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HIGHER AQUATIC PLANTS

Fruit Germination of Some Representatives of the Family *Sparganiaceae* Rudolphi under Laboratory Conditions

E. A. Belyakov and A. G. Lapirov

Institute for Biology of Inland Waters, Russian Academy of Sciences, Borok, Yaroslavl oblast, Nekouzskii raion, 152742 Russia

e-mail: eugenybeliakov@yandex.ru

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Abstract—This paper considers fruit germination patterns in representatives of the family *Sparganiaceae*, which are most widespread in the European part of Russia: *Sparganium emersum* Rehm. (subgenus *Xanthosparganium*), *Sparganium erectum* L., and *Sparganium microcarum* (Neum.) Raunk. (subgenus *Melanosparganium*). It is shown that, after dissemination, fruits of all studied species do not germinate immediately, either intact or after different presowing treatment (scarification). It is found that under laboratory conditions a long period of moist cold stratification positively affects the germination ability of *S. emersum* and *S. microcarpum* fruits, as opposed to fruits of *S. erectum*. In all other experiments, fruits of *S. microscarpum* do not germinate or germinate sporadically. Fruits of *S. erectum* do not germinate, regardless of the different conditions of storage and presowing treatment. Long-term dry storage of *S. emersum* fruits under room conditions leads to a decrease in laboratory germination ability, and only the subsequent stratification of the fruit makes it possible to significantly increase this characteristic.

Keywords: *Sparganium*, germination of fruits, lag time, laboratory percentage of fruit germination, germination period

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INTRODUCTION

Family Sparganiaceae Rudolphi includes 1 genus *Sparganium* L. and ~20 species spread mainly in the temperate zone of the northern hemisphere, as well as in Australia and New Zealand [2].

To perform the study, species common for European Russia were selected: *Sparganium emersum* Rehm. (branched bur reed) (subgenus *Xanthosparganium*), *S. erectum* L. (unbranched bur reed), and *S. microcarpium* (Neum.) Raunk. (bur reed) (subgenus *Melanosparganium* [4]. *Sparganium emersum* grows in shallow waters and banks of rivers, lakes, reservoirs, marshes, and ponds, as well as in ditches, streams, and cuvettes with stagnant and rapidly flowing waters; *S. erecrum* occurs at swamped banks of rivers, oxbows, lakes, reservoirs, and in sedgy marshes with stagnant or weakly flowing water at a depth of 30–60 (100) cm; and *S. microcarpum* grows in the same habitats as the former species, preferring apparently cool waters [4].

The inflorescence of *S. emersum* is frondose–frondulose–bracteose–ebracteose organized as a raceme with floral units–axillary capitate inflorescences from unisexual flowers [3]. The inflorescences of *S. microcarpum* and *S. erectum* are frondose–bracteose–ebractiose, also organized as raceme. The female and male capitate inflorescences of all three species consist of numerous tightly adjoining unisexual flowers located according to a spiral pattern. The number of female flowers in a capitate inflorescence is usually equal to the number of the later formed fruit. The maturation of compound fruits takes place at the end of August.

The authors consider, like I.G. Zubkova and L.K. Shabes [2], the fruit of bur reed to be the dry upper pseudomorphous stone-fruit, understanding this term as an apocarpous or coenocarpous fruit, in the type monospermous, with a drastically pronounced differentiation of the pericarp. Fruits of bur reeds are characterized by relatively powerful endocarp and mesocarp is represented by loosely located parenchymal cells; exocarp is dense with a mechanical layer of sclerified hypoderma [2]. Seeds are fitted with a well developed powdery endosperm, within which there is a small, baculate, weakly differentiated embryo [1]. The embryo is achlorophyllous; its cells contain starch, oil, and protein. Perisperm is one-layered [2].

Data on the germination of fruits of bur reed are scanty, not numerous, and contradictory [9, 16]. In recent years, the germination of fruits of *S. emersum* in the framework of study of specific features of distribu-

tion of this species by different agents (birds, fish, and water) is investigated by Dutch researchers [11–14].

The purpose of our work is to study the specific features of germination of fruits of *S. emersum*, *S. microcarpum*, and *S. erectum* under laboratory conditions depending on different conditions of storage, presowing treatment, and conditions of germination.

MATERIAL AND METHODS

Deciduous fruits of *S. emersum* (population VYa_1) and *S. microcarpum* (population VYa_2) were collected on August 20, 2010, at a dried site of the Velikaya River opposite the village of Obraztsovo (Yaroslavl raion, Yaroslavl oblast) and *S. erectum* (population SYa) on August 18, 2010, in the Sutka River (Nekouzskii raion, Yaroslavl oblast). In addition, ripe fruits of *S. erectum* (population IA) were collected on August 24, 2010, in the lake in the vicinity of the village of Kurchenko (Narimanovskii raion, Astrakhan oblast) and on October 16, 2012, in the Volgograd Reservoir in the estuary of Bay Eruslan (population VV); then they were separated from plants in the laboratory. Conditions, dates of storage, and setting seeds for germination, as well the pattern of their presowing treatment, are presented in Table 1. All variants of experiments were performed in five replications (50 fruits in each). Fruits were placed into a Petri dish on filtered paper wetted by settled tap water. To disturb the integuments of fruits (scarification), emery paper was used, as were concentrated HCl and NaOH. After a short period (during 0.5 min) of impact of the latter, fruits were washed in cold running water for 5 min. They were stratified in a refrigerator at a temperature of 3 ± 2°C and germinated in a luminostat at illumination of 1868 ± 126.2 lux (photoperiod 16/8) and a temperature of 18.2 ± 0.9°C (at night) to 28.7 ± 2.9°C (in the day time).

