The effect of low growth temperature on Hill reaction and Photosystem 1 activities in three biotypes of *Kochia scoparia* (L.) Schrad. with different sensitivity to atrazine and ALS-inhibiting herbicides

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ABSTRACT

A possible influence of low growth temperature on the photochemical activity of isolated mesophyll chloroplasts was studied in three biotypes of kochia [*Kochia scoparia* (L.) Schrad.] that showed either double resistance to both atrazine and acetoacetate synthase (ALS) inhibitors (Bubny biotype), resistance to ALS inhibitors but not to atrazine (Jihlava biotype), or sensitivity to both types of herbicides (Karlín biotype). Plants 5 or 12 weeks old since the date of sowing were examined. The Bubny biotype displayed the lowest values of Hill reaction activity (HRA) among all biotypes examined, and a significantly lower activity of Photosystem (PS) 1 compared to the Karlín biotype; this applied both for the control and low temperature-grown plants and for both plant ages studied. The comparison of HRA and PS1 activity in the Jihlava and Karlín biotypes showed lower values of both parameters for the Jihlava biotype. The HRA of plants grown at low temperature conditions was usually only slightly lower compared to the control plants of all three biotypes examined. The activity of PS1 in the kochia biotypes grown under low-temperature conditions increased markedly and significantly compared to the control plants; this increase was slightly higher in the atrazine-sensitive biotypes Jihlava and Karlín than in the resistant biotype Bubny. The differences between resistant and susceptible biotypes in the HRA diminished under low-temperature conditions whereas the differences in PS1 activity increased.

Keywords: *Kochia scoparia*; ALS inhibitors; atrazine; resistance to herbicides; Photosystem 1 activity; Hill reaction activity; low temperature

Kochia [*Kochia scoparia* (L.) Schrad.] is a weed species native to middle- and eastern Asia steppes, which has been involuntarily introduced into Europe and North America. A rich network of railway lines serves probably as a main route for its expansion in the Czech Republic. The presence of weeds in these localities is usually regulated by a long-term regular or irregular treatment with various herbicides, plants displaying single- or multiple-herbicide resistance can gradually appear there and spread out to other locations. Thus, it is very important to learn more about the nature of this resistance, as this knowledge could be used for a better management of weeds.

During the last three years, kochia biotypes showing double resistance to two classes of herbicides, i.e. PS2 activity inhibitors and acetoacetate synthase (ALS) inhibitors, were found in three localities in the Czech Republic (Chodová and Mikulka 2000b, 2002). The kochia biotype with this type of multiple resistance had been previously described only at two localities in the U.S.A. (Foes et al. 1999, Heap 2003), though various biotypes resistant to either triazines or ALS inhibitors are known from many other locations (for the up-to-date summary of the data on kochia biotypes resistant to various herbicides see Heap 2003).

Triazine herbicides function as inhibitors of Photosystem (PS) 2 electron transport in chloroplast thylakoid membranes and the resistance to them is conferred by a specific change of the structure of the D1 protein in the PS2 reaction center (for reviews see e.g. Powles and Holtum 1994, Oettmeier 1999, Devine and Shukla 2000). However, many other effects are associated with this change in D1 structure and the resistance of weeds to triazines is also influenced by other factors such as the circadian rhythm and plant ontogeny, temperature, light intensity etc. (Dekker 1993). Imidazolinone herbicides belong to the class of ALS inhibitors and as such disrupt the synthesis of essential branched-chain amino acids (valine,
leucine and isoleucine) (Whitcomb 1999, Devine and Shukla 2000). The weed resistance to these herbicides is also associated with the changes in the protein structure and several ALS amino acid substitutions that confer herbicide resistance have been reported from various weed species (Devine and Shukla 2000, Tranel et al. 2003). Similarly to triazine-based herbicides, the ALS inhibitors also show a temperature dependency that can strongly affect the efficacy of herbicide application and could possibly play a role in the manifestation of the plant resistance to these herbicides (Light et al. 1999, 2001).

In most weed species examined to this date (e.g. *Amaranthus* sp., *Conyza canadensis*, *Senecio vulgaris*, *Solanum nigrum*) it seems that the biotypes resistant to triazines are better adapted to low temperatures and worse adapted to heat and high light intensity compared to the sensitive ones (McCloskey and Holt 1991, Dekker 1993, Plowman and Richards 1997, Kremer and Lotz 1998, Dulai et al. 1999, Szigeti and Lehoczki 2003). Kochia is a plant with C4 type of photosynthesis and as such normally prefers warmer temperatures to cold (Nord et al. 1999, Fischer et al. 2000). However, the relationship between a sensitivity/resistance to triazines (or other herbicides) and growth temperature has not been as yet analyzed in this species. The aim of our study was therefore to examine the possible influence of low growth temperature on the photochemical activity of isolated chloroplasts in three biotypes of kochia which showed either double resistance to both atrazine and ALS inhibitors, the resistance to ALS inhibitors but not to atrazine, or the sensitivity to both types of herbicides, and to compare these three biotypes with respect to the efficiency of their photosynthetic electron transport.

