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Environmental Behavior of the Chiral Herbicide Haloxyfop. 2. Unchanged Enantiomer Composition in Blackgrass (*Alopecurus myosuroides*) and Garden Cress (*Lepidium sativum*)

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ABSTRACT: Haloxyfop-methyl is a chiral herbicide against grasses in dicotyledonous crops. In plants and soil, haloxyfop-methyl is rapidly hydrolyzed to haloxyfop-acid, whose *R*-enantiomer carries the actual herbicidal activity. In soil, *S*-haloxyfop-acid is converted within less than 1 day and almost completely into *R*-haloxyfop-acid. In this study, we investigated the possible interconversion of the enantiomers of haloxyfop-methyl and haloxyfop-acid in blackgrass and garden cress. Racemic or enantiopure haloxyfop-methyl was applied to the leaves of plants grown in agar. The metabolism was followed during 4 days using enantioselective GC−MS. In contrast to soils, no interconversion was observed in plants, and metabolism was nonenantioselective. These findings are consistent with the fact that after pre-emergence application to soil and uptake by roots, the observed herbicidal effect is basically independent of the enantiomer composition of the applied substance, whereas after postemergence application, the efficacy clearly is different for the two enantiomers.

KEYWORDS: *haloxyfop, Alopecurus, Lepidium, enantioselective metabolism, enantioselective GC−MS*

INTRODUCTION

Stereoisomers of chiral compounds commonly differ with respect to their biological properties. This may concern not only a desired biological activity of, for example, pharmaceuticals or pesticides, but also undesired, human, or environmental toxicological effects.[1,2] For pharmaceuticals, chiral aspects are meanwhile routinely investigated, whereas for pesticides, the issue of chirality is only slowly gaining importance.[3−5] Most chiral pesticides are still applied as racemic mixtures. According to the European regulation 1107/2009,[6] pesticides shall be classified as candidates for substitution if they contain a significant proportion of nonactive isomers. However, there is currently no guidance on what additional studies are required for registration. For example, to address chirality in the consumer risk assessment, data are needed regarding the stereoselectivity of pesticide metabolism in plants and the stereoisomer composition of residues as well as toxicological endpoints of individual stereoisomers.

Only a few studies have been published on the stereoselectivity of pesticide metabolism in plants (for a review, see ref 7). After application, the stereoisomer composition may change not only as a consequence of preferential, stereoselective metabolism, but also due to possible conversion of one stereoisomer into another. In particular, the second process has hardly ever been investigated in plants since it requires sufficient quantities of pure stereoisomers. For example, experiments with the racemic herbicides dichlorprop and mecoprop showed a faster dissipation of the *S*-enantiomers in most dicotyledonous plants,[8] but this observation may in part also be due to the conversion of the *S*- into the *R*-enantiomer. This may be inferred from the fact that in soils, the enantiomers of dichlorprop and mecoprop were found to interconvert. This process was first overlooked because incubation experiments were performed with racemic test substances only, but it was later demonstrated with experiments using the pure enantiomers.[9,10]

Another group of chiral compounds with even more rapid and preferential interconversion of enantiomers in soils is aryloxyphenoxy-propionate herbicides (the so-called FOP herbicides[11−15]). In our accompanying paper[15] we show that the *S*-enantiomer of haloxyfop is converted within less than 1 day and almost completely into *R*-haloxyfop. This process is advantageous in this case since on enzyme level, only the *R*-enantiomer carries the herbicidal activity. Mode of action is the inhibition of the enzyme acetyl coenzyme A carboxylase (ACCase) that is involved in the synthesis of fatty acids.[16−19] Even though the herbicidal activity is only associated with the *R*-enantiomer, it was shown that after pre-emergence application of many FOPs to soil and uptake by roots, the observed herbicidal effect was basically independent of the enantiomer composition of the applied substance.[11−13,20] This finding can thus be rationalized by the conversion of the *S*- into the active *R*-enantiomer in soil.

