MONITORING SEED VIABILITY OF FIFTEEN SPECIES AFTER STORAGE IN THE IRISH THREATENED PLANT GENEBANK

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ABSTRACT

Germination trials of fifteen rare and endangered Irish plant species, representing twenty-two accessions, were conducted after up to seven years storage in the Irish Threatened Plant Genebank. Seeds had been stored at low moisture content (approximately 5 per cent) and at low temperature ($-18°C$). A variety of results was obtained, with some species showing a significant increase in percentage germination, some showing a significant decrease in percentage germination and others showing no significant change. Within a species, consistent results were not always obtained, with individual accessions sometimes showing varied germination results. Consistent monitoring of seed storage conditions along with regular viability checks is recommended in order to improve the management of the Irish Threatened Plant Genebank. Based on the results presented here, the extrapolation of results from one accession of a species to include all other accessions of the species is not recommended.

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INTRODUCTION

The estimated number of higher plant species in the world exceeds a quarter of a million. These species form the great wealth of the earth's plant genetic resources, contributing towards biodiversity. However, an increasing world human population threatens this biodiversity, and it has been conservatively estimated that one in five plant species could become extinct over the next 50 years (Chin 1994). With the loss of natural habitats, increasing emphasis is being placed on the importance of conserving natural populations of plants for their genetic diversity (Waldren *et al.* 2000). Genetic diversity is also important because of its possible future contribution to improving plants already used for food, shelter and medicine.

Genebanks are one of the most commonly used methods of *ex situ* conservation, and seed genebanks have been used in the conservation of threatened wild species (Hawkes 1987; Linington 2001; Martin *et al.* 2001). They are an efficient and cost-effective way of conserving large amounts of genetic diversity (Waldren *et al.* 2000) as many thousands of seed collections representing different populations or plant taxa can be housed in a small area. In addition, the seeds can be kept safely for hundreds of years (once stored correctly) and require relatively little maintenance and their storage can easily be duplicated in different places, thus limiting their vulnerability. Seed collections provide an invaluable research tool whereby elements of the ecology and species behaviour of plants can be investigated and information on species conservation elucidated. However, as seeds are living material, they require proper storage conditions and continuous monitoring to ensure that viability is maintained. Seeds that are collected and stored in a seed genebank must be of high quality and at maximum viability. Care must also be taken that enough seeds are stored to ensure that the germplasm preserved (i) includes rare and useful genes (Chin 1994) and (ii) reflects the genetic diversity naturally found in the population (Brown and Briggs 1991; Centre for Plant Conservation 1991).

The genetic erosion of material maintained in genebanks is considered a relevant problem at the international level (FAO 1997), and the continuous monitoring of the factors causing genetic erosion in *ex situ* collections is recommended to minimise the loss of genetic diversity (Ruiz *et al.* 1999). One of the main factors causing genetic erosion is the failure to detect loss of germination due to a lack of viability monitoring (FAO 1997). Thus, viability testing through germination is essential for the maintenance of a seed genebank collection and can be a rapid way of identifying problems with the seed storage conditions.

The Irish Threatened Plant Genebank was established in 1994 with the aim of conserving and storing representative collections of Ireland's rare and endangered plant species (see Waldren *et al.*

(2000) for further details). The genebank currently contains 165 collections from all over Ireland, representing 59 plant species. These collections contain representative samples of 50% of Ireland's endangered species, 48% of its vulnerable species and 31% of its rare species. As with all genebanks, any seed stored in the Irish Threatened Plant Genebank are separated into active and base collections. An 'active' collection contains seed available for immediate multiplication, distribution and any associated germination monitoring. A 'base' collection of each seed accession is left untouched and is thus preserved for the long-term future. It may be divided into a base 1 and base 2 collection: the base 1 collection remains in the seed genebank of the host institute, and the base 2 collection is distributed to other seed genebanks for storage as further insurance against loss or damage. The base collection is only touched when trials with the active collection suggest the need for regeneration or re-collection (Waldren *et al.* 2000).

A project was undertaken in 2001 to determine the effect, if any, of long-term storage in the genebank on the germination percentages of stored species.

