Seed germination in *Solidago ×niederederi* (Asteraceae) and its parental species after two different fruit storage periods

Artur Pliszko[1],* & Kinga Kostrakiewicz-Gierałt[2]

[1]Department of Taxonomy, Phytogeography and Paleobotany, Institute of Botany, Jagiellonian University, Kopernika 31, 31-501 Kraków, Poland
[2]Department of Plant Ecology, Institute of Botany, Jagiellonian University, Lubicz 46, 31-512 Kraków, Poland
*corresponding author (e-mail: artur.pliszko@uj.edu.pl)

Abstract. In this paper, we present preliminary results from the study on the effect of fruit storage period on seed germination in *Solidago ×niederederi* and its parental species (*S. canadensis* and *S. virgaurea*). We aimed to test the hypothesis that a long fruit storage period (*i*) reduces the final percentage and speed of seed germination, as well as (*ii*) increases the number of dead seeds among non-germinated ones. We used fruit samples collected in Kraków, southern Poland, in 2013 and 2016, designating two different fruit storage periods of 3.5 years and 0.5 years, under dry, room temperature conditions. Seeds of all the observed species presented remarkably higher final percentage of germination after the short storage period than after the long one. The seeds subjected to the short fruit storage period achieved significantly higher values of Timson's index and coefficient of velocity, as well as significantly lower values of the mean germination time than samples subjected to the long fruit storage period. After the long storage period, a significantly higher number of dead seeds among non-germinated seeds was found in *S. ×niederederi* and *S. virgaurea*. The performed studies showing that the long fruit storage period led to low contribution of germinated seeds in *S. ×niederederi* and its parental species suggested that the longtime seed desiccation had a negative impact on final germination. Considering that the hybrid can be potentially cultivated for scientific, medicinal or ornamental purposes, the suggested fruit storage period, under dry, room temperature conditions, should be shorter than three years. However, we realize that the response to long storage may vary in different populations and, therefore, further investigations are needed.

Key words: achenes, hybrid, seed germination test, *Solidago*, Timson's index

1. Introduction

Hybridization between alien and native plant species is understood as a source of new alien taxa (Pyšek *et al.* 2004) and poses a threat to native biodiversity, especially when spontaneous hybrids show effective competition and introgression (Daehler & Carino 2001; Stace *et al.* 2015). To become an invasive plant, as many other established alien plants, hybrids between alien and native taxa must produce numerous and reproductive offspring able to spread over a large area and cover considerable distances from parental individuals (Pyšek *et al.* 2004). Testing seed germination in hybrids between alien and native congeners is one of the most important first steps in evaluating their potential for naturalization and invasion by seed spreading. However, in many plant hybrids, the generative reproduction is usually limited by reduced pollen viability (Stace *et al.* 2015).

Solidago ×niederederi Khek (Asteraceae), a natural hybrid between the North American *S. canadensis* L. s. l. and European *S. virgaurea* L. s. str., has been reported from several countries in Europe (Nilsson 1976; Pliszko 2013, 2015; Pliszko & Zalewska-Gałosz 2016). However, its establishment and invasive potential are poorly recognized. It usually occurs in anthropogenic habitats such as abandoned fields, disused sand and limestone quarries, roadside slopes, railway embankments and tree plantations, where *S. canadensis* and *S. virgaurea* are found in close proximity (Pliszko 2013; Stace *et al.* 2015; Gudžinskas & Petrulaitis 2016; Pagitz 2016; Pliszko & Zając 2016). It is able to reproduce generatively by wind-dispersed achenes; however, its fruit set is limited due to reduced pollen viability (Migdałek *et al.* 2014; Karpavičienė & Radušienė 2016). Nevertheless, establishment and invasion of *S. ×niederederi* seem to be more dependent on achene production rather than the

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rhizome spreading, since there is no evidence that the hybrid (at least the F_1 generation) forms long rhizomes typical of *S. canadensis* (Pliszko & Kostrakiewicz-Gierałt 2017). In the study conducted by Pagitz (2016), the hybrid showed good fertility with seed germination rate of 31%. However, Pliszko & Kostrakiewicz-Gierałt (2017) evidenced that it can achieve a much higher seed germination rate (91%).

