30th International ESN Symposium
Vienna, Austria, 19-23/09/2010

Session 22. Plant-nematode interactions

Resistance breeding and engineered resistance

Evaluation of R-genes deployment strategies for the durable management of root-knot nematodes

Root-knot nematode

Susceptible plant

Resistant plant

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INRA – Antibes/Sophia Antipolis Research Center - FRANCE
" Biotic Interactions and Plant Health" Unit - Nematology team

Root-knot nematodes *Meloidogyne* spp.

An increasing problem on vegetable crops in France

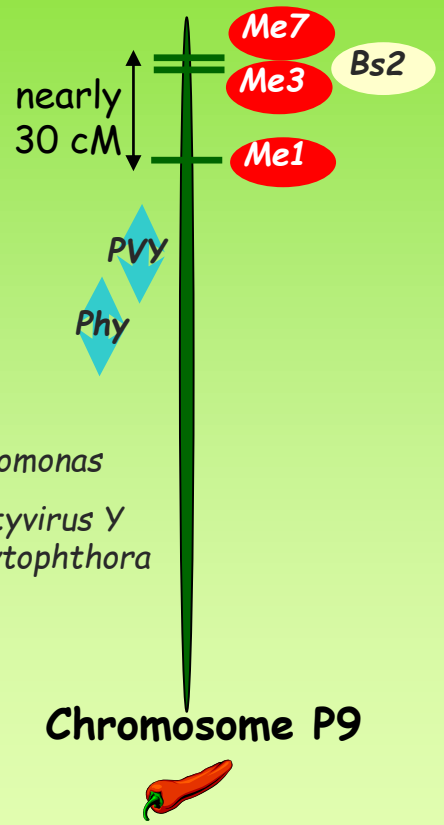
- A survey conducted from 2007 to 2010 :
  a big threat for > 40% of farms producing vegetables in SE France

- Crop rotations with resistant plants : economically efficient and environmentally safe but resistance can be overcome

Resistance to RKN in pepper *(Capsicum annuum)*

Genes Me1, Me3, Me7

from 3 genetically different pepper lines



Bs2 = R *Xanthomonas*

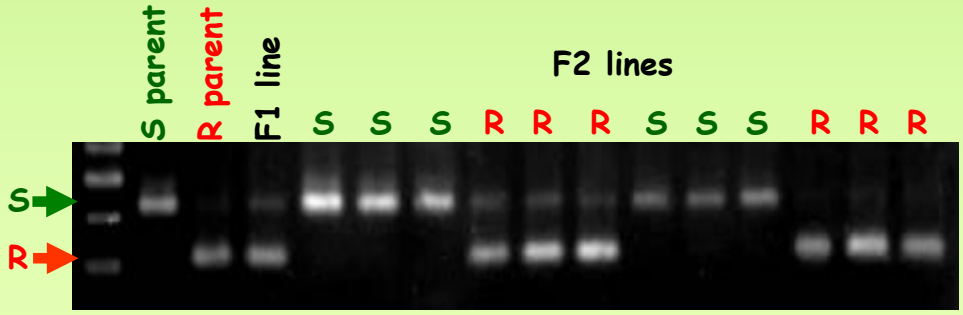
PVY = QTL *potyvirus Y*
Phy = QTL *Phytophthora*

. **Dominant, stable at high T°C**

. **Broad spectrum of action**
- *M. incognita*
- *M. arenaria*
- *M. javanica*
- some *M. hapla*

. **The *Me* genes all linked on P9 in a cluster of R-genes or QTLs**

. **Molecular markers available or in progress for MAS**

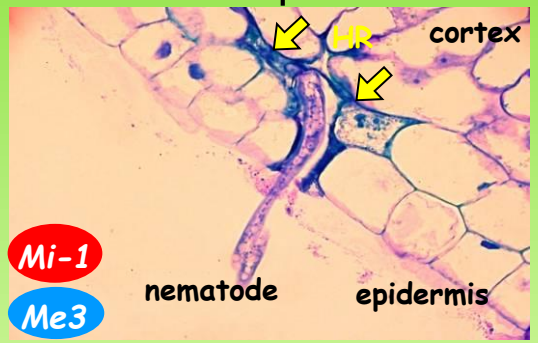


Djian-Caporalino et al, Theor Appl Genet 1999, 2001,2007 and in: K. Niemirowicz-Szczytt (Ed.) 2007

Linkage between *R*-mechanisms and *R*-durability

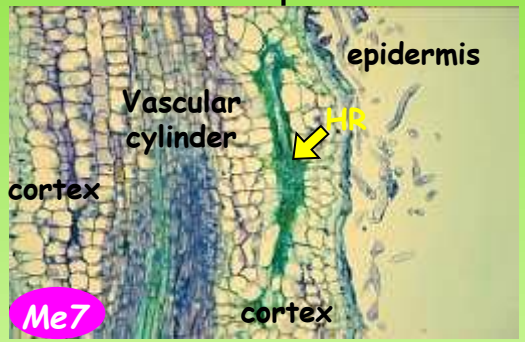
- **Laboratory experiments with high selection pressures**

