

20 01

Effect of herbicide application on N,P,K uptake of maize test plants in pot experiments

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An integrated crop management system requires concerted fertilization-crop protection practice. Few data are available on the interactions between effectivity of pesticides and mineral nutrition of plants. We have studied the manifestation of toxicity in growth and mineral nutrition characteristics (N,P,K uptake, concentrations, ratios) of maize test plants after application of a preemergent herbicide containing 80 % lenacil as active ingredient, in a greenhouse pot experiment, on 3 soils. Application rates were in the range from below to highly exceeding the recommended field rate. P and - to a less extent - N uptake increased, K uptake slightly decreased at increasing herbicide rates. Toxicity caused by too high application rates was reflected also by increased P concentrations, and consequently by decreases in N/P and K/P ratios. Although experimental circumstances were different from field conditions, differences observed on the 3 soils were in agreement with the recommended application practice of the herbicide.

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20 02

Different growth and photosynthetic efficiency of triazine-resistant and susceptible biotypes of *Poa annua* L.

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A reduction of fresh and dry matter of leaves, as well as of fresh and dry matter and length of roots was observed in the resistant biotype of *Poa annua* in the intervals from 4 to 8 weeks after sowing. We did not observe any difference between the number of leaves. The initial fluorescence F_i and F_i/F_p ratio were higher in the resistant biotype. No differences between variants in net photosynthetic rate on leaf area basis were found.

20 03

Selective monitoring of PSII-herbicides at concentrations below 0.5 $\mu\text{g l}^{-1}$ using PAM fluorometry of unicellular green algae

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The European drinking water regulation does not allow that the sum of all pesticides exceeds 0.5 $\mu\text{g l}^{-1}$. To decrease the number of samples for the time-consuming and costly chemical analysis, a fast and cheap monitoring is highly desirable. Since 70 % of all pesticides found in surface waters are PSII-herbicides, a selective screening for these compounds is a valuable instrument for water quality control. For this purpose we developed a bioassay based on pulse-amplitude-modulated fluorescence of intact *Chlorella cells*. This system was able to select the herbicide-sensitive part of total *in vivo* Chl *a* fluorescence to quantify PSII-herbicides. However, the sensitivity did not reach the requirements of the drinking water regulation. Therefore, we coupled our bioassay with a solid phase extraction procedure, resulting in a 3000 times higher sensitivity without any loss of specificity and selectivity of the fluorescence signal. This system can now monitor PSII-herbicides down to concentrations below 0.1 $\mu\text{g l}^{-1}$.

20 04

Nuclear coded protein can be involved in the mechanism of paraquat resistance

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Sensitive and different paraquat resistant horseweed [*Coryza canadensis* (L.) Cronq.] plants were sprayed with inhibitors of different type alone and combined with paraquat. Inhibitor of de novo fatty acid synthesis cerulenin, protein synthesis inhibitors chloramphenicol and cycloheximide, the Cu-chelator diethyl-dithiocarbamate, modifier of lipid composition SAN 9785 were used. Inhibition of the photosynthetic electron transport (PET) chain was characterized by variable fluorescence (F_v). All inhibitors caused short, reversible perturbations in the functioning of PET in the first period after spraying of the resistant plants. Only paraquat treated plants showed the well known transitory inhibition. In all combined treatment the inhibition showed this transitory character with exception of cycloheximide/paraquat spraying. In this case the inhibition became irreversible. It is concluded that probably a nuclear coded protein can play a role in the mechanism of paraquat resistance.

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20 05

***N*-pyridyl-aminomethylene-bisphosphonic acids are potent inhibitors of plastidal 3-deoxy-D-*arabino*-heptulosonate-7-phosphate synthase**

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Aminoalkylphosphonic acids are broadly defined as analogues of amino acids in which carboxylic function is replaced by phosphonic or related function. The disclosure of herbicidal activity of glyphosate (*N*-phosphonomethyl glycine) in 1971 instituted a milestone in biochemistry of these compounds, and initiated an extensive research concerned with the design, synthesis and evaluation of biological properties of new aminophosphonates.

