Low Effectiveness of Prosulfocarb and Mesosulfuron-Methyl + Iodosulfuron-Methyl against *Vulpia myuros*

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Abstract: Due to natural tolerance to most widely used herbicides for grass weed control, prosulfocarb as pre-emergence or early post-emergence herbicide and mesosulfuron + iodosulfuron as post-emergence herbicide are the mainstays of any chemical control program for *Vulpia myuros* in Denmark. However, farmers often report variable efficacy of these herbicides on *V. myuros* compared to other grass weeds. Dose–response experiments were conducted to evaluate the performance of prosulfocarb and mesosulfuron + iodosulfuron on *V. myuros*. Prosulfocarb was sprayed at different plant growth stages to study the influence of plant growth stage on the performance of prosulfocarb on *V. myuros* in comparison with the more susceptible grass weed species *Apera spica-venti*. Doses causing 50% reduction in response variable (ED\(_{50}\)) were estimated from the dose–response analysis. The ED\(_{50}\) values revealed a higher tolerance of *V. myuros* to prosulfocarb and mesosulfuron + iodosulfuron than *A. spica-venti*. The relative difference in the effectiveness of prosulfocarb between *V. myuros* and *A. spica-venti* was constant among plant growth stages studied. The highest levels of *V. myuros* control were achieved when prosulfocarb was sprayed pre-emergence (BBCH 00), while the control substantially declined at later growth stages. The results from the current study document the tolerance of *V. myuros* to prosulfocarb and mesosulfuron + iodosulfuron and highlight the importance of optimization of prosulfocarb spray timing for achieving maximum control of *V. myuros*.

Keywords: natural tolerance; chemical control; integrated weed management

1. Introduction

*Rattail fescue* (*Vulpia myuros* (L.) C.C. Gmel.) is considered a problematic grass weed in parts of Northern Europe including Denmark, where its recent increase in abundance is due to a high frequency of winter cereals in crop rotations and wide adoption of no tillage practices [1]. Recently, *V. myuros* has been reported at densities of several hundred plants/m\(^2\) in winter cereals in Denmark [2]. *V. myuros* also has been reported in other EU countries, for example, in United Kingdom, France, Germany, Romania, and Switzerland, however it is still considered a minor weed in those countries [3].

*V. myuros*, a self-pollinating winter annual grass weed species, has a life cycle very similar to winter wheat [4]. It has a high fecundity and can germinate quickly and under a wide range of conditions [5]. Competition of *V. myuros* can cause significant grain yield losses in a range of crops [6]. A study conducted in Denmark showed that *V. myuros* at 405 plants/m\(^2\) can cause yield losses up to 50% in winter wheat [7].

*V. myuros* traditionally has been managed by plowing in conventional farming systems. However, the adoption of no tillage cropping systems to preserve soil productivity and reduce energy use eliminates the possibility of using tillage to control *V. myuros*. Acetyl-coenzyme A carboxylase (ACCase) and acetolactate synthase (ALS) inhibitors are the most widely used herbicides to control grass weeds in winter cereals. However, *V. myuros* is...
naturally tolerant to ACCase inhibitors, and many ALS inhibitor herbicides are not highly effective against this weed species [2, 8, 9]. Previously, several studies have related the poor performance of foliar applied herbicides on *V. myuros* to low spray retention on the narrow leaf blades of this species [10, 11].

