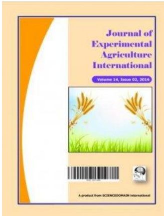

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Sensitivity of Different *Chenopodium album* L. Biotypes to Metamitron

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Author's contribution

The sole author designed, analyzed, interpreted and prepared the manuscript.

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ABSTRACT

Chenopodium album L. populations were tested for sensitivity to the herbicide metamitron. Sensitivity was assessed by a dose-response relationship and ED_{50} values. Seeds were collected from single plants in different districts of Germany and in different types of locations (non-agricultural land, fallow land, conventional farming and organic farming). Additional seeds were collected from single plants within a field in a 12-metre grid space. Results were evaluated for different districts and different types of locations and for a single field. There were only small variations in sensitivity at low application rates. Significant differences could be estimated between districts. All *Chenopodium album* L. populations were sensitive to metamitron. No resistant plants were found.

Keywords: Application rate; Chenopodium album; dose-response relationship; herbicide; sugar beet.

1. INTRODUCTION

Weeds play important roles in agricultural crop production. According to weed occurrence and densities, yield reduction and economic damage can be expected. Weed control is indispensable in securing crop yields. Today, herbicide use is the major weed control method in agriculture. It

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has been found that the susceptibility of weed populations to herbicides varies greatly [1,2]. Therefore knowledge about weed sensitivity is important for resistance risk assessment within herbicide authorization [3]. Following the EPPO standards for the assessment of the inherent resistance risk of a herbicide, the sensitivity variation among different weed populations should be taken into account [4]. *Chenopodium album* L. belongs to the family of plants with the most common distribution worldwide [5] and occurs mainly in temperate and subtropical zones. *Chenopodium album* L. is a dicotyledonous weed frequently found on arable land, in gardens, in vineyards, along roads, along river banks and on non-agricultural land (e.g. dumps).

Chenopodium album L. is an annual spring weed. In Germany, the time of germination is in late spring. Therefore, *Chenopodium album* L. can be found mainly in summer crops. The wide range of occurrence is due to the high seed production, the long lifecycle of seeds and low habitat requirements of *Chenopodium album* L. Under good conditions, the plant is robust and competitive and has a high nutrient appropriation. In sugar beet, the plants reach a natural height of up to 150 cm (root depth up to 100 cm). *Chenopodium album* L. can produce more than 100,000 seeds per plant [6].

In Germany, *Chenopodium album* L. is one of the most serious weeds affecting sugar beet crops and maize. The cropping area of sugar beet accounted for 350,000 ha in 2013. This corresponds to a share of about 3% of the total agricultural land. This is a small portion in comparison to cereal fields. The main areas cultivated with sugar beet are concentrated in few regions. Therefore, sugar beet is cultivated in short crop rotation. Cultivating sugar beet requires high row spaces; due to the slow development of young plants, weed control is one of the main steps saving harvests. In Germany, weed control in sugar beet is done almost exclusively with herbicides. The proportion of acreage with chemical weed control represents 99% [7]. Therefore, it is necessary to determine the period in which the sugar beet must be weed free to avoid yield losses. As a standard procedure, weed control in sugar beet with herbicides in the post-emergence application in the cotyledon growth stage of weeds has proved successful. In general, to reach an acceptable efficacy, 3 herbicide splitting applications at intervals of 1 to 2 weeks are necessary. Weed control comes at a time when there is no competition of weeds. The aim of weed control is a mostly weed-free crop until closing plant row. *Chenopodium album* L. can be well controlled with available herbicides. The active substance metamitron is of great importance in this. Metamitron has been used in weed control in fodder and sugar beet for nearly 40 years. Metamitron is a key herbicide in this crop. In Germany, the authorised amount is 3.5 kg metamitron/ha. *Chenopodium album* L. is normally very sensitive to metamitron. Always high levels of weed control could be estimated.

However, in recent years it was observed that sugar beet fields showed that more weeds were left after metamitron application at recommended rates, especially *Chenopodium album* L., than in the past. Surviving weeds led to heavy weed infestation. The causes were unclear. The successful weed control of *Chenopodium album* L. depends on several factors influencing herbicide efficacy. As well as application rate, these include weather conditions, soil conditions, and appropriate application. Besides insensitivity of biotypes, a reduction in efficacy can be due to various reasons. However, not every surviving plant can be classified as resistant. Also, late weed infestation and late row closing were observed.