Here we report that *N*-pyridyl-aminomethylene-bisphosphonic acids inhibit the activity of the Mn-dependent isoform of the first enzyme of prechorismate pathway, 3-deoxy-D-*arabino*-heptulosonate-7-phosphate (DAHP) synthase [EC 4.1.2.15], partially purified from *Nicotiana plumbaginifolia* cultured cells. Kinetic studies indicated an inhibition of competitive type with respect to the substrate phosphoenolpyruvic acid.

20 06

Effect of imazethapyr on growth and *in vitro* acetolactate synthase activity in *Pisum sativum* L.

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The use of herbicides on crop protection to reduce losses due to weeds has become an important part of modern agriculture. Imazethapyr is a selective herbicide used in several legume crops. This herbicide affects plant growth by inactivating the enzyme acetolactate synthase (ALS, EC 4.1.3.18) that catalyzes the first common step in the biosynthesis of the essential amino acids isoleucine, leucine and valine. The effect of imazethapyr in pea plants was studied under two regimes of nitrogen nutrition: plants growing with nitrate (20 mM) or inoculated with *Rhizobium leguminosarum* bv *viciae*. In both cases, imazethapyr was applied preemergence and in a closed system, without drainage. It could be observed that doses higher than 0.5 ppm produced a general plant growth inhibition (smaller weight and length in shoots and roots), and this effect was more important on pea plants growing with nitrate than on symbiotic plants. The different response to the herbicide treatment could be explained by changes in the target susceptibility. However, ALS activity studies showed similar levels of extractable ALS activity in both sets of plants and a similar response of these enzymes to the herbicide applied *in vitro*.

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20 07

Glyphosate effects on *Bradyrhizobium japonicum* on free living form and on symbiosis with soybean.

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Glyphosate, a broad spectrum herbicide, inhibits aromatic amino acid biosynthesis pathway as a primary mode of action. Secondly, many others targets are affected. Biological nitrogen fixation decreases when legume plants are exposed to sublethal doses of glyphosate and residual doses on soil could affect marginally to nitrogen metabolism on free living microorganisms on soil, plants, and their symbiosis. *Rhizobium* strains have a broad sensitivity to that herbicide. In fact some differential effects found on inhibition, resistance or reversion by final products are strain dependent and these character could be used as a agronomic criterion on glyphosate treated fields. Three *Bradyrhizobium japonicum* strains with distinct herbicide sensitivity to glyphosate were used to infect soybean plants. These strains had similar growth conditions, nitrogen fixation rates and nodulation behavior. Nodule establishment on treated plants with sublethal doses of herbicide was not affected, although nitrogen fixation on those nodules was drastically inhibited. However, that inhibition did not correlate with the sensitivity of the free living strains to glyphosate.

20 08

Effects of herbicides on some physiological and biochemical changes in maize inbred lines

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Maize inbred lines express different degree of susceptibility to herbicides. Therefore the purpose of this study was to observe response of maize inbred plant to effects of two herbicides. Five inbreds were used for this study. Plants were grown both in a field and a growth chamber. The EPTC-dichlormid and metolachlor were applied to soil before and after planting, respectively. Both herbicides were mixed with atrazine. Plants were grown up to 3-leaf stage and up to full maturity in the growth chamber and the field, respectively. The following traits were measured in the field plot: biomass of leaf in 7-8-leaf stage, leaf area after tasseling, plant height and water content in a mature kernel, while the following traits were measured in the growth chamber experiment: total mass of above ground parts and root, leaf area, content of both chlorophyll and total proteins. According to obtained results, it could be concluded that maize inbred lines differ in their response to both effects and kind of herbicides.

