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The first recorded case of herbicide resistance in Estonia: common chickweed (*Stellaria media*) resistant to sulfonylureas

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Herbicide resistance has been insufficiently studied in the Baltic countries. Chickweed (*Stellaria media*), an adaptive and competitive annual weed, poses a significant agricultural threat due to its low and dense canopy. This study aimed to evaluate the level of resistance to acetolactate synthase (ALS) inhibitors in chickweed populations in Estonia and identify potential mechanisms. In the initial dose-response experiment, a biotype from Järva County exhibited resistance to the ALS inhibitor tribenuron-methyl. Molecular analysis identified a mutation at position 574 in the ALS gene, where tryptophan was replaced by leucine. Testing of eight chickweed biotypes for resistance to tribenuron-methyl and the combination herbicide iodosulfuron + amidosulfuron found three resistant biotypes. The Järva County biotype was cross-resistant to sulfonylureas, while two others exhibited resistance only to iodosulfuron + amidosulfuron. The latter two biotypes lacked the ALS gene mutation, suggesting non-target-site resistance mechanisms, although these were not investigated further. These findings highlight the potential for widespread herbicide resistance in Estonia and emphasize the need for more comprehensive monitoring and research into resistance mechanisms.

Key words: ALS inhibitor, tribenuron-methyl, sulfuron, target-site, non-target site

Introduction

Weeds, when present above critical population thresholds, can cause crop yield losses of up to 80% and degrade crop quality (Soltani et al. 2016, Gerhards et al. 2017). Effective crop production, leading to high yields of quality products, relies on an appropriate crop management system, which includes diverse and integrated weed control measures. Herbicides have historically been an efficient tool for weed management in agricultural fields (Kraehmer et al. 2014). Optimal weed control with chemical herbicides can significantly increase yields, especially under high weed pressure.

However, herbicide resistance in arable weeds is increasing rapidly worldwide, becoming one of the greatest challenges to crop production and food security (Ziska 2020). Continuous herbicide use exerts selective pressure on weed populations, leading to a decline in herbicide efficacy, eventually reaching a threshold where effective weed control becomes elusive (Gaines et al. 2020) leading to the evolution of herbicide resistance in hundreds of weed species. Over 500 weed species have developed resistance to one or more herbicides, and this number continues to increase (Heap 2024). Among herbicides, sulfonylureas, a group of herbicides within acetolactate synthase (ALS) inhibitors, classified under Herbicide Resistance Action Committee (HRAC) Group 2 (Beffa et al. 2019), are widely used. Resistance to ALS inhibitors, a longstanding issue, has been documented globally (Duggleby et al. 2008). The widespread reliance on herbicides presents a growing problem, as the evolution of herbicide resistance limits effective weed control.

Resistance can arise through various mechanisms, such as target-site mutations in the herbicide's action site or non-target-site mechanisms like physiological adaptations, gene overexpression, or enhanced metabolic processes, or other factors (Gaines et al. 2020). Non-target-site resistance, challenges control efforts because it makes many herbicides ineffective, regardless of their mode of action (Preston 2004). While ALS-inhibiting herbicides do not directly affect photosynthesis, studies have demonstrated that they, like other herbicides, can inhibit photosynthesis rates (e.g., Košnarová et al. 2021). Common chickweed (*Stellaria media*), an adaptable and competitive annual weed, is a significant threat to both agricultural and horticultural systems due to its dense, low-growing canopy. Resistance to ALS inhibitors in *S. media* was first reported in Europe in the 1980s (Barnwell and Cobb 1989) and persists as a problem today (Linn et al. 2019). In Denmark, *S. media* was the first weed species to be reported as resistant to ALS inhibitors in 1991, with further resistance documented in other species since (Heap 2024). In Norway and Sweden, resistance to ALS inhibitors is now common, and in *S. media*, it has been linked to two

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specific mutations in the ALS protein: Pro 197 and Trp 574 (Laforest and Soufiane 2018) and two mutations in the *S. media* ALS gene (Pro-197-Gln and Trp-574-Leu).

Herbicide resistance monitoring in the Baltic countries and Finland has been limited. However, resistance has been confirmed in Lithuania in silky windgrass (*Apera spica-venti* (L.) P. Beauv.) (Auškalnienė et al. 2020). Additionally, reports of herbicide resistance in *S. media* have been published from Finland (Uusitalo et al. 2013). According to the International Herbicide-Resistant Weed Database (Heap 2024), there are also documented cases of herbicide -resistant common lambsquarters (*Chenopodium album* L.) in Finland, as well as resistance in silky windgrass and *S. media* in Latvia.

