

Azolla

AND ITS MULTIPLE USES
WITH EMPHASIS ON AFRICA

by

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Translated from French

by

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MA-166

Food and Agriculture Organization
of the United Nations

1989

Azolla
and its multiple uses
with emphasis on Africa

Proyecto de gestión
Integrada y Plan
Maestro de la Cuenca
del Rio Pilcomayo
BIBLIOTECA

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PREFACE

The crucial problem of maintaining soil fertility in tropical regions will not be solved one but by many, partial, solutions. The cultivation of the aquatic fern *Azolla* and of diazotrophic symbiont *Anabaena azollae* belongs to the latter. For the past twenty years *Azo* cultivation has remarkably progressed in some Asiatic countries, raising great expectations other regions.

Its high productivity associated with its ability to fix atmospheric nitrogen makes *Azo* an excellent green manure. Its chemical composition makes it an important source of food for various livestock and special cultural practices have made it possible to benefit simultaneously from its nutritive and fertilizing qualities, not to mention many other interesting properties of this fern.

The fact remains, however, that the attention given to *Azolla* in certain spheres nowadays seems to have diminished. The reasons for this should not be generalized, for the author of this pamphlet* has gathered some experience with *Azolla* culture both in Asia and Africa and feels that the ecological and socio-economic conditions which prevail in Africa among other tropical regions call for the development of this culture, especially in small, family-size, mixed agricultural systems where *Azolla's* various properties can best be appreciated.

With this in mind, the present paper was written not only for the agronomists but also for the policy-makers. It describes the different cultural practices and the uses of *Azolla* with the benefits to be expected, provided the constraints and limitations of this culture are adhered to.

We would be very happy if the information in this booklet can contribute, even in a modest way, to the improvement of the living conditions of the small farmers for whom it has been written.

L. Brader

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ACKNOWLEDGEMENTS

I would like to express my gratitude to the Food and Agriculture Organization of the United Nations (FAO) for asking me to write this booklet; to the Université Catholique de Louvain for granting the sabbatical leave which gave me time to concentrate on this paper; to the Fujian Academy of Agricultural Sciences in the People's Republic of China for the warm reception at their National *Azolla* Research Centre during that year; to the Administration Générale de la Coopération au Développement (AGCD) of Belgium which for several years, by its support to the West Africa Rice Development Association and to the International Rice Research Institute has enabled us to continue with our research on *Azolla*.

My thanks also to all the friends who helped me to domesticate *Azolla* : C.C. Liu, T.A. Lumpkin, T. Ton That and I. Watanabe who have shared their rich and long experience of *Azolla* with me; the many collaborators working in Africa, especially H.F. Diara; the scientists, assistants or students of the team at the Plant Physiology Laboratory at the Catholic University of Louvain, with a special mention of P. Godard, T. de Waha Baillonville and D. Desmadryl.

I am grateful to my colleague J.E. Ruelle, who kindly accepted to translate this booklet from French.

And finally a loving thank you to my wife Aline who continually supported me in the preparation of this paper which she typed.

Charles Van Hove

INTRODUCTION

Azolla is a small aquatic fern which has a hereditary symbiotic relationship with *Anabaena azollae*, a diazotrophic or nitrogen-fixing cyanobacteria. The association is characterized by high productivity and a high protein content.

These properties give *Azolla* fertilizing and nutritive qualities, well known and utilized over many centuries in China and Vietnam. However, until the end of the 1950's, azolliculture has been practiced only in very limited areas of these countries, along the coast between 18° and 30° North latitudes, such areas having a humid subtropical climate with wide seasonal variations of temperature.

At that time the governments of these countries decided to promote scientific research on *Azolla*. This quickly resulted in a considerable extension of the area under cultivation, together with the development of several protection techniques against low winter and high summer temperatures as well as against various pests. At the same time the cultural practices improved and this increased the efficiency of azolliculture.

But one had to wait until the seventies and the oil crisis for the scientific world in China and Vietnam to gradually become aware of the potentialities of *Azolla* in agriculture. The Food and Agriculture Organization of the United Nations (FAO) and the International Rice Research Institute (IRRI) have played an important role in this respect. More or less ambitious research programmes with a view to developing azolliculture have ever since been introduced with varying successes in other Asian countries as well as in Africa and America.

In Africa, research efforts in order to develop *Azolla* were sporadic until 1980. They multiplied in the last ten years, mainly under the care of WARDA which has developed a special project for research on *Azolla*, and of FAO which has promoted several projects in various African countries. The Institut Français de Recherche Scientifique pour le Développement par la Coopération (ORSTOM), the International Institute for Tropical Agriculture (IITA) and several national institutions have also contributed to this research effort.

The knowledge accumulated by these researches has confirmed the potentialities of azolliculture and made it possible to specify the advantages to be expected (food, nitrogen, potassium fertilization, weed control in irrigated cultures, improvement in soil structure, reduction of evapotranspiration, energy production...). Various ways of using *Azolla* systems which integrate animal husbandry with crop production have been developed allowing the farmer to make use first of the nutritive value of *Azolla* (linked to its high protein content) and then of its fertilizing value (through animal waste).

Now we come to this paradox : at a time when the acquired experience should make us think that a rational use of *Azolla* is likely to help a lot of peasant farmers, particularly in the regions of Africa, there is a growing skepticism in various spheres with regard to azolliculture and consequently efforts to develop it are being reduced.

Three reasons seem likely to explain this skepticism : the fall of the world market price of chemical fertilizers, the reduction of the use of *Azolla* as green manure in China and the limited success associated with the introduction of azolliculture in many Asian countries.

The first reason is based on the conjuncture and it would therefore not be wise to consider it as permanent.

The reduction in the rice-cultivated areas fertilized by *Azolla* in China is due to local and socio-economical reasons which affect not only *Azolla* but other green manures such as *Astragalus sinicus*. The necessity to occupy and use all of the available land leaves less and less place for such cultures in the cultural calendars; in addition, other economic activities attract an increasing proportion of the labour force from agriculture. As a result increasing use is being made of chemical fertilizers. Finally, even though China is one of the places where azolliculture originally started, the considerable seasonal climatic variations and the abundance of predatory organisms make azolliculture extremely delicate and labour intensive not to mention a productivity that cannot be satisfactory beyond a limited part of the year. It must be emphasized that despite these unfavourable conditions, research on *Azolla* is actively pursued in China, especially for its use as food which is in full expansion.

The reasons mentioned above, or some of them, also explain the meagre results obtained with different development programmes of azolliculture in Asia especially when they are based exclusively on the use of *Azolla* as a fertilizer for irrigated rice. These reasons, however, are not universally true.

It should indeed be noted that :

- many countries have neither nitrogen fertilizer producing industries nor the foreign exchange to import them. Moreover, even when the governments concerned have a policy of importing and possibly subsidizing it, the chemical fertilizer remains in practice inaccessible to many small farmers due to either financial constraints or inadequate distribution. They have therefore no alternative to the use of green manure;
- the problems of agricultural land settlement and the availability of manpower vary considerably from one region to another but are seldom as crucial as in China;
- some climates are clearly more favorable to the culture of *Azolla* than those which characterize the areas where this plant was traditionally developed. Such climates occur in some tropical regions of Africa, for example, where high productivity may be obtained throughout the year because of the slight variations in the temperatures among other things;
- these high and regular productivities greatly facilitate the mastering of azolliculture since special arrangements would not have to be made to conserve *Azolla* in the bad season;
- they also favour a diversification of its uses and therefore a more economic use of the biomass produced. Depending on the time of the year and the type of exploitation this biomass could indeed be used as food or as green manure for rice and market garden

produce, or simultaneously in various complex schemes integrating crop production and animal husbandry.

In these conditions, and considering the fact that *Azolla* strains adapted to various tropical environments have been selected and successfully tested in the field, efforts to introduce azolliculture among peasants in some areas seem to be justified.

The definite aim of this handbook is to give agronomists in tropical regions the basic information with which they will be able to estimate their chances of success with the introduction of *Azolla* in specific local conditions and, should they decide to do so, to select the most appropriate cultural practices.

1

DESCRIPTION OF THE SYMBIOSIS

1.1. MORPHOLOGY OF AZOLLA

An *Azolla* plant, often called a frond, consists of a main rhizome rarely exceeding 3 or 4 cm in length. Floating on the surface of the water it is covered by small alternate leaves closely overlapping and hence hiding the rhizome (fig. 1, p. 6). From this emerge at regular intervals secondary rhizomes having the same general characteristics as the principal rhizome and being able in turn to produce rhizomes of the third order which subsequently produce the fourth. The growth pattern gives the plant a triangular or circular shape depending on the species.

Unbranched, adventitious roots are formed on the lower part of the rhizome towards the water where they grow sinking vertically.

Each leaf is made of two parts called lobes (fig. 3, p. 8). One of these lobes lies beneath the rhizome. Cup-shaped, it is thin without chlorophyll, and gives the plant its buoyancy. The other lobe, thicker and chlorophyllous, develops on the upper side of the stem. It contains an inner cavity which shelters a population of cyanobacteria (blue-green algae) called *Anabaena azollae*.

The leaf cavity is formed by an invagination of the adaxial epidermis of the upper lobe. This epidermis forms two types of pluricellular hair whose cells have the typical structure of transfer cells and probably intervene in the metabolic exchanges between the partners of symbiosis.

1.2. MORPHOLOGY OF ANABAENA AZOLLAE

Anabaena azollae, a procaryotic organism, occurs as unbranched filaments formed by two types of cells. The more numerous, called vegetative cells, measure about 6 μm by 10 μm ; others, called heterocysts, are slightly larger and have thicker cell walls (fig. 3, p. 8).

In the very young leaves, the filaments are made up of vegetative cells only and the number of heterocysts progressively increases during the development of the leaf until it represents 30 to 40 % of all the cells. Finally in some cases, some of the cells change into akinetes; the presence of these conservation forms in the leaf cavity remains unexplained.

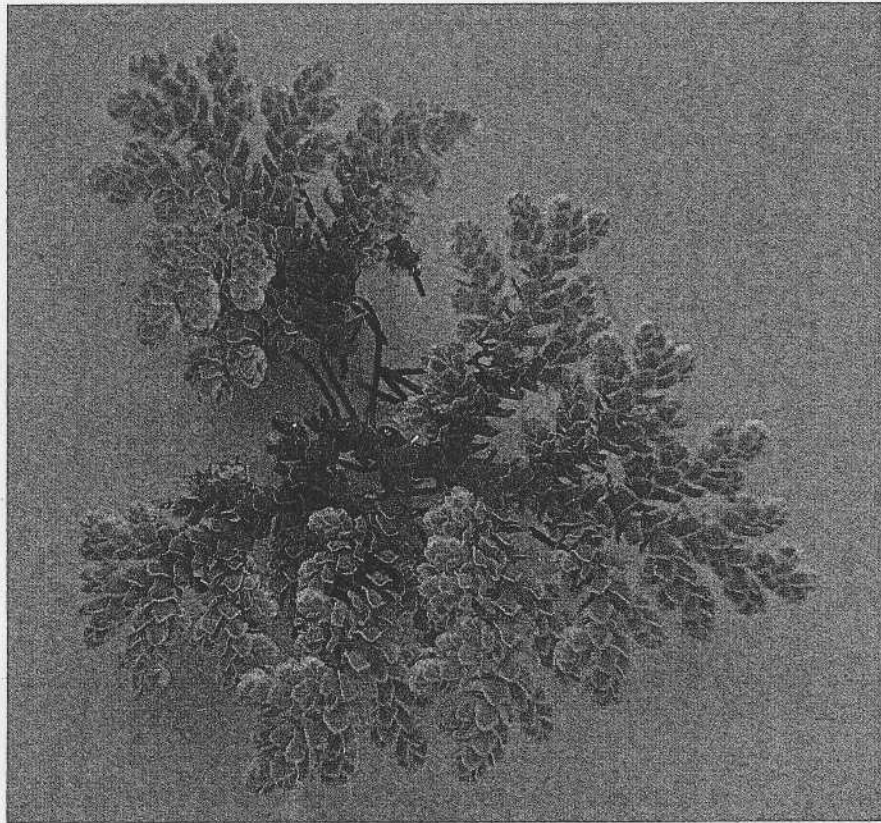


Fig. 1. *Azolla* frond seen from above. The most developed branch is separated from the main plant as a result of the formation of an abscission layer at its base (vegetative multiplication).

1.3. REPRODUCTION OF *AZOLLA*

1.3.1. SEXUAL REPRODUCTION

In certain environmental conditions, as yet ill-defined, *Azolla* undergoes a sexual reproduction of a very complex nature, which will only briefly be described here.

Instead of the lower leaf lobe, the plant produces a group of two (or four) structures called sporocarps which are protected by an uniassial envelope, the involucre. Each sporocarp in turn is made of a biassial envelope, the indusium, which contains either about a hundred of sporangia or a single one (fig. 3, p. 8).

In the first case, each sporangium produces 32 or 64 spores which, because of their minute size, are called microspores; consequently, the sporangia and sporocarps which protect

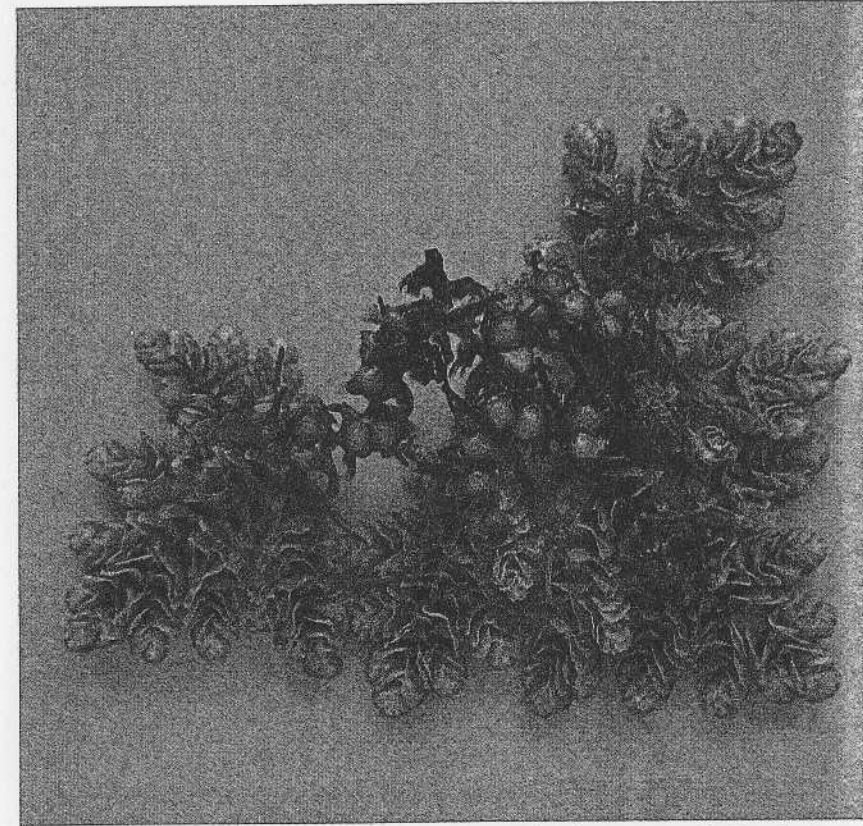


Fig. 2. *Azolla* frond sporulated seen from below. The roots have been cut out to facilitate the observation of the sporocarps. Only the microsporocarps can easily be visible to the naked eye.

them are named microsporangia and microsporocarps. The latter are spherical (about 2 mm diameter). Their colour is yellow and they can easily be seen with the naked eye at the lower side of the plant (fig. 2, p. 7).

In the second case, the lone sporangium produces a single larger spore; hence the name of megaspore, megasporangium and megasporocarp, even though the latter is smaller (± 0.5 mm) than the microsporocarps. At maturity the megasporocarps are pyriform (fig. p. 8). The spherical megaspore at this stage is topped by three or nine spongy structures called floats, which in turn are covered by a conical hood made of the upper part of the indusium which is heavily lignified and protects at its summit a small colony of *Anabaena*. The basal of the indusium disintegrates and reveals the surface of the megaspore which is very thick walled and has a complex structure with specific differences.

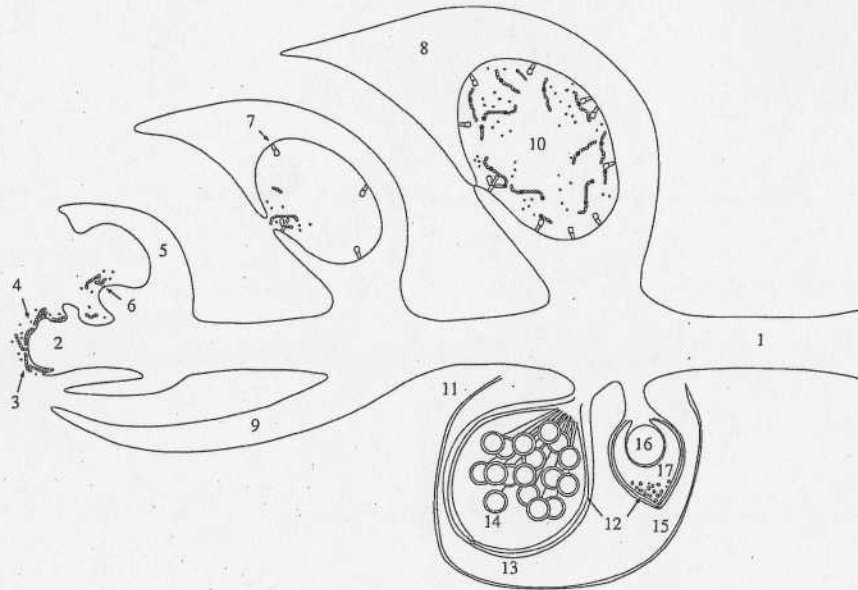


Fig. 3. Leafy stem and sporocarps : longitudinal section 1. Rhizome; 2. Apex; 3. *Anabaena* without heterocysts; 4. *Arthrobaeter*; 5. Leaf primordium with adaxial epidermis invagination; 6. Branched pluricellular hair; 7. Bicellular hair; 8. Upper lobe; 9. Lower lobe; 10. Leaf cavity (*Anabaena* with heterocysts); 11. Involucre; 12. Indusium; 13. Microsporocarp; 14. Microsporangium; 15. Megasporocarp; 16. Megasporangium; 17. Akinetes of *Anabaena*.

