The Effect of Bialaphos on Ammonium-Assimilation and Photosynthesis
II. Effect on Photosynthesis and Photorespiration

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The application of bialaphos (phosphinothricyl-alanyl-alanine) effects a quick photosynthesis inhibition under atmospheric conditions (400 ppm CO_2, 21% O_2). However, under conditions (1000 ppm CO_2, 2% O_2) under which photorespiration cannot occur there is no photosynthesis inhibition. In the previous investigation it could be shown that bialaphos splits in plants into phosphinothricin and alanine. The inhibition of glutamine synthetase through freed phosphinothricin results in an NH_4^+-accumulation and a decrease in glutamine. With the addition of glutamine, photosynthesis inhibition by bialaphos can be reduced. An NH_4^+-accumulation takes place under atmospheric conditions as well as under non-photorespiratory conditions; though in the latter case, in less amounts. After adding glutamine and other amino acids the NH_4^+-accumulation increases especially. This indicates that NH_4^+-accumulation cannot be the primary cause for photosynthesis inhibition by bialaphos. The investigations indicate that for the effectiveness of either bialaphos or phosphinothricin, a process in connexion with photorespiration plays a considerable role. The glyoxylate transamination in photorespiration could be inhibited, which results probably on a glyoxylate accumulation. Corresponding investigations showed inhibition of photosynthesis as well as a direct inhibition of RubP-carboxylase with glyoxylate.

Introduction

Two additional alanine residues distinguish bialaphos from PPT (glufosinate). Our previous investigations into the effect of bialaphos and PPT on NH_4^+-assimilation enzymes show that, through cleavage of the alanine residues in bialaphos upon application to plants, PPT is also the only active herbicidal component in bialaphos [1].

PPT inhibits photosynthesis under photorespiratory conditions (400 ppm CO_2, 21% O_2). Under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2) no inhibition of photosynthesis occurs, although even under these conditions a notable quantity of NH_4^+ is accumulated through GS inhibition [2]. In addition to this NH_4^+-accumulation, a process connected with photorespiration appears to play an important role in the herbicidal effectiveness of PPT.

The purpose of this investigation was to examine the effect of bialaphos on photosynthesis and photorespiration. In addition, differences in effectiveness of bialaphos and PPT were investigated.

Materials and Methods

Plant material

Sinapis alba plants were grown as described [3].

Chemicals

The Na^+-salt of bialaphos (phosphinothricyl-alanyl-alanine) was supplied by Hoechst AG (Frankfurt/Main, West Germany) [1].

Measurement of photosynthesis

Photosynthesis was measured as the CO_2 fixation rate by means of an infrared gas analyser as described [4]. The experiments were performed on entire plants and on excised primary leaves. The excised primary leaves could be fed with bialaphos and other compounds *via* the petiole.

Determination of the content of free ammonium

The assay is based on a reaction between NH_4^+ and phenol in the presence of sodium hypochlorite with formation of a blue phenylquinone monimine complex [5]. The concentration can be determined

spectrophotometrically at a wavelength of 625 nm. Addition of sodium nitroprusside as catalyzer intensifies this reaction.

Measurement of the electron transport rate (Hill reaction)

The isolation of chloroplasts was achieved according to [6].

The electron transport rate of broken chloroplasts was measured as photoreduction of the electron acceptor DCPIP. The final concentrations of the reaction medium contained 50 mM tricine, pH 8.3, 50 mM KCl, 5 mM $MgCl_2$, 5 mM KH_2PO_4, pH 8.3, 30 mM DCPIP, 10.9 ml H_2O. The reaction medium was distributed into two test-tubes. One test-tube was illuminated for 60 s and the other one was stored in darkness for the same time. The DCPIP reduction was determined spectrophotometrically by 605 nm ($\varepsilon = 21 \text{ cm}^2 \cdot \mu\text{mol}^{-1}$).

Determination of RubP-carboxylase

The RubP-carboxylase assay in a crude enzyme extract was described by Braun et al. [7].

Measurement of catalase activity

The method of catalase determination by means of the oxygen electrode was described by Del Rio et al. [8].