1 dai



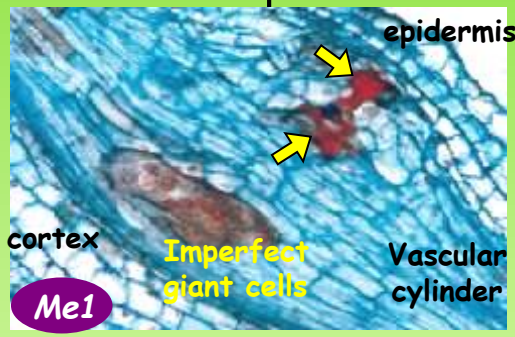
Immediate necrosis

3 dai

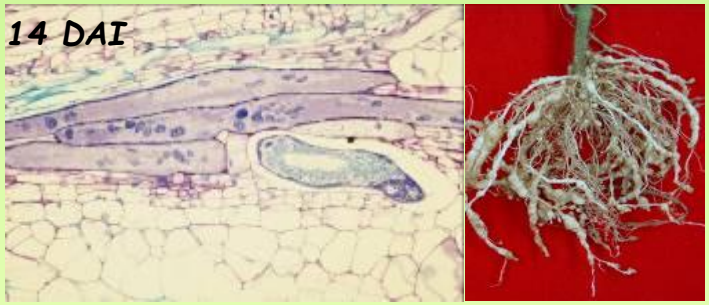


Early necrosis

10 dai



Later necrosis



Genes overcome

Selection of virulent nematodes

Unable to overcome the *Me1* gene

Pegard et al, Phytopathology 2005

Castagnone et al, Plant Breeding 2001

Projects DURANEM in progress

"Durability of resistance to Nematodes"

CTPS — French agriculture ministery and Permanent Technical Committee of the Selection of the crop plants 2007-2010 ➡ ANRT PhD 2010-2013

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

➡ To evaluate the selection pressure of the pepper R-genes on *Meloidogyne spp.* under variable genetic context

Neoleg — INRA PICLeg network, Integrated production of vegetable crops 2009-2011

Sysbiotel — French National Research Agency, project on Ecosystems, living resources, landscapes and agriculture 2009-2012

Valort — EU Interreg Alcotra project, 01/2010-12/2012

➡ To evaluate crop rotations with R-plants under greenhouses and field agronomic conditions

Objectives

Specificity of the virulence? Fitness cost associated?

Dosage effect of R alleles?

Heterozygous lines Me3 or Me1 *versus* homozygous lines Me3/Me3 or Me1/Me1

Quantitative effect of genetic backgrounds?

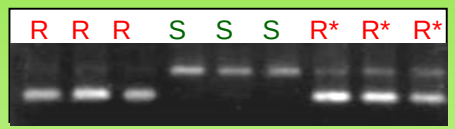
Susceptible (S) *versus* partially resistant (PR) cultivars

Experimental approach

- **Construction of R genotypes (when not yet available)**

  Collaboration with laboratory of Genetics and Plant-Breeding from INRA in Avignon and private breeding companies

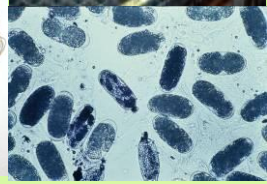
- **Development of co-dominant markers**



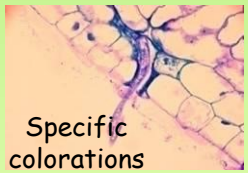
➡ *Sorting homozygous / heterozygous BC lines*

- **Resistance tests in climate-controlled rooms**

  Comparison of numbers of egg-masses/root and eggs per egg-mass



- **Histological studies**



➡ *Linkage between R-mechanisms and R-durability*

- **Selection of virulent variants by repetitive inoculations on R-plants**
- **Evaluation of the fitness of avirulent and virulent nematodes**

➡ *Fitness cost associated to virulence?*

Collaboration with the group of P. Castagnone (IPN team Sophia)

First results : Specificity of the virulence

Several virulent populations : selected or natural

High selection pressure

Avirulent nematodes

tomato Mi-1/Mi-1 — virulents/Mi-1 → pepper Me3/Me3, pepper Me7/Me7, pepper Me1/Me1 ✗

pepper Me3/Me3 — virulents/Me3 → pepper Me1/Me1, tomato Mi-1/Mi-1 ✗

pepper Me7/Me7 — virulents/Me7 → pepper Me1/Me1, tomato Mi-1/Mi-1 ✗

No gall, no egg-mass

Genes alternance seems possible

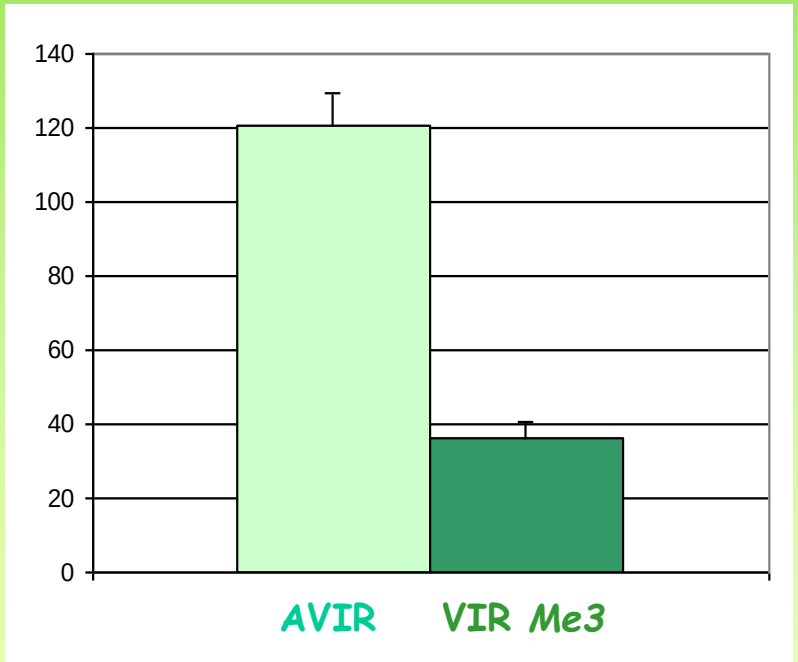
Castagnone-Sereno et al., Plant Breeding 2001

