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ARTYKUŁ ORYGINALNY

Wpływ temperatury na wskaźniki kiełkowania odpornych i wrażliwych na herbicydy biotypów miotły zbożowej (*Apera spica-venti*)

Temperature effects on germination indices in herbicide-resistant and susceptible silky bentgrass (*Apera spica-venti*) populations

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Streszczenie

Miotła zbożowa (*Apera spica-venti*) jest jednym z najbardziej powszechnych chwastów jednoliściennych pszenicy ozimej w Europie Środkowej. Do tej pory zidentyfikowano odporność tego gatunku na różne herbicydy. Celem badania była ocena wpływu temperatury na wskaźniki kiełkowania nasion siedmiu odpornych biotypów miotły zbożowej (reprezentujących odporność na substancje z 1, 2 i 3 grupy HRAC/WSSA) w porównaniu z dwoma biotypami wrażliwymi. Przeprowadzono dwie serie testów na płytkach Petriego, w trzech stałych temperaturach: 8, 18 i 28°C z fotoperiodem dzień/noc: 10/14 h. Obliczono sześć wskaźników kiełkowania na podstawie codziennych pomiarów. Wyniki badań wykazały, że temperatura wpływała na kiełkowanie wrażliwych i odpornych biotypów *A. spica-venti*. Kiełkowanie miotły zbożowej przebiegało najlepiej w temperaturach 18 i 28°C, niezależnie od badanego biotypu. Wyniki takie wskazują na podobną reakcję biotypów odpornych i wrażliwych na działanie temperatury w czasie kiełkowania. Nie stwierdzono wyraźnego związku między rodzajem odporności na herbicydy (pojedyncza, krzyżowa oraz wielokrotna), a kiełkowaniem (wyrażonym wskaźnikami kiełkowania) badanych biotypów *A. spica-venti*.

Słowa kluczowe: końcowy procent kiełkowania, wskaźnik szybkości kiełkowania, wskaźnik intensywności kiełkowania, pierwszy dzień kiełkowania, fitnes

Abstract

Silky bentgrass (*Apera spica-venti*) is one of winter wheat most serious and widespread grass weeds, particularly in Central Europe. Populations of this weed have developed herbicide resistance. This study aimed to assess the effect of temperature on the seed germination indices of seven resistant populations of silky bentgrass biotypes, representing groups 1, 2, and 3, on HRAC/WSSA compared to two susceptible biotypes. Two series of Petri dish experiments were performed at three constant temperatures: 8, 18, and 28°C, with a day/night photoperiod of 10/14 h. Six germination indices were calculated based on daily germination measurements. The results revealed that temperature affects the germination of sensitive and resistant *A. spica-venti* biotypes. Bentgrass germination occurred best at 18 and 28°C. This finding proves the similarities between resistant and susceptible biotypes in response to temperature. No clear connection was found between the type of herbicide resistance (single, cross or multiple) and the germination (expressed by the germination indices) of the tested *A. spica-venti* biotypes.

Keywords: final germination percentage, germination rate index, coefficient of velocity of germination, first day of germination, fitness

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Wstęp / Introduction

Silky bentgrass [*Apera spica-venti* (L.) P. Beauv.] is an annual winter grass considered one of wheat's most serious and widespread weeds, particularly in Europe (Massa and Gerhards 2011; Bitarafan and Andreasen 2020). In Poland, about four million hectares, representing 60% of winter cereal fields, are infested with silky bentgrass. This weed can grow higher than winter wheat and form up to 30 tillers (Krysiak et al. 2011). Seeds germinate in the autumn, when their life cycle and growing conditions concur with winter wheat seeds (Jensen 2009). It is a prolific weed as one mature plant can produce up to 16 000 seeds (Soukup et al. 2006), with soil viability reaching 4 years (Massa and Gerhards 2011). Silky bentgrass can cause up to 30% reduction in wheat yield if uncontrolled (Gehring et al. 2020).

In Poland, farmers usually use chemical methods to control silky bentgrass. However, recurrent application of herbicides with the same mode of action, mostly acetolactate synthase (ALS) inhibitors, has resulted in the evolution of herbicide resistance (Soukup et al. 2006). Globally, the first case of herbicide resistance in *A. spica-venti* was against the photosystem II inhibitor, isoproturon, in Switzerland (Mayor and Maillard 1997). However, the first case of resistance in Poland was reported in 2005 toward ALS inhibitors (Marczewska and Rola 2005). Multiple resistance to herbicide groups with three different modes of action, including acetolactate synthase, acetyl-CoA carboxylase, and photosystem II inhibitors, was developed in Poland, Germany, and the Czech Republic. Resistance to ALS- or ACCase-inhibiting herbicides in silky bentgrass was revealed to be mostly due to target-site alteration (Krysiak et al. 2011; Massa et al. 2013; Hamouzová et al. 2014).