In Estonia, herbicides are the most widely used type of pesticide, accounting for approximately 79% of total pesticide sales over the past decade (Statistical Database 2023). This heavy reliance on herbicides makes resistance development likely, but the potential for herbicide resistance in Estonian weed populations has not been thoroughly investigated. Some studies have explored the effects of reduced herbicide doses in barley (Talgre et al. 2008), and recent research has focused on long-term weed management practices, including crop rotation and cover cropping (Madsen et al. 2023). Although commercial field trials on herbicide efficacy are conducted, the results are not publicly available.

Herbicide resistance mechanisms remain largely unexplored in the Baltic countries. Understanding these mechanisms is crucial for developing effective resistance management strategies. This study represents the first attempt to assess herbicide resistance in *S. media* to sulfonylureas in Estonia, with the primary objective of evaluating the level of resistance and identifying potential resistance mechanisms within Estonian *S. media* populations.

Materials and methods

Investigation of possible resistance of weeds to different herbicides in Estonia is mainly based on the European Guidelines to conduct herbicide resistance tests (2017), this study as well.

Initially, two Estonian biotypes of *S. media* were selected for investigation as part of the routine monitoring of crop pests and diseases in Järva County, Estonia during the summer of 2021. These biotypes were found to be abundant in cereal fields at two locations, designated as SME14 (58.69529N, 25.76610E) and SME18 (58.65130N, 25.56605E). While the precise herbicide usage data for these fields are not available, the farmer at SME14 indicated the application of sulfonylurea herbicides, while at location SME18 mainly MCPA has been used. For comparison, seeds from a previously documented herbicide-sensitive biotype from the Czech Republic (SMCZ) were included as a susceptible control. The SMCZ seeds were collected from a rural area near the Czech University of Life Sciences in Prague, where herbicides had not been applied for an extended period.

Dose-response experiments were conducted in summer 2022 at the Czech University of Life Sciences, Prague. Plants were grown in a semi-open greenhouse with slightly shaded light under natural light regime and temperature conditions. The plants were grown in 7 × 7 cm pots filled with a commercial growing mixture combined with sand, with 4–5 plants per pot and 4 pots per treatment. Herbicide treatments were applied at the 2–3 nodes stage (BBCH stage 32–33, Meier 2001) using a laboratory spray chamber fited with a Lurmark 01F80 nozzle, calibrated to a spray volume of 250 l ha^{-1} and a pressure of 250 kPa. The herbicide Granstar 50SX (FMC-Agro), containing the active ingredient ALS inhibitor tribenuron-methyl (500 g kg^{-1}), with recommended dose 10–30 g ha^{-1}, was used for the experiment. This study set 1N = 10 g ha^{-1}; dose rates of 0.1N, 0.3N, 1N, 3N, 6N and an untreated control for Estonian biotypes were used. For the SMCZ control, doses of 0.003N, 0.01N, 0.03N, 0.1N, 1N, 3N and untreated controls were used.

Chlorophyll fluorescence was measured in both SME14 and SME18 biotypes at three time points (2, 5, and 13 days after treatment) in the dose-response experiment. Both untreated controls and plants treated with the recommended herbicide dose (1N) were examined. For each treatment, measurements were taken from plants in a single pot together with three pots assessed per treatment. Prior to measurement, plants were dark-adapted for 20 minutes. Fluorescence parameters, including baseline fluorescence (Fo) and maximum fluorescence (Fm), were recorded from three leaves per pot using a pulse amplitude modulating imaging fluorimeter (IMAGING-PAM MAXI, Heinz Walz GmbH), according to the manufacturer’s guidelines. A saturation pulse of 2000 µM s^{-1} m^{-2} of photosynthetic photon flux density, at a wavelength of 450 nm, was applied. The mean values per pot were calculated, and the chlorophyll fluorescence ratio Fv/Fm, which represents the maximum quantum yield of photosystem II (PSII), was determined as described by Maxwell and Johnson (2000).

In 2023, new seeds from eight additional Estonian populations were collected during pest monitoring, including one sample from a farmer's complaint (SME134). Preliminary screening of these biotypes was conducted in a glasshouse at the Centre of Estonian Rural Research and Knowledge in Jõgeva, Estonia. In this screening, plants were treated with tribenuron-methyl (Granstar Premia 50SX) and Sekator OD (Bayer CropScience), the latter containing ALS inhibitors amidosulfuron 100 g l^{-1} and iodosulfuron-methyl-Na 25 g l^{-1} as active ingredients. The herbicides were applied at the recommended dose (Granstar Premia 50SX: 1N = 0.15 l ha^{-1}; Sekator OD: 1N = 0.15 l ha^{-1}).