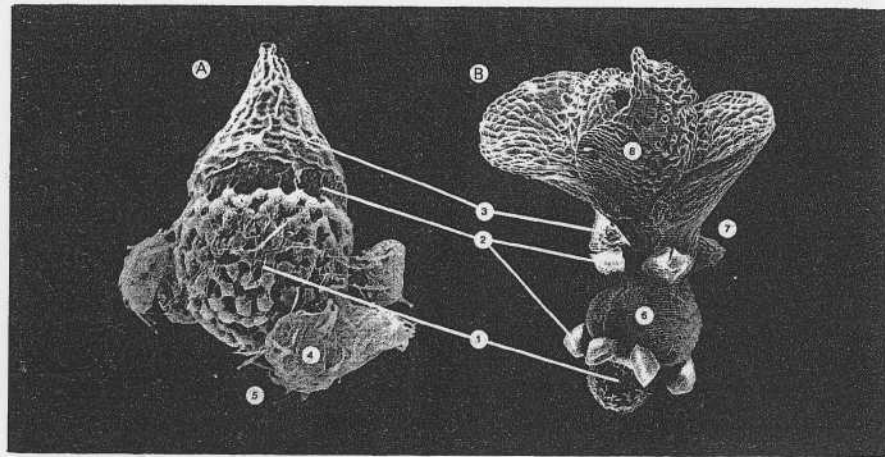


Fig. 4. A. Megasporocarp of *A. filiculoides* covered with massulae (83 x).
B. Young plant of *A. pinnata* emerging from a megasporocarp (35 x).
1. Megaspore; 2. Float; 3. Indusium; 4. Massula; 5. Glochidium; 6. Prothallus; 7. Root; 8. Leaf.

At maturity, the microsporocarps open up and liberate their microsporangia into the water. Each of those will break up into three or four (sometimes more) small spongy mass called massulae which contain the microspores. The massulae bristle with filamentous structures (except in the case of *A. nilotica* unbarbed (*A. pinnata*) or barbed; they are referred to as glochidia. The massulae cling to the megaspore through the glochidia (fig. 4, p. 8).

The massulae are therefore attached to the surface of the megaspore, normally at the bottom of the water. The microspores in the massulae germinate and develop into a small prothallus producing the male gametes (antherozoids), ciliated and mobile. The megaspore germinates in the megasporocarp and becomes a prothallus which produces female gametes oospheres. The antherozoids join with the oosphere through an as yet undescribed process as fertilization takes place. The embryo starts growing and as it grows upwards its apex pushes away the lignified indusium and frees itself from the sporocarp, forming a new plantlet which floats to the surface of the water (fig. 4, p. 8).

Megasporocarps and microsporocarps are resting organs of *Azolla*. By harvesting them with special methods - one can keep the plants for several months without maintenance.

Unfortunately, several *Azolla* ecotypes rarely produce sporocarps and the environmental conditions which induce sexual reproduction are poorly known. Moreover, the harvest of large quantities of sporocarps is difficult. Finally, the production of a sizable *Azolla* biomass from sporocarps is a slow process. It follows that (except in particular cases) it is not interesting to rely on the sexual reproduction of *Azolla* in the cultural practice : one should rather make use of its vegetative multiplication.

1.3.2. VEGETATIVE MULTIPLICATION

The most usual means of multiplication of *Azolla* is a plain fragmentation of the plant. The most developed secondary rhizomes spontaneously break off from the main plant by the formation of an abscission layer at their base, giving rise to new plants (fig. 1, p. 6).

1.4. PERMANENCE OF THE SYMBIOSIS

Contrary to what happens in other diazotrophic symbioses such as that between leguminous plants and *Rhizobium*, the association between *Azolla* and *Anabaena azollae* is permanent; in other words *Anabaena* is present in *Azolla* at all the stages of its life and is automatically transmitted from one generation to the other regardless of whether the multiplication is sexual or vegetative.

The moment the megasporocarps are formed, some *Anabaena* cells present on the surface of the apex of the plant are brought into them by a mechanism still to be defined. They change into akinetes at the bottom of the hood formed by the indusium (fig. 3, p. 8). When, after fertilization, the embryo breaks away from the megasporocarp by pushing through the indusium, its apex comes into contact with the *Anabaena* cells; these attach themselves there and multiply to form a little colony which remains permanently.

Every time a leaf primordium is formed by the apex, it invaginates on the adaxial side of its upper lobe. The invagination develops as the leaf grows and a branched pluricellular hair is formed at its base; acting as a small brush, this hair collects some filaments of *Anabaena* and introduces them into the leaf cavity formed by the invagination. The gradual closing of the cavity goes on with the growth of the leaf while the imprisoned colony of *Anabaena* multiplies and differentiates a large number of heterocysts. At the same time, the epidermal cells around the cavity form about twenty bicellular transfer hairs as well as a second branched pluricellular hair.

The bud primordia produced by the apical bud also inherit some filaments of the cyanobacterium which will play exactly the same role as that played by the colony of the apical bud.

1.5. TAXONOMY AND GEOGRAPHICAL DISTRIBUTION OF AZOLLA

With a little experience, *Azolla* can easily be distinguished from various floating aquatic plants such as *Salvinia* or *Lemna*, but specific determination within *Azolla* can be difficult. The trained taxonomist, who has been using the morphology of the sporocarps as his best identification criterion may find himself at a loss when the material lacks sporocarps, which it does most of the time.

However and although recent data would question this classification, the genus *Azolla* is generally considered as containing two sections with a total of seven species.

I. Section *Rhizosperma* : the megasporocarps have nine floats and the glochidia are absent or unbarbed.

A. nilotica

Native of East Africa, it can be found from Sudan to Mozambique. When this species grows in favourable conditions it is most easily identifiable by its size, which may reach several decimeters. Glochidia absent.

A. pinnata

It is the only species which is subdivided in two varieties : *A. pinnata* var. *pinnata* and *A. pinnata* var. *imbricata*. Glochidia unbarbed.

A. pinnata var. *pinnata* is found throughout Africa south of the Sahel, Madagascar and Australia. It is fairly easy to identify by the morphology of its vegetative structure, shaped like a Christmas tree : indeed, it usually shows a main axis, well defined and carrying regularly arranged secondary branches.

A. pinnata var. *imbricata* : originates from subtropical and tropical Asia. This variety has been used for many centuries in China and Vietnam. It resembles the variety *pinnata* although its general shape is not conspicuously triangular.

II. Section *Azolla* : the megasporocarps have three floats and the glochidia are barbed.

A. caroliniana, *A. mexicana* and *A. microphylla*

All three originate from temperate, subtropical and tropical regions of North and South America. These species differ from each other mainly by the architecture of their megasporocarp wall and thus are often difficult to distinguish at the vegetative stage.

A. filiculoides and *A. rubra*

Native of America and the Far East (Korea, Japan and New Zealand) respectively, the two species differ from the *caroliniana* group by the presence of unicellular trichomes on the abaxial surface of the upper lobe of their leaves; this surface in the other three species bears barbed or at times tricellular trichomes.

1.6. TAXONOMY OF ANABAENA AZOLLAE

The identity of *Anabaena* endophytes within the various *Azolla* species is a problem whose solution has been wanting for a long time. As it is difficult (if not impossible) to cultivate these organisms *in vitro*, the researchers could not investigate their characteristic. Recent works based on monoclonal antibody production techniques have however made it possible to bring out differences in *Anabaena* extracted from different *Azolla* species.

What is more, it has been possible by micromanipulation to create new associations by transferring *Anabaena* from one *Azolla* species to another one which previously had been cleared of its endophyte, and vice versa. The products of these "crossings" have properties which are different from those of the previous associations.

1.7. RELATIONS BETWEEN THE SYMBIOTIC PARTNERS

Clearly, the main relations between *Anabaena* and *Azolla* are the supply to the fern of nitrogenous compounds produced from nitrogen by the bacteria, which in turn are using carbon compounds photosynthesized by the fern.

1.7.1. PRODUCTION OF NITROGEN COMPOUNDS

We know that the association *Azolla-Anabaena* can grow and develop perfectly without any source of nitrogenous compounds. This is made possible by the fact that *Anabaena azollae* like a number of other cyanobacteria, has an enzymatic complex called nitrogenase, capable of transforming N_2 into NH_3 .

As oxygen inactivates nitrogenase and photosynthesis produces oxygen, the two metabolisms are incompatible. They are therefore spatially separated : the production of ammonia from nitrogen occurs in the heterocysts and this not only for their own combined nitrogen requirements but also for those of the vegetative cells and of *Azolla*. The heterocysts

excrete indeed a great deal of the produced ammonia into the leaf cavity, where it is collected by the fern (probably through the transfer hairs).

1.7.2. PRODUCTION OF CARBON COMPOUNDS

Azolla and the vegetative cells of *Anabaena* are photosynthetic. With non-symbiotic *Anabaena* species e.g. *Anabaena cylindrica*, the cyanobacterium must be self-sufficient in carbon compounds and cannot under these conditions transform more than 3 to 5 % of its cells into heterocysts. It has been observed in mature *Azolla* leaves that the proportion of heterocysts exceeds 30 %. This implies that part of the carbon requirements of the cyanobacterium is provided by *Azolla*. There again, it seems that the transfer hairs intervene in the exchanges.

1.7.3. OTHER INTERACTIONS

The relations between *Azolla* and *Anabaena* are apparently subjected to very subtle regulations. It happens for example that in one way or the other, *Azolla* controls the multiplication of *Anabaena* at its apex; the cyanobacteria population develops indeed there just enough to inoculate each new leaf primordium (or each sporocarp) without any anarchic proliferation. *Anabaena* seems to be dependent on *Azolla* in other respects : many *in vitro* cultivation attempts of *Anabaena azollae* have been undertaken but mostly without success (the positive results described in the literature remain questionable). On the other hand it should be noted that many methods make it possible to obtain *Anabaena*-free *Azolla* and these grow normally for as long as they have access to a supply of assimilable nitrogen.

1.8. A THIRD PARTNER OF THE SYMBIOSIS?

It has been known for a long time that bacteria could always be found on the apex as well as in the leaf cavities of *Azolla* (fig. 3, p. 8). Some recent observations indicate that these bacteria, apparently belonging to the genus *Arthrobacter*, are not just accidental contaminants but partners in the symbiosis. Further research is needed to confirm this hypothesis and, if it proves true, to identify the interactions between the three partners of the symbiosis.

2 ECOPHYSIOLOGY

The genus *Azolla* is cosmopolitan and many of its species have very wide geographic distributions. For instance *A. filiculoides* may be found from the North of the United State down to Argentina, through the Equator; and from sea level up to over 4000 meters in altitude. It should thus be expected, and the experience confirms it, that there are many ecotypes*. The rational use of *Azolla* requires the knowledge of ecotype requirements in order to select those ecotypes which are best adapted to a given environment, in other words those with the best productivities. The general reactions of *Azolla* with regard to the principal environmental factors are described below.

2.1. WATER

Azolla is particularly sensitive to a water shortage; this is probably due to the peculiar structure of its stomata that cannot control the transpiration. Without water the plant dies within a few hours.

Azolla is a typical aquatic floating plant. It can also survive rooted in the soil if the soil permanently humid but in that case its productivity is lower; overpopulation occurs indeed rapidly in such conditions, since the fronds are unable to fragment and to disperse.

When the stretch of water is temporarily drained, the survival of the *Azolla* covering varies depending on the species. *A. pinnata* generally is the least able to support this situation not because it is less efficient at holding water but because its root system is less efficient and therefore less able to extract water in those conditions. The practical implications of this behaviour will be described below.

A strip of water not more than a few centimeters deep favours the growth because it provides a good mineral nutrition with the roots not too far from the soil, and also because it reduces the wind effects (see below).

2.2. WIND

The wind accumulates *Azolla* to one side of the stretch of water, creating a premature overcrowded condition and thus slowing down growth. The ways of avoiding this situation are described further on (cf. 5.1.2.5 & 5.2.1.2).

* It has been noted that the specific determination of *Azolla* rests upon tenuous criteria; the problem becomes still more difficult at the sub-specific level. With reference to the bacteriological terminology, *Azolla* cultures from a sample collected in a given locality often are called strains and a number in a reference collection may be attributed to such cultures.

2.3. TEMPERATURE

As suggested by its wide geographical distribution, the genus *Azolla* includes ecotypes adapted to a wide variety of thermic conditions. The analysis of the behaviour of the plants towards temperature is very complex and it should take into consideration not only the absolute temperatures but also the range of daily variations as well as the interaction of other environmental factors such as the light intensity. Our sketchy knowledge about *Azolla* can be summarized as follows :

- as a rule, at constant temperature, the optimum is around 25 °C for all species;
- some strains grow, though slowly, at temperatures below 10 °C and can even survive frost conditions for a few days. But they cannot withstand well temperatures of over 30 °C; this behavior is found in *A. filiculoides* and *A. rubra*.
- On the contrary, some strains survive with difficulty at temperatures below 10 °C but withstand temperatures of over 35 °C or 40 °C. Many ecotypes of *A. pinnata* fall into this group.
- the reactions to the daily range of temperature variation are very diverse.

It should be noted that the very great extension of the area given over to *Azolla* culture in China since 1960 has been made possible by the introduction of exotic species with different temperature requirements and also by the development of methods of protecting *Azolla* against extreme winter or summer temperatures.

2.4. LIGHT

The growth rate of *Azolla* increases with the light intensity up to 1000 $\mu\text{Einstein.m}^{-2}.\text{s}^{-1}$, which corresponds to about 50 klx or 50 % of maximum natural light intensity. Beyond this level, the growth rate remains constant or decreases slowly according to ill-defined interactions with other ecological parameters.

The daily productivity on the other hand is proportional to the hemeroperiod.

2.5. pH

Azolla is particularly insensitive to pH as such. It grows satisfactorily in a pH range from four to ten. Indirect effects however may occur due to the insolubility of certain essential elements and to the proliferation, at high pH levels, of cyanobacteria reducing the growth of *Azolla*.

2.6. SALINITY

The information concerning the tolerance of *Azolla* to salinity is still incomplete. Specific and subspecific differences are evident but electrical conductivity beyond 3 mhos.cm⁻¹ is probably always unfavourable to growth.

Anabaena is more sensitive to salinity than *Azolla* and this allows the production of *Anabaena*-free *Azolla*, very useful for some types of research but obviously inefficient with regard to nitrogen fixation.

2.7. MINERAL NUTRITION

2.7.1. NITROGEN

Azolla grows perfectly without any nitrogenous compound in the medium, all its nitrogen requirements of the symbiosis being met through the diazotrophic metabolism of *Anabaena*.

We know on the other hand the inhibiting effects of inorganic nitrogen (NH_4^+ , NO_3^-) on nitrogenase. This nitrogen is always present, more or less concentrated, in the medium. In the special *Azolla*-*Anabaena* symbiosis the nitrogenase seems to be protected in one way or the other and this protection is more or less complete depending on the species and its ecotypes. This enables *Azolla* to contribute additional nitrogen to the field even in the case of nitrogen fertilization, contrary to what happens with other diazotrophic organisms. However, it should be noted that the competitiveness of *Azolla* towards algae and other aquatic plants is inversely proportional to the concentration of nitrogen in the medium.

2.7.2. PHOSPHORUS

Phosphorus is the only essential element which quite often limits the growth of *Azolla* in a continuous flow, its critical concentration for the onset of deficiency symptoms is about 0.22 ppm. A deficiency in phosphorus (< 0.22 % of the dry matter) is accompanied by a reduction of nitrogenase activity and nitrogen content in *Azolla*.

When enough phosphorus otherwise is available, *Azolla* can accumulate its content up to 1 or 2 % of the dry matter. So enriched, *Azolla* may be transferred to a medium poor in phosphorus where it will carry on proliferating by using its reserves.

The various species and ecotypes differ considerably with regard to their ability to accumulate phosphorus and to their minimal needs of this element.

2.7.3. OTHER ELEMENTS

Positive effects of potassium application on the growth of *Azolla* and also the accumulation of this element by the fern have been observed.

Cases of deficiency in iron and molybdenum (two elements involved in the composition of nitrogenase) as well as cases of iron toxicity have also been observed.

2.8. PESTS

2.8.1. ARTHROPODS

Many insects, especially the larval stages of various *Pyralidae* (*Lepidoptera*) and *Chironomidae* (*Diptera*) can wreak havoc in the *Azolla* populations on which they feed, especially by high temperatures and atmospheric humidity. In some parts of Asia, they are probably the main limiting factor to the use of *Azolla**

Some *Coleoptera*, *Orthoptera* and *Homoptera* as well as two *Acaridia* are known to occasionally feed on *Azolla* but they do not seem to cause as much damage as the above-mentioned insects.

2.8.2. GASTEROPODS

Several (about ten) species in the families *Lymnaeidae*, *Planorbidae* and *Pilidae* have been described as pests on *Azolla*. They may at times become abundant but their impact on *Azolla* populations apparently cannot be as serious as the damage wrought by some insects.

2.8.3. FUNGI

The high temperatures, humidities and population densities plus the grazing of the leaves by various pests induce the proliferation of several fungi (*Rhizoctonia*, *Sclerotium*): this may in some extreme cases lead to the destruction of the culture.

