Final protocol for
Experiment to study interactions between germination environment, soil disturbance and emergence 2007-2009.

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 1:

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 rel. to germination without disturbance	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

Hypothesis 2:

It is hypothesized that the emergence patterns and numbers observed, in both the disturbed and undisturbed plots, are a consequence of the inherent dormancy heterogeneity in the common population, and in the local populations. This inherent dormancy variability interacts with the environment in the individual local common experiments (nurseries). The result of this interaction between dormancy (nature) and environment (nuture) is the observed seedling emergence (and “white thread” counts).

If this hypothesis is true, then the dormancy in the seed populations tested and observed should reflect the environment in which they were selected (cropping system, disturbance) and adapted to, and the emergence pattern is adapted to the locality in which it has been selected. Therefore, the historical disturbances and practices from the seed collection site should be reported.

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 can 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 2007.
Common seedlot to be sent out early October 2007.
Seeds to be sown in autumn 2007.
Disturbances to be made in spring 2008 and 2009.
(Autumn disturbance to be made in autumn 2008. Will be decided later.)
Recording to be done weekly from sowing until and including spring 2009.

4. *Materials needed*:

Substrate – to be mixed at the location.
Material for containing the samples:

- aluminum screen trays (supplied to all participants by Adam Davis).
- Lutrasil or similar material for lining the bottom of the tray.

Logger for soil temperature (can be borrowed from Frank Forcella).
Fencing:

- Either: Wire/aluminum screen 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. 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.

6. Holes:

A hole 4 cm deep 25 cm x 25 cm square is dug in the soil for each sample. This should be done well ahead of time, so that there is not a delay once the seed is harvested/recieved.

7. *Seed containment within samples:*
Adam Davis will supply all participants in the experiment with mesh cut in squares, which must be bent and stapled (do not use copper stables, as this will cause the wire mesh to corrode) at each location to make a cage 25 cm x 25 cm x 5 cm (fig. 1). The size of the holes in the mesh is 1.5 mm diameter. On the bottom, a lining of 25 cm x 25 cm Lutrasil or similar material will be needed to avoid seeds from washing out the bottom of the cage. The cages should be made ready well ahead of time.

Fig. 1. Wire mesh tray for samples.

8. Substrate:
We will be using a substrate made locally at each location. The participants can choose whether they will use local soil, or use the mixture, which they must mix 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 (unfertilized) and 1 part Sand. The loam and peat is sieved through a 9 mm sieve before mixing.
For each cubic metre of mix or soil, add 0.6 kg ground limestone and 1.2 kg superphosphate. The soil type of the loam or local soil used, whether pure or in the mixture, should be recorded (% clay, silt, sand etc.).

After mixing the substrate, it should be spread out to dry at room temperature for easier handling. 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.

This should also be done well ahead of time.

9. *Seed lots*:
Species: *Chenopodium album*.
One common seedlot.
One local seedlot at each location.

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

The common seedlot is harvested into perforated cellophane bags at Research Centre Flakkebjerg. Immediately after harvest, dry seeds at ambient temperature (20 to 25°C) for a week as follows:

1. Lay out harvested seeds as a thin layer on trays to facilitate drying
2. Allow seeds to dry on trays at ambient temperature (20 to 25°C) for one week, avoiding exposure to direct sunlight or to a humid atmosphere during this process.
3. Record temperature throughout drying process using loggers to be used for burial, if this is not possible record temperatures manually at 09.00 h on each working day. If 09.00 h is not practicable, choose another time and make a note.

If you can do so, measure seed moisture content of a sub-sample but if you cannot do so, seal a separate 2 g sub-sample of seed in a single plastic bag and send to Alistair Murdoch for moisture content determination. Seal must obviously be perfect to avoid moisture uptake in transit. Possible solutions for this:

1. ideal is a laminated foil packet as used in the seed trade.
2. for the short period of storage/travel, a simple polythene bag sealer would be great (heat sealing).
3. Otherwise a moisture proof container (small plastic bottle with a washer) which could then be placed in a zipper bag.

