

Strategy to evaluate carrot wild relatives resistant to *Alternaria* species

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Meeting of the Activity “CarrotDiverse” June 04.- 05. 2017, Braga, Portugal





Outline

- Introduction *Alternaria* diseases on carrot
- Some result of earlier evaluation projects
- Overview on new established tools for evaluation *Alternaria* resistance
- Strategy for the *CarrotDiverse* initiative

Impact of carrot infection by *Alternaria* spp.

<i>Alternaria</i> spp.	Diseased plant tissue	Disease incidence and/or damages	References
<i>A. dauci</i>	Infestation of seeds (seedling damping-off)	Reduced seed fecundity (-70-80%)	Schouten et al. 2002 Coles & Wicks 2003, Farar et al. 2004, Koike et al. 2007
	Leaf blight	Yield losses: 40 – 60% (Israel)	E. Ben-Noon et al. 2001
<i>A. radicina</i>	Infestation of seeds (seedling damping-off)	38 seed lots tested (100%) infested 1 to 20% (US, CA) 13 of 19 lots infested 0.25 to 30% 99% of 313 seed lots were contaminated (0.5 to 82.5%), (Po) 18 of 19 lots infested with highest rate of 35% (Australia)	Grogan & Snyder 1952 Maude 1966 Tylkowsa 1992 Coles and Wicks 2003 Pryor et al. 1994
	Black rot or Black crown	DI up to 62% (US, NY) DI between 1-50% (RU) 1-50% (GB) 88% (Australia) 63-99% (US, CA) 82% (US, CA) Significant field and postharvest losses (no statistical indications) Up to 70% of mature carrots are unmarkable if heavily infection. Quality reduction (mycotoxins)	Meier et al. 1922 Vlasova 1986 Greeson et al. 1988 Coles & Wicks 2003 Pryor et al. 1994 Pryor et al. 1998 Ellis and Holliday 1972 Pryor et al. 1998 Schouten et a. 2002 Solfrizzo et al. 2012
<i>A. alternata</i>	Infestation of seeds		Schouten et a. 2002 Krämer et al. , in prep.
	Leaves		K rämer et al. , in prep.
	Root	Mycotoxins	Solfrizzo et al. 2012 Krämer et al. in prep.

Alternaria dauci (Kühn, Groves & Skolko)

Leaf blight

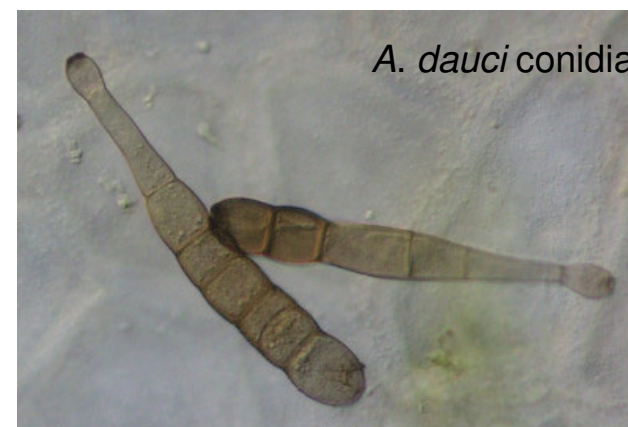
Necrotic lesions, die back of leaves



- First description by Julius Kühn (Germany, 1855)
- Occurrence worldwide
- Transmission by seed
- No resistant carrot cultivars



Losses in yield, quality and marketability



Alternaria radicina (Meier, Drechs. & Eddy)



Black rot

Storage: dry, black lesions



- First description by E. Rostrup (Denmark 1888)
- Occurrence worldwide
- Transmission by seed
- No resistant carrot cultivars



Losses in yield, quality and marketability

Alternaria alternata (Fries) Keissler

Leaves: leaf blight

Storage: dry, black lesions



- First description by E. Rostrup (Denemark 1888)
- Occurrence worldwide
- Transmission by seed
- No resistant carrot cultivars

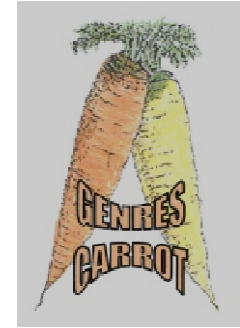
Black rot



Losses in yield, quality and marketability

- *Retrospect of the GenRes 105 project*

GENRES - CT99 - 105



The Future of European Carrot: a programm to conserve, characterise, evaluate and collect carrot and wild species

Task of our group:

Evaluation of 200 genebank accessions
regarding resistance to *Altermaria dauci*

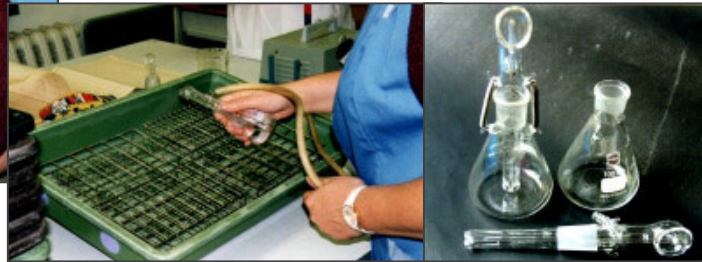
Laboratory test (modified by Bruno1993)



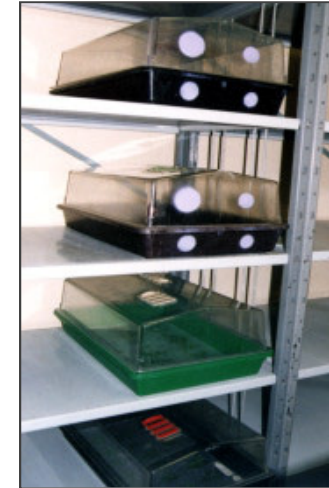
~ 100+/-5 d



- * plant cultivation in a glasshouse
- * pre-evaluation of cutted leaves

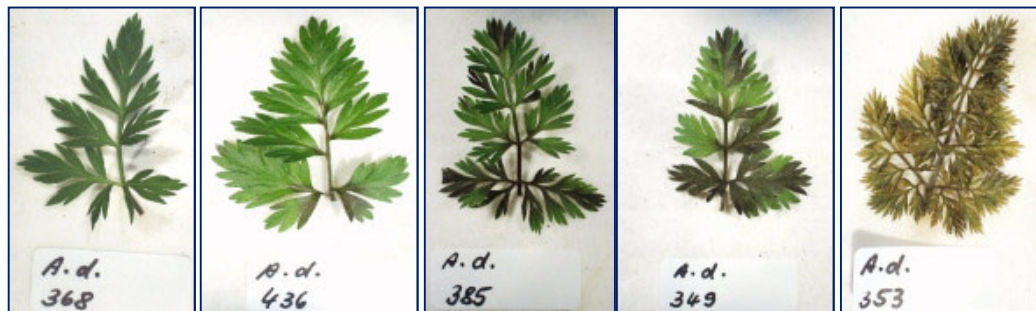


- * inoculation with a glass sprayer
- * inoculum 10^5 spores / ml



- * incubation in plastic boxes (22°C / 95%hum.)

