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Diversified germination strategies of *Centaurea cyanus* populations resistant to ALS inhibitors

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Abstract: *Centaurea cyanus* is an annual weed mostly infesting winter cereals and rape. The aim of the study was to provide insights into the association between the seed germination characteristics and the herbicide sensitivity in *C. cyanus* in the presence of the genetic background control. Four populations of this species resistant to acetolactate synthase (ALS) inhibitors were tested. A germination study was conducted in a growth cabinet. Plants were sprayed at the 2- to 3-leaf stage with a field dose of florasulam (5 g/ha). There were four herbicide treatments dates, which included plants that germinated up to the seventh day, between eight and 12, 13 and 15, 16 and 20 days of the germination study. The germination dynamics of the four tested populations of *C. cyanus* resistant to florasulam was diversified. Three of them reached their maximum germination on the fourth day after sowing, however, the germination of the fourth population was spread over time with the highest number of germinated seeds found seven and twelve days after sowing. The germination time of the plants belonging to the resistant *C. cyanus* populations differentiated their reaction to florasulam. The conducted study indicated that the germination biology of ALS inhibitor-resistant populations of *C. cyanus* is diverse, which makes it difficult to introduce universal management strategies of this species into agricultural practice. Integration of control methods is recommended, including delaying the crop sowing date.

Keywords: cornflower; florasulam; germination dynamics; herbicide resistance management

Herbicide resistance in weeds is one of the greatest challenges facing modern agriculture (Perotti et al. 2020). This phenomenon is defined as the inherited capacity of a plant to survive and reproduce after treatment with a dose of herbicide that is normally lethal to a wild-type plant of the same species (Weed Science Society of America Terminology Committee 1998). Resistant weed biotypes have been selected to many herbicide mechanisms of action, however, biotypes resistant to acetolactate synthase (ALS) inhibitors constitute the most numerous group (Heap 2023). The cornflower (*Centaurea cyanus* L.) is among the weed species where a lack of control with ALS inhibitors has been detected. There are also reports from Poland about populations of this species with resistance to synthetic auxins (Stankiewicz-Kosyl et al. 2021; Heap 2023).

C. cyanus is an annual weed from the *Asteraceae* family that mostly infests winter crops, like cereals

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or rape. It prefers light soils, however, it has also been increasingly recorded on heavier soils. *C. cyanus* occurs in various locations in Europe and Asia and it has also been introduced to North America, where it is treated as an invasive plant (Randall & Marinelli 1996). It has a high potential to compete with crops, its presence can generate a significant reduction in the grain yield of winter wheat. *C. cyanus* reproduces by seeds that are shed before or during crop harvesting, increasing the soil weed seed bank where they stay viable for up to 3 years. In the temperate zone, they mostly germinate in autumn, however, spring germination has also been noted (Guillemin et al. 2017).

Germination is a complex process influenced by genetic and environmental factors. Soil properties, like, for example, the temperature, humidity, access to light, agronomic practices affecting the burial depth or presence of surface residues are factors that could be important for weed seed germination (Tang et al. 2015). Herbicide resistance is one of the genetic factor aspects and, according to the literature, it can correlate with different germination patterns (Délye et al. 2013). Adequate germination timing allows the seedlings to avoid pre-sowing practices and to emerge under the best possible conditions for growth (Riemens et al. 2022). Knowledge concerning the germination process of resistant plants can be helpful in determining the strategy of preventing the selection and eradication of resistant biotypes (Maity et al. 2022).

Adaptations concerning germination are components of a plant's fitness, a set of characteristics crucial for weed success in agricultural ecosystems. It is interesting that resistance to herbicides may not always lead to reduced fitness. Inconsistent results, increased fitness as well as no impact of herbicide-resistance on the weed fitness have been found as well (Babineau et al. 2017; Keshtkar et al. 2019). One of the most common faults in fitness studies is using plant material with a dissimilar genetic background, because it can lead to inconclusive results. Therefore, the control of the genetic background is essential to unambiguously assign fitness costs to those genes that confer resistance (Keshtkar et al. 2019).

Research results are available in the literature showing where the resistant biotypes of *C. cyanus* are noted and what the level of resistance is (Stankiewicz-Kosyl et al. 2021). However, there are no data on the differences in germination between plants with different levels of resistance within a population. Saja et al. (2016) described the germination differences among two ALS resistant biotypes and one susceptible biotype of *C. cyanus*, however, this study does not take the genetic background of plant material into account. Accordingly, the aim of the study was to provide insights into the association between the seed germination characteristics and the herbicide sensitivity in *C. cyanus* in the presence of a genetic background control. Therefore, the herbicide response of plant groups separated within each population according to the germination date was compared.

MATERIAL AND METHODS

Plant material. Four populations of *C. cyanus* resistant to ALS inhibitors were tested in the study. Seeds were collected in 2017–2018 from fields in Poland. At least 100 fully developed *C.cyanus* in florescences were collected from several locations within one field and combined in one sample according to the methodology described by Panozzo et al. (2015). The inflorescences were threshed and the achenes were stored in paper bags until used. To break dormancy, the seeds were placed in a cool room at 4 °C for 7 days. The provenance and characteristics of the populations are given in Table 1. Their level of resistance to ALS inhibitors is expressed by the ED_{50} value, i.e., the dose causing a 50% reduction in the biomass of an herbicide-treated plant. The ED_{50} dose was established during the whole-plant dose-response bioassays (Stankiewicz-Kosyl et al. 2021).