MATERIALS AND METHODS

In total, fifteen species (representing twenty-two accessions) held in the active collection were targeted for repeat germination experiments. The species were chosen using the following procedure. A list of accessions previously tested for germination (Martin 1998) was compared with the active accessions remaining in the genebank. A number of accessions had no active collection, all seeds having been used in previous germination experiments, and so could not be tested in these germination trials. A number of accessions had only one packet of 'active' seed left. As records of seed number stored for each accession were not accurate, it was decided to target species that had more than one accession in the active collection to ensure that an adequate number of seeds were available for testing; the germination results of one accession were then used to extrapolate for the other accessions. Of these fifteen target species, some had been tested for viability after two years storage at -18°C (Martin 1998). Table 1 details the species selected for germination trials, their status in Ireland, those previously tested for germination after two years in storage and those for which dormancy breaking procedures were necessary.

GERMINATION TESTS

Due to constraints on material, germination tests were conducted with one replicate of a maximum of one hundred seeds. The seeds were placed into a Petri dish containing two layers of Whatman no. 1 filter paper, which had been moistened using distilled water. The seeds were arranged in concentric circles on the filter paper, with the aim of maximising the distance between the seeds (Ellis *et al.* 1985) and thereby limiting the spread of possible infections.

Seeds requiring a chilling pretreatment were placed into a cold room (4°C, 12h light/dark, 70–80μmol m^{-2} s^{-1}) for the necessary length of the chilling pretreatment (Martin 1998). Germination of seeds of certain species had previously been shown to benefit from scarification, so the seeds had their testae scored with a scalpel blade before being placed on moist filter paper (Martin 1998). Other species showed increased germination with the application of gibberellic acid (GA_3) (Martin 1998), and seeds from such species were placed on filter paper moistened with GA_3 instead of distilled water. Petri dishes containing pretreated seeds and those seeds that required no pretreatment were placed in a growth room with alternating temperature and light cycles (20/15°C day/night temperature, 13/11h light/dark, 30–40μmol m^{-2} s^{-1}). Alternating temperature cycles have previously been reported to promote germination in many species (Grime *et al.* 1981). Germination was recorded daily for the first two weeks, after which time weekly inspection was considered adequate (Ellis *et al.* 1985). Sufficient distilled water was added to the Petri dishes to keep the filter papers moist, taking care not to overwater. The criterion of germination was visible radicle emergence, and after each count germinated seeds were either discarded or an attempt was made to grow them on, thus providing a 'voucher' specimen for the accession, which would be housed in **TCD**.

DATA ANALYSIS

Any change in cumulative percentage germination was tested for significance using a two-tailed Z-test, which utilises the following equation:

$$Z = (p_1 - p_2)/\sqrt{p(100 - p)(1/n_1 + 1/n_2)}$$

where $p_1 = 100$ times the number of seeds initially estimated to be viable, divided by the number of seeds tested minus 0.5; $p_2 = 100$ times the number of seeds estimated to be viable after storage, divided by the number of seeds tested plus 0.5; p is the mean of p_1 and p_2; and n_1 and n_2 refer to the number of seeds tested before and after storage respectively (Ellis *et al.* 1985).

The Z value calculated was checked for significance using standard normal distribution tables (Daly *et al.* 1995).

RESULTS

The germination percentages for each species tested in 2001 and tested before storage at −18°C (Martin 1998), along with Z-tests results for each accession, are given in Table 2 and germination curves shown in Fig. 1.

Of the species tested, three (*Asparagus officinalis* subsp. *prostratus*, *Erigeron acer* and *Carum verticillatum*) showed significantly decreased germination, although the decline in final germination percentage for *Erigeron acer* was very small compared with the original collection (Table 2). Five species (*Draba incana*, *Frangula alnus*, *Hypericum hirsutum*, *Otanthus maritimus* and *Tuberaria guttata*) showed significantly increased germination, and four (*Kickxia elatine*, *Ligusticum scoticum*, *Sanguisorba officinalis* and *Trollius europeaus*) showed no significant change in germination after storage.

