Hazardous doses of the herbicide imazamox in wild plant species and oilseed rape cultivars

Schädliche Dosen des Herbizids Imazamox bei verschiedenen Wildpflanzenarten und Rapssorten

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DOI: 10.5073/jka.2012.434.045

Summary

Imidazolinones and crops resistant to these herbicides have successfully been introduced recently in some European countries. Imazamox has a high efficacy, moderate persistence and ecotoxicity, but data on hazardous doses (HD) in non-target plants and species sensitivity distributions (SSDs) are still scarce. To screen a larger variety of plant taxa in standardized vegetative vigor tests, 22 wild plant species and 14 oilseed rape (OSR) cultivars were sown into trays filled with a standard soil. When seedlings had reached the three-to-four leaf stage, plants were exposed to a single application of the herbicide Bolero® using a commercial pump sprayer. The five treatments corresponded to rates of 0 (control), 0.4, 4, 20 and 40 g/ha of imazamox with latter representing the recommended dose in Switzerland. Two weeks after the application, five plants per treatment were sub-sampled at random and visible injuries and fresh weights were recorded as endpoints for herbicidal effects. Dose-response curves and effective doses (EDs) were fitted using the drc package of the statistical software R and SSDs were obtained using ETX2.0. ED_{50} varied between 0.006 and 37 g/ha of the active ingredient in *Nigella arvensis* and *Brassica rapa*. Several dose-response curves indicated hormetic effects at a hundredth of the recommended field dose. In OSR, exposure to the field rate (40 g/ha) resulted in growth reductions between 28 and 97 % in the non imazamox-tolerant cultivars and in growth stimulations of up to 20 % in imazamox-tolerant cultivars. Responses were unrelated to leaf thickness, growth rates and the taxonomy of the tested species. Hazardous doses were 0.32 g/ha for HD5 and 3.9 g/ha for HD_{50} indicating that 50 % of the non-target plants would be affected at a tenth of the recommended dose. Based on general herbicide drift values the results suggest that potentially adverse effects may be expected up to a distance of 4 m offsite.

Keywords: Clearfield, non-target plants, plant growth tests, plant functional types

Zusammenfassung

Imidazolinone und gegen diese Herbizide resistente Sorten einiger Kulturarten wurden in den letzten Jahren in einigen Europäischen Ländern erfolgreich eingeführt. Imazamox hat eine hohe Wirksamkeit, moderate Persistenz und Ökotoxizität, aber es gibt bislang nur wenige Informationen zu Schaddosen (hazardous doses, HD) in Nichtzielpflanzen und Art-Sensitivitäts-Spektren (species sensitivity distributions, SSDs). Um eine größere Anzahl von Pflanzenarten in standardisierten Wachstumstests zu überprüfen, wurden 22 Wildpflanzenarten und 14 Rapssorten in mit Standardboden gefüllten Schalen ausgesät. Bei Erreichung des Drei- bis Vierblattstadiums wurden die Pflanzen einmal mittels Pumpsprüher mit dem Herbizid Bolero® behandelt. Die fünf eingesetzten Behandlungen entsprachen den Dosen 0 (Kontrolle), 0.4, 4, 20 und 40 g AS/ha, wobei die letztere in der Schweiz die empfohlene Aufwandmenge darstellte. Zwei Wochen nach der Applikation wurden fünf Pflanzen pro Behandlung nach dem Zufallsprinzip geerntet, wobei sichtbare Schäden und Frischgewichte als Wirkkriterien betrachtet wurden. Dosis-Wirkungs-Kurven und effektive Dosen (EDs) wurden mit Hilfe des drc-Pakets mit der Statistik-Software R berechnet und SSDs wurden mittels ETX2.0 abgeleitet. Die ED_{50}-Werte schwankten zwischen 0.006 und 37 g/ha der aktiven Substanz in *Nigella arvensis* und *Brassica rapa*. Viele der Dosis-Wirkungs-Beziehungen deuteten hormetische Effekte bei einem Hundertstel der empfohlenen Aufwandmenge an. Bei den nicht Imazamox-toleranten Rapssorten wurden bei der empfohlenen Aufwandmenge (40 g/ha) Wachstumsreduktionen zwischen 28 und 97 % beobachtet, während in den Imazamox-toleranten Sorten Wachstumsstimulationen von bis zu 20 % auftraten. Die beobachteten Effekte standen in keinem Zusammenhang mit der Blattdichte, den Wachstumsraten und der Taxonomie der überprüften Pflanzenarten. Die ermittelten Schaddosen betrugen 0.32 g/ha für HD_5 und 3.9 g/ha für HD_{50}, was darauf hindeutet, dass 50 % der Nichtzielarten bei einem Zehntel der empfohlenen Aufwandmenge beeinträchtigt würden. Wenn man die Abdrifteckwerte für Herbizidanwendungen zu Grunde legt, wären potenziell nachteilige Effekte bis zu einer Entfernung von 4 m zum Feldrand zu erwarten.

