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Sulfo technology for multiplication of sunflower hybrids resistant to tribenuron methyl-based herbicides

Cr. Meluca*, N. Pirvu, T. Nistor, R. Sturzi, A. Stoilova

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Abstract. Certain experiments with sunflower hybrids realized through Sulfo technology, resistant to tribenuron methyl 75% based herbicides were performed at Agricultural Research and Development Station (ARDS) Teleorman, Romania. There is a response to the hypothesis that the herbicide application (4–6 real leaves) when night temperatures (after application) are lower than 12°C could provoke higher damages at green tissue level. Damages were registered of 6.5–11.8% and decreased to 0.8–5.6% when temperatures were higher than 12°C. The yielding ability of these sunflower hybrids is not affected as compared to the conventional check hybrid Favorit, its level was, on three year average, of 3652–4985 kg/ha, versus 3735 kg/ha achieved by the Favorit hybrid. It may emphasize that the hybrid Toro showed the highest gain in yield versus the check hybrid Favorit (1250 kg/ha, respectively 33.5%). The yielding ability of the hybrid Toro exceeded by 774 kg/ha (11.8%) the yield achieved by the hybrid PR 64 LE20 (Pioneer registration).

Keywords: Helianthus annuus L., hybrids, herbicides, resistance, phytotoxicity, productivity.

Introduction

Sunflower (Helianthus annuus L.) is one of the most important oilseed crop, grown on a total of over 22 million hectares worldwide (Škoric et al., 2008). The oil has various food and industrial uses. It is superior edible oil, due to high unsaturated fatty acid content (85–91% linoleic and oleic acids). As all oils with melting point below 50°C, it has complete digestibility (Vrînceanu, 2000). The sunflower is a very valuable fodder crop, especially for silos, and a honey plant, too.

Breeding of sunflower in the world had more stages, determined by the achieved progresses in genetic and breeding researches. In the first years of VII decade of the last century, a stage of F1 hybrids released by inbred crossing began, ongoing process of classical sunflower hybrid breeding. At the current stage sunflower herbicide resistant hybrids are developed and introduced through: "clearfield" technology (IMI – resistant hybrids) and "sulfo" technology (hybrids resistant to 75% triburon methyl-based herbicides).

The Sulfo technology of sunflower breeding involves the resistance of hybrids to 75% triburon methyl-based herbicides during vegetation. These are referred to the sulfonylureas (SUs) (Kramer and Schirmer, 2007; HRAC: Herbicide classification, Jan. 2002) and are found to be efficient in controlling a wide range of broadleaved weeds in cereal crops including wheat, barley, oats and rye (Tsyuganov and Potarenko, 2011; Delchev, 2010a; 2010b; 2012). SU compounds interfere with a key enzyme required for weed cell growth – acetolactate synthase (Kolkman et al., 2004). The first registered SU resistant sunflower genetic stocks were developed in USDA-Fargo, North Dakota – SURES-1 and SURES-2 (Miller and Al-Khatib, 2004). From these original populations SU resistance was transferred into a large number of sunflower hybrids (Jocić et al., 2008; 2011).

The aim of this study was to examine the performance of sunflower hybrids resistant to triburon methyl-based herbicides, the phytotoxicity effects as a result of treatment, and to evaluate the adaptability of the applied Sulfo technology under the local conditions.

Material and methods

In 2010–2012 at Agricultural Research and Development Station (ARDS) Teleorman, Romania a series of experimental plots of 20 m2 was performed in the field, by randomized block method, in three replications, with classical and “turbo” hybrids (resistant to triburon methyl-based herbicides) released by S.C. QUALITY AGRO S.R.L. The experiments included recognized “sulfo”-resistant hybrids – Toro and Amigo, and new entries – PR 64 LE20 (Pioneer signature), La Pampa, Sulfosol, Zorba, Bravo, Bond, Rambo, Toledo and Colorado (sulfo-resistant), the check Favorit hybrid (conventional), and “IMI”-resistant Goldimi hybrid. Express-50 herbicide (30 g/ha) was applied on plots sown at different time – on 21st April and on 31st May. Treatment was made in phase 4–6 true leaves, on 12th May (during cold nights, night temperatures <12°C) and on 16th June (higher night temperatures), respectively. Express-50 herbicide is a new and improved triburon-methyl-based herbicide manufactured by Du Pont (DuPont Crop Protection Sulfonylurea Herbicides). It is applied once or twice per growing season at a maximum seasonal rate of 30 g/ha. The phytotoxicity quotient was visually assessed on a scale from 1 to 5 (note 1 – resistant, note 5–susceptible). The results were subjected to ANOVA (Ceapoiu, 1968).

