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ORIGINAL PAPER

Influence of endozoochory and stress conditions on the germination of black cherry *Prunus serotina* Ehrh. seeds outside the natural range

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ABSTRACT

One of the most invasive species in European forest ecosystems is black cherry *Prunus serotina* Ehrh. Although its ecology, invasiveness mechanisms and control methods have attracted considerable forest scientists interest, some issues related to the germination capacity of black cherry seeds require further clarification. Therefore, the aim of this study was to analyse the germination of various seed variants.

The study was conducted in 2019-2022. Black cherry seeds were collected in September 2019 from badger faeces and standing trees. In total, 192 seeds were used for one variant of the experiment. Observations were made every two weeks from the appearance of the first black cherry seedlings. A Pearson's chi-squared test was used to analyse the probability of germination within a particular treatment.

The total number of seeds germinated was 652 (34% of all seeds sown). The highest total share of black cherry seed germination in 2020 was observed in the seeds from badger faeces (65.1%), and the lowest in seeds that were frozen at –70°C (1.04%) and at –18°C (4.17%). In 2021, the highest total share of black cherry seed germination was observed in seeds incubated at 40°C (13.02%), and the lowest in those that were frozen at –70°C (0%). The number of seedlings in 2020 was much lower than in 2021; not a single black cherry seedling appeared in 2022.

Endozoochory increases the invasiveness of black cherry. The pericarp inhibited germination, but only when the seeds in the pericarp were frozen. Black cherry seeds lying in the soil retain their germination capacity for two years.

KEY WORDS

badger, invasive alien species, *Prunus serotina*, seed bank, seed viability

Introduction

One of the most invasive species in European forest ecosystems is the black cherry *Prunus serotina* Ehrh. (Halarewicz, 2011; Jagodziński *et al.*, 2019; Korzeniewicz *et al.*, 2020). Black cherry grows fast, reproduces very easily and occupies the ecological niches of native tree species such as Scots pine *Pinus sylvestris* L. (Bułaj *et al.*, 2017). The expansion of this species is favoured by its high

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germination capacity and seed overproduction, longevity (via its seed bank), high dispersion (via endozoochory), intensive appearance of suckers, fast growth and development of individuals, low requirements in relation to habitat factors and high tolerance to climatic conditions (Bellon, 1977; Faliński, 2004). The black cherry's age to first seed production can be as low as 4-7 years with high light availability (Deckers, 2005). The black cherry creates a seed bank capable of germinating for two years (Namura-Ochalska, 2012) or three years (Marquis, 1975). Animals have a significant role in the spread of black cherries (Kurek, 2011; Baranowska *et al*., 2020). Fruits of the black cherry are eaten mostly by birds, but also by small mammals (Rutkowski, 2002). The common black bird *Turdus merula* L. and common wood pigeon *Columba palumbus* L. are the most important dispersers of black cherry (Deckers *et al*., 2008).

Prunus species have deeply dormant seeds; therefore, black cherry seeds are considered to require stratification (Grisez, 1974). In nature, they are subject to stratification in the soil during winter. Due to delayed germination, a considerable quantity of viable cherry seeds is often stored in the soil (Marquis, 1975). The dissemination and degree of dormancy of black cherry seeds vary from one locality to the other (Phartyal *et al*., 2009). So far, research has been undertaken on the species' ecology (Bonner, 1975; Marquis, 1975; Deckers, 2005; Phartyal *et al*., 2009), zoochory (Kurek, 2011; Baranowska *et al*., 2020) and seed preparation (Huntzinger, 1968; Esen *et al*., 2007). Nevertheless, some issues related to the germination capacity of black cherry seeds outside their natural range and under stress conditions require further clarification. Therefore, the aim of the research was to analyse the germination of various seed variants (with pericarp, without pericarp, stratified, and derived from badger *Meles meles* L. faeces) of black cherry subjected to stress [low temperature (–70°C, –18°C) and high temperature (40°C)]. The following hypotheses were adopted: I) black cherry seeds collected from badger faeces will be characterised by the biggest germination efficiency, II) –18°C will not have a limiting effect on the effectiveness of black cherry germination, III) the pericarp will inhibit germination, IV) the efficiency of germination of stratified seeds will not differ significantly from the efficiency of germination of non-stratified seeds and V) black cherry seeds will retain their 3-year emergence capacity.