Germination study. Fifteen similar-sized seeds of each population were placed on two layers of filter paper (Whatman No. 1, Citiva, UK) in 9 cm

Table 1. Location and characteristics of the *Centaurea cyanus* populations used in the study

Sample ID	GPS	Crop	ED_{50} (g a.i./ha)	
			tribenuron-methyl	florasulam
8401	54°19'19.5"N 21°09'31.2"E	winter wheat	259.74	43.46
8717	51°23'14.3"N 22°17'59.6"E	narrow-leaved lupin	131.67	13.66
9294	52°56'01.0"N 22°52'21.5"E	winter triticale	48.60	30.17
9307	52°43'12.4"N 23°26'02.4"E	winter wheat	151.00	76.61

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glass Petri dishes. The germination tests were initiated by adding 5 mL of deionised water to each Petri dish. The Petri dishes were arranged in a completely randomised design with ten replicates and placed in a growth cabinet (Pol-Eko, Poland) that was adjusted to 14/10 h day/night photoperiods and a temperature regime of 15/10 °C day/night. A photosynthetic photon flux density of 140 μmol/(m^2s) was provided by cool white fluorescent lamps. The experiment was conducted twice. The temperature regime used in the study mimics the range of soil temperatures corresponding to conditions during the sowing of winter wheat in Poland. The germinated seeds were counted as follows: 4, 7, 12, 15, 20 days after sowing (DAS), until no further germination was observed. The seeds were considered germinated when the radicle was visible. The germinated seeds were transferred to multipots for the herbicide response bioassays.

The germination capacity was calculated as the percentage of seeds that germinated out of the initial number of sown seeds. The germination index (GI_{mod}) was computed according to the formula proposed by Ranal and Santana (2006):

$$GI_{mod} = \sum_{i=1}^{k} \frac{|(T_k - T_i) \times N_i|}{N_g}$$

where: T_i – the time from the start of the experiment to the i^{th} interval (day for the example); N_i – the number of seeds germinated in the i^{th} time interval (not the accumulated number, but the number corresponding to the i^{th} interval); N_g – the total number of germinated seeds; k – the total number of time intervals.

Herbicide response bioassays. The germinated seeds originating from the Petri dishes were transferred to multipots with a single pot, 5.5 cm in diameter. The multipots were filled with the vegetable substrate Kronen® (Lasland sp. z o.o. Grądy, Poland) mixed with sand in a proportion of 2 : 1. The plants were cultivated in a greenhouse at 20/15 ± 2 °C and a 14/10 h day/night cycle with supplemental light (180 μmol/(m^2 s). The plants were sprayed at the 2- to 3-leaf stage with a field dose of florasulam (5 g/ha) using Saracen 050 SC, Cheminova (50 g a.i./L) and with distilled water (untreated control). There were four herbicide treatments which included plants that germinated (T1) up to 7th day, (T2) between 8th and 12th day, (T3) between 13th and 15th day and (T4) between 16th and 20th day. For each population, the untreated control consisted of nine plants. The number of herbicide-treated plants was variable and depended on the number of individuals that germinated at a given date. The herbicide was applied using a precision bench sprayer (APORO Sp. z o.o., Poznań, Poland), with a boom equipped with one flat-fan hydraulic Teejet XR 11002 (Teejet Technologies, USA) VP nozzle, and calibrated to deliver 200 L/ha of spray at a pressure of 200 bars. Three weeks after the herbicide application, the above ground plant biomass was cut and immediately weighed (fresh biomass) using a laboratory balance (Radwag, Poland). The above ground biomass reduction for the florasulam-treated plants was expressed as a percentage of the untreated control. The experiment was conducted twice.

Statistical analysis. The effect of the experimental run was not significant; therefore, the data were pooled over the experimental runs. Before the statistical tests were conducted, the data were checked for normality using the Shapiro-Wilk W test. The logarithmic or arcsine transformation of the data was performed, if needed. The results obtained from the germination experiments were analysed statistically using an analysis of variance. The significance of the differences between the means was examined with Tukey's test at $\alpha = 0.05$. The data from the herbicide resistance bioassays were analysed with the Kruskal-Wallis test and then the Dunn test with Bonferroni correction was executed. All the statistical analysis and plots were performed using Statistica software (version 14.0.0).

