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EFFECTS OF PLANT GROWTH REGULATORS AND HERBICIDES ON PHOTOSYNTHESIS

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Summary. In this review on photosynthesis a distinction is made between the direct effects of plant growth regulators and herbicides on the process and the resulting influence on photosynthesis of intact plants.

Predominant in the direct effects is the inhibition of photosynthetic reactions in the chloroplasts at essential sites by various herbicides. There is little information on plant growth regulators in this respect. Some herbicides affect the photosynthetic apparatus by inhibiting the synthesis of carotenoids, whereas stimulation of chlorophyll development has been reported for cytokinins.

Any influence on photosynthesis of intact plants requires absorption by roots or leaves, and accumulation in the mesophyll. Inhibition of carbon dioxide assimilation leads to starvation of the plant, but also to oxidation and disintegration of chloroplasts. A few reports mention stimulation of photosynthesis, but not by herbicides.

There are different types in the selective action on photosynthesis of intact plants. The availability to various plants may be different. Differences in accumulation in the leaves can be attributed to different absorption and translocation, but especially to inactivation in some plant species. Different sensitivity at the chloroplast level has recently been discovered in resistant biotypes of a number of weeds species.

Finally, mention is made of the key position of the stomata. Any change in stomatal aperture will also influence carbon dioxide assimilation and vice versa.

INTRODUCTION

Photosynthesis produces the materials for the growth and development of plants. The rate of the process depends on the plant species, and is subject to the influence of environmental factors such as light, temperature,

CO_2 concentration, availability of water and minerals, etc. The great increase in knowledge on photosynthesis is partly due to the use of specific inhibitors in the study of subreactions of the process. Most of these are herbicides, and there is also considerable information on their influence upon photosynthesis of intact plants. In this review no attempt will be made to provide a complete coverage of the topic because of limited space.

The direct effects on the process will be discussed in the first part. This involves the action of herbicides which interfere with the electron flow in chloroplast reactions. Effects on the photosynthetic apparatus also occur, not only produced by some herbicides, but also by certain growth regulators.

These direct effects have influence on the assimilation of carbon dioxide in the leaves and this will be described in the second part. The remarkable selectivity in the action of herbicides on photosynthesis of various plants will be discussed also. Finally, the interaction between photosynthesis and transpiration in intact plants will be reported.

For common names and abbreviations of herbicides and growth regulators the reader is referred to a list in Weed Research (1979, Vol. 19, pp. 401-405).

DIRECT EFFECTS ON THE PROCESS OF PHOTOSYNTHESIS

More than half of the present herbicides specifically inhibit photosynthetic reactions in the chloroplasts. This inhibition of photosynthesis may lead to disintegration of chloroplasts, but some herbicides also exert a primary effect on the photosynthetic apparatus, sometimes in addition to the effect on the chloroplast reactions. Data on growth regulators are rather limited in comparison to the information which is available on herbicides, but they are included in the discussion on the following aspects:

1. Effects on photosynthetic reactions in the chloroplasts,
2. Effects on the photosynthetic apparatus.

EFFECTS ON PHOTOSYNTHETIC REACTIONS IN THE CHLOROPLASTS

The lamellar-shaped grana in the chloroplasts contain the chlorophylls and other pigments and are embedded in a stroma matrix. The two light reactions in the grana operate in series and drive the electron transport which takes place in the thylakoid membrane of the lamellae and yields NADPH. Simultaneously, ATP is produced in the associated photophosphorylation reactions.

Many herbicides inhibit the photosynthetic electron flow, and the

effects on two or three essential sites are most pronounced (1,2). The majority act on the reducing site of photosystem II, and the effects can be measured as increase in fluorescence. Here the electron flow from photosystem II to photosystem I is interrupted with consequent inhibition of NADP reduction and of coupled phosphorylation. Herbicides acting at this site include the substituted ureas, triazines, triazinones, biscarbamates, uracils, pyridazinones, alkylanilides and others. Trebst (3) distinguished inhibition of electron flow on the site of plastoquinone e.g. with diphenylethers and dinitroanilines. Dipyridilium salts like paraquat act on another site. They deviate the electron flow from NADP reduction because they intercept electrons from photosystem I. This gives rise to a free radical which is reoxidized under the formation of the phytotoxic hydrogen peroxide (4).