Material and methods

The experiment was carried out from September 2019 to September 2022 in the Dendrological Garden of the Poznań University of Life Sciences (52°25'38.8"N, 16°53'36.0"E). Black cherry seeds were collected in the first week of September 2019 at the Forest Arboretum in Zielonka (52°55'52.3"N, 17°10'83.3"E) from badger faeces (2,000 seeds from the discovered latrines) and from three standing trees (2,000 diaspores).

The viability of seeds was assessed via X-ray at the Seed Assessment Station at the Kostrzyca Forest Gene Bank. Radiographic images were generated using a MultiFocus digital radiography system (Faxitron Bioptics LLC, USA). This system is equipped with a complementary metal-oxide semiconductor (CMOS) X-ray sensor coupled with an 11 μm focal spot tube. Additionally, it has up to 8×geometric magnification and provides as high as 6 μm resolution for seed imaging, with a choice of a 48 μm or 24 μm detector. The built-in advanced Automatic Exposure Control selects the appropriate exposure time and kV settings for each sample (Bianchini *et al*., 2021). The development of cherry seed embryos was compared with photos and drawings of embryos from the studies of Willan (1985), Załęski (2000) and Chen *et al.* (2007).

Three X-rays were taken for three batches of seeds (300 pieces) from badger faeces (A, B, C BORSUK) and three batches collected from standing trees (300 pieces; A, B, C ZDROWE). These seeds were weighed on a laboratory scale with an accuracy of 0.01 g. By weighing individual batches of seeds, the average weight for 1,000 seeds was determined. Before sowing, all seeds and fruit were selected for the presence of abiotic (mechanical) and biotic (caused by insects and fungi) damage. The damaged seeds and fruit were not used in the experiment.

Seeds were collected from standing trees. The separate variants of the experiment are described in Table 1. Seeds in variants I and VI were stored in a freezer at –70°C for 3 days after collection, while those in variants II and VIII were stored in a freezer at –18°C for 7 days after collection. Seeds in variant IV were subjected to stratification by hot-cold treatment according to Krüssmann's (1997) recommendations, consisting of 4 weeks at 20°C followed by 18 weeks at 3°C in river sand. Seeds in variant IX were stored at 40°C in a dryer for 3 days after collection. Then, seeds from variants I, II, III and V-IX were sown on 18 October 2019 in multi-pots (32 cells), for which peat substrate (Alonet) was used, except for the stratified seeds (IV), which were sown in river sand on the same day. After a period of stratification on 23 March 2020, the seeds of variant VI were screened with sand and were sown in peat substrate. In total, 192 seeds were used for each variant of the experiment (with a total of 1,920 seeds).

The experiment included full light conditions. Observations were made every two weeks from April to September (in 2020, 2021 and 2022). The germinated black cherry seedlings were removed manually to avoid mistakes in counting. Plants were watered in accordance with the 'Guidelines for the irrigation of forest nurseries in open areas' (Pierzgalski, 2002).

A probability test was used to analyse the data in this experiment. The analyses were performed in the statistical program PQ Stat v 1.8.6.102. None of the seeds were excluded from the analysis (n in total = 1920 = 10 treatments × 192 seeds). A Pearson's chi-squared test (Pearson χ^2) test was used to analyse the probability of germination in a particular treatment. For each variant, this was calculated via the number of germinated seeds in each treatment relative to the total number of available seeds. These results were then analysed using the Pearson's chi-squared test statistic in order to compare two probabilities with fixed hypothesised values. It was hypothesised that the values of probability of germination in all treatments would be the same as for the control (not equal to 0.50), as this is the general assumption. The analyses were performed separately for each of the two years of the study, assuming the probability values resulting from the control in each of the years.

