PLANT RESEARCH INTERNATIONAL

Mycotoxin cluster meeting

3 June 2003, Uppsala

Safe organic vegetables: the carrot-Alternaria model

Ruud W. van den Bulk
coordinator Safe Organic Vegetables project

Safe Organic Vegetables

Objective

To develop strategies which ensure SAFE organic food, by

- Anticipating mycotoxin risks
- Tracing sources of contaminants throughout the food chain
- Reducing the risk factors

Safe Organic Vegetables

Carrot - Alternaria as model system

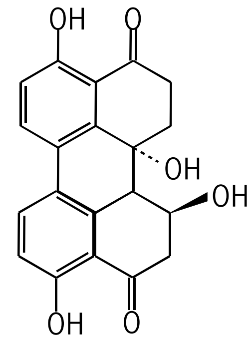
- Carrot is the most popular organic vegetable
- Carrot is a major cash crop for organic farmers
- Alternaria spp. produce various toxic metabolites
- Alternaria occurs throughout the whole production chain of carrots
- Alternaria is difficult to control under organic production conditions

Metabolites of Alternaria spp.

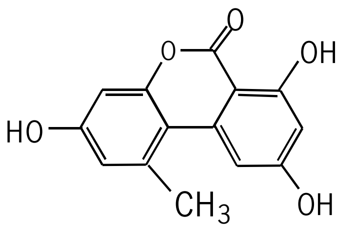
- Over 70 secondary metabolites known
- Carcinogenic + mutagenic properties for culture extracts of *A. alternata*
- Alternariol (AOH), alternariol methyl ether (AME): mutagenic, cytotoxic to bacterial and mammalian cells, suspect carcinogenic
- Altertoxin I (ATX-I): acutely toxic in mice, mutagenic (Ames test), cytotoxic to bacterial and mammalian cells
- Tenuazonic acid (TeA): (sub-)acutely toxic to animal species

Mycotoxins

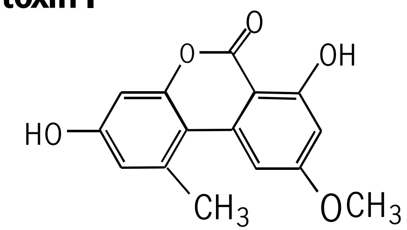
- Altertoxin I
- Alternariol
- Alternariol methyl ether
- Tenuazonic acid
- Radicinin
- Radicinol
  - Fumonisins (FB_1, FB_2)
  - Aal toxins (TA_1, TA_2)



Altertoxin I



Alternariol



Alternariol monomethyl ether

Alternaria toxins in (processed) foods

Fruit	AOH	AME	ALT	ATX-I	TeA	Reference
Apple	+	+	(+)[a]	+	+	77
Apple	+	+	—[b]	—		53
Mandarin	+	+	—	—	+	78, 79
Melon	—	+	—	—	+	78
Olive	+	+	+	—	+	34, 78
Red pepper	+	+	+	—	+	24
Pepper	+	+	—	—	+	78
Tomato					+	80
Tomato	—	—	+			19
Tomato	+	+		—	+	78
Tomato	+	+		—	+	81
Tomato	(+)	(+)			+	32
Tomato	+	(+)	(+)	—	+	77
Tomato	+	+				41
Redcurrant	+	+				41
Raspberry	+	+				41
Strawberry	+	—				41
Gooseberry	+	—				41
Blackberry	+	—				41

[a] (+) = only trace levels and/or very low incidence.
[b] — = toxin looked for but not detected.

Food	AOH	AME	TeA	Reference
Tomato paste			+	31
Tomato products	(+)[a]	—[b]		41
Tomato products			+	21
Tomato paste			+	33
Raspberry drink	(+)	—		41
Apple juice	+	—		40, 69
Apple juice	+	(+)		67
Apple juice concentrates	+	(+)		52
Grape juice, cranberry nectar, raspberry juice, red wine	+	—		67, 69
Prune nectar	+	+		67, 69

[a] (+) = only trace levels and/or very low incidence.
[b] — = toxin looked for but not detected.

