

Final protocol of 26 January 2006 for
Experiment to study interactions between germination environment,
soil disturbance and emergence.

1. *Objective:*

The experiment is designed to determine how magnitude and distribution of emergence of a weed species differs with timing of superficial disturbance of the soil around the time of emergence in undisturbed soil.

In the field, this disturbance could be false seedbed or mechanical weed control (weed harrowing). In both cases, added knowledge about the effects would be beneficial.

One common seedlot is used throughout all locations. A local seedlot is used with the same treatments to compare the variability of the common seedlot between locations with the variability of the common seedlot compared to the local seedlot within a location.

Hypothesis:

Superficial disturbance of the soil at different times relative to emergence in undisturbed circumstances will affect the magnitude and distribution of emergence (see fig. 1 in Rasmussen 1996). Disturbance will affect the following

- Light will trigger more seeds to germinate than without disturbance
- Added oxygen in the soil will facilitate more germination
- Soil moisture will be reduced, which will hamper germination/emergence
- Seeds that have already started to germinate might be killed or buried at a depth from where they cannot emerge

All these factors add up to the expectations shown in the table below:

Time of disturbance rel. to emergence without disturbance	Seeds that germinate	Germinating seedlings that are killed	Total number of seedlings that emerge rel. to emergence without disturbance
Before	More	None	More
Around	More	Some	Equal
After	More	Many	Less

Relation to other experiments:

In the experiment described in Grundy et al. 2003, it was shown that a common (generic) model could be used to predict emergence of weed seeds under different climatic situations. The planned experiment will add knowledge about how the models developed earlier can be used to predict the most relevant timing of soil disturbance.

2. *Size of experiment:*

Number of samples for counting seedlings is 12 for the common seedlot and 12 for the local seedlot (1 seedlot * 6 timings * 2 replicates).

In addition, 6 samples for determination of soil water content but without seedlings must be included. 1 extra sample for determination of onset of emergence with seeds of common seedlot but without counting will also be included. 1 extra sample without seeds for burial of temperature logger. Total number of samples is 32.

3. *Timescale:*

We will initiate the experiment autumn 2005.

Common seedlot to be sent out early October 2005.

Seeds to be sown in autumn 2005.

Disturbances to be made in spring 2006 (and 2007).

Recording to be done weekly from sowing until spring 2007 (2008).

4. *Materials needed:*

Substrate – to be mixed at the location.

Material for containing the samples - Lutrasil.

Fencing:

Either: Wire or nylon mesh lid for samples to prevent birds, small mammals etc. from entering + Small fence around area to keep small mammals out.

Or: Caging the whole area.

Max. hole size in the mesh closest to the samples: 9 mm.

5. *Seed lots:*

Species: *Chenopodium album*.

One common seedlot.

One local seedlot at each location.

6. *Collection, treatment and storage of seed:*

The common seedlot is harvested at Research Centre Flakkebjerg, dried at ambient temperature for a week, stored in sealed plastic bags at 4°C and sent to HerbiSeed. HerbiSeed will clean the seed, establish the seed weight, weigh out seeds for each participant, pack the seed in sealed containers and distribute the seed to the participants. In addition they will obtain a phytosanitary certificate for the seeds needed for most non-EU countries.

The cost for certification and shipment will be payed by the Danish Institute of Agricultural Sciences.

Local populations of seed will have to be collected as local control populations.

- All seeds must be collected from local, reasonably well-established populations, preferable from an agricultural habitat.
- Only mature seeds must be collected, which is ensured by shaking plants in a paper bag and using the seeds that are thus shaken off. Preferably this should be done on the afternoon of a warm, dry day in 2005.
- Collected seeds should be immediately hand cleaned and unwanted chaff etc. should be removed by a blower (particularly important for *C. album*).
- Seeds are to be dried in a room with dim daylight (but not direct sunlight) at ambient temperature for one week (min. 20 °C, max. 25 °C).
- Seeds are to be stored in airtight containers at 4°C until ready for use.