Results and discussion

To demonstrate the resistance of Sulfo hybrids compared to the
conventional check hybrid (Favorit) and IMI check-hybrid (Goldimi) the results obtained after Express 50 herbicide application (30 g/ha), 4-6 real leaves, on plots sown at different times, are presented. The first case that was sown on 21st April emerged on 29th April, herbicide application was on 12th May (lower night temperatures). The second one that was sown on 31 May, emerged on 6th June, herbicide application was on 16th June (higher night temperatures) (Table 1).

The percentage of affected plants of Sulfo-resistant hybrids is 6.5–11.8% at the first sowing stage (night temperatures after herbicide application were 9.0–12.1°C), respectively 0.8–5.6% at the second sowing stage (night temperatures were 14.7–18.4°C after herbicide application). With the classical hybrid Alvarez, the percentage of destroyed plants was 100% at both moments of application, while with IMI hybrid Goldimi, at both application times, three plants resisted, with high degree of branching (as an effect of the herbicide). The percentage of destroyed plants at check is 98.8–99.4% (it is possible that these six IMI–resistant plants could contain the gene resistant to tribenuron methyl).

The reaction of Sulfo resistant hybrids to thermic and hydric stress is presented in Tables 2 and 3. The yielding ability of Sulfo-resistant hybrids was between 3652–4985 kg/ha. Except the hybrids Bravo and Toledo, which registered a level of average yields as with the conventional check Favorit, the tested hybrids achieved statistically distinctly significant and very significant yield gains of 264–1250 kg/ha (7.1–33.5%), compared to the conventional check. One can especially underline the hybrid Toro, with the highest yield gain (1250 kg/ha, respectively 33.5%). The yielding ability of the hybrid Toro exceeds by 774 kg/ha (11.8%) the yield achieved by the conventional check Favorit, the tested hybrids achieved statistically distinctly significant and very significant yield gains of 264–1250 kg/ha (7.1–33.5%), compared to the conventional check. One can especially underline the hybrid Toro, with the highest yield gain (1250 kg/ha, respectively 33.5%).

### Table 1. Behavior of sunflower hybrids regarding the resistance to Express 50 herbicide application, ARDS, Teleorman, 2012

<table>
<thead>
<tr>
<th>Hybrid</th>
<th>Early sowing</th>
<th>Late sowing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sown: 21st April</td>
<td>Sown: 31st May</td>
</tr>
<tr>
<td></td>
<td>Emergence: 29th April</td>
<td>Emergence: 6th June</td>
</tr>
<tr>
<td></td>
<td>Herbicide application: 12th May</td>
<td>Herbicide application: 12th June</td>
</tr>
<tr>
<td>No of rows</td>
<td>No of total plants</td>
<td>No of affected plants</td>
</tr>
<tr>
<td>----------------</td>
<td>-------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>La pampa</td>
<td>15</td>
<td>474</td>
</tr>
<tr>
<td>Toledo</td>
<td>15</td>
<td>610</td>
</tr>
<tr>
<td>Toro</td>
<td>15</td>
<td>600</td>
</tr>
<tr>
<td>Amigo</td>
<td>15</td>
<td>551</td>
</tr>
<tr>
<td>Sulfosol</td>
<td>15</td>
<td>559</td>
</tr>
<tr>
<td>Colorado</td>
<td>15</td>
<td>448</td>
</tr>
<tr>
<td>Alvarez Mt. Clasic</td>
<td>15</td>
<td>542</td>
</tr>
<tr>
<td>Goldimi Mt. Imi</td>
<td>15</td>
<td>516</td>
</tr>
</tbody>
</table>

* at resistant hybrids, the plants were affected at chlorophyll level, having various yellowing degrees followed by recovering during two weeks.