RESULTS

Germination dynamics and capacity of the *C. cyanus* populations. The germination dynamics of the four tested populations of *C. cyanus* resistant to florasulam was diversified (Figure 1). Three of the tested populations reached their maximum germination on the fourth day after sowing. By then, between 45% (8401) and 58% (9307) of the seeds from these populations had germinated. The number of germinated seeds of population 8401 decreased over time. A similar tendency was found in population 9307, with the number of germinated seeds of this population at 15 DAS and 20 DAS being comparable and amounting to 2.7 and 2.3%, respectively. In population 9294, on the seventh day after sowing, a large decrease in the germination was noted compared to the fourth day, but at

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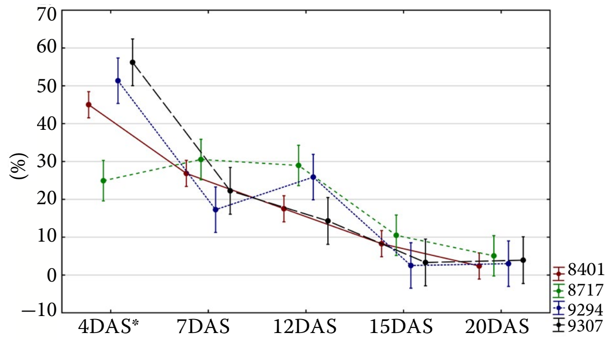

Figure 1. Germination dynamics of the studied populations of *Centaurea cyanus*

Data are mean ± standard error (SE)

*DAS – days after sowing

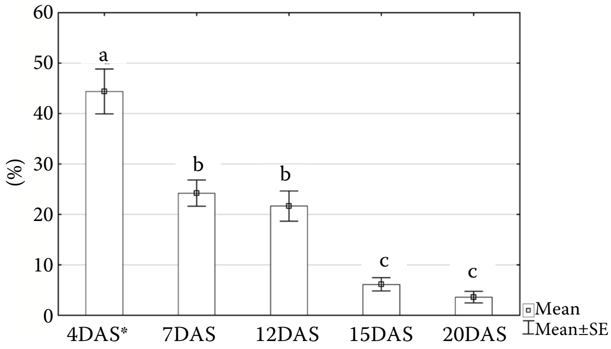

Figure 2. The average of the four populations of the *Centaurea cyanus* germination dynamics

*DAS – days after sowing

Means marked by the same letter do not differ significantly at $P < 0.05$ by Tukey's test

12 DAS, increased seed germination was found compared to the previous date. As in the case of population 9307, the number of germinated seeds in the last two evaluation dates was similar. The germination dynamics of population 8717 was different. Until 12 DAS, the seed germination of this population was uniform, with the highest number of germinated seeds found seven and twelve days after sowing. After this date, there was a significant reduction in the germination of this population.

Analysing the average dynamics of the seed germination of the four tested populations of *C. cyanus* resistant to florasulam, it was shown that, significantly, the largest number of seeds germinated 4 days after sowing (Figure 2). The number of seeds germinated at 7 DAS and 12 DAS was similar, but lower than at 4 DAS by 47% and 54%, respectively. In the last two evaluation dates, the seed germination was significantly the lowest. Among the studied populations, 8717 was characterised by the highest germination capacity (Figure 3). The germination capacity of this population was not significantly different from that observed in population 9307, while the germination of population 9307 was statistically at the same level as that of populations 8401 and 9294.

The germination index calculated for the investigated populations confirmed that the most prolonged seed germination occurred in population 8717, and the value of the index for this population did not differ significantly from populations 8401 and 9294 (Figure 4). The fastest germination was observed in population 9307.

Above ground biomass of the *C. cyanus* plants treated with florasulam in the context of the germination date. The germination time of *C. cyanus* significantly differentiated the seedling biomass of three out of four populations studied 21 days after the florasulam treatment. In the case of population 8401, plants from the last (T4)

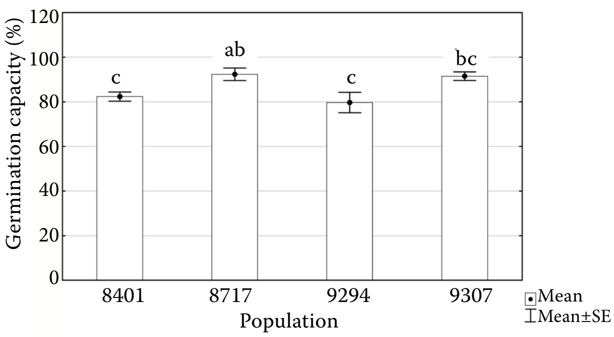

Figure 3. Germination capacity of the *Centaurea cyanus* seeds

Means marked by the same letter do not differ significantly at $P < 0.05$ by Tukey's test

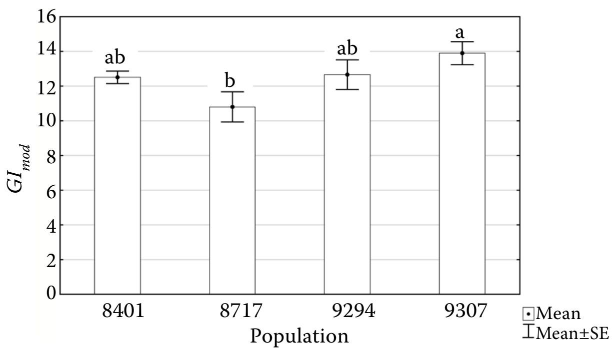

Figure 4. A modified germination index (GI_{mod}) of the *Centaurea cyanus* seeds

Means marked by the same letter do not differ significantly at $P < 0.05$ by Tukey's test

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and first (T1) germination dates significantly produced the largest biomass (Table 2). The biomass of the T1 plants did not differ significantly from that of the T2 plants. The plants that germinated the fastest were the largest subgroup of this population (N = 146) and were characterised by the largest range of the biomass, ranging from 0.612 g to 3.410 g and the largest coefficient of variability (Table 2, Figure 5).

