Soil seedbank analysis of *Ambrosia artemisiifolia*

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Common ragweed (*Ambrosia artemisiifolia* L.) population dynamics is largely influenced by seeds produced and stored in the soil seedbank by the local population. As in many agrestal annual weed species future generations are limited by the availability of seeds.

Soil seedbank analysis is the best measure to evaluate the sustainability of control activities against annual species like common ragweed at the local scale. Additionally, this manual can be used as a standard protocol to estimate ragweed soil seedbank for any ecological study. The implemented viability test procedure can be used for testing any containment of ragweed seeds.

Soil seedbank **varies** considerably in **space** (horizontally, and by depth) and **time** (seasons, years).

Analysis of the soil seedbank for testing the efficacy of any control measure should be performed during the vegetation period between late spring (after germination) and new seed dispersal in late summer. Then you will find the persistent partition of ragweed seeds in the soil.

To predict the annual aboveground population one should count the seed bank in early spring – just before natural germination. At this point in time the losses during the first winter (10-15%) have crystallized already.

Spatial variation can cause high costs if accuracy (number of soil cores) needs to be high.

Generally, a representative sample of the soil seed bank should be based rather on many (small) soil cores than on few big ones. Keep in mind that the likelihood of artefacts like vertical displacement of seeds by drilling increases with a rising number and low diameter of cores. The diameter of soil cores should not be less than 3 cm; between 6 and 8 cm diameter is a good compromise. The bigger the diameter the more effort is needed to perform the drill to deeper soil horizons. Several labs use a minimum number of 20 soil cores per unit with diameters between 5 and 7.4 cm. In case of using lower diameters one should increase the number of cores to 50 or even 100. The 20 soil cores can fit to characterize the soil seedbank for fields of 1 ha with a representative design covering the whole field (diagonals of a rectangular field and some additional cores in the middle of the four sectors).

Of course, the spatial expansion, microsite variability and anthropogenic influences determine the number of soil cores needed. Arable fields or meadows tend to have rather homogenous structures but huge expansions. Soil perturbation by ploughing causes relatively high spatial homogenization of the soil seedbank (at least in direction of the drilling movement). In habitat types with less soil perturbation (riverbanks, ruderal places, roadsides) the spatial structure of ragweed populations may be rather discontinuous and concentrates to the upper soil layers.

Fallows may contain many viable ragweed seeds in deeper soil layers even several years after abandonment. In such cases and in managed arable fields the soil cores should be stratified to lower (10-25 cm) and upper (0-10 cm) soil. Analyzing the dynamics of the soil seedbank to the very details (scientific approach) needs even more vertical segments of cores (depending on the depth of soil tillage (ranging from 15 to 35 cm).

Storage: Soil cores should be collected in plastic bags and stored at low temperatures (below 2°C) in darkness. If sampling is done in spring many seeds may already be stimulated to germinate and will continue even in darkness. Quick further treatment of the samples is recommended to avoid die back of seedlings germinated in the probes.

Seed separation from soil:

Seeds are commonly cleaned from soil by wet sieving (hand washing as well as high pressure wet sieving techniques). At least two mesh diameters are needed: 5 mm for catching coarse material and 1.25 mm (or 1 mm) for catching all ragweed seeds or at least all seed coats. Testing germinability directly from the soil will fail to measure the total number of viable seeds because of dormant seeds remaining in the soil matrix. Also, spreading soil material to flattened dishes needs much space and continuous wetting of the dishes. In dry sandy soils, dry sieving is sufficient for seed selection.

Counting ragweed seeds:

What to count? Often empty seed coats can be found but must be neglected. They can be segregated by soft touch with a pincer. Ragweed siconia can deplete their achene coat and look like a smooth coated rounded nut (Fig. 1). Siconia as well as achenes represent 'intact seeds'.

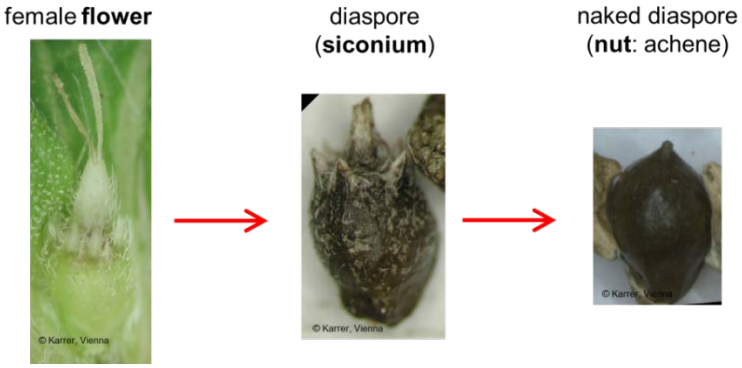

Fig. 1: Development of female flowers of *A. artemisiifolia* to diaspores and achenes

All **intact seeds** remain to be tested for viability (Fig. 2) by germination only, germination and TTC-test, standard TTC-tests (to detect dormant but viable seeds) or simple crush test.

