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Potential of the electrical conductivity of seed soak water and early counts of radicle emergence to assess seed quality in some native species

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Abstract

The potential of the electrical conductivity (EC) test to predict final germination was evaluated in seed lots from seven native species. In four of the seven species tested (*Cyanus segetum*, *Prunella vulgaris*, *Valeriana officinalis* and *Centaurea nigra*), EC was indicative of the final germination (radicle emergence; RE), with high levels of leakage seen for lots with low germination. Single seed measurements of solute leakage from two species confirmed the link between high leakage and the failure to germinate, while in *Cyanus segetum*, high EC was also associated with slow germination (after 42 hours). Reduced EC and earlier RE following a pre-hydration treatment in *C. segetum* supported the hypothesis that metabolic repair may occur during early imbibition. A single early count of RE (at 42 hours) also predicted germination ($R^2 \geq 0.858$) for 12 seed lots of *C. segetum*. Therefore, both measurements of solute leakage from seeds using EC and early counts of RE have potential to predict the germination of seed lots from native species. The use of the EC test may be dependent on the structure of the seed, but the RE test may be applicable to a wider range of species and predict both germination and vigour differences.

Keywords: electrical conductivity, embryo development, native seed, radicle emergence, seed germination, seed quality, seed vigour

Introduction

Seed quality of native, wild species is key to habitat restoration projects as it determines the potential of a seed to germinate and establish a plant. The awareness of its importance is rising globally (Gibson-Roy *et al.*, 2007; Merritt and Dixon, 2011; Broadhurst *et al.*, 2016; Nevill *et al.*, 2016), including amongst contractors and other practitioners involved in the

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large-scale reintroduction of native species (Haslgrübler *et al.*, 2014). However, a recent study by Marin *et al.* (2017) provided experimental evidence of quality problems in the European native seed market, with highly variable values of germination and viability between samples of the same species. Indeed, seed lots containing only non-germinating seeds were found in four of the eight tested species (Marin *et al.*, 2017). Nevertheless, there is still a lack of methods and routine protocols for assessing seed quality in native species (Ryan *et al.*, 2008; Nevill *et al.*, 2016). Laboratory germination testing is the most common approach for seed quality evaluation in native species as it provides information on a range of cues (*e.g.* temperature and light conditions) that, singly or in combination, may be needed, particularly if dormancy is present, for successful germination and emergence of seedlings under field conditions (Gibson-Roy *et al.*, 2007). However, the broad range of germination responses exhibited across native species (Baskin and Baskin, 2014) and the long periods of time necessary to achieve germination (Marin *et al.*, 2017) suggest the need for the development of effective and quicker alternatives.

The tetrazolium test is a valid alternative to germination testing as it enables rapid evaluation of seed quality in native species, with a clear prediction of germination achieved in only two days, regardless of the dormancy status of the seeds (Marin *et al.*, 2017). Recent evidence from agricultural crop seeds points to another potentially applicable test to native species, the electrical conductivity (EC) test. This test measures the leakage of electrolytes from bulks of seeds into soak water and has been developed as a seed vigour test, indicative of the field emergence of lots with high and acceptable levels of normal germination. The EC test has been validated by ISTA for radish and four grain legumes, garden peas, soya beans, *Phaseolus* beans and chickpea (ISTA, 2017). Legumes with large, normally living, cotyledons are good candidates for the EC test to indicate field emergence, because they still germinate in the laboratory even with considerable areas of dead tissue on their cotyledons, provided that critical areas of the embryo remain living (Matthews and Powell, 2006).

Assessments of the leakage of electrolytes using bulk seed samples have also been associated with assessments of normal standard germination. In artificially aged seeds of *Brassica* spp., higher EC was seen from non-germinating seeds and seeds that gave rise to abnormal seedlings (Mirdad *et al.*, 2006). Similarly, commercial seed lots with higher proportions of seeds that failed to produce a radicle, or that produced abnormal seedlings, gave higher EC in bulk tests in cabbage (Demir *et al.*, 2008a) and oilseed rape (Wagner *et al.*, 2012) and in artificially aged radish seeds (Demir *et al.*, 2012). More recently, Mavi *et al.* (2016) showed that in commercial seed lots of radish, the EC of bulks of seed after 1, 3, 5 and 24 hours of soaking was closely related to the proportion of non-germinating seeds and of abnormal seedlings, as well as to seeds that had slower RE. This was confirmed by the significant differences in leakage from single non-germinating and germinating seeds after 3 and 5 hours soaking (Mavi *et al.*, 2016). Similar relationships may also be a feature of some native species.

Other test methods have been developed to assess seed quality in agricultural species. The mean germination time (MGT), which is calculated from frequent counts of germination and is the average delay between imbibition and radicle emergence, was found to be predictive of emergence performance in pepper (Demir *et al.*, 2008b), maize

(Matthews and Khajeh-Hosseini, 2006; Khajeh-Hosseini *et al.*, 2009), watermelon, melon and cucumber (Mavi *et al.*, 2010). A single early count of radicle emergence (RE) was predictive of MGT in oilseed rape (Matthews *et al.*, 2012a) and radish (Mavi *et al.*, 2014) and subsequently of emergence. Therefore, MGT and early counts of RE have potential as useful tests for seed quality evaluation in native species.

The objectives of the current study were to: (1) investigate the potential of the electrical conductivity test to predict final germination of seed lots from seven native species in relation to seed structure; (2) examine the relationship between final germination and the length of the delay to radicle emergence (RE), as indicated by the mean germination time (MGT) and early counts of RE; and (3) determine if EC and single RE counts can be used as quick estimates of seed germination in some native species.

