Azolla, the wonder plant

R. L. Bieleski[1]

It was a bit prophetic that the very first colour photo I ever took, number 0001 in my collection, taken with my trusty Kodak Retina camera and a spectacle lens, was a close-up of *Azolla rubra* at Bethell's Beach, Auckland. Prophetic, because 25 years later I was to spend 9 months on study leave at the University of California, Davis, probing the physiology of this fascinating plant. But before I get to my own small contributions I want to take you for a small tour highlighting some of the things we know about this remarkable plant.

First, the taxonomy. Despite the small size of an individual plant, 1–2 cm across (Fig. 1–2), and its habitat, floating on the top of ponds (Fig. 3–4) usually in company with the monocotyledenous *Lemna* (duckweed, ducksmeat), *Azolla* is actually a fern in its own family, Azollaceae, though current research now places it in the Salviniaceae together with another floating fern, *Salvinia*. *Azolla* has a worldwide distribution in warm, subtropical and tropical regions. Taxonomist's views vary a little, but most centre about there being six species worldwide (Fig. 5), with the scientific name for New Zealand's single native species having done a taxonomic do-si-do from *A. rubra* through *A. filiculoides* and *A. filiculoides* var. *rubra* and back to *A. rubra* (its main Māori name is kārearea). To complicate things in New Zealand, in recent years an exotic species, *A. pinnata*, has come into our country and has been taking over from our *A. rubra* in areas north of Auckland. The jury is out as to whether *A. pinnata* entered naturally (such as on waterbirds' feet) or accidentally through human actions, but it has enjoyed our climate and waterways enough to become classed as an invasive species.

The typical fern structure has been modified in *Azolla* to a point where the stem and rhizome have become reduced to a frequently-branching thread, with leaves and roots coming off at the nodes. The small overlapping scale-like leaves have large air spaces, giving the plant its buoyancy. Reproduction is largely vegetative, with side branches breaking off from the main plant and growing to form a new plant, from which further side branches split off, and so on. What this means in effect is that given ideal conditions, *Azolla* has an exponential growth pattern, and we can talk about its doubling time as a measure of its growth rate. This is not a theoretical concept: in my experiments I grew *Azolla mexicana* under near-optimal conditions, and exponential growth was maintained over five generations, till space in the growth tubs became limiting, causing crowding of the fronds and therefore limiting the exposure of individual fronds to light. The original 500 mg starting weight of inoculum consistently reached ca. 16 g in 11–12 days, giving a doubling time of 2.3 days.

In one stage my growing went through three iterations, meaning that after the 12-day period I took 0.5 g samples from that 16 g to seed new tubs, and then again 12 days later, with exponential growth being maintained right through. If each time I had been able to use the full amount in seeding tubs, I would have had 32 tubs at the second iteration and 1024 at the third, with a final tissue weight around 16 kg. In the field, comparable growth rates are sometimes reached, as doubling times are often spoken of as being as low as 3–5 days. It's hard to comprehend the power of such growth, so can I pose a question for you? Let's begin with my standard inoculum, 500 mg of *Azolla*, about as much as will fit on the end of a teaspoon. If we were to allow that *Azolla* to keep up its exponential growth without limitations of light, nutrients and space coming into play, how long would it take for the weight of our *Azolla* crop to equal the entire

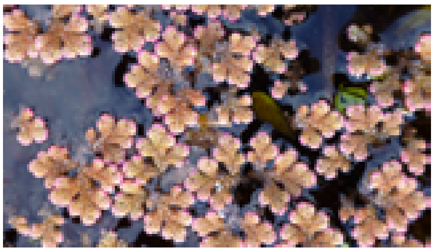

Fig. 1 Individual plants of *Azolla rubra*. Photo: Murray Dawson.