### Table 2. Yielding ability and productivity elements of Sulfo-resistant sunflower hybrids, ARDS, Teleorman, 2010–2012

<table>
<thead>
<tr>
<th>Hybrids</th>
<th>Average yield, kg/ha</th>
<th>Diff. ± check, kg/ha</th>
<th>Relative yield, %</th>
<th>Significant</th>
<th>TKW, g</th>
<th>TW, kg/ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR 64 LE20</td>
<td>4211</td>
<td>+476</td>
<td>112.7</td>
<td>***</td>
<td>41.6</td>
<td>38.3</td>
</tr>
<tr>
<td>Amigo</td>
<td>4114</td>
<td>+379</td>
<td>110.1</td>
<td>***</td>
<td>44.8</td>
<td>37.0</td>
</tr>
<tr>
<td>Toro</td>
<td>4985</td>
<td>+1250</td>
<td>133.5</td>
<td>***</td>
<td>46.0</td>
<td>37.0</td>
</tr>
<tr>
<td>La Pampa</td>
<td>3999</td>
<td>+264</td>
<td>107.1</td>
<td>**</td>
<td>40.4</td>
<td>41.6</td>
</tr>
<tr>
<td>Sulfosol</td>
<td>3920</td>
<td>+185</td>
<td>105.0</td>
<td>***</td>
<td>54.4</td>
<td>37.1</td>
</tr>
<tr>
<td>Zorba</td>
<td>4136</td>
<td>+401</td>
<td>110.7</td>
<td>***</td>
<td>48.8</td>
<td>34.6</td>
</tr>
<tr>
<td>Bravo</td>
<td>3888</td>
<td>+153</td>
<td>104.1</td>
<td>***</td>
<td>47.2</td>
<td>36.2</td>
</tr>
<tr>
<td>Bond</td>
<td>4122</td>
<td>+387</td>
<td>110.4</td>
<td>***</td>
<td>54.8</td>
<td>37.0</td>
</tr>
<tr>
<td>Rambo</td>
<td>4140</td>
<td>+405</td>
<td>110.8</td>
<td>***</td>
<td>44.0</td>
<td>39.0</td>
</tr>
<tr>
<td>Toledo</td>
<td>3652</td>
<td>-83</td>
<td>97.8</td>
<td></td>
<td>45.6</td>
<td>35.5</td>
</tr>
<tr>
<td>Favorit Mt. clasic</td>
<td>3735</td>
<td>Mt.</td>
<td>100.0</td>
<td></td>
<td>44.0</td>
<td>35.0</td>
</tr>
<tr>
<td>Goldimi Mt. IMI</td>
<td>4201</td>
<td>+466</td>
<td>112.5</td>
<td>***</td>
<td>48.5</td>
<td>37.9</td>
</tr>
</tbody>
</table>

LSD 5% 188 5.0
LSD 1% 251 6.7
LSD 0.1% 330 8.8
Table 3. Phenological observations and biometrical determinations of sunflower Sulfo resistant hybrids, ARDS, Teleorman, 2011–2012

