DALE WALTERS

# FORTRESS PLANT

*How to survive when everything wants to eat you*

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*For Beverley*

# ACKNOWLEDGEMENTS

I first thought about writing this book nearly twenty years ago when I was walking the dogs. But then my work commitments increased hugely and, reluctantly, I had to put the idea to one side. When I eventually got round to the book again, not only had the field of plant defence moved on, but I began to question whether any publisher would be willing to take on a book on this subject. Here I got lucky, because I sent some material to Latha Menon at OUP and, to my amazement and delight, she was encouraging. That encouragement led to a contract with OUP, culminating in the volume you now hold in your hands. I am grateful to Latha, not just for taking on this project, but for her support and careful editing. Others at OUP have provided support and answers to my numerous questions, and my thanks go especially to Jenny Nugee, who has the patience of a saint, and to Martha Cunneen, Carrie Hickman, Clare Jones, and Phil Henderson. Thanks also to Elizabeth Stone for her meticulous copy-editing, and Nicola Sangster for proofreading. During the writing of this book I have been helped by a number of people who answered queries, provided images and offered to read what I had written. I am grateful to Pietro Spanu, Angela Overmeyer, Richard O'Connell, Danny Kessler, Jonathan Gershenzon, Ian Grettenberger, Gerald Holmes, Martin Heil, Francis Martin, Mark Brundrett, Simon Walker, Allan Downie, John Randles, Stuart MacFarlane, Harry Evans, Neil Havis, and Graham McGrann. Parts of the text deal with historical aspects of research in specific areas. I have tried my best to get things right but I'm sure not everyone will agree with what I've written. Any errors of fact are mine alone.

When I started writing this book, I was still working and I am grateful to two successive Heads of Department (Bill Spoor and Fiona Burnett) for allowing me the freedom to pursue this venture. My special thanks, however, go to my wife Beverley. She has provided unwavering support and

encouragement through every step of this journey, as she has for the past 43 years of our life together. How she put up with me while I wrote this book, when she herself was ill, is beyond me. Thanks are simply not enough.

**DALE WALTERS**
*Prestwick*
*July 2016*

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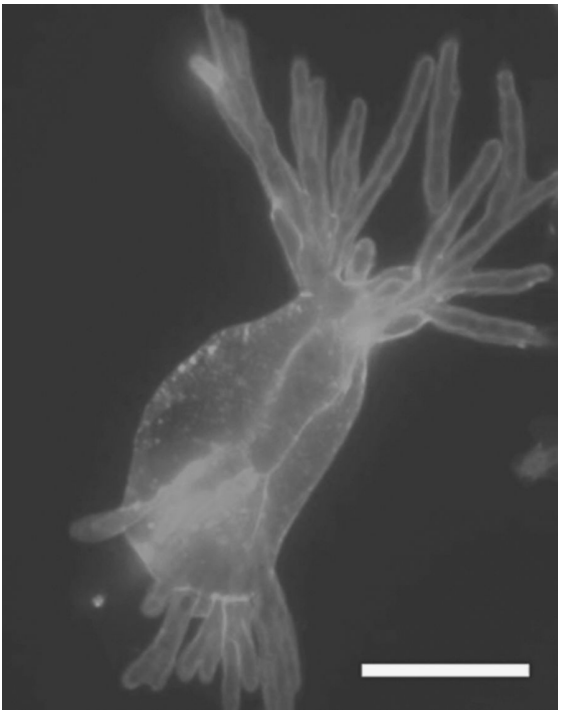
# ABBREVIATIONS

| | |
|---|---|
| **ATP** | Adenosine triphosphate |
| **DAMP** | Damage-associated molecular pattern |
| **ETI** | Effector-triggered immunity |
| **GLR genes** | GLUTAMATE RECEPTOR-LIKE genes |
| **HAMP** | Herbivore-associated molecular pattern |
| **HCD** | Hypersensitive cell death |
| **HR** | Hypersensitive response |
| **JA-Ile** | Jasmonyl-isoleucine |
| **LRR** | Leucine rich repeat |
| **PAMP** | Pathogen-associated molecular pattern |
| **PGIP** | Polygalacuronase-inhibiting protein |
| **PTI** | PAMP-triggered immunity |
| **ROS** | Reactive oxygen species |
| **RNA** | Ribonucleic acid |
| **SAR** | Systemic acquired resistance |
| **TIR** | Toll interleukin receptor |
| **TMV** | *Tobacco mosaic virus* |
| **WASP** | Wound-activated surface potential |



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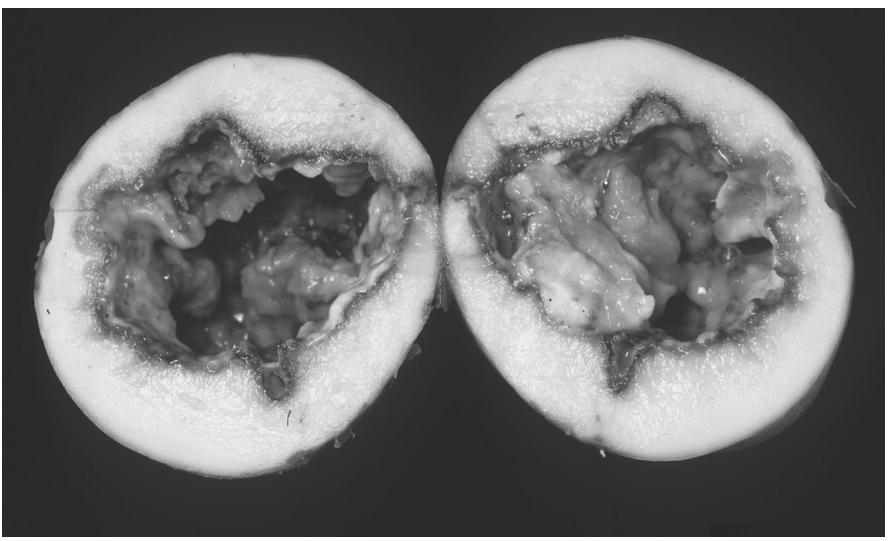

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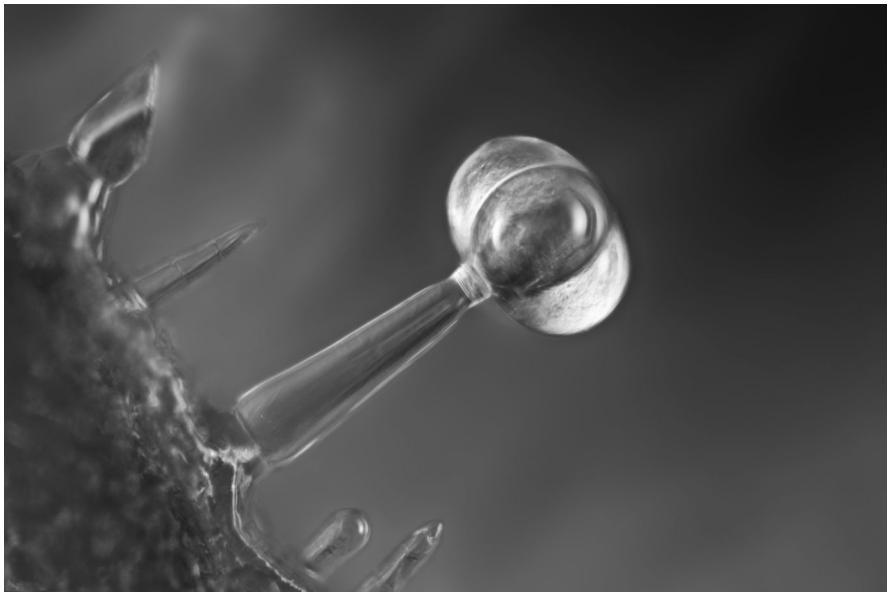

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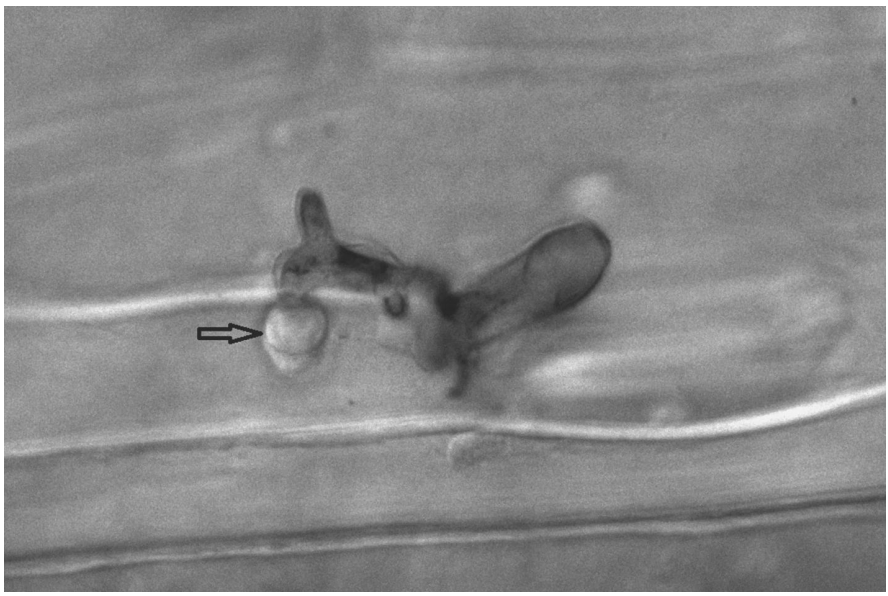

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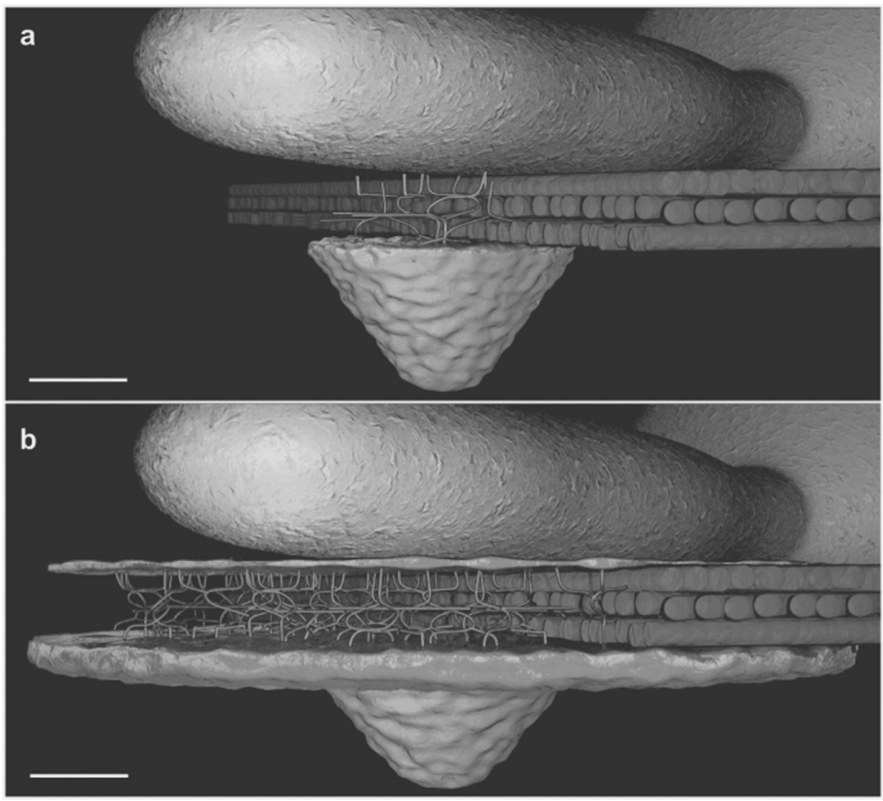