However, after postemergence application to leaves, the herbicidal efficacy is clearly different for the two enantiomers of FOPs and increases with the fraction of applied *R*-enantiomer.[11−13,20] This would suggest that in plants, the inactive *S*-enantiomer is not converted into the active *R*-enantiomer. Experimental evidence is reported in a study where the pure enantiomers of diclofop-methyl were applied to oat coleoptiles[21] (note that FOP herbicides are applied in ester form since uptake of the more lipophilic ester by plants is enhanced). After treatment periods of 1 and 3 h and nearly complete hydrolysis to diclofop-acid in plant tissue, the

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enantiomer composition was not changed. Unfortunately, later sampling was not performed. "Chiral stability" of the enantiomers of diclofop and quizalofop was also observed in studies with freshwater algae.[22,23] A further study was conducted with cole.[24] After foliar treatment, diclofop-methyl and diclofop-acid were both degraded enantioselectively with a preference for the *S*-enantiomers. However, since no experiments were performed with pure enantiomers, no conclusion could be made on possible interconversion.

No experimental data are currently available on the behavior of the enantiomers of haloxyfop-methyl in plants (chemical structure, see Figure 1). In the European registration process, it

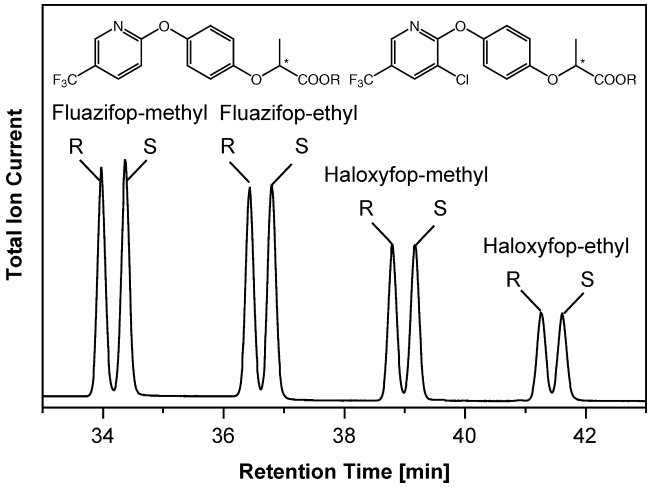

Figure 1. Reconstructed ion chromatogram of eight mass traces showing the elution of the enantiomers of haloxyfop-methyl (concentrations, 1.7 mg/g plant dry weight), haloxyfop-acid (analyzed as ethyl-ester, 1.0 mg/g), and the corresponding internal standards (fluazifop) in an ethylated extract from blackgrass, sampled 24 h after treatment with *rac*-haloxyfop-methyl. Nonenantioselective metabolism was observed for both methyl-ester and acid.

was concluded that "due to the lack of isomeric specificity of the [...] analytical methods, any possible stereochemical inversion in either direction in food of plant and animal origin could not be detected".[25] The selective herbicide is used for the postemergent control of annual and perennial grasses in sugar beet, fodder beet, oilseed rape, potatoes, vegetables, sunflowers, soya beans, vines, strawberries, and other dicotyledonous crops.[26,27] Haloxyfop-methyl is absorbed by leaves and roots and is metabolized to haloxyfop-acid and conjugates with glucose and other sugars.[27]

The present study aims at filling this data gap with regard to metabolism in plants. Our enantiomer-specific, gas chromatographic method allowed us to investigate the enantioselectivity of degradation of haloxyfop-methyl and haloxyfop-acid in plants. Possible interconversion of enantiomers was studied by using pure *R*-haloxyfop-methyl. For our experiments, we selected blackgrass as a typical weed that is controlled with haloxyfop-methyl and garden cress as a dicotyledonous plant that is not sensitive to the herbicide and, from an experimental point of view, was easy to cultivate. The herbicide was applied to the aerial parts of the plants in a way that excluded root uptake so that processes in leaves and shoots could be studied.

MATERIALS AND METHODS

Chemicals and Reagents. Racemic haloxyfop-methyl (purity, 97.5%; CAS name, see Supporting Information, Table S2 in ref 15), racemic haloxyfop-acid (99.5%), and racemic fluazifop-acid (94.5%) were from Ehrenstorfer, Augsburg, Germany. Haloxyfop-P-methyl (99.3%), racemic fluazifop-methyl (97.4%), N-nitroso-N-ethylurea (containing 2% acetic acid as stabilizer), and the nonionic detergent Tween 60 were from Sigma-Aldrich, Steinheim, Germany. A blank formulation (EF-1400) was obtained from Dow AgroSciences, Norfolk, UK. CIPAC standard water D with a hardness of 342 ppm and a pH of 6.9 was prepared as described in ref 28. The mineral fertilizer Kristalon Red Acid was from Yara, Oslo, Norway. LP0013 Agar Technical (No. 3) was purchased from Oxoid, Basingstoke, UK.

