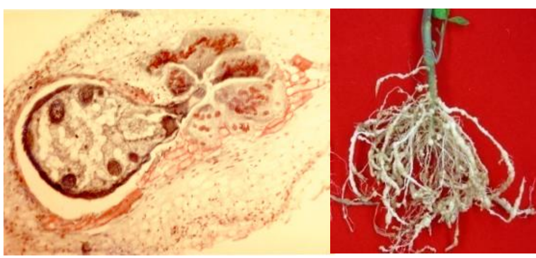
19-21 March 2013 | PalaCongressi - Riva del Garda - Italy

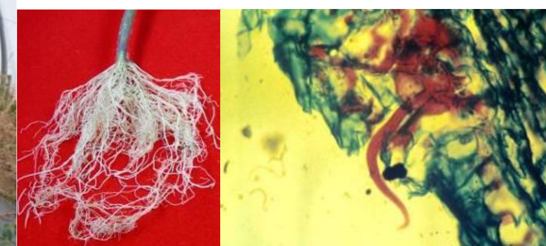
Parallel sessions TOOLS
Session - BREEDING FOR RESISTANCE

Future IPM in Europe

New resistance-genes deployment strategies as non chemical alternatives for the durable management of root-knot nematodes in vegetable crops rotation



Susceptible plant



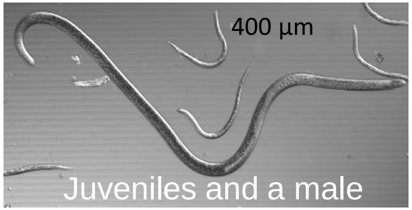
Resistant plant

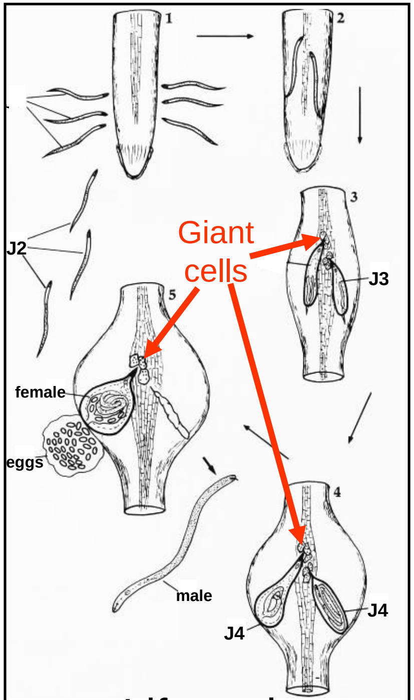
INRA PACA
Plant Nematodes Interaction team
Institut Sophia Agrobiotech

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.

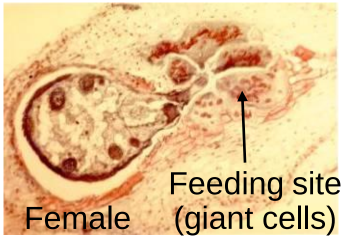
- Microscopic soil borne roundworms (0,2 to 2 mm), obligate sedentary endoparasites





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

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



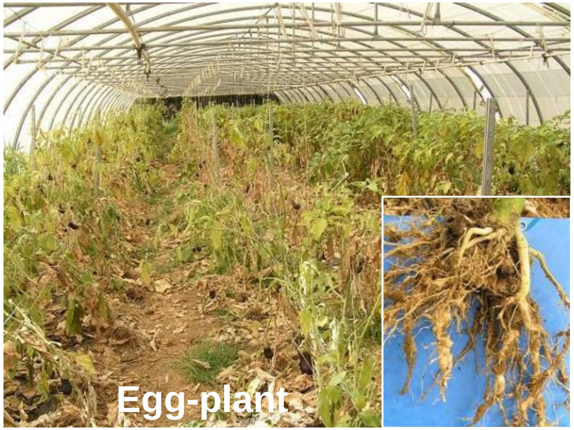
- Extremely polyphagous (> 5,500 host plants)

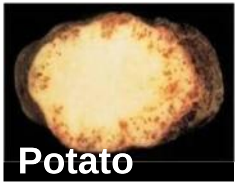
- ~10% of crop losses worldwide *Trudgill & Blok, Annual Review of Phytopathology, 2001*
  (Some species subjected to quarantine regulations in the EU)

- Chemical nematicides *prohibited or restricted* *(Plan Ecophyto 2018)*
  Fumigants : methyl bromide, dichloropropene
  Systemics : e.g. aldicarbe LD_{50}=1ppm

Root-knot nematodes *Meloidogyne* spp.

An increasing problem on vegetable crops in Europe* and 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**

Wesemael et al., Nematology 2011 **Djian-Caporalino, *Phytoma November 2010 & EPPO Bulletin April 2012*

Limitation of the RKN-resistance

Few R-genes identified and breeding for R is long (7-10 years)

In controlled conditions with high pressure of RKN

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

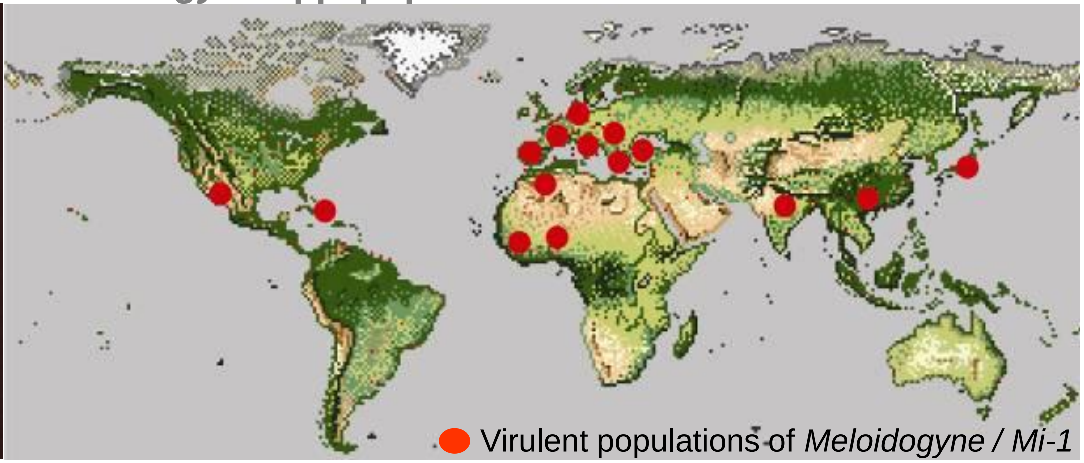
e.g. Jarquin-Barberena *et al. 1991;* Castagnone-Sereno *et al. 1994, 1996, 2001;* Meher *et al. 2009;* Djian-Caporalino *et al., 2011*

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



- **Development of new « robust » *R*-lines**
- **Management of *R*-genes to increase their durability**

Model to study the durability of resistance to RKN

Capsicum annuum

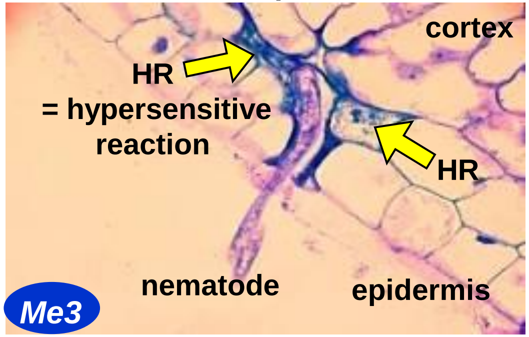
Me1 to Me7, Mech1, Mech2, N

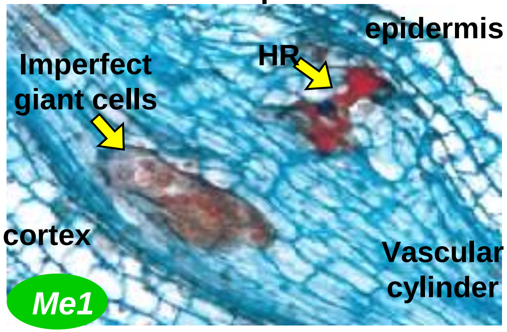
M. incognita
M. arenaria
M. javanica

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*

Results in controlled conditions

French agriculture ministery and Permanent Technical Committee of the Selection of the crop plants, 2007-2010

Europeen network for durable exploitation of crop protection strategies, 2008-2009

. **Choice of the genes** (mode of action)

. **Varietal effect** (genetic background)

. **Combination of genes** (pyramiding)

strongly affected:

➡ the efficiency of the **R** in reducing the reproductive potential of RKNs (total or partial R)

➡ the **durability of R** in preventing the selection of virulent nematodes or lowering the frequency of resistance breakdown

Djian-Caporalino et al., EJPP 2011 ; Barbary et al., submitted to Theor Appl Genet

Experimental approach in natural conditions

Neoleg

INRA PICLeg network, Integrated production of vegetable crops 2008-2012

Sysbiotel

French National Research Agency, project on Ecosystems, living resources, landscapes and agriculture 2009-2013

alcotra VALORT

EU Interreg Alcotra project, 2010-2012

3-years greenhouse and field experiments *(in experimental stations and farms)*

➡ **Validation** with natural nematode populations in protected crop systems

➡ **Development of *R*-plants management strategies 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.