Materials and methods

Seed material

Samples from commercial seed lots of *Centaurea nigra* L., *Cyanus segetum* Hill., *Knautia arvensis* L., *Papaver rhoeas* L., *Prunella vulgaris* L., *Silene vulgaris* (Moench) Garcke and *Valeriana officinalis* L. were obtained from various seed companies in Europe. The species were selected as ones that are widespread and native to Europe, and commonly used in restoration projects, either as single species or in seed mixtures. A total of 83 seed samples were obtained across the species and suppliers. The seed samples will be referred to as seed lots in this paper. The seed lots were stored at 15°C and 15% relative humidity (RH) until the experiments were completed. The 1000 seed weight was measured on the basis of eight replicates of 100 seeds (equilibrated with the storage conditions) for each lot using an electronic 4-place balance.

Germination tests

For all seven species, eight replicates of 25 seeds per lot were placed in 90 mm-diameter Petri dishes on germination paper (Whatman, GE Healthcare Life Sciences) moistened with 2.5 ml gibberellic acid (GA₃; 250 mg l⁻¹) to release any dormancy and enhance germination (Marin *et al.*, 2017). The Petri dishes were then placed in plastic bags to prevent water loss during the test and held under controlled conditions at an alternating temperature of 25/10°C, with a diurnal period of 12 hours-light and 12 hours-darkness. During incubation (four weeks), germination was scored weekly as radicle emergence (RE) and seeds were removed when RE had occurred.

The mean germination time was measured for *Cyanus segetum* during a subsequent germination test. Eight replicates of 25 seeds per lot were placed to germinate as described above and counts of radicle emergence were made twice a day for four weeks. The mean germination time (MGT) was calculated using the formula:

$$\text{MGT} = \Sigma (n_i \times t_i) / N$$

where n_i is the number of seeds that germinated (2 mm radicle emergence) within consecutive intervals of time, t_i the time (hours) between the beginning of the test and

the end of a particular interval of measurements, and N the total number of seeds that germinated. As the above formula illustrates, MGT is the average delay between the start of imbibition and radicle emergence (RE).

Bulk electrical conductivity (EC) measurements

Four replicates of (a) 25 seeds (*Centaurea nigra*, *Cyanus segetum* and *Knautia arvensis*); (b) 50 seeds (*Prunella vulgaris* and *Silene vulgaris*); (c) 100 seeds (*Valeriana officinalis*); or (d) 1000 seeds (*Papaver rhoeas*) per lot were weighed using an electronic 4-place balance and soaked in 10 ml distilled water for 24 hours at 20°C. The conductivity of seed soak water was measured using a conductivity meter (4310, Jenway) after 24 hours and expressed per gram of seeds ($\mu\text{S cm}^{-1} \text{g}^{-1}$). In order to take into account the effect of possible electrolytes on the seed surface, a reading was taken two minutes after adding the water and subtracted from the 24-hour readings. The EC taken after two minutes never exceeded $24.10 \mu\text{S cm}^{-1}$ (recorded for one lot of *Knautia arvensis*). Conductivity readings were taken at 3 and 5 hours, as well as 24 hours for 12 seed lots of *Cyanus segetum*.

Comparison of single seed conductivity measurements and germination

Twenty seeds were drawn randomly from each of the 10 seed lots of *Centaurea nigra* and of 13 lots of *Cyanus segetum*. Single seeds were washed briefly in 8 ml distilled water for a few seconds, the water poured off and a further 8 ml water added to each seed. Electrical conductivity readings were taken after 3 and 5 hours at 20°C and expressed per seed ($\mu\text{S cm}^{-1} \text{seed}^{-1}$). The seeds were then placed in 90 mm-diameter Petri dishes on germination paper moistened with 2.5 ml gibberellic acid (GA_3 ; 250 mg l^{-1}) so that germination performance of each seed could be related to the EC readings. The plastic bags containing the Petri dishes were held under controlled conditions at an alternating temperature of 25/10°C, with a diurnal period of 12 hours-light and 12 hours-darkness. Counts of radicle emergence were made twice a day for four weeks for both species.

Effect of hydration treatment and drying back

Three lots of *Cyanus segetum*, that were characterised as including a proportion of both early and later germinating seeds (before or after 42 hours) were subjected to a hydration treatment in two runs. For each run, 25 seeds, drawn randomly from each lot, were weighed and washed briefly in distilled water for a few seconds. The 25 seeds were then placed in a 90 mm-diameter Petri dish on moist germination paper to hydrate in air for five hours at 25°C in the dark and subsequently allowed to dry back to their original weight on dry germination paper on the bench at 15°C and 15% RH. Twenty five seeds from the same lots that had not been hydrated were used as a control.

The electrical conductivity of single seeds was measured after five hours, followed by germination for both the hydrated seed and the control, as described above. The moist germination papers on which 25 seeds had been hydrated were washed in 40 ml distilled water and the EC of the solution was measured. In order to take into account the EC due to the germination paper only, three replicates of moist germination papers with no seeds were each held in a Petri dish for five hours at 25°C in the dark, subsequently washed and the EC of the solution was measured.

Internal morphology of seeds

Twenty seeds of each species were sown at 20°C in 90 mm-diameter Petri dishes on germination paper moistened with 2.5 ml distilled water. The embryo and endosperm area within the imbibed seeds were determined the following day. Ten seeds from each species were dissected, observations made with a stereomicroscope (Leica MZ FLIII, Leica Microsystems, Germany) and images generated with a microscope camera (Axiocam 506 Color, Zeiss, Germany). Embryo and endosperm area were measured on a median longitudinal section using the software ImageJ (ImageJ 1.46r, NIH, USA). This was to determine if the endosperm was fully consumed during seed development as described by Yan *et al.* (2014) or, if an endosperm layer was present, the embryo:endosperm ratio. In addition, the embryo type of each species was observed and compared with that reported by Martin (1946).