**MATERIAL AND METHODS**

The photochemical activity of isolated mesophyll chloroplasts was measured in three biotypes of kochia (*Kochia scoparia* (L.) Schrad.) derived from three localities in the Czech Republic. The seeds of biotypes Bubny and Jihlava were originally collected from plants growing along the railway tracks and at lay-by areas of the railway stations Praha-Bubny and Jihlava, in 1999. They were sown in April 2000 at the experimental fields of the Research Institute of Crop Production at Praha-Ruzyně (dimensions 14 × 14 m; the fields were separated from the surrounding areas by 2.5 m-wide zones). These fields have been treated with herbicides Gesaprim 500 FW [in a dose corresponding to 500 mg/m² of atrazine as its active ingredient (a.i.)] and Granstar 75 WG (in a dose corresponding to 3.617 mg/m² of tribenuronem as its a.i.) in regular one-year intervals, always in June. In the case of the Jihlava biotype, the experimental field has been treated only with Granstar 75 WG. The plants used for the measurement of photochemical activities of isolated chloroplasts were grown from the seeds collected from these experimental fields in October 2001. The plants of the third biotype examined, i.e. Karlin, were grown from the seeds collected at the road margin and adjacent uncared-for areas at the locality Praha-Karlin also in October 2001. We presume that kochia plants found at this locality came originally from the now-defunct railway track that lies near-by.

The resistance/sensitivity of the plants to ALS inhibitors and atrazine was examined prior to the measurement of photosynthetic characteristics by a bioassay (see Chodová and Mikulka 2000b for details). The results of this test showed that the biotype Bubny is resistant both to ALS inhibitors and atrazine, the biotype Jihlava is resistant to ALS inhibitors but not to atrazine, and the biotype Karlin is sensitive to both types of herbicides.

The plants used for the measurement of photochemical activities of isolated mesophyll chloroplasts were grown at growth chambers in plastic pots containing a 2:1 mixture of topsoil and commercial soil substrate (pH 6.8). The dimensions of pots were 10 × 10 cm. The day/night regime at growth chambers was 12/12 hrs, the light intensity 130 µmol/m²/s and the relative air humidity 65–80%. The temperature was set to either 30 ± 1°C (control) or 10 ± 1°C (low temperature). The first experimental series was conducted 5 weeks after the date of sowing (the average height of plants was 3–5 cm), the second experimental series concerned 12-week-old plants (average height of 10–15 cm). Both series were conducted during the first quarter of 2002 year.

The analysis of the photochemical activity of isolated mesophyll chloroplasts was performed as in Körnerová and Holá (1999). The mixture of leaves from 10–15 plants was used for the isolation of each sample, representing one biotype in one temperature treatment. The Hill reaction activity (HRA) was measured polarographically as the amount of oxygen formed by the chloroplast suspensions in the white light (approx. 750 µmol/m²/s PAR); 0.007M K₃[Fe(CN)₆] was added as an artificial electron-acceptor. The activity of PSI was measured similarly, as a consumption of oxygen by the suspensions of isolated mesophyll chloroplasts. 0.15mM reduced 2,6-dichlorophenolphloroglucinol was used as an artificial donor of electrons and 0.1mM methylviologen as an electron acceptor. The inhibitor of PS2 activity in this case was 0.1mM 3-(3',4'-dichlorophenyl)-1,1-dimethyleurea. The experiments were repeated four times; the differences between the biotypes or between the temperature
treatments were analysed using paired Student’s t-test with a critical probability level 0.05.

RESULTS AND DISCUSSION

The comparison of HRA and PS1 activity in three biotypes of kochia grown either at 30 or 10°C is shown in Figures 1 and 2 and Table 1. The Bubny biotype, which was resistant both to ALS inhibitors and atrazine, displayed the lowest HRA values among all biotypes examined; this applied both for 30°C- and 10°C-grown plants and for both plant ages studied (Figure 1). However, the statistically significant differences in HRA between this biotype and two other biotypes were confirmed only for the 5-week-old plants, whereas in the 12-week-old plants of the Bubny biotype (though also

Figure 1. The relative values of Hill reaction activity (HRA) in two biotypes of *Kochia scoparia* (L.) Schrad. resistant either to both ALS inhibitors and atrazine (biotype Bubny) or resistant to ALS inhibitors but sensitive to atrazine (biotype Jihlava); the values are expressed as percentage of values shown by biotype Karlin, which is sensitive to both ALS inhibitors and atrazine; plants were grown either at 30 or 10°C and were 5- or 12-weeks-old during the measurements of HRA.