Djian-Caporallino, Palloix, Castagnone-Sereno (INRA France), Molinari (CNR Bari, Italy), in preparation

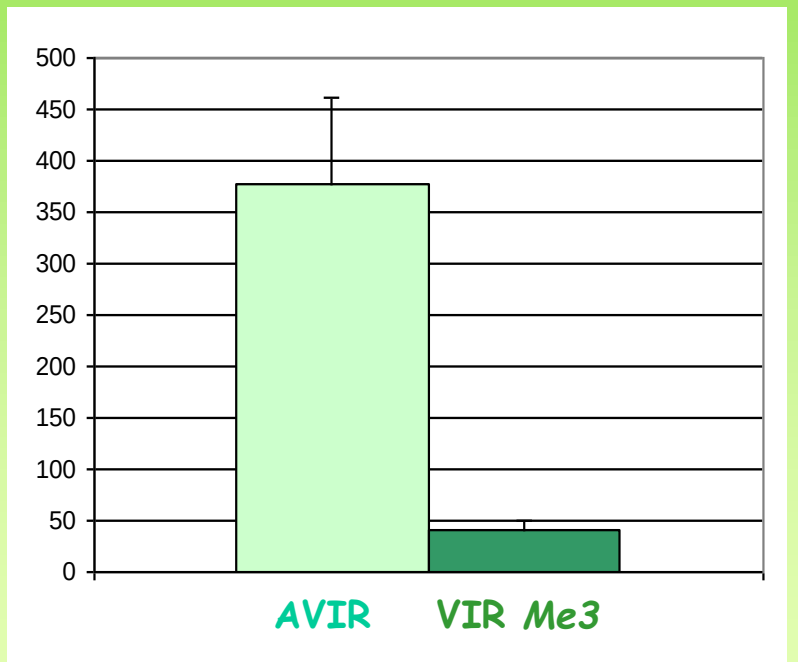
First results : Fitness cost associated to virulence

Inoculation with 500 avirulent or virulent/Me3 *M. incognita*
on DLL (susceptible pepper)

Root Infestation
(**IR** = number of egg masses/plant)



Reproduction Potential
(**RP** = number eggs/number inoculated J2)



15 replicates (IC5%)

A fitness cost seems associated to unnecessary virulence in the nematode

Consequences for field populations?

First results : Dosage effect of R alleles and quantitative effect of genetic background

Inoculation with 5000 avirulents *M. incognita*

HD149 = R parent homozygous for *Me3*

X

DLL = S parent

⬇

BC1 = [(HD149 x DLL) x DLL]

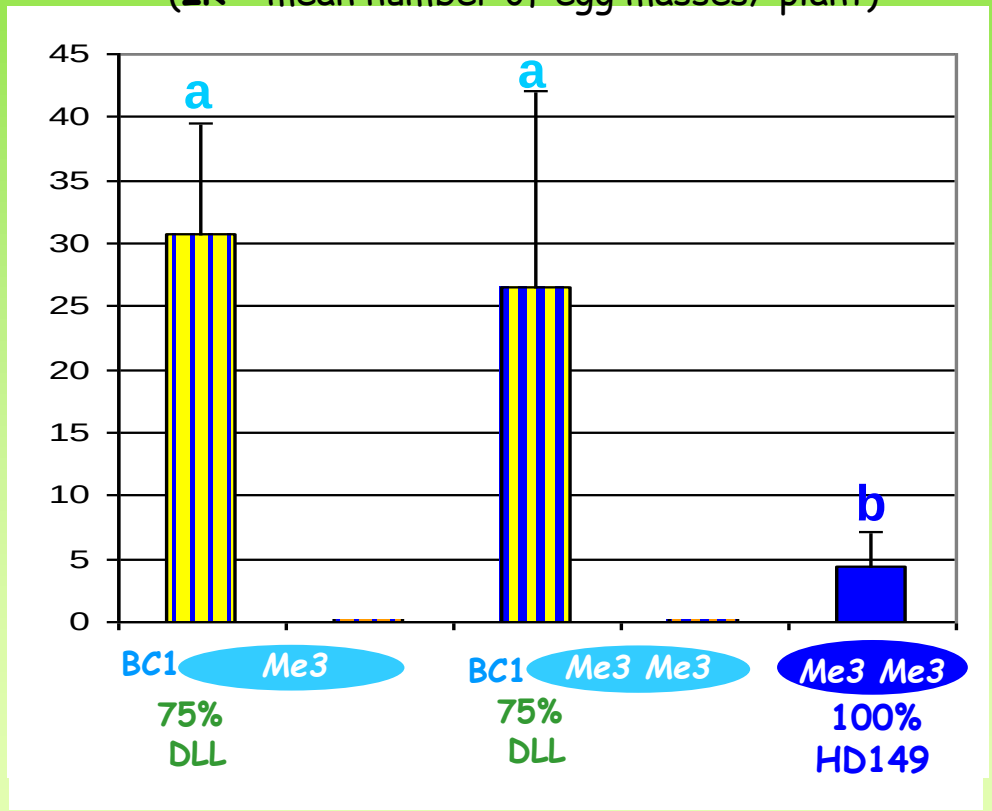
homoz or heteroz lines

75% DLL background

sort with markers

Root infestation

(**IR** = mean number of egg masses/ plant)



