# Electrical Conductivity Test

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# ELECTRICAL CONDUCTIVITY TEST

by

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**CONTENTS**

# 1. INTRODUCTION

Measurement of electrical conductivity of leak water from imbibing tree seeds can be used as a vigour test. A standardized test can provide estimates of seed quality in 24 hours, which can be used as a supplement or a quick alternative to a germination test. Conductivity measurements can also be a useful tool in research into e.g. desiccation tolerance.

The test has so far only been standardized for a few agricultural species although used in research on a large number of species including tree seeds. It should be stressed that standardization for routine testing is very time consuming.

This Technical Note gives practical guidelines on the application and standardization of the test, and it provides the necessary background for evaluating the relevance of the test in different situations. Chapter 2 is a short introduction to 'why' and 'what' seeds leak. The principles behind conductivity measurements and the equipment needed for the test are described in chapter 3. Chapter 4 goes through the actual procedures, step by step, and in chapter 5 the different factors that may affect conductivity are in focus. Instructions on how to standardize the test for new species are given in chapter 6, and, finally, in chapter 7 the general applicability of the test is discussed.

**Viability**

The percentage of seeds that are alive and can germinate under optimal laboratory conditions

**Vigour**

The performance of the seed under varying conditions

Tests: accelerated ageing, speed of germination, electrical conductivity

## 2. SOLUTE LEAKAGE FROM SEEDS

When seeds are dried (naturally or otherwise), the cell membranes tend to lose their ability to keep the solutes within the cells, therefore rehydration is followed by leakage. Normally the membrane integrity is re-established during imbibition. But vigorous seeds probably re-establish the membranes at a faster rate, with subsequent less leakage, than less vigorous seeds. It is therefore assumed that the degree of leakage is correlated with quality of seed. When quality is low, due to many dead seeds and/or seeds that do not manage to re-establish their membranes, leakage is high.

When dry seeds are immersed in pure water, the difference in osmotic pressure between seed and water causes a rapid uptake of water by the seed. At the same time solutes will leak from the seeds into the water. The leakage contains various sugars, amino acids, lipids and other organic acids, as well as inorganic salts with phosphates being the major component. Some of these solutes act as electrolytes and contribute to the electrical conductivity of the water.

Besides giving an indication of the membrane integrity, leakage in itself has negative effects. The seeds are weakened because they lose nutrients, and harmful microorganisms in the environment around the seeds thrive on the leaked solutes (Larson, 1968; Perry and Harrison, 1970; Simon, 1978; AOSA, 1983; Bewley and Black, 1994).

## 3. MATERIAL

**Equipment needed for the conductivity test:**

* Conductivity meter and cell

* Containers

* Constant temperature facilities (e.g. germination cabinet)

* De-ionized or distilled water

* Analytical balance (capable of weighing to 0.01 g)

* Oven for moisture content determination

### 3.1 Conductivity meter

Metals and liquids possess an 'electrical resistance' measured in units of Ohm. The reciprocal of resistance is conductance. Conductivity is the measurement of a material´s ability to conduct electrical current. Two plates are placed in the sample, a potential is applied across the plates (electrodes) and the current is measured.

As conductivity is affected by the geometry of the cell, see figure 1, the measured conductivity (G) is multiplied by L/A (A = area of the plates, and L = length of the column of liquid between the electrodes) to obtain the specific conductivity (C). L/A is also called the cell constant.

$C = G * (L/A)$

The unit of conductance is:

$1/Ohm = Ohm^{-1}$ = Siemens (or sometimes called mho).

The unit of specific conductivity is:

$(1/Ohm)*(cm/cm^2) = Ohm^{-1}*cm^{-1}$, or $Siemens*cm^{-1}$.