For the remaining three species tested, the results obtained are not as clear. In the case of *Campanula trachelium*, *Hypericum canadense* and *Potentilla fruticosa*, more than one accession was tested for germination, and these accessions showed varying results. Two of the *Campanula trachelium* collections showed significantly increased germination (Table 2), one showed significantly decreased germination, and one collection showed no significant change in germination storage (Table 2). In the case of the two *Hypericum canadense* accessions tested, one collection showed significantly decreased germination, while the other collection showed no significant change in germination (Table 2). The two *Potentilla fruticosa* accessions tested also showed contradictory germination results, with one collection showing significantly increased germination and the other showing significantly decreased germination (Table 2).

A number of species were tested for germination two years after they were first placed in storage (see Table 1). Table 3 gives the germination percentages of seeds of these species after two years of storage and in the trials

Table 1—The species chosen for the germination experiment along with the number of seeds used.

Species	*Accession number*	*Status*[1]	*Pre-treatment*	*Number of seeds*
Asparagus officinalis subsp. *prostratus*★	12	Rare	20-day chill	95
Campanula trachelium★	8	Vulnerable	10-day chill	100
Campanula trachelium★	47	Vulnerable	10-day chill	100
Campanula trachelium★	48	Vulnerable	10-day chill	100
Campanula trachelium★	49	Vulnerable	10-day chill	100
Carum verticillatum	117	Not threatened	None	120
Carum verticillatum	120	Not threatened	None	100
Draba incana	99	Rare	$+GA_3$	100
Erigeron acer	105	Vulnerable	None	100
Frangula alnus	156	Rare	S	90
Hypericum canadense★	17	Rare	None	100
Hypericum canadense★	18	Rare	None	100
Hypericum hirsutum	60	Vulnerable	$+GA_3$	100
Kickxia elatine★	108	Vulnerable	S	100
Ligusticum scoticum	95	Rare	None	100
Otanthus maritimus★	11	Endangered	30-day chill	100
Potentilla fruticosa★	59	Rare	20-day chill	100
Potentilla fruticosa★	129	Rare	10-day chill	100
Sanguisorba officinalis★	100	Vulnerable	10-day chill	100
Trollius europaeus★	80	Vulnerable	10-day chill	100
Tuberaria guttata	76	Rare	None	100
Tuberaria guttata★	77	Rare	10-day chill	100

Chill = the number of-days spent undergoing a chilling pre-treatment prior to germination; $+GA_3$ = the application of 500 ppm gibberellic acid; S = scarification; ★ = species previously tested for germination changes after two years in storage. Nomenclature follows Stace (1997).

[1]Status categories used here are as reported in The Irish Red Data Book (Curtis and McGough 1988).

