Sektion 4: Modellierung
Section 4: Modelling

Model for metabolic resistance against ALS inhibitors

Ein Modell für die metabolische Resistenz gegen ALS Inhibitoren

Otto Richter[1]*, Dirk Langemann[2] and Roland Beffa[3]

[1*]Technische Universität Braunschweig, Institute of Geoecology, Langer Kamp 19c, 38106 Braunschweig, Germany
[2]Technische Universität Braunschweig, Institute for Computational Mathematics, Pockelsstr. 14, 38106 Braunschweig, Germany
[3]BAYER Crop Science
*Corresponding author, o.richter@tu-bs.de

DOI 10.5073/jka.2014.443.043

Abstract

Due to herbicide selection pressure metabolic resistance has evolved in many weed species. In this paper we analyse the interaction between the branched chain amino acid (BBC) pathway and detoxifying pathways for herbicide breakdown. The four phase detoxification pathway of herbicides comprising the action of P450, GST, glycosyltransferase and ABC transporter is modelled by a system of coupled enzyme kinetic reactions represented by nonlinear differential equations. The herbicide under consideration inhibits the enzyme ALS, which is the key enzyme for the biosynthesis of branched amino acids. For the kinetics of ALS a Monod approach is employed with a binding site for the inhibitor. Synthetic and detoxification pathways are coupled. The model is used to study the production of branched amino acids under the action of ALS inhibitors for different structures and modes of action of the detoxification pathway. The model is capable of generating typical dose response curves and their shift in dependence of the activity pattern of the enzymes of the detoxification pathway of the inhibitor.

Keywords: ALS inhibitor, branched chain amino acid biosynthesis, enzyme kinetics, metabolic resistance, metabolic network

Zusammenfassung

Metabolische Resistenz beinhaltet die Fähigkeit eines Organismus, toxische Substanzen abzubauen, was zu einer Verschiebung von Dosis-Wirkungskurven führt. In diesem Beitrag wird die Dynamik des Zusammenspiels der Biosynthese der verzweigtkettigen Aminosäuren mit dem Abbauweg für einen ALS Inhibitor anhand eines mathematischen Modelles untersucht. Dieses besteht aus einem System von gekoppelten enzymatischen Reaktionen, dargestellt durch nichtlineare Differentialgleichungen. Die ALS Kinetik wird durch einen Monod Ansatz beschrieben mit Pyruvat als Substrat und einer Bindungsstelle für einen nichtkompetitiven Inhibitor. Das Modell liefert typische Dosis Wirkungskurven und die Verschiebung des ED_{50}-Wertes zu höheren Dosen in Abhängigkeit vom Aktivitätsmuster des Abbauweges für den Inhibitor.

Stichwörter: ALS Inhibitor, Biosynthese von Aminosäuren, Enzymkinetik, metabolische Netzwerke, metabolische Resistenz

Introduction

Herbicide resistance has become a major issue for many weeds (BECKIE, 2006). There are two major groups of resistance mechanisms:

Alteration of the herbicide site of action (target site resistance)

Non-target site resistance comprising e.g. enhanced metabolism, sequestration and restricted translocation

Metabolic resistance can be characterized by enzymatic degradation of pesticides within the target organism (PETIT *et al.*, 2010) resulting in a gradual shift of the dose response curve (TAL *et al.*, 2000; DALY and FISK, 1992). The branched chain amino acids isoleucine, valine and leucine are

synthesized by plants, algae, fungi, bacteria and archaea (McCourt and Duggleby, 2006). The first key enzyme in the biosynthetic pathway is the acetolactate synthase (ALS, EC 4.2.1.16). Thus, ALS inhibitors such as chemicals of the sulfonylurea and imidazolinone families suppress the biosynthesis of the branched chain amino acids. In the case of metabolic resistance, the biosynthetic pathway and the detoxifying pathway of the inhibitor interact.