However, the unit S/cm is very large so conductivity is often measured in mS/cm or µS/cm:

1 µS/cm = 0.001 mS/cm = 0.000001 S/cm = 1 µmho/cm

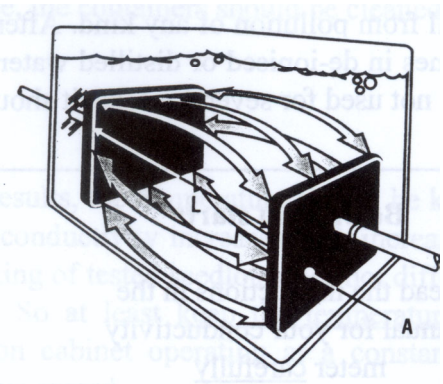


Figure 1. Cell geometry (Cole Palmer, 1997-98)

The specific conductivity of distilled water is around 2 µS/cm. A 20% NaCl solution has a specific conductivity 100,000 times higher than distilled water.

The conductivity of fluids increases rapidly with increasing temperature, around 2% per degree Celcius, hence the measurement must be done under strict temperature control.

Since specific conductivity of water in which seeds are immersed depends on the quantity of seeds and water in addition to the degree of leaking, the test result is expressed relatively to the quantities of seeds (dry weight) and water, for example: 40 $\mu S*cm^{-1}*gr^{-1}*ml^{-1}$.

A number of instruments are available from suppliers of laboratory hardware. The instruments range from handy, but not very accurate field conductivity meters to advanced research conductivity meters. For seed testing, something in between will probably be appropriate in most cases. But the conductivity meter has to be very sensitive and should be able to measure low conductivity levels (10-1400 µS/cm). Some instruments have automatic temperature compensation.

Conductivity of seeds can be determined on bulk basis (e.g. 50 seeds) or on single seeds. Bulk determinations are usually done with a single cell; single seed determinations can also be made by a single cell or with multi-cells that can measure in many small containers at the same time. There are different types of cells e.g. flow- and pipette-cells. The pipette cells can measure on very small volumes of water e.g. 0.6 ml (which is sucked up into the cell), which is an advantage if measurements on single seeds are needed. The flow cells are immersed into the solutions and demands larger quantities of water. The cells are usually constructed from platinum or platinized electrodes in glass tubes. It is necessary to know the cell constant in order to calculate the specific conductivity. $K = 1.0\ cm^{-1} \pm 10\ \%$ is normal, and the exact value will sometimes be printed on the conductivity cell. As the cell becomes contaminated, it may change and can then be found by measuring a solution with a known conductivity, and adjusting the 'cell constant knob' on the meter so that the display shows the right value. Solutions can be made by dissolving salts in water (some examples are given in annex A) or they can be purchased together with the conductivity meter. To obtain the highest accuracy calibration should be carried out with a reference solution that has a conductivity in the expected measuring range.

It is important to protect the dip cell from pollution of any kind. After use, the dip cell should be cleaned by dipping it several times in de-ionised or distilled water. When used frequently, it should be stored moist, but if it is not used for several weeks, it should be stored dry.