In 2024, a dose-response experiment was conducted using Sekator OD on four biotypes selected from the screening (SME125, SME134, SME155, and SME14). Two putatively resistant biotypes, SME 125 and SME 134 (from Järva and Lääne-Viru counties, respectively), one sensitive biotype (SME155) from Jõgeva county, and SME14 as a resistant standard. Plants were grown in a greenhouse under artificial light (16 hours of light, 8 hours of darkness) and treated with Sekator OD at doses of 0.06N, 0.3N, 1N, and 3N. Spraying was carried out manually with a spray bottle, maintaining the spray volume at 250 l ha^{-1}, with treatments applied at the 2–3 nodes stage (BBCH stage 32–33, Meier 2001).

In all experiments, herbicide efficacy was visually assessed and the aboveground biomass harvested 21 days after treatment. The biomass was dried at 95 °C for 48 hours and weighed. Leaf samples were collected from untreated plants in both dose-response experiments. Approximately 50 mg of leaf tissue from each sample was homogenized using a TissueLyzer II, and genomic DNA was extracted using Qiagen DNeasy® Plant Mini Kit, following the manufacturer's protocol. The primers used to amplify the ALS gene, as described by Marshall et al. (2010) and Laforest and Soufiane (2018) are listed in Table 1. PCR amplification was performed using Thermo Scientific™ DreamTaq™ Hot Start Green DNA Polymerase under the following cycling conditions: an initial denaturation at 95 °C for 5 minutes, followed by 35 cycles of 95 °C for 30 seconds, annealing for 40 seconds at a temperature specific to each primer pair, 72 °C for 1 minute, and a final extension at 72 °C for 5 minutes. The PCR products were sequenced by the dideoxynucleotide chain-termination method (Sanger et al. 1977) on the Applied Biosystems capillary electrophoresis system at the University of Tartu, Institute of Genomics, Core Facility of Genomics.

Table 1. The primers used to amplify and sequence *Stellaria media* samples

Primer name	Sequence	Annealing temperature	Amplified nucleotide positions	Reference
StF1	TACCCGGGTGGCGCCTCTTTAG	60	364–965	Marshall et al. 2010
StR1	ATCCCCGTCAATTGAACAAACCTCC			
StF2	GCTGACCTCTAGTGGGCTTG	60	1565–1873	Marshall et al. 2010
StR2	GCCTCCCTAAGATCGGACAC			
StF4	TTAGTATCGGGACTCGCTGA	54	532–1116	Laforest and Soufiane 2018
StR4	CCCAAACGCCAACAACAAA			
StF5	GTATGTTGGAGGAGGGAGTTTG	58	919–1503	Laforest and Soufiane 2018
StR5	CAGCCATTGACGAGGGTTATTA			
StF6	GAGTGGAGGGATGAGTTGAATG	58	1372–1793	Laforest and Soufiane 2018
StR6	ATGGCTGAATCATCGGAAGG			

The experimental data were analysed using the Tukey test, least squares means (for contrasts in chlorophyll fluorescence), and dose-response analysis in R version 4.3.1 (R Core Team 2023), utilizing the packages 'agricolae' (de Mendiburu 2023) and 'drc' (Ritz et al. 2015). The sequence data were aligned and analysed using Mega 11 (Tamura et al. 2021) and BioEdit (Hall 1999).

Results

Three *S. media* biotypes were initially analyzed from Estonia (two biotypes) and the Czech Republic (one biotype). The sensitivity of the two Estonian biotypes was assessed by comparing the reduction in the weed biomass relative to the Czech biotype SMCZ. Results from the dose-response experiment indicated that biotype SME14 exhibited resistance to tribenuron-methyl (see Figure 1). Specifically, the biomass of SM14 decreased to less than 50% of the control only after exposure to three times the recommended dose. Even when exposed to six times the recommended dose, the biomass reduction was less pronounced than in the sensitive biotypes, and the plants remained green and viable. In contrast, biotypes SME18 and SMCZ were found to be sensitive, to the herbicide, displaying a similar decrease in biomass across all tested herbicide concentrations, starting from 0.1 times the recommended dose. The dose-response relationship for SME14 and SMCZ is further illustrated in Figure 2. However, since SME14 and SME18 were not tested with doses lower than 0.1 times the recommended dose, and since the biomass decreased significantly even at concentrations as low as 0.1 times the recommended dose, a dose-response curve for SME18 could not be generated due to convergence issues in the model. The resistance index of SME14 relative to SMCZ was calculated as 32 460. Due to model convergence issues, the resistance index for SME14 relative to SME18 could not be determined.

