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Decreased Fitness of Herbicide Resistant Weeds Suggests Options for Management. Case Study: *Alopecurus myosuroides*

Present by PhD student:

Eshagh Keshtkar

Professor :

Per Kudsk

Senior Scientist:

Solvejg K. Mathiassen

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Introduction

Fitness

- Survival and reproductive success of plant in field
- The proportion of genes that an individual leaves in the gene pool

Fitness cost

When plants are exposed to a stressful situation they exhibit adaptive defense mechanisms such as resistance.

These defense traits (resistance) might cause fitness cost when the stress factor (e.g. herbicide) is not present

Fitness cost and management

Differences between R and S can be used as a tool in management.

Introduction

Blackgrass *(Alopecurus myosuroides)*:

- The 15 top most important herbicide resistant weed worldwide (1)
- The most important herbicide resistant weed in Europe (2)
- Evolved resistance to **6** different sites of action (1)
- Caused wheat grain reduction up to 44% (3)
- Both target-site resistance (TSR) and non-target-site (NTSR) (4)

1. Heap, 2015
2. Lutman et al., 2013
3. Reade and Cobb, 2002
4. Delye, 2005

Aims of the project

Fitness of R & S biotypes with controlled genetic background

A) **Competetive response** (vegetative and reproductive output) to increasing density of wheat

B) **Seedling emergence** of non-dormant seeds at
different temperature regimes
different sowing depths.

Plant material (selection of R & S phenotypes)

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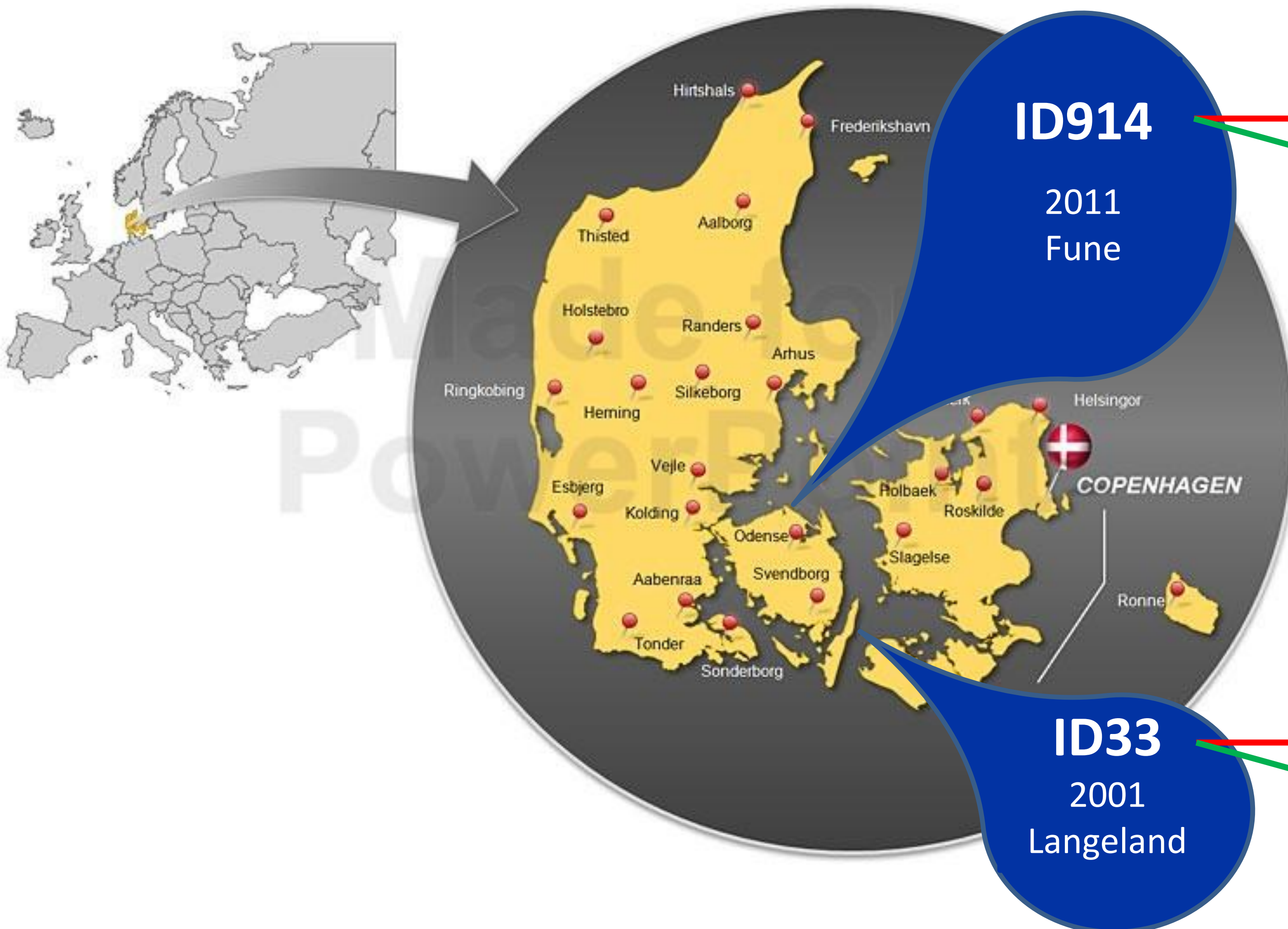
Two fenoxaprop resistance population