It is commonly known that storage conditions can strongly influence seed germination (Gioria & Pyšek 2017). Seed moisture, temperature, and storage periods are among main factors influencing the relationship between seed viability loss during storage and genetic damage accumulation in surviving seeds. Moreover, drying and long-term storage may lead to considerable reduction in germination or even to death of seeds (Pradham & Badola 2012 and the literature cited therein). The influence of fruit storage period on seed germination in *S. ×niederederi* has not been studied so far. Considering that the hybrid can be potentially cultivated for scientific, medicinal or ornamental purposes, similarly to its parental species (e.g., Werner *et al*. 1980; Kołodziej 2008; Mishra *et al*. 2010), it is important to recognize the effect of fruit storage period on its seed germination capacity. In this study, therefore, we aimed to test the hypothesis that the long fruit storage period (*i*) reduces the final percentage and speed of seed germination, as well as (*ii*) increases the number of dead seeds among non-germinated seeds in *S. ×niederederi* and its parental species.

2. Material and methods

2.1. Fruit sampling and storage

Achenes of *Solidago canadensis*, *S. ×niederederi*, and *S. virgaurea* were collected from natural populations (one population per species) in the area of a disused limestone quarry in Kraków (Mydlniki), southern Poland (GPS coordinates: 50°05′N/19°50′E; altitude: 225-243 m a.s.l.), on October 6, 2013 and October 9,

2016. For each species, 10 panicles with mature fruits were randomly sampled and put inside paper bags. Paper bags with panicles were taken to the laboratory and left in a dry, airy place, at room temperature for 5 days. Next, the panicles were threshed manually inside paper bags to obtain a mixture of achenes for further investigation. For each species, sets of 50 achenes in four replications were randomly selected from the samples visually identified as well-developed fruits (with no deformation and damage), using a PZO Warszawa 18890 stereoscopic microscope. Up to a day of sowing (March 31, 2017), achenes were stored in plastic zip lock bags, in a dry, dark room, at the temperature of +25 °C(±5 °C). To be more precise, achenes collected in 2013 were stored for about 3.5 years (long storage period), whereas those collected in 2016 were kept for about 0.5 years (short storage period), under the above-mentioned conditions. The presented storage periods were arbitrarily designated because, in this preliminary study, we wanted to compare two different fruit storage periods in relation to previously published data (Pliszko & Kostrakiewicz-Gierałt 2017), and we did not have any other suitable material for sowing.

2.2. Seed germination test

Sets of 50 achenes in four replications were mixed with 50 g of dry sand as a substrate and placed in 9 cm diameter polystyrene Petri dishes. Next, the substrate, which was distributed uniformly in the dishes at a thickness of about 0.3 cm, was wetted with 5 ml of sterile water, reaching the pH value of ±7.0. All the samples in Petri dishes were kept at room temperature (+25 °C), under 12 h photoperiod (630 lx). The substrate was complemented with 1 ml of sterile water every other day. The samples were checked at one-day intervals counting the germinated seeds up to 21 days from the day of sowing. The seed was determined as germinated when the pericarp of the achene was broken showing radicle, hypocotyl or cotyledons. On the final day of the germination test, non-germinated achenes were visually examined under a PZO Warszawa 18890 stereoscopic

Table 1. Characteristics of seed germination parameters used in the study

Parameter	Abbreviation	Formula for calculation	Description
Timson's index	TI	Σn	n – cumulative daily germination percentage for each day of the test
mean germination time	MGT	$\Sigma(n_i \times d_i)/N$	n_i – number of seeds germinated at day d_i, N – total number of seeds germinated in the test
coefficient of velocity	CV	$100(A_1+A_2+\ldots+A_x)/$ $(A_1 T_1+A_2 T_2+\ldots+A_x T_x)$	$A_1+A_2+\ldots+A_x$ – number of seeds germinated on the first, second and final day of seedling appearance, T_1, T_2 and T_x – number of days between sowing and first, second and final day of seedling recording

microscope to confirm their potential viability. Empty, decayed and covered with fungi achenes were classified as dead, whereas those with a firm, whitish seed were classified as potentially viable, following Baskin & Baskin (2014).

2.3. Data analysis

The speed of seed germination was calculated based on three parameters (Table 1), namely Timson's index (Timson 1965), mean germination time (Orchard 1977) and coefficient of velocity (Baskin & Baskin 2014), which are commonly used in seed germination studies (Al-Mudaris 1998; Baskin & Baskin 2014). According to the above-mentioned authors, a high value of Timson's index (TI) indicates fast seed germination, a high value of mean germination time (MGT) indicates slow seed germination and a high value of coefficient of velocity (CV) indicates rapid seed germination. The parameters of TI and MGT were calculated for a 10-day germination test period, whereas the GVC parameter was calculated for the total time of test period (21 days).

