SUMMARY ACTION C4

The breeding of *Osmoderma eremita*, envisaged by the Life Eremita project, is aimed at obtaining a series of specimens through *ex situ* and *in situ* reproduction, starting from founding individuals taken from natural populations. The breeding makes it possible to have animals - larvae and adults - for reintroduction and repopulation activities during the Life project. This permits a wide action of reinforcement of the populations and the expansion of the species distribution area in Emilia-Romagna in the medium and long term.

The breeding of *Osmoderma eremita* has been subjected to checks by the Ministry of the Environment and by ISPRA (*Higher Institute for Environmental Protection and Research*), as prescribed by Presidential Decree 357/97. To this end, the feasibility study was drawn up (Fabbri et al., 2017) in compliance with national and European guidelines (AA.VV, 2007; IUCN / SSC, 2013; IUCN, 2014).

Before the Life Eremita project was activated, these conservation activities for the *Osmoderma eremita* species had not yet been applied in European projects (Dubois, 2009; Silva et al., 2012). A previous *ex situ* larval breeding experiment was recently conducted in Finland on the *Osmoderma barnabita* species (Motschulsky, 1845) in order to examine the role played by the main substrates present in tree cavities on adult female preferences and larval growth (Landvik et al., 2016).

In Emilia-Romagna, 3 *ex situ* breeding centres were set up and located in the National Park of the Tuscan-Emilian Apennines, in Ligonchio (RE), the National Park of the Casentinesi Forests, Monte Falterona and Campigna, in Santa Sofia (FC), and the Management body for the Parks and Biodiversity Romagna, in Russi (RA). In all three cases, the facilities are also used for educational purposes, for informational activities, and for raising awareness as to the importance of insects and their role in nature. Each breeding facility has some vertical shelves, a table with a lamp and a sink.

The three breeding farms were created from an appropriate number of adult specimens that were withdrawn from natural habitats. As an alternative or in combination with the extraction of adults, one could also proceed to collect larvae in nature. The withdrawal of the founding individuals was carried out only where the ex-ante monitoring has verified the presence of sufficiently large populations, able to guarantee an exemplary ratio captured/census population of about 1/10, in order not to damage the population of origin.

The capture of the founders has been achieved by employing passive fall traps (pitfall traps) and also the intercepting by attraction traps (black cross window traps), or by direct research of adults and larvae in the colonised trees (wood mould sampling) (Ranius & Jansson, 2002; Chiari et al., 2013). The black cross window traps are triggered with a racemic mixture of γ-decalactone, the pheromone emitted in nature by the males of *Osmoderma eremita* to attract females (Larsson et al., 2003).

In order to breed *O. eremita*, the technique employed was based on a set of containers, simulating as many cavities, and containing the *pabulum* (nutrient substrate). The method is effective for obtaining a high number of third-instar larvae and adults to be introduced into the wild. Specimens bred with this system do not show significant differences in average size compared to wild individuals. The containers used for breeding are made of transparent plastic and have the shape of a parallelepiped (about 30 containers per farm, measuring 39x28x28 cm and with capacity of 22 l), equipped with a lid and kept in the dark, to simulate the natural conditions of life inside the cavities of the trees. On the top, on the lid, a large window was opened and covered with a fine plastic net (like a mosquito net) fixed with hot glue. The height of these containers allows an adequate thickness of the alimentary *pabulum* in order to guarantee a more differentiated gradient of humidity and oxygenation between the bottom and the surface, thus

allowing the larvae to move to the most suitable depth. For the preparation of the substrate, a certain amount of litter, which corresponds to about 30% of the finished product, was collected in nature from deciduous forests; subsequently, other materials have been added with the following proportions: 50% of beech wood sawdust, 25% of manure, 25% of soil improver (for example peat), all free of chemical residues and glues. The mixture has been matured in large crates (even 100 litres) for at least 4 months, moistened and stirred every 7 days.