Statistics

All statistical analyses were performed using GenStat 17th edition (VSN International, Hemel Hempstead, UK). Significant differences between experimental groups were assessed with unpaired Student's *t*-test and one-way-ANOVA. The significance of correlations was tested using Spearman's rank correlation, a Two-sample nonparametric test, to assess the strength and direction of any correlation between variables that do not follow a normal distribution. Determination coefficient (R^2) values and regression equations were determined to assess the prediction potential. Significance was evaluated in all cases at $P \leq 0.05$.

Results*Relationship between bulk electrical conductivity and final germination*

The bulk electrical conductivity (EC) test of seed soak water was significantly correlated with total seed germination (radicle emergence) of seed lots from four of the seven species tested (table 1). The Spearman's rank correlations that were significant ($P < 0.05$) were as follows: *Cyanus segetum*, $r = -0.906$ ($P < 0.001$); *Prunella vulgaris*, $r = -0.657$ ($P = 0.005$); *Centaurea nigra*, $r = -0.567$ ($P = 0.022$) and *Valeriana officinalis* $r = -0.479$ ($P = 0.044$). The negative correlation revealed that a greater leakage of electrolytes was associated with lower germination (figure 1). The most significant of the Spearman correlation coefficients was for *Cyanus segetum* with $r = -0.906$ (figure 1A; table 1). This species also had a high coefficient of determination (R^2) of 0.867 ($P < 0.001$).

Early bulk conductivity readings, taken at 3 and 5 hours for 12 seed lots of *Cyanus segetum* were highly predictive of the EC at 24 hours, with R^2 values of 0.913 and 0.943, respectively (figure 2). The remaining three tested species (*Knautia arvensis*, *Papaver rhoeas* and *Silene vulgaris*) did not show a significant correlation between EC and germination (table 1).

Internal morphology of seeds

Seeds from the genera *Centaurea*, *Cyanus*, *Prunella* and *Valeriana* are characterised by a spatulate embryo and lack an endosperm cell layer (table 1). These four species were

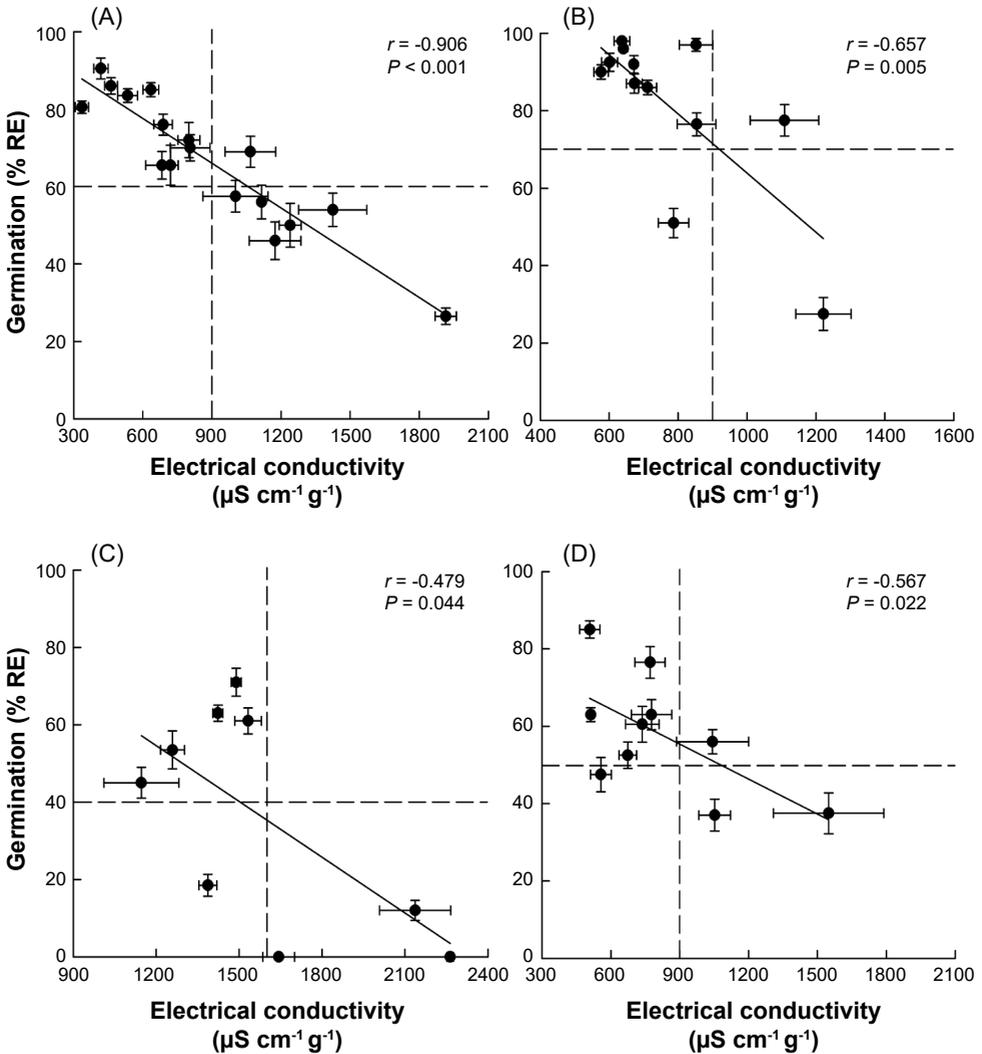


Figure 1. Relationship between bulk electrical conductivity (EC) measurements of (A) 17 seed lots of *Cyanus segetum*, (B) 12 seed lots of *Prunella vulgaris*, (C) nine seed lots of *Valeriana officinalis*, (D) 10 seed lots of *Centaurea nigra* and their germination (percentage radicle emergence). Means are presented \pm s.e. Correlation coefficient, r , and corresponding P -value from Spearman's rank correlation analysis are reported in each panel. The dashed lines indicate the conductivity value (vertical line) below which seeds of different species achieve a specific germination (horizontal line).