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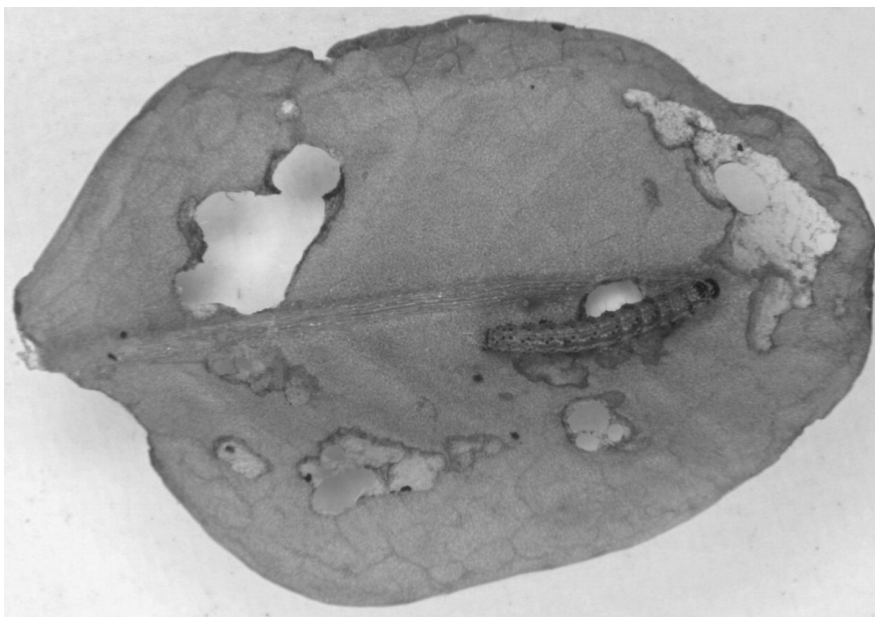

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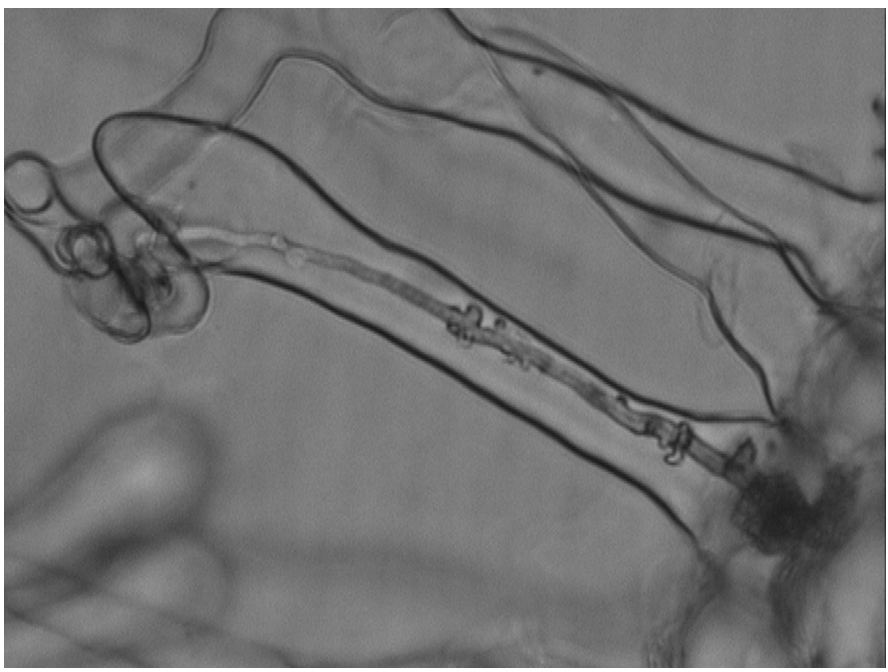

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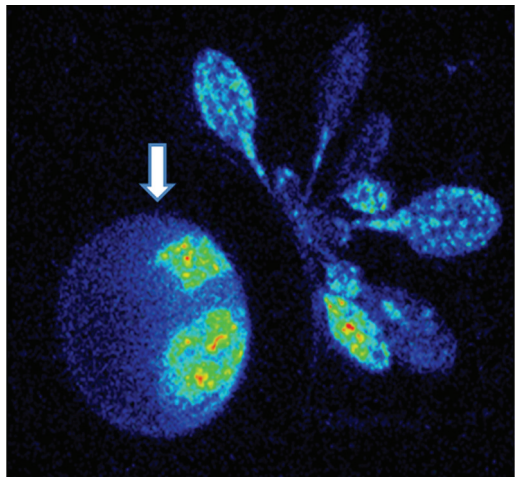

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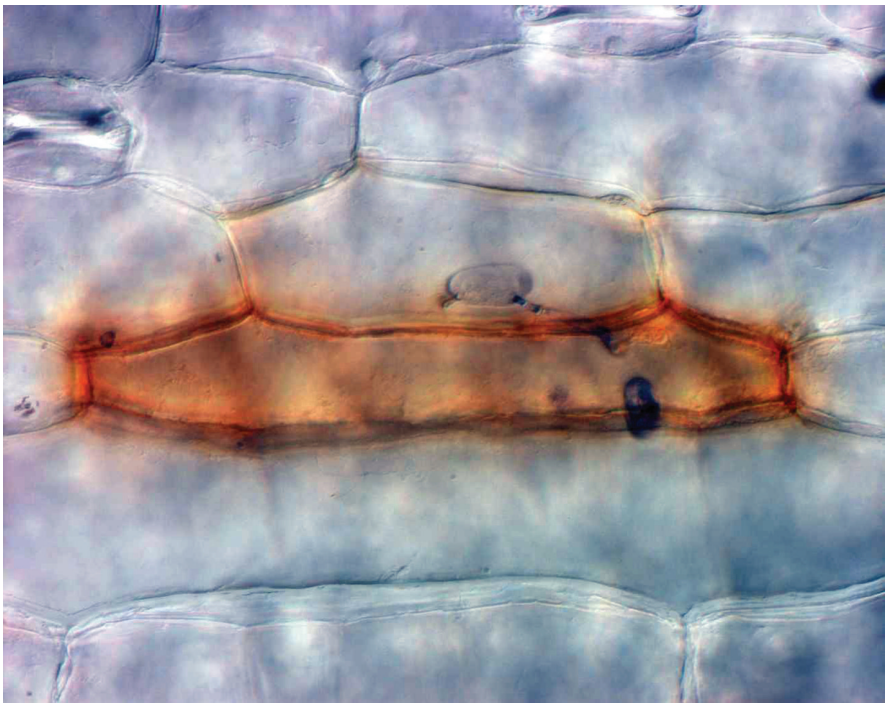

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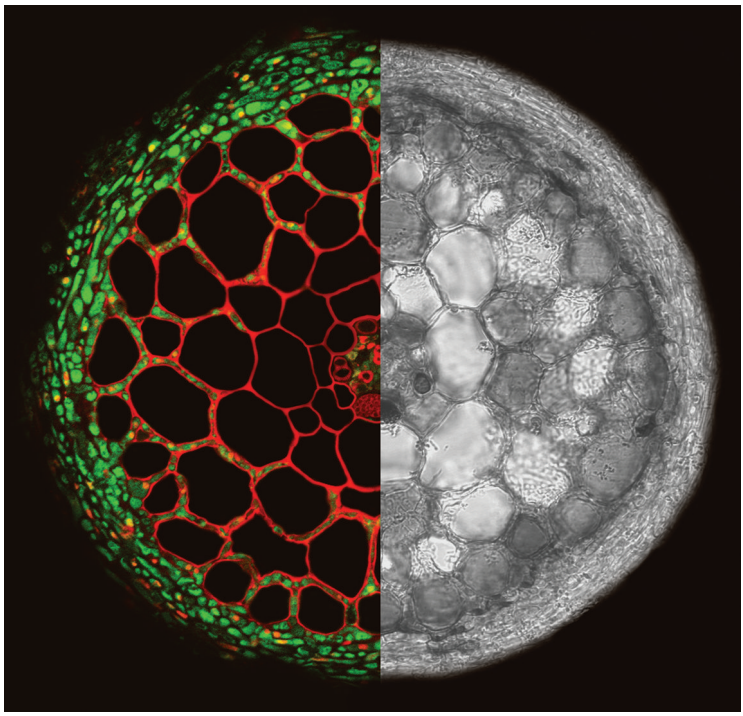

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# Prologue

They alighted silently and after making sure they had the correct target, began their assault. This was swift and efficient—they were expert at this type of warfare. They needed to be expert, because their target possessed the most sophisticated early warning system available, backed up by a formidable arsenal, capable of repelling any attackers. But the invaders were also well equipped. Over long periods of fighting this opponent, they had learned from their mistakes and had developed an effective attack strategy, backed up by state-of-the-art weapons. This ongoing war between the two sides was like a never-ending game of cat and mouse. No sooner had one side found a way of evading the enemy's surveillance systems, the other side would increase the sensitivity of their detection systems. Still, theirs was an essential mission. Their very survival depended on them gaining access to the valuable resources held within the enemy's walls. This time however, their luck ran out. The target was one step ahead of them and they had just begun their attack, using targeted chemical weapons and mechanical strength to breach the outer wall, when all hell broke loose. The defender's early warning system was quick in picking up their attempt to breach the outer walls and responded rapidly by unleashing its own chemical weapons against the attackers. The situation was desperate, as the attackers found their tissues being destroyed by corrosive chemicals, and to make matters worse, the defending side was able to repair damage to the outer walls almost as soon as they were breached. To top it all, any attackers that managed to break through into the defender's territory

found themselves in an unbelievably hostile environment, where the deadly mixture of chemicals deployed by the defender killed them off quickly. The attack was disastrous, but the war was far from over. There were lessons to be learnt from this encounter and the attackers would be much better prepared the next time.

You would be forgiven for thinking that I was describing a siege, with soldiers from an invading force attempting to break through into a fortified city. It could be a battle from the Middle Ages, or from one of the many more recent conflicts that, unfortunately, rage across various parts of the world. But you would be wrong. The skirmish portrayed above is from the plant world, and describes the attempt by a fungus to enter a plant leaf. The attacking fungus is after the food locked up in the cells of the plant, but the plant is well equipped to deal with such attacks. This is a world which is largely invisible to us. Yes, we can see plants and sometimes these plants develop strange spots, or lose chunks out of their leaves, and can keel over and die. But what we don't see is what goes on inside the plant. Here, in a world visible only using powerful microscopes, plants live out their lives in ways which are every bit as sophisticated, awe-inspiring, and wonderful as the lives of animals. And yet most people don't give plants a second thought.

Many think that plants are boring (they don't move!) and all they are good for is eating or walking on. This is a shame, because not only are plants vital for all life on our planet, they are also amazing. In this book, I want to show you just how amazing. I will focus on how plants defend themselves against attack. Plants are food for animals, microbes such as bacteria and fungi, and even for other plants. With plants on the menu for so many other living things on our planet, it is surprising they survive. The fact that plants not only survive, but are to be found in great abundance and variety in most environments, is testimony to their ability to adapt, and to defend themselves against equally adaptable and persistent attackers.

