The Possible Mode of Herbicidal Action of Atrazine Basing on the Gas Exchange and the Mode of Plant Damage after Treatment

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Z. Naturforsch. **34 c**, 923–925 (1979); received May 31, 1979

Herbicidal Action of Atrazine, Gas Exchange, Mode of Damage, Photorespiration, Photooxidation

Under field conditions atrazine inhibits CO_2 fixation but the electron flow seems to be hindered partly only. The remaining capacity of the electron transport chain produces not used reducing electrons. The consequence should be a destructiv photooxidation. This process however is suppressed by photorespiration. When all possible substrats including stored carbohydrates are metabolized by photorespiration photooxidation is not hindered any longer and destroys the plant.

Introduction

The triazines and among them atrazine are world wide used important herbicides. Nevertheless neither the mode of action nor the reasons for the herbicide tolerance of some plants are completely explained. By atrazine spraying over years resistent weeds were selectionated and in susceptible plants a protein on the reductive side of PS II was found to have the binding affinity for atrazine and all the other herbicides which have an inhibitory effect to photosynthesis [1, 2]. *In vitro* the electron transport chain may be blocked completely by the binding of atrazine to this shield protein but the atrazine caused plant destruction and the influence on the germination process cannot be explained by this inhibition only.

Atrazine shows two different kinds of herbicidal action: One in photosynthetic active tissue and the other in photosynthetic inactive tissues. All seedlings of tolerant and susceptible plants react with a stimulated growth of roots after treatment with low atrazine concentrations [3, 4]. There are more lateral roots developed and the primary root is elongated. These reactions are similar to an auxine effect as well as to a cytokinine influence. These assumptions are confirmed by experiments with tissue culture [5]. During the first days seedlings of *maize* which is as an adult plant atrazine tolerant show a stronger growth inhibition than seedlings of susceptible *Phaseolus* or *Triticum* [3, 4].

After atrazine treatment the first observed effect in green plants is the depression of photosynthesis [6, 7]. The velocity of the reaction depends on the mode of application: Water plants with atrazine in the culture medium react very fast [8] just as land plants do after leaf application. But if atrazine is added to the nutrient solution of rooting cuttings it takes days to develop a complete photosynthetic depression [9].

All plants out of the ecophysiological group of C_4 plants seem to be very atrazine tolerant [10]. Therefore atrazine is used as a selective herbicide in the C_4 crops *maize* and *sorghum*. Naturally C_4 weeds are not controlled by atrazine. Therefore after some years of application to the same field an uniform dense under growth of C_4 grasses arises. In contrast the atrazine tolerance due to the C_4 syndrome is generally explained by different detoxification mechanisms in the C_4 crops only [11].

In the first stages of atrazine influence a significant shift from the content of carbohydrates to that of amino acids and proteins is observed. In atrazine susceptible C_3 plants the carbohydrates vanish in the leaves and the proteins are transported to the stems [12]. These processes are accompanied by an augmented nitrate assimilation [4, 13, 14].

Results

Usually after atrazine spraying net leaf photosynthesis vanishes very fast during one to six hours and a respiration arises which is enhanced by light, O_2 and temperature [6, 8]. In *Vallisneria* CO_2

Abbreviations: PS I, II, photosystem I or II; C_3, plants fixing CO_2 by RubPC; C_4, plants fixing CO_2 by PEPC; RubPC, ribulose bisphosphate carboxylase; PEP, phosphoenolpyruvate carboxylase; NADP-H, oxidised or reduced form of nicotinamide adenine dinucleotide phosphate; TNBT, tetra nitro blue tetrazolium chloride (pH 6.8; 0.01 mol).

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0341-0405 / 79 / 1100-0923 $ 01.00/0.

