

GENERATIVE REPRODUCTION OF *EPIPACTIS PALUSTRIS* (L.) CRANTZ  
UNDER EXPERIMENTAL GARDEN CONDITIONS

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**ABSTRACT.** The paper presents the results of 7-year observations of marsh helleborine *E. palustris* growing in an experimental garden. The proportions of flowering shoots, the number of flowers in the inflorescence and fruit setting rate are presented and the annual seedling emergence was recorded, starting from the fourth year since the flowering of the first flowers. The presence of seedlings of this plant in a garden is of paramount importance and their further development needs to be carefully monitored.

**KEY WORDS:** Orchidaceae, *Epipactis palustris*, marsh helleborine, flowering, fruit setting, seeds, germination

## INTRODUCTION

The population size in case of marsh helleborine increases mainly through vegetative reproduction and in terms of this ability it exceeds the other species from genus *Epipactis* (PROCHÁZKA and VELISEK 1983). However, similarly as other orchids, it also shows a huge potential for generative reproduction, which measure is a high proportion of flowering ramets (aboveground shoots), a large number of produced flowers and as a consequence also fruits and seeds. According to WILDHABER (1970), in one capsule of orchids from genus *Epipactis* there are on average 4500 seeds. At the mean number of flowers per raceme in *E. palustris* amounting to 9.4 and at a fruit setting rate of 84.44% (ZIEGENSPECK 1936), one ramet produces approx. 35 thousand seeds.

Despite such a high potential, efficiency of generative reproduction in this species is slight due to weak seed germinability (BATYGINA et AL. 2003). Experiments conducted by KINDEREN (1995) on seed germination in two species of orchids (*Dactylorhiza maculata* and *Epipactis helleborine*) confirmed his assumptions that among thousands of seeds sown every year only several of the germinating seeds reach the autotrophic stage. For the initiation of the germination process (SZLACHETKO and SKAKUJ 1996), as well as in the further stages of protocorm development (KROMER 1998, BATYGINA et AL. 2003), appropriate conditions are required, including the presence of a symbiotic fungus in the substrate (WITHNER 1974, ARDITTI et AL. 1982). Marsh helleborine is considered to be a strongly mycotrophic species in the initial development stages (ARCZEWSKA 1993).

Germination of seeds begins with absorption of water from soil. The apical part of the forming protocorm is composed of smaller cells and it is the apical part of

the shoot, while the basal part, with larger, parenchymal cells, functions as a storage organ for nutrients. No radicle meristem is formed. All roots in orchids appear only following the process of shoot differentiation, thus they are adventitious (VEYRET 1965, STOUTAMIRE 1974, BARABÈ et AL. 1993, BATYGINA et AL. 2003). In the central and basal parts of the protocorm epidermal hairs are formed, through which hyphae of the symbiotic fungus penetrate (RASMUSSEN 1995, 1998). Hyphae are digested by the orchid, in this way becoming a source of food for the development of the protocorm and as a result the formation of the first leaf (JENKINSON 1995). However, HADLEY (1982) presented an assumption that the role of the symbiotic fungus is not limited to the supply of nutrients; infection may lead to more complex processes initiating respective metabolic pathways or provides specific intermediate metabolites.

It is commonly known that territorial occupancy of new habitats by overground orchids, including *E. palustris*, takes places through seeds (ZIEGENSPECK 1936, SUMMERHAYES 1985, LANG 1989), by anemochory (VAN DER PIJL 1972, GANDAWIJAJA and ARDITTI 1983). The effect of seed dispersion over long distances is an important mechanism in the colonization and favouring of these positions, which promote the formation and persistence of a population. However, to date the presence of marsh helleborine seedlings has not been reported either *in situ* or *ex situ*. Problems with finding seedlings *in situ* stem from several reasons, primarily low efficiency of generative reproduction, the origin of plantlets (vegetative or from seed) being impossible to distinguish unless they have been dug out and small seedling size.

