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Eradication of seed-borne plant pathogens

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Many plant pathogens are seed-borne, and their association with seed is an important means of dissemination and carry-over between crops/seasons. The implementation of clean seed policies to exclude inoculum can be an effective means of disease control/management, at national, regional and individual farm levels. There are a number of approaches that can be taken to implement a clean seed policy: (1) produce seed crops in areas known to be free of particular pathogens; (2) test and reject, i.e. test seed lots for the presence of particular pathogens and reject if found to be present; (3) test and treat, i.e. test seed lots and treat if found to be present; (4) treat all, i.e. treat all seedlots regardless of health status. When treating seed in the context of a clean seed policy, the aim is generally that of eradication. The term eradication implies the complete elimination/killing of the target pathogen. However, the success or otherwise of any treatment can only be judged in terms of the assay used to evaluate it. Therefore in the context of seed treatment, we should redefine 'eradication' as a reduction of inoculum to undetectable levels. Thus it is important when comparing different treatments reported in the literature to pay careful attention to the details of the assays used to evaluated them and especially to the numbers of seeds examined as this effectively implies the detection limits. Seed treatments may be chemical, physical or biological and may be targeted at one or all of fungal, bacterial or viral pathogens. During the latter half of the last century most emphasis has been given to fungicidal chemical treatments, and most treatment has been done on the basis of the 'treat all' approach. More recently, as a result of increasing concerns about safety and environmental impact, there has been a reduction in the range of compounds available, their spectrum of activity and in some countries a move to treatment only on the basis of proven need. In organic or ecological production systems, treatment with synthetic fungicides is generally not permitted. As a result there has been an increased interest in physical and biological treatments, and traditional hot-water treatments in particular have seen a revival for some crops. This paper will attempt to review the treatment options available for eradication of the different pathogen types and the 'pros' and 'cons' of different approaches.

Eradication of plant pathogens from seed

XLII Brazilian Phytopathology Congress
Rio de Janeiro
3-7 August 2009

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Inspiration

Maude, R.B. (1996) *Seedborne Diseases and Their Control. Principles and Practice*

Chapter 7 - Disease Control: Eradication and Reduction of Inoculum by Seed Treatment

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Introduction

- Plant Health Solutions
- Independent research, testing and consultancy business
- Based in Warwick, Inglaterra
- Primary focus on seed-borne and bacterial diseases of vegetable crops

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Outline

- Disease management and clean seed
- What does eradication mean ?
- History and types of treatments
- STOVE project
  - Brassicas / *Xanthomonas*
  - Carrots / *Alternaria*

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Disease Management

- The use of 'clean' seed is an important and potentially highly effective means of disease control for seed-borne diseases
- National, regional, individual farm level

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Achieving Clean seed

Options:

1. Produce seed in disease-free regions
2. Test seed for presence of pathogens and only use 'clean' seed lots
3. Treat the seed to 'eradicate' the pathogens
4. Combine 2. and 3. – test and treat only if necessary

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Eradication

- Implies the complete elimination of the target pathogen

BUT

- Efficacy of a treatment can only be judged in terms of the seed test or trials used to evaluate it.
- Important:
  - when comparing different treatments reported in the literature to pay careful attention to the details of the seed test or field trial used to evaluated them and especially to the numbers of seeds examined or sown as this effectively implies the detection limits

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Seed testing

- The problem with seed testing:
  - can never guarantee that a seed lot is completely healthy ('zero tolerance' is not possible)
- Can only test a sample:
  - tolerance std. = minimum % inf. seed which can be reliably detected (depends on sample size)
  - analytical sensitivity = minimum numbers of the pathogen which can be reliably detected (depends on assay design)

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Seed testing

- Probability of a positive test result, P_+ , depends on:
  - the probability of at least one infested seed being contained in the sample:

    $$P_{cont} = 1 - (1 - \theta)^n$$

    where θ is the true proportion of infested seeds, n is the sample size
  - if present, the probability of detecting an infested seed in a sub-sample:

    P_s = analytical sensitivity
- Thus, $P_+ = P_{cont} \times P_s$

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Seed testing

Assuming a perfect test, i.e. $P_s = 1$

True % Infection	Prob. of detection in sample of size n =				
	100	300	1000	3000	30000
1	0.63	0.95	0.99	1.00	1.00
0.1	0.095	0.26	0.63	0.95	1.00
0.01	<0.01	0.03	0.095	0.26	0.95

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Tolerance standards

- What health standard do we want to achieve with our treatment ?
- Depends on:
  - Rate of transmission from seed to seedling
  - Rate of disease spread in the field
  - Economic damage in relation to disease levels
- More information:
  - *Proceedings of 9º Simpósio Brasileiro De Patologia De Sementes, Passo Fundo, Oct 2006* - Thresholds, Standards, Tests, Transmission and Risks.
  - www.planthealth.co.uk

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Seed treatments

Eradication is the aim

Reduction is the reality

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Types of treatments

- Chemical
  - inorganic
  - organic
  - systemic / non-systemic
  - synthetic / natural
  - resistance inducers
- Physical
  - hot water
  - hot air, aerated steam
  - dry heat
  - radiation
- Biological
  - micro-organisms
  - resistance inducers

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The ideal seed treatment

Should:

- reduce the target pathogen(s) to acceptable levels
- not reduce seed germination or vigour
- not reduce longevity/storability of seed
- have low toxicity to humans/animals
- not harm the environment

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A Brief History of Seed Treatments

- 1650s: brining (NaCl), brine/lime (cereals/bunts)
- 1800s: copper sulphate (cereals)
- 1880s: hot water
- 1890s: formaldehyde
- 1900s: mercuric chloride (cereals/Fusarium)
- 1910s: organo-mercury compounds
- 1930s: thiram
- 1940s: copper compounds (vegetables)
- 1950s: captan
- 1960s: aerated steam, dry heat
- 1960/70s: systemic fungicides (carboxin 1966 – basidiomycetes)
- 1990s: biologicals
- 1992: organo-mercurials banned in UK
- 2000s: radiation/electrons

See Maude (1996), dates very approximate

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Location of inoculum

- Superficial
  - on the surface the seed / fruit (most bacteria, many fungi)
- Internal
  - in testa/pericarp (many fungi, some viruses)
  - endosperm/cotyledons (a few fungi)
  - embryonic axis (viruses/smuts)

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Fungi

- Majority of chemical seed treatments have been targeted at fungal pathogens
- Most early seed treatments only affected inoculum on or in the seed coat
- Introduction of systemic fungicides in the 1960s and 70s enabled elimation of more deep-seated inoculum
  - selective, narrow mode of action
  - increased likelihood of resistance
  - most often used in combination/mixtures

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Fungi

- Cereals
  - mostly treated with combinations/mixtures of compounds:
    - also target soil-borne pathogens
    - mainly systemic, different modes of action
  - control generally effective
  - increasingly treatments are applied on the basis of need
    - environmental concerns
    - cost of treatment v. cost of testing
    - esp. for spring sown

Fungi

- Pulses and Vegetables
  - Thiram the most common treatment
    - targets soil-borne damping-off pathogens rather than seed-borne
  - Thiabendazole and/or iprodione added to control particular seed-borne pathogens

Bacteria

- Hot water
- Organo-mercury compounds
- Soaks in antibiotics
  - streptomycin, kasugamycin
- Soaks in disinfectants
  - NaOCl, ClO_2
- Soaks in copper compounds
  - cupric acetate
- Dry heat

Bacteria

- Many papers reporting eradication/control of bacterial pathogens
- Few seem to be widely used. Why ?
  - Antibiotics not permitted
  - Results very variable/contradictory
    - often results based on tests on just one or two seedlots
    - small/differing numbers of seeds tested
  - Phytoxicity
    - varying sensitivity (esp. to physical treatments)
  - Surface treatments
    - apparent success dependent on location of inoculum in the particular seed lots examined

Viruses

- Acid extraction
  - tomatoes/ToMV
- Phosphate soak
  - Na_3PO_4
- Hypochlorite soak
  - tomatoes/PeMV
- Dry heat
  - tomatoes, 70°C for 4 days for TMV

Treatments

- Vast majority of chemical seed treatments have been developed for / targeted at seed-borne fungi on the major cereal crops
- Costs of R & D and registration mean this situation is unlikely to change
- Increased environmental concerns
  - changing European legislation to hazard-based rather than risk-based assessments
- Future - fewer chemical treatment options available for vegetable seeds and other minor crops ?