<table>
<thead>
<tr>
<th>Hybrids</th>
<th>Note of phytotoxicity (note)</th>
<th>Flowering time</th>
<th>Physiological maturity time</th>
<th>Plant height, cm</th>
<th>Empty seeds %</th>
<th>Susceptibility to diseases (% affected plants)</th>
<th>Head position</th>
<th>Head shape, cm.</th>
<th>Res. to lodging in p.v.</th>
<th>Plants lodged to harvest Ing, %</th>
<th>Broken plants under head, %</th>
<th>Vegetation period, days</th>
</tr>
</thead>
<tbody>
<tr>
<td>PR 64 LE20</td>
<td>1</td>
<td>27.06</td>
<td>10.08</td>
<td>169</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>108</td>
</tr>
<tr>
<td>Amigo</td>
<td>1</td>
<td>27.06</td>
<td>10.08</td>
<td>156</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>2.2</td>
</tr>
<tr>
<td>Toro</td>
<td>1</td>
<td>30.06</td>
<td>13.08</td>
<td>159</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>20</td>
<td>1</td>
<td>1.2</td>
<td>0</td>
</tr>
<tr>
<td>La Pampa</td>
<td>2</td>
<td>1.07</td>
<td>14.08</td>
<td>155</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sulfosol</td>
<td>1</td>
<td>30.06</td>
<td>13.08</td>
<td>165</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zorba</td>
<td>2</td>
<td>26.06</td>
<td>8.08</td>
<td>164</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>19</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bravo</td>
<td>1</td>
<td>29.06</td>
<td>10.08</td>
<td>162</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>5</td>
<td>20</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Bond</td>
<td>1</td>
<td>30.06</td>
<td>17.08</td>
<td>152</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>18</td>
<td>1</td>
<td>1.1</td>
<td>3.4</td>
</tr>
<tr>
<td>Rambo</td>
<td>1</td>
<td>30.06</td>
<td>10.08</td>
<td>166</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>21</td>
<td>1</td>
<td>0</td>
<td>5.7</td>
</tr>
<tr>
<td>Toledo</td>
<td>3</td>
<td>25.06</td>
<td>8.08</td>
<td>167</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>4</td>
<td>18</td>
<td>1</td>
<td>0</td>
<td>1.3</td>
</tr>
<tr>
<td>Favorit</td>
<td>-</td>
<td>29.06</td>
<td>10.08</td>
<td>165</td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>5</td>
<td>19</td>
<td>1</td>
<td>1.8</td>
<td>1.0</td>
</tr>
<tr>
<td>Mt.classic</td>
<td>-</td>
<td>2.07</td>
<td>16.08</td>
<td>210</td>
<td>0</td>
<td>2</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>5</td>
<td>20</td>
<td>1</td>
</tr>
</tbody>
</table>

Note: 1 – there is no suffering of plants; 2 – slight yellowing of plants; 3 – obvious yellowing of green tissues, with no necrosis of apical meristem; 4 – pronounced yellowing of green tissues, necrosis, inhibition of apical meristems, which lead to plants branching; 5 – large necrosis of green tissues and apical meristem destroyed, which lead to plant death

Toledo, a more pronounced phytotoxic effect was registered (note 3), but the plants recovered with no other secondary effects (Table 3).

The susceptibility to diseases, expressed by the percentage of affected plants, has registered values of 0% for Plasmodiophora helianthi, 0% for Sclerotinia sclerotiorum, 1–2% for Phoma helianthi and 1–2% for Phomopsis helianthi, compared to hybrid IMI-resistant Goldimi, which registers 2% for Plasmodiophora helianthi, 3% for Sclerotinia sclerotiorum, 4% for Phoma helianthi and 1–2% for Phomopsis helianthi. The tested hybrids had a good position of the head versus stem vertical direction (notes 4–6), a shape which excludes the occurrence of Botrytis cinerea and an adequate head diameter (19–20 cm) (Table 3). All tested hybrids were resistant to lodging, in both vegetation and harvesting stages. The hybrids Bond and Rambo register percent of 3.4%, respectively 5.7% with plant breaking under head, with no losses at harvesting. The hybrid height is medium (159–169 cm), close to conventional hybrid Favorit (165 cm). The shortest hybrid was la Pampa (155 cm) and the highest one was IMI-resistant Goldimi (210 cm). On average, the vegetation period of Sulfo-resistant hybrids is 106–115 days as in Favorit, except the hybrids Toro, La Pampa, Sulfosol and Bond, which reach maturity 3–7 days

Figure 1. Unwanted effects to application of tribenuron methyl–based herbicides: lack of head; modification of head shape
later. The latest hybrid proved to be Bond (115 days), while the hybrids Zorba and Toledo reached maturity 2 days earlier versus conventional hybrid Favorit.

The advantages of the method are: the problem weeds as *Cirsium arvense* L. and *Xanthium strumarium* L. are controlled and absence of remains in the soil. Disadvantages of the method are occurring of phytotoxicity phenomena when herbicide is applied during cold nights (night temperatures <-12 °C) as well as mistakes in herbicide application (inadequate adjustment of equipment, lack of washing, application of another herbicide at a shorter interval) (Figures 1 and 2).

