Ontogeny of the mycoheterotrophic species *Afrothismia hydra* (Burmanniaceae)

STEPHAN IMHOF1* and MOSES N. SAINGE2

1Spezielle Botanik und Mykologie, Fachbereich Biologie, Philipps-Universität, D-35032 Marburg, Germany
2Centre for Tropical Forest Science (CTFS), University of Buea, Department of Plant and Animal Sciences, Buea, Southwest Cameroon

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The complete ontogeny of the mycoheterotrophic *Afrothismia hydra* (Burmanniaceae) from seed to seed dispersal is presented. The oblong–ovoidal seeds are up to 0.7 mm long. They germinate with root tissue only, disrupting the seed coat and developing a primary ovoid root tubercle. At the proximal end of the tubercle, a second tubercle arises and further root initials indicate the sequential growth of more root tubercles with filiform extensions resulting in a small root aggregate. The seed coat often remains attached to this structure. When the root aggregate enlarges, a central axis to which all roots are connected becomes visible. This axis has a growth pole where new root tubercles arise. The same growth pole will later develop into a stem with scale leaves finally terminating in a flower. Flowers develop sympodially when the mature plant is only several centimetres long. After anthesis, the corolla tube disintegrates, leaving a pyxidium which opens by means of a peculiar elongating placenta, here called 'placentophore'. The placentophore elevates the placenta with attached seeds above the flowering level and is interpreted as an adaptation to ombrohydrochory. The reduction of hypocotyl, cotyledon and primary shoot is discussed with regard to the classical germination concepts of monocotyledons and with mycoheterotrophic dicotyledons. © 2008 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2008, 157, 31–36.


INTRODUCTION

The exclusively mycoheterotrophic *Afrothismia* spp. (Burmanniaceae) are peculiar flowering plants, not only for their minute habit and achlorophyll, but also with respect to flower (e.g. Franke, 2004; Franke, Sainge & Agerer, 2004; Sainge, Franke & Agerer, 2005), root structures (e.g. Imhof, 1999a; Maas-van de Kamer, 2003) and mycorrhizal colonization pattern (Imhof, 1999a, 2006). These rare species mostly grow superficially attached to leaf litter or soil surface and only few species are shallowly rooted in the soil. They occur in undisturbed lowland or submontaneous rain forest of central Africa and are highly endangered by habitat destruction (e.g. Cheek, 2003; Franke et al., 2004). Recently, eight species have been discovered (Cheek, 2003; Maas-van de Kamer, 2003; Franke, 2004; Franke et al., 2004; Cheek & Jannerup, 2005; Sainge & Franke, 2005; Sainge et al., 2005; Cheek, 2006), adding to three species and one variety that have been described in the past century (Engler, 1905; Schlechter, 1906; Cowley, 1988). Because of the extensive reduction of morphological characters, the systematic position of the Burmanniaceae has been uncertain for decades (for an overview, see Maas et al., 1986). Today, combined analysis of molecular and morphological data have affirmed their affiliation to the Dioscoreales (Caddick, Rudall & Wilkin, 2000; Caddick et al., 2002a,b; APG, 2003), but the data on the infraordinal relationship of the tribes Burmannieae and Thismieae (sisters according to Caddick et al. (2002b), in contrast to Thismieae + Tacca as sister to Burmannieae + other Dioscoreaceae according to Merckx et al. (2006)) are still ambiguous.

*Afrothismia* spp., like most other mycoheterotrophic plants (MHP), are difficult to cultivate, probably because of the specificity regarding their mycorrhizal fungi (Franke et al., 2006), hence, young stages have

*Corresponding author. E-mail: imhof@staff.uni-marburg.de*
never been depicted. The germination of MHP (Leake, 1994, 2004) other than orchids (e.g. Irmisch, 1853; Bernard, 1899; Weber, 1981; Smreciu & Currah, 1989; Rasmussen & Whigham, 1993; Umata, 1998; Mc-Kendrick et al., 2002; Yagame et al., 2007) and Ericaceae (e.g. Velenovsky, 1907; Christoph, 1921; Francke, 1935; Leake et al., 2004; Bidartondo & Bruns, 2005) remains unknown. This article elucidates the ontogeny of Afrothismia hydra Sainge & Franke, including its germination. As all species of Afrothismia are similar in their root–rhizome system, the development presented here most likely holds for the whole genus.

