

# Covered crop Hygiene

Thank you for inviting me

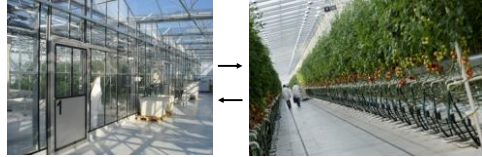


28 July 2015

Ines van Marrewijk  
Groen Agro Control

# Ines van Marrewijk

- For 20 years in horticulture
- For 6 years in research and analyses:
  - Residues of pesticides
  - Fertilizers
  - Microbiology: diagnosis and monitoring
  - Expertise in plant damage cases
  - Research in diseases



# Groen Agro Control

Research, diagnosis and treatments on:

- Water projects for lower emission and good water
- Hygiene and risk analysis
- Pepper: Fruit inner rot (Fusarium), Fruit stem rot (Erwinia), Phytophthora capsici
- Rose: Agrobacterium tumefaciens

### Topics today

- Hygiene related to Crazy Roots
- Virus and disinfectant for hands (cu green mottle virus)
- Fusarium Fruit inner rot in capsicum
- Cleaning before disinfecting, products & concentrations
- Extra's: Phytophthora infestans, Fusarium solani, Verticillium, ToMV, Viroids, GOOD water, White fly, truss stem fungi, Clavibacter

# Disease triangle

### Infection pressure

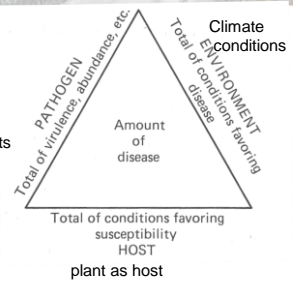
- Bacteria like
- Viruses
- Fungi
- Insects, Nematodes, Etc.

### Can be influenced by:

- Crop protection agents
- Hygiene

### Know how it is spread:

- By air, rain, wind
- By splashing water
- On people, animals and plants
- In living or dead plants
- In the soil
- In or on insects
- By seeds
- By nematodes
- By mechanical means
- Materials etc.



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# Hygiene related to Crazy Roots

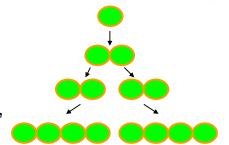


I do not mention it any further, but recirculations of water without UV of Heating it is not safe for the long run

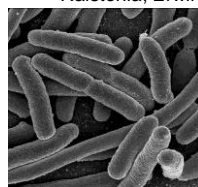


# Bacterial diseases in crops, more and more

- One cell
- Fast reproduction
- Mostly wet rotting spots
- E.g. Clavibacter, Xanthomonas, Pseudomonas, Agrobacteria, Ralstonia, Erwinia



1 bacteria can develop in 24 hours to 8 million bacteria



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## Root Mat (Crazy Roots)



## Development in time

- 1985 UK in Cucumber (own propagation)
- Netherlands 1st: 2002 cucumber, 2006 tomato and eggplant
- 2008: 15 growers 1 propagator
- 2009: 30 growers
- 2010: 50 growers known, but probably about 100 infected
- 2015: >50% of growers
- Not yet known in open field crops, and soil glasshouse crops
- Belgium, Germany, Mexico, N-France, Spain, N-America, Poland, Greece, ...NZ...

## Root mat disease



- Disease organism: *Agrobacterium rhizogenes*
  - infection through wounds on roots
  - wide host range
  - bacterium survives in soil (>15 years) and water
  - spreading of bacterium by water, roots, soil, hands

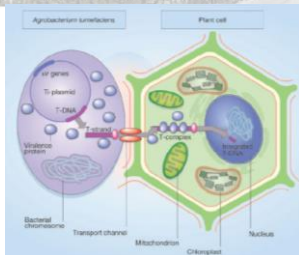
## Agrobacterium



*Agrobacterium tumefaciens*  
 Galls = not really harmful

*Agrobacterium rhizogenes*  
 Root mat disease

## Plasmid determines pathogenicity



Pathogenic properties lie on plasmid (circular DNA). Genetical modification of the DNA of the plant stays.