25 to 50 replicates
(IC5%)

➡ **The number of alleles seems not influence the selection pressure exerted by the R-genes on the RKN populations**

First results : Dosage effect of R alleles and quantitative effect of genetic background

HD149 = R parent homozygous for *Me3*

X

DLL = S parent

⬇

BC1 = [(HD149 x DLL) x DLL]

homoz or heteroz *Me3*

75% DLL background

sort with markers

HD149 = R parent homozygous for *Me3*

X

YW = PR parent

⬇

BC1 = [(HD149 x YW) x YW]

homoz or heteroz *Me3*

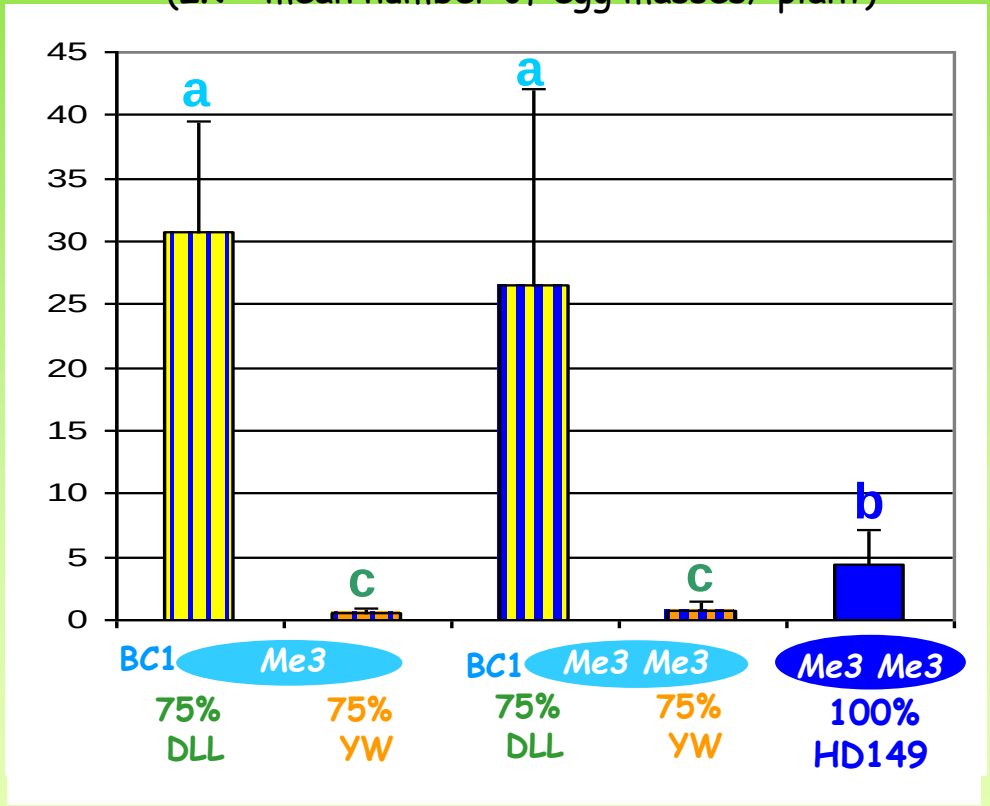
75% YW background

sort with markers

Inoculation with 5000 avirulents *M. incognita*

Root infestation

(**IR** = mean number of egg masses/ plant)



25 to 50 replicates

(IC5%)

➡ **The genetic background influences the selection pressure exerted by the R-gene : R QTLs in YW seem protect the major R-gene *Me3***

Field validation

Vegetable crops rotations :

S salad

R peppers:
Me1, Me3, Me7

R tomatoes:
Mi-1, Mi-3

Experimentations in several places in collaboration with technical centres and private breeding companies

INRA Sophia & Nice - SE France 2009-2011

ANR Systerra & PicLeg

Aubagne – South of France 2011

Interreg Alcotra Valort

Agadir - Moroco 2010-2012

IRD IRD/Azura Maticha

Europe and outside with private seed companies 2010-2012

ANRT PhD

Cifre Conventions

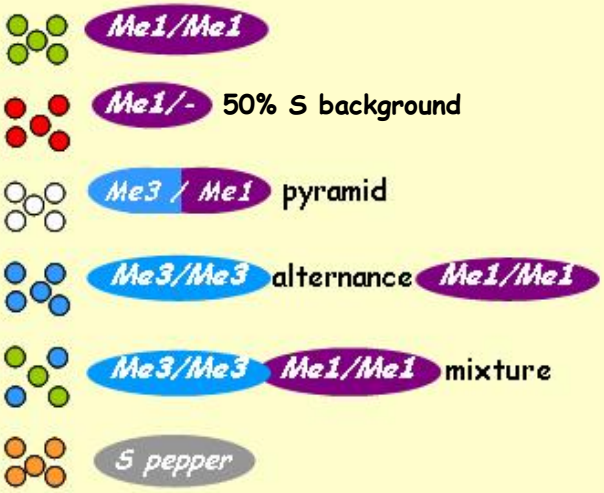
- to determine whether the R-plants will behave the same way facing natural nematode pop
- to assess the time required for the improvement of soil health
- to determine the spatial management of R-plants lowering the risk of emergence of virulent nematodes ; effect of pyramiding vs mixture or vs alternance of R-genes

Example in an experimental station (Nice, SE France) to validate results in agronomic cond.

