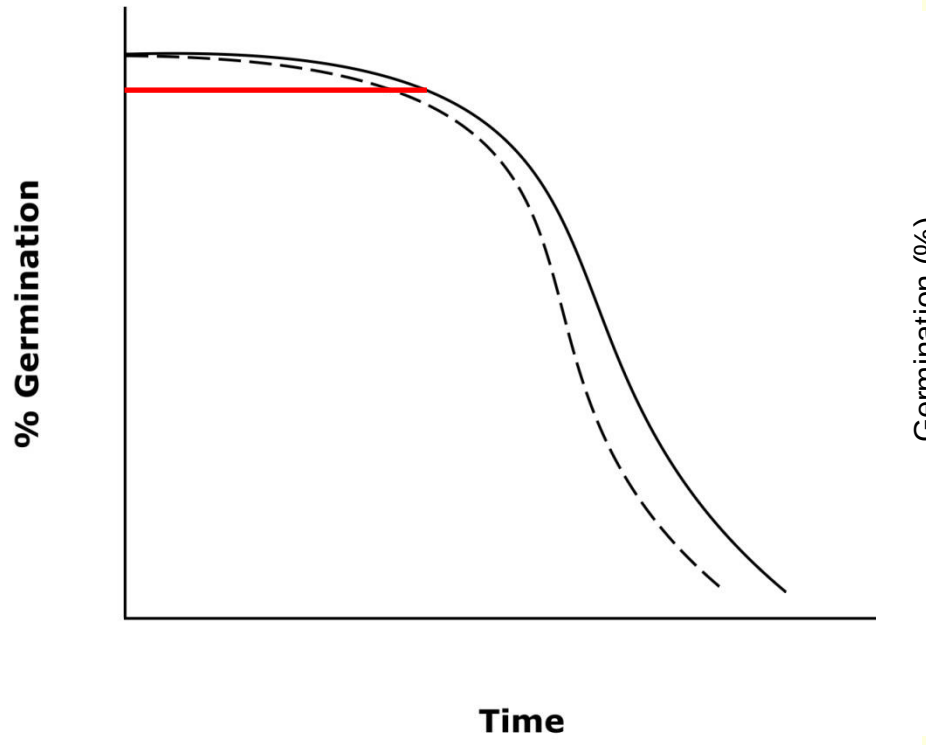
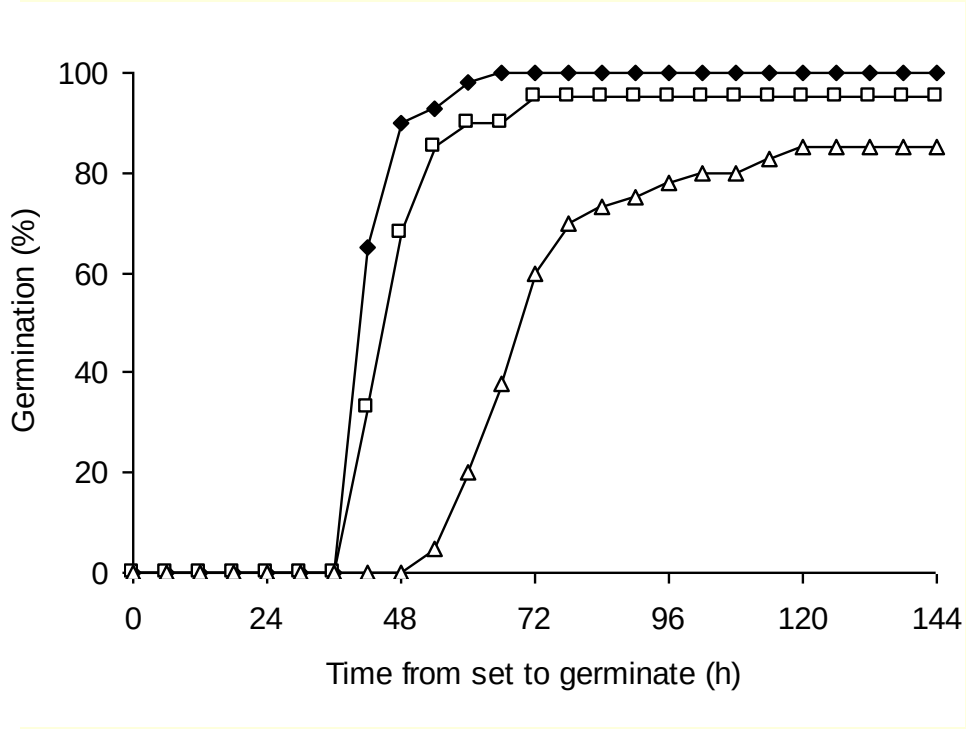
Application of the ageing/repair hypothesis in vigour testing

