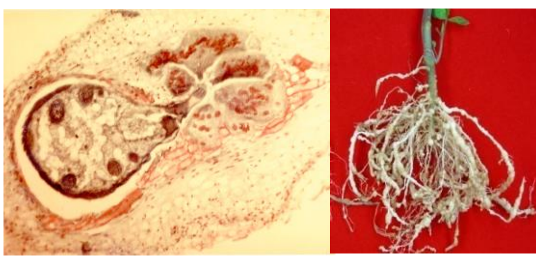
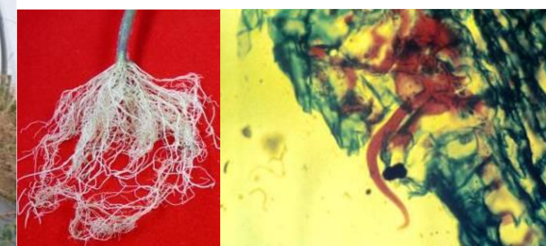
Session 2 : Sustainable and integrated breeding and deployment of genetic resistance

# Experimental evidence of the efficiency of 2 *R*-genes deployment strategies - pyramiding or alternating - for the sustainable management of root-knot nematodes



Susceptible plant

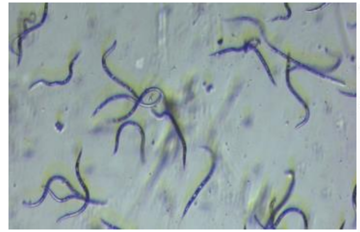
Resistant plant

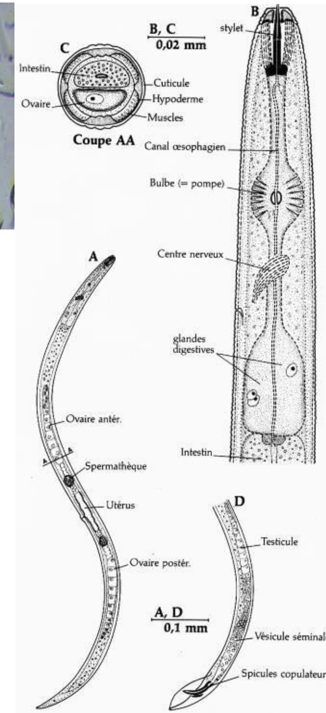
INRA PACA
Plant Nematodes Interaction team
Institut Sophia Agrobiotech

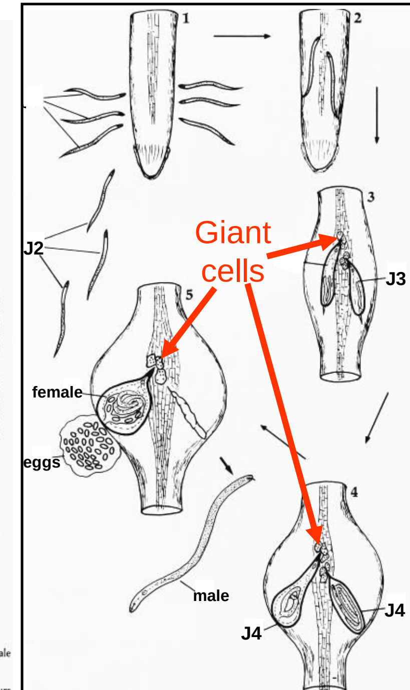
Djian-Caporalino C., Fazari A., Marteu N., Sage-Palloix A.M., Risso S., Lanza R., Palloix A., Castagnone-Sereno P.

# Root-knot nematodes *Meloidogyne* spp.

- Microscopic soil borne roundworms (0,2 to 2 mm), obligate sedentary endoparasites






Life cycle
(3 weeks to 3 months)
Main species parthenogenetic

- Symptoms : galls on roots (thus, sharp decrease in the aerial part ····> death)

- Extremely polyphagous (> 5,500 host plants)

- ~10% of crop losses worldwide *Trudgill & Blok, Annual Review of Phytopathology, 2001*

  (Some species subjected to quarantine regulations in the EU)

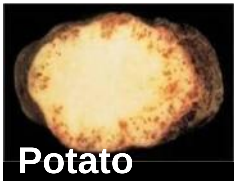
- Chemical nematicides *prohibited or restricted*

  Fumigants : methyl bromide, dichloropropene

  Systemics : e.g. aldicarbe $LD_{50}$=1ppm


# Root-knot nematodes *Meloidogyne* spp.

## An increasing problem on vegetable crops in all Mediterranean regions


- **A survey conducted from 2007 to 2010* :**
  **a big threat for > 40% of farms producing vegetables in SE France**
- **Crop rotations with resistant plants : economically efficient and environmentally safe, but resistance can be overcome**

*Djian-Caporalino, *Phytoma November 2010 & EPPO Bulletin April 2012*

# Limitation of the RKN-resistance

## In controlled conditions with high pressure of RKN

- *Mi-1* in tomato and *Me3* in pepper are overcome

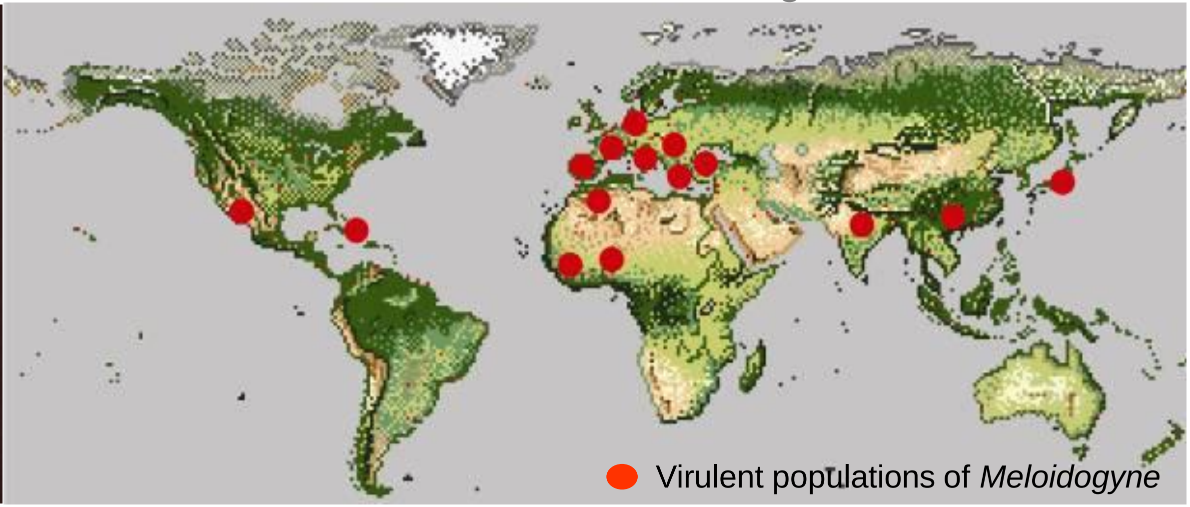
*e.g.* Jarquin-Barberena *et al. 1991;* Castagnone-Sereno *et al. 1994, 1996, 2001;* Meher *et al. 2009;* Djian-Caporalino *et al., 2011*

## In natural conditions

- *Mi-1* in tomato and *N* in pepper cultivars, 60 years of use, are overcome

*e.g.,* Tzortzakakis *et al. 2005, 2008;* Verdejo-Lucas *et al. 2009;* Devran and Söğüt *2010 ;* Thies *2012*

**Worlwide occurrence of *Meloidogyne* spp. populations able to overcome the tomato *Mi-1* R-gene**

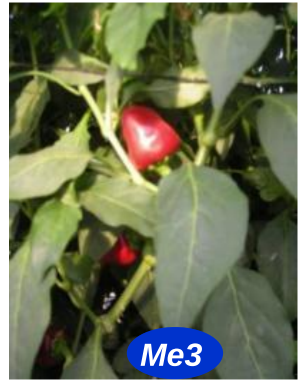

**Develop new « robust » *R*-lines**

**Manage *R*-genes to increase their durability**

# Model to study the durability of resistance to RKN

***Capsicum annuum***

*Me1 to Me7, Mech1, Mech2, N*



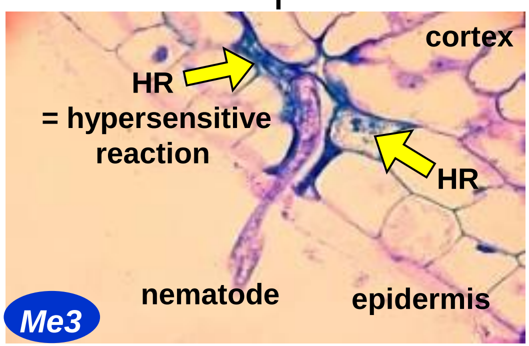


*M. incognita*
*M. arenaria*
*M. javanica*

R stable at high T°

Hendy *et al, Nematologica 1985* ; Hare, *Phytopathology 1956* ;
Thies & Fery, *J Amer Soc Hort Sci 1998 & 2000* ; Thies & Ariss, *EJPP 2009;*
Djian-Caporalino *et al., Theor Appl Genet 1999, 2001, 2007*

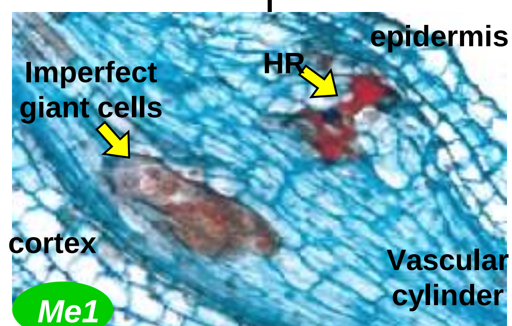
1 dai — /// — 10 dai — day after inoculation




**Early necrosis**

**Gene overcome**

Selection of virulent nematodes




**Later necrosis**

**Difficult to overcome the *Me1* gene**

Castagnone *et al, Plant Breeding 2001* ; Djian-Caporalino *et al., EJPP 2011*

# Experimental approach

## Climate controlled room experiments

. **Strength of the genes** (in several genetic context & with several RKN pop.)