Many researchers conclude that the growing of Sulfo-tolerant sunflowers resulted in effective herbicide control of broadleaved weeds (Zollinger, 2003; Malidža et al., 2006; Kukorelli et al., 2008, 2011a, 2011b, 2012; Delchev, 2013a, 2013b; Pirvu, 2013). Our research confirms that by growing sunflower hybrids resistant to 75% tribenuron methyl-based herbicides the risk is reduced to a very low level, phytotoxicity was very weak and yields were not affected in comparison with conventional and IMI hybrids. Low toxicity of 75% tribenuron methyl-based herbicides in sunflower hybrids was reported by other authors (Kukorelli, 2010; Delchev, 2013a; Pirvu, 2013).

Achieving hybrids adequate to Sulfo technology, resistant to 75% tribenuron methyl-based herbicides, depends on the dominant gene S, discovered in USA. The F1 (SS) hybrids are completely resistant. Kukorelli (2012) reported that the homozygous types had higher level of resistance than the heterozygous ones. Hybrids released through Turbo technology, resistant to tribenuron methyl-based herbicides, in F1 hybrid generation, gene S, which confers resistance, is heterozygous Ss (F1 is completely resistant) (Figure 3). There are some difficulties in Sulfo resistant hybrid multiplication. Due to foreign pollination beside F1 Ss hybrids, s s sterile or fertile plants could occur. Kukorelli (2012) also reported certain types of herbicide-tolerant varieties that showed different levels of resistance.

The discovery of a wild *Helianthus annuus* L. population (in Kansas, USA) resistant to a sulfonyleurea herbicide (tribenuron methyl) (Al-Khatib et al., 1999) and the creation of the first hybrids on this basis (Miller et al., 2000; Jocic et al., 2001; Miller et al., 2004) is a good prerequisite for the development of new modern and improved sunflower hybrids possessing tolerance to tribenuron methyl-based herbicides (Figure 4).

\[
\begin{array}{c|cccccccc}
T_1 & T_2 & M_1 & M_2 & M_3 & M_4 & M_5 & M_6 & T_1 \\
\hline
Form ♀ (SS) x Form ♂ (ss) & \hline
F_1 (SS) & \hline
F_1 (SS) – complete resistance
\end{array}
\]

**Figure 3.** Inheritance of gene S (which confers resistance) in F1 hybrids released through Turbo technology, resistant to tribenuron methyl-based herbicides
Conclusions

Introduction of sunflower hybrids resistant to 75% tribenuron methyl-based herbicides proves the practical and economic importance of Sulfo technology for effective control of the problem broadleaved weeds and its adaptability under our conditions. Hybrids released through Turbo technology, resistant to tribenuron methyl-based herbicides, in F1 hybrid generation, gene S, which confers resistance, is heterozygous Ss (F1 is completely resistant).

The yielding ability of sunflower hybrids released through Turbo technology, resistant to tribenuron 75% methyl-based herbicides, is not affected compared to the conventional check Favorit, on three years average, its level being 3652-4985 kg/ha, the Favorit has realized 3735 kg/ha.

The hybrid Toro is distinguished especially through the highest gain yield compared to the check Favorit (1250 kg/ha, respectively 33.5%). The yielding ability of Toro hybrid exceeds by 774 kg/ha (11.8%) the yield achieved by the hybrid PR 64 LE20 (Pioneer signature).

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DuPont Crop Protection Sulfonylurea Herbicides. Our Commitment to Quality. K-05825 (09/05) Printed in the USA.


Review

Blue-green coloured eggs in *Gallus gallus domesticus*  
H. Lukanov

Genetics and Breeding

Investigation on the resistance of doubled haploid sunflower lines to some biotic factors  
M. Drumeva, P. Yankov, N. Nenova, P. Shindrova

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M. Ilchovska, I. Ivanova

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N. Stancheva, I. Dimitrova, S. Georgieva

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M. Yossifov, L. Kozelov

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D. Dimitrov

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Results

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