Stichwörter: Clearfield, funktionelle Pflanzentypen, Nichtzielpflanzen, Pflanzenwachstumstests

1. Introduction

Since the 1990s, non-transgenic imidazolinone (IMI) tolerant oilseed rape, wheat, sunflower and lentil cultivars have been introduced in America and Australia, and it may well be that such varieties will be placed on the market elsewhere, too (TAN et al., 2005). While one of the complementary herbicides, imazamox, has already been registered in Annex I as a novel plant protection product in the EU (EC 2002a, 2003), the Clearfield® production system will be introduced in European countries in the near future (SCHÖNHAMMER et al., 2010).

Generally, the recommended field application rate of the post-emergence herbicide imazamox is only 40 g/ha, while it is much higher for older compounds. Due to its high efficacy, its moderate persistence and its low ecotoxicity, the chemical is thus regarded as a promising new plant protection product to reduce the weed pressure in various crops. Environmental risk assessment reports on imazamox have been presented by NRA (2000) and SERA (2010). Because of its presumably low environmental concentrations its use has been authorized e.g. in Florida to control invasive aquatic plants in non-agricultural areas (WERSAL and MADSEN, 2007), but non-target aquatic or emergent plants may also be affected (KOSCHNIK et al., 2007). Furthermore, DEEDS et al. (2006) showed strong leaf injury and highly significant growth reductions in off-target wheat at one tenth of the recommended use rate (in their study 35 g a.i. per hectare). In rice, HENSLEY (2009) found adverse effects of the herbicide at even lower doses. While in latter study, effects were larger at later growth stages and caused reduced viability of the harvested seeds, DEEDS et al. (2006) found slightly stronger effects in earlier growth stages but no effects on germination rates of harvested seeds. Both studies, however, contradict the opinion of NRA (2000) that non-target plants will not show phytotoxic responses at 10 % of the recommended field dose. Furthermore, there is some new concern that the incomplete degradation of imazamox in soils may cause phytotoxic effects in follow-up cultures (PANNACCI et al., 2006).

Wild plant species are regarded not to be more or less sensitive than crops (CLARK et al., 2004; CARPENTER and BOUTIN, 2010), but OLSZYK et al. (2008) found evidence for a much higher variability in sensitivity in non-target species than in crops. To date, there is no published information available from both, field and greenhouse experiments on the effects of spray drift and low doses of imazamox on non-target wild plant species. However, species specific differences in sensitivity and the importance of growth stage have been tested in 24 weed species using the imidazolinone herbicide imazethapyr (KLINGMAN et al., 1992). Greenhouse or climate chamber studies using different concentrations of herbicide spray solutions and different plant species are a first valuable indication on potential sub-lethal herbicidal effects on the growth of non-target plants which cannot easily be recognized in field experiments (RIEMENS et al., 2008). During the authorization of plant protection products, such tests are the first step in the risk assessment (tier 1) to generate toxicity-to-exposure ratios (TERs), while there is currently no consensus on how to perform higher-tier tests under field conditions (HEIMBACH, 2007; MONTFORTS and DE JONG, 2007). Generally, the choice of test species and end-points must be representative for the evaluation of risks to non-target plants. Most ecotoxicological studies use fresh or dry weights, i.e. growth after the exposure as an endpoint but long-term studies addressing plant recovery and seed output may yield lower or higher sensitivity of plants to herbicides (CARPENTER and BOUTIN, 2010). For statistical reasons, i.e. to exclude non-randomness of the choice of taxa, it matters how many and which species are tested in such experiments. Furthermore, the ecological importance and rareness of plant taxa or ecological functions of wild non-target plants should be accounted for in pesticide regulatory guidelines and ecotoxicological tests. While EPPO (2003) and OECD (2006) recommend six out of a list of suited species, the US EPA suggests the use of ten different species. If the aim of such studies is to derive species sensitivity distributions (SSDs) and hazardous doses (HD_5) to protect 95 % of the non-target species, many more species, especially more broad leaved than grass species and ecologically important wild plants would have to be tested (BOUTIN and ROGERS, 2000; WHITE and BOUTIN, 2007). In these tests, species from many different plant families should be considered because closely related species, e.g. from the same genus, often show a very similar response (CLARK et al., 2004).

