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Laboratory and Field Assessment of the Frost Resistance of Sosnowsky's Hogweed

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Abstract—The frost resistance of Sosnowsky's hogweed plants (*Heracleum sosnowsky* Manden.) has been evaluated under laboratory and field conditions. The death of seedlings and adult plants observed within a temperature range from –6 to –12°C indicates low frost tolerance of the species. A snow cover provides a stable soil temperature (no less than –3°C) at the depth of renewal bud location and, therefore, provides the sustainability of meristematic potential in cenopopulations of *H. sosnowsky.* A shifting of freezing temperature for a *H. sosnowsky* meristem from –12°C (autumn) to –5 to –7°C (spring) is probably caused by the lack of a true dormancy stage and by changes in the content of cryoprotectors. Plant seeds also demonstrate reduced frost resistance after stratification (overwintering) and increased tissue water content. Field studies were carried out with assistance of volunteers within the framework of the "Moroz" ("Frost") project arranged within the borders of the invasion habitat of the species in European Russia and based on the principles of citizen science. The results of the study show that the destruction of Sosnowsky's hogweed plants after the snow cover removal depends only on weather conditions. Thus, elimination of *H. sosnovsky* stands by freezing can be recommended only for regions where average long-term minimum temperatures in January and February do not exceed –25°C; this method can be relevant for territories where the use of chemical herbicides is limited or prohibited.

Keywords: Heracleum sosnowskyi, invasion, frost resistance, winter hardiness, citizen science, brush destruction

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INTRODUCTION

Competitive properties of Sosnowsky's hogweed (*Heracleum sosnowsky* Manden.) and its domestication resulted in formation of a large invasive habitat of this species, which covers several natural zones in Russia, from forest steppe in the south to forest tundra in the north (Satsyperova, 1984; Vinogradova et al., 2010, 2018; Pergl et al., 2016; Ozerova et al., 2017; Chadin et al., 2017; Ozerova and Krivosheina, 2018; Ebel' et al., 2018).

To date, no effective and environmentally safe methods to control Sosnowsky's hogweed have been developed (Caffrey and Madsen, 2001; Nielsen et al., 2005; *Ecology and Management*..., 2007; Reznik et al., 2008; Krivosheina, 2011; Yakimovich et al., 2013; Dalke et al., 2015). The analysis of measures aimed to map and eliminate undesirable stands of *H. sosnowsky* showed that the most frequent method to control this weed in Russia is chemical control (Dalke et al., 2018). However, use of pesticides and agrochemicals in populated areas is limited; moreover, herbicides cannot be used in water protection zones or in specially protected natural areas (Hygienic Requirements..., 2016).

The sustainability and expansion of *H. sosnowsky* cenopopulations are provided by numerous renewal buds located on the stem-root, as well as by an annually replenished soil seed bank (Dalke et al., 2015; Gudžinskas and Žalneravičius, 2018). Renewal buds of *H. sosnowsky*, *H. lehmannianum*, and *H. ponticum* die if the exit from dormancy occurs during a snowless period (Aleksandrova, 1971) or after severe conditions of the pre-winter period (Khantimer, 1974). This fact makes it possible to eliminate the stands of these plants (to reduce the meristematic potential) by changing a thermal regime of the soil after snow cover removal. In spite of a successful experiment intended to destroy

H. sosnowsky plants using the above-described method (Chadin et al., 2019), the following questions still remained open, such as What temperature is required to destroy *H. sosnowsky* plants by frost? How does the soil temperature change after the removal of the snow cover? What period provides the maximum effect of snow removal? In what regions (climatic zones) can this method of control be applied?

Therefore, the present study was aimed at determining the temperatures causing the death of *H. sosnowsky* plants and the dynamics of the soil temperature regime during the removal of the snow cover in the winter and spring periods, as well as at determining the geographic areas where the snow cover removal can be used to control this invasive species.

MATERIALS AND METHODS

The frost resistance of plants was determined using common field (registration of the number of overwintered specimens per unit area) and laboratory methods (Tumanov, 1979).

Laboratory studies were arranged at the Institute of Biology of the Komi Research Center (Ural Branch, Russian Academy of Sciences, Syktyvkar). Field studies were carried out with assistance of volunteers within the framework of the "Moroz" ("Frost") project (Project "Frost," 2019) on the basis of the principles of citizen science (Silvertown, 2009).

H. sosnowsky seedlings were grown from seeds collected in the autumn of 2018 from 30 plants growing in monospecies stands located in the outskirts of Syktyvkar (61.645961° N, 50.730800° E). The seeds were stratified at 5°C in a moist environment from February 7 to May 27, 2019. By the beginning of the experiment, seeds became fully ripe and formed seedlings with roots 3–4 cm long and cotyledonary leaves. Adult *H. sosnowsky* plants, whose age exceeded one year, were indiscriminately dug out at the end of May along a transect of monospecies stands. During harvesting, adult plants had already formed the first generation of leaves (1–3 leaves), and the stand height was about 50 cm.

The seedlings (100–120 plants) were placed on filter paper in Petri dishes and divided into the control and experimental groups, each consisting of 50–60 plants. Adult specimens (60–70 plants) were washed out to remove soil with the further removal of their aboveground parts (shoot and leaves). The remaining stem-roots were randomly divided into two equal groups (control and experiment). Separate experimental groups of plants (50–60 seedlings and 30–35 adult plants) were used to evaluate the effect of each of the studied temperature ranges.

The seedlings and adult plants were placed into KSh-240 (Russia) and Gram HF-462 (Denmark) refrigerators, respectively. The required temperature was set and maintained using a thermal relay made on the basis of an 8-channel microprocessor timer, a solid state KSA215AC8 relay (Cosmo, China), and a BM8036 thermostat (Master Kit, Russia). The relay operating mode was set within a certain temperature range (for example, from –4 to –5°C). Plants were frozen for 4 h in the chosen temperature range. The temperature in Petri dishes and near plant rhizomes was registered using autonomous TP-1 temperature data loggers (DS1921G-F50, Inzhenernye Tekhnologii, Russia) with the recording frequency of 1–5 min. At the end of the experiment, data obtained from several data loggers were averaged, and the median and minimum temperatures observed during plant exposure to frost were evaluated. In the course of the experiment, we tried to achieve a small (no more than 2°C) difference between the median and minimum values of the freezing temperature.

After the freezing, plants were placed under conditions favorable for their growth (wet environment, PAR 200 μmol/(m^2 s), air temperature 20°C) for 3–5 days. In the case of seedlings, the state of the root and cotyledonary leaves was registered. In adult plants, the growth of shoots and renewal buds was registered. Additionally, the number of surviving plants was calculated.

The water freezing point in the apical part of shoots and in cotyledonary leaves of seedlings was determined using a DSC-60 differential scanning calorimeter (Shimadzu, Japan). Apical parts of buds and seedlings (30–70 mg) were placed into a 90-mm^3 aluminum container and frozen from 0 to –20°C at a rate of 1°C/min. After a completion of measurements, samples were dried at 105°C to a constant weight.