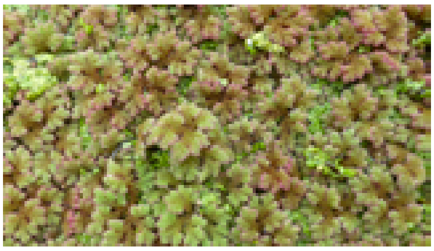

Fig. 2 Close-up of a dense *Azolla rubra* mat, growing in a pond at Halswell Quarry Park, Christchurch. Photo: Murray Dawson.

Fig. 3 *Azolla rubra* plants (red) competing with *Lemna minor* plants (green) in a rāupo wetland, Flaxmere. Photo: Rod Bieleski.

Fig. 4 *Azolla rubra* growing in a drainage channel alongside Pegasus Bay Walkway, north of Christchurch. Photo: Murray Dawson.

Fig. 5 *Azolla* on Lake Macquarie (NSW, Australia). Photo: Rod Bieleski.

[1] 33 William Bond Street, Stanley Point, Auckland 0624, New Zealand; rbieleski@ihug.co.nz

plant biomass of the whole world? A million years? A thousand years? A century? All wrong. It would take about 5 months for the doubling time of 2.3 days, or 9 months for a doubling time of 4 days. Of course limitations do occur, saving us from being buried in *Azolla*, but this does explain why a pond almost free of *Azolla* can become completely covered in less than a month.

Though the growth is primarily vegetative, *Azolla* can behave like more typical ferns and reproduce sexually, but it does have to do it in its own way. The standard fern way is for the diploid generation, the sporophyte that we recognise as our fern, to shed haploid spores into the wide world, there to germinate into separate life stages, the haploid prothalli (small liverwort-like plants) which produce the eggs to be fertilised by the swimming sperm, creating the new diploid (sporophyte) generation. With *Azolla*, spores are not released; instead male and female sporocarps are formed on the underside of the frond, held there, and the fertilisation occurs on the parent diploid sporophyte. In this respect, where there is no physically separate gameophyte generation, the life cycle resembles that of higher plants!

But that is not what makes *Azolla* really special, it is its ability to fix nitrogen, which it achieves by supporting colonies of blue green algae (cyanobacteria), *Anabaena azollae*, living in the air chambers of its fronds. And it is an association that puts all other nitrogen-fixing systems to shame. The growth rates I reported, of a doubling time of 2.3 days, were achieved in the absence of any nitrogen supply in the nutrient medium that the *Azolla* was growing on. Every last skerrick of the nitrogen was fixed by the *Anabaena* and swapped to *Azolla* in the form of NH_4^+ (ammonium), with the *Azolla* providing *Anabaena* with carbohydrate, mainly as fructose. At one stage I got curious as to how much faster *Azolla* could grow if it didn't have to rely on *Anabaena* for its nitrogen and so I supplied it in a normal growth medium. The answer? Growth was actually inhibited a little bit! The consequences of this ability to fix nitrogen were recognised by Chinese rice farmers at least 1500 years ago when they were already using it as a nitrogen fertiliser for their rice paddies (Fig. 6). The earliest known written record of the practice is in a book by Jia Si Xue in 540 A.D. on *The Art of Feeding the People*. Its use in Vietnam dates to the 11th century. By the early 17th century, its use as a compost was being documented in many local Chinese records.

Fig. 6 Chinese farmer inoculating a rice paddy with *Azolla* 1500 years ago.