Results

Based on the X-ray images, it was found that 97.33% of the embryos selected for the sowing of black cherry seeds sourced from badger faeces were correctly developed (A: 99%, B: 96%, C: 97%).

Table 1.
Variants of experiment

Variant	Pericarp	Treatment
I	Yes	–70°C
II	Yes	–18°C
III	No	–
IV	No	Stratification according to Krüssmann's (1997)
V	No	–18°C
VI	No	–70°C
VII	No	seeds extracted from badger faeces
VIII	No	seeds extracted from badger faeces and subjected to the temperature of –18°C
IX	No	+ 40°C,
C	Yes	control

The average weight of the seeds from faeces (n=1,000) was 7.89 g (A: 7.94 g, B: 7.54 g, C: 8.20 g) (Fig. 1a, b, c).

The X-ray images also showed that 99.33% of the embryos selected for the sowing of black cherry seeds sourced from standing trees were correctly developed (A: 100%, B: 100%, C: 98%). The average weight of the seeds from standing trees (n=1,000) was 8.63 g (A: 8.68 g, B: 8.58 g, C: 8.63 g) (Fig. 1d, e, f).

The total number of seeds that germinated from 4 May 2020 to 1 September 2021 was 652 (34% of all seeds sown), while not a single black cherry seedling appeared in 2022 (Table 2).

The highest total share of black cherry seed germination in 2020 was observed in variants VII (badger) (65.1%) and III (without pericarp) (53.13%), and the lowest in variants I (Fruit at –70°C) (1.04%) and II (Pericarp at –18°C) (4.17%) (Fig. 2, Table 3); these values were statistically significant.

In 2021 the highest total share of black cherry seed germination was observed in variant IX (40°C) (13.02%), and the lowest in variant I (Fruit –70°C) (0%) (Fig. 3); both results were statistically significant.

