

THE *AZOLLA*–*ANABAENA* SYMBIOSIS

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INTRODUCTION

Azolla is an aquatic pteridophyte that forms a permanent, hereditary symbiosis with a nitrogen-fixing, heterocyst-forming cyanobacterium, *Anabaena azollae*. The *Azolla*–*A. azollae* symbiosis is the only known mutualistic symbiosis between a pteridophyte and a diazotrophic prokaryote. This association has gained attention in recent decades because of its potential use as an alternative to chemical nitrogen fertilisers and as feed for animals. In this paper, we present an overview of the morphology of the two partners, their life cycle, taxonomy, biogeography and interactions and the practical uses of the symbiosis. More extensive reviews have appeared elsewhere (see Lumpkin and Plucknett 1982; van Hove 1989; Braun-Howland and Nierzwicki-Bauer 1990; Lejeune *et al.* 1999).

MORPHOLOGY OF *AZOLLA* AND *ANABAENA AZOLLAE*

An *Azolla* plant (Pl. I) floating on the surface of the water is roughly triangular or circular in shape and rarely exceeds 3–4 cm (except in the species *Azolla nilotica*). The stems are covered and hidden by small, alternate, imbricate leaves. Adventitious roots are formed on the lower part of the stem and grow vertically in the water. Each leaf is bilobed (Fig. 1), the lower, achlorophyllous lobe ensuring flotation and the upper, chlorophyllous one developing a cavity that remains in contact with the external environment through a structurally sophisticated pore.

The cyanobacterium *Anabaena azollae* (Fig. 1) occurs as filaments located on the plant stem apices and inside the leaf cavities, which are inoculated during their formation with some *Anabaena* from the apex. Up to 30% of the *Anabaena* cells differentiate into heterocysts as the leaves mature. Isolated resting cells called akinetes play a role in the persistence of the symbiosis during sexual reproduction of the plant; they also sometimes appear in leaf cavities.

AZOLLA REPRODUCTION AND PERMANENCE OF THE SYMBIOSIS

Vegetative multiplication by abscission of secondary stems (Pl. I) is the most usual means of

reproduction by the host plant. The permanence of the symbiosis is achieved by the fact that axillary buds formed by the apical bud inherit some cyanobacteria from the apical colony.

Sexual reproduction is complex and occurs under certain ill-defined environmental conditions. Instead of the lower leaf lobe, the plant produces a group of structures called mega- and microsporocarps (Fig. 1), protected by a hood-like structure, the involucre, produced by the upper lobe. Each sporocarp comprises a two-layered indusium containing either a single megasporangium or about 100 microsporangia. During formation of the sporocarps, a few *A. azollae* cells are trapped under the indusium and differentiate into akinetes. The megasporangium will form a single megaspore, and the basal part of the indusium will disintegrate, thus revealing the surface of the megaspore, the top of which is still covered by the upper part of the indusium. The microsporangia will produce 32 or 64 microspores. At maturity the microsporangial content dissociates into three or four (sometimes more) spongy fragments called massulae, which are covered with filamentous, unsagittate or sagittate glochidia. Liberated into water, the massulae will fix on the surface of megaspores through their glochidia. Microspores germinate in the massulae, where they develop into prothalli that produce spermatozoids, whereas the megaspore germinates in the megasporocarp, and the resultant prothallus produces archegonia. The spermatozoids join the eggs by an unknown pathway, and fertilisation takes place. The embryo starts growing and pushes away the indusium. At that time, its apex comes into contact with the akinetes sheltered under the indusium; some of them stick on the apex, where they germinate and form the apical *A. azollae* colony.

TAXONOMY AND BIOGEOGRAPHY

Azolla has a worldwide distribution from temperate to tropical climates; seven species grouped in two sections are classically recognised. The two sections are distinguished by characteristics of the megasporocarps and glochidia. The *Rhizosperma* section comprises *Azolla nilotica*, native to East Africa, and *A. pinnata*, of which two varieties are generally recognised: *A. pinnata* var. *pinnata*, present throughout Africa south of the Sahel, in Madagascar and in Australia, and *A. pinnata* var. *imbricata* from subtropical and tropical Asia. The *Azolla* section comprises, on the one hand, *A. caroliniana*,



Pl. I—Vegetative morphology of *Azolla* seen from above.

