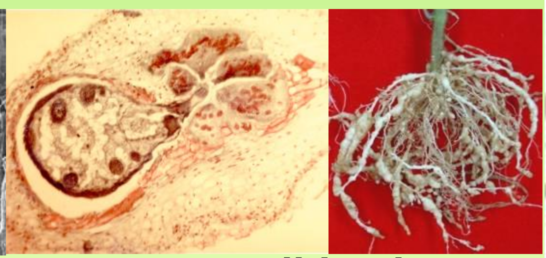
XIV[th] EUCARPIA Meeting on Genetics and Breeding of Capsicum & Eggplant

Valencia, Spain, 30/08-01/09/2010

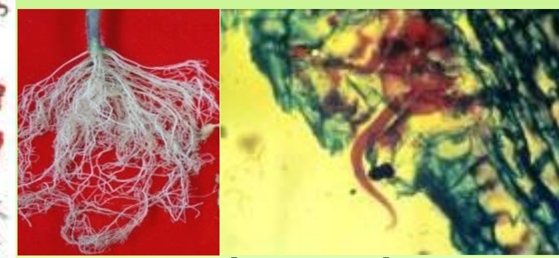
Session II. Breeding for resistance to biotic and abiotic stresses

Durable management of nematodes in pepper using resistant genotypes

Root-knot nematode



Susceptible plant



Resistant plant

Caroline Djian-Caporalino

INRA – Antibes/Sophia Antipolis Research Center - FRANCE

" Biotic Interactions and Plant Health" Unit - Nematology team

Root-knot nematodes *Meloidogyne* spp.

- extremely polyphagous (> 5,500 host plants)

- ~10-40% of crop losses worldwide

- Chemical nematicides *prohibited or restricted*

  Fumigants : methyl bromide, dichloropropene

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

Plant resistance

Typical HR against
M. incognita juvenile

The *Mi-1* R gene from tomato identified in the wild species *Solanum peruvianum*

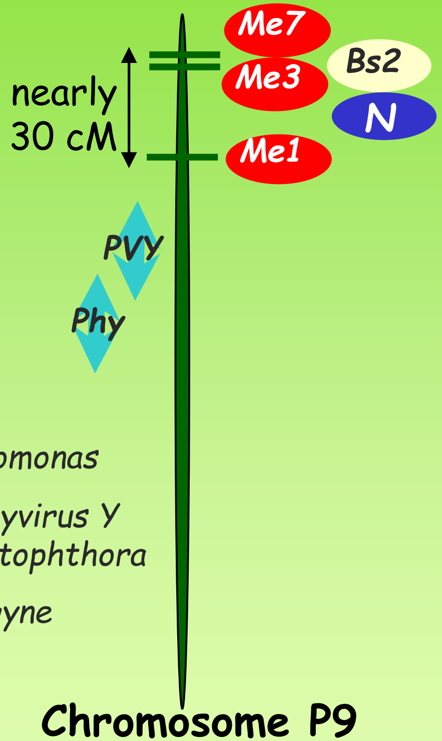
The *Me* and *N* R-genes from pepper identified in wild lines of *Caspicum annuum*

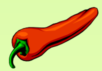
- *efficient*
- *economically competitive*
- *environmentally safe*