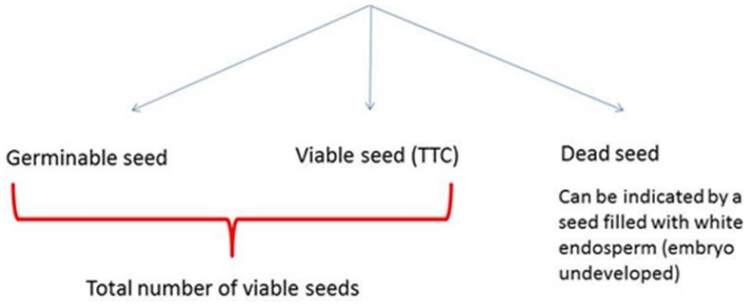

Fig. 2: The viable portion of intact seeds represents the relevant soil seedbank of *A. artemisiifolia*.

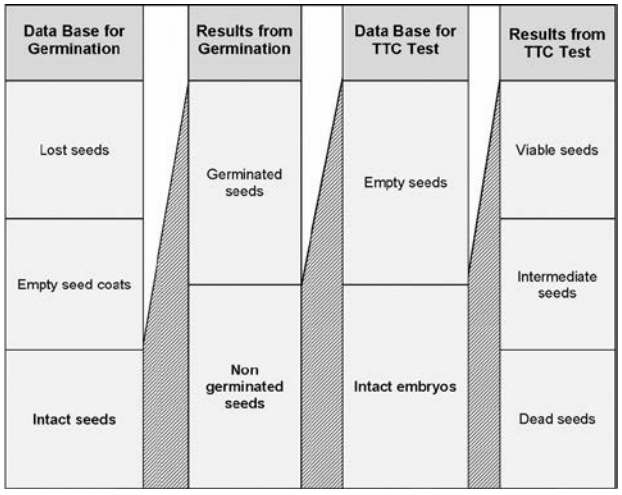

Fig. 3: Hierarchy of *Ambrosia artemisiifolia* seed qualities tested in soil seedbank analysis

Viability test of *Ambrosia artemisiifolia* seeds

Testing the viability of ragweed seeds should follow a standard protocol to be accepted in any case where one needs to know the infestation of any containment (soil seedbank, construction waste, crop seed portions, etc.). It can be relevant i.e. if land parcels are sold in countries where ragweed underlies a legal regulation for control (Switzerland, Hungary). Furthermore, many other European countries also consider implementing such regulation into their legislation.

Based on several tests and joint trials we hereby provide a standard protocol for viability test of ragweed seeds. The optimal test comprises of 2 stages: first, germinability is tested and, secondly, the remaining seeds are tested by a standard staining technique widely used to indicate respiration of living tissue, namely staining with Triphenyl-tetrazolium-chloride (TTC).

Preparation of seeds: Ragweed seeds should be stratified by exposure to low temperature (≤2°C) over minimum four weeks in darkness and moist condition. Seeds from the soil seedbank sampled in late winter or spring can be treated directly because of natural stratification in the field. Wet sieving stimulates germination.

Germination test: should be performed directly after wet sieving in order to avoid losing seeds that started to germinate stimulated by the sieving. Useful environments are '25°C/12h daylight and 15°C/12h darkness' over a period of four weeks. Check for germinated seeds ('visible radicula') every second day and take them off. Seeds that did not germinate should be tested by TTC-test for viability preferably directly after.

Viability test procedures are TTC-staining or crush test. Both provide almost the same results with very young seeds. The older the seeds the more the crush test overestimates viability because old dead seeds also soak up water and give putative 'positive' results. Therefore, we rather recommend the use of TTC-test.

TTC-test: Respiring (living) tissues are able to convert a colorless compound of Triphenyl-tetrazolium-chloride to a carmine-red colored water-insoluble formazan by hydrogen transfer reaction catalyzed by the cellular dehydrogenases. TTC enters both, living and dead cells but only living cells catalyze the formazan. The test is commonly used for testing seed quality (100 intact seeds per population) with various instructions produced by, e.g., the International Seed Testing Association. Certain adaptations for specific seeds are commonly made.