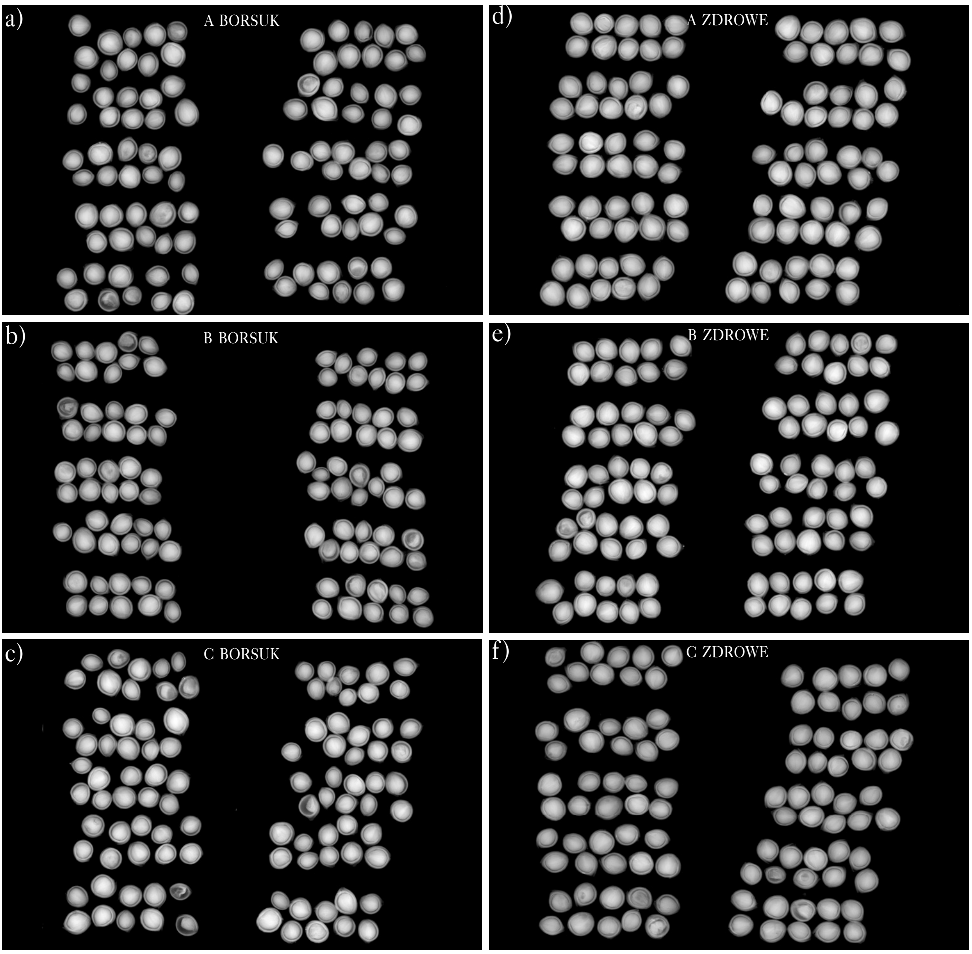

Fig. 1.

X-ray images: a, b, c – black cherry seeds coming from badger faeces; d, e, f – seeds coming from standing trees

Table 2.
Black cherry germination in 2020 and 2021 [pcs.]; variants of experiment described in Table 1

Date of observation	Variants									
	I	II	III	IV	V	VI	VII	VIII	IX	C
5-04-2020	0	0	0	0	0	0	11	0	0	0
17-04-2020	0	1	44	0	3	1	92	14	5	18
1-05-2020	0	4	26	22	38	25	17	45	40	23
14-05-2020	1	1	5	49	0	9	2	11	5	10
31-05-2020	0	1	9	3	3	1	2	2	3	5
12-06-2020	1	1	1	1	2	1	1	3	2	0
25-06-2020	0	0	3	2	0	0	0	0	2	0
14-07-2020	0	0	3	0	2	0	0	0	1	0
30-07-2020	0	0	0	0	0	0	0	0	0	0
9-05-2021	0	0	11	2	13	2	3	10	23	4
23-05-2021	0	0	0	0	0	0	0	1	2	2
6-06-2021	0	0	0	0	0	0	0	0	0	0
20-06-2021	0	1	0	0	0	0	0	0	0	1
2022	0	0	0	0	0	0	0	0	0	0
SUM	**2**	**9**	**102**	**79**	**61**	**39**	**128**	**86**	**83**	**63**

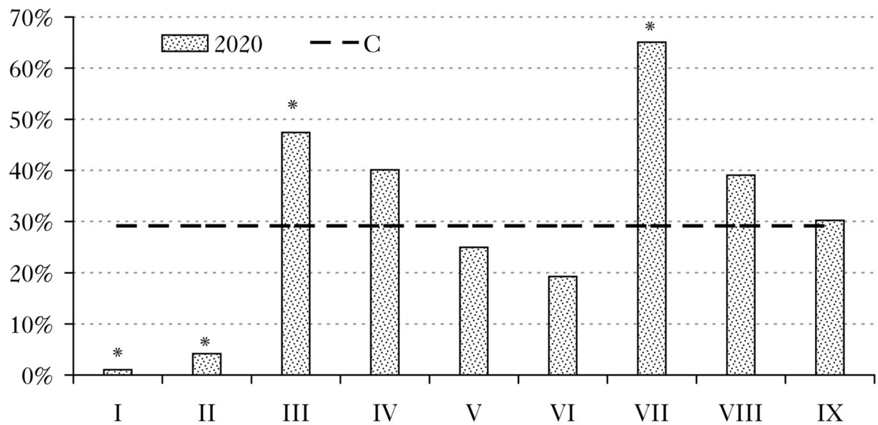

Fig. 2.
The share of germinated black cherry seeds in 2020
* denotes a result statistically different from the control (C). Variants of experiment described in Table 1