**Before you start:**

Read the instructions in the manual for your conductivity meter carefully

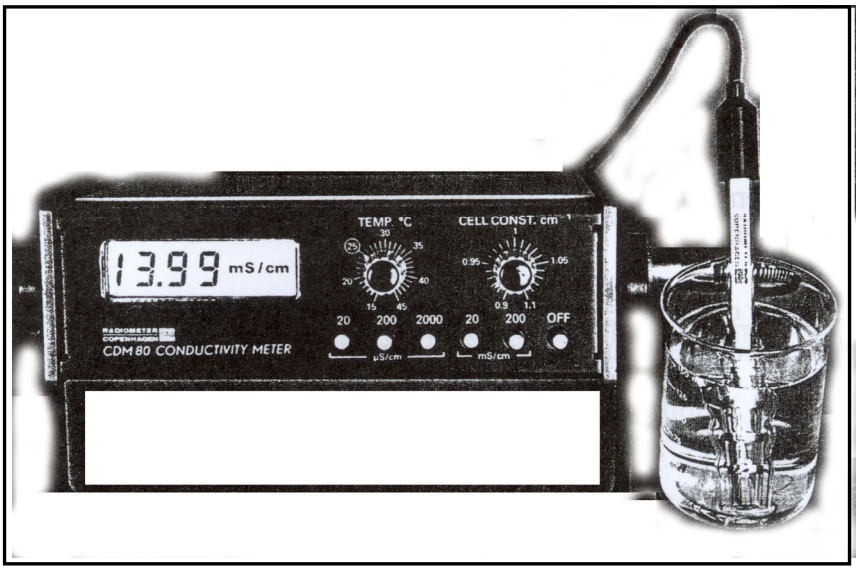


Figure 2. An example of a conductivity meter. CDM80 from Radiometer.

### 3.2 Containers

Containers for seed and water should be made of glass or plastic of the type Erlenmeyer flasks, conical flasks or glass beakers. The size depends on the seeds, 50 ml containers are large enough for small seeds as e.g. pine seeds. As the size of the container influences the results, the volume and dimensions of the containers should always be identical for all seed samples, at least for each species.

During use all containers must be covered with a lid or plastic film to avoid evaporation and dust pollution. Before use, the containers should be cleaned in de-ionized or distilled water.

### 3.3 Temperature

To obtain reproducible results, the temperature should be kept constant during the whole test, and from test to test, as conductivity increases with increasing temperature. However, results have shown that the ranking of tested seed lots does not differ for beans whether tested at 20 or 25°C (Hampton, 1992). So at least keep the temperature constant for the samples being compared. A germination cabinet operating at a constant specified temperature (20°C or 25°C) is very useful in this regard.

### 3.4 Water

The soaking water should preferably be de-ionized water. Distilled water may be used, but the conductivity should be less than 5 $\mu S*cm^{-1}$.

The water should be stored for 24 hours at the specified temperature (20°C or 25°C) before use.

The quantity of water depends on the type of seed and on the minimum quantity of water necessary for measurements with the available conductivity meter. In most cases 20-100 ml per replicate is sufficient.

Remember to set up control containers with water (and no seeds) in each test run. The conductivity of these controls must be subtracted from the sample readings.

### 3.5 Seed sample size

The optimum sample size for a species is found by trying different quantities and choosing one that gives an acceptable variation between replicates. 50 seeds per replicate and 4 replicates can be the starting point. It saves work to use weighed quantities instead of a counted number of seeds. A moisture content determination of the seed is necessary as conductivity is expressed as $\mu S*cm^{-1}$/g dry weight. All samples are weighed to two decimal places.

The samples should be stirred after the water is added and before each conductivity reading.

## 4. TEST PROCEDURE

The quantities of seed, length of soaking period etc in this chapter are examples. The factors that have to be standardized for each species are listed in the box below. Standardization is described in chapter 6.

**Factors you need to standardize for each species**:

* Quantity of seeds per replicate (bulk determinations)

* Number of seeds (single seed determinations)

* Quantity of water per replicate

* Seed treatment before soaking (e.g. washing)

* Length of soaking period

* Temperature

* Initial moisture content

* Correlation with germination tests

**Procedure**

Take out four random samples, e.g. 50 seeds each, from the pure seed fraction of the working sample for bulk determinations. Take out one sample of e.g. 30 seeds, for single seed determinations. Weigh each sample/seed in grammes to two decimal places. Determine moisture content according to ISTA rules on two additional samples (ISTA, 1993).

Rinse each sample/seed once with de-ionized water. Use same quantity of water for each replicate, e.g. 25 ml, and same period of time, e.g. 30 seconds.

Place each sample/seed in a container, e.g. 50 ml, with de-ionized water, e.g. 40 ml or use 1:5 seed and water, and stir. All containers should be of the same size. Ensure that all seeds are completely immersed and evenly distributed. Control containers with the same water as used for the seed samples should be set up as well.