- Ti-plasmid: hormonal shift → galls
- Ri-plasmid: hormonal shift → root mat disease

## Infection



- Via wounds:
  - Mechanical
  - little Pythium damage

## Symptoms in crop



- Excessive rooting → O<sub>2</sub> shortage → Pythium wilting
- More root tips more → cytokinin → more vegetative crop
- Bad fruit setting, misformed fruits

## Damage in tomato crop

- Sometimes up to 90% of plants infected. Or 1%
- Production loss up to 3 kg/m<sup>2</sup>
- Irregular crop → difficult to control and more labour
- Cocktail and cherry most affected

Cucumber: max. 14 weeks crop



Egg plant: not fruits, dead plants



## Infection sources

Research Groen Agro Control 2009 in NL

- Roots
- Substrate, water pipes and (drain) water silo's
- Detected in:
  - Root environment
  - Soil in greenhouse with RMD
  - Drain water
- Not detected in:
  - Soil samples outside greenhouse
  - Starting (fresh) water
  - Dust on roof greenhouse
  - Stems of infected plants (+ in 3cm)

## Suppressing: in the crop

- **Suppress spreading**
  - Disinfect drain water
  - 240mJ/cm<sup>2</sup> UV; 99% efficiency
  - Heater: kills bacterium but plasmid??
- **Suppress symptoms**
  - Cleaning product in water:
    - Na-hypochlorite: 5 ppm max at dripper
    - Peroxide: 15 ppm max at dripper
  - Lowering pH (pH 5.0 drip). Bacteria do not like low pH
  - Prevent root damage (no entry of bacteria)
  - Control of Pythium



## Handling of problems

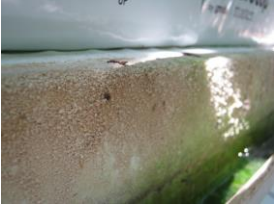
- Chloride in water keeps "root-free" place on block
- Replace dripper to other place or beside block
- Remove foil from block or lift foil up
- Extra drain hole in slab for drainage (last option)
- Generative water management and climate control on plant
- Remove extra leaves
- 'Every' plant with RMD gets an extra stem (tomato)
- Remove roots from drainage system

## Greenhouse clean out

- Gutters: First water, high pressure, brush. Then: disinfect
- Dripper (+tube): keep in pH 1 for 24 hours
- Disinfect also drain system underground
- Empty silo's, remove sludge and disinfect inside
- Maintain and control disinfectant and filters
- Beware of clean equipment of contractor
- Clean also outside and warehouse before deliveries come
- Full field foil to avoid dust from soil, cover gutter
- Delivery of new substrate, do not store on own location
- Chloride, peroxide, per-acid are effective
- 100% clean substrate (do not store in dusty area/hal)



## Risky places For RMD and other diseases



Gutter with rough surface  
Need some ACID for cleaning



Tube to drainsystem  
Difficult to reach

## Risky places



Open soil without foil covering  
DUST -> bacteria everywhere



Dripper: is NOT clean  
Remove -> dipping in pH 2-3

## Risky places



Empty silo's, sludge out, and disinfect

## Risky places



Drain water from slab does not flow away

## Special symptoms



Cut one GOOD drain hole

Perlite drain-holes under slab -> roots under slab, in gutter

## Special symptoms



Rootstock + variety -> NOT yet found a difference

## Special symptoms

Green Agro Control  
AGROCONTROL



Dry surface of block → symptoms IN slab and less ON block

## Special symptoms

Green Agro Control  
AGROCONTROL



Galls on roots and on graft.  
 Perhaps other disease?

## Special symptoms

Green Agro Control  
AGROCONTROL



Active chloride/peroxide in water → symptoms IN slab and less ON block

## Special symptoms

Green Agro Control  
AGROCONTROL



Thick stress-roots in wet substrate are inefficient

## Special symptoms

Green Agro Control  
AGROCONTROL



Water flows over the block → dark/green line on foil  
 Take measures, else shortage in water

## A.r.: Analysis and research

Green Agro Control  
AGROCONTROL

- DNA analysis on DNA is plasmid specific
- Bio-PCR DNA method A.r.: Present or Not Present
- Quantitative DNA-method: infection pressure
- (Analysis on general Agrob is not good enough)

### Research

- Mostly on reducing symptoms, oxidators
- BUT solution lies in crop rotation
- Adding biologicals (antagonists) that influences:
  - Infection
  - Bacterial growth
  - Root growth