The seed germination process is a critical phenomenon in determining the efficiency of a weed species to emerge successfully and compete with the crop (Chauhan et al. 2018). Germination is affected by several environmental factors, in which temperature is the most influential one (Baskin et al. 1998). Temperature requirements can differ among weed species (Van Assche et al. 2002) and may vary within genotypes from the same species (Debeaujon et al. 2000). Several studies have been done on herbicide resistance in different weed species and their effect on seed germination, e.g., resistant to ACCase herbicides *Phalaris minor* (Torres-García et al. 2015) and *Avena sterilis* ssp. *ludoviciana* (Benakashani et al. 2021) or *Echinochloa colona* resistant to triazines (Elahifard and Mijani 2014). It is postulated that the fitness cost, i.e., lower seed germination and production of the resistant (R) populations, is less than that of the susceptible ones in absence of the herbicide but in the presence of environmental stress factor, e.g., lower light intensity, like in the case of *Solanum ptychanthum* (Ashigh and Tardif 2009, 2011).

The current study aimed to compare the effects of temperature on germination indices of seven silky bentgrass (*A. spica-venti*) biotypes collected from different regions in Poland that exhibited cross and multiple resistance to ALS, ACCase, and photosystem II inhibiting herbicides and two biotypes susceptible to herbicides, using ANOVA as the primary model. We hypothesize that the germination indices of silky bentgrass are biotype-specific. Although ALS inhibitors are primarily POST-emergence herbicides, research on the germination of populations resistant to these active substances has significant value for several reasons. First, resistance to ALS inhibitors may be associated with certain biological and physiological changes that affect not only herbicide efficacy after emergence, but also earlier developmental phenophases, including germination and early seedling vigor. Furthermore, understanding the behavior of resistant biotypes during the germination phase is essential for integrated weed management (IWM). If a biotype resistant to ALS inhibitors displays different germination patterns (e.g., a broader temperature range or faster germination), this could allow for more effective application of non-chemical methods and early mechanical control.

Materiały i metody / Materials and methods

Pochodzenie nasion badanych biotypów / Seed origin of the tested biotypes

Seeds of nine biotypes of silky bentgrass were collected during 2013–2017 by research groups of the Institute of Plant Protection – National Research Institute in Poznań and the Institute of Soil Science and Plant Cultivation – National Research Institute in Wrocław, at different locations in Poland. The tested biotypes exhibited herbicide resistance to ACCase (HRAC/WSSA 1) and/or ALS (HRAC/WSSA 2) inhibitors as confirmed in dose-response assays (carried out as part of the BioHerOd* research grant), according to the method by Stankiewicz-Kosyl et al. (2021). The resistance level was expressed as Resistance Index (RI), being a ratio of ED_{50} values of resistant biotype relative to that of a standard susceptible biotype according to Burgos (2015) (tab. 1). Moreover, two herbicide-susceptible biotypes were included in the study. The biotypes in this study were marked according to their herbicide-resistance status. All seeds were stored in the paper envelopes in the dark at 4°C until further studies.

Badanie zdolności kiełkowania / Germination efficacy experiment

Germination of resistant (R) and susceptible (S) biotypes of silky bentgrass was tested in a Petri dish bioassay using a free-standing phytotron chamber (model MDF-500, BIOGENET, Poland). The experiment was carried out in two terms – fall of 2018 and spring of 2019, in a completely randomized arrangement, with three repeti-

Tabela 1. Charakterystyka badanych biotypów *Apera spica-venti*
Table 1. Characteristics of the tested *Apera spica-venti* biotypes

Biotyp Biotype	Typ odporności Resistance type*	Substancja czynna Active substance	Indeks odporności Resistance index RI**	WSSA/ HRAC	Rok zbioru Year collection
B-1	pojedyncza single	chlorosulfuron chlorsulfuron	RRR	2	2013
B-3	pojedyncza single	jodosulfuron metylowy iodosulfuron methyl	RRRR	2	2017
B-4	pojedyncza single	penoksulam penoxsulam	RRR	2	2017
BB-1	krzyżowa cross	chlorosulfuron/jodosulfuron metylowy/ mezosulfuron chlorsulfuron/iodosulfuron methyl/ mesosulfuron	RRR / RRR / RRR	2 2 2	2013
BB-2	krzyżowa cross	chlorosulfuron/jodosulfuron metylowy/ mezosulfuron chlorsulfuron/iodosulfuron methyl/ mesosulfuron	n.d.	2 2 2	2015
AB-1	wielokrotna multiple	fenoksaprop-P-ethylu/chlorosulfuron/ jodosulfuron metylowy/mezosulfuron fenoxaprop-P-ethyl/chlorsulfuron/ iodosulfuron methyl/mesosulfuron	RRR / RRRR / RRRR / RRRR	1 2 2 2	2013
AB-3	wielokrotna multiple	fenoksaprop-P-ethylu/pinoksaden/ jodosulfuron/penoksulam fenoxaprop-P-ethyl/pinoxaden/ iodosulfuron/penoxsulam	RRR / RRR / RRRR / RRRR	1 1 2 2	2017
S1	–	wrażliwy susceptible	S	–	2012
S2	–	wrażliwy susceptible	S	–	2016

n.d. – brak danych – no data
* rodzaj odporności według Adamczewskiego (2014) – resistance type according to Adamczewski (2014)
** indeks odporności według Burgos (2015) i Stankiewicz-Kosyl et al. (2021) – resistance index (RI) according to Burgos (2015) and Stankiewicz-Kosyl et al. (2021)