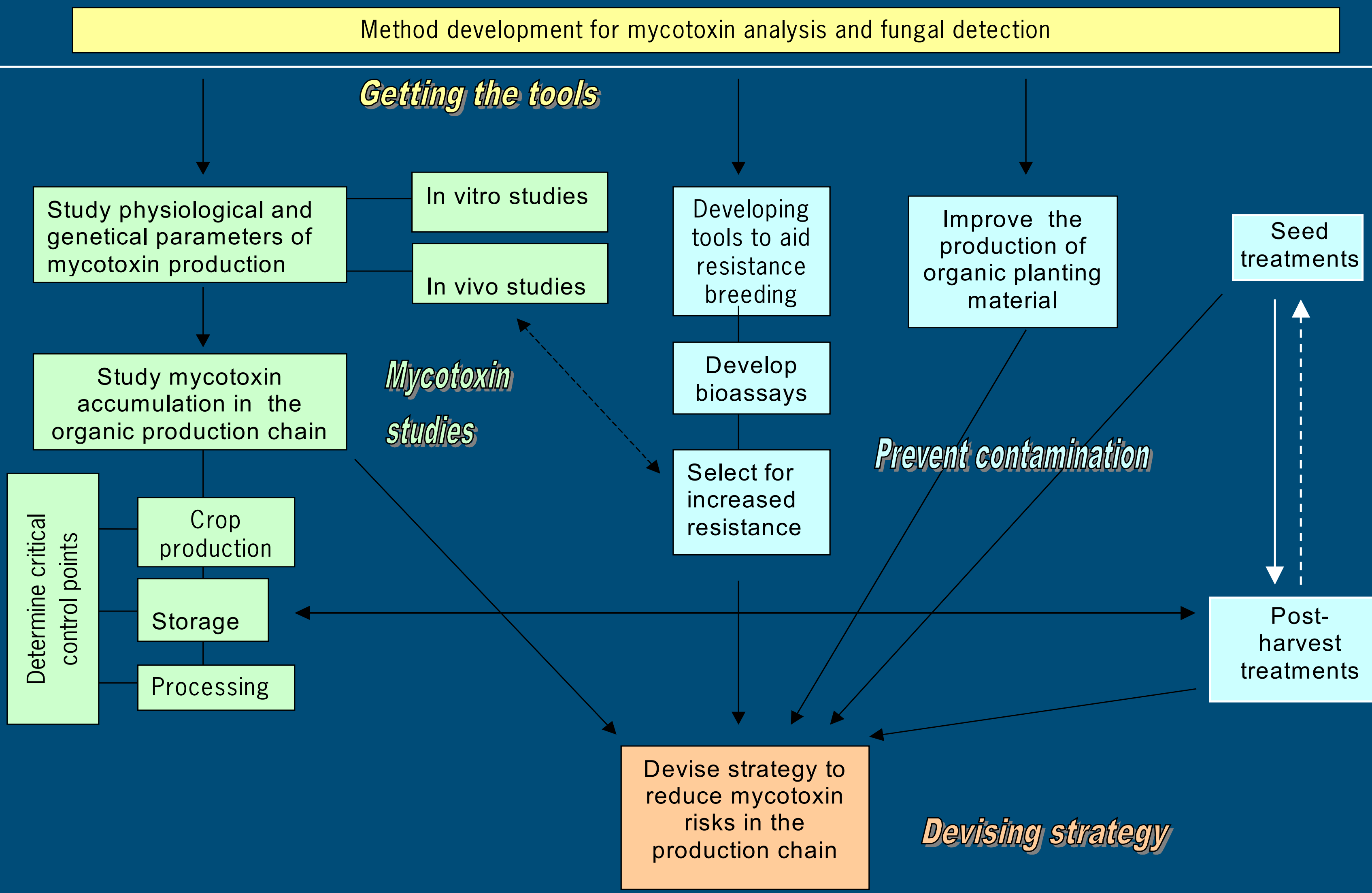
From: Scott, 2001

Alternaria toxins in other foodstuffs

From: Scott, 2001

Foodstuff	AOH	AME	ALT	ATX-I	TeA	Reference
Pecan	+	+				82
Sunflower seed	+	+				62, 78
Sunflower seed	+	+				83
Sunflower seed	+	+			+	84
Wheat, triticale, oats, rye, barley	+	+				85
Wheat, barley,oats	+	+				35
Wheat	+	+	—[a]	—	+	26
Wheat	+	+	—	—	+	25
Wheat	+	+	+	+	+	42
Maize	—	—	+	—	+	42
Barley	—	+	+	—	—	42
Rice plants					+	86
Rice	—	—	+	—	+	42
Wheat bran	+	+	—	—	+	42
Sorghum	—	—	—	+	+	42
Sorghum	—	—			+	26
Sorghum	+	+	(+)[b]	(+)	—	87–89
Sorghum, ragi	—	+	+	—	+	43
Oilseed rape meal	+	+	—	—	+	90
Oilseed rape	—	—		—	—	44, 45

