Establishing quality control in UK wildflower seed production

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Over the past 20 years or so there has been a great increase in the use of seeds of native wildflower species in the United Kingdom, as in other European countries and North America. Wildflowers have been increasingly sown in urban regeneration and civil engineering projects for their attractive appearance and low maintenance requirements (fig. 1). Wild flowers also attract wildlife, thereby increasing biodiversity compared with plantings of traditional amenity grass species. Environmental restoration initiatives have also created a demand for seed, and there is a burgeoning use of wildflower species in gardens and private estates (Laverack et al., 2007).

The increased use of wildflowers has been followed by an increase in the commercial production of seeds and a new trade in approximately 200 species of plants, most of which have not previously been traded. Many of them are now being grown regularly as crops (fig. 2), while some are gathered directly from the wild. The plants range from meadow species (including native grasses) to heath and wetland plants and woodland species, and cover a wide range of families and plant types.

Despite the increase in commercial production, in the UK, the trade remains largely unregulated. Agricultural seed legislation, in the form of the Fodder Seeds Regulations (HMSO, 1993), coincidentally covers some native species which are also traded as 'wild' material (e.g. *Lotus corniculatus* L.), but the requirement for registered cultivars (tested for distinctness, uniformity and stability) means that natural populations cannot possibly conform to the varietal standards. Neither have the standards for purity and germination in these regulations been applied to wildflower seeds. A recent attempt has been made in England to amend the regulations to include wild material, but this applies to the origin of the seed and not to other quality factors.

There has been considerable debate over the last twenty years about the importance of origin (or provenance) of the material of native species, especially as much of the seed has been imported to the UK from other European countries. Even here there is no regulation, but a voluntary code of practice operates (Flora Locale, 2001). In contrast, little work seems to have been done to establish the fundamental requirement for germination testing to determine the planting value of wildflower seeds, and there are neither regulatory levels nor industry standards. Seed is generally traded without germination or purity test results being available.

For some species, the conditions for germination testing are quite straightforward, and a few of the species have germination conditions described in the ISTA Rules, e.g. *Leucanthemum vulgare* (ISTA, 2006). However, these have been developed mainly for cultivated material, and wild populations do not always behave in the same way. A major factor inhibiting the development of germination testing amongst producers, traders and users of wildflower seeds is the presence and range of types of dormancy found in native populations (Baskin and Baskin, 2001), which has inhibited the adoption of routine testing. Dormancy affects wildflower seeds in field and nursery conditions as well as laboratory testing, and in cases of seed failure, the lack of established testing methods often leads to the acceptance that, as the seed quality and behaviour is unknown, the cause just

Figure 1. Annual wildflowers sown on a traffic junction in Scotland in 2007. Species: *Papaver rhoeas, Centaurea cyanus, Chrysanthemum segetum* and *Triplospermum inodorum* (© F. Guest and G. Laverack).

Figure 2. Wildflower seed production plots in Scotland. (© F. Guest and G. Laverack)

cannot be determined. Valuable information on testing conditions and breaking dormancy is now becoming available from the Millenium Seedbank database, and this forms a useful basis for the development of protocols in many species.

The results summarised here are part of a comprehensive study of wildflower seed quality at Scotia Seeds, funded by the Scottish Executive under research and development grants. The work has concentrated on four topics: a) breaking dormancy for germination testing and improvement of field establishment, b) developing germination testing protocols for a wide range of species, c) surveying the quality of seed in the market to determine current standards and d) testing the repeatability of the protocols developed at the Scotia Seeds laboratory with ISTA-accredited laboratories. The results of the survey (topic c) and repeatability work (topic d) are presented here.

Methods

Seed quality survey

The study tested nine wildflower species from eight seed companies (producers and merchants). Seeds were purchased anonymously from the internet. The samples obtained were first examined for purity and then put through germination tests using the protocols developed in the earlier stages of the project (topic b above). Germination tests were set up on germination paper and held at a controlled temperature and light regime. Germination was assessed as total germination, which includes all seeds that have achieved at least physiological germination, i.e. production of a radicle at least 2 mm long.

Comparative germination study

This trial was carried out in collaboration with the ISTA Germination Committee as a comparative germination study of five wildflower species across seven ISTA-accredited seed testing laboratories.

Each of the laboratories was sent samples and instructions for storage prior to test, the period of test and germination protocols (table 1). For two species, two protocols were tested, one that already existed in the ISTA Rules for cultivated species and one proposed by Scotia Seeds for wildflower species. Guidelines for assessment of physiological germination, normal and abnormal seedlings and recording of results were also provided. All data were returned to Scotia Seeds for statistical analysis.