# 1

# How to get your five-a-day

If you think that this is another helping of information on how to grow vegetables or how to cook them, be assured that it is not. There are enough books already dealing with planning your vegetable garden or what to do with the produce once you've harvested it. No, this chapter is going to look at the variety of other organisms that rely on plants for their nutritional requirements. A great many things eat plants or use plants as food. Some are very familiar: cows, sheep, goats, rabbits, deer, elephants, hippos. Much smaller animals also eat plants. I am referring to insects, and those of us with an interest in gardening will be all too familiar with the damage insects can inflict on our plants in their quest for nutritional fulfilment. Most of us will be aware of the abundance of insects of all different types, sizes, and shapes. There seem to be so many of them. In fact, some 751,000 different species of insect have been identified,[1] representing more than half of all catalogued species of organism on this planet (1,438,769 species).[2] That's a lot of insects, but apparently this figure is an underestimate. It has been estimated that there are 30 million species of arthropods (arthropods include insects, spiders, crustaceans, centipedes, etc.) in tropical forests alone, of which the great majority are insects. A good many of these insects will live off plants, so it is just as well that plants are quite abundant too. There are 224,244 species of higher plant currently known,[2] the majority of these comprising the *angiosperms* or flowering plants. These include the *monocots* (those plants with one cotyledon or seed leaf in the embryo) such as the grasses; and the *dicots* (plants

with two cotyledons in the embryo) ranging from beans and potatoes to sunflowers and roses. Also included in the higher plants are *gymnosperms* such as pine trees (these are plants where the seeds are unprotected and open to the environment; the name gymnosperm means 'naked seed'), ferns, bryophytes (mosses, liverworts), and so on. Insects consume just about every part of plants, from leaves to flowers and fruits. Some of these insect pests—certain caterpillars and locusts, for example—consume large quantities of leaves, while others, such as aphids, are more dainty and insert a straw-like stylet into leaves in order to take up sap (Plates 12, 14, 15). But the exploitation is not all one way, for insects can also be useful to plants, with many plant species depending on insects for pollination and reproduction.

Of course it's not just animals that use plants as food, though they are certainly conspicuous, and in many cases we can actually see plant parts or even whole plants being consumed, often very quickly. But there are much smaller organisms that depend on plants for their nutrition—micro-organisms or microbes. Those that cause damage to plants (pathogens) include fungi, bacteria, and viruses.

To date, 44,368 species of fungi have been catalogued.[2] These remarkable and adaptable organisms can be found in all environments. Fungi play a crucial role in the biosphere and are the most important degraders of dead organic matter. But many fungi are parasitic, attacking animals, plants, and even other fungi. Those that interact with plants aren't all bad. Indeed, as we shall see later, some—called mycorrhizal fungi—exist in a symbiotic state with plants, with both partners benefiting from the relationship. Walk through the woods in the autumn and you will come across dozens of mycorrhizal fungi, although you will probably not realize it. Most of the body of these fungi—the mycelium, consisting of very thin threads called hyphae—will be associated with the roots of trees in the wood, with only the reproductive parts of the fungi, their fruiting bodies (mushrooms and toadstools), appearing above the soil surface. But our main concern here are those fungi that parasitize plants. Roughly speaking they can be split into two groups depending on how they obtain their food from the plant. The biotrophs, such as powdery mildews and rusts (Plate 16), need to keep

the host plant alive, since if the plant (or even the plant cell they are trying to infect) dies, so too will the fungus. These fungi have a very subtle relationship with the plant cells, using highly specialized feeding structures called haustoria (Plate 1) to absorb the nutrients they require for their growth and reproduction. So intimate and specialized is this relationship, that the haustoria never actually penetrate the membrane surrounding the plant cell. They are in this respect remarkably similar to the feeding structures used by mycorrhizal fungi, but more of that later. In contrast to the civilized approach to parasitism practised by biotrophs, the other group of fungi, called necrotrophs (the name gives away their lifestyle; Plate 13), don't need to keep the plant cells or tissues alive, since they are able to live off the dead tissue. Indeed, for many of these fungi, death of the cells they are infecting can't happen quickly enough, since the more rapidly they kill the plant cell, the less likely it is to mount a defence. These fungi use all manner of armaments to subdue the plant cells—enzymes that degrade the walls surrounding the cells, toxic compounds which can put the cell machinery out of action, or even both. This seems a terribly uncivilized approach to getting food from plants, although it does away with having to go to the trouble of keeping the plant alive.

Some pathogens are not all they seem. It's not that they are not good at being pathogens—because they are—it's just that although they look like fungi, they are not fungi. I am referring to the Oomycetes. This is a large group of land-living and aquatic organisms that resemble fungi in the way they grow (they have hyphae and mycelia) and obtain their food, but which, in fact, are grouped together with brown and golden algae and diatoms.[3] Included in the Oomycetes are the water moulds, which cause diseases of fish and other aquatic vertebrates. The terrestrial members are mainly pathogens of plants and include the downy mildews that affect hosts as diverse as grapes and sorghum, damping-off, the scourge of seeds and seedlings, and the devastating late blight of potato, *Phytophthora infestans*. This group of organisms has a seriously impressive pedigree as pathogens, having caused both massive crop losses and considerable human suffering.

All the organisms we have looked at so far are eukaryotes. These are organisms whose cells contain their nuclei (the region of the cell containing the chromosomes) bounded by a membrane. Eukaryotic cells also have other internal compartments that, like the nucleus, are surrounded by membranes. For example, all such cells contain mitochondria, the so-called powerhouses of the cell, which convert the energy trapped in food substances into a form the cell can use for all of its various activities. In addition, plant cells contain chloroplasts. These amazing organelles use energy from light, captured by the green pigment chlorophyll, together with carbon dioxide from the air, to make sugars. In contrast to these eukaryotes, the single cells of prokaryotes lack nuclear and other membrane-bound internal compartments (Figure 1). Prokaryotes can be divided into two domains, the bacteria and the Archaea, of which some 11,500 have been catalogued.[2] The prokaryotes have the most ancient

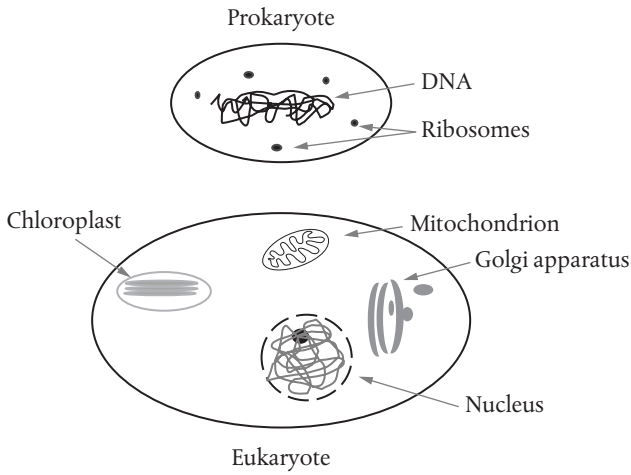


**Figure 1** Diagram illustrating the relatively simple cell structure of a prokaryote and the more complex cellular makeup of a eukaryote. In the prokaryotic cell, the single chromosome containing the DNA is situated within the cytoplasm. The eukaryotic cell is considerably more complex: the chromosomes are housed within the nucleus, and there are also organelles (mitochondrion and, in the case of plants, chloroplast) and an endomembrane system (Golgi apparatus). Note that prokaryotic cells are typically much smaller than eukaryotes (the drawings are not to scale).

origins of any group of organisms still present on Earth today, with fossil records dating back 3.5 billion years. They were the only living organisms on an otherwise sterile planet for more than 2 billion years, adapting to the ever changing environment of the Earth. They are outstandingly successful organisms, having spread to every conceivable habitat. If success is measured by numbers of individuals, then the bacteria in one person's mouth outnumber all the humans who have ever lived! These are obviously very small organisms, such as *Escherichia coli*, a bacterium inhabiting our intestines, measuring just 2 μm in length and 0.8 μm in diameter (1 μm = one millionth of a metre). Most bacteria play positive and important roles, including converting atmospheric nitrogen into inorganic forms of nitrogen that can be used by plants, known as 'nitrogen fixation'. Only a small number of the known species of bacteria parasitize animals or plants, but those that do cause great damage and suffering. Although bacteria use natural openings like stomata (the pores at the leaf surface that can open and close, letting in carbon dioxide for photosynthesis, but allowing water to escape in transpiration) and wounds (scars created when leaves naturally fall off the plant, for example), they use similar methods to fungi to obtain food from plant cells. Many bacteria attacking plants will use enzymes to degrade cell walls and toxins to subdue the plant cells, leading to some impressive and sometimes smelly damage to plants. A good example is the bacterium *Pectobacterium carotovorum,* which causes a soft rot on potatoes, vegetables, fleshy fruits and ornamentals (Plate 2). This pathogen is a favourite in plant pathology practical classes, where students are set the task of inoculating wounded potatoes or carrots with the bacterium in order to obtain infection. The plant material is inoculated quite simply by transferring some of the bacteria from a culture growing in a nutritious liquid into the wound, placing the inoculated potatoes or carrots into a container with some moist tissue paper, and waiting until their next practical class. The stench in the lab once the students have opened the containers of now well-rotting potatoes is stomach-turning. But it does illustrate the damage that can be caused by a microscopic organism. Pathology, whether animal or plant, is truly a gruesome business.

If bacteria are small, viruses are much smaller (the largest virus is about 500 times smaller than a small bacterium) and simpler in structure than bacteria (see Figures 2 and 3). In fact, viruses are so simple that they cannot be called cells (and they can barely be regarded as living). Viruses consist of a core of nucleic acid (either RNA or DNA) surrounded by a coat of protein. This nucleic acid core contains the genetic information for making more virus particles, but since the virus does not have the cellular machinery to do this itself, it must subvert the genetic machinery of the host cell to make new copies of itself. It is, in effect, a pirate; a very successful one.[4]

Like bacteria, viruses don't have the means of getting into a plant unaided. They need help, and many viruses get this help from animals. So insects—aphids for example—can pick up virus particles when feeding on a plant and transfer the virus to other plants when they move on to the next meal. Viruses can also be carried, and spread, by nematodes (small roundworms living in the soil, some of which parasitize plants), and believe it or not, by fungi. The fungus *Spongospora subterranea,* which causes a disease of potatoes called powdery scab (a common disease, familiar I'm sure to most of us who regularly peel potatoes), is a vector of the *Potato mop top virus.*

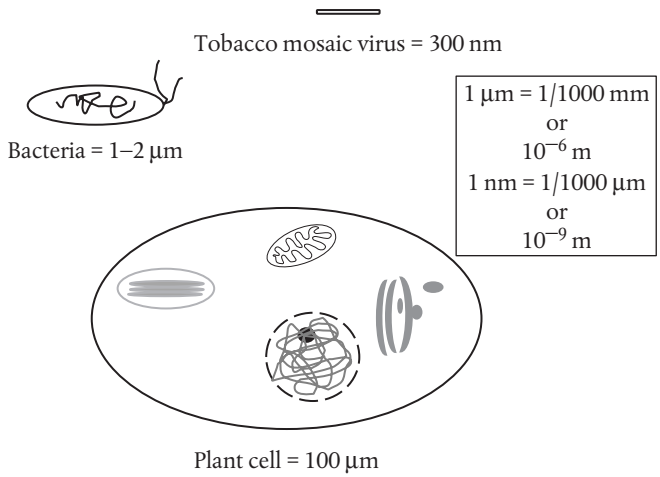


**Figure 2** Relative sizes of a plant cell, a bacterial cell, and a virus particle (*Tobacco mosaic virus*).

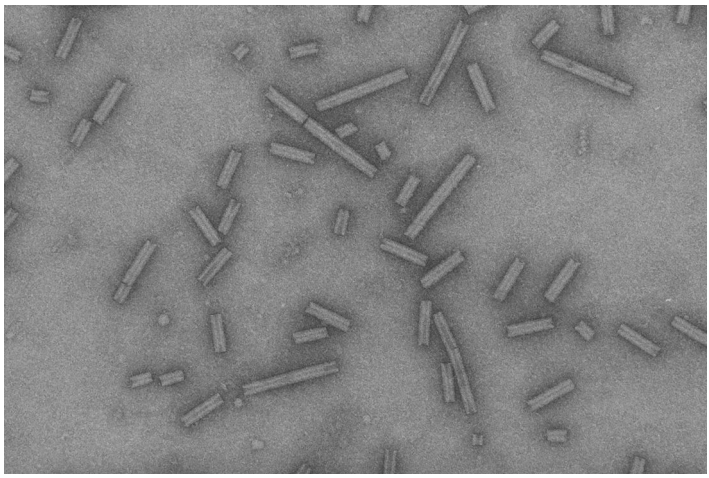

**Figure 3** Electron microscopy image of particles of the *Pea early-browning virus*. The long particles of the virus are about 215 nm in length and the shorter particles are about 105 nm in length. There are also some small (probably broken) particles in view.