A. mexicana and *A. microphylla* from temperate, subtropical and tropical America respectively and, on the other hand, *A. filiculoides* and *A. rubra* from America and the Far East respectively. These two groups are separated by characteristics of leaf trichomes. However, the taxonomy of the plant and of its cyanobiont is still confusing. Recent phylo-

genetic studies based on molecular methods show three groups of *Azolla*: one with *A. pinnata*, one with *A. nilotica* and a third group consisting of two subgroups, one with *A. caroliniana*, *A. mexicana* and *A. microphylla* and the other with *A. filiculoides* and *A. rubra*. Such studies also show a co-evolution between the different species of *Azolla* and their

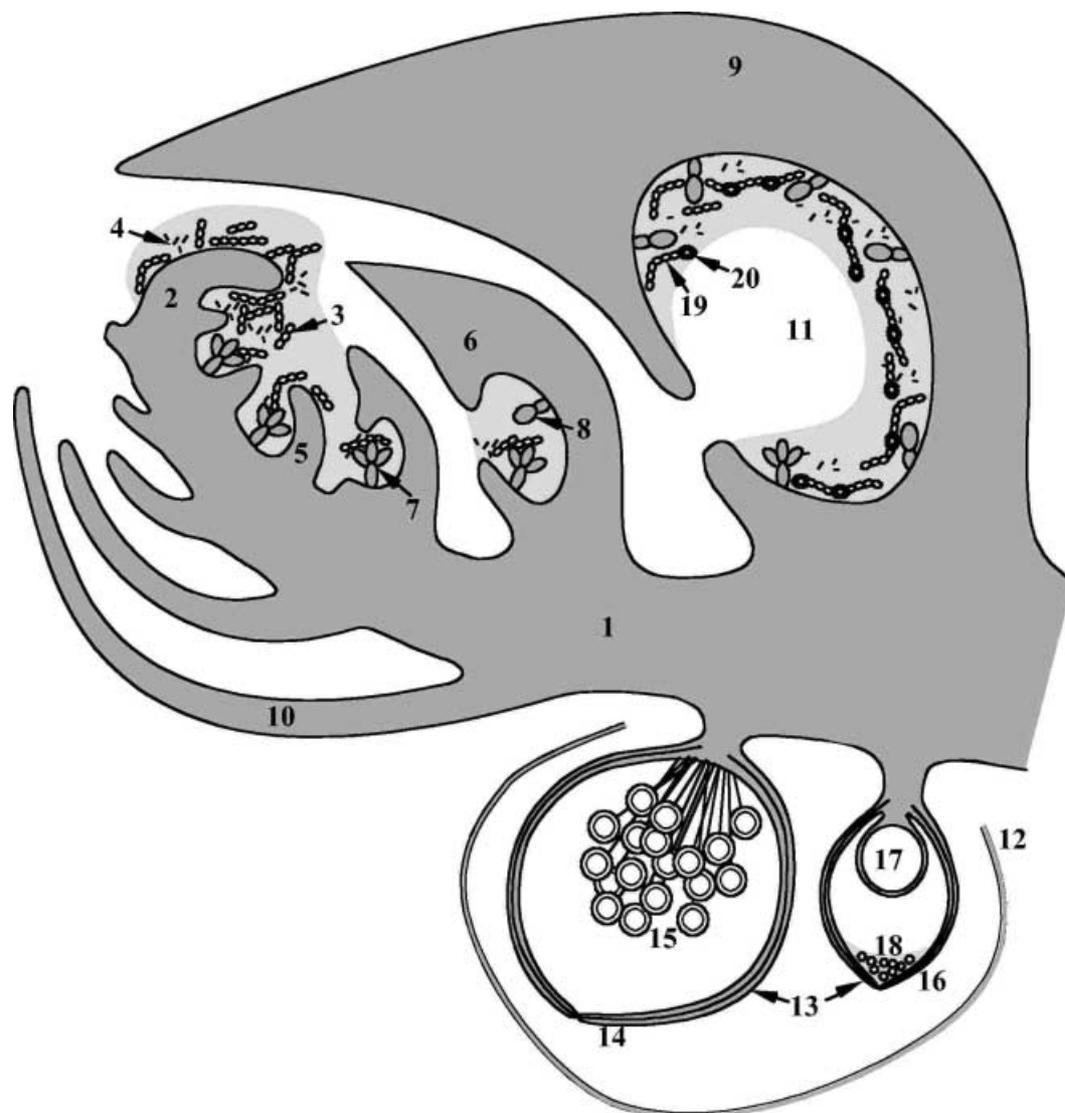


Fig. 1—Morphology of *Azolla* stem and sporocarps (longitudinal section). 1. Stem; 2. Stem apex; 3. Apical *Anabaena* colony without heterocysts; 4. Other bacteria; 5. Leaf primordium; 6. Young leaf; 7. Pluricellular branched hair; 8. Bicellular single hair; 9. Upper leaf lobe; 10. Lower leaf lobe; 11. Leaf cavity (showing a central gaseous region and a peripheral mucilaginous region); 12. Involucre; 13. Indusia; 14. Microsporocarp; 15. Microsporangia; 16. Megasporocarp; 17. Megasporangium; 18. Akinetes of *Anabaena*; 19. Vegetative cell of *Anabaena*; 20. Heterocyst. (Adapted from van Hove 1989)

symbiotic *Anabaena*, raising questions about the systematics of the symbiont, *A. azollae*, which was considered a unique species until now. Its very inclusion in the genus *Anabaena* has also been questioned.

INTERACTIONS BETWEEN THE PARTNERS

The *Azolla*-*A. azollae* association can develop on a medium devoid of nitrogen compounds because of the ability of *A. azollae* to reduce N_2 to NH_3 . Some of the ammonia is supplied to the fern, and

the fern supplies the cyanobacterium with photosynthetic assimilates. *Anabaena*-free *Azolla* are able to develop when provided with combined nitrogen, but they are not competitive under natural conditions. A number of other bacteria reside in the leaf cavity, but it is not known if they play a role in the symbiosis.