Objectives of the study were to screen different plant species and crop cultivars in standardised growth tests for the phytotoxicity of imazamox and to derive effective doses (EDs). In addition, we used a number of spring and winter oilseed rape (OSR) cultivars to see whether there are differences in the sensitivity within the same crop species. In addition to the controls receiving no herbicide treatment and a treatment simulating the recommended field application rate, two IMI-tolerant OSR cultivars were included in the experiment. These cultivars were used as an internal standard to which the observed growth responses could be related to within and between individual experiments. We also intended to derive a sensitivity ranking of the different OSR cultivars, which may hint to potential damage created by imazamox drift from herbicide resistant OSR fields to neighboring intolerant OSR cultures. Moreover, rare arable weeds including some red list taxa, were used to address the effects of low doses of imazamox on non-target plants. We used seed material from Germany and assumed that none of the tested seed origins was resistant to ALS-inhibiting herbicides. By combining effective doses for a number of wild species after the method of VAN VLAARDINGEN et al. (2004), we aimed to derive a species sensitivity distribution (SSD) and values for hazardous doses (HD_5) that would significantly affect 5 % of the tested species. This is a widely accepted method to describe the ecotoxicological risk of pollutants on plant populations and non-target plants also performed in higher-tier tests. The screening of a larger variety of rare and more uncommon arable species may also hint to taxonomic differences in the efficacy of imazamox and helps to explain whether plant functional traits (e.g. specific leaf area, SLA, and relative growth rate, RGR) are related to the phytotoxicity of the herbicide.

2. Materials and methods

2.1 Plant growth tests

In order to study the effect of different doses of imazamox applied above-ground as a post-emergence herbicide, plants of twelve non-resistant OSR cultivars and 22 wild plant species (Tab. 1) were grown in trays in a greenhouse. The herbicide screening with seedlings was based on OECD (2006) and EPPO (2003). Because we intended to screen a large number of cultivars and species and due to the lack of greenhouse space we did not use individual pots with single plants in these experiments. Instead, six rows with 30 seeds per row for each cultivar (one row = one cultivar) were sown in plastic trays (40 * 60 cm). A few days after germination, plants were thinned to 10 plants per row to leave enough space between individual plants and rows. Five trays with the same OSR cultivars or plant species (six rows) were used for the five concentrations (application rates) of the imazamox herbicide. The standard soil used in the experiments was a LD80 Fruhstorfer Erde® (Hawita GmbH, Germany), which contains a defined amount of slow release fertilizers. During the four experimental runs over a time period of five months, daily temperatures varied between 20 and 30 °C, while night temperatures were set to 15 °C. Temperature and relative humidity were recorded during the experiments. To prevent bias and to avoid placement effects, test and control trays were randomly rearranged every three to five days. Plants were watered on demand using a multifunctional sprayer.

Seven to ten days after germination plants had achieved the three to four leaf stage. At this time, ten seedlings per species were harvested at random (two from each of the five trays) and fresh weights were determined to have a mean reference fresh weight (start weight). At the same day, the herbicide Bolero® (BASF) was supplied with a Gardena® spray flask yielding 0.5 mL on average per lift. Ten lifts of solutions with different herbicide concentrations (0, 0.05, 0.5, 2.5 and 5 ml/L equaling to 0, 0.002, 0.02, 0.1 and 0.2 ml/L a.i.) were sprayed per tray. The five spray applications were calculated to correspond to application rates of 0 (control), 0.4, 4, 20 and 40 g/ha a.i. Latter application rate is the recommended dose for field sprayings in Switzerland. After spraying, the occurrence of visible symptoms was noted on a two to three day routine. 14 days after the treatment, shoot masses of five randomly selected plants from each of the five treatments were harvested and fresh weights of individual plants were determined as the relevant end point. By subtracting the start weight (day 0) from the end weight, absolute growth rate (AGR) could be determined. Furthermore, relative growth rates (RGR) were determined by subtracting the logarithm of the start weight from the logarithm of the end weight and dividing this value by 14 (duration in days of the experiment). Furthermore, leaves from the control plants were randomly sampled and their leaf area and dry weights were determined to calculate specific leaf area (SLA).

Tab. 1 Overview of the taxonomy, conservation status, specific leaf area (SLA), relative (RGR) and absolute growth rates (AGR) of 22 wild plant species used in the phytotoxicity screening. Effective doses refer to the doses of imazamox creating a growth reduction of 5 (ED_5) and 50 % (ED_{50}). "[1]" in the column on conservation status refers to data from www.floraweb.de with "+" not endangered, "3" endangered, "2" severely endangered, "1" nearly extinct and "0" extinct. "nd" refers to not determined.

