

Efforts towards a harmonized EB detection, results of the first *Alternaria* ring test

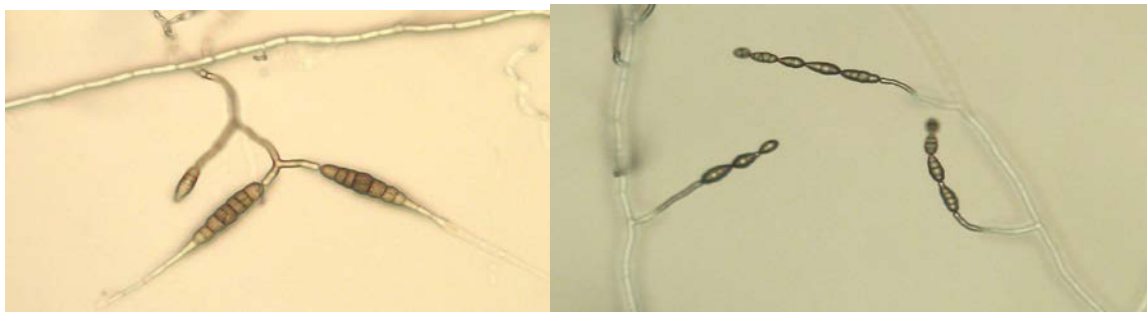
Jürgen Leiminger (TUM)

Jan Spoelder (HLB)

Bert Evenhuis and Marieke Förch (WUR)

EuroBlight *Alternaria* subgroup meeting, 24 and 25th May 2014, Freising

- Collection of *Alternaria* infected leaves from different countries
- Shipment to different labs, sample preparation and evaluation of *Alternaria* species
- Participating Labs: Bert Evenhuis and Marieke Förch (WUR, The Netherlands)
Jan Spoelder (HLB, The Netherlands)
Jürgen Leiminger and Anna Livic (TUM, Germany)
- each lab will analyze leaf samples according to their own lab protocols
- analysis of *Alternaria* species according to spore formation (microscope)



Harmonized sampling and shipment

1. sample leaves, preferably from plots, which were not treated with *Alternaria* specific fungicides (app. 10 leaves)
2. dry leaves and put them between paper towels for shipping
3. add following information
location, variety, contact person, part of the plant from which the samples were taken, disease severity



Alternaria ring test 2014

Contact person: _____

Country: _____

Tested location: _____

Nearest city: _____

Sampling date: _____

Variety (if know): _____

Estimation of the approximate
EB disease severity (%) _____

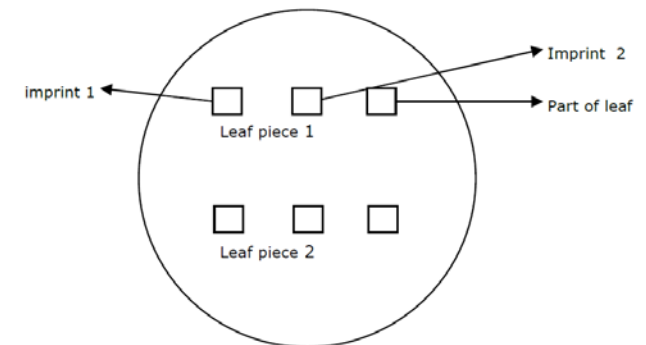
Fungicide treated against EB Yes No

Please indicate out of which plant
section the samples were taken
(lower, middle, higher leaf section) _____

Additional information _____

Nr.	country	sample sent by	field location	leaf level	variety	fungicide treatment	disease severity	sampling date
1	Germany	Jürgen	Freising	low-middle	Agila	untreated	?	15.07.2014
2	Germany	Jürgen	Freising	low-middle	Maxilla	untreated	?	15.07.2014
3	Germany	Jürgen	Straßmoos	middle	unknown	Mancozeb	moderate	25.07.2014
4	Belgium	Pieter	Passionistenstraat, Kruishoutem 9770	middle	Bintje	untreated	5%	08.08.2014
5	The Netherlands	Jan	Wijster	?	Festien	untreated	10%	11.08.2014
6	Sweden	SLU	Nymö	?	Kuras	untreated	?	31.07.2014
7	Sweden	SLU	Nymö	?	Kardal	untreated	?	07.08.2014
8	Sweden	SLU	Nymö	?	Kuras	untreated	?	19.08.2014
9	Germany	Jürgen	Niedersunzing	high	unknown	treated	moderate to high	24.08.2014
10	Belgium	Pieter	Passionistenstraat, Lozer	Middle-high	Bintje	untreated	0.5-1%	28.08.2014
11	The Netherlands	Marieke	Westmaas	higher	unknown	treated	?	01.09.2014
12	The Netherlands	Marieke	Valthermond		Aveka	untreated	20%	03.09.2014
13	The Netherlands	Marieke	Lelystad	higher	Bintje	untreated	75%	02.09.2014