To date it has not been determined under what conditions the development of a plant from seed occurs and how long it takes in nature (RASMUSSEN 1992), or how

many years the underground stage of protocorm development takes (ARCZEWSKA 1993, RASMUSSEN 1998). All data concerning germination come from laboratory analyses and obviously the course of the process does not necessarily have to be identical as under natural conditions. Factors breaking seed dormancy *in situ* are not known (STOUTAMIRE 1974). According to Dressler (1981), most overground orchids do not produce chlorophyll for several months from seed germination, since appropriate phytohormones, supplied by the symbiotic fungus, are required for the activation of the photosynthetic system. The time interval during which the underground protocorm is transformed into a juvenile plant is species-specific (BATYGINA et AL. 2003). In marsh helleborine it is estimated at 2-3 years from seed germination (SUMMERHAYES 1985, SZLACHETKO and SKAKUJ 1996).

The aim of the study was to observe marsh helleborine under *ex situ* conditions, at a lack of competition from other plant species. The reproduction potential of this species, as well as the manner of expansion and territorial occupancy by ramets have been investigated.

#### MATERIAL AND METHODS

After a permit from the Ministry of the Environment had been obtained for the collection of marsh helleborine from positions in the city of Poznań, in the autumn 1999 (A1-OD) and in the autumn 2000 (RP-OD), a small number of plants were transplanted to the experimental garden. In order to improve the chances for successful transplantation of plants, which are considered to be highly sensitive to changes in environmental conditions, single shoots were not collected, but rather small lumps of soil (20 × 20 × 20 cm) were collected together with rhizomes they contained. This ensured the presence of mycorrhizal fungi on the new sites.

Samples were collected from two populations in the city of Poznań. One of them is located on an embankment, man-made in 1970 as a result of the construction of a railroad line encircling the city (ATPOL square BC-99). Thus it occupies an anthropogenic position (A1). Helleborine is found there in the flowering, calciphilous meadow community *Galietum borealis* Nowiński 1928 (syn. *Galio borealis-Molinietum* (W. Koch 1926) Philippi 1960) from the *Molinion* association. The other population is located in the Różany Potok (RP) valley (ATPOL square BC-99). Helleborine is found in a moist molinia meadow represented by the *Selino carvifoliae-Molinietum caeruleae* Kuhn 1937 association, from the *Molinion* community.

Collected samples were placed in an insolated position, on separate plots marked A1-OD and RP-OD, respectively. Plant cultivation measures consisted in abundant watering of plots during vegetation periods and hand weeding.

In the years 2000-2006 all ramets were counted, the structure of their age status was determined, flowers were counted and in 2000, 2005 and 2006 also set fruits were counted. The number of ramets which appeared in the first year after planting was adopted as the initial number for comparative analyses in successive years.

In order to investigate the age structure the age status method was used, determining the proportions of ramets in individual phases of ontogenetic development. The following development stages of helleborine were adopted after KAMIŃSKI and SAROSIEK (1990): juvenile – for plants with 1-2 leaves; immature – for plants with 3-4 leaves; full-grown vegetative (virginile) – for plants with five and more leaves, but non-flowering; and generative.

Maps of ramet distributions in a 1:10 scale were prepared each year for the purpose of observations of expansion and territorial occupancy in plot A1-OD (KWIATKOWSKA and SYMONIDES 1978, 1980). Distinguished development stages were marked on these maps using appropriate graphic symbols (NIECKUŁA 1987). In this way information on the spatial and age structure was obtained.

#### RESULTS AND DISCUSSION

Analysis of the age structure (Table 1, Figs 1 and 2) indicates that the proportions of fertile shoots and the number of flowers in inflorescences increased from year to year, which suggests good adaptation of plants to the conditions found in the garden (Phot. 1). In this respect we need to focus on plants from plot RP-OD, which in the first year after planting (2001) did not form any fertile shoot, while five years later (2006) the mean number of flowers per raceme was 22.4 (for 397 plants), which is an impressive result for this species. In this garden a maximum, previously not recorded, unit number of flowers in a raceme was observed, amounting to 37 flowers. Under natural conditions the maximum recorded number of flowers in a raceme was 35 (BERNACKI 1999), with a mean of 9.4 (ZIEGENSPECK 1936), 10-15 (WYRZYKIEWICZ-RASZEWSKA 2006). Fruit setting rate in plots was high and ranged from 60.72 to 98.76%. In populations from natural habitats fruit setting was recorded at 48.5-72.6% (BAUMANN et AL. 1998), over 80% (SUMMERHAYES 1985), 80-90% (MÜLLER 1988), while in anthropogenic positions from 85% (TAŁAŁAJ 2004) to 100% (WYRZYKIEWICZ-RASZEWSKA 2006).