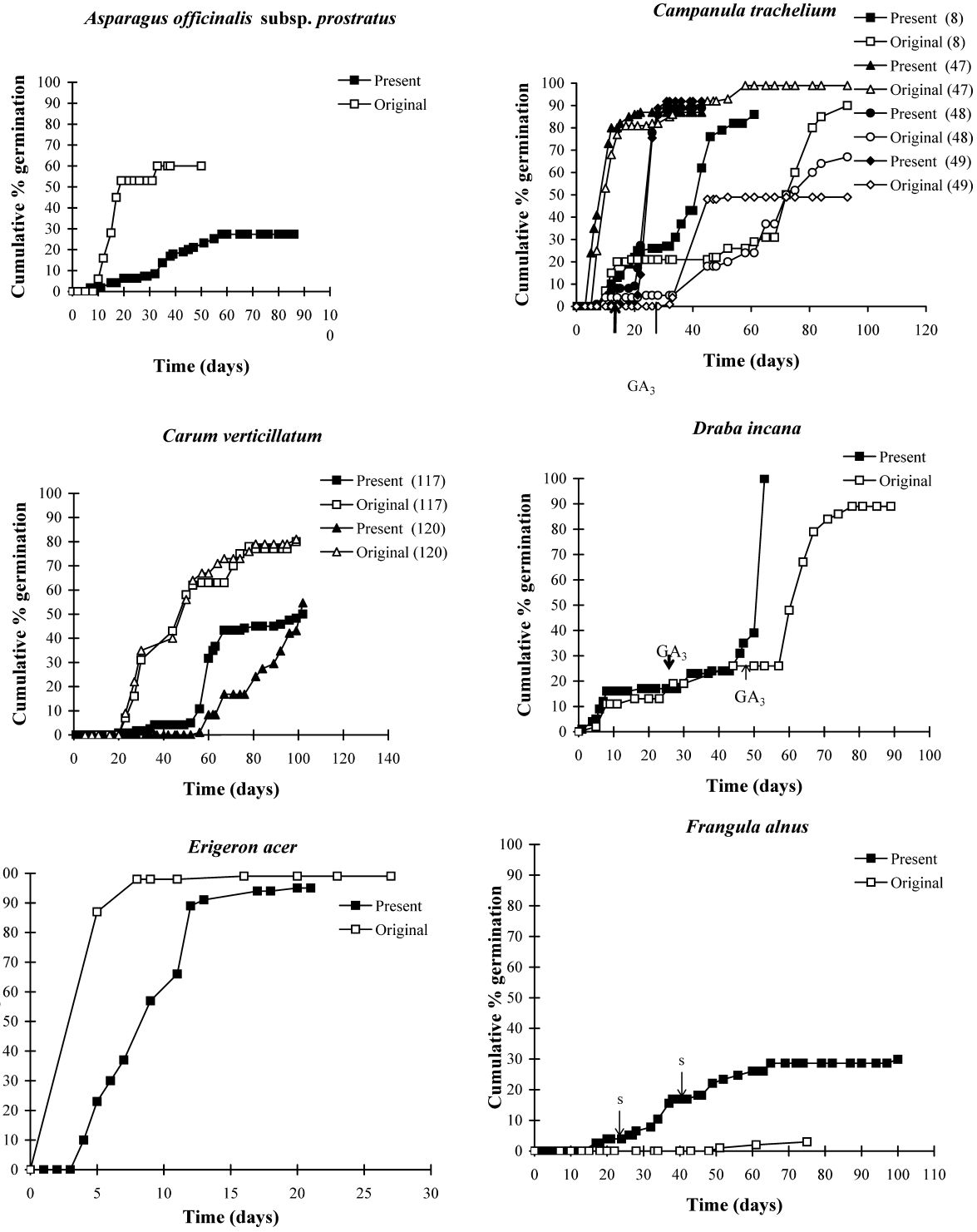

Fig. 1 (above, opposite and overleaf)—Percentage germination graphs for 15 species, representing 22 accessions, held in The Irish Threatened Plant genebank. Graphs represent the original germination percentages (original) for these species before storage at −18°C and the germination percentages after up to seven years in storage (present). The application of gibberellic acid is indicated by an arrow and the symbol GA_3, the letter 's' above or below an arrow indicates the day when seeds were scarified. Bold arrows relate to closed symbol germination graphs, while non-bold arrows relate to open symbol germination graphs. Where more than one collection of seeds for a species was germinated, the collections are distinguished by their accession numbers.

