Research Article

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Germination requirements for 29 terrestrial and wetland wild plant species appropriate for phytotoxicity testing

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Abstract

BACKGROUND: Species selected for phytotoxicity testing have been limited to a few standard crop species owing to restrictive recommendations at the regulatory level. However, guidelines by the Organisation for Economic Development and Cooperation (OECD) were recently amended in 2006 to include a list of herbaceous non-crop plant species suitable for testing. The objective of this study was to outline the optimum germination requirements for a selection of wild species for which seeds were readily available from commercial suppliers.

RESULTS: Of the 29 herbaceous terrestrial and wetland species included in this study, all achieved 50% germination and 23 reached >70% germination to meet the criterion outlined in the OECD guidelines. Most species attained their maximum germination within 14 days or less. Cold stratification of imbibed seeds improved germination for 14 species. Increasing sowing soil depth did not improve seed germination. The variance attained in this experiment between replicates was low, especially for species with >70% germination (standard error ∼5%).

CONCLUSION: The present study showed that 23 of the 29 species tested required minimal pretreatments and produced consistent, reliable and uniform germination reaching at least 70%. The inclusion of wild plant species in regulatory testing should be given real consideration.

Keywords: non-target plant testing; regulatory studies; pesticide assessment guidelines; non-crop plant species; herbaceous plants

1 INTRODUCTION

Originally, pesticide registration guidelines, such as those published by the United States Environmental Protection Agency (EPA)[1,2] and the Organisation for Economic Cooperation and Development (OECD),[3] aimed to protect agronomically important plants such as crops and ornamentals from accidental exposure to herbicides. A limited number of annual herbaceous crop species have been relied upon to represent the herbicide sensitivity of all plants, including non-crop species. However, the risk of herbicide exposure posed to natural habitats (e.g. field margins) is of increasing concern, and the associated negative effects have been well documented.[4–11] As evidence of the detrimental effects on plant species adjacent to areas of herbicide application mounts, the topic of species selection in pesticide regulatory guidelines has been brought to the forefront.

There has been much discussion and controversy surrounding the use of crop species as surrogates for the highly diverse group of terrestrial plant species that exist in natural habitats. Whether or not crops can adequately represent non-crop species with regard to herbicide sensitivity is a long-standing debate. Emphasis has been placed on whether differences in herbicide sensitivity exist between crops and wild species,[12–18] as well as whether non-crop species are more difficult to germinate and/or propagate than crops.[19–21]

There are many advantages to using crop species in phytotoxicity tests for regulatory purposes: they are readily available, require no special treatment prior to sowing, and usually have consistent and reliably high rates of germination.[19] As crops are easy to work with and have been relied upon exclusively in regulatory risk assessments, there has been resistance to including non-crop species in regulatory protocols. This resistance is mainly due to the frequently unknown (or not widely available in the open literature) germination and emergence characteristics (e.g. uniformity, reliability, speed of germination) of wild plant species. The new OECD terrestrial plant testing guidelines (208 and 227)[22,23] have been amended to include a list of 52 herbaceous non-crop plant species suitable for phytotoxicity testing in addition to the crop species previously utilised. All of the species added to the new OECD guidelines were selected from peer-reviewed studies, where they were successfully used in phytotoxicity testing.

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Table 1. Herbaceous plant species included in germination tests. Nomenclature from Gleason and Cronquist.[35] Species are further indicated as dicotyledons (dicot) or monocotyledons (monocot). Life span is represented as annual (A), biennial (B) or perennial (P). Habitat categories are defined as follows:[36] WET = almost always found in wetlands under natural conditions (>99% probability); FACW = usually occurs in wetlands, but occasionally found elsewhere; FAC = equally likely to occur in wetlands or non-wetland habitats; FACU = occasionally occurs in wetlands but usually found elsewhere; UPL = almost never occurs in wetlands under natural conditions (<1% probability)