7. *Number of seeds:*

The *C. album* will be sown with 200 seeds per cm of depth. The chosen depth is 5 cm that requires 1000 seeds per sample, 12000 seeds for 12 local seed samples. To ensure enough seeds, each local seedlot should consist of at least 25000 seeds.

For the common seedlot, 13000 seeds are required for each location that participates. We expect approximately 12 locations to participate, which results in a total need for at least 165000 seeds of the common lot. 200000 would be better.

8. Characterisation of seedlots:

Alistair Murdoch will carry out some characterisation of the seedlots – this requires about 5000 extra seeds per lot. This is included in the above calculations. Please send app. 10 g seeds to:

Alistair Murdoch
Seed Science Laboratory
Department of Agriculture
The University of Reading
Earley Gate, PO Box 237
Reading RG6 6AR, U.K.
United Kingdom

9. Distribution of seeds:

HerbiSeed will distribute the common seedlot to all at the time we request. Seeds should be inside sealed containers for all types of distribution, and for mail, these containers should be inside bubble plastic envelopes or other type of insulated package.

10. Counting and weighing of seeds:

At each location, the thousand-grain weight of the local seed sample is determined by counting and weighing 5 samples of 1000 seeds. Seed portions of 13*1000 seeds of the common seed and 12*1000 of the local seed are weighed out on an electronic balance with a precision of ± 0.1 mg.

11. Substrate:

We will be using a substrate made locally at each location. The participants must make the mixture themselves according to the recipe for seed compost at

http://www.gardeningdata.co.uk/soil/john_innes/john_innes.htm:

2 parts Loam, 1 part Peat and 1 part Sand. The loam and peat is sieved through a 9 mm sieve before mixing. For each cubic metre of mix, add 0.6 kg ground limestone and 1.2 kg superphosphate.

After mixing the substrate, it should be spread out to dry at room temperature for easier handling. All the finished substrate should be sieved through a 2.00 mm sieve, and only what passes through the sieve should be used. Around 100 L of sieved substrate should be sufficient.

The liter-weight of the substrate is established by compacting 1 L as much as possible and weighing 5 different samples.

12. Holes:

A hole 5 cm deep with a diameter of 25 cm is dug in the soil for each sample.

13. Soil temperature recording:

In an extra sample without seeds the soil temperature should be measured by installing a "Tid-Bit-Logger" or similar device logging the temperature at least hourly. The device

should be buried at a depth of 2 cm in the sample. Mark the position if the device must be removed annually to record data (applies to Tib-Bit- loggers borrowed from Frank Forcella). For those who do not have such a logger, Frank Forcella will probably be able to lend them one.

14. Seed containment within samples:

A fine mesh large enough to fit into the holes and extend at least 2 cm above the holes, that is at least 40 cm diameter. Strings can be attached to facilitate easy upheaval. We can suggest using a double layer of Lutrasil Thermoselect 18, which is commonly used in horticultural production and should be easy to obtain (see http://www.nonwovens-group.com/bereiche/Lutrasil_18_engl.pdf).

15. Seed and soil mixture:

1000 seeds for each sample are mixed thoroughly with 2.5 L of air-dry substrate, weighed out from the liter-weight determined earlier. The mixing can be done by enclosing soil and seeds in a closed container and shaking this around in all directions for at least 5 minutes. This mixture is then placed on top of the cloth/mesh in the hole. The soil should be compacted with a disc and a weight on top, until it is level with the soil surface. The soil is watered with deionised water. Each sample is watered with 100 ml each of the first 3 days after initiation.

1	2	3	4	Extra	Extra
5	6	7	8	Extra	Extra
9	10	11	12	Extra	
13	14	15	16	Extra	
17	18	19	20	Extra	
21	22	23	24	Extra	

Extra Extra Extra Extra

Extra Extra Extra Extra

Fig. 1. Placement of samples with seeds and possible placement of extra samples for soil moisture determination, seed emergence timing and soil temperature measurement.

