Mundulla Yellows — a new tree-diebback threat

A recently described tree-killing disease, Mundulla Yellows, has been found in Western Australia. It has the potential to seriously affect a number of our native plant species. What is Mundulla Yellows and what can be done to combat the disease?

by Dagmar Hanold, Mike Stukely and John W Randles

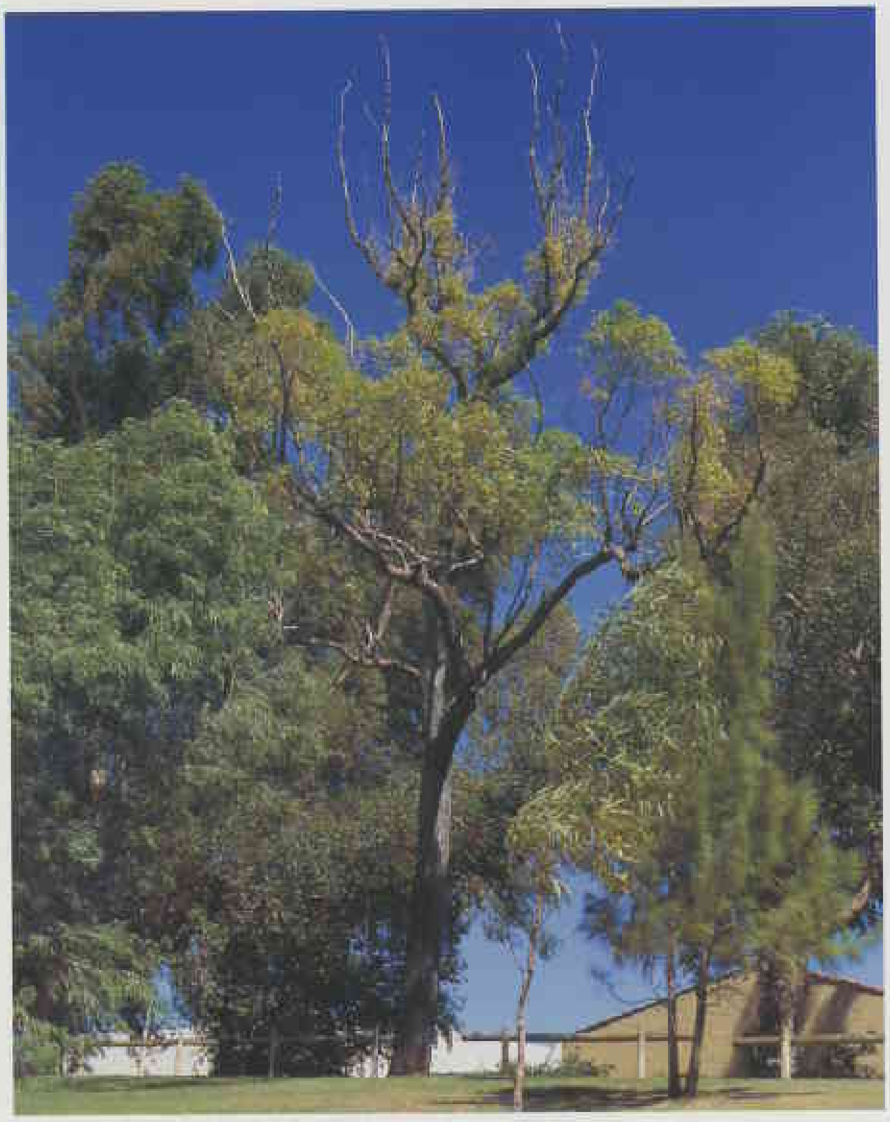
In Western Australia, the term 'dieback' generally refers to the devastating disease of many native plants, caused by the root-rotting slime mould *Phytophthora cinnamomi*. But there are other diseases of trees, caused by a variety of living organisms or environmental factors, that also cause tree crowns to die back. A little-known and only recently described disease, Mundulla Yellows, has the potential to seriously affect a number of our native plant species, as well as revegetation plantings on farms and possibly some eucalypt plantations. Mundulla Yellows is a progressive slow dieback and yellowing disease of many varieties of eucalypts, now suspected of being caused by a virus-like agent. It has been reported in trees of all ages. Once symptoms appear, the affected trees do not recover, and die within a few years.

Research is in progress to identify the cause of Mundulla Yellows, to develop a rapid diagnostic test, and to determine its distribution and how it is spread, so that strategies can be developed to manage the disease.