Evaluation 7dpi and 9dpi



1	3	5	7	9
0-10%	11-30%	31-50%	51-80%	81-100%

Test repetition after 14d

Statistics & Selection

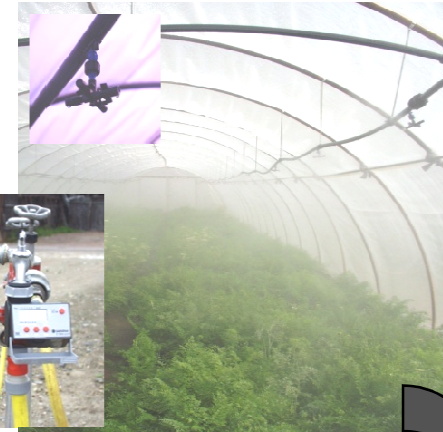
Semi-Fieldtest



Cultivation (~100d)



Inoculation



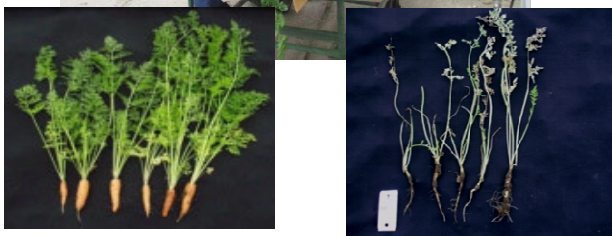
Irrigation



Harvest of all individual plants



Incubation ca.30d

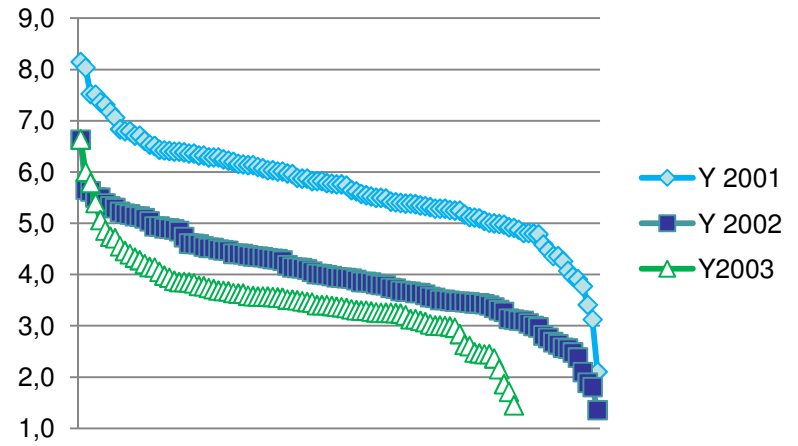
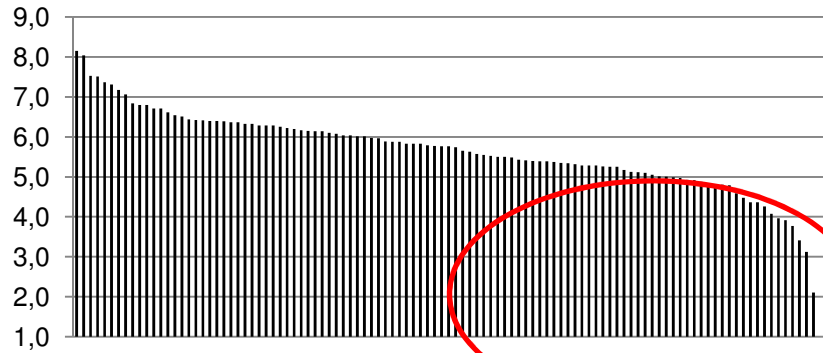


Evaluation

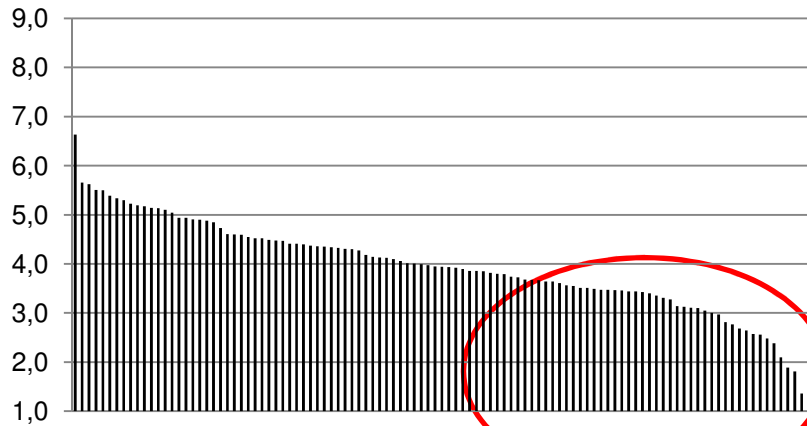
GENRES105

Alternara dauci; n=200 acc. – cvs., LR, WR

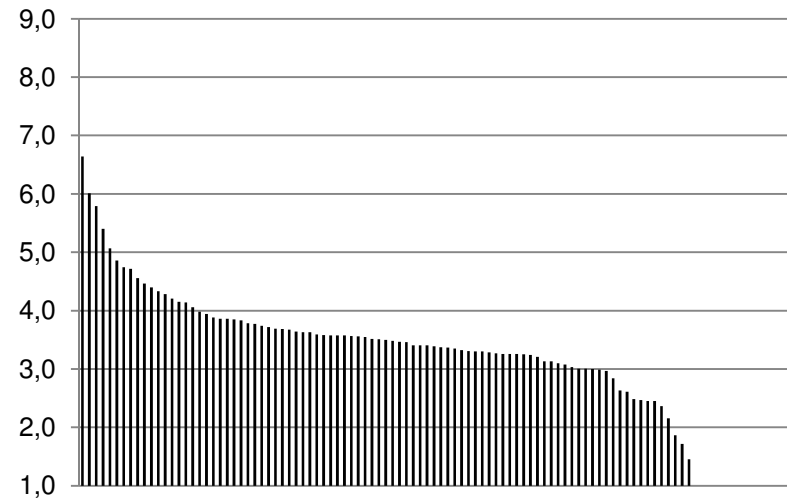
Acc-ave-LT 2001



Acc-ave-LT 2002



Acc-ave-LT 2003



- Evaluation the resistance behavior to *Alternaria* spp. via Bioassay



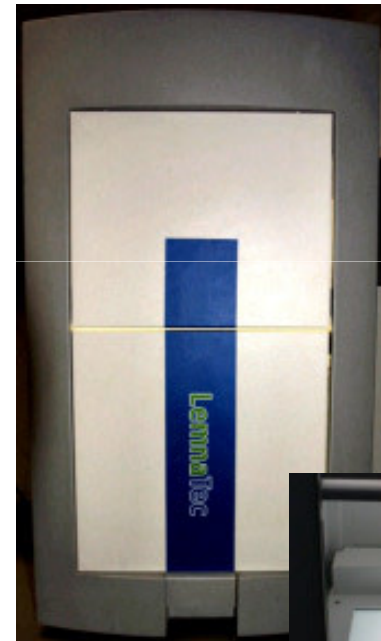
A main problem of disease evaluation in past was:
The objective reproducible calculation of the
disease symptoms



The solution was a DIAS



Scanalyser (LemnatTec, Aachen, Germany)



- first generation, 2006
- a lot teething problems

Calibration

Farbschema: GM2 (GM1\4\PD2+)

Farbklassen

alle anzeigen

healthy

diseased

green

Analysieren...

