

# Nematostatic N<sub>2</sub>-fixing *Crotalaria* for sustainable greenhouse vegetable production

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**Background**  
 Tunnel greenhouse vegetable production is rapidly expanding in Mediterranean Countries  
 High humidity  
 High temperature  
 Short duration of the cultures } → Pest problems (nematodes)

No satisfying treatment against nematodes (Chemicals against nematodes are toxic to the users & soon banned in Europe)  
 Common practice : 3 month sanitary period with empty greenhouse (fallow)  
 Action of solar rays / xylene treatment  
 Tourteau of castor-oil plant

**Objectives**  
 ➤ to improve vegetable production (yield, sustainability, quality) by exploiting the agronomic & biological properties of *Crotalaria* spp. plants  
 • nitrogen-fixing root nodules  
 • Arbuscular Mycorrhizas (AM) symbioses  
 • control root-knot nematode populations (nematostatic compounds production)  
 ➤ To reduce the use of chemical fertilizers and pesticides.  
 ➤ field application and transfer to farmers,  
 ➤ To develop cost-effective, environment-friendly and sustainable management practices

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graph TD
    Crotalaria["Crotalaria sp. (Legumes)"] --> Mycorrhiza
    Crotalaria --> NitrogenFixation
    Crotalaria --> ActiveMolecules["Active Molecules (Flavonoids, alkaloids)"]
    Crotalaria --> BioActivities["Biological activities (phytoparasitic nematodes)"]
    ActiveMolecules --> PlantNutrition["Plant nutrition (N, P)"]
    ActiveMolecules --> NematodeControl["Nematode control"]
    BioActivities --> NematodeControl
    
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**Assay schedule in Noves in 2011-2012**

|                     |        |
|---------------------|--------|
| Soil sampling       | May 31 |
| Crotalaria seedling | Jun 7  |
| weeding             | Jun 28 |
| Inoculation         |        |
| weeding             |        |
| Plant sampling      | Jul 6  |
| Cutting and digging | Jul 29 |
| Lettuce 1 planting  | Sep 30 |
| Lettuce 1 Harvest   | Nov 17 |
| Lettuce 2 planting  | Jan 1  |
| Lettuce 2 Harvest   | Mar 19 |

Crotalaria plant sample measures:  
 height, dry matter, nodule number & rhizobial content  
 15N analysis

In lab

**Strategy**

|          |     |     |     |
|----------|-----|-----|-----|
| Bloc IV  | CSO | CSO | CSO |
|          | CSB | CSB | CSB |
| Bloc III | CCC | CCC | CCC |
|          | CSB | CSB | CSB |
| Bloc II  | CCC | CCC | CCC |
|          | CSB | CSB | CSB |
| Bloc I   | CSB | CSB | CSB |
|          | CCC | CCC | CCC |

Tunnel Assay  
 3-year Rotation  
 1/1/13  
 Complete randomized block design

28 m

Jan-Mar Lettuce  
 Mar-Jul Melon  
 Aug-Sep Sorghum or *Crotalaria*  
 Oct-Dec Lettuce

A single nematode application, 18 months earlier, of Telone C5 (Dow AgroSciences)

**Crotalaria seedling**

**Inoculation**

**1 week**

**1 month**

**Drying**

**Nodulation**

**Lettuce**

**Harvest**

**Healthy roots**

**Root Galls**

**Microbiological impact of *Crotalaria* on soil and roots**

comparison of mycorrhizal intensities according to soil origins

microscopic observations of finer root mycorrhizal structures from plant roots

bioassays of arbuscular mycorrhizal fungi with gramineous plants (e.g. *Lolium perenne*)

soil

**Crotalaria plant growth & root nodulation (2011)**

Plant growth and nodulation of *C. juncea* after 6 weeks growing in the 4 blocks of the experimental design set up in Noves (2011)

| Block   | Plant number (approx.) |
|---------|------------------------|
| Block 1 | ~50                    |
| Block 2 | ~60                    |
| Block 3 | ~65                    |
| Block 4 | ~60                    |

15N natural abundance, N concentration and percentage of N<sub>2</sub> fixed in *C. juncea* after 6 weeks growing in the 4 blocks of the experimental design set up in Noves (2011)

| Block   | 15N natural abundance | N concentration | % N <sub>2</sub> fixed |
|---------|-----------------------|-----------------|------------------------|
| Block 1 | ~0.002                | ~0.15%          | ~10%                   |
| Block 2 | ~0.002                | ~0.15%          | ~10%                   |
| Block 3 | ~0.002                | ~0.15%          | ~10%                   |
| Block 4 | ~0.002                | ~0.15%          | ~10%                   |

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**Inoculum strain molecular characterization**

**16S-23S ITS PCR-Amplification**

5 strains

OHS 16S  
 OHS 1629  
 OHS 1810  
 OHS 165  
 OHS 1615

800pb 600pb

**Sequence Alignment**  
 The 5 strains are *Bradyrhizobium* spp.

**CONCLUSION & PERSPECTIVES**

- Very positive green manure effect of *Crotalaria juncea* on lettuce biomass in both sites and within two successive campaigns in Noves
- Δ<sup>15</sup>N measurements are in progress to evaluate the part of nitrogen fixation in this effect (transfer).
- Reduced gall attack was observed in all cases when lettuce was preceded by *Crotalaria*
- Possible remnant effect of *Crotalaria* will be examined in next years
- N transfer from *C. juncea* necromass to lettuce: analysis in progress
- An additional experiment is being settled in Murcia (Spain) in 2012, with tomato

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