Place the containers in a germinator at the constant specified temperature at which the cell has been calibrated (20°C or 25°C). Cover the containers to avoid pollution and evaporation of

water. After e.g. 24 hours of incubation, specific conductivity is measured. The measurement should be made immediately after the removal of the samples from the incubator. A change in temperature of the soaking solution due to exposure to a different room temperature will affect the conductivity readings.

The seeds are stirred and the electrical specific conductivity is measured with the cell, either between the seeds or after the seeds have been removed from the water. The conductivity of the control containers are measured and the mean value is subtracted from the readings for the seed samples. Between readings, the dip cell should be rinsed in de-ionised water.

Prevent pollution of the solution and equipment during reading, i.e. from dust, skin contact and the like.

Specific conductivity per gramme of dry seed is calculated and recorded on the data sheet (see annex B).

## 5. FACTORS INFLUENCING CONDUCTIVITY

### 5.1 Seed moisture content

Initial moisture content will influence conductivity. Hampton et al. (1994) measured the conductivity of *Lotus corniculatus* L and *L. uliginosus* seeds with a moisture content between 5 and 17%. Conductivity was almost constant between 11 and 17 %, whereas it was much higher at 5 and 8 %. The conclusion was to adjust the moisture content to a level between 11 and 17 %. Sørensen (1995) did not find a higher conductivity of dry *Picea abies* seeds at 6-8% moisture content compared to 15 and 20 % moisture content.

Higher conductivity at low moisture contents may be due to imbibition damage. It is therefore important to investigate the relationship between moisture content and conductivity, and find a level where small changes in moisture content does not have a significant effect on conductivity.

If dry seeds must be rehydrated, it should be done by exposure to high relative humidity or on wet filterpaper, to limit the leakage from the seeds prior to the test. The weight increase will have to be monitored to stop rehydration at the desired moisture content.

If seeds with high moisture content have to be dried, this should be done in a way that does not damage the seed, e.g. in a desiccator over silica gel at a moderate temperature.

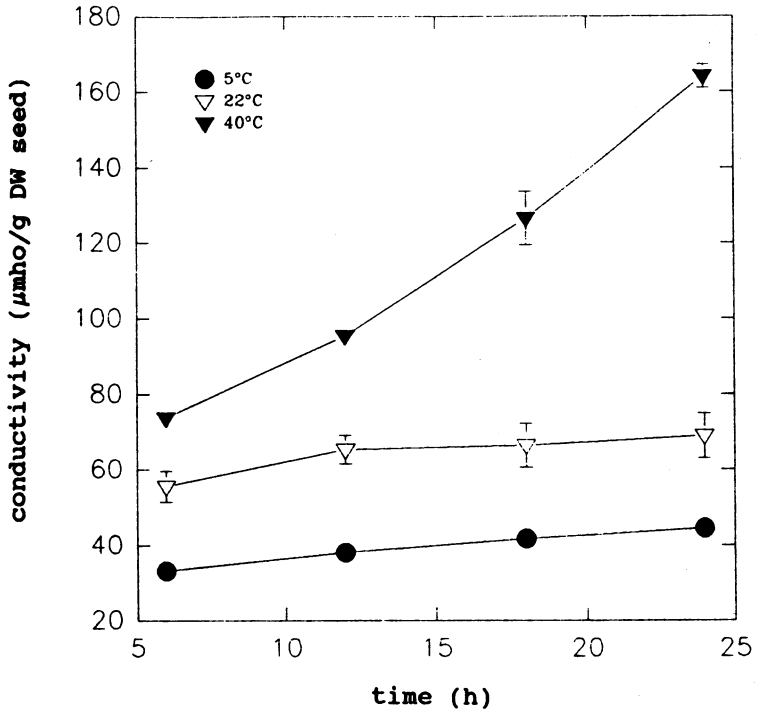


Figure 3. Conductivity of *Picea abies* seeds soaked in water at different temperatures (5, 22 and 40°) (Sørensen, 1995).