Results

Effect of bialaphos on photosynthesis

Measurement with entire plants. Entire *Sinapis alba* plants were sprayed with either bialaphos solution or PPT solution and placed in darkness overnight. Then the photosynthetic rate was measured. After 15 min a significant decrease of photosynthesis occurred in bialaphos treated as well as PPT treated plants (Fig. 1). The measurements were taken under atmospheric conditions (400 ppm CO_2, 21% O_2).

However, under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2) there were no photosynthetic inhibitions in plants treated with either bialaphos or PPT (Fig. 2).

Measurement on excised primary leaves. The photosynthetic rates of bialaphos treated excised primary leaves were measured under various concentrations of CO_2 and O_2 (Fig. 3). A 1 mM bialaphos solution was applied through the petiole.
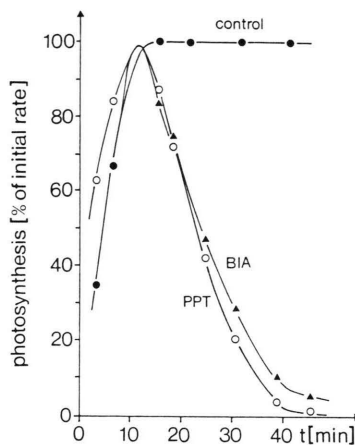

Fig. 1. Photosynthetic activity of bialaphos and PPT treated *Sinapis alba* plants under atmospheric conditions (400 ppm CO_2, 21% O_2). ● = Control (untreated plants); ○ = PPT treated plants; ▲ = bialaphos-treated plants.
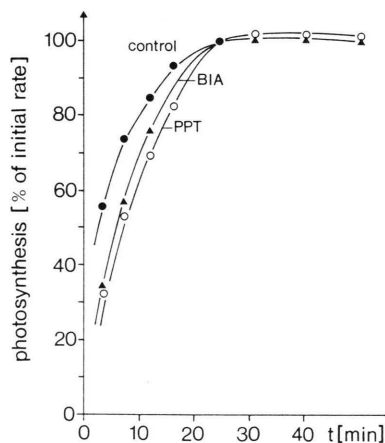

Fig. 2. Photosynthetic activity of bialaphos and PPT treated *Sinapis alba* plants under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2). ● = control (untreated plants); ○ = PPT treated plants; ▲ = bialaphos treated plants.

Under atmospheric conditions (400 ppm CO_2, 21% O_2) a strong inhibition of photosynthesis occurred very rapidly after application of herbicide solution. About 80 min after application, photosynthesis was almost completely inhibited.

However, under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2) no inhibition by bialaphos was detected.

Further measurements of photosynthesis were performed under the following conditions after adding bialaphos: 1000 ppm CO_2/21% O_2; 400 ppm CO_2/
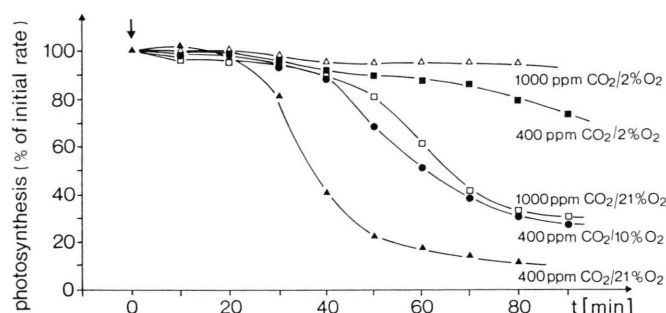
Fig. 3. Photosynthetic activity of bialaphos treated excised mustard primary leaves under different CO_2 and O_2 conditions. The 100% value of photosynthesis was calculated from the photosynthetic rate at the beginning of the experiment. Bialaphos was added at zero time (↓).

10% O_2; 400 ppm CO_2/2% O_2. The degree of intactness of photosynthesis can be taken as an indicator for the suppression of photorespiration. The greatest decrease in photorespiration could be reached under 400 ppm CO_2 and 2% O_2. Total suppression of photorespiration could be achieved through simultaneous increase of CO_2 content to 1000 ppm and decrease of O_2 content to 2%.