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*

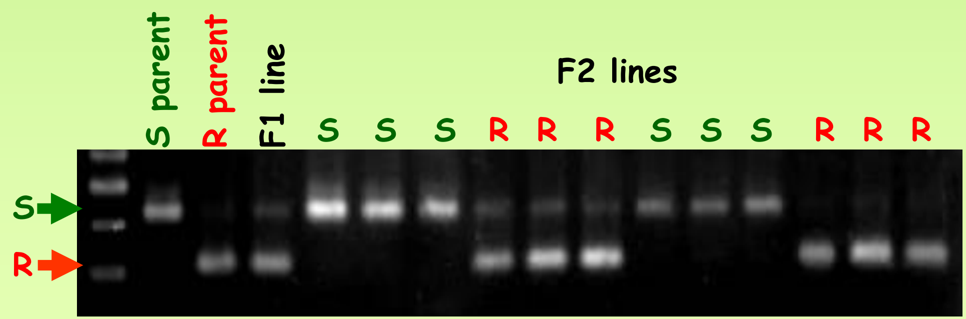
N = R *Meloidogyne*

. Dominant, stable at high T°C

. Broad spectrum of action: *M. incognita*, *M. arenaria*, *M. javanica*

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

. Molecular markers available or in progress for MAS

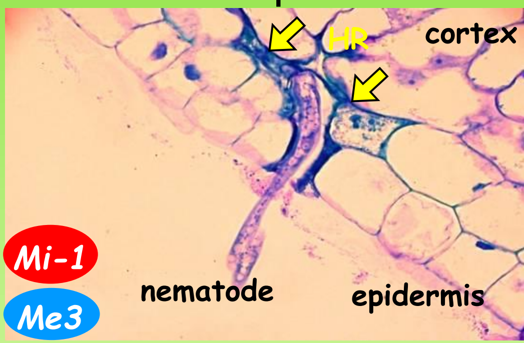


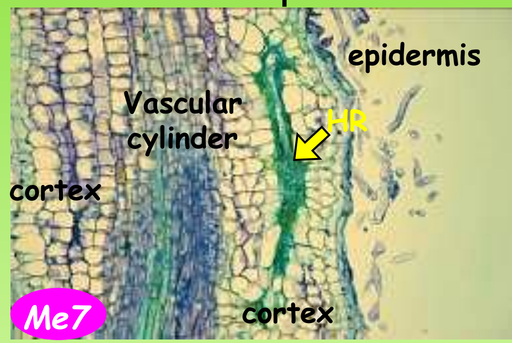
Djian-Caporalino et al, Theor Appl Genet 1999, 2001,2007 and in: K. Niemirowicz-Szczytt (Ed.) 2007
Djian-Caporalino, Palloix (INRA France) & Wang (IVF Beijing, China), in preparation

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

- **Laboratory experiments with high selection pressures**

1 dai — 3 dai — // — 10 dai

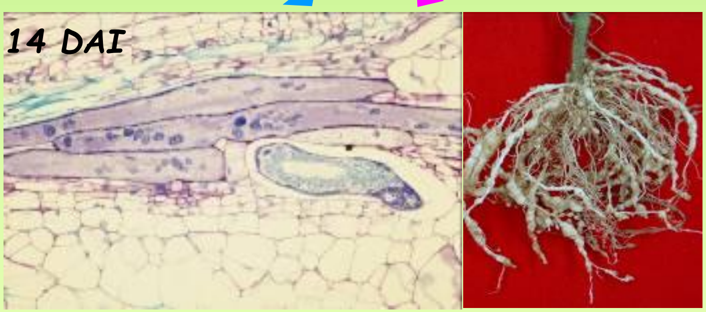




Immediate necrosis

Early necrosis

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

Limitation of the RKN-resistance

- **In natural conditions**
  - RKN *R-genes* are rare
  - *Mi-1* (in tomato cultivars) has been the only nematode *R*-gene used for 60 years (since 1950)
  - the first overcoming was noticed in 1970

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

➤ **New « robust » *R*-lines**

➤ **Management of *R*-genes to increase their durability**

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 Cifre 2011-2014

Endure — Europeen network for durable exploitation of crop protection strategies 2008-2010

- **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 — *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?

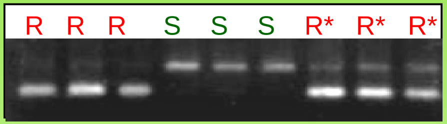
Susceptible (S) *versus* partially resistant (PR) cultivars

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

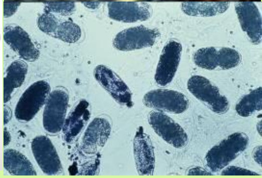
- **Development of co-dominant markers**



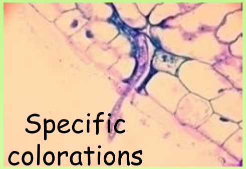
Sorting homozygous / heterozygous BC lines

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

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

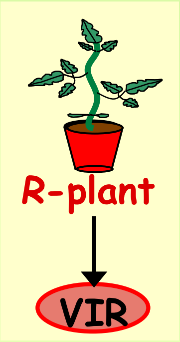


- **Histological studies**



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?

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

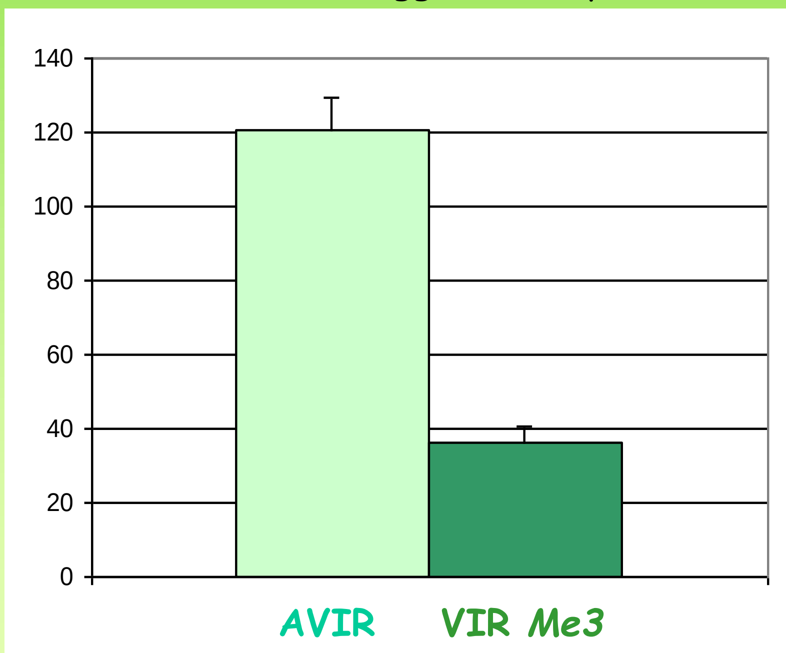
Genes alternance seems possible

Castagnone-Sereno et al., Plant Breeding 2001
Djian-Caporallino, Palloix, Castagnone-Sereno (INRA France), Molinari (CNR Bari, Italy), in preparation