Nice (SE France)


Aubagne (SE France)

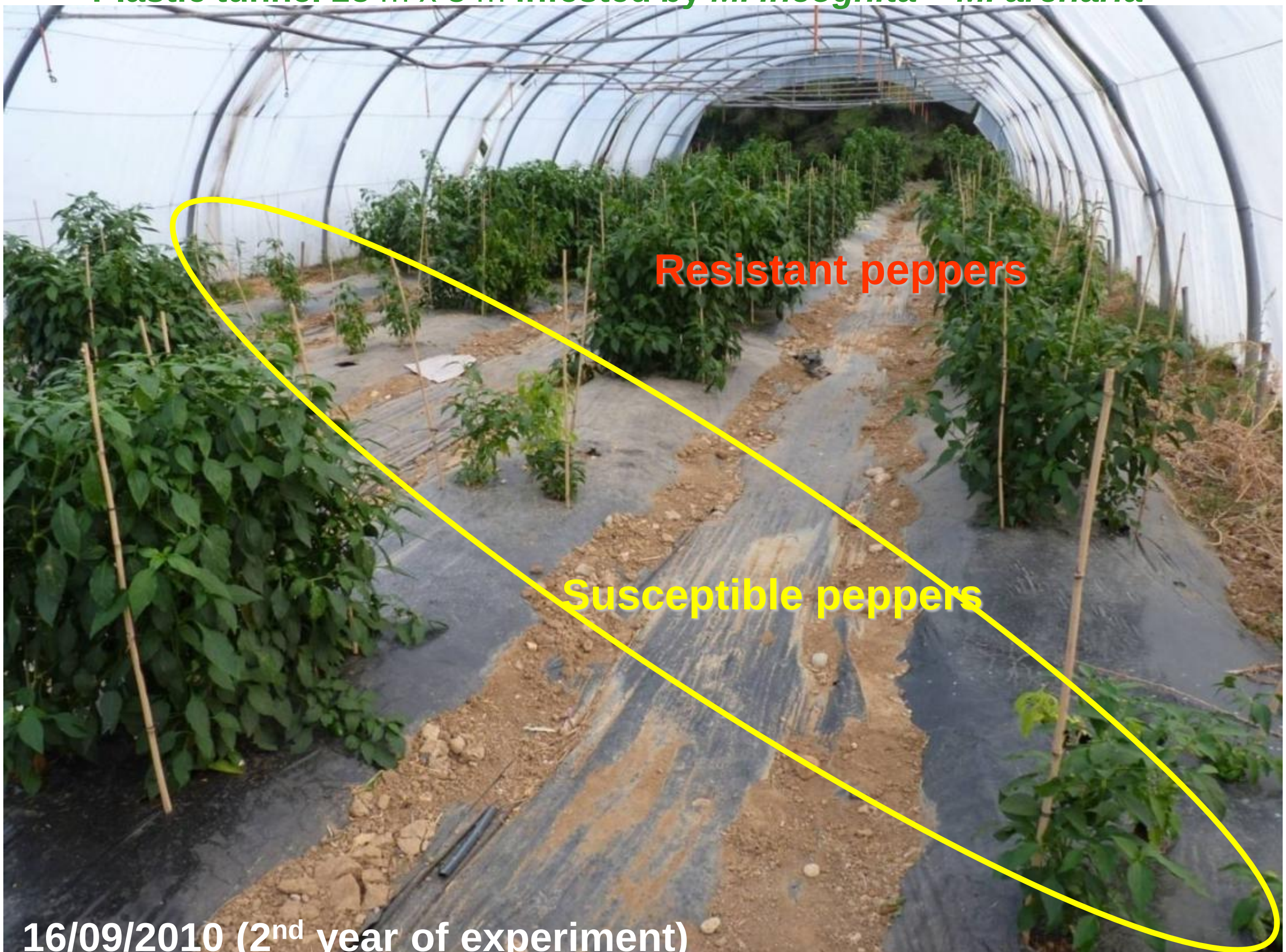
Agadir (Morocco)

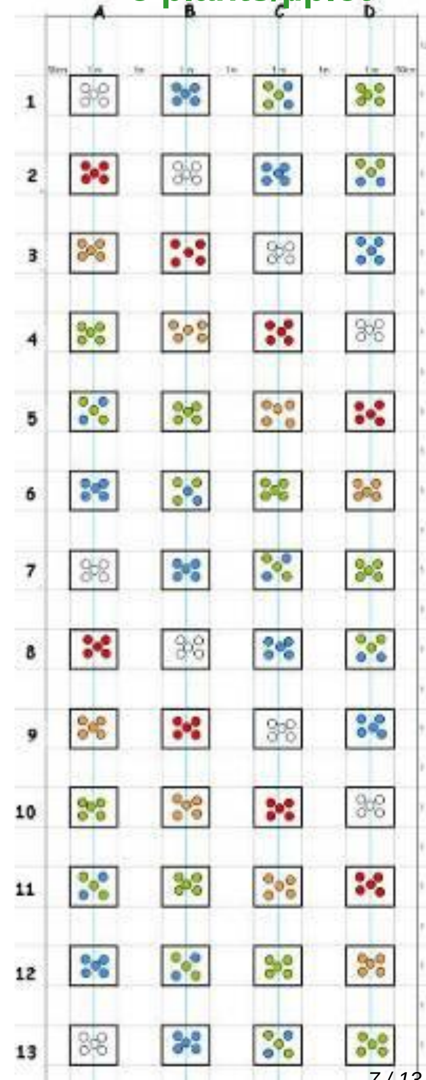
Experimental approach in natural conditions

Exemple of an experiment in a farm belonging to the Chamber of Agriculture of Alpes-Maritimes (technical institute) in Nice (SE France)

224 m^2, 52 µplots, 5 plants/µplot

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



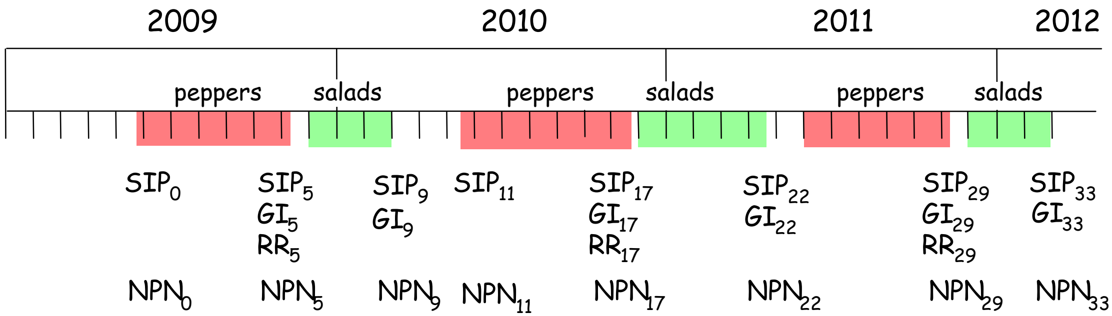


Experimental approach in natural conditions

Material and methods

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

Schedule and Infestation parameters



Susceptible cultivar DLL (control)

R inbred line *Me1*

R hybrid [DLL x *Me1*]