those in which EC was predictive of germination. Seeds from the genera *Silene*, *Knautia* and *Papaver* are characterised by a peripheral, spatulate and rudimentary embryo type, respectively, and include an endosperm cell layer in the mature seed. The embryo:endosperm ratio ranged from 0.76 ± 0.04 in *Silene vulgaris* to 0.22 ± 0.02 in *Papaver rhoeas* (table 1).

Table 1. Coefficient of correlation, r , and P -value from Spearman's rank correlation analysis between bulk electrical conductivity (EC) measurements of seed soak water and germination (percentage radicle emergence) for seeds of seven native species from six families. Species, families, number of seed lots, 1000 seed weight, embryo type and embryo:endosperm ratio are reported. n.s. indicates the lack of a significant correlation between variables ($P > 0.05$).

Species	Family	Number of lots	r	P	1000 seed weight (g)	Embryo type	Embryo: endosperm ratio†
<i>Cyanus segetum</i>	Asteraceae	17	-0.906	<0.001	4.14 ± 0.12	Spatulate	n.a.
<i>Prunella vulgaris</i>	Lamiaceae	12	-0.657	0.005	0.79 ± 0.04	Spatulate	n.a.
<i>Centaurea nigra</i>	Asteraceae	10	-0.567	0.022	2.46 ± 0.12	Spatulate	n.a.
<i>Valeriana officinalis</i>	Valerianaceae	9	-0.479	0.044	0.64 ± 0.04	Spatulate	n.a.
<i>Silene vulgaris</i>	Caryophyllaceae	9	-0.283	n.s.	0.87 ± 0.12	Peripheral	0.76 ± 0.04
<i>Knautia arvensis</i>	Caprifoliaceae	11	-0.273	n.s.	6.30 ± 0.61	Spatulate	0.72 ± 0.02
<i>Papaver rhoeas</i>	Papaveraceae	15	0.070	n.s.	0.12 ± 0.004	Rudimentary	0.22 ± 0.02

† n.a. indicates that the species does not include an endosperm cell later.

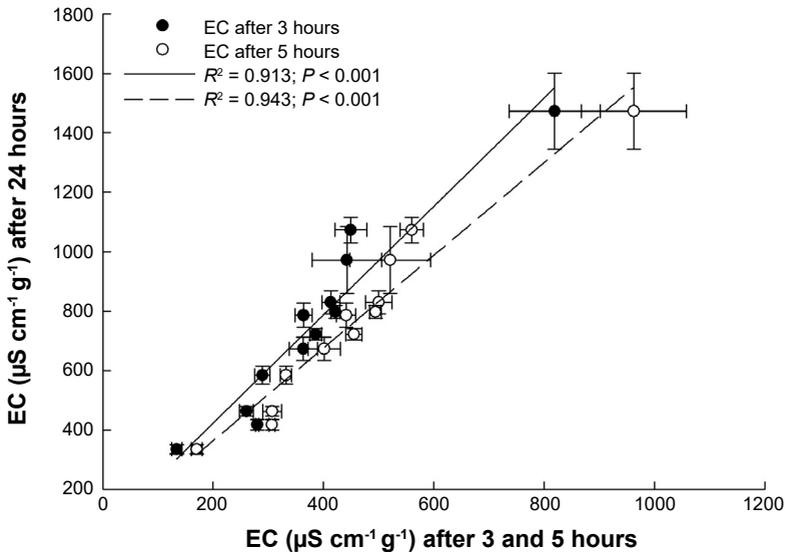


Figure 2. Linear regression between electrical conductivity (EC) readings of bulks of seeds from 12 seed lots of *Cyanus segetum*, after three (●, solid line) and five (○, dashed line) hours, and EC after 24 hours. Means are presented \pm s.e. The determination coefficient, R^2 , and P -value are reported.

Comparison of EC measurements from single seeds and their subsequent germination

Levels of electrolyte leakage from single seeds were measured for two Asteraceae species (*Cyanus segetum* and *Centaurea nigra*) and the EC of different categories of seeds (germinated or non-germinated) were compared. For both species, the overall mean EC was significantly higher for seeds that did not produce a radicle ($P < 0.001$) and was consistently greater for non-germinating seeds for each seed lot in both species (tables 2 and 3). Slower radicle emergence was also related to higher levels of leakage for *C. segetum* (table 2). This is clearly revealed by a comparison of leakage from the earlier germinating seeds (germination at and before 42 hours) with that from later germinating seeds (germination after 42 hours). Indeed, for the mean of all lots, EC was significantly greater for later germinating seeds ($P < 0.001$; table 2). No differences in EC were seen in relation to the timing of RE in *Centaurea nigra* (table 3).

Prediction of final germination by mean germination time and radicle emergence

Both the length of the average delay to RE (mean germination time, MGT) and an early count of RE predicted final germination of *Cyanus segetum* (figure 3). The MGT for the 12 lots of *C. segetum* ranged from 37 to 98 hours (figure 3A) and was significantly correlated with final germination ($r = -0.727$), with an R^2 of 0.638. The early count of RE at 42 hours was, in turn, significantly correlated with MGT ($r = -0.818$; $P < 0.001$; figure 3B). Consequently RE at 42 hours was highly predictive of final germination with a highly significant correlation coefficient (figure 3C) and an R^2 of 0.858.