Plants. Seeds of blackgrass, *Alopecurus myosuroides* (LS-Number Fors000021), were from Appels Wilde Samen, Darmstadt, Germany, and garden cress, *Lepidium sativum* (UFA 50280), was from Fenaco, Winterthur, Switzerland. The plants were grown on agar, which was either poured on sterile polystyrene Petri dishes (diameter, 9 cm) or filled into 40 mL clear glass vials (diameter, 2.8 cm, Figure 2). Agar-

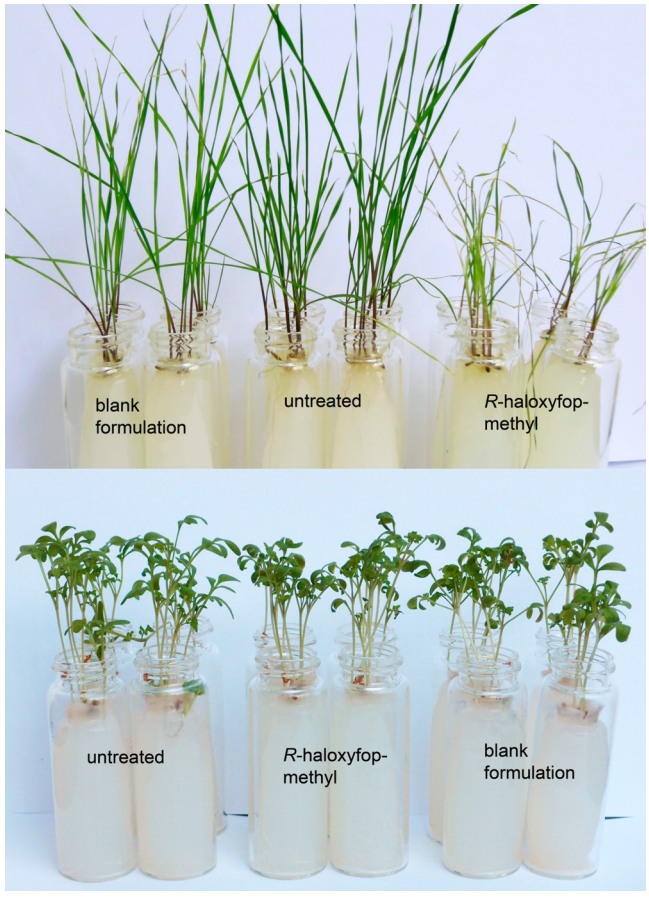

Figure 2. Blackgrass (top) and garden cress (bottom) 96 h after treatment with formulated *R*-haloxyfop-methyl, a blank formulation, and grown without treatment. The herbicide has selective activity on true grasses (*Poaceae*).

medium for Petri dishes was prepared with tap water at a concentration of 12 g/L, the medium for vials with a nutrient solution (0.1% Kristalon Red Acid, pH ≈ 7) at a concentration of 15 g/L. Agar was autoclaved at 121 °C for 15 min.

The germination rate of blackgrass after 1 week was about 30−40%. Therefore, seeds were first sown on agar plates, and germinated seedlings were then transplanted into agar vials. In a clean air bench, 25 seeds were spread on each plate and covered with a polystyrene lid. To suppress growth of fungi in the agar, blackgrass seeds were first

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disinfected. For that, seeds were placed into a tea strainer and dipped in aqueous acetic acid (7%, with addition of ∼1 g/L of the surfactant Tween 60) for 15 min. Thereafter, the seeds were thoroughly rinsed in tap water for 2 h. After sowing, the agar plates were kept in a greenhouse chamber at 80% relative humidity and a temperature of ∼25 °C (day) or ∼18 °C (night). The seedlings were exposed to natural sunlight (November−January at 47° N, attenuated by the windows of the greenhouse) and, as necessary, artificial light (high pressure sodium lamps, type Master SON-T PIA Plus 400 W, Philips) to give a total irradiance of ∼250 W/m^2 (photoperiod, 14 h per day). After about 7 days, five seedlings were transplanted into a 40 mL glass vial filled with nutrient agar (planting depth of seeds, ∼5 mm; the roots of the seedlings were completely pushed into the agar). These vials were kept in the greenhouse for another 10 days. After this time, the grass seedlings typically had developed three leaves with a length of ∼10−15 cm.