2.9. PRODUCTION OF ANTHOCYANINS

The production of anthocyanins, giving the *Azolla* carpet a reddish colour of varying intensity often signals unfavourable conditions such as excessive temperature or light intensity, or unbalanced nutrition.

Given the diversity of factors inducing this colour, no precise diagnosis can be formulated *a priori*; the experienced user shall however learn to recognize the usual cause of this phenomenon in the local ecological conditions and will be able to react in time, e.g. by the application of the appropriate fertilizers, by the addition of fresh water, by shading, etc....

Finally it should be noted that while certain *Azolla* species do not produce anthocyanins, others do so in conditions assuring a satisfactory growth.

* So far, this has not been the case in Africa. One should, however, acknowledge the possibility that this disturbing phenomenon may appear with the development of azolliculture, especially in the humid tropics.

3

AGRONOMIC INTEREST OF AZOLLA

The capacity to fix atmospheric nitrogen is undoubtedly the ground for the interest given to the *Azolla-Anabaena* symbiosis which has many other interesting properties as well. Some recent trends in research aim at developing cultural practices through which several beneficial properties will simultaneously be exploited. We shall now briefly describe the benefits one can expect from azolliculture.

3.1. NITROGEN SUPPLY

Because of its diazotrophic nature, *Azolla* brings nitrogen to the environment in which it develops. This nitrogen will be freed and eventually made available to cultivated plants after the death and decay of the fern. Even though many factors may influence the extent of this contribution, it could be estimated that the burying of an *Azolla* carpet of good quality generally has effects similar to those obtained by applying thirty to forty units of nitrogen per hectare.

3.2. IMPROVEMENT OF THE SOIL STRUCTURE

The high productivity of *Azolla* allows large quantities of organic matter to be incorporated (by ploughing in) to the soil, whose structure consequently will be improved. Because of the mineralization which is generally rapid in tropical soils, significant changes can only be expected after massive and repeated applications.

3.3. POTASSIUM ACCUMULATION

Azolla can better accumulate this element in a potassium-poor environment than does rice. This potassium returns to the soil with the decomposition of the fern, which thus indirectly acts as a potassium fertilizer.

3.4. INHIBITION OF WEEDS

The photosynthetic activity under the carpet of *Azolla* is highly reduced for want of light. As a result, many young plants (those whose seeds are poor in reserves) die before being able to pierce this screen and assure their autotrophic metabolism. This herbicidal effect depends on the type of dominant weeds but is often spectacular and greatly appreciated by the peasants, whose weeding chores are largely reduced. In the same way, *Azolla* prevents the proliferation of algae harmful to the development of irrigated cultures.

3.5. WATER SAVINGS

The evapotranspiration of a water surface covered by *Azolla* is inferior to the evaporation of a non-covered water surface. This effect should be taken into consideration when there is a shortage of water or when water is expensive.

3.6. TEMPERATURE REGULATION

The presence of an *Azolla* carpet has a buffering effect on the daily temperature fluctuations in the water under it. This effect will be positive or negative for the crop associated with *Azolla* according to the circumstances.

3.7. FOOD SOURCE

Azolla serves as a valuable food for many types of livestock and its use in this field is expanding very fast. (cf. 5.5 & 5.6).

3.8. ENERGY SOURCE

It is possible to feed a biomethane digester with *Azolla* only or with the fern plus other materials such as rice straw; the effluents can be collected and used as fertilizer. The experience in this field, although positive, is however very limited and complementary research is needed.

4

CRITERIA OF AZOLLA SELECTION

Given the place and the season, the ecotype(s) of *Azolla* to be cultivated must be selected according both to the environmental conditions and to the intended use(s). We describe herewith the main selection criteria that, in our present state of knowledge, should be considered.

4.1. PRODUCTIVITY

The value of *Azolla* depends in the first place on its productivity which in turn is determined by a large number of environmental factors to which the ecotypes react differently.

The growth of a population of *Azolla* at the water surface in stable conditions (light, water and air temperatures, pH and nutritive components of the medium...), could be represented by a sigmoid (S-shaped) curve, slightly asymmetrical, with the inflexion point (i) generally situated before the biomass reaches half of its maximum value (fig. 5, p. 20).

Starting with a quantity of *Azolla* which only partially covers a stretch of water, the first period to be noticed is one of exponential growth (phase I): the isolated fronds float freely on the water surface without any constraint. This phase continues, depending on the species, until the biomass covers the totality of the available surface, or even slightly beyond, which represents a quantity of fresh matter of about 1 kg.m^{-2} . From this moment, the growth becomes practically linear (phase II); this phase continues, depending on the ecotypes and the environmental conditions until the biomass reaches about 2 kg.m^{-2} in some cases and up to 8 kg.m^{-2} in others (though this is exceptional).

With this overcrowding, however, the fronds must progressively either come on top of one another or develop an erect bearing; these phenomena gradually slow down (phase III) and eventually stop the growth. The maximum biomass - or ceiling value - is reached and maintained for some time (Phase IV). It foretells the degeneration of the population (phase V). The corresponding productivity curve (biomass produced by unit of time) indicates clearly that the highest values are obtained during the linear phase, with a maximum at the inflexion point of the growth curve.

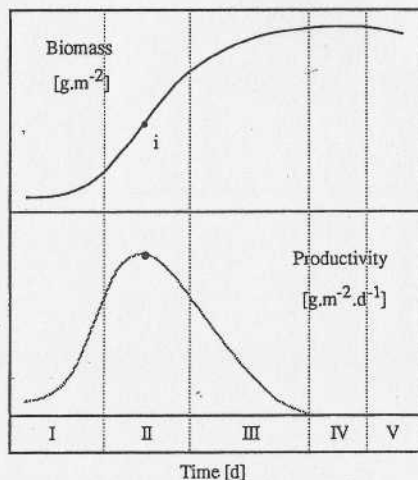


Fig. 5. Growth and Productivity Curves.

The growth curves vary according to the environmental conditions and the *Azolla* ecotypes. They can be distinguished by the value of the maximum productivity which will in the best cases reach some $300 \text{ g.m}^{-2}.\text{d}^{-1}$, by the length of the linear phase as well as by the maximum biomass, the latter being usually of 2 to 4 kg.m^{-2} but reaching 12 kg.m^{-2} exceptionally.

When the goal is to produce the largest possible quantity of *Azolla*, which is most often the case, the principal criterion of selection is the maximum productivity. In that case, the excess biomass is regularly removed in order to maintain the density of the population close to that corresponding to the inflexion point and in any case at the linear phase. The frequency of these removals depends on a compromise between the desire to assure maximum productivity and that of saving the labour required for the harvests. It must also take into consideration the length of the linear growth phase of the cultivated *Azolla* ecotype.

In some cases however, the criterion of selection would not be the maximum productivity but the maximum biomass; such is the case when the culture will not be harvested but ploughed in on the spot (cf. 5.2.1.6).

It should be noted that, especially in regions with contrasting seasons, it is often useful to plant a mixture of two or three different ecotypes, each of them characterized by a high productivity during a different part of the year and thus dominating during that period (fig. 6, p. 31).

4.2. CHEMICAL COMPOSITION

Another selective criterion is the chemical composition of *Azolla* which varies according to the species and ecotypes but also with the ecological conditions and the phase of growth, other words the population density. Various aspects of the chemical composition can be considered, depending on whether *Azolla* will be used as green manure or as food. Comparative studies on the chemical composition of the different species and ecotypes; unfortunately still very incomplete and as a result only general information on that subject will be given here.

4.2.1. PERCENTAGE OF DRY MATTER

Two problems are encountered in estimating this percentage with *Azolla*: one is the need for a standardized way of draining the surface water and the other is caused by the mineral and organic matter which clings, sometimes abundantly and tenaciously, to the root system. The particles must be thoroughly washed away before proceeding with the analysis. Consequently there is little agreement between the published data on this subject; values of 5 to 7 % can however, be taken as fair estimates.

The point must be stressed that an improper washing of the samples before drying falsifies not only the estimation of the percentage of dry matter but also all the subsequent analyses made on that dry matter (and sometimes very seriously). Cases are known of impurities attached to the root system, particularly soil colloids, that may account for up to 50 % of the dry matter.

4.2.2. ASH CONTENT

The ash content generally measures between 10 and 20 % of the dry matter. When it is higher, because of the presence of impurities as mentioned above, the food value of *Azolla* will be negatively affected.

4.2.3. NITROGEN CONTENT

The nitrogen content varies considerably, roughly from 2 to 7 % of the dry matter. It is influenced by a lot of factors, particularly phosphorus deficiencies and sub-optimal temperatures which have a very negative effect. In natural conditions, values near 3.5 % are frequent. The two varieties of *A. pinnata* are generally poorer in nitrogen than the other species.

It should be noted that the nitrogen content only gives a rough indication of the value of *Azolla*. This nitrogen comes, in fact, partially from the atmosphere (N_2) and partially from the soil (NH_4^+ , NO_3^-), but only the fraction taken from the atmosphere corresponds to a significant contribution of nitrogen to the system. However, this fraction varies with the species and ecotypes; some continue to take the largest fraction of their nitrogen (> 75 %) from the atmosphere even when there is a high concentration of mineral nitrogen in the water, while other ecotypes have their nitrogenase activity strongly inhibited in the same conditions.

One should therefore select ecotypes that are less sensitive to combined nitrogen but this requires expensive experimentation. Here again, the performance of *A. pinnata* seems in general to be weaker than that of other species.

Finally it should be noted that if the nitrogen content in *Azolla* does not vary much in relation to the population age, the non protein nitrogen content, linked to the cell wall compounds, sharply increases at the end of the linear phase and thereby reduces the food value. The harvest of *Azolla* during the linear growth phase as recommended above (cf. 4.1) is once again justified.

4.2.4. AMINO ACID CONTENT

The food value of *Azolla* is largely determined by its amino acid composition. The amino acid content increases during the linear growth phase and falls sharply when growth slows down. The essential amino acids represent about 55 % of the total. Considerable specific and subspecific differences exist concerning the proportions of the different essential amino acids. This probably explains why the published data show little agreement on this matter. At least some *Azolla* strains have well balanced amino acid contents with the exception of slight methionine, cystein and sometimes lysine deficiencies, and constitute good quality food if cultivated in good conditions.

4.2.5. CARBON CONTENT

The carbon content is fairly constant at about 43 %. The Carbon/Nitrogen ratio, which determines the rate of decomposition at the time of the ploughing-in, is consequently conditioned by the nitrogen content and has thus a high variability (from 6 to 20 %) but is often about 12 %.

4.2.6. CARBOHYDRATES AND LIPIDS CONTENT

The soluble carbohydrates represent about 3.5 %, starch 4 to 10 % and lipids 3 to 6 % of the dry matter. There is little information on the interspecific differences.

4.2.7. CELL WALL COMPOUNDS CONTENT

Azolla has a relatively weak cellulose content in comparison with other fodders; it is usually between 10 and 20 % of the dry matter. The hemicellulose percentage is of the same order.

The lignin concentration varies considerably from one species to the other and as a function of the density of the population. Young populations generally contain ± 5 % of lignin whereas in phase IV the content can exceed 30 %, strongly reducing the digestibility of *Azolla*.

4.2.8. PRESENCE OF TOXINS

No toxins have ever been detected in *Azolla*.

4.3. SENSIBILITY TO PESTS

In the regions with pest problems, the criterion of susceptibility to them must of course be taken into consideration (cf. 5.1.3.3).

4.4. SURVIVAL CAPACITY AFTER DRAINING OFF THE WATER

When temporary breaks in the water supply are to be feared, which is often the case in African rice fields, or when the eradication of pests requires momentary drainage, it is advisable to cultivate *Azolla* species that can cope with the condition, particularly *A. carolinian*, *A. microphylla* or *A. filiculoides*.

4.5. APPETENCY

When *Azolla* is cultivated for food purposes, the preferences manifested by the animals for the different species or strains should be taken into consideration. The information on this subject, sketchy though it is, indicates for instance that *A. pinnata* is generally not very well liked whereas other species are highly appreciated. Differences exist at the infraspecific level and in addition the different animals concerned do not necessarily react in the same manner.

5

DIFFERENT WAYS OF USING *AZOLLA*

Whatever *Azolla* is to be used for, it must generally be kept in a permanent vegetative state in order to have it available and ready for use when needed. As we have already mentioned, it is almost impossible to obtain spores which would permit the conservation of the material in a dry state and even when this is possible, the time required to produce a sufficient biomass from spores is usually too long.

In the first place the basic methods of *Azolla* culture will be described and then the different uses of the cultivated *Azolla* will be considered.

5.1. MAINTENANCE NURSERIES

5.1.1. INTRODUCTION

Any stretch of standing water could normally be used for the maintenance of an *Azolla* population. In favourable conditions, *Azolla* develop spontaneously in the paddy fields as soon as they are flooded, either from spores of the previous season or mostly from plants which have survived in situ. In other cases, *Azolla* populations stay permanently in marigots (fig. 7 p. 31), in irrigation or drainage channels (fig. 8, p. 34) or on the banks of lazy streams. In such cases, the interested peasants would only have to harvest these *Azolla* when needed. The spontaneous persistence of *Azolla* throughout the year is however not the general rule and one will often have to keep them in nurseries especially designed for that purpose (fig. 9, p. 34).

5.1.2. NURSERY LAYOUT

5.1.2.1. Care-taking of nurseries

Depending on the social structure of agriculture and the kind of uses to which *Azolla* is put, the planning and the maintenance of the nurseries will be carried out either individually or collectively. In the South Philippines, for instance, where azolliculture was introduced with success some years ago, village communities entrust one of their members with the responsibility of taking care of the nurseries. This person receives the necessary inputs and has the responsibility of assuring on a permanent basis the maintenance of a healthy *Azolla*.

population. The other villagers can get inoculums there at the appropriate time and, in turn, multiply them.

5.1.2.2. Localization

The site preferably should be selected in the immediate proximity of the caretaker's home or work place so that a daily supervision can be secured.

5.1.2.3. Dimensions

The area to be used for the nursery depends on many factors but usually a few hundred square meters should be sufficient. A depth not exceeding 5 to 10 cm is desirable but requires frequent readjustment of the water level.

5.1.2.4. Shading

Even though *Azolla* perfectly tolerates direct, even intense sunlight under certain conditions, experience has shown that slight shading is sometimes favourable! It will therefore be prudent to initially cover part of the nursery with a screen which can be removed if proving useless. The use of nutritious creepers obviously will be recommended.

5.1.2.5. Wind protection

If *Azolla* starts being pushed to one side of the nursery by prevailing winds that thereby check its growth, hedges for wind protection could be planted (fig. 9, p. 34). The nursery can also be subdivided by small dikes perpendicular to the dominant winds or a thin sheet of water may be kept on a roughly ploughed soil; these measures reduce the effects of the wind.

5.1.3. MAINTENANCE

5.1.3.1. Thinning out

We should remember that it is in the linear growth phase that *Azolla* productivity is highest. Thus it is useful to maintain on a permanent basis a complete cover of *Azolla*, say at least 1 kg.m⁻². Moreover, this prevents the development of other organisms such as *Pistia*, *Salvinia*, *Lemna*, *Marsilea*, *Eichhornia*, or various algae. On the other hand, overcrowding must be avoided since it reduces the productivity of *Azolla*. The excess biomass should thus be thinned out or removed regularly, for instance once a day or at least once a week, depending on the use to which the harvested *Azolla* will be put. There are several efficient harvesting methods; two will be described here :

- the *Azolla* carpet is pushed with a bamboo pole or a plank towards one of the banks; being thus concentrated, it can easily be harvested by hand or with wide-mesh wicker baskets and poured out into bottom-holed containers which will let out the excess water;
- the water surface is scraped with a net about 3 m long and 50 cm high, stretched perpendicularly to the water level by two persons. At regular intervals the net is raised

and the *Azolla* mass is brought to the bank (fig. 9, p. 34). This method is suitable only where the water level is high enough to prevent retrieving large quantities of soil with *Azolla*.

After harvesting, the *Azolla* left on the water surface should be uniformly scattered using a wide flat broom (made, say, from bamboo twigs or sorghum stems), with which the *Azolla* carpet can be lightly tapped.

5.1.3.2. Fertilization

Since biomass exportations are regularly carried out, nutritive elements (save for nitrogen) must be supplied. This is particularly true for phosphorus but also, in the long run, for the other essential elements. Recipes are hard to formulate in this matter since the requirements depend on the importance of the exportations - that is to say on the productivity - on one hand and on the underlying soil quality on the other. However, with a little experience the need of fertilizing material is easy to recognize : indeed, the appearance of the *Azolla* carpet is modified, the plants become smaller and change their colour and at the same time their productivity falls. A means of maintaining the fertility is to regularly dump organic matter into the nursery (manure and plant waste) as well as domestic ashes.

With regard to phosphorus, the nursery caretaker should store some superphosphate, to be applied when needed and preferably in frequent small doses rather than large infrequent ones : a weekly exportation of, say 1 kg of *Azolla* must be compensated by the input of about 500 mg phosphorus.

5.1.3.3. Control of harmful organisms

A. Insects

As mentioned earlier (2.8.1), some insects may cause serious damage to *Azolla* cultures. If and when they are detected in the nursery one must react immediately. One of the nursery caretaker's duties is to detect as early as possible the signs of insect attack. These signs vary according to the species involved. The *Pyralidae* larvae cover themselves in a cylindrical sheath made from *Azolla* fronds and move on the water surface, leaving on the carpet behind a sinuous trail which is easy to recognize. The *Chironomidae* larvae, which resemble those of mosquitoes, hide under the carpet where they consume the roots of *Azolla* and induce the decomposition of the plants : they can be detected by briskly lifting a handful of *Azolla*, from which they will beat a hasty retreat.