In the case of population 8717, the highest biomass after the florasulam treatment was found in the plants from the fourth (T4) and second (T2) dates of germination, while the biomass of the plants from T1, T2 and T3 did not differ significantly from each other (Table 3). At T4, both the range of the plant biomass and the coefficient of variation were the highest, while their lowest values were found at T3 (Table 3, Figure 6).

The highest median value of the biomass was found in the plants of population 9294 from the second (T2) and third (T3) germination dates (Table 4). The biomass of the plants originating from the dates T1, T3 and T4 were at a similar level, with the subgroups from the last two germination dates being less numerous than from the first two dates. The largest range of the biomass and coefficient of variation was found for the T2 plants (Table 4, Figure 7).

Population 9307 was the only one among the four studied populations in which the time of the germination did not significantly differentiate the plant

Table 2. Median values, quartile range, P-value and coefficient of variation (CV) of the above ground biomass (in grams) produced by the florasulam treated plants of population 8401 of *Centaurea cyanus* in dependence on the germination date

Term	N	Median	Quartile range	P-value	CV (%)
T1	146	0.83835^{ab}	0.44330	0.0001	42.2
T2	38	0.74250^{bc}	0.23500	0.0001	24.8
T3	17	0.67300^{c}	0.09700	0.0001	21.6
T4	3	1.41400^{a}	0.77600	0.0001	41.9

Medians marked by the same letter do not differ significantly at P < 0.05 by Tukey's test

T1 – up to 7th day; T2 – 8–12th day; T3 – 13–15th day; T4 – 16–20th day

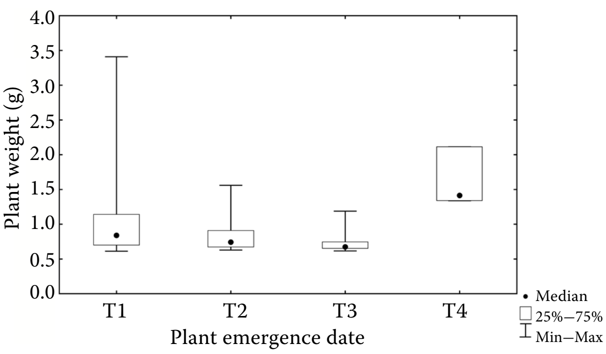

Figure 5. Above ground biomass produced by the florasulam treated plants of population 8401 of *Centaurea cyanus* in dependence on the plant germination date

Table 3. Median values, quartile range, P-value and coefficient of variation (CV) for the biomass plants (in grams) of population 8717 of *Centaurea cyanus* in dependence on the plant germination date

Term	N	Median	Quartile range	P-value	CV (%)
T1	136	0.63900^{b}	0.044800	0.0340	8.1
T2	57	0.65900^{ab}	0.079000	0.0340	9.0
T3	24	0.63850^{b}	0.059000	0.0340	7.9
T4	11	0.66200^{a}	0.087000	0.0340	13.3

Medians marked by the same letter do not differ significantly at P < 0.05 by Tukey's test ; for terms explanation see Table 2

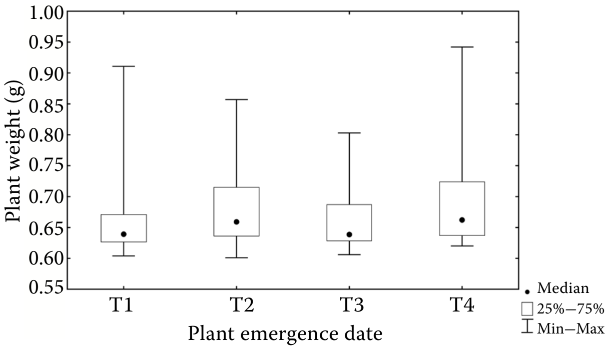

Figure 6. Biomass plants of population 8717 of *Centaurea cyanus* in dependence on the plant germination date

For terms explanation see Table 2

Table 4. Median values, quartile range, P-value and coefficient of variation (CV) for the biomass plants (in grams) of population 9294 of *Centaurea cyanus* in dependence on the plant germination date

Term	N	Median	Quartile range	P-value	CV (%)
T1	142	0.438000^{b}	0.036700	0.0001	6.7
T2	46	0.470000^{a}	0.049000	0.0001	11.1
T3	3	0.461000^{ab}	0.024000	0.0001	2.7
T4	4	0.417000^{b}	0.024000	0.0001	3.3

Medians marked by the same letter do not differ significantly at P < 0.05 by Tukey's test; for terms explanation see Table 2

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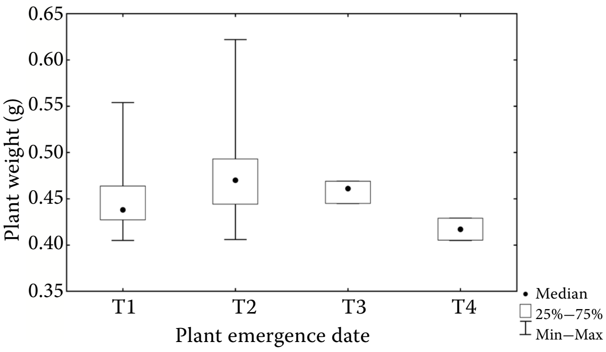

Figure 7. Biomass plants of population 9294 of *Centaurea cyanus* in dependence on the plant germination date
For terms explanation see Table 2

biomass after treatment with florasulam (Table 5). The largest range of the plant biomass and the coefficient of variation were found in the fourth germination date (T4) (Table 5, Figure 8).