Garden cress was directly sown into the glass vials since germination was nearly 100%. Five seeds were placed ∼3 mm deep into the nutrient agar. After 7 days in the greenhouse, the cress seedlings had two leaves (cotyledons) and shoot lengths of ∼4−7 cm.

Herbicide Application on Blackgrass. Haloxyfop-methyl has a low water solubility of 9 mg/L at 25 °C.[27] To improve its solubility in the application solution and its uptake by weeds, commercial herbicides (e.g., Gallant) additionally contain adjuvants, aromatic solvents, and emulsifiers. For our experiments, we dissolved ∼50 mg of racemic haloxyfop-methyl (or ∼25 mg *R*-haloxyfop-methyl) in ∼400 mg blank formulation, an emulsifiable concentrate with the code EF-1400 (composition confidential). This solution was then mixed with 30 mL of CIPAC standard water D. The resulting, milky emulsion thus contained 1.6 g/L *rac*-haloxyfop-methyl or 0.8 g/L *R*-haloxyfop-methyl. Assuming that 200 L are sprayed per hectare, these concentrations correspond to application rates of 320 g/ha *rac*-haloxyfop-methyl or 160 g/ha *R*-haloxyfop-methyl, the latter being the currently approved, maximum application rate in Switzerland. For a preliminary experiment, an application solution was prepared with haloxyfop-methyl, composed of [*R*]:[*S*] = 2:1, at a concentration of 1.5 g/L (note that, based on the label, the test substance should have been racemic). To investigate possible herbicidal effects of the adjuvants, the blank formulation was also diluted with CIPAC water as described above but without the addition of haloxyfop-methyl.

The application method was designed to ensure that the herbicide is only taken up through leaves (and not by roots via agar) and that it is distributed as evenly as possible on the leaves. For that, the grass leaves were inserted overhead into an empty test tube (160 mm × 16 mm), which was then filled with application solution. Within less than 5 s, the leaves were pulled out of the test tube, and the application solution was allowed to drip off the leaves, still overhead (we assume that the tips of the leaves received somewhat higher amounts of active substance and formulation). After 2 min, the treated plants were placed into a climate chamber at 21−22 °C and 65% relative humidity and illuminated by two types of lamps simulating natural sunlight (three tubular low-pressure mercury-vapor fluorescent lamps, type TL40*W*/05, Philips, emitting radiation between 300 and 460 nm; and three tubular fluorescent lamps, type F40*W*/125-RS, Sylvania, emitting radiation between 400 and 650 nm). Grass leaf samples were collected immediately after application and 2, 5, 24, 32, 48, 72, and 96 h later. At each sampling time, five leaves were harvested and extracted as described later. Extractions and analyses were performed in triplicate at all time-points.

Herbicide Application on Garden Cress. For garden cress, a more diluted application solution was prepared (reason, see Results and Discussion section): ∼6 mg of racemic haloxyfop-methyl (or ∼3 mg of *R*-haloxyfop-methyl) was dissolved in ∼50 mg of EF-1400 and mixed with 30 mL of CIPAC standard water D (resulting concentrations, 0.2 g/L *rac*-haloxyfop-methyl or 0.1 g/L *R*-haloxyfop-methyl). The cress leaves were directly dipped into the application solution, pulled out within less than 5 s, and allowed to drip off overhead for 2 min. Then, the plants were placed into the climate chamber (conditions as described for blackgrass). Leaf samples were taken immediately after application and 2, 5, 24, 32, 48, 72, and 96 h later (in triplicate). All leaves of a particular vial were used for extraction.