20 09

Paraquat-induced intracellular acidification as revealed by fluorescent probes in intact leaves

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The effect of the contact foliar herbicide paraquat on the intracellular pH was studied in intact leaves of the aquatic macrophyte *Elodea canadensis*, by means of the pH-sensitive fluorescent probes 9-aminoacridine and BCECF. Both dyes revealed intracellular acidification induced by 10^{-5} M paraquat. The effect was detectable after 10 min and remained present until the cells died. It is suggested that intracellular acidification is an until now overlooked component of the phytotoxic action of paraquat.

20 10

Interaction of herbicides and new inhibitors with *de novo* fatty acid biosynthesis in chloroplasts

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Plastids (etioplasts, chloroplasts) of higher plants possess all enzymes for *de novo* biosynthesis of fatty acids from acetate or pyruvate and thus represent a very suitable test-system for *de novo* fatty acid biosynthesis and its specific inhibitors. The two acetyl-CoA providing enzymes in the plastids - the acetyl-CoA synthetase (ACS) and the pyruvate dehydrogenase complex (pPDHC) - are specifically inhibited by new compounds. Ethyl-AMP blocks ACS and acetylmethylphosphinate (AMPI) the pPDHC. The activity of the pPDHC decreases during greening, whereas the activity of the ACS rises parallel to the transformation of etioplasts into chloroplasts which is confirmed by the inhibitor studies. The relative contribution of the enzymes ACS and pPDHC to *de novo* fatty acid biosynthesis is strongly dependent on the developmental stage of the plastids. The next step in *de novo* fatty acid biosynthesis is the acetyl-CoA carboxylase (ACC) which can specifically be inhibited by the graminicides diclofop, sethoxydim and cycloxydim. Other inhibitors, such as thiolactomycin and cerulenin, block the β -ketoacyl-ACP synthases (KAS I, II or III) of the fatty acid synthetase (FAS). Various open questions on the regulation of plants' *de novo* fatty acid biosynthesis will be solved applying the herbicides and inhibitors and help to isolate the corresponding genes of sensitive and resistant plants.

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20 11

Photosynthetic O₂ evolution and chlorophyll fluorescence in *Vicia faba* plants treated with methabenzthiazuron

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Photosynthetic O₂ evolution and chlorophyll fluorescence in *Vicia faba* leaves were studied during the first weeks of growth in order to evaluate the damage caused by methabenzthiazuron (MBT), applied in preemergence at two recommended doses (2.5 kg.ha⁻¹ and 4 kg.ha⁻¹), under field conditions. During the first weeks after plant emergence both doses of herbicide were followed by a drop in O₂ evolution and in some fluorescence parameters, such as the optimal quantum yield (F_v/F_m), the effective quantum yield ($\Delta F/F_m'$), and photochemical and non-photochemical quenching, q_p and q_n . Increases in minimal fluorescence (F_0 and F_0') and variable and maximal fluorescence (F and F_m'), were also observed on application of saturating pulses. Fluorescence parameters gave the best indication of the damage caused to the photosynthetic system by MBT doses. Plant tissues detoxified the herbicide and, one month after plant emergence, photosynthesis was fully recovered in herbicide-treated plants; biomass production recovered and even increased at the end of the growth period.

20 12

Physiological mechanism of some herbicides interaction in binary complex

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It has been shown, that herbicides, germination inhibitors (GI), with the exception for thiocarbarnate, decreased the phytotoxicity of herbicides, photosynthesis inhibitors (PI), in binary complexes. This action is only partly connected with GI effect on PI accumulation in plants. The main reasons of plants resistance increase, perhaps, are changes in lipids peroxidation reactions and phytohormonal balance, induced by GI action.