The χ^2 test was used to check the statistical significance of differences in the percentage of germinated and non-germinated seeds between species (the 3×2 contingency table) and between storage periods (the 2×2 contingency table). Normal distribution of the untransformed data concerning germination parameters (TI, MTG and CV), as well as the number of dead and potentially viable seeds among the non-germinated seeds of *S. ×niederederi, S. canadensis*, and *S. virgaurea* subjected to short and long storage periods was tested using the Kolmogorov-Smirnov test, while homogeneity of variance was tested using the Levene test at the significance level of $P < 0.05$. As the values in some groups were not consistent with normal distribution and the variance was not homogenous, the analysis was based on the non-parametric tests. The Mann-Whitney U test was applied to check if there were significant differences in mean values of germination parameters

and the number of dead and potentially viable seeds among the non-germinated seeds of particular species between samples subjected to short and long storage periods. The Kruskal-Wallis H test with multiple comparisons was used to assess if there were significant inter-species differences in mean values of germination parameters and in the number of dead and potentially viable seeds among the non-germinated seeds subjected to short and long storage periods. The χ^2 test was calculated using the interactive computer programme (Preacher 2001), while other statistical tests were performed using a Statistica 13 software package.

3. Results

Seeds of all the observed species presented remarkably higher final percentage of germination after the short storage period than after the long one (Table 2). After the long storage period, the highest value of the mean share of germinated seeds was noted in *S. canadensis* and the lowest one – in *S. virgaurea* ($\chi^2 = 17.9$, p = 0.001, *fe* for non-germinated seeds of all species = 75.66, *fe* for germinated seeds for all species = 24.33), whereas after the short storage period, the highest value of the mean share of germinated seeds was found in *Solidago virgaurea* and the lowest one in *S. canadensis* ($\chi^2 = 20.7$, p = 0.0003, *fe* for non-germinated seeds of all species = 9.66, *fe* for germinated seeds for all species = 90.33).

The seeds of *S. ×niederederi, S. canadensis,* and *S. virgaurea* subjected to the short fruit storage period achieved significantly higher values of Timson's index (Table 3) and coefficient of velocity (Table 4), as well as significantly lower values of the mean germination time (Table 5), than samples subjected to the long fruit storage period.

Considering the interspecific differences, the mean values of Timson's index (Table 3), mean germination

Table 2. The mean final percentage (±SD) of non-germinated and germinated seeds of *Solidago ×niederederi* and its parental species after two different fruit storage periods, calculated on the basis of four replications, and level of statistical significance of differences (χ^2 test)

Species	Type of seeds	Mean final percentage of seeds (±SD)		χ^2 test			
				Expected value (f_e)		χ^2 value	P value
		long storage period (3.5 years)	short storage period (0.5 years)	long storage period (3.5 years)	short storage period (0.5 years)		
Solidago ×niederederi	non-germinated	68.5 (±6.0)	9.0 (±8.2)	38.75	38.75	74.5	0.0
	germinated	31.5 (±6.0)	91.0 (±8.2)	61.25	61.25		
Solidago canadensis	non-germinated	68.0 (±32.4)	19.5 (±4.1)	43.75	43.75	47.7	0.0
	germinated	32.0 (±32.4)	80.5 (±4.1)	56.25	56.25		
Solidago virgaurea	non-germinated	90.5 (±4.4)	0.5 (±1.0)	45.50	45.50	163.3	0.0
	germinated	9.5 (±4.4)	99.5 (±1.0)	54.50	54.50		

time (Table 5) and coefficient of velocity (Table 4) differed significantly only in the case of the short fruit storage period.

Irrespective of the storage period, the number of dead seeds in *S.* ×*niederederi* and *S. virgaurea* prevailed on potentially viable ones, whereas in *S. canadensis* an opposite trend was observed (Table 6). After the long storage period, a significantly higher number of dead seeds among non-germinated seeds was found in *S.* ×*niederederi* and *S. virgaurea*. The number of dead and potentially viable seeds also showed inter-species variability. After the long storage period, the highest number of dead seeds was noticed in *S. virgaurea*, whereas the lowest one – in *S. canadensis* and the highest number of potentially viable seeds was recorded in *S.*

canadensis, whereas the lowest one – in *S.* ×*niederederi* (Table 6). After the short storage period, the number of dead seeds did not differ significantly among the species and, moreover, the highest number of potentially viable seeds was observed in *S. canadensis*, whereas the lowest one – in *S. virgaurea* (Table 6).