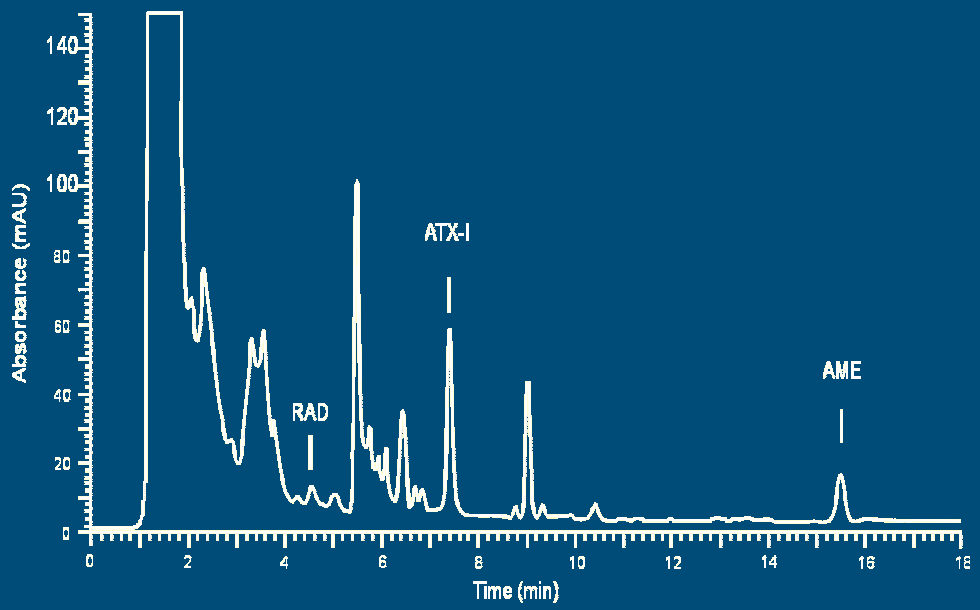
[a] — = toxin looked for but not detected.
[b] (+) = only trace levels and/or very low incidence.



PLANT RESEARCH INTERNATIONAL

Mycotoxin analysis of carrot

- HPLC method in CEN-format available
- Purify by C18 reversed-phase column (RAD, ATX I, AME) or OASIS polymeric reversed-phase column (TeA)



HPLC chromatogram of carrot spiked with 0.5 µg/g of RAD, ATX-I, and AME (UV diode array detector at 256 nm).

Performance characteristics

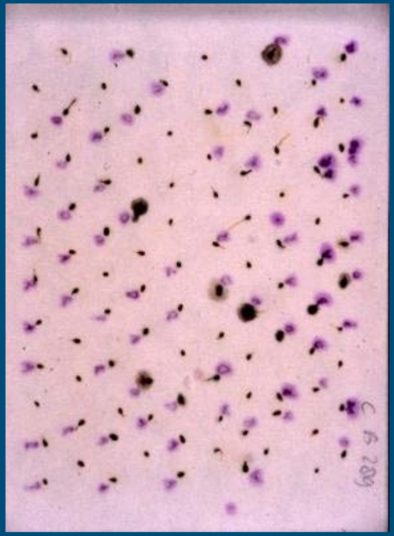
	TeA	ATX-I	AOH	AME	RAD
RICE					
Recovery (%)	87	87	94	86	92
Repeatability (RSDr %)	2	6	3	4	12
Limit of quantification (µg/g)	0.7	0.7	0.3	0.1	0.4
CARROT					
Recovery (%)	69	71	-	91	35
Repeatability (RSDr %)	13	5	-	4	6
Limit of quantification (µg/g)	0.05	0.05	-	0.03	0.01