Due to natural tolerance to most widely used herbicides, the options for chemical control of *V. myuros* are limited compared with other grass weeds. In Denmark, farmers rely on residual herbicides such as prosulfocarb applied pre-emergence or early post-emergence and post-emergence application of mesosulfuron + iodosulfuron in autumn and/or spring for the control of *V. myuros* in winter cereals. However, some reports suggest that the typically used rates of prosulfocarb and mesosulfuron + iodosulfuron provide more inconsistent control of *V. myuros* than of other grasses [2, 8, 12]. The effectiveness of control with prosulfocarb depends on the growth stage of *V. myuros*. For example, previous studies have shown that some level of *V. myuros* control could be achieved if prosulfocarb was applied pre-emergence, but effectiveness tended to decrease rapidly when sprayed at later growth stages [8, 13]. However, the information on prosulfocarb performance as an early post-emergence application on *V. myuros* is limited. To optimize herbicide application timings, information on prosulfocarb effectiveness on *V. myuros* at different growth stages is required. Reports suggest that *V. myuros* can tolerate the recommended rates of mesosulfuron + iodosulfuron better than other grass weed species such as *A. spica-venti* [2], but there is no information on the level of tolerance in *V. myuros* against mesosulfuron + iodosulfuron. The objective of this study was to assess the performance of prosulfocarb and mesosulfuron + iodosulfuron and evaluate the impact of plant growth stages on the performance of prosulfocarb on *V. myuros*. *Apera spica-venti* was used as a susceptible control species in the study.

2. Materials and Methods

2.1. Seed Source

Seeds of *V. myuros* were collected from a non-agricultural area at Flakkebjerg Research Centre, Denmark, where there has been no known history of herbicide application. Seeds of susceptible populations of *A. spica-venti* originated from several locations in Denmark and were mixed together in the same proportion to create a meta-population [14].

2.2. Prosulfocarb Bioassay

Twenty seeds of *V. myuros* and *A. spica-venti* were sown in 1 L pots filled with field soil (sandy loam). The pots were placed in an unheated glasshouse or on outdoor tables depending on the time of the year when the experiment was conducted. Groups of pots were sown on different dates to ensure different plant growth stages could be sprayed at the same time. After seedling emergence, the number of plants per pot were thinned to a pre-set number. Pots treated pre-emergence were not thinned. Pre-emergence treatments were carried out one day after sowing. Plants were treated with a range of prosulfocarb doses (0, 25, 50, 100, 200, 400, 800, 1000, 2000, and 4000 g/ha) (Boxer, 800 g L$^{-1}$ prosulfocarb, Syngenta Crop Protection A/S, Denmark). Herbicide application was carried out in a spray cabinet equipped with a boom fitted with two flat-fan nozzle (HARDI ISO F-110-02). The nozzles were operated at a pressure of 300 kilopascal and velocity of a 5.2 km h$^{-1}$ delivering spray volume of a 152 L ha$^{-1}$. A few hours after application of prosulfocarb, 30 mL of Milli Q water was applied to the soil surface of each pot to ensure a uniform distribution of prosulfocarb in the upper soil layer. The plants were sprayed at BBCH (Biologische Bundesanstalt Bundessortenamt and Chemical Industry) 00 to 13 (Table 1). The prosulfocarb dose–response study was repeated four times. As plants were sprayed at different growth stages in four repeats, thus making the interpretation of results straightforward. Experiments 1–4 hereafter refer to the four runs of the dose–response study (Table 1). The four experiments were sprayed on 22 May 2018 (Experiment 1), 23 October 2018 (Experiment 2), 18 March 2019 (Experiment 3), and 15 April 2019 (Experiment 4). After spraying, the pots were placed in growth chamber at 14/10 °C day/night with 16 h photoperiods.
for Experiment 1, in a cold glasshouse for Experiments 2 and 3, and outdoors for Experiment 4 (Table 1). There were three replicates per treatment plus an untreated control in a complete randomized block design. Dry foliage weights were recorded 3–4 weeks after herbicide treatment.

Table 1. A table displaying the set-up of different experiments carried out to examine the level of the lower performance of prosulfocarb and mesosulfuron-methyl + iodosulfuron-methyl in *V. myuros*.

