The Effect of Phosphinothricin (Glufosinate) on Photosynthesis
I. Inhibition of Photosynthesis and Accumulation of Ammonia

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Phosphinothricin (glufosinate) is an irreversible inhibitor of glutamine synthetase in plants. Enzyme inhibition results in substantial light-dependent accumulation of ammonia, inhibition of photosynthesis and ultimately the death of the plants. First of all the metabolic processes in which NH_3 is liberated were established. Comparative investigations under photorespiratory and non-photorespiratory conditions with *Sinapis alba* plants revealed that about 60% of the accumulated NH_3 derives from photorespiration. Trials with PPT and KNO_3 showed that nitrate assimilation is insignificant as a source of NH_3, and so the remaining 40% is accumulated in catabolic or anabolic processes. Under photorespiratory conditions CO_2 fixation after treatment with 1 mM PPT finally fell below the compensation point, and this was independent of the addition of nitrate. In non-photorespiratory conditions, by contrast, photosynthesis continued at a level of over 80% for several hours in entire plants and in severed leaves, although at the same time substantial amounts of ammonia had accumulated.

Introduction

Phosphinothricin, a phosphinic acid analogue of glutamate, inhibits GS in bacteria [1], algae [2] and higher plants [2, 3]. As shown recently by means of an isolated enzyme of wheat and mustard, this is an irreversible inhibitor [4]. GS, the key enzyme in the now generally recognized GS/GOGAT pathway, plays a crucial role – at least in higher plants – in the (re)assimilation of NH_3 [5, 6]. Thus in earlier investigations with MSO, a structural analogue of PPT that is similarly familiar as a GS inhibitor [7], elimination of NH_3 was observed in bacteria and aquatic plants [8, 9], whereas accumulation was noted in higher plants [10, 11]. Various authors have been able to show that this accumulation of ammonia is in fact the direct result of GS inhibition by MSO [12–14].

The primary aim of this paper is to quantify the inhibition of photosynthesis of higher plants that occurs as a result of the application of PPT. The second point investigated is the metabolic processes in which the accumulated ammonia occurs. There are three major potential sources: the assimilation of inorganic nitrogen, photorespiration, and catabolic or anabolic processes. Previous studies of the effect of MSO have produced contradictory results. Apart from the groundwork done in our paper [15] no results involving PPT are yet available. Therefore firstly the influence of nitrate assimilation and secondly the role of photorespiration as a cause of ammonia accumulation will be clarified.

Abbreviations: GDH, glutamate dehydrogenase; GOGAT, glutamine: 2-oxoglutarate aminotransferase; GS, glutamine synthetase; MSO, methionine sulphoximine; PPT, phosphinothricin.

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Material and Methods

Plant material and chemicals

16- to 18-day-old plants of *Sinapis alba* (white mustard) were used for investigating the toxic effect of the herbicide; for the growing conditions see [15].

The active ingredient PPT was supplied by Hoechst AG (Frankfurt/Main, West Germany) (code no. Hoe 35956). This is a phosphinic acid analogue of glutamate. Chemical name (IUPAC): DL-homoalanin-4yl (methyl)phosphinic acid. The NH_4^+ salt of this compound (Code No. Hoe 039866, common name: glufosinate-ammonium) was described as a new non selective herbicide [16, 17].

Measurement of photosynthesis

Photosynthesis was measured as the CO_2 fixation rate by means of an infrared gas analyser, as described in [18]. The experiments were conducted on entire plants and on severed primary leaves. The latter could be fed with PPT via the petiole.

The experiments with individual leaves involve the risk of impairment of the transpiration stream when the leaves are severed: the resultant closure of the stomata leads to inhibition of photosynthesis. Whether this was the case or whether the toxic effect of the PPT caused the rate of photosynthesis to drop had to be proved. This could be achieved by tracing the resistance to water vapour via a humidity sensor (Nova Sina, München) as PPT showed no effect on the behaviour of stomata. Experiments in which the stomatal resistance increased were discarded.