The germination time of plants belonging to the resistant *C. cyanus* populations differentiated in their reaction to florasulam (Figure 9). In the first emergence date (T1), the lowest median of the above ground biomass reduction compared to the untreated control, amounting to only 18.7%, was noted for population 8401. The reduction in the biomass accumulation medians of populations 8717, 9294 and 9307 ranged from 25.3% to 30.3%. At T2, the greatest biomass reduction (30.8%) was found in the plants from population 9307, significantly greater than the plants from populations 8717 and 9294. The statistical analysis showed no significant difference between the populations after the florasulam treatment in the biomass of the plants that emerged at T3. The biomass reduction medians ranged from 21.4% (9294) to 34.7% (8401). Also, at T4, no significant differences in the biomass reduction medians between the populations were found. The above ground biomass of the plants from population 8401 and 9307 was 21.0% and 34.1% higher than observed for the untreated control, respectively.

Table 5. Median values, quartile range, *P*-value and coefficient of variation (CV) for the biomass plants (in grams) of population 9307 of *Centaurea cyanus* in dependence on the plant germination date

Term	*N*	Median	Quartile range	*P*-value	CV (%)
T1	117	0.76480[a]	0.084600	0.2304	18.3
T2	33	0.75900[a]	0.087000	0.2304	12.6
T3	6	0.79000[a]	0.255000	0.2304	33.9
T4	6	0.92400[a]	0.572000	0.2304	55.2

Medians marked by the same letter do not differ significantly at $P < 0.05$ by Tukey's test
For terms explanation see Table 2

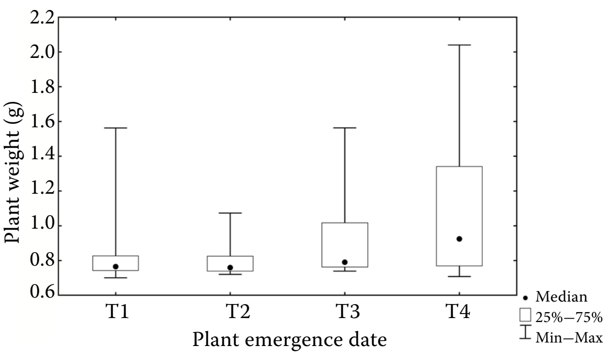

Figure 8. Biomass plants of population 9307 of *Centaurea cyanus* in dependence on the plant germination date
For terms (T1–T4) explanation see Table 2

DISCUSSION

Germination is a key developmental stage in the plant life cycle and plays a significant role in the survival of species in arable communities (Thompson et al. 2021). In the present study, the germination ability of ALS-resistant populations of *C. cyanus* was high, ranging from 80% for population 9294 to 92% for population 8717. According to Dyer et al. (1993), in populations resistant to ALS inhibitors, the application of herbicides from this group leads to an increase in the content of free amino acids such as valine, leucine, and isoleucine, which affect higher germination rates at lower temperatures, ranging from 4.6 to 13.2 °C. This gives the resistant populations an advantage over susceptible ones in the autumn or spring, during the most intense weed emergence in crops. However, it should be noted that the development of an adaptive mechanism, allowing populations to survive herbicide treatments, may come at the expense of other biological traits that reduce their competitiveness against the crop plant and susceptible populations. This phenomenon is described as the "fitness cost" (Keshtkar et al. 2019).

Studies by various authors indicate that the weed germination is influenced by the habitat of the mother plant, which affects the length of the seed dormancy (Steadman et al. 2004). The populations evaluated during this study originate from different soil and climatic conditions and from different crop plants. Three of them were collected in winter