Metamitron belongs to the chemical family of triazinone and is a photosystem II inhibitor. Herbicide resistance to photosystem II inhibitors has been known since the beginning of the 1980s. Reasons for this include the increasing use of herbicides in group C1 of the HRAC classification in crop rotation (e.g. metribuzin, atrazine, simazine, terbuthylazine, bromoxynil, isoproturon, linuron).

Experiments by Merchant et al. [8] showed herbicide resistance to metamitron of *Chenopodium* biotypes in Belgium. Often, a cross-resistance to atrazine could be estimated. DNA analysis showed a serine264 to glycine mutation for these Belgian biotypes [9]. Aper et al. [10] investigated the local spread of metamitron-resistant *Chenopodium album* L. in Belgium and found genetic similarity between different fields. Ulber et al. [11] studied the variability in the reaction of different herbicidal substances and dosages for *Chenopodium album* L. and *Amaranthus retroflexus* L. Populations with resistance to terbuthylazine were detected; some populations showed a cross-resistance to metamitron. According to

Neve et al. [12], many studies compared resistant and susceptible weed populations.

In sugar beet cultivation, the use of herbicides containing metamitron is a standard method of weed control. The periodic use of herbicides of the same substance group in tight crop rotations leading to the selection of resistant weed biotypes cannot be excluded. Due to the previously mentioned reasons, *Chenopodium album L.* is particularly vulnerable.

The objectives of this study were to describe the natural variation in herbicide response of weed populations to metamitron below application rates which are relevant for herbicide resistance.

2. MATERIALS AND METHODS

To test the sensitivity of *Chenopodium album* L. to metamitron, seeds of single plants were collected from different land use areas. Dose relationships were estimated under standardized experimental conditions. A better understanding of *Chenopodium album* L. sensitivity against metamitron presents a number of opportunities. For example, this information could be used to optimize application rates in weed control.

2.1 Seed Collection in Different Districts

For the investigations of *Chenopodium album* L. (CHEAL) regarding their sensitivity to the active substance metamitron (Goltix 700 SC), seeds were harvested from single plants in five different districts in Germany in the Braunschweig area. The mean annual temperature in this area is 8.8°C, and the mean annual rainfall is between 600 and 650 mm. CHEAL biotypes come from the connected districts of Braunschweig (BS), Peine (PE), Wolfenbüttel (WF), Helmstedt (HE) and Gifhorn (GF). Sampling points can be separated into four types of location (L-type): non-agricultural land (L-type1), fallow land (L-type2), conventional farming (L-type3), and organic farming (L-type4). Sampling points were randomly selected and distributed over the entire district. More details regarding the types of location are described in Tables 1 and 2. Seeds were collected at the end of the vegetation period from single plants. Seeds were cleaned and air-dried in a greenhouse and then stored in plastic bags at 5°C. It is to be expected that metamitron was used for weed control in conventional farming with sugar beet in the crop rotation.

2.2 Seed Collection within a Field

For the investigation of CHEAL in their sensitivity within a field to metamitron, seeds were collected in a 12-metre grid space. Samples were taken from single plants within a single sugar beet field. Seeds were collected at the end of the vegetation period from single plants and cleaned and air-dried in a greenhouse before being stored in plastic bags at 5°C.

Table 1. *Chenopodium album* L. – biotypes – type of location

Type of location	Code	Areas
Non-agricultural land	L-type1	Strips of lawn, paths, wasteland, rail tracks, excavation, parking area
Fallow land	L-type2	Flower strips, field edge, fallow, silo area
Conventional farming	L-type3	potato, sugar beet, maize, rape, orchards, asparagus, grass reseeding
Organic farming	L-type4	Clover-grass, carrots, potato, onion, soybean

Table 2. *Chenopodium album* L. – biotypes – crop plants and number of tested fields in conventional and organic farming

Type of location	Crop plants	Number of fields
Conventional farming	sugar beet	77
	maize	8
	potato	8
	others	10
Organic farming	potato	3
	clover	3
	soybean	3
	carrots	3
	fallow	3

2.3 Bioassay

Seeds of CHEAL were sown in germination trays under greenhouse conditions and the soil was kept moist. Soil from the Julius Kühn-Institute location was used (loamy sand, 49.7% sand; 38.1% silt, 12.2% clay; pH 6.4; organic carbon 0.9%). The soil was sieved to 2.5 mm, steamed and adjusted to 60% of maximum water capacity. At the one to two-leaf growth stage (BBCH 11-12), five plants were selected and placed in bioassay pods (7x7x8 cm). Herbicide application was performed at the 2 to 3 leaf stage (BBCH 12-13) of the plants. The selected application rates were 0; 1.56; 3.13; 6.25; 12.5; 25, 50 and 100% according to the standard application rate (Goltix 700 SC with 5 L/ha, 700.59 g metamitron/l).

