PLANT RESEARCH INTERNATIONAL

Seed health management

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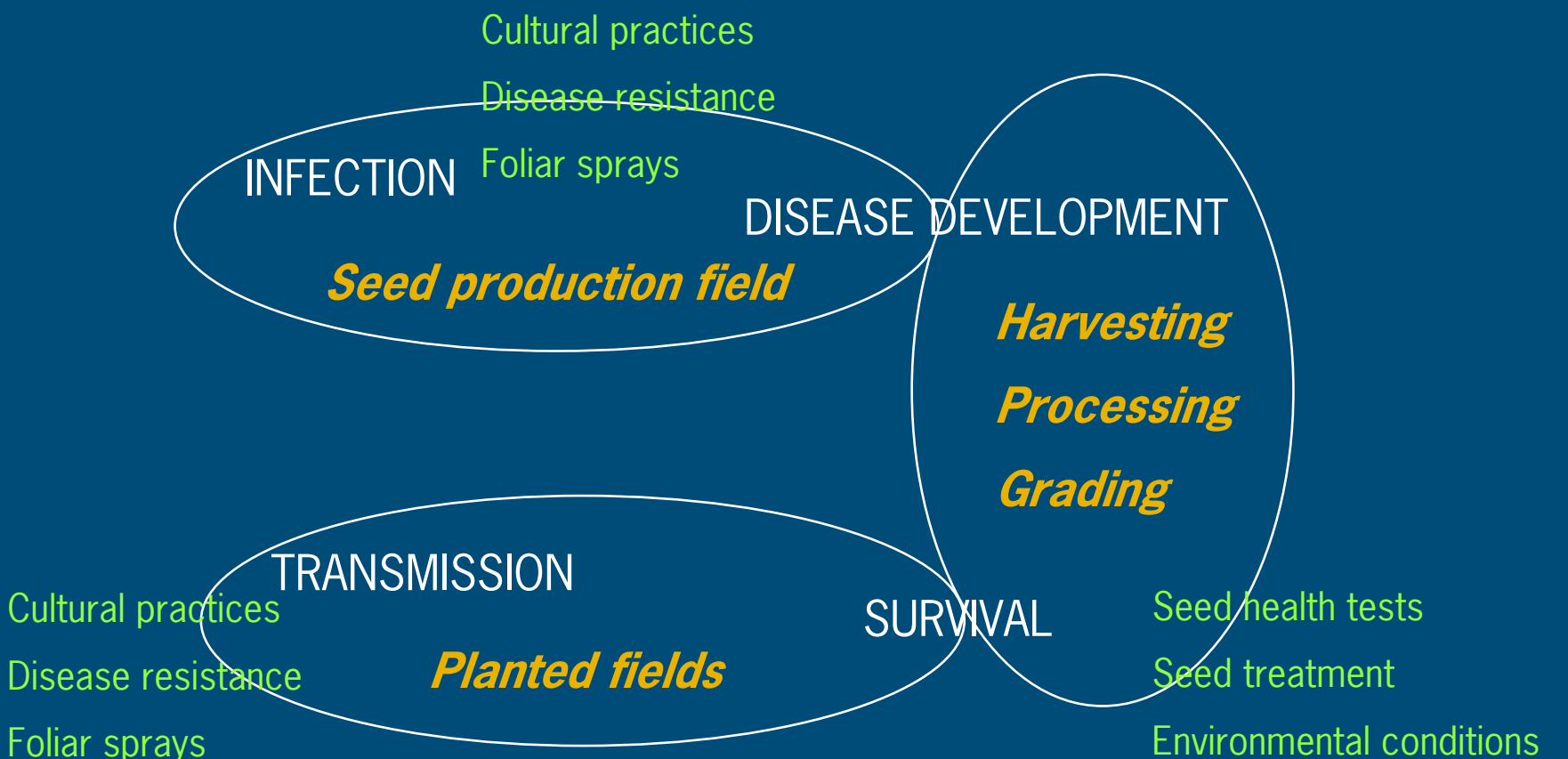
Seed health management

- Integrated approach aimed at the entire production system
  - In planted fields
  - In seed production fields
  - During harvest, processing, grading and storage