Material and Methods

Notations

ALS: Acetolactate synthase EC 4.1.3.18

P450: Cytochrome P450 monooxygenase

GST: Glutathion-S-transferase EC 2.5.1.18

ABC: ATP binding cassette transporter

KARI: Keto-acid-reductoisomerase EC1.1.86

DH: Dihydroxy-acid dehydratraseEC4.2.1.19

TA: Transaminase EC2.6.1.42

$I_1,...,I_4$: ALS inhibitor and its metabolites

$x_1,..x_5$: metabolites of isoleucine biosynthesis

$y_1,...,y_5$: metabolites of valine biosynthesis

$z_1,...,z_4$: metabolites of leucin biosynthesis

v_i: enyzmatic law of reaction i

K_m: Michaelis constant

K_i: general: binding constants

K_{eq}: equilibrium constant

n_{ij}: elements of stochiometric matrix

V_{max}: maximal velocity

S_i: substrate concentration

L: allosteric constant

General model framework

The dynamics of metabolic networks consisting of m substances and r enzymatic reactions is modelled by a system of differential equations for the metabolite concentrations of the form

$$\frac{dS_i}{dt} = \sum_{j=1}^{r} n_{ij} v_j(S_1,..,S_m) \qquad i = 1,...,m \quad (1)$$

where the coefficients n_{ij} are the elements of the stochiometric matrix and the v_j are the reaction velocities expressed by enzymatic laws which may depend on other metabolite concentrations of the network. In our model we employ the following enzymatic forms:

Michaelis Menten kinetics:

$$v_i(S) = \frac{V_i S}{K_i + S} \qquad (2)$$

The index *i* denotes the enzyme. Reactions involving cofactors such as ATP or are assumed to be saturated with respect to the cofactors.

Monod model for one substrate and two inhibitors

This model applies to the key enzyme acetolactate synthase ALS. A Monod model for ALS kinetics was published by Vinogradov *et al.* (2006) and implies two active binding sites for pyruvate and

two binding sites for valine, which is one of the end product of branched chain biosynthesis. ALS is feed back regulated by valine, which acts as an inhibitor. This model was extended to a further inhibitor, a herbicide, which is assumed to have also two binding sites.

$$v_{ALS}(S,V,I) = \frac{V_{\max}\alpha(1+\alpha)}{L'(1+c_s\alpha^2)+(1+\alpha)^2}, \quad \alpha = \frac{S}{K_s}, \quad \beta = \frac{V}{K_V}, \ \gamma = \frac{I}{K_I} \qquad (3)$$

$$L' = L\frac{(1+c_V\beta)^2(1+c_I\gamma)^2}{(1+\beta)^2(1+\gamma)^2}$$

Figure 1 shows the ALS reaction velocity in dependence of herbicide concentrations at different levels of pyruvate.

Branched chain amino acids and detoxification pathway

The metabolic network for branched chain amino acid biosynthesis was extended to the degradation pathway of the herbicide. The latter comprises the following phases:

Phase I: Oxidation by cytochrome P450 Monooxygenase

Phase II: Glutathione conjugation catalysed by glutahione-S-transferase (GST)

Phase III: vacuolar transport by ATP binding cassette transporter (ABC) and subsequent breakdown. The combined reaction schemes are shown in figure 2.