4. Discussion

The results obtained in this study (Table 2) allow us to accept our hypothesis that the long fruit storage period reduces the percentage of seed germination in *Solidago* ×*niederederi* and its parental species and suggest that the longtime seed desiccation has a negative impact on final germination. It should be pointed out that several

Table 3. The mean value of Timson's index (TI) in *Solidago* ×*niederederi* and its parental species after two different fruit storage periods, calculated on the basis of four replications. The different letters in superscripts denote statistical differences between the species

Species	TI		Level of statistical significance (U value; p value)
	long storage period (3.5 years)	short storage period (0.5 years)	
Solidago ×*niederederi*	127.5	626.0[ab]	U=0.0; p=0.03
Solidago canadensis	118.5	524.5[a]	U=0.0; p=0.03
Solidago virgaurea	43.0	717.5[b]	U=0.0; p=0.03
Level of statistical significance (H value; p value)	H=3.2; p=0.2	H=8.3; p=0.01	

Table 4. The mean value of coefficient of velocity (CV) in *Solidago* ×*niederederi* and its parental species after two different fruit storage periods, calculated on the basis of four replications. The different letters in superscripts denote statistical differences between the species

Species	CV		Level of statistical significance (U value; p value)
	long storage period (3.5 years)	short storage period (0.5 years)	
Solidago ×*niederederi*	13.6	24.3[ab]	U=0.0; p=0.03
Solidago canadensis	16.1	21.2[b]	U=0.0; p=0.03
Solidago virgaurea	15.3	26.7[a]	U=0.0; p=0.03
Level of statistical significance (H value; p value)	H=1.4; p=0.5	H=6.5; p=0.03	

Table 5. The mean value of mean germination time (MGT) in *Solidago* ×*niederederi* and its parental species after two different fruit storage periods, calculated on the basis of four replications. The different letters in superscripts denote statistical differences between the species

Species	MGT		Level of statistical significance (U value; p value)
	long storage period (3.5 years)	short storage period (0.5 years)	
Solidago ×*niederederi*	6.2	3.7[a]	U=0.0; p=0.03
Solidago canadensis	6.0	4.3[b]	U=0.0; p=0.03
Solidago virgaurea	6.5	3.7[a]	U=0.0; p=0.03
Level of statistical significance (H value; p value)	H=0.7; p=0.6	H=5.9; p=0.05	

Table 6. The mean number of dead and potentially viable seeds among the non-germinated seeds in *Solidago ×niederederi* and its parental species after two different fruit storage periods, calculated on the basis of four replications. The different letters in superscripts denote statistical differences between the species

Species	Type of non-germinated seeds	The mean number of seeds		Level of statistical significance (U value; p value)
		long storage period (3.5 years)	short storage period (0.5 years)	
Solidago ×niederederi	dead	32.00[a]	4.00	U=0.0; p=0.03
	potentially viable	2.25[a]	0.50[ab]	U=1.5; p=0.08
Solidago canadensis	dead	5.00[a]	1.50	U=1.5; p=0.08
	potentially viable	29.00[b]	8.25[b]	U=0.0; p=0.03
Solidago virgaurea	dead	42.75[b]	0.25	U=0.0; p=0.03
	potentially viable	2.50[a]	0.00[a]	U=2.0; p=0.11
Level of statistical significance of the dead seed number (H value; p value)		H=9.8; p=0.007	H=3.9; p=0.1	
Level of statistical significance of the potentially viable seed number (H value; p value)		H=7.9; p=0.02	H=9.4; p=0.009	

experiments provided evidence that the long storage in room conditions contributes to final seed germination reduction in many species from the Asteraceae family, for example, *Centaurea cyanus* L.*, Echinacea purpurea* (L.) Moench*, Rudbeckia hirta* L. (Aghilian *et al.* 2014), and *Onopordum acanthium* L. (Qaderi *et al.* 2005). However, Walck *et al.* (1997) showed a positive relationship between storage time and final germination of seeds in *S. altissima* L.*, S. nemoralis* Aiton, and *S. shortii* Torr. & Gray. Taking into consideration that *S. ×niederederi* can be potentially cultivated for scientific, medicinal or ornamental purposes, the suggested fruit storage period, under dry, room temperature conditions, should be shorter than three years. However, we realize that the response to long storage may vary in different populations and, therefore, further investigations are needed. In the future, we plan to collect fruit samples from several populations of the hybrid and its parental species and test their seed germination abilities at one-year intervals during a storage period of five years. Moreover, we also plan to test seed germination of the hybrid in natural conditions, because we agree with Gioria & Pyšek (2017) that high percentage of seed germination evidenced in the laboratory is not a consistent predictor of invasiveness.