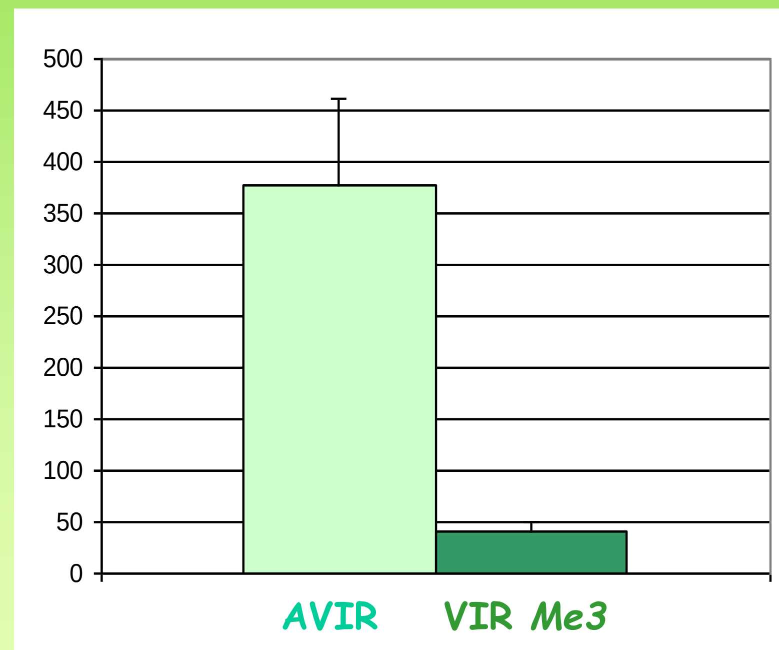
First results : Fitness cost associated to virulence

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

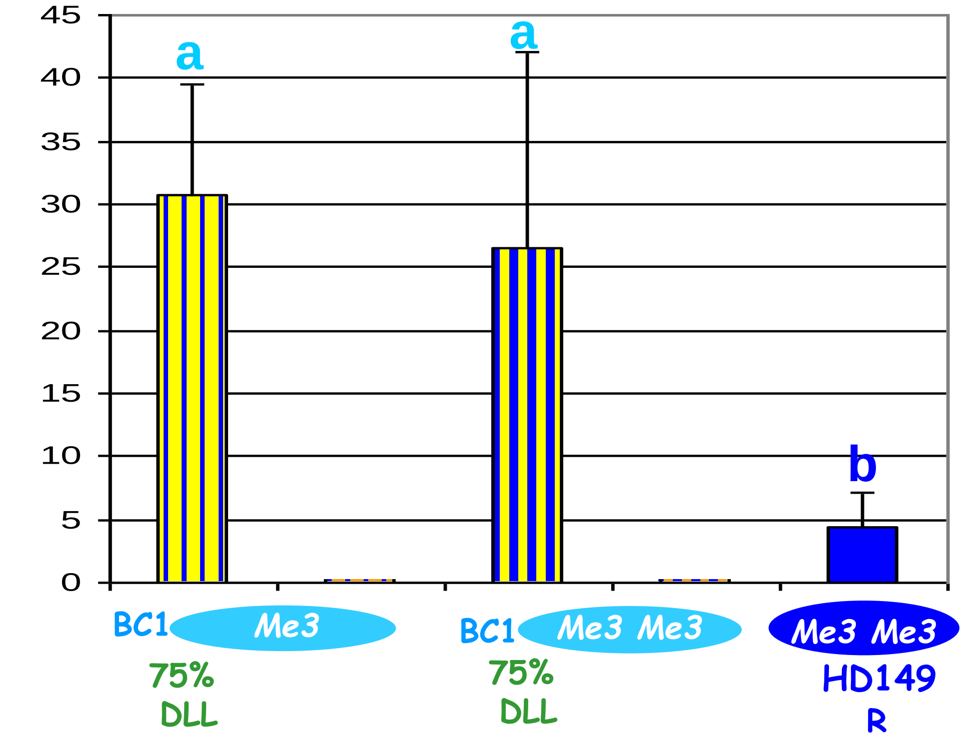
Consequences for field populations?

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*
- DLL = S parent
- BC1 = [(HD149 x DLL) x DLL]
  25% S
  25% homoz *Me3*
  50% heteroz *Me3*
  75% DLL background

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



25 to 50 replicates
(IC5%)