The beginning of a water–ice phase transition was determined using the TA 60 ver. 1.33 software package for the DSC-60 calorimeter. The amount of water that underwent the phase transition was calculated by the area of the exothermic peak using the value of the specific heat of water crystallization (330 J/kg). The percentage of frozen water ($P_{\mathrm{frozenH_2O}}$, %) was calculated using the following formula:

$$P_{\mathrm{frozenH_2O}} = \left(\frac{V_{\mathrm{H_2O}} - V_{\mathrm{frozenH_2O}}}{V_{\mathrm{H_2O}}}\right) \times 100, \qquad (1)$$

where $V_{\mathrm{H_2O}}$ is the water content in the apex (mg) and $V_{\mathrm{frozenH_2O}}$ is the amount of frozen water (mg) determined by the DSC-60 calorimeter.

The water content in plant samples (WC, %) was determined using the following formula:

$$\mathrm{WC} = \left(\frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{FW}}\right) \times 100, \qquad (2)$$

where FW and DW represent the weight (mg) of a fresh and dried sample, respectively.

The field evaluation of the frost resistance of *H. sosnowsky* plants was carried out from September 2018 to May 2019 in 14 areas having typical *H. sosnowsky*

Fig. 1. Average long-term minimum air temperature in January for the period of 1970–2000 (dotted lines) in Russia (according to Fick and Hijmans, 2017). The locations of plots are indicated with asterisks.

stands and situated within the zone between 55–61 degrees north latitude and 30–49 degrees east longitude (Fig. 1). The experimental areas were located on the territory of Yaroslavl, Moscow, Kaluga, Leningrad, and Nizhny Novgorod oblasts, Komi Republic, Udmurt Republic, Republic of Tatarstan, and also Moscow and St. Petersburg sities.

In each region, control and experimental plots of the recommended size (4 × 4 m) were arranged. The plots were located within the borders of uniform cenopopulations of Sosnowsky's hogweed, so an equal density of plants on both control and experimental plots was considered at the beginning of the experiment. In the case of experimental plots, the snow cover was removed down to the ground surface, while control plots remained intact. The snow was removed manually and/or mechanically. In the Komi and Udmurt Republics and the Moscow region, several control and experimental plots were arranged; in other regions, only one control and one experimental plot were used. The working process is described in detail in individual working journals of the "Moroz" project participants (Project "Frost," 2018–2019).

Using a uniform protocol, the data on the state and the number of Sosnowsky's hogweed plants on eight plots located in five regions were collected in April–May 2019. The number of seedlings and sprouts was determined in a series of 5–12 sampling measurements arranged at two diagonals of a record plot using a frame with the area between 0.025 and 0.04 m^2. The number of plants was recalculated to plants/m^2. The number of adult plants, whose age exceeded one year, was calculated for the whole plot. The number of surviving plants on an experimental plot was expressed as the percentage of the same value measured for the control plot (100%).

The soil temperature at a depth of the renewal bud location (15 cm) was measured only at the plots located in Syktyvkar and Kazan. The measurements were carried out every 4 h in a continuous mode starting from the beginning of field experiments and up to their completion; for this purpose we used autonomous TP-1 temperature data loggers.

The snow depth and the air temperature were characterized using the data of hydrometeorological stations closest to the control and experimental plots. The data were obtained from open archives of the US National Centers for Environmental Information (U.S. National Environmental..., 2018, 2019), as well as from the website Reliable prognosis (Reliable Prognosis, 2004–2019) and the website Automated Information System for Processing of Regime Information (AISPRI), (2019).

The statistical description of data was performed on the basis of such parameters as the mean, standard deviation, and median. Results obtained for the control and experimental variants were compared using the nonparametric Kruskal–Wallis test. Statistical calculations were performed in the MS Excel package and the R environment (R Core Team, 2017). The

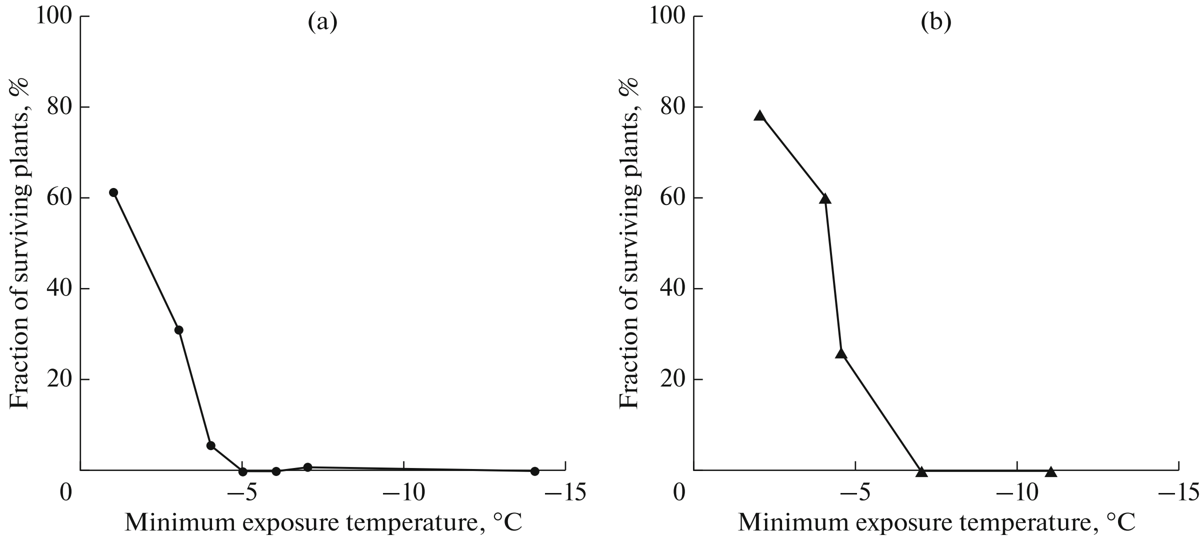

Fig. 2. Survival rate of (a) seedlings and (b) adult plants of Sosnowsky's hogweed after their freezing for 4 h at different temperatures. The effect of each temperature was determined by calculation of the number of surviving individuals in groups consisting of 50–60 or 30–35 plants (for seedlings and adult plants, respectively).

results are shown as the mean ± standard deviation. The data obtained in the field and laboratory experiments on the freezing of *H. sosnowsky* plants are available in the repository Zenodo (Zenodo, 2019).

RESULTS

According to our experiments, exposure to below-zero temperatures for 4 h negatively influenced the viability of *H. sosnowsky* seedlings and adult plants. Freezing at –1°C killed up to 40% of seedlings. Freezing at –2°C killed 50% of plants. Finally, freezing at –5°C or below caused the death of all seedlings (Fig. 2a).