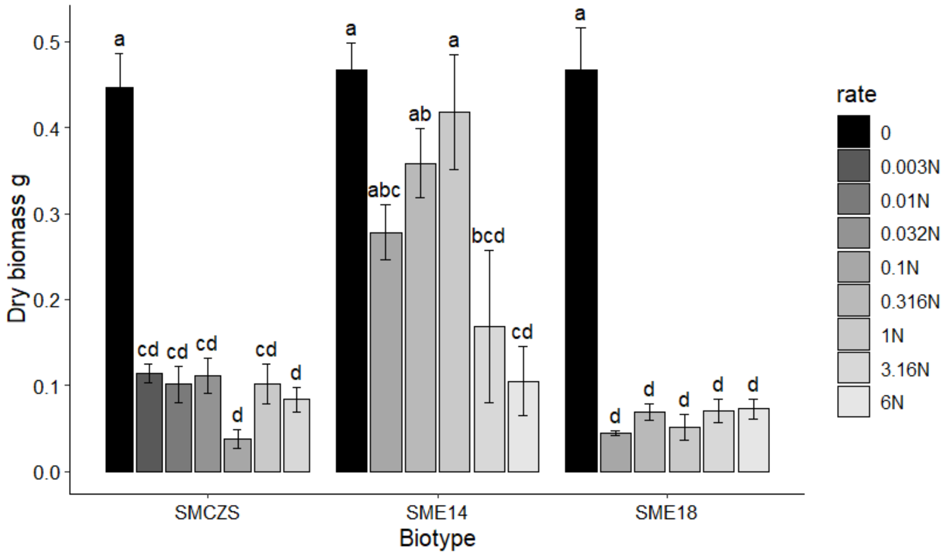


Fig. 1. The effect of different doses of tribenuron-methyl on the dry biomass of the *S. media* biotypes SME14 (resistant), SME18 and SMZS (susceptible standard from the Czech Republic). Statistically significant differences in biomass between the biotypes are indicated by different letters, based on the results of the Tukey test with Bonferroni correction for multiple comparisons.

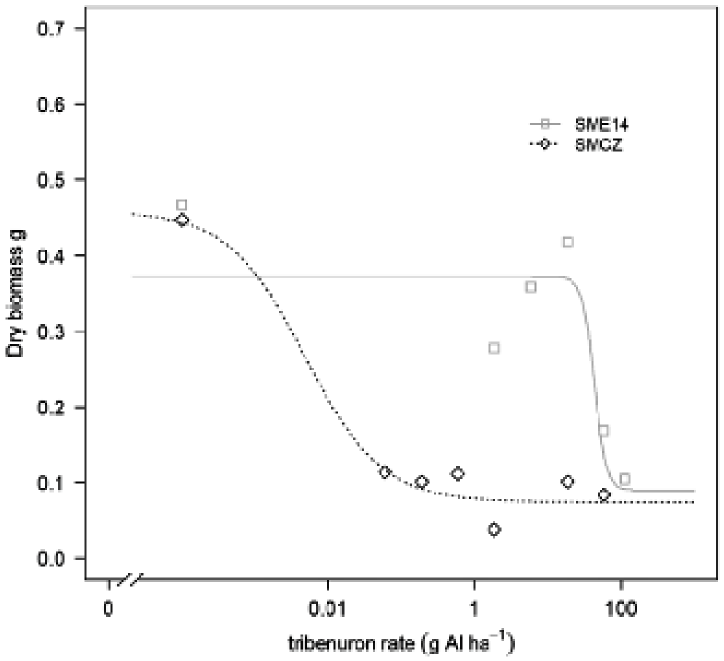


Fig. 2. The dose-response curves for the biotypes SME14 (resistant) and SMCZ (sensitive) in response to tribenuron-methyl were constructed using a fixed four-parameter log-logistic model with BoxCox transformation (model fit F = 2.81, p = 0.029). The estimated effective dose (ED50) for SME14 was 48.69 g ai ha^{-1} while for SMCZ, it was 0.0015 g ai ha^{-1}.

The dose-response data from the 2022 experiment were additionally confirmed through the analysis of chlorophyll fluorescence, which is indirectly influenced by ALS inhibitors. In most cases, pairwise comparisons of the least squares means of the maximum quantum yield of photosystem II were not statistically significant. However, four pairwise contrasts were statistically significant: SME14treated 13d vs. SME18treated 13d (*p*= 0.022), SME14 untreated 13d vs. SME18treated 13d (*p*= 0.020), SME18treated 5d vs. SME18treated 13d (*p*= 0.039), and SME18treated 13d vs. SME18untreated 13d (*p*= 0.022). These results suggest that the fluorescence of the herbicide treated sensitive biotype, SME18, on the 13th day post-treatment differed significantly from that of SME14 (Fig. 3). This indicates a notable effect of the treatment on the sensitive biotype but not on the resistant one. The means and standard deviations of the maximum quantum yield of photosystem II for both treated and untreated biotypes, based on repeated measurements, are given in Table 2. Due to the clear differences observed on day 13, and the near desiccation of the sensitive plants, measurements were discontinued at that point. This observation highlights the delayed response of ALS inhibitors, as evidenced by the significant difference in photosynthetic activity of the sensitive biotype between days 5 and 13, indicating no effect was yet detectable on day five.