. **Varietal effect** (genetic background)

. **Combination of genes** (pyramiding)


## 3-years greenhouse and field experiments

. **Validation** with natural nematode populations

. **Deployment strategies of *R*-plants lowering the risk of emergence of virulent nematodes :**

i) alternance of *R*-genes in rotation,
ii) mixture of different *R*-genotypes in the same plot
iii) pyramiding of 2 *R*-genes in one genotype.

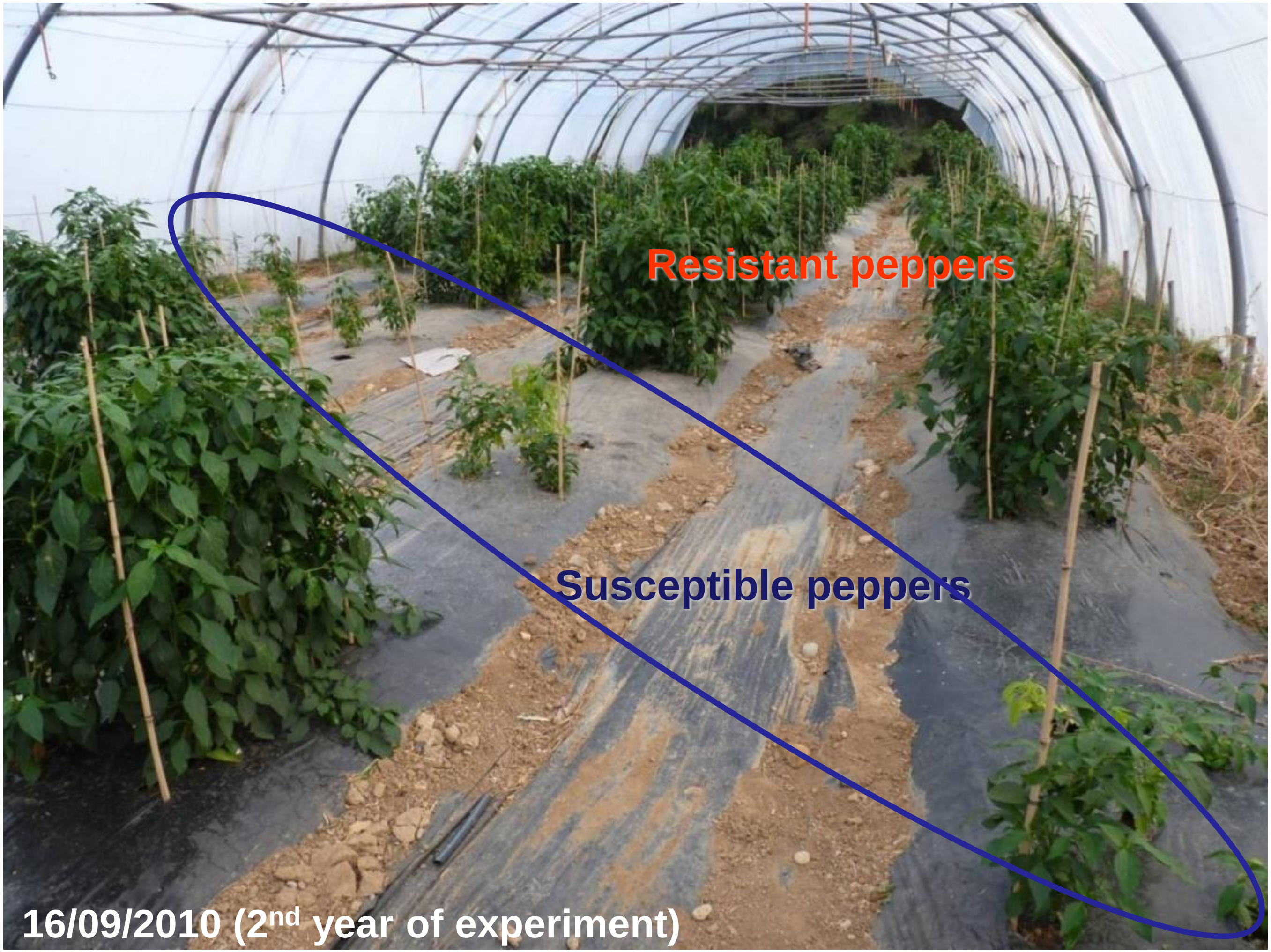


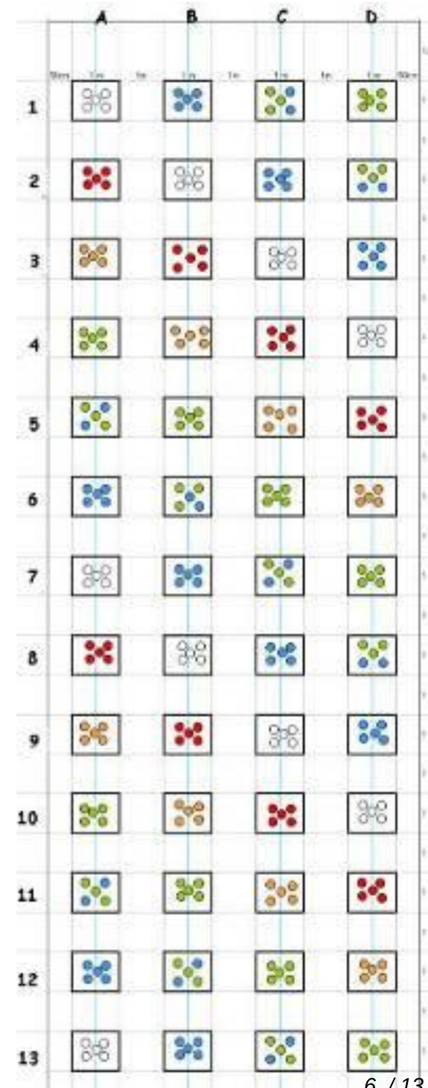

# Example of an experiment in natural condition

Nice, SE France

**250 m², 52 µplots, 5 plants/µplot**

**Plastic tunnel** 28 m x 8 m **infested by *M. incognita* + *M. arenaria***



# Example of an experiment in natural condition

Nice, SE France

**6 MODALITIES**
**8 to 9 µplots/modality**
**x 5 plants/µplot**
**= 40 to 45 plants/modality**

**Susceptible cultivar DLL (control)**

**R inbred line *Me1***

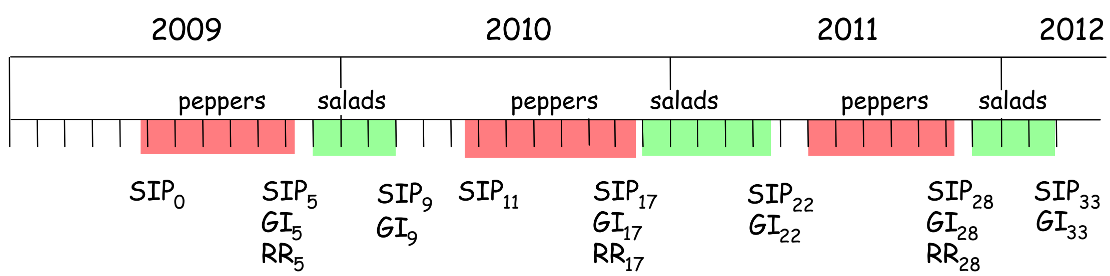