Starting from 2004, i.e. four years after the first *E. palustris* plant flowered, in the vicinity of plot A1-OD seedlings of this species started to appear every year. The seedling from 2004 wintered and in the spring of 2005 renewed its development. However, as a result of drought and hot weather in July, the aboveground part dried up. In 2005 towards the end of July and in the beginning of August further seven seedlings appeared. Photographs 2 and 3 show that they represent different development stages, which is rather striking since they appeared in the same year. Four of them, best developed, continued their growth in the following year (2006); however, only two survived till autumn, because one seedling did not survive hot summer weather and one was accidentally pulled out.

In September 2006 three new seedlings appeared and at the beginning of October there were two more, followed by the next four in 2007 and in 2008 there appeared three more. In the period from 2004 to 2008 a total number of 20 seedlings appeared. At present the oldest is four years old and in 2008 it formed seven leaves.

TABLE 1. Fertile shoots, the number of flowers and fruits in racemes of *E. palustris* in the years 2000-2006 on plots A1-OD and RP-OD

Year	Plot	Fertile shoots		No. of flowers in raceme			No. of fruits in raceme			Fruit set (%)
		number	proportion (%)	min.	max.	mean	min.	max.	mean	
2000	A1-OD	1	4.2	8	8	8	7	7	7	87.5
	RP-OD	*	*	*	*	*	*	*	*	*
2001	A1-OD	9	27.28	6	14	10.50				
	RP-OD	0**	0.00	0	0	0.00				
2002	A1-OD	29	59.20	7	26	14.80				
	RP-OD	16	35.56	5	19	11.10	1	18	7.94	71.35
2003	A1-OD	49	57.65	4	24	14.60				
	RP-OD	70	73.68	3	28	15.20				
2004	A1-OD	110	79.13	2	25	15.92				
	RP-OD	167	91.76	3	31	19.30				
2005	A1-OD	160	74.07	4	22	13.32	0	19	8.09	60.72
	RP-OD	264	88.29	6	31	21.38	3	31	21.11	98.76
2006	A1-OD	137	50.93	5	30	12.20	5	30	10.12	82.95
	RP-OD	397	84.47	5	37	22.40	3	37	21.76	97.14

\* No data available, plants on plot RP-OD were planted a year later than on plot A1-OD.

\*\* In the first year after planting there were no fertile shoots, only vegetative shoots grew.

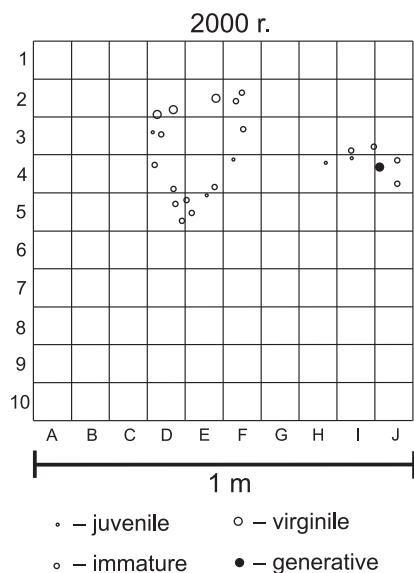


FIG. 1. Spatial and age structure of *E. palustris* (L.) Crantz on plot A1-OD, in the first year after planting (2000)

