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Investigation of the Resistance of *Amaranthus retroflexus* L. to Atrazine Through the Method of Leaf Fluorescence

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Abstract: It has been investigated the resistance of various of *Amaranthus retroflexus* L. to atrazine by the method of leaf fluorescence and the determination of quantum yield. Effects of varying concentrations of atrazine and intervals of its incubation with leaves on secondary kinetics of chlorophyll **a** fluorescence and quantum yield in both treated and untreated plants, were evaluated. It was shown that atrazine produced a significant infibitory effect on photosynthesis, depressed the quantum yield markedly, and blocked the transport of electrons, thus promoting the yield of chlorphyll **a** fluorescence and reducing O_2 evolution from the susceptible biotype. The resistant biotype of *Amaranthus retroflexus* L. behaves like an untreated plant..

Key words: resistance, *Amaranthus retroflexus* L., atrazine, methods of leaf fluorescence.

Introduction

The tolerance of some and sensitivity of other herbicides to the applied ones has been the subject matter of numerous authors ever since the herbicides were in use. The differences existing between the same plant species biotypes stem from morphological or physiological characters or from the herbicide metabolite phytotoxicity occurring over its metabolism.

Le Baron (1987, cit. Mallory–Smith et al., 1990b) defined weed resistance to the herbicides as the ability of a specific biotype to survive herbicide amounts lethal to the individuals of the normally sensitive population.

The herbicide impact is expressed in a linked or an interaction manner between an active substance and one or more proteins, negatively affecting the plant metabolism and growth. The plants may become resistant to the herbicide effect by modifying the proteins that are reducing or eliminating herbicide ability to bind or interact with them.

Weed resistance to herbicides has become a crucial problem in the conditions of agricultural production where the same herbicides or those of the same mechanism of impact are successively used.

As an answer to such a strong selection impact and due to the fact that one herbicide or those having the same mechanism of impact are successively used, the genetic composition of weed populations changes in that the frequency of resistant allells and individuals increases. Re–use of the same herbicide or of that with the same impact mode disrupts the sensitive and makes the resistant populations viable so that the characteristics of resistance may further be transferred to the next generation.

Despite being the subject matter of numerous authors, this problems remains to be solved in terms of genetic, biochemical and physiological bases inducing weed resistance to the herbicides. In these plants, resistance is the result of biochemical changes where metabolic activity takes place rather than that of absorption, translocation or of the sped up plant metabolism.

Only relevant, precise and quick methods of great precision can implement this assignment fully, i.e. to answer whether the biotype is resistant or not.

Weed resistance to herbicides was first noticed with triazines (Abel, 1954) and the methods of resistant types to triazines determinations were frequently used. However, since the weed resistance to the hugely turned over ALS inhibitors has been the problem of economicity for the last ten years, new and more convenient methods for determining and surveying weed resistance to these herbicides are sought.

The majority of researchers pointed out that resistance to ALS inhibitors developed very fast, soon after a few treatments with the same impact mechanism herbicides had been applied (Adkins et al., 1997; Uchino et al., 1999; Wolf et al., 2000). Thus, Saori et al., (1994 cit. Sprague et al., 1997) confirmed that resistance to these herbicides usually developed after 4–7 successive treatments with ALS inhibitors. Horak and Peterson (1995 cit. Sprague et al.1997) established that the species *Amaranthus rudis* Sauer had developed resistance to *imazetapir* immediately after its second application.

These authors also pointed out that resistance was likely to be the result of the highly present resistant individuals within a natural population, or if the weed seeds come from the place other than that of the resistant population. Additionally, (*Amaranthus retroflexus* L.) careless weed developed resistance to *sulfomethuron–methyl* after the third year of continual use in combination with simazine (Sibony et al., 1992) cit. Sibony et al., 2001.) In general, all the species of the strain *Amaranthus* are thought to be extremely sensitive to many ALS inhibitors due to their numerous characteristics related to the outcome of resistance in the populations

indigenous to this strain. This has been well–confirmed on a range of careless weed *Amaranthus palmeri* S.Wats (Horak and Peterson, 1995; Gaedderi et al., 1997; Sprague et al., 1997), A. retroflexus L. (Sibony et al., 1992; Gerwick et al., 1993; Saari et al., 1994), A. blitoides S.Wats (Saari et al., 1994), A. rudis (Horak and Peterson, 1995; Lovell et al., 1996; Hinz and Owen, 1997; Sprague et al., 1997; Wagner et al., 2002a, 2002b), A lividus L. (Manley et al., 1996) and *A. hybridus* (Manley et al., 1996, 1999; Poston et al., 2000).