**R hybrid [DLL x *Me1*]**

**Alternation *Me3* then *Me1***

**Mixture *Me3* and *Me1***

**Pyramiding *Me3 Me1***

**Schedule and Infestation parameters**

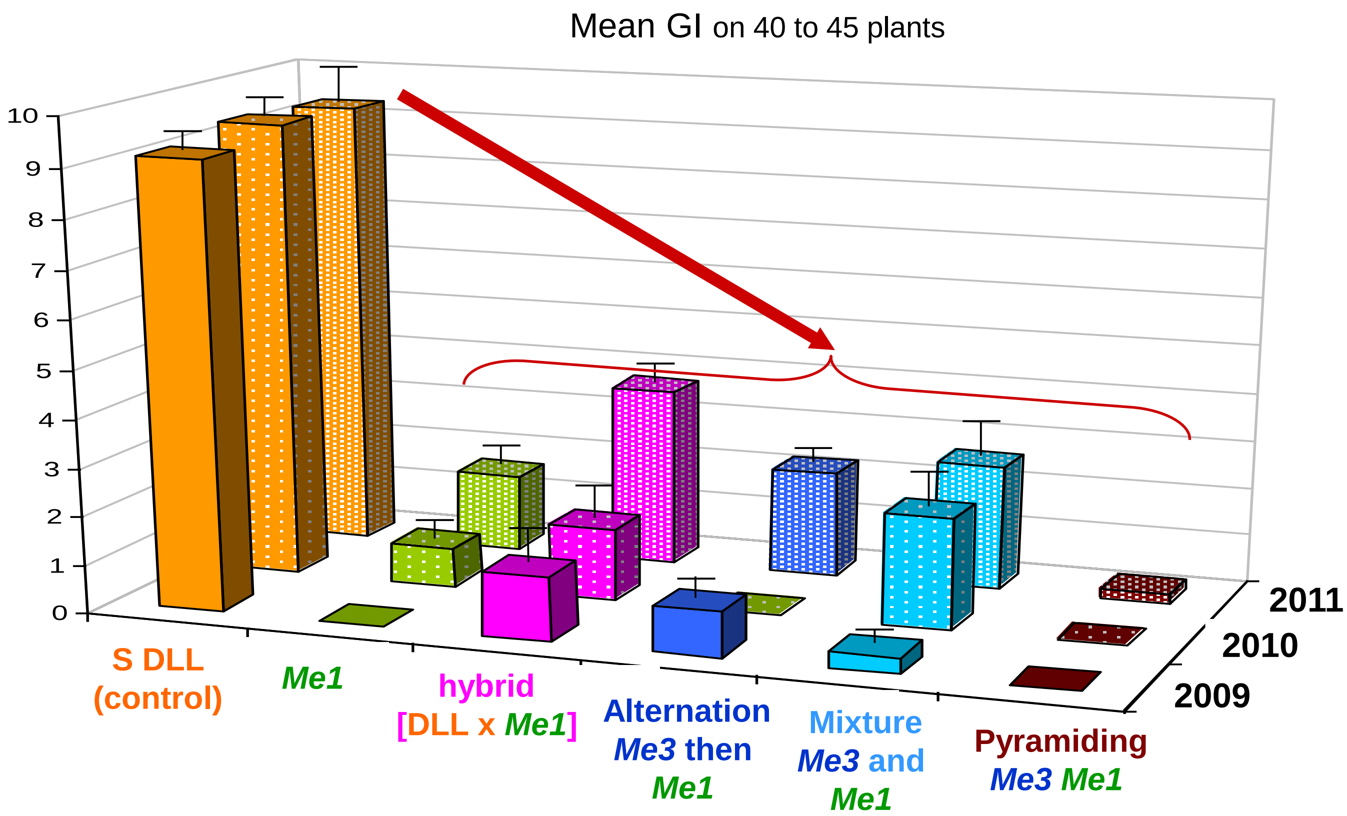



**SIP = soil infection potential** *(number of Meloidogyne J2 /kg of soil)*
**GI = gall index** *(on peppers and salads)*
**RR = reproduction rate of virulent nematodes**
**if egg masses detected on *R*-peppers**

*(number of eggs produced /J2 inoculated on R-peppers in controlled conditions)*

# Example of an experiment in natural condition

**GI = gall index on peppers** ***after 5 months of culture in summer***




**GI on *S*-peppers nearly maximum and very high compared to the *R*-peppers**

# Example of an experiment in natural condition

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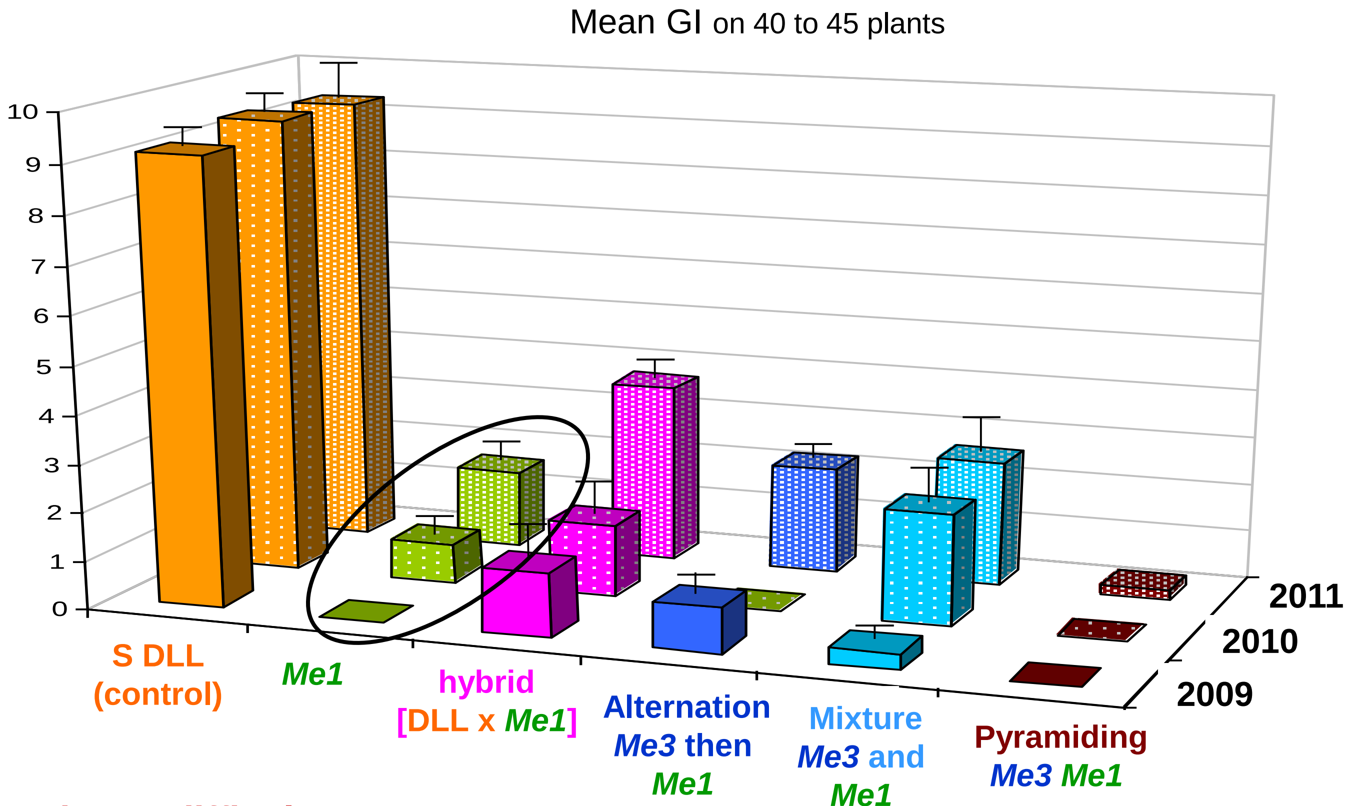
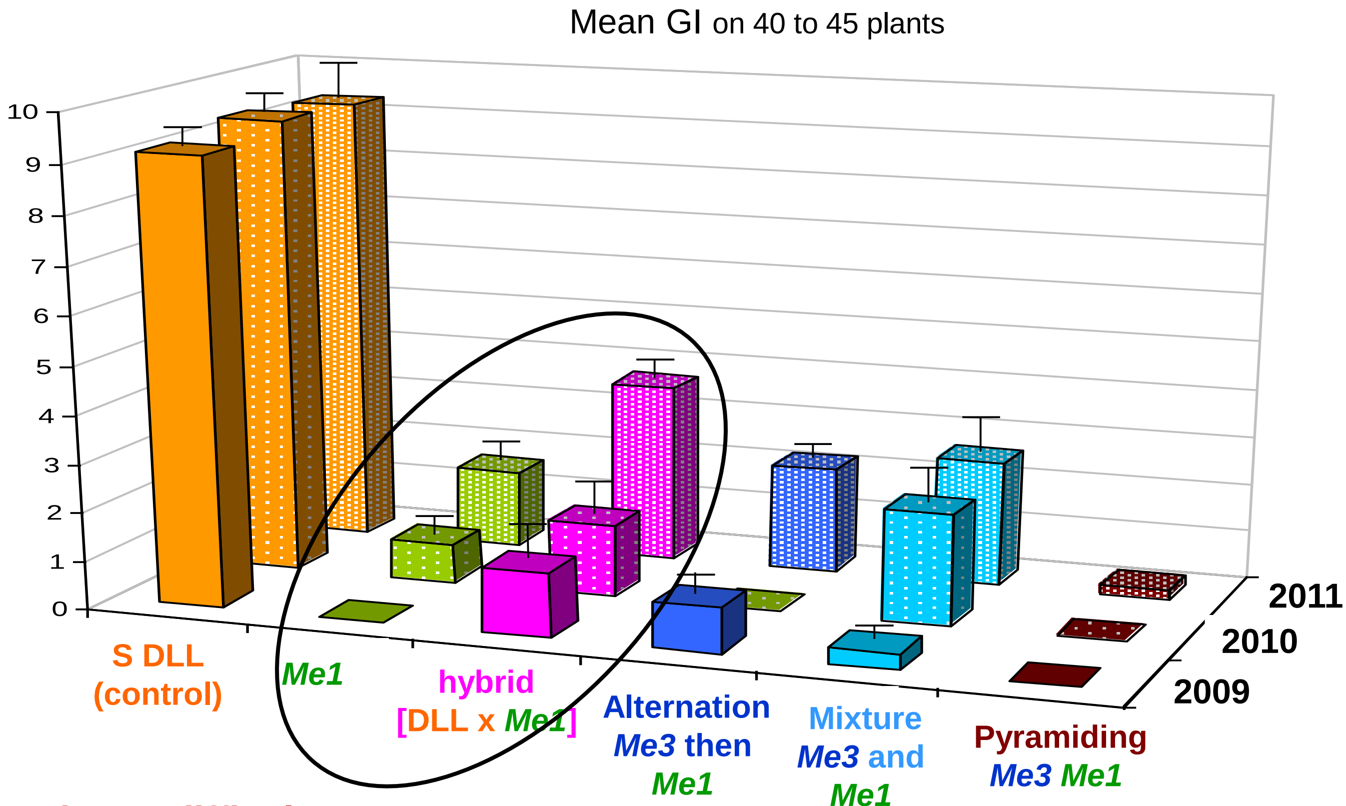