Scientific name	Common name	Family	Group	Life span	Habitat	Average weight seed^{-1} (mg)
Abutilon theophrasti Medikus	Velvet-leaf	Malvaceae	dicot	A	FACU	9.75
Asclepias incarnata L.	Swamp milkweed	Asclepiadaceae	dicot	P	WET	5.29
Asclepias syriaca L.	Common milkweed	Asclepiadaceae	dicot	P	UPL	5.32
Aster lateriflorus L. Britton	Goblet-aster	Asteraceae	dicot	P	FACW	0.33
Avena fatua L.	Wild oats	Poaceae	monocot	A	UPL	18.01
Bellis perennis L.	English daisy	Asteraceae	dicot	P	UPL	0.13
Centaurea cyanus L.	Cornflower	Asteraceae	dicot	A	UPL	3.81
Chelone glabra L.	White turtlehead	Scrophulariaceae	dicot	P	WET	0.46
Chrysanthemum leucanthemum L.	Ox-eye daisy	Asteraceae	dicot	P	UPL	0.47
Circaea lutetiana L.	Common enchanter's nightshade	Onagraceae	dicot	P	FACU	2.61
Digitalis purpurea L.	Common foxglove	Scrophulariaceae	dicot	B/P	UPL	0.11
Echium vulgare L.	Blue-weed	Boraginaceae	dicot	B	UPL	2.63
Elytrigia dasystachya (Hook.) A. Löve	Thick-spike wheatgrass	Poaceae	monocot	P	FACU	2.83
Eupatorium maculatum L.	Spotted Joe-pye weed	Asteraceae	dicot	P	WET	0.30
Euthamia graminifolia (L.) Nutt	Common flat-topped goldenrod	Asteraceae	dicot	P	FACW	0.06
Galium aparine L.	Cleavers	Rubiaceae	dicot	A	FACU	10.27
Geum canadense Jacq	White avens	Rosaceae	dicot	P	FAC	0.13
Inula helenium L.	Elecampane	Asteraceae	dicot	P	UPL	1.86
Ipomoea hederacea Jacq	Ivy-leaved morning glory	Convolvulaceae	dicot	A	FAC	30.70
Lycopus americanus Muhl	American water-horehound	Lamiaceae	dicot	P	WET	0.16
Lythrum salicaria L.	Purple loosestrife	Lythraceae	dicot	P	WET	0.05
Phalaris arundinacea L.	Reed canary-grass	Poaceae	monocot	P	FACW	0.89
Prunella vulgaris L.	Self-heal	Lamiaceae	dicot	P	UPL	0.74
Rudbeckia hirta L.	Black-eyed Susan	Asteraceae	dicot	B/P	FACU	0.24
Rumex crispus L.	Curly dock	Polygonaceae	dicot	P	FAC	1.25
Solanum nigrum L.	Black nightshade	Solanaceae	dicot	A	FAC	0.45
Solidago canadensis L.	Common goldenrod	Asteraceae	dicot	P	FACU	0.07
Verbascum thaspus L.	Common mullein	Scrophulariaceae	dicot	B	UPL	0.12
Verbena hastata L.	Common vervain	Verbenaceae	dicot	P	FACW	0.28

In order for a species to be considered suitable for use in regulatory risk assessments, one of the most important criteria is that the minimum germination requirement (>70%) be reached under standardised conditions.[22,23] There are concerns that, although this level of germination is easily achievable for most crop species, attaining the same level of success with non-crop species will be more difficult. The quantity of work that has focused on establishing standard germination protocols for regulatory guideline purposes is quite limited,[19–21] in spite of the wealth of literature devoted to determining the germination requirements of wild plant species for a range of different experimental purposes.[24–29] Previous work that has investigated the germination requirements of wild species for consideration in regulatory guidelines has had variable success.[19–21]

The objective of the present study was to determine the germination requirements for a selection of non-crop species for possible inclusion in regulatory phytotoxicity testing, using minimal or no pretreatment under standardised conditions. The species selected represented a wide taxonomic group that includes herbaceous species with differing life cycles and habits, as well as species from the new OECD guidelines.[22,23]

2 EXPERIMENTAL METHODS

Seed germination tests were performed using 29 herbaceous wild plant species representing 15 different families (Table 1). All seeds were obtained from various commercial seed suppliers from different regions of North America and Europe, except for two species, *Verbena hastata* L. and *Circaea lutetiana* L., which were personally gathered by the authors.