Importieren...

Exportieren...

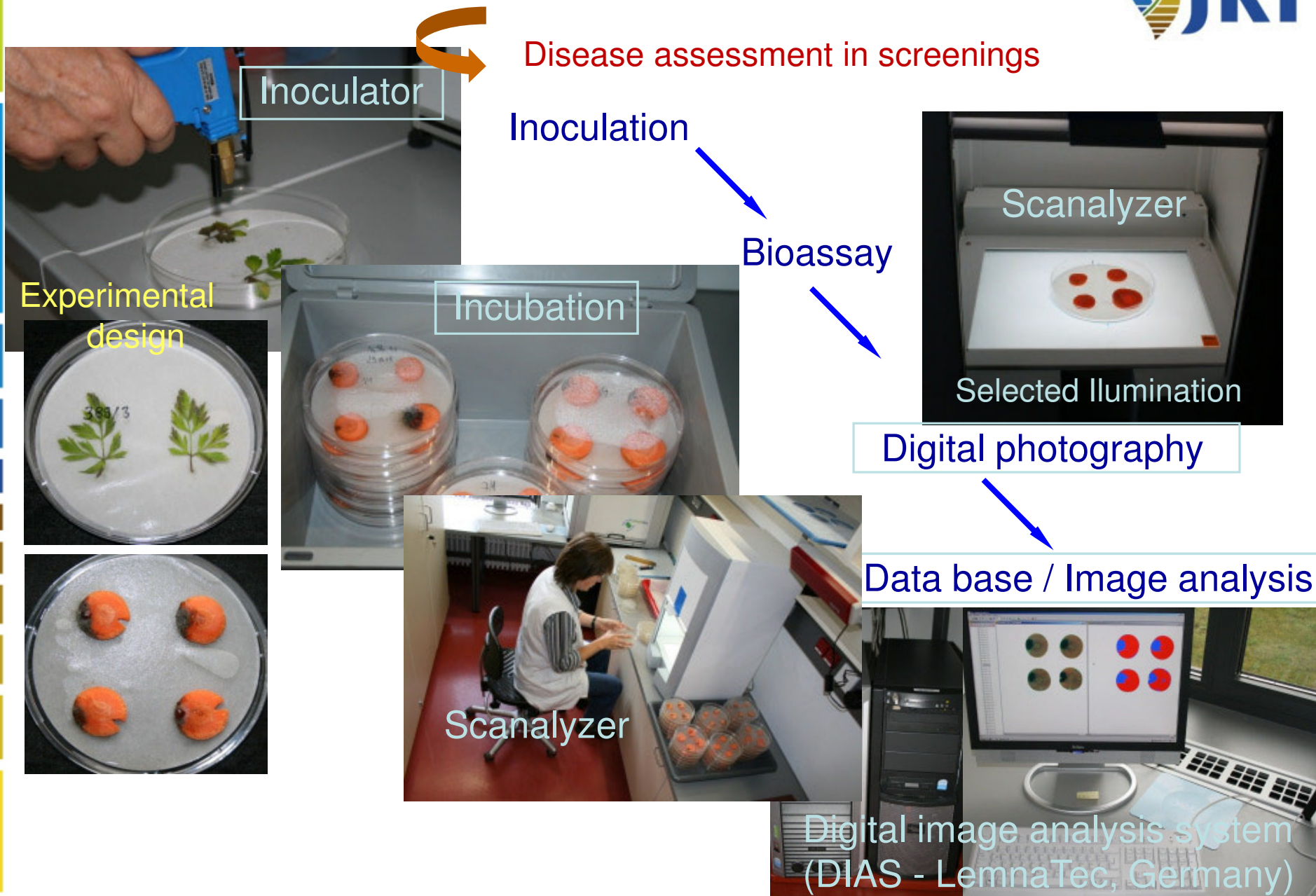
Bezeichnung

GM2

OK

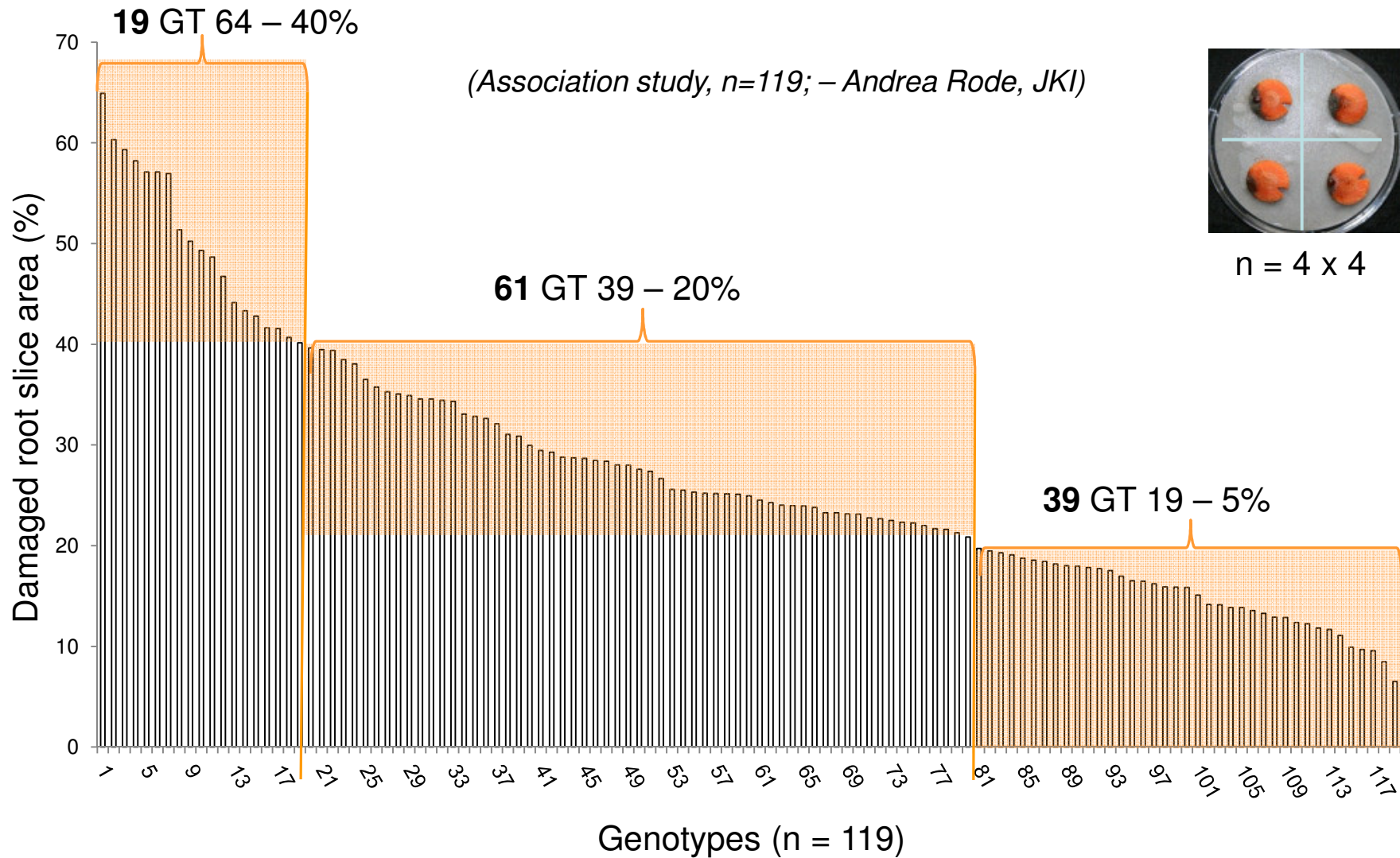
Abbrechen

Improvement of resistance to fungal pathogens in carrot



AIF – FuE satimex project (2010-2012)