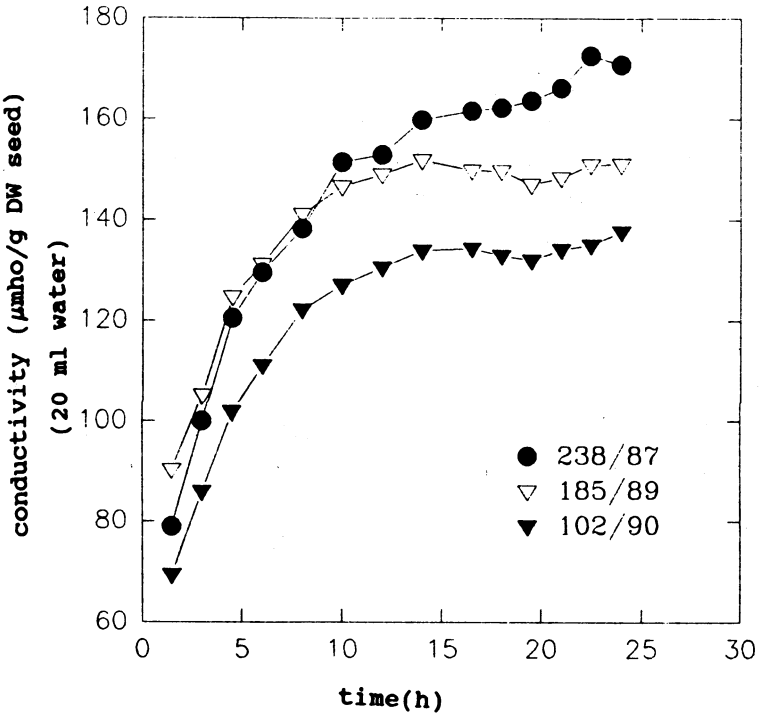


Figure 4. Conductivity curve for 3 *Picea abies* seed lots soaked in water (Sørensen, 1995).

### 5.2 Soaking temperature

Temperature greatly influences the amount of solutes leaking from seed soaked in water. Figure 3 shows the development over time of conductivity in soaking water of *Picea abies* seeds at three different temperatures. Leakage increases with increasing temperature. A possible cause may be a change in the viscosity of the soaking water (Murphy and Noland, 1982).

### 5.3 Length of soaking period

Leakage from the seeds will first increase rapidly and then level off. Figure 4 shows leakage of three different *Picea abies* seed lots over time. When conductivity measurements are used as a test, usually only one determination of conductivity is made. It is therefore important to measure conductivity in the horizontal part of the curve. At least four to six different seed lots should be used to make these curves upon which soaking period is determined. The period will typically be 16-24 hours.

### 5.4 Empty seeds and impurities

Empty seeds may disturb the results. Leakage from empty seeds is low, as in high quality seeds, hence the test may overestimate the quality of a seed lot in a bulk determination. If it is difficult to sort out empty seeds, a cutting test prior to the conductivity test may be a good idea.

Abnormal seeds will leak as much (or as little) as normal seeds (Bonner, 1989), and will also contribute to an overestimation of the quality.

Removal of dead seed parts (wings etc.) must be done very carefully before soaking as the solute leakage from these parts is very high. Small impurities, fungicides and insecticides can also cause variation in the conductivity measurements so a standard preliminary wash in distilled water may be necessary. It is important that the wash is standardized and used on all seed lots, as the seeds will leak solutes during the process.

### 5.5 Mechanical injuries

When seeds with mechanical injuries are soaked in water, leakage will be high. The presence of just a few seeds with mechanical injuries in a seed lot will cause a higher level of conductivity for the seed lot, resulting in an underestimate of the quality of the lot.

However, removal of injured seeds from the test cannot be recommended as selection would be very subjective.