Discussion

This study showed that black cherry seeds collected from badger faeces (with destroyed pericarp) were characterised by the highest germination capacity, congruent with the results of Baranowska *et al.* (2020). Also in line with Baranowska *et al.* (2020), the results indicate that the process of germination of black cherry seeds is characterised by high disturbance at the initial stages of the experiment. It was confirmed the hypothesis that black cherry seeds collected from badger faeces would be characterised by higher germination efficiency in the first year after sowing than non-stratified seeds. Endozoochory offers several advantages to the ingested seed in the form of potential long-distance dispersal, germination facilitation thanks to seed coat

Table 3.
Results of the statistical analyses (the Pearson χ^2 test). After the test statistic F, the probabilities are provided in brackets. Abbreviations: df – degrees of freedom

Variant	df	2020 – Pearson χ^2	df	2021 – Pearson χ^2
I	192	87.909 (p<0.0001)	190	No germination
II	192	68.891 (p<0.0001)	183	8.491 (p=0.0036)
III	192	35.932 (p<0.0001)	90	8.101 (p=0.0044)
IV	192	6.048 (p=0.0139)	113	2.944 (p=0.0862)
V	192	0.095 (p=0.7585)	131	5.164 (p=0.0231)
VI	192	13.608 (p=0.0002)	153	5.0581 (p=0.0245)
VII	192	99.814 (p<0.0001)	64	0.068 (p=0.7942)
VIII	192	12.497 (p=0.0004)	106	5.064 (p=0.0244)
IX	192	9.450 (p=0.0021)	109	1062.354 (p<0.001)

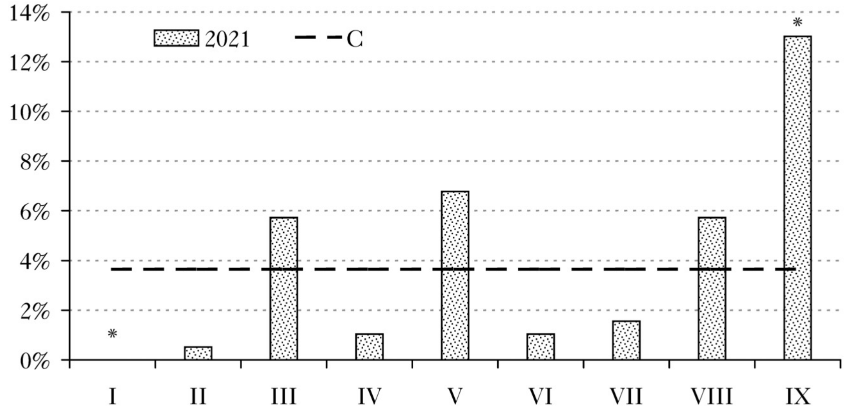

Fig. 3.
The share of germinated black cherry seeds in 2021
* denotes a result statistically different from the control (C). Variants of experiment described in Table 1

abrasion in the gut, and a fertilising effect of the faeces at the time of seedling establishment (Shiferaw *et al*., 2004). Phartyal *et al*. (2009) showed that seeds of black cherry collected from the faeces of herbivorous mammals were vigorous and germinated at a higher percentage compared to seeds collected directly from the tree, which is consistent with the results. This may be because inhibitors of seed germinations may be leached out during the passage of seeds through the digestive system of animals (Phartyal *et al*., 2009). Animal-mediated seed dispersal in general – and directed dispersal in particular – may play a role in facilitating invasive species' spread (Malo and Suarez, 1997; Wenny, 2001).

According to an early report, black cherry exhibits delayed germination: seeds from one crop germinate over a period of 3 years (Marquis, 1975). It was estimated that hundreds of black cherry seeds accumulate in the soil in any given year (Marquis, 1975). Each spring, about one-half of these germinate (Uchytil, 1991). As seeds, black cherry is able to enter closed-canopy forests and form a long-living seed bank (Closset-Kopp *et al*., 2007). According to Krüssmann (1975) the germination capacity of seeds of the *Prunus* genus is 1-2 years, which is consistent with my finding. According to Phartyal *et al*. (2009) black cherry does not form a persistent soil seeds bank in temperate forests of Western Europe, analogous to in its native range in North America (Beatty, 1991; Brown, 1992). We have not confirmed the hypothesis that black cherry seeds will retain the 3-year emergence capacity. It should be emphasised that the number of seedlings in 2020 was much lower than in 2021.