Effect of a hydration treatment on single seed conductivity and radicle emergence

A hydration treatment followed by drying back of three lots of *Cyanus segetum* resulted in reduced leakage per seed in each of two repeat runs of the treatment, compared with a non-hydrated control (table 4). The electrical conductivity of single seeds that produced a radicle was significantly lower for the hydrated seeds (mean $2.38 \mu\text{S cm}^{-1} \text{ seed}^{-1}$) compared with the control seeds ($3.51 \mu\text{S cm}^{-1} \text{ seed}^{-1}$). Lower leakage from hydrated seeds was accompanied by an increase in early RE (at or before 42 hours) with the overall mean for the three lots / runs increasing from 47% in the control to 62% following the hydration treatment. This was particularly evident following the first hydration treatment in lot no 17, when the percentage of early germinating seeds increased from 42 to 91%. The reduction in leakage was also seen for the seeds that did not produce a radicle, from a mean for all lots / runs of $4.30 \mu\text{S cm}^{-1} \text{ seed}^{-1}$ for the control to $2.74 \mu\text{S cm}^{-1} \text{ seed}^{-1}$ for hydrated seed. The EC of the washings from the germination papers on which 25 seeds had been hydrated averaged 17.76, 19.66 and $20.02 \mu\text{S cm}^{-1}$, for lots no 1, 14 and 17, respectively. The EC of the washings from the germination papers with no seeds, used as controls, averaged $14.03 \mu\text{S cm}^{-1}$. Thus an estimate of leakage from individual seeds during hydration was low per seed and would have had little effect on the differences between the hydrated and control seed.

Table 2. Mean electrical conductivity (EC, $\mu\text{S cm}^{-1} \text{ seed}^{-1}$) of seed soak water (8 ml) of all single seeds from 13 seed lots of *Cyanus segetum* after five hours at 20°C and for seeds that either germinate (produce a radicle) or fail to do so in their subsequent germination test. Also shown is the EC ($\mu\text{S cm}^{-1} \text{ seed}^{-1}$) for seeds that produce a radicle before or after 42 hours. Values in parentheses are the number of seeds contributing to each value. Statistically significant differences between categories were tested using unpaired Student's *t*-test.

Lot no.	Electrical conductivity after three hours					Electrical conductivity after five hours				
	All seeds	No radicle produced	Germinated seeds	Seeds with radicle emergence in ≤ 42 hours	Seeds with radicle emergence at > 42 hours	All seeds	No radicle produced	Germinated seeds	Seeds with radicle emergence in ≤ 42 hours	Seeds with radicle emergence at > 42 hours
1	2.85 (20)	2.96 (5)	2.82 (15)	2.77 (5)	2.84 (10)	3.23 (20)	3.57 (5)	3.11 (15)	3.01 (5)	3.17 (10)
2	3.40 (20)	3.69 (13)	2.86 (7)	2.56 (1)	2.91 (6)	3.97 (20)	4.41 (13)	3.16 (7)	2.81 (1)	3.22 (6)
3	6.02 (20)	6.43 (17)	3.73 (3)	- (0)	3.73 (3)	7.20 (20)	7.69 (17)	4.41 (3)	- (0)	4.41 (3)
4	2.59 (20)	2.67 (4)	2.57 (16)	2.60 (15)	2.14 (1)	2.99 (20)	3.10 (4)	2.96 (16)	2.98 (15)	2.64 (1)
5	3.40 (20)	3.49 (15)	3.12 (5)	- (0)	3.12 (5)	3.95 (20)	4.09 (15)	3.51 (5)	- (0)	3.51 (5)
7	3.10 (20)	3.22 (14)	2.84 (6)	- (0)	2.84 (6)	3.56 (20)	3.72 (14)	3.20 (6)	- (0)	3.20 (6)
8	3.01 (20)	3.10 (8)	2.95 (12)	3.15 (8)	2.55 (4)	3.71 (20)	4.10 (8)	3.44 (12)	3.69 (8)	2.96 (4)
9	2.99 (20)	3.01 (16)	2.92 (4)	2.72 (3)	3.50 (1)	3.49 (20)	3.53 (16)	3.33 (4)	3.08 (3)	4.09 (1)
10	3.02 (20)	3.31 (6)	2.90 (14)	2.83 (10)	3.07 (4)	3.44 (20)	3.85 (6)	3.26 (14)	3.16 (10)	3.52 (4)
11	3.13 (20)	4.00 (7)	2.66 (13)	2.40 (10)	3.52 (3)	3.56 (20)	4.67 (7)	2.96 (13)	2.66 (10)	3.96 (3)
14	4.05 (20)	4.43 (12)	3.47 (8)	3.38 (4)	3.56 (4)	5.05 (20)	5.48 (12)	4.41 (8)	4.38 (4)	4.44 (4)
15	1.89 (20)	2.04 (5)	1.84 (15)	1.85 (14)	1.82 (1)	2.17 (20)	2.35 (5)	2.11 (15)	2.11 (14)	2.07 (1)
17	3.59 (20)	3.85 (12)	3.19 (8)	2.99 (4)	3.40 (4)	4.33 (20)	4.70 (12)	3.76 (8)	3.39 (4)	4.13 (4)
Mean	3.31 (260)	3.81 (134) †	2.78 (126) †	2.60 (74) ‡	3.04 (52) ‡	3.89 (260)	4.56 (134) †	3.19 (126) †	2.97 (74) ‡	3.50 (52) ‡

† indicates a statistically significant difference at $P < 0.001$ between seeds that germinated and those that did not produce a radicle.