Herbicides were applied with the use of a moving-nozzle cabinet sprayer equipped with a flat-fan nozzle tip (TeeJet 8002EVS, TeeJet Technologies GmbH, Ludwigsburg, Germany) calibrated to deliver 300 L ha^{-1} of spray solution at 210 kPa in a single pass over the soil.

The plants were cultivated in a climate chamber with 16 h light at temperatures from 20 to 24°C during the day and 15 to 16°C at night. A relative air humidity of 50 to 55% was recorded during the day and 55 to 60% during the night. The pots were irrigated as needed for 14 days and the fresh weight of the plants was determined by weighing for each pot. The experimental layout was always a completely randomized design with 4 replicates. From the dose relationship, ED_{50} values (effective dose for 50% growth reduction) were calculated. A log-logistic model (sigmoidal symmetric responses on log-dose) was used [13].

All CHEAL biotypes were tested in comparison to a resistant biotype of Belgium (Kortessem F1).

2.4 Statistical Analysis

Statistical analyses were performed in Statgraphics centurion XV. Dose-response curves were conducted by the excel macro BIOASSAY97 [13]. The effective concentration (ED_{10}, ED_{30}, ED_{50} and ED_{90} values) was assessed using the statistical program R version 3.03 [14].

3. RESULTS AND DISCUSSION

In the experiments, the sensitivity of CHEAL biotypes to the herbicide metamitron was investigated. For the different CHEAL biotypes, the dose-response relationship was determined and ED_{50} values were calculated. Fig. 1 shows a typical dose-response relationship found for a CHEAL biotype from an organic farming area. Fresh matter yield is shown in comparison to the application rate. It is notable that very low application rates (<10% of maximum registered rate) caused high efficacy. The ED_{50} value is about 4.08% of the maximum application rate. An efficacy of 90% will be reached at application rates below 20%. Therefore, this CHEAL biotype shows a high sensitivity towards the herbicide.

Fig. 2 shows the dose-response relationship of two different CHEAL biotypes (CHEAL 81 = sugar beet field and CHEAL 90 = fallow land) in comparison to a resistant biotype from Belgium (Kortessem-F1). The curve shapes show a significant shift in herbicide efficacy. The resistant biotype is clearly less sensitive to metamitron compared to the other biotypes. The resistance factor was calculated from ED_{50} values of the resistant and sensitive biotypes (CHEAL 90) resulting in a factor of 6.7. Biotype CHEAL 81 from a sugar beet field was less sensitive in comparison to the CHEAL biotype from fallow land.

The investigated CHEAL biotypes come from different areas which are summarized according to types of location (Table 1). Fig. 3 shows the herbicide sensitivity of CHEAL from these locations. Overall, there are only slight differences visible between the different locations. Statistically significant differences were not detected. Plants from location type 4 (organic farming) showed a slightly higher sensitivity compared to location type 3 (conventional farming).

Comparing the CHEAL sensitivity of different arable uses in conventional farming, there were no significant differences in the sensitivity of sugar beet fields and other fields (e.g. maize, rape, potato). Therefore, the assumption is that on the other arable land, sugar beet will be cultivated in crop rotation with the use of metamitron in weed control.

A comparison of herbicide sensitivity (ED_{50} values) of CHEAL in different districts with different soils, crops and crop rotations is shown in Fig. 4. The most insensitive biotypes were found in the districts PE and HE. The most sensitive plants to metamitron were found in the districts BS and GF, both with a low percentage of sugar beet in crop rotation. The biotypes of BS were significantly different from all others. The CHEAL biotypes of GF were significantly different to PE and HE. Overall, the differences between all districts showed a range of low variation in sensitivity.

Besides the herbicide sensitivity of CHEAL at the district level, herbicide sensitivity to metamitron was also investigated at the field level for conventional farming with sugar beet in crop rotation. Bioassay results of different CHEAL biotypes in comparison to a resistant biotype are shown in Fig. 5. Dose rates of 6.25 and 12.5% of the maximum dose rate (5 L Goltix 700 SC/ha = 3.5 kg metamitron/ha) were investigated. At the lowest dose rate, significant differences in sensitivity were clearly visible. Fresh weight yield ranged from 4 to 72% compared to the control. At the second dose rate (12.5%), differences in fresh weight were smaller, ranging from 2 to 15%. A different picture is provided by looking at the resistant CHEAL biotype. At both application rates, the fresh weight yield exceeds 80%. Overall, there is a low variability in herbicide sensitivity of the selected field.