Effect of glutamine on bialaphos toxicity

Measurements were carried out, in which different glutamine concentrations were applied in addition to bialaphos (1 mM).

The measurements of primary leaves showed that, with increasing glutamine concentrations, the photosynthesis inhibition through bialaphos was reduced (Fig. 4). The measurements were taken under atmospheric conditions (400 ppm CO_2, 21% O_2).

Effect of other amino acids on bialaphos toxicity

Measurements of excised primary leaves were carried out in which asparagine, glutamate and glycine were added to the bialaphos solution. The amino acids showed a slight decrease in inhibition of the photosynthesis with bialaphos.

Glutamine however, causes the strongest decrease in inhibition of photosynthesis with bialaphos (Table I).

Ammonium-accumulation after treatment with bialaphos

Immediately after the gas exchange measurements, the NH_4^+-content of bialaphos treated primary leaves was measured. The treated leaves showed a clear increase of NH_4^+-concentration (Fig. 5). There was an NH_4^+-accumulation under normal atmospheric conditions (400 ppm CO_2, 21% O_2) as well as under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2). After addition of various amino acids, there was a particularly high NH_4^+-accumulation.

Effect of glyoxylate on photosynthesis

Instead of bialaphos, glyoxylate was given to the excised primary leaves. The glyoxylate concentrations consisted to 10 mM and 30 mM and the measurements took place under atmospheric conditions (400 ppm CO_2, 21% O_2).

With 10 mM glyoxylate, the photosynthesis inhibition was only slight. With 30 mM glyoxylate a strong inhibition occurred (Table II).
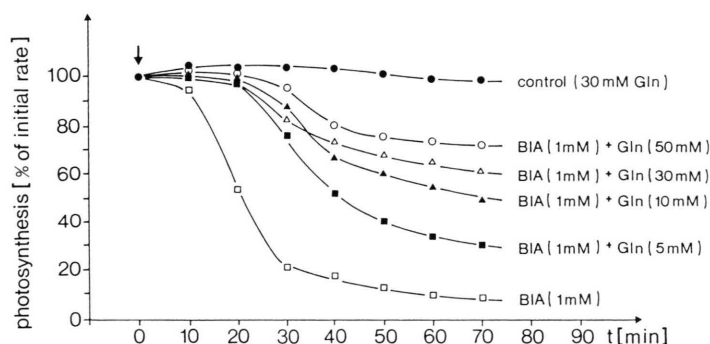

Fig. 4. Photosynthetic activity of bialaphos treated excised mustard primary leaves. In addition to bialaphos (1 mM), different glutamine concentrations were applied. Measuring occurred under atmospheric conditions (400 ppm CO_2, 21% O_2).
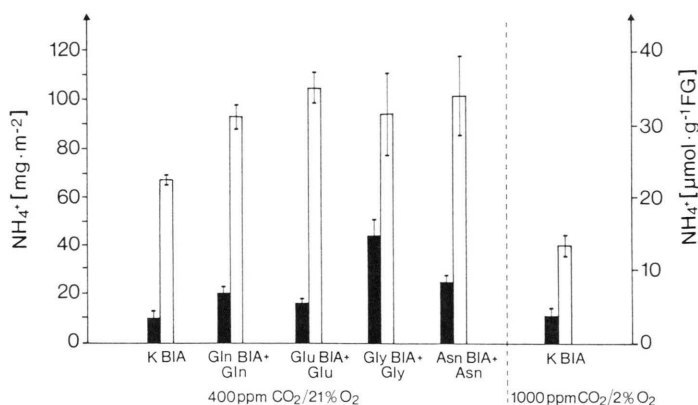
Fig. 5. NH_4^+-accumulation of mustard primary leaves after bialaphos treatment: under atmospheric conditions (400 ppm CO_2, 21% O_2), under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2), after the addition of different amino acids (asparagine, glutamate, glutamine, glycine).