Extraction. The harvested plant material was cut into pieces of ∼2 cm and extracted with 10 mL of methanol containing 10 μg of racemic fluazifop-methyl and fluazifop-acid as internal standards (chemical structures, see Figure 1). Extraction was facilitated by sonication for at least 5 min or until visually all chlorophyll was extracted and the leaves were bleached. An aliquot of the extract was then removed for spectrophotometric analysis of chlorophyll a at wavelengths of 665, 652, and 750 nm, as described in ref 29. With tweezers, the bleached leaves were removed from the extract and dried in an oven at 60 °C for 1 h to determine their dry weight (3−6 mg per five blackgrass leaves, 6−18 mg per five cress plants, depending on their age).

Ethylation of Haloxyfop-Acid, Cleanup. To analyze haloxyfop-acid and fluazifop-acid by GC−MS, it was necessary to derivatize. Ethylation was considered appropriate as it allowed, in contrast to methylation, distinction of haloxyfop-methyl and -acid, analyzed as ethyl ester. Prior to derivatization, the methanolic extract was reduced to a volume of ∼100 μL on a heating plate (80 °C) with a gentle flow of N_2. The concentrated extract was acidified with 2−3 drops of 0.5% trifluoroacetic acid in methanol. Haloxyfop-acid and fluazifop-acid were then derivatized by addition of ∼750 μL of diazoethane in methyl *tert*-butyl ether (MTBE).

Diazoethane was prepared as follows. In a 50 mL glass bottle, 1.5 g of N-nitroso-N-ethylurea was dissolved in 30 mL of MTBE and placed on ice. To this solution, 6 mL of 40% aqueous potassium hydroxide was added, and the bottle was loosely covered so that gas formed during reaction could escape. After gas formation had ceased (∼1 h), the orange colored organic layer containing the diazoethane was transferred to a 50 mL glass bottle containing a few potassium hydroxide pellets and stored in the freezer (−20 °C) until use (usually within 1 week). Note that diazoethane is toxic by inhalation and by contact with skin or eyes, and thus, all handling was strictly done in a fume hood. Likewise, the compound is explosive, and contact with sharp glassware must be avoided. Prior to disposal of any waste, excess diazoethane was reacted with dilute acetic acid in methanol.

No methyl ester was formed from ethylation of the acid in methanol/MTBE. After derivatization and evaporation of the solvents, the residues were dissolved in ∼100 μL of ethyl acetate. For cleanup, the derivatized extract was passed through a silica mini-column (5 mm i.d. Pasteur pipet filled with ∼60 mm of silica gel 60, Merck, Darmstadt, Germany, dried at 140 °C for 24 h, deactivated with 5% water, and topped with 10 mm of anhydrous sodium sulfate). The analytes were eluted with 10 mL of ethyl acetate. The eluate was carefully concentrated with a gentle flow of N_2 and mild heating to a final volume of 1 mL and quantitatively transferred into a 2 mL autosampler glass vial.

Enantioselective GC−MS Analysis. The enantiomers of haloxyfop-methyl, haloxyfop-ethyl, and the corresponding internal standards (fluazifop-methyl and fluazifop-ethyl) were separated on a chiral GC column coated with permethyl-β-cyclodextrin (15%) in OV1701 (BGB 171, 20 m, 0.25 mm i.d., 0.15 μm film, BGB, Boeckten, Switzerland). GC conditions were as follows: 1 μL split/splitless injection (240 °C, initial 48 s splitless); temperature program, 70 °C, 2 min isothermal, 25 °C/min to 150 °C, 1 °C/min to 200 °C, isothermal hold at 220 °C; constant flow, 2 mL/min helium. The GC was coupled to a Voyager single quadrupole MS (Finnigan, Manchester, UK) operated in electron impact ionization (70 eV, 180 °C) and selected-ion-monitoring mode. Haloxyfop-methyl, haloxyfop-ethyl, fluazifop-methyl, and fluazifop-ethyl were quantified using the ions *m*/*z* 375 (316 and 288 for confirmatory purposes), 389 (316, 288), 341 (282, 254), and 355 (282, 254), respectively. The enantiomer resolution of the four analytes was ∼1.3, 1.2, 1.4, and 1.3, respectively (defined as $R = 2\,(t_{r2} - t_{r1})/(w_1 + w_2)$, where t_{ri} is the retention time of enantiomer i, and w_i is the width at the base of peak i). A representative chromatogram of a plant extract is shown in Figure 1. The elution order of the enantiomers was *R* prior to *S* for all compounds as determined with pure reference substances. Quantification was based on peak area ratios relative to the internal standards

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J. Agric. Food Chem. 2015, 63, 2591−2596

and in reference to suitable standard solutions of the racemic compounds. For more details on the analytical method, see ref 15.