tions, twice each term. Thirty seeds were placed uniformly, using sterile tweezers, in each glass Petri dish (11 cm dia meter) previously surface disinfected by washing with sterile gauze soaked in ethyl alcohol (99.8% concentration, POCH, Gliwice). Two layers of sterile paper discs (soft quality filters, EUROCHEM, Tarnów), previously moistened with 5 cm^3 of distilled water, were placed in each dish. Silky bentgrass seeds were surface sterilized in a 2.5% ethyl alcohol solution (concentration 99.8%, POCH, Gliwice) for 5 minutes and then rinsed abundantly with distilled water to wash residues of the alcohol. The dishes were kept in the growth chamber for 10 days. The tested silky bentgrass seeds were germinated at three constant temperature regimes: 8, 18, or 28°C, with a day/night photoperiod of 10/14 h, and the humidity was maintained at a relatively constant level of 60%. Constant temperature does not mimic natural conditions, but it acts as a controlled experimental tool for understanding the basic physiological responses of seeds, identifying optimal conditions, and distinguishing differences between populations. If the experiment were conducted with day-night temperature cycles, it would be much harder to determine the specific factors influencing differences in germination – whether it is temperature, rhythm, moisture, or other factors. Distilled water was added to Petri dishes to ensure the seeds' constant moisture. Germinating seeds were counted daily and removed. Seeds were considered to be germinated when the radicle had emerged (BBCH 05) (Bochenek et al. 2016).

Wskaźniki kiełkowania / Germination indices

The germination indices of the tested silky bentgrass biotypes were analyzed based on daily measurement of the number of germinated seeds. Seed germination was expressed as a percentage of total viable seeds, and cumulative germination percentages were calculated independently for each experiment. The germination dynamic of the tested silky bentgrass biotypes was determined using the temperature model of 8–18–28°C. Six germination indices were measured and calculated according to the methodology developed by Kader (2005). These indices were as follows: final germination percentage (FGP), mean germination time (MGT), germination rate index (GRI), coefficient of velocity of germination (CVG), germination index (GI), and first day of germination (FDG) (tab. 2).

Analiza statystyczna / Statistical analysis

The calculated germination indices of the tested silky bentgrass seeds were subjected to statistical analysis using the STATISTICA 13.0 software (TIBCO Software Inc., CA, USA). A two-factor analysis of variance (ANOVA) was performed for a completely random design, with two factors: germination temperature and silky bentgrass biotype. The analysis of variance was preceded by testing the compliance of the empirical distribution with the normal distribution using the Kolmogorov-Smirnov normality test and Levene's test of homogeneity of variances. If the homogeneity of variance was not met, a logistic or square root transformation of the data was performed. Data transformation was performed for the following indices:

• FGP to Bliss degrees: FGP = arcsin (sqrt (FGP/100)) (equation 1)

• FDG index with the tangent function: FDG = tan (FDG) + 4 (equation 2)

Tabela 2. Opis analizowanych wskaźników kiełkowania
Table 2. Description of the analyzed germination indices

Wskaźniki kiełkowania Germination indices	Skrót Abbreviation	Jednostka Unit	Opis Description	Wzór Equation
Końcowy procent kiełkowania Final germination percentage	FGP	%	Zdolność kiełkowania nasion w danej partii/populacji. Im wyższa wartość wskaźnika, tym wyższa zdolność kiełkowania nasion. Seed germination capacity in a given batch/population. The higher the index value, the higher the seed germination capacity.	FGP = (liczba nasion kiełkujących w danej partii/wszystkie nasiona w partii) × 100 FGP = (the number of seeds germinating in a given seed batch/all seeds in the batch) × 100
Średni czas kiełkowania nasion Mean germination time	MGT	dni days	Dzień, w którym wykiełkowała największa liczba nasion w partii/populacji. Im niższa wartość wskaźnika, tym wcześniej (szybciej) nasiona kiełkowały. The day the largest number of seeds germinated in a given batch/population. The lower the indicator value, the earlier (faster) the seeds germinated.	MGT = $\Sigma f \cdot x/\Sigma f$ f – liczba nasion skiełkowanych w dniu x – number of seeds germinated per day x
Wskaźnik szybkości kiełkowania Germination rate index	GRI	%/dzień %/day	Dzienny procent kiełkowania nasion w danej partii/populacji. Im wyższa wartość wskaźnika, tym szybciej nasiona kiełkują i mają większą zdolność kiełkowania. Daily percentage of seed germination in a given batch/population. The higher the value of the indicator, the faster the seeds germinate and the greater their germination capacity.	GRI = $\frac{G_1}{1} + \frac{G_2}{2} + \cdots + \frac{G_x}{x}$ G_1, G_2 – procent kiełkowania w pierwszym/drugim dniu po siewie × 100 – germination percentage on the first/second day after sowing × 100 G_x – procent kiełkowania w x dniu po siewie × 100 – germination percentage on x day after sowing × 100
Wskaźnik intensywności kiełkowania Coefficient of velocity of germination	CVG	–	Czas potrzebny do osiągnięcia maksymalnego kiełkowania nasion w danej partii/populacji. Osiąga tym wyższe wartości im liczba kiełkujących nasion jest wyższa, a czas potrzebny na ich skiełkowanie krótszy. Wskaźnik osiągnąłby wartość 100, gdyby wszystkie nasiona wykiełkowały w pierwszym dniu. The time needed to achieve maximum seed germination in a given batch/population. The higher the number of germinating seeds, the shorter the time needed for germination. The index would reach 100, if all the seeds germinated on the first day.	CVG = $N_1 + N_2 + \ldots + N_x/100 \cdot N_1T_1 + \ldots + N_xT_x$ N – liczba nasion skiełkowanych danego dnia – the number of seeds germinated on a given day T – liczba dni od wyłożenia na szalkę do skiełkowania – number of days from placing on the plate to germination