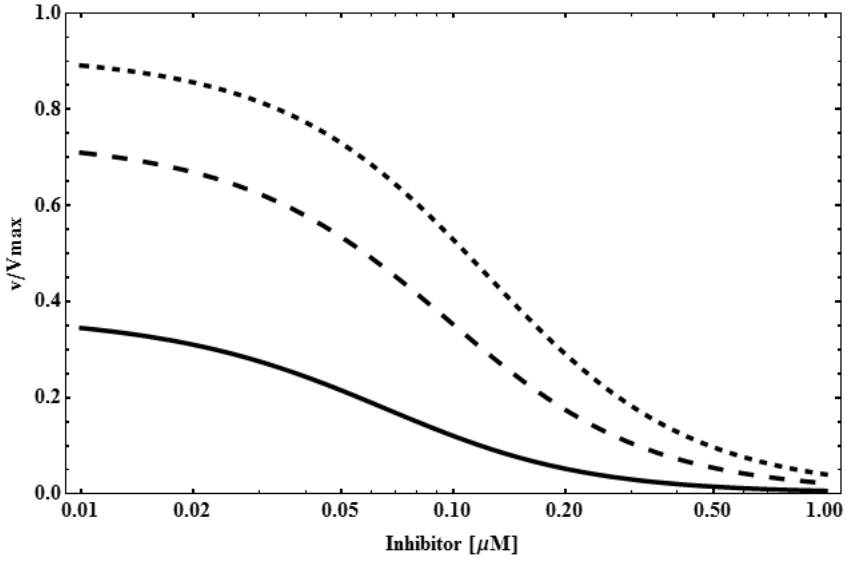

Fig. 1 Velocity of ALS dependent on inhibitor concentrations for different levels of pyruvate.
(_____) 2mM pyruvate, (- - -) 10 mM pyruvate, (---) 50 mM pyruvate**.**

Abb. 1 *ALS Kinetik in Abhängigkeit eines Inhibitors bei drei Pyruvatkonzentrationen.*

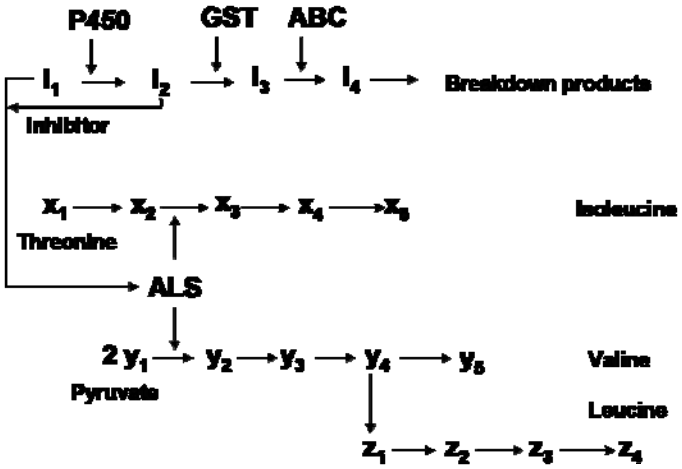

Fig. 2 Branched chain amino acid pathway and pathway for the breakdown of a ALS inhibitor. Note that phase I products may still act as inhibitor to ALS (WERCK-REICHARDT *et al.*, 2000).

Abb. 2 *Syntheseweg der verzweigtkettigen Aminosäuren ergänzt durch den Abbauweg eines ALS Inhibitors. Man beachte, dass Phase I Reaktionsprodukte noch eine inhibitorische Wirkung haben können (WERCK-REICHARDT et al., 2000).*

The following equations describe the dynamics of the detoxifying pathway under the assumption that the enzymes are saturated with respect to cofactors ATP and GST.

$$\frac{dI_1}{dt} = -\frac{V_{P450} I_1}{I_1 + K_P} + \sum D_i \delta(t - t_i) \qquad (4)$$

Oxidation of herbicide by cytochrome P450 monooxygenase (1.term) and pulse uptake under the dosage regimen {D_i, ti}, i. e. concentration D_i enters the system at time t_i.

$$\frac{dI_2}{dt} = \frac{V_{P450} I_1}{I + K_P} - \frac{V_{GST} I_2}{I_2 + K_G} \qquad (5)$$

Gluthatione conjugation of oxidized herbicide

$$\frac{\mathrm{dI_3}}{\mathrm{dt}} = \frac{\mathrm{V_{GST} I_2}}{\mathrm{I_2 + K_G}} - \frac{\mathrm{V_{ABS} I_3}}{\mathrm{I_3 + K_{ABC}}} \qquad (6)$$