Alternation *Me3* then *Me1*

Mixture *Me3* and *Me1*

Pyramiding *Me3 Me1*

SIP = soil infection potential *(number of Meloidogyne J2 /kg of soil)*

GI = gall index *(on peppers and salads)*

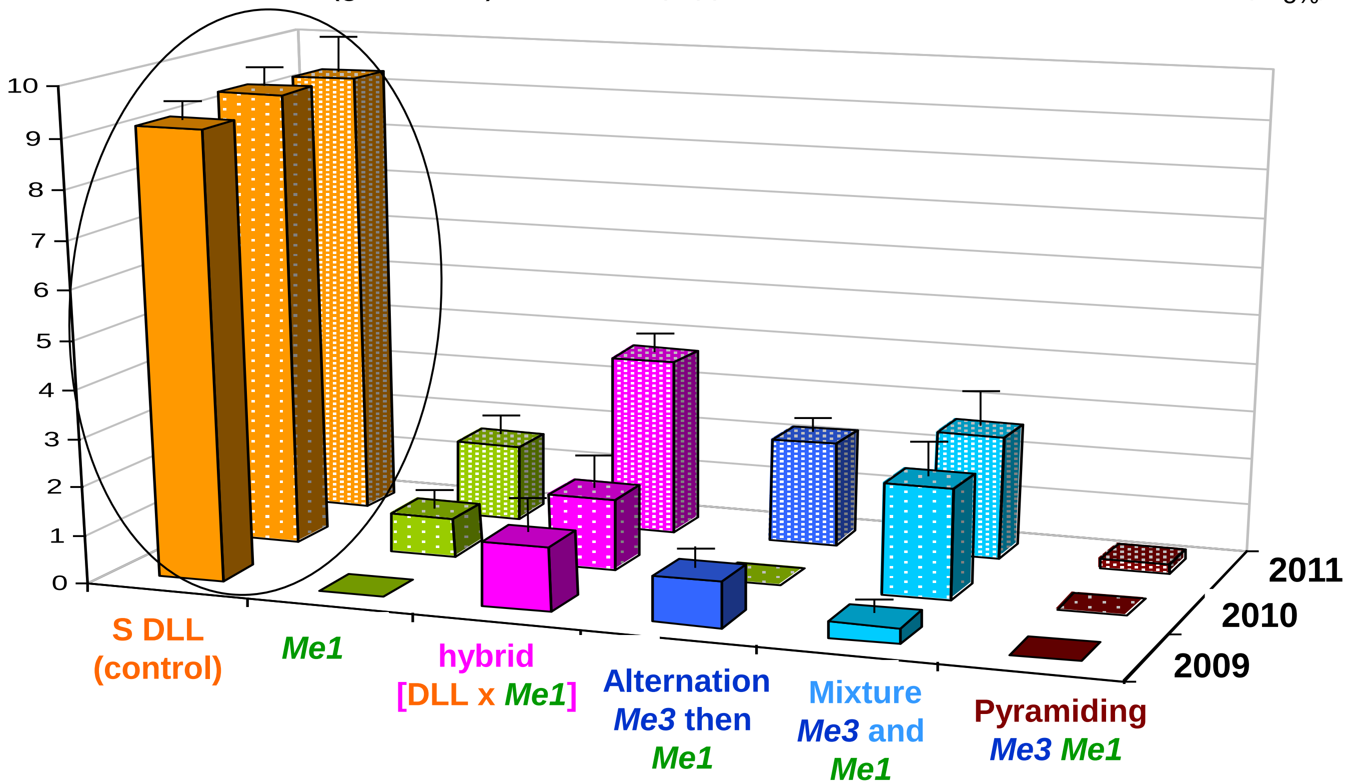
RR = reproduction rate of virulent nematodes if egg masses detected on *R*-peppers
(number of eggs produced /J2 inoculated on R-peppers in controlled conditions)

NPN = soil content with non parasitic nematodes
(identification of species and number of nematodes/dm3 of soil)

Experimental approach in natural conditions

Results on strength and durability of resistances

Mean GI (gall index) on 40 to 45 peppers after 5 months of culture in summer ($IC_{5\%}$)

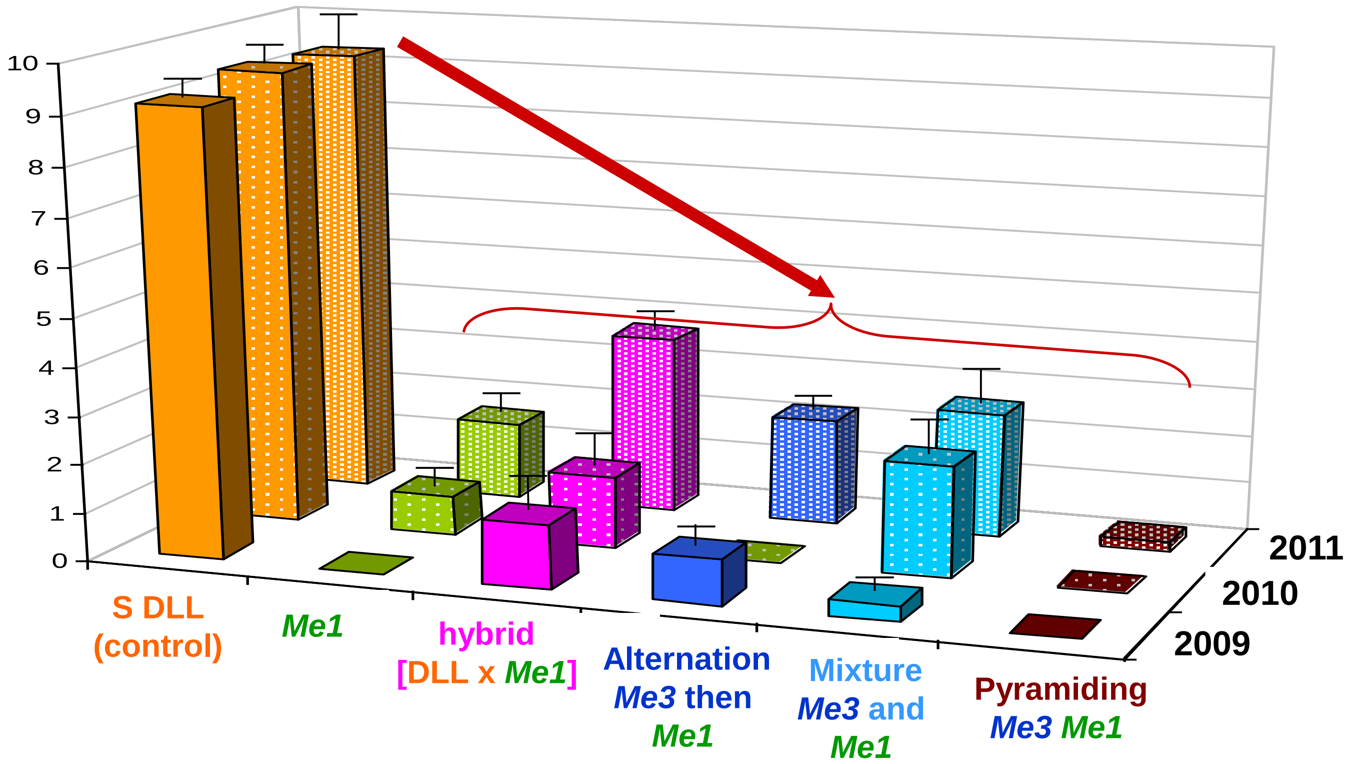


GI on *S*-peppers nearly maximum

Experimental approach in natural conditions

Results on strength and durability of resistances

Mean GI (gall index) on 40 to 45 peppers after 5 months of culture in summer ($IC_{5\%}$)

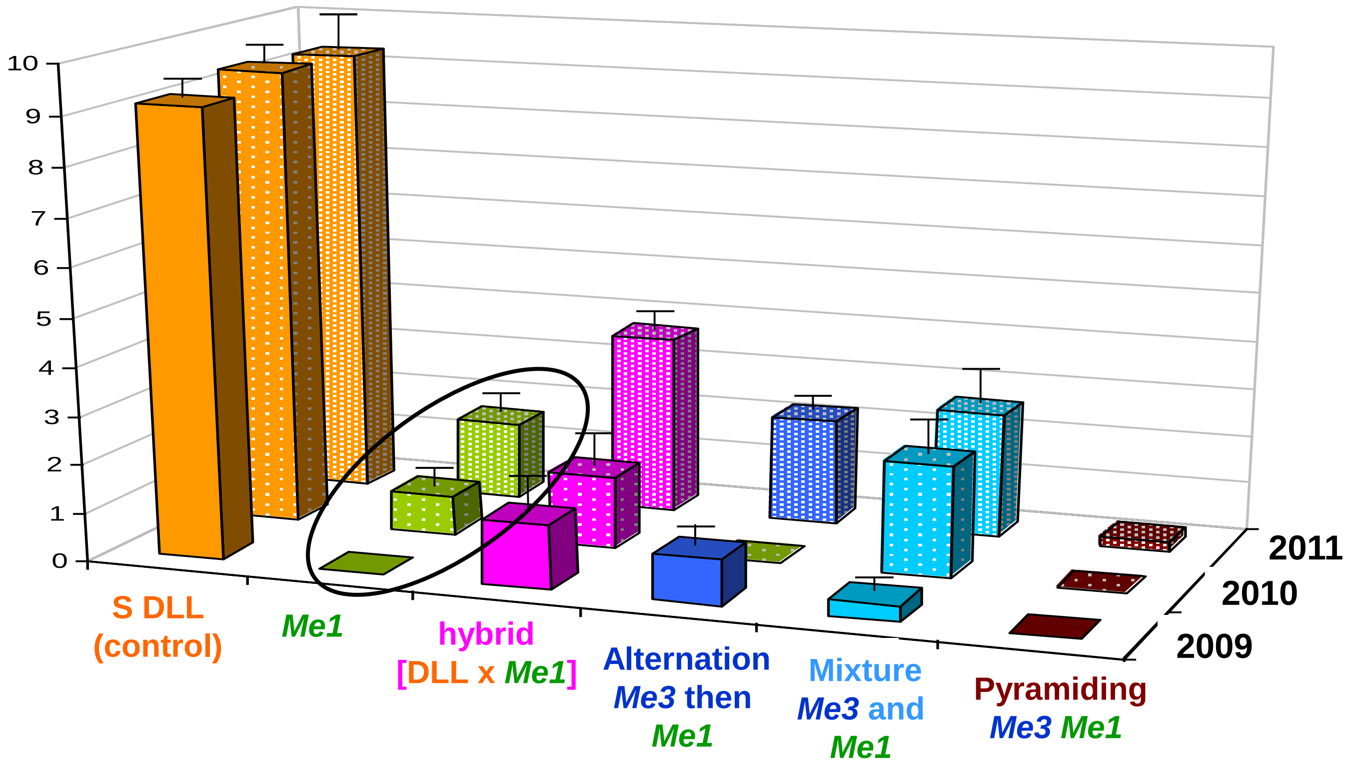


GI on *S*-peppers nearly maximum and very high compared to the *R*-peppers

Experimental approach in natural conditions

Results on strength and durability of resistances

Mean GI (gall index) on 40 to 45 peppers after 5 months of culture in summer ($IC_{5\%}$)