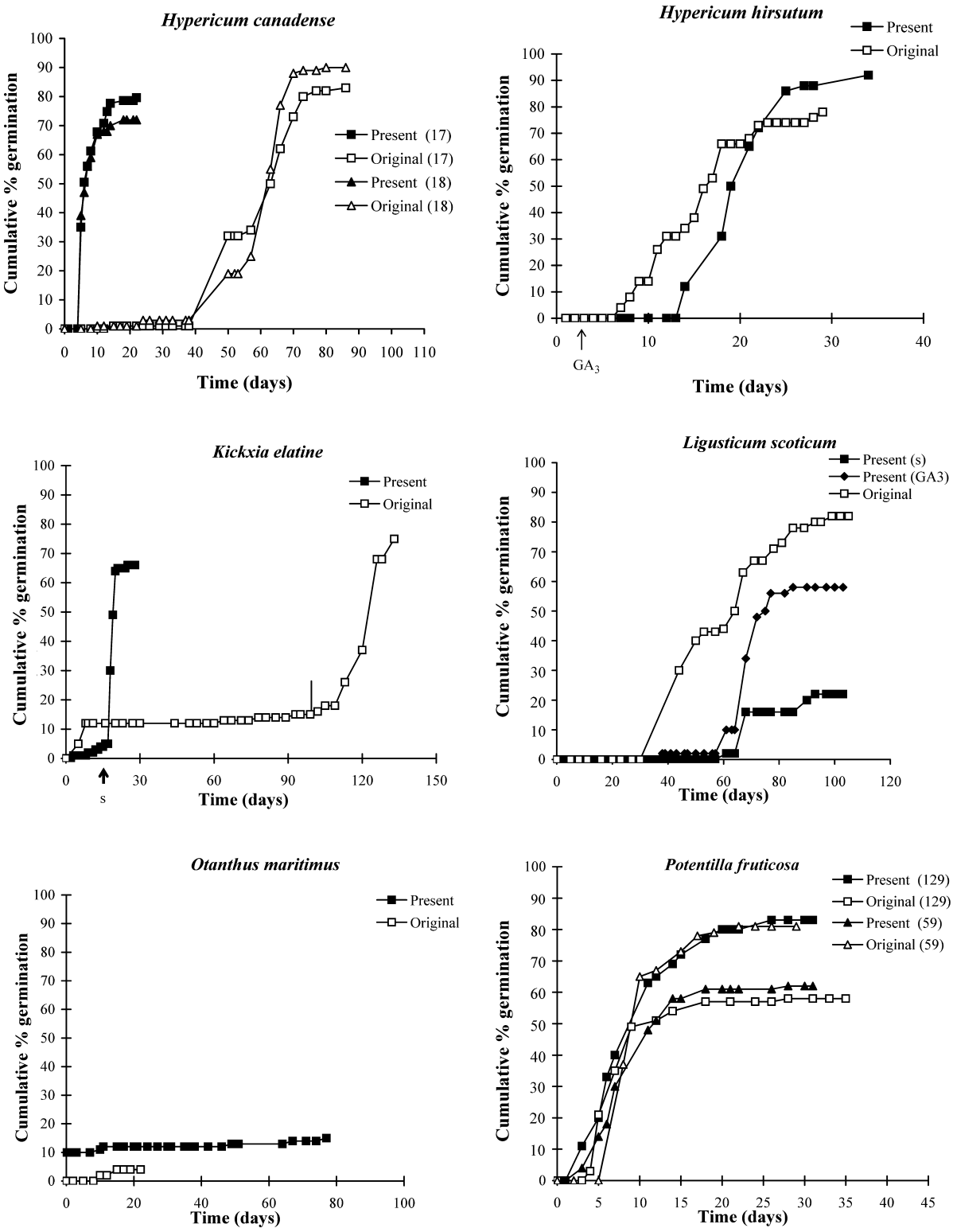
Hypericum canadense
Cumulative % germination
Time (days)
Present (17)
Original (17)
Present (18)
Original (18)
Hypericum hirsutum
Present
Original
GA3
Kickxia elatine
s
Ligusticum scoticum
Present (s)
Present (GA3)
Otanthus maritimus
Potentilla fruticosa
Present (129)
Original (129)
Present (59)
Original (59)

Fig. 1 contd.