In the case of adult plants frozen within the temperature range from –4 to –5°C, renewal buds remained viable in 30–60% of plants (Fig. 2b). A freezing for 4 h at –7°C or below killed all adult plants. In contrast to the control plants, no visible signs of the growth of shoots, petioles, and renewal buds were observed in dead plants the next day after their freezing. The longitudinal sections of stem-roots and shoots showed tissue darkening and destruction.

Prior the freezing procedure, the average water content in seedlings and renewal buds of adult plants was 73 and 86%, respectively. The results of a differential scanning calorimetry showed that the water–ice phase transition in these plant tissues occurred at the same temperature (–6.2 ± 0.4°C in seedlings and –6.2 ± 1.6°C in renewal buds). Exposure to this temperature froze 87% of free water contained in plant tissues, which resulted in the death of plants.

Regions in which the field studies were arranged significantly differed in the minimum temperatures of the coldest month (January). Changes in the temperature regime between regions were the most obvious in a longitudinal direction (from east to west, Fig. 1). The average annual data for 30 years of observations indicate that the minimum January temperature in the warmest (Moscow oblast) and coldest (Komi Republic) regions reaches –13 and –29°C, respectively.

A preliminary analysis of the temporal series of average daily air temperatures in November–March showed that the coldest period in the regions chosen for field studies came in the second half of January (Hogweed Plant, 2019). Therefore, the removal of the snow cover in January was carried out with allowance for a short-term local weather forecast. As a result, the snow cover on the experimental plots was completely removed prior the expected drop of the air temperature. The frequency of such treatments of experimental plots varied from one time (Komi Republic, the middle of January 2019) to eight times (Republic of Tatarstan, November 2018–March 2019) (Table 1).

During the period of expected frosts and snow removal (January–February), the average air temperature in regions located in the center of European Russia (Moscow oblast, Nizhny Novgorod oblast) was about –5°C, while the minimum temperature did not fall below –20°C (Table 1). Other regions located in the east (Republic of Tatarstan) and northeast (Udmurt Republic, Komi Republic) of the European part of Russia were characterized by significantly lower air temperatures: the average air temperature in January–February 2019 reached –10°C, while the minimum temperature varied from –25 to –31°C. In all studied regions, the average snow depth at the beginning of January reached 30 cm and doubled by the end of February (except for Moscow oblast).

Table 1. Air temperature, snow depth, and survival rate of Sosnowsky's hogweed plants at the places of field experiments

Region of observations	Plot	Weather station index	Number of snow cover removals	Air temperature for the period of Jan. 1, 2019–Feb. 28, 2019, °C		Snow depth, cm		Density of seedlings, plant/m^2		Density of adult plants, plant/m^2		Surviving plants, % of the control	
				average	minimum	Jan. 1, 2019	Feb. 28, 2019	control	experiment	control	experiment	seedlings	adult plants
Komi Republic	1	23804	2	−11.6	−31.0	35	71	346	72	49	9	21	18
Komi Republic	2	23804	1	−11.6	−31.0	35	71	453	213	19	11	47	58
Komi Republic	3	23804	2	−11.6	−31.0	35	71	175	5	14	7	3	50
Udmurt Republic	1	28411	3	−10.6	−27.0	22	60	133	0	13	3	0	23
Republic of Tatarstan	1	27595	8	−9.4	−25.0	33	64	799	236	3	1	30	33
Nizhny Novgorod oblast	1	27459	2	−6.8	−19.9	37	61	–	–	2	2	–	100
Moscow, Moscow oblast	1	27612	1	−4.1	−19.8	24	26	605	120	14	9	20	64
Moscow, Moscow oblast	2	27612	2	−4.1	−19.8	24	26	3469	1592	10	12	46	120

Air temperature and snow depth are shown according to the data of the corresponding service (U.S. National Environmental..., 2019). Plots where no snow cover removal was performed are indicated as "control" and "experiment" means plots where the snow cover was removed. A dash indicates that the number of plants was not calculated.

Field observations in spring (April–May 2019) showed that *H. sosnowsky* plants from the experimental plots demonstrated a significantly slower growth and leaf formation compared to the plants from the control plots (intact snow cover). The flowering of generative plants survived after the freezing started one week later than in the control (data obtained for the Komi Republic and Republic of Tatarstan).

The averaging of data from all regions included into the study showed that the snow cover removal followed by frosts resulted in a survival of ~24% of seedlings and 58% of adult plants (Table 1). In the case of regions with a warmer winter (Moscow, Moscow oblast, and Nizhny Novgorod oblast), the survival rate of adult plants reached 95%. In the case of more severe climatic conditions (Udmurt Republic), up to 100% of seedlings and 77% of adult plants were killed by frosts. On the majority of the studied plots, plants formed a dense canopy in June 2019 with a leaf area index exceeding 1.

A study of the thermal regime of the soil showed that, in the case of a stable snow cover exceeding 10 cm in depth, the soil temperature at a depth of 15 cm remains stable (no less than –1°C), in some cases falling to –3°C (Fig. 3). Under conditions of the Komi Republic, after a single snow cover removal performed at the end of January and followed by the air temperature drop to –31°C, the soil temperature at a depth of 15 cm fell to –6°C (Fig. 3). At the beginning of February 2019, the snow depth at this site exceeded 10 cm, so the soil temperature at a depth of 15 cm rose to –1°C and remained at this level until the snow melted at the beginning of April (Fig. 3).

After repeated (8 times) snow cover removal from the experimental plot located near Kazan, the snow depth at this plot did not exceed 10 cm. The only exception occurred in the second half of December 2018, when the snow depth reached 25 cm for a short time (Fig. 4). After the fifth snow removal performed in the second ten days of January 2019, the soil temperature at a depth of 15 cm reached –5°C. The further frosts down to –25°C (January 26, 2019) resulted in a sharp drop of soil temperature to –10.5°C (Fig. 4); however, the further increase in the air temperature and in the snow depth caused a rise of the soil temperature. In the middle of February 2019 and until the snow melting in spring, the soil temperature at a depth of 15 cm did not fall below –3°C.

Low frost resistance of *H. sosnowsky* plants under laboratory conditions was confirmed by the results of field observations. The removal of the snow cover in the case where the air temperature dropped to −20°C or below caused the freezing and killing of plants. To achieve this result, the stem-roots of *H. sosnowsky* should be frozen to −7°C under natural conditions, while seedlings should be frozen to at least −5°C.