MATERIALS AND METHODS

During an expedition to Cameroon, following an invitation from Dr George B. Chuyong (University of Buea, Cameroon), and under the guidance of Vincent Merckx (Leuven, Belgium), we were able to collect flowering material of Afrothismia hydra from Korup National Park near Chimpanzee Camp (5°3′50.20″N/8°51′25.23″E, c. 136 m above sea level, 9 September 2006, no. 188 Imhof & Sainge) and Rengo Rock Camp (5°2′11.16″N/8°49′50.58″E, c. 120 m above sea level, 10.09. 2006, no. 189 Imhof & Sainge). After 2 weeks of fixation in FPA (37% formalin : propionic acid : 50% ethanol at a ratio of 0.5 : 0.5 : 9), specimens were transferred into 70% ethanol for permanent storage (Merckx, Leuven, Belgium), we were able to collect flowering material of Afrothismia hydra from Korup National Park near Chimpanzee Camp (5°3′50.20″N/8°51′25.23″E, c. 136 m above sea level, 9 September 2006, no. 188 Imhof & Sainge) and Rengo Rock Camp (5°2′11.16″N/8°49′50.58″E, c. 120 m above sea level, 10.09. 2006, no. 189 Imhof & Sainge). After 2 weeks of fixation in FPA (37% formalin : propionic acid : 50% ethanol at a ratio of 0.5 : 0.5 : 9), specimens were transferred into 70% ethanol for permanent storage at the Philipps-University Marburg. Unexpectedly, dissection under the microscope revealed very young plant stages within the few earth clumps that accompanied the larger specimen and that could be ordered according to the time passed after germination. Morphological investigation and photography was carried out using a Leica SD6 microscope in combination with a digital photo device (Leica DFC280).

RESULTS

Seeds of Afrothismia hydra are shaped like a grain of rice, 600–700 μm long and 250–300 μm wide. The two ends of the seeds are slightly dissimilar; one is rather rounded, the other more truncated. The truncated side is attached to the placenta. The outer seed coat is hyaline, encompassing a light-brown inner structure with dark tips (Fig. 1).

The youngest stage found after germination is barely longer than the seed itself and shows disruption of the seed coat through the emergence of a first tubercle, yet missing a tail-like extension. The seed coat opened at the rounded tip of the seed, whereas the truncated end shows a hyphae firmly connected to the seed. The young tubercle, with the exception of the outer one or two cell layers, contains whitish material (Fig. 2). The next stage (Fig. 3) shows that the initial tubercle has grown a short tail-like extension (only about 150 μm long) and is fully packed with the whitish material, which can now be interpreted as the mycorrhizal fungus (see arrow in Fig. 3). A second tubercle has developed from the proximal part of the first tubercle – the part that was initially hidden in the disrupted seed coat. The new tubercle is not completely filled with fungal material; the distal part appears hyaline. Its tail-like extension has grown to 1 mm in length. Close to the proximal part of both tubercles, where the seed coat is still attached, the beginning of a third tubercle arises (Fig. 3). This pattern of root tubercle development repeats several times, resulting in a star-like structure, the developmental sequence of which is still detectable (Fig. 4). At this stage, the longest filiform root extension was 9 mm long. In the course of further tubercle development, the proximal parts of the roots build a common axis. This axis is the first visible shoot organ (rhizome), having a distal growth pole where new tubercles arise and a proximal end sometimes indicated by the still attached seed coat (Fig. 5). The distal pole also rarely develops scale leaves (Fig. 6). Intracellular raphides are visible externally because of their distinctive light reflection and the root extensions have grown to a length of 13 mm in the root–rhizome system in Figure 6.