The founding adults were placed at the beginning of the summer (2018) in the containers already filled 2/3 full of soil, whose surface was covered with pieces of trunk or branches (to facilitate the walking of adults), and closed at the top with the lid and a fine-knit net. To prevent the larvae from being attacked by pests or predators, in fact, every single container with soil has been covered with a fine plastic net. The animals are fed with fresh fruit, fruit purees and also with artificial jellies produced for beetles' sustenance. Following the reproduction and hatching of the eggs, one waits for the larvae to reach the second growth stage (L2), which is generally achieved in a few weeks. At this stage of development, to avoid cannibalism, the larvae are moved to other containers of the same capacity, about 20 larvae per container. These containers are filled for about 3/4 of soil and litter (2/4 of soil at the bottom and above it 1/4 litter). Here they are bred until complete development.

The sets of containers are checked every seven days; in particular, the checks focus on the structure, the soil production, and the containers that host insects in the extra-reproductive period. In the period of reproduction and oviposition, however, it is necessary to inspect the containers in which the adults are housed daily and, subsequently, the larvae. During any operation, disposable gloves are used to minimise the risk of entry of any diseases through contact with the operators.

Through manual inspection, any larvae of elateridae or other potential predators are removed.

During the inspections the correct degree of humidity of the soil and the possible need to replace a part of it are verified. The food is renewed in the containers of adults, and the larvae containers are sample checked to verify the good health of the larvae themselves and any signs of disease (e.g. presence of dark necrotic lesions on the tegument, finding dead larvae, etc.) or stress and suffering (e.g.: observation of larvae emerging and remaining on the surface). During the checks and the change of the soil, the contents of the containers are emptied into large plastic basins (30-40 l type). A 2 l pressure sprayer is used to humidify the breeding containers. The soil is replaced approximately once a month from the end of the second year of breeding or, anyway, when faecal pellets exceed 50% of the volume. Whenever the soil is changed, the counting and weighing of the specimens and their division by stages are also carried out. The weighing is done using a digital precision scale. The checking and emptying of the terrariums are strictly suspended from the beginning of October until the end of December, to avoid the risk of damaging the newly formed cocoons.

Before each release action, a check is planned on the health status of the specimens and to divide larvae by sex (the determination of the sex of the specimens is done by looking for the organ of Herold that distinguishes the male). Before the release, all adults will be measured with a digital or analogue precision calliper, weighed and provided with a numbered label (e.g. numbers to mark queen bees).

The farms are also equipped with some transparent plastic containers that are smaller than those used for breeding (28x19x14 cm and 5 l volume), for various uses such as the isolation of individuals for various reasons, the temporary accumulation of individuals during counting and measuring operations, educational purposes, transportation, etc.

The farms are equipped with a paper register. All visits and checks carried out by operators and other employees are recorded in the register. Also recorded are the environmental conditions

detected by multifunction probe or data-logger, the number of specimen after the count, the number of active containers (with live specimens), the dates of movements, the dates of eclosions and hatches, the number of eggs observable against the light, the operations performed (soil humidification, substitution of litter and/or soil, etc.), the annotation of any particular phenomena (presence and elimination of pests, development of fungi and other organisms, formation of excessive condensation, ice formation in winter, and death of larvae or pupae, etc.).
All phases of *ex situ* breeding are documented and photographed.
Since in Italy the natural cycle of *Osmoderma eremita* is above all biennial, at the end of the first year of breeding it is possible to obtain young third-stage larvae (L3) and, in the second year of breeding, mature third-stage larvae (L3), ready to cocoon in the soil and remain in the pre-pupa stage until the spring of the following year (third year of breeding), when the adults hatch. The cycle can be synchronised to the different picking altitudes of the founders, by adjusting the temperature inside the breeding and therefore speeding up or delaying it.
The management of each breeding farm is carried out by an expert entomologist assisted by an expert operator and involves a work commitment of about 4 hours daily for 70 days/year.