# Cucumber Green Mottle Virus

- Better disinfectant for hands than skim milk
- Mix of cucumber plant juice with virus + disinfectant
- Bio test on plants
- Growth for 2 weeks -> Elisa test on virus



# Used are....

All "milk" products: solution of 3,5% protein (eiwit in table)  
Need to be low fat, else not active

Type	Gegevens	Oplossen per 1L	Conc	Eiwit g/L
Magere melk	puur (3,5 g eiwit / 100 ml)	nvt	3,5% eiwit	35
Melk Poeder	poeder ca.35% eiwit (35g/100gr)	100 gram / L	3,5% eiwit	35
Wei-eiwit	poeder 129g eiwit /kg (12,9%)	271 gram / L	3,5% eiwit	35
VirkonS	poeder	10 gram / L	1% VirkonS	-
Water	controle met water als middel	-	-	-

Virkon in 1% Cowmilk = 3,5% protein  
= 80% caseine 20% whey-protein

# VirkonS colour after adding juice

Although colour is green, it still works. But pink is good indicator



plantsap:middel	plantsap:middel	plantsap:middel	plantsap:middel	plantsap:middel	plantsap:middel
1:100	2:100	3:100	5:100	10:100	50:100
ok	ok	ok	ok	ok	besmettelijk

# Werkzaamheid middel

Skim milk already "out" with 2 % juice 98 milk: NOT OK  
Milk low-fat milk powder and whey protein GOOD

Virkon S is best up to 50%/50% working: PERFECT

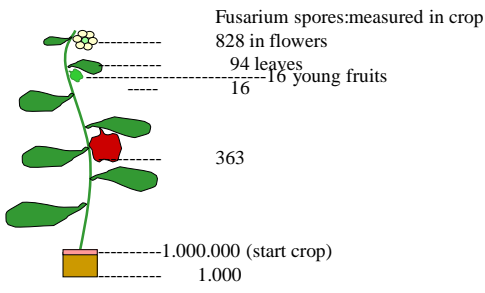
Middel	sap/middel	Elisa	Plant	Uitslag
Magere melk	1 / 99	+ sv	ok	ok
	2 / 98	+ sym	besmettelijk	ok
	3 / 97	+ sym	besmettelijk	ok
	5 / 95	+ sym	besmettelijk	ok
	10 / 90	+ sym	besmettelijk	ok
Melk Poeder	50 / 50	+ sym	besmettelijk	ok
	1 / 99	+ sv	ok	ok
	2 / 98	+ sv	ok	ok
	3 / 97	+ sv	ok	ok
	5 / 95	+ sv	ok	ok
Wei-eiwit	10 / 90	+ sv	ok	ok
	50 / 50	++ sv	besmettelijk	ok

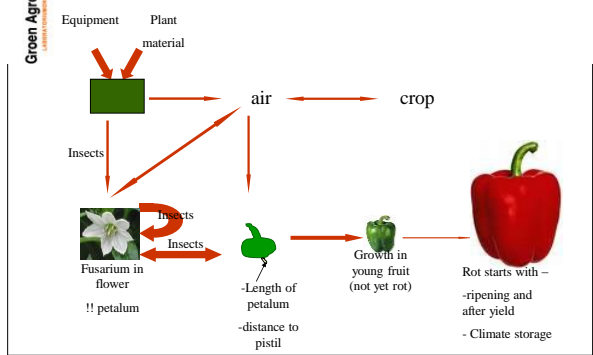
Middel	sap/middel	Elisa	Plant	Uitslag
VirkonS	1 / 99	- sv	ok	ok
	2 / 98	- sv	ok	ok
	3 / 97	- sv	ok	ok
	5 / 95	- sv	ok	ok
	10 / 90	- sv	ok	ok
Controle	50 / 50	+ sym	besmettelijk	ok
	1 / 99	+ sym	besmettelijk	ok
	2 / 98	+ sym	besmettelijk	ok
	3 / 97	+ sym	besmettelijk	ok
	5 / 95	+ sym	besmettelijk	ok
50 / 50	+ sym	besmettelijk	ok	
	+ sym	besmettelijk	ok	
	+ sym	besmettelijk	ok	

sv= symptoomvrij sym=symptomen virus

# Fusarium Inner rot: Capsicum



# Inner Rot: what items play a role





# 1: cleaning by hand and water

- Crop: do not shredder when having virus of bacteria
- Gutters: remove all 'green' stuff with water + high pressure + brush + water