RESULTS AND DISCUSSION

Rapid Ester Cleavage of Haloxyfop-Methyl to Haloxyfop-Acid in Blackgrass. After application on blackgrass, *rac*-haloxyfop-methyl was rapidly metabolized to haloxyfop-acid, the herbicidally active compound (Figure 3a). Within 24 and

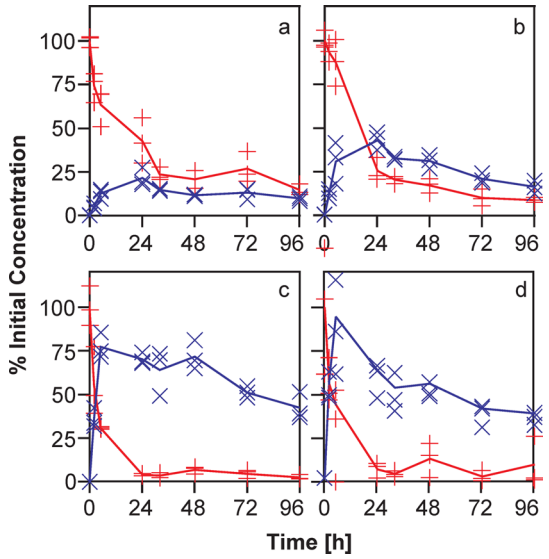

Figure 3. Metabolism of haloxyfop-methyl and haloxyfop-acid in blackgrass (a,b) and garden cress (c,d) after treatment with *rac*-haloxyfop-methyl (a,c) or *R*-haloxyfop-methyl (b,d). Symbols depict individual concentration data for haloxyfop-methyl (+) and haloxyfop-acid (×); the lines connect mean concentrations of three replicates. Residues are based on plant dry weight and those of the metabolite in parent equivalents. The enantiomer composition remained constant throughout the experiments.

32 h, concentrations of haloxyfop-methyl decreased by more than 50% and 75%, respectively (residues based on plant dry weight). Residues of haloxyfop-acid reached a maximum of ∼22% (parent equivalents) 24 h after treatment, which suggested that the acid was further metabolized (or that other primary metabolites were formed besides). Moreover, plant growth also resulted in somewhat lower concentrations (dilution effect, less than a factor of two).

After 32 h, metabolism of parent and metabolite slowed down considerably (Figure 3a). At the same time, the treated grass showed first withering symptoms (see Figure 2 for grass treated with *R*-haloxyfop-methyl). Within about 1 week, the leaves turned completely brown. Concurrently, the chlorophyll a content decreased from ∼20 mg/g (dry weight) before treatment to less than 3 mg/g 96 h after treatment (data not shown). In the control experiment, where blackgrass was treated with blank formulation, without the active compound, no striking herbicidal injuries were observed. Only the leaf tips slightly turned brown, whereas untreated grass showed no symptoms at all (Figure 2).

Nonenantioselective Metabolism of Haloxyfop-Methyl and Haloxyfop-Acid in Blackgrass. The enantiomer composition of haloxyfop-methyl in blackgrass did not change within the 96-h duration of the experiment (Figure 1). The enantiomer fraction (defined as EF = [*R*]/([*R*] + [*S*]), where [*R*] and [*S*] are the concentrations of *R*- and *S*-haloxyfop-methyl, respectively) varied between 0.48 and 0.51, which indicated that metabolism was nonenantioselective. The enantiomer composition of the acid metabolite also remained constant within 96 h, which suggested that not only the formation, but also the further transformation was nonenantioselective (Figure 1). EF values ranged from 0.47−0.54. Enantiomer fractions showed clearly less variation than the concentrations of the individual enantiomers since it was not possible to apply the test substance in a way to ensure that all plants received exactly the same amount of herbicide (see Materials and Methods section), whereas the enantiomer composition in the application solution was, of course, reproducible.