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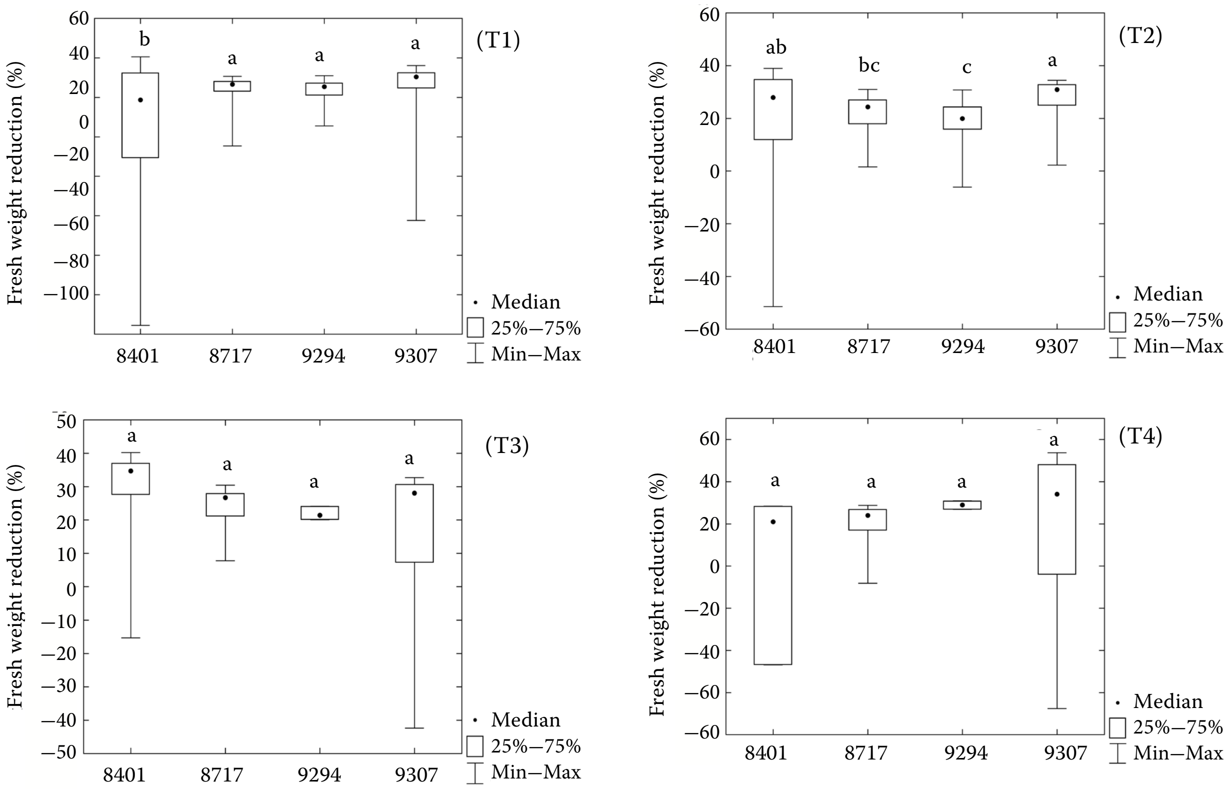

Figure 9. Above ground biomass reduction of the florasulam treated plants expressed as a percentage of the untreated control depending on the germination date (T1, T2, T3, T4)

Medians marked by the same letter do not differ significantly at $P < 0.05$ by Tukey's test

cereals, and one in a spring legume. The populations originating from winter wheat or winter rye showed similar germination dynamics. The highest proportion of seeds from these populations, 45–58%, germinated at 4 DAS. In contrast, the germination of population 8717 originating from a narrow-leafed lupin was prolonged, and the maximum germination rate of 30% was reached at 7 DAS. These results confirm the previously described correlation between seed germination and the habitat of the mother plant. The conducted study demonstrated significant differences between the *C. cyanus* populations in their germination ability and germination index. However, significant differences in the germination index were observed only between populations 8717 and 9307, while population 8717 significantly differed from populations 8401 and 9294 in terms of its germination ability. Similarly, Saja et al. (2016) found differences in the germination dynamics and germination index among resistant populations of *C. cyanus*. The results of other authors' studies on the germination biology of resistant populations of other weed species are diverse. Recasens et al. (2007) did not find differences in the germination ability, index, speed of germination, and emergence between two ACC-inhibitor-resistant populations of *Lolium rigidum* originating from different, geographically distant locations. Differences in the germination strategy among resistant populations of *Kochia scoparia* were demonstrated by Kumar and Jha (2017). The populations differed in germination ability and required different times to reach 50% cumulative germination capacity (I50) under constant and variable temperatures. Kumar et al. (2018) confirmed the different germination strategies of this species under field conditions as well. Among the studied populations, there were difference in the time and dynamics of the emergence. The diverse emergence pattern of *Kochia scoparia* populations confirms the need for adopting diverse and location-specific weed control tactics. In the study of Babineau et al. (2017), *Apera spica-venti* resistant to ALS inhibitors exhibited differences in the maximal germination compared to the sensitive population of this species, both at low tem-

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peratures (10 °C) and higher temperatures (16 °C and 22 °C). However, differences in the germination rate were only observed at the lowest temperature. It should be noted, though, that some weed populations showed varying germination dynamics at lower temperatures only. In the experiment conducted by Dyer et al. (1993), differences in the number of germinated seeds after 48 hours were observed in populations of *K. scoparia* resistant to ALS inhibitors compared to the sensitive population, but only at a low temperature of 4.6 °C. The impact of the temperature on the germination rate of resistant and sensitive populations was dependent on the biotype. Park et al. (2004) confirmed that differences in the germination process between R and S populations of *Bromus tectorum* increased with a decreasing temperature. These results provided a basis for conducting the present research at variable, low temperatures (10/15 °C), which, on the one hand, simulate natural field conditions during the germination of this species and, on the other hand, induce the occurrence of greater differences in the germination biology of the species.

