

Benthamiana rhapsody

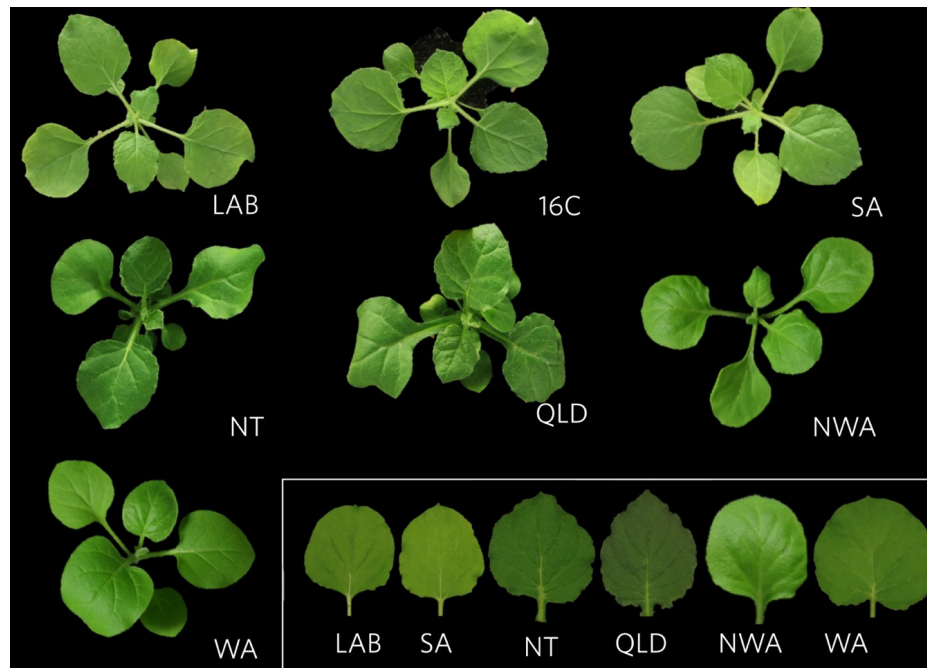
How benthi—this wild tobacco plant from the Australian outback—became a model experimental system that is accelerating research and saving lives.

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The Australian outback circa 1994.

If you're a natural history buff like I am, you will love Australia. Back in the 90s, a younger version of myself and my partner, Saskia Hogenhout, spent months travelling around Australia in a Ford Falcon. We took this clunky rusty old car all over the continent, 35,000 kilometres. We were studying beetles, of all things. In fact, we published a paper on the tiger beetle, as the fastest running insect in the world. We clocked flightless species of this beetle at over 100 body lengths per second—that's about twice as fast as the typical cockroach.



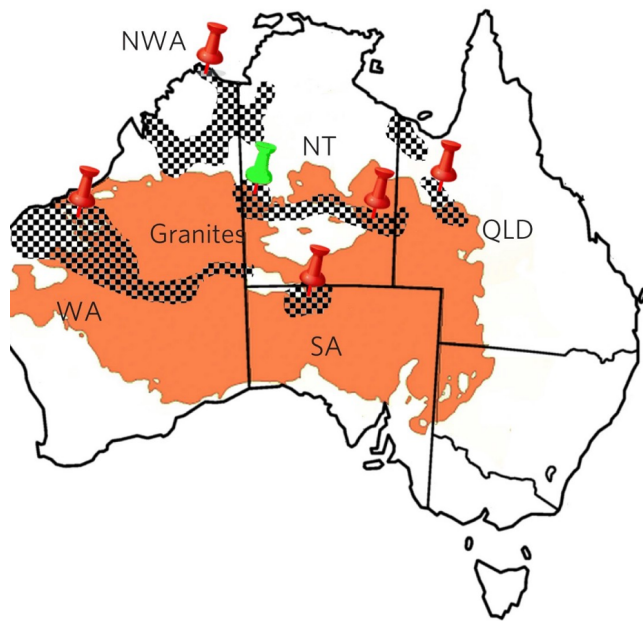
Nicotiana benthamiana aka benthi — the Australian wonder plant. LAB is the commonly used laboratory line is compared here to wild accessions. Source: Bally et al. 2015.