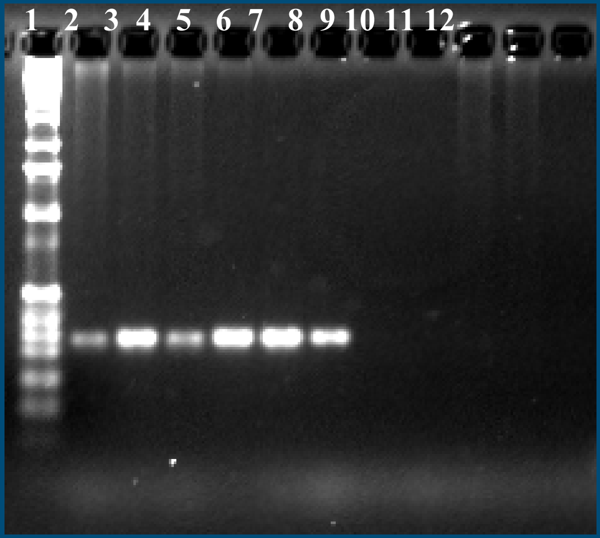
Methods for fungal analysis

Blotter test

Plating on ARSA

PCR



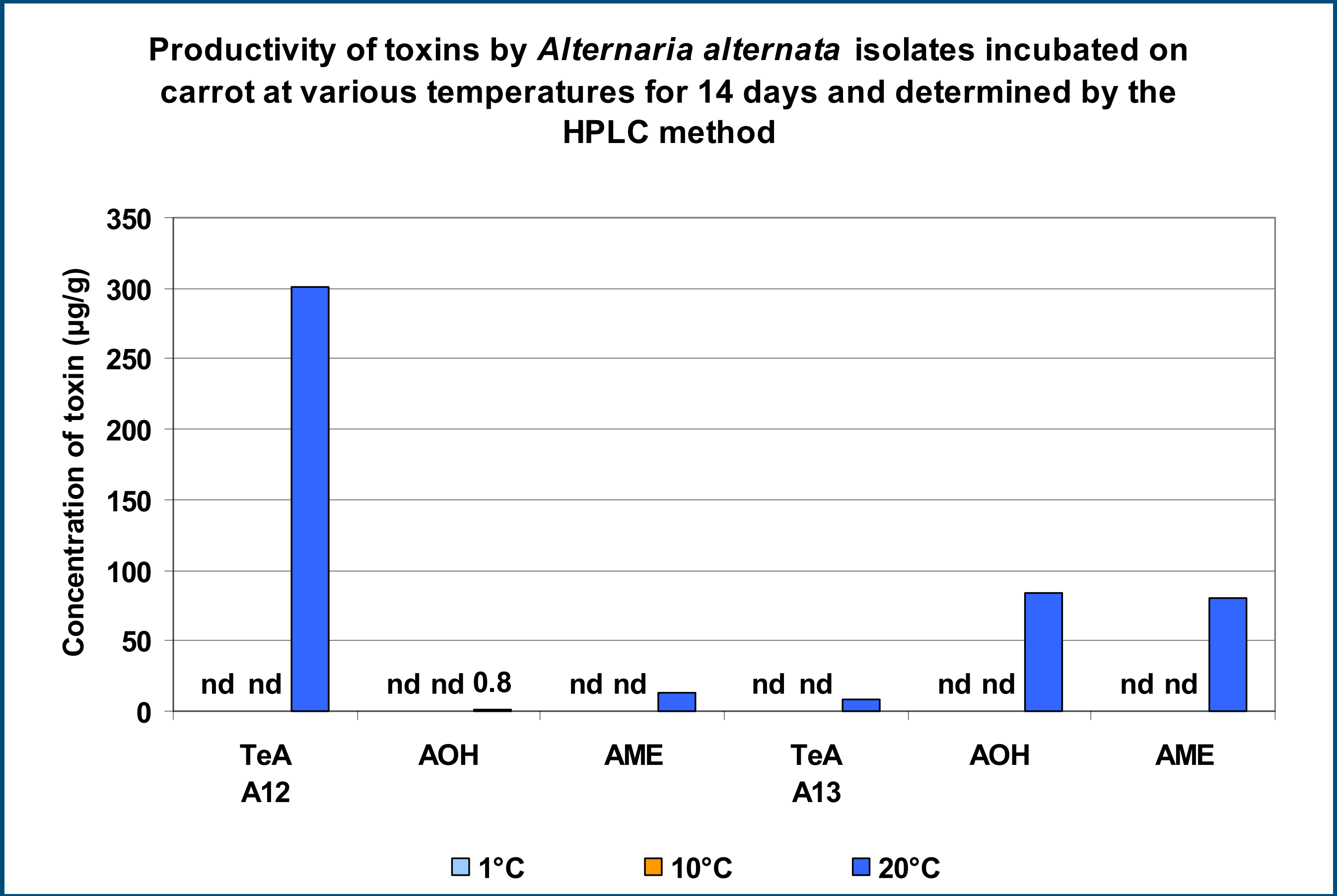


Insight in physiology / genetics of mycotoxin production

Toxigenicity of A. alternata strains on rice grains

Source of strain	Toxin content (µg/g)			
	TeA	AOH	AME	ATX I
Carrot	6283	269	303	12
Wild carrot	2180	254	123	9
Celery	Nd	212	80	31
Parsley	5235	190	111	17