But viruses are still very large indeed compared to viroids. A viroid contains a single strand of the nucleic acid RNA and does not even have a protective protein coat. They are exceptionally small, being about one-thousandth the size of the smallest virus. Viruses are so small, they are measured in nanometres (nm), where 1 nm is $1 \times 10^{-9}$ m, or one thousandth of a micrometre (µm). As a guide, particles (or rods as they are called) of *Tobacco mosaic virus* are 300 nm long by 15 nm in diameter. At one-thousand times smaller than this, viroids really are very small.[4] But, as you are only too well aware by now, small can still be mean. As far as we know, at least forty plant diseases are caused by viroids, including cadang-cadang (dying) disease of coconut. This disease has been responsible for the loss of more than 30 million coconut palms since the 1930s. Even today, over eighty years later, 1 million coconut palms are killed by cadang-cadang every year. The frightening aspect of this disease is that no control measures work, and the disease is spreading from infected areas at the rate of about 500 metres every year.[5]

As if it's not enough that plants are on the menu for animals and microbes, they are not even safe from other plants. About 1% of angiosperms, some 3,000 species, parasitize other plants.[6] These freeloaders include the European mistletoe (*Viscum album*), dodder (*Cuscuta*), and witchweed (*Striga*), the latter of which wreaks havoc on crops in various parts of the world. Some parasitic plants, such as dodder, don't have chlorophyll and so cannot carry out photosynthesis. This makes them completely dependent on other plants for their supply of carbohydrates. Other parasitic plants, of which *Striga* is a good example, are green and are able to photosynthesize, but still need other plants to provide most of their carbohydrates. Once these parasites have entered the host plant, via the root or the stem, for example, they link up with the vascular system of the host—either the xylem (the water-conducting vessels in plants) or the phloem (the vessels responsible for carrying carbohydrates and other materials from leaves to the rest of the plant). This enables the parasite to obtain its supply of water, carbohydrates, and other goodies from the host plant. This diversion of water and foodstuffs away from the plant and into the freeloader is bound to affect the host. In fact, *Striga* can remove so much water and carbohydrate from its host that whole crops can be virtually wiped out. If wiping out its host seems to be a silly strategy for the *Striga*, think again. *Striga* is an annual parasite, like its host, and as long as it produces seed before the host perishes, all is well (for the *Striga* at any rate).

Plants are under attack by all the organisms we've looked at in this chapter, and by others we have not dealt with, phytoplasmas and spiroplasmas, for example. Perversely, it is quite often the smallest of these attackers than cause the greatest death and destruction. How can plants possibly survive such an onslaught? The fact that plants continue to thrive is testament to the ingenious methods they use to deal with potential diners. The trick is to recognize that you're being attacked and then do something about it. This sounds simple enough, but the recognition has to be quick, otherwise the unsuspecting plant will be overrun by the attacker.

To make life more difficult, many of these attackers have stealth on their side. Many years of battling with their host plants have enabled them to come up with various means of evading recognition. So it seems that recognition of the enemy is a vital first step for the plant to defend itself. Let's take a closer look at how plants are able to recognize the baddies.

# 2

# Recognizing the enemy

Faced with the threat of being eaten, most animals move away from the source of danger as quickly as possible. Of course, not all animals can move at great speed, in which case, some form of external protection is handy. The hare can speed away from trouble, leaving the poor old tortoise far behind. Still, what the tortoise lacks in speed, it makes up for in body armour. Getting through that thick shell can't be easy. For a plant faced with the threat of being eaten, things look rather bleak. After all, they can't run away, even very slowly, and most of them have no obvious external protection. Come to think of it, it's hard to imagine that plants can sense much at all—and certainly not an enemy that is microscopically small. Micro-enemies of plants include fungi such as powdery mildews, which can be blown on to leaves in the wind. A fungal spore landing on a leaf surface might as well be landing on a table top—it's surely not going to be detected. Or is it?

Plants are well known for responding to mechanical stimuli. Charles Darwin was fascinated by how plants respond to external stimuli, describing, for example, how roots of different plants change their direction of growth upon encountering a physical barrier.[1] One aspect of the physical environment that plants must contend with is wind. A common sight on exposed coasts are trees, alive and well, with trunks bent, growing in the direction of the prevailing wind. Plants also respond to more gentle breezes and, in fact, plant responses to such mechanical stimuli, including touch, are well documented. Most of us will be aware of the sensitive plant,

*Mimosa pudica,* the leaves of which fold within a second of being touched, or the Venus flytrap, whose leaves have trigger hairs that cause the trap to shut with similar rapidity when they are disturbed. These particular plants have specialized sensory cells that respond rapidly to mechanical stimulation. The responses of most plants to wind, or other mechanical stimuli, take rather longer and result in changes in growth. In some plants, the response is to become shorter and sturdier, while in others, there is a relaxation of the stimulated part of the plant. Both of these responses are thought to help the plant cope with mechanical stresses such as wind. This phenomenon, known as thigmomorphogenesis, is a slow response of plants to mechanical stimulation.[2]

## Early warning systems

Clearly, plants can respond to mechanical stimuli such as wind, but are they able to detect a potential attacker, a fungal spore for example, on the surface of one of their leaves? Experiments conducted at the University of Fribourg in Switzerland provide a tantalizing glimpse of just how sensitive leaf surfaces are. Using thale cress (*Arabidopsis thaliana*), a model plant much used in plant science, researchers applied what they called a 'soft mechanical stress' to leaves.[3] This involved gently rubbing the leaf between thumb and forefinger without pressing the thumb. Treating the leaves in this way made them more resistant to attacks by the grey mould fungus (*Botrytis cinerea*). This is remarkable enough, but amazingly, signalling changes within the treated leaf were detected within seconds of applying the soft mechanical stress and changes in the expression of genes usually associated with mechanical stress were detected within thirty minutes. It's incredible to think of all of this activity taking place inside the leaf so quickly and all because the leaves were being rubbed very gently. So how does this work? How can simply rubbing a leaf gently, without causing any overt damage, lead to all these changes within the leaf, and more importantly, make them better able to fend off the grey mould fungus? The researchers found that

the soft mechanical stress led to subtle changes in the cuticle, the waxy layer that forms the outer surface of leaves. Normally, the cuticle acts as a reasonably effective barrier to most external agents. However, the changes to the cuticle following the gentle rubbing made it more permeable, allowing substances to pass through it, when ordinarily they would not be able to. These substances might be from the damaged cuticle itself or even from the fungal spore, and as we shall see shortly, once they get inside the leaf, the plant's surveillance systems detect them, setting in motion a series of events leading ultimately to fending off the attacker.[4]

Under normal circumstances, fungal spores are not lucky enough to have kindly plant scientists paving the way for them by gently rubbing leaves. Rather, once they find themselves on a leaf surface, many fungi will need to force their way into the leaf. To us, leaves tend to be small and flimsy, but the leaves of some plants are tough—just think of holly leaves, for example. To a microscopic fungus on the surface of a leaf, getting inside that leaf must be the equivalent of us wanting to enter a brick building by trying to force our way directly through the wall. Sure, we could open the door to the building, and in a sense, this is what some fungi and most bacteria do, because they enter the leaf via natural openings in the plant. Such openings include lenticels, which can be found on stems, roots, and fruits of plants, and also on potato tubers, and stomata, the pores on leaves which open during the day and close at night, and when open, allow carbon dioxide to enter for photosynthesis. Some pathogens, bacteria for example, can detect molecules released from stomata as they go about their normal business of letting carbon dioxide into the leaf and, in turn, losing water by evaporation to the surrounding air (a process is known as transpiration). This allows the bacteria to locate open stomata. Other pathogens—rust fungi are a good example—seem to use a combination of chemical and topographical cues on the leaf surface to find stomata, while *Cercospora zeae-maydis*, the cause of grey leaf spot of maize, requires light in order to perceive stomata.[5] It used to be thought that pathogens landing on a leaf surface 'found' stomatal openings by chance. We now know that this is not true and that pathogens which typically gain entry to

leaves via stomata have navigational equipment that leaves nothing to chance. Spores of rust fungi, once they have landed on leaves of a suitable host plant, germinate, producing germ tubes. These germ tubes 'sense' the leaf surface, using both topographical and chemical cues to find their way to stomatal openings, through which they infect the plant.[6]

## Danger signals

Other fungi opt for the hard way—straight through the leaf. This sounds a great deal more challenging than entering via a stomatal opening. So how do these fungi actually get through an intact leaf surface? The answer, for many of these fungi, is a two-pronged approach: first, the tissue is softened up by secreting enzymes onto the leaf surface and then, once the leaf surface is more yielding, brute force is used to push the fungus through into the leaf (see Figure 4). This certainly works, but it comes at a cost, because in the process of blasting its way through the outer surface of the leaf, fragments of damaged cuticle and underlying plant tissue are released—these fragments are known as damage-associated molecular patterns (DAMPs). At any rate, such fragments should not be found inside the leaf. In fact, the plant is able to detect these DAMPs and recognizing that all is not well, a series of events is triggered, leading eventually to a defensive response.[7]

DAMPs are used, not just by plants, but by multicellular organisms in general, as an indicator of the damaged self. One DAMP that has been well-studied in both animals and plants is extracellular ATP. Adenosine triphosphate (ATP) is the energy-carrying molecule of all cells. It captures energy from the breakdown of food and releases it to fuel other activities within the cell. It is a nucleotide consisting of three main components: a nitrogenous base—adenine; a sugar—ribose; and a chain of three phosphate groups bound to the ribose. It is this phosphate tail of the ATP molecule that is the power source tapped by the cell. ATP belongs within the cell and if an animal cell is damaged, any ATP found outside the cell is quickly

