STOVE
Seed Treatments for Organic Vegetable Production

Final Workshop, Darmstadt, 13-14 September 2006

Xanthomonas on carrots and brassicas

presented by
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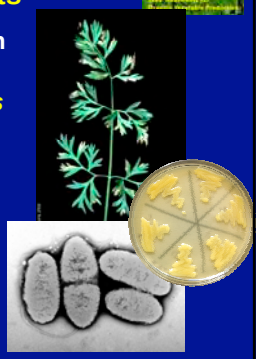
PLANT HEALTH SOLUTIONS

Bacterial blight of carrots

- Dark / brown lesions with chlorotic halo.
- Caused by *Xanthomonas hortorum* pv. *carotae* (*Xhc*)
- V. diff to distinguish from Alternaria blight in the field, mixed infections



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

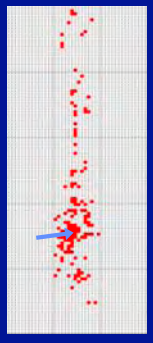
Seedborne bacterial pathogens

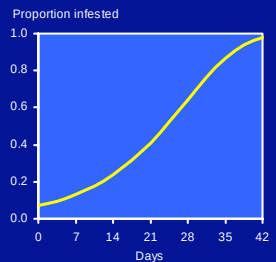
- Experimental difficulties:
  - Relatively low (but epidemiologically significant) levels of infestation found in naturally infested seedlots
    - 0.1% inf., 20% trans. -> 15,000 seeds for 1 inf. seedling (in untreated)
    - large numbers needed in grow-out tests or field trials
  - Direct testing of individual seeds not feasible
    - group testing of thousands of seeds

Black rot - seedling symptoms

Spread in brassica transplants

Symptoms (39 days)





1 inf. seedling → ~5,000 inf. transplants

Spread in brassica transplants

- Conservative estimates using spread model suggest a tolerance standard for seed health of:

  1 in 25,000 (0.004%)

  for module-raised transplants

- To achieve this standard requires testing of 75,000 seeds (P=0.95)

Seed testing for *Xcc* / *Xhc*

1. Soak seeds overnight in saline (*Xhc*) or 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 15 cfu/ml (*Xhc*), 1.5 cfu/ml (*Xcc*) (P=0.95)

Binomial model

- Probability of one or more infected seeds being in a sample of size, n, from a seedlot with a true infestation level, θ, is given by:

$$p = 1 - (1 - \theta)^n$$

Max. likelihood - Interpretation

- Previously:
  - the probability of a positive result, given that we know the true infection level θ.
- Maximum likelihood is the reverse:
  - what is the infection level which is most likely to have given the result obtained ?
- Sub-sample size constant:

$$\theta = 1 - \exp\left[\frac{\ln(1-r/k)}{n}\right]$$

  r = number of positives, k = number of samples, n = sample size

- Sub-sample size variable:
  - use a computer program like *STPro*™

Test design

- Sequential or multi-stage design used for both pathogens, e.g.:
  - 1st round - 1 x 10,000 seeds
  - 2nd round - 5 x 2,000 seeds
  - 3rd round - 8-10 x 10,000 seeds
- Results analysed by max. likelihood using *STPro* to estimate infestation levels *and* likelihood-based 95% confidence levels

STpro (Version 1.0)
==========
Datafile: TEST.DAT
Time: 12:17 on 11/02/03

Seedlot 1

No. seeds in sample	No. samples tested	No. positive	Fitted value
2000	3	0	0.1
9000	5	1	0.9

Estimated proportion of infected seeds = 0.000022
95.0 % confidence limits 0.0000012 to 0.000095

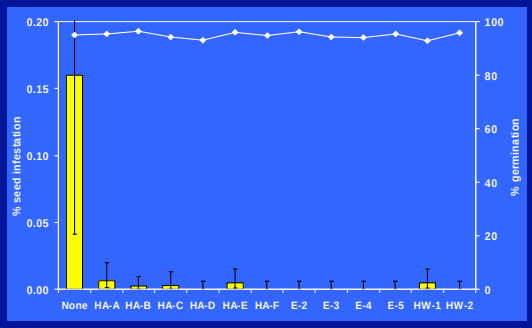
STOVE project outline

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

Brassica / *Xcc*: optimising physical treatments



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BCAs and natural products

- Initial screening of potential biological control agents (BCAs) was done *in vitro*.
  - unknown effects of seed test methods
  - numbers required
- Essential oils were also screened *in vitro*

In vitro screening I

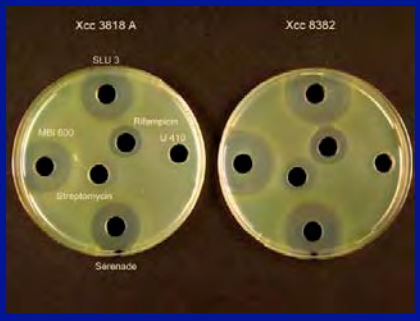
Inhibition of growth of *Xcc* on agar by culture supernatants