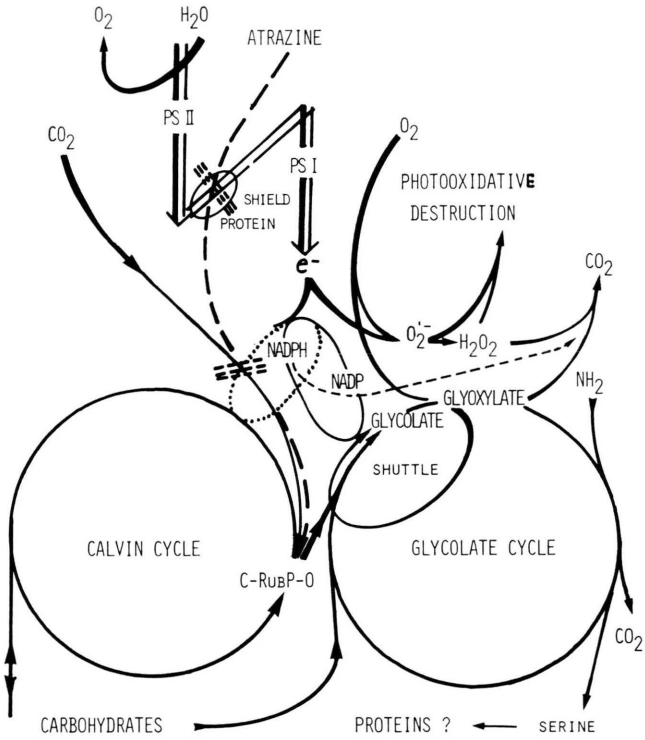
intensifies the atrazine caused O_2 consumption at 15° and 25° but reduces it at 35 °C and high light intensities [8]. Up to 12 hours this respiration in light is approximately 1.4 times higher than the dark respiration which seems to be augmented also by atrazine some times. In *Phaseolus* parallel to a slow change from light to dark the light stimulated respiration vanishes and at very low light intensities a weak CO_2 consumption occures which seems to be generated by the small amounts of PEPC contained by each cell. With the proceeding diminution of light normal dark respiration starts slowly [6]. In *Vallisneria* after a rapid change from dark to light with 5×10^{-7} mol atrazine an O_2 outburst is registered in the first 20 minutes of illumination while the control plants show a slow declining O_2 consumption only. In low atrazine concentrations the binding to the shield protein seems to have a stimulating effect to the water splitting system at the other end of PS II. This can be measured because in light no dark respiration occurs and the photorespiration has not jet been induced. In 5×10^{-6} mol atrazine there is also a small O_2 output but after 15 minutes O_2 and CO_2 are consumpted by this water plant simultaneously up to 6 hours [8]. After having transfered the plants into atrazine free water an irreversible influence of the herbicide should be detected. *Vallisneria* shows good recreation only at low temperature and at low light intensities after having remained not more than 1 hour in 5×10^{-6} mol atrazine [8]. In contrast the water moss *Fontinalis* recovers net leaf photosynthesis up to 60% after having been treated in 10^{-6} mol for one week [6]. It is hard to understand that all these reactions should be due to a simple inhibition of the electron transport. Therefore it is not surprising that atrazine sprayed *Phaseolus* shows only a very weak depression of PS II activity in the photosynthetic active tissues controlled after 12 hours by TNBT vital staining. The estimated cell internal atrazine concentration might be not higher than 10^{-7} to 10^{-6} mol which is not enough to inhibit the electron transport completely [15], nevertheless no CO_2 uptake is measured anymore.

Similar to these observations the mode of plant damage by atrazine does not comply well with the theory that the mode of action of this herbicide is determined only by a total inhibition of the electron flow and the detoxification capacity of the plants. The plant destruction is similar to the destruction of plants cultivated with strong light in CO_2 free air but not similar to the damage of plants by starvation when cultivated in complete dark over long time. This was proved in long time dark experiments when no typical atrazine caused destruction was observed, treated plants died a little bit earlier and in a different way from untreated ones. The plant destruction in light seems to be caused by a photooxydative process, which destroys the photosynthetic pigments first (bleaching) and afterwards the whole cell [7, 16]. This reaction is similar to high light injury.