WHERE IS IT?

Mundulla Yellows was first reported in the late 1970s in the south-east of South Australia, by bee keeper Geoff Cotton. There is evidence that the disease also occurs in other States, including Western Australia, but typical Mundulla Yellows symptoms have not been reported outside Australia. In South Australia, Mundulla Yellows is present in scattered sites throughout an area estimated to exceed 25,000 square kilometres—and it is spreading! Many of the sites show high incidence of the disease.

Mundulla Yellows is named after the town of Mundulla in South Australia (see box). Its symptoms have been observed in a wide range of eucalypts growing in modified landscapes, as well as in remnant natural vegetation. Sheoaks (the *Allocasuarina* spp.), banksias (*Banksia* spp.) and wattles (*Acacia* spp.) show similar symptoms, which suggests that the disease may not be confined to eucalypts. Mundulla Yellows has so far been observed mainly in sites that have undergone significant disturbance, such as farmland, roadsides and urban parks. However, it has been seen occasionally in natural forest in eastern Australia.



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Main: A jarrah leaf showing interveinal yellowing—a sympton of Mundulla Yellows.
Photo – Mike Stukely
Inset: Salmon gum with medium stage Mundulla Yellows symptoms on a roadside in the WA Wheatbelt.
Photo – Allan Wills

Above: A jarrah (*Eucalyptus marginata*) showing medium to late stage Mundulla Yellows symptoms, in a Perth suburban park. Dead branches can be seen at the top, while the dense epicormic shoots that have sprouted lower down are clearly yellow. Only a small amount of green foliage remains. The other nearby eucalypts still appear healthy.
Photo – Mike Stukely

The first sightings of Mundulla Yellows in WA were made by Frank Podger (see box). Recent surveys by the Department of Conservation and Land Management in WA have located additional species with Mundulla Yellows symptoms. They include river redgum (*Eucalyptus camaldulensis*) and lemon-scented gum (*Corymbia citriodora*)—both of which are cultivated—as well as the WA natives salmon gum (*E. salmonophloia*) and York gum (*E. loxophleba*). Affected trees occur in a scattered distribution, and mostly in coastal areas, although Mundulla Yellows symptoms have been observed as far inland as York, Williams and Boyup Brook. In the Perth area, sheoaks also appear to be affected by Mundulla Yellows. The declines of wandoo (*E. wandoo*) and flooded gum (*E. rudis*) in the Wheatbelt and of tuart (*E. gomphocephala*) south of Perth do not, however, appear to be associated with Mundulla Yellows.

AN ENVIRONMENTAL CONCERN

The origin of Mundulla Yellows is unknown. Is Mundulla Yellows an exotic disease that has only recently

Right: Pricklybark (*Eucalyptus todtiana*) with Mundulla Yellows symptoms in a Perth suburban park. Dead limbs have been removed for safety reasons, but this treatment does not stop the disease, and clear yellowing is now spreading to other parts of the crown.
Photo – Mike Stukely

Centre right: Buckingham Old Coach Road, South Australia, showing healthy river redgums photographed during a flood in 1906.
Photo – Courtesy Mr Adrian Packer

Below right: The same site in 1999 with tree deaths due to Mundulla Yellows.
Photo – Courtesy The Border Chronicle

arrived in Australia? If so, where did it come from, and why is it already so widespread when its rate of spread appears slow? Alternatively, has the disease been present for a long time as a latent infection in some plants, causing little or no damage, and has its recent increase been caused by environmental changes favouring disease development or perhaps due to mutations in the pathogen(s)? Further investigation is needed.

Mundulla Yellows is now acknowledged as a threat of national significance to natural biodiversity and ecological sustainability. It causes the irreversible loss of natural vegetation, including ancient gum trees. Since a wide range of eucalypts and other species of native flora, which comprise natural Australian ecosystems, appear to be affected, it poses potential danger to natural wildlife habitats and, thus, the conservation of threatened plant and animal species. It has evoked considerable public concern, as well as much speculation as to its cause.

The potential of Mundulla Yellows to cause damage to our native flora may be exacerbated by climatic changes predicted for the coming decades. It is potentially detrimental to a number of industries and parties, including commercial tree growers, bee keepers, tourism, native cut flowers, public amenity, land and forest managers, and local government. It could jeopardise revegetation programs, salinity and groundwater level control, and input on the international carbon credits policy,

MUNDULLA YELLOWS: A SHORT HISTORY (BY FRANK PODGER)

In 1979, bee keepers and farmers Geoff Cotton, Bill Hunt and Sandy Mathison, from the Tatiara district of South Australia, noticed abnormal patches of bright yellow foliage in small numbers of large river redgums (*E. camaldulensis*) around Mundulla and in South Australian blue gum (*E. leucoxylon*) near Keith, close to the border with Victoria.

