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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Under field conditions atrazine inhibits CO₂ 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 destructive photooxidation. This process however is suppressed by photospiration. When all possible substrates including stored carbohydrates are metabolized by photospiration 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 resistant 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 cytokinin 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 C₄ plants seem to be very atrazine tolerant [10]. Therefore atrazine is used as a selective herbicide in the C₄ crops maize and sorghum. Naturally C₄ weeds are not controlled by atrazine. Therefore after some years of application to the same field an uniform dense under growth of C₄ grasses arises. In contrast the atrazine tolerance due to the C₄ syndrome is generally explained by different detoxification mechanisms in the C₄ 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 C₃ 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, O₂ and temperature [6, 8]. In Vallisneria CO₂

Abbreviations: PS I, II, photosystem I or II; C₃, plants fixing CO₂ by RubPC; C₄, plants fixing CO₂ by PEPC; RubPC, ribulose bisphosphate carboxylase; PEPC, 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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intensifies the atrazine caused $O_2$ consumption at $15^\circ$ and $25^\circ$ but reduces it at $35^\circ$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 occurs 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 $5 \times 10^{-7}$ 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 $5 \times 10^{-6}$ mol atrazine there is also a small $O_2$ output but after 15 minutes $O_2$ and $CO_2$ are consumed by this water plant simultaneously up to 6 hours [8]. After having transferred 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 $5 \times 10^{-6}$ 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, 15]. 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, destrucit photo-oxidation 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 photorespitoric 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 CO₂ fixation but blocks the e⁻ transport to some extent only: a) NADPH is not used in the CO₂ fixation process anymore but it can be consumed by photorespiratory processes as in the glycolate glyoxylate shuttle, b) e⁻ are transferred to O₂: Superoxide dismutase forms H₂O₂ out of the O₂ radicals. H₂O₂ oxidizes glyoxylate to CO₂ possibly by consuming NADPH. c) The oxygenase reaction of the RubPC is stimulated by O₂ radicals, by H₂O₂ or by atrazine. At low or no CO₂ fixation, normal high O₂ tension and high light intensity and high temperature the leaf tissue seems to be protected against photooxidative damage by the different processes of photorespiration also.

of the plastids as carotins, fatty acids and photorespiration is sufficient to hinder serious

photooxidative destruction caused by the blocked CO₂ fixation. Most of the stored carbohydrates are metabolized to CO₂ or to amino acids and proteins to some extent. By spraying glucose and sucrose 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 CO₂ fixation therefore C₄ plants might by more atrazine tolerant in general. There is much evidence that CO₂ fixation by PEPC in the mesophyll cells is more atrazine tolerant. The sensibility of the CO₂ fixation by RubPC in the bundle sheat cells is compensated by the much higher CO₂ 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.