Physiology / genetics of mycotoxin production

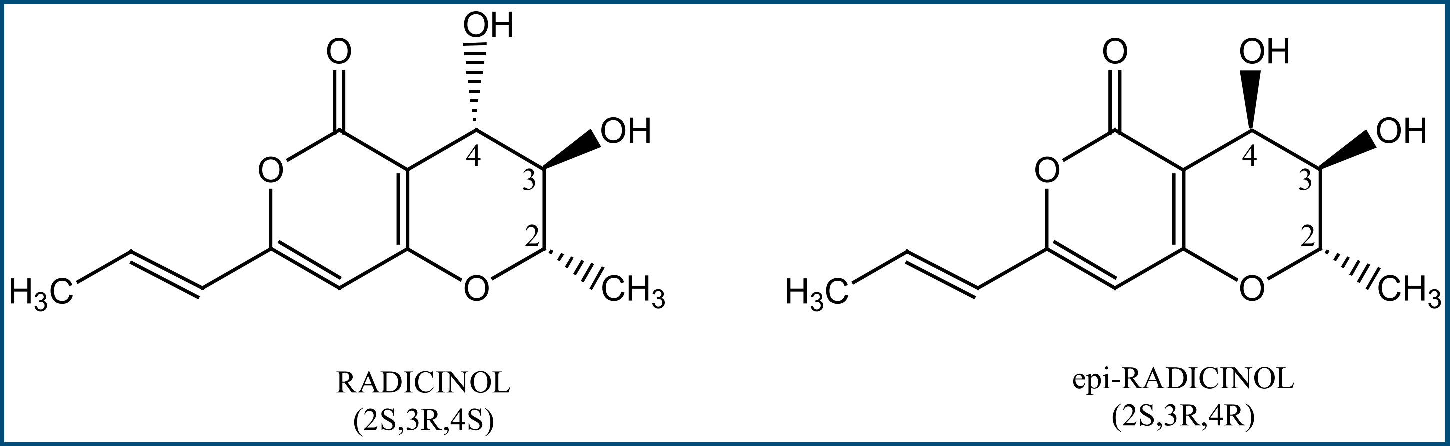


PLANT RESEARCH INTERNATIONAL

HPLC result of A. radicina on carrot

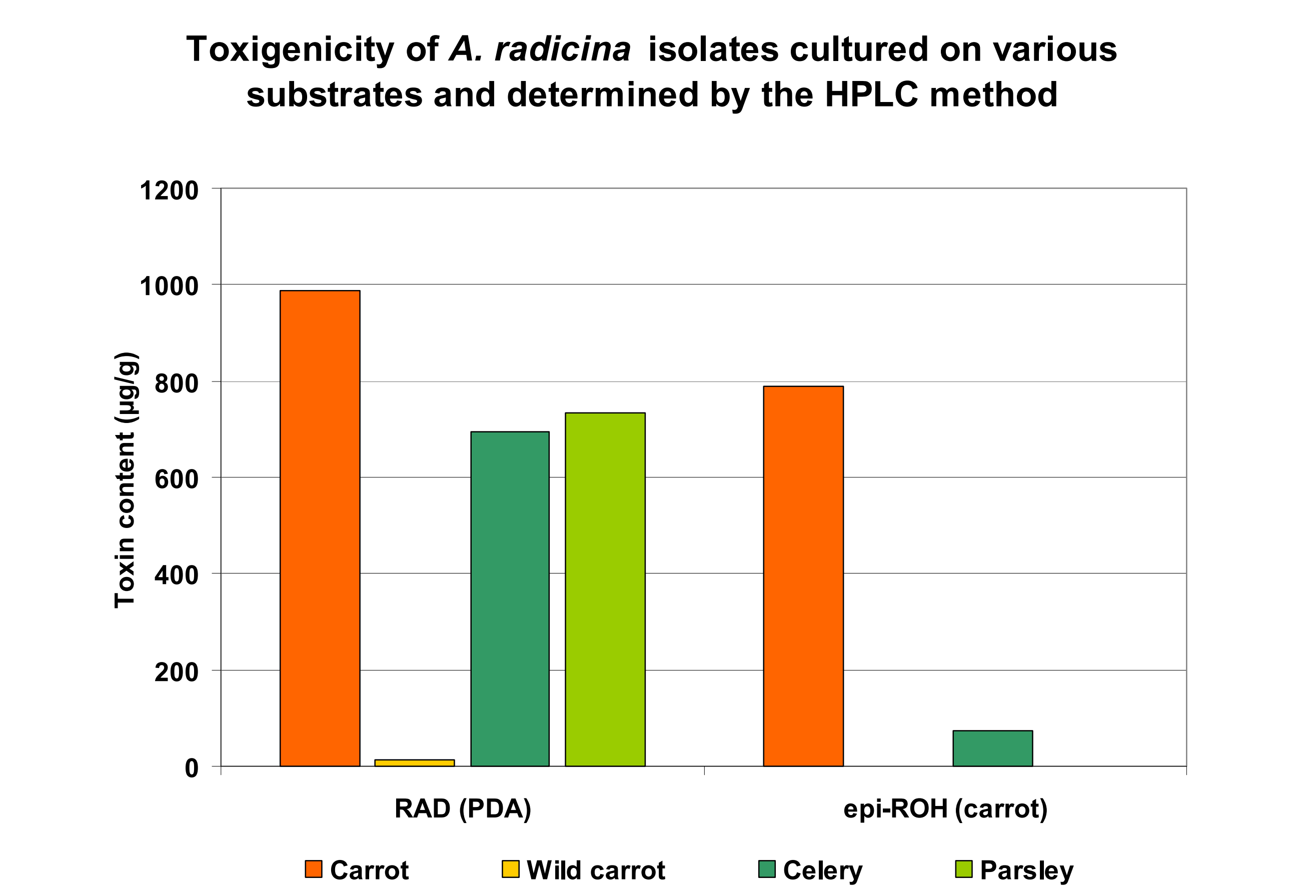
Identification of 2 unknown compounds by reversed phase LC/MS, GC-MS, ^{13}C-NMR, ^{1}H-NMR

- 1. Epi-radicinol, non-toxic in *Artemia salina* and erythroblastoid human cell line test



- 2. Radicinin-like, still under study

Physiology / genetics of mycotoxin production



PLANT RESEARCH INTERNATIONAL

Monitoring fungal infection in the production chain

Fungus (isolated from crown part)	Denmark production			Netherlands production			France production		
	A	B	C	A	B	C	A	B	C
A. alternata	14	10	16	6	5	2	45	15	24
A. radicina	7	10	13	20	55	56	2	1	4
A. alternata + A. radicina	1	1	1	2	1	1	1	0	0

