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Production of gametophytes by *Hippochaete* (*Equisetaceae*) hybrids

Tvorba gametofytů ze spor hybridů rodu *Hippochaete* (*Equisetaceae*)

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The production of gametophytes from spores of the following species and hybrids of *Hippochaete* (*Equisetaceae*) taxa is reported: *H. ramosissima* (Desf.) Börner, *H.* × *meridionalis* (Milde) Holub (= *H. ramosissima* × *H. variegata*), and *H.* × *moorei* (Newm.) H.P. Fuchs (= *H. hyemalis* × *H. ramosissima*). The gametophytes were obtained from spores collected on plants occurring in two localities. Evolutionary consequences of the absence of complete hybrid sterility are discussed.

Keywords: *Hippochaete*, *Equisetum*, gametophytes, hybrids

Introduction

The genus *Hippochaete* differs from the genus *Equisetum* in a number of characters: apiculate cones; stomata sunken below the surface of the epidermis, forming a single line on the side of the furrows (Hauke 1963); different gametophyte morphology and development (Duckett 1972, 1973, 1979). Both genera (considered also as subgenera by some authors) also differ in their ability to hybridize. Hybridization is known within these genera, but no hybrids between both have been reported so far (e.g. Holub 1972, Stace 1985, Jermy et al. 1990). Hybridization between *Hippochaete* species is more common than that between species of *Equisetum*. Novák (1972) suggested that both genera represent two parallel evolutionary lines isolated probably from the end of the Palaeozoic.

The common occurrence of hybrids between most of the co-existing species together with the absence of a clear distinction between individual species are characteristic features of *Hippochaete* (Hauke 1963). The existence of hybrids was confirmed by experimental hybridization (Duckett 1979). The hybrids possessed mostly abnormal spores and are reported to be sterile (Hauke 1963). Hrouda et Krahulec (1982) found about 10% of spores in *H.* × *meridionalis* (*H. ramosissima* × *H. variegata*) to be green and normal; these spores seemed to be morphologically functional. Page et Barker (1985) reported the existence of green spores in *H.* × *trachyodon* (*H. hyemalis* × *H. variegata*). Dubois-Tylski et Girerd (1986) reported green spores in specimens resembling *H.* × *moorei* (*H. hyemalis* × *H. ramosissima*). By evaluating these types as belonging to *H. hyemalis* they corrected the description of this species.

The hybrids are not morphologically homogeneous. Some are of intermediate character, but there are also nothomorphs close to one of the putative parents; the latter are usually described as varieties (Novák 1972, Hrouda et Krahulec 1982). This pattern cannot be

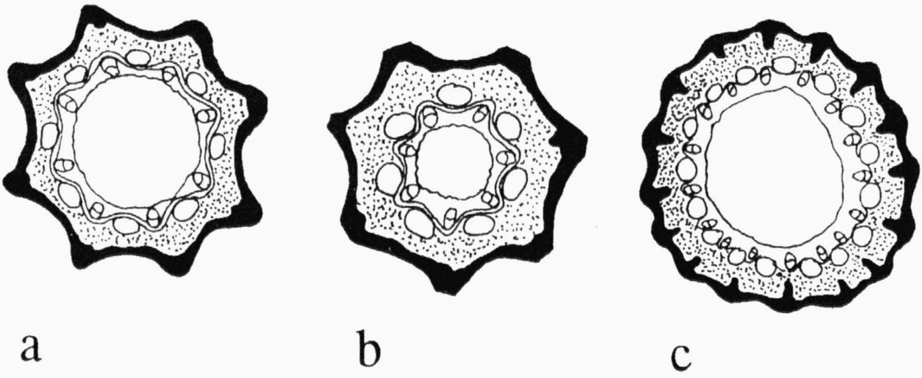

Fig. 1. Stem cross-sectionsof the taxa studied:
a – *Hippochaete ramosissima*; b – *H.* × *moorei*; c – *H.* × *meridionalis*.

Table 1. – Characters distinguishing particular *Hippochaete* taxa studied.