***Me1* robust : difficult to overcome**

# Example of an experiment in natural condition

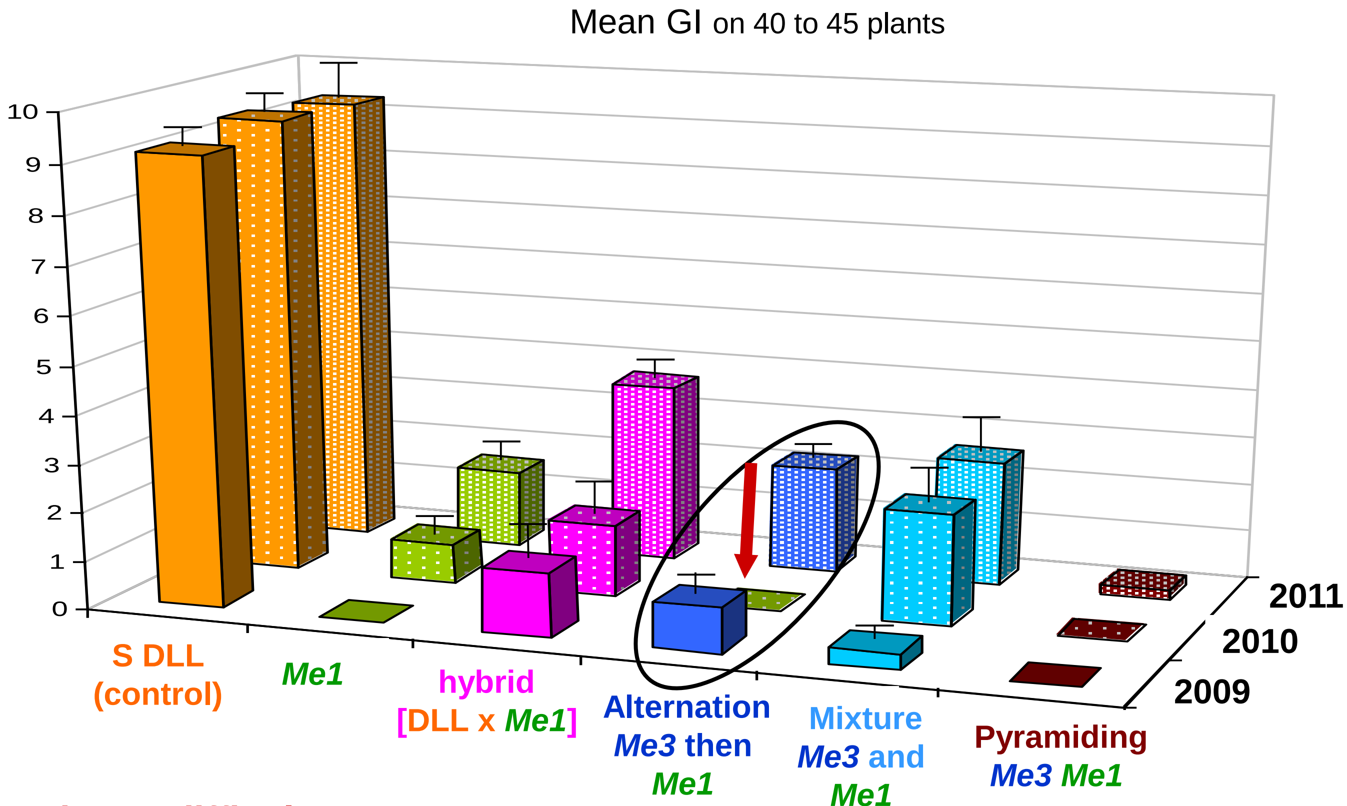
**GI = gall index on peppers** ***after 5 months of culture in summer***




- ***Me1*** **robust : difficult to overcome**
- **F1 hybrid (*Me1* in S background) less R than *Me1 R*-parent**

# Example of an experiment in natural condition

**GI = gall index on peppers** ***after 5 months of culture in summer***

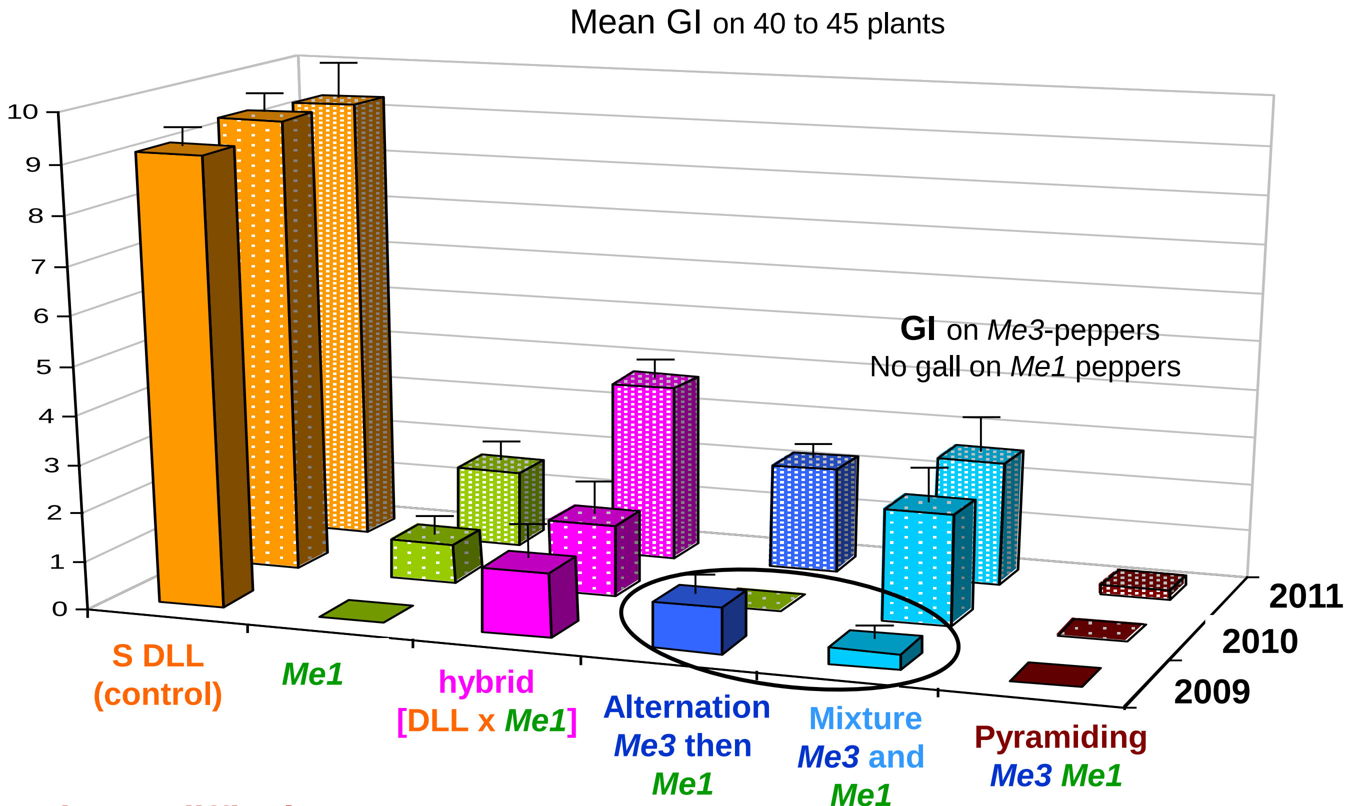



- ***Me1*** **robust : difficult to overcome**
- **F1 hybrid (*Me1* in S background) less R than *Me1 R*-parent**
- ***Me3*** **overcome   but specificity of virulence confirmed : alternation *Me3* with *Me1* interesting**