1. How the hypothesis explains vigour and vigour test results
2. Summary of connections between test results
3. Vigour test development since 2001
4. Test development and validation
5. How vigour tests are used

Basis of vigour and vigour tests
Two key concepts



Seed survival curve

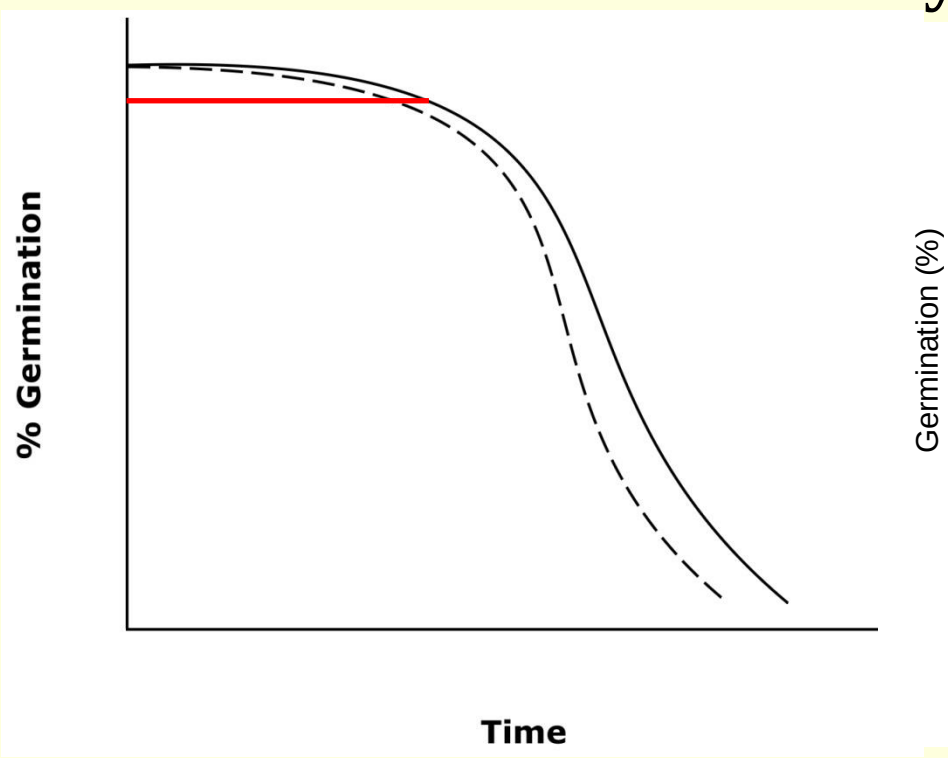


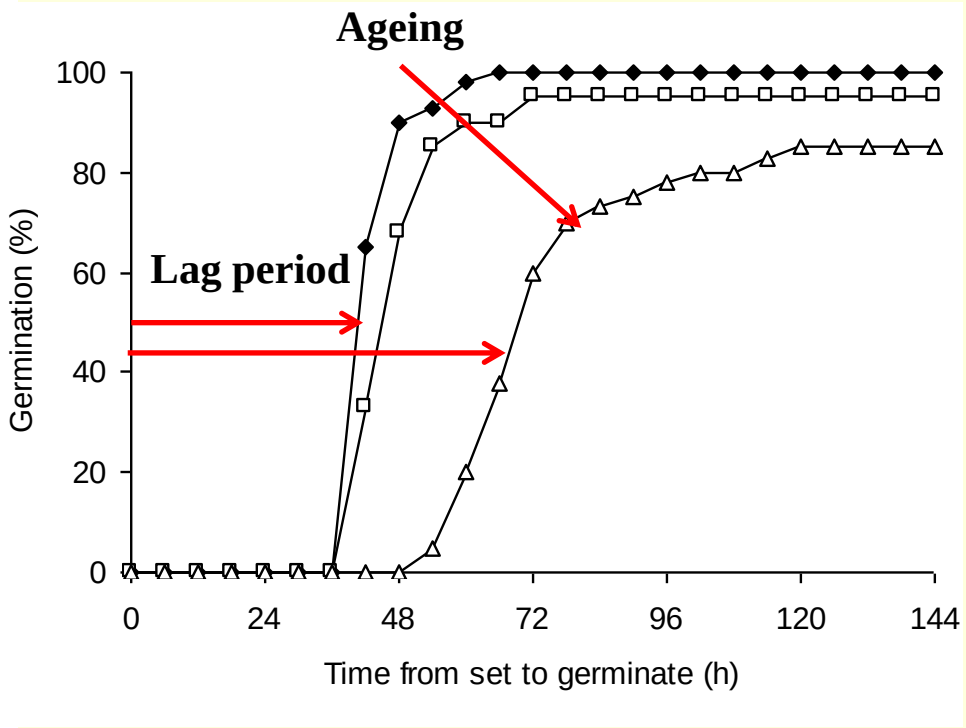
Germination progress curve

Our aim:

To illustrate how these two concepts help provide an integrated explanation for differences in vigour and vigour tests

Basis of vigour and vigour tests
Two key concepts





Seed survival curve

Position on curve determined by seed ageing

- **Accelerated ageing**
- **Controlled deterioration**
- **Electrical conductivity**
- **Tetrazolium staining**

Germination progress curve

Metabolic repair during lag period

- **Radicle emergence**

Need for metabolic repair greater in aged seeds i.e. longer before RE

Interpretation of vigour tests based on ageing / repair hypothesis

ISTA validated tests i.e. in ISTA Rules

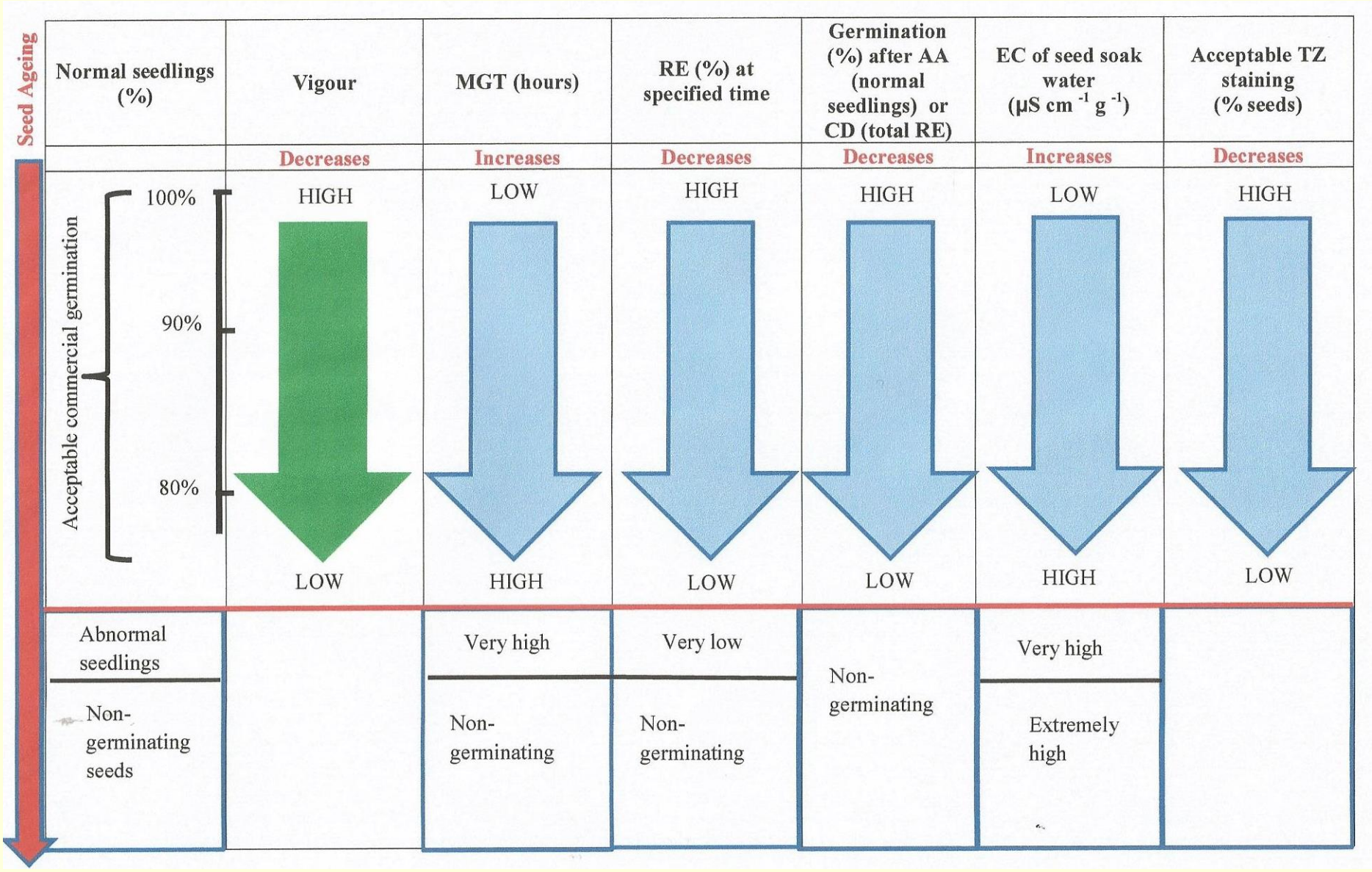
Vigour test	Species	Result reported	Interpretation
Accelerated Ageing (AA)	*Glycine max*	% Normal seedlings	Ageing and position of seed lot on the survival curve
Controlled deterioration (CD)	*Brassica spp*	% total germinated seeds (normal plus abnormal seedlings) Or Conductivity of seed leachate(µS $cm^{-1}g^{-1}$)	
Conductivity test (EC)	*Pisum sativum* *Phaseolus vulgaris* *Glycine max* *Cicer arietinum* *Raphanus sativus*	Conductivity of seed leachate(µS $cm^{-1}g^{-1}$)	Leakage from dead seeds and dead / damaged tissue following ageing and imbibition damage
Radicle emergence (RE)	*Zea mays* *Brassica napus* *Raphanus sativus*	% radicle emergence	Time for repair of ageing
Tetrazolium (TZ)	*Glycine max*	% vigorous seeds	Pattern of living / dead tissue