In 2019, after a single snow cover removal performed in the coldest period (January) at the experi-

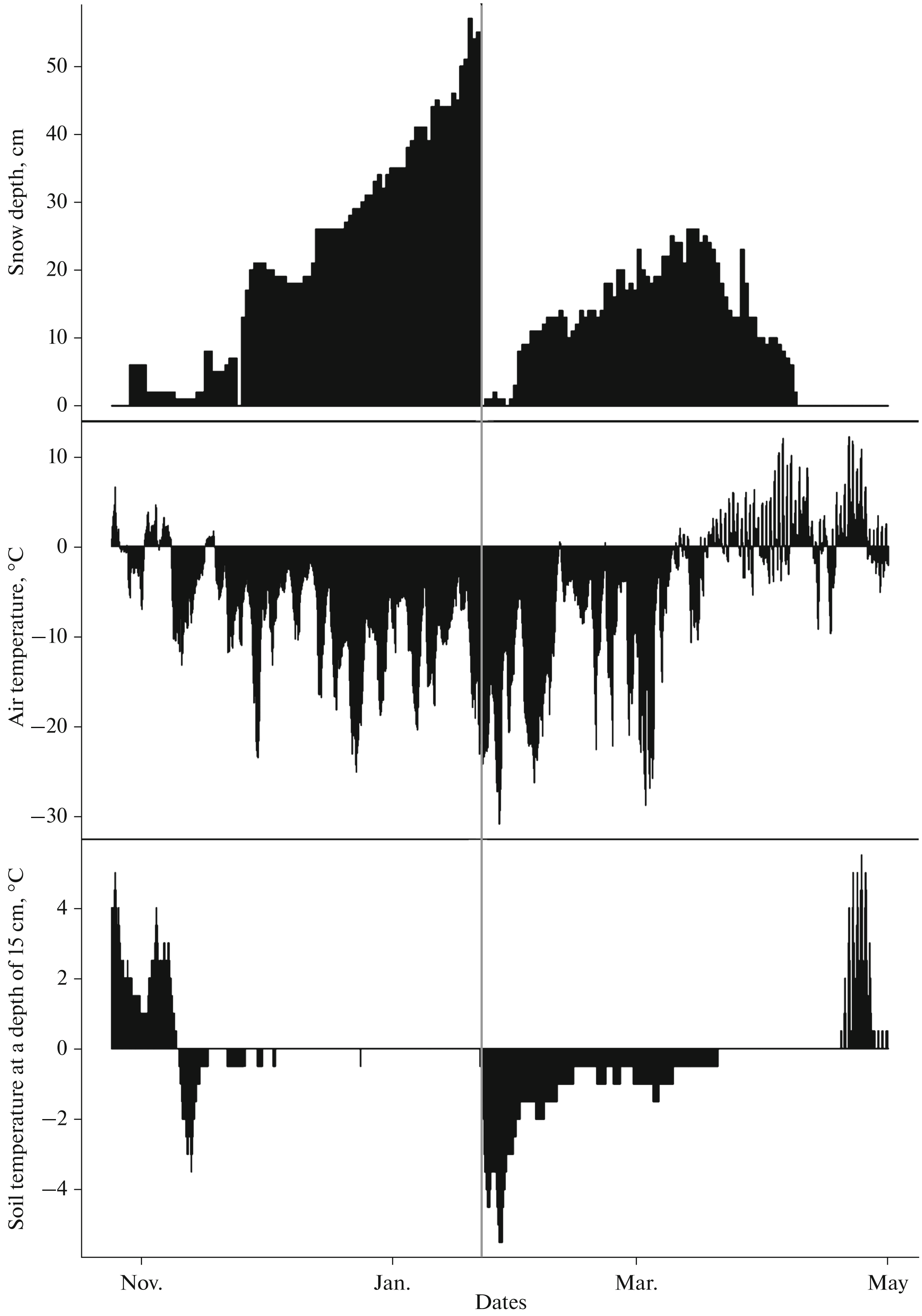

Fig. 3. Snow depth, air temperature, and soil temperature at a depth of 15 cm measured on the experimental plots before and after snow cover removal (Syktyvkar, Komi Republic). The date of the snow cover removal is indicated by a vertical line.