Three pretreatments involving a 0, 1, or 2 month cold, wet stratification period and three different environments (a greenhouse and two growth chambers that differed in light intensity and temperature) were utilised. Light and temperature differences between the three different environments are shown in Table 2. Each species was subjected to every combination of stratification period and environment for a total of nine experimental treatments. For each experimental treatment, three replicates of 50 seeds each were included; for three species (*Asclepias syriaca* L., *Galium aparine* L. and *Ipomoea hederacea* Jacq), 30 seeds per replicate were used in some treatments owing to a depleted seed supply. In the case of *A. syriaca*, 30 seeds per petri dish were used only in the no-stratification treatment in growth chambers. For *G. aparine,* 30 seeds per replicate were used for the 1 month stratification in both growth chambers and all locations in the 2 month stratification treatment. Thirty seeds

Table 2. Minimum and maximum temperature (°C) and the average amount of photosynthetically active radiation (PAR) (photons cm^{-2} s^{-1}) found in each experimental location used for germination tests. A 16 : 8 hour light/dark photoperiod was provided in all growing environments

Location	Average minimum temperature	Standard deviation	Average maximum temperature	Standard deviation	PAR
Greenhouse	18.6	2.7	38.3	6.8	1.8×10^{16}
Growth chamber 1	15.4	0.7	28.1	1.4	1.4×10^{16}
Growth chamber 2	15.3	0.2	25.1	0.9	1.3×10^{16}

per petri dish were used in all replicates with *I. hederacea*, except in the 1 month stratification in both growth chambers, where 50 seeds were used. In all cases, three replicates per treatment were used as recommended by Baskin and Baskin.[28]

Seeds were surface sown in extra-deep petri dishes (100 mm diameter × 20 mm deep) filled with 1 sand : 3 Promix (containing approximately 9.5% organic matter and consisting of soil that is 75% sand, 17% silt and 8% clay; Premier Horticulture Inc., PA), following procedures described by Baskin and Baskin.[28] Dishes were top watered as required to ensure that neither the seeds nor the soil dried out, and monitored for a 28–31 day test period. For replicates that underwent stratification, seeds were sown as described above, and plastic covers were placed on each dish and secured with laboratory film to prevent moisture loss during the stratification period. Stratification occurred in a dark refrigerator at 2–4 °C, and, once complete, the petri dishes were placed in the appropriate environments and monitored in the same way as non-stratified replicates. Germination was recorded every 2 or 3 days and at the end of the test period. Seeds were removed as they germinated.

In the case of five species (*Abutilon theophrasti* Medikus, *Asclepias incarnata* L., *Avena fatua* L., *Chelone glabra* L. and *Elytrigia dasystachya* (Hook.) A. Löve), an additional treatment was performed to determine if soil covering improved germination. The same protocol was used, the only modification being that seeds were evenly covered with approximately 15 mL of the aforementioned soil mixture (enough to cover the seeds adequately). Based on the germination achieved in the initial round of testing, a stratification period was selected for a total of three additional experimental treatments (three locations) for the species selected for further evaluation. Of the five species that underwent additional testing, *A. fatua* (both dormant and non-dormant seeds) and *E. dasystachya* were not stratified. *Abutilon theophrasti, A. incarnata* and *C. glabra* were subjected to a month-long stratification period.

Upon completion of testing, the average total percentage germination for each of the species in each of the experimental treatments was determined. The data were arcsin transformed in order to meet the assumptions of homogeneity of variance and normality of residuals, and germination differences between experimental treatments were determined for each species using a two-way analysis of variance (ANOVA). Data were also summarised to indicate the minimum number of days required to reach both 50% and 70% germination (if applicable).