Australian animals get a lot of attention with good reason—if you don’t pay attention, they might kill you. But I’d like to sing the praises of a cool Australian plant, *Nicotiana benthamiana*, which is a wild relative of the tobacco some people smoke (*Nicotiana tabacum*). In many plant biology labs, we call *Nicotiana benthamiana* ‘benth’ or ‘benthi’ because we simply love this plant. We use it a lot in our research on plant pathology and we’re not the only ones—if you scan the literature, particularly the plant–microbe interactions literature, you’ll come across benthi all the time.

Transient expression: the key to benthi’s success

Why is benthi so popular? Well, it’s because of this method called agroinfiltration. Agroinfiltration allows you to transiently express genes in benthi, using the plant pathogen *Agrobacterium tumefaciens*. By doing that, you can very quickly study the function of these genes. This helps us address one of the big problems of the post-genomics era, where it’s relatively easy to find a gene (or a lot of genes), but hard to figure out what a particular gene does.

Let’s look at how we perform agroinfiltration to express a gene of interest in benthi. This is a movie that a student of mine, Jing Song, made years ago. It illustrates how to perform an agroinfiltration



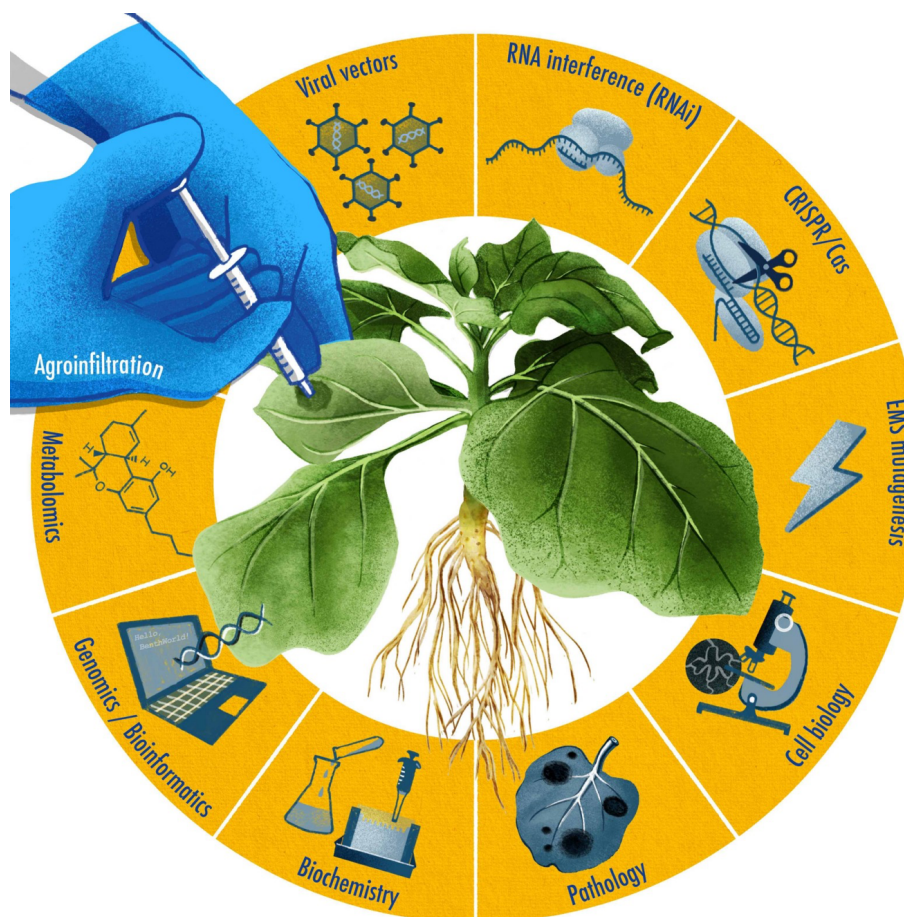
Collection sites (pins) of *Nicotiana benthamiana* accessions studied by Bally et al. 2015. Green pin is collection site of LAB accession.

experiment. You take a syringe, you fill it up with a suspension of agrobacteria. These agrobacteria carry a DNA construct that is designed to express a specific gene that you are interested in testing. Using the syringe, you push the suspension of agrobacteria into the open spaces within the leaf. There, the agrobacteria transfer the DNA construct into the plant cells, where it can be expressed.

Plant Biology: Agroinfiltration of the wild to...