| Study                  | Experiment | Treatments (Herbicide Dose and/or Plant Stages)                                                                 | Environment                                    |
|------------------------|------------|---------------------------------------------------------------------------------------------------------------|------------------------------------------------|
| Prospulfocarb bioassay | Experiment 1 | Prosulfocarb rate ranging from 0 to 4000 g ha\(^{-1}\); BBCH 00, BBCH 11, BBCH 12, BBCH 13                | Growth chamber at 14/10 °C day/night with 16 h photoperiods |
|                        | Experiment 2 | Prosulfocarb rate ranging from 0 to 4000 g ha\(^{-1}\); BBCH 00, BBCH 10, BBCH 11, BBCH 12                | Unheated glasshouse                             |
|                        | Experiment 3 | Prosulfocarb rate ranging from 0 to 4000 g ha\(^{-1}\); BBCH 00, BBCH 10, BBCH 13                | Unheated glasshouse                             |
|                        | Experiment 4 | Prosulfocarb rate ranging from 0 to 4000 g ha\(^{-1}\); BBCH 11, BBCH 12                | Outdoor under natural conditions               |
| Mesosulfuron-          | Seed       | Mesosulfuron-methyl + iodosulfuron-methyl concentrations ranging from 0 to 144 mg/L were added to agar medium containing weed seeds | Laboratory                                     |
| methyl + iodosulfuron- | germination |                                                                                                              |                                                |
| methyl bioassay        | assay      |                                                                                                              |                                                |
| Seedling assay         |            | Mesosulfuron-methyl + iodosulfuron-methyl concentrations ranging from 0 to 144 mg/L were added to agar medium containing weed seedlings | Laboratory                                     |

2.3. *Mesosulfuron-Methyl + Iodosulfuron-Methyl* Bioassay

2.3.1. Seed Germination Assay

The plant agar medium (Duchefa) (10 g/L) (1% w/v) was boiled in a microwave and allowed to cool to 35–40 °C. A stock solution of mesosulfuron-methyl + iodosulfuron-methyl (Atlantis OD, 10 g/L mesosulfuron-methyl + 2 g/L iodosulfuron-methyl + 30 g/L mefenpyr-diethyl, Bayer CropScience, Denmark) in the commercial form was prepared. Using this stock solution, different concentrations of mesosulfuron-methyl + iodosulfuron-methyl were prepared. Mesosulfuron-methyl + iodosulfuron-methyl assay concentrations were 0, 0.009, 0.018, 0.036, 0.072, and 0.144 mg/L. The herbicide concentrations were added to agar medium using micropipette. Twenty milliliters of agar medium plus herbicide solution were poured into quadratic plastic dishes (245 mm × 245 mm). Fifty seeds of *V. myuros* and *A. spica-venti* were placed in each dish. The dishes were covered with lids and placed in the greenhouse. The experiment was arranged in a completely randomized design with three replicates. Coleoptile and radicle lengths were measured 10–15 days after treatment.

2.3.2. Seedling Assay

Plants of *V. myuros* and *A. spica-venti* were grown in plastic trays using potting mixture containing soil, peat, and sand (2:1:1 by weight). The plastic trays were placed in an unheated glasshouse on the table with automatic watering system. The plant agar media and herbicide concentrations were prepared as described above in the seed germination assay. Seedlings of *V. myuros* and *A. spica-venti* at the 2–3 leaf stage (BBCH 12–13) were harvested from the plastic trays and washed under tap water to remove any particles of potting mixture from roots. The seedling tips (growing points of leaves and roots) were excised. Roots of seedlings were transplanted into plastic dishes (245 mm × 245 mm) containing a plant agar media using forceps. The plastic dishes were covered and placed in the heated glasshouse. The experiment was arranged in a completely randomized design with ten plant seedlings in each dish and three replications. Herbicide efficacy
was recorded as seedling survival by visually assessing the new roots and shoots 15 days after transplanting.

2.4. Statistical Analysis

A nonlinear log-logistic model was used to analyze the prosulfocarb and mesosulfuron + iodosulfuron dose–response studies [15]:

\[
Y = \frac{d - c}{1 + \exp[b(\log(t) - \log(e))]} \tag{1}
\]

where \(Y\) is the response variable (represents the percent fresh weight, germination, coleoptile length, and radicle length relative to untreated control); \(c\) and \(d\) denote the lower and upper asymptote, respectively; \(e\) is the dose causing 50% reduction in response variable (ED\(_{50}\)); and \(b\) is the slope of the curve. The model was checked by residual plots and with lack of fit test (\(p > 0.05\)). If the value of \(c\) was zero, the four-parametric model was reduced to a three-parametric one as:

\[
Y = \frac{d}{1 + \exp[b(\log(t) - \log(e))]} \tag{2}
\]

The analyses were performed using R version 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria, http://R-project.org) with drc package [16]. The value of \(e\) (ED\(_{50}\)) was compared between the species using post hoc \(t\)-tests. Tolerance index (TI) was estimated as \(V.\ myuros\) to \(A.\ spica-venti\) ED\(_{50}\) ratios to compare the responses from a population of \(V.\ myuros\) with those of \(A.\ spica-venti\).