Effect of epi-brassinolide on CO₂ assimilation and chlorophyll fluorescence in *Vicia faba* plants treated with photosynthesis-inhibiting herbicides

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Chlorophyll fluorescence and CO₂ assimilation in *Vicia faba* leaves were studied during the first weeks of growth in order to evaluate the effects of epi-brassinolide (BR) on the damage caused by methabenzthiazuron (MBT) (1.4-5.6 kg.ha⁻¹), simazine (1.5 kg.ha⁻¹) and cyanazine (0.75-1.25 kg.ha⁻¹). Faba bean seeds were incubated in BR solution (10⁻³-10⁻² ppm) during 24 h, at room temperature, before sowing. Herbicides were applied in preemergence. Fluorescence parameters and CO₂ assimilation were measured on the youngest fully expanded leaf during 1-2 month after plant emergence. During the first weeks all herbicides caused a drop in CO₂ assimilation and in some fluorescence parameters, such as the optimal quantum yield (F_v/F_m), the effective quantum yield (ΔF/F_m'), and photochemical and non-photochemical quenching, q_p and q_N. At the concentration tested, BR did not modify MBT effect. Nevertheless, the effect of both simazine and cyanazine were greatly reduced by BR. BR effects were mainly apparent when actinic light was increased. Thus, our data clearly showed that a BR pretreatment caused a faster recovery of triazine-treated plants.

Effects of sulfonylurea herbicides in some maize inbred lines

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In this paper we investigated the influence of sulfonylurea herbicide on some physiological processes in three maize inbred lines. Leaf water potential (pressure chamber), transpiration, conductance of stomata and WUE (IRGA), and leaf abscissic acid (ABA) were measured 15, 30 and 45 days after the treatment. Obtained results indicated the herbicide induced differences between inbreds as well as the investigated parameters.

20 15

Development of herbicide resistance in weeds

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Attention is given to the remarkable development of resistance of weeds to various herbicides during the last two decades. The number of weed species with resistant biotypes increased above 100. In this number those resistant to photosynthesis-inhibiting triazines comprise more than 50%. Various weed species developed resistance against paraquat and diquat which interact in a different way with the same process. The phenomenon also arised for dinitroanilines inhibiting cell division, for lipid synthesis inhibitors, and for some other herbicides. Resistance to inhibitors of acetolactate synthase, an essential enzyme in the synthesis of some branched amino acids, appeared rather fast in a number of weed species. Frequently, the susceptibility of the herbicide target in the resistant biotype is reduced, but increased detoxification of the herbicide in resistant plants is another mechanism. The resistance problem is complicated by cross-resistance to other herbicides.

20 16

Triazine resistance: why is this resistance correlated with reduced productivity?

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Triazine-resistant plants have a mutation in the D1 protein of the reaction center of photosystem II at site 264: serine is replaced by glycine. There are several reports in literature that triazine-resistant (R) plants have a lower photosynthetic capacity than susceptible (S) plants. We have observed that R and S plants have equal photosynthetic performance when grown at low light irradiance. However when grown at high irradiance, R plants have a lower photosynthesis rate and lower dry matter production compared with S plants. These effects are not due to a lower rate of electron flow in photosystem II in R plants, because the whole chain electron flow is the same in R and S.

The lower photosynthetic performance in R plants grown under high light appears to be caused by higher damage by photoinhibition, which is probably related with different mechanisms of photoprotection like non-photochemical quenching and xanthophyll cycle.

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Genotype-dependent mechanism of interactions between maize and S-triazines

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Maize is known as a stable plant to S-triazines due to the work of a glutathione-S-transferase and to possibility of peptide-conjugation of a herbicide and its metabolites. The last, or additional stage of inactivation of xenobiotics is a matter of discussion as if short peptides not only glutathione like are expressed, as concerning the only role of short peptides to conjugate stranger molecules. The significant quantities of peptides may be registered by amino acid composition of plant tissue if proteins or free amino acids accumulation don't take place. Thus, experiments with herbicide exposure were made, following by electrophoretic investigations. It was shown that uptake of large doses of S-triazines led to significant changes of amino acid composition of maize leaves. It looks as the main adaptive reaction in this experiments is expression of peptides, which composition depended on genotype of a plant. For example, experimental plants of ordinary lines of maize had more glutamate than control, that was not shown on opaque-2 maize plant. Pool of free amino acids wasn't changed during this experiments. We may propose content of adaptive peptides according to difference in amino acid composition of control and experimental plants, but the last answer may be given only by HPLC procedures and further investigations.