Conventional seed

- Relies on the use of fungicides for disease management both during seed production and treatment of the harvested seed
- Rare to find un-treated conventional seed
  - easier to treat all seed than to test and treat on the basis of need
  - lack of tolerance standards or treatment thresholds

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Organic Seeds

- Seed health more important for organic production where there are few options for control in the field
- Seed health standards should be more stringent for seed used in organic production
- Fewer options for disease management during seed production
- Renewed interest in physical, biological, natural treatments…..

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STOVE project

Seed Treatments for Organic VEgetable production

- EC co-funded project
  - QLK5-2002-02239
- ~4 yrs
- Web-site: www.stove-project.net
- Aim:
  - to identify effective, organically acceptable seed treatments for a range of host/pathogen combinations

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STOVE - partners

- BBA, Germany (co-ordinator)
- PRI, Netherlands
- HDRA/PHS, UK
- Gothenburg University, Sweden
- Nunhems (Hild), Germany
- University of Turin, Italy
- Findus, Sweden

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STOVE – hosts/pathogens

Fungi

- Lamb's lettuce - *Phoma valerianellae*
- Carrot - *Alternaria dauci* (leaf blight), *A. radicina* (black root rot)
- Brassicas - *Alternaria* sp. (dark leaf spot)
- Parsley - *Septoria petroselina*
- Bean - *Colletotrichum lindemuthianum* (anthracnose)
- Pea – *Ascochyta pisi* (leaf, pod spot)

Bacteria

- Brassicas - *Xanthomonas campestris* pv. *campestris* (bacterial black rot)
- Carrot - *Xanthomonas hortorum* pv. *carotae* (bacterial blight)

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Treatments examined

- Physical
  - Hot water, Aerated steam (hot air), Electron bombardment
- Natural products - Essential oils
  - E.g. Oregano oil, Thyme oil, Clove oil, ….
- Microbial
  - commercial products (e.g. Serenade, Cerall….)
  - experimental strains (from Turin, SLU, BBA….)
- Resistance Inducers
  - E.g. Milsana, Chitoplant, Comcat, ….

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Project outline

Year 1

- Hot water, hot air and electron treatments
  - Optimisation for different pathosystems
  - Effects on seed viability
- Micro-organisms, resistance inducers, plant extracts
  - Effects on seed-borne pathogens and on plant growth

Year 2

- Comparative testing in the glasshouse and in the field
  - Hot water- | Hot air- | Electron- | Micro-organisms | Resistance inducers | Plant extracts

Year 3/4

- Field / glasshouse testing of combinations of selected physical methods with selected microorganisms, plant extracts and resistance inducers

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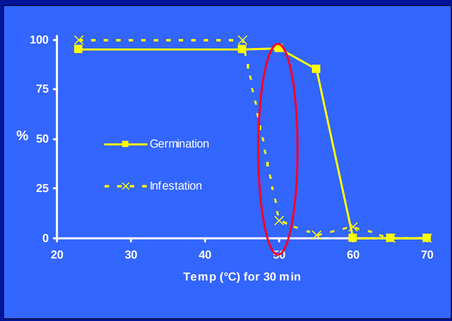
Physical treatments - optimisation

Principle



Hot water

- Used for ca. 100 yrs
- Simple, relatively 'low tech'
- Big disadvantage that seed needs drying after treatment
- Problem of variation in sensitivity

Hot air - *ThermoSeed*®

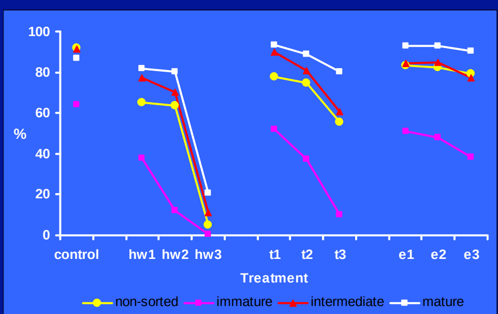
- Treatment with hot, humid air for a short time with precise control of:
  - *Temperature*
  - *Air humidity*
  - *Treatment time*