## 6. STANDARDIZATION OF THE TEST ON A NEW SPECIES

The procedure described in chapter 4 is used during standardization of the test on a new species. Basically, this includes simultaneous conductivity and germination testing of samples of several seed lots, preferably of different age and quality. If the technique of accelerated ageing is mastered, it will be useful to age a number of samples for varying periods to obtain different vigour levels.

**Calibration, step by step**

The more seed lots that provide the background for the test the better, but during the preliminary investigation of the factors involved, the work can be based on few seed lots. If possible at least three seed lots should be used, but for some of the steps it might be enough to base the investigation on a single seed lot. Always use at least three replicates per treatment during standardization so that it is possible to calculate standard deviation and assess variation. In routine testing duplicate measurements might be sufficient.

Calculations of mean germination time, standard deviation and moisture content can be found in annex B.

**1. Seed samples**
Obtain samples of a number of seed lots with different quality.

**2. Viability and vigour**
Determine viability of the seed lots by a germination test and use mean germination time as a measure of vigour.

**3. Is there a correlation between conductivity and viability/vigour ?**
Wash and soak samples from three seed lots, preferably with very different quality, in a suitable amount of water for 24 hours, measure conductivity and see if there seems to be correlation between germination percentage/mean germination time and conductivity.

**4. Is the test destructive ?**
From a seed lot with medium quality, germinate seeds which have been soaked for 16, 24 and 36 hours respectively and compare both germination percentage and mean germination time with the initial germination test.

**5. Test the effect of initial moisture content**
Rehydrate seed samples from three different lots to obtain samples with different moisture content. Soak these samples for 24 hours and measure conductivity. The results will show whether imbibition damage occurs at low initial moisture contents.

**6. What is optimal temperature ?**
18, 20 and 25°C seem to be the agreed standard temperatures for measuring conductivity. Check at your moisture meter what your options are, and choose one of these temperatures. You can also soak some seed samples at different temperatures and use conductivity measurements to determine optimal temperature.

**7. Ratio between seeds and water**
Try different ratios of seeds and water for three seed lots. Use the ratio you started out with as reference and find the optimal ratio.

**8. Soaking period**
Measure conductivity at regular intervals (e.g. every 30 minutes or every hour) over 36 hours for three seed lots to determine how long the soaking period should be.

**9. Final development of the test**
Apply the conductivity test according to the procedures determined through the calibration of a large number of seed lots. Plot the corresponding germination and conductivity results against each other and determine the relationship between conductivity and seed quality (see some examples in chapter 7).

Make a note of the test procedure for future use.

**Time consumption**

Standardization of the test is time consuming (the number of germination and conductivity tests are listed in table 1). If the test is going to be used as a routine test, standardization should be done very carefully including all steps. However, if conductivity is measured as part of research into e.g. desiccation tolerance, it might not be possible to spend the time and/or seeds on going through all the steps. In this case temperature, moisture content etc. must be determined from experience. It is however a good idea to go through at least step 8, to ensure that conductivity is measured after the curve has levelled off.

**Table 1. Number of tests during standardization**

| Step | Germination tests (no) | Conductivity tests (no) |
|---|---|---|
| 2. Germination test | 3 | 3 |
| 3. Conductivity test | | 3 |
| 4. Soaking-damage | 3 x 3 | 3 x 3 |
| 5. Moisture content | | 3 x 5 |
| 6. Temperature | | 3 x 2-3 |
| 7. Ratio of seed and water | | 3 x 2-3 |
| 8. Soaking period | | 3 x 3 * |
| 9. Final development | 5-10 | 5-10 |

* measured at regular intervals over 36 hours

# 7. INTERPRETATION AND USE OF CONDUCTIVITY TESTS

## 7.1 Applicability of the test to different types of seed

The electrical conductivity test can be used as a routine supplement or a quick alternative to the germination test. It can also be used more selectively, e.g. in cases where poor quality is suspected, or as a research tool e.g. when investigating seed maturation or desiccation tolerance.