R-ID914
S-ID914

R-ID33
S-ID33

Plant material (selection of R & S phenotypes)

Seeds of each population were sown separately in tray.

Parent Clone

Clones and parent plant labeled.

Clones were **sprayed** with 69 g a.i. ha $^{-1}$ fenoxaprop at 2-3 tiller stage

Parent were kept in glasshouse for **seed production**.

Plant material (selection of R & S phenotypes)

Clones (sprayed plants):

Resistant (R)

Semi-resistant (SR)

Susceptible (S)

Parent (unsprayed plants):

Classified according to the responses of their clones.

4 isolated phenotypes:

ID33 (S , R)
ID914 (S , R)

Exp.1. Resistance profile of phenotypes

Whole plant dose response experiment

Four seedlings were kept per pot.
At 3-4 leaf stage pots were sprayed.

ACCase inhibitor

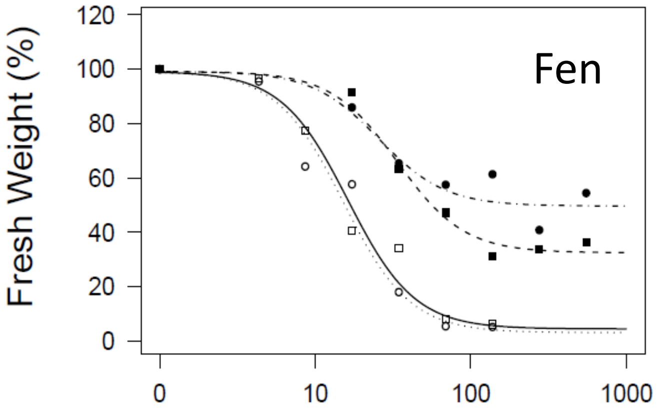
Fenoxaprop (4.35 to 552 g a.i ha $^{-1}$)
Clodinafop (1.9 to 120 g a.i ha $^{-1}$)
Cycloxydim (1.25 to 38 g a.i ha $^{-1}$)

ALS inhibitor

Flupyrsulfuron (1.25. to 20 g a.i ha $^{-1}$)

Fresh weight was measured 3 weeks after spraying.

Exp.1. Resistance profile of phenotypes



S: ○ □

R: ● ■

ID914: ● ○

ID33: ■ □

Exp.2. Molecular assay

5 known mutations at ACCase gene of *A. myosuroides:*

Ile-1781
Trp-2027
Ile-2041
Asp-2078
Gly-2096

2 known mutations at ALS gene :

Pro-197
Trp-574

- None of the known mutations were detected.
- **So, the mechanism of resistance is NTSR.**

Exp.3. Response of R & S phenotypes to wheat

A target-neighborhood design was conducted
for 2 years in greenhouse and 1 year in field.

To:
Compare competitive response of S and NTSR phenotypes
to increasing density of winter wheat.

No ecological fitness cost were observed in vegetative and reproductive output of R phenotypes under normal and biotic stressful condition

Exp.4. Germinability of R & S phenotypes (in Petri-dish)

50 seeds were germinated in Petri-dish with 4 rep.

Petri-dishes were placed in incubator
(14/10 h day/light (175 µmol m^{-2} s^{-1}))

2 temperature regimes: 17/10°C
10/5°C (day/night)

Petri-dishes were arranged in CRD.

Germinated seeds were recorded at consecutive times until no further germination was observed.