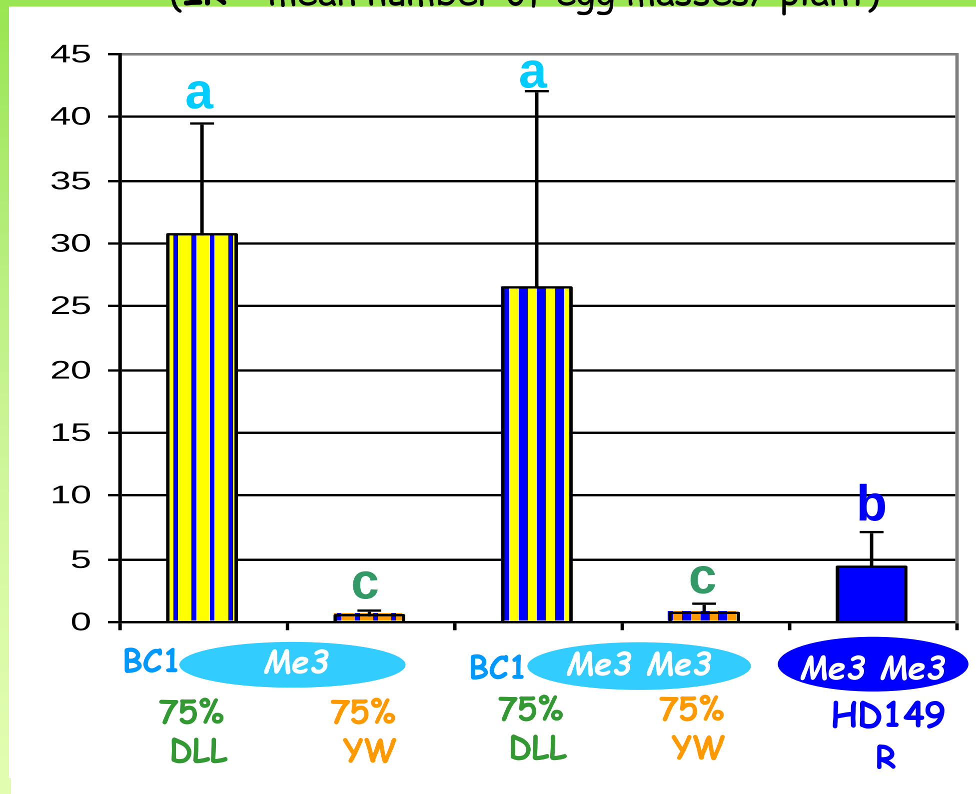
The number of alleles does not influence the selection pressure exerted by the R-genes on the RKN populations

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

Inoculation with 5000 avirulents *M. incognita*

- HD149 = R parent homozygous for *Me3*
- DLL = S parent
- BC1 = [(HD149 x DLL) x DLL]
  25% S
  25% homoz *Me3*
  50% heteroz *Me3*
  75% DLL background
- YW = PR parent
- BC1 = [(HD149 x YW) x YW]
  25% S
  25% homoz *Me3*
  50% heteroz *Me3*
  75% YW background

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/Azura Maticha

Europe and outside with private seed companies 2010-2012

ANRT PhD

Cifre Conventions

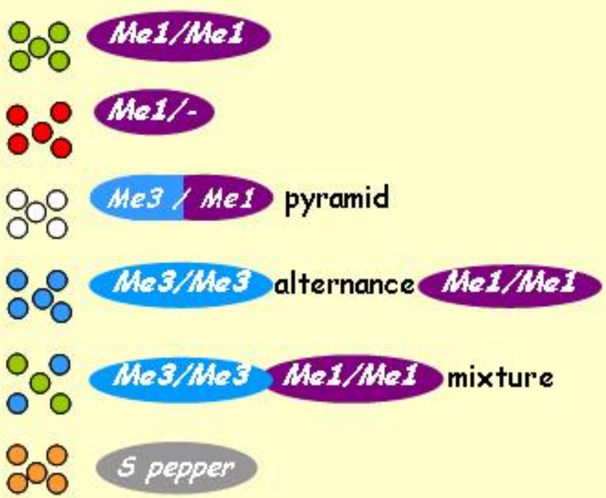
- to determine whether the *R*-plants will behave the same way facing natural nematode pop
- to assess the time required for the improvement of soil health
- to determine the spatial management of *R*-plants lowering the risk of emergence of virulent nematodes ; effect of pyramiding vs mixture or vs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

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

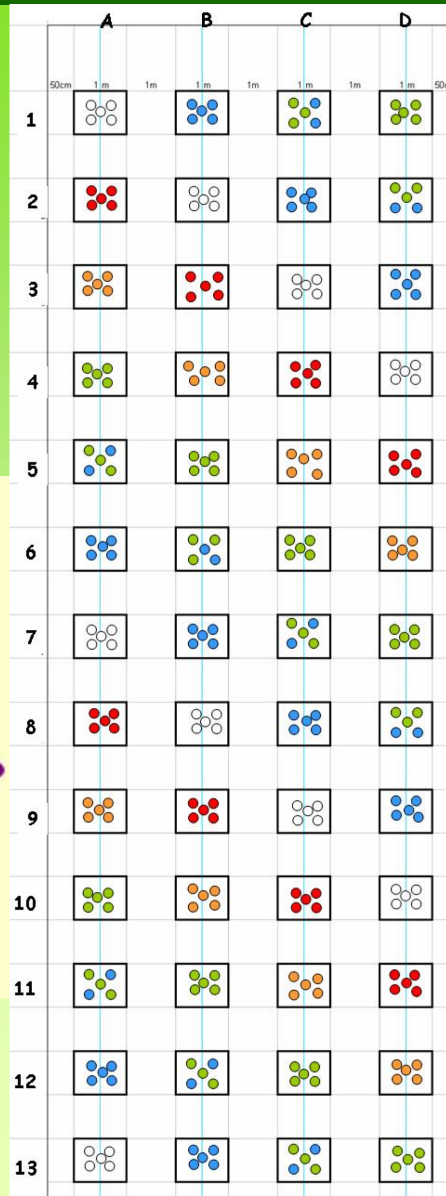
A plot of 250 m2 highly infested
M.incognita + M.arenaria + M. hapla