Moreland (2) distinguished further uncouplers which dissociate electron transport from ATP formation, energy transfer inhibitors which act directly on phosphorylation, and inhibitory uncouplers which inhibit electron transport and also uncouple phosphorylation. A number of herbicides including dinitrophenols and benzonitriles are considered to act as inhibitory uncouplers.

Research on photosystem II inhibition by herbicides is now concentrated around the nature and characterization of the binding site for various herbicides at the outside of the chloroplast thylakoid (3,5,6,7), lately also with chloroplasts from resistant biotypes (8,9).

There is a great contrast between the abundant information about the influence of herbicides on the electron flow in chloroplast reactions, and the limited and sometimes conflicting reports on plant growth regulators in this respect. Higgins and Jacobsen (10) collected some information on stimulated photophosphorylation and coupled electron transport by auxin, but this has not always been confirmed (11). It is possible that abscisic acid or its metabolite phaseic acid inhibits electron flow in addition to other effects on photosynthetic reactions (10). There are conflicting data about the activity of photochemical reactions in relation to gibberellic acid (12, 13) and cytokinins (13, 14).

The photosynthetic energy accumulated in NADPH and ATP in the grana is used for the fixation of carbon dioxide in the stroma, where the necessary enzymes are located also. There are different pathways for CO_2 fixation. In the Calvin-Benson pathway operating in C_3 plants, carbon dioxide is bound to ribulose diphosphate (RuDP) from which two molecules of phosphoglyceric acid are formed. In the Hatch and Slack pathway in the mesophyll cells CO_2 is bound to phosphoenolpyruvate (PEP) yielding C_4

dicarboxylic acids which are presumably transported to the bundle sheath cells. There CO_2 is released and bound to RuDP from which also phosphoglyceric acid is formed. This system operates in C_4 plants.

Inhibitors and in particular herbicides have been used relatively little in studying the processes involved in the fixation of carbon dioxide. Here, the knowledge is primarily obtained with radioactive CO_2 (15). Only a slight inhibition of the activity of RuDP-carboxylase was observed with dinoseb, but not with other electron transport inhibitors (16). A specific inhibitor of PEP-carboxylase, 3-mercaptopicolinic acid, has also been reported (17). Treharne (18) presented evidence that gibberellins and cytokinins could activate RuDP-carboxylase, but they are probably not specific (10). In some species abscisic acid lowered the RuDP-carboxylase activity, but not in others, while increase in the activity of some other photosynthetic enzymes (typical of the C_4 pathway) was also observed (10).

EFFECTS ON THE PHOTOSYNTHETIC APPARATUS

The specific inhibition of light reactions in chloroplasts by a number of herbicides as described in the previous section will disrupt the existing photosynthetic apparatus and seriously affect the intact plant (see next section). At sublethal concentrations, however, these herbicides may induce the formation of shade-type chloroplasts being especially characterized by a smaller number of thicker grana, more xanthophyll and chlorophyll b, and less quinones and carotenes (19, 20).

Rather different is the effect of some herbicides on the synthesis of carotenoids. These pigments protect chlorophyll against photooxidation by dissipating efficiently excessive light energy that cannot be passed on to the photoreaction centres and would otherwise produce singlet oxygen (21). They can also rapidly quench the excess energy of this singlet oxygen, when produced.

The mechanism of action of the chlorosis-inducing herbicide amitrole remained obscure for a long time, but now most workers agree that it inhibits the carotenoid synthesis, as do dichlormate, pyrichlor, norflurazon, fluridone and difunone (22). Probably the desaturation reactions from phytoene to phytofluene and lycopene are inhibited so that the final carotenes are not formed.

One of these herbicides, fluridone, increased the sensitivity to gibberellic acid, and this was explained as an effect on carotenoids which are probably precursors of abscisic acid, an antagonist to gibberellic acid. Treharne (18) collected the evidence for the stimulation of chloroplast development by cytokinins. Kinetin treatment increased chlorophyll

and other pigment systems involved in the light reactions of photosynthesis (14, 23). Cytokinins also retarded chlorophyll degradation in senescing or detached leaves (10).