Tabela 2. Opis analizowanych wskaźników kiełkowania – cd.
Table 2. Description of the analyzed germination indices – continued

Wskaźniki kiełkowania Germination indices	Skrót Abbreviation	Jednostka Unit	Opis Description	Wzór Equation
Indeks kiełkowania Germination index	GI	–	Procent kiełkujących nasion w danej partii/populacji i ich tempo kiełkowania. Nadaje poszczególnym dniom kiełkowania wagi, gdzie najwyższa waga występuje w pierwszym dniu kiełkowania, a najniższa w ostatnim. Im wyższa wartość wskaźnika, tym wyższa zdolność i szybkość kiełkowania nasion. The percentage of germinating seeds in a given batch/population and their germination rate. Gives weight to individual days of germination, with the highest weight occurring on the first day of germination and the lowest on the last day. The higher the value of the indicator, the higher the ability and speed of seed germination.	$GI = (10 \cdot n1) + (9 \cdot n2) + \ldots + (1 \cdot n10)$ n1, n2 ... n10 – liczba kiełkujących ziarniaków w pierwszym, drugim i kolejnych dniach do dziesiątego dnia – the number of germinating seeds in the first, second and subsequent days up to the tenth day 10, 9 ... 1 – wagi odpowiadające liczbie kiełkujących ziarniaków w pierwszym, drugim i kolejnych dniach kiełkowania – weights corresponding to the number of germinating seeds on the first, second and subsequent days of germination
Pierwszy dzień kiełkowania First day of germination	FDG	dzień day	Szybkość kiełkowania nasion w danej partii/populacji. Im niższa, tym szybciej nasiona kiełkują. Speed of seed germination in a given batch/population. The lower it is, the faster the seeds germinate.	–

Means were differentiated using Fisher's NIR test at the significance level of $\alpha = 0.05$.

An analysis of canonical variables was performed to present the diversity of the biotypes in a multi-indice manner. The graphical distribution of silky bentgrass biotypes (qualitative variable), described by germination indices (quantitative predictor), is presented in the system of the first two canonical variables. In addition, a discriminant analysis was performed to determine which independent variables best divide a given set of cases into naturally occurring groups, described by a qualitative dependent variable. The analysis was performed for all six germination indices with or without germination temperature ranges. The analysis was performed using the GenStat 18 statistical program.

Wyniki i dyskusja / Results and discussion

An analysis of variance conducted for the germination of *A. spica-venti* biotypes showed significant differences (at $p < 0.001$) for the investigated factors (temperature and biotype) and their interaction for all analyzed germination indices (tab. 3).

Tabela 3. Średnie kwadraty z dwuczynnikowej analizy wariancji przeprowadzonej dla wskaźników kiełkowania biotypów *Apera spica-venti*
Table 3. Mean squares form the two-way analysis of variance performed for the germination indices of *Apera spica-venti* biotypes

Źródło zmienności Source of variation	d.f.	GRI	GI	FGP	MGT	CVG	FDG
Temperatura – Temperature	2	143.1***	24752.0***	5771.4***	101.2***	6717.2***	32.45***
Biotyp – Biotype	8	88.75***	38484.7***	2682.66***	9.18***	488.3***	1.874***
Temperatura × Biotyp Temperature × Biotype	16	10.20***	2622.1***	357.21***	1.52***	178.0***	1.250***
Błąd – Residual	297	0.622	280.3	42.93	0.094	21.39	0.133

*** poziom istotności – level of significance $p < 0.001$

The germination rate index (GRI) was presented graphically (fig. 1). Silky bentgrass biotypes germinated rapidly at 18°C. The average GRI of biotypes at this temperature was 7.31%/day, 30% higher than at 8°C, where the GRI was the lowest (5.08%/day). On average, among the tested silky bentgrass biotypes, the highest average values of the GRI were recorded for the AB1 biotype (8.4%/day), and the lowest was recorded for the biotypes BB1 (4.5%/day) and S2 (4.7%/day). The BB2 biotype at 18°C had the highest GRI (10.4%/day), and the BB1 biotype at 8°C had the lowest GRI, of 3.3%/day.