Diagram integrating pathogen, environment and disease management strategies



Modified after McGee, 1995

Seed health testing programmes

Legislative measures

- Quarantine measures
  - prevent entry
  - following outbreaks, prevent further spread + eradicate pathogen
- Inoculum thresholds for infected seeds in certification programmes
  - establish tolerance levels
  - standardise and establish certification programmes for seed health testing

Tolerance levels

	Group of pathogens	Host	Infection source	Tolerance (%)	Problem	Control
1	*Clavibacter michiganensis* subsp. *michiganensis*	Tomato	Seed	0-tolerance	Q-organisms	Selection/ Field inspection Seed treatment
2	*Phoma*	Bean Cabbage	Seed, plant debris	0.01 –0.1%	Yield and quality loss	Seed and crop treatments
3	*Botrytis*	Onion	Seed, plant debris	0.1 – 2%	Yield and quality loss	Seed and crop treatments
4	*Colletotrichum* *Alternaria* *Ascochyta*	Bean Onion Carrot Pea	Seed, plant debris	2 - 5 %	Yield and quality loss	Seed and crop treatments
5	*Fusarium* *Alternaria* *Phoma betae*	Variable Variable Beet	Seed, plant debris, soil, weeds	5 – 25 %	Quality loss seed or plant products	Seed and crop treatments