Different patterns of the germination timing within and between populations indicate the development of adaptive traits by plants in response to different environmental conditions in which they grew, and may suggest the influence of crop management practices on the selection of germination and emergence of weed strategies (Owen et al. 2010). The dynamics of germination and emergence are dependent on the seed dormancy period, which is longer in herbicide-treated populations than in populations occurring in ruderal habitats (Maity et al. 2022). In our study, part of the plants from T4 belonging to populations 8401 and 9307, showed the smallest reduction in biomass after the florasulam application, indicating that they originated from seeds with a longer dormancy period. These plants produced the highest biomass following the herbicide treatment, indicating their greater resistance to florasulam compared to plants derived from seeds germinating in earlier periods.

In our study, plants within populations that exhibited varying degrees of florasulam sensitivity (greater or smaller reduction in the biomass after the herbicide application) depending on the germination timing have been compared. The diversity of strategies among resistant populations is noteworthy. In the case of population 8401, two peaks of significantly higher resistance were observed (the biomass in T4 and T1 was significantly higher than T3), while population 8717 showed one peak (the biomass in T4 was significantly higher after the herbicide application than T1 and T3), and population 9294 had a significant increase in the biomass in T2 compared to T1 and T4. Growth stimulation was observed in some plants at each timing. Population 8401 exhibited growth stimulation in all the timings, while population 9307 showed it in three timings. Hormesis following the herbicide treatment has been observed in resistant populations of other weed species, both at subtoxic doses and at the recommended label dose (Beltz et al. 2022; Wrochna et al. 2023). Biological tests have demonstrated that populations 8401 and 9307 are highly resistant to tribenuron methyl and florasulam (Stankiewicz-Kosyl et al. 2021). In the case of populations highly resistant to a specific herbicide, the recommended dose can stimulate the plant biomass growth (Beltz et al. 2022; Wrochna et al. 2023).

The conducted studies indicate that the germination biology of ALS inhibitor-resistant populations of *C. cyanus* is diverse, making it challenging to implement universal preventive strategies and control methods for resistant populations of this species in agricultural practice. Developing diverse weed control programmes is the only effective way to reduce the abundance of resistant weed populations (Menchari et al. 2008). Early emergence allows weeds to gain a competitive advantage over crop plants, while later emergence allows for the avoidance of mechanical and chemical weed control measures (Delye et al. 2013). Considering that the highest plant biomass after the herbicide application was observed in the latest emergence timing for 3 out of the 4 tested populations, delayed crop sowing is recommended in agricultural practice to eliminate late-emerging weed plants with a high level of herbicide resistance. Similar recommendations have been formulated for *Alopecurus myosuroides* (Gerhards et al. 2022).

CONCLUSION

The study showed differences in the germination between florasulam-resistant *C. cyanus* populations. In three of the four populations, the most numerous germination levels were noted four days after sowing, while one population had germination spread over time and no clear culmination was observed. Differences in the germination index and germina-

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tion capacity between populations were also found. The time of the germination of *C. cyanus* affected the reaction of plants to florasulam. In three of the four populations, the plants that germinated the latest (T4) had the highest biomass after the herbicide application. After the application of florasulam, the median range of the plant biomass reduction, originating from the examined dates of germination, was comparable, however, plants within populations exhibited varying degrees of herbicide sensitivity. Stimulation of plant growth after the herbicide application (hormesis) was observed at each time. Differences in the germination between populations of *C. cyanus* resistant to ALS inhibitors and different responses to the plant herbicide within the population indicate the need for further research in this area. Understanding the process of germination and emergence of as many resistant *C. cyanus* populations as possible will allow the development of guidelines for managing these populations in crops in order to reduce their number.

REFERENCES

Babineau M., Mathiassen S.K., Kristensen M., Kudsk P. (2017): Fitness of ALS-inhibitors herbicide resistant population of loose silky bentgrass (*Apera spica-venti*). Frontiers in Plant Science, 8: 1660. doi: 10.3389/fpls.2017.01660

Belz R.G., Carbonari C.A., Duke S.O. (2022): The potential influence of hormesis on evolution of resistance to herbicides. Current Opinion in Environmental Science and Health, 27: 100360. doi: 10.1016/j.coesh.2022.100360

Délye C., Menchari Y., Michel S., Cadet E., Le Corre V. (2013): A new insight into arable weed adaptive evolution: mutations endowing herbicide resistance also affect germination dynamics and seedling emergence. Annals of Botany, 111: 681–691.

Dyer W.E., Chee P.W., Fay P.K. (1993): Rapid germination of sulfonylurea resistant *Kochia scoparia* L. accessions is associated with elevated seed levels of branched chain amino acids. Weed Science, 41: 18–22.

Gerhards R., Messelhäuser M.H., Sievernich B. (2022): Suppressing Alopecurus myosuroides in winter cereals by delayed sowing and pre-emergence herbicides. Plant, Soil and Environment, 68: 290–298.

Guillemin J.P., Bellanger S., Reibeil C., Darmency H. (2017): Longevity, dormancy and germination of *Cyanus segetum*. Weed Research, 57: 361–371.