‡ indicates a statistically significant difference at $P < 0.001$ between seeds that produced a radicle before and after 42 hours.

Table 3. Mean electrical conductivity (EC, $\mu\text{S cm}^{-1} \text{ seed}^{-1}$) of the seed soak water (8 ml) of all single seeds from 10 seed lots of *Centaurea nigra* and for seeds that either germinate (produce a radicle) or fail to do so in their subsequent germination test. Also shown is the EC ($\mu\text{S cm}^{-1} \text{ seed}^{-1}$) for seeds that produce a radicle before or after 72 hours. Values in parentheses are the number of seeds contributing to each value. Statistically significant differences between categories were tested using unpaired Student's *t*-test.

Lot no.	Electrical conductivity after three hours					Electrical conductivity after five hours				
	All seeds	No radicle produced	Germinated seeds	Seeds with radicle emergence in ≤ 72 hours	Seeds with radicle emergence at > 72 hours	All seeds	No radicle produced	Germinated seeds	Seeds with radicle emergence in ≤ 72 hours	Seeds with radicle emergence at > 72 hours
1	2.52 (20)	2.60 (8)	2.46 (12)	- (0)	2.46 (12)	2.77 (20)	2.87 (8)	2.70 (12)	- (0)	2.70 (12)
2	2.48 (20)	2.75 (4)	2.41 (16)	2.30 (6)	2.48 (10)	2.93 (20)	3.37 (4)	2.82 (16)	2.76 (6)	2.85 (10)
3	2.73 (20)	3.47 (5)	2.49 (15)	2.48 (11)	2.51 (4)	3.23 (20)	4.38 (5)	2.85 (15)	2.84 (11)	2.87 (4)
4	2.56 (20)	2.76 (7)	2.45 (13)	2.69 (3)	2.37 (10)	2.79 (20)	2.93 (7)	2.72 (13)	2.94 (3)	2.66 (10)
5	2.58 (20)	2.65 (16)	2.31 (4)	2.21 (1)	2.34 (3)	2.84 (20)	2.94 (16)	2.48 (4)	2.44 (1)	2.49 (3)
6	2.81 (20)	3.37 (8)	2.43 (12)	2.62 (3)	2.37 (9)	3.22 (20)	3.88 (8)	2.78 (12)	2.93 (3)	2.73 (9)
7	3.09 (20)	3.12 (18)	2.78 (2)	2.92 (1)	2.64 (1)	3.43 (20)	3.48 (18)	2.94 (2)	2.99 (1)	2.88 (1)
8	2.15 (20)	2.28 (6)	2.10 (14)	2.16 (3)	2.09 (11)	2.45 (20)	2.72 (6)	2.33 (14)	2.33 (3)	2.33 (11)
9	2.22 (20)	2.44 (4)	2.17 (16)	2.16 (11)	2.20 (5)	2.53 (20)	2.84 (4)	2.46 (16)	2.43 (11)	2.52 (5)
10	2.61 (20)	2.83 (14)	2.09 (6)	2.02 (1)	2.10 (5)	3.18 (20)	3.55 (14)	2.32 (6)	2.24 (1)	2.33 (5)
Mean	2.57 (200)	2.85 (90) †	2.34 (110) †	2.36 (40)	2.34 (70)	2.94 (200)	3.30 (90) †	2.64 (110) †	2.67 (40)	2.63 (70)

† indicates a statistically significant difference at $P < 0.001$ between seeds that germinated and those that did not produce a radicle.

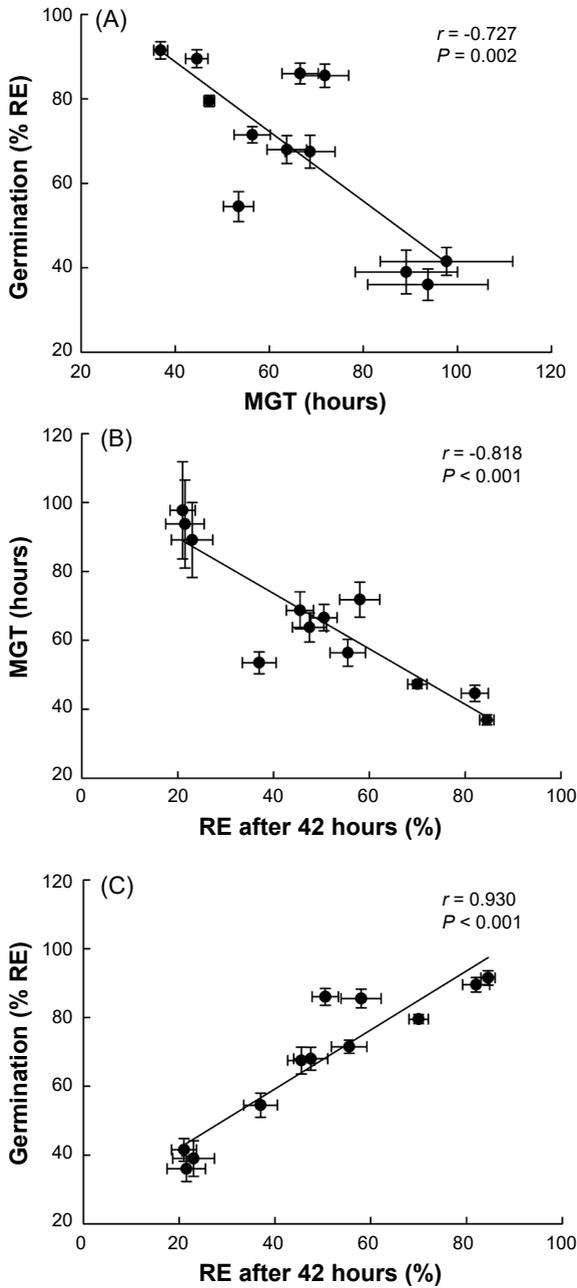


Figure 3. Relationship between (A) mean germination time (MGT) and final germination (percentage radicle emergence); (B) radicle emergence (RE) at 42 hours and MGT and (C) RE at 42 hours and final germination for 12 seed lots of *Cyanus segetum*. Means are presented \pm s.e. Correlation coefficient, r , and corresponding P -value from Spearman's rank correlation analysis are reported in each panel.