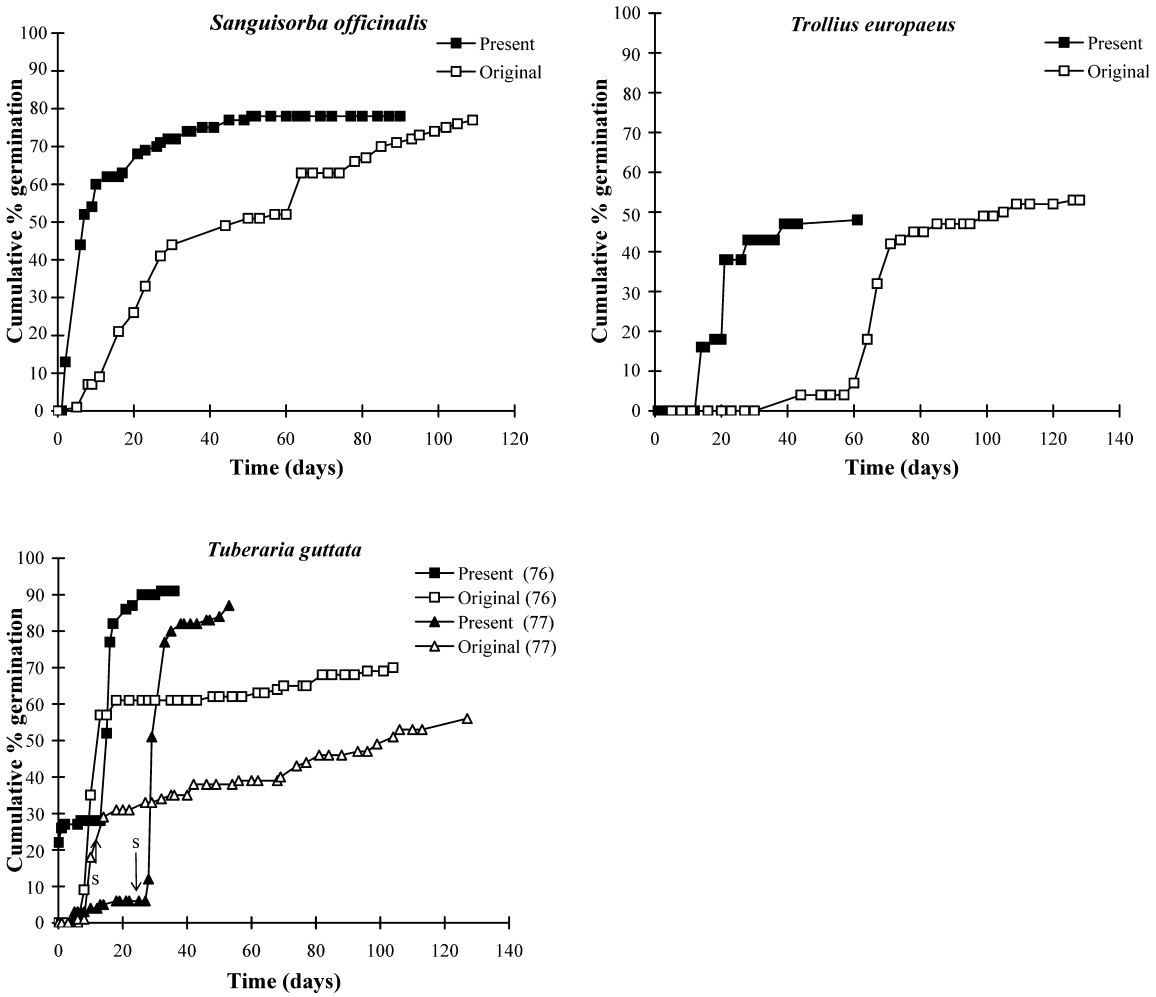

Fig. 1 contd.

conducted in 2001, along with the results of the Z-tests for each of these species and accessions.

Of the nine species tested, three of the *Campanula trachelium* accessions, one of the *Potentilla fruticosa* accessions and *Kickxia elatine*, *Sanguisorba officinalis* and *Tuberaria guttata* showed no significant change in germination percentage over the past five years. *Otanthus maritimus*, both accessions of *Hypericum canadense*, one *Potentilla fruticosa* accession and *Trollius europaeus* all showed significantly increased germination percentages, while the remaining *Campanula trachelium* accession and *Asparagus officinalis* subsp. *prostratus* showed significantly decreased germination percentages over the past five years.

DISCUSSION AND CONCLUSIONS

Testing the viability of stored seed through germination trials at regular intervals is an integral part of maintaining a seed genebank as it allows the monitoring of genetic 'erosion' during storage (Ruiz *et al.* 1999). Seed accessions have to be regenerated when their percentage germination falls below certain critical values (Chin 1994), and Hawkes (1987) suggests a viability test every three years and the regeneration or re-collection of a species if the viability falls below 85%. The results obtained in the course of the germination trials carried out here support the regular monitoring of seed viability, although at sufficiently spaced intervals to ensure accurate viability measurements.