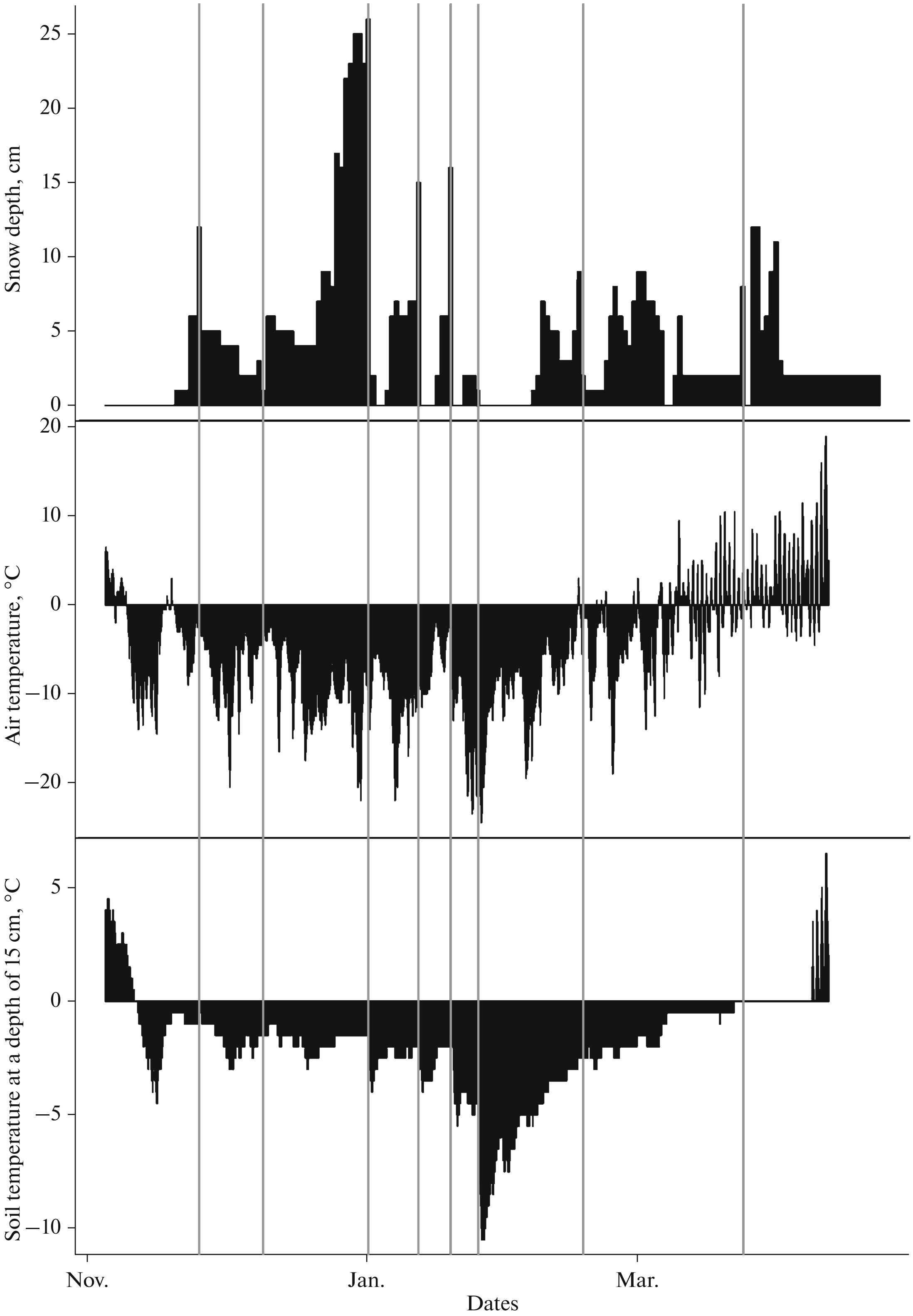

Fig. 4. Snow depth, air temperature, and soil temperature at a depth of 15 cm measured on the experimental plots before and after snow cover removal (Kazan, Republic of Tatarstan). The date of the snow cover removal is indicated by a vertical lines.

Table 2. Effect of exposure to below-zero temperatures during the dormancy period on the number of Sosnowsky's hogweed plants in the outskirts of Syktyvkar

Groups of plants in different years of observation	Plant density (median), plant/m^2		p*
	control	experiment	
Seedlings (first-year plants):			
May 12, 2018**	107	0	<0.0001
May 18–19, 2019	453	213	<0.0001
Adult plants (one-year-old plants or older):			
May 12, 2018**	9	2	0.0210
May 18–19, 2019	19	11	0.0002

* *p*-Value according to the Kruskal–Wallis test. ** Data obtained by Chadin et al. (2019) for the winter period of 2017–2018.

mental plot near Syktyvkar, only a short-term fall of soil temperature to −4 to −6°C was observed. As a result, only some seedlings and adult plants were killed by frost, and the stand of Sosnowsky's hogweed recovered during the vegetation period.

A complete elimination of *H. sosnowsky* plants was realized for the first time in the Komi Republic under conditions of an extremely cold March 2018 (Chadin et al., 2018). During this period, the air temperature dropped below −20°C at least seven times, so the soil temperature at a depth of 15 cm could have dropped below −10°C, since experimental plots were permanently maintained in a snow-free state from the beginning of March to the snow melting. In the course of the project implementation in 2019, this hypothesis was confirmed for the variant with snow cover removal multiple times (Republic of Tatarstan). A systematic snow removal during the whole winter period (8 times in total) with the subsequent air temperature drops to −20°C resulted in a drop of the soil temperature to the critical value (−10.5°C). However, the further rise in temperature and precipitation increased the soil temperature again. After a triple snow removal at the experimental plot in the Udmurt Republic and the subsequent air temperature drop to −29°C occurring at the end of January, all seedlings and 77% of adult *H. sosnowsky* plants were eliminated (Table 1).

The impact of weather conditions on the death of *H. sosnowsky* plants caused by soil freezing can be estimated by comparing experiments arranged in the outskirts of Syktyvkar in 2018 and 2019 (Table 2). After a snow removal in 2018, the number of seedlings of this species decreased to zero, while the number of adult plants decreased fivefold. In spring 2019, the number of plants growing on experimental plots decreased only by a factor of two. At the same time, the density of seedlings and adult plants in the Sosnowsky's hogweed stand in 2019 was ~200 and 11 plants/m^2, respectively.

In Syktyvkar, the average air temperature near the ground in January and February 2018 and 2019 (–10 to –12°C) was 2–7°C higher than the average annual values calculated for the base period of 1961–1990. The minimum air temperatures reached –31°C (Table 3). March 2018 was very cold, and the average air temperature dropped 5°C below the average long-term values. At the same time, the early spring 2019 was very warm, and the average monthly air temperature in March doubled the long-term average. The sum of below-zero temperatures for the period of January–March 2018 was –980°C; for the same period of 2019, it was –813°C.

The winters of 2017–2018 and 2018–2019 were rather warm with temperature anomalies; deviations from the average temperature of the base period (1961–1990) were +2.5 and +2.1°C, respectively (Climate Change, 2018b; Climate Change, 2019). However, 2018 and 2019 significantly differed in relation to the early spring weather. In March 2018, very cold conditions prevailed in the European part of Russia. This temperature anomaly averaged over the territory of Russia was −0.49°C, while in the Komi Republic this deviation reached −6°C (Climate Change, 2018a). As a result, after a snow cover removal in March 2018, followed by series of air temperature drops to −29°C, almost all *H. sosnowsky* plants were killed by frost.

According to the data of the Hydrometeorological Center of Russia (Hydrometeorological Center of Russia, 2019), spring 2019 was ranked fourth among the highest temperature values for the whole history of meteorological observations. On the almost the entire territory of the Russian Federation, the average seasonal air temperature exceeded the norm by 2°C or more. As a result of such positive temperature anomalies (insufficient sum of below-zero temperatures), soil temperatures reached critical values only for the half of the plants of the *H. sosnowsky* cenopopulation on average. In spring 2019, surviving seedlings and adult plants restored 100% of the percent cover on the experimental plots.

Table 3. Weather conditions in the outskirts of Syktyvkar in 2018 (numerator) and 2019 (denominator)

Parameter	January	February	March
Average annual air temperature for the base period (1961–1990)*	–17	–14	–6
Average air temperature, °C	–10 –12	–12 –10	–11 –3
Minimum air temperature	–25 –31	–30 –27	–29 –29
Sum of below-zero temperatures, °C	–301 –390	–345 –296	–335 –125
Average snow depth, cm	39 48	69 68	80 74

Parameters were calculated according to the data from the Syktyvkar weather station (BMO 23804; U.S. National Environmental..., 2018, 2019). *Parameters were calculated according to the data from another source (Automated Information System..., 2019).

DISCUSSION

Frost resistance is the ability of plants to survive at temperatures below 0°C without significant damage. Frost resistance is determined by the genotype, rate of temperature decrease, and conditions preceding the frost and influencing the character of ice formation. The development of frost resistance in plants is promoted by a complex of biochemical changes occurring during the hardening process (Tumanov, 1979; Klimov, 2001; Trunova, 2007). The main criterion for evaluation of frost resistance is calculation of the number of plants surviving after their exposure to hypothermia. The "low" and "high" grades of frost resistance are rather conditional. Fruit and berry plants, whose buds are damaged at temperatures between −1 and −7°C, are considered as crops with low frost resistance (Solov'eva, 1988; Knyazev et al., 2006; Krasova et al., 2009; Ozherel'eva, 2018). However, plants with low frost resistance also include winter wheat varieties which die at −19°C (Ivanisov and Ionova, 2016).