	H. ramosissima	*H.* × *moorei*	*H.* × *meridionalis*
Stem cross-section	continuous collenchyma and chlorenchyma	chlorenchyma interrupted by narrow carinal collenchyma extending almost to vascular bundles	continuous collenchyma and chlorenchyma
Stem sheaths	without median furrows	with median furrows in upper part of the sheath	with median furrows
Sheath teeth	teeth acute, not mucronate with narrow scarious border or almost absent; tooth bases sometimes with black spot	teeth often acute, with twisted long tips and narrow scarious border, usually dark, sometimes with black band in the centre at base of sheath	teeth with broad scarious border and a black band in the centre; black colour common in upper part of the sheaths
Stems	with furrows, ribs convex with transverse tubercles	with shallow furrows, ribs mildly convex to flat	with furrows, ribs flat

Table 2. – Composition of growing medium used for gametophyte cultivation. Growing medium: 1 l of tap water supplied with 1 ml of WUXAL super, 10 g of $CaCl_2$ and 15 g of Agar.

WUXAL super	(g per 1 l):				
N (NH_4NO_3)	98	B	0.12	Mn*	0.16
P_2O_5	98	Cu*	0.085	Mo*	0.01
K_2O	73	Fe*	0.19	Zn*	0.06

* these elements used in chelatized form

explained by other factors (e.g. matroclinity) and indicates the existence of backcrossing; this means that there is probably no absolute sterility of primary hybrids.

To test the sterility of hybrids we collected cones of one species and two hybrids at two localities and carried out germination experiments.

Material

The cones of hybrids and of *H. ramosissima* were collected at the end of July 1994 at two localities:

1. Slovak Republic, SW Slovakia, district Bratislava-vidiek, small pine wood at the railway N of Zohor (latitude 48°20' N, longitude 16°59' E). *H. ramosissima*, *H.* × *moorei* (plants No. 1–8).
2. Slovak Republic, Central Slovakia, distr. Banská Bystrica, 1.5 km north of Slovenská Lupča (latitude 48°47'N, longitude 19°16' E). *H. ramosissima*, *H.* × *meridionalis*, and *H.* × *moorei* (plants No. 10–24).

The hybrids were characterized by a combination of morphological characters and by stem anatomy (Fig. 1, Table 1). Each taxon was represented in our set several times, but we cannot exclude the possibility that, for example, hybrids from one locality were represented only by one clone.

Methods

Cones from individual plants were isolated directly in the field. In the laboratory spores from one sporangium were carefully (i.e. with the highest possible degree of sterility) put into a small amount of distilled water which was spread over the surface of agar plates supplied with nutrient solution (Table 2). The Petri dishes were cultivated at room temperature under a regime of 14 hours light, 10 hours darkness. After 10 days we started recording the number of germinating spores. To prevent fungal infection the culture of gametophytes was treated with Amphotericin B after seven weeks.

Results

We succeeded in obtaining germinating spores, which developed into gametophytes, from *H. ramosissima* as well as from both hybrids (Table 3, Fig. 2). The frequency of germinating spores was two to three orders lower in the hybrids than in *H. ramosissima*, but the results clearly show that at least the hybrids studied (*H.* × *meridionalis*, *H.* × *moorei*) produce spores capable of germinating and developing into gametophytes.

Discussion

The existence of green spores of *Hippochaete* hybrids has been described during the 1980s in at least two different hybrids (*H.* × *meridionalis*, *H.* × *trachyodon*). In this paper we report on the production of gametophytes from *H.* × *meridionalis* and *H.* × *moorei*. This fact is additional evidence that *Hippochaete* hybrids are not fully sterile as has been thought. It seems highly probable that gametophytes of hybrid origin can cross with each other or with their parents and that this is the reason why the species are not so clearly delimited in this genus as in *Equisetum* (Hauke 1963, Holub 1972) and why the hybrids are polymorphic. It cannot be excluded that existing gene flow and recombination may lead to the formation of fully fertile types resembling a hybrid in some characters, as described by Dubois-Tylski et Girerd (1986). It is highly probable that existing gene flow leads to the loss of a clear distinction between species on the one hand and to the increase in their ecological plasticity on the other. The same may be true for those hybrids which are known to persist at localities where only one of the parents is present (or both parents absent). These hybrids sometimes grow in habitats not occupied by any of their parents,