Vacuolar transport of oxidized and conjugated herbicide

$$\frac{dI_4}{dt} = \frac{V_{ABS} I_3}{I_3 + K_{ABC}} - k_b I_4 \qquad (7)$$

Vacuolar breakdown

In this study we consider the valine pathway, which is modelled by the following equations system

$$\frac{dy_1}{dt} = -2v_{ALS}(y_1, I, y_5) - k' y_1 \qquad (8)$$

Pyruvate: 1. term ALS reaction, 2. term unspecific consumption in other reactions

$$\frac{dy_2}{dt} = v_{ALS}(y_1, I, y_5) - v_{KARI}(y_2) \qquad (9)$$

2-acetolactate

$$\frac{dy_3}{dt} = v_{KARI}(y_2) - v_{DH}(y_3) \qquad (10)$$

2,3-dihydroxy-3-methylvalerate

$$\frac{dy_4}{dt} = v_{DH}(y_3) - v_{TA}(y_4) \qquad (11)$$

2-ketoisovalerate

$$\frac{d\,y_5}{dt} = v_{TA}(y_4) \qquad (12)$$

valine

The inhibitor I comprises the herbicide I_1 and the phase I product I_2, which may also act as an inhibitor to ALS.

$$I = I_1 + a\,I_2 \qquad (13)$$

where the factor a weighs the rest toxicity of I2.

Results

Single runs

The model is capable of simulating the time course of concentrations of the BBC reaction chain following the application of an ALS inhibitor. The following figures show the time courses of ALS activity and concentrations of metabolites both of the biosynthetic pathway of valine and the detoxifying pathway of the inhibitor.

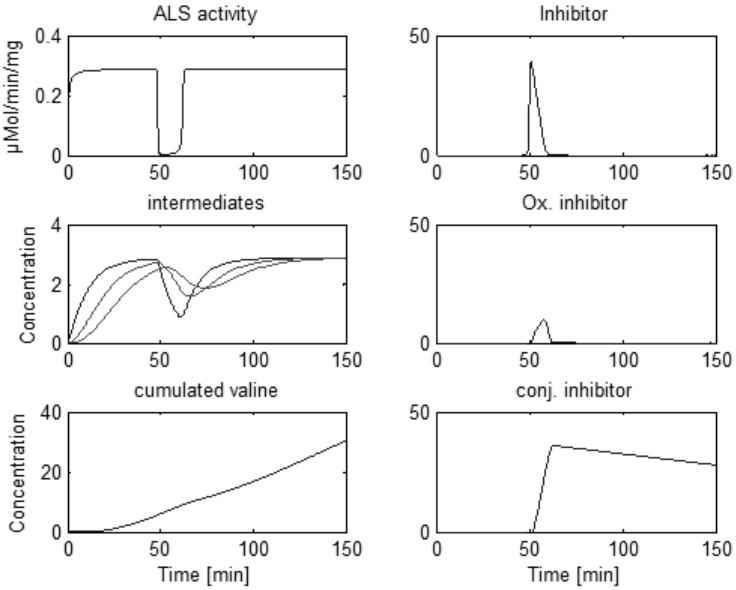

Fig. 3 Response of the valine pathway to the addition of a ALS inhibitor in the case of high metabolic resistance. The inhibitor is immediately removed and acts only a short time interval.