3. Results

3.1. Prosulfocarb Bioassay

Data from all experiments were analyzed separately as plants were sprayed at different growth stages and significant differences were found in the regression parameter estimates between experiments. The tolerance index (TI), i.e., ED\(_{50}\) value of \(V.\ myuros\) relative to ED\(_{50}\) value of \(A.\ spica-venti\), expressing the level of herbicide tolerance in \(V.\ myuros\) relative to \(A.\ spica-venti\), was calculated by dividing the estimated ED\(_{50}\) values for \(V.\ myuros\) by the corresponding values for \(A.\ spica-venti\) (Figures 1–4). TI’s values showed that \(V.\ myuros\) was more tolerant to prosulfocarb than \(A.\ spica-venti\) at all growth stages studied (Table 2 and Figure 5). \(V.\ myuros\) showed 3.4-, 2.1-, 2.1-, and 3.3-fold tolerance at the BBCH 00 (pre-emergence), BBCH 11, BBCH 12, and BBCH 13 stages, respectively, in Experiment 1. \(V.\ myuros\) exhibited 2.8-, 3.5-, 3.2-, and 1.4-fold tolerance at the BBCH 00, BBCH 10, BBCH 11, and BBCH 12 stages, respectively, in Experiment 2 (Table 2). The TI for \(V.\ myuros\) was 2.5-, 2.7-, and 1.6-fold at the BBCH 00, BBCH 10, and BBCH 13 stages, respectively, in Experiment 3 (Table 2). In Experiment 4, \(V.\ myuros\) showed 4.0- and 1.5-fold tolerance at the BBCH 11 and BBCH 13 stages, respectively.

Application rates of prosulfocarb required to suppress 50% shoot biomass of \(V.\ myuros\) was lowest at BBCH 00 stage and, with a few exceptions, tended to increase at more advanced growth stages. Generally, a similar trend was observed for \(A.\ spica-venti\). For instance, the estimated ED\(_{50}\) value was lower at the BBCH 00 stage than at BBCH 11, BBCH 10, and BBCH 13 in Experiments 1–3, respectively. In Experiment 4, the plants were sprayed only at two growth stages (BBCH 11 and BBCH 13), where the rates of prosulfocarb for 50% shoot biomass reduction of \(V.\ myuros\) was lower at BBCH 11 than at BBCH 13, although the difference was non-significant. For \(A.\ spica-venti\), the estimated ED\(_{50}\) values were significantly lower at BBCH 11 than at BBCH 13 in Experiment 4. In general, moving towards more advanced growth stages, higher ED\(_{50}\) values were estimated than at early growth stages, and these results are consistent in the two species and four trials.
3.2. Mesosulfuron-Methyl + Iodosulfuron-Methyl Bioassay

3.2.1. Seed Germination Assay in Agar Medium

Coleoptile and radicle lengths were inhibited by herbicide treatments for both grass weed species, but the inhibition was significantly higher for *A. spica-venti* than for *V. myuros* (Figure 6A,B). Estimated ED$_{50}$ values for *V. myuros* and *A. spica-venti* were 0.04 and 0.01 mg/L for coleoptile growth and 0.05 and 0.01 mg/L for radicle growth, respectively (Table 3). The ED$_{50}$ of *V. myuros* was 3.0 and 4.4 times (TI) higher than *A. spica-venti* for coleoptile and radicle growth, respectively.