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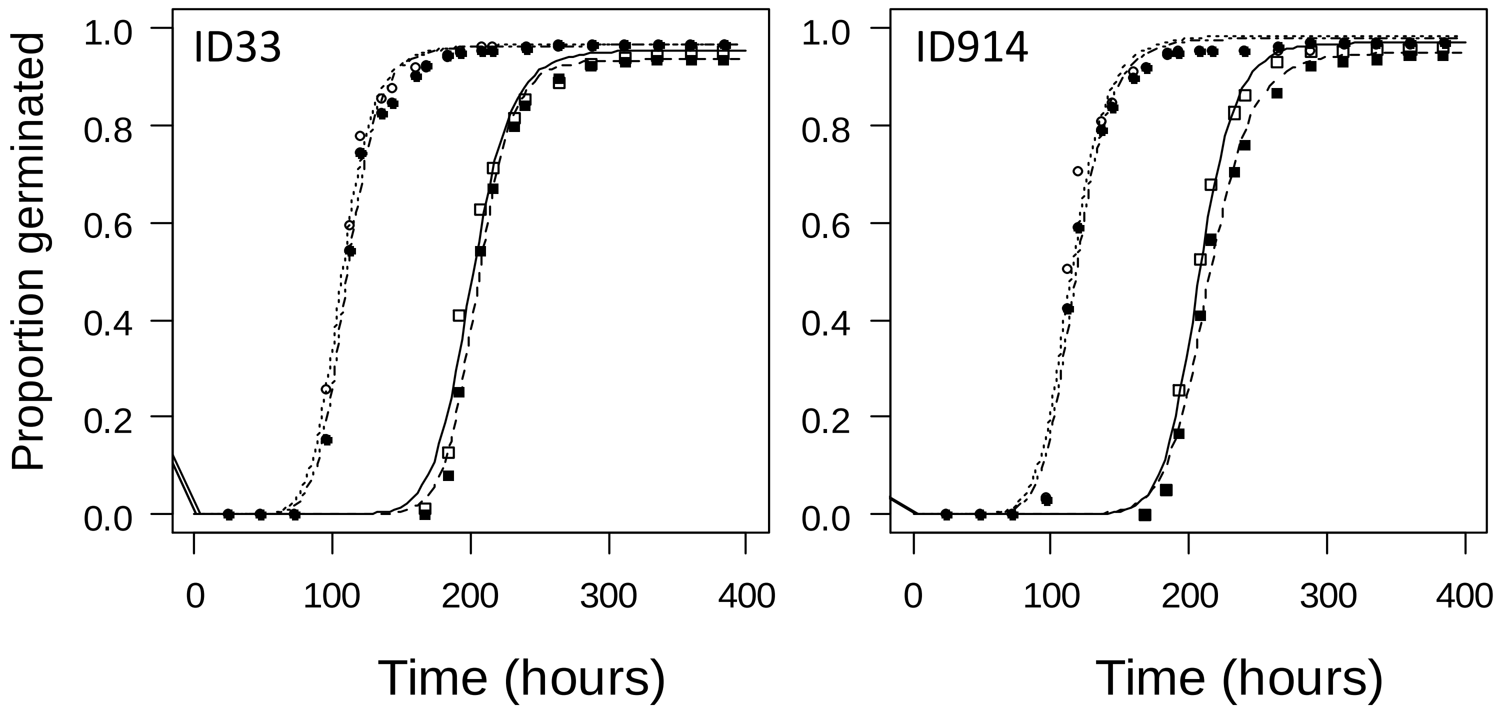
Exp.4. Germinability of R & S phenotypes (in Petri-dish)

A three parameters log-logistic model was fitted to the cumulative germinated seeds according to the event-time approach (Ritz et al., 2013)

$$E(t) = \frac{d}{1 + \exp[b(\log(t) - \log(T_{50}))]} = \frac{d}{1 + \left(\frac{t}{T_{50}}\right)^{b}}$$

E : the cumulative seedling emergence (germination) at time *t*,
d : upper limit parameter (maximum germination)
T_{50}: the time to reach 50% of the maximum germination (*d*)
B: the slope of the curve at T_{50}

Exp.4. Germinability of R & S phenotypes (in Petri-dish)



S: ○ □
R: ● ■

Optimal (17/10° C): ● ○
Sub-optimal(10/5 °c): ■ □

Final germination: **R=S**

Speed of germination of ID914: **S > R**
Speed of germination of ID33 : **S = R**