Peppers as summer crops,

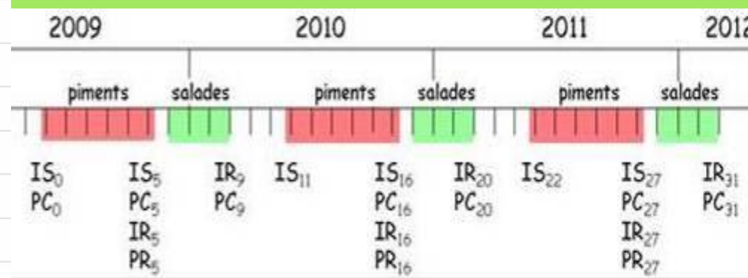
6 modalities,
52 µplots (1 m2),
5 plants/µplot



Susceptible salads
as winter crops



Infestation parameters



IS = soil infestation

IR = root infestation (gall index)

PR = reproduction potential of virulents (if detected)

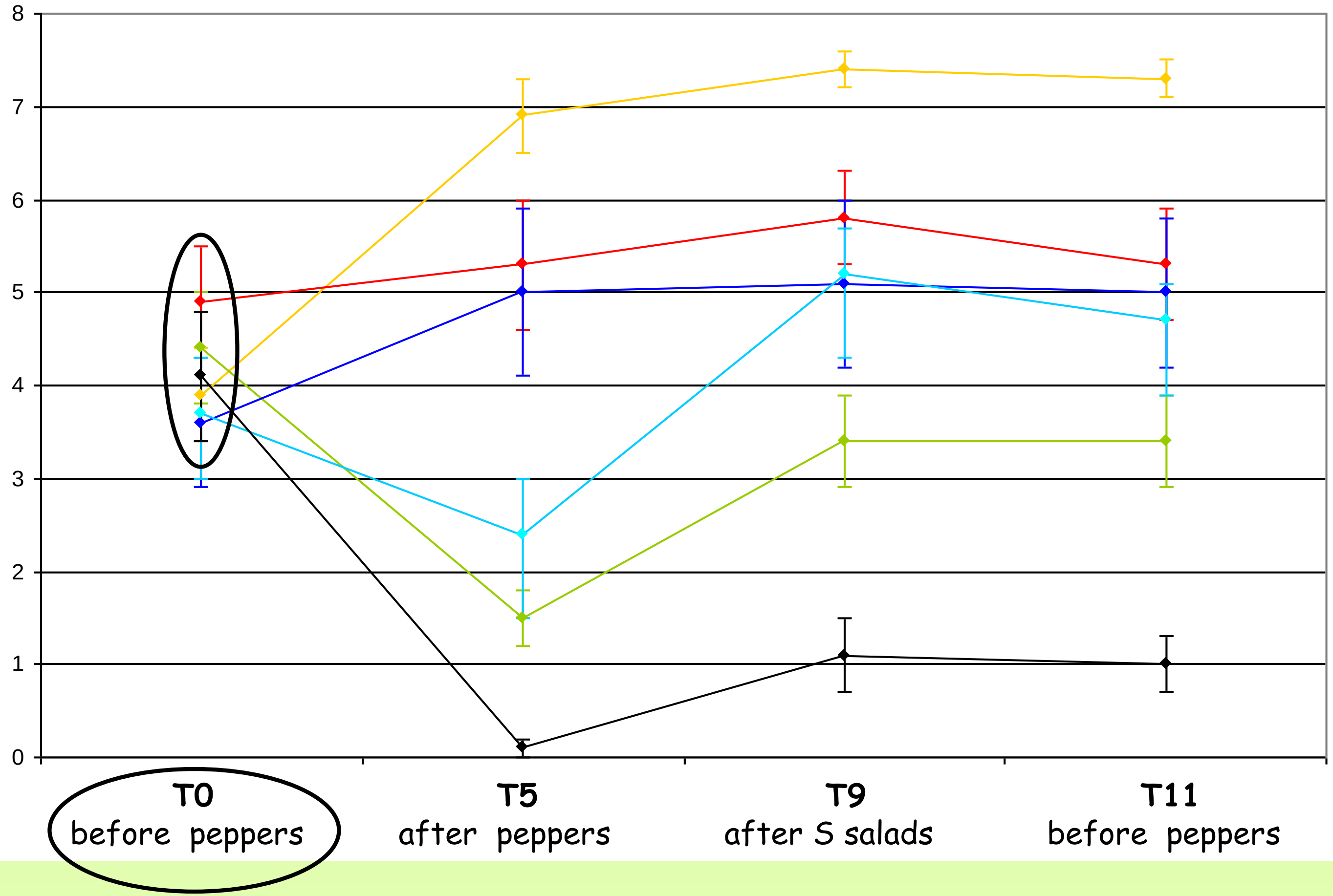
PC = nematode communities


12/05/2009
02/07/2009
28/08/2009
Me3/Me3
Me1/Me1
S pepper
Me1/-
Me3 / Me1
09/10/2009
Salads
16/02/2010