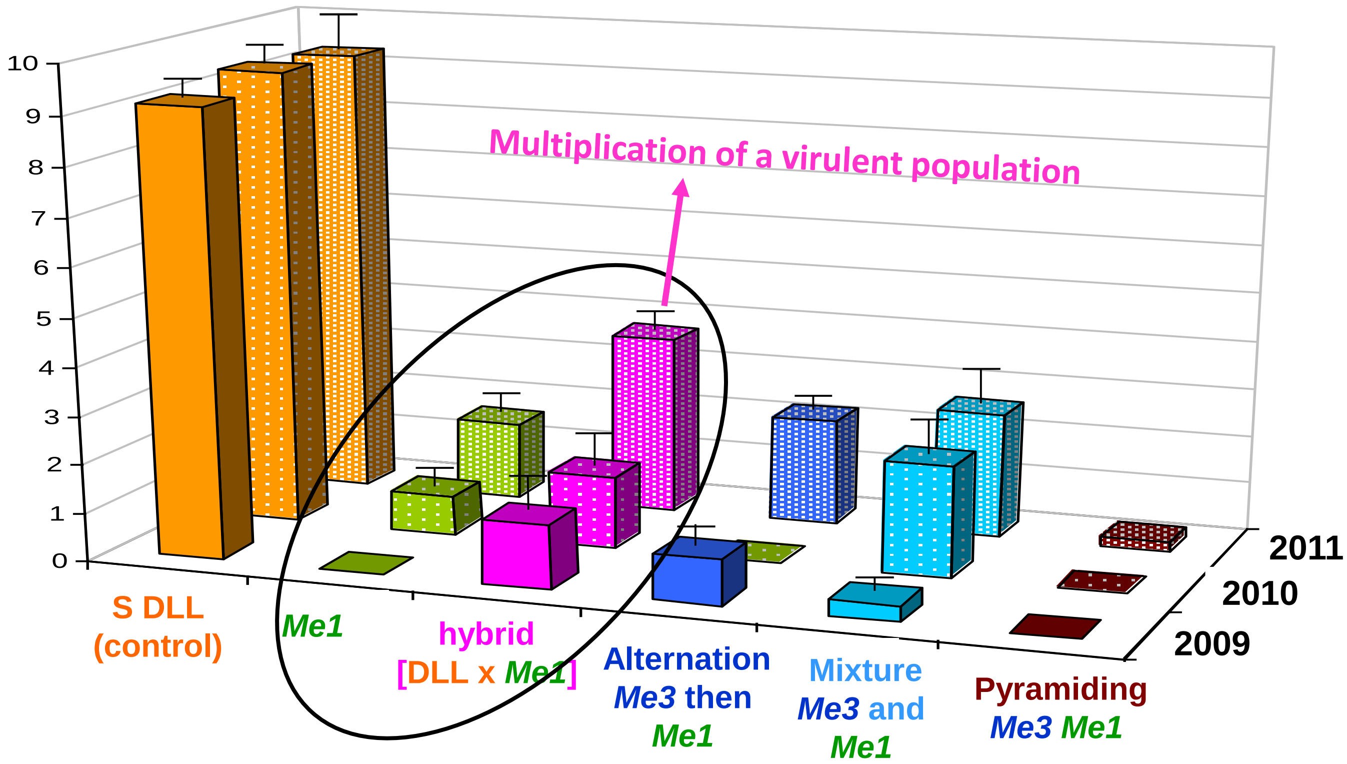


Me1 **robust : difficult to overcome even in natural conditions**

Experimental approach in natural conditions

Results on strength and durability of resistances

Mean GI (gall index) on 40 to 45 peppers after 5 months of culture in summer ($IC_{5\%}$)

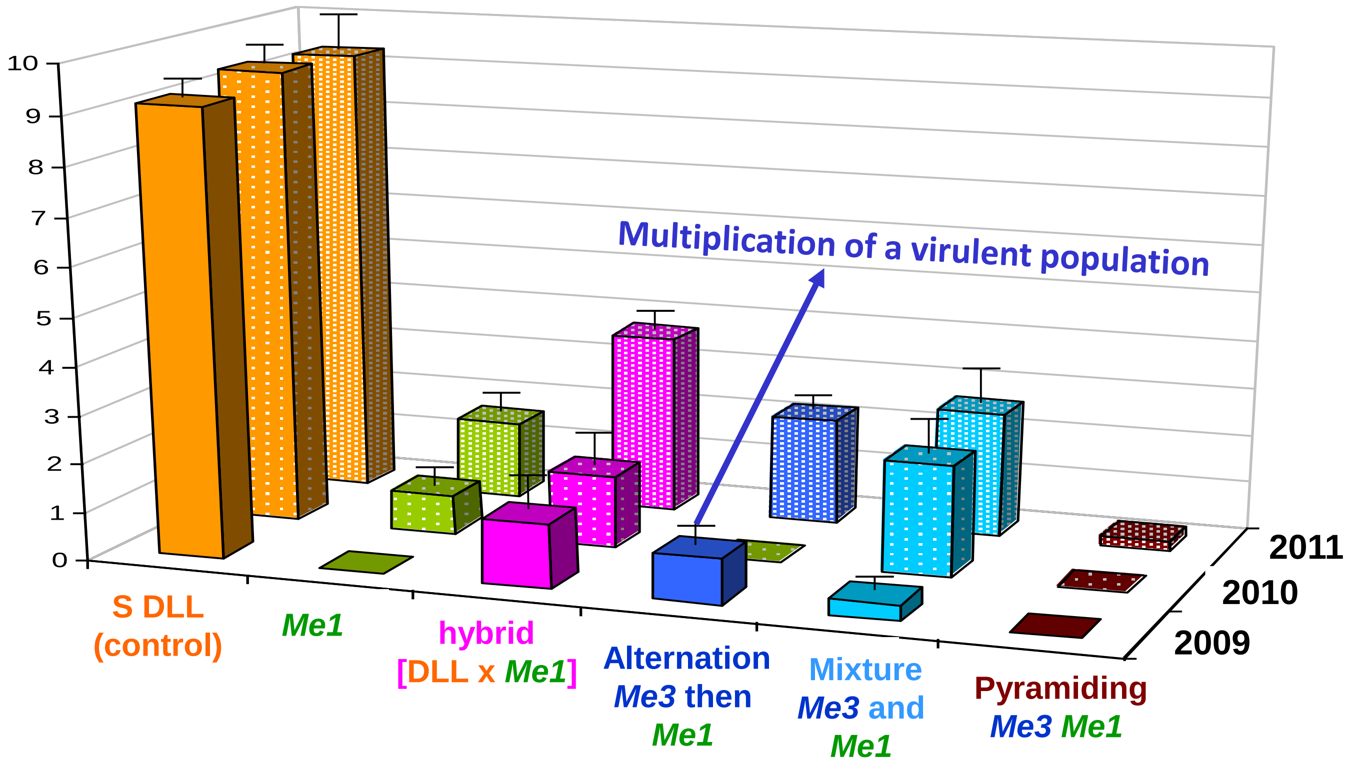


F1 hybrid (*Me1* in S background) less R than *Me1 R*-parent

Experimental approach in natural conditions

Results on strength and durability of resistances

Mean GI (gall index) on 40 to 45 peppers after 5 months of culture in summer ($IC_{5\%}$)