To measure moisture content:

1. Measure fresh weights of two reps of approx. 1 gram of seed with an accuracy of 0.001 g. Each replicate of seeds should be placed in glass or metal containers with lids (glass Petri dishes are ideal)
2. dry containers plus fresh seeds in a ventilated oven at 130-133°C for one hour – timing should only commence after oven has got back up to 130°C; ensure lids are removed while in oven!!
3. after drying period, replace lids and then transfer to a desiccator to cool for 30 minutes before weighing again with an accuracy of 0.001 g. Desiccator should contain dried silica gel.

After drying, seeds should be stored in a single sealed plastic bag in a refrigerator (3-5°C). The main seed sample should be sent immediately after drying to HerbiSeed. HerbiSeed will clean the seed, pack the seed in sealed containers and distribute the seed to the participants. The seeds are to be stored in the sealed container in a refrigerator (3-5^{o}C) until burial.

HerbiSeed will obtain a phytosanitary certificate for the seeds needed for most non-EU countries.The cost for certification and shipment will be shared between all participants.

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

- All seeds must be collected from local, reasonably well-established populations, preferably from an agricultural habitat. Collect seeds from a single population in a single field. Please record the date of harvest and the latitude and longitude of the sampling point. Try to obtain the most recent cropping history of the field.

- Only mature seeds must be collected, which is ensured by shaking plants in a paper or perforated cellophane bag and using the seeds that are thus shaken off. Preferably this should be done on the afternoon of a warm, dry day in 2007.
- Collected seeds should be immediately dried as indicated for the common seed lot above, i.e. seeds are to be dried in a room with dim daylight (but not direct sunlight) at ambient temperature for one week (min. 20 ^{o}C, max. 25 °C). Record temperature during drying if you can.
- After drying, seed lot should be hand cleaned by sieving etc. and unwanted chaff etc. should then be removed by gently rubbing the seeds and then by using a blower (particularly important for *C. album*).
- If you can, measure seed moisture content as above for common seed lot, If not possible, do not worry unduly as Alistair will measure on receipt.
- Dried seeds are to be stored in airtight containers and if they are not to be buried immediately, they should be placed in a refrigerator at 3-5°C until ready for use.
- On removal from the refrigerator, you must allow seeds to warm to ambient temperature for about two hours before opening their airtight container. This will prevent any increase in moisture due to condensation. As soon as you open the container, please withdraw a 12 g subsample and reseal it in another airtight container for seed characterisation (see below).
- Seeds should be buried as soon as possible after collection and receipt of the common seedlot. Both seedlots should be buried at the same time.

For all seedlots, record date and location of harvesting. Supply data on historical disturbances and practices and local climate in the data sheet supplied for results.

11. Number of seeds:

The *C. album* will be sown with 1000 seeds per sample, 12000 seeds for local seed samples and 12 g for seed characterisation. To ensure enough seeds, each local seedlot should consist of at least 30000 seeds.

For the common seedlot, 13000 seeds are required for each location that participates + 1000 seed for the maternal environment experiment for those who participate in this. We expect approximately 12-15 locations to participate and need an extra portion for seed characterisation, which results in a total need for at least 240000 seeds of the common lot. 300000 would be better.

12. Characterisation of seedlots:

Alistair Murdoch will carry out some characterisation of the seedlots – this requires about 13000 extra seeds per lot. This is included in the above calculations. Immediately before (i.e. on the same day) you bury the seeds, take a subsample of approx. 12 g seeds and seal in an airtight container. It is vital that seeds are completely airtight to prevent changes in moisture content.

1. ideal is a laminated foil packet as used in the seed trade.
2. for the short period of storage/travel, a simple polythene bag sealer would be great (heat sealing).
3. Otherwise a moisture proof container (small plastic bottle with a washer) which could then be placed in a zipper bag.

Send as soon as you have buried the 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

13. Distribution of seeds:
HerbiSeed will distribute the common seedlot to all as soon as they have received it, cleaned it and obtained the phytosanitary certificates. 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.