Fruits were germinated according to rules for work [5]. The following indices were used: lag-time (*L*)—time (day) between the beginning of the experiment and beginning of germination; final germination (*G*)—percentage of germinated seeds at the end of the experiment [15] or "laboratory percentage of fruit germination" [6, 7]; and germination period (*P*)—time (day) of germination of seeds [15].

RESULTS

Fresh fruits (Table 1, variants 1–3) of the studied species of bur reeds after using different methods of presowing treatment (scarification) do not germinate, like intact seeds (Table 1, variant 4a; Table 2). A long period of moist cold stratification (variant 4b) positively affected germination of fruits of *S. emersum*; at a short lag-time, a sufficiently high laboratory percentage of fruit germination was obtained (Table 2). In fruits that did not germinate in variant 4b, although they were stratified for 2 months (variant 4c), a lower laboratory percentage of fruit germination was recorded (Table 2). A drastic decrease in this index was caused by prolonged dry storage of fruits of *S. emersum* under room conditions (Table 1, variant 5a; Table 2). Subsequent stratification of fruits that did not germinate in variant 5a made it possible to considerably increase the laboratory percentage of fruit germination (Table 1, variant 5b; Table 2). Another result was obtained after a long storage (2 years 1 month) of dry fruits of *S. emersum* in the refrigerator (Table 1, variant 6a; Table 2). Despite the subsequent stratification of fruits that did not germinate in variant 6a (Table 1, variant 6b; Table 2), no increase in germination was obtained.

In *S. microcarpum*, only after prolonged cold stratification (Table 1, variant 4b), a relatively high result was obtained: *L*, 4.2 ± 3.3 days; *G*, $16.6 \pm 9.7\%$; *P*, 4.6 ± 4.4 days. In the remaining variants of the experiment, fruits of *S. microcapum* germinated singly or did not germinate at all. Regardless of conditions and dates of storage and pattern of presowing treatment, a similar picture was observed in all variants of experiments with fruits of *S. erectum* collected in Yaroslavl, Astrakhan, and Volgograd oblasts.

DISCUSSION

In nature the authors found no germination of fruits of bur reeds. All attempts to restore the populations of these plants using fruits were unsuccessful, which is explained by some researchers by their slow and irregular germination (Martin and Uder, 1939, cited from [16]). The latter is related to specific features of the pericarp structure of the fruit [9, 16] (dense envelopes), which is considered the main factor inhibiting germination. A group of Japanese researchers [8] notes the appearance of sprouts of *S. erectum* var. *erectum* in spring and autumn with a wide annual fluctuation, although seedlings of *S. erectum* var. *microcarpum* were not found by them in natural habitats [8].

Under laboratory conditions it was shown by the authors that fresh fruits of *S. emersum*, *S. microcarpum*, and *S. erectum*, both preliminarily treated by different methods (treatment by emery paper; impact by concentrated HCl and NaOH) and intact, do not germinate (Table 2). The same was noted in paper [8]; fruits of *S. erectum* var. *erectum* and *S. erectum* var. *macrocarpum* do not germinate immediately after dissemination. Steinbauer and Neal [16, p. 36] noted that for some species of *Sparganium* (*S. minimum* Fries, *S. chlorocarpum* Rydb, *S. americanum* Nutt, *S. fluctuans* (Morong) Robinson, and *S. eurycarpum* Engelm.) "submergence of seeds into concentrated sulfuric acid for several minutes was partially successful with respect to the stimulation of germination," unlike their treatment with aqueous solutions of thiourea and ethylene chlorohydrin of different concentrations.