The results did not fully confirm earlier reports (Huntzinger, 1968; Baranowska *et al.*, 2020) that the pericarp inhibits the germination of black cherry seeds. Camacho-Morfin (1994) reported inhibitors in seeds of *P. serotina* subsp. *capuli*. Chemically dormant seeds do not germinate due to the presence of inhibitors in the pericarp, and they require removal or leaching of these inhibitors to break this dormancy. In a study by Pairon *et al.* (2006), black cherry fruit without a mesocarp (presumably eaten and dispersed by birds) were 17.7% more viable than fruit with mesocarp. Smith (1975) also found a higher percentage (up to 28.7%) of viable fruit among droppings and regurgitated fruit for some months throughout the year, which is similar to my findings. It was confirmed the hypothesis that the pericarp would inhibit germination, but only in variant II, where the seeds in the pericarp were frozen.

It was generally accepted that black cherry seeds require stratification. In nature, they are subject to stratification on the forest floor during winter (Marquis, 1990) and they may show delayed germination extending up to 3 years after being deposited (Marquis, 1975). According to Krüssmann (1978) the germination capacity of seeds of the *Prunus* genus is 1-2 years, which is consistent with results. The results of my research also indicate that black cherry seeds do not require any special preparation for sowing. More important than classic stratification is the removal of the pericarp.

Climate change is expected to have a more pronounced effect during the early stages of plant development – germination and seedling establishment – than in the adult stages (Fernández--Pascual *et al.*, 2015). Germination of ingested seeds exposed to high temperatures is either inhibited or prevented. Thermoinhibited seeds can germinate immediately under favourable temperatures; however, thermodormancy induced by prolonged exposure to higher temperatures may not allow germination even when the temperatures are lowered. This phenomenon has been reported in several crop species (Leymarie *et al.*, 2009; Huo *et al.*, 2013; Geshnizjani *et al.*, 2018), and several forest species, including *Pinus* spp. (Guo *et al.*, 2018). With rising global temperatures and increasingly variable precipitation, more crops are expected to become susceptible to either thermoinhibition or thermodormancy during germination (Reed *et al.*, 2022). As a result of ongoing climate change, the black cherry in its natural range of occurrence is likely to become a 'loser' species, whose area of occurrence is decreasing (Iverson *et al.*, 2008). However, the prognosis of its occurrence in Europe – especially in Western Europe – is quite different: the black cherry appears to be resistant to climate change outside its natural range.

Conclusions

It was found that endozoochory increases the invasiveness of black cherry by improving its germination capacity. The pericarp inhibited emergence only when the seeds were frozen. Seeds of black cherry in the soil retain their germination capacity for two years. The results of my research also indicate that black cherry seeds do not require any special preparation for sowing. More important than classic stratification is the removal of the pericarp.

Conflict of interest

Author declare there is no conflict of interest.

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STRESZCZENIE

Wpływ endozoochorii i warunków stresowych na zdolność kiełkowania nasion czeremchy amerykańskiej *Prunus serotina* Ehrh. poza naturalnym zasięgiem jej występowania

Jednym z najbardziej inwazyjnych gatunków w ekosystemach leśnych Europy jest czeremcha amerykańska *Prunus serotina* Ehrh., co wynika m.in. z wysokiej efektywności jej rozmnażania,

a także zoochorii. Czeremcha amerykańska tworzy bank nasion, które w korzystnych warunkach mogą zachować 2-3-letnią zdolność kiełkowania. Rośliny rodzaju *Prunus* mają głęboko uśpione nasiona, dlatego uważa się, że wymagają one stratyfikacji przed siewem.