Table 2. The mean values and standard deviations of the maximum quantum yield of photosystem II were calculated based on repeated measurements for the *S. media* biotypes SME14 and SME18. The treated plants received the recommended dose of tribenuron-methyl (10 g ai ha^{-1}), and both treated and untreated plants were measured on the second, fifth, and thirteenth days (d) after treatment.

biotype	treatment	time days	avg	sd
E18untreated	control	2	0.686861	0.02032
E18treated	tribenuron	2	0.572971	0.180673
E18untreated	control	5	0.612861	0.052661
E18treated	tribenuron	5	0.621389	0.018634
E18untreated	control	13	0.640216	0.043254
E18treated	tribenuron	13	0.452054	0.102219
E14untreated	control	2	0.694041	0.006388
E14treated	tribenuron	2	0.682585	0.01082
E14untreated	control	5	0.657539	0.001924
E14treated	tribenuron	5	0.632312	0.025729
E14untreated	control	13	0.649511	0.067532
E14treated	tribenuron	13	0.642839	0.030618

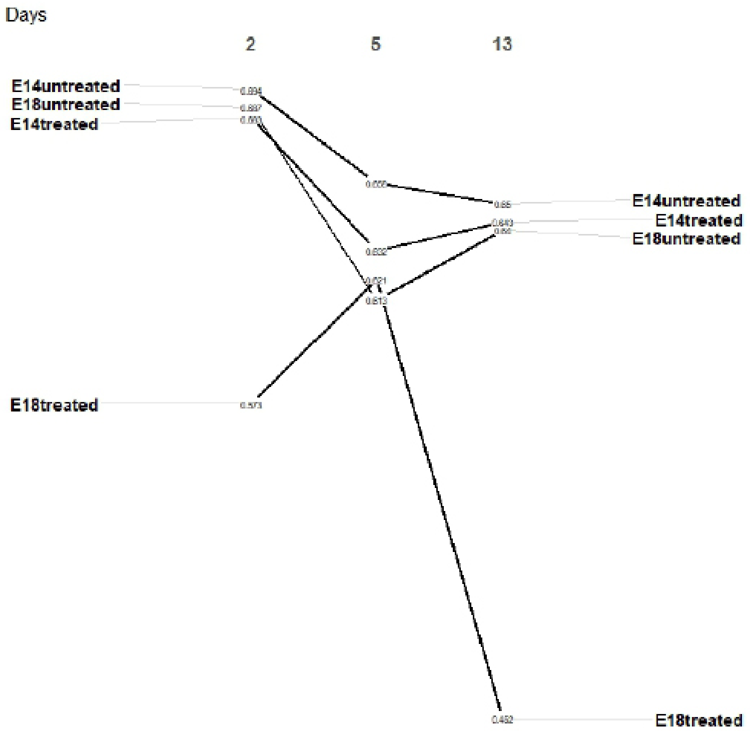


Fig. 3. The mean PSII values of plants from biotypes SME14 and SME18, treated with recommended dose of tribenuron-methyl (10 g ai ha^{-1}) and untreated controls, were measured on the second, fifth, and thirteenth days after treatment.

In 2023, a screening of eight populations from various counties in Estonia indicated that tribenuron-methyl resistance is not widely prevalent across the country. However, the study suggested a potential resistance to amidosulfuron + iodosulfuron in biotype SME14, as well as two other biotypes, SME125 and SME134, which originated from Järva and Lääne-Viru counties, respectively (Fig. 4). The dose-response experiment involving the amidosulfuron-iodosulfuron mixture and these three biotypes (with biotype SME155 from Jõgeva county serving as a sensitive standard) confirmed resistance in all three biotypes. Resistance was most pronounced in SME14, while SME125 and SME134 exhibited somewhat weaker resistance (Fig. 5). The ED50 for amidosulfuron + iodosulfuron was 90.29 g ai ha^{-1} for SME14, 41.56 g ai ha^{-1} for SME125, and 48.46 g ai ha^{-1} for SME134, compared to a recommended dose of 18.75 g ai ha^{-1}. Due to convergance issues in the model, the resistance index of these biotypes relative to the sensitive SME155 could not be calculated.