Figure 2. The relative values of Photosystem (PS) 1 activity in two biotypes of *Kochia scoparia* (L.) Schrad. resistant either to both ALS inhibitors and atrazine (biotype Bubny) or resistant to ALS inhibitors but sensitive to atrazine (biotype Jihlava); the values are expressed as percentage of values shown by biotype Karlin, which is sensitive to both ALS inhibitors and atrazine; plants were grown either at 30 or 10°C and were 5- or 12-weeks-old during the measurements of PS1 activity.

Table 1. Comparison of Hill reaction activity (HRA) or Photosystem (PS) 1 activity in three biotypes of *Kochia scoparia* (L.) Schrad. sensitive or resistant to ALS inhibitors or atrazine; biotype Bubny shows double resistance to both ALS inhibitors and atrazine, biotype Jihlava is resistant to ALS inhibitors and sensitive to atrazine, and biotype Karlin is sensitive to both types of herbicides; plants were grown either at 30 or 10°C and were 5- or 12-weeks-old during the measurements of HRA and PS1 activity; the values of statistical significancy (p) resulting from t-test are shown.

<table>
<thead>
<tr>
<th>Comparison</th>
<th>5-weeks-old plants</th>
<th>12-weeks-old plants</th>
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<tr>
<td></td>
<td>30°C</td>
<td>10°C</td>
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<tr>
<td>Hill reaction activity</td>
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<tr>
<td>Bubny – Karlin</td>
<td>0</td>
<td>0.038</td>
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<tr>
<td>Bubny – Jihlava</td>
<td>0.001</td>
<td>0.057</td>
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<tr>
<td>Jihlava – Karlin</td>
<td>0.002</td>
<td>0.566</td>
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<tr>
<td>Photosystem 1 activity</td>
<td></td>
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<tr>
<td>Bubny – Karlin</td>
<td>0.049</td>
<td>0.004</td>
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<tr>
<td>Bubny – Jihlava</td>
<td>0.555</td>
<td>0.100</td>
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<tr>
<td>Jihlava – Karlin</td>
<td>0.004</td>
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displaying lower HRA than the sensitive Karlin biotype) this difference was not statistically significant, and the HRA values of the Bubny and Jihlava biotypes apparently did not differ at all (Figure 1, Table 1).

Our finding that the triazine-resistant biotype of kochia shows lower efficiency of PS2 electron transport is not much surprising. The resistance to triazines is associated with the reduced binding affinity of these herbicides to the Q<sub>b</sub> site of the D1 protein, which is most commonly conferred by a structural change due to the substitution of glycine to serine at residue 264 of this protein (Foes et al. 1993, Oettmeier 1999, Devine and Shukla 2000, Kumata et al. 2001, Sibony and Rubin 2003, Varadi et al. 2003). Though this structural change still allows for the plastoquinone binding to the Q<sub>b</sub> site, which is necessary for a successful transport of electrons between PS2 and cytochrome b<sub>6</sub>f complex of chloroplast thylakoid membranes, the efficiency of the photosynthetic electron transport is reduced. Many reports of this phenomenon exist for triazine-resistant biotypes of various weeds (e.g. Ort et al. 1983, Dekker 1993, Chodová et al. 1994, 1995, Körnerová et al. 1998, Devine and Shukla 2000).

However, our results suggest that this difference between the triazine-resistant and -sensitive biotypes could decrease with advancing ontogeny of plants, at least under optimum growth conditions. In most plant species, the reproductive phase of ontogeny is accompanied by a decrease in photosynthetic efficiency (Evans 1993). Such was the case for the double-sensitive Karlin biotype and, less markedly, for the ALS inhibitors-resistant, atrazine-sensitive Jihlava biotype grown at 30°C, which at the age of 12 weeks were already flowering. The HRA values measured in chloroplasts isolated from 12-week-old plants of Karlin or Jihlava biotypes were about 80 or 87% of those measured in 5-week-old plants, respectively. On the other hand, the HRA of the double-resistant Bubny biotype did not change with the plant age (the HRA values measured in chloroplasts isolated from 12-week-old plants were 99% of those measured in 5-week-old plants) when plants were grown at 30°C. In 10°C-grown plants, this decrease of HRA with the plant's age was not much evident (the HRA values measured in chloroplasts isolated from 12-week-old plants were about 98, 90 and 98% of those measured in 5-week-old plants for the Bubny, Jihlava and Karlin biotypes, respectively), which was probably due to the fact that at this temperature even 12-week-old plants did not yet start flowering. The changes in the efficiency of photosynthetic electron transport observed in the triazine-resistant biotype of kochia could therefore be accompanied also by the changes in its ontogenetic course.