The results from this trial were subjected to an ANOVA, Z score analysis and ISO-5725 analysis (tables 3, 4; figs. 1, 2).

Table 1. Germination protocols for wildflower seeds

Species	Pretreatment	Method of germination	Temperature of germination (°C)	Light regime
Achillea millefolium (proposed protocol*)	None	TP, H_2O	25	12/12
Achillea millefolium (existing protocol*)	None	TP, H_2O	20–30	8/16
Leucanthemum vulgare (proposed protocol*)	None	TP, H_2O	20	12/12
Leucanthemum vulgare (existing protocol*)	None	TP, H_2O	20–30	8/16
Chenopodium album	None	TP, KNO_3	15	12/12
Vicia cracca	Scarify: chip seed coat with scalpel (approx. 2 mm)	PP, H_2O	20	12/12
Hypochaeris radicata	None	TP, H_2O	20	12/12

* Proposed protocol for testing wild flower species; Existing protocol in the ISTA Rules for cultivated species.

The individual species were analysed by a one-way ANOVA, and the laboratory and species means were analysed by two-way ANOVA. The Z score analysis was used to give an indication of the performance of each of the participating laboratories in comparison to all others. The ISO 5725 was used to assess the tendencies of laboratories to over- or underestimate results, and further to assess the variation between repeats and reproducibility across laboratories.

Results

Seed quality survey

Large differences in the percentage of total germination were found between samples of the same species (table 2). For example, in the case of *Primula veris*, germination ranged from 0 to 90%, and for *Leucanthemum vulgare* germination ranged from 7 to 87%. Of the 60 samples tested, 13 had germination below 25%. One sample of *Achillea millefolium* reached the maximum 100% total germination (i.e. all seeds achieved physiological germination or more), whereas in *Papaver rhoeas* the maximum was only 50%. Since all the germination data were based on total germination, it is likely that the normal germination percentage of the samples would be lower than shown here.

There were also differences in the overall quality of seed from different companies with, for example, company 5 supplying seeds with better overall germination rates than company 4.

Purity testing also showed differences in the proportion of inert matter found in some of the samples. In the case of *Leucanthemum vulgare* purity ranged from 86.3 to 100% and for *Ranunculus acris* and *Papaver rhoeas* 82.6 to 100% and 82.3 to 100%, respectively.

Table 2. Comparison of total germination* and purity of wildflower seeds from different online sources

a) Germination

Company source	Species*								
	A	B	C	D	E	F	G	H	I
1	49	64	3	20	93	22	–	45	95
2	61	61	58	3	92	74	–	81	76
3	48	86	52	28	–	88	–	–	67
4	0	20	20	–	88	30	44	–	13
5	90	87	60	23	85	92	–	83	78
6	34	69	65	–	3	57	–	88	75
7	51	7	–	9	98	–	37	92	56
8	81	78	77	50	52	74	9	100	–

b) Purity

Company source	Species								
	A	B	C	D	E	F	G	H	I
1	100	97.4	99.2	99.2	97.4	100	–	96.5	99.8
2	100	98.7	99.4	98.8	96.3	99.4	–	97.2	99.5
3	98.7	91.5	97.3	88.3	–	97.9	–	–	95.4
4	99.1	99.2	82.6	–	95.6	100	85	–	99.2
5	100	86.3	97.0	82.3	93.7	97.7	–	91.9	99.4
6	92.4	95.2	99.7	–	93.5	98.8	–	98.3	98.7
7	92.4	95.4	–	100	97.7	–	100	100	95.5
8	100	100	99.3	100	100	100	100	99.3	–

* Total germination = all seeds that achieve at least physiological germination, i.e. a 2 mm radicle. A = *Primula veris;* B = *Leucanthemum vulgare;* C = *Ranunculus acris;* D = *Papaver rhoeas;* E = *Prunella vulgaris;* F = *Silene dioica;* G = *Ajuga reptans*; H = *Achillea millefolium;* I = *Galium verum.*

Table 3. ANOVA on normal germination assessed by 7 different laboratories (%)

Species	Laboratory							Mean[1]
	1	2	3	4	5	6	7	
Achillea millefolium (Proposed protocol*)	92^{a}	93^{a}	95^{a}	78^{b}	69^{c}	92^{a}	94^{a}	88^{a}
Achillea millefolium (Existing protocol*)	90^{a}	93^{a}	91^{a}	90^{a}	64^{b}	94^{a}	90^{a}	87^{a}
Leucanthemum vulgare (Proposed protocol*)	59^{ab}	57^{b}	65^{a}	61^{ab}	59^{ab}	61^{ab}	66^{a}	61^{b}
Leucanthemum vulgare (Existing protocol*)	58^{a}	61^{a}	60^{a}	48^{b}	61^{a}	61^{a}	57^{a}	58^{b}
Chenopodium album	47^{c}	19^{d}	76^{a}	27^{d}	63^{b}	48^{c}	54^{c}	48
Vicia cracca	75^{abc}	80^{ab}	71^{c}	77^{abc}	74^{abc}	74^{bc}	80^{a}	76
Hypochaeris radicata	57^{a}	64^{a}	59^{a}	44^{b}	58^{a}	62^{a}	50^{b}	56
Mean[1]	68^{BC}	67^{C}	74^{A}	61^{E}	64^{DE}	70^{B}	70^{B}	