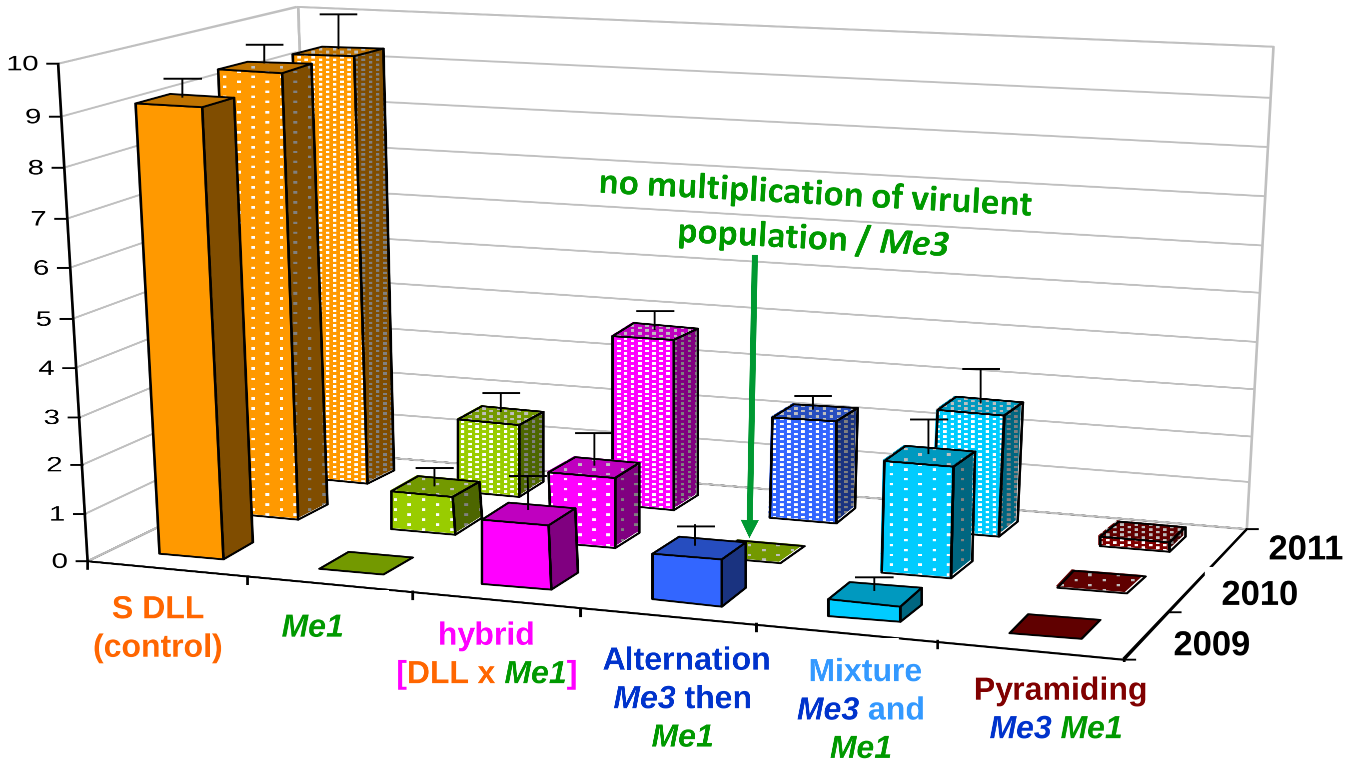


***Me3* overcome the first year**

Experimental approach in natural conditions

Results on strength and durability of resistances

Mean GI (gall index) on 40 to 45 peppers after 5 months of culture in summer ($IC_{5\%}$)



_Me3_ overcome the first year but specificity of virulence confirmed

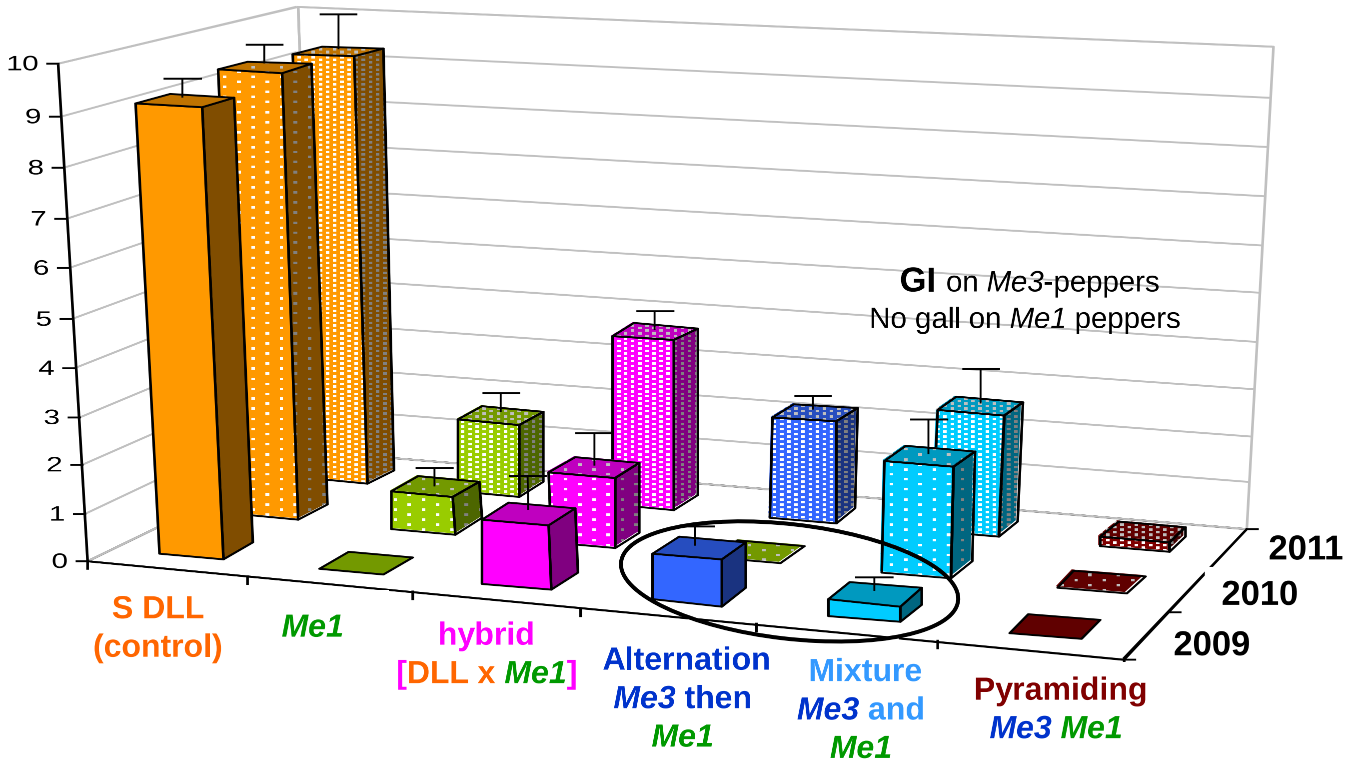
Djian-Caporalino *et al., EJPP 2011*

=> alternation _Me3_ with _Me1_ interesting to stop _Me3_ virulent population (recycling an ineffective R-gene)

Experimental approach in natural conditions

Results on strength and durability of resistances

Mean GI (gall index) on 40 to 45 peppers after 5 months of culture in summer ($IC_{5\%}$)



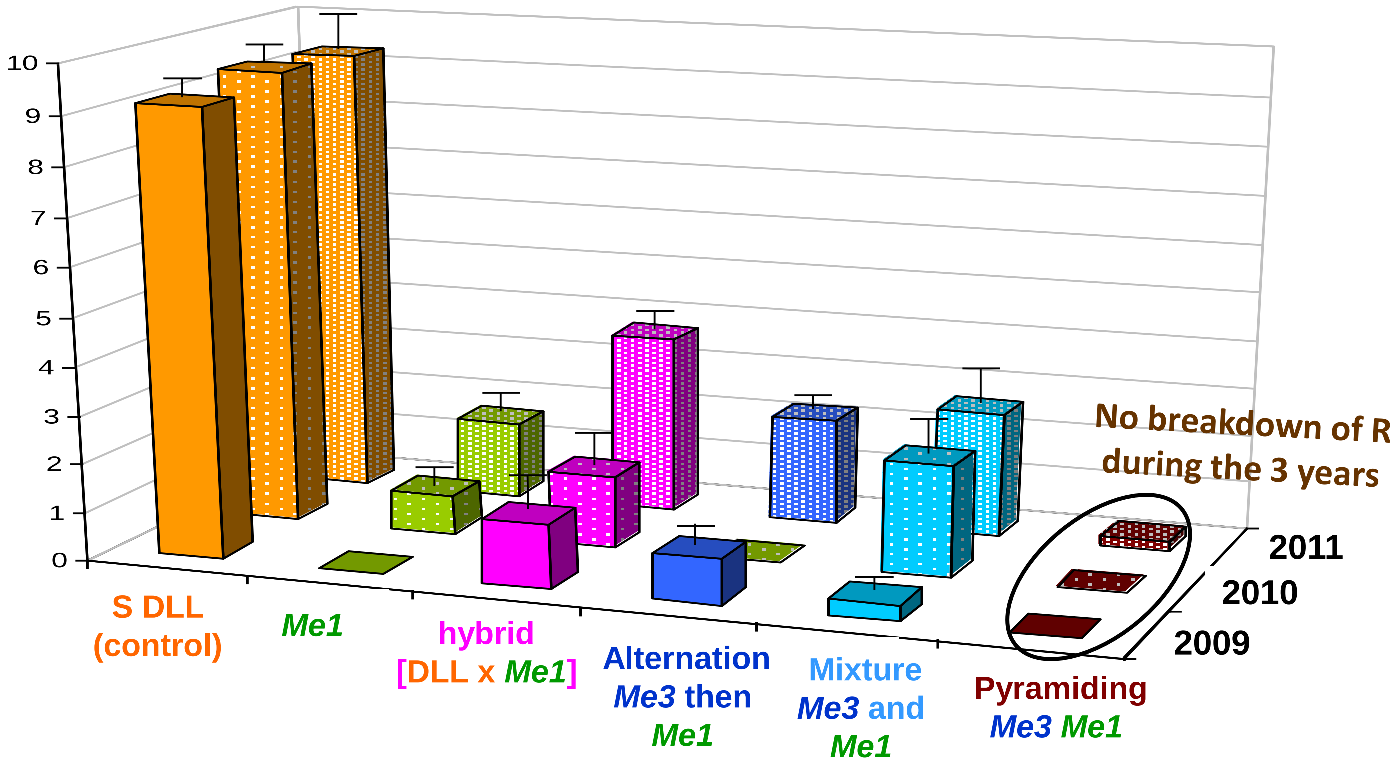
***Me3 R*-peppers seem protected by *Me1 R*-peppers**