The experiments performed with sensitive and resistant biotypes *Amaranthus retroflexus* L. indicated chloroplasts to be the points of selective tolerance to atrazine (Arntzen et al., 1979).

Since the phyto–toxic effect of atrazine inhibits photosynthetic transport of electrons, measuring the chlorophyll fluorescence yield *a* and release of O_2 may provide the quantitative measure of such an inhibition.

Bearing in mind the approaches made to this field, the degree of resistance of the different biotypes *Amaranthus retroflexus* L. to atrazine using leaf fluoroescence has been attempted.

Materials And Method

Plant seeds of differing biotype *Amaranthus retroflexus* L. were collected from the areas where triazine herbicides have intensively been used for more than ten years. The seeds of the sensitive biotype are from those areas on which no herbicides have been used. The plants were grown in the controllable conditions: 12 hours of light of intensity ($\mu E\ m^{-2}\ s^{-1}$) at 22^0C and 12 hours without light presence at 18^0C. Relative moisture in the phytotronic chamber accounted for 75%.

Depending on their age and position, the leaves were submerged in the atrazine solution (10 and 20 mg/l) or in the water without herbicides. Incubation time of the leaves was designated for each experiment in the section "Results and discussion". Over the incubation, the leaves were not exposed to light (for being in the dark). For the chlorophyll fluoroescence *a* measurement and oxygen release, $10cm^2$ pieces of leaves were cut and placed in the measuring compartment.

The speed of photosynthetic release of O^2 was measured by modified electrode for leaf (LD–2 Hansatech) (Deviev and Walker, 1983) at satirizing CO_2. The intensity of actinic light on the leaf spot was 200 or 400 $\mu E^{-2}S^{-1}$ as the experiment might have been.

The light was passed through blue filter (Coming 4–96). *Bjorkman lamp* (50 W/12V) and used as a source of light. Fluorescence was defined by interfering filter (740 nm) and measured by photodiode.

Quantum yield was measured and computed by computer in the programme made at the *Research Institute for Photosynthesis*, Sheffield (Walker, 1978). Different intensities of light were obtained by means of neutral filters.

The results were obtained by measuring photosynthesis in its steady state. The discs of the leaves were 20 min. without light, that is, in the dark (in the chamber) then exposed to light for 5 min. to be in the dark for 1 min. and again, when the chlorophyll fluoroescence *a* was measured and O_2 released.

Results and Discussion

The experiments with sensitive and resistant biotypes *Amaranthus retroflexus* L., indicated that chloroplasts presented the loci of selective tolerance to atrazine (*Arntzen et al*., 1979). Since the phyto-toxic effect of atrazine is based on the inhibition of photosynthetic electron transport, measurements of the chlorophyll fluorescence yield *a* and release O_2 may give the quantum measure of inhibition.

Slow kinetics of the chlorophyll fluorescence *a* is used as a general diagnostic probe to give information about photosynthetic apparatus. Considering that fluorescent signal is highly sensitive even to slight changes in the physiological state of the leaf fluorescence, when measured, leaf fluorescence, enables us to easily differentiate the leaves that look the same with naked eye. Therefore, measurement of the fluorescence kinetics is used to study the impact of stress on the plants just as well that of herbicides, as the factor of stress.

Fig. 1 presents the results of measuring the chlorophyll fluorescence *a* and photosynthetic release O_2 in the usual state on the pieces of the *Amaranthus retroflexus* L. The leaves were 1 hour incubated in the atrazine solution of 20 mg/l concentration.

The atrazine of 20 mg/l concentration has been chosen as the lowest one exhibiting measurable effects on the plant leaves and therefore used for the rest of the experiment. The measurements indicated that 1–hour leaf incubation in 10 mg/l atrazine concentration caused neither changes in the chlorophyll fluorescence *a* nor those in the speed of O_2 release. Release speed of the plant treated with O_2 was decreased by about 20% compared to that of the untreated sensitive plant. Since the herbicidal impact of atrazine needed light, the degree of damage was likely to be proportional to the light intensity (Aschton, 1965).