Table 4. Mean electrical conductivity (EC, $\mu\text{S cm}^{-1} \text{ seed}^{-1}$) of the soak water of single seeds from three lots (1, 14 and 17) of *Cyanus segetum* after five hours soaking at 20°C. Seeds were either given a 5-hour hydration / drying treatment or were non-hydrated control seeds. The hydration treatment was performed twice and data were compared with the control. Seeds were identified as those that did or did not produce a radicle (number of seeds in parentheses) in a subsequent germination test. The percentage of germinated seeds that produced a radicle at, or before, 42 hours is also reported.

Lot no.	Treatment	Electrical conductivity after five hours ($\mu\text{S cm}^{-1} \text{ seed}^{-1}$)			Seeds with radicle emergence in ≤ 42 hours (%)
		All seeds	No radicle produced	Germinated	
1	Control	3.70 \pm 0.25 (25)	3.72 \pm 0.67 (4)	3.70 \pm 0.27 (21)	43%
	5-hour hydration 1 st	2.09 \pm 0.08 (25)	2.27 \pm 0.19 (6)	2.04 \pm 0.09 (19)	47%
	<i>P</i> -value	< 0.001	0.037	< 0.001	
	5-hour hydration 2 nd	2.35 \pm 0.09 (25)	2.56 \pm 0.43 (4)	2.30 \pm 0.08 (21)	57%
	<i>P</i> -value	< 0.001	0.193	< 0.001	
14	Control	3.81 \pm 0.24 (25)	4.20 \pm 0.40 (13)	3.38 \pm 0.16 (12)	58%
	5-hour hydration 1 st	2.76 \pm 0.11 (25)	2.75 \pm 0.15 (14)	2.77 \pm 0.15 (11)	45%
	<i>P</i> -value	< 0.001	0.002	0.011	
	5-hour hydration 2 nd	2.91 \pm 0.13 (25)	3.25 \pm 0.17 (12)	2.60 \pm 0.15 (13)	77%
	<i>P</i> -value	< 0.001	0.047	0.002	
17	Control	3.96 \pm 0.26 (25)	4.57 \pm 0.41 (13)	3.30 \pm 0.16 (12)	42%
	5-hour hydration 1 st	2.47 \pm 0.10 (25)	2.66 \pm 0.15 (14)	2.23 \pm 0.06 (11)	91%
	<i>P</i> -value	< 0.001	< 0.001	< 0.001	
	5-hour hydration 2 nd	2.50 \pm 0.10 (25)	2.62 \pm 0.13 (16)	2.71 \pm 0.21 (9)	67%
	<i>P</i> -value	< 0.001	< 0.001	0.037	
Mean	Control	3.82 \pm 0.14 (75)	4.30 \pm 0.26 (30)	3.51 \pm 0.14 (45)	47%
	5-hour hydration	2.51 \pm 0.05 (150)	2.74 \pm 0.08 (66)	2.38 \pm 0.05 (84)	62%
	<i>P</i> -value	< 0.001	< 0.001	< 0.001	

Discussion

Electrolyte leakage from bulk samples of seed, assessed by the electrical conductivity (EC) of seed soak water, was indicative of the final germination (radicle emergence; RE) of four out of the seven species tested (*Cyanus segetum*, *Prunella vulgaris*, *Valeriana officinalis* and *Centaurea nigra*), with high levels of leakage (high EC) seen for lots with low germination. Single seed measurements of solute leakage from two species confirmed the link between high leakage and the failure to germinate. In *Cyanus segetum*, the relationship between the length of the delay to radicle emergence (mean germination time; MGT) and an early (after 42 hours) count of RE suggested a single RE count would also predict final germination. The observation of reduced EC and earlier RE following a pre-hydration treatment in three seed lots of *Cyanus segetum* supported the hypothesis that metabolic repair may occur during early imbibition.

The EC of seed soak water clearly predicted differences in the final germination (percentage radicle emergence) in 17 commercially available seed lots of *Cyanus segetum* (figure 1A) and the seed lot of highest quality (91% RE; 418 $\mu\text{S cm}^{-1} \text{g}^{-1}$ EC) could be clearly distinguished from the one with the lowest final germination (27% RE; 1915 $\mu\text{S cm}^{-1} \text{g}^{-1}$ EC). Seed lots with a relatively high level of germination (above 60%) were identified as having EC readings of less than 900 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (figure 1) for all but one lot of *Cyanus segetum*. An EC reading below 900 $\mu\text{S cm}^{-1} \text{g}^{-1}$ also identified seed lots of *Prunella vulgaris* and *Centaurea nigra* having more than 70% and 50% germination, respectively, while lots of *Valeriana officinalis* with above 40% germination had EC readings of less than 1600 $\mu\text{S cm}^{-1} \text{g}^{-1}$ (figure 1). These levels of final germination would be considered low in crop species, but can occur frequently in commercial lots of native species (Ryan *et al.*, 2008; Marin *et al.*, 2017). In contrast to the situation for crop species in many countries, there are no minimum levels of germination below which seeds of wild species cannot be sold. Furthermore the level of germination achieved by seed lots of wild species from different sources can vary markedly (Ryan *et al.*, 2008; Marin *et al.*, 2017). Our work suggests that EC measurements could be used by seed producers to identify and eliminate the lower germinating seed lots in some species. The bulk EC of *Cyanus segetum* after 24 hours was significantly correlated with earlier readings after 3 and 5 hours (figure 2), an observation also made in radish (Mavi *et al.*, 2016). This suggests that even more rapid prediction of germination is possible (figure 2). The association between high levels of leakage and both slow and poor germination was supported by the higher EC readings from single seeds after 3 and 5 hours soaking for seeds of *Cyanus segetum* that subsequently germinated later (after 42 hours), and from non-germinating seeds of both *C. segetum* (table 2) and *Centaurea nigra* (table 3).