Organic amendment the first year => the roots were well developped and intercrossed between *Me1* and *Me3* peppers the first year

Experimental approach in natural conditions

Results on strength and durability of resistances

Mean GI (gall index) on 40 to 45 peppers after 5 months of culture in summer ($IC_{5\%}$)

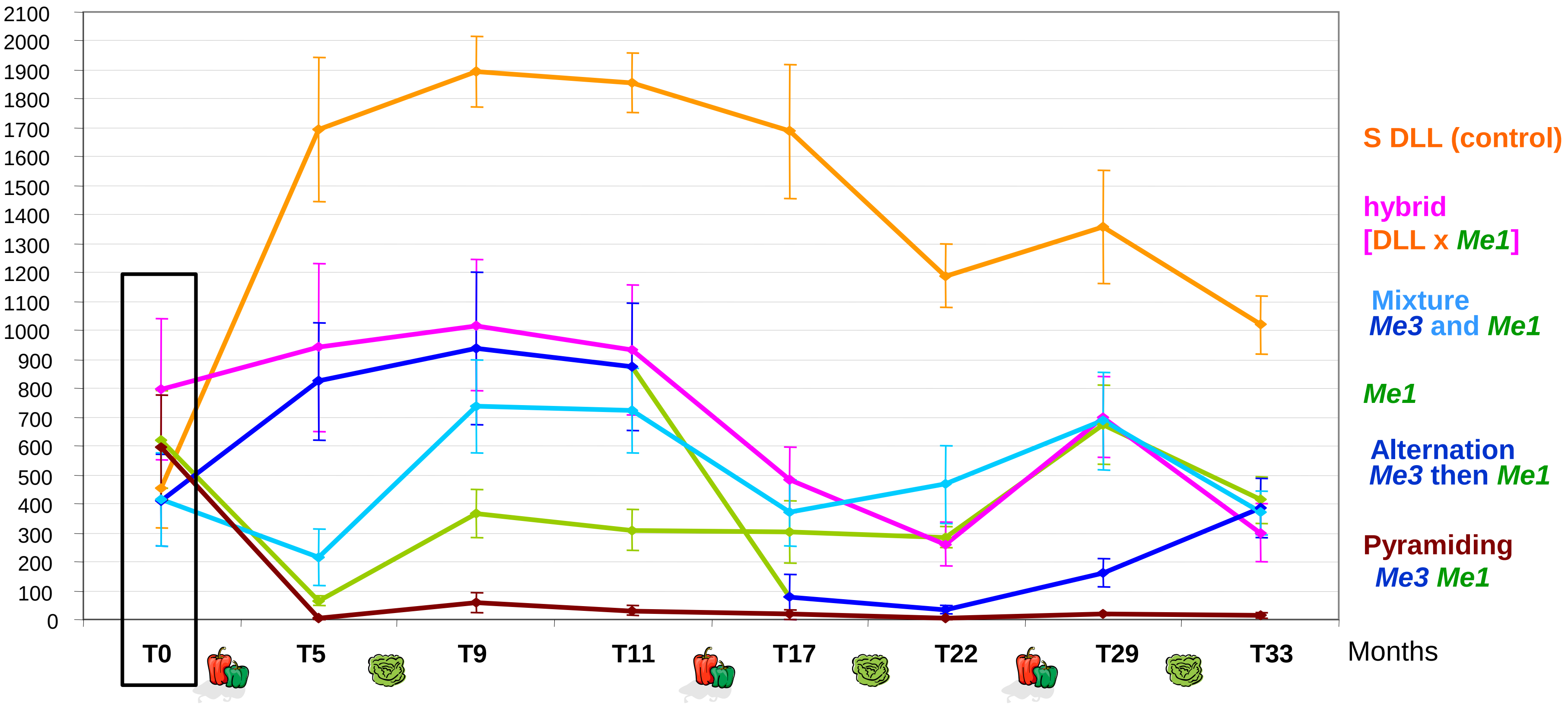


***Me3Me1 R*-peppers never infested : the best modality of deploying the R-genes**

Experimental approach in natural conditions

Results on reduction of the soil infection potential ("trap" effect)

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

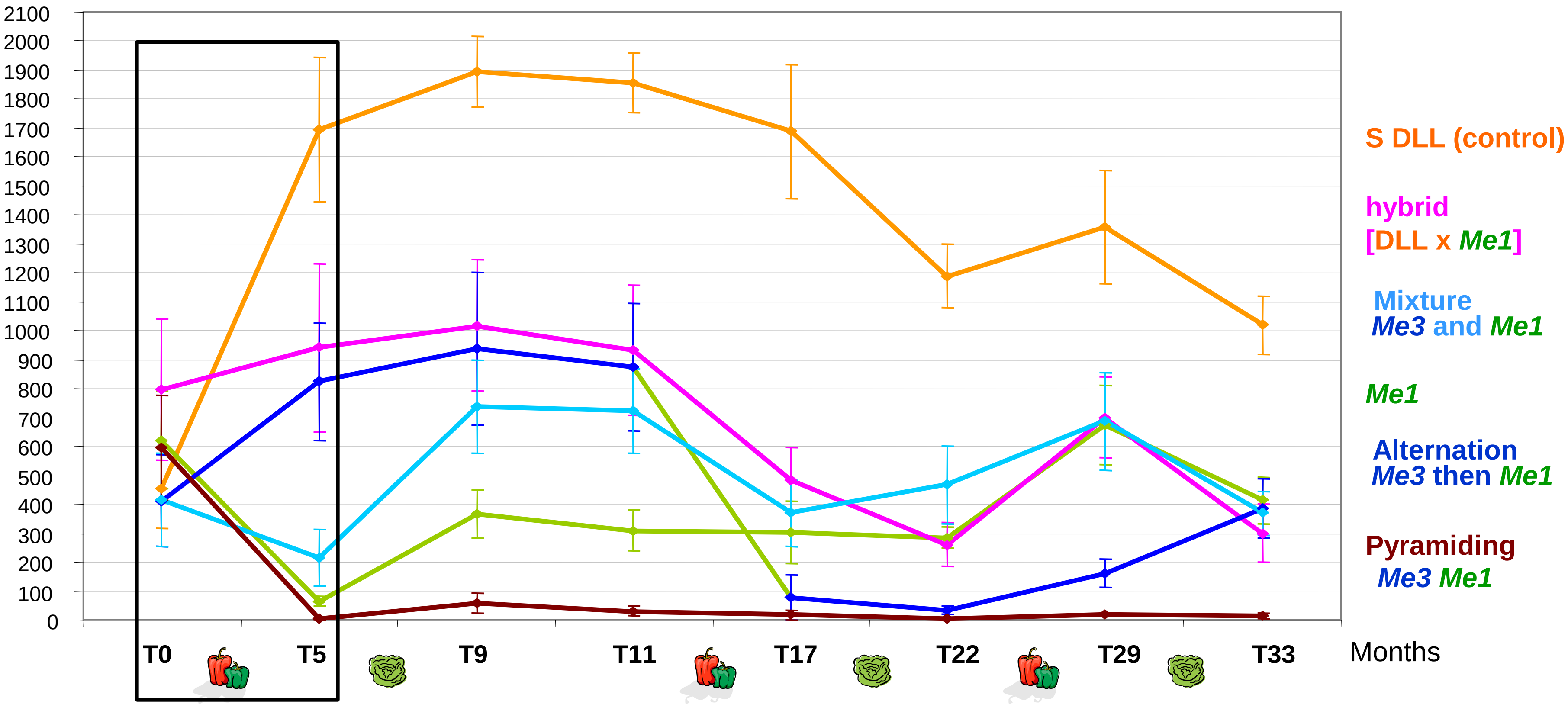


Before peppers : SIP was high and homogenous in each microplot (GI > 5)