The gene you use can be an effector (virulence gene) from a plant pathogen, a plant immune receptor gene, or any host protein you're interested in. It can be a gene from another plant, even distantly related ones like rice and wheat. The gene can encode a protein that is tagged, for example, with a fluorescent protein. It can be mutant to test the effect of the mutation on the gene function. It can be a mixture of two, three or more genes to find out how they affect each other. Essentially, you can do all sorts of things. The system allows you to unleash your experimental creativity.

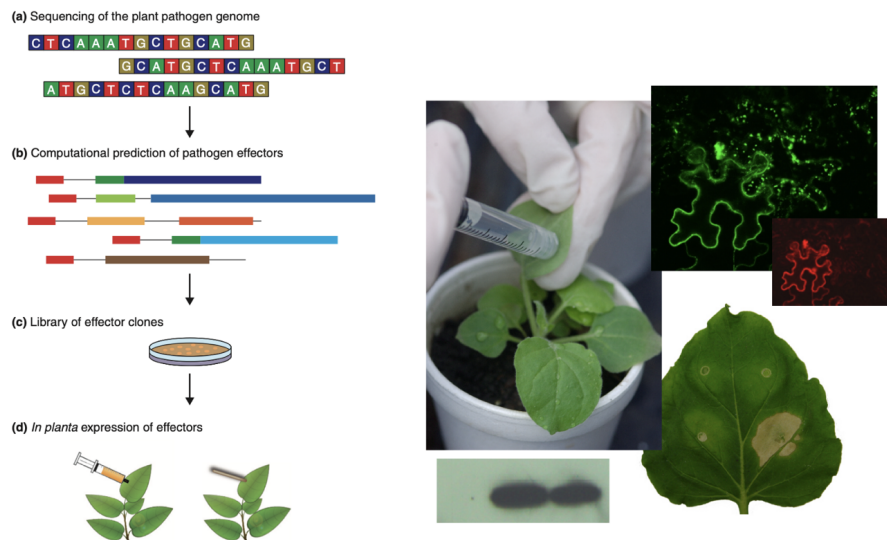
Once you infiltrate the leaf with the agrobacteria, after a couple of days, the leaf cells will be expressing the gene of interest. You can do all sorts of experiments with that leaf. You can look at the localization of a fluorescently tagged protein. You can check if the bacterial effector altered metabolism in the plant cell. You can even challenge that leaf with a pathogen to find out if the infection is affected or monitor the cell biology of the interaction.



Benthiana — the workhorse of plant biology. Image art by Hsuan Pai. Adapted from Figure 1 of Derevnina et al. 2019.

A new model system

Functional analysis by transient expression has become a very popular tool in the era of genomics. Because basically, in the post-genomics era, when we have genomes of pathogens and plants, we need new ways of doing business to link genes to biological activities. We need pipelines that allow us to quickly go from gene sequence to function. And Benthiana is just perfect for this.



New way of doing business

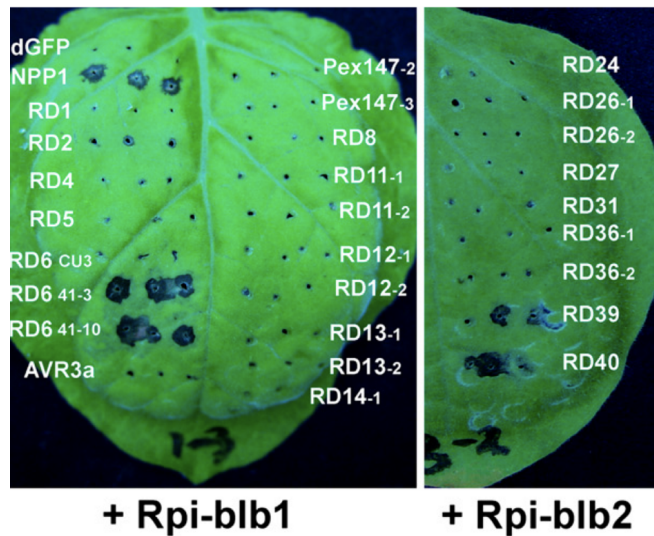
Discovery pipelines
Effectors and immune receptors

Benthi is particularly appropriate in the post-genomics era, for example to perform high-throughput screens to connect plant pathogen effectors to biological activities. Adapted from Pais et al. 2013.