Supernatant	Medium	Xcc3818A
MBI 600	TSB	++++
Serenade	TSB	+++
FZB 24	TSB	++++
MSMX	TSB	-
Fus. 351/2	PDB	-
RG 6	NYDB	-
RG 68	NYDB	-
R 11	NYDB	-
M 8	NYDB	-
M 29	NYDB	-
SLU 1	TSB	-
SLU 2	TSB	-
SLU 3	TSB	+++
SLU 4	TSB	-
SLU 5	TSB	-

Supernatant	Medium	Xcc3818A
I 112	TSB	-
E 11	TSB	-
E 183	TSB	-
I A 6	TSB	-
I 124 b	TSB	-
II 79/2	TSB	-
U 407	TSB	++
U 410	TSB	+++
G12	TSB	++
G 53	TSB	-
T 69039	PDB	-
MAS 35	PDB	-
IK 726	PDB	-
FZB 53	TSB / GYM	-

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

In vitro screening II



In vitro screening III

Xanthomonas incorporated into the agar

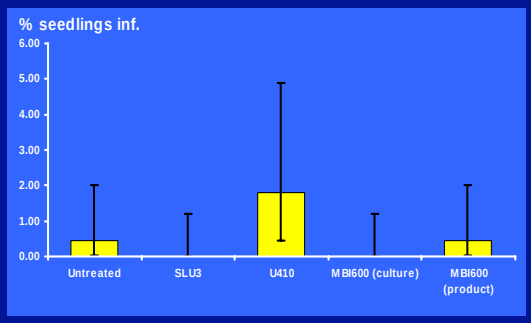
Supernatant	Xcc3818A	Xcc3882	Xhc3856	Xhc9000
MBI 600	++++ / +++*	+++	+++	+++
Serenade	+++ / +++*	+++	+++	+++
SLU 3	+++ / +++*	+++	+++	+++
FZB 24	++++ / -*	-*	-*	-*
U 410	+++ / -*	-*	n.t.	n.t.
G 12	++ / -*	-*	n.t.	n.t.

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

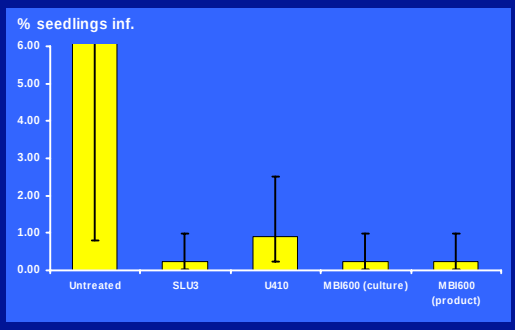
Transmission experiments

- 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 5 min, diluted and plated on selective media
- Sub-culture and confirm identity
- Estimate infection by maximum likelihood methods

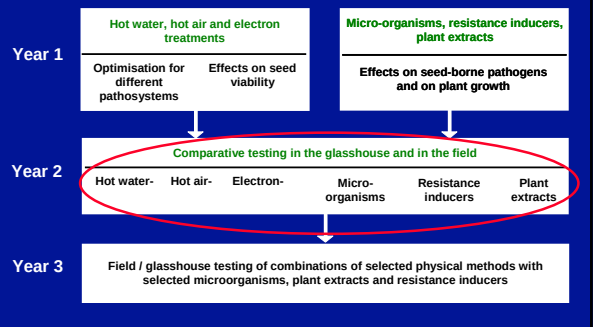
Brassica / *Xcc* - biologicals



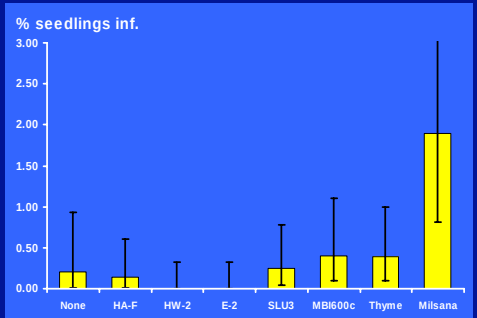
Carrot / *Xhc* - biologicals



STOVE project outline



Carrot / *Xhc* – Transmission



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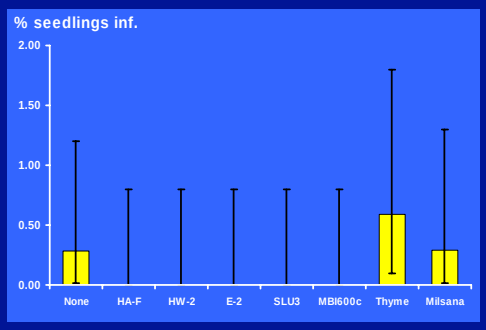

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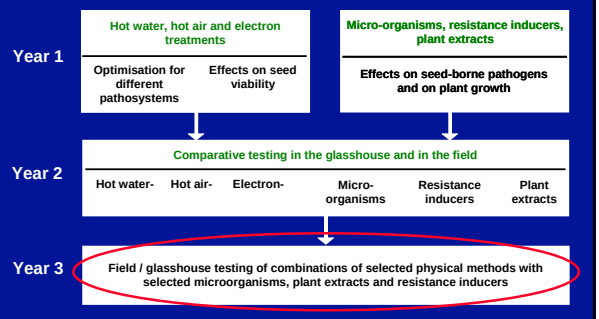