It is these magnificent old trees that lend an essential Australian character to the landscape, forever captured in the art of Hans Heysen. These ancient trees slowly declined and, over the next decade, died. Neighbouring trees also became affected, and discrete new centres of disease gradually appeared elsewhere in the landscape.

Despite Cotton's growing apprehension, most landholders took little notice of the problem until 1991, when Cotton first showed examples of Mundulla Yellows to David Paton of Adelaide University. In 1992, vital though modest research grants from the Honey Bee Research Council and from the Save the Bush Fund allowed Paton and his students to begin a disease monitoring program. Over the next five years, this work showed greater than expected rates of deterioration within affected trees, and further spread of the disease to healthy neighbours was much greater than expected.

The name 'Mundulla Yellows' was adopted in 1997 by Podger, Cotton, Choate and Randles, and is now in general use. The name refers to the small and lovely rural town of Mundulla (the name given by indigenous people)—the place where the disease was first recognised and where strong support for subsequent action originated—and the disease's distinctive and striking set of symptoms, particularly the early appearance of small clusters of bright yellow leaves in otherwise healthy crowns.

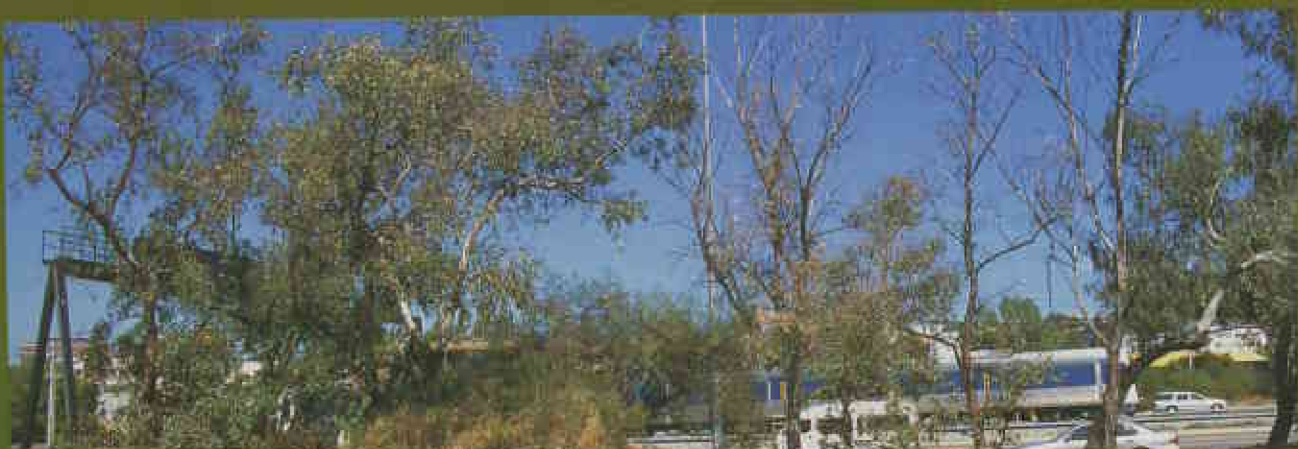
The connection with Western Australia first emerged soon after a conversation I had with Stan Bellgard in early 1993. He was then researching *Phytophthora megasperma* for the WA Department of Conservation and Land Management. He told me of a report from South Australia of the possible involvement of *P. megasperma* with widespread deaths of river redgum. To me, however, it seemed that several features of Mundulla Yellows, and of the climatic regime at Mundulla, did not fit the published requirements of *P. megasperma*. In August 1993 I visited the Tatiara district for the first time and was directed to Geoff Cotton. We took soil samples at five diseased sites around Mundulla, none of which yielded any species of *Phytophthora*. Most importantly, no evidence of rot in even the smallest secondarily thickened roots was observed in five excavated saplings of diseased river redgums.