Results AS 2012: Root slice - Test -*Alternaria radicina* –

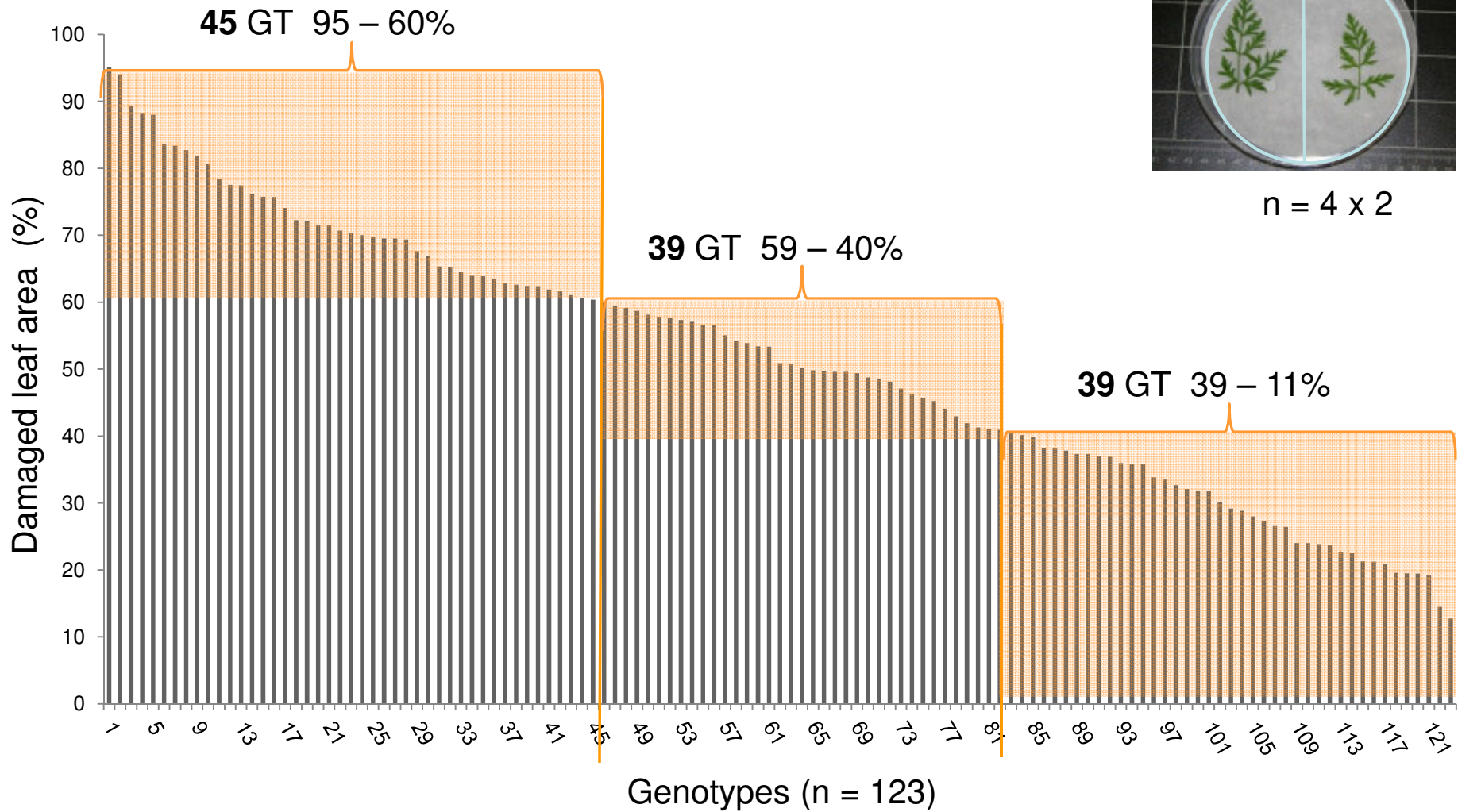


Results AS 2012: Leaf segment -Test - *Alternaria dauci* -

(Association study, n=123, - Andrea Rode, JKI)



n = 4 x 2



- Strategy for the UmbelWG initiative on Daucus WCR

ECPGR Umbellifer WG wild relatives project.

(11.03.2013)

3. Project details per partner and action

JKI Quedlinburg	
Institution	Julius Kühn-Institute, Institute for Breeding Research on Horticultural and Fruit Crops
WG member	Thomas Nothnagel
Action	Evaluation of carrot wild relatives against <i>Alternaria dauci</i>
Description	<p>During the Quedlinburg meeting, we have been agreed to evaluate carrot wild relatives from different partner countries for various traits e.g. <i>D. carota carota</i> collected in , GB, ... to test against <i>Alternaria dauci</i>. The proposed action is to test approximately 30 accessions against <i>Alternaria dauci</i> in a laboratory test. I need at least 100 seeds/ accession is needed.</p> <p>Accessions provided by C. Allender (16 accessions / carrot diversity set), S. Solberg (wild carrot from NGRC), E. Geoffriau (wild carrot France)</p>
Period	2013

Material

CWR Project 2013-2015



Source	Carrot Wild Relatives	
	Obtained	Evaluated
Agrocampus Ouest INHP (Angers, France)	7	7
GAT (IPK - Gatersleben, Germany)	4	4
NGB (Alnarp , Sweden)	5	5
HRIGRU (Wellesbourne, Warwick, UK)	12	12
VIR (St. Petersburg, Russia)	7	1 (2)
LRCAF (Lithuania)	1	1
Sum	36	30 (2)

Control: *Daucus carota* ssp. *sativus* cv. 'Nevis' (Bejo, The Netherlands)
Daucus carota ssp. *sativus* cv. 'Rotin' (Sperli, Germany)

Methods

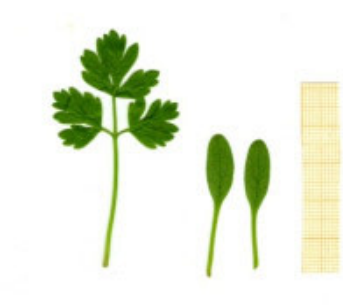
- Pre-evaluation the CWRs using the EPGRI guideline (PGRI. 1998. Description for wild and cultivated Carrots (*Daucus carota* L.))
- Estimation the genetic distance using SSR markers and NT-SYS-UPGMA analysis
- Evaluation of the resistance behavior to A.d, A.r., A.a.
- Analysis of volatile and non-volatile pattern via HS-SPME GC, HPLC, RAMAN analysis