16. Arrangement of samples:

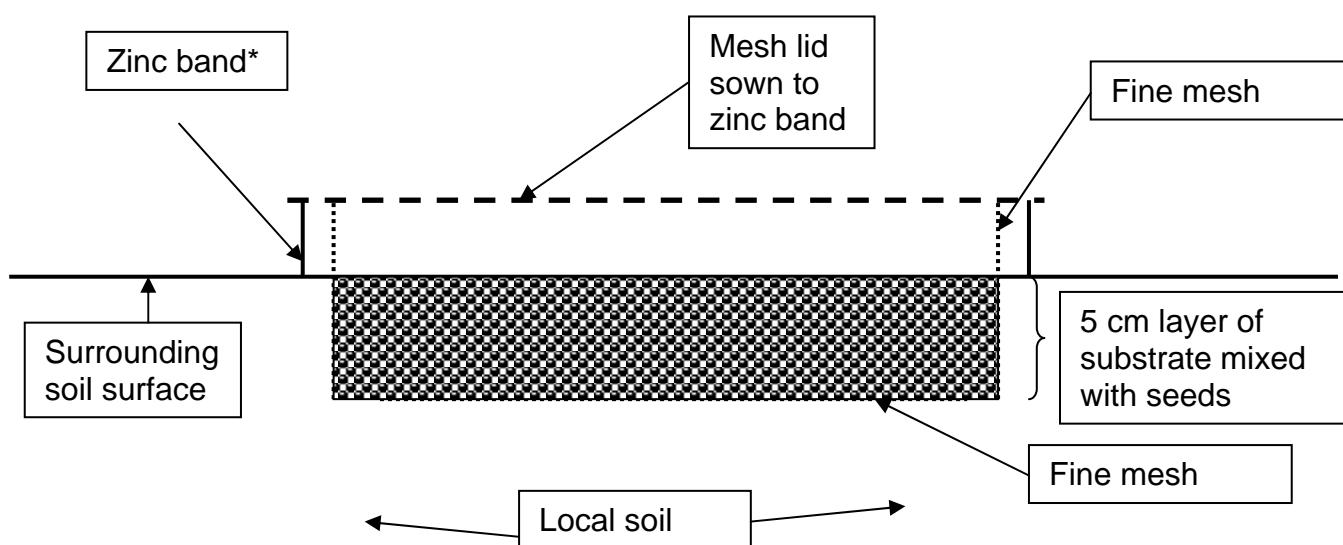
Andrew Mead has made an experimental design for the experiment, please consult the powerpoint and excel-files attached. Those samples which are not included in the plan (without seeds for soil moisture measurements and with seeds for determining time of onset of emergence) can be placed adjacent to the experimental samples however it fits best at each location, see examples in fig. 1.

17. Location of experiment:

The samples should be placed at least 2 m from any wall, hedge or other factor that might alter the microclimate locally, not placed in shade etc. A rim of minimum 50 cm of unplanted ground is suggested around the collection of experimental samples. Between individual samples is only required room enough for the person recording the emerging weeds.

18. Sample burial:

Each sample shall be buried in the ground so that the top of the soil in the sample is level with the surrounding soil surface, see fig. 2.



* Lids for each sample could be replaced with netting or caging the whole area

Fig. 2. Schematic representation of sample in soil.

19. Mesh/nylon lids:

Lids of mesh or nylon with 9 mm square holes tied to a band, which is possible to remove (see fig. 3), around the rim of the sample are placed on each in order to keep out birds, mammals etc. It is equally acceptable to make one large lid for the whole collection of samples.



Fig. 3. Example of lid for one sample.



Fig. 4. Example of netting/caging the whole experimental area.

20. Fence/caging:

A small fence should be installed around the samples in order to keep small mammals out. An alternative could be to cage in the whole area. With small enough mesh (9 mm), this would render the lids for each sample superfluous, see fig. 4.

21. Irrigation

If surrounding fields are irrigated, and this is a common practise, irrigation may be applied to the samples also. Record amount of irrigation.

22. Prediction of emergence

To predict the onset of emergence, a glass plate will be placed on top of the extra seed sample in the early spring of 2006, allowing room for ventilation. The date should be noted when the first *C. album* seedlings are observed emerging in this glass plate covered sample. This date marks the initiation of the treatments.