![Figure 1](image_url)

**Figure 1.** Prossulfocarb dose–response on aboveground fresh weight represented as percentage of untreated control of *Vulpia myuros* and *Apera spica-venti* in Experiment 1 at: BBCH 00 (A); BBCH 11 (B); BBCH 12 (C); and BBCH 13 (D).
3.2.2. Seedling Assay

The plant agar was found to be a suitable media for growing *V. myuros* and *A. spica-venti*, where individuals from both *V. myuros* and *A. spica-venti* developed new roots and shoots. New roots and shoots of both species were suppressed more in dishes with herbicide compared to untreated controls, but the suppression of new roots and shoots was significantly higher in *A. spica-venti* than in *V. myuros* (Figures 6C and 7). For instance, the ED$_{50}$ values for *V. myuros* and *A. spica-venti* were 0.14 and 0.04 mg/L, respectively, indicating that *V. myuros* was 3.2 times (TI) more tolerant to mesosulfuron + iodosulfuron than *A. spica-venti* (Table 3). The results from both seed germination and seedling assay show that *V. myuros* is more tolerant to mesosulfuron + iodosulfuron than *A. spica-venti*.

![Figure 2](image-url)  
*Figure 2.* Prosulfocarb dose–response on aboveground fresh weight represented as percentage of untreated control of *Vulpia myuros* and *Apera spica-venti* in Experiment 2 at: BBCH 00 (A); BBCH 10 (B); BBCH 11 (C); and BBCH 12 (D).
Figure 3. Prosulfocarb dose–response on aboveground fresh weight represented as percentage of untreated control of *Vulpia myuros* and *Apera spica-venti* in Experiment 3 at: BBCH 00 (A); BBCH 10 (B); and BBCH 13 (C).
Figure 4. Prosulfocarb dose–response on aboveground fresh weight represented as percentage of untreated control of *Vulpia myuros* and *Apera spica-venti* Experiment 4 at: BBCH 11 (A); and BBCH 12 (B).

Table 2. Prosulfocarb doses (g ha\(^{-1}\)) providing 50% reduction in fresh weight of *V. myuros* and *A. spica-venti* estimated using log-logistic model. Standard errors are presented in parentheses.