- Developed in Sweden by *Acanova* for cereals (www.acanova.se)
- Now being applied as an alternative to chemical treatments for conventional production – 1000 t/week plant in operation

Electron treatment

- Mobile system
- Based on TV technology
- Seed falls past a beam of electrons
- Voltage and dose adjusted to penetrate only the seed coat

Effect of seed sorting on sensitivity to hot water, Thermoseed®, electron treatments in *B. oleracea*



Sorting on the basis of chlorophyll fluorescence

Black rot of brassicas

- V-shaped chlorotic, yellow lesions with blackened veins
- Caused by *Xanthomonas campestris* pv *campestris* (*Xcc*)
- Systemic infection - stunted or dead plants
- Premature defoliation, secondary soft rots
- At least six races

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Seed testing for *Xcc*

1. Shake 2.5 h and centrifuge 5 min (*Xcc*)
2. Dilute and plate on selective media
3. Sub-culture suspect colonies
4. Confirm identity by pathogenicity test
5. Estimate infestation levels by maximum likelihood methods

Theoretical sensitivity 1.5 cfu/ml (P=0.95)

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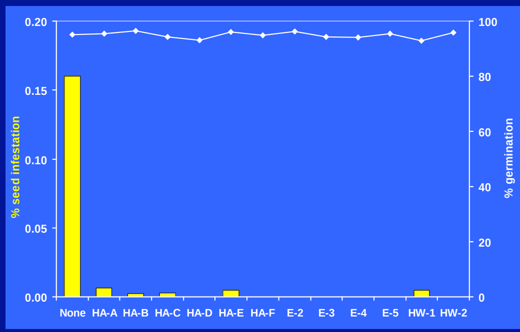
Brassica / *Xcc*: optimising physical treatments



Up to 50,000 seeds tested

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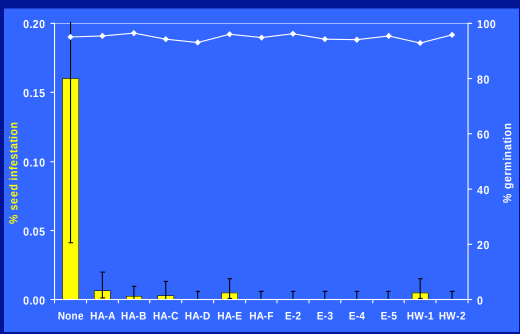
Brassica / *Xcc*: optimising physical treatments



Up to 50,000 seeds tested

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Biologicals - *In vitro* screening

Xanthomonas **incorporated into the agar**

Supernatant	Xcc3818A	Xcc3882	Xhc3856	Xhc9000
MBI 600	++++ / +++*	+++	+++	+++
Serenade	+++ / +++*	+++	+++	+++
SLU 3	+++ / +++*	+++	+++	+++
FZB 24	++++ / -*	-*	-*	-*
U 410	+++ / -*	-*	n.t.	n.t.
G 12	++ / -*	-*	n.t.	n.t.

- = no inhibition zone, + = ≤ 11 mm, ++ = ≤18 mm, +++ = ≤36 mm, ++++ = > 36 mm* =

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Transmission experiments

- Batches of 500 seeds sown in seed trays
- Grown in glasshouse for 4-5 weeks
- Samples of seedlings harvested from each tray
- Samples then stomached, diluted and plated on selective media
- Sub-culture and confirm identity
- Estimate infection by maximum likelihood methods