According to the data of differential scanning calorimetry, water contained in seeds and buds of Sosnowsky's hogweed plants included in the study froze at −12°C (Malyshev, 2018), killing the plants. Seedlings harvested under snow in March froze already at −7 to −8°C (Malyshev, 2019). A direct measurement of the survival rate of adult plants performed at the beginning of the vegetation season showed their 100% death at −7°C. Complete death of seedlings was observed at −5°C. The results of differential scanning calorimetry confirmed that the ice formation in the tissues of seedlings and buds of *H. sosnowsky* plants in spring occurred at −6°C.

In comparison with the frost resistance measurement in other plant species (Van Huystee et al., 1967; Senser and Beck, 1977; Alaudinova et al., 2007; Malyshev and Atoyan, 2018), a shift in the freezing temperature of Sosnowsky's hogweed buds was rather small (from −12 to −6°C).

Frost resistance reduction occurring during overwintering can be explained by changes in the content and composition of carbohydrates, which act as cryoprotectors stimulating the processes of growth, development, and adaptation of wintering buds and plant rhizomes (Kaurin et al., 1981; Maslova et al., 2007; Zhivet'ev et al., 2011; Pomortsev et al., 2013). Similar changes in frost resistance for different vegetation periods were also shown for cloudberry buds and rhizomes (Kaurin et al., 1981).

Temperatures lethal for *H. sosnowsky* seedlings and buds in spring are comparable with the temperatures of recurring spring frosts (−1 to −7°C). Fruit and berry plants, whose buds are damaged at these temperatures, are classified as plants with low frost resistance (Solov'eva, 1988; Knyazev et al., 2006; Krasova et al., 2009; Ozherel'eva, 2018). Low frost resistance of *H. sosnowsky* is determined by the lack of a deep dormancy period of its renewal buds and a high water content in these buds (5–7 mg H_2O/mg of dry weight) (Alexandrova, 1971; Dalke et al., 2018a; Malyshev, 2018, 2019). The same reason was shown in the case of leek plants, which died when the soil was frozen to −6°C (Palkin et al., 2017). Reduction of the water content in buds of woody and shrub plants to 1 mg/mg of dry mass provides their frost resistance down to −40°C (Alaudinova, 2007; Malyshev and Atoyan, 2018).

According to our data, snow cover provides a stable (no less than −3°C) soil temperature at a depth of location of *H. sosnowsky* renewal buds, which completely prevents their killing by frosts (Alexandrova, 1971) and provides a high winter resistance of the species (Satsyperova, 1984). In spite of low frost resistance of terminal and axillary *H. sosnowsky* buds located at a depth of 10–15 cm, snow cover provides their good protection from cryostress and also from mechanical injuries during the vegetation period (Dalke et al., 2015; Klima and Synowiec, 2016).

Frost resistance of *H. sosnowsky* seeds is associated with very low water content (0.5 mg/mg of dry weight on average) (Malyshev, 2018). After winter stratification, which is completed prior snow melting, the water content in seeds increases tenfold. During this period, removal of the snow cover in combination with a subsequent drop in air temperature may kill a significant part of the soil seed bank. Winter resistance of the species is dictated by the snow cover, which provides stable temperature conditions for the overwintering of plants and preservation of the meristematic potential of *H. sosnowsky* cenopulations.

The regions of performed studies significantly differed in their temperature conditions during the winter period. According to the average annual minimum temperature in January (Fick et al., 2017), the potential possibility to use freezing of *H. sosnowsky* plants after the removal of snow cover as a method for its control decreases in the following series: Komi Republic > Udmurt Republic > Republic of Tatarstan > Nizhny Novgorod oblast > Moscow and Moscow oblast.

In spite of the fact that the effect of below-zero temperatures on the viability of *H. sosnowsky* plants was confirmed under both laboratory and field conditions, it is difficult to achieve similar results on large areas. The main problem is a strong dependence of the efficiency of such a method on weather conditions. Moreover, a general warming in the spring and winter periods has been registered in the Northern Hemisphere over the last 40 years. For the period of 1976–2018, the linear trend of the average air temperature in Russia was +0.39°C/10 years for winter and +0.61°C/10 years for spring (Climat Change..., 2018a, 2018b). Snow-free winters able to kill plants wintering under snow cover were observed in Russia in the period of 1939–1979; since the late 1980s, positive extrema prevail (Kruglov, 2005). In recent years, only the spring season of 2018 was characterized by negative cold extrema, which made it possible to eliminate undesirable *H. sosnowsky* stands after a snow cover removal (Chadin et al., 2019).

Thus, the performed evaluation of *H. sosnowsky* resistance to below-zero temperatures under laboratory and field conditions demonstrated a low frost resistance of the species. The seedlings and adult plants of this species completely died at –5 and –7°C, respectively. On the basis of the results obtained, we can recommend freezing as a method to eliminate *H. sosnowsky* stands only for the areas where the average long-term minimum temperatures in January and February do not exceed –25°C. In such regions, this method can be relevant for territories with the limited or prohibited use of chemical herbicides.

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COMPLIANCE WITH ETHICAL STANDARDS

Conflicts of Interest

The authors declare that they have no conflict of interest.

Statement of the Welfare of Animals

This article does not contain any studies with humans or animals as the objects of studies.

REFERENCES

Alaudinova, E.V., Simkina, S.Yu., and Mironov, P.V., Seasonal changes in water content in meristematic tissues of *Picea obovata* L. and *Pinus sylvestris* L. buds and its distribution in cells, *Khvoinye Boreal'noi Zony*, 2007, vol. 24, nos. 4–5, pp. 487–491.

Aleksandrova, M.I., Some species of the hogweed plants in the mid-flight zone of the Komi ASSR, *Extended Abstract Cand. Sci. (Agric.) Dissertation*, Kirov, 1971.

Automated Information System for Processing of Regime Information (AISPRI), 2019. http://aisori.meteo.ru/ClimateR. Accessed July 11, 2019.

Caffrey, J.M. and Madsen, J.D., The management of giant hogweed in an Irish River catchment, *J. Aquat. Plant Manage.*, 2001, vol. 39, pp. 28–33.

Chadin, I.F., Dalke, I.V., and Malyshev, R.V., Evaluation of *Heracleum sosnowskyi* frost resistance after snow cover removal in early spring, *Russ. J. Biol. Invasions*, 2019, vol. 10, pp. 83–91.

Chadin, I., Dalke, I., Zakhozhiy, I., et al., Distribution of the invasive plant species *Heracleum sosnowskyi* Manden. in the Komi Republic (Russia), *PhytoKeys*, 2017, vol. 77, pp. 71–80. https://doi.org/10.3897/phytokeys.77.11186

Climate Change 2018, Spring: March–May, Overview of the State and Trends of Climate Change in Russia, Moscow, 2018a. http://climatechange.igce.ru/index.php?option=com_docman&task=doc_download&gid=242&Itemid=75&lang=ru. Accessed July 11, 2019.