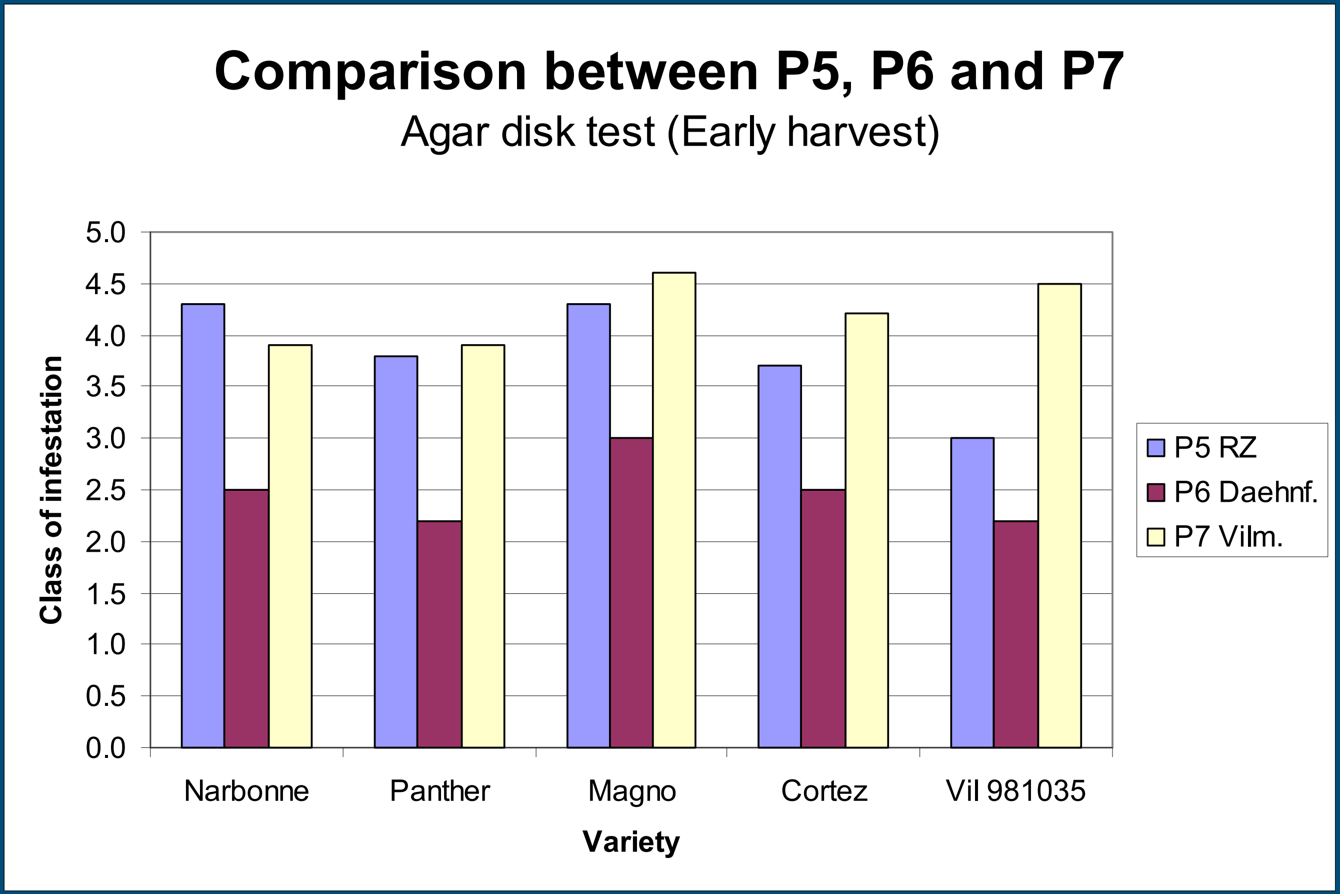
Monitoring mycotoxins in the production chain

- Samples from various stages are scored for fungus, blended, and analysed by HPLC for mycotoxins
- So far, 1 sample out of 109 field samples contaminated with *epi*-radicinol (44 µg/kg)
- 15 A. alternata infected samples (F) showed no contamination
- 2 samples out of 24 A.radicina infected samples were contaminated with RAD and *epi*-ROH (up to 137 µg/kg)
- 62 carrot products tested negative for toxins