14. 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. Thousand-grain weight of the common sample will be supplied from Denmark. Record seed weight (mean and variation) on an electronic balance with a precision of $\pm$ 0.1 mg. Seed portions of 13 $*$ 1000 seeds of the common seed and 12 $*$ 1000 of the local seed are determined by weighing out seeds according to the mean seed weight. 1000 seeds should be put in each pot!

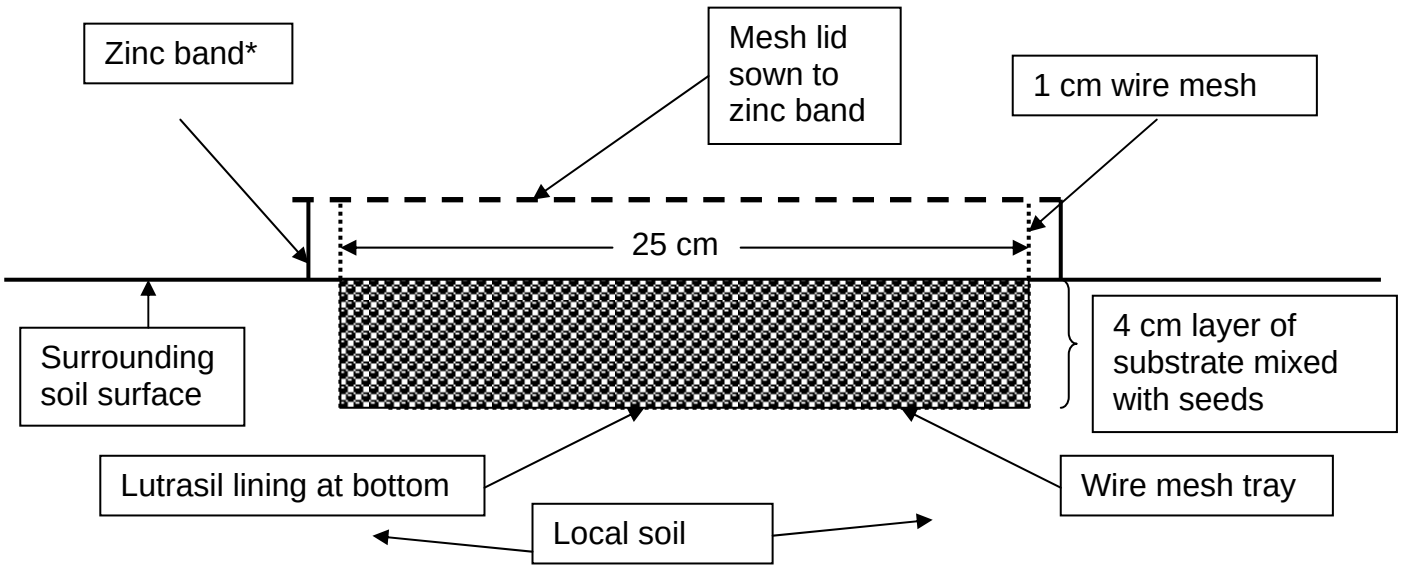
1	2	3	4
5	6	7	8
9	10	11	12
13	14	15	16
17	18	19	20
21	22	23	24
25	26	27	28
29	30	31	32

Fig. 2. Placement of samples with seeds and possible placement of extra samples for soil moisture determination, seed emergence timing and soil temperature measurement.

15. 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. If you don't have a data-logger that works outside, please take this offer to borrow one.

16. Seed and soil mixture:
1000 seeds for each sample are mixed thoroughly with 2.5 L of air-dry substrate or local soil, 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.

17. Arrangement of samples:
Andrew Mead will make an experimental design for the experiment, please consult the powerpoint and excel-files when they are forwarded. For the general lay-out, see fig. 2. The extra samples for moisture measurement etc. could be either placed between the two replicates (13-20) or at the end (25-32).



* Optional. Lids for each sample could be replaced with caging the whole area

Fig. 3. Schematic representation of sample in soil.

18. *Sample burial:*
Each cage shall be buried in the ground so that about 1 cm of the cage extends above the ground, see fig. 3. This mixture is then placed in the cage. The soil should be compacted with a 25 cm x 25 cm board and a weight on top, until it is level with the soil surface, 1 cm below the rim of the cage. No water is applied.