Climate Change 2018, Winter: December 2017–February 2018, Overview of the State and Trends of Climate Change in Russia, Moscow, 2018b. http://climatechange.igce.ru/index.php?option=com_docman&task=doc_download&gid=226&Itemid=75&lang=ru. Accessed July 11, 2019.

Climate Change 2019, Winter: December 2018–February 2019, Overview of the State and Trends of Climate Change in Russia, Moscow, 2019. http://climatechange.igce.ru/index.php?option=com_docman&task=doc_download&gid=248&Itemid=75&lang=ru. Accessed July 11, 2019.

Dalke, I.V., Chadin, I.F., and Zakhozhii, I.G., Control of Sosnowskyi's hogweed (*Heracleum sosnowskyi* Manden.) invasion on the territory of the Russian Federation, *Russ. J. Biol. Invasions*, 2018a, vol. 9, pp. 331–334.

Dalke, I., Malyshev, R., and Maslova, S., Growth of *Heracleum sosnowskyi* Manden. plant in indoor conditions after end of vegetation period, *Zenodo*, 2018b. https://doi.org/10.5281/zenodo.1244757. Accessed July 11, 2019.

Dalke, I.V., Chadin, I.F., Zakhozhiy, I.G., Malyshev, R.V., Maslova, S.P., Tabalenkova, G.N., and Golovko, T.K., Traits of *Heracleum sosnowskyi* plants in monostand on invaded area, *PLoS One*, 2015, vol. 10, no. 11: e0142833. https://doi.org/10.1371/journal.pone.0142833

Ebel', A.L., Zykova, E.Yu., Mikhailova, S.I., Chernogrivov, P.N., and Ebel', T.V., Colonization and naturalization of the invasive species *Heracleum sosnowskyi* Manden. (Apiaceae) in Siberia, *Mater. IV Mezhd. nauch. konf. "Ekologiya i geografiya rastenii i rastitel'nykh soobshchestv," Yekaterinburg, 16–19 aprelya 2018 goda* (Proc. IV Int. Sci. Conf. "Ecology and Geography of Plants and Plant Communities," Yekaterinburg, April 16–19, 2018), Yekaterinburg, 2018, pp. 1065–1070.

Ecology and Management of Giant Hogweed (*Heracleum mantegazziannum*), Pyšek, P., Cock, M.J.W., Nentwig, W., and Ravn, H.P., Eds., Wallingford: CABI Publ., 2007.

Fick, S.E. and Hijmans, R.J., WorldClim 2: new 1-km spatial resolution climate surfaces for global land areas: new climate surfaces for global land areas, *Int. J. Climatol.*, 2017, vol. 37, no. 12, pp. 4302–4315. https://doi.org/10.1002/joc.5086

Gudžinskas, Z. and Žalneravičius, E., Seedling dynamics and population structure of invasive *Heracleum sosnowskyi* (Apiaceae) in Lithuania, *Ann. Bot. Fenn.*, 2018, vol. 55, pp. 309–320. https://doi.org/10.5735/085.055.0412

Hogweed Plant, 2019. http://proborshevik.ru/archives/4193. Accessed July 11, 2019.

Hydrometeorological Center of Russia. https://meteoinfo.ru/novosti/16117-vesna-2019-v-rossii-byla-ochen-teploj. Accessed, July 7, 2019.

Hygienic Requirements to Safety of Testing, Storage, Transportation, Sale, Application, Neutralization, and Utilization of Pesticides and Agrochemicals: SanPiN 1.2.2584-10. http://docs.cntd.ru/document/902204851. Accessed July 11, 2019.

Ivanisov, M.M. and Ionova, E.V., Frost tolerance of the varieties and lines of soft winter wheat, *Int. Res. J.*, 2016, no. 9 (51), part 3, pp. 110–113.

Kaurin, A., Junttila, O., and Hanson, J., Seasonal changes in frost hardiness in cloudberry (*Rubus chamaemorus*) in relation to carbohydrate content with special reference to sucrose, *Physiol. Plant.*, 1981, vol. 52, no. 2, pp. 310–314. https://doi.org/10.1111/j.1399-3054.1981.tb08512.x

Khantimer, I.S., *Sel'skokhozyaistvennoe osvoenie tundry* (Agricultural Development of Tundra), Leningrad, 1974.

Klima, K. and Synowiec, A., Field emergence and the long-term efficacy of control of *Heracleum sosnowskyi* plants of different ages in southern Poland, *Weed Res.*, 2016, vol. 56, no. 2, pp. 377–385. https://doi.org/10.1111/wre.12214

Klimov, S.V., Pathways for plant adaptation to low temperatures, *Usp. Sovrem. Biol.*, 2001, vol. 121, pp. 3–22.

Knyazev, S.D., Golyaeva, O.D., Nikolaev, A.V., and Panfilova, A.A., Features of winter blackcurrant plant damage, in *Plodovodstvo i yagodovodstvo Rossii* (Fruit and Berry Production in Russia), *Nauch. Tr. All-Russian Breed. Technol. Inst. Hortic. Nursery Manag.*, 2006, vol. 16, pp. 131–134.

Krasova, N.G., Ozherel'eva, Z.E., and Galasheva, A.M., Apple tree flower resistance to spring frosts, *Vestn. Orlov. Gos. Agrar. Univ.*, 2009, no. 6 (21), pp. 50–53.

Krivosheina, M.G., Insect pests of Sosnowsky hogweed (*Heracleum sosnowskyi*) in Moscow region and the prospects of their usage in biological control, *Russ. J. Biol. Invasions*, 2011, vol. 2, nos. 2–3: 99.

Kruglov, N.M., Direct freezing as a method of evaluating the frost resistance of fruit plants, *Mater. mezhd. nauchno-metod. konf. "Sostoyanie i perspektivy selektsii i sortorazvedeniya plodovykh kul'tur," Orel, 12–15 iyulya 2005 g.* (Proc. Int. Nauchno-Metod. Conf. "Status and Prospects of Breeding and Varieties of Fruit Cultures," Orel, July 12–15, 2005), Orel, 2005, pp. 359–363.

Malyshev, R.V., Freezing point of water, water content and proportion of freezing water in *Heracleum sosnowskyi* plants, *Zenodo*, 2018. https://doi.org/10.5281/zenodo.1475913. Accessed July 11, 2019.

Malyshev, R.V., Freezing point of water, water content and proportion of freezing water in *Heracleum sosnowskyi* seedlings with radicles, *Zenodo*, 2019. https://doi.org/10.5281/zenodo.2636095. Accessed July 11, 2019.