Regarding the Mann-Whitney U test results (Tables 3-5), we can accept our hypothesis that long fruit storage period reduces the speed of seed germination in *S. ×niederederi* and its parental species. On the other hand, after a long fruit storage period, the germination parameters in the hybrid can be very similar to those observed in its parental species (Tables 3-5). This observation proved that seeds of *S. ×niederederi* can show germination abilities found in its parental species, providing an intriguing speculation that seed germination speed

is heritable as evidenced in many other plant species (Baskin & Baskin 2014 and the literature cited therein). However, it should be stated that statistically significant interspecific differences in mean values of the Timson's index, mean germination time and germination velocity coefficient of shortly stored seeds revealed in this study suggest the need for further investigation.

After the long fruit storage period, a significantly higher number of dead seeds among non-germinated seeds was found in *S. ×niederederi* and *S. virgaurea* (Table 6), thus our hypothesis that the long storage period might increase seed mortality can be accepted only for these two taxa. The domination of dead seeds over viable ones observed in *S. ×niederederi* and *S. virgaurea* after the long fruit storage period (Table 6) might be the result of the sensitivity of achenes to decreasing moisture content. On the other hand, data from the Seed Information Database of the Royal Botanic Gardens, Kew (http://data.kew.org/sid) suggest that *S. virgaurea* seeds, similarly to the majority of the species (Hay & Probert 2013), are orthodox and they can be dried without any damage. According to Shaban (2013), the viability of orthodox seeds is relatively easily sustained by lowering their moisture contents and storage temperature, but they gradually deteriorate and, finally, die even under such suitable conditions for storage. However, the occurrence of a low number of degenerated seeds, especially after the long storage period, which was noticed exclusively in *S. canadensis*, suggests that dry storage at room temperature might preserve seed viability in this species.

References

Aghilian S., Khajeh-Hosseini M. & Anvarkhah S. 2014. Evaluation of seed storage potential in forty medicinal plant species. Intl. J. Agri. Crop. Sci. 7: 749-759.

Al-Mudaris M. A. 1998. Notes on various parameters recording the speed of seed germination. Der Tropenlandwirt. Beitr. Trop. Landwirtsch. Veterinarmed. 99: 147-154.

Baskin C. C. & Baskin J. M. 2014. Seeds: ecology, biogeography and evolution of dormancy and germination. 2nd ed. 1586 pp. Academic Press/Elsevier, San Diego, CA, USA.

Daehler C. C. & Carino D. A. 2001. Hybridization between native and alien plants and its consequences. In: J. L. Lockwood & M. L. McKinney (eds.). Biotic homogenization, pp. 81-102. Kluwer Academic/Plenum Publishers, New York, USA.

Gioria M. & Pyšek P. 2017. Early bird catches the worm: germination as a critical step in plant invasion. Biol. Invasions 19: 1055-1080. http://dx.doi.org/10.1007/s10530-016-1349-1.

Gudžinskas Z. & Petrulaitis L. 2016. New alien plant species recorded in the southern regions of Latvia. Bot. Lith. 22: 153-160. http://dx.doi.org/10.1515/botlit-2016-0016.

Hay F. R. & Probert R. J. 2013. Advances in seed conservation of wild plant species: a review of recent research. Conserv. Physiol. 1: 1-11. http://dx.doi.org/10.1093/conphys/cot030.

Karpavičienė B. & Radušienė J. 2016. Morphological and anatomical characterization of *Solidago ×niederederi* and other sympatric *Solidago* species. Weed Sci. 64: 61-70. http://dx.doi.org/10.1614/WS-D-15-00066.1.

Kołodziej B. 2008. Effect of agrotechnical factors on the yield of goldenrod (*Solidago virgaurea* L. ssp. *virgaurea*). Herba Polonica 54: 28-34.