To clarify the question of nitrate assimilation as a cause of NH_3 accumulation the leaves were treated via the petiole with the pure herbicide in a concentration of 1 mM as well as with the same substance plus 50 mM KNO_3. In order to clarify the role of photorespiration, experiments under normal atmospheric, *i.e.* photorespiratory, conditions were compared with those under non-photorespiratory conditions.

Measurement of ammonia

The ammonia was determined by the Berthelot detection method, in which the blue dye complex phenylquinone monimine is formed. The application method is based on investigations by Weatherburn [19].

Results

Fig. 1 shows the rates of photosynthesis of entire plants that were sprayed with a PPT dose equivalent to 1 kg/ha and then exposed to photorespiratory or non-photorespiratory conditions. To create non-photorespiratory conditions the O_2 partial pressure was reduced to 2% and in addition the carbon dioxide level was raised from 400 to 1000 ppm.

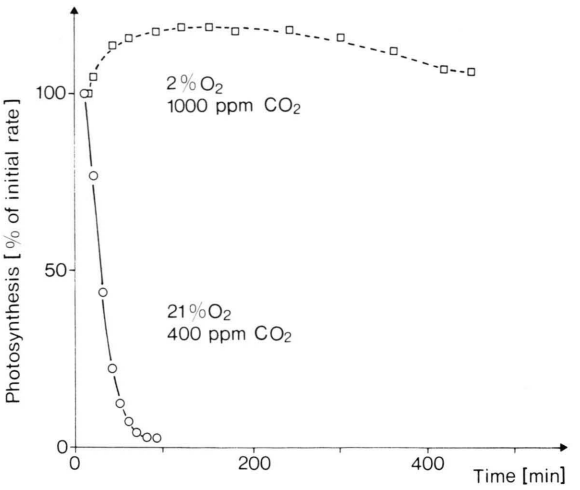

Fig. 1. Photosynthetic activity of mustard primary leaves with and without photorespiration after the application of 1 kg/ha PPT. PPT was sprayed 16 h before exposure to light. During the light period the leaf (still attached to the plant) was in an assimilation chamber at 25 °C, 56% relative humidity, 190 W m^{-2} photosynthetically active radiation (PhAR), with different composition of the air flowing through the leaf cuvette: ○ = 400 ppm CO_2, 21% O_2; □ = 1000 ppm CO_2, 2% O_2.

It is evident that under normal CO_2 and O_2 conditions the initial rate of photosynthesis fell by 50% after only about 30 min, whereas it remained fairly constant for 7.5 h with 2% O_2. The controls which were sprayed only with wetting agent, are omitted in Fig. 1 for the sake of clarity. They showed that CO_2 fixation was fairly constant and that photosynthesis even rose slightly during the trial period (from 100% to 111% under photorespiratory conditions and from 100% to 128% under non-photorespiratory conditions).

Table I illustrates the effect of PPT on the rates of photosynthesis of severed leaves. The absolute initial

Table I. Time course of photosynthetic rates (in % of a H_2O control) of severed mustard primary leaves under photorespiratory and non-photorespiratory conditions after petiolar feeding with 1 mM PPT, 50 mM KNO_3, and both. Means from 10 experiments. Standard deviations were usually about 4%. Further conditions in the assimilation chamber as in Fig. 1.

Treatment	Conditions	Photosynthetic rate 10	20	30	40	60	90	120	min
PPT −	photorespiratory:	100	99	80	38	8	0	−2	
PPT + KNO_3	21 % O_2 +	100	98	95	91	71	17	−2	
− KNO_3	0.04% CO_2	99	98	96	94	89	89	91	
PPT −	non-photorespiratory:	93	94	92	88	84	85	79	
PPT + KNO_3	2% O_2	93	97	100	100	95	89	84	
− KNO_3	0.1% CO_2	88	95	102	103	99	93	92	

values for CO_2 fixation under photorespiratory conditions were 46 ± 8 mmol CO_2 m^{-2} h^{-1} (treated) and 47 ± 6 mmol CO_2 m^{-2} h^{-1} (control); under non-photorespiratory conditions they were 72 ± 4 mmol CO_2 m^{-2} h^{-1} (treated) and 74 ± 5 mmol CO_2 m^{-2} h^{-1} (control). This difference is attributable firstly to the Warburg effect and secondly to the fact that the limiting factor is eliminated by raising the CO_2 concentration from 400 to 1000 ppm. When measurements are carried out with 21% O_2 the rate of photosynthesis falls to zero over a period of about 80 min. At 2% O_2 however it falls by only about 25% after more than two hours.