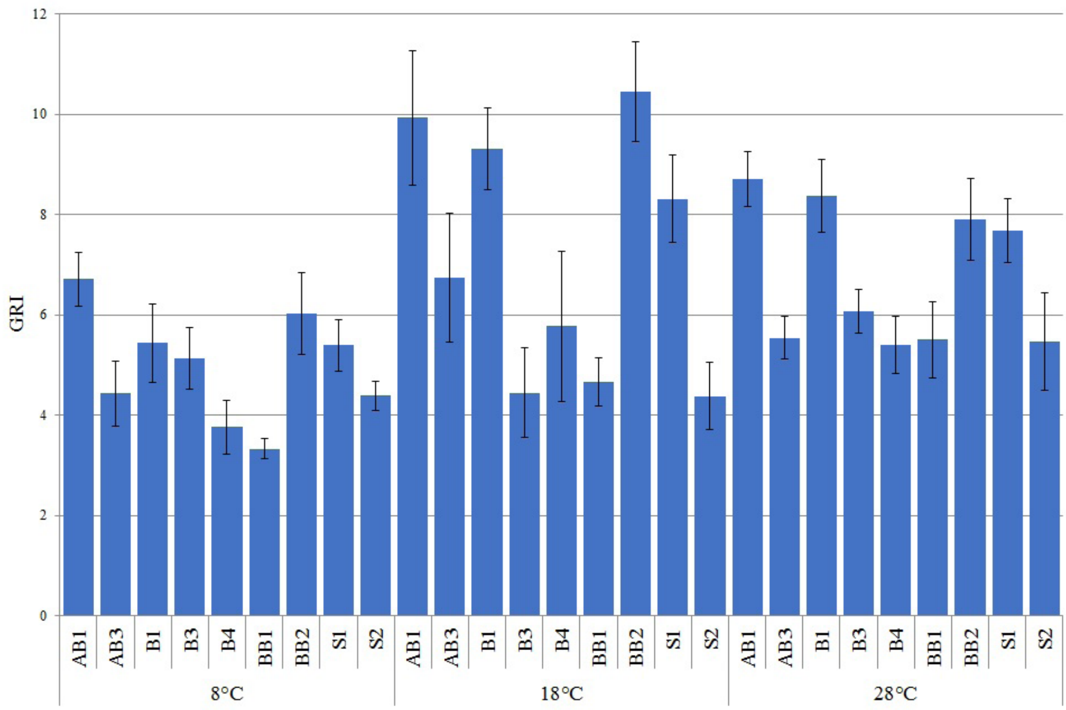
A similar trend was observed for the germination index (GI), an indice that combines the percentage and time of germination. Bentgrass biotypes showed the highest germination index at 28°C, where the average GI for biotypes was 164.7 (tab. 4). The lowest average GI was recorded at 8°C (135.6), which was 18% lower than at 28°C. Among the tested silky bentgrass biotypes, the highest average GI were recorded for AB1 (199.9) and BB2 (188.7) biotypes, while the lowest was recorded for the BB1 (118.6) and S2 (119.9). The BB2 biotype at 18°C had the highest GI (211.0), and the BB1 biotype at 8°C, the lowest (93.3).

Apera spica-venti biotypes achieved the highest final germination percentage (FGP) at a temperature of 8°C (tab. 4). The average FGP at this temperature was 89.0, and was 16% higher than at 18°C (74.4) and 10% higher than at 28°C. Among the tested silky bentgrass biotypes, the highest average FGP values were recorded for the AB1 biotype (93.0), while the lowest was recorded for the AB3 (74.1) and S2 (72.9) biotypes. The BB2 biotype at 8°C had the highest FGP (95.7), and the B3 biotype at 18°C had the lowest (54.1).

Silky bentgrass biotypes achieved the fastest of mean germination time (MGT) at 18°C, with an average of 3.6 days (tab. 4). The slowest MGT was at 8°C, equal to 5.5 days. On average, among the tested silky bentgrass biotypes, the AB1 (3.6) and BB2 (3.8) biotypes showed the fastest MGT, while the BB1 (5.2) biotype showed the slowest. The BB2 biotype achieved the shortest mean germination time at 18°C (2.6), and the longest was recorded for the BB1 biotype at 8°C (6.4).

Apera spica-venti biotypes germinated most intensively at 8°C, reaching an average CVG of 31.84 (tab. 4). The lowest average value of the CVG was at 18°C, equal to 16.40, and at 28°C – 21.34. Among the tested silky bentgrass biotypes, the AB1 and B1 biotypes showed the highest average CVG, of 27.8 and 27.5, respectively. The lowest CVG was for the AB3 (19.0) and S2 (19.4) biotypes. The B3 biotype at 8°C had the highest CVG (36.3), and at 18°C recorded the lowest CVG (9.0).