Upon my return to Perth, I saw that Mundulla Yellows was widely established there. However, the degree of damage and the stages of symptom development seemed to indicate a later arrival in Perth than at Mundulla, perhaps by a decade or more. Soon afterwards, I encountered a report of work in Italy, which described somewhat similar symptoms in young eucalypts. The authors had attributed that disease to a phytoplasma. This led me to contact with Adelaide University's distinguished plant virologist John Randles at the Waite Institute. His subsequent interest has led to the first significant funding to support the assignment of Dagmar Hanold to research the possible role of phytoplasmas and viruses, and to the advances reported in this article.

In the course of private surveys from 1993, over more than 20,000 kilometres, I have encountered Mundulla Yellows at many places across the length and breadth of Australia. The principal features of distribution of the disease, noted throughout these travels, are:

- it is rarely seen in essentially undisturbed natural vegetation, and then at the very margin with cleared land and roadways;
- in WA it occurs in a broken pattern that extends northward from Walpole in the far south-west to include infested sites in Bunbury, Perth, Geraldton, and on to the wet-dry tropics at Broome, Derby, Kununurra and, only in the last two years, Wyndham; and
- in country towns and cities, where it is most commonly encountered, it is most frequent in well-watered parks, road verge plantings subject to run-off, and well-cared-for home gardens. It is very uncommon at places where dry season moisture status is not ameliorated by some source of supplementary water.

Twenty-three eucalypt species native to Western Australia have been recorded as affected by Mundulla Yellows. Within Western Australia, these include tropical species such as long-fruited bloodwood (*Corymbia polycarpa*), woollybutt (*Eucalyptus miniata*) and Darwin stringybark (*E. tetrodonta*), which are highly susceptible, as are the southern temperate marri, red-flowering gum (*C. ficifolia*), jarrah, blackbutt (*E. patens*) and pricklybark (*E. todtiana*). Some other species natural to semi-arid areas of WA, and used in exotic plantings here and elsewhere, are also affected. A great many more eastern Australian native species are often severely damaged in exotic cultivation around Australia.



A group of river redgums planted alongside Perth's Mitchell Freeway, and affected by Mundulla Yellows. Some have died, others are near death, but some still appear healthy. Photo – Mike Stukely

and could impact on quarantine practices.

Mundulla Yellows has symptoms different from those of previously reported diseases of eucalypts. To define the problem—and to distinguish Mundulla Yellows from yellowing due to other factors—symptoms have been described in *A Field Guide to Mundulla Yellows* by D Hanold and J W Randles, published in 1999 by the University of Adelaide. The disease passes through three stages: early, medium and late.

FINDING THE CAUSE

When investigations into the cause of Mundulla Yellows using molecular methods began, in January 2000, a living organism was thought to be the cause (see box on page 47). This was because affected trees occurred in mixed stands with, or immediately adjacent to, unaffected trees. If environmental factors—such as nutrient imbalances, herbicide spraying and high soil salinity—were the cause, they would be more likely to affect most trees in an area. Also, some disease symptoms had developed in previously healthy plants grafted with patches of bark taken from Mundulla Yellows-affected trees. This method is used widely by plant pathologists to see if a contagious agent is present.

Unlike *Phytophthora*-induced dieback, there was no evidence to suggest that disease-causing organisms such as fungi, bacteria or nematodes were associated with Mundulla Yellows. So researchers began to investigate the hypothesis that Mundulla Yellows was caused by a micro-organism that could not live or replicate outside the host cells (obligate intracellular pathogen).

Plant pathogens in this category can belong to the virus, viroid or phytoplasma groups. So far, more than 1000 viruses, 30 viroids and about 200

Top photos: Stages of Mundulla Yellows symptom development in river redgum.
Top left: Early stage. Uneven yellowing affects the outer parts of one limb or a segment of the crown. These zones comprise fully expanded leaves with interveinal yellowing (chlorosis).
Top centre: Medium stage. Dieback of affected shoots occurs with progression of yellowing inwards towards the centre of the crown. Flowering and seed production decline on the affected branches. Neighbouring limbs subsequently develop the same symptoms. Part of the crown is still unaffected.
Top right: Late stage. Yellow 'panic growth' (epicormic shoots) below the dying zones gives a denser appearance. Seed production ceases. Dieback progresses. Eventually the whole crown shows dieback and the tree dies.
Photos – Dagmar Hanold

Right: A jarrah leaf showing interveinal yellowing (top) compared with one that is healthy.
Photo – Mike Stukely

phytoplasmas are known to infect plants. Phytoplasmas and viroids do not vary much in their structure, but viruses can be very diverse. They can vary in the structure and biochemical properties of their genome (consisting of nucleic acids, i.e. DNA or RNA), and the composition, shape and size of their particles (consisting of proteins). Consequently, there is a vast range of potential candidates for this investigation.