- Northern locations: the glass plate should be placed over the sample when snow and frost has finished, but before farmers in the surrounding area have begun to till their fields
- Southern locations: for those, where the glass plate may not be appropriate for determining the start of the treatments. For these locations, the first treatment will be initiated when farmers in surrounding fields are beginning to till their fields.

23. Treatments:

Treatments are initiated in spring 2006. Both the common seedlot and the local seedlot will receive the same treatments as follows:

Treatment 1. Undisturbed (no disturbance)

Treatment 2. Disturbance when the first *C. album* seedlings are observed emerging in the sample under the glass plate cover.

Treatment 3. Disturbance when the first *C. album* seedlings of the spring flush are observed emerging in the untreated sample (Treatment 1). Note: some locations will have observed small numbers of seedlings emerging during the winter months which should be ignored for this purpose. Instead, wait until there is a significant seedling emergence event denoting the start of the spring flush.

Treatment 4. Disturbance the first day when 50 day degrees have been accumulated after the date of Treatment 3.

Treatment 5. Disturbance the first day when 100 day degrees have been accumulated after the date of Treatment 3.

Treatment 6. Disturbance the first day when 200 day degrees have been accumulated after the date of Treatment 3.

The day degrees are calculated as

$$\sum_i^t (((T_{\min} + T_{\max}) / 2) - 3)$$

where T_{\min} is the daily minimum temperature, T_{\max} is the daily maximum temperature, and 3 is the base temperature for *C. album*; i is the day when treatment 3 is carried out and t is

the number of days after this. During this period, the day degrees must be calculated daily, to make sure the treatments are done at the right time.

For the purpose of disturbance, the cloth/mesh containing the soil/seed mixture should be carefully lifted out of the hole in the surrounding local soil. Then, outside in full daylight, the soil/seed mixture should be sieved through a sieve with 4.00 mm holes. Once the soil/seed mixture has been sieved it should be returned to the mesh-lined hole in the local surrounding soil from where it was lifted.

Record and remove all seedlings that are found at this time ("white threads").

24. Determination of soil moisture

6 extra samples with soil are used to determine moisture content. One sample is the "untreated", and one corresponding to each treatment time. A 2 cm ring at least 5 cm high is used to extract a sample of soil from the "untreated" (treatment 1) and the "disturbed" (treatments 2-6) at each time of disturbance. In treatments 2-6 this is done the first time immediately after disturbance and replacement, then at each new disturbance in the other treatments. After disturbance has ceased, soil moisture is measured at weekly intervals until the moisture content is the same in all treatments due to precipitation or draught.

The soil is weighed, dried at 100 °C for 24 hours and weighed again. Deionised water is added to return to the water content before drying, and the ring of soil is returned to the sample. The location is marked, so the same soil is not used again.

25. Recording data:

- Weekly emergence is recorded for each sample. Before recording, a wire ring with a diameter 4 cm less than the sample at the soil surface is centered in the sample (see fig. 5). Seedlings within the ring are counted, this is the basic count. Seedlings around the ring are also counted, but recorded separately (secondary count). At recording, seedlings are removed by being cut off at the surface, endeavouring to disturb the soil as little as possible. At peak emergence times, 2-3 weekly counts may be necessary.
- OPTIONAL: at first emergence in treatment 1 and at first emergence after disturbance in treatments 2-6, 1 plant outside the wire ring is left to grow. At each recording, the height and leaf numbers of the plant are recorded. At 25 cm the plant is cut at soil surface.
- Standard spreadsheets will be provided to enter the data.
- Daily maxima and minima of air temperature should be recorded 1 m above local ground, together with the daily rainfall. In case this is not possible, the closest possible information should be used.

26. Fate of experimental samples after first year:

Depending on the results of the first year the participants in the experiment will decide whether to repeat it exactly next year or whether to carry out some alterations, e.g. doing less disturbances, but some in daylight and some during night-time.

References:

Grundy, A.C., Peters, N.C.B., Rasmussen, I.A., Hartmann, K.A., Sattin, M., Andersson, L., Mead, A., Murdoch, A.J., Forcella, F. (2003): Emergence of *Chenopodium album* and *Stellaria media* of different origins under different climatic conditions. *Weed research* 43(3), 163-176.

