

Project plan re-introduction of *Spiranthes aestivalis* in The Netherlands

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- I. Objective is to re-introduce *S. aestivalis* in a sustainable fashion at a historical growing site in The Netherlands.
 - II. In 1873 *Spiranthes aestivalis* was discovered in The Netherlands for the first time. Until 1936 it was found in damp oligotrophic habitats in the Southern part of the country. In total 13 sites were identified. *S. aestivalis* disappeared in 1936 due to dewatering and overfertilisation. In recent times some of the original habitats have strongly been improved by effective nature management and are considered suitable again for *S. aestivalis*. The best site has - by thorough investigation - proven to be the 'Urkhovense Zeggen', a natural reserve East of the city of Eindhoven. The vegetation (including other orchid species) resembles the historic *S. aestivalis* sites, it is oligotrophic with a weak basic seepage and has characteristics of the *Campylio – Caricetum dioicae* association. Moreover the soil is rich in (orchid) mycorrhizae. It is realised that for a successful re-introduction the availability of more sites would be desirable. We are in serious search for such sites and have several candidates in mind.
 - III. It is unlikely that *S. aestivalis* will return by itself, so re-introduction will be indispensable. To this end we have been looking for suitable seed-donor locations in nearby North-West Atlantic areas of which the most nearby have been found in France. For desired genetic diversity two sites at least would be required which have been found in Normandie and in Pays de la Loire. In Normandie a number of sites are known in les Landes de Lessay with over 1000 plants of *S. aestivalis* growing in moist heather fields. About Pays de la Loire the French orchidologists Prof. Marc Andre Selosse and Chantal Griveau informed us on locations in La Flèche (Sarthe) with relatively small populations of 100 plants at maximum.
 - IV. Since *S. aestivalis* is a strictly protected species a permission from the French authorities (CBNBrest) is required to collect seeds. The relevant procedure is ongoing. For our part we are committed to minimise the effect of seed collection on the present populations.

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Therefore the amount of seeds to be collected should be minimised, especially for the case of Sarthe. We therefore propose to collect a maximum of 2 seed-pods in the case of La Flèche and a maximum of 5 stems with ripe pods for Lessay spread over the grounds. For La Flèche only one ripe pod will be cut off per plant by means of scissors and collected in a marked dry paper sack. For Lessay the stems will be cut with scissors and also marked and collected in dry paper sacks.

- V. Sowing of the seeds will be done in The Netherlands immediately after harvesting in:
1. Pots with soil obtained from the location Urkhovense Zeggen, La Flèche⁵ and Lessay. The reason to sow in different soils is to maximise the chance of success of cultivating mature plants which would serve as source of additional seeds that could eventually be used to sow directly in the intended site. The pots will be kept in a cold greenhouse in the dark until protocorm formation becomes visible. Pots will then be placed in moderate light (regime following outdoor conditions).
 2. Pots with soil obtained from the location Urkhovense Zeggen, La Flèche and Lessay mixed with grinded egg carton and moistened with liquid from a preculture of 1 L tap water, 10% sucrose and 100 g soil from the locations Urkhovense Zeggen, La Flèche and Lessay, respectively. Pots will be kept in a cold green house in the dark until protocorm formation becomes visible. Pots will then be placed in moderate light (regime following outdoor conditions).
 3. In vitro (BM1 medium with Nitsch vitamin mixture⁴, 20g.L⁻¹ sucrose and 5g.L⁻¹ agar, pH 5.5) in the dark. When roots and shoot formation has started, in vitro seedlings will be replanted on 1/5 MS medium supplemented with 20g.L⁻¹ sucrose, 5g.L⁻¹ agar and 20g. L⁻¹ grinded unripe banana, pH 5.5 and placed under dim light conditions. When tuber formation has taken place plantlets will be transferred to pots with soil from the Urkhovense Zeggen location and pots are then placed in a cold green house in moderate light (regime following outdoor conditions).
- Flowering can be expected after 2- 4 years and the seeds will be used
- a. for further propagation under controlled (greenhouse) conditions
 - b. for direct sowing outdoor in the intended nature area. Also a selection of mature plants can be planted in the said nature area.
 - c. Naturely, the further development of the plants will thoroughly be monitored by the Dutch Society for European Orchids, in cooperation with Naturalis Biodiversity Centre, Leiden, the Wageningen University & Research, Science4Nature and the Eindhoven Municipality Authority. The results of which will be communicated with the licensing authority (CBNBrest).
- VI. Organisations involved in the project are
1. WEO (Dutch Society European Orchids, NL);
 2. Levend Archief (Dutch organisation for the preservation of plant seeds NL);
 3. Naturalis Biodiversity Center, Leiden (NL);
 4. Radboud University, Nijmegen (NL);
 5. VOV (Dutch Society Propagation of Orchids, NL);
 6. CBNBrest; DREAL Normandie; DREAL des Pays de la Loire; Lessay, Commune de La Feuille (Manche, France); La Flèche, Commune de la Flèche (Sarthe. France)
 7. Gemeente Eindhoven (NL);

⁵ Only a small amount is required, maximum two dozen cm³. The soil will be transported in a small closed container under ambient conditions.

⁴ Vitamins, mg/l: Biotin, 0.05. Folic acid, 0.50. Glycine, 2.00. myo-Inositol, 100.00. Nicotinic acid, 5.00. Pyridoxine HCl, 0.50. Thiamine HCl, 0.50.

8. Science4Nature (NL);

VII. Project management and responsibilities

1. Project management rests with the WEO (dr. J. ten Dam, dr.ir. J. Lammers). The WEO has taken the initiative of the project for re-introduction of *S. aestivalis* in the southern part of The Netherlands. They have identified a suitable re-introduction natural reserve and source fields. Also they have organised the scientific support and propagation possibilities. It is WEO's responsibility to manage effective communication between the parties involved, to coordinate the successive steps and to obtain permissions where required.
2. Levend Archief (prof. Dr. J.Schaminée, dr. G.Oostermeijer, expert biologists) will advise on the scientific, technical and organisational aspects of this project
3. Naturalis Biodiversity Center, Leiden (NL) (prof. dr. B. Gravendeel), takes care of part of the scientific support of the project. Naturalis investigated the biological properties of the soil that are necessary for germination of orchid seeds and growing-up of the plants. They have carried this out for both one of the source fields (Lessay) and the target field (Urkhovense Zeggen, UZ). They will also assist in the collection of seeds via a suitable contact in France (prof. Marc-André Selosse) and assist in arranging the permission of collecting seeds.
4. The VOV (dr. Wagner) is specialised in the propagation of orchids. They will apply proven techniques for this purpose. They will receive seeds of *S. aestivalis* from Naturalis. They will prepare suitable germination-beds of UZ soil and sow and grow up the plants (V).
5. CBNBrest will advise on the final permission for seed collection. DREAL des Pays de la Loire and DREAL Normandie will provide for the respective final permissions. Commune de La Feuille and Commune de la Flèche will be involved and will be requested for permission of collecting soil samples.
6. Gemeente Eindhoven. The city of Eindhoven is owner of the natural reserve 'de Urkhovense Zeggen' (UZ). The WEO is responsible to ask permission for collecting soil and for the introduction of the plants by direct sowing and planting of mature plants.
7. Science4 Nature (dr. G. Oostermeijer, dr. S. Luyten). They have greenhouses at their disposition. The WEO/VOV will contact them for assistance in growing-up seedlings of *Spiranthes aestivalis*.

VIII Demande de Dérogation (separately), for Pays de la Loire and for Normandie.