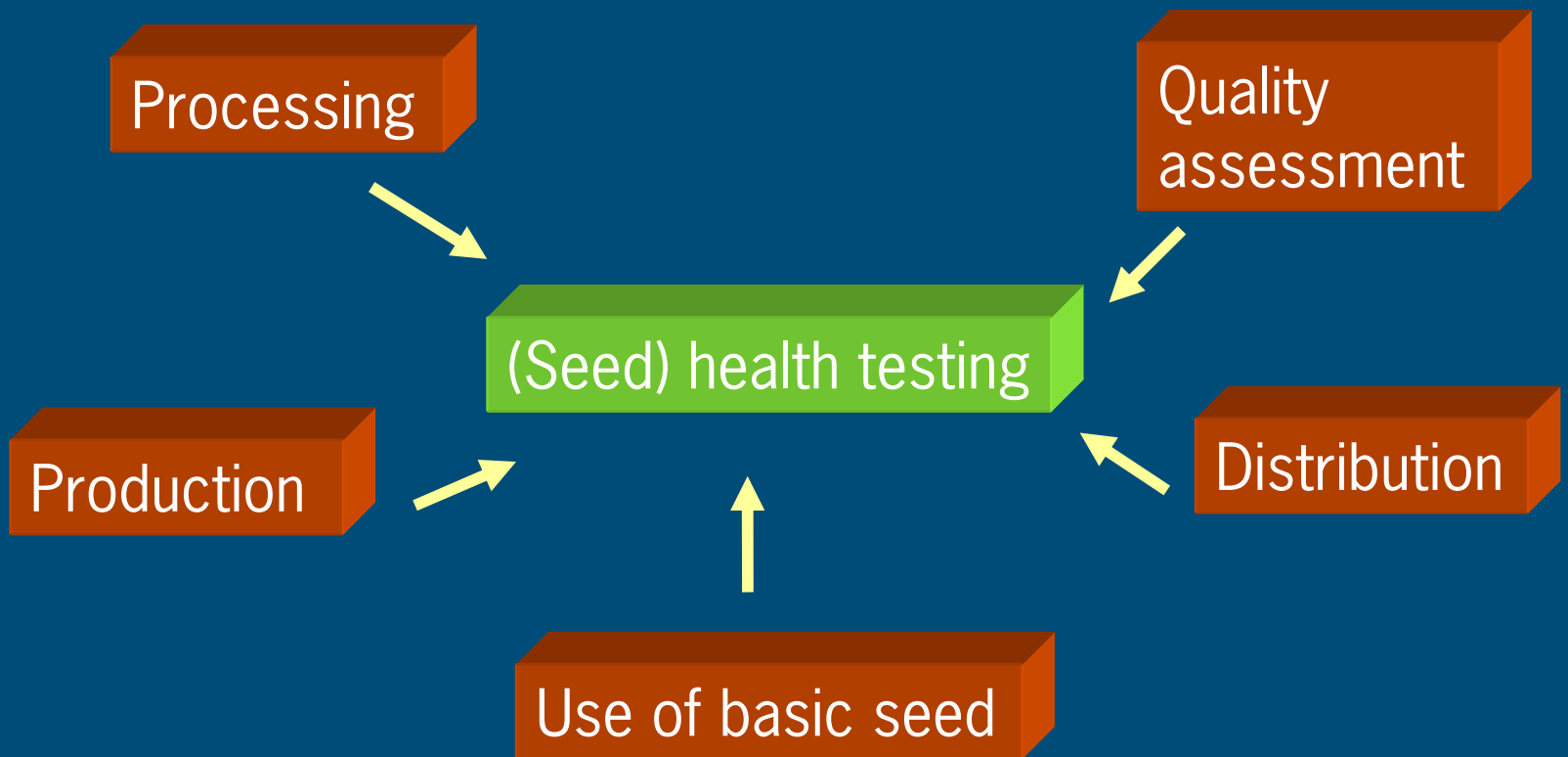
Tolerance levels

- Have been established under temperate climate conditions
- Tolerance levels in different climates in Indonesia (low vs high lands) are largely unknown

Why seed health testing?

- Seed borne inoculum may cause progressive disease in the field and reduce the commercial value
- Imported seed lots may introduce diseases into new regions
- Testing may elucidate seedling evaluation and causes of poor germination
- Testing may indicate the necessity to perform seed lot treatment

The role of (seed) health testing



International Seed Testing Association

- Objectives
  - Develop, adopt and publish procedures for sampling and testing seeds
  - Promote uniform application of these procedures in international trade (International seed sample certificates for accredited laboratories)
  - Promote research in the field of seed science and technology

- Designated authority → designated by a government of a country
- Designated members → persons or laboratories engaged in seed testing designated by their designated authority
- Accredited Laboratory: member laboratory accredited b the executive committee

International Rules for Seed Testing

Annexe to Chapter 7 Seed Health testing

- ISTA Seed Health Validation Programme
  - To improve the ability of ISTA to respond to technological changes in seed health testing
- International Rules for Seed Testing: effective accordong to specialists in the seed health validation programme

ISTA official Seed Health Testing Methods

Host	Pathogen	Host	Pathogen
Daucus carota	*Alternaria dauci*	*Oryza sativa*	*Drechslera oryzae*
Daucus carota	*Alternaria radicina*	*Oryza sativa*	*Pyricularia oryzae*
Helianthus annuus	*Botrytis cinerea*	*Oryza sativa*	*Alternaria padwickii*
Brassicaceae	*Leptosphaeria maculans*	*Hordeum vulgare*	*Ustilago nuda*
Pisum sativum	*Ascochyta pisi*	*Triticum aestivum*	*Septoria nodorum*
Phaseolus vulgaris	*Colletotrichum lindemuthianum*	*Festuca arundinacea*	*Neotyphodium coenophialum*
Linum usitatissimum	*Botrytis cinerea*	*Clycine max*	*Phomopsis complex*
Picea engelmannii/glauca	*Colyscypha fulgens*	*Linum usitatissimum*	*Alternari linicola*
Pinus taeda/elliottii	*Fusarium moniliiforme*	*Linum usitatissimum*	
		Brassica spp.	*X. c. pv. campestris*

International Seed Health Initiative

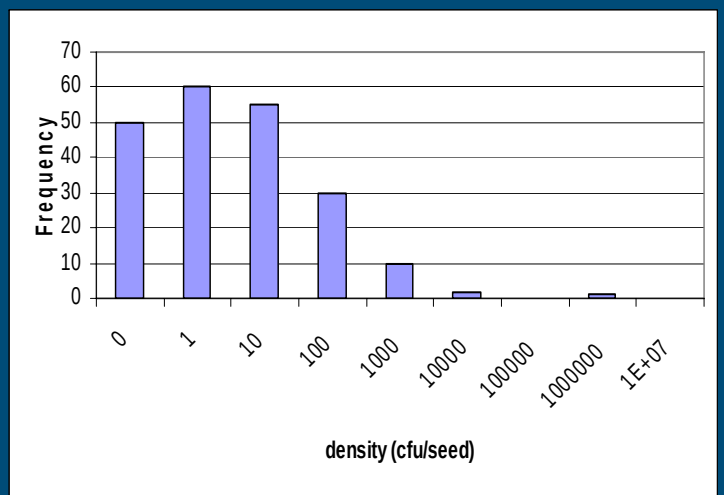
- Initiative of the Vegetable Seed Industry (1993)
- Members: The Netherlands, France, USA, Japan and Israel
- Membership open for other countries (contribution via the international seed federation (ISF))
- Development of general standard methods are developed within the International Technical Groups (ITG)