Example of results from the experimental station

Soil infestation (IS) 8 to 9 replicates

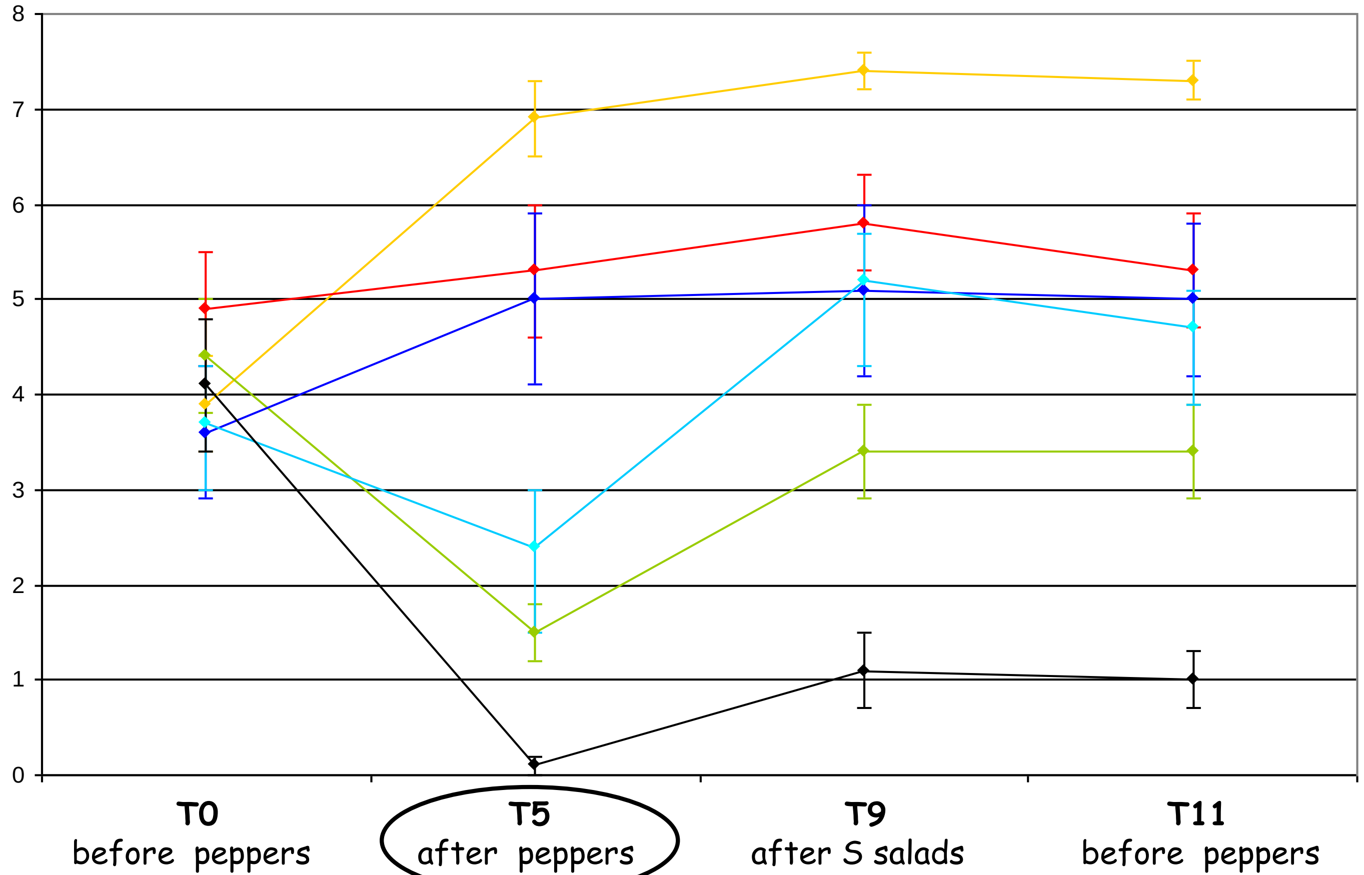
Mean of gall index (0 to 10) per susceptible tomato plant inoculated with 1kg of soil (IC5%)