3 RESULTS

Of the 29 herbaceous species included in the present study, all of them achieved a minimum 50% germination and 23 of them reached >70% to meet the criterion outlined in the new OECD terrestrial plant guidelines[22,23] (Table 3).

Most of the 29 species reached their maximum germination in 7 days or less. As noted above, all species tested reached a minimum 50% germination, 22 of them in 1 week or less, with six species taking up to 14 days. The only exception was *Geum canadense* Jacq, which took 31 days to reach 50%. Of the 23 herbaceous species tested that reached the 70% criterion of the new OECD terrestrial plant guidelines, 18 of them achieved this in a week or less, with an additional five species reaching >70% in 8–14 days (Table 3). Variability among the replicates was generally quite low for the majority of species reaching >70% germination, exhibiting a standard error of <5%.

The stratification period proved to be an important factor in germinating the seeds of 14 of the species tested; six had significantly higher germination after a 1 month cold stratification period, with the remaining eight species preferring at least 2 months of cold stratification. For the other 15 herbaceous species tested, cold stratification did not significantly improve germination and is therefore not required (Table 3). It is important to note that, for two of the latter species, *I. hederacea* (Table 3b) and *Inula helenium* L. (Table 3c), cold stratification had a significantly negative effect on germination and is not recommended.

Interestingly, most of the wetland species tested required a minimum 1 month cold stratification period (Table 3a), while a large number of upland and facultative species preferred no cold treatment for optimal germination (Tables 3b and c). Lifespan did not seem to affect whether cold stratification was needed for optimal germination when considering perennial species. However, it should be noted that the majority of short-lived species (annual and biennial) did not require cold stratification to achieve their highest germination.

In general, the environment (locations with various light intensities and temperature regimes) in which the seeds were grown did not significantly affect germination for the majority of herbaceous species included in the present study (Table 3). However, if considering the natural habitats of the species tested (wetland versus upland species), the artificial growing environments were an important factor in attaining maximum germination. For the wetland species (Table 3a), four of the nine species preferred lower light conditions. In contrast, all of the facultative species (Table 3b) and most of the upland species (Table 3c) did not germinate significantly better in one environment compared with another.

A significant interaction existed between environment and cold stratification periods for some of the species tested (Table 3). The interaction between the two factors indicated that the effect of one factor was not independent of the influence of a particular level of the other factor. For example, germination of *A. incarnata*

and *Aster lateriflorus* (L.) Britton was enhanced in all locations when stratified for 2 months, but varied considerably in the different locations when not stratified or stratified for 1 month. A significant interaction also denoted some level of variability in response. For instance, although *Phalaris arundinacea* L., *Chrysanthemum leucanthemum* L. and *Verbascum thapsus* L. germinated perfectly well under all conditions, some variation existed among the different treatments.

None of the five species responded positively when seeds were covered (Table 3). For three species (*A. incarnata, C. glabra* and *E. dasystachya*), the purpose of this additional experiment was to determine if the stratification period could be reduced by covering seeds. All three species germinated better after 2 months stratification, although no significant difference occurred among treatments for *E. dasystachya*. In all three cases, covering the seeds with soil did not lessen stratification time. The two other species tested (*A. theophrasti* and *A. fatua*) did not reach the 70% germination threshold with uncovered seeds, and neither showed any improvement in germination when covered with soil (Table 3c). Moreover, for *A. fatua* (both dormant and non-dormant seeds) and *C. glabra*, covering the seeds significantly reduced germination compared with seeds sown on the surface, regardless of environment, and is not recommended (Table 3c).