A positive impact of scarification on preliminarily stratified (6 months in a moist state) fruits of *S. emersum* was found by Pollux et al. [14]. In their experi-

Table 1. Pattern of presowing treatment of fruits of *Sparganium emersum*, *S. microcarpum*, and *S. erectum* and conditions under which the experiments were performed

Experimental variant	Population	Fruit characteristics	Presowing treatment of fruits	Date of placing for germination	Duration of germination, days
1	VYa_1	Freshly collected	Scarification with the use of emery paper	26.VIII 2010	28
	VYa_2				
	IA				
2	VYa_1	″	$HCl_{(concentr)}$	″	28
	VYa_2				
	IA				
3	VYa_1	″	$NaOH_{(concentr)}$	″	28
	VYa_2				
	IA				
4a	VYa_1	″	Absent	″	28
	VYa_2				
	IA				
4b	VYa_1	Nongerminated in variant 4a	Stratification on moist filtered paper during 2 years and 1 month	22.X 2012	32
	VYa_2				
	IA				
4c	VYa_1	Nongerminated in variant 4b	Stratification on moist filtered paper in 54 days	14.I 2013	32
	VYa_2				
	IA				
5a	VYa_1	Freshly collected	Storage under room conditions in paper packets over 2 years and 2 months	22.X 2012	32
	VYa_2				
	SYa				
5b	VYa_1	Nongerminated in variant 5a	Stratification on moist filtered paper in 54 days	14.I 2013	32
	VYa_2				
	SYa				
6a	VYa_1	Freshly collected	Stratification in paper packets over 2 years and 1 month	22.X 2012	32
	VYa_2				
	SYa				
6b	VYa_1	Nongerminated in variant 6a	Stratification on moist filtered paper in 54 days	14.I 2013	32
	VYa_2				
	SYa				
7	VV	Freshly collected	Storage under room conditions in paper packets for 28 days	14.XI 2012	32
8	VV		Storage under room conditions in paper packets for 28 days, then stratification in paper packets for 60 days	14.I 2013	32

Table 2. Main indices of germination of fruits of *Sparganium emersum*

Index	Variants of experiments						
	4a	4b	4c	5a	5b	6a	6b
L, days	0	5.8 ± 1.0	4.6 ± 0.4	11.4 ± 9.1	6.6 ± 0.8	10.2 ± 4.4	4.8 ± 1.8
G, %	0	78.4 ± 13.3	21.7 ± 17.4	1.2 ± 0.9	48.9 ± 8.0	2.0 ± 0.8	5.7 ± 2.1
P, days	0	15.2 ± 2.5	16.0 ± 10.0	0.6 ± 0.5	20.6 ± 2.6	1.6 ± 1.4	3.6 ± 1.9

Average values and their errors are listed.

ments, the aggregate percentage of germination of these fruits (~94) for 60 days of observations was considerably higher than in the control group (~46%, stratified nonscarified fruits). In addition, an increase in the proportion of germinated fruits of *S. emersum* by 12.6% (compared to the control) was recorded after they were passed through the gastrointestinal tract of fish [11, 12]. The disturbance of the rest of fruits a year after the removal of pericarp was also recorded in *S. erectum* var. *erectum* and *S. erectum* var. *macrocarpum* [8].

In the experiments of the authors, the scarification of fruits of *S. emersum*, *S. microcarpum*, and *S. erectum* immediately after dissemination caused no germination, apparently because of the more powerful structure of the fruit pericarp and seed integument, which are considerably affected by geographic and ecological conditions of growing.

According to the opinion of Pollux et al. [12], the disturbance of the rest of fruits of *S. emersum* requires a prolonged moist cold stratification, which is supported by studies of the authors (Table 1, variant 4b; Table 2). For instance, after prolonged dry storage of fruits of *S. emersum* under room conditions and refrigerator, the main indices of germination were low (Table 1, variants 5a and 6a, Table 2), unlike subsequent moist cold stratification of nongerminated fruit for 2 months, which exerted a positive impact (Table 1, variants 5b and 6b; Table 2). In the former case, fruits of *S. emersum* apparently lapse into a state of secondary rest that was far deeper than the initial [6]. Close results were obtained by Muenscher [9], who performed experiments with five species of bur reeds (*S. angustifolium*, *S. americanum*, *S. chlorocarpum*, *S. fluctuans*, and *S. eurycarpum*),

According to the opinion of the authors, the low germination of fruits of *S.microcarpum* obtained in experiments after prolonged cold stratification (Table, variant 4b) and the absence of germination of fruits of *S. erectum* in all variants of the experiment are related to the structure of pericarp. In both species it consists of multilayer exocarp with a ring of mechanical tissue with uniformly thickened and lignified integuments underlying the epiderm and a powerful endocarp [2]. In addition, the integument of the fruits of *S. erectum* is represented by spongy tissue and a lipid membrane that allows them to swim [10] but hinders the penetration of water to the embryo and, hence, subsequent germination.

CONCLUSIONS

Immediately after dissemination, fruits of three species of bur reeds (*Sparganium emersum*, *Sparganium microcarpum*, and *Sparganium erectum*) are in a state of organic rest, since they do not germinate either preliminarily treated by different methods (emery paper and impact of concentrated HCl and NaOH) or intact. For the disturbance of rest of fruits of *S. emersum* and *S. microcarpum*, prolonged moist cold stratification turned out to be the most efficient. Fruits of *Sparganium microcarpum* in all remaining variants of the experiment did not germinate. A complete absence of germination was recorded in fruits of *S. erectum*. Low indices of germination of *S. microcarpum* and the inability of fruits of *S. erectum* to germinate are related to the structure of pericarp and seed integument.

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