KRAEHMER A., BOETTCHER C., RODE A., NOTHNAGEL T., SCHULZ H.: Quantifying biochemical quality parameters in carrots (*Daucus carota* L.) - FT-Raman spectroscopy as efficient tool for rapid metabolite profiling. *Food Chemistry* **212**:495-502 (2016)

NOTHNAGEL T., BUDAHN H., KRÄMER R.: Resistance characterization of carrot wild relatives (*Daucus carota* ssp. *carota*) to *Alternaria* spp. *Acta Hort.* **1153**:251-257 (2017)

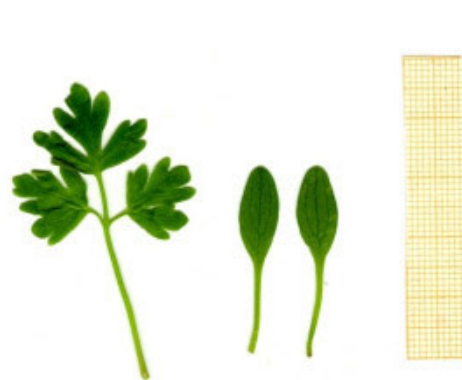
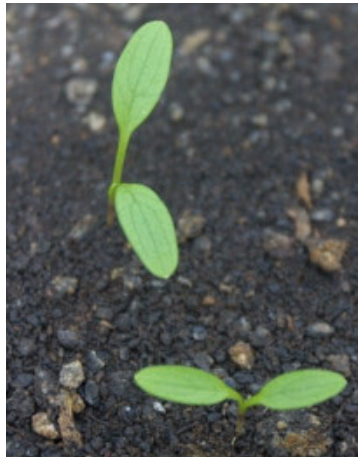
Pathogen	Plant age	Plant tissue	Inoculation concentr.	amount	Incubation
<i>Alternaria alternata</i>	92 d	Leaflets	3.0×10^5	40 μ l/L	7 d
<i>Alternaria dauci</i>	100 d	Leaflets	3.0×10^5	40 μ l/L	9 d
<i>Alternaria radicina</i>	106 d	Root slices	1.0×10^6	3 μ l/RS	21 d

CWR 2013-01 - ACC. 2570 - VIR / Tadschikistan



Conium maculatum (Gefleckter Schierling)

CWR 2013-02 - ACC. 2571 - VIR / Tadschikistan



Conium maculatum (Gefleckter Schierling)

CWR 2013-06 - 2580 - VIR / Tadschikistan



Pimpinella peregrina
(Fremde Bibernelle)



CWR 2013-07



CWR 2013-08



CWR 2013-09

CWR-2013-07	2582	Daucus	carota	VIR / Tadschikistan
CWR-2013-08	2591	Daucus	carota	VIR / Armenien
CWR-2013-09	2592	Daucus	carota	VIR / Armenien



CWR 2014-14



CWR 2014-18

CWR-2013-14	CWR-2014-40	NGB 21705.1	<i>Daucus carota</i>	Agersø (South Denmark) SS0704
CWR-2013-18	CWR-2014-44	ACO-IRHS 880	<i>Daucus ssp.carota var.carota</i>	France, Center Verger D52 Chanonat-Varennnes