4 DISCUSSION

In order for a species to be suitable for inclusion in regulatory guidelines, seeds must be readily available, require minimal

Table 3a. Results of germination tests involving wetland and facultative wetland species. Location refers to the greenhouse and the two growth chambers with different light intensities and temperature regimes. Stratification involves cold treatment for 0, 1 or 2 months. Sowing depth refers to covering seeds with soil or not. Statistics (two-way ANOVA) and recommendations for testing are presented

Species	Maximum germination (%)	Std error (%)	Minimum days to 50%	Minimum days to 70%	Experimental treatment	df	*F* value	*P* value	Recommendations
A. incarnata	77.3	0.7	4	11	Location	2	12.60	<0.001	Seeds germinate best in high light conditions with minimum 2 month stratification
					Stratification	2	50.18	<0.001	
					Interaction	4	4.83	0.008	
	69.3	2.4	8	n/a	Location*	2	3.81	0.052	Covering seeds does not improve germination, regardless of location
					Sowing depth*	1	22.37	<0.001	
					Interaction*	2	9.47	0.003	
A. lateriflorus	90.7	0.7	4	4	Location	2	7.26	0.005	Seeds germinate best in growth chambers. 2 month stratification recommended
					Stratification	2	30.99	<0.001	
					Interaction	4	4.79	0.008	
C. glabra	84.7	2.4	11	11	Location	2	16.27	<0.001	Seeds germinate best in low light conditions with minimum 2 month stratification
					Stratification	2	32.46	<0.001	
					Interaction	4	2.34	0.094	
	38.0	7.6	n/a	n/a	Location*	2	1.75	0.215	Covering seeds significantly reduces germination and is not recommended
					Sowing depth*	1	110.53	<0.001	
					Interaction*	2	6.34	0.013	
E. maculatum	56.7	4.8	8	n/a	Location	2	5.19	0.017	Seeds germinate best in low light conditions with minimum 1 month stratification required
					Stratification	2	17.32	<0.001	
					Interaction	4	3.41	0.031	
E. graminifolia	78.0	7.0	8	8	Location	2	1.71	0.209	Neither location nor stratification period significantly affected germination
					Stratification	2	1.17	0.332	
					Interaction	4	1.38	0.281	
L. americanus	84.7	2.7	4	7	Location	2	25.77	<0.001	Seeds germinate significantly better in the greenhouse than in the other locations. 1 month stratification gives best results
					Stratification	2	14.10	<0.001	
					Interaction	4	5.58	0.004	
L. salicaria	88.7	2.9	6	8	Location	2	7.56	0.004	Seeds germinated best in low light conditions with minimum 1 month stratification
					Stratification	2	24.64	<0.001	
					Interaction	4	0.64	0.642	
P. arundinacea	94.0	3.1	4	7	Location	2	71.24	<0.001	Some variability in germination but always greater than 75% under all conditions. Stratification not required
					Stratification	2	37.28	<0.001	
					Interaction	4	36.52	<0.001	
V. hastata	92.0	2.3	7	7	Location	2	0.91	0.422	Location did not affect germination. Minimum 1 month stratification required. Seeds only remain viable for <12 months
					Stratification	2	275.15	<0.001	
					Interaction	4	0.82	0.528	

* Results of the alternative treatment (where applicable).

Table 3b. Results of germination tests involving faculative species (found in both wetland and terrestrial habitats). Location refers to the greenhouse and the two growth chambers with different light intensities and temperature regimes. Stratification involves cold treatment for 0, 1 or 2 months. Sowing depth refers to covering seeds with soil or not. Statistics (two-way ANOVA) and recommendations for testing are presented

Species	Maximum germination (%)	Std error (%)	Minimum days to 50%	Minimumdays to 70%	Experimental treatment	df	*F* value	*P* value	Recommendations
G. canadense	58.7	7.7	31	n/a	Location	2	2.99	0.075	Location did not affect germination.
					Stratification	2	4.26	0.031	2 month stratification significantly
					Interaction	4	4.27	0.013	improves germination
I. hederacea	96.7	3.3	3	3	Location	2	0.95	0.405	Location did not affect germination.
					Stratification	2	274.96	<0.001	Stratification had a significantly negative
					Interaction	4	2.28	0.100	effect on germination and is not recommended
R. crispus	97.3	1.8	6	6	Location	2	0.29	0.752	Neither location nor stratification
					Stratification	2	0.97	0.399	significantly affected germination
					Interaction	4	2.23	0.106	
S. nigrum	66.7	2.7	8	n/a	Location	2	0.29	0.751	Neither location nor stratification
					Stratification	2	0.59	0.567	significantly affected germination
					Interaction	4	1.07	0.402	