Abb. 3 *Dynamisches Verhalten der Valin Biosynthese nach Zugabe eines ALS Inhibitors im Falle einer stark ausgeprägten metabolischen Resistenz. Der Inhibitor wird schnell abgebaut und kann nur eine kurze Zeit wirken.*

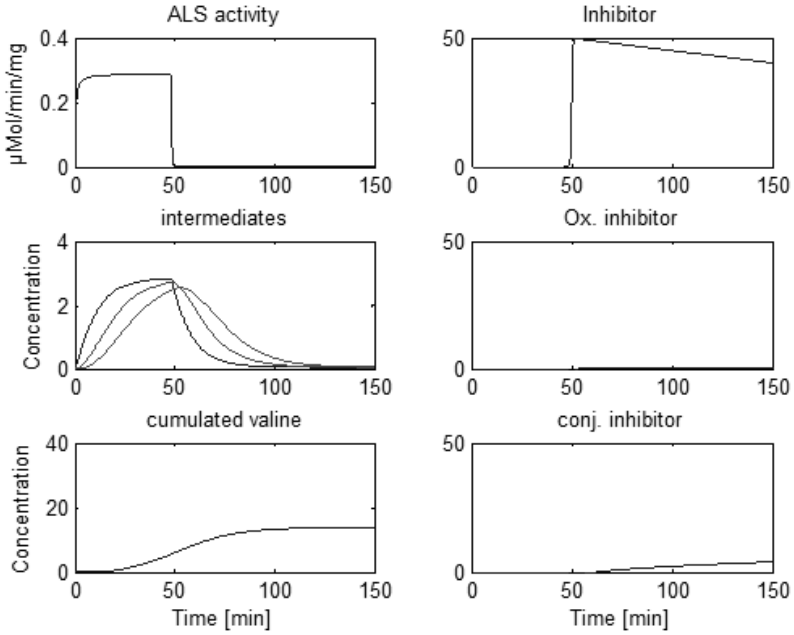

Fig. 4 Response of the valine pathway to the addition of a ALS inhibitor in the case of low metabolic resistance. Removal of the inhibitor is slow and ALS activity is prevented for a long time interval.

Abb. 4 *Dynamisches Verhalten der Valin Biosynthese nach Zugabe eines ALS Inhibitors im Falle einer schwach ausgeprägten metabolischen Resistenz. Der Inhibitor wird nur langsam abgebaut und kann daher eine nachhaltige Störung des Metabolismus bewirken.*

The inhibitor is added at time t = 50 min. Figure 3 shows time courses of concentrations of metabolites of the valine pathway together with metabolites of the herbicide degradation pathway under a high detoxifying capacity, i.e. for high V_{max} values of P450 and GST. The disruption of valine biosynthesis is only short and the system soon returns to its previous mode of action. In the case of low metabolic resistance, the damage of valine synthesis is prolonged (Fig. 4). Metabolites of the detoxifying pathway might be still toxic, which was shown to be the case for products of the P450 reaction (WERCK-REICHARDT *et al.,* 2000). To demonstrate this effect we have simulated a system with high P450 activity but with low activity of the GST reaction. As can be seen from the simulations results shown in Figure 5, the primary inhibitor soon disappears, whereas the product accumulates causing a sustained disruption of valine biosynthesis. Therefore, a fine tuning of the detoxification pathway is necessary for efficient metabolic resistance.