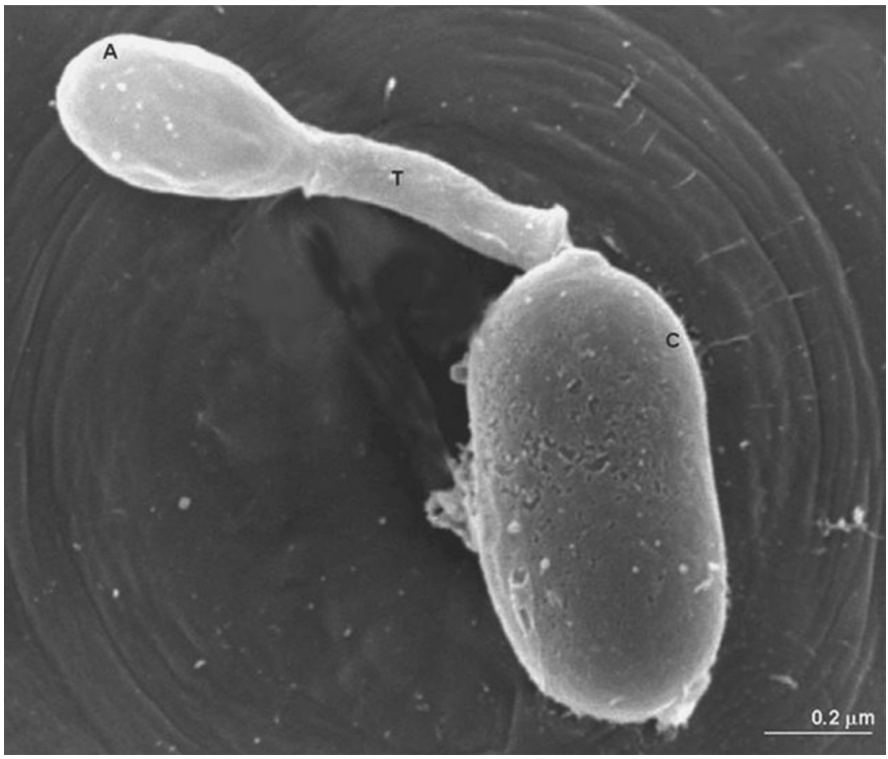


**Figure 4** The fungus *Colletotrichum kahawae* on the surface of a coffee berry. The visible structures are the fungal spore (conidium, C), the germ tube emerging from the conidium (T), and the appressorium (A) from which the fungus infects the host.

detected by receptors located on the cell membrane. Although animal scientists have been studying extracellular ATP for more than sixty years, its study in plants is much more recent. Nevertheless, in 2014, researchers discovered a receptor for ATP on the cell membrane of *Arabidopsis*, demonstrating that extracellular ATP serves as a signal for the damaged self in plants.[8]

When the plant cell wall is damaged, bits of it—such as oligogalacturonides (fragments of pectin)—can act as DAMPs. However, cell wall damage can also lead to the formation of rather more unusual DAMPs. Plant cell walls are comprised mostly of cellulose fibres, which provide it with

strength and flexibility. Another form of cellulose, hemicellulose, cross-links with the cellulose fibres to provide additional strength—plant cell walls really *do* need to be strong. Pectins in the cell wall form a sort of hydrated gel, cementing everything together. Some of the pectins are methyl-esterified—methyl groups are added to it—to provide protection against attack by pathogen enzymes. Some pathogens are not keen on this, since it makes getting through the cell wall more difficult. Their solution is to remove these methyl groups, which they do using enzymes called pectin methyl esterases. This releases two products—oligogalacturonides, which we already know act as DAMPs, and methanol. This is where it gets interesting, because there is more to methanol than meets the eye. When released in the plant, methanol can act as a DAMP.[9] In fact, methanol also acts as a DAMP following herbivore attack. Researchers found that if they genetically silenced the activity of pectin methyl esterase in plants, not only was less methanol produced, but the plants were also more susceptible to insect attack.[10]

## Detecting the non-self

Plants are also able to detect the invading microbes themselves. Specifically, they can recognize molecules that form an important part of the microbe, such as chitin, which is a component of the cell walls of fungi, and conjugates of lipids and sugars known as lipopolysaccharides, which are present in the outer membranes of many bacteria. These molecules are called microbe-associated molecular patterns (MAMPs) or, if the microbe is a pathogen, pathogen-associated molecular patterns (PAMPs), and are recognized by proteins located on the external face of the plant cell. These proteins, known as receptors, or more accurately as pattern recognition receptors, are sentries, on the lookout for microbes in search of the goodies locked away inside the plant cell (we will return to these receptors shortly). Recognition of a PAMP by one of these sentries triggers an influx of calcium ($Ca^{2+}$) ions into the cell and also leads to the production of

various reactive oxygen species (ROS), including hydrogen peroxide. ROS are useful molecules to have around, since they are antimicrobial and can also act as secondary signals. These events occur rapidly following recognition, with ROS being produced within five minutes, and leads to the activation of a signalling pathway, which in turn activates genes responsible for defences. This gene expression occurs in two phases. The first occurs within twenty minutes, is independent of ROS production, and is responsible for producing proteins involved in regulation and signalling of defence. The second phase is dependent on ROS accumulation and produces enzymes responsible for synthesizing defence components, including defensive weapons and further signalling molecules. The aim of this rapid triggering of a barrage of defences is to stop the intruder in its tracks and it is effective against most pathogens, especially those not adapted to growing on a particular plant. It goes by the impressive-sounding name of PAMP-triggered immunity (PTI) and represents the basal defence system of plants.[11]

Now, PTI is all well and good but as we know, nothing in life stays the same. For some pathogens, PTI is just a barrier to overcome, and in time, that is exactly what some pathogens achieve. These malicious microbes develop molecules, called effectors, which are instrumental in their counter-defensive strategy. These effectors can either block the initial recognition of PAMPs on the surface of the plant cell, or they are transferred into the cell, where they suppress the activation of plant defences. In other words, the effectors sabotage host defences, thereby allowing the pathogen to go unchallenged by the sentries guarding the cell. Slipping by unrecognized and unmolested, the pathogen can enter the cell and plunder the resources within. The battleground can be highly localized, with all the action taking place at the spot where the pathogen is poised to breach the plant cell wall. Researchers studying infection of *Arabidopsis* by the fungus *Colletotrichum higginsianum* found that the pathogen secreted its effectors at the very site of intended entry, in what would appear to be an attempt to quell the plant's defensive machinery, prior to the major push through the cell wall.[12]

In plants and other organisms, precisely cut fragments of ribonucleic acid (RNA) known as small RNAs are involved in controlling the function of messenger RNA (messenger RNA is responsible for carrying the code for making a specific protein from the DNA in the nucleus to the cell's cytoplasm, where the protein is eventually made). One type of small RNA is microRNA (miRNA). This is formed when longer RNA molecules are chopped into smaller chunks by an enzyme known as Dicer. In 2006, researchers showed that when *Arabidopsis* is under attack by the bacterial pathogen *Pseudomonas syringae*, perception of bacterial PAMPs leads to the production of a miRNA which modulates the defence response, helping to make the plant resist the onslaught.[13] The bacterium, in turn, has evolved effector molecules capable of suppressing the activity of the miRNA.[14]

But this is not the end of the line for the plant. Like the pathogen, the plant can also adapt, and over time, develops specific proteins, known as Resistance or R proteins, able to recognize the pathogen-produced effectors, preventing them from doing their job. This is known as effector-triggered immunity (ETI; also known as *R* gene-mediated resistance), and leads to the rapid activation of very effective plant defences, including suicide by the cell under attack. This might seem rather extreme, but as we shall see in Chapter 5, this strategy can also kill the pathogen.

In an interesting twist, recent research suggests that many R proteins do not directly recognize pathogen effector molecules. Pathogen effectors work by modifying the plant's defensive response and it appears that many plant R proteins recognize these modifications and not the effector molecule itself. In a sense, R proteins could be viewed as guarding a specific part of the plant's basal defence system. However, not all R proteins work in this way and some do interact directly with pathogen effectors.[11] A good example is in tomato, where an R protein actually binds to an effector produced by the bacterial pathogen, *Pseudomonas syringae* pv. *tomato*.

Now I'm sure you know what's coming next. The plant won't maintain the upper hand for long, because the pathogen will evolve and adapt, and

when, after a few years, it does so, the plant will begin the process of adapting to the latest change in the pathogen. And so the game of cat and mouse, begun in the mists of evolutionary time, continues, ever evolving, adding layer upon layer of biological complexity to the struggle between plant and pathogen.

So what mechanisms do pathogens use to thwart the plant's surveillance systems and defensive armoury? The approaches adopted are varied, but always remarkable. For example, many bacteria involved in causing disease in mammals and plants, such as various *Pseudomonas* species, possess a flagellum, a whip-like structure, which can propel the bacterium towards attractants. One such bacterium is *Pseudomonas aeruginosa*, which can infect a wide variety of hosts, ranging from humans to plants. A major component of the flagellum is the lipopolysaccharide flagellin, a PAMP recognized by receptors on the cell surfaces in mammals and plants. Researchers at the University of Utrecht in the Netherlands discovered a novel mechanism used by *P. aeruginosa* to avoid flagellin recognition.[15] These bacteria are able to degrade any flagellin that is not part of the structure of the flagellum. This blocks recognition, while maintaining the integrity and function of the flagellum and essentially hides the bacterium from the immune systems of its mammalian and plant hosts. Another *Pseudomonas* bacterium, *P. syringae*, adopts a different approach to subverting plant defences. This pathogen enters the leaf via open stomata (see Figure 5). However, plants under attack can detect PAMPs from the bacterium and close their stomata, effectively shutting the door in the face of the intruder. Not to be outdone, the bacteria evolved the ability to overcome this barrier by producing a toxin, coronatine, which can reopen the stomata, allowing entry to the leaf once more. And then there is the fungal pathogen *Cladosporium fulvum*, which causes leaf mould in tomato. Chitin fragments from the cell wall of this fungus are recognized by receptors in the plant, triggering defences. But the fungus has adapted to this by producing a protein which binds to the chitin fragments, preventing recognition by the plant and enabling the fungus to get by undetected.[16]

Of course, not all microbes are thieves. Some are plant-friendly and have an interaction with the plant by which both partners benefit. But there is a

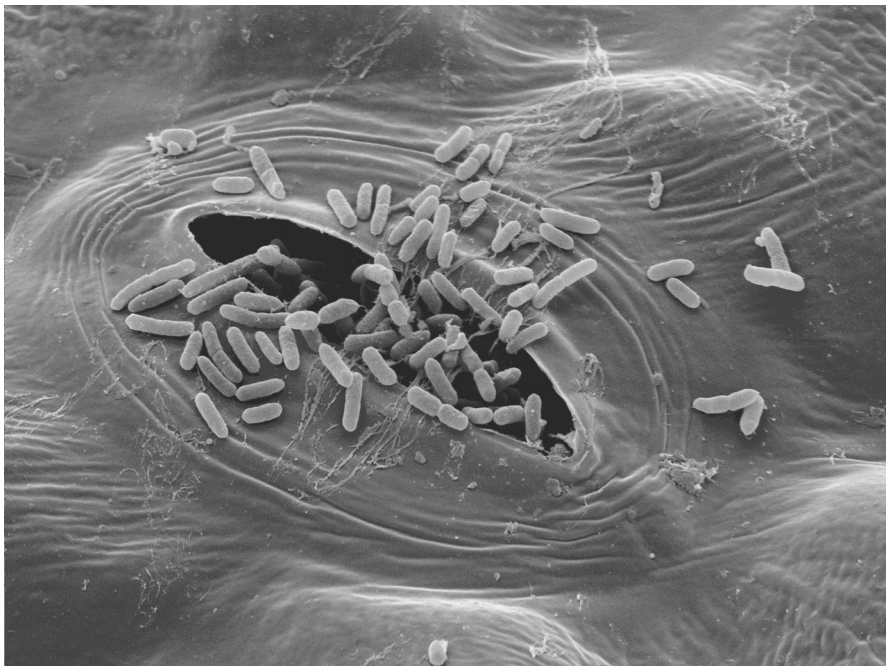

**Figure 5** *Escherichia coli* bacteria around a stomatal opening on a lettuce leaf.

potential problem—many MAMPs are widespread among microbes, and beneficial microbes and pathogens possess similar MAMPs. On the face of it, it would appear that the beneficial microbes don't stand a chance—their MAMPs will be recognized by the plant, defences will be triggered, and it will be game over. Not quite, for although beneficial microbes are perceived as invaders and defences are activated, the microbes are able to suppress the plant's defences.[17] However, beneficial microbes can also evade plant recognition. *Rhizobium* bacteria, for example, form a symbiotic relationship with leguminous plants by which nodules are formed on the roots. The bacteria reside in these nodules and fix atmospheric nitrogen, converting it to organic forms of nitrogen such as amino acids which the plant can use, in return for a supply of sugars from the plant. When the *Rhizobia* bacteria encounter the plant root, they are, at first, perceived as a threat and so evading recognition and/or suppressing defences is necessary if a symbiotic

relationship is to be established. Some *Rhizobia* species, such as *Sinorhizobium meliloti*, produce flagellin molecules that do not trigger plant defences and moreover, various studies have demonstrated that *Rhizobia* are capable of suppressing plant defence responses.[17]