General principles for seed testing

- Methods should be adequate for the target pathogen
  - Sample size, number of subsamples, techniques
- Results will be influenced by seed treatments
- Storage and packing can influence the seed health status
  - Some fungi (Rhizopus) can rapidly spread on blotter tests)
- A negative test result does not quarantee that the seed lot is pathogen-free, only that the seed sample tested was found to be negative

General principles for seed testing

- Validated methods should be used that are:
  - Specific: distinguish target pathogen from all organisms
  - Sensitive: the ability to detect pathogenic organisms in a low incidence in seed stocks
    (Beware: Poisson distribution)
  - Speed
  - Simplicity: minimize the number of stages
  - Cost effectiveness
  - Reliability: robust, repeatable



Nr. of seeds tested in seed lots with various threshold levels of infection and the probability for false negatives

Threshold infection (%)	Probability (%) of false-negatives	No. of seeds to test
0.01	5	30.000
0.01	1	46.000
0.05	5	6000
0.05	1	9200
0.1	5	3000
0.1	1	4600

Field inspections

Alternaria/ tomato

Xanthomonas/cabbage

TMV/pepper

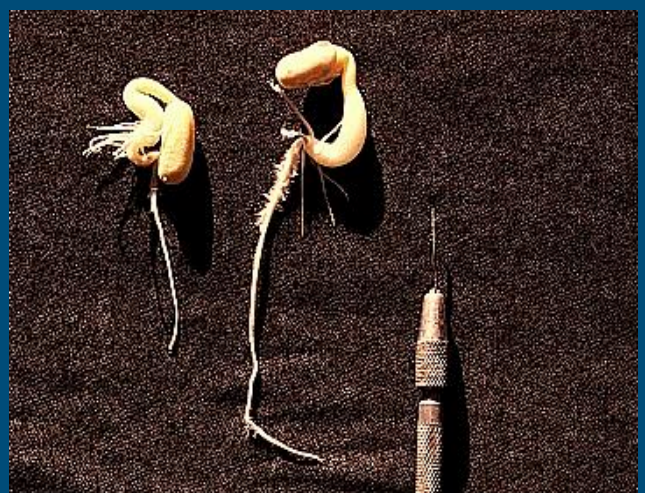
Visual selection seed

Colletotrichum/bean

Ascochyta/pea

Mosaic virus/pea

Pathogenicity assays



Pseudomonas syringae pv. *phaseolicola* in bean

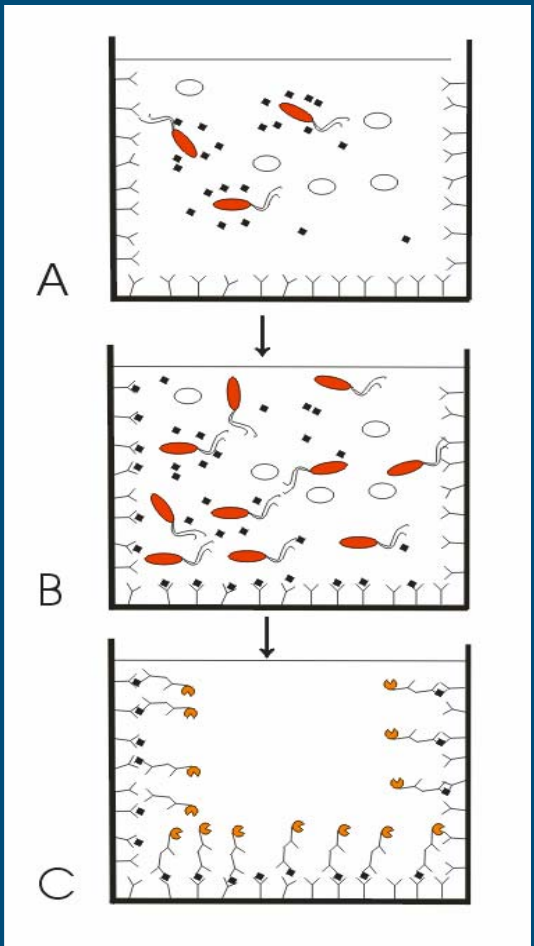
Dilution plating on growth media

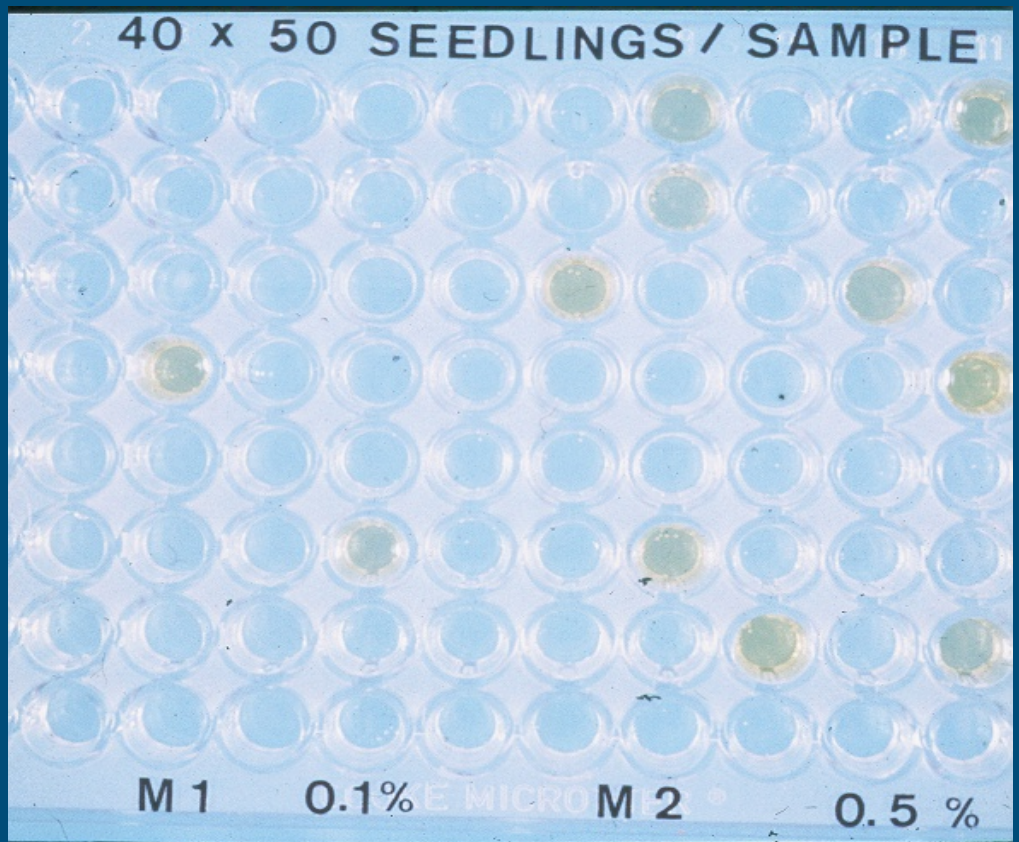
P. s. pv. phaseolicola/King's B

Blotter tests and microscopical methods

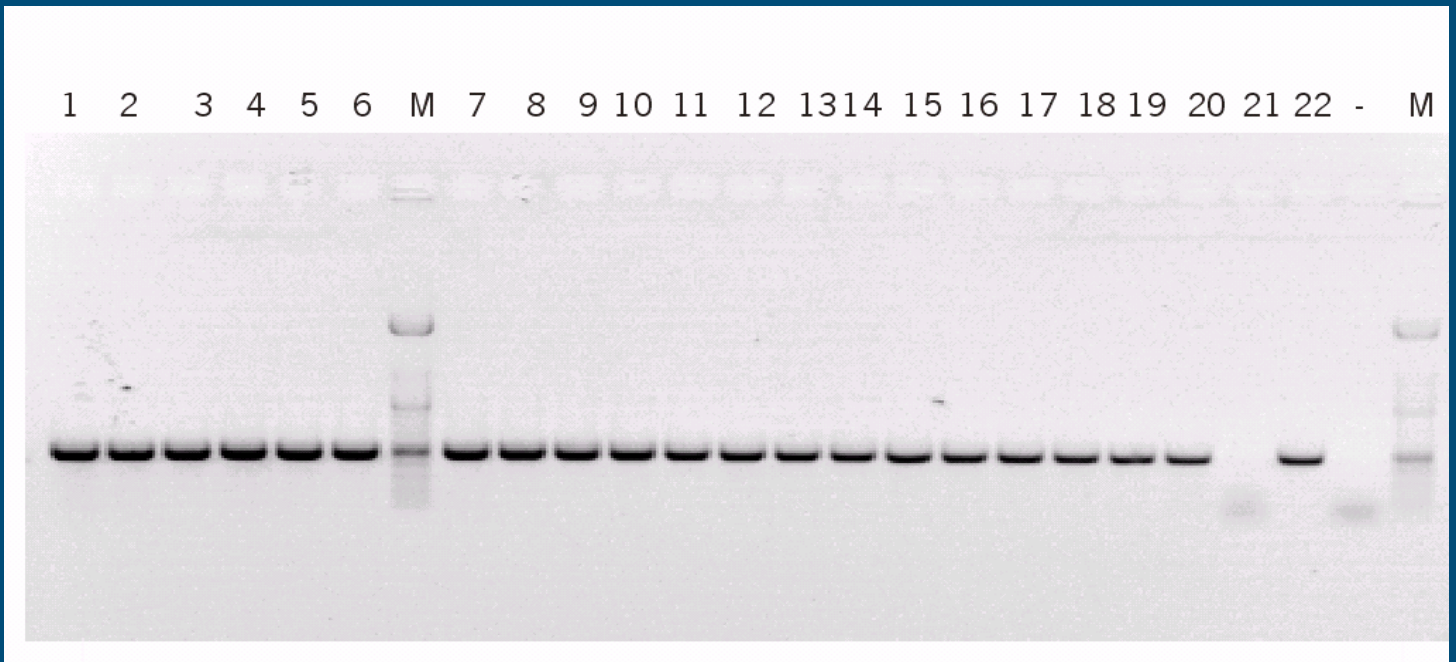
Alternaria dauci/carrot

Enzyme Linked Immunosorbent Assay





Detection of specific DNA by PCR amplification



The tools for seed health testing

Method	Viruses	Bacteria	Fungi
Field inspections	++	++	++
Visual selection seed	++	++	++
Pathogenicity test	++	++	++
Growth media	-	++	++
Microscopical techniques	+/-	++	++
Blotter test	-	-	++
ELISA	++	+	+/-
Immunofluorescence assay	-	++	-
PCR-amplification	+	+	+/-

Selection of seed production area's

- Preferably in arid or semi-arid climates
  - many fungi and bacteria are readily spread under cool moist conditions and by rain
    - e.g. *X. axonopodis pv. vesicatoria, C. michiganensis subsp. michiganensis, A. solani, C. capsici, B. aclada*
- Greenhouse production
  - Specially for high grade seed material
  - for control of insect- and wind-transmitted pathogens
    - e.g. *A. dauci*, *B. aclada*
  - to allow effective biological control

Culture measures and sanitation practices

- Crop rotations
- Sowing date
  - Crop may outgrow pathogen at higher temperature
- Spacing of crops
  - Use of disease free areas (green houses) for production of basic seed
  - No use of infected seed in disease free-areas
- Crop density
  - leave wet period determines largely infection risks
- Appropriate fertilisation
- Harvest time: maturity of seed
  - 15 days earlier harvest improved health of barley and wheat seed considerably with respect to infections with *Bipolaris sorokiniana* (Olvang, 2004)
- Roguing of diseased plants
- Control of weed and crop plant debris

Culture measures and sanitation practices

- Appropriate irrigation practices (furrow better than overhead)
- disinfection of equipment and machineries
- Avoid wounding of plant material
- Use of fungicides and insecticides to control diseases and pests
- Use of wind breaks