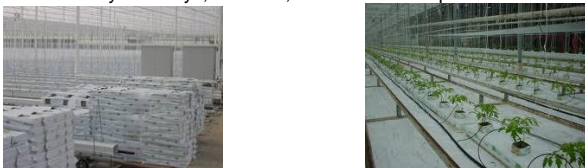
# 2: Disinfect

- Reinigen kas met middel, evt herhalen -> inwerktijd
- Dompelen: stekers, slangetjes, slangen (zuur pH 1)
  - Goten: water+middel afspuiten
  - Drainafvoersysteem, koppelstukken, drainput
  - Ruimte: Formaline of H2O2+perazijnzuur
  - Karren e.a materiaal ontsmetten



# 3: Prepare for new crop

- Clean up the yard and barn
- Disinfectant slabs at the ENTREE of plants, people
- New substrate in when ALL is clean
- Slabs in – making plant holes – fill up slabs
- Tapes of drippers in, etc etc
- 2 days before planting spray full area with low concentration Chloride
- Trolley and trays; clean IN, and as clean as possible OUT.



# Antagonist active or not?



- MycoStop (ECOstyle) 0,1% on medium,
- Should do something against Fusarium
- After 14 day...
- MycoStop is Streptomyces griseoviridis Strain K61 a micro-organism.
- Only some action
- Nopath (Koppert) 0,5% in medium
- After 14 day
- First days very active.
- After 8 days stopped
- Nopath is plant oil (mint?)

# Hygiene

Do not pass .. Unless disinfect

Clean?



# Cleaning and disinfect

Name	Bacteria	Fungi	Virus	pH 123456789	Disinf In-org.M	Disinf. Organ. M
Per-acids (Jet 5)	++	+	+	➡	+/-	+
Quaternair ammonium (Menno ter forte)...3h/-	++	-	-	█	+	++
Benzoic acid (menno clean)	+	+	+	█	+	+
Alcohol 70%	++	+	+		-	-
Virkon S	++	++	++		-	?



## Cleaning and disinfect

Name	Bacteria	Fungi	Virus	pH 1 2 3 4 5 6 7 8 9	Disinf. In-orgM	Dininf. Org.M
Peroxide (+ acid ) Easy clean, horti clean )	+	+	+	▶	-	+
Sodiumhypochloride	++	+	+	▶	-	++
Fluor (glass etching)	--	--	--			
Formaline	++	+	+	▶	-	++
Strong Acids, Bases	++	+	+	■	■	+

## Concentration oxidator: Chloride

Notice that you need to use the same units for A and B  
Do not make A ppm and B % or vica verca

making a solution Peroxide		please enter amounts
needed volume of chemical	1000	(ml)
Concentration chemical for dilution (packing)	12,500	%(ppm)
needed concentration after dilution	0,001	%(ppm)
necessary volume chemical needed	0,08	(ml)
necessary amount of water needed	999,92	(ml)

In case of sodium hypochlorite for example, please realize that the chemical you start with is 12,5% or 125000 ppm. A few suppliers sell different concentrations. E.g. 15% (or 4% household bleach)

De formule:  $\{(concentratie\ q * ml\ B) / concentratie\ p\} =$  hoeveelheid A die verdund moet worden

Maximum input at unit	10-15 ppm	5000 ppm is 0,5%
Maximum out op drripper, young plant	2 ppm	1000 ppm is 0,1%
Maximum out of drripper, >2 month plant	5 ppm	100 ppm is 0,01%
2 days before planting, greenhouse/slabs/gutter	50 ppm	10 ppm is 0,001%
Disinfecting drainsystem/silo: flush with water after	500 ppm	Always first CLEAN with water
Disinfecting greenhouse	50-100 ppm	Always first CLEAN with water

## Concentration oxidator: Peroxide

Notice that you need to use the same units for A and B  
Do not make A ppm and B % or vica verca

making a solution Peroxide		please enter amounts
needed volume of chemical	1000	(ml)
Concentration chemical for dilution (packing)	35,000	%(ppm)
needed concentration after dilution	0,001	%(ppm)
necessary volume chemical needed	0,03	(ml)
necessary amount of water needed	999,97	(ml)