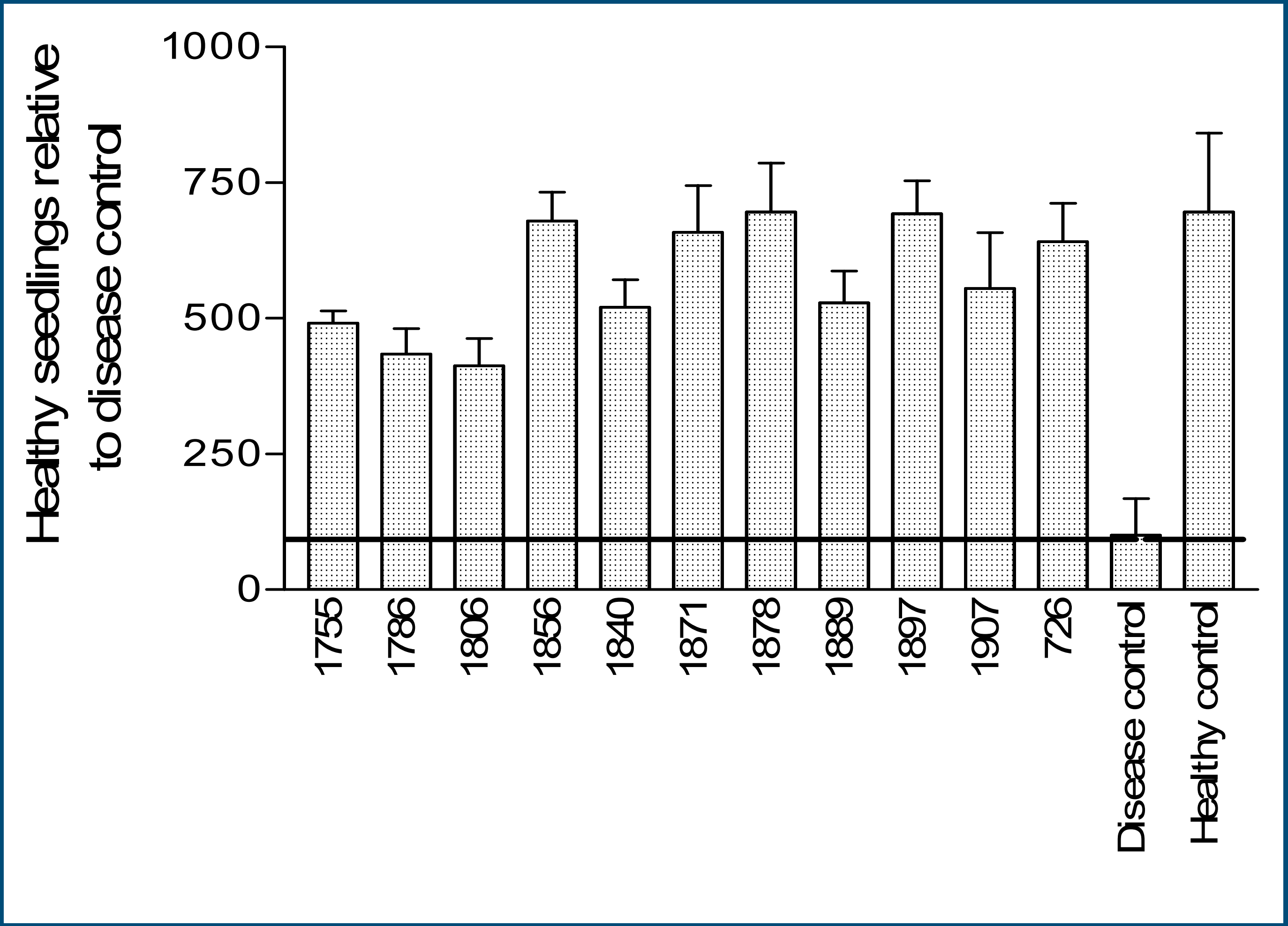
Developing control measures

- Developing tools for resistance breeding
- Improved production of organic planting material
- Seed treatments
- Post-harvest treatments

Developing tools for resistance breeding



Seed treatments with antagonists



Cytotoxicity of antagonists on human cells

Fungal isolates	Peat bran CD50 (mg/ml)	Rice CD50 (mg/ml)
Control (blank)	>10	>20
1755 Plectosporium tabacinum	>10	>20
1889 Gliocladium solani	7	>20
726 Clonostachys rosea	0.51	1.8
1871 C. rosea	1.5	1.5
1897 C. rosea	0.38	1.2
1878 G. catenulatum	1.6	2.7
1806 Acremonium strictum	1.3	6

Conclusions / remarks

- Methods for detection of Alternaria + analysis of Alternaria toxins in place; need for ring testing
- A. alternata toxins found in inoculation experiments; so far not found in naturally contaminated samples or products
- Status of A. radicina toxins not clear yet
- Insight in physiology/genetics: at low temperatures no risk
- Do we know enough as basis for risk assessment?
- Prevention strategies are worthwhile
  - starting material must be healthy
  - breeding for resistance is difficult
  - seed treatments have potential, post-harvest treatments do not
- Extrapolation to other mycotoxin producers possible?

Project partners

- Plant Research International, Netherlands
- Poznan Agricultural University, Dept. of Chemistry and Dept. of Seed Science and Technology, Poland
- Royal Veterinary and Agricultural University, Dept. of Plant Biology, Frederiksberg, Denmark
- CNR Institute of Science of Food Production, Italy
- Rijk Zwaan B.V., The Netherlands
- Daehnfeldt A/S, Denmark
- Vilmorin, France