## The nitty-gritty of receptors

A few pages ago we saw that an elicitor (a PAMP, for example) is recognized by a receptor protein, usually located in the plasma membrane of the cell. Finding these receptors has not been easy, but by searching for mutant plants that cannot respond to PAMPs, some have been identified. A good example is the discovery of the receptor that recognizes the bacterial PAMP, flagellin. The action of flagellin is due to a particular sequence of twenty-two amino acids in the protein known as Flg22. This amino acid sequence is highly conserved across bacterial species, that is, it is the same in a diverse range of bacteria. Researchers identified a mutant of *Arabidopsis* that did not activate defences when treated with Flg22 and found subsequently that it carried a mutation in a gene (*FLS2*) which codes for a protein in the plasma membrane—a transmembrane protein. The part of this protein on the outside of the membrane consists of repeated short sequences of amino acids containing a lot of the amino acid leucine—this is known as a leucine-rich repeat (LRR). This is the part of the receptor that binds to Flg22, thereby recognizing the invader and triggering off the signalling pathway leading to defence. The LRR domain is commonly found in proteins involved in defence against pathogens in both plants and animals.[18]

Like the LRR domain on the receptor that recognizes flagellin (or recognizes the Flg22 portion of flagellin, to be precise), the tomato resistance protein Cf has an extracellular domain that is responsible for recognizing the fungal pathogen *Cladosporium fulvum*.[19] However, most resistance proteins with a LRR domain are not extracellular, but are intracellular, facing the inside of the cell. These proteins deal with pathogen effectors that act within the cell.

Some plant resistance proteins also possess, in addition to a LRR domain, another domain known as the Toll/interleukin-1 receptor (TIR) domain. TIR domains are found in receptor proteins in animals—the Toll-like receptors—which are involved in recognizing pathogen-associated molecules and activating immune responses in the animal. They are usually found in sentinel cells such as macrophages and dendritic cells. Once a microbe has breached physical barriers in the animal such as the skin or intestinal tract mucosa, they are recognized by the Toll-like receptors, which then activate immune responses. The similarities between the receptors of PAMP- and effector-triggered immunity in plants and those of the innate immune system in animals suggests that these proteins have an ancient evolutionary origin, maybe even predating the split between animals and plants[7]—roughly 1.6 billion years ago.[20]

## Detecting bugs

If plants can detect and recognize pathogens, they ought to be able to detect the considerably larger insects that come to dine on them. Since insects are so much bigger than microbes, might plants be able to detect their physical presence? Some plants are very good at this. I am referring to carnivorous plants such as the Venus flytrap and the sundew, which respond with lightning speed to an insect landing on one of their leaves. These plants are clearly specialists, with the sophisticated machinery necessary to detect and ensnare their prey with great speed. The Venus flytrap is a good example. There are nectar glands all around the rim of the lobes on the trap and these secrete a sugary solution. On visiting a trap, an insect will start feeding on this solution and will then wander around in search of more. Each lobe has three trigger hairs and although touching it once will not trigger the trap, touching the same hair or one of the others within quick succession will and the trap springs shut. If the tip of a trigger hair is touched, it bends to a point where the hair narrows at the base. This is the point which generates the signal for the trap to shut. In fact, work

published in 2011 showed that touching the trigger hairs generates an action potential (essentially a wave of electrical discharge that travels along the membrane of a cell) and once two action potentials are detected within 15–20 seconds of each other, the trap shuts within a fraction of a second.[21] More recently, researchers discovered that more than three action potentials are required to trigger expression of the genes coding for the digestive enzymes. The workers also found that the flytrap possesses a sodium channel that acquires the sodium released from the trapped and decomposing prey. The activity of this sodium channel is dependent on the number of action potentials detected by the trap—the more the victim struggles, the more action potentials are fired, helping the Venus flytrap identify the insect as worth the effort of secreting digestive enzymes.[22]

But what about ordinary plants, the normal run-of-the-mill plants that don't eat insects? Most plants possess hairs known as trichomes on their aerial surfaces. If they occur on a leaf surface in sufficient density, they can physically impede the movement of the insect and disrupt feeding (see Plate 3). This would appear to be a purely passive process, with the plant being completely unaware of the presence of an insect on its surface. Such an assumption would be wrong, certainly in the case of tomato plants. Researchers at Pennsylvania State University in the USA discovered that movement of caterpillars or moths on the surface of tomato leaves ruptured trichomes, resulting in the rapid triggering of plant defences. The trichomes were found to contain all of the necessary signalling components, thereby allowing a signalling cascade to be triggered rapidly and alerting the plant to the presence of the insect herbivore. This ability to detect the movement of an insect on the leaf surface provides the plant with early warning of impending attack, allowing it to prepare suitable defences, well before eggs are laid or the insect starts feeding.[23]

Some insects chew and munch their way through plant tissue and this feeding activity generates vibrations. If a caterpillar is feeding on a leaf, the vibrations generated in that leaf will be transmitted to other parts of the plant, at speeds of up to 100 m per second. Now that is quick, and means

that distant, and as yet unattacked parts of the plant, can be put on alert before trouble arrives. In case you think this is rather far-fetched, please be assured that it does not belong to the realms of science fiction. In some ingenious and elegant experiments, researchers at the University of Missouri recorded vibrations made as caterpillars of the small white butterfly (*Pieris rapae*) fed on leaves of *Arabidopsis* plants.[24] They then played these recorded vibrations back to plants (using piezoelectric actuators attached to leaves) and found that chemical defences were triggered both in the leaf receiving the playback of the vibrations and more distant leaves. Amazingly, the plants were also able to distinguish between vibrations caused by insects chewing and those caused by wind or insect song, proving that plants, like discerning humans, appreciate good vibrations.

The table manners of some insect diners can be quite unsavoury. Beetles, grasshoppers, and caterpillars of moths and butterflies seem to have insatiable appetites as they chew on their plant tissue of choice. In contrast, insects such as aphids and whiteflies are considerably more refined and dainty in their approach to dining. Unlike their uncouth cousins, their mouthparts are different, and rather than munching on their host, they insert a proboscis or stylet into the plant to suck out a liquid meal. As they enjoy their food, both types of diner produce saliva, but once again, the biting, ripping, and tearing brigade just have to take things too far. Whereas aphids and whiteflies secrete saliva incrementally as the stylet moves through the host tissue, chewing insects such as lepidopteran caterpillars deposit small quantities of both saliva and regurgitant, known as oral secretions, on the plant tissue as they eat. Yes, you did read that correctly—regurgitant. As they feed, caterpillars of butterflies and moths deposit compounds from the gut onto the leaf surface by regurgitation. This was demonstrated clearly by researchers at the Max Planck Institute for Chemical Ecology in Jena, Germany, who found that feeding caterpillars of the African cotton leafworm (*Spodoptera littoralis*; Plate 17) regurgitated constantly onto leaves.[25] Why caterpillars should regurgitate onto leaves is still hotly debated, especially, as we will see later, since compounds in these oral secretions can betray its presence to the plant. However, these oral

secretions also contain compounds capable of suppressing plant defences. It certainly puts a new spin on spit.

Of course, chewing insects aren't the only herbivorous animals. As any gardener will know, slugs eat plants, and a plethora of mammals also have a partly or wholly vegetarian diet. One thing is common to all of these chewing herbivores—by taking various sized chunks of plant tissue, they damage or wound the plant, and in so doing, release fragments of plant cell wall, as well as the contents of the damaged cell, and even partly digested bits of plant tissue. As we saw earlier with pathogen attack, the appearance of parts of the plant that are outside their usual compartments is not normal and sets alarm bells ringing. This recognition of the damaged self was investigated by Martin Heil and his colleagues at CINVESTAV in Irapuato, Mexico, who found that plants ranging from tomato and lima bean to sesame and maize, displayed damaged-self recognition. They suggested that it could represent, in evolutionary terms, an ancient mechanism used by plants to detect and respond to attack by a wide range of chewing herbivores—a sort of general response.[26] This makes good sense, but plants can also detect specialist herbivores—the pernickety ones—those whose diet is largely (or even entirely) a particular type of plant.

And here we come back to saliva, for it seems that specialist insect herbivores are given away by their spit. The oral secretions that the insect deposits on or in the plant tissue contain molecules that can betray both its presence and its identity. Analysing insect spit seems like a strange way to spend one's working day. Nevertheless, I bet you are wondering how an eager researcher might collect spit from an insect. Well, most studies in this area have used insect regurgitant, rather than spit per se, and this is collected by gently squeezing the caterpillar with forceps, just behind its head. Apparently, this causes immediate regurgitation, which perhaps is not surprising.

Some components of these insect oral secretions are perceived by plants as a signal of herbivore attack, while, as we will see later, other components interfere with the triggering of defences. These components, including compounds as diverse as fatty acid–amino acid conjugates, fragments of cell walls, and peptides released from digested proteins, are known as

Herbivore-associated molecular patterns (HAMPs). One of these components, a fatty acid derivative named volicitin, was discovered in the regurgitant of beet armyworm caterpillars (*Spodoptera exigua*).[27] When deposited on the surface of maize leaves as the caterpillar feeds, it induces the plant to release volatile chemical signals that attract parasitic wasps, which are natural enemies of the caterpillars. The regurgitant of another type of armyworm, the fall armyworm (*Spodoptera frugiperda*), contains compounds called inceptins, which induce the release of volatile chemicals from cowpea plants.[28] Although most of the HAMPs discovered so far were found in insect regurgitant, it appears that saliva can also contain them. In fact, when researchers studied feeding of the fall armyworm on maize leaves, they found that HAMPs were present in both saliva and regurgitant. On maize leaves, the caterpillars of the fall armyworm don't produce much regurgitant, and so the source of most of the HAMPs is saliva.[29]

We saw earlier that damage to plants caused by pathogen attack or an insect herbivore releases ATP from cells. Receptors on the plant cell membrane detect the ATP, which should not be outside the cell, and so the ATP is acting as a DAMP. Research in Gary Felton's lab at Pennsylvania State University in the USA discovered that leaves treated with saliva from larvae of the corn earworm contained less ATP than untreated leaves. When the researchers looked for the underlying mechanism for this observation, they found that the earworm's saliva contained enzymes capable of degrading ATP. Moreover, they found that these ATP-hydrolysing enzymes suppressed the plant's defence responses and were therefore acting as effectors. One of the ATP-degrading enzymes was an apyrase, which are ubiquitous components of the saliva of blood-sucking arthropods such as mosquitos. In fact, the enzyme present in saliva from the earworm and that from mosquito saliva were very similar, suggesting a much broader evolutionary role for these salivary enzymes than was thought previously.[30]