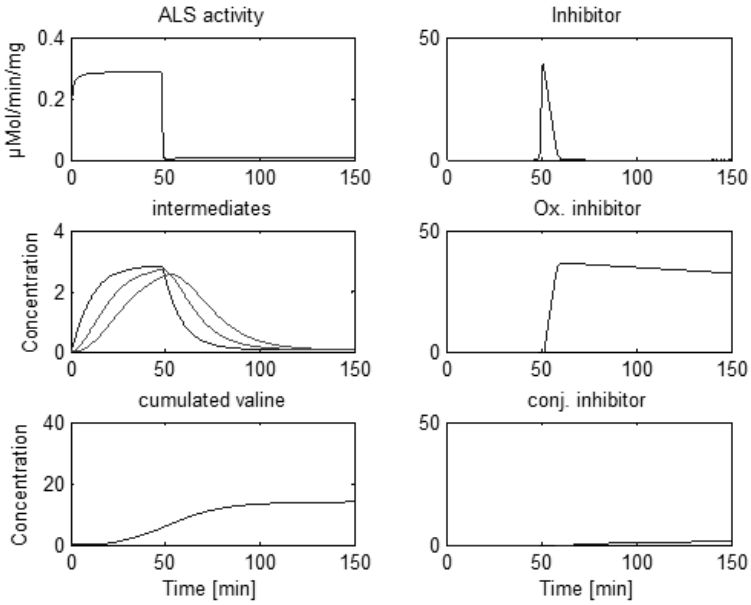

Fig. 5 Response of the valine pathway to the addition of a ALS inhibitor in the case of high P450 but low GST activity. Although the degradation of the primary inhibitor is fast, the rest toxicity of the product causes sustained disruption of the ALS reaction.

Abb. 5 *Dynamisches Verhalten der Valin Biosynthese nach Zugabe eines ALS Inhibitors im Falle einer schwach ausgeprägten Aktivität des Enzyms GST. Obwohl der primäre Inhibitor schnell abgebaut wird, sorgt die Resttoxizität des Produktes für eine anhaltende Unterbrechung der ALS Reaktion.*

Dose response curves

Dose response curves are obtained by performing "experiments" with the model system, i.e. the system of differential equations is solved for a large number of initial herbicide concentrations at a given pyruvate initial concentration. The response is measured as the cumulated production of valine. Response curves were generated for different activities of the detoxifying pathway expressed by the Vmax values of P450 and GST. For each dose response curve 6000 initial value problems were solved. Some results are shown in Figure 6. Because of the (assumed) rest toxicity of the oxidized inhibitor detoxification is sensitive to the coordination of both enzymes as was also shown in the previous simulation studies presented in figures 3 to 5. The form of the simulated dose response curves are similar to measured dose response curves and can be described by the by the log-logistic model (KNEZEVIC *et al.,* 2007)

$$S(d) = \frac{1}{1 + \exp[b(\log(d) - \log(e)]} \quad (14)$$

S(d) is defined as the ratio of maximum cumulated valine production (no inihibitor) and valine production under the presence of the inhibitor at concentration d, the parameter b determines the steepness of the curve and e is the ED_{50} value, i.e. $S(e)=0.5$. Figure 7 shows the fit of simulated data obtained by the model with superimposed normal error to the log-logistic model. Although the fit looks quite well with an adjusted R^2 of 0.99, one can see slight systematic deviations at higher doses.

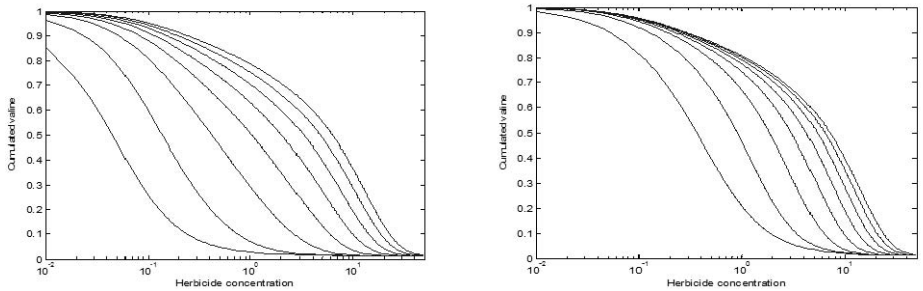

Fig. 6 Series of dose response curves for increasing activity of P450 with constant activity of GST (left) and for increasing activity of GTS with constant activity of P450 (right).