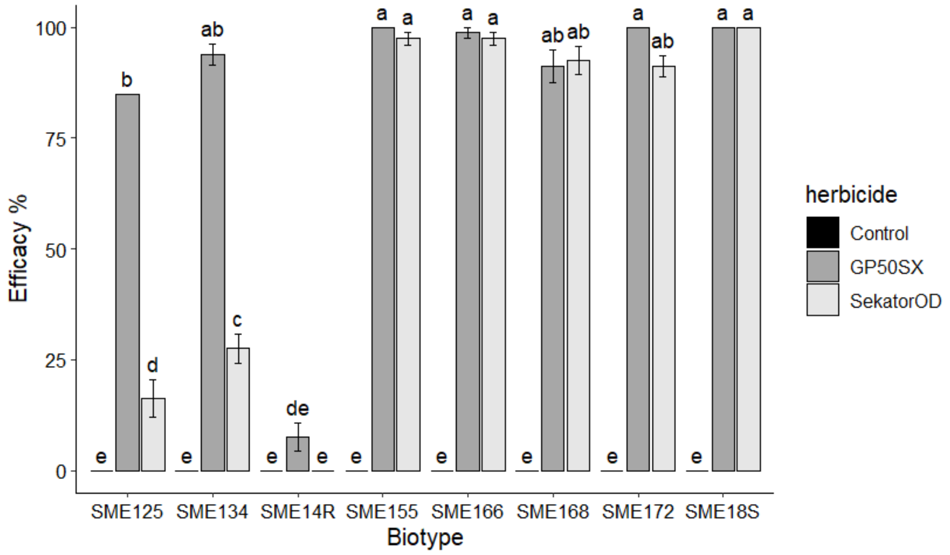


Fig. 4. The estimated efficacy of ALS inhibitors in eight Estonian *S. media* biotypes was evaluated using the recommended field doses of two ALS-inhibiting herbicides: tribenuron-methyl (with a recommended dose of 12.5 g ai ha^{-1} used in the experiment) and mix of amidosulfuron and iodosulfuron-methyl-Na (with a recommended dose of approximately 18.75 g ai ha^{-1} used in the experiment). Statistically significant differences in biomass between treatments were determined using the Tukey test with Bonferroni correction for multiple comparisons (α = 0.05), with different letters indicating significant differences.

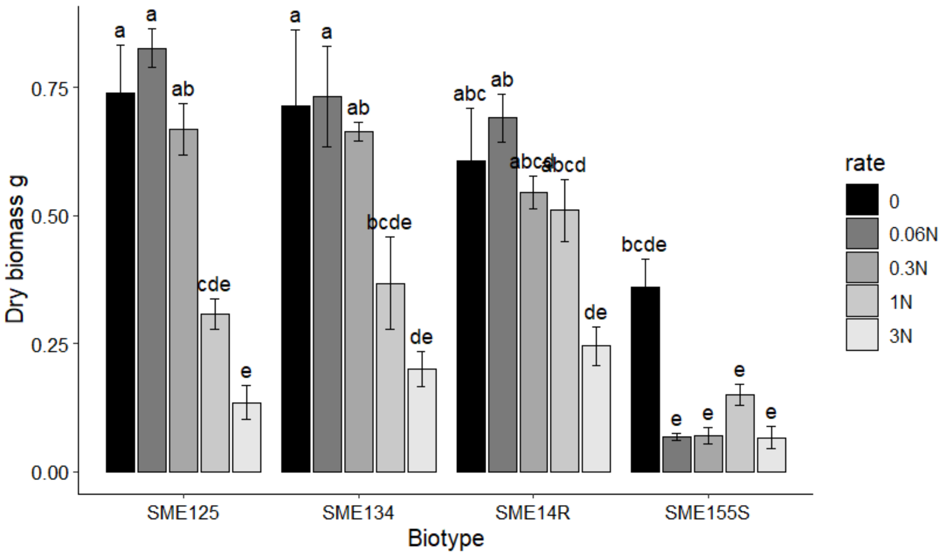
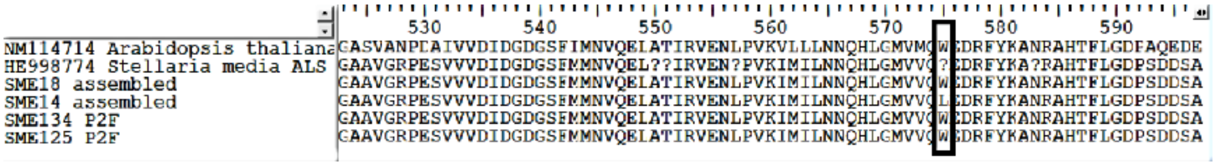


Fig. 5. The effect of different rates of amidosulfuron + iodosulfuron with a recommended field dose of 18.75 g ai ha^{-1} (denoted as 1N), on the dry biomass of the *S. media* biotypes SME125, SME134, SME14 (resistant) and SME155 (susceptible) was assessed. Statistically significant differences in biomass are indicated by different letters, based on the results of the Tukey test with Bonferroni correction for multiple comparisons.