Seed health management post harvest

Culture measures and sanitation practices

- Avoid cross-contamination during harvest, processing and storage
  - Seed grading and sorting may remove light, shrinkled or spotted seeds which may be contaminated, but each step includes the risk for mearing
- Optimise storage conditions to suppress the progress of seed infections

Upgrading health condition

Action	Aim	Risk
Standard cleaning by separation	Removal debris, sclerotia, hollow seeds	Smearing inoculum over equipment/seeds
Size grading	Removal small potentially infected seeds	Loss of healthy seeds Smearing inoculum over equipment/seeds
Liquid cleaning	Removal light seeds	Smearing inoculum over equipment/seeds
Electric eye cleaning	Removal spotted seeds	Masking invisible infections
Physical treatment (i.e. heat)	Eradication of pathogens	Reduction vitality of seeds
Chemical treatment	Suppression of pathogens	Unwanted shifts microflora

Seed treatment to eradicate and reduce inoculum

- Chemical (+ natural bio-active compounds)
- Physical
- Biological

Efficacy of seed treatments

- Contact with active ingredients
  - land: 10.000 m^2
  - in furrow: 500 m^2
  - seed: 58 m^2
- + less impact on non-target organisms and drift

Grouping of fungicides

- 'Modern fungicides':
  - Non systemic:
    - limited penetration in seed
    - not mobile within tissues of (germinating) seeds
    - can protect against invasion of soil borne diseases
    - eradicate pathogens superficially present on seed
    - broad range of action
    - low risk for resistance development (multisite inhibitor, cell poison)
  - Systemic:
    - prevent disease development away from the site of application
    - eradicate deep seated seed infections
    - translocated in sprouting seeds
    - narrow range of action
    - high risk for resistance development (single site activity)