**Djian-Caporalino *et al., EJPP 2011***

# Example of an experiment in natural condition

**GI = gall index on peppers** ***after 5 months of culture in summer***

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- ***Me3 R*****-peppers seem protected by *Me1 R*-peppers**

# Example of an experiment in natural condition

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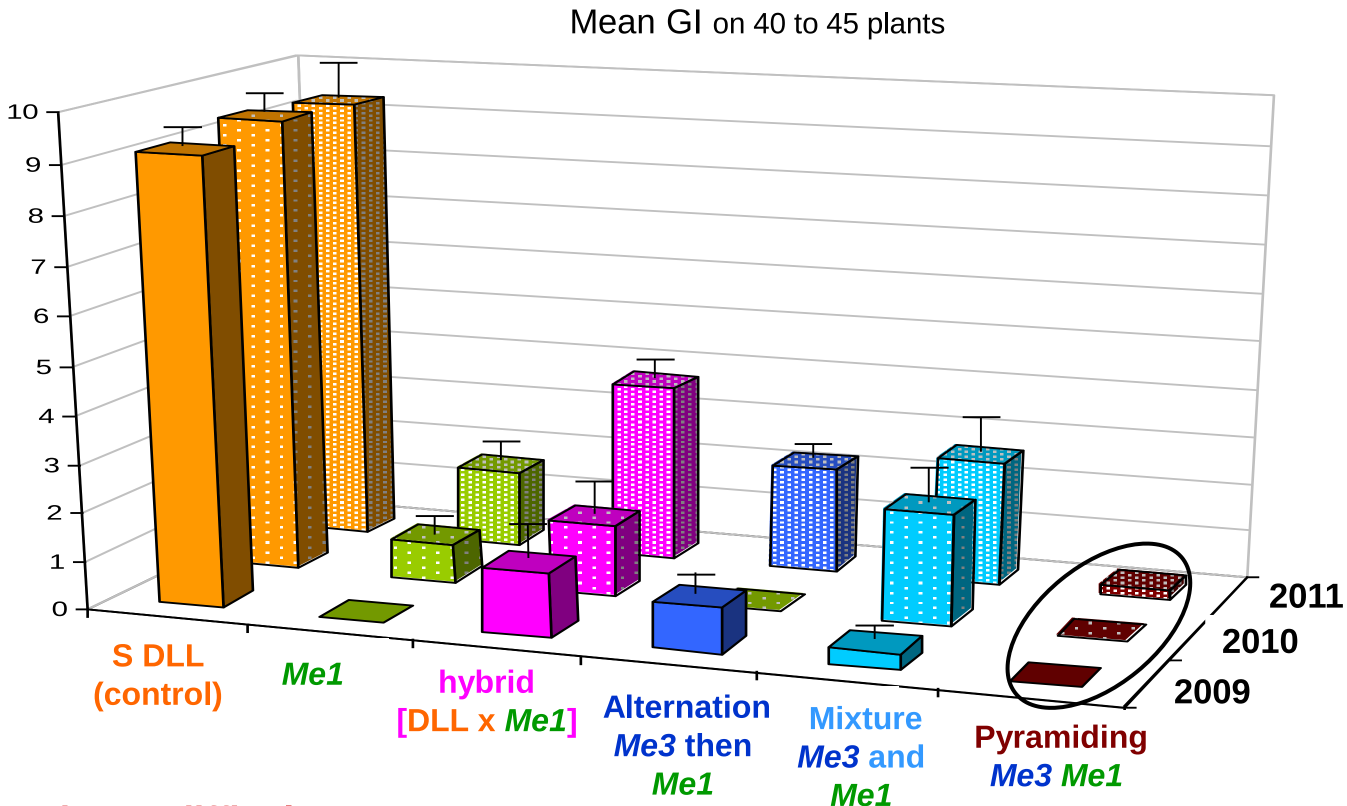


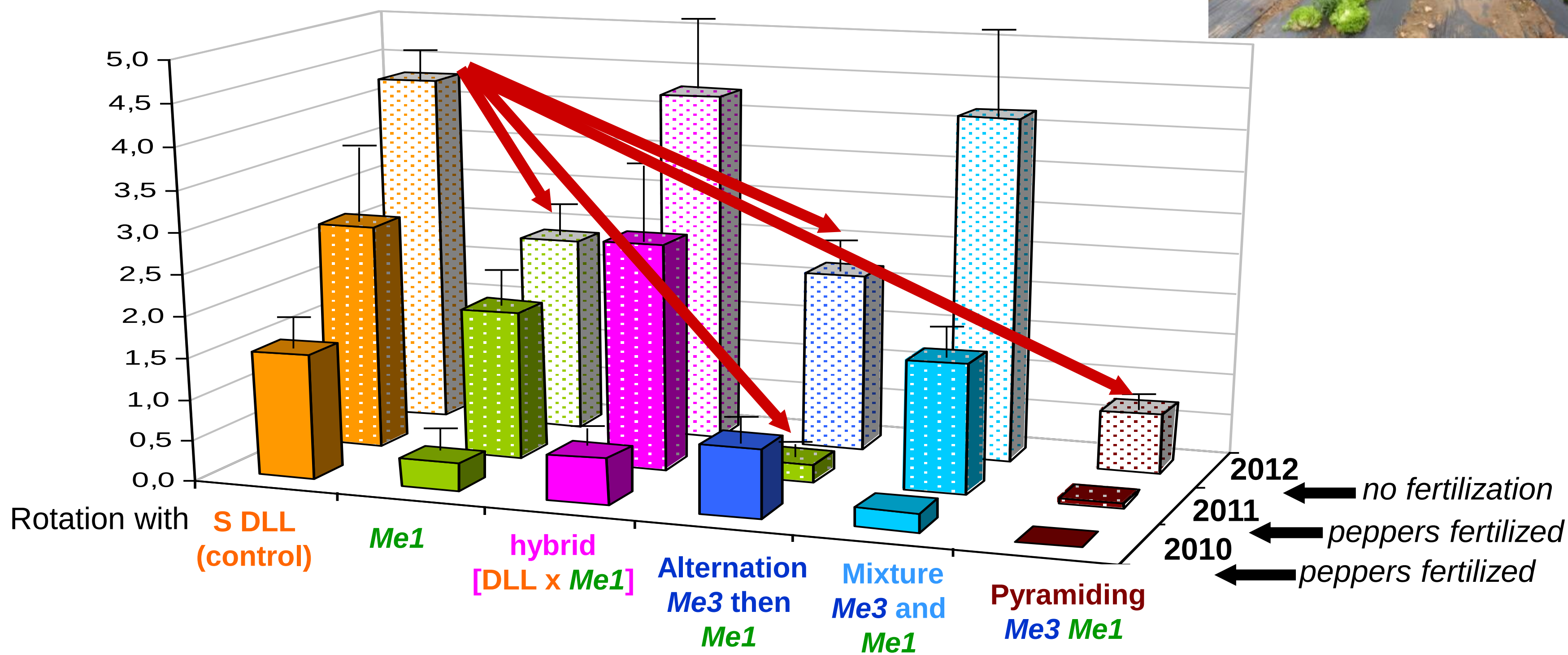

- ***Me1*** **robust : difficult to overcome**
- **F1 hybrid (*Me1* in S background) less R than *Me1 R*-parent**
- ***Me3*** **overcome but specificity of virulence confirmed : alternation with *Me1* interesting**
- ***Me3 R*****-peppers seem protected by *Me1 R*-peppers**
- ***Me3Me1 R*****-peppers not infested : the best modality**