Experimental approach in natural conditions

Results on reduction of the soil infection potential (“trap” effect)

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

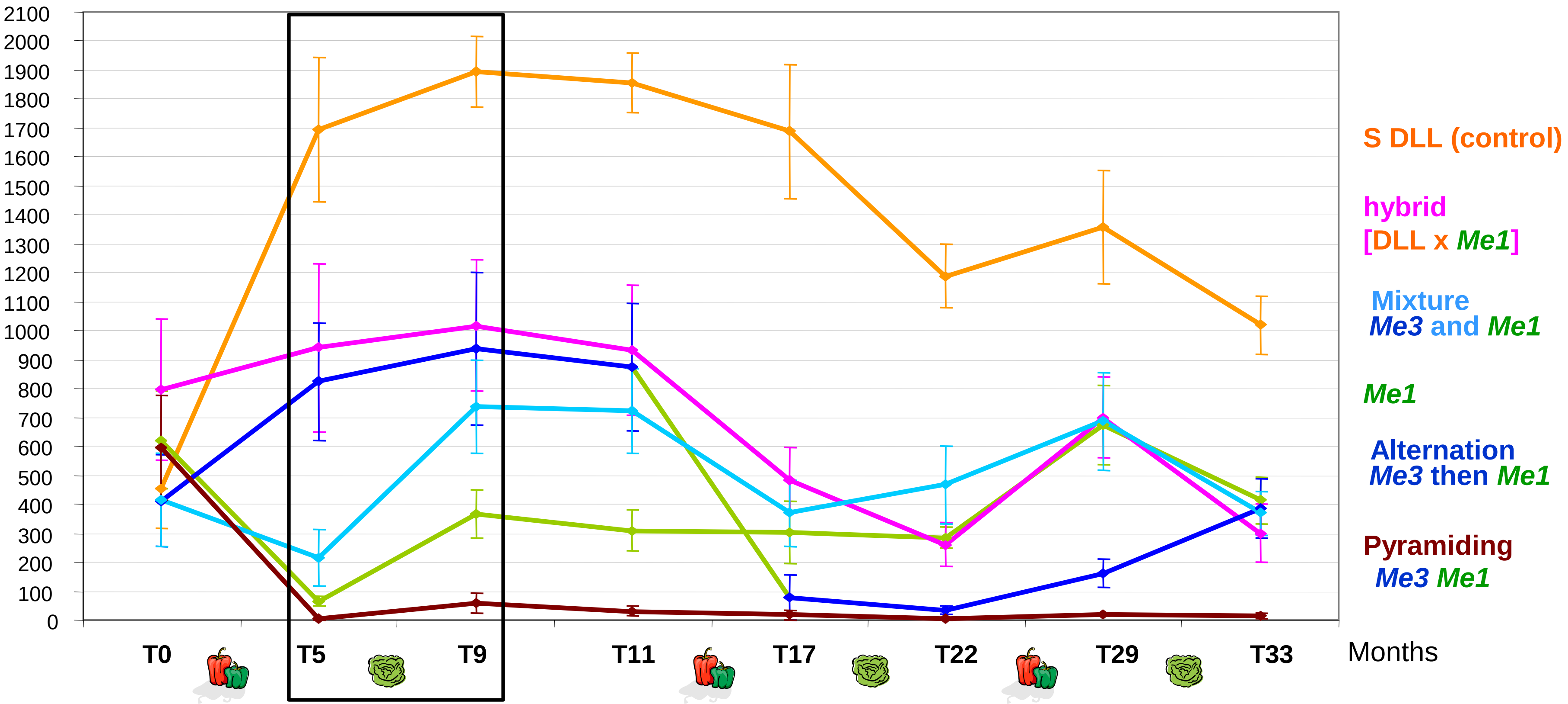


- **S DLL strongly increased the SIP ; no significant for hybrid [DLL x *Me1*] and *Me3***
- **Mixture *Me3 and Me1* reduced the SIP**
- ***Me1* and particularly the pyramided *Me3Me1* peppers strongly reduced the SIP: >90% !**

Experimental approach in natural conditions

Results on reduction of the soil infection potential (“trap” effect)

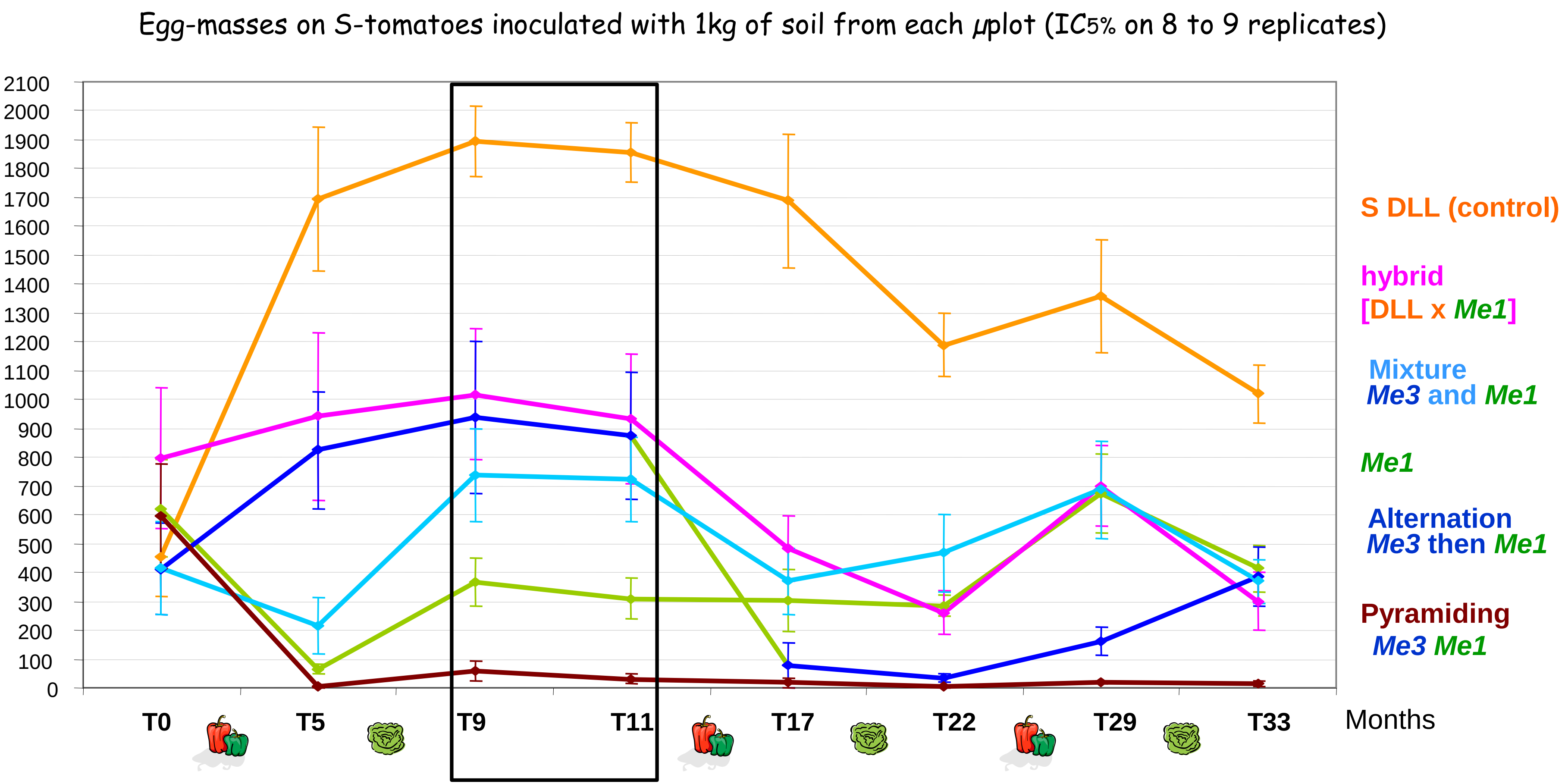
Egg-masses on S-tomatoes inoculated with 1kg of soil from each µplot (IC5% on 8 to 9 replicates)



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

Experimental approach in natural conditions

Results on reduction of the soil infection potential ("trap" effect)

