

# Sethoxydim-Uptake by Leaf Slices of Sethoxydim Resistant and Sensitive Grasses

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Leaf slices of the sethoxydim sensitive grasses *Poa pratensis* and *Festuca ovina* and the sethoxydim resistant grasses *Poa annua* and *Festuca rubra* take up the herbicide at similar rates. Uptake is almost linear with concentration up to 2 mM, independent of light and little affected by temperature between 0 and 25 °C. Uptake is highly pH sensitive. At pH 7.0 rates of uptake are only about 20% of the rates observed at pH 3.5. Time course of uptake suggests a rapid equilibration of the compartments taking up the herbicide within 2 h. It is suggested that sethoxydim uptake by the grass-leaf tissue is a nonspecific process involving passive lipid diffusion and lipid equilibration of the non-dissociated weak acid sethoxydim ( $pK_a = 4.6$ ).

## Introduction

Sethoxydim is a herbicide with the tradename Poast<sup>®</sup>, which is widely used with a dose of 0.03 to 0.60 kg active ingredient/ha against grasses in dicotyledonous crops, especially soybeans [1–3]. Dicotyledonous plants are not affected. Grasses are susceptible, and it is assumed that sethoxydim is taken up by leaves, transported in the phloem to the based meristem and there inhibits lipid biosynthesis [3–5]. Leaves cease growing and become necrotic.

However, among the grasses there are also resistant species. Sensitive and resistant species occur within the same genus, e.g. *Poa pratensis* is sensitive and *Poa annua* resistant, *Festuca ovina* is sensitive and *Festuca rubra* resistant [6, 7]. This specificity poses a fascinating problem to the plant physiologist. In the present study we used these pairs to investigate the uptake of sethoxydim. Experiments suggest that uptake is a passive process due to lipid diffusion of the non-dissociated weak acid sethoxydim and equilibration of the herbicide in lipid membrane phases. Uptake is similar in both grass species. Hence, the differential susceptibility must have reasons other than uptake.

## Materials and Methods

Seeds of *Poa pratensis* L., *Poa annua* L., *Festuca rubra* L. and *Festuca ovina* L. were obtained from the stock of BASF-AG D-6703 Limburgerhof, FRG.

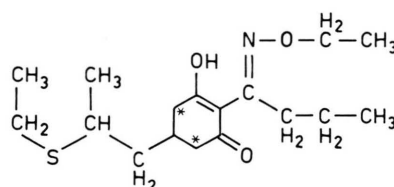
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Plants were grown in soil culture in a glasshouse until they were 3 to 4 weeks old and leaves about 12 cm long.

For incubation with sethoxydim solutions leaf slices of 1 to 2 mm were cut with razor blades. The upper and lower 2 to 3 cm of the leaves were discarded. Leaf slices were washed for 19–24 h in the dark in a solution of 1 mM KCl, 1 mM  $\text{Ca}(\text{NO}_3)_2$ , 0.25 mM  $\text{MgSO}_4$ , 0.904 mM  $\text{NaH}_2\text{PO}_4$  and 0.048 mM  $\text{Na}_2\text{HPO}_4$  at pH 5.7 (i.e.  $1 \times$  solution of ref. [8]).

Sethoxydim is a cyclohexanone derivative; 2-(1-(ethoxyimino)butyl)-5-(2-(ethylthio)-propyl)-3-hydroxy-2-cyclohexene-1-one:



Nonlabelled and  $^{14}\text{C}$ -labelled sethoxydim was obtained from BASF-AG. Labelling was in the positions indicated by asterisks, specific activity was  $0.48 \times 10^{12}$  Bq/mol. Stock solutions of sethoxydim were kept under an  $\text{N}_2$ -atmosphere.

For uptake experiments sethoxydim was dissolved in ethanol and diluted with  $1 \times$ -solution to the desired final concentration.

Uptake experiments were performed in 25 ml Erlenmeyer flasks shaken on a plexiglass rack in the water bath of a Warburg apparatus at 25 °C or 0 °C in air and in darkness or in the light of krypton lamps



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(Osram) at about  $400\text{--}500\ \mu\text{E m}^{-2}\text{ s}^{-1}$ . Leaf slices, 200 mg fresh weight, were incubated in 5 ml of sethoxydim solutions at varied concentration and specific activities between  $0.74 \times 10^6$  and  $7.4 \times 10^6\ \text{Bq/mmol}$ . Experiments were started by exchange of the  $1 \times$  solution for the  $[^{14}\text{C}]$ sethoxydim solutions. Uptake was stopped by rapidly rinsing the leaf slices and then washing them for 10 min in unlabelled sethoxydim solutions of a 2-fold lower sethoxydim concentration as during uptake in a shaking ice bath. After methanol extraction radioactivity was measured with a liquid-scintillation spectrometer (Packard Tri-Carb 460 CD or Betaszint BF 5000) in a Beckman MP Ready-Solv scintillation cocktail. Rates of uptake were calculated on the basis of the radioactivity of the leaf slices and the specific activity of the uptake solutions.