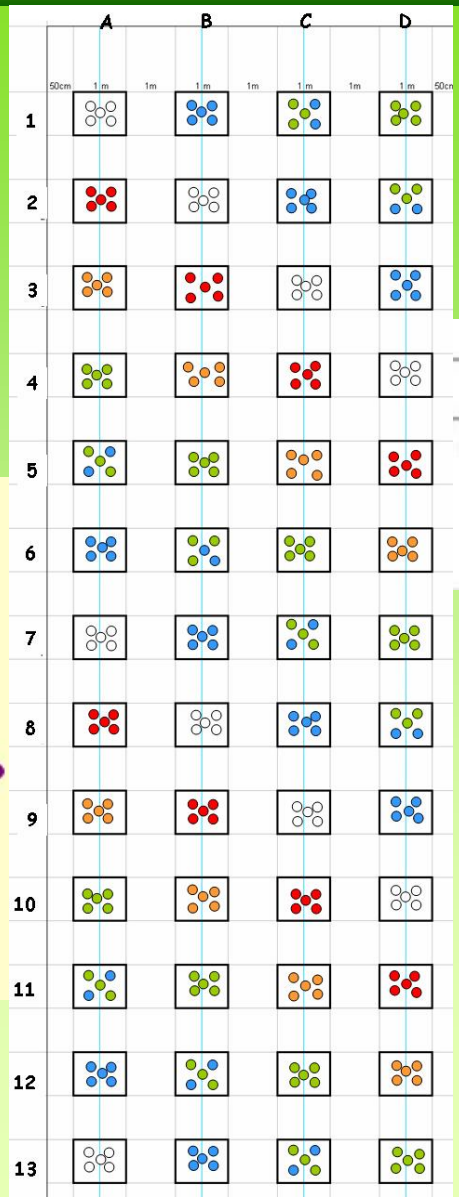
A plot of 250 m2 highly infested *M.incognita* + *M.arenaria* + *M. hapla*

Peppers as summer crops,

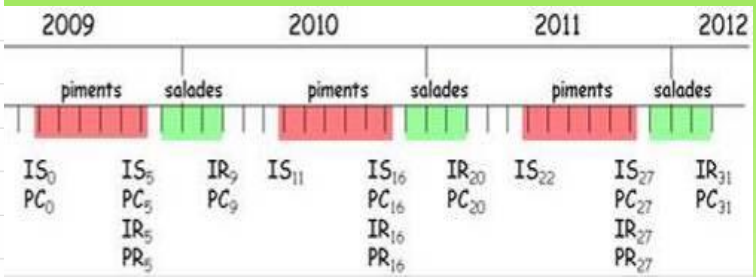
6 modalities,
52 µplots (1 m2),
5 plants/µplot



Susceptible salads as winter crops



Infestation parameters



IS = soil infestation

IR = root infestation (gall index)

PR = reproduction potential of virulents (if detected)

PC = nematode communities

Example of results from the experimental station

1rst year

- S peppers DLL with mean GI nearly max
- A few galls on *Me1xDLL* R hybrids (50% S background), but no virulent population obtained
- *Me3Me3* peppers could be overcome: vir. pop.
- *Me3Me3* peppers seemed protected by *Me1Me1* peppers
- *Me1Me1* and *Me3Me1* peppers were not overcome

Root infestation on peppers at T5

(RI: 0 to 10)