A first way of controlling these insects is to drain the nursery of water for about a week as the larvae need free water to survive. This is a somewhat tricky procedure, for *Azolla* itself is very sensitive to dryness, depending more or less on the species (cf. 2.1). Success therefore depends on the *Azolla* strain present and on the water retention capacities of the soil. In addition, it is essential that no heavy rain fall on the nursery during the treatment; unintended flooding would defeat the purpose of the operation.

Insecticidal spraying (carbofuran, methomyl, monocrotophos, diazinon...) is an alternate method which should not raise economical or ecological problems, with the small area involved. However, with nurseries used as fish ponds or being made accessible to livestock or

poultry, the insecticide selected obviously must be non-toxic to vertebrate animals. Finally the dosage has to be adjusted to the local conditions and the type of *Azolla* cultivated, since some insecticides are more or less phytotoxic depending on the *Azolla* species.

It is also known that some insects do prefer certain species of *Azolla* while they cannot develop normally on others, particularly on *A. pinnata*. Unfortunately, the relevant data are not very abundant and precise recommendations cannot be given. The grower confronted with insect attacks would have to test several strains of *Azolla* in order to select those that develop in these conditions.

Biological control is a third method; it works in principle by introducing and favouring the multiplication of organisms which attack the *Azolla* pests. Some of these organisms are known but research programmes with a view to enhancing their beneficial effects have up to now been neglected.

Finally, one can think of introducing in *Azolla*, by genetic manipulation, genes which will make it resistant to these insect pests.

B. Gasteropods

Whenever a decrease in the number of molluscs would be useful, the following control measures can be taken :

- periodic harvest and destruction of the adults and of their egg masses;
- pesticide application : however, many molluscicides are toxic to *Azolla* and especially to the fishes;
- introduction of carnivorous fishes.

C. Fungi

Fungal attacks are to be expected on extremely dense *Azolla* carpets and by high temperature and humidity. The wounds inflicted on the fern by predatory insects and molluscs also favour fungal infection.

In such cases the first measure to be taken is to harvest and destroy the contaminated parts of the *Azolla* carpet; in addition, *Azolla* shall be carefully thinned out so as to keep it in the linear growth phase. If necessary insects and molluscs will be controlled. Only when these measures prove ineffective shall the use of fungicides be considered.

D. Algae and aquatic plants

As these organisms are likely to hamper the development and harvest of *Azolla*, their growth should be prevented. The best way is to assure a permanent complete cover of the water surface in order to inhibit photosynthesis in the algae and plantlets under the carpet.

One should also avoid excess nitrogen in the dosage of fertilizers that are periodically added to the nursery.

Finally, manual weeding can be necessary in some cases.

5.1.4. VALORIZATION OF THE BIOMASS PRODUCED

The first raison d'être of maintenance nurseries is to supply rice fields or fish ponds or other water stretches with inoculums intended for *Azolla* multiplication on a much larger scale (fig. 11, p. 35). The need for such supplies is, however, only seasonal whereas the nursery constantly produces an excess biomass. In satisfactory ecological conditions, for instance, the thinning out of a 10 ares (1000 m²) surface yields between 100 and 200 kg of fresh matter daily.

This biomass must of course be put to beneficial uses in order to make nursery maintenance a tempting proposition. It can for one thing serve as food complement to fish breeding or animal husbandry (poultry, rabbits, pigs...) that will be started close to the nursery; it also can be composted to fertilize nearby situated market gardens or dried for the preparation of food to be consumed elsewhere or, again, fermented in a biogas digester. More will be said later about these applications (cf. 5.4 to 5.6).

5.2. AZOLLA, GREEN MANURE IN IRRIGATED RICE FIELDS

5.2.1. MONOCROPPING AZOLLA

5.2.1.1. Introduction

When occupation of the paddy field days or weeks before planting out is not on the calendar, and if water is available during that period, an *Azolla* monoculture can be started in the paddies. A few days before the rice is to be transplanted, *Azolla* will be ploughed in, so that its decomposition brings a good supply of nitrogen, with phosphorus and potassium as well, to the growing rice during the first weeks.

5.2.1.2. Field preparation

Water is brought to the paddy without any previous preparation of the soil. The persistence of the last crop's stubbles prevents to a large extent the accumulation by the wind of *Azolla* to one side of the rice field. If for some reason the ground had to be ploughed before the introduction of *Azolla*, its uneven surface will have the same effect as the stubbles in the first case.

However, the soil preparation (ploughing, harrowing and leveling) will normally be carried out at the end of the monoculture when the *Azolla* carpet is being incorporated into the soil.

The water level should not exceed 5 cm in order to enable *Azolla* to keep close to the soil without taking root.

5.2.1.3. Inoculum preparation

Eight days before the inoculum is taken from the nursery, the latter shall be thinned out and then fertilized with about 10 kg of superphosphates per hectare. This will be followed by a second application two or three days before harvesting; with this enrichment *Azolla* will be able

to multiply considerably in the rice fields. The fertilizer applied in the nursery has not been wasted since it eventually will be used by the rice plants after the decomposition of *Azolla*.

If necessary, an insecticide may be applied a few days before harvesting. A cheaper way would be to apply an appropriate pesticide to the heaped-up *Azolla* on the nursery banks just after harvesting; once impregnated, the *Azolla* plants can be used a couple of days later as inoculum.

5.2.1.4. Inoculation density

The choice of the inoculation density is a matter of compromise. For it should be noted that :

1. with a given quantity the highest production/time ratio is obtained on an area which is not limiting (exponential phase, population density less than 1 kg.m^{-2});
2. with a given area the highest production/time ratio is obtained if the biomass stays between 1 and 2 to 4 kg.m^{-2} (linear phase);
3. low densities are favourable to the development of weeds;
4. the time required to reach the incorporation biomass (if possible 2 to 4 kg.m^{-2}) and therefore the duration of field occupancy and water consumption is inversely proportional to the inoculation density;
5. the adequate nursery size with respect to the inoculation of a given area of rice fields is proportional to the inoculation density;
6. the man-power required for the inoculation (harvesting, carrying and distributing *Azolla*) is proportional to the inoculation density.

With these factors in mind, the recommended densities vary from less than 0.1 to 1 kg.m^{-2} . Very low quantities may be justified when time and water are not limiting factors and danger of weeds, algae and pests proliferation is low; these conditions are rarely met with in tropical regions and are closer to the winter conditions in subtropical zones, e.g. in the north of Vietnam or the south of China where the monoculture of *Azolla* has been developed in winter, in rotation with two crops of rice each year. It should be noted that this practice is diminishing because the farmers often prefer to grow market gardens which are more profitable during that season even if they must use commercial fertilizers to meet the requirements of rice cultivation.

In tropical regions, and during the hot seasons of the year in subtropical regions, it would be advantageous to increase the inoculation density in order to avoid the competition of weeds and algae.

Doses between 250 and 500 g.m^{-2} generally represent a good compromise which should only be exceeded in exceptional cases.

It should be noted that an inoculation density of 500 g.m^{-2} implies, with a nursery that admittedly can supply 2 kg of *Azolla* per m^2 with each removal, a ratio of 1 to 4 between the areas of the nursery and the rice field. Clearly this is usually unrealistic and an intermediate stage should be provided, viz the setting up of multiplication nurseries, most often in the midst of the rice paddies.

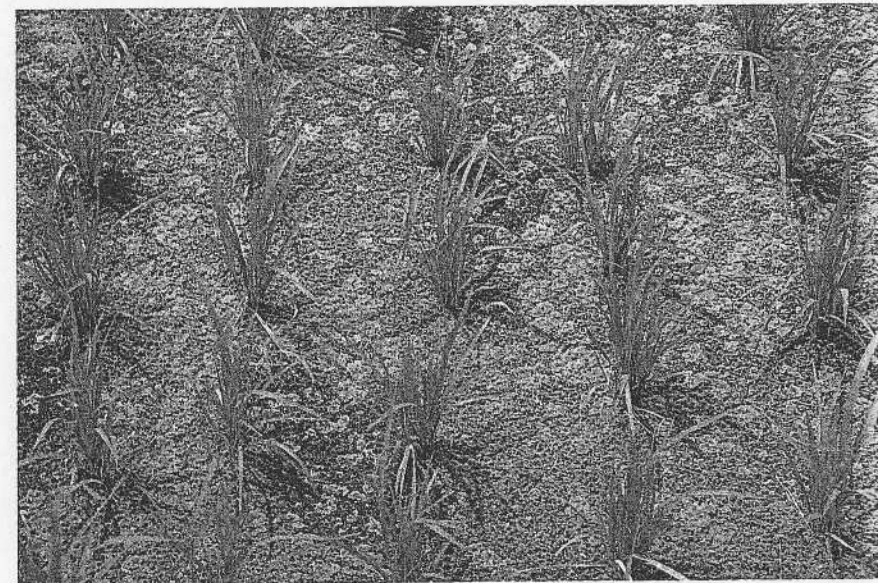


Fig. 6. Rice-*Azolla* intercrop. The simultaneous presence of three *Azolla* ecotypes increases the probability of a high productivity. Note the absence of weeds (China).

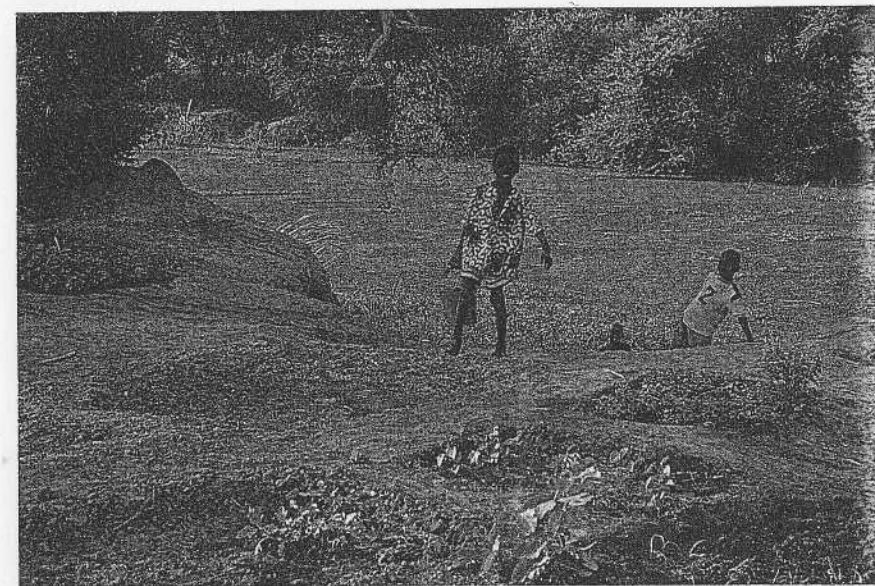


Fig. 7. Vegetable garden being grown on the banks of an *Azolla* covered marigot. During the preparation of the beds *Azolla* are harvested and buried (Senegal).

For example, imagine a nursery of 100 m² with a peak density of 3 kg.m⁻², whose production should suffice to inoculate ten rice paddies of 1000 m² each. The nursery supplies 200 kg of *Azolla*. One could bring 20 kg (20 g.m⁻²) to each paddy but such an operation entails a serious risk of having *Azolla* supplanted by other organisms on one hand and on the other it means that all the paddies must be flooded from the beginning of the operation.

It is therefore preferable to inoculate a single paddy field at a density of 200 g.m⁻² or even half a paddy field at 400 g.m⁻² or in extreme cases a quarter of a field at 800 g.m⁻²; as soon as the density in the paddy reaches the limit of the linear growth phase, the biomass will be distributed among the neighbouring paddies from which it can be further propagated. While this operation is being carried out, it goes without saying that the surplus biomass from the nursery can also be brought to the paddies. Finally, it should be remembered that in certain ecological conditions the flooding of the rice paddies leads to a spontaneous development of *Azolla* either from spores or from small islands of population which survive from one season to the other.

5.2.1.5. Ways of inoculation

A. Inoculation by broadcasting

When sown by broadcasting, *Azolla* does not spontaneously spread out evenly on the surface of the water. It has a tendency to pile up into compact clumps which were formed in the harvesting and transporting stages. This hampers the growth and leads to considerable waste. The dispersion of the plants is improved by striking these clumps with a broom (cf. 5.1.3.1 & fig. 10, p. 35).

B. Inoculation by flowing

If the layout of the land is convenient, man-power can be spared by establishing the nursery upstream; the excess *Azolla* then will simply be carried away by the stream to the first field at flooding time. Upon saturation of the first, a gate shall be opened to the second paddy and so on (fig. 12, p. 38).

5.2.1.6. Duration of the monoculture

How long it takes for an *Azolla* culture to get ready for ploughing-in depends on the inoculation density and on the productivity. Starting from an inoculum of 300 g.m⁻², corresponding to the exponential growth phase, the biomass may require no more than 48 hours to double, in optimal conditions; three to four days will more often be necessary. At a density of 1200 g.m⁻² the growth enters its linear phase with a more or less constant yield of 100 to 200 g.m⁻².d⁻¹. In the best of these circumstances, the biomass will therefore need 8 days to reach a density of 2 kg.m⁻² and 13 days for 3 kg; these figures climb to 16 and 26 days respectively in the less favourable conditions.

It should be remembered that when the time for the monoculture is not a limiting factor, it would be an advantage to use an *Azolla* type which has a high maximum biomass.

5.2.1.7. Incorporation of *Azolla* into the soil

As soon as the *Azolla* carpet reaches a sufficient density (2 to 4 kg.m⁻² or more), the rice paddy is drained and *Azolla* is incorporated into the soil. Depending on the resources available and the soil characteristics, the incorporation is done either by hand, by stamping, by hoes, by rotary or conical weeders, by ploughing or harrowing with an animal or engine (fig. 13, p. 38). The shape of the weeders and harrows sometimes must be adjusted for this particular use.

The extent of the preliminary draining is a determining factor for a successful incorporation. If rice transplanting follows the *Azolla* monoculture, the incorporation must be carried out in such a way that about 20 % of the *Azolla* will float again upon flooding and grow a new carpet between the rice plants, while the bulk - 80 % - of its biomass shall remain underground and act as a fertilizer (cf. 5.2.2). If on the other hand direct sowing is practiced, all of *Azolla* must be incorporated lest a new carpet would suffocate the rice seedlings before they emerge. This delicate operation calls for a thorough drainage and an incorporation followed by a few days without water in the fields.

5.2.1.8. Advantages and constraints of monoculture

Due to the rapid decomposition of *Azolla* in most of the tropical soils, the fertilizing effect of the monoculture is more apparent during the first few weeks of the development of rice. It is generally comparable to an application of 30 units of nitrogen. In some cases it would nevertheless be useful to provide a small dose of chemical fertilizer when transplanting, in order to assure the initial development of the seedlings.

While functioning as green manure *Azolla* monoculture also leads to the destruction of a number of unwanted weed seedlings (cf. 3.4).

As previously pointed out, the monoculture monopolizes the land between a period of from 10 days to more than one month, depending on the situation; and it requires water. It is therefore compatible with certain cultural calendars only and when water is both available and inexpensive.

Some extra man-power is sometimes required (not considering the maintenance of the nursery) for the possible transportation of the inoculum from the nursery to the field and also in certain cases for the erection of temporary small dykes intended to maintain satisfactory population densities at the beginning of the culture.

The work of incorporating *Azolla* into the soil is done at the same time as the ploughing and leveling which is required even in the absence of *Azolla*.

5.2.2. INTERCROPPING AZOLLA WITH RICE AFTER AZOLLA MONOCULTURE

5.2.2.1. Intercrop

As previously indicated, when *Azolla* is incorporated into the soil after monoculture, some of it (ideally about 20 %) returns to the surface of the water when the rice field is flooded again and it grows while the transplanted-rice develops; by doing so, it inhibits the growth of weeds and limits evapotranspiration (fig. 6, p. 31).



Fig. 8. Harvesting *Azolla* from an irrigation canal for chicken and duck feeding. The scoop is made with a forked branch and a piece of mosquito net. The *Azolla* is stocked in a wicker-work basket which allows the water to drain off (Senegal).



Fig. 9. Thinning of an *Azolla* nursery. A *Prosopis* hedge (background) acts as a wind-shelter. Harvesting is done by two people scraping the water surface with a net (Senegal).



Fig. 10. Spreading *Azolla* with a broom made of twigs. This operation is indicated in the nurseries after the harvest of excess *Azolla*, and also on the freshly inoculated stretches of water (China).



Fig. 11. Harvesting of *Azolla* bound to inoculate the ponds of a pig and fish-breeding farm (China).

Besides, after a period which varies with the importance of the initial population and the environmental conditions - normally 15 days to 3 weeks - the new *Azolla* carpet in turn can be incorporated.

In some regions of China a special transplantation pattern applies to the plant association practice. Instead of spacing the plants 20 by 20 cm cross-wise, the rows will be separated alternately by 12 and by 50 cm, while in the rows the plants will be put only 13 cm apart; this should give more light to *Azolla* and favour its growth without reducing the rice density.

5.2.2.2. Ways of incorporating *Azolla* into the soil

Azolla is incorporated into the soil either by hand, or by stamping or again with a rotary weeder. This will be done at the time of weeding (fig. 14, p. 39). The incorporation is made easier by a preliminary drainage of the paddy. It is often necessary to modify the shape of the weeder's blades to ensure a satisfactory incorporation. The shape will be adjusted according to the structure of the soil and the quality of the drainage.

It should be noted that the special transplantation scheme described above (cf. 5.2.2.1) also facilitates the incorporation effort.

5.2.2.3. Number of incorporations

The incorporation can usually be repeated only once (rarely twice) while the rice is growing, for this growth impairs the development of *Azolla* by decreasing light intensity and increasing humidity under the canopy. The fern thus becomes susceptible to fungal attacks, which is considered beneficial at this stage since these attacks lead to the decomposition of *Azolla* and the liberation of its components that can be absorbed by the rice. After the second (or third) incorporation, *Azolla* is left to develop on its own, carrying on with its herbicidal and water-saving functions before withering.