Tab. 1 *Übersicht über die Taxonomie, den Schutzstatus, die spezifischen Blattflächen (SLA), relativen (RGR) und absoluten (AGR) Wachstumsraten der 22 in der Phytotoxizitätsstudie untersuchten Wildpflanzenarten. Effektive Dosen beziehen sich auf die Imazamoxraten, welche eine Wuchsminderung von 5 (ED_5) bzw. 50 % (ED_{50}) hervorriefen. „[1]" in der Spalte zum Schutzstatus bezieht sich auf Angaben von www.floraweb.de mit "+" nicht gefährdet, „3" gefährdet, „2" stark gefährdet, „1" fast ausgestorben, und „0" ausgestorben. „nd" bedeutet nicht erfasst.*

Scientific name	EPPO Codes	Plant Family	Conservation Status [1]	SLA $cm^2\ g^{-1}$ d.m.	RGR $g\ g^{-1}\ d^{-1}$ f.m.	AGR $g\ 14d^{-1}$ f.m.	Effective doses $g\ ha^{-1}$ imazamox ED_5	ED_{50}
Agrostemma githago	AGOGI	Caryophyllaceae	1	378	0,119	1,13	0,005	7,64
Anagallis foemina	ANGCO	Primulaceae	+	437	0,162	0,13	1,48	2,5
Anchusa arvensis	LYCAR	Boraginaceae	+	327	0,103	0,54	0,907	1,47
Anthemis arvensis	ANTAR	Asteraceae	+	350	0,164	0,11	10,77	14,6
Brassica juncea	BRSJU	Brassicaceae		470	0,138	0,25	4,9	17,9
Brassica nigra	BRSNI	Brassicaceae		395	0,022	0,05	2,11	25,06
Brassica rapa	BRSRR	Brassicaceae		309	0,161	3,38	0,02	37
Camelina alyssum	CMAAL	Brassicaceae	0	466	0,108	0,28	1,5	3,7
Camelina sativa	CMASA	Brassicaceae	+	510	0,086	0,25	1,56	3,22
Centaurea cyanus	CENCY	Asteraceae	+	399	0,142	0,19	1,8	3,52
Euphorbia exigua	EPHEX	Euphorbiaceae	+	325	0,099	0,03	2,27	4,75
Filago arvensis	FILAR	Asteraceae	3	275	0,149	0,07	0,22	0,68
Hirschfeldia incana	HISIN	Brassicaceae		347	0,188	0,15	1,8	3,07
Legousia spec.-veneris	LEGSV	Campanulaceae	3	244	0,028	0,03	2,88	3,48
Mercurialis annua	MERAN	Euphorbiaceae	+	301	0,123	0,28	1,49	1,84
Nigella arvensis	NIGAR	Ranunculaceae	2	471	0,106	0,03	0,0009	0,068
Papaver argemone	PAPAR	Papaveraceae	+	362	0,103	0,03	10,3	15,8
Sherardia arvensis	SHRAR	Rubiaceae	+	367	0,153	0,21	3,56	5,51
Silene linicola	SILLI	Caryophyllaceae	0	505	0,134	0,39	1,009	3,93
Silene noctiflora	MELNO	Caryophyllaceae	+	596	0,187	0,23	0,342	23,04
Sinapis arvensis	SINAR	Brassicaceae		345	0,165	1,51	0,015	0,233
Spergularia rubra	SPBRU	Caryophyllaceae	+	316	nd	nd	2,51	5,48

2.2 Data analyses

Because we did not have enough greenhouse space to screen a large number of different plant species in single pots, we used trays in which rows of different plant species were sown. Seedlings had enough space to grow, but we are aware of the problems related to the sampling of five individuals grown in the same experimental unit. However, the lack of true replicates (for statistical problems of sub-sampling and pseudoreplication refer to ONOFRI et al. (2010) in these initial trials may be tolerable as the intention was to perform a multi-species test. After entering the data into Excel worksheets, descriptive statistical analyses were performed. Growth of the plants subjected to the different treatments was related to the growth of plants from the control. In order to derive effective doses (EDs) and to visualize dose-response curves the biomass data were analyzed with the ´drc´ package (Version 1.9-3) using the R-software. Log-logistic models were fitted and ED values for growth reductions of 5, 10, 50 and 90 % were calculated for each cultivar and species according to RITZ and STREIBIG (2005, 2010). Furthermore, species sensitivity distributions (SSD´s) and hazardous concentrations (HC´s) that adversely affect 5 and 50 % of the tested species were visualized and calculated according to the methodology specified by van Vlaardingen et al. (2004). To do so, ED_{50} values of all species were entered into the program ETX 2.0.