Values with different superscripts are significantly different.
* Proposed protocol for testing wild flower species; existing protocol in the ISTA Rules for cultivated species.
[1] Means of species and laboratories analysed by 2-way ANOVA; lab means for single species analysed by 1-way ANOVA.

Comparative germination tests

The overall means of the seven laboratories involved in the comparative test revealed small, but significant differences in normal germination between laboratories (table 3). However, analysis of the total germination data reported by the laboratories (not shown) showed fewer differences. This suggested that some of the differences seen in the overall means of normal seedlings may be attributable to assessment of normal seedlings in often unfamiliar species.

For the individual species tested, comparative germination test results (table 3) for *Achillea millefolium* and *Leucanthemum vulgare* showed no significant differences between the existing protocol for cultivated species and the proposed protocol for wild species. Similarly, the results for *Vicia cracca* and *Hypochaeris radicata* showed no significant differences across laboratories. Only *Chenopodium album* gave unacceptable differences across laboratories.

The Z scores (table 4) revealed that in only 2 out of 49 instances were the data outside acceptable limits, and these were for existing protocols.

The H values (fig. 3) showed small, and acceptable over- and underestimations across all laboratories and all species. One laboratory (Lab 5, fig. 3) showed significant underestimation of germination for *Achillea millefolium* (existing) and another (Lab 4) did so for *Leucanthemum vulgare* (existing). The K values (fig. 4) showed small, acceptable variations across all laboratories and species. One laboratory (Lab 3; fig. 4) showed significant variation for *Hypochaeris radicata*.

Table 4. Z score analysis of normal germination results (%)

Species	Laboratory						
	1	2	3	4	5	6	7
Achillea millefolium (Proposed protocol*)	0.47	0.52	0.7	–0.95	–1.86	0.45	0.67
Achillea millefolium (Existing protocol*)	0.03	0.55	0.33	0.24	–2.24*	0.62	0.24
Leucanthemum vulgare (Proposed protocol*)	–0.56	–1.32	1.18	–0.18	–0.56	–0.03	1.48
Leucanthemum vulgare (Existing protocol *)	0.01	0.59	0.43	–2.11*	0.7	0.64	–0.26
Chenopodium album	–0.02	–1.45	1.44	1.07	0.75	0.03	0.31
Vicia cracca	–0.02	1.21	–1.47	0.27	–0.53	–0.59	1.28
Hypochaeris radicata	0.09	1.09	0.38	–1.74	0.24	0.85	–0.91

* Proposed protocol for testing wild flower species; existing protocol in the ISTA Rules for cultivated species.

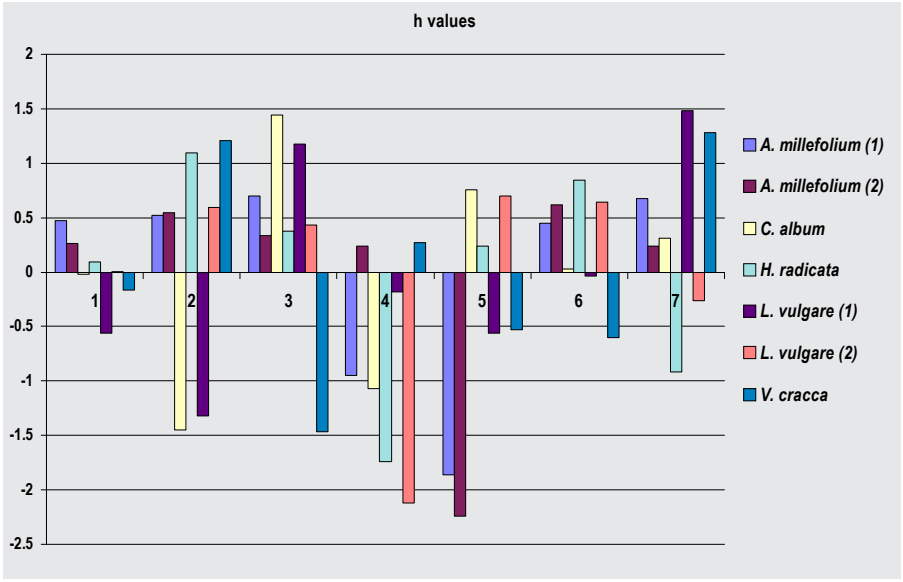

Figure 3. Results of ISO 5725 analysis (H values) for normal germination of 5 species (2 alternative protocols) tested in 7 laboratories. Note: species followed by number 1 denotes proposed protocol, number 2 denotes existing protocol.