The results obtained in the course of our tests show that changes in germination percentages varied considerably among genera, for example *Asparagus officinalis* subsp. *prostratus* showed significantly decreased germination after the seven years in storage, while *Otanthus maritimus* showed a significant increase in germination over the same time period. This difference in germinability between species may reflect significant differences in storability of individual species (Ruiz *et al.* 1999), which should be taken into account before

species are stored. Changes in percentage germination also varied considerably among accessions of a single species, e.g. the four accessions of *Campanula trachelium* showed very different results, with two accessions showing significantly increased germination, one showing significantly decreased germination and one accession showing no significant change in percentage germination after seven years in storage (Table 2). These variations again may reflect differences in storability even within the same species. They may also reflect differences in operator technique, growth room conditions or pretreatment application.

From the germination results obtained after two years in storage, some accessions showed significantly increased germination percentages from initial values, e.g. *Campanula trachelium* accessions 48 and 49 and *Tuberaria guttata* (Fig. 1), with no further significant increase in the following years to 2001 (Table 3). An increase in germination was also found by Ruiz *et al.* (1999) for cereal grains tested for viability after ten years in storage. The authors explained this phenomenon as a result of dormancy being broken after one to two years in storage. This appears to be the case with the *Campanula trachelium* and *Tubararia guttata* accessions tested here, as, especially in the case of *Campanula trachelium*, the final germination percentages for accessions 48 and 49 were of the same order as the final germination percentages obtained for accessions 8 and 47. Changes in germination percentages of this type, i.e. an increase after a period in storage, suggest that the initial viability of some of the species stored in the Irish Threatened Plant Genebank could have been underestimated due to seed dormancy, and a more complete viability investigation should be carried out in the future for any species with relatively low germination percentages before storage. Conversely, changes of this type also suggest that storage of seed with a relatively low initial germinability may also be advantageous for conservation purposes, as increases in germinability may occur during storage.

Table 2—Germination percentages for 15 species (representing 22 accessions) of plant at collection and after storage at −18°C. Differences in germination percentages were tested using a two-tailed Z–test.

Species/collection number	*% germination*		*Years in storage*	*Increase/decrease significance level*
	at collection	*2001*		
Asparagus officinalis	60	27	7	↓ ***
Campanula trachelium (8)	90	86	7	ns
Campanula trachelium (47)	99	87	7	↓ ***
Campanula trachelium (48)	67	89	7	↑ ***
Campanula trachelium (49)	49	92	7	↑ ***
Carum verticillatum (117)	80	50	6	↓ ***
Carum verticillatum (120)	81	55	6	↓ ***
Draba incana	89	100	6	↑ **
Erigeron acer	99	95	6	↓ *
Frangula alnus	3	30	6	↑ ***
Hypericum canadense (17)	82	80	7	ns
Hypericum canadense (18)	90	72	7	↓ ***
Hypericum hirsutum	81	92	7	↑ **
Kickxia elatine	75	66	6	ns
Ligusticum scoticum	82	80	6	ns
Otanthus maritmus	4	15	7	↑ **
Potentilla fruticosa (59)	81	62	7	↓ **
Potentilla fruticosa (129)	58	83	6	↑ **
Sanguisorba officinalis	77	78	6	ns
Trollius europaeus	53	48	6	ns
Tuberaria guttata (76)	56	87	6	↑ ***
Tuberaria guttata (77)	70	91	6	↑ ***

For species with more than one accession, the accessions are distinguished by their accession numbers. ↑ = increase in germination; ↓ = decrease in germination; ***$P \leq 0.001$; **$P \leq 0.01$; *$P \leq 0.05$; ns = not significant.