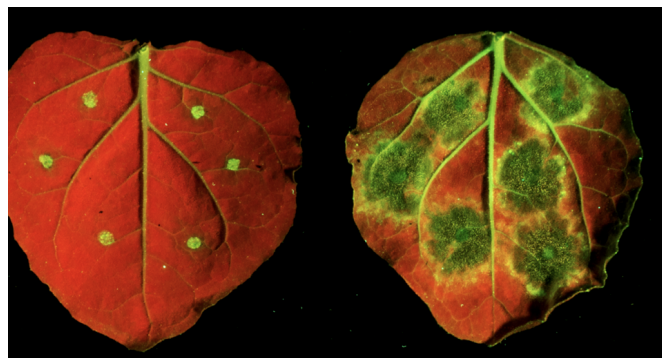
One weakness of *N. benthamiana* has been genetics. Where *Arabidopsis* and maize are optimal genetic systems, benthi has been limited in part because of a lack of mutants. This has recently changed. We can now use CRISPR to make mutants and the quality of the genome sequence of *N. benthamiana* has dramatically improved, thanks in large part to work by a consortium led by Peter Waterhouse, Queensland University of Technology, Brisbane, as well as a genome assembly produced by the Boyce Thompson Institute for Plant Research. Mark that as another tool in the benthi toolbox, allowing benthi to take its well deserved place as a model system along with classical systems like maize, rice and *Arabidopsis*. These resources and the growing literature illustrate the rise and rise of benthi as a model system.

Benthi in the lab: fast-forward functional analysis

How does benthi impact my own research? In one popular application, we predict particular genes from genomes based on sequence features. This step is purely computational. These genes can be pathogen effectors, or they can be the immune receptors that plants use to detect the effectors. Then, we co-express the effectors and the receptors in a benthi leaf using agroinfiltration and look for combinations that yield an immune response: notably, the striking hypersensitive cell death



Expression of effectors from the plant pathogen *Phytophthora infestans* in the potato plant immune receptors Rpi-blb1 and Rpi-blb2 reveal the effectors that are detected by these immune receptors. Each effector is expressed in triplicate. Immune response is the dark hypersensitive cell death around the inoculation site. Source: Oh et al. 2009.



The potato immune receptor (aka R gene) Rpi-blb2 confers resistance to *Phytophthora infestans* (left leaf) when expressed in *Nicotiana benthamiana*. The leaf on the right is a control showing the infection lesions caused by the pathogen. Source: Kamoun Lab.

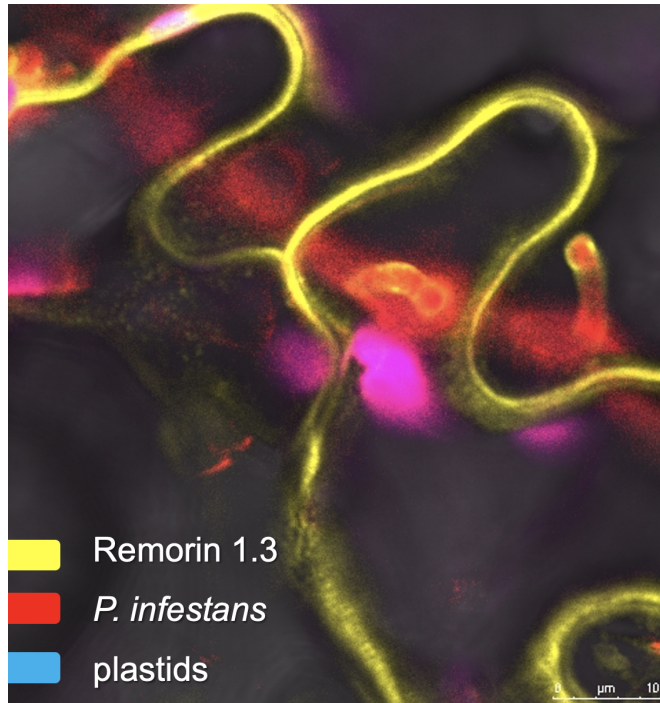
response. This allows us to screen for the effectors that are detected by these immune receptors, by co-expressing the immune receptor with a panel of different effectors or the effector with a panel of receptors. This is a high-throughput method and it has enabled us and many others to screen through large collections of effectors. So, we couldn't resist naming the tech 'effectoromics'.