"Chiral Stability" of Haloxyfop-Methyl and Haloxyfop-Acid in Blackgrass. After application of *rac*-haloxyfop-methyl on blackgrass, residues thus remained racemic. However, the observed, constant enantiomer fractions of 0.5 may, in principle, also be the result of a fast racemization in the leaves. To investigate a possible interconversion of enantiomers, further blackgrass plants were treated with pure *R*-haloxyfop-methyl. These experiments confirmed that no *S*-haloxyfop-methyl and no *S*-haloxyfop-acid were formed from the corresponding *R*-enantiomers. The plant extracts only contained traces of the *S*-enantiomers that were already present in the reference material (∼1%). The opposite process, conversion of the *S*- into the *R*-enantiomers, could also be excluded since metabolism was nonenantioselective in experiments with the racemic herbicide. Therefore, additional experiments with pure *S*-haloxyfop-methyl were not performed as they were not considered necessary.

Appearance and degree of necrosis symptoms and reduction of the chlorophyll a content were similar after treatment with *R*-haloxyfop-methyl as in the experiments with *rac*-haloxyfop-methyl (Figure 2). Note that the same amount of the *R*-enantiomer was applied (*R*-haloxyfop is the herbicidally active form).

Concentration-Dependent Formation of Haloxyfop-Acid. The rate of degradation/dissipation of *R*-haloxyfop-methyl was similar to that of *rac*-haloxyfop-methyl (Figure 3a,b). However, in the experiments with pure *R*-haloxyfop-methyl, concentrations of *R*-haloxyfop-acid reached a maximum of ∼43% (parent equivalents), which is twice as high as in the corresponding experiments with the racemic compound (∼22%). In a further experiment, where blackgrass was treated with haloxyfop-methyl, composed of [*R*]:[*S*] = 2:1, the maximum concentration of the acid was just between the above values, ∼32% (data not shown). A simple explanation for these differences in relative formation cannot be given as it is obviously not due to preferential conversion of enantiomers nor due to obvious differences in senescence. Formation of the acid may, however, depend on the concentration of applied haloxyfop-methyl. Indeed, when considering further data from experiments with garden cress (see below), which were conducted with clearly lower herbicide concentrations, a good correlation was found between maximum formation of haloxyfop-acid and initial concentration of haloxyfop-methyl in the leaves (Figure 4). Nonetheless, the underlying biochemical explanation for this observation remains unclear.

Phytotoxic Injuries on Garden Cress Caused by the Blank Formulation. Haloxyfop has selective activity on true grasses (*Poaceae*) but no activity on other monocotyledonous and dicotyledonous plants.[12] Garden cress was selected as representative dicotyledonous plant since it has a high

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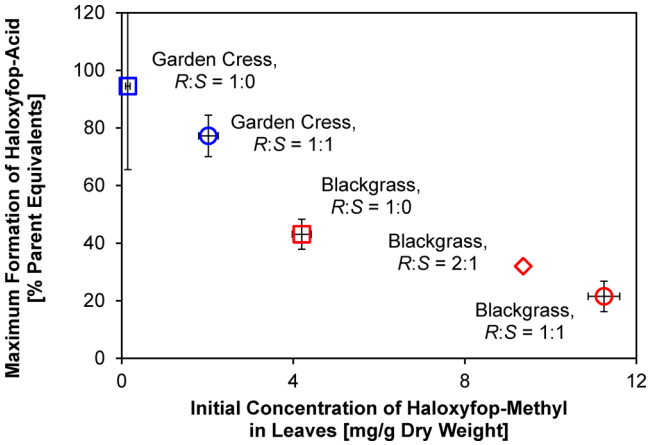

Figure 4. Maximum formation of haloxyfop-acid in experiments with blackgrass and garden cress treated with haloxyfop-methyl at different concentrations and enantiomer composition in the application solution; thus, different initial concentration in the leaves. Error bars indicate the standard deviation of three replicate experiments. The maximum formation of haloxyfop-acid is given in parent equivalents in relation to the mean initial concentration in the leaves. Note that these values are not corrected for plant growth.

germination rate and can easily be cultivated. In preliminary experiments, garden cress was treated with the same solution/emulsion of formulated haloxyfop-methyl as used for blackgrass. However, the plants showed severe withering symptoms already after 24 h. The control experiment, in which plants were treated with blank formulation only, indicated that the observed phytotoxicity was caused by the formulation rather than by haloxyfop. The amount of water used to dilute the formulated herbicide was chosen as it would be done in agricultural practice, but we assume that with the treatment procedure (dipping of leaves in the application solution to avoid uptake by roots), considerably higher amounts of active substance and adjuvants were finally applied to the leaves than with a spray application under normal field conditions. Therefore, a more diluted application solution (8×) was prepared for the definitive experiments with garden cress. Phytotoxic injuries were now clearly reduced, and only the most tender leaves showed minor symptoms. After 96 h, treated (formulation with and without haloxyfop-methyl) and untreated cress plants looked quite similar (Figure 2).