In order to determine the influence of nitrate assimilation on NH_3 accumulation the plants were treated in a further series of trials with KNO_3 in a concentration of 50 mM as well as with PPT (Table I). As a result the rate of photosynthesis of the controls falls slightly (Fig. 2A). This is undoubtedly attributable to the unnaturally high nitrate content, which clearly involves osmotic stress for the plants. At a concentration of 5 mM KNO_3, which is in any case closer to the normal natural conditions in the plant [20], photosynthesis in fact remains constant over a period of 100 min. In previous work with MSO and nitrate the concentrations normally used were between 3 and 21 mM, though concentrations up to 100 mM were also employed [14, 20–22]. A nitrate concentration of 50 mM was chosen despite the unnatural conditions so as to ensure that the NO_3^- content was high enough for nitrate assimilation to be investigated as a source of NH_3.

In these experiments the absolute initial rates under photorespiratory conditions were 48 ± 10 mmol CO_2 m^{-2} h^{-1} (treated) and 44 ± 2 mmol CO_2 m^{-2} h^{-1} (control) and under non-photorespiratory conditions 78 ± 8 mmol CO_2 m^{-2} h^{-1} (treated) and 77 ± 4 mmol CO_2 m^{-2} h^{-1} (control).

The rate of photosynthesis of the PPT/KNO_3-treated plants was similarly completely reduced under normal atmospheric conditions – it finally fell below the CO_2 compensation point to the respiratory values. However, inhibition of CO_2 fixation was longer in setting in than in the case of plants that received only PPT (Table I). Incidentally, there was no major difference between the experiments with PPT and those with PPT/KNO_3 at 2% O_2.

Reduction in photosynthesis is accompanied by accumulation of ammonia when plants are treated with phosphinothricin. Fig. 2 illustrates this for a series of trials on leaves to which PPT/KNO_3 was applied.

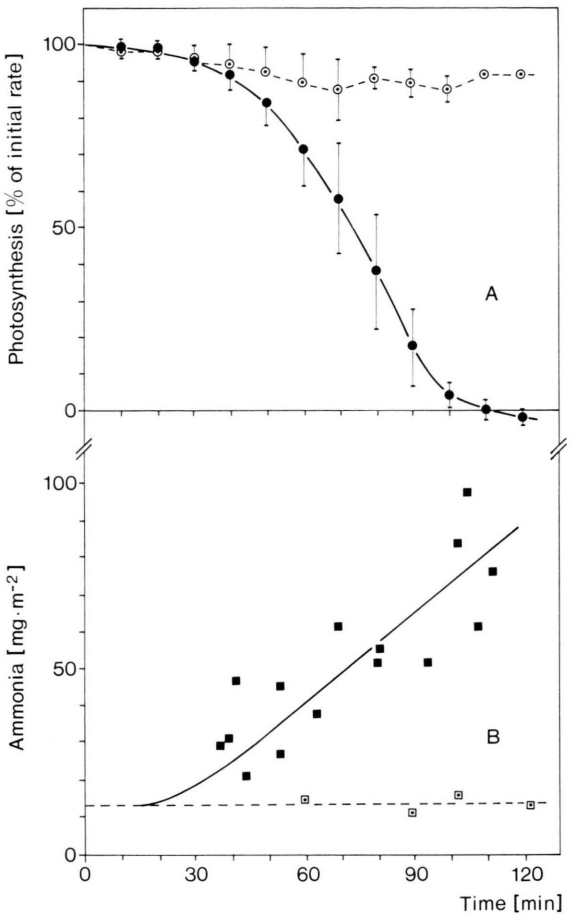

Fig. 2. Photosynthesis and ammonia accumulation of mustard primary leaves after petiolar feeding with 1 mM PPT and 50 mM KNO_3 under photorespiratory conditions (400 ppm CO_2, 21% O_2). A, photosynthesis: ● = treated, ⊙ = control; B, ammonia content: ■ = treated, ⊡ = control.