Peroxide: mostly buy 35%

is result, do not change

Maximum input at unit	20-30 ppm	5000 ppm is 0,5%
Maximum out op drripper, young plant	5 ppm	1000 ppm is 0,1%
Maximum out of drripper, >2 month plant	10 ppm	100 ppm is 0,01%
Disinfecting concentration	50-100 ppm	10 ppm is 0,001%

## Concentrations oxidators

Calculation tool for making the right dilution  
And always keep checking:

- Volume water given : used volume oxidator
- Quick check with reagent or colouring paper (Mercq)
- Plant growth
- Germ count (before and after adding ppm)

## Thanks for your attention! Work together with pleasure, and stick to your well prepared plans

Extra's: GOOD water, White fly, Truss stem rot tomato, TMV, Erwinia Fruit stem rot capsicum, Fusarium, Clavibacter

## Research GOOD water

Limits drip water: why	Parameter: Chemical	Specific	From	Effect on crop
Root exudate	Salicylic acid	Roots	Roots	not known
Root exudate	2,4-Dichloorbenzoë-acid	Roots	Roots	not known
Organic matter-all	Rest material after UV	MicroBio, org matter	Rockwool	not known
Wetting Agent	Grodan Plantop	Rockwool	Rockwool	Possible growth reduction
Heavy metal	Nickel	Ca generator	Ca generator	damage at high conc
Heavy metal	Chrom	Ca generator	Ca generator	damage at high conc
Heavy metal	Aluminium	Ca generator	Ca generator	damage at high conc
Biocide	Fluor	Crop rotation (Glass)	Crop rotation (Glass)	partly known
Biocide	Peracetic-acid	Crop rotation	Crop rotation	partly known
Biocide	Formaldehyde	Crop rotation	Crop rotation	partly known
Parameter: Biologic	Specific	From	Effect	
germ count	Bacteria Fungi	Micro	O2 and antagonism	





## Trial with mini units



## Result GOOD water

Limits and practical values in dripwater			
For cucumber, tomato, pepper			
Parameter: Chemical	Specific	Unit	Praktice Limit
Root exudate	Salicylic acid	mg/l	<0,05 7
Root exudate	2,4-Dichlorobenzoë-acid	mg/l	<0,05 1
Heavy metal	Nickel	ug/l	1-20 3.000
Heavy metal	Aluminium	ug/l	5-200 25
Heavy metal	Chrome	ug/l	1-20 250
Biocide	Fluor	mg/l	0,2 1
Biocide	Paraselsic-acid	mg/l	0,5 1
Biocide	Formaldehyde	mg/l	0 5
Biocide	Peroxide	mg/l	5 10
Biocide	Sodium Hypochloride	mg/l	2 5
Gas in water	Oxygin (not influenced by T)	%	60-100 >80
Gas in water	Oxygin (influenced by T)	mg/l	2-12 >6
Gas in Bore-water	Methane (CH4)	mg/l	0,1-12 0,2
Parameter: Biological	Specific	Unit	Praktice Limit
Organic matter	Sterile from drain	gl	<1 2
Germ Count	Bacteria	kve/ml	100.000 500.000
Germ Count	Fungi	kve/ml	<10 20



## White fly

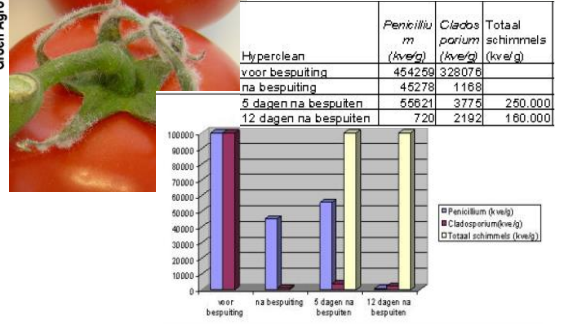
Chemicals.. Bio  
New: Mycotal+Addit eggs and larve.  
Side effect on spidermite