Discussion

The survey results revealed quality problems in a high proportion of the wildflower seed lots being sold in the UK market, with some lots being clearly unsuitable for planting because of very low (or no) germination. This was the case even though the less demanding criterion of total germination (production of at least a 2 mm radicle) was used in the germination test. In some species, even the highest levels of germination found were quite low, and work to establish whether there is potential to improve on these levels may be worthwhile. Most of the suppliers provided seed with poor germination in at least one species, suggesting that they could all improve quality control, and some offer seed which is inferior to other companies overall. The variability within species may be due to differences in field, processing or storage factors, and there is a clear need to identify the causes of the problems. Variation in purity may also be partly due to field factors, but is also strongly affected by processing, and there is clearly potential for improvement in the case of some suppliers. This data suggests that there is a strong case for the introduction of more regular quality control in the wildflower seed trade in the UK to prevent inferior seeds being sold.

The comparative germination trial demonstrated that reliable and repeatable methods for testing the germination of wildflower seeds can be developed. For *Achillea millefolium* and *Leucanthemum vulgare*, the results showed that the proposed protocols could be used as an alternative to the existing protocols in the ISTA Rules. Similarly, the protocols developed

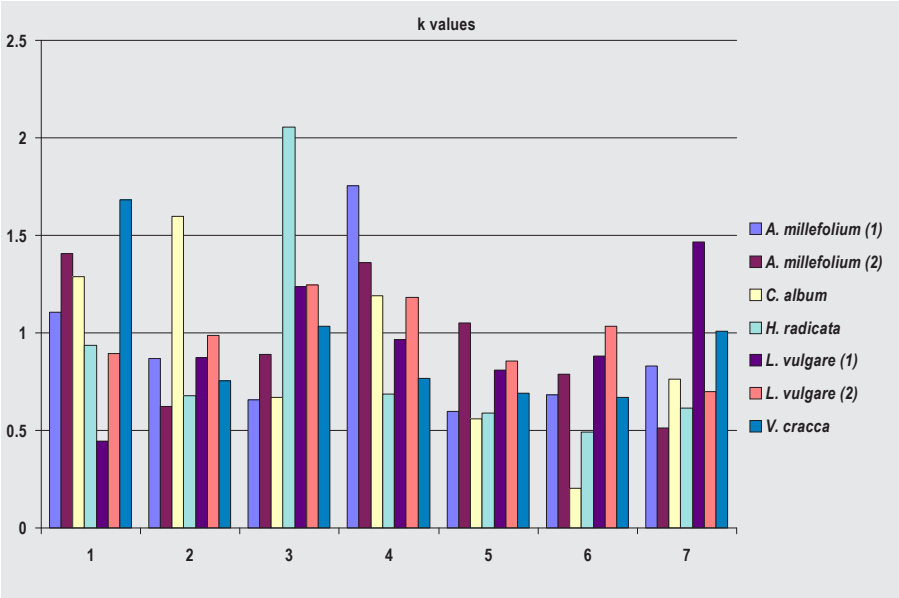

Figure 4. Results of ISO 5725 analysis (K values) for normal germination of 5 species (2 alternative protocols) tested in 7 laboratories. Note: species followed by number 1 denotes proposed protocol, number 2 denotes existing protocol.

for *Vicia cracca* and *Hypochaeris radicata* could be adopted for the testing of these species. The tests for *Chenopodium album* showed unacceptable differences between laboratories, and further work is necessary. It may be that the dormancy was not consistently broken in this species, or that small differences in the germination testing procedure used by different laboratories (e.g. germination paper, moisture regime) may have influenced germination. In species such as these, for which there is little testing experience, the inclusion of total germination, which includes any seed that achieves physiological germination (2 mm radicle), is particularly useful. Thus, where there are differences between laboratories in the percentage of normal germination, the observation of similar total germination results from the same laboratories suggests that the laboratories differ in their evaluation of normal and abnormal seedlings.

The establishment of appropriate and repeatable germination protocols for wild flower species is the first step towards quality control in these species. In our work to date we have developed protocols for germination testing of 150 UK wildflower species. This could form the basis for establishing quality control in UK wildflower seed production.

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