Weeds vary in their sensitivity, both between and within populations; this natural variation must be understood before shifts in sensitivity can be assessed [4]. Until now, the natural sensitivity of weed species to herbicides has not been well investigated. Studies on herbicide sensitivity are primarily carried out in connection with herbicide resistance. The results of resistant biotypes are compared to susceptible biotypes. Due to the widespread use of herbicides, it is not always possible to obtain unaffected baseline data from field populations [4].

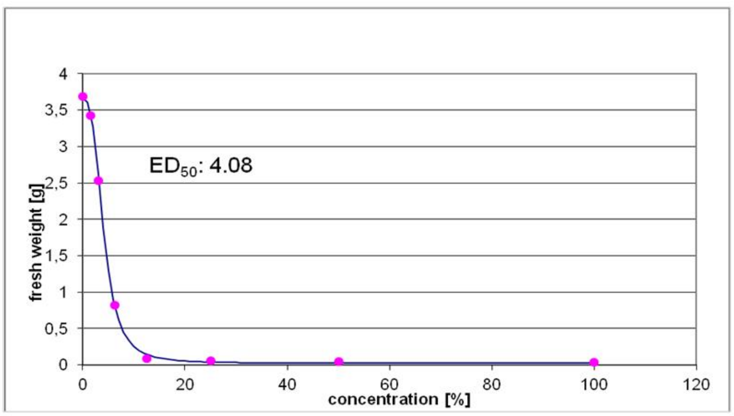

Fig. 1. Herbicide sensitivity of *Chenopodium album* L. (100 = 5 L Goltix 700 SC/ha = 3.5 kg metamitron/ha) – origin organic farming – typical dose-response curve

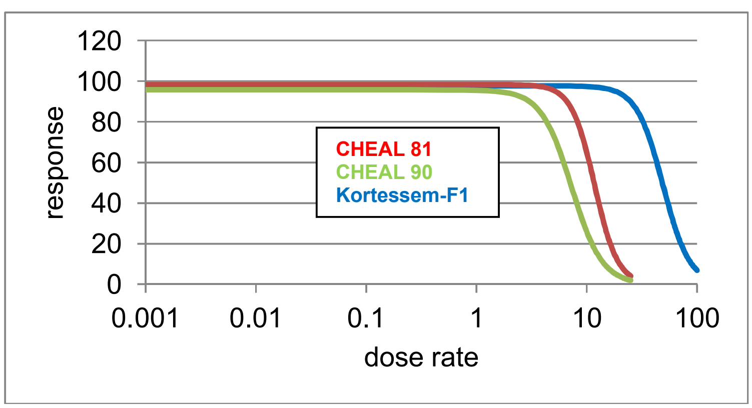

Fig. 2. Herbicide sensitivity of *Chenopodium album* L. (100 = 5 L Goltix 700 SC/ha = 3.5 kg metamitron/ha) – Fresh weight in relation to control – red = CHEAL81, green = CHEAL90, blue = Kortessem-F1

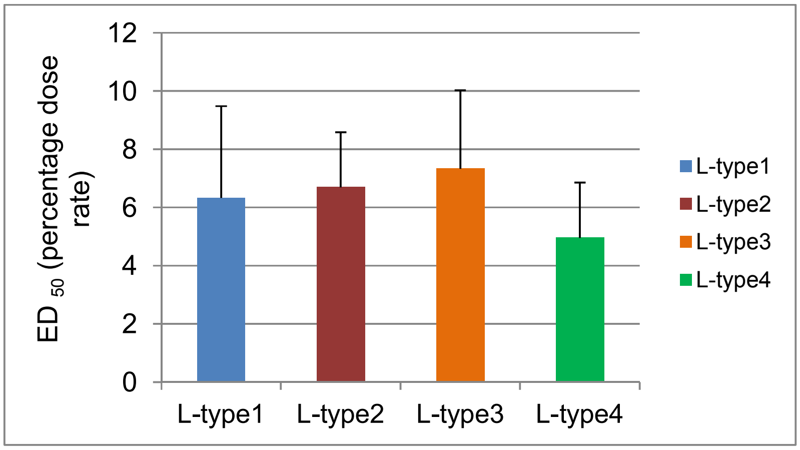

Fig. 3. Herbicide sensitivity of different types of the location of *Chenopodium album* L.