Admiral	Pyriproxiifen	11828 N
Admira	Imidacloprid (! Bij spu)	11483 N
Botanigard WP	Beauveria bassiana	12612 N
Savona	Organische zuren	nub lijst
Mycotal	Lecanitolium muscarum stam Veg	10989
Calypso (regen larven)	Thiacloprid	12452 N
Carax	Pyridaben	11101 N
Decis EC	Deltamethrin	7774 N
Kohinor 70 WG (larven)	Imidacloprid (! Bij spu)	13831 N
Normolt (larven)	Teflubenzuron	9914 N
Oberon	Spiromesifen	12588 N
Pierum 50 WG	Pymetrozine	12491 N
BrifeFall	Paecilomyces F. (bio)	12694 N

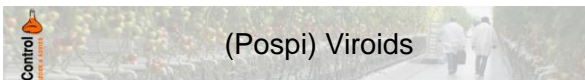
DELPHIBUG  
Encarsia formosa  
ENERMIX  
Encarsia formosa  
EN-STRIP  
Encarsia formosa  
+ Eretmocerus eremicus  
ERCAL  
Eretmocerus eremicus  
LIMONICKA  
Amblydromalus limonicus  
(=Typhlodromalus limonicus)  
MIRICAL  
Macrolophus pygmaeus  
(= Macrolophus caliginosus)  
SAVONA  
MYCOTAL  
Verticillium lecanii-m  
SWIRSKI-MTE  
Amblyseius swirskii  
SWIRSKI-MTE LD  
Amblyseius swirskii (takes time)  
SWIRSKI-MTE PLUS  
Amblyseius swirskii



## Tomato truss stem



Fungi: Penicillium, Cladosporium, other fungi

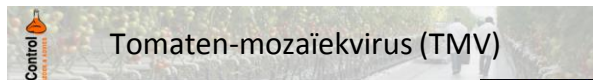


## (Pospi) Viroids

- Smallest plant pathogen, smaller than virus
  - Is only some genetic material
  - For reproduction depending on host plant.
- PSTVd potato spindle tuber viroid**
- In EU in Petunia, Physalis, tomato and Solanum jasminoides
  - Q-organism because of potatoes in EU very !!
  - "Bushy" top of the plant
  - Can be asymptotically in ornamental plants
  - Yellowing, necrotic spots, shrinking leaves



Solanum jasminoides  
Solanaceae, ornamental  
PSTVd in tomato (left) and healthy (right)



## Tomaten-mozaïekvirus (TMV)


TomV (TMV): Resistant varieties

Infection sources, highly contagious:

- Seeds
- Soil / Substrate not well disinfected / recirculation-water
- Crop handling, equipment, clothing, hands etc
- Can contaminate a whole glasshouse within some weeks
- Host plants: - tomato, tabacco, pepper. - Petunia, delphinium
- Symptoms: different per virus-strain
  - Colored spots on leaves, needle-like leaves (*herbicide*)
  - Black/brown necrotic spots on stem and leaves
  - Misshapen fruits (bubbling, sunken spots)
  - Stunted growth, bad development of roots
  - Depending on: Temp, age, day lenght, lightsum/day




## Tomaten-mozaïekvirus (TMV)



Tomato Mosaic Virus      Herbicide Injury

© P. H. Aggenbroek, Plant & Organism, Wageningen UR

## Pepper fruit-stem rot



Erwinia stem rot      Fusarium rot on cut

Rhizopus      Penicillium

**Erwinia stem rot**

- Soft crop/stem
- Damage at yield
- Damage in container
- Dirty container (Erwinia) and machines

**Plant-Stem rot Tomato**

- Erwinia
- Xanthomona
- Pseudomonas
  - P. syringae
  - P. corrugata
- Open wounds
- Hands, knives

**Pseudomonas syringae pv. Actinidiae (PSA) kiwi**

- !! Spore by air

## Fusarium foot rot


- *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL)
- Spores spread through water and air
- Symptoms: root and foot rot; brown vascular bundles



Spores (micro- and macroconidia, 100x)      root rot      foot rot      brown vascular bundles


## Fusarium wilting disease

- *Fusarium oxysporum* f.sp. *lycopersici* (FOL)
- Growth via roots to vascular bundles
- Spores spread through water and air
- Symptoms: browning vascular system, hollow stem, wilting
- Resistant tomato cultivars exist



