Patterns of cross-resistance to ACCase-inhibitor herbicides in winter wild oat (Avena ludoviciana) populations

By: Hamidreza Sasanfar
The Problem?
Winter wild oat (*Avena ludoviciana* Durieu.) is the prevailing *Avena* species in Iran which is frequently found at high densities in winter wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.)
ACCase inhibitors are a group of herbicides enabling efficient management of grass weed species such as wild oat.

But...

Many wild oat populations have been reported to have become resistant to ACCase inhibitors in the world.

Seefeldt et al. 1994; Murray et al. 1996; Bourgeois and Morrison 1997; Beckie et al. 2002
## Herbicide Resistant Weeds in Iran (Heap, 2015)

<table>
<thead>
<tr>
<th>#</th>
<th>Species</th>
<th>Common Name</th>
<th>First Year</th>
<th>Site of Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td><em>Phalaris minor</em></td>
<td>Little seed Canary grass</td>
<td>2004</td>
<td>ACCase inhibitors (A/1)</td>
</tr>
<tr>
<td>2</td>
<td><em>Avena sterilis ssp. ludoviciana</em></td>
<td>Sterile oat</td>
<td>2006</td>
<td>ACCase inhibitors (A/1)</td>
</tr>
<tr>
<td>3</td>
<td><em>Lolium rigidum</em></td>
<td>Rigid Ryegrass</td>
<td>2007</td>
<td>ACCase inhibitors (A/1)</td>
</tr>
<tr>
<td>4</td>
<td><em>Avena fatua</em></td>
<td>Wild Oat</td>
<td>2007</td>
<td>ACCase inhibitors (A/1)</td>
</tr>
<tr>
<td>5</td>
<td><em>Phalaris paradoxa</em></td>
<td>Hood Canarygrass</td>
<td>2007</td>
<td>ACCase inhibitors (A/1)</td>
</tr>
<tr>
<td>6</td>
<td><em>Avena sterilis</em></td>
<td>Sterile Oat</td>
<td>2008</td>
<td>ACCase inhibitors (A/1)</td>
</tr>
<tr>
<td>7</td>
<td><em>Avena sterilis</em></td>
<td>Sterile Oat</td>
<td>2009</td>
<td>ALS inhibitors (B/2)</td>
</tr>
<tr>
<td>8</td>
<td><em>Echinochloa colona</em></td>
<td>Junglerice</td>
<td>2009</td>
<td>Photosystem II inhibitors (C1/5)</td>
</tr>
<tr>
<td>9</td>
<td><em>Sinapis arvensis</em></td>
<td>Wild Mustard</td>
<td>2009</td>
<td>ALS inhibitors (B/2)</td>
</tr>
<tr>
<td>10</td>
<td><em>Avena sterilis ssp. ludoviciana</em></td>
<td>Sterile oat</td>
<td>2009</td>
<td>ALS inhibitors (B/2)</td>
</tr>
<tr>
<td></td>
<td><strong>Multiple Resistance: 2 Sites of Action</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td><em>Avena sterilis ssp. ludoviciana</em></td>
<td>Sterile oat</td>
<td>2010</td>
<td>ACCase inhibitors (A/1)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ALS inhibitors (B/2)</td>
</tr>
<tr>
<td>12</td>
<td><em>Rapistrum rugosum</em></td>
<td>Turnipweed</td>
<td>2010</td>
<td>ALS inhibitors (B/2)</td>
</tr>
<tr>
<td>13</td>
<td><em>Phalaris brachystachys</em></td>
<td>Shortspike Canarygrass</td>
<td>2014</td>
<td>ACCase inhibitors (A/1)</td>
</tr>
</tbody>
</table>
Objective

- It is necessary to detect resistance as early as possible.
- Management strategies must be developed to prevent selection and spread of resistance.
- To quantify levels of resistance and investigate patterns of cross-resistance in winter wild oat populations to:
  
  clodinafop, sethoxydim and pinoxaden
Method of research
# Plant Material Collection