So far, the test has mainly been used on orthodox seeds without dormancy. But there is no reason to believe that it could not be applied on dormant or recalcitrant species. For recalcitrant species the test can be very relevant in establishing whether a seed lot has suffered desiccation damage or not. With regard to dormant species, a quick test which correlates to germination is very useful, because a germination test is very time consuming.

It has been shown for several species (e.g. *Pinus sylvestris*, Sahlen and Gjelsvik, 1993) that there is a correlation between seed maturity and conductivity. The method has a potential to establish optimal time of harvest.

Hard seeds may pose a problem. If the seeds are totally impenetrable to water, some kind of scarification will be necessary before the test. This scarification should of course be standardized.

## 7.2 Is the method non-destructive?

It is sometimes assumed that the conductivity test is non-destructive, and consequently the conductivity and germination test have been made on the same seeds during the standardization process. But some seeds may be damaged by the long soaking period, therefore the germination test should not be made on seed samples used for measuring conductivity.

## 7.3 Seed anatomy

The different components of a seed, e.g. embryo, endosperm and covering structures may contribute to the leakage in different ways.

For example, leakage from the pericarps of maize kernels (*Zea mays*) is substantial, especially in the first hours of imbibition. It does not correlate with seed quality and depends on the variety (Bruggink et al. 1991).

If a seed coat proves to be a large source of variation in a conductivity test, it can be considered to remove it. This should however be done very carefully in order not to damage the seed and thereby influence leakage.

## 7.4 Single seed or bulk determinations?

Electrical conductivity can be determined on bulk basis (i.e. samples) or on individual seeds. The disadvantage of making bulk determination is that for example a few dead seeds in a sample may have too large an influence on the result of the test. On the other hand,

determinations on single seeds are either very time consuming or require equipment that can make many single seed determinations at the same time (multi-electrode). Bonner (1989) compared the two methods on pine seeds and found that bulk determinations were as precise as those made with the multi-electrode and that price and maintenance problems of the multi-electrode favoured bulk-determinations.

### 7.5 Conductivity and germination

In this note conductivity is related to seed germination in the laboratory. Ultimately the relationship between conductivity and germination in the field should be established. But as field conditions are difficult to standardize, the laboratory germination test is obligatory. To give an idea of which levels of conductivity to expect two examples are given.

***Picea abies*** (Norway spruce)
The conductivity values corresponding to seed quality, germination percentage and mean germination time were estimated from the results of experiments involving 30 different seed qualities made from 3 different seed lots by controlled deterioration (The seeds were imbibed to 15 and 20% moisture content respectively, put in sealed plastic bags and incubated in a water bath at 43°C for 1-3 days). Thereafter, conductivity was measured on three replicates of 2 g per treatment, soaked in 40 ml water, 16 hours at 20°C. The standard deviations on germination percentage and mean germination time were 10.5 % and 0.76 days (Sørensen, 1995).

| **Seed quality** | **Germination (%)** | **Conductivity (mS/g/40 ml)** | **Mean germination time (days)** | **Conductivity (mS/g/40 ml)** |
|---|---|---|---|---|
| High | 85-100 | < 69.2 | < 5.6 | < 70.6 |
| Medium | 65-85 | 69.2 - 87.9 | 5.6 - 6.7 | 70.6 - 87.5 |
| Low | 40-65 | 87.9 -111.3 | 6.7 - 8.1 | 87.5 - 109.0 |
| Poor | <40 | > 111.3 | > 8.1 | > 109.0 |

***Pisum sativum*** (Vining peas)
Matthews and Powell (1987) developed a standardized electrical conductivity test for this species. 50 seeds per replicate (two replicates) are soaked in 250 ml de-ionized water at 20°C for 24 hours.