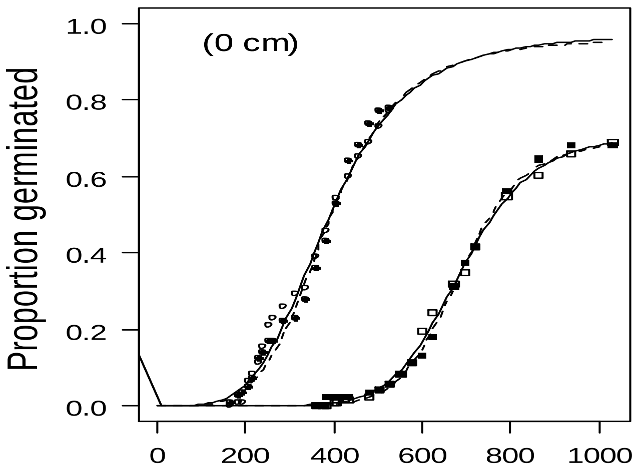
Exp.5. Seedling emergence of R & S plants (in pot)

36 uniform size seeds of each phenotype were selected.

4 sowing depths:
- 0 cm
- 1 ”
- 3 ”
- 6 ”

2 temperature regimes:
- 17/10°C (day/night)
- 10/5°C ”

Exp.5. Seedling emergence of R & S plants (in pot)



ID33

S: ○ □
R: ● ■

Optimal (17/10° C): ● ○
Sub-optimal(10/5 °c): ■ □

Final Ger.:
R = S
Except at 6cm & low Temp.

Speed of Ger.:
S > R
except at 0cm

Time (hours)

Exp.5. Seedling emergence of R & S plants (in pot)

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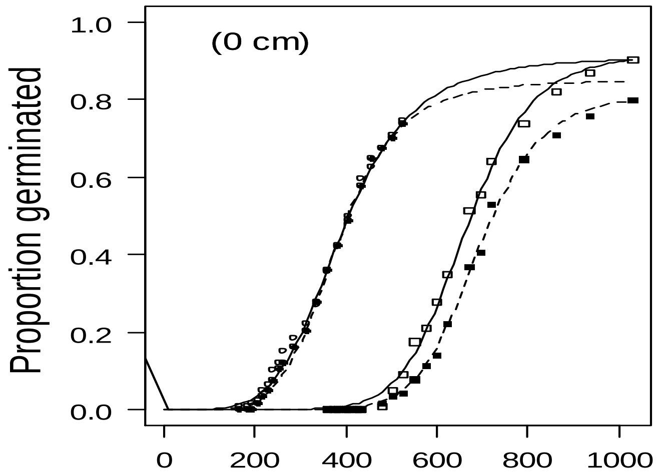

Time (hours)

ID914

S: ○ □
R: ● ■

Optimal (17/10º C): ● ○
Sub-optimal(10/5 ºc): ■ □

Final Ger.:
S > R

Speed of Ger.:
S > R

Conclusions

- No fitness cost considering to biomass and seed production.
- Seedling emergence fitness cost at sub-optimal condition in R-914.
- Subtle seedling emergence fitness cost at sub-optimal condition in R-33.
- Stressful condition magnified fitness cost considering to (seedling emergence)

Conclusions

Management suggestion :

- **Deeper soil cultivation**
- **Delayed sowing of autumn sown crops**

Can create an unfavorable environment for R phenotypes

Perspective

- Check accuracy of seedling emergence results in field condition.
- Modeling to predict the fate of resistant plants in population.
- If frequency of R plants would be low,
  how long does it take to get ride of them?

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PhD student:

Eshagh Keshtkar

(Eshagh.Keshtkar@agro.au.dk)

(ikeshtkar@gmail.com)

'he germination parameters of four phenotypes selected within two populations under different temperature regimes estimated by the event-time model (Equation 2) from Exp. and 2. Standard errors are in parentheses.