Under field conditions *Azolla* can accumulate up to 2–4 kilograms of nitrogen per hectare per day, 1.1 tonnes of nitrogen per hectare per year, and almost three times the performance of legumes such as clover at around 400 kg of nitrogen per hectare per year. It is no wonder, therefore, that use of *Azolla* has become a major tool in the growing of rice in China and Vietnam. A major push for expanding the use of *Azolla* began in those two countries in the early 1960s. In 1980, *Azolla* was being grown as a green manure on about 1.3 million hectares of rice in China alone. In the paddy fields, the typical pattern is for some of the *Azolla* to die, as the mat becomes fully shaded by the rice, sink and decompose – its rapid decomposition means that its nitrogen and other nutrients are made available for uptake by rice during grain development. Besides this straightforward contribution as a nitrogen fertiliser, *Azolla* is also important after rice harvesting in trapping nutrients out of the water that might otherwise be washed away and also in smothering weed (and mosquito) growth. I have had trouble finding out its current use as a biological fertiliser, and its use on rice may be declining. However, uses as supplements for animal and bird feed are being explored and expanded, and small lots of *Azolla* growing in canals and ponds as food for pigs and ducks are now ubiquitous throughout southern China. A factor in the use of *Azolla* as a green manure and an animal feed is its low carbon to nitrogen ratio of about 10:1, meaning that when used as a green fertiliser its nitrogen supply cannot be swamped by bacterial activity. As an animal feed, it has a protein content between 13 to 24% on a dry weight basis, making it a very high quality plant protein source.

So where did I come into the act? At the time, in 1979, Professor D. W. Rains of the Department of Agronomy and Range Science (University of California, Davis) was heading a large program trying to understand the biology of *Azolla*, with a view to using it more skilfully and further expanding its agricultural uses. A problem that showed up was that *Azolla* seemed to have a high requirement for phosphorus, with phosphorus supply often limiting growth. A typical finding was that even when supplemental phosphorus was provided, less than 20% was actually utilised by the *Azolla*, so that even though the in-plant N:P ratio was around 10, the yield of N fixed to P supplied was more like 1:1 to 2:1. As an old hand at studying phosphorus nutrition of plants, I was given the job of trying to uncover some basic facts about phosphate transport and utilisation in *Azolla*, to see if ferns were "different" to the higher plants I had worked on up till then. The basic setup I had available in which to grow my plants was a growth cabinet which gave near-optimal conditions for *Azolla*: 27°C, 16 hour day, about 25% of full daylight, standard plant nutrient solution minus any nitrogen source, in 14 cm × 14 cm × 20 cm plastic tubs, with aeration. Movement of phosphate in experimental tests was followed using the radioisotope P^{32}. If you want to know the gory details, see Bieleski and Läuchli (1992).

The most basic thing I found out about *Azolla* was that its ability to accumulate phosphate was not in any way inferior to that found in higher plants. Rates depended on whether the plants were being grown in an ample supply of phosphate (0.2 mmolar) or were put into a phosphate starvation regime, when the plants developed an additional "low phosphate accumulation system" and were able to accumulate phosphate about two times faster. Under the starvation regime, *Azolla* was still able to show a net uptake of phosphate when the external concentration fell as low as 0.1 μmolar, 1/2000th of that in the growth solution and 1/30th that in typical soil water. The phosphate pump was very powerful: the concentration gradient between the environment supplying the phosphate at 0.1 μmolar and the tissue content, 10 mmolar, was 1:100,000. To put it in context, 0.1 μmolar phosphate is what you would get by dissolving a heaped tablespoon of sodium phosphate in an Olympic swimming pool, and 10 mmolar by dissolving that heaped tablespoon in a large bucket. The next question I attacked was "how much of the phosphate is taken up by the roots, and how much by the fronds?" The roots were about three times as efficient as the fronds, on average, at taking up phosphate. However there was a greater proportion of frond tissue, so that the total uptake into the plant carried out by the fronds themselves ranged from 70% of the total when phosphate was in abundant supply (100 μm) to 45% when phosphate was limiting (1 μmolar) and at a concentration more typical of field conditions. Nonetheless, all the uptake studies said that *Azolla* was very efficient at extracting phosphate from the water, and its tissue content was not unusual, and so the reason for the apparent high demand for phosphate had to be sought elsewhere.