Abb. 6 *Schar von Dosis-Wirkungskurven für wachsende Aktivität von P450 bei konstanter Aktivität von GSt (links) und für wachsende Aktivität von GST bei konstanter Aktivität von P450 (rechts).*

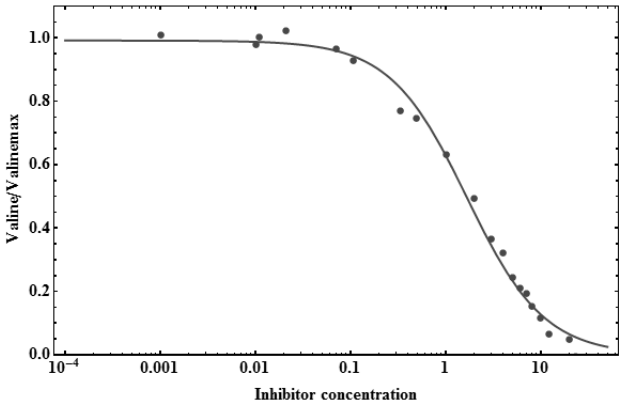

Fig. 7 Fit of simulated data with superimposed normal error to the log-logistic dose response curve.

Abb. 7 *Anpassung simulierter Daten mit normal verteiltem Fehler an die log-logistische Dosis- Wirkungsfunktion.*

Discussion

The model reproduces the dynamics of valine production under the influence of an ALS in a qualitative manner. It allows the study of metabolic resistance at the level of biochemical reaction pathways. The simulations show that metabolic resistance is effective if both enzymes, P450 and GST are present and act in a concerted manner. For quantitative analyses, model parameters pertaining to one organism should be available. Up to now, we have taken enzymatic parameters from the literature involving different species. Apart from a realistic parameterization the model has to be extended to incorporate also the leucine and isoleucine pathways. To analyse the evolution of metabolic resistance, the model can be integrated into a genetic model for polygenic inheritance and population genetics such as the approach of RENTON *et al.* (2011) identifying the abstract model parameters with parameters of the detoxifying pathway of the inhibitor.

References

BECKIE, H. J., 2006: Herbicide-Resistant Weeds: Management Tactics and Practices. Weed Technology **20**, 793–814.

DALY, J. C. and J. H. FISK, 1992: Inheritance of metabolic resistance to the synthetic pyrethroids in Australian Helicoverpa armigera. Bulletin of Entomological Research **82**, 5-12.

KNEZEVIC, S. Z., J. STREIBIG and C. RITZ, 2007: Utilizing R Software Package for Dose-response Studies: The Concept and Data Analysis. Weed Technology **21**, 840-848.

MCCOURT, J. A. and R. G. DUGGLEBY, 2006: Acotohydroxyacid synthase and its role in the biosnyrhetic pathway for brachend-chain amino acids. Amino Acids **31**,173-210.

PETIT, C., B. DUHIEU, K. BOUCANSAUD and C. DÉLYE, 2010: Complex genetic control of non-target-site-based resistance to herbicides inhibiting acetyl-coenzyme A carboxylase and acetolactate-synthase in Alopecurus myosuroides Huds. Plant Science **178**, 501–509.

POWLES, S. and Q. YU, 2010: Evolution in Action: Plants Resistant to Herbicides. Annual Review of Plant Biology **61**, 317-349.

RENTON, M., A. DIGGLE, S. MANANLI and S. POWLES, 2011: Does cutting herbicide threaten the sustainablity of weed management in cropping systems? Journal of theoretical Biology **283**, 14-27.

TAL, A., E. KOTOULA-STYKA and B. RUBIN, 2000: Seed-bioassay to detect grass weeds resistant to acetyl coenzyme A carboxylase inhibiting herbicides. Crop Protection **19**, 467–472.

VINOGRADOV, V., M. VYAZMENSKYA, S. ENGEL, I. BELENKYA, A. KAPLUNA, O.KRYUKOVA, Z. BARAKA and D. M. CHIPMAN, 2006: Acetohydroxyacid synthase isozyme I form E. Coli has unique catalytic and regulatory properties. BBA **1760**, 356-363.

WERCK-REICHHART, D., A. HEHN and L. DIDIERJEAN, 2000: Cytochromes P450 for engineering herbicide tolerance. Trends in plant science **5**, 3, 116-123.