The first day of germination (FDG) refers to the day of the first germinated seed. Silky bentgrass biotypes germinated faster at 18°C and 28°C, achieving an average FDG of 2.0 days (tab. 4). At 8°C, the average FDG was



Rys. 1. Wartości wskaźnika szybkości kiełkowania (GRI) dla biotypów *Apera spica-venti* kiełkujących w różnych temperaturach
Fig. 1. Values of germination rate index (GRI) for *Apera spica-venti* germinating in different temperature regimes

Tabela 4. Wartości wybranych wskaźników kiełkowania *Apera spica-venti*
Table 4. Values of selected germination indices of *Apera spica-venti*

Temperatura Temperature	Biotyp Biotype	GI		FGP		MGT		CVG		FDG	
		średnia mean	s.d.	średnia mean	s.d.	średnia mean	s.d.	średnia mean	s.d.	średnia mean	s.d.
8°C	AB1	180.8 d	10.18	95.44 a	4.664	4.405 f	0.248	33.22 ab	4.115	2.417 ef	0.5149
8°C	AB3	122.2 jk	15.51	82.53 ef	5.671	5.472 c	0.5199	26.79 d	4.597	2.75 cd	0.6216
8°C	B1	151.7 ef	17.84	94.17 ab	4.252	5.164 de	0.4911	35.21 a	7.545	3.25 b	0.6216
8°C	B3	141.5 fghi	15.72	91.97 ab	5.774	5.494 c	0.292	36.29 a	5.14	3.167 b	0.7177
8°C	B4	104.6 lm	15.74	80.61 fg	4.928	6.042 b	0.4991	26.59 d	2.987	3.25 b	0.4523
8°C	BB1	93.3 m	5.26	82.49 ef	4.55	6.393 a	0.3033	26.73 d	5.35	4.25 a	0.7538
8°C	BB2	161.3 e	18.58	95.68 a	5.12	4.937 e	0.242	35.09 ab	6.048	2.25 fg	0.4523
8°C	S1	147.2 fgh	13.16	91.98 ab	6.011	5.282 cd	0.2563	35.2 a	5.585	2.667 de	0.4924
8°C	S2	117.9 kl	8.31	85.94 cde	5.359	5.848 b	0.4367	31.46 bc	7.973	3 bc	0
18°C	AB1	209.8 a	24.08	88.91 bcd	7.662	3.039 m	0.3076	21.22 ef	4.552	2 g	0
18°C	AB3	148.1 efgh	25.79	69.7 ij	10.598	3.53 kl	0.3318	14.29 ijk	5.472	2 g	0
18°C	B1	194.7 bc	16.45	84.33 def	5.536	2.996 m	0.2018	17.91 fghi	3.558	2 g	0
18°C	B3	105.3 lm	17.1	54.15 l	7.739	3.932 hi	0.443	8.99 l	3.204	2.25 fg	0.4523
18°C	B4	131.9 ij	30.06	66.62 jk	13.712	3.817 ij	0.379	13.3 jk	6.017	2 g	0
18°C	BB1	122.2 jk	11.24	73.59 hi	5.022	5.1 de	0.2835	22.22 e	4.865	2.083 fg	0.2887
18°C	BB2	211 a	19.7	85.49 def	7.607	2.607 n	0.2099	16.79 ghij	4.476	2 g	0
18°C	S1	184.7 cd	18.71	83.33 ef	5.808	3.412 l	0.2612	20.31 efg	3.724	2 g	0
18°C	S2	109.1 kl	15.37	63.77 k	5.963	4.459 f	0.2618	12.56 kl	3.237	2 g	0
28°C	AB1	209.2 a	11.88	94.69 a	4.63	3.619 jkl	0.0885	29.11 cd	2.996	2 g	0
28°C	AB3	136.8 ghi	10.26	70.01 ij	6.535	4.069 gh	0.1378	16.04 hijk	3.193	2 g	0
28°C	B1	202.3 ab	15.88	93.53 ab	5.521	3.754 ijk	0.2234	29.36 cd	4.227	2 g	0
28°C	B3	149.6 efg	10.15	76.36 gh	5.295	4.097 gh	0.12	19.29 efgh	2.426	2 g	0
28°C	B4	135.4 hij	13.65	72.77 hi	5.585	4.277 fg	0.2872	17.65 fghi	4.412	2.083 fg	0.2887
28°C	BB1	140.3 fghi	17.26	73.5 hi	6.14	4.138 gh	0.2081	17.33 ghi	3.221	2.25 fg	0.4523
28°C	BB2	193.7 bcd	17.94	90.86 abc	5.592	3.764 ijk	0.2124	27.03 d	4.171	2.083 fg	0.2887
28°C	S1	182.2 cd	11.42	83.17 ef	4.143	3.616 jkl	0.2276	22.03 e	2.308	2 g	0
28°C	S2	132.8 ij	21.07	69.09 ij	8.708	3.961 hi	0.2719	14.22 ijk	3.716	2.167 fg	0.3892
NIR LSD	Temperatura Temperature	4.484		1.8		0.08		1.2		0.1	
	Biotyp Biotype	7.766		3		0.14		2.1		0.17	
	Temperatura × biotyp Temperature × biotype	13.451		5.3		0.25		3.7		0.29	

s.d. – odchylenie standardowe – standard deviation
Wartości średnie w kolumnach oznaczone różnymi literami różnią się statystycznie – Mean values in columns followed by different letters differ significantly

3.0 days. Among the tested silky bentgrass biotypes, the BB2 biotype showed the lowest FDG (2.1), while the BB1 biotype showed the highest FDG (2.8). FDG > 3 was recorded at 8°C for biotypes: B1, B3, B4 and BB1.

Data presented in table 5 indicate that, except for the FGP, all other competitiveness indices determined for the seeds of the analyzed silky bentgrass biotypes had a significant impact on variable cV_1. This impact was negative (GRI and GI) and positive (MGT, CVG, and FDG). On the other hand, four out of six indices (GRI, GI, FGP, and CVG) had a significant impact on the second variable (cV_2).