So let's look at the field situation. Maximum field growth rates of *Azolla* are usually obtained only after heavily fertilising paddies with phosphate, and this is what has led to suggestions that *Azolla* may have a high specific phosphorus requirement to support growth and nitrogen fixation. Though the phosphorus content of *Azolla* at 0.3–0.6% dry weight (5–10 μmoles phosphorus per g fresh weight), is a bit higher than the normal values for most plants, 0.2–0.25% dry weight, that difference can be entirely put down to the very low proportion of non-living and structural tissues in *Azolla*. No wood, damn all xylem, it's all living and active tissue, and the concentrations are not at all high for young plant tissues. Instead, I feel that the reason for the sensitivity of *Azolla* to phosphate supply can be found in the relationships between the plant, the water in which it is growing, and the soil of the pond beneath. In a paddy field, the water may be 15 cm deep and contain 5 mmoles per cubic metre of inorganic phosphorus in solution at the time of inoculating with *Azolla.* It means that it can only support growth of about 10 g fresh weight per square metre before the water becomes completely depleted of phosphate, whereas a dense *Azolla* cover is more like 200 g fresh weight per square metre. For further growth, phosphate has to be supplied from elsewhere – i.e., released by the soil. The nature of the relationship between the soil minerals and inorganic phosphate is such that the phosphate is strongly bound, to the point that the equilibrium concentration with the surrounding soil water is typically around 3 μmolar. That is the concentration that typical plant roots face (and explains why the phosphate accumulation pumps are so effective). With plants growing in soil, the root hairs are never more than a millimetre or two away from the phosphate-binding soil particles. But *Azolla* roots do not penetrate the soil and are not in close contact with the soil particles. Thus, for much of its growth period the *Azolla* crop must be completely dependent on diffusive transfer of inorganic phosphate from the paddy soil particles to the adjacent water, but then through a 15 cm layer of water to the root and frond surfaces. This can be expected to be a major rate-limiting process (Bieleski and Ferguson, 1983). A root system that penetrates the soil offers a much more effective interface for phosphate transfer than the thick layer of water that the *Azolla* must depend upon. It has been noted that a water level which allows *Azolla* roots to touch the soil will often cause phosphorus deficiency to disappear (Lumpkin and Plucknett, 1980). So there it is: the *Azolla* is phosphate-limited not because of its physiology, but simply because it is floating free on top of the pond, and so its roots don't penetrate the soil like those of most other plants.

What of the future? From what I have found, we can suggest ways to attack the problem of phosphate shortage in the life of *Azolla*. We could breed *Azolla* with very much longer roots, or decrease the water level of paddies to a point where the *Azolla* roots can access the soil. Neither seems practical. Instead of putting phosphate into the paddy in a single dressing, we could dribble it in over the growing season – a bit complicated, but doable. Or we could aerate the pond, stirring the water and breaking down the diffusion barrier between soil and water. Or we could go further and agitate the surface of the pond soil to also increase the ease of transfer of its bound phosphate into the pond water. And will *Azolla* have a developing future in agriculture, or will its use fade out with the decline in peasant farming in India, Vietnam and China? I hope not. At present, almost all nitrogen fertiliser used in agriculture comes from the Haber process, which is very energy-intensive. In total, global Haber ammonia synthesis uses of the order of 1.5% of the world's annual energy supply. So as we move towards reducing our use of energy in a drive to slow global warming, *Azolla* offers a small lifeline (Fig. 7A–B).

Fig. 7A–B Research appears to have continued at an *Azolla* production and demonstration farm at the Philippine Rice Research Institute (Muñoz, Nueva Ecija). Photos: "Judgefloro" (CC-BY-SA-4.0).

I finally move to a sting in the tail of the tale about *Azolla*. Right now we are in a geological period where the actions of a single organism, human beings, are driving the world's climate into a new state. Global warming is real, and it has been brought about by us humans releasing fossil carbon into the atmosphere. For this, geologists have coined the word "Anthropocene" to describe what is shaping up as a new geological era.