* Results of the alternative treatment (where applicable).

pretreatments and produce consistent, reliable and uniform germination reaching at least 70%.[22,23] A majority of species used in this experiment exhibited the required seed characteristics acceptable for phytotoxicity testing.

Seeds selected for this study were accessible from commercial seed sources or easy to collect (*V. hastata* and *C. lutetiana*). Although wild-collected seeds were utilised for these two latter species, they are also available from commercial suppliers. Seeds of wild species were often smaller in size than those of many crops; however, their handling was never problematic. It should be noted that seeds of lettuce (*Lactuca sativa* L., 0.001 g seed^{-1}), carrot (*Daucus carota* L., 0.001 g seed^{-1}) and onion (*Allium cepa* L., 0.004 g seed^{-1}), for example, are much smaller than those of many of the wild species used in this study (Table 1).

Basic seed treatments were necessary for most species included in the present study in order to obtain maximum germination. For instance, many wild species require a period of cold treatment prior to germination, which is an adaptation to seasonal variability typical of temperate regions. Also, species with small seeds usually require light to germinate, and light intensity requirements are often related to the type of habitat in which the species usually grow. Specific germination conditions were uncomplicated to achieve and did not require expensive equipment or extensive additional effort.

Most species tested germinated in less than 14 days. This is acceptable for the usual 3–4 week study duration of the seedling emergence test.[22,23] For the vegetative growth test, the time to germinate is unproblematic if extra seeds are sown and additional seedlings are discarded. The authors agree with Cole *et al.*[13] that 14 days is an acceptable length of time for germination to occur.

Of the 29 species tested in this study, 23 met the criteria outlined in the OECD guidelines with regard to the acceptable level of germination. Contrary to the mostly annual crop species typically used in phytotoxicity testing, the wild species selected in the current study encompassed a wide variety of life histories. Many were long-lived species, including 16 herbaceous perennials, and only a few were short-lived species (including three annuals and four biennial and/or short-lived perennials). Species exhibiting different morphologies were also represented. For example, small low-growing species such as *Bellis perennis* L. and *Prunella vulgaris* L., tall species such as *Solidago canadensis* L., *V. thapsus* and *Lythrum salicaria* L. and also grass species such as *E. dasystachya* and *P. arundinacea* were included. All the species selected for testing represented various types of habitat, ranging from terrestrial to wetland environments. Eleven families were represented among the 23 species that met the OECD criteria, many of which are not included when only crops are tested (e.g. Asclepiadaceae, Boraginaceae, Convolvulaceae, Lamiaceae, Lythraceae, Rubiaceae, Scrophulariaceae and Verbenaceae). The present study showed that it is possible to meet all of the aforementioned OECD guideline criteria using wild plant species, and hence their inclusion in regulatory testing should be given real consideration.

One of the species for which sufficient germination was not attained, *A. fatua*, has previously been considered to be a good candidate for phytotoxicity testing.[13] Fresh seeds of this species will germinate readily, but, when allowed to dry out, embryo water content decreases, dormancy is induced and germination is more difficult to obtain.[28] In the present study, seeds considered non-dormant by the suppliers germinated more rapidly than the dormant seeds, although for both batches the 70% threshold was not achieved. Conversely, several of the species that germinated satisfactorily in this study had not exhibited the same success in previous attempts.[19–21] In all cases, the discrepancy was probably due to a lack of basic pretreatment conditions and the utilisation of incorrect sowing depth. Contrary to most crop species, many wild species will germinate better when surface sown under specific light regimes, as was observed in this study.