Celem badań była analiza wschodów nasion czeremchy (z owocnią, bez owocni, stratyfikowanych i pochodzących z odchodów borsuka *Meles meles* L.) poddanych wpływowi różnych czynników stresowych: niska temperatura (–70°C, –18°C) i wysoka temperatura (+40°C). Przyjęto, że: I) nasiona czeremchy z odchodów borsuka będą charakteryzowały się najwyższą efektywnością wschodów, II) temperatura –18°C nie będzie hamowała efektywności wschodów czeremchy, III) owocnia będzie hamowała kiełkowanie, natomiast IV) efektywność kiełkowania nasion stratyfikowanych będzie zbliżona do efektywności kiełkowania nasion niestratyfikowanych, V) nasiona czeremchy zachowają 3-letnią zdolność wschodów.

Badania prowadzono od września 2019 r. do września 2022 r. w Ogrodzie Dendrologicznym Uniwersytetu Przyrodniczego w Poznaniu (52°25'38,8"N, 16°53'36,0"E). Nasiona czeremchy zebrano we wrześniu 2019 r. w Arboretum Leśnym w Zielonce (52°55'52,3"N, 17°10'83,3"E) z odchodów borsuka (2000 nasion z latryn) oraz z 3 stojących drzew (2000 diaspor). Żywotność zebranych nasion oceniano metodą rentgenowską. Ustalono średnią masę 1000 nasion. Uszkodzonych nasion i owoców nie wykorzystano w doświadczeniu.

Opis wariantów doświadczenia zawarto w tabeli 1. Nasiona wariantów: I, II, III oraz V-IX wysiano 18.10.2019 r. w kasety w podłoże torfowe, z wyjątkiem nasion stratyfikowanych (IV), które tego dnia wysiano w piasek rzeczny. Do jednego wariantu doświadczenia użyto 192 nasion. Od początku kwietnia do września 2020, 2021 i 2022 r. obserwowano momenty wschodów. Obserwacje prowadzono co 2 tygodnie.

Test χ^2 Pearsona wykorzystano do analizy prawdopodobieństwa kiełkowania w konkretnym wariancie. Obliczenia wykonano, biorąc pod uwagę wschodzące nasiona z każdego wariantu w stosunku do całkowitej liczby dostępnych nasion. Postawiono hipotezę, że wartości prawdopodobieństwa wschodów we wszystkich obiektach były takie same jak dla kontroli. Analizę przeprowadzono oddzielnie dla 2 lat badań (2020 i 2021), przyjmując wartości prawdopodobieństwa wynikające z liczby wschodów nasion w każdym roku.

Stwierdzono, że 97,33% zarodków nasion czeremchy pochodzących z odchodów borsuka było rozwiniętych prawidłowo (ryc. 1a, b, c), natomiast w przypadku nasion drzew stojących było to 99,33% (ryc. 1d, e, f). Średnia masa 1000 nasion z odchodów borsuka wynosiła 78,9 g, a z drzew stojących 86,3 g. Łączna liczba nasion, które skiełkowały w okresie od 4.05.2020 r. do 1.09.2021 r., wyniosła 652, co oznacza 34% wszystkich nasion. W 2022 r. nie pojawiła się ani jedna siewka czeremchy (tab. 1, 2).

Najwyższy udział wschodów w 2020 r. (statystycznie istotny) odnotowano w wariancie VII (borsuk) – 65,1% i III (bez owocni) – 53,13%, a najniższy (statystycznie istotny) w wariancie I (owoce –70°C) – 1,04 % i II (mróz owocnia –18°C) (ryc. 2). W 2021 r. największy udział (statystycznie istotny) wschodów odnotowano w wariancie IX (+40°C) – 13,02%, a najniższy w wariancie I (owoce –70°C) – 0% (ryc. 3; tab. 2).

Endozoochoria wpływa na zwiększenie inwazyjności czeremchy amerykańskiej. Owocnia hamowała wschody czeremchy amerykańskiej, ale tylko wtedy, kiedy nasiona były przemrożone. Przelegujące w glebie nasiona *P. serotina* zachowują 2-letnią zdolność kiełkowania. Wyniki badań wskazują, że nasiona czeremchy nie wymagają specjalnego przygotowania do siewu, poza usunięciem owocni hamującej kiełkowanie.