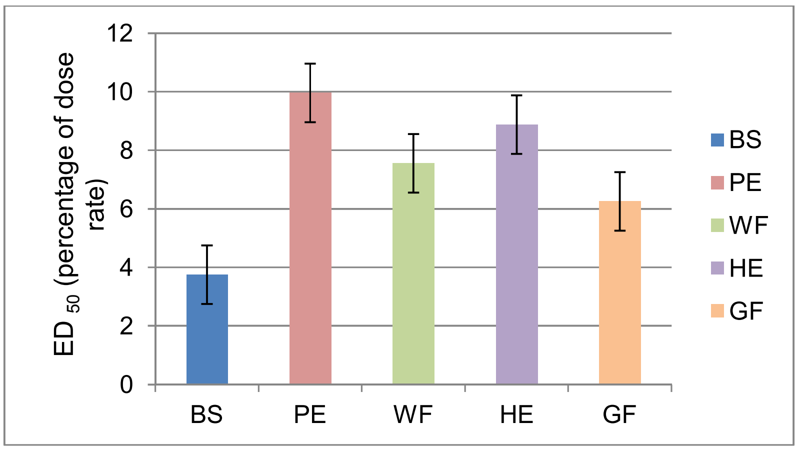

Fig. 4. Herbicide sensitivity (ED_{50}-values) of *Chenopodium album* L. in different districts

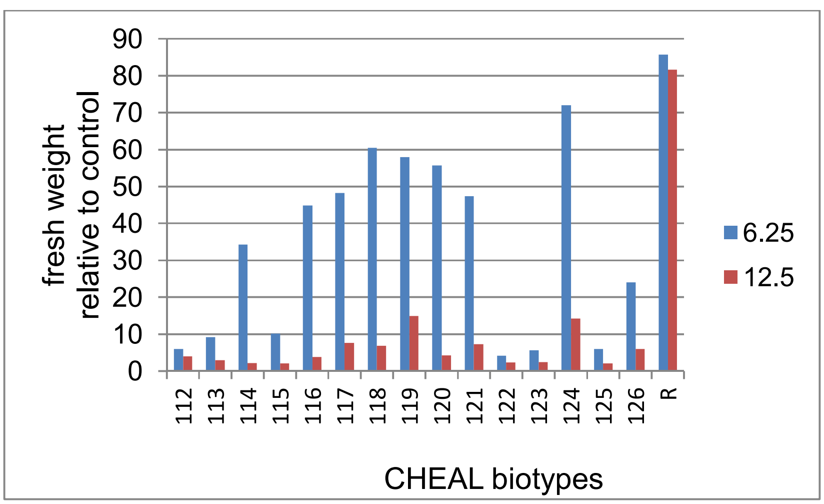

Fig. 5. Herbicide sensitivity of *Chenopodium album* L. on field level at application rates of 6.25 und 12.5% (100 = 5 L Goltix 700 SC/ha = 3.5 kg metamitron/ha)

It is assumed that herbicide resistance is a selection process due to unilateral herbicide use. Furthermore, it can be assumed that weed biotypes with different herbicide sensitivity are present within a natural weed population but also in populations influenced by herbicides.

The experiments presented here sought to explore and explain differences in CHEAL sensitivity against metamitron. The aim of the baseline monitoring project was to understand the natural variation in response to metamitron in diverse populations of CHEAL. Sensitivity data show trends towards herbicide resistance [3].

In the experiments, there were significant differences when comparing herbicide sensitivity of CHEAL in districts with different arable uses and crop rotations. No significant differences were estimated when comparing the sensitivity of sugar beet fields to other field uses in the year of seed sampling (e.g. maize, rape, potato). It must be stated that all crops are in rotation with sugar beet; therefore, metamitron-free fields cannot be expected. In contrast to other studies [8,9], CHEAL biotypes show high susceptibility to metamitron. This is possibly caused by the history of crop cultivation and herbicide use.

4. CONCLUSION

In general, it can be concluded that there is no evidence of differences in herbicide sensitivity. Overall, all CHEAL biotypes were susceptible to metamitron, but only small differences were visible at low dosages. This assessment leads to the conclusion that resistance is not expected at the time of the investigations. This is of practical benefit for crop cultivation, crop rotation, and herbicide selection. Further development is required and resistance management is recommended to avoid herbicide resistance.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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