5.2.2.4. Advantages and constraints of intercropping

Rice-*Azolla* association has many advantages : it limits the growth of weeds and the loss of water; it fertilizes the rice fields. The fertilizing effect is comparable to a split application : each incorporation corresponds more or less to the application of 30 units of nitrogen per hectare.

However the associated culture as described above entails some risks. If the growth of *Azolla* is rapid soon after the transplanting of the rice, following an insufficient mastery of the incorporation, the development of the rice could be impaired because of competition for the nutritive elements on one hand and because a dense *Azolla* carpet has a negative effect on the tillering. In the case of important fluctuations in the level of the water, the *Azolla* moreover tend to stay attached to the rice seedlings when the level of the water falls and damage the seedlings when they rot on them.

The incorporation of *Azolla* is sometimes difficult without preliminary drainage of the rice paddy; but this is not always practicable. Besides, some peasants who practice the intercropping technique do not incorporate *Azolla*, which they leave to decompose

spontaneously. In this case, the fertilizing role of *Azolla* is of course highly reduced - though not nullified - and its other favourable effects still persist.

Finally, it should be noted that intercropping is not very compatible with direct sowing: for in this case the inoculation of *Azolla* can only be envisaged after complete spearing of the rice, otherwise the seedlings will be suffocated; what is more, the incorporation is not possible.

5.2.3. INTERCROPPING AZOLLA WITH RICE WITHOUT PREVIOUS MONOCULTURE

If for one of the reasons previously invoked (cf. 5.2.1.8) the monoculture of *Azolla* cannot be undertaken, the rice paddy can be inoculated after the planting out of the rice seedlings by using *Azolla* taken from the nursery (fig. 15, p. 42). A basal application of nitrogen fertilizer is then desirable to provide for the needs of the rice at the beginning of its development.

In this case, the risks associated with intercropping preceded by monoculture, as described above (cf. 5.2.2.4), can be avoided.

5.3. ASSOCIATION OF AZOLLA WITH OTHER IRRIGATED CULTURES

It goes without saying that what has been said with regard to monoculture practices and rice-*Azolla* plant association, is equally applicable with some adjustments to other cultures like taro or lotus.

5.4. AZOLLA, GREEN MANURE IN NON IRRIGATED CULTURES

If the use of *Azolla* as green manure is generally envisageable for any such culture, it applies essentially to cultures that are located very close to the *Azolla* production site considering the problems of transporting the biomass.

One example of application in this domain is that of the fertilization of vegetable gardens situated near *Azolla* nurseries, marigots, fish ponds or any stretch of water which favours the growth of *Azolla* (fig. 7, p. 31). Contrary to what happens in the large scale farming, the vegetable gardens are characterized by a succession of different cultures spread over the whole year and therefore allowing the regular use of the excess biomass produced. This is worked into the soil during ploughing of the beds before the new culture is undertaken. *Azolla* can also be applied as mulch on the surface of the soil at the base of the plants.

Another interesting example is that of the cultures on ridges set up in low-lying marshes. *Azolla* can be produced on the surface of the channels and worked into the soil of the ridges.



Fig. 12. Inoculation of rice paddies by draining. Only the first paddy has been inoculated. As soon as it is saturated, the dyke separating it from the next paddy will be opened in order to inoculate it too and the process is repeated from paddy to paddy (Philippines).



Fig. 13. Incorporation of an *Azolla* monocrop with a power-driven cultivator (Sierra Leone).



Fig. 14. Incorporation of intercropped *Azolla* by foot-stamping after partial drainage of the rice field (Rwanda).

In the case of temporary overproduction, *Azolla* can be turned into compost. To this effect, it is advisable to mix *Azolla* with earth and to add superphosphate to reduce the loss of nitrogen. The compost consisting of a mixture of *Azolla* and rice straw apparently gives good results too.

5.5. AZOLLA AS FOOD

5.5.1. INTRODUCTION

If the exploitation of *Azolla* as green manure in rice culture has been dramatically developed in Asia from the late 1960's to the early 1980's, more and more peasants now grow *Azolla* with a view to using it as food for various breeding animals. This however is still largely

based on empirical findings : research on the subject has been lagging behind, compared to what is being done with regard to the fertilizing value of *Azolla*.

This paper will therefore include only a brief review of the ways in which *Azolla* presently is being used in animal nutrition; these ways are more often justified by the farmers experience than by experimental research, even though the latter is making remarkable progress.

5.5.2. VARIOUS FORMS OF FOOD PRESENTATION

Azolla can be fed to animals fresh or dried and if dried, just as it is or mixed with pellets, or eventually after ensilage. It should be remembered that all the necessary precautions must be taken against the presence of any toxic pesticide in the biomass used. Finally, an adaptation period can be anticipated, as is often the case with the introduction of a new kind of food into animal rations.

5.5.2.1. Fresh *Azolla*

This way of presenting *Azolla* neither demands a transformation process nor entails any denaturation of the compounds, but it does not allow storage and its very high water content renders its transportation over long distances very expensive; besides, it does not suit all animals. If such is the case, it is however easy to obtain a partial tending by spreading the *Azolla* over a drying area; a few hours will normally be enough to reduce the water content from 95 % to less than 50 %.

5.5.2.2. Dried *Azolla*

With the particularly high water content of *Azolla*, it is not economically advisable to envisage any dehydration process other than sun-drying. This however implies the availability of adequate drying areas near the harvesting site, appropriate climatic conditions and finally enough man-power. The operation requires an initial spreading of the biomass in a layer not more than 10 cm thick which is then turned over two or three times a day, usually for three to four days.

The drying, done in good conditions, only modifies to a small extent the nutritive value but it seems to have a negative effect on the organoleptic qualities, at least for some animals. It allows storage and transportation over long distances as well as the production of pellets.

5.5.2.3. Ensiled *Azolla*

Some Chinese breeders ensile the excess biomass from spring production and keep it to feed their animals, mostly pigs, during winter. The method described below seems to give good results but variations are certainly possible.

The *Azolla* to be kept in silos should be clean and slightly dry (water content around 60 %). They are stamped into silos made of cement or in plastic bags. When the layer reaches 30 cm, salt and maize flour are sprinkled on it at the rate of 5 g and 50 g respectively per kg of

Azolla before a new layer of *Azolla* is piled up and the process is repeated. The silo is then covered. The fermentation takes less than a month and its product can be kept for two years.

5.5.3. FOOD FOR FISHES

Azolla is consumed by many species of fish which often prefer it to other aquatic plants. This is the case of typical macrophytophagous species like *Tilapia rendalli* or *Ctenopharyngodon idellus*, but it also occurs with rather omnivorous fishes or planktonophages such as *Oreochromis niloticus* (= *Tilapia nilotica*) or *Cyprinus carpio*.

In the first case, *Azolla* directly contributes to a gain of weight of the fishes concerned. In the second, the fishes gain very little from eating *Azolla* but their waste enriches the medium and this allows the growth of organisms which are in turn consumed by the fishes.

On the other hand, with an *Azolla* carpet over a fish pond, the physicochemistry of the underlying medium is modified and it is essential to avoid complete covering of the water surface. In practice, the *Azolla* can easily be confined to one portion (± 50 %) of the pond with floating barriers made of, for example, bamboo (fig.16, p. 42).

It is moreover important to maintain an equilibrium between the population of fish and that of *Azolla* either by introducing, when necessary, a supplementary biomass of *Azolla* collected elsewhere, or by harvesting the excess biomass in order to keep the *Azolla* population in the linear growth phase. The excess *Azolla* can of course be put to good use in one way or the other.

Finally, it should be understood that the *Azolla* contribution to pisciculture, interesting though it may be, cannot alone ensure high productivities.

5.5.4. FOOD FOR PIGS

Azolla is now being used for pig breeding to a very large extent in China, from the south to the northern provinces of Manchuria, although the climatic conditions in the latter restrict the production of *Azolla* to about a hundred days per year.

Azolla is most often supplied in fresh condition at a daily rate of 1-4 kg depending on the age of the pigs and either as such or mixed with other food. The introduction of ensiled *Azolla* in the ration of pigs should be progressive until it reaches 1 to 1.5 kg per day. According to some estimates, 100 kg of fresh *Azolla* would allow the production of 1 kg of meat.

A nursery of 1000 m² with a daily output of 100-200 kg can therefore provide complementary food to a piggy of 30 to 60 pigs.

5.5.5. FOOD FOR CHICKENS

They consume either fresh or dried *Azolla*. According to some estimates, the feed supplied at the rate of about 100 to 300 g per day can be substituted for 20 % of the commercial feed ration.



Fig. 15. Inoculation of *Azolla* in an associated culture not preceded by monoculture (Senegal).

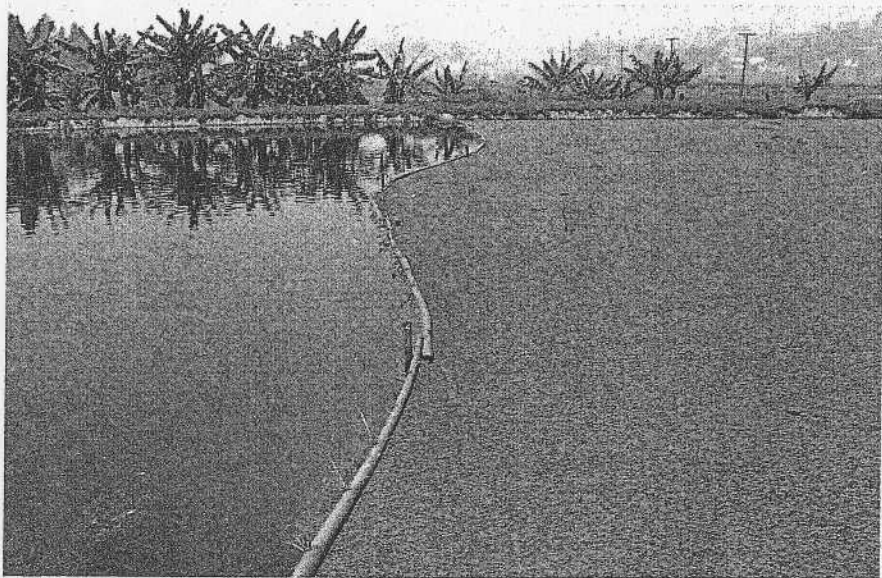


Fig. 16. Fish-pond. A barrier made of floating bamboo prevents the fern from proliferating all over the water surface (China).

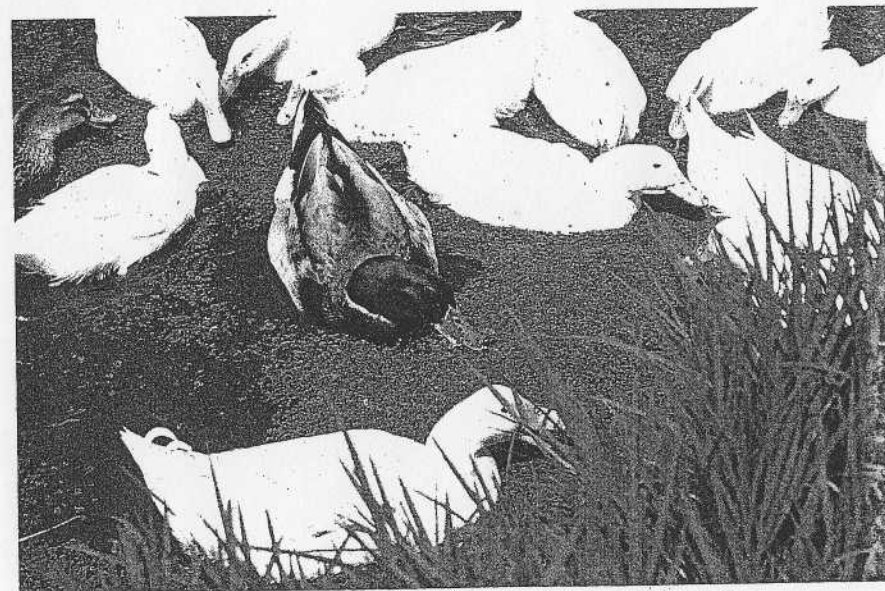


Fig. 17. Ducks with their daily ration of *Azolla* taken from a nearby pond (Ivory Coast).

5.5.6. FOOD FOR DUCKS

Ducks are known to like *Azolla* but we have very little information about the benefits they derive from feeding on it. When they are bred in enclosures, it does not seem possible to maintain an *Azolla* population in the pond allotted to them; the *Azolla* quickly disappears. The problem can be solved by cultivating *Azolla* on one side of the water, protected by a bamboo barrier or a mesh so that the excess biomass will flow into the part of the pond reserved for the ducks. In other cases, a regular supply of *Azolla* collected from a neighbouring pond is fed to the ducks (fig. 17, p. 43).

When the ducks are bred in semi-liberty, which is most frequently the case, it is good to encourage the growth of *Azolla* in nearby pools and flooded fields over which they roam.

5.5.7. FOOD FOR GEESE

Geese bred in pens receive a daily ration of 100 to 200 g of maize flour and 500 to 1000 g of fresh *Azolla*.

5.5.8. FOOD FOR RUMINANTS

Although data from the literature suggest the possibility of using *Azolla* in association with straw as cattle fodder, the ruminants do not seem to particularly appreciate *Azolla* and

besides, the usually available quantities would feed only a few individuals, not a herd. Complementary research on this subject would however be worthwhile.

5.5.9. FOOD FOR RABBITS

If rabbit breeding has not been widespread up to now in most tropical and subtropical regions, it is beginning to expand.

Rabbits provide good quality meat with a high efficiency, not to mention the by-products, such as skin and hair. Their growth cycle is relatively short and they are very prolific. With their small size and catholic food tastes, they arguably deserve being considered for family-size breeding enterprises without major investments.

Research on the use of *Azolla* as food for rabbits is very recent and has not yet been applied in the peasant farming environment. The preliminary results are however encouraging and lead us to believe that by selecting species on the basis of their nutritive value and organoleptic qualities, *Azolla* could be introduced into the food ration at a rate of 20 to 30 %. It probably will be better accepted as a constituent of food pellets, for its high water content when fresh does not suit the rabbit very well and its pulverulent condition in the dry state may offend the animal's very sensitive nose. One could also add *Azolla* to mashed food.

5.5.10. FOOD FOR MOLLUSCS

As already mentioned (cf. 2.8.2) several Gasteropods feed on *Azolla*. At least one species, *Pomacea canaliculata*, is edible and even prized as a delicacy by man. It was introduced for breeding purposes from America to some Asian regions a few years ago; unfortunately it escaped to the rice fields where it has become an occasional major nuisance as it eats rice too. One can, however, still be interested in this snail if only strict control measures are taken to prevent its dissemination into cultivated lands.

5.5.11. FOOD FOR MAN

There are several appealing recipes based on *Azolla* : soups, *Azolla*-plus-meat balls to be fried or boiled, spring rolls, etc.... These recipes are as yet unpublished; only a small group of researchers engaged in the study of *Azolla* have had the pleasure of serving these dishes to their guests.

Undoubtedly, this use could bring more value, provided precautions are taken to cultivate *Azolla* in satisfactory hygienic conditions. Plants in full growth and with less developed roots should always be chosen.

5.5.12. COMPLEMENTARY REMARKS

It follows from the above that *Azolla* could be exploited as a source of food. Complementary research is still needed to optimize and eventually diversify its use in this domain but this does not prevent an immediate exploitation of *Azolla*.

On the other hand, it may be useful to stress that *Azolla* can be produced without using elaborate technical means; many Chinese villagers, as soon as they can have a small pond of a few square meters, grow *Azolla* which they use to feed their poultry.

5.6. COMPLEX WAYS OF USING AZOLLA

5.6.1. INTRODUCTION

Different complex production systems using *Azolla* and integrating either arable farming and livestock or diversified animal husbandry have recently been developed. With a good technical knowledge, these production methods seem particularly efficient and moreover, some of them are already being widely used in China.

5.6.2. RICE-AZOLLA-FISH COMPLEX

A few years ago, the Academy of Agricultural Sciences of Fujian in the People's Republic of China developed a system based on a convenient layout of the fields, which will allow the simultaneous development of rice, *Azolla* and different fishes with complementary nutritional requirements.