<table>
<thead>
<tr>
<th>Population</th>
<th>Location</th>
<th>Crops planted</th>
<th>Herbicide applied</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>Marv Dasht</td>
<td>Wheat-corn</td>
<td>Clodinafop (6 yr)</td>
</tr>
<tr>
<td>M2</td>
<td>Marv Dasht</td>
<td>Wheat-corn-oilseed rape</td>
<td>Clodinafop (3 yr), fenoxaprop (2 yr), sethoxydim (1 yr)</td>
</tr>
<tr>
<td>M3</td>
<td>Marv Dasht</td>
<td>Wheat-corn</td>
<td>Clodinafop (4 yr), fenoxaprop (2 yr)</td>
</tr>
<tr>
<td>M4</td>
<td>Marv Dasht</td>
<td>Wheat-corn</td>
<td>Not available</td>
</tr>
<tr>
<td>F2</td>
<td>Fasa</td>
<td>Wheat-corn</td>
<td>Clodinafop (4 yr), fenoxaprop + clodinafop (1 yr), fenoxaprop (1 yr)</td>
</tr>
<tr>
<td>F3</td>
<td>Fasa</td>
<td>Wheat-corn</td>
<td>Clodinafop (4 yr), clodinafop + fenoxaprop (1 yr), fenoxaprop (1 yr)</td>
</tr>
<tr>
<td>S1</td>
<td>Sepidan</td>
<td>Wheat-corn</td>
<td>Not available</td>
</tr>
<tr>
<td>S2</td>
<td>Sepidan</td>
<td>Wheat-corn-barley-onion</td>
<td>Clodinafop (2 yr), fenoxaprop (2 yr), fenoxaprop + difenzoquat (1 yr)</td>
</tr>
<tr>
<td>S3</td>
<td>Sepidan</td>
<td>Wheat-corn-rice</td>
<td>Clodinafop (4 yr), diclofop (2 yr)</td>
</tr>
<tr>
<td>S4</td>
<td>Sepidan</td>
<td>Wheat-corn</td>
<td>Not available</td>
</tr>
<tr>
<td>ES</td>
<td>Estahban</td>
<td>Wheat-corn</td>
<td>Clodinafop (2 yr)</td>
</tr>
<tr>
<td>ES4</td>
<td>Estahban</td>
<td>Wheat-corn-oilseed rape</td>
<td>Clodinafop (4 yr), sethoxydim (1 yr)</td>
</tr>
<tr>
<td>S</td>
<td>Sepidan</td>
<td>susceptible population</td>
<td>(Seeds were collected from an area with no history of herbicide use)</td>
</tr>
</tbody>
</table>
Materials and Methods

Sepidan

Marvdasht

Estahban

Fasa
# Materials and Methods

## Herbicides

<table>
<thead>
<tr>
<th>Chemical family</th>
<th>Common name</th>
<th>Formulation</th>
<th>Recommended label rate X (per ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aryloxyphenoxypropionate (FOPs)</td>
<td>clodinafop</td>
<td>EC 8%</td>
<td>64 g a.i.</td>
</tr>
<tr>
<td>Cyclohexanedione (DIMs)</td>
<td>sethoxydim</td>
<td>EC 12.5%</td>
<td>375 g a.i.</td>
</tr>
<tr>
<td>Phenylpyrazolin (DENs)</td>
<td>pinoxaden</td>
<td>EC 10%</td>
<td>45 g a.i.</td>
</tr>
</tbody>
</table>

Eight doses: 0, 0.25X, 0.5X, 1X, 2X, 4X, 8X and 16X
Materials and Methods

Iranian Research Institute of Plant Protection

2010-2011

Whole plant

Seed bioassay
Materials and Methods

Whole-Plant Bioassay

Dose-response experiment in the greenhouse

- clodinafop
- sethoxydim
- pinoxaden
Materials and Methods

Seed Bioassay

1- Determination of discriminatory concentration
2- Dose response in petri dish
**Materials and Methods**

**Statistical analysis**

Survival, biomass and coleoptiles length were expressed as a percentage of the nontreated control.

\[
Y = \frac{d - c}{1 + \exp \left\{ b(\log(x) - \log(e)) \right\}}
\]
Observations
Dose-response:
Clodinafop-propargyl (g a.i. ha$^{-1}$)