Analysis of potential target-site resistance in the ALS gene revealed a mutation at position 574 in the ALS cross-resistant biotype SME14, where aminoacid tryptophane was replaced by leucine. In contrast, this mutation was not present in the partially resistant biotypes SME125 and SME134, nor in the sensitive biotypes SME18 and SME155. The sequence fragments of these biotypes around position 574 are presented in Figure 6.



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                                      530       540       550       560       570       580       590
NM114714 Arabidopsis thaliana GASVANPEAIVVDIDGDGSFIMNVQELATIRVENLPVKVLLLNNQHLGMVMQWEDRFYKANRAHTFLGDFAQEDE
HE998774 Stellaria media ALS  GAAVGRPESVVVDIDGDGSFMMNVQEL??IRVEN?PVKIMILNNQHLGMVVQ?EDRFYKA?RAHTFLGDPSDDSA
SME18 assembled               GAAVGRPESVVVDIDGDGSFMMNVQELATIRVENLPVKIMILNNQHLGMVVQWEDRFYKANRAHTFLGDPSDDSA
SME14 assembled               GAAVGRPESVVVDIDGDGSFMMNVQELATIRVENLPVKIMILNNQHLGMVVQLEDRFYKANRAHTFLGDPSDDSA
SME134 P2F                    GAAVGRPESVVVDIDGDGSFMMNVQELATIRVENLPVKIMILNNQHLGMVVQWEDRFYKANRAHTFLGDPSDDSA
SME125 P2F                    GAAVGRPESVVVDIDGDGSFMMNVQELATIRVENLPVKIMILNNQHLGMVVQWEDRFYKANRAHTFLGDPSDDSA
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Fig. 6. The fragments of aligned ALS protein sequences translated from DNA sequences sequenced in this study (SME18, SME14, SME125 and SME134) and reference sequences of *Arabidopsis thaliana* and *Stellaria media* from NCBI nucleotide database. The position 574 is marked and the replacement of tryptophane with leucine can be seen.

Discussion

This study provides the initial characterization of herbicide resistance in the Estonian *S. media* population to sulfonylureas. Target-site resistance to tribenuron-methyl and cross-resistance to other sulfonylureas were confirmed in the Estonian *S. media* biotype SME14 from Järva county. In contrast, two other biotypes exhibited non-target-site resistance and weaker resistance to the mix of amidosulfuron and iodosulfuron . Previous research has shown that *S. media* often develops cross-resistance (Hall and Devine 1990). Thus far, no additional tribenuron-methyl-resistant biotypes have been identified, suggesting that such resistance is not widespread in Estonia. This suggests that the resistant biotype likely evolved on-site rather than spreading through seed dispersal. No precise data is available about the herbicide usage of these fields. However, the farmers indicated that sulfonylureas have been used at the field of SME14, while mainly MCPA at the field of SME18. That can probably explain the resistance of SME14 to ALS inhibitors. On the other hand, resistance to to the mix of amidosulfuron and iodosulfuron was observed in three out of eight biotypes analyzed in Estonia. Given that the mix of amidosulfuron and iodosulfuron is one of the most commonly used herbicides among Estonian farmers for controlling dicotyledonous weeds, this may help explain the higher prevalence of resistance to these herbicides.

Neighboring countries have encountered issues with *S. media* much earlier than Estonia. Resistance to sulfonylureas in *S. media* was detected in Finland over a decade ago (Uusitalo et al. 2013), and resistant *S. media* populations have also been identified in Latvia and Sweden (Heap 2024). In Denmark, resistance to sulfonylureas in *S. media* was observed more than 30 years ago (Kudsk et al. 1995). In addition to *S. media*, several other weed species resistant to ALS-inhibiting herbicides have been identified globally. These include *Apera spica-venti* in the Czech Republic (Hamouzova et al. 2011) and Lithuania (Auškalniene 2020) and many other countries including Latvia, Denmark, and Sweden (Heap 2024); *Tripleurospermum inodorum* in Poland (Adamczewski et al. 2014, 2019) and many other countries; *Papaver rhoeas* in many European countries, Sweden and Denmark being among them; *Chenopodium album* in several European countries, including Finland and Norway; and *Alopecurus myosuroides* across much of Europe, though it has yet not spread to the northernmost countries, like Norway, Finland, and Latvia (Heap 2024). It is possible that resistance to sulfonylureas is also more widespread in Estonia, as there have been reports from farmers concerning *Centaurea cyanus* (cornflower) and its response to sulfonylurea herbicides.