Interpretation of vigour tests based on ageing / repair hypothesis

Other, non validated tests

Vigour test	Species	Result reported	Interpretation
Saturated salt accelerated ageing	Small seeded species	% Normal seedlings	Ageing and position of seed lot on the survival curve
Cold test (CT)	*Zea mays*	% normal and abnormal seedlings; % dead seeds	Aged seeds need repair; Incomplete repair at low temperature (especially in anaerobic conditions) increases abnormal seedlings
Cool test (18^{o}C)	Cotton	% based on number of normal seedlings with hypocotyl + root length >4cm	Incomplete repair at lower temperature slows RE giving smaller seedlings
Seedling size and uniformity	Various species	Varies e.g. seedling dry weight, length or uniformity	Differences in the timing of RE

Connections between vigour assessments



Vigour test development since 2001
Progress to validation and vigour tests in the ISTA Rules

Vigour test	Species / modification of method	Validated (in ISTA Rules)	Test length
Accelerated Ageing	*Glycine max*	2001 (2002)	11 days
Conductivity test	*Pisum sativum*	2001 (2002)	24 hours
	Phaseolus vulgaris *Glycine max* *Cicer arietinum* *Raphanus sativus*	2010 (2011) – 2013 (2014)	24 hours
Controlled deterioration	*Brassica spp*	2009 (2010)	10 – 17 days(3 days + germination test)
	Modified moisture raising method	2016 (2017)	10 – 17 days(3 days + germination test)
	EC as alternative to germination test to assess deterioration	2016 (2017)	5 days
Radicle emergence Automation potential	*Zea mays*	2011 (2012)	66 hours: 20 ± 1°C; 144 hours: 13 ± 1°C
	Brassica napus	2014 (2015)	30 hours: 20 ± 1°C
	Raphanus sativus	2016 (2017)	48 hours: 20 ± 1°C
Tetrazolium	*Glycine max*	2016 (2017)	24 hours

How do some non-validated tests compare?

Saturated salt accelerated ageing	Small seeded species	48 –140 hours ageing plus 7-21 days germination, depending on species	
Cold test (CT)	*Zea mays*	10 – 13 days	**RE a possible alternative:** • **Quicker,** • **Easier standardisation** • **Automation potential**
Seedling size and uniformity	Various species	Length of germination test	
Cool test (18^{o}C)	Cotton	7 days	

Test development

- **Research**
  - Tests a hypothesis – does a potential test reveal differences in the lab and in the field / glasshouse ?
  - May use selected / manipulated seed material
- **Development**
  - Uses commercial seed lots with acceptable high germination
  - Establishes relationship to practical expression of vigour
  - Determines abiotic and biotic sources of variation
  - Establishes if repeatability and reproducibility are possible
- **Validation**
  - ISTA Method Validation Programme

How are vigour tests used?

- **Seed companies**
  - Sell to suitable market
  - Storage decisions
  - Seed treatment decisions
- **Extension / advisory services**
  - Advice to farmers on sowing risks

What next in the Vigour Committee?

- Addition of more species to:
  - RE test
  - EC test
- Automation of tests
  - RE test

Thank you!

- All Vigour Committee members for their contributions
- The Japanese Organising Committee for their invitation
- Olga Stöckli for her help and support