**Whole-Plant Bioassay**
17th EWRS Symposium, 23-26 June 2015, Montpellier, France

(Marvdasht)
- S (Marvdasht)

(Fasa)
- S (Fasa)

(Sepidan)
- S (Sepidan)

(Estahban)
- S (Estahban)

Dry weight (% of nontreated control)

Clodinafop-propargyl (g a.i. ha$^{-1}$)
Whole Plant Bioassay

16th EWRS Symposium, 23-26 June 2015, Montpellier, France

Control  0.25 X  0.5 X  1 X  2 X  4 X  8 X  16 X

M2 (The highest resistant biotype)

S (Susceptible biotype)
**Dose-response**:

Sethoxydim (g a.i. ha⁻¹)

(Marvdasht)

(Fasa)

(Sepidan)

(Estahban)

Dry weight (% of nontreated control)

Dry weight (% of nontreated control)

Dry weight (% of nontreated control)

Dry weight (% of nontreated control)

Sethoxydim (g a.i. ha⁻¹)

Sethoxydim (g a.i. ha⁻¹)

Sethoxydim (g a.i. ha⁻¹)

Sethoxydim (g a.i. ha⁻¹)
Pinoxaden (g a.i. ha$^{-1}$)

Dry weight (% of nontreated control)

Marvdasht

Fasa

Sepidan

Estahban

(Dry weight (% of nontreated control))

Pinoxaden (g a.i. ha$^{-1}$)
Results

M2

Dose-response: Pinoxaden Whole-Plant Bioassay

2017th EWRS Symposium, 23-26 June 2015, Montpellier, France

0.25 X 0.5 X 1 X

S4

1 X 2 X 4 X
Table 1. The estimated herbicide rate required for 50% reduction (ED$_{50}$) in shoot dry matter of 12 resistant and one susceptible winter wild oat populations and their corresponding resistance ratios (R/S) to three ACCase inhibitor herbicides.

<table>
<thead>
<tr>
<th>Population</th>
<th>Clodinafop</th>
<th>Sethoxydim</th>
<th>Pinoxaden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ED$_{50}$ (g ai L$^{-1}$)</td>
<td>R/S</td>
<td>ED$_{50}$ (g ai L$^{-1}$)</td>
</tr>
<tr>
<td>M1</td>
<td>74.6</td>
<td>7.6</td>
<td>131.8</td>
</tr>
<tr>
<td>M2</td>
<td>&gt;336.26</td>
<td>&gt;34.1</td>
<td>170.7</td>
</tr>
<tr>
<td>M3</td>
<td>&gt;336.26</td>
<td>&gt;34.1</td>
<td>201.5</td>
</tr>
<tr>
<td>M4</td>
<td>336.26</td>
<td>34.1</td>
<td>305.4</td>
</tr>
<tr>
<td>F2</td>
<td>30.9</td>
<td>3.1</td>
<td>672.1</td>
</tr>
<tr>
<td>F3</td>
<td>204.7</td>
<td>20.7</td>
<td>454.5</td>
</tr>
<tr>
<td>S1</td>
<td>66.8</td>
<td>6.8</td>
<td>107.4</td>
</tr>
<tr>
<td>S2</td>
<td>41.2</td>
<td>4.2</td>
<td>413.5</td>
</tr>
<tr>
<td>S3</td>
<td>105.8</td>
<td>10.7</td>
<td>145.8</td>
</tr>
<tr>
<td>S4</td>
<td>67.2</td>
<td>6.8</td>
<td>385.2</td>
</tr>
<tr>
<td>ES</td>
<td>15.1</td>
<td>1.5</td>
<td>73.5</td>
</tr>
<tr>
<td>ES4</td>
<td>38.1</td>
<td>3.9</td>
<td>762.5</td>
</tr>
<tr>
<td>S x</td>
<td>9.9</td>
<td>1.0</td>
<td>62.7</td>
</tr>
</tbody>
</table>

$^z$ ED$_{50}$ values were estimated from the log-logistic model (Eq1).