Resistance to tribenuron-methyl in *S. media* is attributed to target-site mutations, such as the Trp574Leu mutation identified in resistant biotypes. This mutation has previously been associated with resistance to various ALS-inhibiting herbicides in the UK (Marshall et al. 2010). The Pro-197-Gln mutation is known to confer resistance to sulfonylureas, while the Trp-574-Leu mutation confers resistance to both sulfonylureas and triazolopyrimidines (Marshall et al. 2010, Laforest and Soufiane 2018). In addition to target-site resistance, effective non-target site resistance mechanisms appear to be present in the Estonian population. While non-target-site resistance in *S. media* has not been documented, such mechanisms have been observed in closely related species such as *Myosoton aquaticum* (Bai et al. 2019) a widespread and competitive winter weed of wheat in China, has evolved resistance to many classes of herbicides. Bai et al. (2019) suggested that these mechanisms could be related to enhanced P450-mediated metabolism, based on experiments with malathion. They also noted that target-site and non-target-site mechanisms can co-occur, a possibility that may apply to the Estonian biotype SME14. However, the specific resistance mechanisms of *S. media* to the mix of amidosulfuron, and iodosulfuron have not been studied nor were they analysed in the present study. It is also plausible that multiple non-target-site resistance mechanisms are involved, as Loubet et al. (2023) but its precise genetic determinisms remain fairly unclear. Full-transcriptome sequencing had previously been implemented to identify NTSR genes. However, this approach had generally been applied to a single weed population, limiting our insight into the diversity of NTSR mechanisms. Here, we sought to explore the diversity of NTSR mechanisms in common ragweed (*Ambrosia artemisiifolia* L.) demonstrated that multiple non-target site resistance mechanisms can evolve within a single species (in their case, *Ambrosia*).

The combination of target-site and non-target-site resistance mechanisms in *S. media* could accelerate the spread of resistance across fields, especially considering that sulfonylureas, particularly amidosulfuron+iodosulfuron, are among the most commonly used herbicides for controlling dicotyledonous weeds in Estonia. Although *S. media* is not a tall plant and may not pose a significant problem in terms of light competition, it can form dense canopies that strongly compete for water and nutrients. In fact, *S. media* was considered one of the most agriculturally significant weeds in Europe as of 2010 (Marshall et al. 2010). Consequently, the issue of resistance may be more widespread in Estonia and neighboring countries than currently recognized. Although herbicide resistance has only recently been recognized in Estonia, farmers have reported numerous cases involving various species, particularly *S. media*, with a focus on sulfonylurea herbicides. ALS-inhibiting herbicides, especially sulfonylureas, are commonly used in Estonia for controlling dicotyledonous weeds. The future significance of *S. media* as a weed remains uncertain, thought climate change and minimal tillage practices could potentially contribute to its broader and faster spread. Given the high genetic diversity within the genus and the limited number of herbicides available for selective control, the rapid development of resistance a real concern.

To better understand the extent of herbicide resistance in *S. media* in Estonia, further research is needed to elucidate the distribution and underlying mechanisms of resistance. More extensive investigation of potentially resistant biotypes, along with broader monitoring efforts, are planned for 2025. However, *S. media* is not the only problematic weed species in the region. Therefore, it is imperative to expand monitoring and research efforts on herbicide resistance in Estonia in the coming years, including the exploration of resistance mechanisms. To prolong the effective use of herbicides, it is essential to rotate herbicide products and use them in mixtures whenever possible, ideally incorporating different modes of action. However, the selection of herbicide products is often limited, and Estonian commercial farms typically rely on the same products for weed control. Monitoring herbicide resistance can help minimize the use of herbicides that are at risk of resistance development without compromising weed control in the fields. This strategy would enable farmers to adjust their spraying programs and adopt sustainable, localized management practices before herbicide resistance becomes entrenched, which could lead to a decline in field performance.

Additionally, monitoring herbicide resistance is essential to prevent the use of ineffective products and help identify alternative strategies to reduce weed infestations. Alternative methods for controlling weeds, such as improving crop competitiveness and integrated weed management solutions, should be further explored. Current recommendations for managing herbicide resistance include using herbicide mixtures and rotating active ingredients, prioritizing preemergence over postemergence herbicides, developing crop cultivars with enhanced weed competitiveness, expanding harvest weed seed control practices, and advancing site-specific or precision weed management techniques (Beckie et al. 2019).

Conclusions

Resistance to the ALS inhibitors has been identified in several *S. media* biotypes in Estonia. Both target-site and non-target-site resistance mechanisms are involved in this resistance. Sulfonylureas are one of the most problematic herbicide groups for controlling dicotyledonous weeds in Estonia. Herbicide resistance in Estonia is likely more widespread, affecting a greater range of species and herbicide modes of action. There is a need for more extensive herbicide resistance monitoring and research in Estonia.

Acknowledgements

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