3. Results and Discussion

While all of the chosen OSR cultivars germinated readily, only 22 out of the 40 wild species selected initially achieved germination rates of over 50 %. We are not sure whether seed pre-treatment would have lead to higher germination rates, but the results indicate that most of the chosen rare arable weeds can be used in phytotoxicity tests. Also WHITE et al. (2009) found that germination requirements for terrestrial and wetland non-crop species are normally met in greenhouse or chamber trials, so that a large variety of wild plant species may well be included in regulatory testing.

Foliar symptoms looked similar in OSR cultivars and the tested wild plant species. Two to three days after the spraying, first visible effects, yellowing and leaf crinkling, occurred in the youngest leaves, while the two oldest leaves were still growing. Lowest effects were observed in the two imazamox-tolerant cultivars while growth reductions exceeded 55 % on average (28-97 %) in the twelve tested non-tolerant OSR cultivars. In contrast, both growth stimulations and reductions were observed in the imazamox-tolerant cultivars when sprayed with the recommended field dose. It is unclear, however, whether the modified AHAS gene that confers the tolerance to imazamox is expressed effectively and at the same rate under different environmental conditions. As has been reported by TAN et al. (2005) highest tolerance to imazamox will occur in OSR when both of the two original mutations are stacked within the genome, but we have no information on the genetic status of the tested cultivars. Even when no complete tolerance to imazamox is conferred, imazamox-tolerant plants will recover from the herbicide while non-tolerant cultivars will sooner or later die. Figure 1 shows dose response relationships for growth reductions in 22 wild plant species and Table 1 lists the calculated effective doses ED_{50} and ED_5 of imazamox that will reduce growth by 50 and 5 %, respectively.

Most of the curves revealed a sigmoidal shape and lack-of-fit tests were highly insignificant in most cases, indicating that the regression models were acceptable. Still, lack-of-fit tests were significant ($p < 0.05$) for the results obtained with *Anchusa arvensis*, *Centaurea cyanus* and *Silene linicola*. Interestingly, in almost half (10 out of 22) of the tested species the lowest dose of 0.4 g a.i. per ha, i.e. a hundredth of the recommended dose, created a slight growth stimulation relative to the control. We suggest that these hormetic effects might be related to adaptive responses which have also been reported for other herbicides (e.g. glyphosate), (CEDERGREEN, 2008). Although such positive biological effects are not much understood, ecotoxicological concepts may have to be adapted in the future (CALABRESE, 2005). In the next step, a species sensitivity distribution (SSD) was constructed using the ED_{50} values derived for the 22 tested wild plant species and the ETX 2.0 software. Hazardous doses were calculated to be 0.32 g/ha for HD5 and 3.9 g/ha for HD50. Latter finding indicates that 50 % of the non-target wild plant species would be affected at a tenth of the recommended field dose. Taking into account the general herbicide drift values given in EC (2002b) the results from present study suggest that up to a distance of 4 m from sprayed fields the non-target vegetation would adversely be affected.

Finally, the growth reductions observed in the imazamox spraying experiments were also related to the different growth characteristics of the tested species. While the responsiveness of plant species was unrelated to their taxonomy, specific leaf areas (SLA, i.e. leaf thickness, Tab. 1) and relative growth rates (RGR) a slight negative relationship ($R^2 = 0.23$) was observed between ED_5 and the absolute growth. It appears that "internal doses" of the herbicide were greater in plants with higher metabolic activity, while the uptake and herbicide efficacy was lower in plants producing less biomass. While BOUTIN et al. (2004) recommend including growth rates of species as an important criterion for the choice of test species, our results indicate that absolute growth may affect the responsiveness of seedlings in the early life stage.