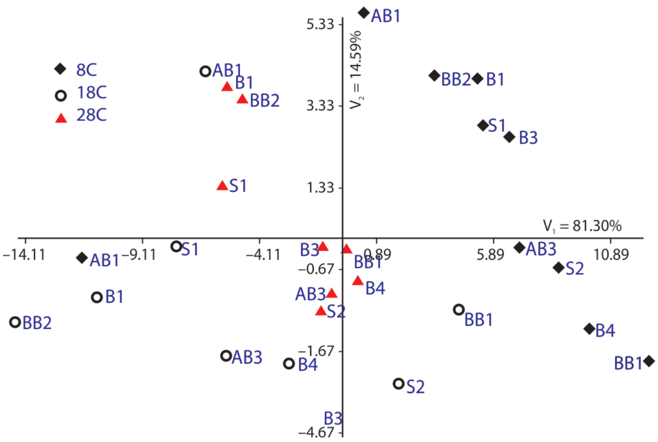
The first two canonical variables, V1 and V2, explained 95.89% of the variability in the analyzed dataset (fig. 2).

Tabela 5. Współczynniki korelacji między dwiema pierwszymi zmiennymi kanonicznymi, a wskaźnikami kiełkowania testowanych biotypów *Apera spica-venti* z uwzględnieniem trzech zastosowanych temperatur kiełkowania

Table 5. Correlation coefficients between the first two canonical variables and germination indices of the tested *Apera spica-venti* biotypes, at the three germination temperatures

Wskaźniki kiełkowania Germination indicators	cV_1	cV_2
GRI	–0.913***	0.295*
GI	–0.799***	0.551**
FGP	0.001	0.848***
MGT	0.985***	0.050
CVG	0.450*	0.800***
FDG	0.783***	0.022

cV_1 – współczynnik korelacji dla pierwszej zmiennej kanonicznej – correlation coefficient for the first canonical variable
cV_2 – współczynnik korelacji dla drugiej zmiennej kanonicznej – correlation coefficient for the second canonical variable
poziom istotności – level of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$



Rys. 2. Analiza zmiennych kanonicznych przeprowadzona dla wszystkich wskaźników kiełkowania biotypów *Apera spica-venti*, uwzględniająca trzy temperatury kiełkowania

Fig. 2. Canonical variable analysis conducted for all germination indices of *Apera spica-venti* biotypes, considering three germination temperatures

The influence of temperature on germination, as determined by the calculated germination indices, was the main criterion for grouping the tested weed biotypes. On the contrary, a grouping of biotypes based on the type of herbicide resistance was not observed.

All examined germination indices significantly impacted variable cV_1, with a negative relationship for MGT and FDG (tab. 6). The second variable, cV_2, was not significantly associated with any indices.

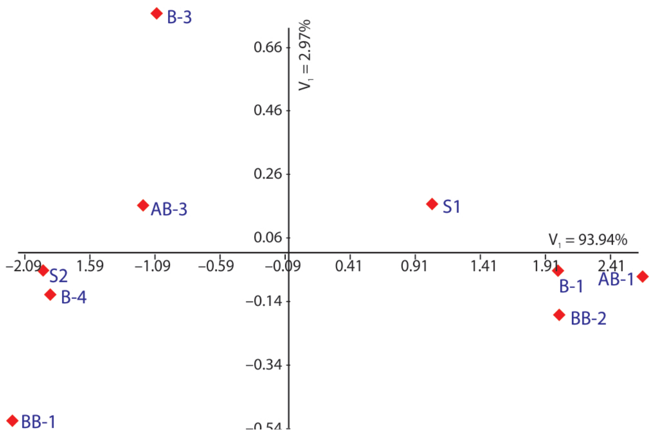
Canonical variable analysis conducted for all germination indices of silky bentgrass biotypes, regardless of germination temperature, explained 96.91% of the variability in the analyzed biotypes (fig. 3).

Tabela 6. Współczynniki korelacji między dwiema pierwszymi zmiennymi kanonicznymi, a wskaźnikami kiełkowania testowanych biotypów *Apera spica-venti* niezależnie od zastosowanej temperatury kiełkowania

Table 6. Correlation coefficients between the first two canonical variables and germination indices of the tested *Apera spica-venti* biotypes regardless of the applied germination temperature

Wskaźniki kiełkowania Germination indicators	cV_1	cV_2
GRI	0.995***	–0.038
GI	0.998***	–0.038
FGP	0.973***	–0.201
MGT	–0.942***	–0.187
CVG	0.935***	0.127
FDG	–0.673*	–0.267

cV_1 – współczynnik korelacji dla pierwszej zmiennej kanonicznej – correlation coefficient for the first canonical variable
cV_2 – współczynnik korelacji dla drugiej zmiennej kanonicznej – correlation coefficient for the second canonical variable
poziom istotności – level of significance * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$



Rys. 3. Analiza zmiennych kanonicznych przeprowadzona dla wszystkich wskaźników kiełkowania biotypów *Apera spica-venti*, niezależnie od temperatury kiełkowania

Fig. 3. Canonical variable analysis conducted for all germination indices of *Apera spica-venti* biotypes, regardless of germination temperature

The position of individual biotypes in the canonical variable system indicates that, based on indices such as GRI, GI, FGP, and CVG, the biotypes BB2, B1, and AB1 germinated best. Meanwhile, the MGT and FDG indices indicated that the biotypes AB3 and B3 germinated the poorest (fig. 3). Furthermore, comparing the discriminant analysis of germination indices with the dynamics of germination for individual biotypes, regardless of temperature, it was found that the results of both analyses coincided for the biotypes BB2, B1, and AB1 as the best germination performance, and the biotype AB3 as the weakest.