# Example of an experiment in natural condition

**GI = gall index on salads**

***after 3 months of culture in winter***

Mean GI on 40 to 45 plants




**Some *R*-peppers modalities protected the salads, significantly**

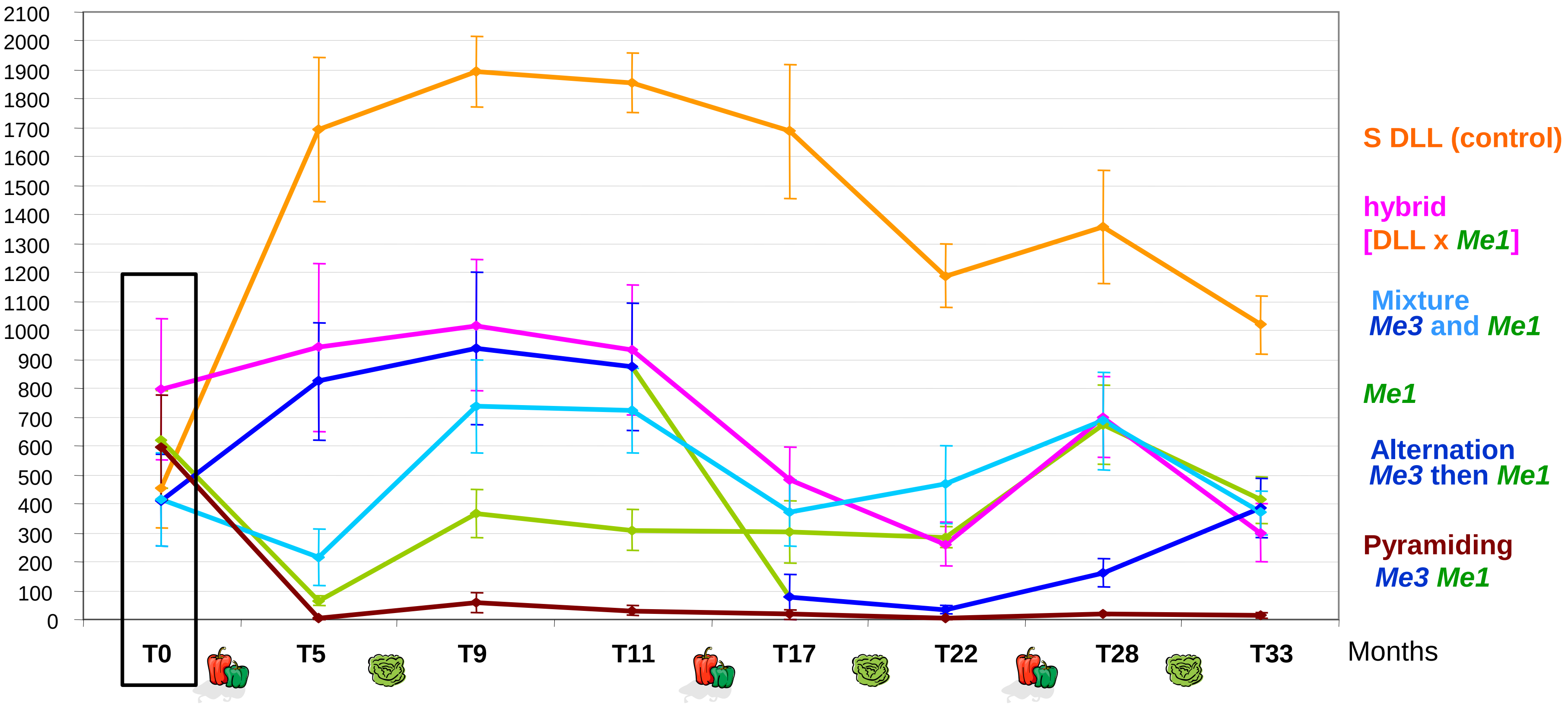
***Me3Me1 R*-peppers gave the best protection to the salads in the rotation**

*note : the third year, the peppers were not fertilized by the grower => they were not sufficiently developped to trap a lot of nematodes*

# Example of an experiment in natural condition

**SIP = soil infection potential**

Egg-masses on S-tomatoes inoculated with 1kg of soil from each µplot (IC5% on 8 to 9 replicates)

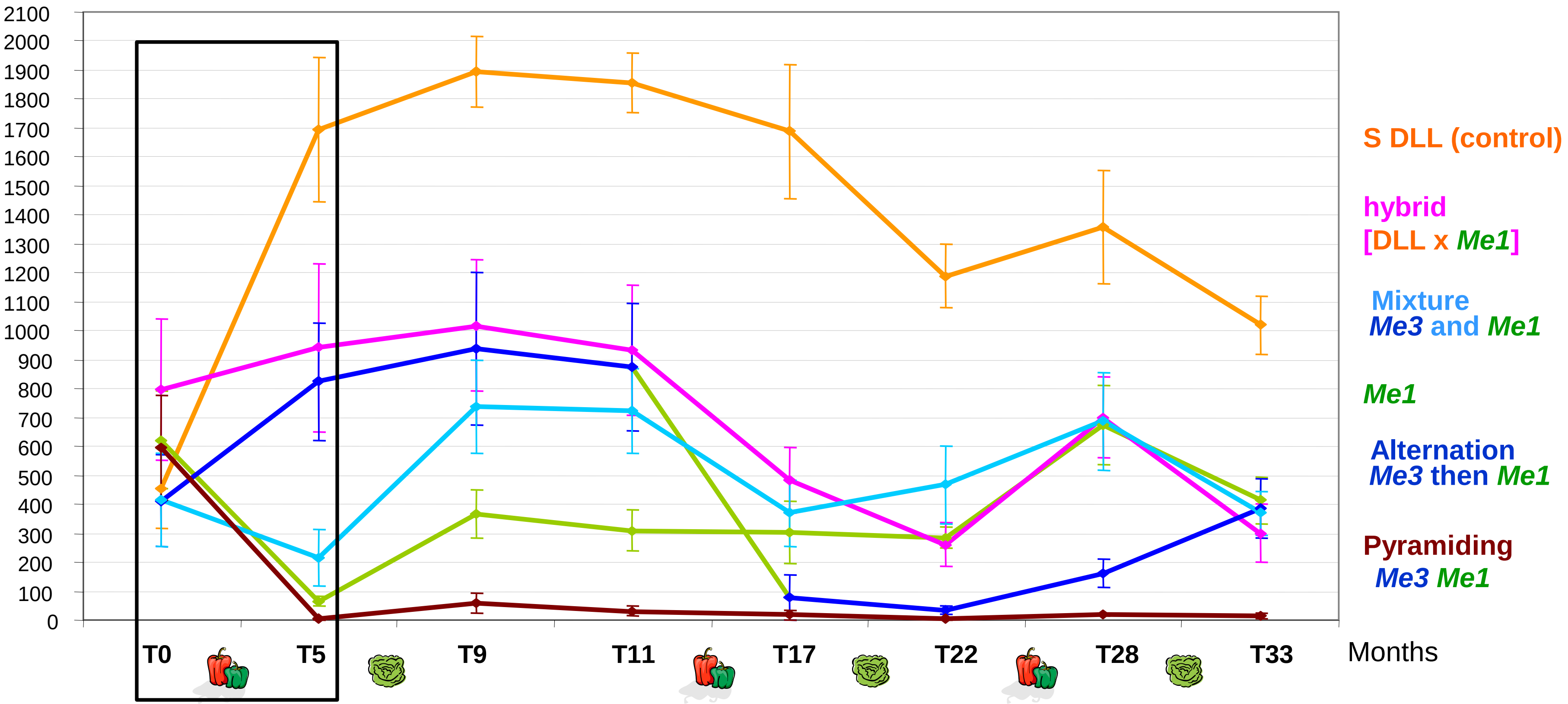



**Before peppers : SIP was high and homogenous in each microplot**

# Example of an experiment in natural condition

## SIP = soil infection potential

Egg-masses on S-tomatoes inoculated with 1kg of soil from each µplot (IC5% on 8 to 9 replicates)