| Growth Stage | BBCH 00 (1) | BBCH 11 (2) | BBCH 12 (3) | BBCH 13 (4) | Significance Level between Growth Stages |
|--------------|-------------|-------------|-------------|-------------|----------------------------------------|
| **Experiment 1** |             |             |             |             |                                        |
| *V. myuros*   | 199 (43.9)  | 438 (87.6)  | 293 (26.6)  | 926 (252.2) | 1 vs. 2, \(p < 0.001\); 1 vs. 3, \(p = 0.074\); 1 vs. 4, \(p < 0.001\); 2 vs. 3, \(p = 0.144\); 2 vs. 4, \(p = 0.005\); 3 vs. 4, \(p < 0.001\); |
| *A. spica-venti* | 59 (10.4)   | 212 (19.3)  | 141 (11.7)  | 278 (48.3)  | 1 vs. 2, \(p = 0.04\); 1 vs. 3, \(p = 0.002\); 1 vs. 4, \(p < 0.001\); 2 vs. 3, \(p = 0.006\); 2 vs. 4, \(p = 0.086\); 3 vs. 4, \(p < 0.001\); |
| Tolerance indices | TI | p = 0.017 | p = 0.026 | p < 0.001 | p = 0.038 |
| **Growth stage** | BBCH 00 | BBCH 10 | BBCH 11 | BBCH 12 |                                        |
| **Experiment 2** |             |             |             |             |                                        |
| *V. myuros*   | 110.2 (8.9) | 170 (18.6)  | 184 (12.6)  | 241 (17.0)  | 1 vs. 2, \(p = 0.17\); 1 vs. 3, \(p = 0.004\); 1 vs. 4, \(p < 0.001\); 2 vs. 3, \(p = 0.686\); 2 vs. 4, \(p = 0.041\); 3 vs. 4, \(p = 0.038\); 3 vs. 4, \(p < 0.001\); |
| *A. spica-venti* | 39 (5.3)   | 48 (4.03)   | 58 (5.1)    | 167 (15.6)  | 1 vs. 2, \(p = 0.17\); 1 vs. 3, \(p = 0.004\); 1 vs. 4, \(p < 0.001\); 2 vs. 3, \(p = 0.076\); 2 vs. 4, \(p < 0.001\); 3 vs. 4, \(p < 0.001\); |
| Tolerance indices | TI | 2.8 | 3.5 | 3.2 | 1.4 |
| **Growth stage** | BBCH 00 | BBCH 10 | BBCH 11 | BBCH 13 |                                        |
| **Experiment 3** |             |             |             |             |                                        |
| *V. myuros*   | 223 (46.9)  | 223 (50.9)  | 802 (54.6)  | 1 vs. 2, \(p = 0.948\); 1 vs. 3, \(p < 0.001\); 2 vs. 3, \(p < 0.001\); |
| *A. spica-venti* | 89 (12.4)  | 82 (11.7)   | 513 (90.8)  | 1 vs. 2, \(p = 0.669\); 1 vs. 3, \(p < 0.001\); 2 vs. 3, \(p < 0.001\); |
| Tolerance indices | TI | 2.5 | 2.7 | 1.6 | 1.5 |
| **Growth stage** | BBCH 11 | BBCH 12 | BBCH 13 | BBCH 13 |                                        |
| **Experiment 4** |             |             |             |             |                                        |
| *V. myuros*   | 353 (63.5)  | 447.3 (135.8) | 1 vs. 2, \(p = 0.3716\); |
| *A. spica-venti* | 87.1 (7.3)  | 302.4 (58.7) | 1 vs. 2, \(p < 0.001\); |
| Tolerance indices | TI | 4.0 | p < 0.001 | 1.5 | p = 0.3751 |
Figure 5. Photographic illustration of prosulfocarb dose–response for the *Vulpia myuros* and *Apera spica-venti* sprayed on: pre-emergent seeds (A); and germinating seedlings (B). The normal dose (N) of prosulfocarb was 800 g/ha.

Figure 6. Dose–response of mesosulfuron-methyl + iodosulfuron-methyl on germination and coleoptile (A) and radicle lengths (B) and seedling survival (C) of *V. myuros* and *A. spica-venti* in agar-based bioassay.
Table 3. Mesosulfuron-methyl + iodosulfuron-methyl-sodium rates (mg/L) providing 50% reduction of response variable using log-logistic model for V. myuros and A. spica-venti in agar-based seed germination and seedling assay. Standard errors are presented in parentheses.

| Experiments         | Species     | V. myuros | Apera Spica-Venti | Tolerance Index |
|---------------------|-------------|-----------|-------------------|-----------------|
| Seed germination    | Coleoptile  | 0.04 (0.007) | 0.01 (0.003) | 3.0 p = 0.021  |
|                     | length      |           |                   |                 |
| Seed germination    | Radicle     | 0.05 (0.008) | 0.01 (0.002) | 4.4 p = 0.006  |
|                     | length      |           |                   |                 |
| Seedling assay      | Seedling    | 0.14 (0.023) | 0.04 (0.009) | 3.2 p = 0.017  |
|                     | survival    |           |                   |                 |

Figure 7. Photographic illustration of V. myuros and A. spica-venti populations assayed as untreated control and at 0.072 mg/L mesosulfuron + iodosulfuron in the agar-based seedling assay.

4. Discussion

Due to natural tolerance to most of the widely used herbicides, prosulfocarb and mesosulfuron + iodosulfuron are considered important chemical control options for V. myuros. Nonetheless, farmers have reported that the two herbicides are not highly effective in controlling V. myuros compared with other grass weeds such as A. spica-venti. To evaluate the level of tolerance of V. myuros to prosulfocarb and mesosulfuron + iodosulfuron, we carried out outdoor, glasshouse, and laboratory studies on a V. myuros population and compared the responses to a meta-population of A. spica-venti.
4.1. Prosulfocarb Bioassay