- imbibe seeds for 12-15 h in tap water (only in case of dry seeds)
- prepare a 1% TTC solution (1 g in 100 ml demineralized water)
- cut each seed into halves (using a nail clipper or medical scalpel is advised)
- check for the presence of an intact embryo to be used further
- put seeds with an intact embryo (both halves) into 1 to 5 ml glasses with 1% TTC solution individually (1 seed into 1 glass); very appropriate are plates which are used in PCR-analyses. Incubation for 24h in darkness at 30°C
- assign the embryos to staining intensity classes "alive", "dead", and "intermediate" according to Fig. 4.
- two independent observers desirable
- If there is no time for immediate count after 24 hours, you can store the colored seeds at 4°C and darkness for some time.

Fig. 4: Classification of *Ambrosia artemisiifolia* seeds; alive = fully stained (a-c), intermediate = partially stained (d-g), dead: = not stained (h-i), or = degraded embryo (j)

Fully stained seeds are counted as 'viable'. Intermediate staining is variable and hard to interpret. If you want to know the number of seeds that are really viable count only fully stained as such. If you aim to estimate the risk of eventually germinating ragweed seeds you should include the intermediates to the 'viables'. No staining indicates for dead seeds (Fig. 4). Intermediates from older seed lots tend to be partially stained overall reddish or orange, but the seeds do not perform well in the germination test. Seeds of 3 to 5 years in age tend to have delayed germination and are not able to develop further forming a well-developed primary root with root hairs. In case of fresh seeds (less than one year in age), the team found them to develop rather normally.

Consequently, intermediate seeds should be counted 'alive' when seeds are fresh, but counted 'dead' when more than one year in age. In case of soil seedbank samples of natural populations, regularly several seed cohorts from different years are present, and 'intermediates' have to be evaluated 'dead' in the ecological sense.

Crush test: can be applied to assess whether seeds contain live cells by squeezing out potential liquid of seed that have been cut in half. The manual was proposed for the population dynamics task force comprising the following steps (see Fig. 5):

- seeds should have a dry surface; you can let them dry in a petri dish at room temperature
- hold the seed firmly between tweezers in a petri dish (to avoid it jumping away), while cutting it longitudinally (from top to base) with a scalpel or razor knife
- place a filter paper on a glass slide, and then place the biggest half seed on the filter paper with the cut side touching the paper
- crush it, by placing another glass slide on top of it, and pressing it firmly all the way down to the paper
- take the seed away, and immediately observe the paper, holding it against the light. If the filter paper is wet, the seed is considered 'alive'.

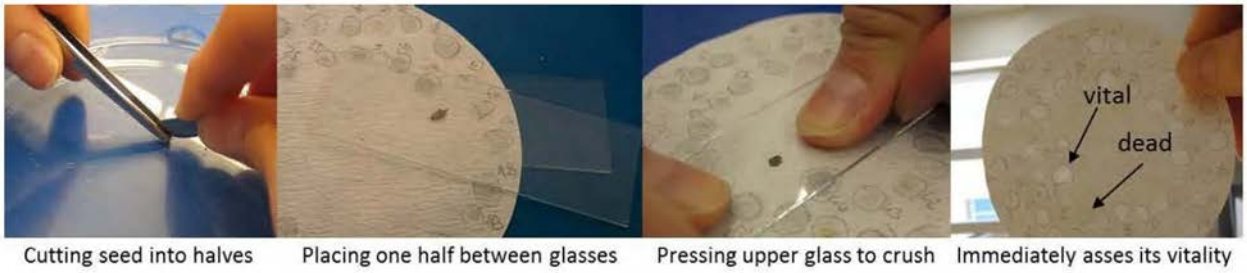

Fig. 5: Crush test to assess viability of seeds (from Lommen & al 2014, unpubl.)

Generally, crush test gives higher proportions of 'viable' seeds and overestimates viability. The overestimation is almost zero when fresh seeds are tested. Older seeds with damaged or reduced embryo also deliver liquids and are counted 'alive' erroneously. Consequently, we propose to use the crush test as a cheap and quick testing procedure only with fresh seeds but not with old seed lots like ones from soil seedbank.

Long time storage of ragweed seeds:

Avoid storage of soil seedbank samples for a longer period. At least, storage under wet conditions leads to decay of ragweed seeds within a few months/years. If soil cores are stored subsequent to drying the survival rates seem to be higher (maximum of 5 years for quantitative analyses).

Readings:

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