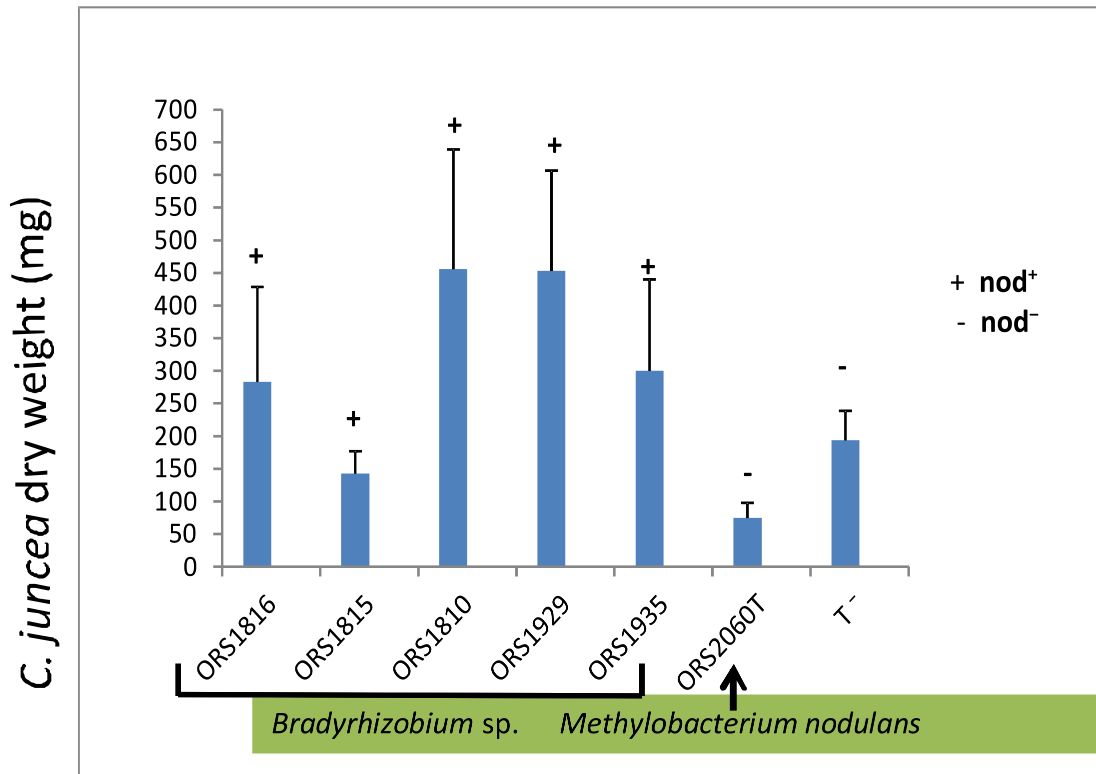
Methylobacterium nodulans

ORS 2060 (origin *C. podocarpa)*

Efficiency potential of the 5 *Bradyrhizobium* *Inoculant* strains on *C. juncea*

Contrasting strains

P value < 0,05



Molecular approach

Inoculant Rhizobial strain molecular characterization & tracing in soil and time

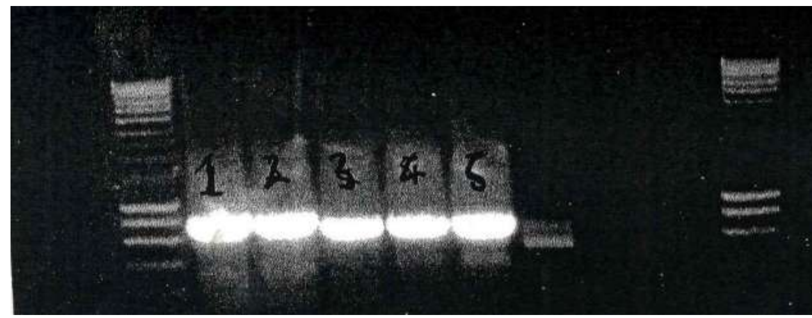
5 inoculant strains

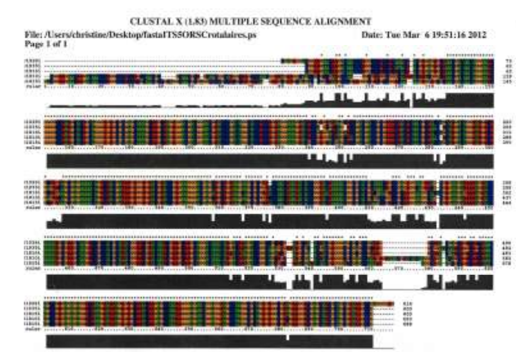
16S-23S ITS PCR-Amplification

ORS 1816 ORS 1929 ORS 1810 ORS 1935 ORS 1815

800pb

600pb





- The 5 strains are *Bradyrhizobium* spp.
- ITS polymorphism among the inoculant strains
- No natural *Crotalaria* nodulating strain in experimental soils