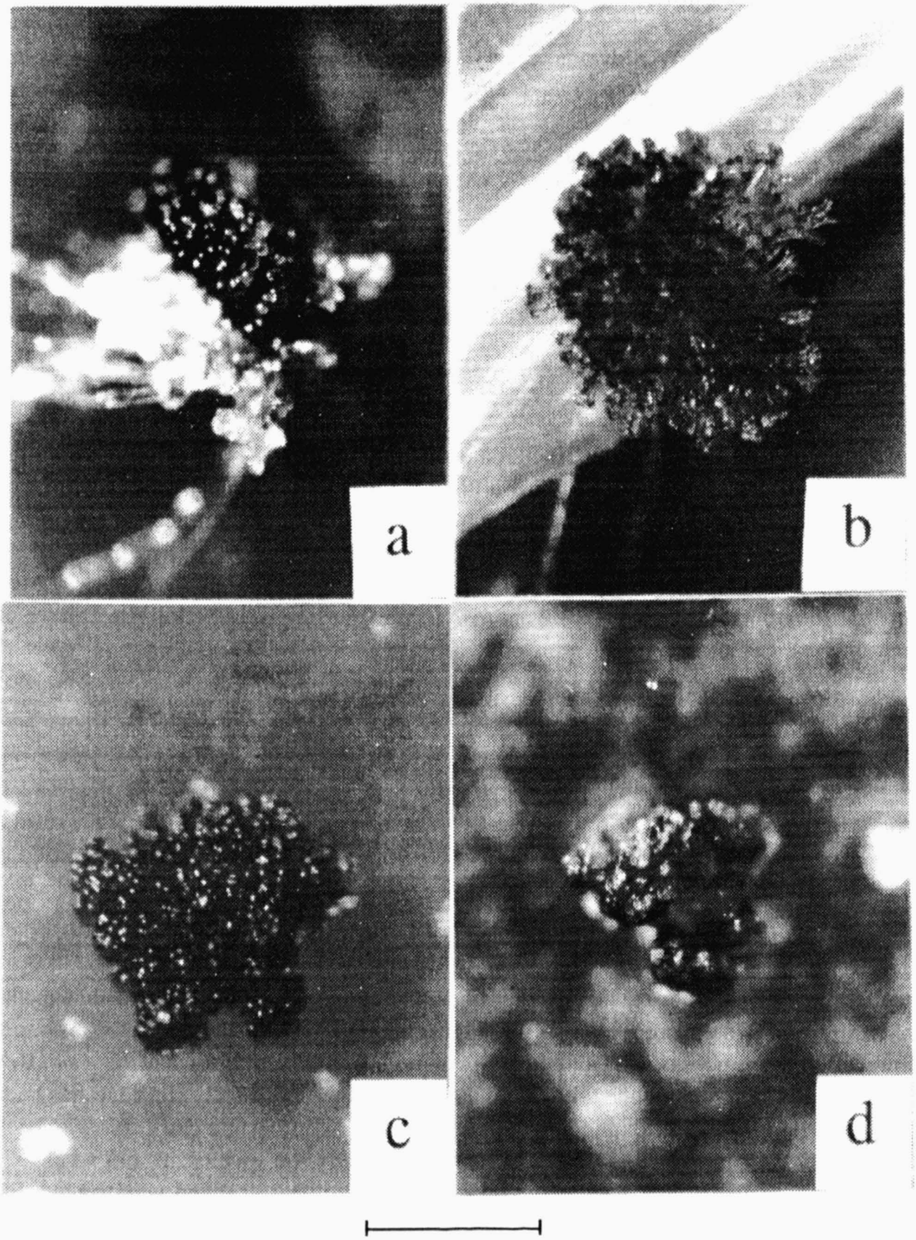

Fig. 2. Young gametophytes of *H. ramosissima* (a, b), *H.* × *meridionalis* (c) and *H.* × *moorei* (d). bar = 1 mm