Variability among populations or ecotypes of the different study species was not measured in the present study. Preliminary data suggest that variability among ecotypes may exist. These differences may be due to seed storage conditions and/or age, as well as inherent genetic differences. More research is warranted on the variability in dormancy and germination response, which may be due to differential genetic and environmental conditions. In most crop species, dormancy has been removed to ensure rapid

Table 3c. Results of germination tests involving upland and faculative upland species. Location refers to the greenhouse and the two growth chambers with different light intensities and temperature regimes. Stratification involves cold treatment for 0, 1 or 2 months. Sowing depth refers to covering seeds with soil or not. Statistics (two-way ANOVA) and recommendations for testing are presented

Species	Maximum germination (%)	Std error (%)	Minimum days to 50%	Minimum days to 70%	Experimental treatment	df	*F* value	*P* value	Recommendations
A. theophrasti	68.0	3.5	3	n/a	Location	2	37.93	<0.001	Germination best in higher light
					Stratification	2	48.12	<0.001	locations. 1 month
					Interaction	4	2.76	0.059	stratification recommended
	63.3	1.8	3	n/a	Location*	2	3.05	0.085	Covering seeds does not
					Sowing depth*	1	29.04	<0.001	improve germination,
					Interaction*	2	0.45	0.649	regardless of location
A. syriaca	86.7	2.4	6	6	Location	2	19.57	<0.001	Germination best if seeds grown
					Stratification	2	117.42	<0.001	in growth chambers rather
					Interaction	4	0.80	0.542	than greenhouse. Stratification for 2 months recommended
A. fatua (dormant seeds)	63.3	4.1	6	n/a	Location	2	0.45	0.642	Slightly higher germination with
					Stratification	2	3.57	0.049	no stratification. Location
					Interaction	4	0.80	0.539	does not significantly affect germination
	32.7	0.7	n/a	n/a	Location*	2	0.76	0.491	Covering seeds significantly
					Sowing depth*	1	63.00	<0.001	reduces germination and is
					Interaction*	2	0.53	0.600	not recommended
A. fatua (non-dormant seeds)	64.0	4.2	1	n/a	Location	2	0.34	0.715	Location did not significantly
					Stratification	2	8.38	0.003	affect germination.
					Interaction	4	0.59	0.676	Stratification not required
	33.3	2.4	n/a	n/a	Location*	1	0.10	0.762	Covering seeds significantly
					Sowing depth*	1	27.30	0.001	reduces germination and is
					Interaction*	1	1.11	0.324	not recommended
B. perennis	92.0	2.0	3	3	Location	2	13.36	<0.001	Germination best if seeds grown
					Stratification	2	12.96	<0.001	in low light conditions with
					Interaction	4	2.74	0.061	cooler temperatures. Stratification not required
C. cyanus	91.3	1.8	2	2	Location	2	0.16	0.855	Neither location nor
					Stratification	2	2.02	0.161	stratification period
					Interaction	4	0.69	0.606	significantly affected germination
C. lutetiana	60.0	14.2	14	n/a	Location	2	1.25	0.310	Location does not significantly
					Stratification	2	43.51	<0.001	affect germination.
					Interaction	4	2.29	0.099	Stratification for 2 months recommended. Seeds probably require maturation period of 12 months
C. leucanthemum	100.0	0.0	4	4	Location	2	7.90	0.003	Germination best if seeds
					Stratification	2	25.24	<0.001	stratified for 2 months, but
					Interaction	4	6.49	0.002	good germination under all conditions
D. purpurea	94.7	0.7	10	13	Location	2	0.49	0.620	Location did not significantly
					Stratification	2	4.26	0.030	affect germination.
					Interaction	4	1.49	0.248	Stratification not required
E. vulgare	90.7	3.5	6	6	Location	2	6.92	0.006	High temperature and light
					Stratification	2	5.59	0.013	levels significantly improve
					Interaction	4	14.87	<0.001	germination. Stratification for 1 or 2 months significantly improved germination
E. dasystachya	75.3	1.8	1	1	Location	2	0.86	0.438	Location did not significantly
					Stratification	2	0.59	0.566	affect germination.
					Interaction	4	1.79	0.175	Stratification not required