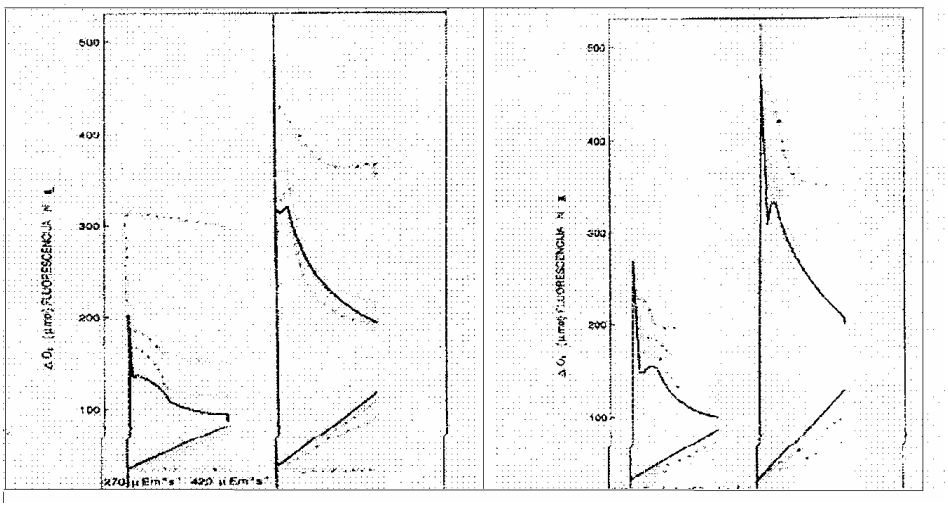
Therefore, re–lighting of leaf segments with the higher intensity light is likely to cause higher (by 10%) inhibition of O_2 release, as well as a higher relative fluorescence in the sensitive biotype.

When incubated for 3 hours in atrazine solution of the same concentration, the leaves of the only sensitive biotype have been completely inhibited in terms of photosynthesis, which is absolutely reasonable, because, the longer the time of exposing, the greater atrazine amount will be taken up by leaf from the solution. In the resistant biotype, the leaves of which were also exposed to the 3–hour long impact of atrazine of invariable concentration, a slightly inhibited photosynthesis could be noticed.

The sensitive biotype had a higher fluorescence than the resistant one did over 1 hour of either of the biotypes leaves incubation in atrazine solution. After 3 hours of incubation, the fluorescence of the sensitive type remained at the level P–maximum, which was in correlation with the inhibition of O_2 release. The fluorescence of the resistant biotype increased slightly. At the higher light intensity, the differences between the sensitive and resistant biotype were even more pronounced.

By inhibiting transport of electrons, atrazine affected the efficiency of the photosynthetic apparatus while using the energy of light, as could be observed when measuring quantum yield (-number of moles O_2 released per mole photons absorbed by photosynthetic apparatus).

Fig. 1.,2. Simultaneous measurement of chlorophyll a fluorescence



(____) the control of susceptibile biotype
(--------) susceptibile biotype whouse leaves were incubated in solution of atrazine (20 mg/l) for 3 hour

Quantum yield was computed from the slopes of the curves denoting the dependence of the photosynthetic O_2 release on the light intensity. At low light intensity, where the speed of photosynthesis is directly proportional to the light intensity, quantum yield is constant and maximal, representing the measure of efficiency, with which the light was converted into the stable photosynthetic products. Assuming that plants are using an invariable photosynthetic mode equally efficiently converting photons into the energy of chemical bonds, their invariable quantum yield is expected unless the system functional integrity is disrupted. Thus, the quantum yield determination may help quantitatively determine the effects of atrazine and other herbicides, as the inhibitors of photosynthesis, on the photosynthetic apparatus.

Figs 3. and 4. have shown the dependence of the photosynthetic O2 release on the light intensity. Maximal speed of O_2 release (at the highest light intensity) in resistant biotype is twice as high that in the susceptible one, the leaves of which were incubated in atrazine solution for 3 hours.

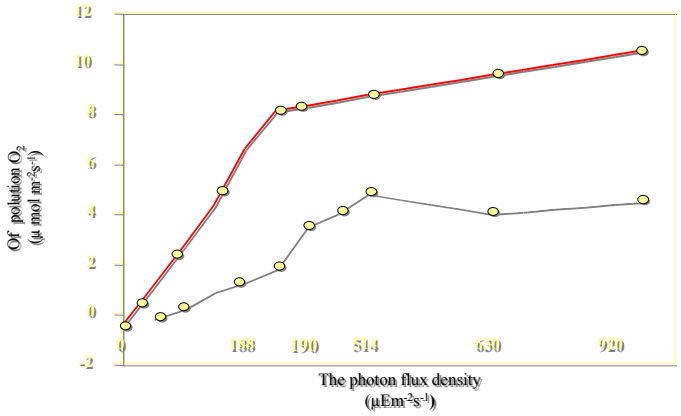
As can be seen from the tab.1., the quantum yield of the sensitive biotype's *Amaranthus retroflexus* L. photosynthesis treated with atrazine is twice as low that in the resistant biotype. The quantum yield of the resistant biotype has undergone no changes under the influence of atrazine in the amount applied.

These measurements have shown great differences to exist in the degree of resistance of *Amaranthus retroflexus* L. to atrazine. This weed has exhibited resistance due to a long–term use of the triazine herbicides.