## Fusarium solani: Foot rot


- Spores spread: air, water, seed. Optimal T 28°C
- Spores survive >1 year.
- Can affect *F. oxysporum lycopersici*-resistant plants
- Symptoms:
  - dark brown lesions on foot
  - interveinal chlorosis and necrotic spotting on young leaves
  - browning of vascular system



Foot rot, sweet pepper

Perithecia (fruiting bodies)

## Fusarium solani



## Measures against Fusarium

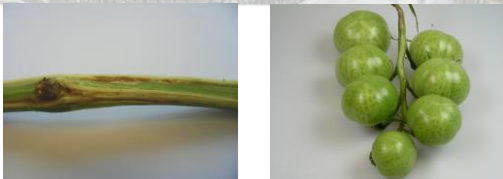
- Use resistant cultivars if possible
- Disinfect seed (high temperature)
- Start with disease-free plant material
- Remove affected plants or parts in plastic bags
- Fungicides..carbendazin (NL not allowed Tom)
- Apply antagonists..\*\*
- Disinfect recirculation water
- Strict hygiene
  - Cleaning equipment
  - Crop handling (gloves, logical order)
  - Employees / visitors

## Clavibacter



- Clavibacter michiganensis* subsp. *michiganensis*
- Bacterial wilt, bacterial canker
- Quarantine organism in EU:
  - import and spreading of infected material is prohibited
  - findings should be notified at national authorities (NI and EU)
  - infected plants should be removed and destroyed

## Symptoms Clavibacter



- Glazy brown-yellow spots on leaves
- Wilting of leaf edges and wilting of whole plant (Verticillium?)
- Browning of vascular bundles
- White netted stripes on unripe fruits
- Significant production loss

## Properties Clavibacter

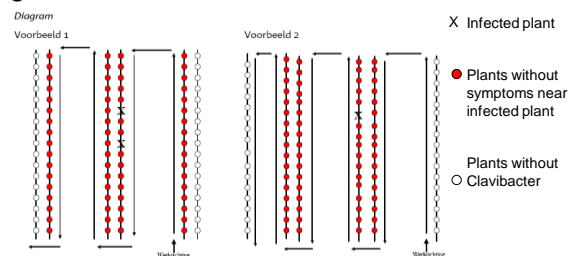
- Spreading via:
  - seed (fast in vascular bundles) Protocols in Seeds and Propagation
  - crop handling (wounds)
  - water
  - visitors
- Bacteria is drought resistant
- Bacteria can survive in soil and substrate up to 8 months
- Optimal climate for disease: high RH and temp. 24-32°C
- Incubation time: about 6 weeks
- Host plants:
  - tomato
  - pepper
  - egg plant
  - weeds (Solanaceae)

## Measures on Clavibacter

- Plants with symptoms
  - remove dripper to dry the plant for 2-3 days
  - remove plants (slabs later) in plastic bags
  - disinfection of whole area
  - re-plant is an option
- Isolated\* area around removed plants (\* 1-3 spots)
  - 1 plant found -> isolate about 4-10 rows
  - one person does all the work in disposable overall..end of day
  - with equipment/material that stays in this area
  - hygiene measures on hands, equipment ....
  - yield: put fruits directly in carton-trays



## Removing infected plants





## Removal of plants

And than.. Rest of crop?

Just good hygiene

- Make a plan for 2 weeks
- Act according to plan
- Check
  - Scouting on symptoms
  - Labour does a good job
  - Plan needs changes



## Hygiene in crop

- Disinfect hands (every 4-10 plants) and shoes
- Use disposable gloves if hands get irritated
- Disposable coats for visitors
- Change and disinfect knives and scissors after each row
- CI+: every 4-10 plants
- No: mobile phones, smoking, tomatoes at lunch

Virkon S: better in white bucket



## Clean crop

- Hygienic measures during crop season
  - Thorough greenhouse clean-out
  - Shall not be found next year
- WHEN cleaning up is done properly

No chemicals are allowed to control Clavibacter in the crop.  
Spraying solutions does not help to prevent the spread Cl.



## Control on Clavibacter

- GSPP certification:
  - Seeds: seed companies are ready
  - Plants: some propagators have certification
- Growers hygiene protocol:
  - Protocol for greenhouse clean-out and in crop
  - Closed-door policy
- Sensitive bio-PCR analysis (Groen Agro Control)
  - Crop with symptoms
  - In propagation without symptoms