The pH of the uptake medium in the experiments of Fig. 3 and 4 were adjusted with phosphate buffer at concentration 1 mM or 10 mM.

Alloxydim derivatives are known to disintegrate into various compounds, both spontaneously and within plant cells and tissues, the half time is given as about 11 h [3]. In the present study in most uptake experiments experimental periods were only 20 or 30 min, so that this problem was minimized. Only the time course of Fig. 1 extended up to 25 h, but nothing much changed after 2 h.

We suggest that sethoxydim disintegration was not a problem in the present study.

Error, where given, are standard errors.

## Results and Discussion

Fig. 1 gives the amount of  $[^{14}\text{C}]$ sethoxydim taken up by leaf slices of both *Poa* species in relation to incubation time and shows that uptake rapidly leads to a constant level of label in the tissue. After 2 h uptake ceases entirely or continues only at a very low rate. The difference between uptake by the sethoxydim sensitive *P. pratensis* and the sethoxydim resistant *P. annua* (at 0.1 mM sethoxydim) is not significant.

Rates of sethoxydim uptake by leaf slices of the different grass species are given in Fig. 2. The sensitive and the resistant grasses take up the herbicide at very similar rates. The concentration dependence of uptake is almost linear up to the rather high concentration of 1 mM. There is only a slight curving at the higher sethoxydim concentrations. Uptake is inde-

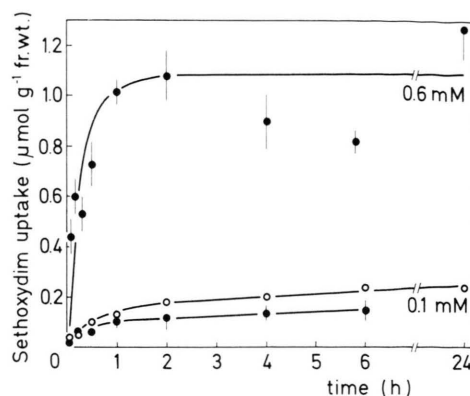


Fig. 1. Time course of  $[^{14}\text{C}]$ sethoxydim uptake by leaf slices of the sensitive grass *P. pratensis* (open circles) and the resistant grass *P. annua* (closed circles) at two different sethoxydim concentrations as indicated on the graphs and at pH 5.7.

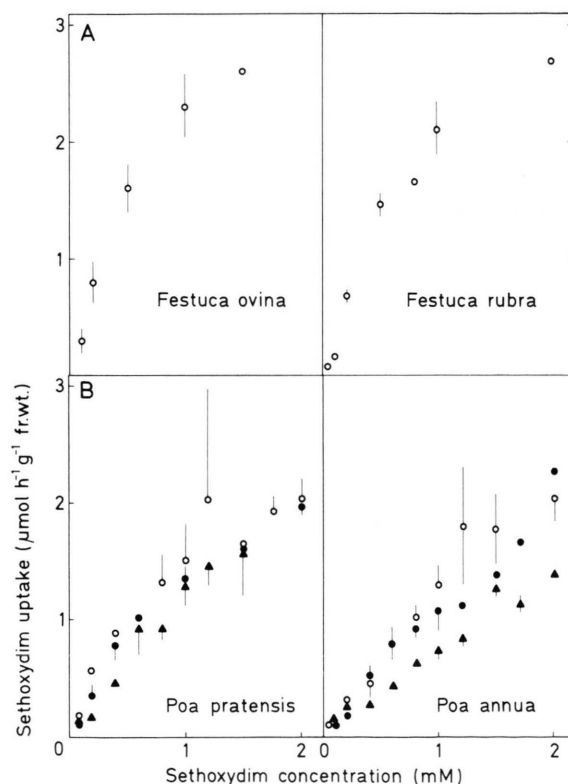


Fig. 2. A. Dependence of  $[^{14}\text{C}]$ sethoxydim-uptake rates by leaf slices of the sensitive grass *P. pratensis* and the resistant grass *P. annua* on sethoxydim concentration, light and temperature at pH 5.7. Open circles: light, 25 °C, closed circles: dark 25 °C; closed triangles: dark, 0 °C.

B. Dependence of  $[^{14}\text{C}]$ sethoxydim-uptake rates by leaf slices of the sensitive grass *F. ovina* and the resistant grass *F. rubra* on sethoxydim concentration at pH 5.7, light and 25 °C.

pendent of light and in *P. pratensis* also of temperature. In *P. annua* there was a small temperature effect, rates of uptake being slightly smaller at 0 °C than at 25 °C. The rapid completion of sethoxydim uptake, its linear concentration dependence up to 1 mM, the small temperature effects and the insensitivity to light suggest that sethoxydim uptake by the sensitive and resistant grass species is a passive and non-specific process.