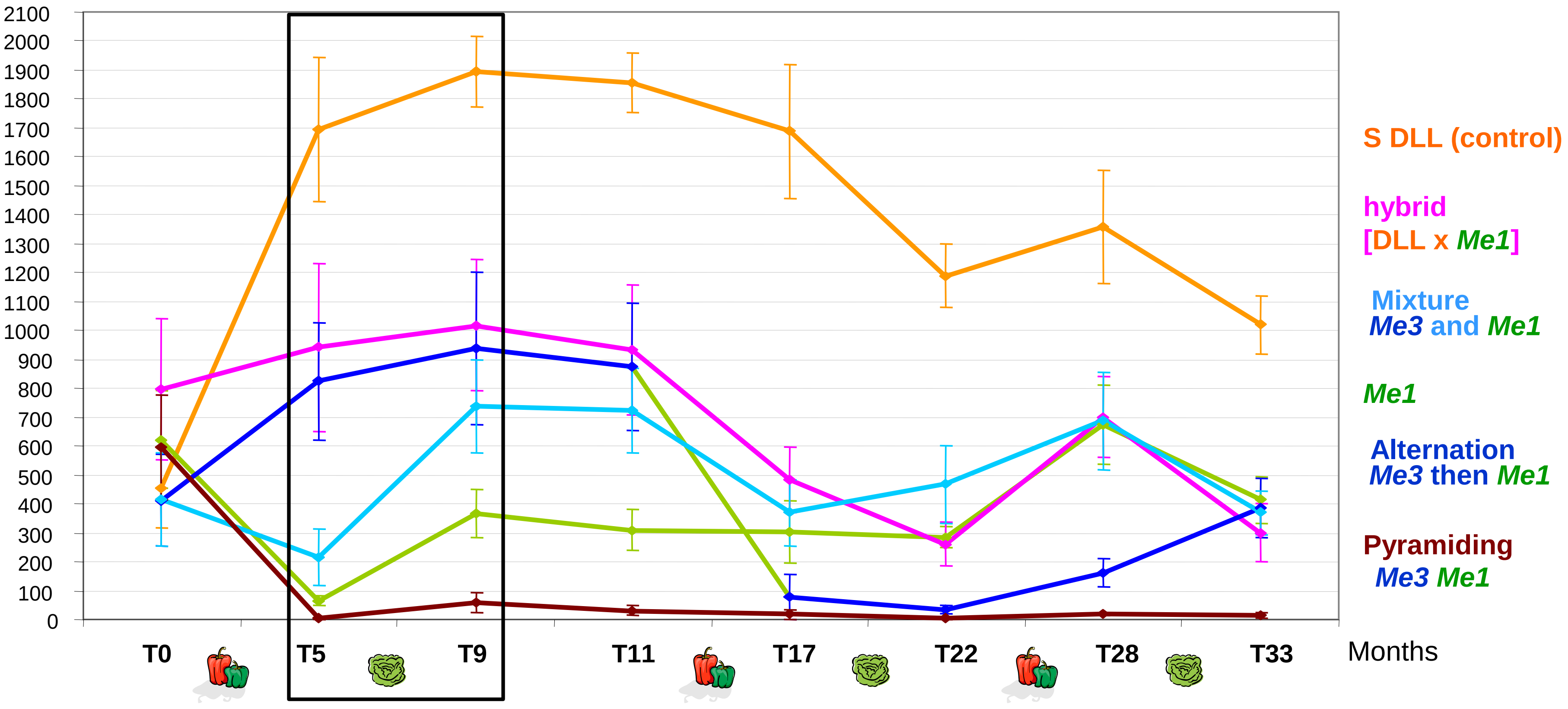



- **S DLL strongly increased the SIP ; no significant for hybrid [DLL x *Me1*] and *Me3***
- **Mixture *Me3 and Me1* reduced the SIP**
- ***Me1* and particularly the pyramided *Me3Me1* peppers strongly reduced the SIP: >90% !**

# Example of an experiment in natural condition

## SIP = soil infection potential

Egg-masses on S-tomatoes inoculated with 1kg of soil from each µplot (IC5% on 8 to 9 replicates)

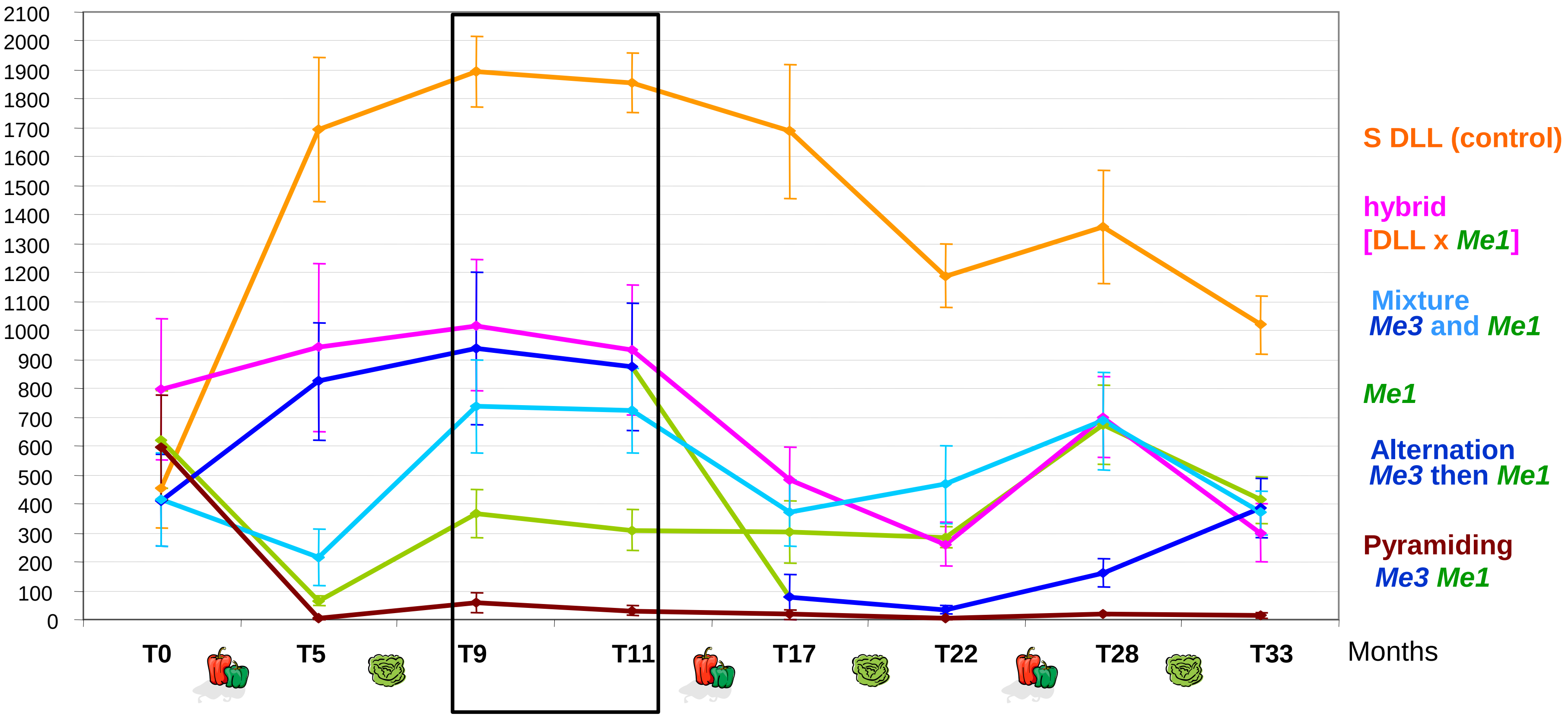



***S*-salads allowed the multiplication of nematodes in each microplot**

# Example of an experiment in natural condition

## SIP = soil infection potential

Egg-masses on S-tomatoes inoculated with 1kg of soil from each µplot (IC5% on 8 to 9 replicates)




**After 2 months without any culture, no significant evolution of SIP**

# Example of an experiment in natural condition

## SIP = soil infection potential

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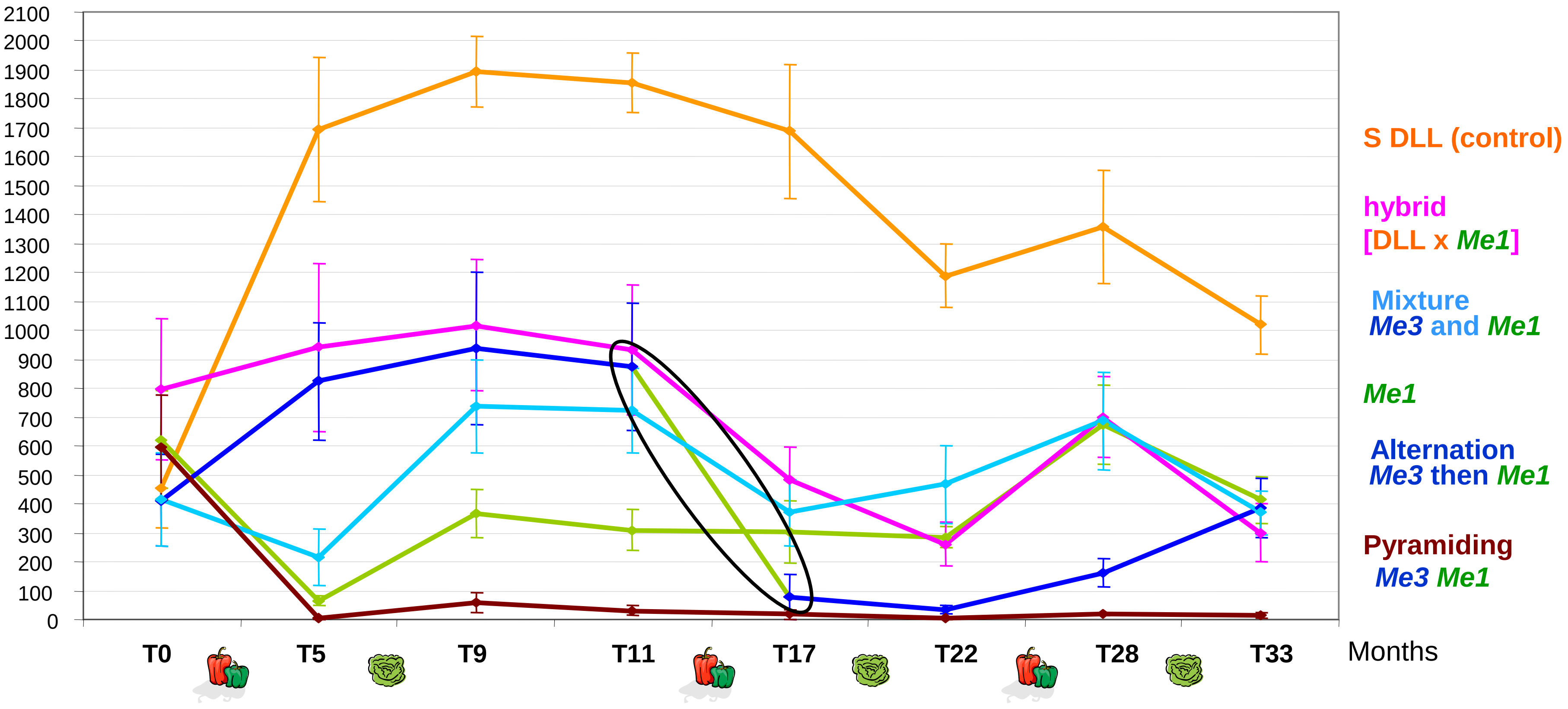