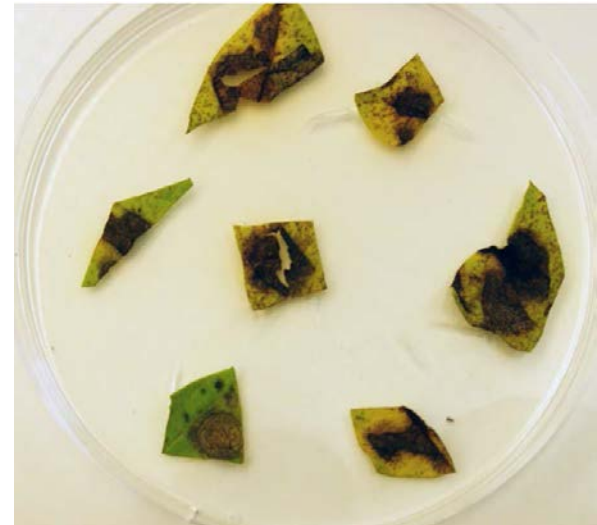
- cut out of little infection sites (1 cm²)
- no surface sterilization
- water agar 1.5% with ampicillin (200 mg/l agar)
- two imprints of both sides onto the agar plus leaf piece
- two different pieces per infected leaf
- incubation in climate room, 15 °C, 16/8h photoperiod, (no UV light)
- Analysis of spore formation after two weeks



- Remark:
- Spores will be formed on the imprint or on the leaf
- The formation of *A. solani* spores can take longer than that of *A. alternata*
- if no spores are formed after two weeks, plates will be incubated for another week


- cut out EB infected leaf pieces bearing a single lesion
- No surface sterilization
- transfer necrotic leaf cuts to petri dishes containing water agar (WA)
+ 50ug/ml streptomycin
- incubate agar plates for 3-8 days at 20°C, 16hr light/8hr dark, (no UV light)
- check agar plates for sporulation at 3 and 7 days after incubation.
The 7 day check serves as a control for the 3 day check

- cut out of infected lesions from infected leaflets (0.5 to 1 cm size)
- Surface sterilization in 5% NaOCl, 1 min, followed by washing step in sterile water
- Slight nutritious (SN-) Agar for production of spores
- Incubation for 3-6 days at 20 °C under UV light, 12/12 h photoperiod
- Analysis of sporulating species



identification of *A. solani*

Nr.	field location	sampling date	identified species		
			TUM	WUR	HLB
1	Freising	24.07.2014	A.s	missing	missing
2	Freising	24.07.2014	A.s +	A.s.	A.s.
3	Straßmoos	25.07.2014	A.s ++	A.s.	A.s ++
4	Passionistenstraat, Kruishoutem 9770	08.08.2014	A.s +	A.s +	A.s ++
5	Wijster	11.08.2014	A.s +	A.s.	A.s +
6	Nymö	31.07.2014	A.s ++	A.s +	A.s ++
7	Nymö	07.08.2014	A.s ++	A.s.	A.s ++
8	Nymö	19.08.2014	A.s +	A.s +	A.s ++
9	Niedersunzing	25.08.2014	A.s +	A.s.	A.s
10	Passionistenstraat, Lozer	28.08.2014	A.s.	A.s.	A.s ++
11	Westmaas	01.09.2014	A.s +	A.s +	A.s ++
12	Valthermond	03.09.2014	A.s +	A.s +	A.s ++
13	Lelystad	02.09.2014	A.s ++	A.s +	A.s ++

 Species not found

identification of *A. alternata*

Nr.	field location	sampling date	identified species		
			TUM	WUR	HLB
1	Freising	24.07.2014	A.a.	A.a.	A.a.
2	Freising	24.07.2014	A.a.	missing	A.a.++
3	Straßmoos	25.07.2014	A.a.	missing	A.a.+
4	Passionistenstraat, Kruishoutem 9770	08.08.2014	A.a.	A.a.	A.a.++
5	Wijster	11.08.2014	A.a.	A.a.	A.a.
6	Nymö	31.07.2014	A.a.	A.a.	A.a.
7	Nymö	07.08.2014	A.a.+	missing	A.a.+
8	Nymö	19.08.2014	A.a.	A.a.	A.a.
9	Niedersunzing	25.08.2014	A.a.	missing	missing
10	Passionistenstraat, Lozer	28.08.2014	missing	missing	A.a.
11	Westmaas	01.09.2014	A.a.	A.a.	A.a.+
12	Valthermond	03.09.2014	A.a.	A.a.	A.a.
13	Lelystad	02.09.2014	A.a.	A.a.	A.a.+