Nonenantioselective Metabolism of Haloxyfop-Methyl and Haloxyfop-Acid in Garden Cress. In garden cress, metabolism of haloxyfop-methyl was faster than in blackgrass, with a half-life of ∼3 h (Figure 3c,d for treatment with *rac*- and *R*-haloxyfop-methyl). However, the residues of the methyl-ester did not decline further between 24 and 96 h after treatment. Apparently, this small residue fraction (<10%) is not available for metabolism, for example, it may be associated with lipophilic waxes in the cuticle of the leaves.

Maximum formation of the acid metabolite was observed already 5 h after treatment (Figure 3c,d). Up to ∼95% (average of three replicates) was formed from *R*-haloxyfop-methyl and ∼77% from the racemic compound. The correlation shown in Figure 4 suggests that maximum formation depends on the initial concentration of haloxyfop-methyl in the leaves (see Discussion section above). In experiments with garden cress, variability of the measured concentrations in the three replicates was quite high, in particular at early sampling times (Figure 3c,d). In bent leaves (Figure 2), small droplets of application solution adhered instead of a homogeneous, thin film, and consequently, application to cress leaves was less reproducible than with blackgrass. After 5 h, the concentration of the acid slowly decreased (Figure 3c,d) not only due to further metabolism, but also of course due to plant growth.

In experiments with *rac*-haloxyfop-methyl applied on garden cress, the enantiomer fractions ranged from 0.44−0.57 for the parent compound and from 0.47−0.52 for the acid metabolite. The EF values thus slightly deviated from 0.50, in particular, at low residue levels of haloxyfop-methyl but without a clear temporal trend. Again, in experiments with *R*-haloxyfop-methyl, no formation of *S*-haloxyfop-methyl and *S*-haloxyfop-acid was observed, which suggests that metabolism of parent and metabolite was not or only slightly enantioselective and that no conversion of enantiomers occurred.

Remarkable Contrast Between Chiral Stability in Plants and Rapid Interconversion of Enantiomers of Haloxyfop-Acid in Soil. Our experiments with blackgrass and garden cress showed that the enantiomer composition of haloxyfop-methyl and its major metabolite haloxyfop-acid was not changed in plants either by enantioselective metabolism or by interconversion of enantiomers. Blackgrass is susceptible to the herbicide, whereas the dicotyledonous garden cress is not susceptible. These findings with regard to chiral stability can thus probably be generalized to most mono- and dicotyledonous plants. Consequently, it may be expected that residues in crops have the same enantiomer composition as in the applied product provided that the compound is applied postemergent and primarily taken up through leaves. In rotational and succeeding crops, uptake of haloxyfop-acid from soil occurs via roots, and potential residues should then predominantly contain the *R*-enantiomer. However, because of the relatively fast degradation of the acid in soils (typical half-lives under field conditions, 5−27 days[25]), measurable residues would only be expected when the succeeding crop is planted shortly after application of the herbicide, for example, after failure of the preceding crop.

Chiral stability in plants strongly contrasts the observed, rapid conversion of *S*- to *R*-haloxyfop-acid in soils[12,15] and mammals.[25] In soils, this transformation is biologically mediated (probably via deprotonation at the methine carbon[7,15]), whereas in plants, apparently, no such enzymes are present or accept haloxyfop-acid as substrate. It was speculated that in plants, enzyme-mediated interconversion of enantiomers of chiral pesticides is less common than in soil,[7] but at the same time, it was also stated that more plant metabolism studies would be needed to support such a conclusion.

The present study shows that the use of the herbicidally active, pure *R*-haloxyfop-methyl rather than the racemic compound is advantageous from an environmental perspective. It allows 50% lower application rates at similar field performance, results in lower residues in crops and environment, and may lead to fewer side effects on nontarget organisms.

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Notes

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