Heap I. (2023): International herbicide-resistant weed database. Available at: www.weedscience.org. (Accessed May 2, 2023).

Keshtkar E., Abdolshahi R., Sasanfar H., Zand E., Beffa R., Dayan F.E., Kudsk P. (2019): Assessing fitness costs from a herbicide-resistance management perspective: a review and insight. Weed Science, 67: 137–148.

Kumar V., Jha P. (2017): Effect of temperature on germination characteristics of glyphosate-resistant and glyphosate-susceptible kochia (*Kochia scoparia*). Weed Science, 65: 361–370.

Kumar V., Jha P., Dille J.A., Stahlman P.W. (2018): Emergence Dynamics of Kochia (*Kochia scoparia*) Populations from the U.S. Great Plains: A multi-site-year study. Weed Science, 66: 25–35.

Maity A., Lujan Rocha R., Khalil Y., Bagavathiannan M., Ashworth M.B., Beckie H.J. (2022): Concurrent evolution of seed dormancy and herbicide resistance in field populations of dominant weed species in Western Australian cropping systems. Weed Science, 70: 309–318.

Menchari Y., Chauvel B., Darmency H., Délye C. (2008): Fitness costs associated with three mutant acetyl-coenzyme A carboxylase alleles endowing herbicide resistance in black-grass *Alopecurus myosuroides*. Journal of Applied Ecology, 45: 939–947.

Owen M.J., Michael P.J., Renton M., Steadman K.J., Powles S.B. (2010): Towards large-scale prediction of *Lolium rigidum* mergence. II. Correlation between dormancy and herbicide resistance levels suggests an impact of cropping systems. Weed Research, 51: 133–141.

Panozzo S., Scarabel L., Collavo A., Sattin M. (2015): Protocols for robust herbicide resistance testing in different weed species. Journal of Visualized Experiments, 101: 1–10.

Park K.W., Mallory-Smith C.A., Ball D.A., Mueller-Warrant G.W. (2004): Ecological fitness of acetolactate synthase inhibitor-resistant and-susceptible downy brome (*Bromus tectorum*) biotypes. Weed Science, 52: 768–773.

Perotti V.E., Larran A.S., Palmieri V.E., Martinatto A.K., Permingeat H.R. (2020): Herbicide resistant weeds: A call to integrate conventional agricultural practices, molecular biology knowledge and new technologies. Plant Science, 290: 110255. doi: 10.1016/j.plantsci.2019.110255

Ranal M.A., de Santana D.G. (2006): How and why to measure the germination process? Brazilian Journal of Botany, 29: 1–11.

Randall J.M., Marinelli J. (1996): Invasive Plants: Weeds of the Global Garden. New York, Science Press.

Recasens J., Caimons O., Torra J., Taberner A. (2007): Variation in seed germination and early growth between and within acetolactate synthase herbicide resistant and susceptible *Lolium rigidum* accessions. Seed Science and Technology, 35: 32–47.

Riemens M., Sønderskov M., Moonen A.C., Storkey J., Kudsk P. (2022): An integrated weed management framework: a pan-European perspective. European Journal of Agronomy, 133: 126443. doi: 10.1016/j.eja.2021.126443

Saja D., Rys M., Stawoska I., Skoczowski A. (2016): Metabolic response of cornflower (*Centaurea cyanus* L.) exposed to tribenuron-methyl: one of the active substances of sulfonylurea herbicides. Acta Physiologiae Plantarum, 38: 168. doi: 10.1007/s11738-016-2183-x

Stankiewicz-Kosyl M., Haliniarz M., Wrochna M., Synowiec A., Wenda-Piesik A., Tendziagolska E., et al. (2021): Herbicide resistance of *Centaurea cyanus* L. in Poland in the context of its management. Agronomy, 11: 1954. doi: 10.3390/agronomy11101954

Steadman K.J., Ellery A.J., Chapman R., Moore A., Turner N.C. (2004): Maturation temperature and rainfall influence seed dormancy characteristics of annual ryegrass (*Lolium rigidum*). Australian Journal of Agricultural Research, 55: 1047–1057.

Tang W., Xu X., Shen G., Chen J. (2015): Effect of environmental factors on germination and emergence of aryloxyphenoxy propanoate herbicide resistant and susceptible Asia minor bluegrass (*Polypogon fugax*). Weed Science, 63: 669–675.

Thompson M., Mahajan G., Chauhan B.S. (2021): Seed germination ecology of southeastern Australian rigid ryegrass (*Lolium rigidum*) populations. Weed Science, 69: 454–460.

Weed Science Society of America Terminology Committee (1998): Herbicide resistance and herbicide tolerance definitions. Weed Technology 12. Available at https://wssa.net/wssa/weed/resistance/herbicide-resistance-and-herbicide-tolerance-definitions/ (Accessed May 2, 2023)

Wrochna M., Stankiewicz-Kosyl M., Wińska-Krysiak M. (2023): Stimulation of early post-emergence growth of *Alopecurus myosuroides* and *Apera spica-venti* following spray application of ACCase inhibitors. Agriculture, 13: 483. doi: 10.3390/agriculture13020483

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