The activity of PS1 measured in the suspension of mesophyll chloroplasts isolated from the double-resistant Bubny biotype was significantly lower compared to the double-sensitive Karlin biotype; this applied for both temperature treatments and for both plant ages analysed (Figure 2, Table 1). The difference in this parameter between the Bubny and Jihlava biotypes was statistically significant only for 12-week-old plants grown at 10°C; for the other three temperature-plant age combinations studied we did not find any difference in the PS1 activity between this pair of biotypes (Table 1). The decreased activity of PS1 in the triazine-resistant biotype could be theoretically associated with the lowered availability of reduced plastoquinones (and, consequently, with the reduction in the rate of entire photosynthetic electron transport). However, this can hardly be the case in our experiments where measurements of PS1 activity were done on isolated chloroplasts supplied with sufficient amount of artificial electron acceptors and donors. On the other hand, the decrease in the ratio of reduced/oxidized form of plastoquinone, which should result from the lowered efficiency of the PS2 photosynthetic electron transport, could lead to the changes in the redox state of chloroplasts, and such changes are known to have far-reaching consequences on the quantity and function of various components.

The comparison of HRA and PS1 activity in the ALS inhibitors-resistant, atrazine-sensitive Jihlava biotype with the Karlín biotype (sensitive to both types of herbicides) showed that the values of both parameters measured for the Jihlava biotype were lower than those measured for the Karlín biotype. However, while for HRA this difference was statistically significant only in 5-week-old plants grown at 30°C, the significance of the difference between this pair of biotypes in PS1 activity was high at almost all temperature-plant age combinations examined (with the exception of 12-week-old plants grown at 30°C) (Figures 1 and 2, Table 1). Biotypes of weeds resistant or susceptible to ALS inhibitors usually do not differ in growth parameters, seed production or general character of ontogeny (Thompson et al. 1994, Christoffoleti et al. 1997, Sibony and Rubin 2003), but the resistance to this class of herbicides is often accompanied by a decreased feedback inhibition of ALS by valine, leucine or isoleucine, and increased levels of free branched-chain amino acids in seeds or leaves (Dyer et al. 1993, Eberlein et al. 1999, Chodová and Mikulka 2000a, Devine and Shukla 2000). Whether and/or how this phenomenon could be reflected in the decreased efficiency of photosynthetic electron transport, and why PS1 seems to be more affected compared to PS2, remains yet to be resolved.

Kochia belongs to the C4 photosynthesis class of higher plants and we have therefore expected to find a decrease of its photosynthetic efficiency (particularly of PS2 function) due to the low-temperature treatment of the plants. However, the HRA measured in mesophyll chloroplasts isolated from the plants grown at 10°C was usually only slightly lower compared to those found for the 30°C-grown plants of all three biotypes examined (Figure 3). These differences were statistically non-significant with the exception of 5-week-old plants of the double sensitive Karlin biotype (Table 2). A possible explanation of this phenomenon could lie in a relatively low light intensity (130 µmol/m²/s) used for the cultivation of the plants. As negative effects of low-temperature stress on plants are often associated with the photoinhibition and photodamage (McKersie and Leshem 1994, Wise 1995, Sonoike 1998, Pessarakli 1999), it is possible that under the relatively irradiance used for the cultivation of our plants no such damage to the photosynthetic apparatus occurred and the function of PS2 was not much affected.