We've already seen that during pathogen or herbivore attack, the plant is able to detect the presence of the attacker by recognizing various DAMPs, including ATP, methanol, and oligogalacturonides. A plant under attack can also produce other danger signals, the production and release of which are

under tight control by the plant. One such signal is the peptide systemin (a peptide is essentially a small protein), which is produced by tomato plants following wounding. Systemin was discovered in 1991 by a team at Washington State University in Pullman led by the inspirational Clarence 'Bud' Ryan. It was the result of more than thirty years of research by Ryan and his co-workers and was a landmark discovery, since systemin was the first peptide hormone discovered in plants, the first polypeptide hormone in animals, insulin, having been discovered by Banting and Best at the University of Toronto in 1922.[31] In the early 1970s, work in Ryan's lab had shown that when tomato leaves were damaged by the Colorado potato beetle, *Leptinotarsa decemlineata*, a specific defence was activated, not just at the wound site, but also in tissues remote from the site of damage.[32] This defence involved the accumulation of proteinase inhibitors, which prevent the insect from digesting proteins in their diet. To Ryan, this suggested the existence of a signal, generated at the wound site, which would travel to distant tissues to activate the defence. This was ground-breaking stuff back in 1972 and led to a long search for the signal, ending with the identification of systemin nearly twenty years later. We now know that when a tomato plant is wounded, such as during insect attack, systemin is formed at the wound site from a precursor molecule, prosystemin. Systemin is rapidly distributed throughout the wounded leaf and reaches distant leaves via the phloem within a couple of hours.[33] Further signalling is then initiated (which we cover in Chapter 3), leading to the activation of defences. The importance of systemin in defending the tomato plant against herbivores was uncovered using plants genetically manipulated to produce altered levels of the peptide. Plants engineered to produce low levels of systemin were less able to defend themselves against chewing insects such as the tobacco hornworm, *Manduca sexta*.[34] In contrast, in plants with elevated systemin levels, defences were continually switched on, whether the plant was wounded or not.[35]

In tomato, the systemin receptor is a membrane-spanning protein with an LRR domain similar to the resistance proteins recognizing PAMPs that we came across earlier. In fact, the systemin receptor, like many of the PAMP receptors, is similar to the Toll-like receptors found in animals,

providing further evidence of a shared and ancient evolutionary history for these receptors.[36]

As one might expect, tomato is not the only plant to possess a peptide with elicitor activity. In 2006, researchers reported the existence of a family of plant elicitor peptides (Peps) from *Arabidopsis* and just one year later another Pep was discovered in maize. In fact, Peps have been found in a wide range of plant species.[37] Like systemin, the Peps in *Arabidopsis* are also perceived by receptors with an LRR domain, suggesting that the LRR domain might be a common component of receptors whose job is to detect peptide DAMPs. All in all, this represents a powerful danger detection system, especially since the perception of these peptide DAMPs by the plant triggers resistance not just against insect attackers, but also to bacterial and fungal pathogens.[38]

Herbivorous insects don't just feed on plants, they also lay their eggs on plants. From the plant's perspective, this is not good news, since the eggs will hatch into voracious plant-eating machines. An ability to detect eggs on, for example, the leaf surface, would appear to be a useful advanced warning system. It will come as no surprise therefore, that plants possess mechanisms capable of detecting eggs on leaf surfaces and mount appropriate defences as a result.[39] So how do plants detect eggs? Oviposition (the laying of eggs) can vary among insects, with some eggs attached tightly to the plant surface, while others are attached loosely, and yet others can be inserted into a cavity once the insect has scratched the waxy cuticle on the leaf surface. Those eggs that are not glued to the plant surface nonetheless become covered in secretions as they move through the insect oviduct, and compounds in these secretions can be detected by the plant, alerting it to the presence of the eggs on its surface. The first of these elicitors from eggs was discovered in eggs produced by bruchid weevils and were subsequently called 'bruchins'. Eggs themselves also contain compounds that can be perceived by plants and interestingly, elicitors from eggs belonging to a range of different insect species activate the same responses in plants. This bears a remarkable similarity to the detection of PAMPS (those elicitors from bacterial and fungal pathogens)

and suggests that plants respond similarly, at least at the molecular level, to microbes and insect eggs.[40]

Surveillance and detection is central to any defence strategy, since it gives the plant advanced warning of an impending attack, allowing it to prepare its defences and respond quickly. Time is of the essence, since the more rapidly the plant can mount its defences, the more likely it is to ward off the attacker successfully. Now, it's all well and good to have a sophisticated alarm system, but this must be matched by an equally robust system of defences, capable of debilitating the enemy, or even better, killing it. If you thought plant alarm systems were ingenious, just wait until to see what plant defences are capable of. Prepare yourselves, because, as you will discover in Chapters 4 and 5, plants can fight dirty. But before weapons can be deployed, the plant still has an important job to do, for having recognized the attacker, it must coordinate its signalling so that other parts of the plant are made aware of the danger.

# 3

# Call to arms

Imagine this scenario. A settlement becomes established in an area with considerable natural resources and, in time, its inhabitants become wealthy. Other less well-off settlements, on hearing of the riches being enjoyed by their fortunate neighbours, become envious. The prosperous settlement is well defended—they are surrounded by solid fortifications and have a large contingent of well-armed soldiers. They also have sentinels, posted some distance from the settlement, to sound the alarm if any threat approaches. One day, the sentinels spot armed warriors marching towards them and decide to alert their compatriots back in the settlement. But the sentinels are quite some distance away and, being on foot, cannot deliver their warning quickly enough. The result is disastrous, for despite their formidable armoury, the settlement's inhabitants are caught unprepared and are overrun. Their envious, but poorly equipped neighbours have defeated a superior force and taken their wealth. If only the sentinels had horses, they could have warned their compatriots quickly and avoided the terrible consequences.

As we saw in Chapter 2, plants have sophisticated means of detecting and recognizing the enemy, and as we shall see, they also possess an arsenal bristling with weapons. Although they have defensive barriers, such as the cuticle and cell wall, without the means to connect the surveillance system with the formidable array of inducible weaponry (i.e. weapons that only come into play following attack), plants would be at great risk. In fact, plants have excellent systems linking enemy recognition with

defence deployment, involving short- and long-distance signalling within the attacked plant and signalling to neighbouring plants. When a plant cell is attacked, defences are activated, not just in that cell, but also in surrounding cells, and some defences are also activated in other parts of the plant; this is known as systemic resistance. If one part of the plant is under attack, it makes good sense to get defences ready in other parts of the plant, just in case. But it might be wasteful to trigger these remote defences straight away, since the attack might not spread that far. What happens instead is that the distant plant tissues are put on alert, or primed, so that they can be ready to mobilize defences rapidly if they are attacked. This can be viewed as a sort of plant immunization and is far less wasteful of energy and resources than firing off defences when an attack might never materialize.[1,2]

Putting the rest of the plant on alert suggests the movement of a signal from the initial site of attack to the remote parts of the plant. The nature of the signal depends on the type of systemic resistance that is activated. Attempted infection by some types of pathogen, especially biotrophs (those that need to keep the plant cells alive), can result in death of the cell under attack. This rather drastic course of action is known as a hypersensitive response, but there is a positive side to it, since it will kill the invader and halt the infection. Another positive consequence of these events is the development of systemic acquired resistance (SAR), where the distant plant tissues not yet under direct attack are put on alert. The signal linking the cells under direct attack with the remote tissues of the plant has been elusive. Nevertheless, one signalling molecule is crucial to the establishment of SAR—salicylic acid.

## Pain relief and plant defence

Salicylic acid is the main constituent of extracts of various trees, most notably willow, but is also found in a number of fruits and vegetables. The pain-relieving attributes of willow tree extracts were appreciated as long ago as 2000 BC by the Sumerians, who used the extracts to treat fever,

pain, and inflammation. The father of modern medicine, Hippocrates (460–375 BC), recommended chewing on willow bark for relief of fever and pain and also advised his women patients to drink a tea brewed from willow bark to relieve the pain experienced during childbirth.[3] More than 2,000 years were to pass before the first systematic clinical studies were conducted using willow bark. At the meeting of the Royal Society of London on 2 June 1763, a letter from the Reverend Edward Stone of Chipping- Norton in Oxfordshire to the Society's President was read to the assembled Fellows.[4] The letter described Mr Stone's studies of the effects of willow bark on patients suffering from ague, the symptoms of which included intermittent fever, pain, and fatigue. These symptoms were commonly associated with malaria, for which the standard treatment at the time was Peruvian bark (from trees belonging to *Cinchona* spp.). The good reverend had accidentally tasted willow bark and found its bitter taste similar to that of Peruvian bark. He put two and two together and wondered whether willow bark might also alleviate the symptoms of ague. Fuelled by curiosity, he set about collecting some willow bark, which he dried next to a baker's oven and then pounded and sifted it until he was left with a powder. He tested his willow bark powder on fifty ague sufferers over a five-year period and found that symptoms were greatly alleviated without any obvious side effects. Willow bark was not quite as effective as Peruvian bark in treating ague, but this should come as no surprise since the active ingredient in Peruvian bark is quinine, which acts directly on the malarial parasite, while the active ingredient in willow bark is salicin, which alleviated the symptoms of ague. Pure salicin was eventually isolated from willow and meadowsweet (*Spirea ulmaria*) in the 1830s and was eventually named salicylic acid in 1838, following extraction of a more potent acid form of the willow bark extract by the Italian chemist Raffaele Piria.[3]

The use of salicylic acid increased considerably, but it was associated with some unpleasant side effects, particularly gastric irritation. In the search for a less irritant substitute for salicylic acid, chemists synthesized a derivative, acetylsalicylic acid. This was found to have fewer side effects than salicylic acid and was eventually marketed as Aspirin.[3]

There is more to salicylic acid and aspirin than pain relief, for both are capable of triggering defence responses in plants. Before we look at salicylic acid and the signalling involved in plant defence, let us go back in time.

## Immunizing plants—you've got to be joking!

In 1901, two French botanists, Jean Beauverie and Julien Ray, working independently, produced the earliest known reports on what has come to be called induced resistance. They worked on plants that were susceptible to the grey mould fungus *Botrytis cinerea* and found that plants that were given an initial challenge with the pathogen displayed considerably enhanced resistance to subsequent inoculations.[5,6] Investigations by others over the next thirty years showed that these observations were not flukes and led to the assessment that plants were capable of expressing 'induced acquired immunity'. In 1961, Frank Ross, working at Cornell University in Ithaca, New York, published the results of experiments using tobacco and the *Tobacco mosaic virus* (TMV).[7] He showed that inoculation of one lower leaf of the tobacco plant increased resistance of the upper leaves on that plant to subsequent TMV infection. Amazingly, the enhanced resistance in the upper leaves was not confined to TMV, since these leaves also developed resistance to fungal and bacterial pathogens. It appeared therefore, that plants that became infected with a pathogen developed a broad spectrum resistance to subsequent infections. This was startling stuff and became even more so when it was confirmed and extended by other researchers, most notable of whom was Joe Kuć of the University of Kentucky in the USA. Joe was already a plant pathologist of considerable standing when he started working on induced resistance. He had established a solid reputation for his research on protective compounds in plants, especially phytoalexins (of which, more later). As an undergraduate studying plant pathology in the mid-1970s, I was well aware of Joe's work on phytoalexins. A few years later, following my PhD studies, I came across Joe Kuć again, or rather, various papers he had published in the '70s on

induced resistance. The idea fascinated me and I became hooked. This was the early 1980s and I was just starting out on my own as an independent researcher and looking for promising research topics. Thus started a thirty-year obsession with induced resistance. I had just started working on induced resistance when I bumped into a colleague who had recently returned from an international plant pathology conference. He told me of a scientist who claimed that resistance could be induced in plants—that plants could be 'immunized'. According to my colleague, the idea was crazy and, apparently, most people at the conference thought likewise. The scientist in question was Joe Kuć and the reality was that induced resistance was not taken seriously by plant pathologists for a long time, in spite of the large volume of meticulous research carried out by Joe and his PhD students and co-workers over the years. Attitudes began to change in the mid-1990s with the advent of increasingly sophisticated molecular technologies and today, induced resistance is a hot topic, with a great many laboratories worldwide devoted to unravelling the complexities of what was once thought to be 'mistaken'.