Migdałek G., Kolczyk J., Pliszko A., Kościńska-Pająk M. & Słomka A. 2014. Reduced pollen viability and achene development in *Solidago ×niederederi* Khek from Poland. Acta Soc. Bot. Pol. 83: 251-255. http://dx.doi.org/10.5586/asbp.2014.025.

Mishra D., Joshi S., Bisht G. & Pilkhwal S. 2010. Chemical composition and antimicrobial activity of *Solidago canadensis* Linn. root essential oil. J. Basic Clin. Pharm. 1: 187-190.

Nilsson A. 1976. Spontana gullrishybrider (*Solidago canadensis × virgaurea*) i Sverige och Danmark. Svensk Bot. Tidskr. 70: 7-16.

Orchard T. 1977. Estimating the parameters of plant seedling emergence. Seed Sci. Technol. 5: 61-69.

Pagitz K. 2016. *Solidago ×niederederi* (*S. canadensis × S. virgaurea* ssp. *virgaurea*) in the Eastern Alps. In: C. Ries & Y. Krippel (eds.). Biological invasions: interactions with environmental change. Book of abstracts. NEOBIOTA 2016 – 9th International Conference on Biological Invasions, pp. 194. Vianden, Luxembourg.

Pliszko A. 2013. A new locality of *Solidago ×niederederi* Khek (Asteraceae) in Poland. Biodiv. Res. Conserv. 29: 57-62. http://dx.doi.org/10.2478/biorc-2013-0008.

Pliszko A. 2015. Neotypification of *Solidago ×niederederi* (Asteraceae). Phytotaxa 230: 297-298. http://dx.doi.org/10.11646/phytotaxa.230.3.10.

Pliszko A. & Kostrakiewicz-Gierałt K. 2017. Resolving the naturalization strategy of *Solidago ×niederederi* (Asteraceae) by the production of sexual ramets and seedlings. Plant. Ecol. http://dx.doi.org/10.1007/s11258-017-0762-6

Pliszko A. & Zając M. 2016. Current and potential distribution of *Solidago ×niederederi* (Asteraceae) in Poland. In: C. Ries & Y. Krippel (eds.). Biological invasions: interactions with environmental change. Book of abstracts. NEOBIOTA 2016 – 9th International Conference on Biological Invasions, pp. 163. Vianden, Luxembourg.

Pliszko A. & Zalewska-Gałosz J. 2016. Molecular evidence for hybridization between invasive *Solidago canadensis* and native *S. virgaurea*. Biol. Invasions 18: 3103-3108. http://dx.doi.org/10.1007/s10530-016-1213-3.

Pradham B. K. & Badola H. K. 2012. Effect of storage conditions and storage periods on seed germination in eleven populations of *Swertia chirayita*: a critically endangered medicinal herb in Himalaya. Sci. World J., 9 pages. http://dx.doi.org/10.1100/2012/128105.

Preacher K. J. 2001. Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence (computer software), 2001. http://quantpsy.org.

Pyšek P., Richardson D. M., Rejmánek M., Webster G. L., Williamson M. & Kirschner J. 2004. Alien plants in checklists and floras: towards better communication between taxonomists and ecologists. Taxon 53: 131-143.

Royal Botanic Gardens Kew, Seed Information Database (SID), version 7.1, 2017. http://data.kew.org/sid.

Qaderi M. M., Presti A. & Cavers P. B. 2005. Dry storage effects on germinability of Scotch thistle (*Onopordum acanthium*) cypselas. Acta Oecol. 27: 67-74. http://dx.doi.org/10.1016/j.actao.2004.09.004.

Shaban M. 2013. Aging in orthodox seeds is a problem. Int. J. Adv. Biol. Biom. Res. 1: 1296-1301.

Stace C. A., Preston C. D. & Pearman D. A. 2015. Hybrid flora of the British Isles. 501 pp. Botanical Society of Britain and Ireland, Bristol.

Timson J. 1965. New method of recording germination data. Nature 207: 216-217. http://dx.doi.org/10.1038/207216a0.

Walck J. L., Baskin J. M. & Baskin C. C. 1997. A comparative study of the seed germination biology of a narrow endemic and two geographically-widespread species of *Solidago* (Asteraceae). 1. Germination phenology and effect of cold stratification on germination. Seed Sci. Res. 7: 47-58.

Werner P. A., Gross R. S. & Bradbury I. K. 1980. The biology of Canadian weeds: 45. *Solidago canadensis* L. Can. J. Plant Sci. 60: 1393-1409. http://dx.doi.org/10.4141/cjps80-194.