The response of HRA to low temperature was very similar in all three biotypes (Figure 3). It is now widely accepted that the resistance of plants to triazines, caused by the specific mutation(s) at the Q₆ site of the D1 protein, is associated not only with the lowered efficiency of photosynthetic electron transport but also with the increased sensitivity.
of plants to photoinhibition and the decreased efficiency of some protective processes (e.g. the light-induced formation of zeaxanthin) (Darko et al. 1996, Plowman and Richards 1997, Devine and Shukla 2000, Kumata et al. 2001, Szigeti and Lehoczki 2003, Varadi et al. 2003). The triazine-susceptible biotypes are therefore usually better adapted to the growth under high light conditions compared to the resistant ones. Plowman and Richards (1997), who examined the effect of high and low light and three temperature regimes on atrazine-susceptible and -resistant Brassica rapa, have found that under high light conditions the susceptible biotype showed a greater photon yield and relative competitive ability than the resistant biotype when grown at high or medium temperatures, but at low temperature the situation was reverse. Under low light conditions, the chlorophyll fluorescence parameters of both biotypes did not differ in either temperature regime examined, which agrees well with our results. Kumata et al. (2001), who examined simazine-resistant and -susceptible biotypes of Poa annua also did not find any differences in photosynthetic performance of these biotypes when grown under shaded conditions, whereas under full-sunlight conditions the resistant biotype showed a reduced capacity of photosynthesis.

The activity of PS1 in the kochia biotypes grown under low-temperature conditions increased markedly and significantly compared to the 30°C-grown plants, both in 5-week-old and 12-week-old plants (Figure 4, Table 2). This agrees with the results of other experiments made on cold-stressed plants, where the increased efficiency of electron transport around PS1 complex was observed (Sonoike 1998, Pessarakli 1999). PS1 is probably less susceptible to low temperature-induced damage compared to PS2, particularly under low-light conditions when no photodamage to this complex should occur (Nie and Baker 1991, Körnerová and Holá 1999, Sonoike 1998, Pessarakli 1999). All three biotypes examined responded to the cultivation at 10°C by an increase of the PS1 activity; this increase was slightly higher in the atrazine-sensitive biotypes Jihlava and Karlin than in the resistant biotype Bubny (Figure 4). It is possible that the changed efficiency of photosynthetic electron transport and/or other changes of chloroplast function, which are characteristic for the triazine-resistant biotype, affect also the response of photosynthetic apparatus to low temperature.

Our study has shown that a) the biotypes of Kochia scoparia (L.) Schrad. resistant to the triazines and/or to ALS inhibitors display lower efficiency of photosynthetic electron transport compared to the sensitive biotype; b) this difference depends on the plant age and ontogenetic stage; c) the low-temperature treatment does not necessarily have a negative effect on the photosynthetic efficiency in this species presumed that plants are grown under conditions of low light; d) the differences between resistant and susceptible biotypes in the HRA diminish under such conditions whereas the differences in PSI activity increase.

REFERENCES


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ABSTRACT

Vliv nízké růstové teploty na aktivitu Hillovy reakce a fotosyntézu 1 u tří biotypů bytelu metlatého [Kochia scoparia (L.) Schrad.] s odlišnou citlivostí vůči atrazinu a ALS inhibujícím herbicidům

Byl sledován vliv nízké růstové teploty na fotochemickou aktivitu izolovaných chloroplastů tří biotypů bytelu metlatého [Kochia scoparia (L.) Schrad.] s dvojnásobnou rezistencí vůči atrazinu a inhibitorům acetolaktátsyntázy (ALS)
– biotypu Bubny, s rezistencí vůči inhibitorům ALS a citlivostí vůči atrazinu – biotypu Jihlava nebo s citlivostí vůči oběma herbicidům – biotypu Karlín. Hodnocení proběhlo za 5 a 12 týdnů od výsevu semen. Biotyp Bubny vykazoval nejnižší hodnoty aktivity Hillovy reakce (HRA) ze všech zkoumaných biotypů a průkazně nižší aktivitu fotosystému (PS) 1 ve srovnání s biotypem Karlín u rostlin kontrolních i pěstovaných při nízké teplotě v obou fázích růstu. Porovnání aktivit HRA a PS 1 u biotypů Jihlava a Karlín ukázalo nižší hodnoty obou parametrů u biotypu Jihlava. Aktivita HRA rostlin, pěstovaných při nízké teplotě, byla jen mírně nižší oproti kontrole u všech tří biotypů. Aktivita PS 1 u biotypů bytelu metlatého pěstovaných za nízké teploty byla průkazně zvýšena v porovnání s kontrolními rostlinami, a to o něco více u biotypů citlivých vůči atrazinu Jihlava a Karlín než u rezistentního biotypu Bubny. Diference mezi biotypy rezistentními a citlivými v aktivitě HRA se zmenšily při nízkých teplotních podmínkách, zatímco diference u PS 1 aktivity se zvětšily.

**Klíčová slova:** Kochia scoparia; inhibitory ALS; atrazin; rezistence vůči herbicidům; aktivita fotosystému 1; aktivita Hillovy reakce; nízká teplota

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