Effect of bialaphos on the electron transport rate

The electron transport rate was measured after exposing bialaphos treated mustard plants to light for different periods of time.

The measurements show that the photosynthetic electron transport *via* DCPIP is not inhibited by bialaphos (Table III).

Table I. The effect of different amino acids on bialaphos toxicity to photosynthesis under atmospheric conditions (400 ppm CO_2, 21% O_2). The excised primary leaves were fed with the compounds *via* the petiole. Photosynthetic rate in %.

min	Bialaphos	Bialaphos+ Asn (30 mM)	Bialaphos+ Glu (30 mM)	Bialaphos+ Gly (30 mM)	Bialaphos+ Gln (30 mM)
0	100	100	100	100	100
10	101 ± 1	102 ± 1	103 ± 3	102 ± 3	101 ± 3
20	98 ± 2	98 ± 1	102 ± 4	102 ± 3	99 ± 5
30	81 ± 6	81 ± 6	91 ± 1	98 ± 2	84 ± 8
40	40 ± 6	59 ± 8	68 ± 3	75 ± 4	74 ± 8
50	23 ± 3	43 ± 5	55 ± 2	50 ± 4	69 ± 6
60	18 ± 2	32 ± 7	37 ± 2	40 ± 4	66 ± 6
70	15 ± 2	23 ± 7	29 ± 2	30 ± 3	62 ± 5

Table II. The effect of glyoxylate on photosynthesis under atmospheric conditions (400 ppm CO_2, 21% O_2). The excised primary leaves were fed *via* the petiole. Photosynthetic rate in %.

min	Glyoxylate (10 mM)	Glyoxylate (30 mM)
0	100	100
10	100 ± 2	100 ± 6
20	98 ± 3	87 ± 17
30	93 ± 4	68 ± 22
40	88 ± 4	52 ± 20
50	84 ± 6	42 ± 18
60	78 ± 8	34 ± 15
70	74 ± 8	28 ± 15
80	70 ± 9	23 ± 13

Table III. Electron transport rate per chlorophyll of *Sinapis alba* leaves after treatment with bialaphos (0.05%) · 1 = H_2O, control; 2 = bialaphos treated.

| | 90 min | | 180 min | | 300 min | |
	1	2	1	2	1	2
Electron transport rate [μmol e⁻ · mg⁻¹ Chl·h⁻¹]	328.6 ±61	342.4 ±53	314.0 ±56	332.8 ±53	291.3 ±75	294.3 ±51

Discussion

As was shown in the previous investigation into the effect of bialaphos, bialaphos splits in plants into PPT and alanine [1]. Accordingly, PPT appears to be the only herbicidal active substance in bialaphos application. It was therefore concluded that the herbicidal effects of bialaphos and PPT are the same.

Investigations on the effect of bialaphos and PPT on photosynthesis in whole plants have shown that, under atmospheric conditions (400 ppm CO_2, 21% O_2), the inhibition of photosynthesis with PPT and bialaphos proceeds quickly (Fig. 1). Under atmospheric conditions bialaphos as well as PPT [9] show a rapid inhibition of photosynthesis in excised primary leaves (Fig. 3). However, photosynthesis is not inhibited with either bialaphos (Fig. 2, Fig. 3) or PPT (Fig. 2) under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2). Thus, photorespiration seems to play an important role for the effect of both bialaphos and PPT. This becomes particularly obvious after measurements of photosynthesis using bialaphos in different CO_2/O_2 concentrations. The inhibition of photosynthesis by bialaphos decreases strongly with increasing photorespiratory suppression. Similar results were previously obtained with PPT (Wild *et al.*, unpublished results). In different CO_2/O_2 concentrations, the extent of inhibition of photosynthesis can be taken as an indicator for the intensity of photorespiration.