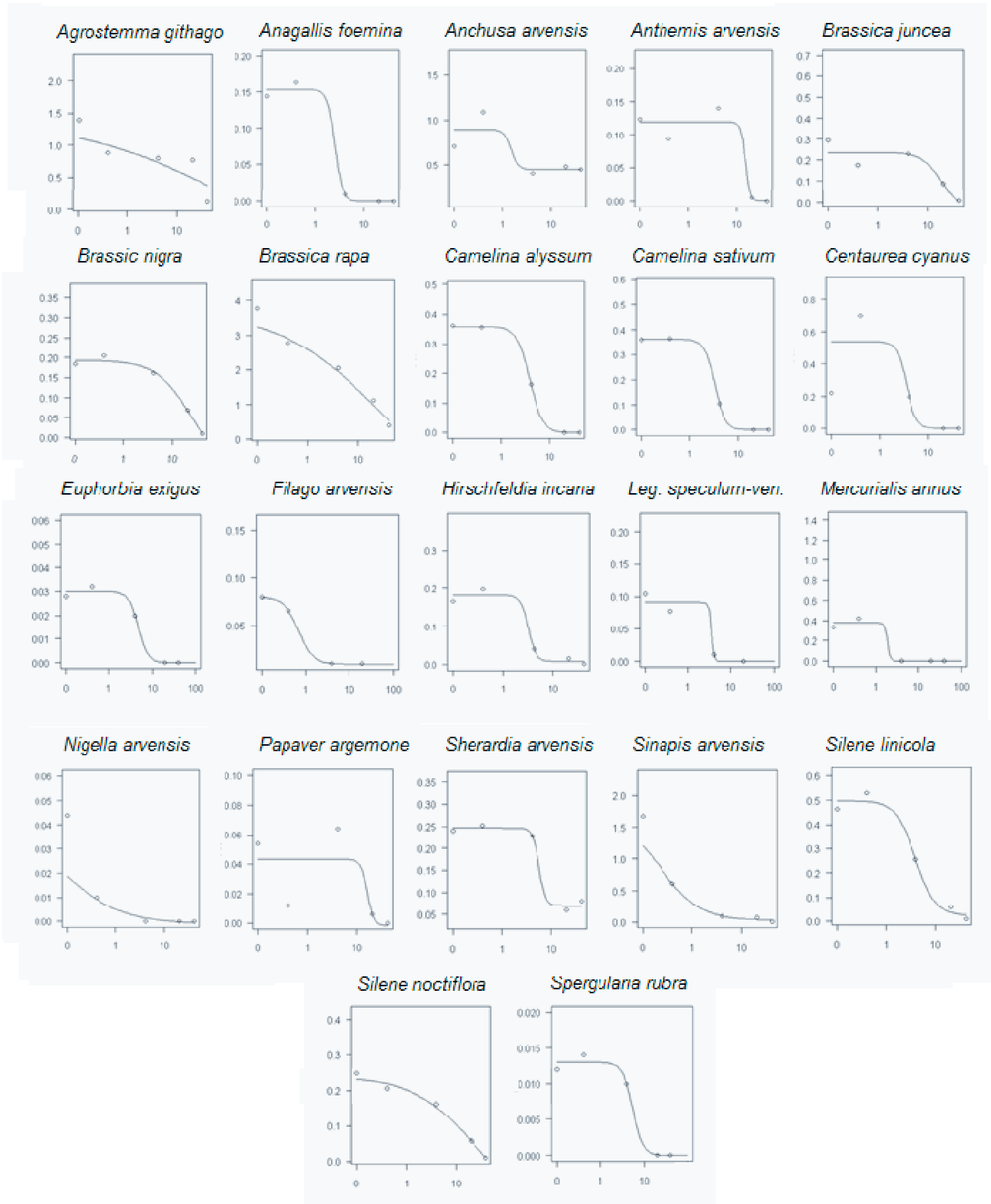

Fig. 1 Dose-response relationships for the reduction of fresh weights (g fresh matter, y-axes) by the herbicide imazamox (dose in g/ha, x-axes) in 22 wild plant species. Log-logistic curves were fitted with the drc package, refer to RITZ and STREIBIG (2010). For effective doses that were derived from the non-linear regressions refer to Table 1.

Abb. 1 *Dosis-Wirkungs-Beziehungen für die Reduktion der Frischgewichte (g Frischsubstanz, y-Achse) durch das Herbizid Imazamox (Dosis in g/ha, x-Achse) bei 22 Wildpflanzenarten. Die log-logistischen Kurven wurden mit dem drc Paket angepasst, siehe RITZ and STREIBIG (2010). Die effektiven Dosen, welche mittels nicht-linearer Regressionen abgeleitet wurden, sind in Tabelle 1 enthalten.*

Acknowledgements

The experiments were supported by a grant from the Geschwister Stauder Stiftung (Stuttgart). Various commercial seed suppliers and Prof. Dr. Zwerger and Dr. Hans-Peter Söchting of the Julius Kühn-Institut (JKI), Institute for Plant Protection in Field Crops and Grassland (Braunschweig) are thanked for providing seed material of OSR cultivars and wild plant species.

References

BOUTIN, C. AND C.A. ROGERS, 2000: PATTERN OF SENSITIVITY OF PLANT SPECIES TO VARIOUS HERBICIDES – AN ANALYSIS WITH TWO DATABASES. ECOTOXICOLOGY **9**, 255-271.

BOUTIN, C., N. ELMEGAARD AND C. KJAER, 2004: TOXICITY TESTING OF FIFTEEN NON-CROP PLANT SPECIES WITH SIX HERBICIDES IN A GREENHOUSE EXPERIMENT: IMPLICATIONS FOR RISK ASSESSMENT. ECOTOXICOLOGY **13**, 349-369.