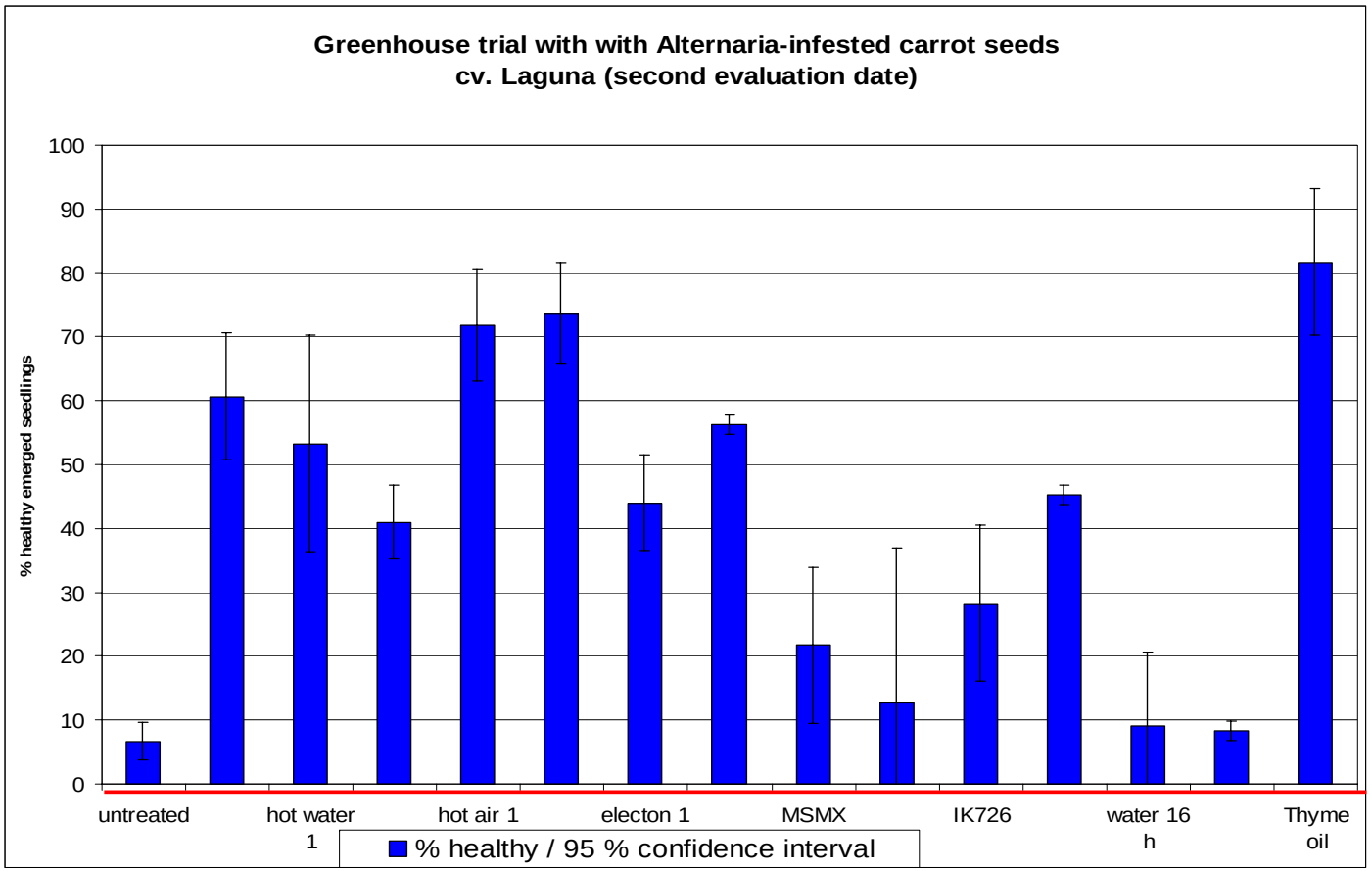
Treatment of seeds

- Loading: correct ratio chemical to seed
- Distribution: uniform division between seeds
- Retention: strong adherence to seeds
- Hazard: no risk for operator
- Contamination: no pollution

Physical methods

- Hot water
- Aerated steam (+ ultrasound treatment=Sono-Steam)
- Dry heat
- Radiation
- Electron treatment (E-dressing)

Effect physical treatments on Alternaria in carrot seed



Warm water treatment (1)

- Activity based on coagulation of proteins in cells
- Efficacy dependent on temperature regime: quick temperature raise should be followed by quick temperature fall
- Most effective against superficial organisms
- Variations to improve efficacy:
  - presoaking in cold water
  - addition of acidified cupric acetate
  - addition of acidified zinc sulphate


NPK founders, India

Warm water treatment (2)

- Relative simple and cheap

- Frequently incomplete eradication of bacterial and fungal pathogens
- Ineffective against internal infections in larger seeds (e.g. *Ascochyta* in pea)
- Risk for seed damage
- Requirement for redrying of treated seeds
- Only small amounts can be treated at one time

Hot water treatment for seed borne diseases

Crop disease/ Pathogen	Treatment
Brassica canker/ *Leptosphaeria maculans*	30 min, 50 °C
Cereal loose smut/ *U. segetum* var. *tritici*	6 h, 41 °C
Rice Bakanae disease/ *Gibberella fujikuroi*	7 min, 57 °C
Brassica black rot/ *X. c*. pv. *campestris*	30 min, 50 °C
Tomato canker/ *C. m. michiganensis*	60 min, 53 °C
Pea blight *P. s*. pv. *pisi*	15 min, 55 °C

Biological methods

Often poor ecological competence in the spermosphere and therefore:

- Often less effective than chemical agents
- Often more variation
- Often short term protection

Priming in relation to seed health

Priming: controlling imbibition to initiate germination, but to prevent radicle emergence

- Results in faster and more synchronous germination
- Allows addition of fungicides (but may also spread diseases)
- Types of seed priming methods
  - Osmotic priming: PEG
  - Drum priming: slow hydration in a drum
  - Solid matrix priming: solid material and water in known proportion

Conclusions

- Preventive measures are preferred
  - Use pathogen free basic seed
  - Grow in (semi) arid or contained area's
  - Use hygienic protocols strictly
- Use curative seed treatments if appropriate
  - Upgrade seed quality by sorting procedures
  - Treat seeds with suitable fungicides
  - Use physical treatments to control bacterial diseases

  In all stages of seed production: control health quality by reliable seed testing methods