Further studies should be more devoted to the resistance distribution determinations of *Amaranthus retroflexus* L. in the country, equally on the basis of differing atrazine and other herbicide amounts as the inhibitors of photosynthesis. By establishing 50% inhibition (T_{50}), the resistance factor, which

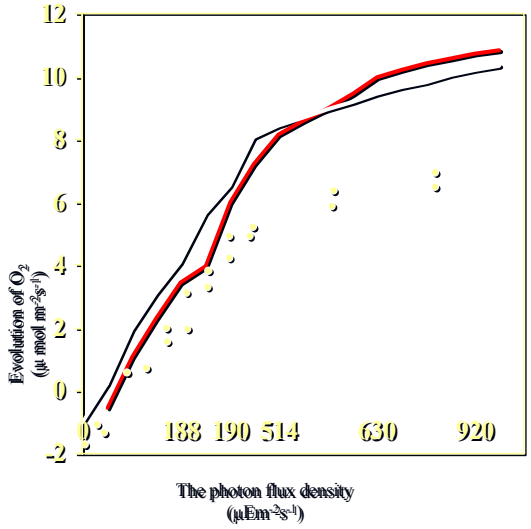
would directly signify the resistance degree to differing and frequently used herbicides in this and in other weeds, as well, should be revealed.

Fig. 3. The dependence of O_2 evolution (μE $m^{-2}s^{-1}$) on the photon flux density (μE $m^{-2}s^{-1}$)



(——) the control of susceptible biotype
(-------) susceptible biotype whose leaves were incubated in solution of atrazine (20mg/l) for 3 hour

Fig. 4. The dependence of O_2 evolution (μE $m^{-2}s^{-1}$) on the photon flux density (μE $m^{-2}s^{-1}$)



(——) the control of resistant biotype
(-------) resistant biotype whose leaves were incubated in solution of atrazine (20mg/l) for 3 hours

Tab. 1. The effect of atrazine (20 mg/l) on quantum yield (ξ) and the rate on O_2 ($\mu E\ m^{-2}s^{-1}$)

Biotype	Quantom yield (ξ)			Maximum rate of O_2 evolution		
	Time of incubation (hours)					
	0	1	3	0	1	3
Susceptible	0,06	0,02	0,02	11,22	5,49	1,10
Resistant	0,05	0,05	0,04	9,45	10,55	11,21

Conclusion

The study of resistance of various biotypes *Amaranthus retroflexus* L. to atrazine, using the leaf fluorescence method and measuring quantum yield suggested certain conclusions:

- atrazine was found to significantly inhibit photosynthesis of the sensitive biotype *Amaranthus retroflexus* L., whereas the resistant one behaved as if it had been the plant unexposed to atrazine effect. The increased yield of the chlorophyll fluorescence *a* and the decreased O_2 release confirmed that atrazine had blocked photosynthetic electron transport. Quantum yield affected by atrazine was largely decreased in the sensitive and retained at the control level in the resistant biotype.
- the resistance of *Amaranthus retroflexus* L. to atrazine has been confirmed for the first time to be the consequence of the long–term herbicide use on the same area. Taken geographically, the distribution of the resistant biotype *Amaranthus retroflexus* L. as well as that of the other important weeds' resistant biotypes toward herbicides in the country had to be established.
- using the method of leaf fluorescence, even unobservable effects of some low herbicide concentrations could be detected on the plants quite early. In order to efficiently use fluorescence method for resistance to herbicides, as the inhibitors of photosynthesis, fluorescence should be calibrated in relation to photosynthesis, which would enable fluorescence method to be used as a diagnostic probe in the field conditions without measuring the remaining photosynthesis parameters.

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IZUČAVANJE FENOMENA REZISTENTNOSTI A*maranthus retroflexus* L. PREMA ATRAZINU PRIMENOM METODE FLUORESCENCIJE LISTOVA

- originalan naučni rad -

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Rezime

U radu je proučavana rezistentnost različitih biotipova *Amaranthus retroflexus* L. prema atrazinu primenom metode fluorescencije listova i merenjem kvantnog prinosa. Praćen je efekat različitih koncentracija atrazina i vremena inkubacije listova na sekundarnu kinetiku fluorescencije hlorofila **a** i kvantni prinos biljaka. Pokazano je da atrazin značajno inhibira fotosintezu, smanjuje kvantni prinos i blokira transport elektrona i, na taj način, kod osetljivog biotipa, utiče na povećan prinos fluorescencije hlorofila **a** i smanjeno oslobađanje O_2. Rezistentni biotip *Amaranthus retroflexus* L. se ponaša kao biljka koja nije prethodno tretirana.