Sethoxydim uptake by the leaf slices is highly pH dependent as depicted for *Poa* in Fig. 3 (Results for *Festuca* were similar, but are not shown). Based on total sethoxydim concentration in the uptake medium uptake is highest at pH 3.5 and decreases considerably with increasing pH. pH-values lower than 3.5 in the medium were not feasible without risking to damage the tissue. It was likely, that the pH effects shown in Fig. 3 were due to dissociation of the weak acid sethoxydim having a  $pK_a$  of 4.6 [9]. Fig. 4 shows the dissociation curve of sethoxydim. For comparison the relative rates of sethoxydim uptake by the four grass species are also presented. For this purpose the data of Fig. 3 were normalized on the basis of the assumption that the non-dissociated acid is the only species taken up. It is evident that at

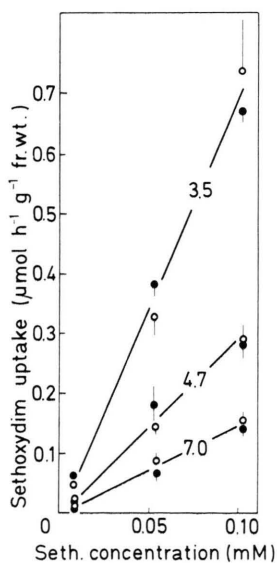


Fig. 3. Dependence of [ $^{14}\text{C}$ ]sethoxydim-uptake rates by leaf slices of the sensitive grass *P. pratensis* (open circles) and the resistant grass *P. annua* (closed circles) on sethoxydim concentration at different pH of the medium as indicated on the graphs.

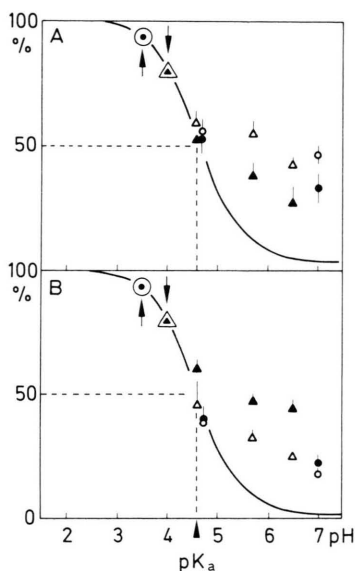


Fig. 4. Comparison of [ $^{14}\text{C}$ ]sethoxydim uptake at varied pH with the dissociation curve of the weak acid sethoxydim ( $pK_a = 4.6$ ). Open symbols: leaf slices of the sensitive grasses (A: *F. ovina*; B: *P. pratensis*); closed symbols: leaf slices of the resistant grasses (A: *F. rubra*; B: *P. annua*). The ordinate gives both dissociation of sethoxydim showing % of the non-dissociated acid (curve) and normalized sethoxydim-uptake rates (symbols). At pH 3.5 the non-dissociated acid is 92.6% of the total sethoxydim concentration present in the medium, and rates of uptake observed at this pH were set equal to 92.6% for the experiment of the circles (arrow). For the experiment of the triangles this normalization was performed at pH 4.0 where the non-dissociated acid is 79.9% of the total sethoxydim concentration (arrow). Total concentration in the medium varied between 8 and 103  $\mu\text{M}$ . For the normalisation in this figure all experiments at a given pH and various external sethoxydim concentrations were averaged. The small errors (S.D.) show that sethoxydim concentration did not affect the relative rate of uptake as determined by pH.

low pH, *i.e.* approximately up to the  $pK_a$ , uptake follows the dissociation curve but at higher pH values rates of uptake are larger.

This confirms the assumption that the molecular species taken up predominantly indeed is the non-dissociated acid. However, if uptake was occurring exclusively *via* transport of the non-dissociated acid, it should follow the entire dissociation curve as given in Fig. 4. That this is not the case can be explained in two ways. First, there could be some passive permeability also for the univalent sethoxydim anion explaining the uptake at higher pH. Second, at higher pH of the medium the pH at the site of uptake, *i.e.* in

the cell walls and close to the plasmalemma, might be somewhat lower than in the medium and in consequence relative concentrations of the non-dissociated acid may be somewhat larger. This would explain the uptake of sethoxydim at higher pH in compatibility with the hypothesis that only the non-dissociated species is taken up. Weak organic acids can diffuse in the lipid phase of membranes much more readily than the electrically charged anions [10]. Distribution of sethoxydim in a two-phase system between octanol and water was pH dependent but concentration independent. Partition coefficients, *i.e.* concentration in octanol:concentration in water, were  $51 \pm 5$  at pH 2.0,  $18 \pm 1$  at pH 5.7 and  $2 \pm 0$  at pH 8.0 ( $\bar{x} \pm SD$ ,  $n = 3$ ).

The time course of sethoxydim uptake by the grass-leaf slices (Fig. 1) is consistent with a rapid equilibration of lipid phases of membranes with this herbicide.

Clearly, uptake at the membranes cannot be responsible for the different susceptibilities of the four grass species towards the herbicide, since uptake is very similar.

In contrast to the results obtained here for short-term uptake, during longer application to intact plants uptake of sethoxydim is reduced with increasing light intensity and stimulated 7.5-fold by a temperature increase from 5 to 35 °C [11]. This may, however, be related to long distance transport and metabolism of sethoxydim rather than initial uptake.

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