Malyshev, R.V. and Atoyan, M.S., Water content and temperature of water–ice phase transition in tree buds, *Vestn. Inst. Biol. Komi Nauch. Tsentra, Ural. Otd., Ross. Akad. Nauk*, 2018, no. 2 (204), pp. 2–7. https://doi.org/10.31140/j.vestnikib.2018.2(204).1

Maslova, S.P., Tabalenkova, G.N., Kurenkova, S.V., and Plyusnina, S.N., Seasonal changes in anatomical and morphological structure and the content of phytohormones and sugars in underground shoots of a long-rhizome perennial grass *Phalaroides arundinacea, Russ. J. Plant Physiol.*, 2007, vol. 54, pp. 491–497.

Nielsen, C., Ravn, H.P., Nentwig, W., and Wade, M., *The Giant Hogweed Best Practice Manual, Guidelines for the Management and Control of an Invasive Weed in Europe*, Hørsholm: Forest & Landscape, 2005.

Ozerova, N.A. and Krivosheina, M.G., Patterns of secondary range formation for *Heracleum sosnowskyi* and *H. mantegazzianum* on the territory of Russia, *Russ. J. Biol. Invasions*, 2018, vol. 9, pp. 155–162.

Ozerova, N.A., Shirokova, V.A., Krivosheina, M.G., and Petrosyan, V.G., The spatial distribution of Sosnowsky's hogweed (*Heracleum sosnowskyi*) in the valleys of big and medium rivers of the East European Plain (on materials of field studies 2008–2016), *Russ. J. Biol. Invasions*, 2017, vol. 8, pp. 327–346.

Ozherel'eva, Z.E, Accelerated evaluation of resistance to spring frosts of strawberry varieties, *Sovrem. Nauch. Issled. i Razrabotki*, 2018, vol. 3, no. 4 (21), pp. 303–305.

Palkin, Yu.F., Mokshonova, I.M., and Rachenko, M.A., Frost resistance of *Allium porrum* plants in the Eastern Siberia, *Vestn. Ross. S-khoz. Nauki*, 2017, no. 4, pp. 23–24.

Pergl, J., Sádlo, J., Petrusek, A., Laštůvka, Z., Musil, J., Perglová, I., Šanda, R., Šefrová, H., Šíma, J., Vohralík, V., and Pyšek, P., Black, Grey and Watch Lists of alien species in the Czech Republic based on environmental impacts and management strategy, *NeoBiota*, 2016, vol. 28, pp. 1–37. https://doi.org/10.3897/neobiota.28.4824

Pomortsev, A.V., Grabel'nykh, O.I., Dorofeev, N.V., Peshkova, A.A., and Voinikov, V.K., Relation between frost-resistance of winter grains, their respiration rate and water-soluble carbohydrates content in autumn–spring period, *J. Stress Physiol. Biochem.*, 2013, vol. 9, no. 4, pp. 115–121.

Project "Frost," 2018–2019. http://proborshevik.ru/moroz-2018-2019-prodolzhaetsya. Accessed July 11, 2019.

Project "Frost," 2019. http://proborshevik.ru/proekt-moroz. Accessed 11, 2019.

R Core Team R: A Language and Environment for Statistical Computing, Core Team, Vienna: R Foundation for Statistical Computing, 2017. https://www.R-project.org. Accessed July 11, 2019.

Reliable Prognosis 2004–2019. https://rp5.ru. Accessed July 11, 2019.

Reznik, S.Ya., Dolgovskaya, M.Yu., Zaitsev, V.F., David'yan, G.E., and Nentvig, V.O, On the possibility of the use of the weevil *Nastus fausti* Reitter (Coleoptera, Curculionidae, Entiminae, Nastini) for biological control of invasive species of giant hogweeds (*Heracleum* spp.), *Entomol. Obozr.*, 2008, vol. 87, no. 3, pp. 489–502.

Satsyperova, I.F., *Borshcheviki flory SSSR—novye kormovye rasteniya* (Hogweed Species of the USSR Flora—New Fodder Plants), Leningrad: Nauka, 1984.

Senser, M. and Beck, E., On the mechanisms of frost injury and frost hardening of spruce chloroplasts, *Planta*, 1977, vol. 137, no. 3, pp. 195–201. https://doi.org/10.1007/BF00388150

Silvertown, J., A new dawn for citizen science, *Trends Ecol. Evol.*, 2009, vol. 24, no. 9, pp. 467–471. https://doi.org/10.1016/j.tree.2009.03.017

Solov'eva, M.A., *Atlas povrezhdenii plodovykh i yagodnykh kul'tur morozami* (Atlas of Fruit and Berry Plants Damage by Frost), Kiev, 1988.

Trunova, T.I., *Rastenie i nizkotemperaturnyi stress. 64-e Timiryazevskoe chtenie* (Plant and Low Temperature Stress, the 64th Timiryazev Lecture), Moscow: Nauka, 2007.

Tumanov, I.I., *Fiziologiya zakalivaniya i morozostoikosti rastenii* (Physiology of Hardening and Frost Resistance of Plants), Moscow: Nauka, 1979.

U.S. National Environmental Information Center Archive, 2018. https://ftp.ncdc.noaa.gov/pub/data/gsod/2018. Accessed July 11, 2019.

U.S. National Environmental Information Center Archive, 2019. https://ftp.ncdc.noaa.gov/pub/data/gsod/2019. Accessed July 11, 2019.

Van Huystee, R.B., Weiser, C.J., and Li, P.H., Cold acclimation in *Cornus stolonifera* under natural and controlled photoperiod and temperature, *Bot. Gaz.*, 1967, vol. 128, nos. 3–4, pp. 200–205. https://www.jstor.org/stable/2472925

Vinogradova, Yu.K., Mayorov, S.R., and Khorun, L.V., *Chernaya kniga flory Srednei Rossii: chuzherodnye vidy rastenii v ekosistemakh Srednei Rossii* (Black Book of Flora of the Central Russia: Alien Plant Species in the Ecosystems of Central Russia), Moscow: GEOS, 2010.

Vinogradova, Y., Pergl, J., Essl, F., Hejda, M., van Kleunen, M., and Pyšek, P., Invasive alien plants of Russia: insights from regional inventories, *Biol. Invasions*, 2018, vol. 20, no. 8, pp. 1931–1943. https://doi.org/10.1007/s10530-018-1686-3

Yakimovich, E.A., Yasyuchenya, O.A., and Ivashkevich, A.A., *Metodicheskie rekomendatsii po primeneniyu gerbitsidov dlya bor'by s borshchevikom Sosnovskogo* (Methodical Recommendations for the Use of Herbicides to Control Sosnowsky's Hogweed (*Heracleum sosnowskyi*) Plants Growth), Minsk: Inst. Zashch. Rast., 2013.

Zhivet'ev, M.A., Graskova, I.A., Pomortsev, A.V., and Voinikov, V.K., Water and carbohydrate content at leaves of plants used in medicine during vegetation, *J. Stress Physiol. Biochem.*, 2011, vol. 7, no. 4, pp. 69–79.

Zenodo. https://doi.org/10.5281/zenodo.3368583. Accessed July 11, 2019.

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