S pepper DLL : RI5 = 9

Me1xDLL : RI5 = 1,5

Me3Me3 : RI5 = 1

Me3Me3 + *Me1Me1* : RI5 = 0,3 on *Me3Me3*

Me1Me1 : RI5 = 0

Me3Me1 : RI5 = 0

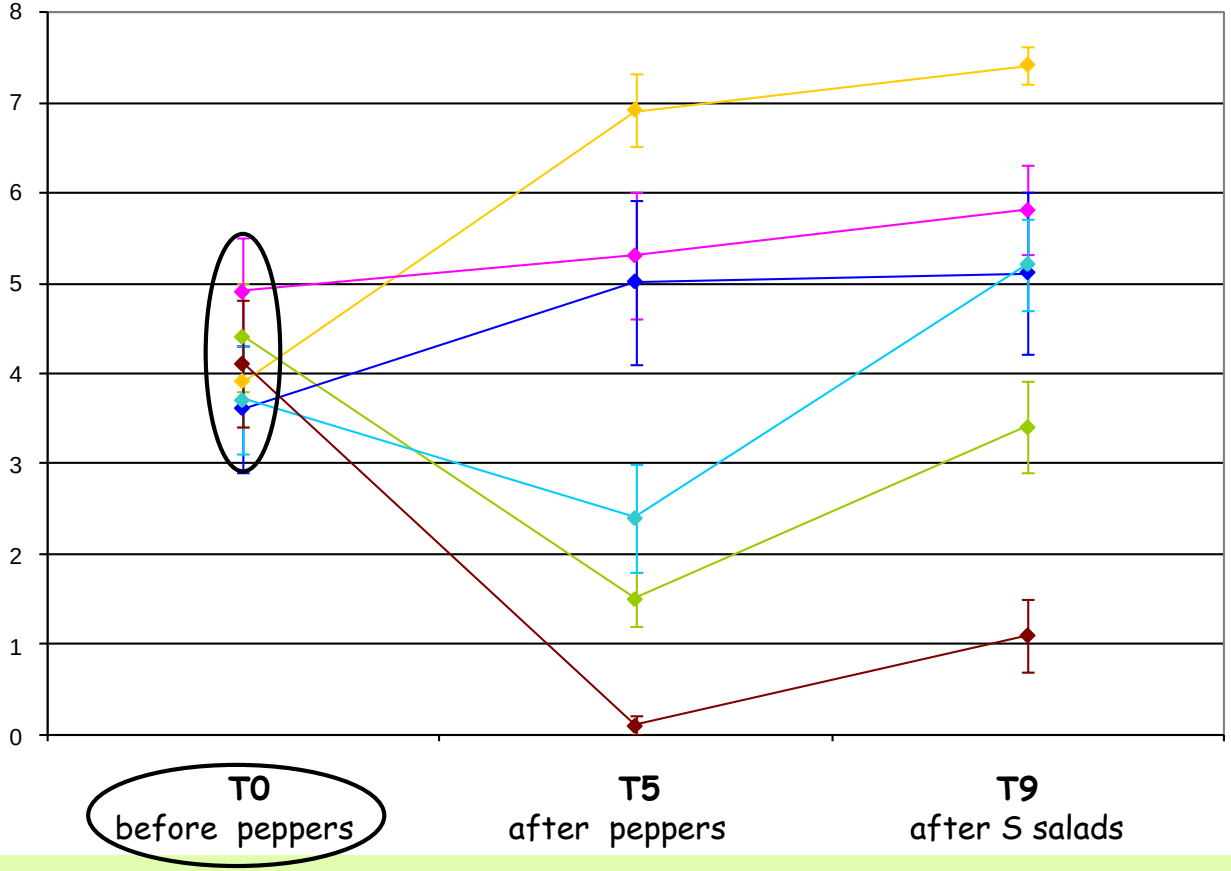
40 to 45 replicates

Example of results from the experimental station

1rst year

Soil infestation (SI) 8 to 9 replicates

Mean of gall index (0 to 10) per susceptible tomato plant inoculated with 1kg of soil (IC5%)



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Me1Me1 : RI5 = 0

Me3Me1 : RI5 = 0

40 to 45 replicates

before peppers : SI was high in each microplot (4-5)

Example of results from the experimental station

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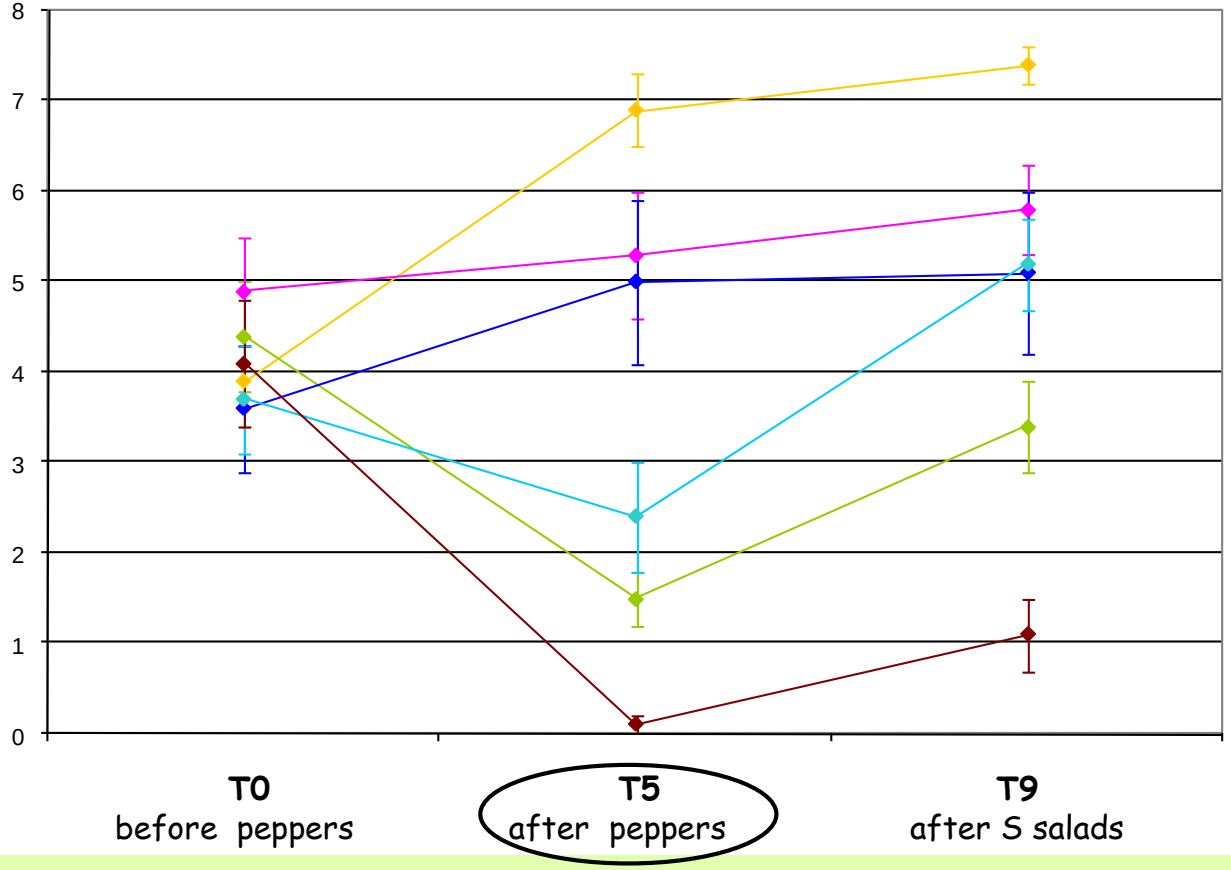