CALABRESE, E.J., 2005: PARADIGM LOST, PARADIGM FOUND: THE RE-EMERGENCE OF HORMESIS AS A FUNDAMENTAL DOSE RESPONSE MODEL IN THE TOXICOLOGICAL SCIENCES: ENVIRONMENTAL POLLUTION **138**, 379-412.

CARPENTER, D. AND C. BOUTIN, 2010: SUBLETHAL EFFECTS OF THE HERBICIDE GLUFOSINATE AMMONIUM ON CROPS AND WILD PLANTS: SHORT-TERM EFFECTS COMPARED TO VEGETATIVE RECOVERY AND PLANT REPRODUCTION. ECOTOXICOLOGY **19**, 1322–1336.

CEDERGREEN, N., 2008: HERBICIDES CAN STIMULATE PLANT GROWTH. WEED RESEARCH **48**, 429-438.

CLARK, J., L.S. ORTEGO AND A. FAIRBROTHER, 2004: SOURCES OF VARIABILITY IN PLANT TOXICITY TESTING. CHEMOSPHERE **57**, 1599-1612.

DEEDS, Z., K. AL-KHATIB, D.E. PETERSON AND P.W. STAHLMAN, 2006: WHEAT RESPONSE TO SIMULATED DRIFT OF GLYPHOSATE AND IMAZAMOX APPLIED AT TWO GROWTH STAGES. WEED TECHNOLOGY **20**, 23-31.

EC, 2002A: IMAZAMOX. SANCO/4325/2000 –FINAL, 29 NOVEMBER 2002.

EC, 2002B: GUIDANCE DOCUMENT ON TERRESTRIAL ECOTOXICOLOGY UNDER COUNCIL DIRECTIVE 91/414/EEC. SANCO/10329/2002 REV 2 FINAL, 39 PP.

EC, 2003: COMMISSION DIRECTIVE 2003/23/EC OF 25 MARCH 2003 AMENDING COUNCIL DIRECTIVE 91/414/EEC TO INCLUDE IMAZAMOX, OXASULFURON, ETHOXYSULFURON, FORAMSULFURON, OXADIARGYL AND CYAZOFAMID AS ACTIVE SUBSTANCES, OFFICIAL JOURNAL OF THE EUROPEAN UNION L 81/39, 4 PP.

EPPO, 2003: EUROPEAN AND MEDITERRANEAN PLANT PROTECTION ORGANIZATION, 2003. ENVIRONMENTAL RISK ASSESSMENT SCHEME FOR PLANT PROTECTION PRODUCTS. CHAPTER 12, NON-TARGET TERRESTRIAL HIGHER PLANTS. EPPO STANDARDS, BULLETIN 33, PARIS, FRANCE. 239-244.

HEIMBACH, F., 2007: ENTWICKLUNG DER ÖKOTOXIKOLOGIE VON PFLANZENSCHUTZMITTELN IN DEN LETZTEN 25 JAHREN: VON ERSTEN DISKUSSIONEN ÜBER PRÜFORGANISMEN ZUR PROBABILISTISCHEN RISIKOANALYSE. UWSF – Z. UMWELTCHEMIE UND ÖKOTOXIKOLOGIE **19**, 43-38.

HENSLEY, J.B., 2009: EFFECTS OF SIMULATED DRIFT OF GLYPHOSATE, IMAZETHAPYR, GLUFOSINATE AND IMAZAMOX TO NON-TRANSGENIC RICE. DISSERTATION AT THE SCHOOL OF PLANT, ENVIRONMENTAL, AND SOIL SCIENCES, 130 PP, HTTP://ETD.LSU.EDU/DOCS/AVAILABLE/.

KLINGMAN, T.E., C.A. KING AND L.R. OLIVER, 1992: EFFECT OF APPLICATION RATE, WEED SPECIES, AND STAGE OF GROWTH ON IMAZETHAPYR ACTIVITY. WEED SCIENCE **40**, 227-232.

KOSCHNIK, T.J., M.D. NETHERLAND AND W.T. HALLER, 2007: EFFECTS OF THREE ALS-INHIBITORS ON FIVE EMERGENT NATIVE PLANT SPECIES IN FLORIDA. JOURNAL OF AQUATIC PLANT MANAGEMENT **45**, 47-51.

MONTFORTS, M.H.M.M. AND F.M.W. DE JONG, 2007: FIELD STUDIES IN PESTICIDE REGISTRATION: QUESTIONING THE ANSWERS. INTEGRATED ENVIRONMENTAL ASSESSMENT AND MANAGEMENT **3**, 150-153.