(For readings above 30 μS $cm^{-1}$ $g^{-1}$ the difference between two replicates must not exceed 5 μS$cm^{-1}$ $g^{-1}$. If below 30 μS $cm^{-1}$ $g^{-1}$, the difference must not exceed 4 μS $cm^{-1}$ $g^{-1}$).

| **Conductivity (μS $cm^{-1}$ $g^{-1}$)** | **Seed quality** |
|---|---|
| 24 or less | nothing to indicate that the seed is unsuitable for early sowing under adverse conditions |
| 25 - 29 | seed may be suitable for early sowing, but there is some risk of poor performance under adverse conditions |
| 30 - 43 | seed not suitable for sowing especially under adverse conditions |
| 44 or more | seed not suitable for sowing |

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**ANNEX A**

CALIBRATION OF CONDUCTIVITY METERS

**Table 1.** Preparation of solutions with a known conductivity for calibrations of conductivity meters. (For preparation of solutions, use de-ionized or distilled water with conductivity less than 2 µS/cm).

| Solution | Preparation |
|---|---|
| **A** | Dilute 100 ml of solution C to 1000 ml at 20°C |
| **B** | Weigh out (in air) 500 mg NaCl and add water to give 1 kg solution (0.05 weight percent NaCl) |
| **C** | Weigh out (in air) 0.7440 g KCl and dilute with water to 1000 ml at 20°C |
| **D** | Weigh out (in air) 7.4365 g KCl and dilute with water to 1000 ml at 20°C |
| **E** | Weigh out (in air) 74.2640 g KCl and dilute with water to 1000 ml at 20°C |

**Table 2.** Measuring range and conductivity at 18 and 25°C for the solutions prepared according to the directions in table 1. (* to the value specified in the table should be added the conductivity of the water used for the preparation).

| Measuring range | Solution | Conductivity * | |
|---|---|---|---|
| | | **at 18°C** | **at 25°C** |
| 0-20 µS/cm | | Calibrate in the range 0-200 µS/cm | |
| 0-200 µS/cm | **A** | | 147 µS/cm |
| 0-2000 µS/cm | **B** | 873 µS/cm | 1015 µS/cm |
| | **C** | 1221 µS/cm | 1409 µS/cm |
| 0-20 mS/cm | **D** | 11.17 mS/cm | 12.86 mS/cm |
| 0-200 mS/cm | **E** | 97.8 mS/cm | 111.3 mS/cm |

Radiometer (1993)

**ANNEX B**

CALCULATIONS

M.c. = moisture content

DW = dry weight

FW = fresh weight

**M.c./% (FW basis)** = ((FW-DW)/FW) * 100 %

**Dry weight**: DW (g) = FW-((m.c./100)*FW)g

**Specific conductivity, when measuring on a seed sample:**

Conductivity (µS) for each replicate = $\mu S*cm^{-1}*g^{-1}*ml^{-1}$
Seed DW (g) * quantity of water (ml)

(Remember to subtract conductivity value for control container)

**Mean germination time/days** :

(NSG = Number of seeds germinated)

(NSG day 1*1) + (NSG day 2*2) +... (NSG day n*n (days)) = Mean germination time/days
total number of germinated seeds (seed)

**Standard deviation:**

$$\sqrt{\frac{n\left(\Sigma x^2\right)-\left(\Sigma x\right)^2}{n\left(n-1\right)}}$$

x = conductivity reading
n = number of replicates
Σ = sum

**ANNEX C**

MANUFACTURERS (examples)

Single cell:

Radiometer
Valhøjs Allé 176
2610 Rødovre
Denmark

Tel: +45 38 27 28 29
Fax: +45 38 27 27 12

Jenway
Gransmore Green Felsted,
Dunmow, Essex, CM6 3LB,
England

Tel: +44 1371 820122
Fax: +44 1371 821083

Cole-Parmer Instrument Company
625 East Bunker Court
Vernon Hills,
Il 60061
USA

Fax: 847 549 1700

Multi probes:

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