Variability

Anthocyanin biosynthesis



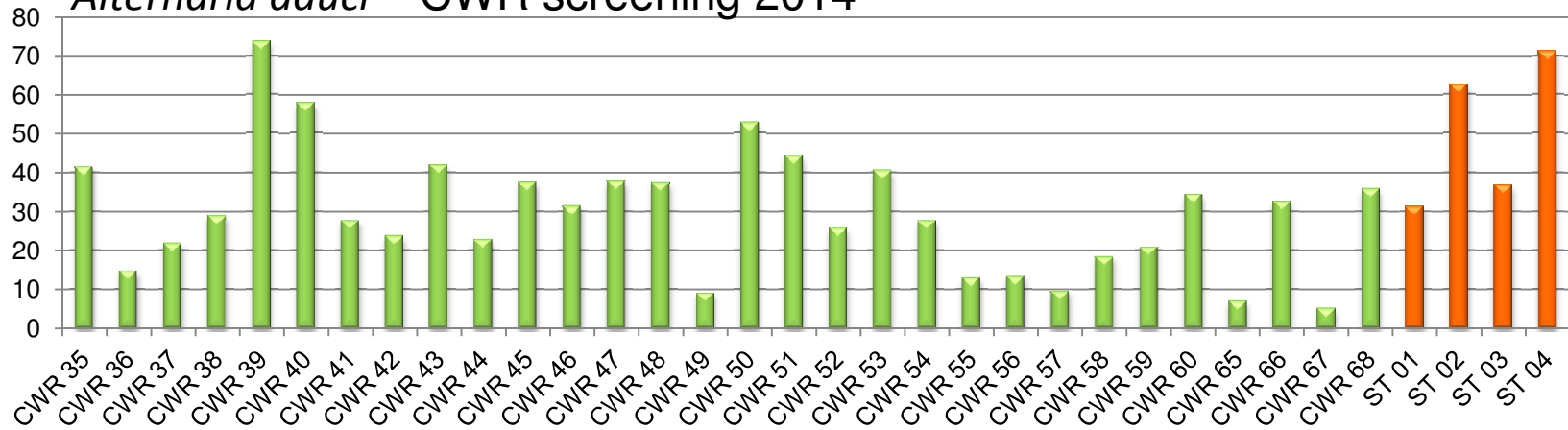
CWR 2014-32

Bolting behavior

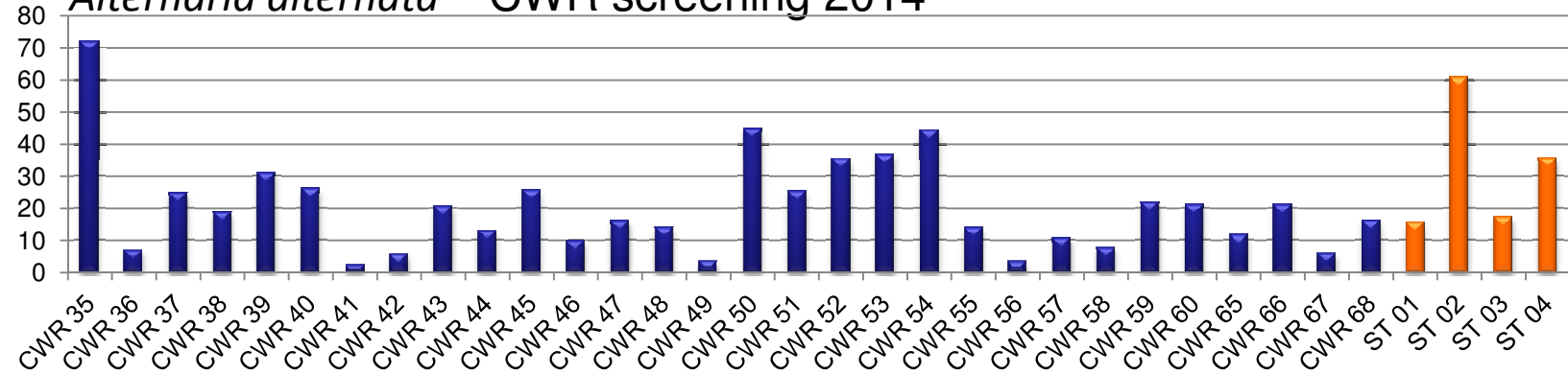


CWR 2014-26

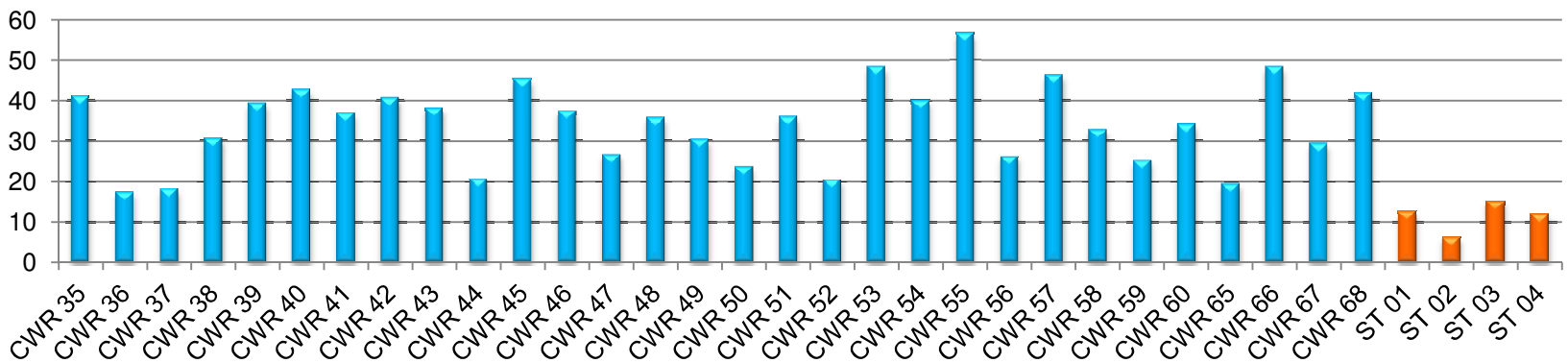
Alternaria dauci – CWR screening 2014



Alternaria alternata – CWR screening 2014



Alternaria radicina – CWR screening 2014

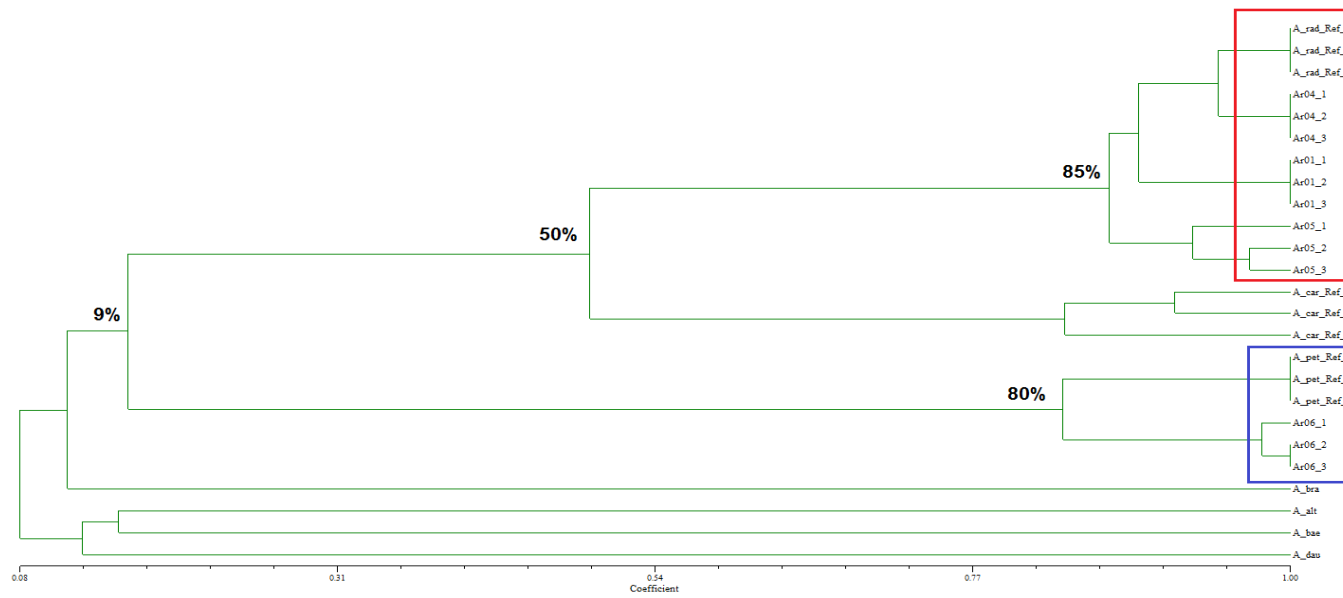
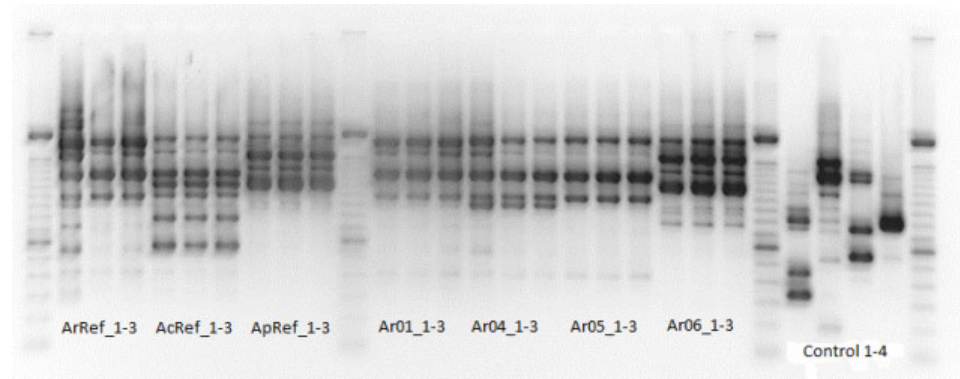
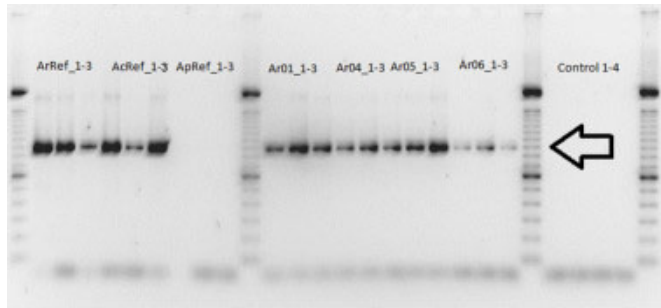