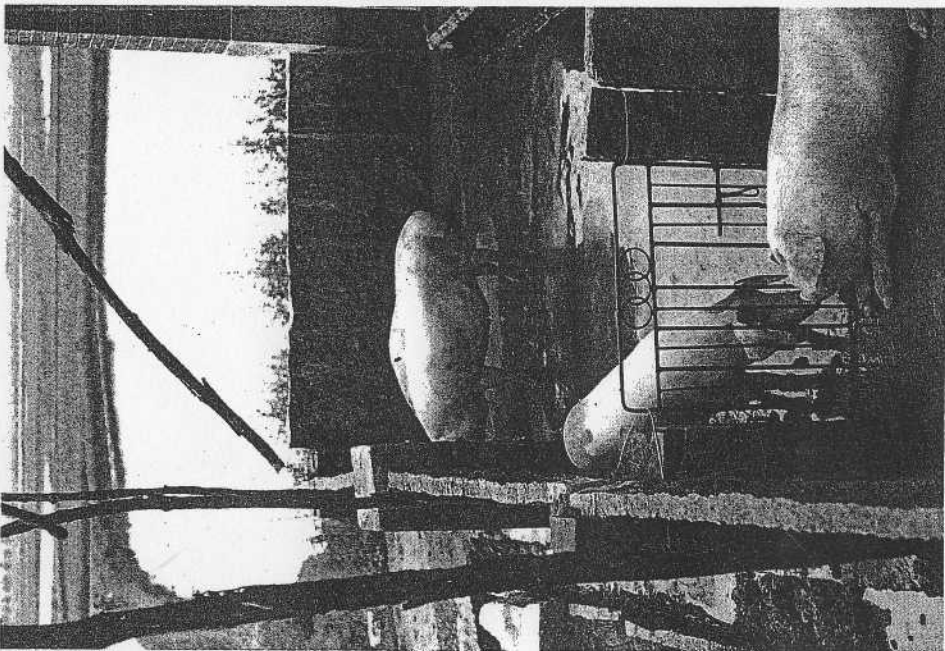
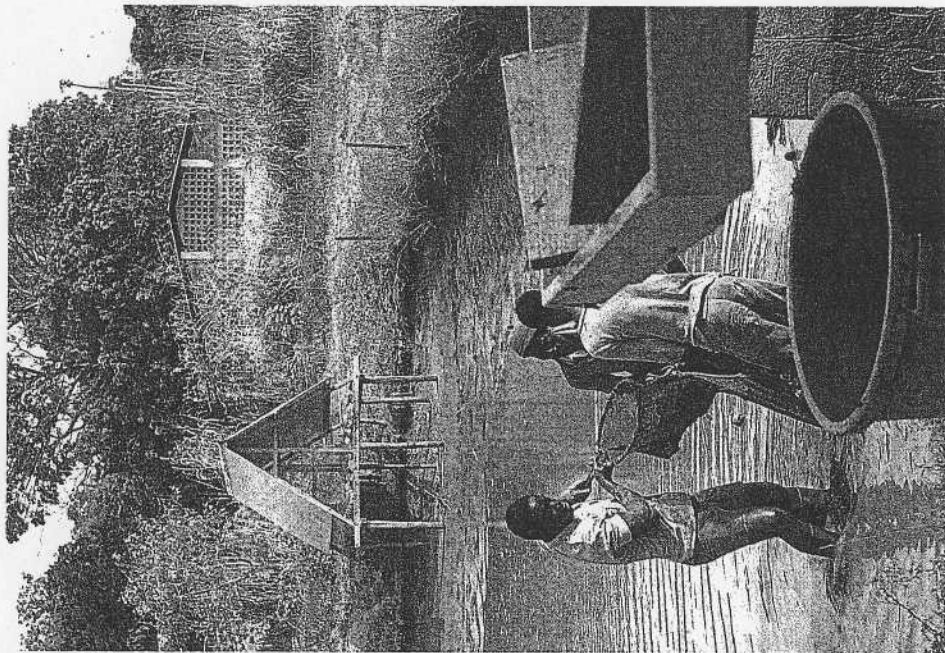
It is a remarkable instance of ecological agricultural layout in which each of the partners contributes to the equilibrium of the system. The fishes (a correct mixture of planktonophages, macrophytophages and polyphages) derive a benefit from *Azolla*, more or less, depending on the species; their wastes promote the proliferation of plankton which is consumed by some of the fishes, and also fertilize the rice. The polyphagous fishes protect the rice and *Azolla* from a number of insect and mollusc pests.

No complete description of the method is needed here, since the FAO has just published a detailed report on this subject : "The Rice-*Azolla*-Fish System", RAPA Bulletin, Vol. 4 (1988). A copy of this report can be obtained by writing to the following address :

*Regional Soil Management and Fertilizer Use Officer
FAO Regional Office for Asia and the Pacific
Maliwan Mansion, PHRA ATIT Road
Bangkok, Thailand.*

5.6.3. PIG-AZOLLA-FISH COMPLEX

This system combines a piggery with a fish pond partially covered with *Azolla* (fig. 18 p. 46). The waste produced in the piggery is drained off into the fish pond lower down. *Azolla* thus is continuously fertilized but the plankton also will proliferate and fishes both macrophytophagous and planktonophagous can multiply. In addition some *Azolla* is harvested daily and fed to the pigs, as described above (cf. 5.5.4).



5.6.4. POULTRY-AZOLLA-FISH COMPLEX

This system is similar to the previous one except that the piggery is replaced by poultry sheds (chicken, guinea fowl...) built on piles at the edge of the fish pond, with a latticed floor through which the excrements will fall into the pond (fig. 19, p. 46).

5.6.5. BIOGAS AND FERTILIZER PRODUCTION

The anaerobic fermentation of *Azolla*, or better of a mixture of *Azolla* and rice straw, in a biogas digester results in the production of methane which can be used for lighting or heating. The effluent from the digester, which contains all the mineral elements of the initial biomass (apart from a small proportion of nitrogen lost in the form of ammonia), will serve as fertilizer for the neighbouring cultures. Practical know-how in this field is still very scarce but positive results have recently been obtained in the Philippines.

Fig. 18. A breeding farm. The piggery overhangs the fish ponds, part of which (in the background) is covered by *Azolla*; each day, the excess *Azolla* is harvested and fed to the pigs, while the waste flowing out by the channel (visible on the left) enriches the waters of the ponds (China).

Fig. 19. The emptying of a pond in a mixed breeding of chickens and *Tilapia*. The chickens receive a daily ration of *Azolla* taken from a nearby nursery and the pond is enriched by the fowl's excrements.

6

CONCLUSION

Our knowledge of *Azolla* did greatly increase in the last few years, at the fundamental as well as at the applied research level. From the 1970's we particularly want to recall the following researches :

- the localization of the nitrogenase at the level of the heterocysts in *Anabaena*;
- the detailed description of the development cycle of *Azolla*;
- a better understanding of many aspects of the relationship between the partners of the symbiosis;
- the importance of the phosphorus requirements;
- the refined methods of estimation of *Azolla*'s contribution of nitrogen to the soil;
- the confirmation of the "herbicidal" effect of *Azolla*;
- the analysis of the chemical composition and nutritive value of *Azolla*;
- the study of *Azolla*'s decomposition kinetics in the soil;
- the identification of the principal ecological as well as socio-economic constraints, the in some cases limit the possibility of valorizing azolliculture.

During that decade also the first exotic species were introduced in China, particularly *A. filiculoides* (1977) which rapidly became widely distributed.

The 1980's were equally successful :

- systematic building of large reference collections, principally at the International Rice Research Institute (Philippines), the National *Azolla* Research Centre (China) and the Université Catholique de Louvain (Belgium);
- perfecting methods of selecting the most performant strains in given ecological situations (it may be worthwhile to stress the fact that exotic species and ecotypes often perform better than indigenous populations);
- obtaining the first *Azolla* hybrids and carrying out the first transfers of *Anabaena* from one *Azolla* species to the other, thus paving the way to more performant symbioses;
- progress in establishing the specific and subspecific characters of *Azolla* and *Anabaena* by the methods of classical taxonomy, chemotaxonomy, molecular genetics and monoclonal antibody production; a more thorough knowledge in this field is needed in order to build reliable reference collections and to enhance their value;

- demonstrating *Azolla*'s ability to accumulate phosphorus and potassium, as well as the relative insensitivity of nitrogenase in *Anabaena azollae* to nitrogenous compounds, and identifying the best ecotypes with regard to these performances;
- comparative study of *Azolla*'s nutritive value (chemical composition and appetency), which shows considerable differences depending on the environmental conditions but also on the species, ecotypes and population densities;
- starting to work on the production of biogas;
- attempt at characterizing the bacteria found in the leaf cavities;
- demonstrating the advantages of azolliculture on humid soils under certain conditions;
- diversification and improvement of the cultural practices that valorize *Azolla*, especially the development of production systems integrating livestock and arable farming, as such systems enable one to simultaneously benefit from the fertilizing contributions and food qualities of *Azolla*;
- development of research programmes aimed at valorizing *Azolla* outside its traditional areas of cultivation, e.g. in the Philippines and in various African countries.

The 1980 's were also characterized by a remarkably improved international collaboration : organization of conferences, training courses and regional or international coordinated research programmes, exchange of research workers and of *Azolla* ecotypes between the main research centres; publication by IRRI of "*Azolla* Newsletter", a contact periodical for research workers.

From this accumulated knowledge it is obvious that the peasants of many subtropical and tropical regions could take advantage of the numerous uses of *Azolla*, provided they are supplied with the appropriate strains and taught the cultural practices adapted to their local conditions. For this purpose it should be intervened at different levels, as outlined below.

6.1. THE ROLE OF INTERNATIONAL RESEARCH CENTRES

More research is needed in order to optimize azolliculture and one can be expected to rely for this on international research centres or universities. Besides, these centres have a role to play in the transmission of accumulated research experiences by publishing scientific papers and popularizing literature, and by organizing scientific meetings and training courses. However, they obviously cannot take up the management of actions that would be started at the national or local level.

6.2. THE ROLE OF NATIONAL RESEARCH CENTRES

As is always the case with agriculture, the development of new practices in a given region must be refined and adapted to local conditions. In the case of *Azolla*, instead of repeating experiments to show its value as fertilizer or food (the accumulated data in this field are

convincing enough), the agronomical research stations of the interested countries should work hard on the selection of the most performant ecotypes and the most efficient ways of using *Azolla* considering the cultural calendar, the availability and control of water, the available manpower and the types of culture and breeding practiced. Research on the needs and methods of fertilization, on the equipment to be used or on the pests will often be necessary as well.

In several countries, among which African ones, the knowledge acquired in this domain is, moreover, already sufficient to justify trials in peasant farming environments and even to planify extensions.

6.3. EXTENSION PROGRAMMES

Experience shows that a supervised training is essential; the form to be taken will depend on the existing structures (national propaganda networks, cooperatives, villages, non governmental organizations...). In any case, it seems necessary at first to have a staff especially and solely employed for the development of azolliculture and having received the necessary preliminary training. This staff will be in charge of the setting up and care of maintenance nurseries, and of the distribution of *Azolla* to the interested peasants (for psychological reasons it might be advisable to demand payment, even if costs are not covered, before supplying the *Azolla* inoculums). The staff will also watch the way in which the peasants use *Azolla* and they will be available to give them advice.

6.4. THE MANAGEMENT OF AZOLLICULTURE BY THE PEASANTS

Wherever *Azolla* has been introduced with positive results, it should be possible for the supervision to be gradually dispensed with. In environments where *Azolla* can survive without any difficulty all year round, each peasant should be able to assure his own production. But if the conservation of *Azolla* becomes problematic at certain times of the year, it will be advisable to entrust some specialized farmers with the management of maintenance nurseries.

6.5. THE INTERVENTION OF POLITICAL LEADERS

With reference to the countries where *Azolla* is at the moment effectively used on relatively large scale (China, Vietnam, The Philippines), it will be noticed that the development of azolliculture in each case was promoted by public authority in a very straightforward manner. That such supports would be useful (to say the least) in the initial stages is easily understandable : the farmers do not perceive immediately the benefits to be reaped from such unusual cultural practices which moreover cannot be mastered without some sophisticated initiation.

One has therefore to acknowledge that the public authorities (or possibly some non-governmental organizations) must agree to an initial investment if they want to develop azolliculture. But it may be pointed out here that most African governments subsidize the purchase of nitrogen fertilizers : now these are recurrent expenses from which, on the other hand, the small farmer away from the distribution centres is not very likely to benefit. With the development of azolliculture no foreign exchange is needed, the initial expenses do not recur and the disadvantaged peoples will benefit from it.

Spending will be on a minor scale anyway, especially if such a strategy is started and tested in some limited areas where ecological conditions, water availability, and mind openness from the peasants' side seem favourable. But success also depends on a well-trained staff of supervisors, willing to work in the field and enjoying an adequate scientific and technical support. The creation of regional networks undoubtedly would be useful; in this case it would facilitate concerted actions and promote the exchange of information between research workers and agronomists, to their mutual benefit.

ANNEXES

1. BIBLIOGRAPHY

About 1500 publications treating of *Azolla* have been published since 1980 and even a selected list of references would exceed the scope of this paper.

The reader looking for complementary information would do well to consult the excellent monography "*Azolla* as a green manure : Use and management in crop production" by T.A. Lumpkin and D.L. Plucknett, Westview Tropical Agricultural Series, N° 85, Westview Press, Boulder, Colorado, 280 pp (1982).

Another monography, in Chinese, will be available soon : "*Azolla* in China" by C.C. Liu and W.W. Zheng. Agriculture Publishing House, Beijing, China (1989); an English language version is being considered.

The International Rice Research Institute has moreover edited "The International Bibliography on *Azolla*" (1979) and "The International Bibliography on *Azolla*, 1983 Supplement" (1984).

Finally, the reader in need of any clarification or precision about the subjects treated in this paper may consult the author, whose library contains most of the publications on *Azolla*.

2. THE SUPPLY OF AZOLLA STRAINS

Researchers wanting *Azolla* strains can contact the author, indicating the ecological characteristics of the region in which they hope to introduce *Azolla* as well as the use to which *Azolla* is to be put.

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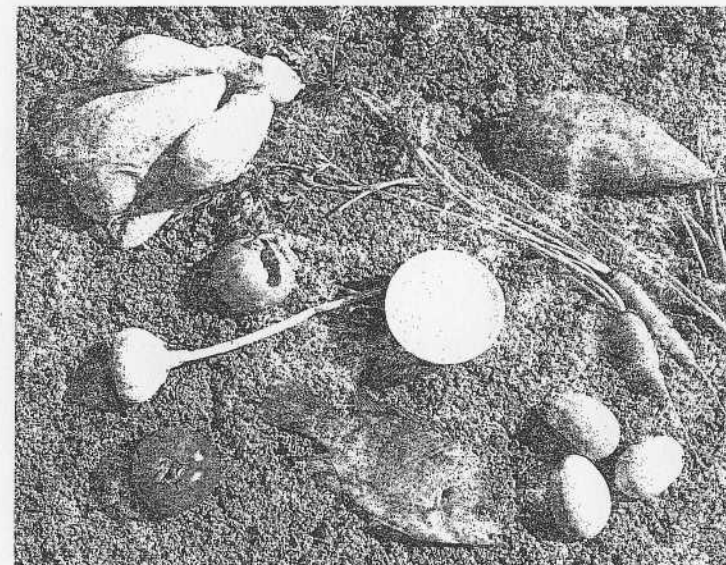
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- comment carbon est un PA et + pour montrer ΔG ?
- comment ΔG est exode pour AgCl , I_2 (Eubromine (marque)).
- comment hypofluorure en solut.
- dimorphisme entre A Ti = orange, facile pour Ti - et A carb -
- comment contrôler adéquatement un ou plusieurs paramètres
- faut-il pour changer P ou V et R et R -
- Nombres = 5 major à par variation de l'ordre mixte.
- Nombres: 5 pour?
- comment de votre suite

36400
Azolla

Azolla

**AND ITS MULTIPLE USES
WITH EMPHASIS ON AFRICA**



Food and Agriculture Organization
of the United Nations

The aquatic fern *Azolla* as feed for fish

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Abstract

When fed with commercial pellets at a daily rate of 2.5% of its body weight, *Tilapia (Oreochromis) nilotica* grows faster than *T. rendalli*, but has no significant influence on *T. nilotica*. The chemical composition of the two fish species is very similar and does not seem affected by the three diets tested.

KEYWORDS: Fish nutrition, Growth, Biochemical composition, Aquatic fern.

Introduction

Azolla, an aquatic floating fern, is known to be consumed by various fish species. Its introduction in semi-intensive agropiscicultural systems, for improving their nitrogen status, has been suggested (Barbier et al., 1985; Micha, 1985; Vincke and Micha, 1985).

Based on previous results bearing on selective appetency of *Tilapia (Oreochromis) nilotica* for various *Azolla* species (Antoine et al., 1986, 1987; Micha et al., 1987; Wery et al., 1987), *A. microphylla* has been used in this experiment which compares biological parameters of two African Cichlidae, *T. (O.) nilotica* and *T. rendalli* fed with three different diets (100% pellets; 50% pellets + 50% pellets). Finally, biochemical composition of fish fed with these contrasted regimes is compared.

Materials and methods

Azolla production

A. microphylla (strain ADUL-69-MI), cultivated on a modified N-free Hoagland solution, were maintained at the linear phase of their population growth curve (Van Hove et al., 1985): fresh samples were collected daily to feed the fish. The

fern's dry weight represents 5.1% of its fresh weight.

Fish rearing

Ten fish, all females (20g ± 2g), individually marked were reared in 100 l (length: 93.5cm; width: 28.8cm; height: 35.0cm) aquariums in which the water (26°C ± 0.5°C) is completely renewed daily. Two aquariums were used for each treatment.

Diets

Three diets were tested:

- 100% Trouvit K30-1 crushed pellets;
- 50% Trouvit K30-1 crushed pellets, 50% (on dry weight basis) fresh minced *Azolla*;
- 50% Trouvit K30-1 crushed pellets.

Ration

The following rations were used:

- 5% of fish weight as Trouvit K30-1 crushed pellets, 6 days a week;

Table I. Chemical composition of diets as percentage of fresh or (dry) matter

Components	Azolla % in the diet			
	0%		50%	
Crude proteids	30.1	(34.3)	27	(28.1)
Crude lipids	6.3	(7.2)	0.5	(5.0)
Crude fibers	3.5	(4.0)	0.9	(9.8)
Ash	8.5	(9.7)	1.5	(15.7)
N-free extract	39.3	(44.8)	40	(41.4)
Moisture	12.3	(0.0)	90.4	(0.0)

- 2.5% of fish weight as Trouvit + 2.5% fresh Azolla (on dry weight basis);

- 2.5% of fish weight as Trouvit.

Azolla was presented in the morning, pellets 4 times a day, in equal parts.

Chemical analyses

Azolla and fish were frozen and dried before analysis; chemical composition of commercial pellets, Azolla and fish were calculated on a dry weight basis as obtained by desiccation for 24h at 105°C in a ventilated oven. The following parameters were determined:

- Moisture: weight after 24h at 105°C in a ventilated oven;
- Total nitrogen: according to Kjeldahl;
- Ash content: weight after 12h at 450°C;
- Crude lipids: cold extraction with CCl₄ for 48h;
- Crude fibers: according to Kurschner;
- N-free extracts: by calculation.