The results obtained in the prosulfocarb dose–response studies showed a higher tolerance of *V. myuros* to prosulfocarb compared with *A. spica-venti* (Table 2 and Figures 1–4). Other researchers also found that prosulfocarb is not highly effective in controlling *V. myuros*, and often maximum authorized doses were needed for achieving some level of control [8,17]. The results of the three experiments are not comparable because the experiments were carried out at different times of the year and, thus, under different climatic conditions. The primary aim of this study was to evaluate the level of susceptibility to prosulfocarb of different growth stages of *V. myuros* to that of *A. spica-venti* at the same growth stages. The results are consistent across experiments with *V. myuros* being less susceptible than *A. spica-venti*. To visualize the relative increase in prosulfocarb dose required to suppress 50% biomass at later growth stages in *V. myuros* and *A. spica-venti*, the estimated relative ED$_{50}$ values with the increased growth stages were pooled across experiments and plotted against BBCH growth stage for the two weed species (Figure 8). The ED$_{50}$ values from Experiment 4 are not included in the plot because in Experiment 4 plants were not sprayed at the pre-emergence stage, and, therefore, it was not possible to calculate the relative ED$_{50}$ values. The plot between relative ED$_{50}$ values and BBCH growth stages showed that relative difference in the effectiveness of prosulfocarb between *V. myuros* and *A. spica-venti* tends to remain similar when sprayed at different plant growth stages, which indirectly indicate that tolerance in *V. myuros* is inherent and tends to remain constant at different growth stages. More research is needed to investigate the mechanism of tolerance in *V. myuros* in detail and should include absorption, translocation, metabolism, and vacuolar sequestration studies.

![Figure 8](image-url)  
**Figure 8.** The relationship between relative difference in ED$_{50}$ values with the increased growth stages and BBCH growth stages in *V. myuros* and *A. spica-venti*. The ED$_{50}$ values with the increased growth stages were pooled across Experiments 1–3. Vertical bars represent standard errors of the mean.

The results show that weed growth stage at the time of spraying had a significant effect on plant sensitivity to prosulfocarb. Pre-emergence application of prosulfocarb at the rate of 800 g/ha resulted, on average, in an 80–90% biomass reduction of *V. myuros*, while the same herbicide dose provided 70–80% dry matter reduction when sprayed at
emerged seedling stages (BBCH 10 to BBCH 11). Similar to our results, Hull et al., [8] also found a lower efficacy of prosulfocarb when applied early post-emergence compared to pre-emergence applications.

In the current study, the estimated ED$_{50}$ doses required to control _V. myuros_ and _A. spica-venti_ were lower than the typically used field doses. Pot-grown weed plants are generally more susceptible to herbicides than plants grown in the field and, interestingly, results from the four experiments are consistent, indicating lower susceptibility of _V. myuros_ to prosulfocarb, regardless of plant growth stage. Farmers often experience failures when applying prosulfocarb for the control of _V. myuros_ under field conditions, and field observations have shown that significantly higher doses of prosulfocarb are needed for effective control. In contrast, prosulfocarb provided effective control of _A. spica-venti_ with ED$_{50}$ values of less than 100 g ha$^{-1}$ at pre-emergence (BBCH 00) and BBCH 10 and less than 200 g ha$^{-1}$ at BBCH 12. However, beyond BBCH 12, control of _A. spica-venti_ with prosulfocarb tended to decline. Our findings are in line with those of Adamczewski et al. [18] who also reported satisfactory control of _A. spica-venti_ with recommended rates of prosulfocarb when applied pre-emergence under field conditions.