Population	Phenotype	Experiment 1				Experiment 2			
		T_{G50} (hours)[a]		G_{max}[b]		T_{G50} (hours)[a]		G_{max}[b]	
		10/5 °C	17/10 °C	10/5 °C	17/10 °C	10/5 °C	17/10 °C	10/5 °C	17/10 °C
ID33	S	201 (1.8)	107 (1.4)	95.5 (1.4)	96.5 (1.2)	197 (1.8)	110 (1.1)	97.0 (1.2)	98.0 (0.9)
	R	205 (1.6)	110 (1.4)	93.5 (1.7)	96.4 (1.3)	205 (2.0)	112 (1.2)	96.0 (1.4)	98.0 (0.9)
ID914	S	207 (1.6)	114 (1.5)	97.0 (1.2)	98.5 (0.8)	223 (2.1)	111 (1.0)	95.6 (1.4)	96.5 (1.3)
	R	214 (2.0)	118 (1.5)	95.0 (1.5)	98.0 (1.1)	230 (2.0)	116 (1.2)	92.4 (1.9)	97.0 (1.2)

Speed of germination i.e time to reach 50% germination
Final germination= *d* parameter×100

he seedling emergence parameters of S and R phenotypes selected within two populations (ID33 and ID914) under increasing sowing depths and two temperature regimes. he parameters obtained by fitting the event-time model (Equation 2) to the data of experiment 1. Standard errors are in parentheses.

woing depth (cm)	E_{max}[a]								T_{E50} (hours)[b]							
	10/5 °C				17/10 °C				10/5 °C				17/10 °C			
	ID33		ID914		ID33		ID914		ID33		ID914		ID33		ID914	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
0	70.9	69.3	91.9	81.3	97.5	96.2	91.0	85.0	689	686	663	690	386	388	390	380
	(4.1)	(4.0)	(2.6)	(3.5)	(9.3)	(8.3)	(8.1)	(6.1)	(15)	(13)	(12)	(12)	(25)	(21)	(21)	(15)
1	57.0	56.3	75.8	52.3	70.8	72.9	68.1	60.4	456	478	488	504	217	219	218	234
	(4.1)	(4.1)	(3.6)	(4.2)	(3.8)	(3.7)	(3.9)	(4.1)	(10)	(10)	(10)	(13)	(2)	(2)	(3)	(3)
3	50.7	50.7	71.6	52.2	78.5	71.5	73.3	55.6	491	512	461	516	221	227	215	236
	(4.2)	(4.2)	(3.8)	(4.2)	(3.4)	(3.8)	(3.7)	(4.1)	(10)	(10)	(10)	(11)	(2)	(2)	(3)	(4)
6	12.5	6.3	32.4	3.7	19.1	15.6	25.1	14.0	621	687	693	742	302	296	287	303
	(2.8)	(2.0)	(4.0)	(1.6)	(3.3)	(3.1)	(3.6)	(2.9)	(17)	(28)	(19)	(76)	(15)	(18)	(9)	(14)

Final seedling emergence = *d* parameter×100
Speed of seedling emergence i.e. time to reach 50% of seedling emergence

e seedling emergence parameters of S and R phenotypes selected within two populations (ID33 and ID914) under increasing sowing depths and two temperature regimes.
e parameters obtained by fitting the event-time model (Equation 2) to the data of experiment 2. Standard errors are in parentheses.

voing depth (cm)	E_{max}[a]								T_{E50} (hours)[b]							
	10/5 °C				17/10 °C				10/5 °C				17/10 °C			
	ID33		ID914		ID33		ID914		ID33		ID914		ID33		ID914	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
0	80.6 (3.4)	87.1 (2.8)	90.2 (2.9)	68.3 (4.3)	85.6 (3.1)	91.6 (2.4)	80.5 (4.2)	62.0 (5.3)	639 (11)	636 (9)	608 (14)	712 (17)	314 (6)	307 (5)	375 (9)	382 (13)
1	61.1 (4.1)	65.3 (4.0)	78.6 (3.4)	43.3 (4.2)	70.8 (3.8)	73.6 (3.7)	68.8 (3.9)	54.9 (4.1)	427 (7)	444 (7)	474 (10)	523 (16)	212 (2)	223 (4)	208 (3)	217 (4)
3	56.3 (4.1)	61.1 (4.1)	71.6 (3.8)	48.0 (4.2)	69.4 (3.8)	77.1 (3.5)	65.3 (4.0)	59.0 (4.1)	471 (7)	474 (7)	469 (9)	533 (11)	213 (2)	217 (2)	208 (2)	219 (2)
6	18.9 (3.3)	11.5 (2.7)	45.9 (4.2)	15.5 (3.1)	13.3 (2.8)	14.6 (2.9)	nd	nd	632 (23)	691 (38)	555 (10)	615 (31)	276 (13)	259 (9)	nd	nd

nal seedling emergence = d parameter×100
peed of seedling emergence i.e. time to reach 50% of seedling emergence
. not determined

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