Eventually, the rhizome elongates without developing root tubercles, but bears scale leaves and finally terminates in a flower (Fig. 7). Flower buds show the tepal lobes and the urceolate inferior ovary early, but the corolla tube is still short (Fig. 8). At this stage the pre-anthetic flower is still encompassed by the subtending bract. The axes of the scale leaves bear the beginnings of side shoots (Figs 8, 10), the uppermost of which will continue the inflorescence for each branch of the monochasial cyme. These side shoots always develop additional root tubercle aggregates at their bases (Fig. 9).

After anthesis the corolla tube and lobes disintegrate and only the capsule crowned with the style remains. The ovary wall is translucent and the developing seeds are visible, attached to the column-like placenta connected to the basal (and initially also the upper) part of the capsule (Fig. 10). As a result of elongation of the basal part of the placenta, the connection to the upper part of the capsule is disrupted; the operculum separates from the proximal part of the fruit (thus characterized as a pyxidium, Fig. 10) and the seeds, arranged like the feathers in a feather duster, are exposed (see inset of Fig. 10). Placental elongation elevates the seeds above flowering level (Fig. 10).

DISCUSSION

This article presents a process never previously depicted. Among MHP, only orchids (reviewed by Arditti et al., 1990; Peterson, Uetake & Zermer, 1998) and Monotropoideae (for literature, see Introduction)
Figure 1–6. Fig. 1. Seed of Afrothismia hydra. A, truncated side; B, rounded side. Scale bar, 0.2 mm. Fig. 2. Germination of Afrothismia hydra, the seed coat disrupted as a result of primary root tubercle development. A hypha is firmly attached to one end of the seed (arrow). Scale bar, 0.2 mm. Fig. 3. A second root tubercle (2) has developed, not yet fully colonized by the root fungus. The first tubercle (1) shows distinct hyphal coils (arrow) in its tissue. First filiform root extensions (re) occur. The seed coat (sc) is still attached and close to the attachment point a third root initial (3) is visible. Scale bar, 0.2 mm. Fig. 4. More root tubercles and initials have developed, the sequence of which (1–5) is still detectable. Scale bar, 0.2 mm. Fig. 5. A primary shoot axis (s) becomes visible; at the growth pole (gp) new tubercles arise. The seed coat (sc) is still attached. Scale bar, 0.5 mm. Fig. 6. The shoot axis (s) with further tubercles has grown; a scale leaf (sl) is visible at the growth pole. The filiform root extensions (re) in this specimen reach 13 mm in length. Scale bar, 0.5 mm.

have been examined for their early ontogeny. Caddick et al. (2000) described floral ontogeny in Burmanniaceae and, only once, young stages (but not the germination) of *Voyria tenella* Hook. (Gentianaceae) have been shown (Imhof, Weber & Gómez, 1994). In the light of the results presented here, the seedling concepts for monocotyledons (Tillich, 1992, 1995) are difficult to apply in *Afrothismia*. No cotyledon, hypocotyl or primary shoot becomes visible during germination; instead, *Afrothismia hydra* seems to germinate with a root only. The root character of the first-emerging structure becomes obvious when it develops into a tubercle with filiform extension; this structure has been proved to be a root in other studies (Imhof, 1999a, 2006). Clearly, because of the difficulties in applying classical germination concepts, the comparable initial