Rasmussen, J. (1996): Mechanical weed management. In: Second International Weed Control Congress, Copenhagen 1996, p. 943-948.

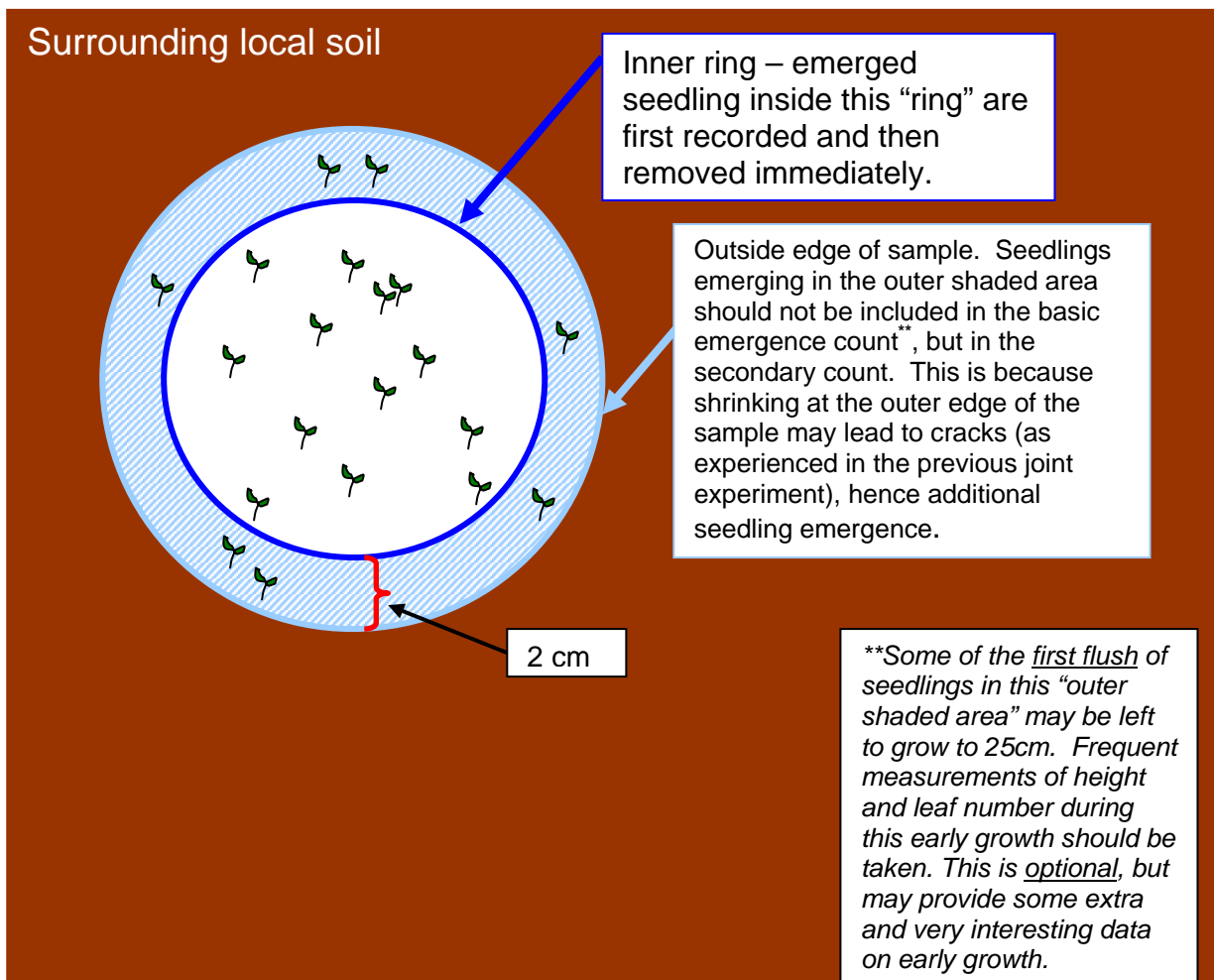


Fig. 5. Placement of wire ring before counting seedlings.