The results we obtained do not clearly indicate that the type of herbicide resistance, either single, cross, or multiple, can have a significant effect on germination characteristics (expressed by the germination indices) of collected *A. spica-venti* biotypes. The resistant biotypes achieved the average values of germination rates, which were similar to those of the sensitive biotypes. Thus, no clear conclusions can be drawn regarding the differences in the germination of resistant and sensitive silky bentgrass biotypes. Information available in the literature on germination characteristics of herbicide-resistant and susceptible *A. spica-venti* is not distinct. Numerous reports revealed differences between the germination of R and S weed biotypes (Chen et al. 2020; Ghazali et al. 2020). For example, Babineau et al. (2017) studied germination patterns between ALS-resistant and susceptible *A. spica-venti* populations from Denmark at three different temperatures (10, 16 and 22°C) using growing degree days (GDD) as the measurement unit. The resistant population consistently germinated earlier than the susceptible population across all tested temperatures, with differences ranging from 9 to 20 growing degree days. This earlier germination occurred regardless of environmental temperature conditions, indicating a robust phenological advantage for resistant biotypes. The study found no differences in emergence rates between temperatures, but significant variation existed between populations, with emergence rates varying between 4 and 43%. Studies by Massa and Gerhards (2011) on silky bentgrass collected from various locations in Germany, the Czech Republic, and Poland exhibited three times higher germination rates in the biotype resistant to ALS, ACCase, and PSII inhibitors than in the sensitive biotype. Additionally, no germination differences were found among the tested R biotypes, which are resistant to ACCase and PS II inhibitors. In turn, Stankiewicz-Kosyl and Ciepka (2014) found that the S biotype of *A. spica-venti* had a higher germination capacity than R, regardless of the temperature regime. Likewise, germination of (S) populations of *Lolium rigidum* was greater and faster than the R populations (Zamani et al. 2023). Germination was also reduced in glyphosate-resistant *Kochia scoparia* biotype compared with the sensitive one (Kumar and Jha 2017). Other studies showed no differences in the germination of resistant and sensitive weed biotypes, whether monocotyledonous *Echinochloa colona* (Mutti et al. 2019; Sheng et al. 2019) or dicotyledonous *Conyza bonariensis* or *Raphanus* sp. (da Silva Amaral et al. 2020; Vercellino et al. 2021).

In our own research we observed that the temperature affects the germination dynamics of silky bentgrass. In a lower temperature range, the germination indices were poorer, as compared to 18°C. Research by many authors indicated that the indices of weed seed germination can change depending on temperature (Ismail et al. 2007; Babineau et al. 2017), and also whether the germination temperature is constant or variable. For example, Pedroso et al. (2019) reported that at temperatures below 19.3°C, three R biotypes of *Cyperus difformis* germinated earlier than the S biotypes. Park et al. (2009) reported that germination of *Bromus tectorum* biotypes resistant to ALS inhibitors was comparable to that of the susceptible one at constant temperatures of 5, 15 and 25°C. At a temperature of 5°C, the resistant biotype germinated approximately 27 h earlier than susceptible one and achieved a final germination percentage of 60%. They also stated that, as the seed incubation temperature decreases, differences in germination processes between sensitive and resistant biotypes may become visible. Wu et al. (2016) showed no difference in the final germination rate between the R and S biotypes of *Alopecurus japonicus* under different temperature conditions. Our data agree with several authors that the germination of weed biotypes resistant and sensitive to herbicides is characterized by high variability and heterogeneity. It is assumed that the fitness cost of the resistant population is less than that of the susceptible one in the absence of the herbicide (Ashigh and Tardif 2009, 2011). Resistant weeds can germinate in a wide range of temperatures and under different environmental factors while achieving high germination. Contradicting results regarding the presence or absence of fitness costs in resistant biotypes may be due to genetically different mechanisms of resistance in the tested seeds (Keshtkar et al. 2019). Various point mutations responsible for endowing resistance to herbicides may alter weed biotypes' competitive abilities and fitness costs (Zangeneh et al. 2018). It is also often difficult to obtain genetically homogeneous weed seeds due to the complicated process of conferring resistance genes, which is additionally influenced by various environmental conditions (Ghazali et al. 2020).

Wnioski / Conclusions

1. No clear connection was found between the type of herbicide resistance (single, cross or multiple) and the germination (expressed by the germination indices) of the tested *A. spica-venti* biotypes.
2. The germination indices of resistant biotypes were (on average) similar to those of sensitive ones.
3. The significant influence of temperature on the germination dynamics of silky bentgrass was confirmed.

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