**Figure 7–10.** Fig. 7. Young flowering plant of *Afrothismia hydra*. Scale bar, 5 mm. Fig. 8. Flower bud of *Afrothismia hydra* enveloped in a bract (b). Corolla lobes (cl) and inferior ovary (o) are already differentiated. A scale leaf below (sl) bears an initial (i) for a side shoot. Scale bar, 1 mm. Fig. 9. Sympodium of *Afrothismia hydra* with six successive sympodial branches (1–7); the terminal parts of the older are already disintegrated (1*–4*). The bases of the side shoots possess a dense mass of root tubercles. Scale bar, 5 mm. Fig. 10. Close-up of Figure 9 showing an open (op) and a closed pyxidium (cp) and a developing flowering shoot (s). The seeds of the open pyxidium are exposed by the elongating basal part of the placenta (pp, placentophore). The young flowering shoot bears a flower bud (fb) as well as the first additional root tubercles (r) at the basal part. Inset: the elongation of the placentophore pushes the lid from the pyxidium. Scale bars, 1 mm.
structures of orchids are termed ‘protocorms’ (Treub, 1890; Bernard, 1909) and Velenovsky (1907) coined the term ‘procaulom’ for the corresponding organ in the Ericaceae. ‘Protocorm’ is still in use as it represents an organ distinct from the rest of the corm (Leroux, Barabé & Vieth, 1997). In contrast, contemporary publications on the germination of Monotropoideae avoid the expression ‘procaulom’, but also hesitate to call the germinating organ a ‘root’, despite its identical appearance to mature roots and the immediate development of the hyphal mantle that is characteristic of roots (Leake et al., 2004; Bidartondo & Bruns, 2005). However, in Voyria tenella, the youngest stage found (2 mm in length) consisted entirely of root tissue, including a central vascular cylinder and the specific mycorrhizal colonization pattern, without any trace of shoot tissue. Only when a little star-like root clump has developed (Imhof et al., 1994; Imhof, 1997). The results of the present study represent an analogous germination strategy. Considering the tiny seeds with few storage compounds and the fact that, in both Afrothismia spp. (Imhof, 1999a, 2006) and Voyria spp. (Imhof, 1997, 1999b), only the root tubercle parenchyma is able to break down hyphae for carbon supply, immediate development of roots during germination is essential in combination with an early colonisation by the appropriate fungus (see Fig. 2). These constraints similarly hold for Monotropoideae and, hence, their germinating organ is supposedly also a root. Rauh (1937) also addressed the complete reduction of cotyledons and even hypocotyl in Monotropa hypopitys L. and Pyrola uniflora L. (Ericaceae) and explicitly called the germinating structures first found by Francke (1935), as well as Velenovsky (1907) and Christoph (1921), ‘roots’.

The formation of the shoot axis that eventually bears the flower is obscure. There are two conceivable origins. It could be a root-borne shoot, similar to those of, for example, Pyrola spp. (Rauh, 1937) or Voyria truncata (Standl.) Standl. & Steyerm. (Imhof et al., 1994). However, this would require concerted development by several distinct root tubercles to form a single shoot axis, which assumedly is a complex process. A less complicated and more plausible interpretation is that an indefinite small portion of the germ represents a hypocotyl, having the potential to generate several shoot-borne roots (Figs 3, 4) and, eventually, when the carbon supply is assured, to form the rhizome and flowering shoot. The additional root aggregates at the bases of the sympodial branches are interpreted as organs of local carbon supply for the flowering side shoots (see Imhof, 1999a).

Another unusual feature is the elongating placenta, already depicted in the taxonomic description (Sainge & Franke, 2005). Considering the enormous extent of this elongation, to about four or five times the length of the pyxidium (see picture in Sainge & Franke, 2005), it seems to be the result of meristem growth rather than cell elongation. Future anatomical investigations will help to clarify this hitherto unknown phenomenon. Ecologically, uplift of the seeds helps to widen seed dispersal when the seed puff is hit by raindrops. Hence, in accordance with Stone (1980), who assumed Thismia clavigera (Beeck.) F.v.M. to be dispersed by rain splash, we believe Afrothismia hydra to be ombrohydrochorous and, in analogy to gynophore and carpophore, we suggest the expression ‘placentophore’ for the unique structure lifting the seeds from the pyxidium.

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