 species not found

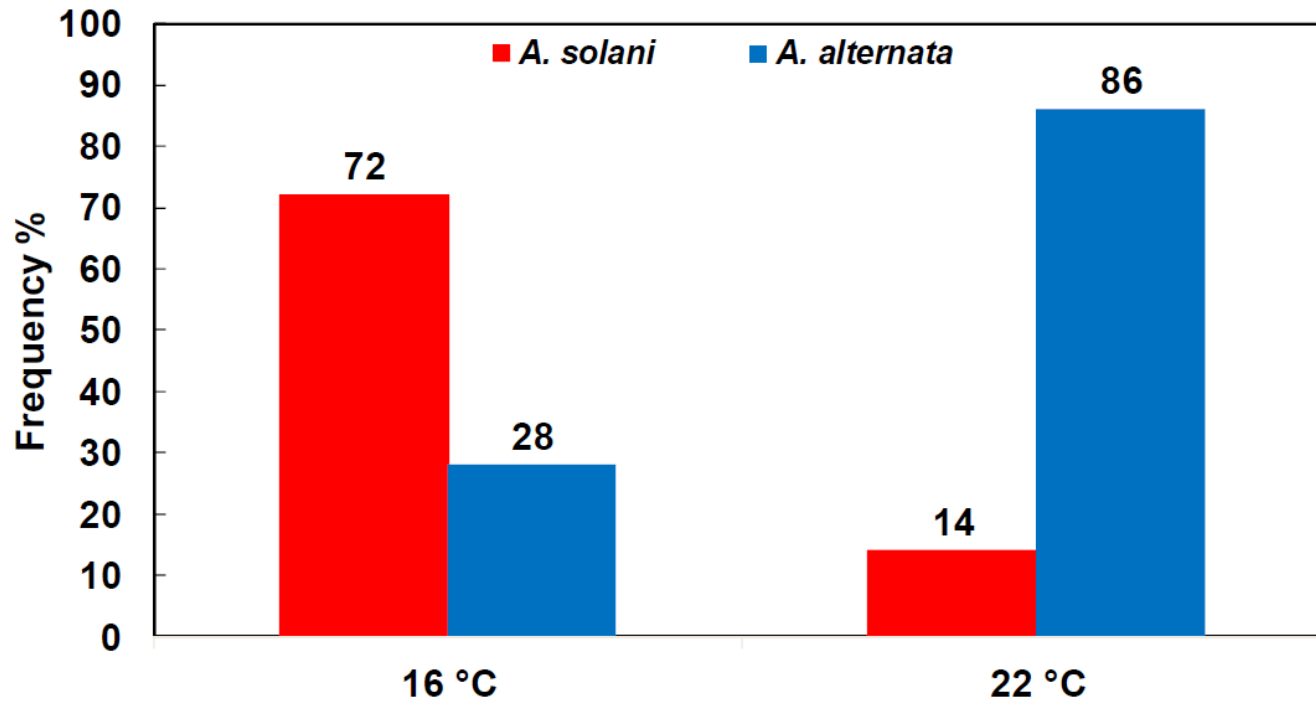
- *A. solani* was found in all leaf samples, high recovery rates at all lab protocols
- although it is known that *A. solani* needs strong induction for sporulation and/or UV, UV light was not necessarily needed
- *A. alternata* is known to sporulate easily and does not need triggering
- however, greater difficulties in the recovery of *A. alternata*
- influence of the temperature on sporulation and species recovery (Stammler, 2014)



Low temperatures during isolation process increases frequency of *A. solani*, high *A. alternata*

10 samples, 161 isolates analysed

16 °C and 22 °C at incubation of leaves in moist chamber before isolation



Stammler, 2014



- Sample leaves (app. 10 leaves), preferably from plots, which were not treated with *Alternaria* specific fungicides
- In the case that leaves will be shipped make sure that leaves are dried put leaves between paper towels
- cut out of little infection sites (1 cm²), one lesion per leaf
- surface sterilization is NOT obligatory, however it helps to reduce fungal/bacterial contamination (5% NaOCl, 1 min)
- Medium: no particular recommendations (water agar 1.5% with antibiotica or SNA)
- Incubation of petri dishes in climate room, temperature (15) 20°C, photoperiod (16/8h) is requested
- UV light is NOT obligatory but will support sporulation
- spore formation can be visualized with a binocular within 3 to 7 days

Thank you for your attention!