NRA, 2000: NATIONAL REGISTRATION AUTHORITY FOR AGRICULTURAL AND VETERINARY CHEMICALS EVALUATION OF THE NEW ACTIVE IMAZAMOX IN THE PRODUCTS RAPTOR HERBICIDE & RAPTOR WG HERBICIDE, 57 PP.

OECD, 2006: OECD GUIDELINE 227 FOR THE TESTING OF CHEMICALS: TERRESTRIAL PLANT TEST: VEGETATIVE VIGOUR TEST. 21 PP.

OLSZYK, D., T. PFLEEGER, E.H. LEE, C. BURDICK, G. KING, M. PLOCHER AND J. KERN, 2008: SELECTING AND EVALUATING NATIVE PLANTS FOR REGION-SPECIFIC PHYTOTOXICITY TESTING. INTEGRATED ENVIRONMENTAL ASSESSMENT AND MANAGEMENT **4**, 105-117.

ONOFRI, A., E.A. CARBONELL, H.-P. PIEPHO, A.M. MORTIMER AND R.D. COUSENS, 2010: CURRENT STATISTICAL ISSUES IN WEED RESEARCH. WEED RESEARCH **50**, 5-24.

PANNACCI, E., A. ONOFRI AND G. COVARELLI, G., 2006: BIOLOGICAL ACTIVITY, AVAILABILITY AND DURATION OF PHYTOTOXICITY FOR IMAZAMOX IN FOUR DIFFERENT SOILS OF CENTRAL ITALY. WEED RESEARCH **46**, 243-250.

RIEMENS, M.M., T. DUECK AND C. KEMPENAAR, 2008: PREDICTING SUBLETHAL EFFECTS OF HERBICIDES ON TERRESTRIAL NON-CROP PLANT SPECIES IN THE FIELD FROM GREENHOUSE DATA. ENVIRONMENTAL POLLUTION **155**, 141-149.

RITZ, C. AND J. STREIBIG, 2005: BIOASSAY ANALYSIS USING R. JOURNAL OF STATISTICAL SOFTWARE **12**, 1-22.

RITZ, C. AND J. STREIBIG, 2010: PACKAGE ´DRC´. ANALYSIS OF DOSE-RESPONSE CURVES, 138 PP. HTTP://WWW.R-PROJECT.ORG, HTTP://WWW.BIOASSAY.DK.

SCHÖNHAMMER, A., M. PFENNING AND S. CHENEVIER, 2010: INNOVATIVE POSSIBILITIES OF WEED CONTROL IN OILSEED RAPE WITH CLEARFIELD. JULIUS-KÜHN-ARCHIV **428**, 329-330.

SERA, 2010: IMAZAMOX - HUMAN HEALTH AND ECOLOGICAL RISK ASSESSMENT. FINAL REPORT. SYRACUSE ENVIRONMENTAL RESEARCH ASSOCIATES, INC., NEW YORK. SERA TR-052-24-02A, 169 PP.

TAN, S., EVANS, R.R., DAHMER, M.L., SINGH, B.K., SHANER, D.L. (2005): IMIDAZOLINONE-TOLERANT CROPS: HISTORY, CURRENT STATUS AND FUTURE. PESTICIDE MANAGEMENT SCIENCE **61**, 246–257.

VAN VLAARDINGEN, P.L.A., T.P. TRAAS, A.M. WINTERSEN AND T. ALDENBERG, 2004: ETX 2.0. A PROGRAM TO CALCULATE HAZARDOUS CONCENTRATIONS AND FRACTION AFFECTED, BASED ON NORMALLY DISTRIBUTED TOXICITY DATA. RIVM REPORT 601501028/2004, 68 PP., RIVM BILTHOVEN, THE NETHERLANDS.

WERSAL, R.M. AND J.D. MADSEN, 2007: COMPARISON OF IMAZAPYR AND IMAZAMOX FOR CONTROL OF PARROTFEATHER (*MYRIOPHYLLUM AQUATICUM* (VELL.) VERDC.). JOURNAL OF AQUATIC PLANT MANAGEMENT **45**, 132-136.

WHITE, A.L. AND C. BOUTIN, 2007: HERBICIDAL EFFECTS ON NON-TARGET VEGETATION: INVESTIGATING THE LIMITATIONS OF CURRENT PESTICIDE REGISTRATION GUIDELINES. ENVIRONMENTAL TOXICOLOGY AND CHEMISTRY **26**, 2634-2643.

WHITE, A.L., C. BOUTIN, R.L. DALTON, B. HENKELMAN AND D. CARPENTER, 2009: GERMINATION REQUIREMENTS FOR 29 TERRESTRIAL AND WETLAND WILD PLANT SPECIES APPROPRIATE FOR PHYTOTOXICITY TESTING. PEST MANAGEMENT SCIENCE **65**, 19-26.