AZOLLA IN AGRICULTURE

The *Azolla*-*A. azollae* symbiosis is the only agronomically promoted cyanobacterial symbiosis. It has long been used by farmers, mainly in Asia, as

feed for their animals and as green manure. *Azolla* is one of the most nutritive aquatic plants, owing to its high crude protein and carotenoid contents and generally good amino-acid profile. It can be incorporated into the feed of fish, pigs, poultry, rabbits and even humans. A number of laboratory and field studies have shown beyond any doubt the beneficial effect of *Azolla* as an organic nitrogen fertiliser, mainly in terms of increasing rice grain yield. In addition, the presence of an *Azolla* mat on the surface of the water body has been shown to significantly reduce weed development, limit evapotranspiration, reduce volatilisation of applied N fertilisers and purify water. Recent research has focused on the use of *Azolla* in integrated farming systems, mainly rice–fish–*Azolla* and pig–poultry–fish–*Azolla*. Positive results of the use of *Azolla* in rice and fish production should prompt investigations into its benefits in a range of ecosystems. *Azolla* has occasionally been reported as a weed: although some of the negative effects are indisputable, others require confirmation and quantification.

CONCLUSIONS

Knowledge of *Azolla*–*A. azollae* biology and agronomy has progressed considerably during the

past 30 years. A number of areas still warrant further investigation. These include the still confusing taxonomy of *Azolla* and *A. azollae* and the factors that induce the sporulation process. From a more applied perspective, more research is needed on the selection of *Azolla* strains adapted to various environments and the use of *Azolla* in integrated farming systems, including the control of its predators. A better evaluation of its nutritive value and its effect on NH₃ volatilisation is necessary. Furthermore, the control of *Azolla* itself, where it is considered as a pest, requires investigation.

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