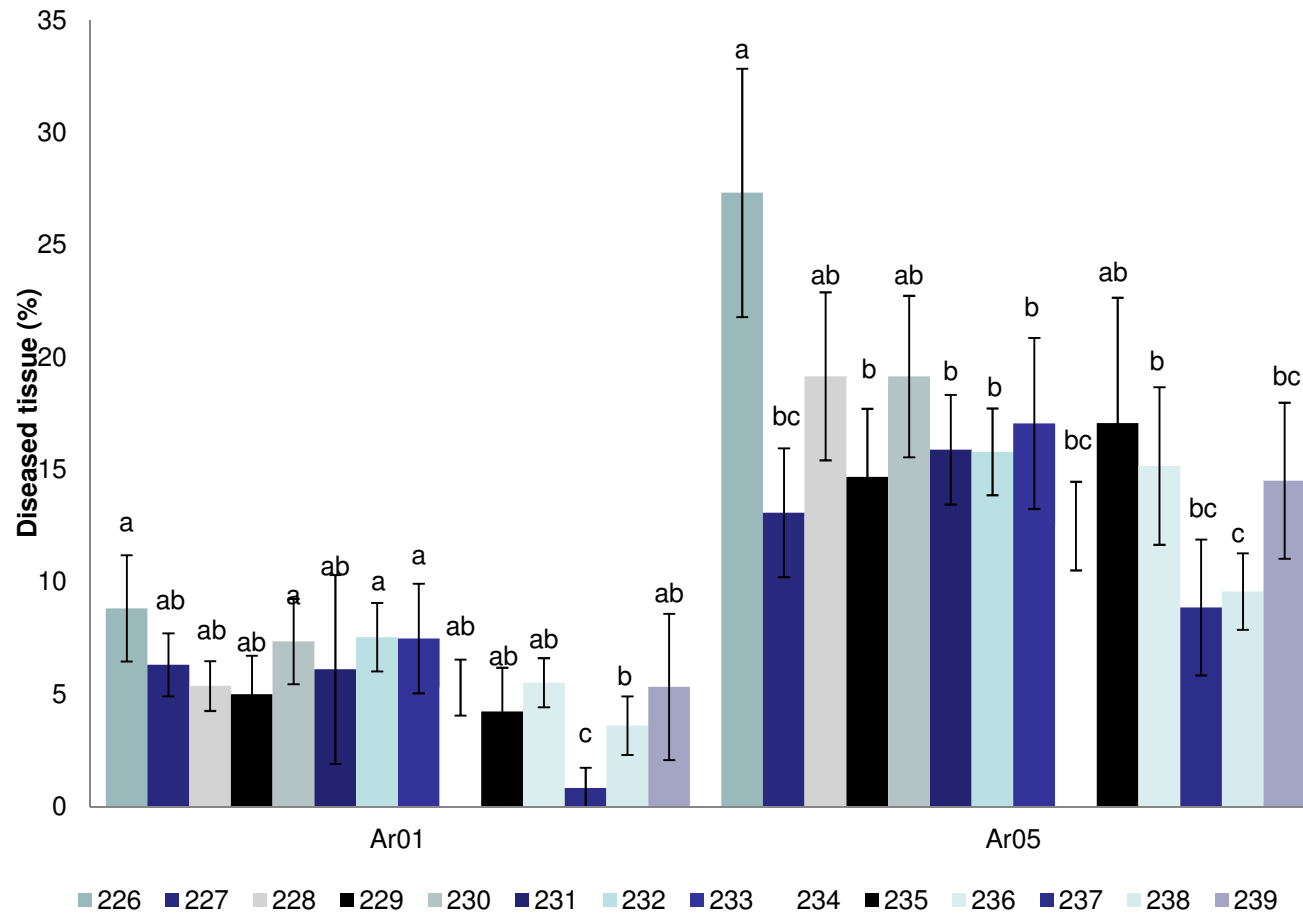
NO	DAUCUS	GENE BANK	GEO. ORIGIN	STATUS	BOT	LD	AP	RB	A.d.	A.a.	A.r.
CWR-35	<i>D. carota</i> ssp. <i>carota</i>	VIR	TA	CWR	3	5	5-7	5-7			
CWR-36	<i>D. carota</i> ssp. <i>carota</i>	NGB	SWE	CWR	3	7	3-5	3			
CWR-37	<i>D. carota</i> ssp. <i>carota</i>	NGB	DK	CWR	3	5	3-5	3			
CWR-38	<i>D. carota</i> ssp. <i>carota</i>	NGB	N	CWR	3	7	5	3			
CWR-39	<i>D. carota</i> ssp. <i>carota</i>	NGB	DK	CWR	3	5	5	3			
CWR-40	<i>D. carota</i> ssp. <i>carota</i>	NGB	DK	CWR	3	5	5	3			
CWR-41	<i>D. carota</i> ssp. <i>carota</i>	IRHS	FRA	CWR	3	7	3-5	3-5			
CWR-42	<i>D. carota</i> ssp. <i>carota</i>	IRHS	FRA	CWR	7	5	3	3-5			
CWR-43	<i>D. carota</i> ssp. <i>carota</i>	IRHS	FRA	CWR	7	7	1-3	3			
CWR-44	<i>D. carota</i> ssp. <i>carota</i>	IRHS	FRA	CWR	5	5	1-3	3			
CWR-45	<i>D. carota</i> ssp. <i>carota</i>	IRHS	FRA	CWR	5	5	5-7	3			
CWR-46	<i>D. carota</i> ssp. <i>carota</i>	IRHS	FRA	CWR	7	3	5-7	1-3			
CWR-47	<i>D. carota</i> ssp. <i>carota</i>	IRHS	FRA	CWR	3	3	5	3			
CWR-48	<i>D. carota</i> ssp. <i>carota</i>	HRIGRU	RUS	CWR	5	5	3	3			
CWR-49	<i>D. carota</i> ssp. <i>carota</i>	HRIGRU	GEO	CWR	7	3-5	3-5	3			
CWR-50	<i>D. carota</i> ssp. <i>carota</i>	HRIGRU	PTL	CWR	7	5	5	3-5			
CWR-51	<i>D. carota</i> , wild species	HRIGRU	UK	CWR	5	7	1-3	3-5			
CWR-52	<i>D. carota</i> , wild species	HRIGRU	GRE	CWR	7	3	5-7	3			
CWR-53	<i>D. carota</i> , wild carrot	HRIGRU	ESP	CWR	7	7	5	3			
CWR-54	<i>D. carota</i> , wild species	HRIGRU	ESP	CWR	7	7	3-5	3-5			
CWR-55	<i>D. carota</i> ssp. <i>carota</i>	HRIGRU	ITA	CWR	7	5	3-5	3			
CWR-56	<i>D. carota</i> , wild species	HRIGRU	UK	CWR	5	5	1-3	3-5			
CWR-57	<i>D. carota</i> , wild species	HRIGRU	CHE	CWR	7	3	5	3			
CWR-58	<i>D. carota</i> , wild species	HRIGRU	GER	CWR	3	7	3-5	3-5			
CWR-59	<i>D. carota</i> , wild species	HRIGRU	POL	CWR	3	7	3	5			
CWR-60	<i>D. carota</i> ssp. <i>carota</i>	LIT	LIT	CWR	3	5	3	5-7			
CWR-65	<i>D. carota</i> ssp. <i>carota</i>	GAT	GER	CWR	5	5	1-3	3-5			
CWR-66	<i>D. carota</i> ssp. <i>carota</i>	GAT	ITA	CWR	7	3	3-5	3-5			
CWR-67	<i>D. carota</i> ssp. <i>maximus</i>	GAT	ITA	CWR	5	3	3-5	3-5			
CWR-68	<i>D. carota</i> ssp. <i>carota</i>	GAT	GER	CWR	5	3	1-3	3-5			
ST 01	<i>D. carota</i> ssp. <i>aurantius</i>	VIR	ARM	LR, o	3	7	1-3	1			
ST 02	<i>D. carota</i> ssp. <i>aurantius</i>	VIR	ARM	LR, o	3	5	1	1			
ST 03	<i>D. carota</i> ssp. <i>sativus</i>	SPERLING	GER	OP, o	3	7	1	1			
ST 04	<i>D. carota</i> ssp. <i>sativus</i>	BEJO	NL	F1, o	3	5	1	1			