INFLUENCE ON PHOTOSYNTHESIS OF INTACT PLANTS

Any effect on chloroplast reactions or on the photosynthetic apparatus as such would influence photosynthesis of intact higher plants, but compared to isolated chloroplasts the situation is much more complex. Exogenous applied plant growth regulators or herbicides must be taken up (e.g. by roots) and translocated before they are sufficiently accumulated in the leaf mesophyll to affect photosynthesis. More than in isolated chloroplasts the compounds are subject to metabolism. An intermediate step between chloroplast and higher plant studies is the use of cell cultures to study the effects of various compounds (24), but the interpretation of the results and their translation to the situation in intact plants could be difficult (24, 25). The same applies to the use of unicellular algae. The following aspects will be considered:

1. CO_2 assimilation in the leaves,
2. Selective action in photosynthesis,
3. Interaction with transpiration.

CO_2 ASSIMILATION IN THE LEAVES

Most herbicides which affect photosynthesis are absorbed by the roots and need translocation to the leaves before action, but some exert also or exclusively a contact action on the leaves. There are indications that in addition to the mobility of the herbicide in the plant the transpiration rate is a determining factor for root absorption and xylem translocation to the leaves (26). Consequently, accumulation of these herbicides in the leaves increases with light intensity, temperature and availability of water to the roots, but decreases with air humidity. In how far this also applies to plant growth regulators is unknown. They are translocated both in the phloem and in the xylem system.

The inhibiting effects of herbicides on chloroplast reactions lead to suppression of carbon dioxide reduction. This raises the intercellular CO_2 concentration in the mesophyll and diminishes the influx of carbon dioxide into the leaves. The CO_2 exchange can be measured quantitatively by the infrared gas analyzer technique (see e.g. 27). Control of environmental conditions is desirable in connection with the accumulation in the mesophyll. Obviously, effects on the photosynthetic apparatus may also

induce a suppression in CO_2 assimilation because of decrease (or increase) in absorbing pigments.

There are many data on the inhibition of CO_2 assimilation in intact plants or leaves by various herbicide groups such as phenylureas, triazines, triazinones, uracils, biscarbamates, dinitrophenols, benzonitriles, diphenylethers, bipyridilium compounds and others (26). Most of these compounds are active as root application, but some also as leaf sprays. A decrease in CO_2 assimilation is usually observed within a few hours, especially with leaf sprays and treatment of the roots in nutrient solution under favourable environmental conditions for transpiration. The rise in CO_2 concentration in the mesophyll resulting from the inhibition of photosynthesis will decrease stomatal aperture so that transpiration also decreases, but usually to a smaller extent than photosynthesis. Reversely, there is also a secondary effect on photosynthesis when stomatal aperture (and transpiration) are reduced, but this will be discussed in the last section.

In contrast to the numerous data on inhibition or complete suppression of photosynthesis by herbicides at low concentration, stimulation (on a leaf area basis) has not been observed (26), not even at very low concentrations. The growth stimulation which sometimes occurs at low rates of triazine and urea compounds is ascribed to increased nitrogen uptake and protein synthesis (28).

The inhibition of carbon dioxide assimilation and of carbohydrate synthesis leads to starvation of the plant, since energy-requiring processes continue, but this is not the only reason for phytotoxicity of these herbicides (29). This is distinctly so with the bipyridilium herbicides (4). Here electron acceptance leads to the formation of reactive free radicals which are reoxidized and give rise to hydrogen peroxide so that chloroplast membranes are destroyed. This explains the very rapid effects.

However, evidently also with other types of photosynthesis inhibitors starvation alone cannot explain phytotoxicity. It has been observed that the inhibition is followed by disintegration of chloroplasts in the light (30) and that the development of symptoms is proportional to light intensity (26). Other information indicates that this should be explained by photooxidation of the pigments, because the transfer of absorbed energy to the electron flow in the photosynthetic reactions drops by the presence of inhibitors (2, 31). The phytotoxic effect of a herbicide would even be greater when besides inhibition of electron transport, synthesis of carotenoids is affected. Then the pigments would be overloaded earlier because of rapid expiration of the protective function of the carotenoids.

The knowledge on the influence of plant growth regulators on photo-

synthesis of intact plants is much more limited, while the information collected by Higgins and Jacobsen (10) indicates that the results are not always unanimous. Auxins have been reported to stimulate CO_2 assimilation of leaves and leaf cells, and this was attributed to stimulation of photophosphorylation. Diverse effects on photosynthesis of leaves have been observed for gibberellins, ranging from inhibition to stimulation, and apparently depending upon the development of the tissues or upon environmental conditions (10). There are a number of reports that cytokinins have a positive effect on CO_2 assimilation of leaves, and this could be ascribed to delayed senescence of the tissues (10). The influence of abscisic and phaseic acid is usually negative, and probably indirect due to the action on stomatal aperture. However, non-stomatal inhibition of photosynthesis has also been demonstrated, and might be brought back to effects on chloroplast activity.