Obligate intracellular plant pathogens cannot spread independently, but need either biological vectors or mechanical means of transmission. Sap-sucking insects, nematodes, fungi, pollen, seed and plant sap carried on tools are examples identified in the past as possible modes of spread.

BREAKTHROUGH

Thanks to advances in biotechnology, there is a range of methods available for testing whether such a pathogen is responsible for Mundulla Yellows. Tissue samples from normal eucalypts were compared with those affected by Mundulla Yellows using different means.

Karen Gibb and Nuredin Habili carried out tests that detected phytoplasmas in eucalypts; however, they were found with similar frequency in Mundulla Yellows-affected trees and healthy trees, so did not appear to be a cause of Mundulla Yellows. Virus-like particles could be observed occasionally in leaf tissue by means of electron microscopy, but their role as a cause could not be confirmed by this method.

Left: Dieback, leaf distortion and stunting symptoms in a river redgum seedling in the glasshouse after bark patch grafting, with normal seedlings behind.
Photo – Dagmar Hanold

As no viruses or viroids of eucalypts had previously been characterised, a molecular test for virus-like agents in eucalypt tissue first had to be developed to test Mundulla Yellows-affected trees. Purified leaf extracts would be expected to comprise both general 'background' components from the host plant's cells and additional unusual components from pathogens if they were present. A breakthrough was made with this approach. Unusual nucleic acids (MY-RNAs) were found in Mundulla Yellows-affected river redgums (*E. camaldulensis*) in the south-east of South Australia. Indications from the size and biochemical characteristics of these nucleic acids pointed to a possible association with a virus or viroid.

MY-RNAs were detected in the leaf tissue of affected trees in South Australia before symptoms developed. This suggests that MY-RNAs are more likely to be associated with a cause rather than an effect of the disease. It also indicates that there may be a significant lag period between infection and appearance of disease symptoms. MY-RNAs have been detected in trees within revegetation sites and plantations less than a year after they were planted into areas previously without Mundulla Yellows. They have also been found in nursery-grown seedlings in the glasshouse. This suggests that the widely used practice of raising seedlings in one area for planting elsewhere may carry a risk of spreading Mundulla Yellows.

SURVEY

A survey conducted in the southern Australian states in 2000–2001 detected MY-RNAs in more than 30 species of eucalypts, as well as in sheoaks and bottlebrushes (*Callistemon* spp.), at a number of locations. However, it appears that symptoms may vary, perhaps due to environmental or host factors, or possibly variations in the MY-RNAs. In samples taken from trees with Mundulla Yellows symptoms in Western

Australia, MY-RNAs have so far been detected in marri (*Corymbia calophylla*), salmon gum, York gum and cultivated river redgum in the south-west, as well as in Darwin box (*E. tectifica*) from the tropical north.

There is preliminary evidence that insects may spread Mundulla Yellows. Field sites are being monitored for the development of symptoms and spread of MY-RNAs. Since symptoms take time to develop, and the spread of the disease appears to be slow, samples need to be collected regularly over at least five years.

With the above evidence, we are now investigating the hypothesis that Mundulla Yellows is caused by a virus-like agent. MY-RNAs now need to be characterised to test their association with a potential virus-like pathogen. A sensitive, specific and fast routine test suitable for screening large numbers of samples from a wide range of plant species needs to be developed. This will be essential for testing nursery material and planting stock for infected material, and to identify possible sources of genetic disease resistance for use in breeding programs. It will also be essential for investigating mechanisms of disease expression, modes of spread (such as different insect species) and other features of the disease cycle.

THE FUTURE

In the case of *Phytophthora* dieback in WA, it took more than 40 years from the first records of symptoms to the discovery of the cause—and, by then, huge areas of vegetation had been devastated. Groundbreaking advances in Mundulla Yellows research during the past two years have opened the way towards identifying the cause of this disease. Only when the disease cycle is known can specific strategies be designed to disrupt it and thus control the spread of the disease. Until more specific knowledge is available, general plant hygiene practices will help to minimise the risk through human activity of spreading diseases, including Mundulla Yellows, from plant to plant and, most importantly, into new areas. An integrated research program towards nationwide control of Mundulla Yellows has been devised, but funding still needs to be secured.