**Alternating *R*-genes in rotation efficient to decrease virulent populations in the field** *(specificity of virulence)*

# Example of an experiment in natural condition

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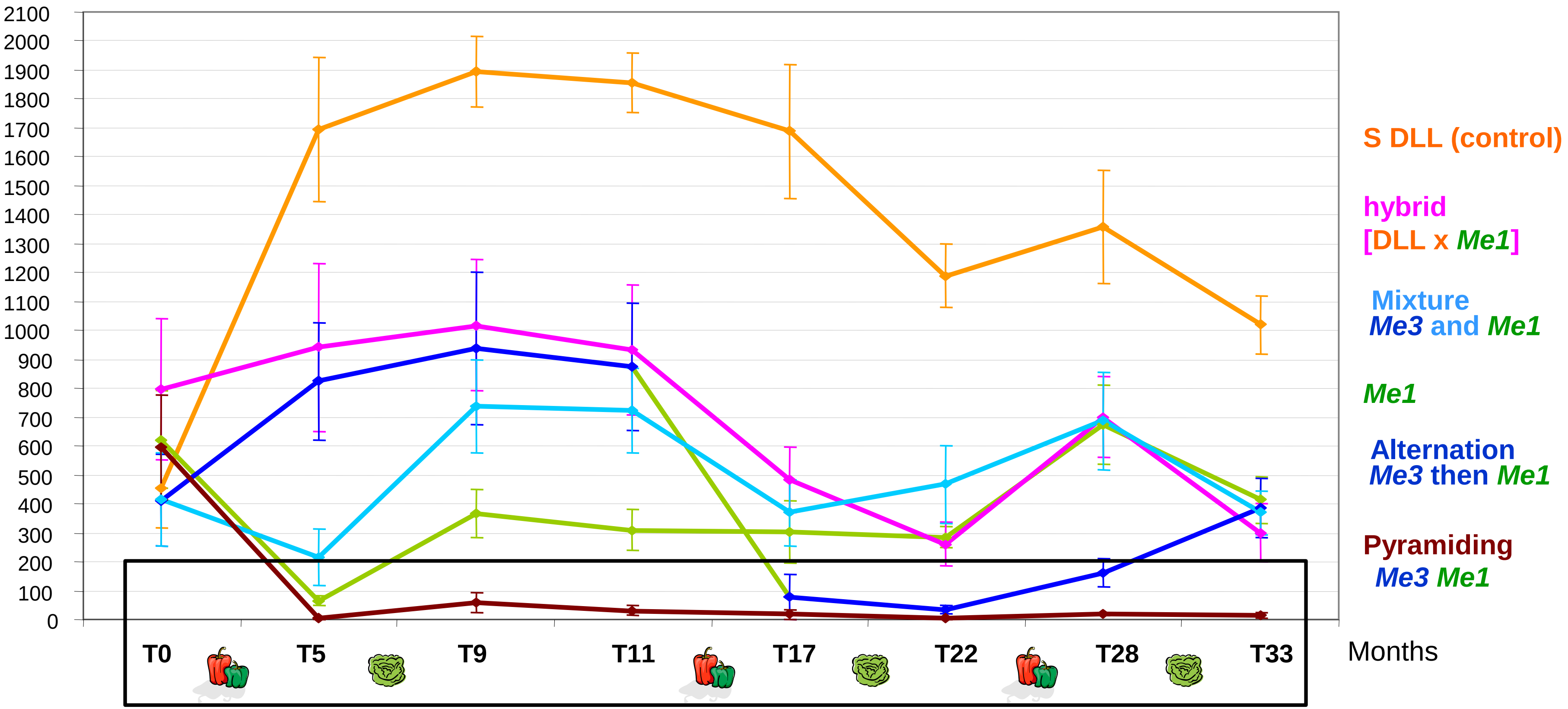



**Alternating *R*-genes in rotation efficient to decrease virulent populations in the field** *(specificity of virulence)*

**Pyramiding *R*-genes in one pepper genotype : best modality as trap crop and to suppress the emergence of virulent isolates**

# Conclusions

## Strategies to strengthen and increase the *R* durability

### At the plant level *(plant breeders)*

- **Choice of the *R*-genes** (the more robust, linked to the *R*-mechanism)
- **Choice of the genetic background** (in which the *R*-gene is introgressed)
- **Combination of *R*-genes** (pyramiding)

⇨ *To prevent the selection of virulent nematodes*

### At the field and rotation level *(farmers)*

- **Diversification of *R*-plants** (alternating *R*-genes)

⇨ *To reduce the selection pressure of R-genes on the pathogens*

- **Use *R*-genes pyramiding with a good fertilization** (increase their "trap" effect)

⇨ *To decrease the amount of pathogens*

in good agreement with concepts recently developed for pepper-virus, rapeseed-fungus, rice-bacteria

**Palloix *et al., New Phytol* 2009, Brun *et al., New Phytol* 2010 Yoshimura *et al. Mol Breeding* 1995 ; Hittalmani *et al. Theor Appl Genet* 2000; Singh *et al., Theor Appl Genet* 2001**

# Perspectives

**The GEDUNEM project** : Varietal and technical innovations for the sustainable and integrated management of RKN in protected vegetable cropping systems.

INRA **metaprogramme SMaCH (Sustainable Management of Crop Health)**

*Action PRESUME (Plant REsistance SUstainable ManagEment) 02/2012*

*Gedunem labelled by*

PEIFL *07/2011* PICLég *09/2012*

**Combination of *R*-plants and cropping techniques** : intercultural management (green manure, prophylactic treatments), biological control, multicrop rotations with bad host plants, and *R*-plants (alternance *Mi*-tomatoes, *Me3*-peppers)



***Thurs, Oct 18, 2nd Poster Session***

**Analysis of partial resistance factors (QTL**, quantitative trait locus) that could explain the effect of the genetic background on major *R*-genes

Cifre Conventions ANRT PhD

**PhD Arnaud Barbary 01/04/2011-2014**

RIJK ZWAAN · GAUTIER semences · syngenta · TAKII · Vilmorin · CLAUSE · SAKATA

***Thurs, Oct 18, 2nd Poster Session***

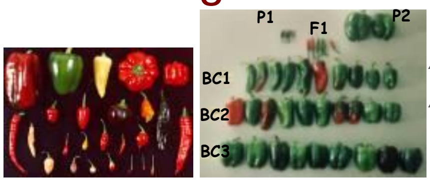
*The plant genetic background plays an important role on durability of major R-genes to nematodes*

# Collaborative network

- **INRA** Sophia Antipolis (SE France)


*Philippe Castagnone-Sereno*
*Caroline Djian-Caporalino*
*Ariane Fazari (techn)*
*Nathalie Marteu (techn)*
*Arnaud Barbary (PhD)*
*Delphine Angella (CDD)*

- **INRA** Avignon and Montpellier (SE & SW France)

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*Marc Tchamitchian*
*Mireille Navarrete*
*Mathilde Chapuis (student)*
*Amélie Lefevre*
*Laure Pares (techn)*

- **IRD** Montpellier (SW France)

*Thierry Mateille*
*Johannes Tavoillot (techn)*

- **Farmers' associations and technical centres** (SE France)

CETA du Pays d'Aubagne — Agricultures & Territoires, Chambres d'Agriculture — APREL — Ctifl

- **Research Group in Organic Farming** (SE France)

GRAB — Groupe de Recherche en Agriculture Biologique

- **AZURA group,** Maraissa company (Agadir, Morocco)
  Vegetable producer and experimental station for integrated management

Azura — Exigeants pour vous, Exigeants pour nous

- **Private breeding companies** (Syngenta, Vco, Gautier, Takii, Sakata, Rijkzwaan)

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# *Thank you for your attention*

***Financial support :***

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