Example of results from the experimental station

Soil infestation (IS) 8 to 9 replicates

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



Root infestation at T5 (IR: 0 to 10)
40 to 45 replicates

S pepper DLL : IR5 = 9

Me1/- : IR5 = 1,5

Me3Me3 : IR5 = 1

Me3Me3 + *Me1Me1* : IR5 = 0,3 / *Me3Me3*

Me1Me1 : IR5 = 0

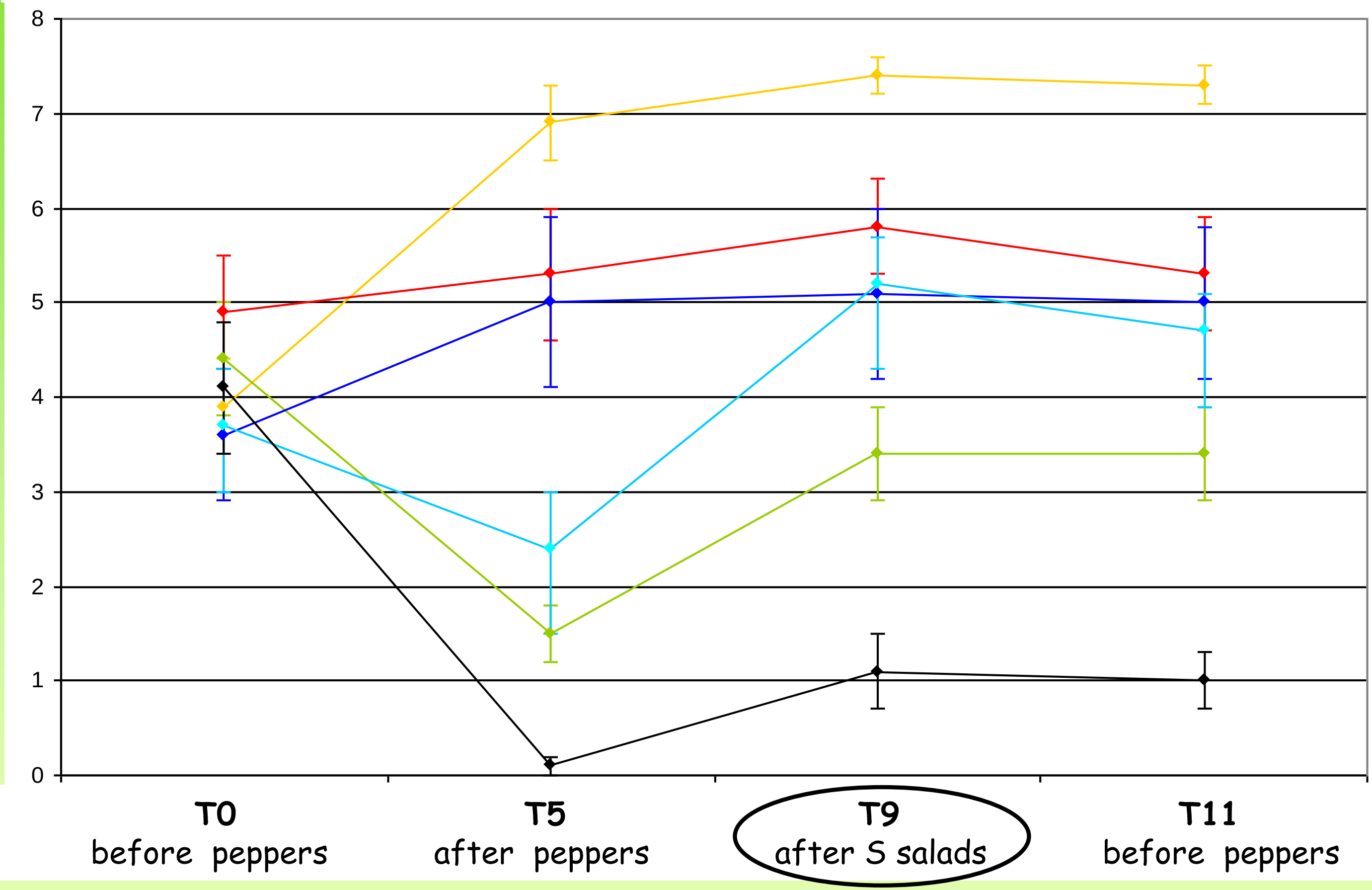
Me3Me1 : IR5 = 0

- R pepper *Me3Me3* and **F1 hybrid *Me1* x S pepper DLL** could be overcome
- R pepper *Me3Me3* seemed protected by R pepper *Me1Me1*
- R peppers *Me1Me1* and *Me3Me1* not overcome and strongly reduce the IS