Table II shows the average NH_3 accumulation rates. Contrary to our expectation even less NH_3 was accumulated in plants that received nitrate in addition to the herbicide. Comparison of the experiments under photorespiratory conditions with those under non-photorespiratory conditions reveals however that with 2% O_2 the amount of ammonia formed was only about 40% of that formed under normal atmospheric conditions.

The ammonia accumulation that takes place somewhat more slowly in the experiments with PPT/KNO_3

Table II. Ammonia accumulation of mustard primary leaves provided with PPT or PPT/KNO_3, respectively, treated under conditions with or without photorespiration.

Treatment	Conditions	Ammonia accumulation rate (μmol NH_3 g^{-1} fr wt hr^{-1})	% of ammonia at photorespiratory conditions
PPT	21% O_2, 400 ppm CO_2	31.1 ± 6.3 (13)[1]	100.0
Control (H_2O)		4.9 ± 0.5[2] (4)	
PPT	2% O_2, 1000 ppm CO_2	11.6 ± 2.7 (12)	37.3
Control (H_2O)		3.5 ± 0.7 (4)	
PPT/KNO_3	21% O_2, 400 ppm CO_2	21.7 ± 5.7 (15)	100.0
Control (KNO_3)		5.5 ± 0.9 (4)	
PPT/KNO_3	2% O_2, 1000 ppm CO_2	9.6 ± 4.3 (11)	44.2
Control (KNO_3)		4.7 ± 0.9 (4)	

[1] Numbers in parentheses represent the number of experiments.
[2] Ammonia content of the controls did not alter during the course of the experiments. This is the reason, why the control rates are given in μmol NH_3 g^{-1} fr wt.

does correlate with the delayed inhibition of CO_2 fixation of these plants.

The rates of photosynthesis in relation to the ammonia content thus prove to be basically identical in the leaves that were treated only with PPT and those that received KNO_3 in addition. Under photorespiratory conditions CO_2 exchange decreases at about 35 mM NH_3 to the respiratory value (Fig. 3). The trials with 2% O_2 were conducted for a considerably longer time than those with 21% O_2, and so in these conditions enormous quantities of ammonia were accumulated in the course of time (Fig. 4). The plants treated with PPT and PPT/KNO_3 displayed the same characteristics.

NH_3 accumulation is, furthermore, light-dependent. This became clear in trials with PPT-sprayed plants, in which only one primary leaf was exposed whilst the other was kept in the dark. It proved that the ammonia content in the exposed leaf was many times higher than that in the leaf kept in the dark (data not shown). Compared to the controls, however, even in the dark some NH_3 had been accumulated. Consequently, light-dependent processes are responsible largely though not exclusively for ammonia accumulation.