The four species in which EC was related to final germination (figure 1) all had non-endospermous seeds with relatively large embryos (table 1). *Cyanus segetum* had the largest embryo and showed the strongest correlation ($r = -0.906$, figure 1). It may be that a relatively large embryo is needed before differences in the extent of damaged and dead areas can be detected by seed leakage. The three species to which EC could not be applied showed a range in embryo:endosperm ratio, with a maximum of 0.76 for *Silene vulgaris*. It may be that there is a critical ratio above which the EC can be applied as the relative volume of living tissue in the seed increases. In recent work on the same seed lots (Marin *et al.*, 2017), tetrazolium staining revealed a wide variation between lots of all species and that germination was correlated with the proportion of seeds showing complete staining of the embryos. This was suggested as a test indicative of germination in just two days. The EC test appears to be restricted only to seed with appropriate seed morphology, but even so can be completed within 3, 5 or 24 hours (figure 2) to give a prediction of germination. Further testing of other species showing similar morphology to *Cyanus segetum* would be a useful next step in developing this method of predicting germination further.

The significant relationships between the mean germination time for *Cyanus segetum* and both germination (figure 3A; $R^2 = 0.638$) and a single early count of RE at 42 hours (figure 3B; $R^2 = 0.801$), resulted in a highly significant relationship between the 42-hour RE count and germination (figure 3C; $R^2 = 0.858$). This suggests that a 42-hour RE count can predict germination of *Cyanus segetum*, as seen for a 48-hour RE count in radish (Mavi *et al.*, 2016).

In previous work, early RE counts have been used to indicate potential differences in emergence, that is vigour, of maize, radish and oilseed rape (Matthews *et al.*, 2011, 2012a; Mavi *et al.*, 2014). Seed lots at risk of poor emergence can be identified by a low early RE count and the RE test is included in the ISTA Rules as a validated vigour test for maize, radish and oilseed rape (ISTA, 2017). The use of early counts of RE to predict vigour in crop seeds suggests the possibility that similar counts in *Cyanus segetum* may not only indicate the ability to germinate, as shown in the present work, but also emergence in the field. Evidence for this would be a useful future objective, since the overall aim of the producers of native seed species is to provide seeds that will emerge well and become lasting components of vegetation (Kiehl *et al.*, 2014).

The negative correlation found between EC and final germination resulted from greater leakage of non-germinating seeds, as seen by the single seed conductivity measurements for both *Centaurea nigra* and *Cyanus segetum* (tables 2 and 3). The two categories of seeds were clearly identified by EC measurements after 3 and 5 hours, confirming that early readings are indicative of differences in final germination as seen for bulk samples of seed. These observations are in agreement with previous work on radish, oilseed rape and cabbage (Demir *et al.*, 2008a, 2012; Wagner *et al.*, 2012). In the case of *Cyanus segetum* higher single seed conductivities were seen for seeds that were slower to radicle emergence, as observed for radish (Mavi *et al.*, 2014, 2016).

The significant reduction in electrolyte leakage and earlier radicle emergence following the pre-hydration treatment (table 4) can be explained in terms of the ageing / repair hypothesis (Matthews and Khajeh-Hosseini, 2007; Matthews and Powell, 2011). Osborne (1983) suggested that all seeds undergo a period of DNA repair before germination. The ageing / repair hypothesis proposes that in aged seeds the slower germination, or prolonged lag period before radicle emergence, reflects the time needed to repair the deterioration that has accumulated during ageing (Matthews and Khajeh-Hosseini, 2007; Matthews *et al.*, 2012b; Powell and Matthews, 2012). The present work has shown that a reduced time to radicle emergence is also associated with reduced solute leakage from the seeds, as seen in radish (Mavi *et al.*, 2016). This raises the question whether metabolic repair before germination includes the repair of membranes, as well as of DNA.

Our results showed a significant reduction in leakage following the hydration treatment for seeds that failed to produce a radicle, as well as for the germinating seeds (table 4). This could be explained by the fact that a large proportion of viable tissue may still be found in non-germinating seeds. Indeed, damage may affect parts of the embryo that are essential for germination which therefore fails to occur, even though other areas of the seed may remain alive (ISTA, 2011). The living tissue in such seeds may therefore have undergone repair during hydration reducing the leakage from these parts of the seed.

In summary, we have shown that both measurements of solute leakage from seeds using EC and early counts of RE have potential to predict the germination (radicle emergence) of seed lots from several native species. Further characterisation of EC data for other species and in relation to seed traits, particularly seed structure, is needed before EC can be applied to predict germination of diverse species. However, the RE test could well be applicable to a wide range of species and predict both germination and vigour differences.

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