It is an irony that this warming has recently given us evidence of another event where the global climate has been changed by the actions of a single organism. Here it is the onset of the Paleocene ice age, and the organism is our humble *Azolla*. In 2004, the reduced ice cover in the Arctic allowed the Arctic Coring Expedition drillship *Vidar Viking*, supported by the Swedish and Russian icebreakers *Oden* and *Sovetskyi Soyuz*, to sail close to the North Pole and drill deep boreholes in sediments of the Lomonosov Ridge beneath the Arctic Ocean seabed. The cores it recovered were sediments recording events stretching back to the age of the dinosaurs 80 million years ago when global temperatures were much warmer than those of today, and temperate conditions extended right out to the earth's poles. We know that this greenhouse climate ended abruptly when atmospheric levels of CO_2 suddenly fell from 3500 ppm in the early Eocene to 650 ppm during a brief period 49 to 47 million years ago, resulting in a sudden temperature fall in the Earth's climate into an ice age with large icecaps at each pole, still partly with us today. And those ACEX cores turned up a remarkable finding. Sediments in that critical period, 49 million years ago, revealed that thick organic deposits more than 8 m deep, composed almost entirely of *Azolla* fossils, extended along the 1800 km ridge. The finding created a storm of interest amongst geologists. Extant *Azolla* can only tolerate slight salinity, so why was there a huge production chain of *Azolla* plants in the middle of the Arctic Ocean, and why were they there precisely when the Earth experienced one of the most dramatic climatic changes in its history? Was there a connection between the two events?

Many geologists think the answer is "yes", drawing the following picture. About 50 million years ago, the Arctic Ocean was largely land-locked and centred on the North Pole, as it is today, but with much warmer temperatures than those seen today. Its only significant marine connection was through a long narrow seaway called the Turgay Strait, which extended southwards across western Siberia to the equatorial Tethys Ocean. Then, 49 million years ago, the shallow Turgay Strait became blocked, and the Arctic Ocean was isolated from the other oceans, becoming stratified with little vertical mixing of its water, so that its bottom waters became anoxic (deprived of oxygen). Rainfall was high: rivers discharged large volumes of freshwater, creating layers of surface freshwater extending out into the ocean (similar freshwater layers spread out from the Amazon River today), explaining the ability of *Azolla* to grow there. As the floating *Azolla* mats became waterlogged or as storms fragmented them, the plants died and sank to the anoxic sea floor where there was no decay and the carbon became fossilised and removed from circulation. Calculations show that with 800,000 years of *Azolla* blooms and a 4,000,000 km² basin to cover, easily enough carbon could have been sequestered by *Azolla* alone to account for the observed 80% drop in CO_2 concentration in the atmosphere. Of course other organisms and other processes were involved, but *Azolla* is fingered as the main driver, with this period of the Earth's history now being called the "Azolla Event". After that time the atmospheric CO_2 concentration continued to fall slowly over the next 49 million years due to geological processes that sequestered even more carbon dioxide. Present values are just above 400 ppm, having risen from pre-industrial values in the mid-18th Century of about 280 ppm.

So there it is. The humble little *Azolla*, almost ignored when we talk about our native flora, merits the title "superorganism" given to it by some of those describing these dramatic events. Just Google "Azolla Event" to get a fuller account of the story. And show some respect the next time you pass a red-surfaced pond.

References

Bieleski, R.L. and Ferguson, I.B. (1983). Physiology and metabolism of phosphate and its compounds. In *Encyclopedia of Plant Physiology NS 15A: Inorganic Plant Nutrition* (eds: Läuchli, A.A. and Bieleski, R.L.), pp. 422–449. Springer-Verlag, Berlin.

Bieleski, R.L. and Läuchli, A.A. (1992). Phosphate uptake, efflux and deficiency in the water fern, *Azolla*. *Plant, Cell & Environment 15*: 665–673.

Lumpkin, T.A. and Plucknett, D.L. (1980). *Azolla*: botany, physiology and use as a green manure. *Economic Botany 34*: 111–153.