Results

Chemical composition of the diets

For a same quantity of food (dry matter), Table I shows clearly that the Azolla-containing diet had high fiber and ash dry contents and was poor in proteids and lipids. Its quality was, however, still negatively affected by its very high water content.

Growth

Statistical analysis showed no significant aquarium effect, the data presented are mean values for the two aquariums and for each treatment.

Fig. 1 shows the growth curves of *T. (O.) nilotica* as affected by diets. The 100% pellets treatment with or without Azolla give approximately the same bad results: the fish reached respectively 56 and 48g after 10 weeks. Fig. 2 shows the growth curves of *T. rendalli* as affected by diets. The 100% pellets treatment gave the best result (the fish reached 66g after 10 weeks). Replacing 50% pellets by Azolla decreased the growth but the final score was still acceptable (62g after 10 weeks) and certainly better than the 50% pellets treatment (43g after 10 weeks).

Body composition

It appears from Table II, that incorporating 50% Azolla in the diet has no effect on body water-content ($\pm 75\%$) for both species. Crude lipids did not seem affected by the including Azolla in the diet but the lipids content was a little lower for *T. rendalli* than for *T. (O.) nilotica*. Crude proteid occurred in almost the same concentrations in both species.

Discussion

From previous results (Antoine et al., 1987; Micha et al., 1987) on smaller fish (5g) fed at a low level (3.6% of body weight) it appeared that incorporating Azolla in the diet tended to decrease the growth and the body quality of *T. nilotica* and *T. rendalli*; *T. rendalli* was, however, less affected than *T. nilotica*.

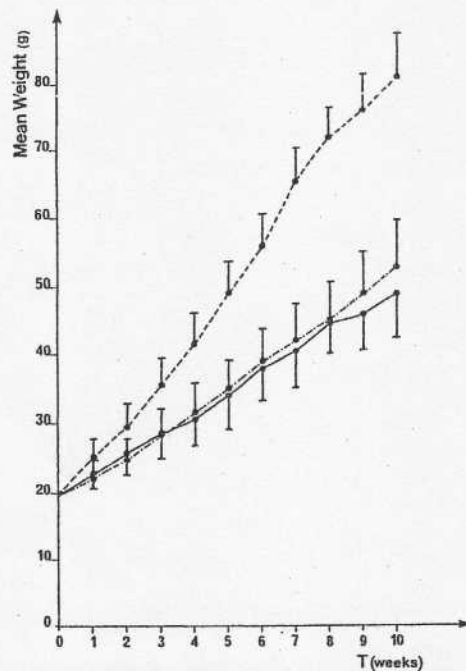


Fig. 1. Compared growth curves of *T. (O.) nilotica* fed with three different diets (feeding intensity level: 5%). (---) 100% pellets (Trouvit K30-1); (-.-.-) 50%; 50% *Azolla microphylla* (dry matter); (—) 50% pellets (Trouvit K30-1); (L) standard deviation.

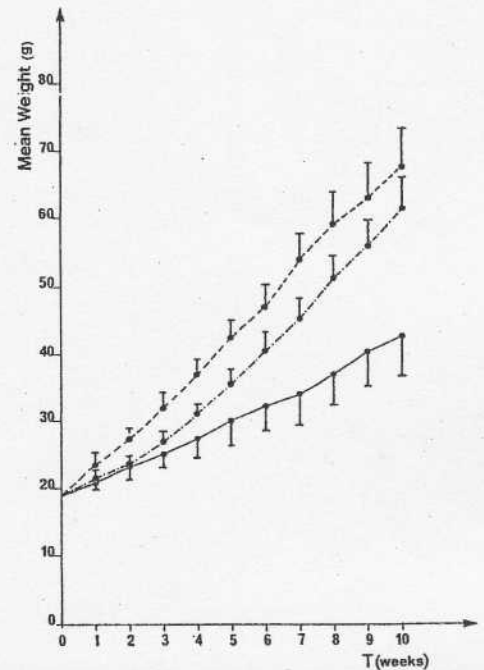


Fig. 2. Compared growth curves of *T. rendalli* fed with three different diets (feeding intensity level: 5%). (---) 100% pellets (Trouvit K30-1); (-.-.-) 50% pellets, 50% *Azolla microphylla* (dry matter); (—) 50% pellets (Trouvit K30-1); (L) standard deviation.

The present results with larger fish (20g) fed at a higher level (5% of body weight) show that a diet including 50% *Azolla* compared to a 100% pellet diet tends to decrease the growth but not the body quality of both species and confirm previous results. The data presented above show, however, that *T. rendalli* is not only less affected than *T. (O.) nilotica* by *Azolla* incorporation in the diet but also that the growth rate of *T. rendalli* is markedly increased when the *Azolla* ration is added in equal part to a pellets maintenance ration.

From these experiments, carried out under controlled conditions, it appears that the direct effect of *Azolla*, when mixed with pellets, has a valuable direct effect on the growth of the macrophytophagous species and not on the microplanktonophagous one. That is the reason

why it seems more advantageous to rear both species (*T. nilotica* and *T. rendalli*) on a diet including *Azolla* in tropical ponds and in agro-piscicultural systems. In this way, *Azolla* will contribute directly to the improvement of the fish production and indirectly to the quality of the environment. Indeed, through decomposition of the faeces nitrogen among others is released in the water.

Conclusion

These results show that *T. rendalli*, a herbivorous species, is able to make direct profit of *Azolla microphylla*, whereas *T. (O.) nilotica*, a planktonophagous species, is not. Consumption of *Azolla microphylla* by the latter species will nevertheless indirectly increase the productivity

Table II. Body-composition comparison of two Cichlidae fed three different diets

Fish species	Diet	Moisture (%)	Crude lipids (%) dry matter	Crude proteids (%) dry matter	Ash (%) dry matter
<i>T. (O.) nilotica</i>	100% pellet	75.0	14.2	64.6	20.5
	Pellets (50%) + <i>Azolla</i> (50%)	76.1	14.4	66.0	19.5
	50% pellets	75.3	13.3	64.7	20.0
<i>T. rendalli</i>	100% pellets	75.2	12.2	65.3	21.5
	Pellets 50% + <i>Azolla</i> (50 %)	76.0	11.0	67.4	20.3
	50% pellets	75.2	12.2	65.9	21.5

in agro-piscicultural systems, by improving their organic matter, especially the nitrogenous one.

Acknowledgements

We thank Prof. M. Vanbelle, Prof. W. Vervack, and B. Toussaint for their help with the biochemical analyses. Prof. P. Andre and P. Lhoir have permitted a complementary *Azolla* production in their laboratory. U. Falter and L. de Backer (Prof. G. Thines) cooperated to produce fingerlings to verify the health of the fish, and provided help with the experiments.

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EVALUACION DE LA DIGESTIBILIDAD APARENTE DE LA AZOLLA *Azolla filiculoides* COMO INGREDIENTE PRINCIPAL EN LA FORMULACION DE DIETAS EN ALEVINOS DE CACHAMA BLANCA *Piaractus brachypomus*

ANA ISABEL SANABRIA OCHOA*

RESUMEN

El ensayo se realizó en la estación piscícola La Terraza del Instituto Nacional de Pesca y Acuicultura (INPA) en Villavicencio Meta.

El propósito de esta investigación fue estimar la digestibilidad aparente de la *Azolla filiculoides* en alevinos de cachama blanca para tener un indicativo de la disponibilidad de nutrientes de esta materia prima susceptible de ser utilizada como fuente alternativa de proteína en la formulación de dietas para el levante de alevinos de *Piaractus brachypomus*.

La digestibilidad aparente se estimó indirectamente usando óxido de cromo como marcador externo. Como dieta de referencia se utilizó una formulación elaborada en los laboratorios de PURINA S.A. de alta calidad nutricional, con un tenor protéico del 25% y 1% de óxido de cromo; las dietas experimentales se elaboraron sustituyendo el 20, 30 y 40% de la dieta de referencia por la materia prima a probar (*Azolla filiculoides*).

Los resultados obtenidos se sometieron a un análisis irrestricto al azar para determinar cuál de las dietas fue la mejor y al no encontrar diferencias significativas ($p < 0.005$) entre éstas se realizó la prueba de Kruskal-Wallis con un nivel de significancia de ($p < 0.005$) para corroborar los resultados.

Los diferentes porcentajes de inclusión de la Azolla no presentaron diferencias significativas entre los coeficientes de digestibilidad de los principios nutritivos de la materia prima probada, lo que indica en términos generales que el helecho acuático *Azolla filiculoides* puede ser utilizado en la formulación de alimentos para las especies del género *Colossoma*.

ABSTRACT

This research was realized in the station of ictyological studies "La Terraza" of the Instituto Nacional de Pesca y Acuicultura (INPA) in Villavicencio, department of Meta, Colombia.

The purpose of this investigation was to measure apparent digestibility of *Azolla filiculoides* in the spawn of the cachama blanca *Piaractus brachypomus* in order to obtain an indication of its use as a primary food source, with specific emphasis on its ability to provide alternative sources of protein for growth and sustenance.

The apparent digestibility is estimated directly by using chromium oxide as an external marker. As a base diet, a food source from the laboratories of PURINA S.A. was selected without concern for cost. It chosen for its high nutritional quality, with a content of 25% protein and 1% chromium oxide. The experimental diets were varied by substituting 20, 30, and 40% of the diets with the test material (*Azolla filiculoides*).

The results obtained were analyzed using ANOVA to determine which of the diets, and no significant difference was encountered ($p < 0.005$) between them. This was done using the Kruskal-Wallis process with a level of significance of

0.995 to secure the results.

The different percentages of *Azolla filiculoides* included did not present significant differences ($p < 0.005$) between the coefficients of digestibility of the primary nutrients of the base food source this indicates in general terms that aquatic ferns like *Azolla filiculoides* can be used in the formulation of food sources for fish of the genus *Colossoma*.

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INTRODUCCION

En América latina la acuicultura ha sido identificada como una alternativa eficiente que contribuye al desarrollo rural principalmente en aquellas zonas promisorias para el cultivo de especies hidrobiológicas (Hernandez, 1989).

En Colombia la piscicultura se ha convertido en una rentable alternativa de producción pecuaria al ser integrada a las actividades económicas propias de cada región (Vásquez, 1988).

Sin embargo la industria piscícola en la actualidad tiene una serie de obstáculos que limitan su desarrollo entre los cuales se destaca la nutrición y alimentación de los peces, pues éste insumo es el de mayor importancia para la obtención de buenos niveles de producción, por lo tanto, el desarrollo y rentabilidad de un cultivo intensivo de peces depende entre otros aspectos de la obtención de dietas que satisfagan los requerimientos de nutrientes esenciales (proteínas, lípidos, carbohidratos, etc.) y energía y sean suministrados en cantidades adecuadas para asegurar un rendimiento óptimo. Según Lovell, (1977), es importante en piscicultura contar con una dieta balanceada y un adecuado manejo alimenticio, a fin de conseguir la producción máxima de proteína muscular (crecimiento) de las especies cultivables como la cachama blanca. Para conseguir esto es necesario alimentar los animales con dietas que contengan niveles relativamente altos de Proteína (22-55%), y de alta calidad (Walton, 1987).

En acuicultura tradicionalmente se ha utilizado la harina de pescado como principal fuente protéica en alimentos comerciales para peces, pero hoy día dada su escasez y alto precio se ha venido sustituyendo por otras fuentes protéicas; además los altos requerimientos protéicos de los peces unido a que la proteína sea proporcionalmente el nutriente más costoso de la dieta, hace necesario la obtención y valoración de fuentes protéicas alternativas disponibles en la región que disminuyan los costos de producción y aseguren altos rendimientos en la producción (De la Higuera, 1987).

Es así como la primera tarea que se debe efectuar al valorar el potencial nutritivo de cualquier materia prima que se considere apta para ser incluida

en una dieta es medir su digestibilidad, entendiéndose como digestibilidad la diferencia que existe entre la cantidad de alimento ingerido y la cantidad de alimento que aparece en las heces. Este termino implica dos procesos digestión y absorción (Crampton, 1979).

El conocimiento de la digestibilidad de diferentes materias primas es esencial en la formulación de dietas prácticas para peces; al estimar la digestibilidad se tiene un indicativo de la disponibilidad de nutrientes presentes en las materias primas evaluadas, permitiendo así sustituirlas en las dietas. Estas sustituciones son importantes para la obtención de dietas de mínimo costo dependiendo del precio de mercadeo, la disponibilidad y la composición de dicha materia prima (Cho, 1983; Cantelmo 1989).

AZOLLA (*Azolla filiculoides*)

La Azolla es un género de pequeños helechos acuáticos nativos de Asia, África y América. Son plantas pequeñas que crecen en lagunas, canales, campos inundados y otros cuerpos de agua dulce.

La característica más sobresaliente de éste helecho es su relación simbiótica con el alga verde-azul *Anabaena azollae*. El helecho suministra nutrimentos y una cavidad foliar protectora para el alga, la que a su vez suministra nitrógeno al helecho.

En Colombia ésta planta presenta una amplia distribución geográfica que va desde los 3700 m.s.n.m. en el páramo de Sumapaz (reportado por Cleef) hasta los 50 m.s.n.m. en el departamento de Córdoba (reportado por Idrobo), pasando por los 175-200 m.s.n.m. en los Llanos Orientales (reportado por Jaramillo).

COMPOSICIÓN DE LA AZOLLA

Aunque la relación peso seco/peso fresco sea delicada de medir debido a la dificultad de estandarizar los métodos de eliminación del agua adherida a la superficie de las plantas, numerosos datos de literatura están de acuerdo en situar el porcentaje de materia seca entre 5-7%, las cenizas representan el 10-24% del peso seco, la materia grasa representa entre el 3-6% de la materia seca, el almidón el 6%. Los componentes parietales en base seca citados para la celulosa varían entre 5.6-15.2%, para la hemicelulosa 9.89-17.9%, para la lignina entre el 3-6% de la materia seca. El contenido de proteína se ha evaluado entre el 13-30% (Van Hove & López 1983).

En 1991 en el departamento de nutrición animal de la Universidad Nacional de Colombia se hizo un análisis bromatológico de la Azolla encontrando los siguientes resultados:

Azolla en % de M.S.	
PROTEINA	27.66
LIBRA CRUDA	10.31
CENIZA	14.13
GRASA	4.63
HUMEDAD	96.00

CALIDAD DE LA PROTEINA DE LA AZOLLA

El termino calidad protéica se refiere a la variedad y proporción de los aminoácidos, cuanto más completa sea su gama y más cerca las proporciones de las necesidades fisiológicas de una especie, más alta será la calidad de la proteína (Lloyd, 1982). En general se acepta que cuanto más se aproxime la composición de aminoácidos indispensables de una proteína a los requerimientos de aminoácidos indispensables al animal, con tanto mayor eficacia es utilizada por éste, pues el organismo no es capaz de sintetizar algunos aminoácidos que están presentes en sus proteínas por lo tanto la proteína dietaria debe ser de una naturaleza tal que pueda proveerlos (ver tabla 1).

TABLA 1. Comparación de una proteína óptima con proteína de la Azolla.

Como (%) de	Proteína	Azolla
Ucina	Óptima	
Met + Cys	63	63
Treonina	72	72
Triptófano	18	31
Valina	75	103
Isoleucina	60	83
Leucina	114	138
Fenil + Tir	120	149

FUENTE: Van Hove y López, 1983

Utilización en alimentación animal

Como forraje para porcinos es utilizada ampliamente en la China donde se le considera semejante por su calidad a la batata, con efectos favorables sobre el crecimiento y características de la carne de los animales. Comparada con otros forrajes se estima que la alimentación con Azolla puede ahorrar cerca del 40% de los costos de producción en la alimentación porcina.

En Colombia se ha utilizado la Azolla para la alimentación de vacas lecheras en el Valle del Cauca, mediante el suministro de 40 Kg de king-grass, 3Kg de harina de sorgo, 2 Kg de melaza y 7 Kg de Azolla que reemplazaron 4 Kg de alimento completo comercial. Las vacas que recibieron la Azolla presentaron una ligera tendencia a aumentar la producción.

Se han realizado experimentos con pollos de engorde, gallinas ponedoras, patos y peces, con resultados que muestran las grandes posibilidades de la Azolla en la alimentación animal utilizándola como fuente de proteína de bajo costo.

El *Azolla* es bien aceptada por muchas especies de peces herbívoros como la carpa herbívora, la carpa israelí, la tilapia mosambica, quienes prefieren la *Azolla* como fuente de alimento sobre la mayoría de las especies de plantas acuáticas (Lumpkin & Plucknet, 1982). En algunos ensayos se ha mostrado que la *Tilapia nilotica* puede consumir del 50-80% de su peso en *Azolla* por día con un porcentaje de digestión cercano al 60% (Becerra, 1991).

MATERIALES Y METODOS

La investigación se realizó en la estación piscícola "La Terraza" del Instituto Nacional de Pesca y Acuicultura (INPA) en la ciudad de Villavicencio.

El sistema experimental propuesto para el desarrollo de esta investigación fue diseñado por Cortez y Quintero (1991), tomando como base el sistema Guelph-Cyag-2 desarrollado por Cho (1975-1982). Este sistema consta de 24 acuarios con una capacidad de 60 l cada uno, están provistos de un sistema de aireación que garantiza cantidades deseables y permanentes de oxígeno, un suministro de agua que puede ser ajustado para asegurar la velocidad de circulación y piso inclinado para minimizar la sedimentación y maximizar el movimiento del material fecal, que drena a través de un tubo de desagüe que conduce el agua y las heces a una columna de sedimentación común para cada dos acuarios, en la que por decantación el material fecal llega al fondo, lugar donde se halla un dispositivo para la recolección de las muestras.

Los animales experimentales empleados fueron seleccionados de los producidos en la estación buscando una uniformidad en la talla y con pesos promedios entre los 15-20 g. Se sometieron a un tratamiento profiláctico, posteriormente se pesaron y colocaron en los acuarios con una densidad en biomasa de 1500 g y se sometieron a un período de adaptación tanto al sistema como al alimento de 3 días.