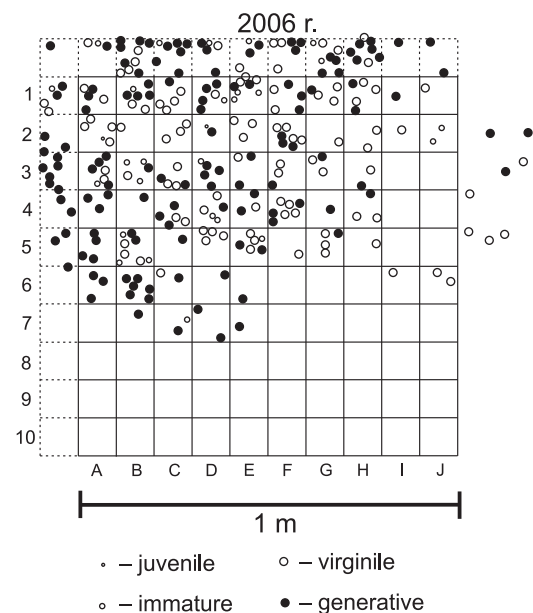


FIG. 2. Spatial and age structure of *E. palustris* (L.) Crantz on plot A1-OD, in the 7th year after planting (2006)

RASMUSSEN (1992), based on his laboratory experiments, assumed that seedlings should appear *in situ* in the spring, since seeds require preliminary maturation and an overcooling period, during which the testa has to be at least partly damaged. However, all seedlings in the tested plots appear in late summer and in the

beginning of autumn. It needs to be explained whether seeds germinate immediately after sowing, in the autumn of the same year and their underground development lasts through spring and summer of the following year, or whether they germinate as late as spring and in late summer they are already visible above ground



PHOT. 1. Flowering shoots of *E. palustris* on plot RP-OD (photograph taken 13.07.2005)

PHOT. 2. Seedling no. 4 (photograph taken 12.08.2005)



PHOT. 3. Seedlings nos. 6 and 7 (photograph taken 12.08.2005)

as seedlings. HARVAIS and HADLEY (1967) pointed out to the fact that seeds of European overground orchids germinate best at a temperature of 23°C or more, and thus they do not begin growth until the next season after sowing.

FALIŃSKA (2002) also assumed that seedlings should appear between maternal plants due to the presence of the mycorrhizal fungus in the soil. In turn, in the conducted investigations it was found that despite much space being potentially available around maternal plants, seedlings grew at a certain distance (approx. 1.5-2 m), which definitely shows that they are not of vegetative origin. Moreover, it proved that seeds from which they grew did not come from the seed bank of the soil in which transplanted rhizomes were found. Their appearance at some distance from maternal plants also means that they found an appropriate fungal partner in the garden soil. According to WITHNER (1974), for most overground orchids the most typical symbiotic fungus is *Rhizoctonia solani*, a species found relatively commonly in the soil. This author also pointed out the strongly pathogenic properties of this fungus, but he also explained that symbiotic associations with orchids are possible thanks to their ability to produce phytoalexins, which inhibit parasitic attempts of the fungus. This might be the reason why seedlings may also be found in different sites than maternal plants, since it is not very difficult for them to find the fungal partner.

In the opinion of the author, seedlings appear also among maternal plants, but they remain undetected as plants formed in the generative manner, since they do not differ in appearance from plants propagated vegetatively. It may be shown by the fact that the number of ramets counted twice within a year, in the spring and autumn, is sometimes higher in the autumn. This difference may be the effect of the appearance of generatively formed plants, since all vegetative shoots appear in the spring.

## CONCLUSIONS

1. Analyses from the experimental garden showed that intensive vegetative propagation of marsh helleborine shoots is supplemented by generative reproduction.

2. Seedlings appearing every year in the garden comprise clear evidence that generative reproduction of marsh helleborine is not as unusual as it is commonly believed and no special conditions are required, apart from those generally needed for growth and development of this species.

3. In the conducted experiments seedlings – as aboveground shoots, do not appear in spring, as it has been suggested to date, but rather in late summer and autumn.

4. The maximum time required by marsh helleborine seeds, from sowing to the formation of the first assimilating leaf, is four years; however, it may not be excluded that this period may be shorter, since seeds coming not from the year 2000, but the following years, could have germinated in the analysed plots.

5. In the garden a maximum number of flowers in a raceme, not previously observed in marsh helleborine,

and amounting to 37 flowers, as well as a very high mean number of flowers in the raceme of 22.4 were found.

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