In addition, we can take genes from crop plants, like potato, and express them in benthi. For example, we can take a disease resistance gene, so-called *R* gene, from a wild potato, and introduce it in benthi. As you can see in this figure, the gene we chose is functional and provides resistance against the potato blight pathogen, just as it did in the original wild potato plant. We can even take resistance genes from monocot plants, from grasses like rice, wheat, and study them in *N. benthamiana*. We can co-express them with pathogen effectors and find out whether they can detect the pathogen. [My colleagues Juan Carlos De La Concepcion and Mark Banfield at](#)

[John Innes Centre and Ryohei Terauchi at Kyoto University did just that to find out which rice resistance genes function against the blast fungus and make mutants that have improved activities.](#)

In my lab, a few years back, [Maria Eugenia Segretin](#) and then [Artemis Giannakopoulou](#) used benthi to screen for slightly over-active mutants of a resistance gene that functions against oomycete and fungal pathogens. These variants were just a bit more active than the original resistance protein. We called them 'trigger-happy', and yes, they were ready to fire

faster and would trigger a stronger immune response than you would normally get with the wild-type sequence. I can foresee how some of these mutants, first identified in benthi, could then be introduced by CRISPR-based gene editing into a crop to make slightly more effective.



Fast-forward cell biology of the plant-pathogen interface. The image shows the pathogen hypha in red and its protruding haustoria that are invading the plant cell cavity and surrounded by the Remorin 1.3 plant protein. Adapted from Bozkurt et al. 2014. For more information, see Bozkurt and Kamoun 2020.

Another application that was developed by my colleagues Sebastian Schornack and Tolga Bozkurt is combining agroinfiltration with pathogen inoculations to do cell biology on the infection interface between pathogen and plant. All you have to do is tag the proteins of interest with fluorescent markers, agroinfiltrate them in a benthi leaf, inoculate with the pathogen (say, the potato blight pathogen *Phytophthora infestans*). Then, using a confocal laser microscope, you can light up the fluorescent protein and study the interface between the pathogen and the plant. Where does the protein accumulate? Is it perturbed by the infection? You can see in this example that the plant protein remorin,

labelled in yellow, is highlighting the intimate interface between the pathogen *Phytophthora* and the invaded plant cell of *N. benthamiana*.

Tolga Bozkurt went on to build his lab at Imperial College London around this fast-forward cell biology method. In recent years, his team showed how plant autophagy pathways, chloroplasts and even plant immune receptors can home in on the pathogen point of infection (the haustorial interface).

What a wonderful, amazing system, right? All those things we can do with benthi. And so quickly. In my lab, they call Friday ‘infiltration day’. That’s when they rush to the plant growth facility to agroinfiltrate benthi plants. Then, by Monday the leaves are ready to process, and by the end of the week you have your results. This is why we call it fast-forward plant biology. It’s so quick that it allows us to test dozens of hypotheses in a time-efficient way. The rapid turn-around enabled by this

experimental system is critical for the iterative process of hypothesis-driven research that can lead us to long-lasting impactful discoveries.

Benthi for the future

Benthi is even used to produce vaccines or new drugs, in what is known as 'biopharming'. One Canadian company, Medicago, even has a few products in its pipeline. In December 2021, Medicago and GSK reported that their benthi produced COVID-19 vaccine is ~75% effective against preventing disease of any severity from the Delta variant of SARS-CoV-2. The impact of this odd Aussie plant goes way beyond plant pathology and plant-microbe interactions.

The benthi toolbox has accelerated the pace of research in plant pathology and plant-pathogen interactions and impacted work on the crops that feed humankind: potato, rice, wheat, and others. We are using this unexpected experimental system to solve the global plant health problems that we face. From curiosity-driven research and diversity studies in remote parts of Australia, we get an experimental plant to study molecular mechanism of plant disease biology.



Nicotiana benthamiana in its wild habitat in Western Australia. Source: Royal Botanical Gardens Kew.

Let me conclude by taking a moment to appreciate the work of experts like Sandy Knapp of the Natural History Museum and Mark Chase of Kew Gardens who have spent their careers exploring and describing plant biodiversity. They are our modern day plant hunters and much more. We need scientists like Sandy and Mark to understand better the

biodiversity of the world we live in, and we need to preserve it. Who knows what other surprises will come out of the natural world. I'm excited to find out, just give the keys to that Ford Falcon.

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