New established tools for evaluation *Alternaria* resistance

- New defined *Alternaria* isolates (Aggressivity tests)
- ELISA assay (*Alternaria* specific Immunoassay)
- New LemnaTec machine (VIS, Fluo)





Resistance test with 14 carrot cultivars and two *Alternaria radicina* isolates: DIAS estimated diseased leaf tissue (%) (Different letters indicated significance, Tukey, $P < 0.05$, mean $n = 10$, subdivided for both isolates Ar01 (7 dpi) and Ar05 (5 dpi)).

Assessment of *Alternaria* infestation

Digitale Image Analysis (DIAS)

- Disease symptoms on leaves, petiols, root slices
- Dimesion, epidemiology

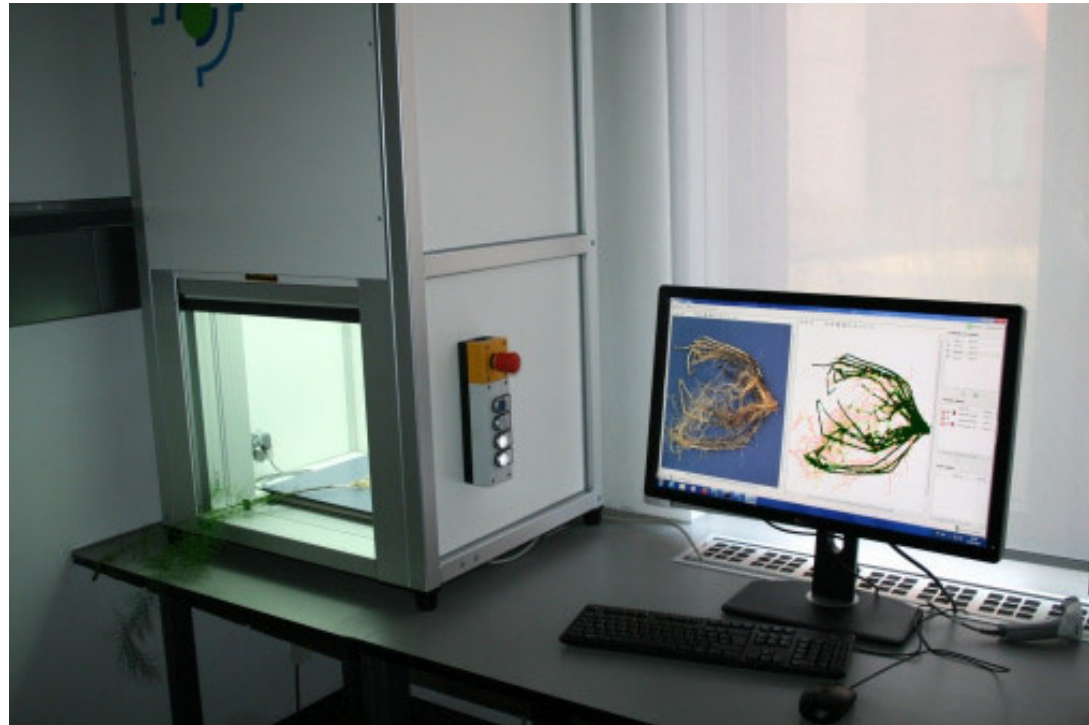
Detection of *Alternaria* within the plant tissue

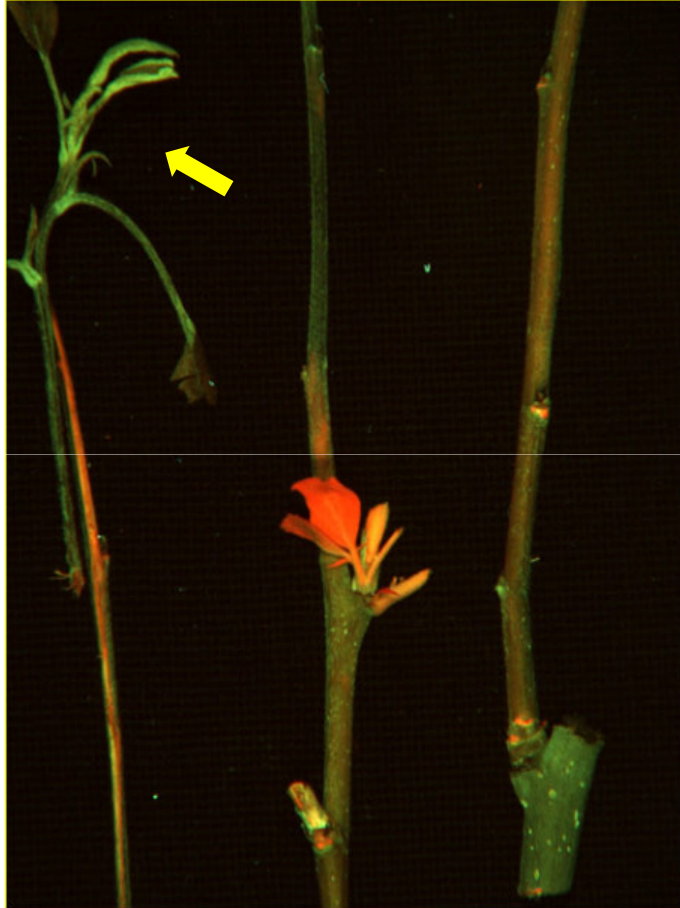
- Enzym Linked Immunosorbent Assay (ELISA) – *Alternaria* specific antibodies
- PCR, RT-PCR, qPCR (partially established or in prepartion)

Lemnatec

**Digitale
Image
Analysis
System**

*VIS camera system
UV camera system*





Gfp-labelled fire blight *Erwinia amylovora*
on *Malus*

Strategy and timetable alternaria resistance evaluation - CarrotDiverse

Carrot Diverse

Daucus collections



Characterisation



Carrot diversity set
96 acc.

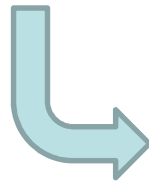


Genotyping by sequencing
(GbS)



Genom wide association
(GWAS)

Resistance evaluation



Strategy and timetable alternaria resistance evaluation - CarrotDiverse

Pre-evaluation:

- Germability
- Growth habit & size
- Vigor
- Bolting behavior
- Common plant healthy

Oct 17 – Dec 17

(96 Acc. X 10 Plants = 960 plants incl. 2 Standards)

Resistance evaluation:

Laboratory test
DIAS / ELISA analysis

Jan 2018 – May 2018

(96 Acc.x 5 Plants = 480 incl. 2 Standards)

Data availability & data transfer:

July 2018

Publication: (*J Phytopathology; Gen Res Crop Evo ???*)

End of 2018