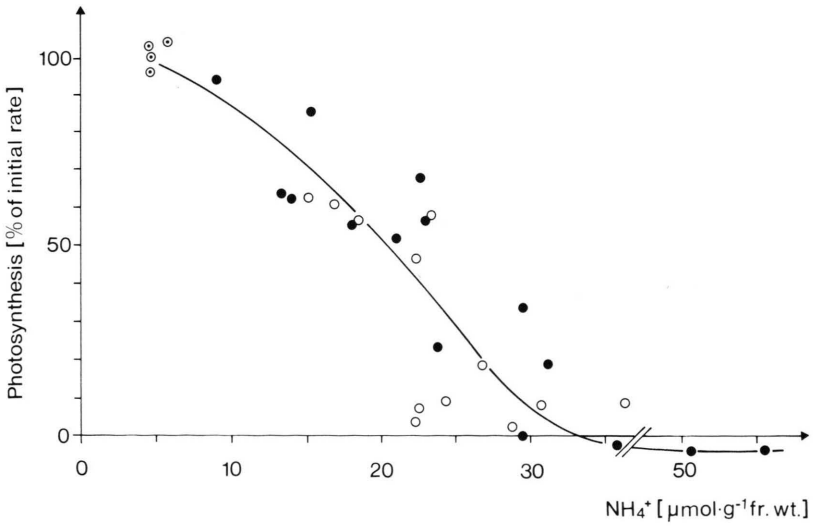

Fig. 3. Photosynthesis as a function of ammonia content of mustard primary leaves provided with PPT or PPT/KNO_3, respectively, under photorespiratory conditions. Ammonia content of the controls with KNO_3 is given in Table II. ○ = PPT, ● = PPT/KNO_3, ⊙ = control (H_2O).

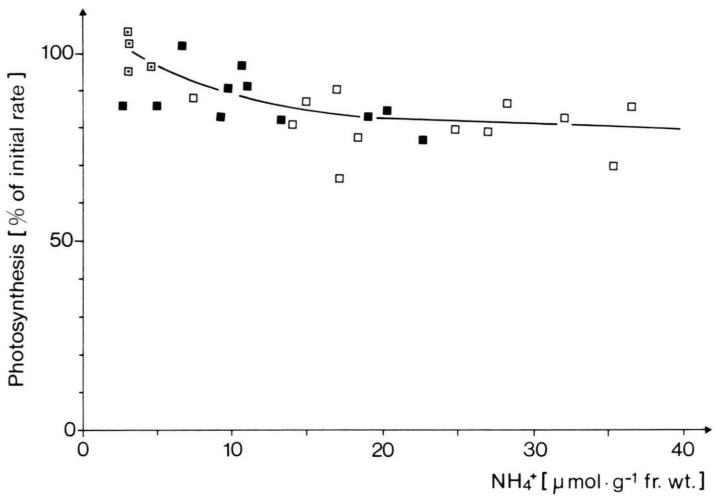

Fig. 4. Photosynthesis as a function of ammonia content of mustard primary leaves provided with PPT or PPT/KNO_3, respectively, under non-photorespiratory conditions (1000 ppm CO_2, 2% O_2). Ammonia content of the controls with KNO_3 is given in Table II. □ = PPT, ■ = PPT/KNO_3; ⊡ = control (H_2O).

Discussion

At normal atmospheric conditions, PPT treatment results in inhibition of photosynthesis. This is shown in the trials in which the herbicide is sprayed direct onto the leaves and in the experiments with the active ingredient applied *via* the petiole.

PPT at a dose equivalent to 1 kg/ha was used for spraying entire plants; the CO_2 fixation rate fell to zero within about 80 min (Fig. 1).

Photosynthesis was also inhibited completely in the case of plants with severed leaves. It was recently confirmed that the PPT concentration of 1 mM applied to the petiole was sufficient to inhibit GS completely [23]. On adding 0.25 mM PPT the GS of extracts of leaves had a residual activity of only about 20% after only 2 min [4].

The photosynthesis of leaves to which PPT/KNO_3 was applied fell below the compensation point within 2 h. By adding both nitrate and PPT the CO_2 fixation is however reduced more slowly than when the herbicide is used on its own (Table I). This was also observed in experiments with MSO and KNO_3 [22].

Completely different results can be observed under non-photorespiratory conditions. In entire plants and in severed leaves photosynthesis continues for several hours at more than 75% of the initial rate. This behaviour is independent of any addition of NO_3^-. In previous work with the GS inhibitor MSO there are contrasting data on this point, though a number of authors were able to observe similar findings with MSO [21, 24–26]. Evidently the interruption of photorespiration in any way is crucial to the inhibition of photosynthesis.

PPT treatment of plants causes high ammonia accumulation in the tissues [15]. There may be three major potential sources for the ammonia. In the C_2 pathway of photorespiration the glycine decarboxylase reaction produces not only CO_2 but also an equivalent quantity of ammonia. Another metabolic process forming ammonia is the evolution of NH_3 in the course of nitrate assimilation by reduction of the externally absorbed NO_3^-. Thirdly, ammonia also occurs in catabolic and anabolic processes, such as in the breakdown of proteins or nucleotides and in the oxidative deamination of phenylalanine or tyrosine. In each case the NH_3 liberated is reassimilated *via* the glutamine synthetase. Inhibition of the GOGAT cycle at any point therefore results in accumulation of ammonia.