FINDING THE CAUSE OF A NEW PLANT DISEASE

Finding the cause of a new disease is rather like detective work. Evidence must be critically examined and possible suspects eliminated by means of specialised investigation methods. The approach we have used successfully in the past includes the following steps:

1. *Define the disease* and provide a range of descriptors to differentiate it from other diseases or disorders.
2. *Separate facts from fiction.* Usually a mixture of opinions and valid observations exists when starting to investigate a disease of unknown cause. It is necessary to critically assess this mixture to determine what facts are available to build on.
3. *Formulate a working hypothesis* on the most likely cause. The disease may be due to living organisms or environmental factors.
4. *Test this hypothesis* by designing experiments to establish whether it is true or false.
5. *Identify the cause of the disease* by proving or disproving the hypothesis. A set of rules established by the early microbiologist R Koch (Koch's Postulates) is used to verify whether the cause has been found. If the first hypothesis turns out to be wrong, a different one may have to be established and tested.
6. *Investigate the disease*, if the cause is a living organism, by: a) characterising its causal organism(s); b) determining the host range and the events leading to the disease's appearance and spread, i.e. the disease cycle; and c) examining the effects of environmental factors on disease expression.
7. *Design control or management strategies.* Once the disease cycle is known, strategies can be designed to disrupt it. This could involve controlling vector insects or preventing transmission by other means; identifying genetic resistance for use in breeding programs to establish resistant lines for replanting; or, in the case of some viruses, designing mild strains to inoculate host trees and thus prevent infection by virulent ones.

Depending on the nature of the pathogen and the host plants under investigation, this complete process could take many years. However, it prepares a sound base of scientific knowledge on which to build strategies to deal with a disease problem. In the long term, it saves resources from being wasted on unsuccessful trial-and-error approaches, and it may provide wider benefits by increasing our knowledge of plant pathogens. We are applying this approach to investigate the cause of Mundulla Yellows.

by Dagmar Hanold and John W Randles

Dagmar Hanold is a molecular biologist specialising in plant viruses and viroids of trees. She has been developing molecular diagnostic methods for new diseases and has conducted extensive disease surveys on palms in the Pacific area.

John W Randles is a plant virologist with a special interest in viroids and the characterisation and epidemiology of plant viruses. He has identified the viral causes of a number of new diseases in a range of plant species.

Both work at the Waite Institute (University of Adelaide), Department of Applied and Molecular Ecology, Glen Osmond, SA 5064. They can be contacted on (08) 8303 7307 or by email (dagmar.hanold@adelaide.edu.au).

Mike Stukely is a Research Scientist with the Department of Conservation and Land Management, based at Kensington. Mike can be contacted on (08) 9334 0299 or by email (mikes@calm.wa.gov.au).

Frank Podger has worked for almost 50 years on diseases of native vegetation in Australia, particularly in WA, where in 1967 he determined the cause of the devastating disease then called jarrah dieback. He later helped to develop strategies and policies for its management. He has had a keen interest in Mundulla Yellows from almost ten years ago, and maintains it in retirement. Frank can be contacted by email (frankpod@tpg.com.au).

Diseases and Pathogens of Eucalypts, published by CSIRO and edited by Keane, Kile, Podger and Brown, provides excellent further reading.

Winner of the Alex Harris Medal for excellence in science and environment reporting.

LANDSCOPE

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An exciting range of recreational opportunities are being offered in some national parks, creating employment for locals. See page 28.

Native animals need tree hollows and people need wood. How are these conflicting uses managed? See page 20.

The search to find out the cause of a new tree killer known as Mundulla Yellows. See page 41.

Declining water levels threaten a remarkable community of cave-dwellers in Yanchep National Park. Turn to page 34.

Re-discovering the long-forgotten memoirs of a Kimberely pioneer. See page 48.

FEATURES

REGULARS

COVER

Leafy seadragons are occasionally seen in the seagrass around the Busselton Jetty (see page 10). They are in the same family as seahorses but, unlike seahorses, they have leafy appendages that make them almost invisible in their surroundings. The male carries the eggs in the skin beneath his tail. After hatching, the young swim off to fend for themselves.

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Editors: David Gough, Carolyn Thomson-Dans.
Story editor: Verna Costello.
Scientific/technical advice: Andrew Burbidge, Keith Morris, Kevin Kenneally, Paul Jones and staff of Science Division.
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Illustration: Gooitzen van der Meer.
Cartography: Promaco Geodraft
Marketing: Estelle de San Miguel ☎ (08) 9334 0296 Fax: (08) 9334 0498.
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