Los peces experimentales fueron alimentados con 4 dietas una vez por día a razón del 1% del peso vivo. El alimento se suministró inmediatamente después de la recolección del material fecal evitando así la contaminación de las heces con partículas alimenticias.

Como dieta de referencia se utilizó una formulación elaborada en los laboratorios de Purina S.A. sin limitaciones de costo y de alta calidad nutricional con un tenor protéico de 25% y 1% de óxido de cromo como indicador externo.

Las dietas experimentales se elaboraron sustituyendo el 20,30 y 40% de la dieta de referencia por la materia prima a probar (*Azolla filiculoides*). Tanto la dieta de referencia como las dietas experimentales fueron elaboradas en el

laboratorio de nutrición de la estación piscícola La Terraza.

La recolección de las heces se realizó tomando el material fecal del dispositivo del tubo sedimentador, luego se centrifugaron a 10000 revoluciones por gramo de material recogido durante 20 minutos, el sobrenadante se descartó y la muestra se almacenó a 5°C hasta terminar el ensayo; luego se secaron a 60°C durante 24 horas aproximadamente hasta que la muestra alcanzó un peso constante; se pulverizaron y congelaron para las posteriores determinaciones.

Los análisis de laboratorio se realizaron en las instalaciones de Purina en Mosquera (Cundinamarca) según el esquema de Wendee y siguiendo la metodología de Bateman (1970).

Los coeficientes de digestibilidad aparente para un nutriente dado o materia seca se calculan según la siguiente fórmula:

$$\text{CDAs} = \frac{\%Cr, O_2 \text{ en dieta} - \% \text{ nutriente en heces}}{\%Cr, O_2 \text{ en heces} - \% \text{ nutriente en dieta}} \times 100$$

Los resultados obtenidos en los tres ensayos se sometieron a un análisis irrestricto al azar para determinar cuál de las dietas ofreció mejores resultados, con un nivel de significancia de $p < 0.005$, al no encontrar diferencias entre las dietas se realizó una prueba de Kruskal-wallis para corroborar estos resultados con un nivel de significancia de $p < 0.005$.

RESULTADOS Y DISCUSION

-Sistema experimental: El sistema experimental utilizado para desarrollar el presente ensayo funcionó como se esperaba, permitiendo superar algunos de los inconvenientes que con frecuencia se han presentado en este tipo de estudios como son: la recolección de una cantidad representativa de heces, uso de peces en crecimiento y/o de cualquier talla, escasa manipulación de los animales y el uso de marcadores inertes como el óxido de cromo para determinar por un método indirecto el coeficiente de digestibilidad aparente de los principios nutritivos de la materia prima utilizada.

-Animales experimentales: Los animales experimentales fueron seleccionados buscando un peso y talla uniforme disminuyendo así las interacciones sociales como: jerarquía social, alto nerviosismo y aumento en la agresividad que afecta su comportamiento alimenticio que comúnmente se presenta en peces que en su medio natural presentan un comportamiento de cardumen como la cachama blanca.

Posteriormente los animales se sometieron a un tratamiento profiláctico para

prevenir posibles enfermedades de tipo bacteriano y/o micótico causado por la manipulación de los peces.

El experimento se inició con la fase de adaptación al sistema y al alimento, la cuál duro tres días como lo recomienda Cho (1979). Al cavo de esta fase los peces se mostraron más dóciles al momento de suministrar el alimento, lavar el equipo y tomar la muestra.

- Alimento: La formulación de la dieta de referencia se hizo con base en la determinación del contenido de aminoácidos presentes en el músculo dorsal de la cachama blanca hecho por Ramírez (1991), el cual permitió establecer los requerimientos protéicos de esta especie ofreciéndole un alimento adecuadamente balanceado y de buena calidad, como se observa en la tabla 2.

TABLA 2. Aminograma comparativo de la Cachama blanca y la Azolla.

AA	CACHAMA BLANCA "AZOLLA"	
	valores en % g/100g de proteína	
TREONINA	4.5	4.70
VALINA	5.1	5.75
METIONINA	0.8	1.88
ISOLEUCINA	4.8	5.28
FENILALANINA	4.2	5.64
LISINA	7.8	6.45
HISTIDINA	3.5	2.31
TRIPTOFANO	—	2.01
Ac. ASPARTICO	9.7	9.39
Ac. GLUTAMICO	15.6	12.72
SERINA	3.6	4.10
PROLINA	6.0	4.46
GLICINA	7.0	5.72
ALANINA	6.5	6.45
CISTEINA	0.6	2.26
TIROCINA	3.5	4.10
LEUCINA	10.6	9.05
ARGININA	5.4	6.62

FUENTE: * Ramírez, 1992.

** Buckingham et al., 1975

Sobre la base de la dieta de referencia se elaboraron las dietas experimentales que solamente difieren de la primera por la inclusión de la materia prima a evaluar (*Azolla filiculoides*). Una norma importante que se tuvo en cuenta en todo el conjunto de las dietas experimentales fue la calidad de los ingredientes que no vario durante todo el experimento; para conseguir y evitar variaciones en la composición y disponibilidad de los nutrientes de las dietas, estas fueron preparadas en cantidades suficientes para el experimento completo.

Se observó una buena aceptación del alimento por parte del animal tanto en la superficie como en el fondo del acuario aun que el alimento con el 40% de inclusión de Azolla no fue consumido tan vorazmente.

-Hece: La recolección del material fecal se realizó una vez por día tratando

de disminuir el tiempo de permanencia del pellet fecal en el fondo del tubo sedimentador para evitar la pérdida de material por filtración.

-Control del peso: Los animales experimentales fueron alimentados con una ración diaria del 1% del peso vivo para mantener su metabolismo basal. Se llevo un registro al inicio y al final de cada ensayo observándose una ligera ganancia de peso, la cual se presenta como manifestación de un adecuado balance entre el factor protéico y energético de la dieta, utilizando la energía para su metabolismo basal y actividad voluntaria asegurando así la utilización de la proteína para el crecimiento.

-Calidad del agua: En piscicultura se define la calidad del agua como la disponibilidad y adecuabilidad del agua para la sobrevivencia y crecimiento de los peces y ésta determinada principalmente por la temperatura, oxígeno, pH, etc.

Se ha observado que la temperatura modifica la tasa metabólica, requerimientos de proteína y energía, ingesta total, velocidad de crecimiento e índices de utilización nutritiva, por esto la temperatura es uno de los factores a tener en cuenta cuando se trate de realizar estudios de nutrición en peces.

En el presente ensayo se llevó un registro diario de la temperatura hallándose un valor promedio de 24.4 °C el cual esta dentro del rango óptimo establecido para el género *Colossoma* (23-30°C), (Ramos, 1987).

El oxígeno disuelto en el agua es uno de los indicadores de la calidad del agua que necesita ser mantenido en un nivel adecuado por su posible influencia sobre la ingesta, requerimientos y utilización nutritiva. Durante el presente ensayo se presentaron valores entre 4.37-4.69 mg/l, estando dentro del rango óptimo establecido para el cultivo de peces de aguas cálidas (4-12 mg/l).

Los valores letales de pH para peces de cultivo están por debajo de 4 y por encima de 11, aunque los peces puedan sobrevivir en valores cercanos a estos extremos, se observa un crecimiento lento. Para el cultivo de peces de aguas cálidas como la cachama se recomienda valores entre 6-7.5.

-Pruebas de laboratorio: El óxido de cromo es un marcador inerte no digerible que pasa por el sistema digestivo con una velocidad más o menos constante y no es absorbido; es lógico esperar que todo el óxido crómico adicionado al alimento aparezca en las heces sin embargo, aunque se encuentra la misma cantidad de indicador, el porcentaje relativo de este en las heces es mucho mayor que el que estuvo en relación con el alimento. Esta diferencia se debe a la absorción de nutrimentos durante el tiempo que permanece el alimento

en el sistema digestivo, y esta diferencia es la que hace posible calcular el coeficiente de digestibilidad aparente sin necesidad de hacer una colección total de las heces.

Los coeficientes de digestibilidad de los componentes nutricionales de las diferentes dietas se muestran en la tabla 3.

TABLA 3. Coeficientes de digestibilidad aparente para los componentes de la dieta.

ENSAYO 1	PROTEINA	FIBRA	CENIZA
TRATAMIENTO			
TESTIGO	86.99	5.55	71.50
TESTIGO+A20	73.84	8.71	70.87
TESTIGO+A30	85.73	11.38	73.74
TESTIGO+A40	64.35	10.53	72.28

ENSAYO 2	PROTEINA	FIBRA	CENIZA
TRATAMIENTO			
TESTIGO	71.51	13.59	70.90
TESTIGO+A20	47.30	10.83	54.12
TESTIGO+A30	62.01	9.86	63.13
TESTIGO+A40	51.65	1.31	66.02

ENSAYO 3	PROTEINA	FIBRA	CENIZA
TRATAMIENTO			
TESTIGO	78.60	-10.19	71.39
TESTIGO+A20	66.99	4.55	71.09
TESTIGO+A30	71.55	12.67	75.77
TESTIGO+A40	74.98	17.48	72.30

Los diferentes porcentajes de inclusión de *Azolla* en las dietas no presentaron diferencias significativas con un nivel de $p < 0.005$ para los coeficientes de digestibilidad de la proteína, fibra y cenizas; como tampoco se observaron diferencias significativas entre las repeticiones. La capacidad nutritiva de un alimento ésta dada no solamente por la calidad de sus ingredientes sino también por la interacción que existe entre los nutrientes presentes en esta. Así posiblemente los altos niveles de fibra presentes en las dietas especialmente en el alimento con el 40% de sustitución influyo en la digestibilidad de este ingrediente y de la proteína. Como lo reportado por Kitamikado (1964) quien determino la digestibilidad de la proteína de cuatro dietas que contenían 10, 20, 30, 40% de almidón y agregó 90, 80, 60 y 40% de harina de pescado respectivamente. La disponibilidad de la proteína disminuyo desde un alto porcentaje (81%) en la dieta que contenía el 10% de almidón, (63%) a 32% en la dieta que contenía 20% de almidón. Inaba *et al* (1963), Nose (1967), Page & Andrews (1973) y Austreng *et al* (1977) citados por N.R.C. (1991) reportaron un decrecimiento de la digestibilidad de la proteína de las dietas con un alto contenido de fibra.

Según Hilton (1983) los altos niveles de fibra cruda influyen en la digestibilidad de este ingrediente y de los otros nutrientes que conforman la dieta como consecuencia del aumento de la motilidad gastrointestinal que provoca una disminución del tiempo de permanencia del alimento en el tubo

digestivo expuesto a la actividad enzimática haciendo que la digestibilidad de la materia seca disminuya.

La celulosa que existe de forma natural en los alimentos es un componente de la pared de las células vegetales. Si esta pared no se rompe los materiales del interior de la célula no pueden ser atacados por las enzimas digestivas y por lo tanto su digestibilidad disminuye.

La cantidad de fibra que es digerida difiere de una especie a otra incluso entre individuos de la misma especie, debido a la diversidad de composición y tamaño de su flora microbiana. Sobre la composición tiene gran influencia el tipo de alimento ingerido ya que no todos los microorganismos del tracto intestinal atacan la celulosa y la hemicelulosa (Lloyd *et al.*, 1982).

De los componentes fibrosos la lignina es muy resistente a la acción de los microorganismos y por lo tanto es menos digestible; la celulosa es desdoblada con mayor facilidad y la hemicelulosa como grupo se considera como la más digestible, la celulosa es el principal componente de la fibra cruda, en consecuencia la digestibilidad de la fibra depende en gran parte de la digestibilidad de la celulosa.

El coeficiente de digestibilidad de la proteína del helecho acuático *Azolla filiculoides* (70%) es comparable con los valores reportados por Carneiro & Castagnolli (1983) en sus experimentos de alimentación de cachama blanca realizados con cuatro dietas con diferentes niveles de proteína (14,16,22 y 26%) sus respectivos valores de digestibilidad fueron 77.6, 70.8, 87.0 y 78.4%.

Aunque Ogino *et al* (1973) asegura que el valor de la digestibilidad de la proteína de los alimentos para peces debe fluctuar entre 95.2 y 95.5%. Cho *et al* (1979) obtuvo con truchas coeficientes de digestibilidad inferiores al 91% con niveles de proteína en la dieta de 30%; por otro lado en sus investigaciones Smith (1971) obtuvo coeficientes de digestibilidad mucho más bajos con niveles protéicos considerablemente más altos.

Otros estudios en alimentación de peces han reportado coeficientes de digestibilidad de proteína de 80.3% por Ateack (1979), 83.5% por Ufodike & Matty (1983), según la R.N.C. la carpa puede asimilar hasta el 95% de proteína pero este valor puede variar dependiendo de la fuente protéica.

Los resultados del presente ensayo sugieren que la calidad nutricional de la proteína en términos generales fue buena teniendo en cuenta que el valor de la digestibilidad de la proteína fue (70%) siendo esto un indicativo de la disponibilidad digestiva individual de los aminoácidos, reflejada en el incremento de peso de los animales experimentales como se observa en la tabla 4.

TABLA 4. Ganancia de peso

ENSAYO 1				
T	NP	PI	PF	IP
AT	108	3005.2	3150	144.8
A20	99	2997.4	3051.9	54.5
A30	108	3003.7	3024.8	21.1
A40	106	3012.3	3003.7	20.01

ENSAYO 2				
T	NP	PI	PF	IP
AT	145	3006	3092.6	85.6
A20	126	3001.7	3064.3	66.8
A30	124	3003.4	3002.5	29.1
A40	145	3019.3	3023.7	4.4

ENSAYO 3

T	NP	PI	PF	IP
AT	107	3021.1	3224	202.9
A20	109	3003.9	3064.3	60.4
A30	105	2999.8	3020.7	13.1
A40	112	3008.1	2986.5	32.7

Los valores obtenidos para los coeficientes de digestibilidad de los principios nutritivos de la materia prima probada demuestra que la digestibilidad de los nutrientes no depende del nivel de inclusión en la dieta de referencia por lo cual se sugiere la utilización del 30% del nivel de sustitución para la alimentación de alevinos de cachama blanca.

La conversión alimenticia depende, de la temperatura, tamaño del pez y sus preferencias alimenticias. En este ensayo los peces experimentales fueron alimentados con una ración diaria del 1% del peso vivo durante 9 días consiguiéndose una conversión alimenticia de 2,25, 5,86 y 5,82 para las dietas experimentales con 20, 30 y 40% de inclusión de Azolla respectivamente. Estos valores son comparables con los obtenidos en la estación piscícola de Repelón utilizando dietas artificiales balanceadas de 25% de proteína y un suministro diario del 3% del peso vivo.

En Venezuela se ha utilizado alimento para truchas y conejos los que han dado buenos resultados en el cultivo de *Colossoma*; con el uso de alimento para aves en estanque en tierra se consiguieron índices de conversión de 1.7.

En Brasil se desarrollaron trabajos de nutrición en mono y policultivos de *Colossoma* y *Piaractus*, los que fueron alimentados con raciones para gallinas que contenían 29-35% de proteína, los datos de conversión alimenticia para cachama negra fueron de 2.8-3.0 y para cachama blanca 2.5-3.7 (Cantelmo, 1989).

CONCLUSIONES

El alto potencial nutricional de la *Azolla filiculoides* junto a su gran abundancia en los Llanos Orientales de Colombia, permiten utilizar este helecho como una fuente proteica alternativa en la formulación de dietas de alta calidad y bajo costo para el levante de alevinos de cachama blanca.

La calidad proteica de la *Azolla filiculoides* se reflejó en el cubrimiento de los requerimientos proteicos de la cachama manifestándose como un incremento del (10.88%) del peso corporal de los animales experimentales.

La digestibilidad de los nutrientes de la *Azolla filiculoides* fueron buenas (proteína 66.54%, fibra 9.82%, ceniza 68.87%), (dieta testigo Proteína 78.37%, fibra 9.81%, ceniza 68.87%) exceptuando la fibra bruta; al aumentar el nivel de la fibra ejerció una influencia negativa sobre la digestibilidad de los otros nutrientes, lo que hace necesario realizar estudios más profundos sobre este punto en especial.

En general la digestibilidad de la proteína fue buena (66.54%) (dieta testigo 78.37%), independiente del nivel de inclusión de la materia prima y a pesar de que solamente el 7.5% del total de proteína fue de origen animal (harina de pescado).

Un alimento a base de *Azolla filiculoides* puede ser utilizado eficientemente por la cachama blanca, gracias a las características anatómicas y fisiológicas de su tracto digestivo y hábitos alimenticios.

La valoración global de la calidad nutricional de la *Azolla filiculoides* en términos de conversión alimenticia fue buena (4.6) con un suministro de alimento diario del 1% del peso vivo durante 9 días.

El sistema experimental permitió evaluar con gran precisión la utilización nutritiva de las dietas experimentales por parte de los peces al emplear agua carente de plancton.

AGRADECIMIENTOS

Agradezco a todas las personas y entidades que colaboraron en el desarrollo de esta investigación y en particular a:

Al Dr. ALEJANDRO URIBE PERALTA, Médico Veterinario, Ph. D. Por su extraordinaria colaboración en el desarrollo de esta tesis como director.

Al Dr. FRANCISCO JOSÉ DÍAZ, Ingeniero Agrónomo División de Investigaciones INPA, por su apoyo.

Al Dr. HERMES ORLANDO MOJICA, Biólogo Marino, Director estación piscícola La Terraza. Por sus oportunas apreciaciones como coordinador de este trabajo.

A PURINA COLOMBIANA S.A. En especial a los Drs. FRANCISCO PEÑA y LUZ MARINA MEDINA. Por su valiosa colaboración.

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