Table 3c. (*Continued*)

Species	Maximum germination (%)	Std error (%)	Minimum days to 50%	Minimum days to 70%	Experimental treatment	df	*F* value	*P* value	Recommendations
	69.3	5.8	8	n/a	Location*	1	0.48	0.508	Covering seeds does not improve
					Sowing depth*	1	143.07	<0.001	germination, regardless of
					Interaction*	1	0.27	0.619	location
G. aparine	81.1	2.9	1	1	Location	2	0.33	0.726	Location did not significantly affect
					Stratification	2	268.58	<0.001	germination. Stratification for
					Interaction	4	1.05	0.412	2 months recommended
I. helenium	87.3	0.7	3	3	Location	2	0.09	0.914	Location did not significantly affect
					Stratification	2	9.52	0.002	germination. Stratification
					Interaction	4	0.26	0.902	significantly reduces germination and is not recommended
P. vulgaris	96.0	1.2	3	3	Location	2	0.46	0.639	Location did not significantly affect
					Stratification	2	0.24	0.791	germination. Stratification not
					Interaction	4	0.12	0.972	required
R. hirta	92.0	1.2	4	4	Location	2	35.94	<0.001	High temperature and light levels
					Stratification	2	112.16	<0.001	significantly improve
					Interaction	4	15.49	<0.001	germination. Stratification not required
S. canadensis	96.7	0.7	4	4	Location	2	0.18	0.838	Location does not affect
					Stratification	2	15.33	<0.001	germination. Stratification for
					Interaction	4	7.53	0.001	2 months recommended
V. thaspus	99.3	0.7	4	4	Location	2	0.18	0.838	Location does not affect
					Stratification	2	15.33	<0.001	germination. Germination >90%
					Interaction	4	7.53	0.001	in all treatments with slight variability in response

* Results of the alternative treatment (where applicable).

and even germination when sowing.[28] An additional concern prevailing in phytotoxicity testing and risk assessments relates to the range in responses which shapes the statistical variability. The variance attained in this experiment between replicates was usually low (<5%) when greater than 70% germination was reached.

Twelve of the species tested in this study were part of the proposed list of recommended species in the new OECD guidelines.[22,23] In ten cases, the percentage and time to germination achieved were well within the criteria deemed acceptable for phytotoxicity testing. This confirms the relevance of these species as good candidates, and, additionally, many have been used in other studies and as such were deemed amenable to testing in greenhouses.[22,23,30] Nonetheless, further work needs to be undertaken in order to expand the list of strong candidate species appropriate for phytotoxicity testing. Wild species that could be considered neglected candidates are agricultural weeds. These species germinate easily (which is not unexpected for species that rely on high germination for population survival), and they deploy rapid growth and complete their life cycle within a short time. They are often used in plant screening for pesticide efficacy testing, which gives them an extra advantage as candidates, since their germination and growth requirements are well known. In addition, many agricultural weed species are considered to be important food sources or shelter for wildlife.[31,32]

5 CONCLUSIONS

Species selection has been recognised as a major problem for all phytotoxicity tests currently required and submitted.[30,33,34] A satisfactory equilibrium is required in the use of agronomic and wild species, a balance that should take into account the broad variability that occurs among species, among ecotypes of wild species and also among crop cultivars.[18] The present study showed that 23 of the 29 wild species tested required minimal pretreatment, and produced consistent, reliable and uniform germination reaching at least 70%. If the goal of phytotoxicity testing is the protection of wildlife habitat and biodiversity as well as plants of agronomic interest, the range of testable species must be expanded, and a reference base for a comparative risk analysis must be established.

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