When compared with the initial germination percentages, i.e. germination percentages obtained before seeds were placed in storage, some species and accessions (e.g. *Potentilla fruticosa* accession 59 and *Hypericum canadense* accession 18) showed significantly decreased germination after storage (Table 2). However, when germination percentages after two years and approximately seven years in storage were compared, *P. fruticosa* showed no significant change in germination, while *H. canadense* showed a significantly increased germination percentage, although not to initial levels (Table 3). Changes of this nature in seed viability over time reinforce the need for regular seed testing over sufficient intervals, as results obtained after two years may not accurately reflect the actual seed viability of certain species.

The significantly increased germination of *Otanthus maritimus* after two and seven years in storage was perhaps not unexpected as after seven years of storage, all germination occurred during the chilling pretreatment, i.e. when the seeds were in continuous darkness in the cold room. *O. maritimus* is of Mediterranean origin and has previously been shown to exhibit photoinhibition of seed germination (Thanos *et al.* 1991). However, Thanos *et al.* only obtained 7.6% total germination compared with 15% total germination reported in this study (from the viability tests carried out by Martin (1998), the total viability of the *O. maritimus* seeds placed in storage was 16%). In general, *O. maritimus* has a low germinability, although the progressive increase in percentage germination seen for this species in the course of the trials carried out in 1994 and 2001 and reported here would suggest that the species benefits from prolonged exposure to freezing temperatures. Further work would need to be carried out to investigate this response.

It should also be remembered that the germinability of an accession does not necessarily reflect its viability, and changes in germinability with time should be viewed with caution if used as a measurement of viability. For example, the increased germination of *O. maritimus* with time cannot be due to an increase in viability, but must reflect breaking of dormancy by extended storage at low temperature. Some of the changes in germinability may also reflect different degrees of operator error, as the staff performing the germination trials changed over time. During germination trials for routine experimental work, *A. officinalis* subsp. *prostratus* showed very low germination ($< 10\%$) when lightly scarified, with significantly increased germination percentages when the seeds were more fully abraded (S. McSweeney and S. Waldren, unpublished data). It is also likely that growth room conditions were not identical for the different germination trials. These potential differences in operators and environmental conditions may have affected germination, and the establishment of unvarying

Table 3—Cumulative germination percentages for 9 plant species after 2 years storage in the Irish Threatened Plant Genebank at −18°C and in 2001. Differences in germination percentages were tested for significance using a two-tailed Z-test.

Species	*Cumulative % germination*		*Number of years between tests*	*Increase/decrease significance level*
	After 2 years	*2001*		
Asparagus officinalis	66	27	5	↓ ***
Campanula trachelium (8)	92	86	5	ns
Campanula trachelium (47)	97	87	5	↓ **
Campanula trachelium (48)	91	89	5	ns
Campanula trachelium (49)	92	92	5	ns
Hypericum canadense (17)	55	80	5	↑ ***
Hypericum canadense (18)	55	72	5	↑ *
Kickxia elatine	55	66	4	ns
Otanthus maritmus	6	15	5	↑ *
Potentilla fruticosa (59)	66	62	5	ns
Potentilla fructicosa (129)	58	83	4	↑ ***
Sanguisorba officinalis	80	78	4	ns
Trollius europaeus	19	48	4	↑ ***
Tuberaria guttata (77)	84	91	4	ns

For species with more than one accession, the accessions are distinguished by their accession numbers. ↑ = increase in germination; ↓ = decrease in germination; ***$P \leq 0.001$; **$P \leq 0.01$; *≤ 0.1; ns = not significant.

germination protocols for each species is vital for the accurate monitoring of germinability over time.

Nevertheless, the results obtained from the monitoring tests indicate that the storage conditions employed in the Irish Threatened Plant Genebank are suitable for the preservation of the majority of the fifteen species investigated. Only three species showed significantly decreased germination percentages when compared with the germination percentages before storage. The remaining twelve species showed either a significantly increased germination percentage or no significant difference in germination percentage after seven years in storage. However, viability differs greatly between species and within a species, as does the response to storage. Therefore, the results for one species should not be used to extrapolate and hypothesise results for another. Full and complete monitoring of all species contained in a genebank should be carried out at regular intervals, using a strictly repeatable protocol to assess the effects of storage, thus enabling accurate preservation of an invaluable resource.

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