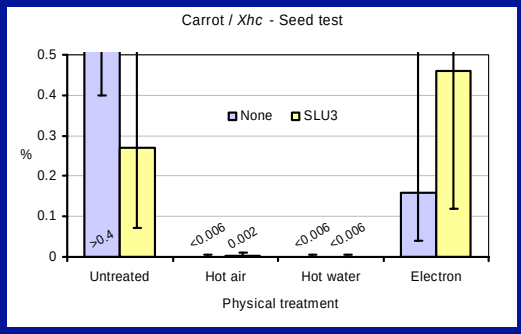
Final stage

- **Selected for trials:**
  - **Best of each of the three physical treatments in combination with the experimental BCA SLU3**

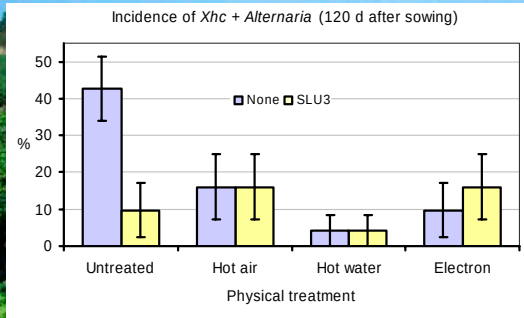
Final stage

- **Carrots / *Xhc***
  - **Seed tests**
    - up to 50,000 seeds
  - **Field trials**
    - approx. 5,000 seeds per plot (3 plots 3.6 x 8 m)
- **Brassica / *Xcc***
  - **Seed tests**
    - up to 50,000 seeds
  - **Field trials not feasible due to numbers/area req.**
  - **Glasshouse transmission experiments**
    - 6,000 seeds per treatment

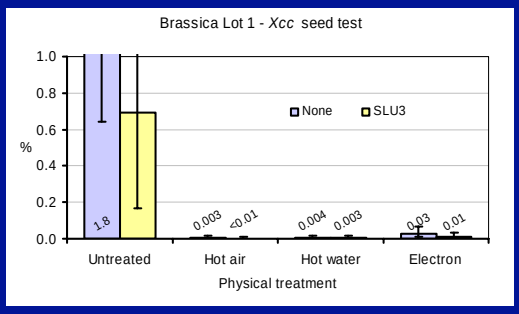
Carrots – seed test



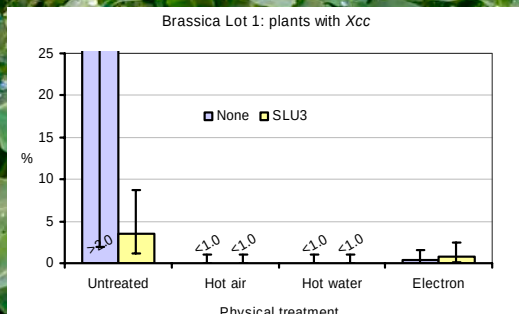
Carrots - field trial



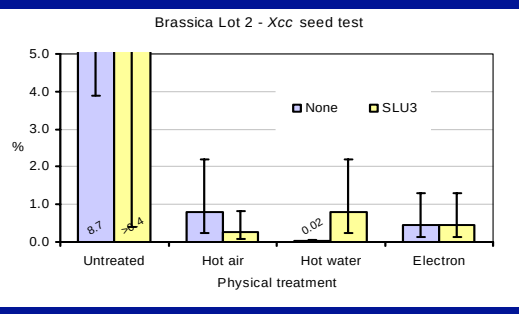
Brassicas – seed test



Brassicas – transmission



Brassicas – seed test



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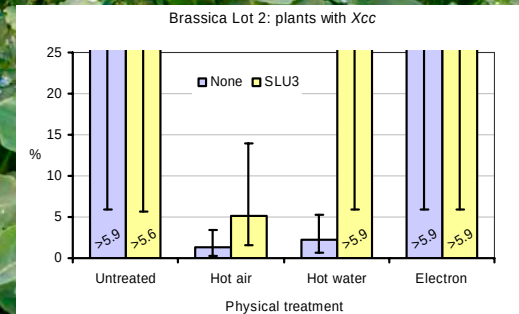

Bacteria - conclusions

- **Physical treatments:**
  - **hot air and hot water consistently reduced seed infestation levels;**
  - **did not always 'eliminate' the pathogens;**
  - **practical value will depend on initial infestation level.**
- **BCA:**
  - **some evidence of a slight reduction when used alone;**
  - **no benefit as a combination treatment.**

Bacteria - cautions

- **Due to practical constraints:**
  - **fewer treatments, doses and treatment combinations examined than for fungal pathogens**
  - **e.g. Thyme oil only tested on seed at 0.1%, may be effective at higher concentration ?**
  - **only one BCA tested in the field**