Photorespiration is discussed separately at this symposium, and it will only be mentioned here briefly in relation to photosynthesis. Inhibitors of photorespiration such as glycidate, hydroxy-2-pyridine methanesulphonic acid and isonicotinyl hydrazide appeared to stimulate photosynthesis for short periods under suitable conditions (32), but the same compounds inhibited photosynthesis in other studies (33, 34).

SELECTIVE ACTION IN PHOTOSYNTHESIS

Photosynthesis is a universal process. The light reactions in the chloroplasts of various plant species proceed very similarly while the assimilation of carbon dioxide differs only between a few large groups. This raises the question how to explain the diversity in the effects of herbicides on photosynthesis of various plant species. Three types of differences between plants should be distinguished in this selective action, viz. different availability to the plant surface, differences in degree of accumulation in the chloroplasts, and different sensitivity at the chloroplast level (35).

Different availability to the plant surface with foliage treatments may arise from differences in interception and retention of the spray solution. This is the case when crop and weeds are separated in time or space, but also because of different morphological or anatomical properties of the plants. Likewise, differences in availability may occur with root exposure, because of different depths of germination and rooting horizon in combination with low penetration of the herbicide into the soil.

Differences in degree of accumulation may sometimes be attributed to different absorption by the foliage (cuticle structure and composition) or to differences in root absorption and translocation to the leaves. There

is evidence for differences in one of these or both processes, e.g. for phenylureas in different plant species. Transpiration rate may be a factor here.

An important reason for selectivity of these herbicides is inactivation in the leaves of certain plant species. Various ways leading to loss of phytotoxicity in certain plants have been elucidated e.g. for triazines, substituted ureas and pyridazinones (36). Inactivation of photosynthesis-inhibiting herbicides in the leaves can be demonstrated by measuring photosynthesis during and after a short, temporary exposure of the roots to herbicides in nutrient solution (26). Herbicide inactivation is reflected in the recovery from inhibition of photosynthesis.

Chloroplasts of many tolerant crops and susceptible weeds were equally sensitive to a number of herbicides (35). However, recently a different sensitivity at the chloroplast level has been observed in resistant biotypes of sensitive weed species. This occurred after yearly applications of triazines and is now evident in 14 plant species (37). In these resistant biotypes several times the normal concentration (up to 100) was required for inhibition of photosynthesis. The resistance is attributed to modification in the binding site for the herbicide (8) where changes occurred in the protein of the photosystem II-complex responsible for herbicide binding (9). Meanwhile diuron-resistant strains of unicellular algae have also been found (e.g. 38). The paraquat resistance of some perennial ryegrass cultivars seems also located in the chloroplast (39).

INTERACTION WITH TRANSPIRATION

The influence of plant growth regulators on transpiration is fully discussed in a separate lecture, and here the interaction with photosynthesis will only be illustrated in some examples. There is a close connection between transpiration and photosynthesis, because the stomata not only influence the rate of CO_2 fixation in the mesophyll cells, but this rate in the mesophyll tissue may also determine stomatal aperture because of its regulation by the intercellular CO_2 concentration (40, 41). This is illustrated with photosynthesis-inhibiting herbicides (electron flow inhibitors). Here, inhibition of photosynthesis is usually followed by a relatively smaller decrease in transpiration (26) indicating that stomatal closure is a secondary effect due to increased intercellular concentration of CO_2.

Cytokinin stimulated transpiration and stomatal opening in a number of plant species, but others did not respond (42). A direct effect on stomatal aperture by decreasing epidermal and mesophyll turgor or sustaining

potassium ion uptake in the guard cells has been suggested. Others believe that the effect is secondary because in detached leaves senescence and chlorophyll degradation are retarded. This keeps the photosynthetic rate high, the intercellular CO_2 concentration low and the stomata open. However, studies with intact plants indicate a direct effect on stomatal opening (42).

The opposite is observed with abscisic acid (10, 18). The stomata close at low concentration of the growth regulator. This increases the diffusion resistance for CO_2 so that photosynthesis becomes inhibited. Here the inhibition of photosynthesis is not the primary effect, because the intercellular CO_2 concentration decreasesin response to abscisic acid, whereas increase is observed with a photosynthesis inhibitor (42). Apparently, the main effect of abscisic acid is on stomata rather than on chloroplasts (see also 43).

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