Discussion

Under field conditions atrazine possibly inhibits to a certain extent only the electron transport by binding to the shield protein at PS II but inhibits CO_2 fixation totally. There is a surplus production of reducing electrons at the end of the electron transport chain at PS I. The result of this situation is an oxygen radical formation with all connected reactions ([17, 18], see figure). One of the consequences should be an abnormal, destructiv photooxidation of the leaf tissue. This seems to be hindered by photorespiration *p. e.* in activating the superoxide dismutase and in helping the catalase to split the arising amounts of H_2O_2. Photorespiration may be also a result of the glycolate glyoxylate shuttle which is strongly enhanced by not used NADPH or it may be due to the oxygenase reaction of the RubPC which is stimulated by H_2O_2 or O_2 radicals as may be under normal conditions [7, 19, 20]. Due to the in part inhibited electron flow after atrazine application photorespiration has to be less intensiv than under normal conditions. The protective function of photorespiration seems to be so important that stored carbohydrates also could be used in later stages of atrazine influence [21]. A result of photorespiratoric activity is a consumption of carbohydrates which are metabolized to some extent to amino acids and proteins. When all available carbohydrates are photorespired the photooxidativ destruction is not hindered any more and the plant damage is light dependent and is enhanced by high light intensities and high temperatures. In contrast at low O_2 tensions (5%) and increased CO_2 tensions (1000 ppm) atrazine caused plant destruction is delayed generally.

The mode of herbicidal action is divided into 2 steps: During stage one the whole protective equip-



Hypothetical scheme: Atrazine inhibits CO_2 fixation but blocks the e^- transport to some extent only: a) NADPH is not used in the CO_2 fixation process anymore but it can be consumpted by photorespiratoric processes as in the glycolate glyoxylate shuttle. b) e^- are transfered to O_2: Superoxide dismutase forms H_2O_2 out of the O_2 radicals. H_2O_2 oxidizes glyoxylate to CO_2 possibly by consumpting NADPH. c) The oxigenase reaction of the RubPC is stimulated by O_2 radicals, by H_2O_2 or by atrazine. At low or no CO_2 fixation, normal high O_2 tension and high light intensity and high temperatur the leaf tissue seems to be protected against photooxidatic damage by the different processes of photorespiration also.

ment of the plastids as carotins, fatty acids and photorespiration is sufficient to hinder serious photooxidative destruction caused by the blocked CO_2 fixation. Most of the stored carbohydrates are metabolized to CO_2 or to amino acids and proteins to some extent. By spraying glucose and succrose on the leaves the duration of this period is delayed. This stage lasts for one day up to 1 week. If atrazine can be removed during this period as it is possible in experiments with water plants sometimes total recreation is observed.

During stage two the plastid pigments are photooxidized first, then the typical leaf defect appears and subsequently the plant is irreversible damaged. No photorespiration occurs any longer and only dark respiratoric processes remain. This period two lasts for 5 days up to 5 weeks until the plant dies. The length of this stage just as the length of stage one depends on the physiological conditions of the plant and on ecological parameters as light intensity and temperature.

The atrazine plant destruction is due to an inhibition of the CO_2 fixation therefore C_4 plants might by more atrazine tolerant in general. There is much evidence that CO_2 fixation by PEPC in the mesophyll cells is more atrazine tolerant. The sensibility of the CO_2 fixation by RubPC in the bundle sheat cells is compensated by the much higher CO_2 concentration in these plastids. This mode of atrazine tolerance works only when supported by a good detoxification mechanism which hinders a cell internal accumulation of the herbicide.

By concluding that atrazine induced plant damage is a result of photooxidation and by postulating that photorespiration is a protectiv process against this destruction the light dependency of atrazine caused plant damage and most of the reactions due to atrazine influence in photosynthetic active tissues can be explained.

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