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Me1Me1 : RI5 = 0

Me3Me1 : RI5 = 0

40 to 45 replicates

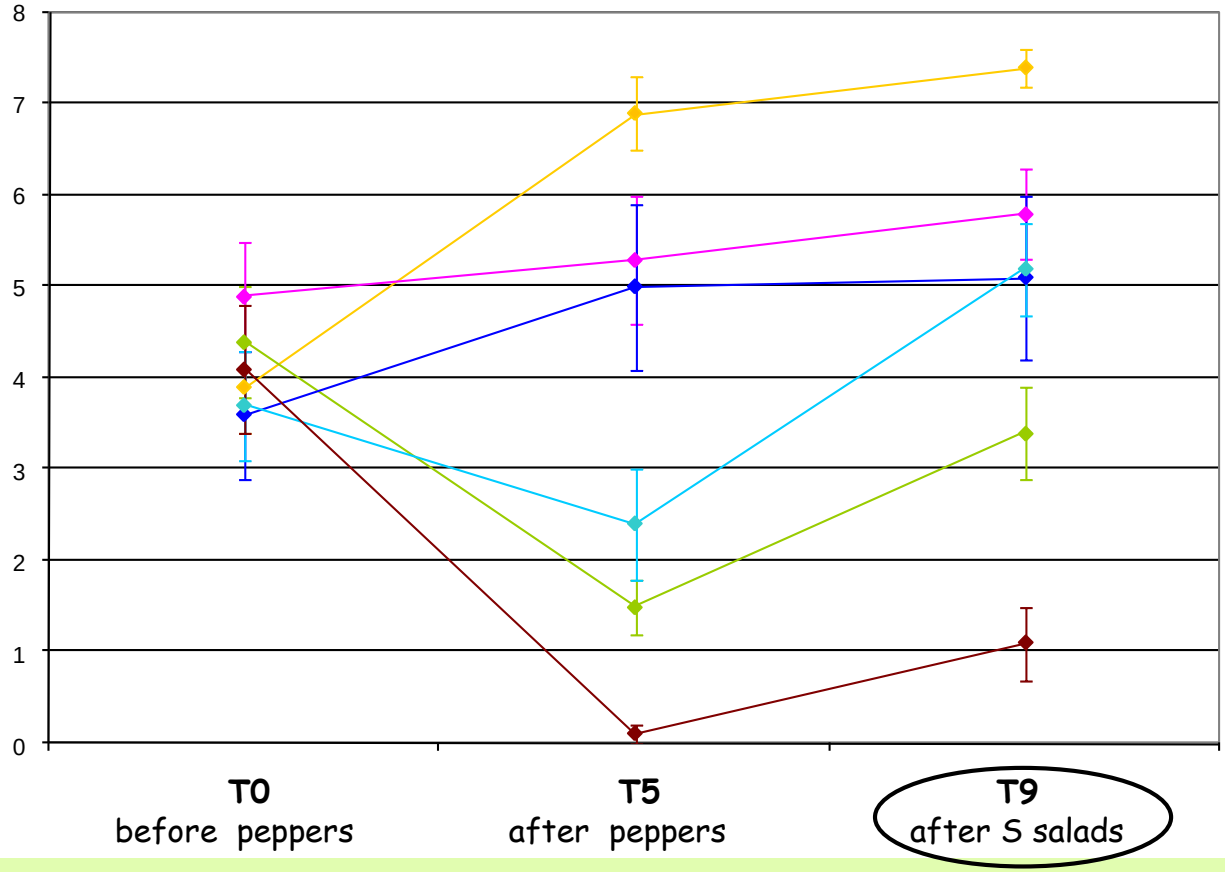
- S peppers DLL strongly increased the SI
- R *Me3Me3* + *Me1Me1* peppers reduced the SI
- R *Me1Me1* and *Me3Me1* peppers strongly reduced the SI

Example of results from the experimental station

1rst year

Soil infestation (SI) 8 to 9 replicates

Mean of gall index (0 to 10) per susceptible tomato plant inoculated with 1kg of soil (IC5%)



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Me3Me3 + *Me1Me1* : RI5 = 0,3 on *Me3Me3*