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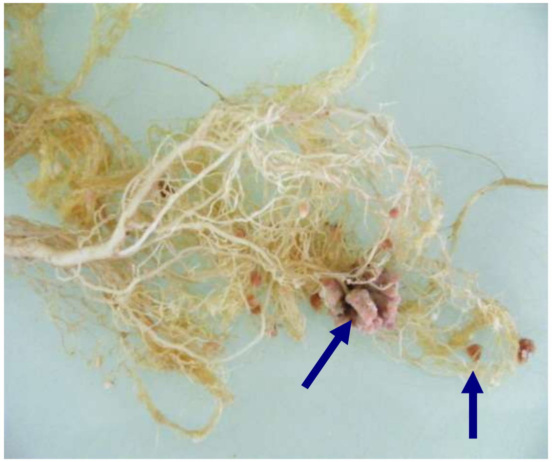
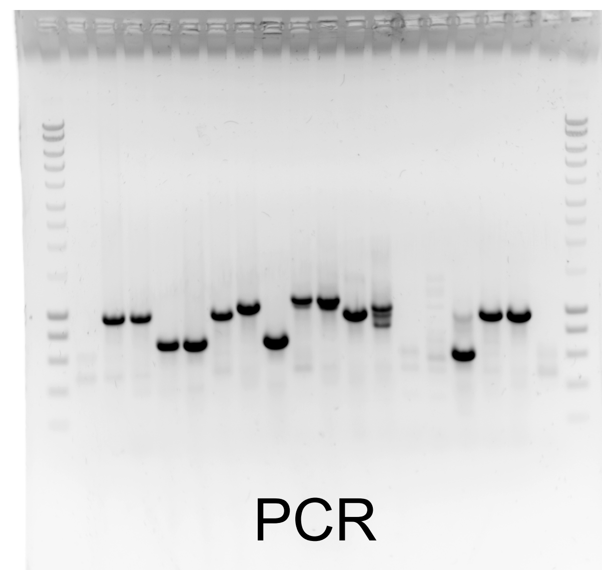
Noves, 1 year later…



C. juncea nodules
(Noves samples)

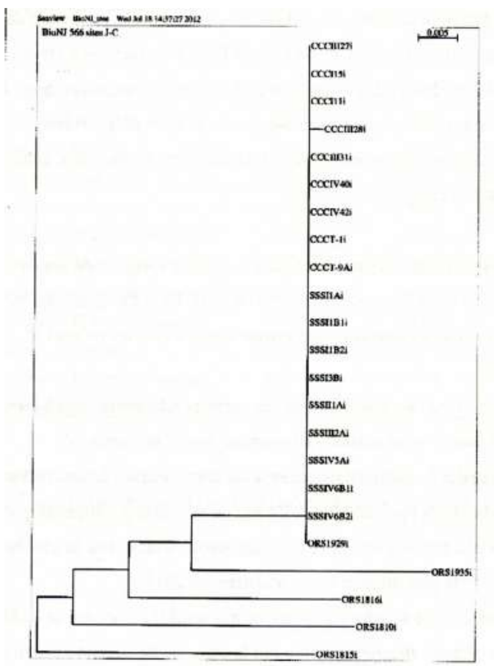
Bacterial
isolation

DNA extraction



16S-23S ITS rDNA
sequencing

Bradyrhizobium sp
A. radiobacter
Rhizobium spp



Noves & Archena

After 1 year & in all plots :
100% nodule *Bradyrhizobium* isolates identical to ORS 1929

ORS 1929 = sole strain able to survive ?
Most competitive ?

Archena (Spain)
Assay in a Canarian shelter

Archena (Spain)
Assay in a Canarian shelter

Nodules

Archena
tomato after *Crotalaria*
(green manure)

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↑

Archena
Tomato &
Crotalaria
associated
cultures →

Galls on tomato roots

Harvest daily registration

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Archena (Spain) tomato harvest

2012 (Year 1) & 2013 (Year 2)
- *Crotalaria* / tomato rotation
- *Crotalaria* + tomato co-culture

Half yield compared to traditional
Good quality tomato fruits

Farmer

Take home message

⇨ *C. juncea* in Mediterranean tunnel conditions exhibits a rapid growth with abundant biomass (> Sorghum)

⇨ The biological nitrogen fixing potential of *C. juncea* may be similar to bean (50 Kg N /ha / 3 months)

⇨ Positive Green Manure effect of *Crotalaria juncea* on lettuce biomass (+ 10%)

⇨ Reduced gall attack observed after *Crotalaria* (lettuce/tomatoe ; France & Spain)

⇨ Soil mycorhizogenous potential may be higher after *C. juncea* (>>> ray grass)

⇨ *Bradyrhizobium* spp. inoculant strains may persist and fix N_2 in mediterranean conditions (France & Spain)

- ⇨ Impact of *C. juncea* culture on soil bacterial / fungal community
- ⇨ Nitrogen transfer to following crop ? (in progress over 3 years)
- ⇨ Soil chemical composition / fertility after 3 years ?

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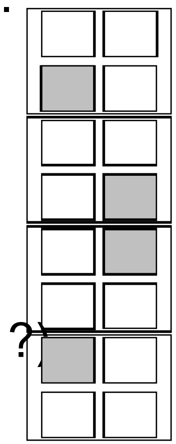
After 3 years in Noves (France)

➢ Vegetable production is more **homogenous**, more **abundant**, **good quality**.

➢ In year 2 and 3 (2012 et 2013) all **surrounding greenhouses** faced **caterpillar** & nematode attacks, but not the trial greenhouse: *Crotalaria* might have a **zonal effect** larger than the unit plot.

➢ **Convinced** to go on / switch from *Sorghum* to *Crotalaria* interculture (seeds ?)



After 2 years in Archena (Spain)

➢ **Surprised** to harvest good quality tomatoes, even half yield

➢ (Year -1 = 0 harvest)

➢ *Crotalaria* = **barrier** effect against nematodes ?

➢ Year 3 : in progress

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