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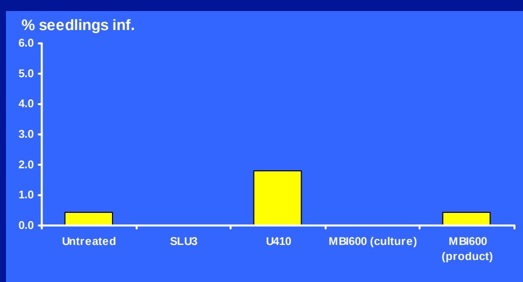
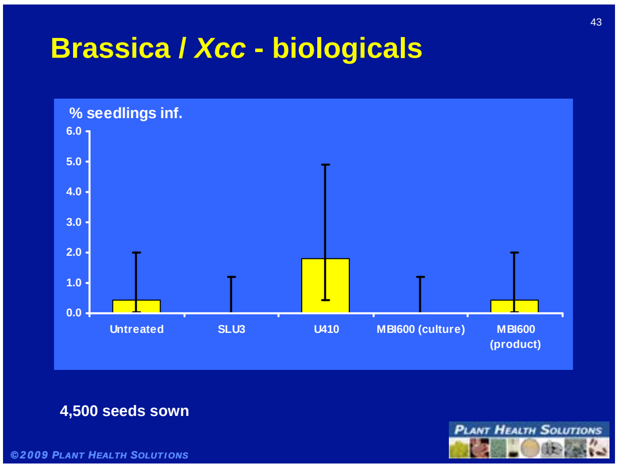
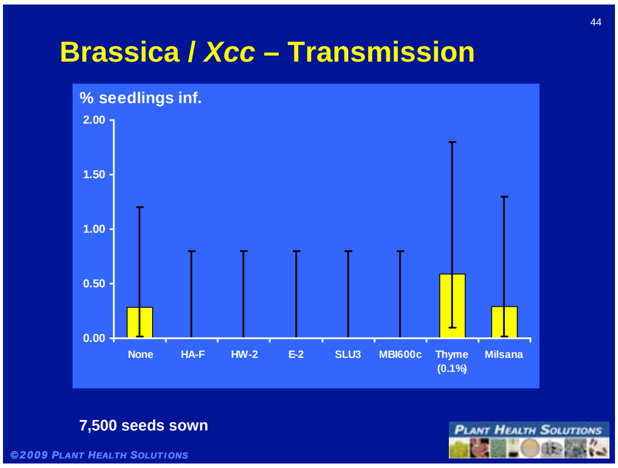
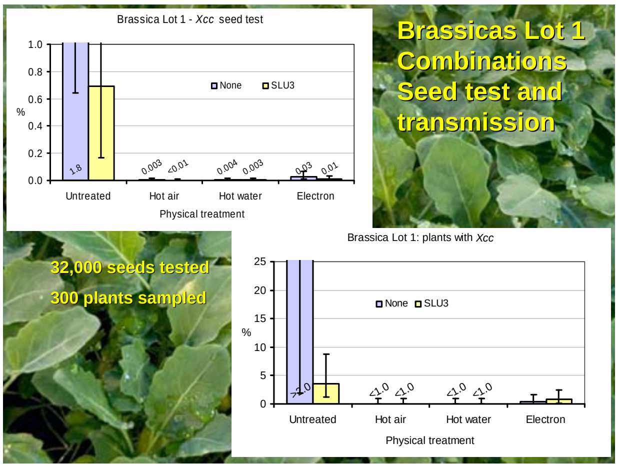
Brassica / *Xcc* - biologicals