Example of results from the experimental station

Soil infestation (IS) 8 to 9 replicates

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



Root infestation at T5 (IR: 0 to 10)
40 to 45 replicates

S pepper DLL : IR5 = 9

Me1/- : IR5 = 1,5

Me3Me3 : IR5 = 1

Me3Me3 + Me1Me1 : IR5 = 0,3 / ***Me3Me3***

Me1Me1 : IR5 = 0

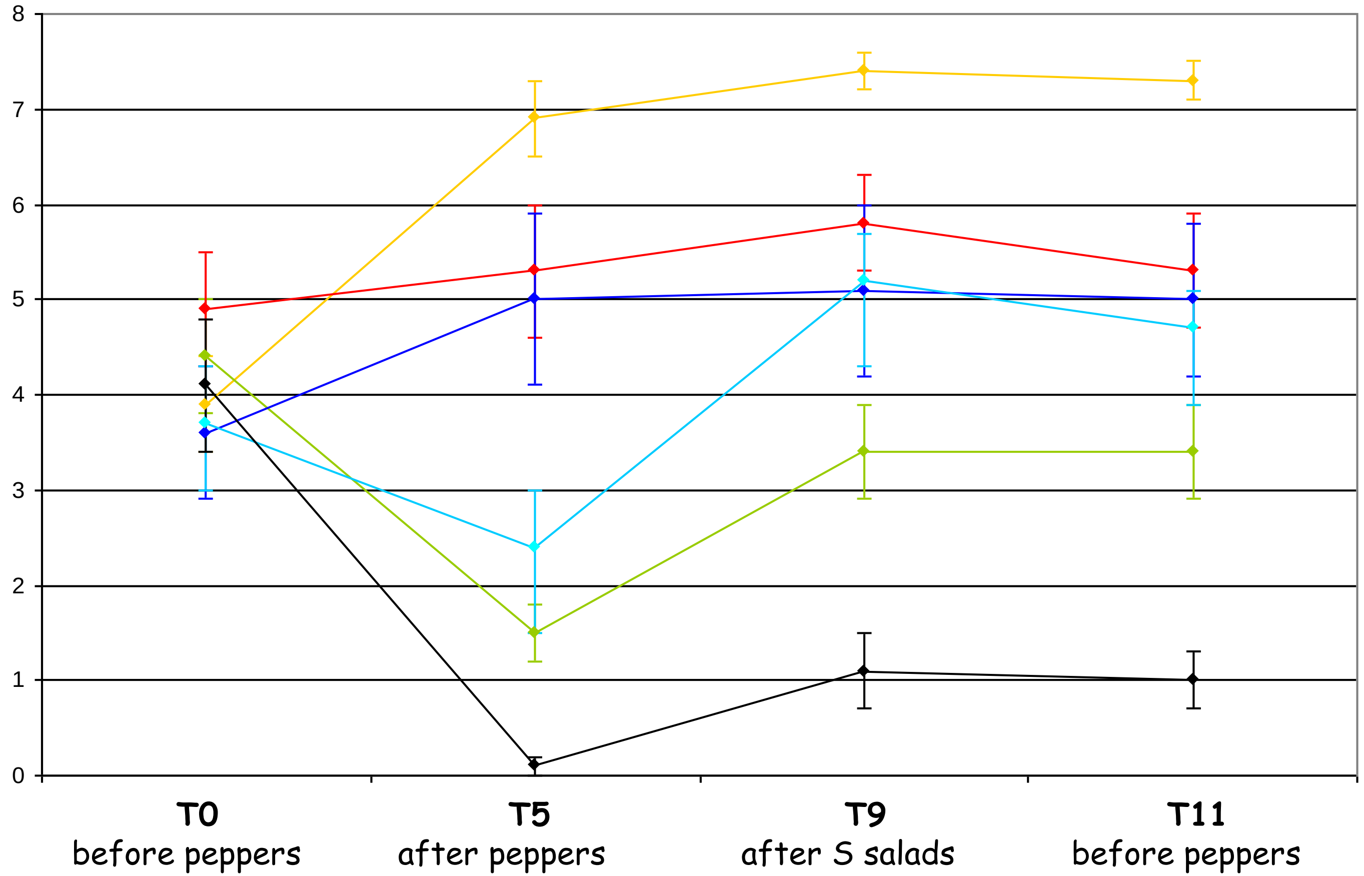
Me3Me1 : IR5 = 0

- R pepper ***Me3Me3*** and **F1 hybrid *Me1* x S pepper DLL** could be overcome
- R pepper ***Me3Me3*** seemed protected by R pepper ***Me1Me1***
- R peppers ***Me1Me1*** and ***Me3Me1*** not overcome and strongly reduce the IS

Example of results from the experimental station

Soil infestation (IS) 8 to 9 replicates

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



Root infestation at T5 (IR: 0 to 10)
40 to 45 replicates

S pepper DLL : IR5 = 9

Me1/- : IR5 = 1,5

Me3Me3 : IR5 = 1

Me3Me3 + *Me1Me1* : IR5 = 0,3 / *Me3Me3*

Me1Me1 : IR5 = 0

Me3Me1 : IR5 = 0

- R pepper *Me3Me3* and **F1 hybrid *Me1* x S pepper DLL** could be overcome
- R pepper *Me3Me3* seemed protected by R pepper *Me1Me1*
- R peppers *Me1Me1* and *Me3Me1* not overcome and strongly reduce the IS

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)

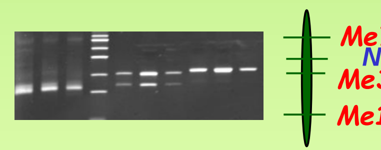
Dr Alain Palloix
Anne-Marie Sage-Palloix (ing)
Ghislaine Nemouchi (technician)

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

Dr Sergio Molinari, Aurelio Cianco (techn)

✓ **IVF,** Institut of vegetables and flowers (Beijing, China)

Dr Lihao Wang



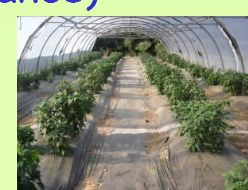
✓ **IRD**, CBGP (Montpellier) Maroc/Azura

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