4.2. Mesosulfuron-Methyl + Iodosulfuron-Methyl Bioassay

The agar-based assay showed that the lengths of coleoptiles and radicles and seedling survival of _V. myuros_ and _A. spica-venti_ were suppressed by increasing doses of mesosulfuron-methyl + iodosulfuron-methyl. Abdurruhman et al. [19] reported suppression of coleoptile and radicle lengths and establishment of new shoots and roots in wild oat (_Avena sterilis_ L.) populations treated with mesosulfuron-methyl + iodosulfuron-methyl in agar media. Previously, many studies have shown that the agar-based assay can be used to detect resistance in grass weeds such as _Sorghum halepense_, _Alopecurus myosuroides_, and _Lolium rigidum_ [20–22]. This method has proven to be a reliable and quick way to detect the differences between resistant/tolerant and susceptible grass weed biotypes for pre-emergence and post-emergence herbicides [19,23]. The results obtained in agar-based assay show that the ED$_{50}$ values for _V. myuros_ were 3–4.4 times higher than for _A. spica-venti_. The results confirm that _V. myuros_ is tolerant to mesosulfuron-methyl + iodosulfuron-methyl, as it is to other herbicides. Other researchers found that mesosulfuron-methyl + iodosulfuron-methyl is not highly effective in controlling _V. myuros_, and often higher than the maximum recommended rates were needed for satisfactory control of this weed species [8]. It is often anticipated that ineffective control of _V. myuros_ with post-emergence herbicides can be attributed to low spray retention due to its narrow and erect leaves [10,11]. The results of the agar-based test show that _V. myuros_ is naturally tolerant to mesosulfuron-methyl + iodosulfuron-methyl and that differences in spray deposition can only partly be the cause for the observed differences in susceptibility. This assumption is further supported by the finding of Akhter, [24], who showed that low spray retention is not the cause of low performance of glyphosate on _V. myuros_. Yu et al. [9], studying the closely related species _V. bromoides_, proved its tolerance to ACCase and several ALS inhibitors and concluded that an insensitive ACCase and enhanced metabolism were the likely mechanisms of tolerance to the two modes of action. Here, we document for first the time that _V. myuros_, similar to _V. bromoides_, is naturally tolerant to mesosulfuron-methyl + iodosulfuron-methyl compared to a population of _A. spica-venti_. In the current study, we did not attempt to study the _V. myuros_ tolerance mechanism against mesosulfuron-methyl + iodosulfuron-methyl, but the available information shows that the response of _V. myuros_ and _V. bromoides_ to various herbicides are similar. Hence, it can be speculated that the mechanism of tolerance in _V. myuros_ to ALS inhibiting herbicides including mesosulfuron-methyl + iodosulfuron-methyl might be due the same mechanism as found for _V. bromoides_ [2].

According to the current study, pre-emergence application of prosulfocarb can provide some level of _V. myuros_ control but prosulfocarb has a narrow window of application as the activity declined significantly at more advanced growth stages. Moreover, the soil half-life of some residual herbicides is short, for example, the half-life of prosulfocarb is
around 10 days [25], and, therefore, pre-emergence herbicide spraying potentially cannot control late germinating V. myuros plants, particularly in years with mild winters and high dormancy. Changing from a single application to sequential applications of herbicides can overcome this shortcoming and provide more effective control of other grass weeds. Nonetheless, the results from the current study indirectly indicate that V. myuros control with sequential treatments of herbicides, i.e., combining a pre-emergence application of prosulfocarb with a post-emergence treatment of mesosulfuron-methyl + iodosulfuron-methyl, would be less than acceptable, especially on dense stands. In the absence of effective chemical control methods, V. myuros, due to its profound growth behavior, can quickly take over the field and cause significant grain yield losses [7]. It appears that management of V. myuros will require other inputs in addition to herbicides. Integrated weed management strategies with several preventive and cultural control measures such as tillage, crop rotation, delayed crop sowing, and crop competition applied in combination with available herbicides can facilitate sustainable weed management of V. myuros.

5. Conclusions

The current study revealed that V. myuros is tolerant to prosulfocarb and mesosulfuron-methyl + iodosulfuron-methyl compared with A. spica-venti, and the relative differences in the effectiveness of prosulfocarb between V. myuros and A. spica-venti tend to remain constant among the plant growth stages studied. The results further show that the highest level of V. myuros control with prosulfocarb is achieved if the herbicide is applied pre-emergence or on germinating seedlings, whereas control tends to decline substantially beyond these growth stages. The information on the level of V. myuros tolerance to herbicides documented here will help for planning a proper chemical weed control program as part of integrated weed management strategies against this species.

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