Table II shows that the total amount of ammonia liberated under normal atmospheric conditions is 31 μmol NH_3 h^{-1} g^{-1} fr wt. Assuming that 334 μmol CO_2 h^{-1} g^{-1} fr wt are reduced at an average rate of photosynthesis, this value at 9% of the apparent CO_2 fixation is quite low. However, one possible assumption is that the internally produced and reassimilated amount of NH_3 is higher [27, 28]. If so, the plants have developed detoxification mechanisms by employing the enzymes GDH and aspartate-synthetase. With the aid of these mechanisms they are able to eliminate not only the normally occurring concentrations of NH_3 but also much higher ones. In any case

other authors have shown in more recent work that the ammonia concentration accumulated in the presence of a GS inhibitor can not be used as a measure of the normal intensity of photorespiration [22, 29].

If the main cause of ammonia accumulation lies in photorespiration, it should be reduced accordingly by inhibiting this process. In fact the NH_3 concentration at 2% O_2 and 1000 ppm CO_2 was only 37% of the value at 21% O_2 and 400 ppm CO_2. The numerous results published so far with MSO as the source of the ammonia differ for one and the same plant species: some authors found that the majority of the accumulated NH_3 in wheat and spinach is formed under non-photorespiratory conditions [22, 30]; other findings, similarly on wheat [21, 26] but also on oats [20], pepper [24, 25] and barley [21] point to photorespiration as the primary source of NH_3: in these cases the maximum amount of ammonia accumulated under non-photorespiratory conditions is 40% of that accumulated under photorespiratory conditions. Contradictory results were also obtained in comparing C_3 and C_4 plants: some authors observed in maize and sorghum only about 1/4 of the ammonia content of C_3 plants [21, 31], whereas others observed equivalent NH_3 concentrations [2, 32]. The effect of O_2 and CO_2 on NH_3 accumulation in the green algae *Ankistrodesmus* and *Chlamydomonas* and the effect of isonicotine hydrazide and MSO on *Chlamydomonas* and on higher plants illustrate clearly the role of photorespiration in this connection [33, 34]. According to our results, too, 63% of the accumulated ammonia is derived from the glycolate pathway.

Investigation of nitrate as a possible source of ammonia in PPT-treated plants produced some surprising results. If NO_3^- is assumed to be the source of ammonia nitrogen, then a plant ought to accumulate more NH_3 if fed with additional nitrate. In our experiments however the exact opposite was observed: the PPT/KNO_3-treated plants contained about 30% less ammonia than the plants to which only PPT was applied. In each case it is clear that at least within the trial period of two hours – the early effects of PPT action were therefore observed here – more ammonia does not occur as a result of reducing the nitrate. Similar results were obtained with MSO on various higher plants [21, 30].

With regard to the source of nitrogen for the accumulated ammonia it can therefore be said that nitrate reduction is unimportant at an early stage of photosynthesis inhibition, that photorespiration is responsible for 63% of the NH_3 formed and that therefore 37% has to originate from catabolic or anabolic processes.

These findings tally well with the results of the experiments on the light dependence of NH_3 accumulation. Confirmation was gained here of what other authors have already observed with MSO [35, 36], namely that after the application of PPT the majority of the ammonia is formed in the light – this would correspond to the ammonia from the light-dependent glycolate pathway; but as this paper has shown, part of the NH_3 is liberated in the dark- this would be attributable to the light-independent catabolic and anabolic processes.

The correlation between photosynthesis and ammonia concentration always shows similar values regardless of the addition of nitrate. At first glance it could appear that ammonia accumulation is responsible for inhibiting photosynthesis, as many authors have so far postulated for MSO. On the other hand, CO_2 fixation under non-photorespiratory conditions remains largely intact despite high NH_3 concentrations. The cause of photosynthesis inhibition by the influence of PPT will therefore investigated at greater length in a second part of this paper [37].

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