$^\gamma$ Shoot dry weight reductions were less than 50% over the range of clodinafop application rates, thus the ED$_{50}$ was not estimable.

$^x$ Susceptible population had never been treated with any of herbicides tested.
Dose-response: Clodinafop-propargyl (mg a.i. L$^{-1}$)

- **Marvdasht**
  - Clodinafop-propargyl:
    - 0.00
    - 0.08
    - 0.16
    - 0.32
    - 0.64
    - 1.28
    - 2.56
    - 5.12
  - Coleoptiles length (% of nontreated control):
    - 0
    - 20
    - 40
    - 60
    - 80
    - 100

- **Fasa**
  - Clodinafop-propargyl:
    - 0.00
    - 0.08
    - 0.16
    - 0.32
    - 0.64
    - 1.28
    - 2.56
    - 5.12
  - Coleoptiles length (% of nontreated control):
    - 0
    - 20
    - 40
    - 60
    - 80
    - 100

- **Sepidan**
  - Clodinafop-propargyl:
    - 0.00
    - 0.08
    - 0.16
    - 0.32
    - 0.64
    - 1.28
    - 2.56
    - 5.12
  - Coleoptiles length (% of nontreated control):
    - 0
    - 20
    - 40
    - 60
    - 80
    - 100

- **Estahban**
  - Clodinafop-propargyl:
    - 0.00
    - 0.08
    - 0.16
    - 0.32
    - 0.64
    - 1.28
    - 2.56
    - 5.12
  - Coleoptiles length (% of nontreated control):
    - 0
    - 20
    - 40
    - 60
    - 80
    - 100
Results

M2

<table>
<thead>
<tr>
<th>Control</th>
<th>0.25 X</th>
<th>0.5 X</th>
<th>1 X</th>
<th>2 X</th>
<th>4 X</th>
<th>8 X</th>
<th>16 X</th>
</tr>
</thead>
</table>

S
Dose-response:
Sethoxydim

Seed Bioassay (Marvdasht)

Sethoxydim (mg a.i. L$^{-1}$)
0.0 0.5 1.0 2.0 4.0 8.0 16.0 32.0
Coleoptiles length (% of nontreated control)
0 20 40 60 80 100
M1 M2 M3 M4 S

Fasa

Sethoxydim (mg a.i. L$^{-1}$)
0.0 0.5 1.0 2.0 4.0 8.0 16.0 32.0
Coleoptiles length (% of nontreated control)
0 20 40 60 80 100 120
F2 F3 S

Sepidan

Sethoxydim (mg a.i. L$^{-1}$)
0.0 0.5 1.0 2.0 4.0 8.0 16.0 32.0
Coleoptiles length (% of nontreated control)
0 20 40 60 80 100 S1 S2 S3 S4 S

Estahban

Sethoxydim (mg a.i. L$^{-1}$)
0.0 0.5 1.0 2.0 4.0 8.0 16.0 32.0
Coleoptiles length (% of nontreated control)
0 20 40 60 80 100 ES ES4 S
Dose-response: Pinoxaden

Seed Bioassay (Marvdasht)

Pinoxaden (mg a.i. L\(^{-1}\))
0.00 0.10 0.20 0.40 0.80 1.60 3.20 6.40
Coleoptiles length (% of nontreated control)
0 20 40 60 80 100
M1 M2 M3 M4 S

(Fasa)

Pinoxaden (mg a.i. L\(^{-1}\))
0.00 0.10 0.20 0.40 0.80 1.60 3.20 6.40
Coleoptiles length (% of nontreated control)
0 20 40 60 80 100
F2 F3 S

(Sepidan)

Pinoxaden (mg a.i. L\(^{-1}\))
0.00 0.10 0.20 0.40 0.80 1.60 3.20 6.40
Coleoptiles length (% of nontreated control)
0 20 40 60 80 100
S1 S2 S3 S4 S

(Estahban)

Pinoxaden (mg a.i. L\(^{-1}\))
0.00 0.10 0.20 0.40 0.80 1.60 3.20 6.40
Coleoptiles length (% of nontreated control)
0 20 40 60 80 100
ES ES4 S
Results