Earlier investigations have shown that bialaphos [1], similar to PPT [10], inhibits GS *in vivo*. A glutamine shortage and an NH_4^+-accumulation results through inhibition of GS. The glutamine deficiency could be compensated by supplying exogenous glutamine. Accordingly, the photosynthesis inhibition through bialaphos decreases significantly with increasing exogenously supplied glutamine concentrations (Fig. 4). Again, similar results were obtained with PPT and glutamine, the photosynthesis inhibition is likewise greatly reduced [2]. Glutamine reacts with 2-oxoglutarate catalyzed by the GOGAT to form two molecules of glutamate, the substrate of transamination reactions. Thus glutamine deficiency results *e.g.* in general amino acid deficiencies. However, in contrast to glutamine, amino acids as for instance asparagine, glycine, serine, glutamate show only a slight compensating effect on the inhibition of photosynthesis through bialaphos (Table I).

The NH_4^+-concentration of bialaphos treated plants was measured. There was a high NH_4^+-accumulation for bialaphos (Fig. 5) and PPT [9] under atmospheric as well as non-photorespiratory conditions. An even further rise in NH_4^+-concentration was measured after adding various amino acids to the bialaphos solution (Fig. 5). Under non-photorespiratory conditions photosynthesis is not inhibited. Nevertheless, the NH_4^+-concentration rises. Then, after adding glutamine to bialaphos or PPT solution there is a strong decrease in photosynthesis inhibition, though the NH_4^+-accumulation increases a great deal (Fig. 4, Fig. 5).

Our results indicate that a toxic NH_4^+-accumulation is not the sole cause of the effectiveness of either bialaphos or PPT. A process occurring during photorespiration must play an important role in the inhibition of photosynthesis by either bialaphos or PPT. In order to gain more precise information, the following experiments were carried out:

It was investigated whether the catalase activity is inhibited by bialaphos. Catalase destroys toxic H_2O_2 produced in photorespiration. However, catalase activity is not influenced by bialaphos (data not shown).

As stressed above GS inhibition results in glutamine and consequently in amino acid deficiency. Thus the glyoxylate transamination in photorespiration could be inhibited, which results in a glyoxylate accumulation.

Glyoxylate should inhibit RubP-carboxylase [11, 12] and the regeneration of RubP [13], which leads to a photosynthesis inhibition.

In a corresponding investigation it was shown that glyoxylate inhibits photosynthesis, provided it was applied in high concentrations.

Preliminary experiments indicate that the inhibition of RubP-carboxylase by glyoxylate could be the cause of photosynthesis inhibition. The plants were treated with 20 mM glyoxylate. After an acting period of 60 min there was a 56% RubP-carboxylase inhibition.

Figure 6 gives a preliminary overview of the course of the herbicide effect of bialaphos based on the investigation results until now.
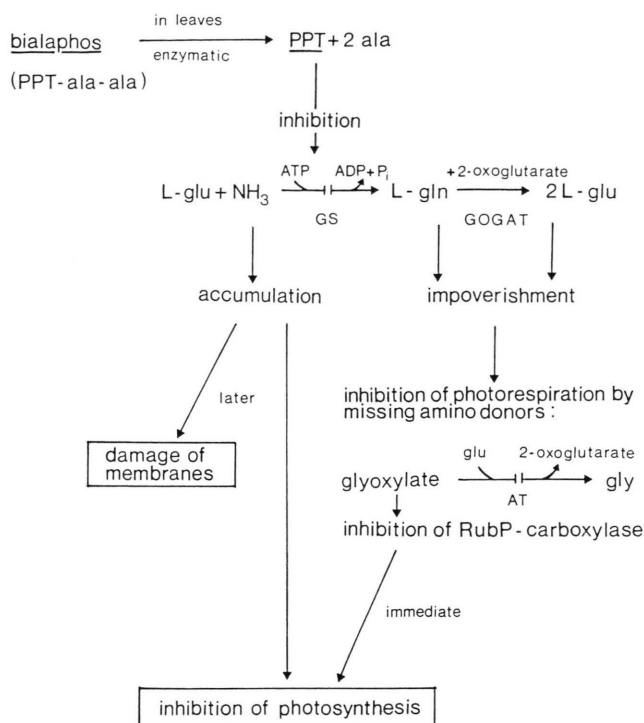
Fig. 6. The action of bialaphos in plants.

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