Table 3. – Germination of spores in *Hippochaete ramosissima* and two hybrids, *H.* × *moorei* and *H.* × *meridionalis*. Spores were sown on 21 July in Petri dishes of 56.7 sq. cm in size. Numbers represent individual plants from which spores from two sporangia (a, b) were sown. Germinating spores were counted on two dates (1 August 1994, 17 August 1994) in five sq. cm (second and fourth column). Total of germinating spores for the whole Petri dish at a given date is shown in the following column. For values >1000 the total represents only a rough estimate.

	No	1 August	Total	17 August	Total
H. ramosissima	1a	7	12	2	15
	1b	0	5	3	5
	2a	453	6000	491	6500
	2b	589	8000	557	7500
	5a	46	130	31	160
	5b	152	270	111	300
	6a	505	1400	461	1300
	6b	296	500	275	450
	7a	512	1500	377	1100
	7b	369	850	376	800
	20a	0	0	0	0
	20b	0	0	0	0
	23a	0	0	0	0
	23b	0	0	0	15
	24a	0	0	0	0
	24b	0	0	0	0
H. × *moorei*	4a	0	0	0	0
	4b	0	2	0	2
	8a	0	0	0	16
	8b	0	0	0	2
	19a	0	0	0	3
	19b	0	0	0	0
	21a	1	1	0	0
	21b	1	1	0	0
H. × *meridionalis*	10a	0	0	0	0
	10b	0	0	0	1
	11a	0	0	0	0
	11b	0	0	0	0
	12a	1	1	1	1
	12b	0	0	0	0
	13a	0	0	0	0
	13b	0	0	0	0
	14a	0	0	0	0
	14b	0	0	0	0
	15a	0	0	0	1
	15b	0	0	0	5
	16a	0	0	0	0
	16b	0	0	0	0
	17a	0	0	0	0
	17b	0	0	0	0
	18a	1	1	0	1
	18b	0	0	0	0
	22a	0	0	0	0
	22b	0	0	0	0

as described by Hrouda et Krahulec (1982), who found *H.* × *meridionalis* growing on conglomerate rocks. More often, the hybrids vigorously colonize disturbed habitats, as reported recently for *H.* × *meridionalis* and *H.* × *moorei* (Hrouda et Krahulec 1982) or for *Equisetum (Hippochaete)* × *ferrisii* (Moran 1983, Rutz et Farrar 1984). It seems that disturbed habitats encourage the development and survival of *Hippochaete* hybrids.

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Souhrn

Hybridy z rodu *Hippochaete* (*Equisetaceae*) byly dosud známy jako úplně sterilní. Jejich variabilita však ukazovala, že je velmi pravděpodobné zpětné křížení: kromě intermediárních rostlin jsou nacházeny v hybridních populacích i rostliny podobné více jednomu či druhému z rodičů. V průběhu 80. let byl u dvou hybridů (*H.* × *meridionalis*, *H.* × *trachyodon*) nalezen malý podíl morfologicky funkčních, nedeformovaných spor. To naznačovalo, že sterilita hybridů nemusí být úplná. Tento příspěvek přináší výsledky z experimentu, ve kterém byly vypěstovány gametofyty ze spor dvou hybridů: *H.* × *meridionalis* (*H. ramosissima* × *H. variegata*) *a H.* × *moorei* (*H. hyemalis* × *H. ramosissima*). Těchto gametofytů bylo přibližně o dva až tři řády méně než vyrostlo gametofytů ze spor rodičovského druhu *H. ramosissima*. Skutečnost, že hybridní rostliny nejsou úplně sterilní, vysvětluje existenci více morfotypů hybridů a má i další evoluční následky. Existující genový tok může vysvětlit i méně ostré hranice jednotlivých druhů tohoto rodu, zejména ve srovnání s rodem *Equisetum*, stejně jako jejich ekologickou přizpůsobivost.

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