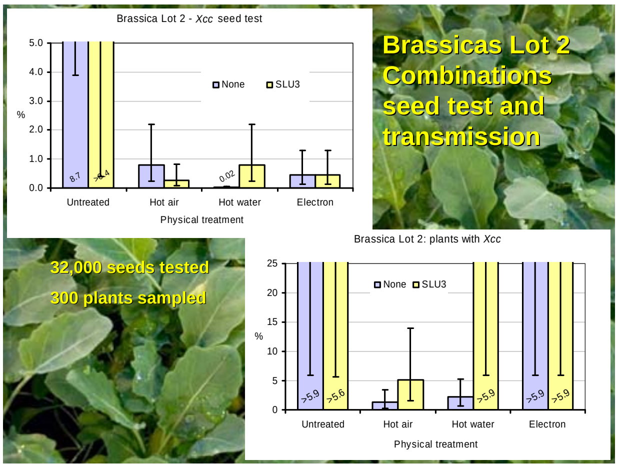


4,500 seeds sown





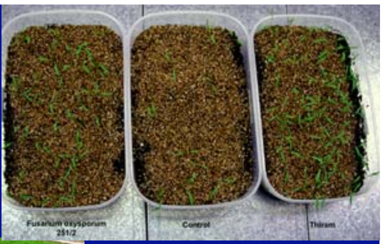


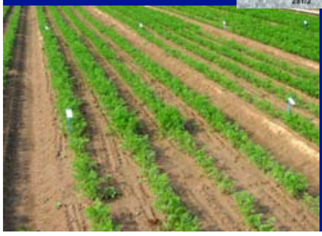


Xcc - conclusions

- Physical treatments:
  - hot air and hot water consistently reduced seed infestation levels;
  - did not always 'eradicate' the pathogen;
  - practical value will depend on initial infestation level.
- Biological
  - some evidence of a reduction when used alone;
  - no benefit as a combination treatment.
- Thyme oil only tested on seed at 0.1%
  - subsequent work suggests it to be effective at higher concentration

Efficacy testing under controlled conditions





Field trials

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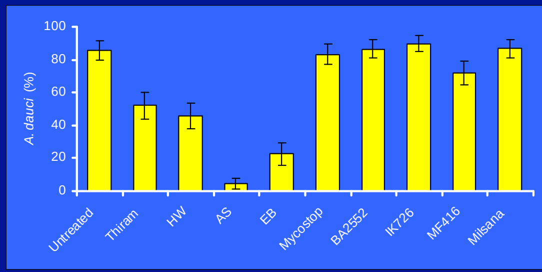
Carrots / *Alternaria*

Single treatments evaluated in blotter tests



300 seeds tested

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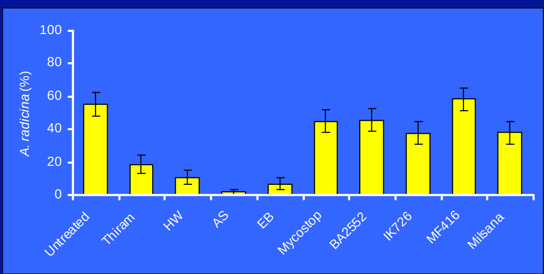
Carrots / *Alternaria*

Single treatments evaluated in blotter tests



300 seeds tested

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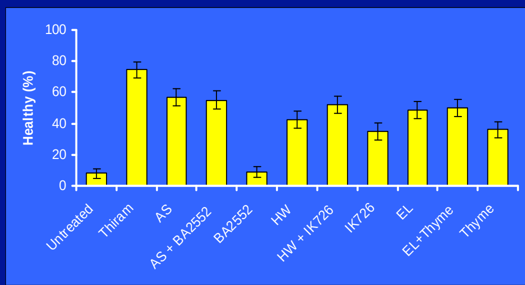
Carrots / Alternaria - Glasshouse

Single and combination treatments



300 seeds sown

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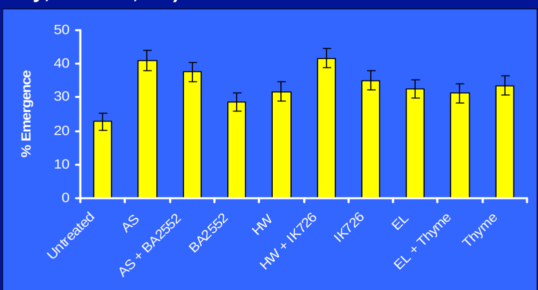
Carrots / Alternaria Field trials

Combined data from 5 sites in 4 countries (Germany, Italy, Sweden, UK)



~4200 seeds per site – minor foliar symptoms only at one site

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Carrot / *Alternaria* - Conclusions

- **Blotters:**
  - **no treatment gave 'eradication'**
  - **physical treatments showed big effects, but not biologicals**
- **Glasshouse trials:**
  - **treatments not differentiated in a less infested seed lot**
  - **both physical and biological effects seen in heavily infested lot**
- **Field trials:**
  - **Improved emergence with all treatments**
  - **Aerated steam the most effective single treatment**
  - **Additive effect from hot water + IK726**
  - **No foliar symptoms, or storage rots – two interpretations**
    - **treatments effective**
    - **plot size too small (1400 per plot but ~1.4 million seeds per ha)**

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Seed-borne plant pathogens

Eradication is the aim

Reduction is the reality

Lack of 'tolerance standards' for many crop/pathogen combinations limits progress

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Acknowledgements

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The End

Thank you for listening

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