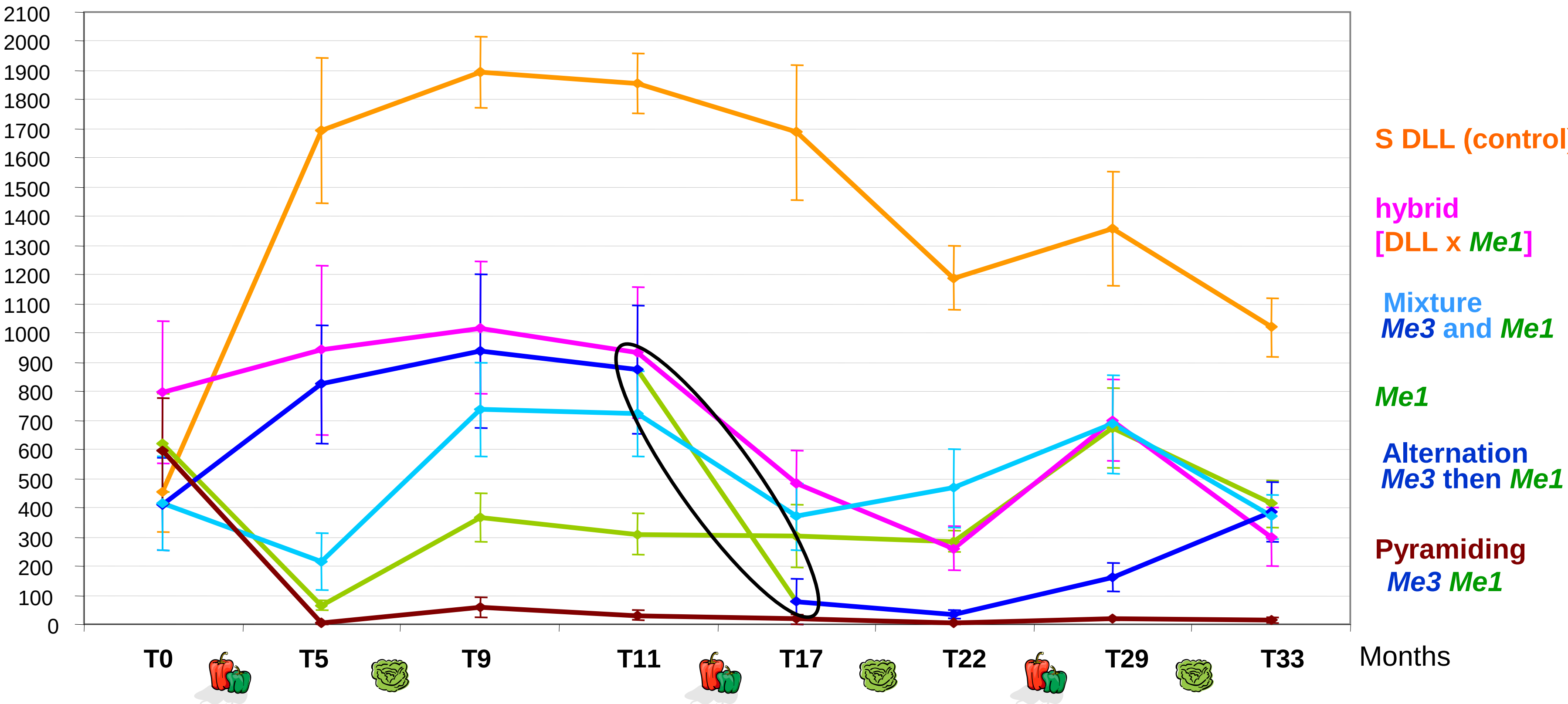


After 2 months without any culture, no significant evolution of SIP

Experimental approach in natural conditions

Results on reduction of the soil infection potential ("trap" effect)

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

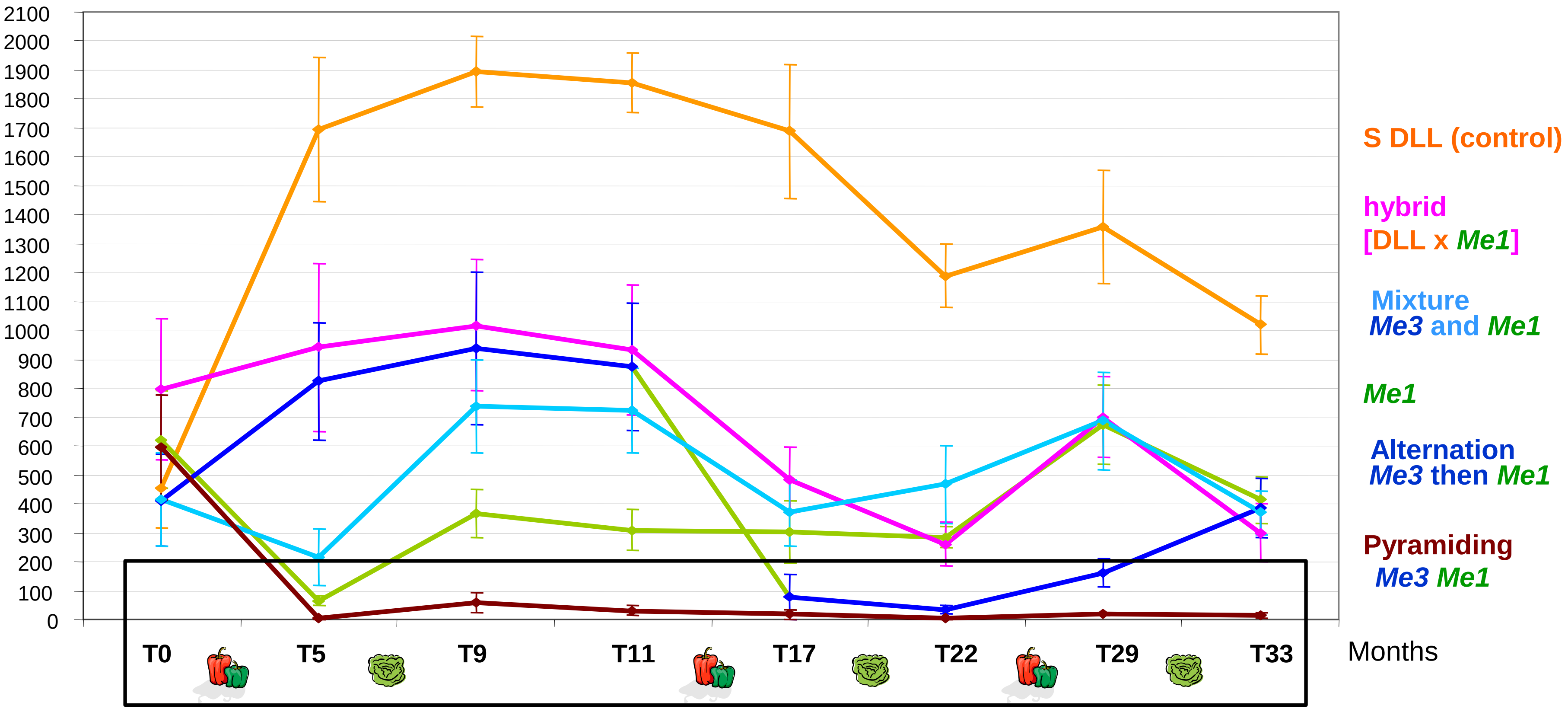


Alternating *R*-genes in rotation efficient to decrease virulent populations in the field ***(specificity of virulence)***

Experimental approach in natural conditions

Results on reduction of the soil infection potential ("trap" effect)

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



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

Strategies to strengthen and increase the R durability and to limit the use of chemicals

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 probability of mutation of pathogens, thus the selection of virulents*

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 : allows to recycle ineffective R-genes in successive cycles of selection*

- **Use *R*-genes pyramiding with a good organic amendment** (increasing their "trap" effect)

⇨ *To decrease the amount of pathogens in the soil*

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.

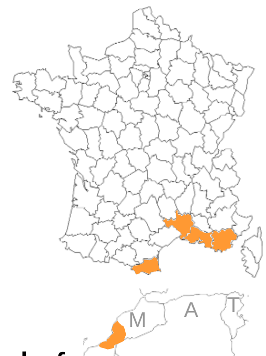
INRA **metaprogramme SMaCH (Sustainable Management of Crop Health)**

Action PRESUME (Plant REsistance SUstainable ManagEment) **02/2012 – 02/2016**

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)

1 experimental station and 4 farms to evaluate consequences of such systems at farm level not only from agronomical and pathological points of view, but also as regards to ecologic and socioeconomic consequences (impact on soil health and soil microbial communities, land occupation, labour organisation, economic and commercial consequences...)

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 VEGETABLE SEEDS SAKATA

Collaborative network

INRA

- **Centre PACA**
  - UMR ISA, équipe IPN **Sophia Antipolis**
  - UR GAFL, UR EcoDev, UR PaVe **Avignon**
- **Centre Montpellier** UE DEAR **Alénya Roussillon**
- **Centre Dijon** UMR MSE
- **Centre Rennes** UMR Bio3P

IRD **Montpellier** UMR CBGP

Farmers associations and technical institutes

- **APREL** (Association for vegetable research and experimentation) **St Rémy de Provence**
- **GRAB** (Research Group in Organic Farming) **Avignon**
- **Chambres d'agriculture** **CA 06 & CA83**
- **Society Azura, Group Maraissa** **Maroc**
- **CTIFL** (Interprofessional technical center for fruits and vegetables) **Balandran**

Private breeding companies

Farmers **La Baronne-Nice (06), Six-Fours (83), Lambesc (13), Marguerittes (30)**

Thank you for your attention

Financial support

CTPS

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Endure

INRA · PICLég · Neoleg

ANR Sysbiotel

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