Control

1 X

2 X

4 X

8 X

16 X

Dose-response: Pinoxaden

Seed Bioassay
Table 2. The estimated herbicide rate required for 50% reduction ($ED_{50}$) in coleoptiles elongation of 12 resistant and one susceptible winter wild oat populations and their corresponding resistance ratios (R/S) to three ACCase inhibitor herbicides

<table>
<thead>
<tr>
<th>Population</th>
<th>Clodinafop</th>
<th>Sethoxydim</th>
<th>Pinoxaden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$ED_{50}$ (mg ai L$^{-1}$)</td>
<td>R/S</td>
<td>$ED_{50}$ (mg ai L$^{-1}$)</td>
</tr>
<tr>
<td>M1</td>
<td>0.95</td>
<td>12.83</td>
<td>0.53</td>
</tr>
<tr>
<td>M2</td>
<td>&gt;1.95 $^\gamma$</td>
<td>&gt;26.3</td>
<td>0.49</td>
</tr>
<tr>
<td>M3</td>
<td>1.38</td>
<td>18.57</td>
<td>0.42</td>
</tr>
<tr>
<td>M4</td>
<td>0.55</td>
<td>7.48</td>
<td>1.03</td>
</tr>
<tr>
<td>M5</td>
<td>0.70</td>
<td>9.49</td>
<td>1.18</td>
</tr>
<tr>
<td>F1</td>
<td>1.21</td>
<td>16.39</td>
<td>6.43</td>
</tr>
<tr>
<td>S1</td>
<td>1.95</td>
<td>26.26</td>
<td>0.44</td>
</tr>
<tr>
<td>S2</td>
<td>0.50</td>
<td>6.73</td>
<td>1.04</td>
</tr>
<tr>
<td>S3</td>
<td>0.30</td>
<td>4.06</td>
<td>0.31</td>
</tr>
<tr>
<td>S4</td>
<td>0.37</td>
<td>4.93</td>
<td>0.58</td>
</tr>
<tr>
<td>ES</td>
<td>0.05</td>
<td>0.70</td>
<td>0.26</td>
</tr>
<tr>
<td>ES4</td>
<td>0.61</td>
<td>8.16</td>
<td>1.28</td>
</tr>
<tr>
<td>S $^x$</td>
<td>0.07</td>
<td>1.00</td>
<td>0.41</td>
</tr>
</tbody>
</table>

$^z$ $ED_{50}$ values were estimated from the log-logistic model (Eq. 1).

$^\gamma$ Plant survival was higher than 50% over the range of clodinafop application rates, thus the $ED_{50}$ was not estimable.

$^x$ Susceptible population had never been treated with any of herbicides tested.
Conclusion
## Patterns of cross-resistance

<table>
<thead>
<tr>
<th>Population</th>
<th>FOPs</th>
<th>DIMs</th>
<th>DENs</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>**</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>M2</td>
<td>****</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>M3</td>
<td>****</td>
<td>*</td>
<td>**</td>
</tr>
<tr>
<td>M4</td>
<td>****</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>F2</td>
<td>*</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>F3</td>
<td>****</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>S1</td>
<td>**</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>S2</td>
<td>*</td>
<td>**</td>
<td>****</td>
</tr>
<tr>
<td>S3</td>
<td>***</td>
<td>*</td>
<td>*</td>
</tr>
<tr>
<td>S4</td>
<td>**</td>
<td>**</td>
<td>****</td>
</tr>
<tr>
<td>ES</td>
<td>*</td>
<td>*</td>
<td>-</td>
</tr>
<tr>
<td>ES4</td>
<td>*</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>S</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*, **, *** and **** showed resistance index (RI) <5, 5-10, 11-20 and >20, respectively.
The resistance levels of most population to clodinafop were considerably higher than those to sethoxydim.

The selective pressure from other ACCase inhibitors can result in cross-resistance to pinoxaden.

A pinoxaden's phenylpyrazolin chemistry and proposed different ACCase binding domains can not necessarily indispose the risk of pinoxaden resistance.
Thanks for your attention

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