Me1Me1 : RI5 = 0

Me3Me1 : RI5 = 0

40 to 45 replicates

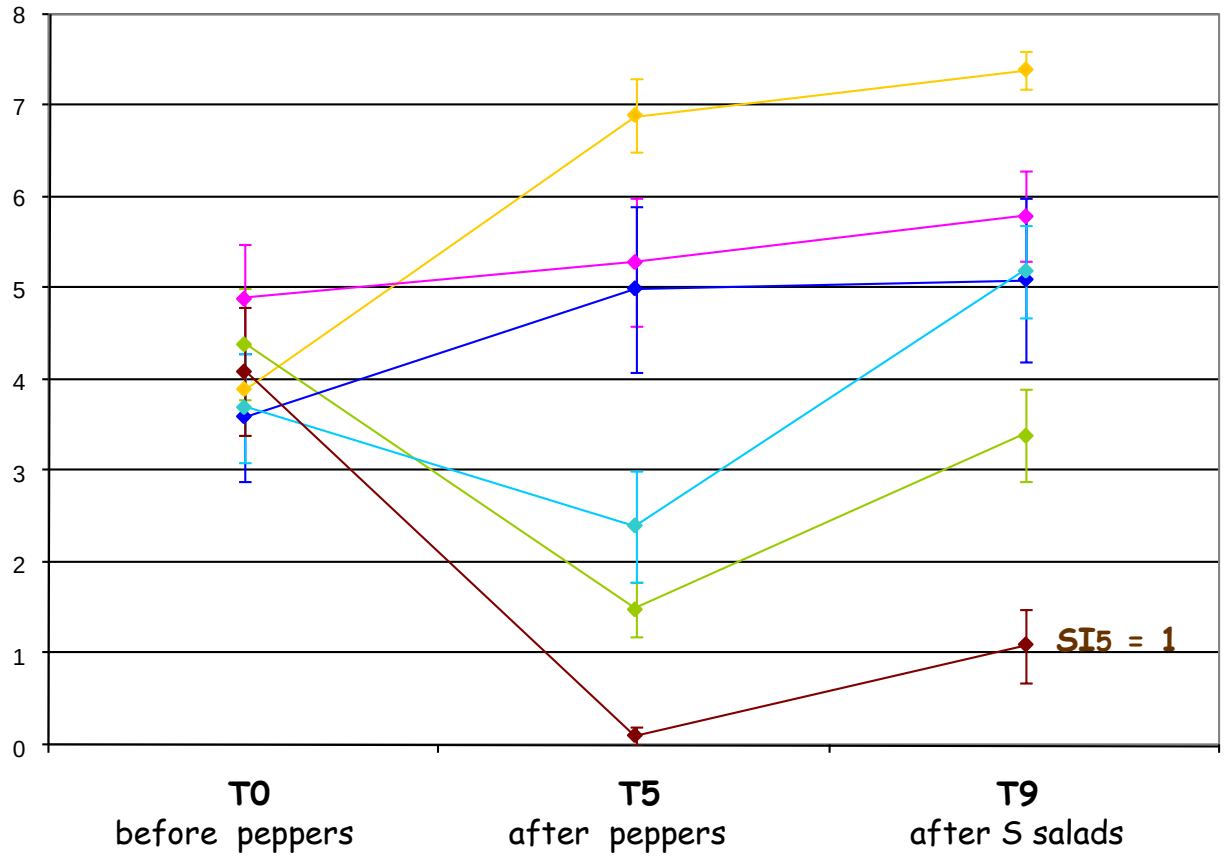
S salads allowed the multiplication of nematodes in each microplots

Example of results from the experimental station

1rst year

Soil infestation (SI) 8 to 9 replicates

Mean of gall index (0 to 10) per susceptible tomato plant inoculated with 1kg of soil (IC5%)



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Me3Me3 + *Me1Me1* : RI5 = 0,3 on *Me3Me3*

Me1Me1 : RI5 = 0

Me3Me1 : RI5 = 0

40 to 45 replicates

➡ R *Me3Me1* peppers are definitively the better modality = « traps crops »

Example of results from the experimental station

2nd year

16/09/2010

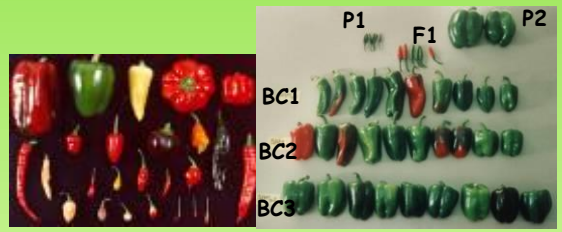
Organization and collaborations

✓ **INRA,** UMR IBSV, IPN (Sophia)

Dr Caroline Djian-Caporalino
Dr Philippe Castagnone-Sereno
Ariane Fazari (technician)
Nathalie Marteu (technician)
Ulysse Portier (technician)
& several students

✓ **INRA,** UR GAFL (Avignon)

Dr Alain Palloix
Anne-Marie Sage-Palloix (ing)
Ghislaine Nemouchi (technician)



✓**CNR,** Istituto per la Protezione delle Plante (Bari, Italie)

Dr Sergio Molinari

✓ **IRD**, CBGP (Montpellier)

Dr Thierry Mateille, Johannes Tavoillot (techn)

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

✓ **Private seed companies** (Syngenta, Vco, Gautier, Taki, Sakata, Neunhems, Rijkzwaan)

ANRT PhD Cifre


Thank you for your attention