19. Mesh/nylon lids:
Lids of mesh or nylon with 9 mm square holes tied to a band, which is possible to remove, 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.

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.

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 help predict the onset of emergence, a glass plate or fiber (e.g. lutrasil) will be placed on top of the extra seed sample in the early spring of 2008, allowing room for ventilation. The date should be noted when the first *C. album* seedlings are observed emerging in this glass plate or fiber covered sample.
- Northern locations: the glass plate/fiber 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: the glass plate may not be appropriate for determining the start of the treatments.

Since this method has shown not to be totally appropriate, is it only used as a help. At all locations, treatments should start in the spring 2008, when either one of these conditions is met:

- emergence is seen in at least two samples, whether local or common, with or without glass or fiber cover

or

- when farmers start to till the surrounding fields.

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. DO NOT initiate disturbance until after 31 January 2008.

23. Treatments:
Treatments are initiated in spring 2008.
DO NOT initiate treatments in the autumn of 2007, even if emergence is seen at this time. Instead, emergence should be recorded as described below.

Both the common seedlot and the local seedlot will receive the same treatments as follows:

Treatment 1. Undisturbed (no disturbance in spring).
Treatment 2. Disturbance when the first *C. album* seedlings are observed emerging or farmers begin to till fields, as described above.
Treatment 3. Disturbance the first day after 50 day degrees have been accumulated after the date of Treatment 2.
Treatment 4. Disturbance the first day after 100 day degrees have been accumulated after the date of Treatment 2.
Treatment 5. Disturbance the first day after 150 day degrees have been accumulated after the date of Treatment 2.
Treatment 6. Disturbance the first day after 200 day degrees have been accumulated after the date of Treatment 2.

The day degrees are calculated as

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

where T_{min} is the daily minimum air temperature, T_{max} is the daily maximum air temperature, and 3 is the base temperature for *C. album*; i is the day when treatment 2 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 cage 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 turned upside down into a big container. "White threads" should be carefully extracted (see later). The soil/seed mixture should be mixed carefully by hand so that all the soil/seed mixture receives full daylight. Once the soil/seed mixture has been mixed by hand, it should be returned to the cage in the local surrounding soil from where it was lifted.

No compacting should be carried out afterwards, but attempt to even out the pile to fit with the cage.

Record and remove all seedlings that are found at this time ("white threads"). If possible, record white threads, which have previously been cut (and thus already counted as seedlings) seperately. Do not include white threads in the seedling count.

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 ^{o}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. If there has been precipitation during the drying period, this amount of water should also be added to the core. The location is marked, so the same soil is not used again.

25. Recording data:

- Emergence is recorded approximately weekly for each sample from the burial of the samples until December 2009. If the soil is snow-covered or frozen, recording is not needed. If recording is done, but no seedlings were found, 0 should be put in the spreadsheet. 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.
- Recording should always be carried out immediately before disturbance.
- If non-sterilized soil is used, background emergence is recorded at the same times in the pots for soil moisture determination.
- 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 collected.

26. Fate of experimental samples after first year:
(Recording should be carried out until autumn tillage (e.g. plowing, harrowing) is carried out in the surrounding fields. At this time, all samples should be disturbed in the same way disturbance was carried out in the spring. If no autumn tillage is carried out in surrounding fields, the disturbance should be done in the experiment when surrounding fields are harvested and emergence has ceased. Contact Ilse if in doubt. In spring 2009 the treatment procedure from spring 2008 will be repeated.) This will be decided upon later.

27. *Participants* *Date of initiation*
Czech Republic: Bozena Sera
Denmark: Ilse A. Rasmussen
Finland: Jukka Salonen
Italy: Francesco Tei + Federica Graziani
Norway: Kirsten Tørresen
Portugal: Edite Sousa
Spain: Jose M. Urbano + Jose L. Gonzalez Andujar
Spain: Alicia Cirujeda
Sweden: Lars Andersson
United Kingdom: Paul Neve
USA: Frank Forcella
USA: Adam Davis
USA: Jack Dekker

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.