## Aspirin is not just for headaches

The search for the signal involved in SAR has been long and involved a great many experiments on cucumber plants, much used by Joe Kuć in his studies on induced resistance. Aside from being an important crop plant, and one afflicted by a debilitating disease called anthracnose (caused by the fungus *Colletotrichum orbiculare*), cucumber plants have thick stems and large leaves, ideal for studying the movement of signals between leaves. Kuć and his colleagues in Kentucky carried out numerous experiments involving grafting and petiole girdling in an attempt to determine the nature and source of the signal, and in so doing, laid the foundations for much of research that followed.[8,9] In 1990, two groups of researchers, one based at Rutgers University in New Jersey working on tobacco and the other based at the biotechnology and chemical company Ciba-Geigy in Switzerland

(now Syngenta) working on cucumber, provided tantalizing evidence that salicylic acid might be the elusive signal.[10,11] We now know that salicylic acid is not, in fact, the mobile signal that moves in the phloem from the sites of initial infection to warn other plant parts, but we also know that it is essential for SAR to occur. Evidence for the importance of salicylic acid in SAR comes from experiments using a bacterial gene (*nahG*) encoding an enzyme that breaks down salicylic acid, which is introduced into plants to be studied. Some elegant experiments were conducted on tobacco plants, involving grafting, where the upper (and younger) parts of normal tobacco shoots were grafted onto the lower (and older) parts of tobacco plants containing the *nahG* gene and vice versa. The older leaves on the grafted plants were then inoculated with TMV and several days later, the younger leaves were inoculated. In those plants whose older leaves were unable to accumulate salicylic acid because of the *nahG* gene, the younger leaves exhibited strong resistance to TMV, indicating that SAR had developed normally. In plants where the younger leaves contained the gene and so could not accumulate salicylic acid, these leaves were susceptible to TMV, showing that SAR was not induced. These results demonstrate that salicylic acid is essential for SAR to become established in parts of the plant remote from the site of initial infection. It also shows that leaves that cannot produce salicylic acid are still able to produce a signal that can activate SAR elsewhere in the plant.[12] Subsequent research revealed that the signal that moves in tobacco from infected to uninfected leaves is actually methyl salicylate. On arrival in the distant tissues, the methyl salicylate is converted to salicylic acid, which then triggers SAR.[13]

## Help! I've been wounded

The idea that plants might respond actively to wounding by producing a chemical was suggested as long ago as 1892.[14] Another twenty-nine years were to pass before the presence of a wound hormone was demonstrated in potato tubers and then, in 1939, the wound hormone was isolated from

runner beans, purified, and named traumatin.[15] It turned out to be a fatty acid derivative. Research on wound signalling then hit the doldrums, but the field was rejuvenated in the 1970s following a discovery made in Clarence Ryan's lab at Washington State University. As we saw in Chapter 2, work by Ryan and his colleagues suggested that the signal responsible for alerting distant tissues in a tomato plant of insect attack is the peptide systemin. They managed to make systemin in the lab and found that if it was applied to wounds on tomato plants, it was translocated in the phloem to other tissues.[16] The problem was that when it was applied to plant tissues, it was not very good at activating defence responses, in this case the accumulation of proteinase inhibitors (which inhibit the ability of the insect to digest proteins in its gut). As the search for the wound signal continued in Ryan's lab, it became apparent that certain fatty acid derivatives were pretty good at activating defences. Prominent among these compounds was jasmonic acid, first recognized in 1962 as an essential oil contributing to the fragrance of jasmine flowers and found subsequently to be an important regulator of a variety of processes in plants. Ted Farmer was working in Ryan's lab in the late 1980s and early 1990s and he began to study the effects of the methyl ester of jasmonic acid, methyl jasmonate, on activation of plant defences.

Farmer found that spraying tomato plants with a dilute suspension of methyl jasmonate led to a massive accumulation of the defensive proteinase inhibitors. This spectacular result was accompanied by an unusual observation—control plants in the same room, which had not been treated with methyl jasmonate, also produced some proteinase inhibitors. This suggested to Farmer that the methyl jasmonate had volatilized and acted as an airborne signal, activating defences in leaves of neighbouring control plants. It was thought that the methyl jasmonate had entered the leaves of the neighbouring plants via open stomata, after which the methyl group was removed, releasing jasmonic acid and activating the defence.[17]

Applying methyl jasmonate to plants and looking at defence activation is one thing; to prove that methyl jasmonate/jasmonic acid is involved in defence signalling and activation *in vivo* is quite another and requires a

different approach to experimentation. The new tools for unravelling the complexities of jasmonates and defence came in the form of plants that were altered in their ability to make jasmonates or to respond to them. The first to get going were Greg Howe and colleagues working in Clarence Ryan's lab, who used a tomato line unable to accumulate jasmonic acid.[18] These plants showed greatly increased susceptibility to larvae of the tobacco hornworm, *Manduca sexta*. Even more impressive were the results obtained by Michele McConn and her colleagues, who demonstrated that *Arabidopsis thaliana* plants that were unable to accumulate jasmonic acid suffered huge levels of damage by a fungus gnat (*Bradysia impatiens*). In contrast, damage to these plants was minimal provided they were treated with methyl jasmonate beforehand.[19] More recent experiments from Ted Farmer's lab in Lausanne have extended these findings to vertebrate herbivores. They found that the Eastern Hermann's tortoise (*Eurotestudo boettgeri*) preferred to eat *Arabidopsis* plants that were either unable to make jasmonic acid or to perceive it and largely ignored wild type plants with fully functioning jasmonic acid manufacture and perception.[20] From the insect experiments, it is clear that plants deficient in jasmonic acid cannot produce the defensive proteinase inhibitors and so are more susceptible to herbivory. With the tortoise study however, exactly why the plants with altered jasmonate manufacture or perception should be more palatable to the vertebrate herbivore remains a mystery.

It turns out that defences are not activated by jasmonic acid itself, but by modified forms of jasmonic acid in which it is conjugated to amino acids. One such compound is jasmonoyl-isoleucine (JA-Ile). So what actually happens in a leaf when it is wounded by insect attack? In *Arabidopsis* plants, first in the sequence of events following wounding is the rapid synthesis of jasmonic acid. This occurs within just 30 seconds at the wound site and is followed just 15 seconds later by accumulation of jasmonic acid at undamaged sites near the wound. This is followed quite quickly by the synthesis of JA-Ile which then does the job of activating the defences.[21]

In his book *Leaf Defence*, Ted Farmer observes that most garden herbivores, such as slugs and caterpillars, consume a small amount of leaf tissue and then move to an undamaged leaf to continue feeding. He suggests that this might be the result, in part at least, of the synthesis of jasmonic acid and the activation of defences at the feeding site and not long thereafter in other as yet undamaged leaves.[22] The herbivore thus has limited time on a leaf before plant defences make feeding unpleasant and even very dangerous. It makes sense therefore for the slug or caterpillar to move, preferably to a different and hopefully undamaged plant. So how long does the herbivore have on a leaf before its life becomes miserable? Just how long does it take for the wound signal to move from the wound site to other leaves on the plant? Some researchers have tackled this question and addressed it by determining the time between wounding and the accumulation of jasmonic acid or JA-Ile in distal leaves. These experiments have yielded speeds of 3–8 cm per minute, or as Ted puts it 'roughly the equivalent of the walking speed of a small invertebrate herbivore'.[23]

## Larval feeding and WASPs

It will not have escaped your notice that we still have not identified the mysterious wound signal—the signal that actually travels from the wound site to distant plant tissues to activate defences. Research published in 1992[24] suggested that the signal might be electrical and then, twenty-one years later, work from Ted Farmer's lab demonstrated a link between electrical signals produced upon wounding and the activation of defences. The researchers recorded changes in electrical potentials on the surface of *Arabidopsis* leaves when larvae of the Egyptian cotton leafworm (*Spodoptera littoralis*) were placed on the leaves to feed—referred to as wound-activated surface potential changes (WASPs) by the researchers. No changes in electrical potential were observed when the larvae were just walking on the leaf, but things changed once the larvae began to feed. As soon as a

larva began to munch on the leaf, electrical signals were generated near the wound site and then spread to other leaves at a speed approaching 9 cm per minute. Crucially, the workers also found that at sites receiving the electrical signals, genes responsible for defences were turned on. What's more, in plants unable to perceive jasmonic acid, larval feeding generated electrical signals but defences were not activated, demonstrating that the generation and propagation of the electrical signal are crucial for activating defences at sites remote from the attack.[25]

It turns out that the electrical signals are generated by ion channels belonging to a family of proteins known as the glutamate receptor-like (GLR) ion channel proteins.[25] These are related to ion channels best known for their role in rapid excitatory synaptic transmission in the mammalian nervous system. When it was discovered, in 1998, that plants possess a large family of *GLR* genes,[26] the first question was why should plants, which do not have a nervous system, possess such genes? Answering this question is taking time, but the possibility that GLRs might be involved in plant defence surfaced in 2006, when researchers discovered that *Arabidopsis* plants overexpressing one of these genes exhibited enhanced resistance to the fungal pathogen *Botrytis cinerea.*[27] Later work, published in 2013,[28] demonstrated that knocking out one of these genes increased susceptibility of *Arabidopsis* plants to the bacterial pathogen *Pseudomonas syringae*. In the work from Ted Farmer's lab, knocking out two of these *GLR* genes prevented transmission of the electrical signal and activation of defence genes in remote leaves following wounding.[25] It seems, therefore, that when an insect herbivore chomps on a leaf, an electrical signal is generated through the activity of GLRs, and the signal is then transmitted to other leaves where jasmonic acid is produced, which in turn, activates defences.

When an insect feeds on a leaf, an electrical wave is generated by a continuous relay of cell-membrane depolarizations, in a process that is similar to the propagation of excitatory signals in animals. This raises the intriguing possibility that these ion channel proteins existed before the divergence of animals and plants, generating long-distance warning signals in our common ancient ancestors.

## The light side of caterpillar feeding

As we saw in Chapter 2, one of the messengers triggered rapidly in cells after an attack by pathogens is calcium. This early messenger is also triggered following insect attack and is important in regulating signal transduction and, as a result, exerts an indirect control on the plant's defences. It is often said that 'seeing is believing' and although minute changes in calcium within cells and tissues can be measured with great accuracy, being able to visualize the changes would provide a different perspective on what goes on in the plant following an attack. Researchers at the Max Planck Institute for Chemical Ecology in Jena and the Martin Luther University in Halle-Wittenberg, Germany, have now found a way of visualizing changes in calcium following insect feeding. Victoria Kiep, Jyothilakshmi Vadassery, and their colleagues used *Arabidopsis* plants that produce a protein that breaks down once it has bound calcium ions.[29] This process emits light, the amount of which corresponds to calcium concentrations in the plant's cells and tissues. They used a highly sensitive camera system to follow these changes in calcium and were able to visualize the actual changes in calcium after every caterpillar bite (Plate 18). They found that the changes in calcium were rapid, but rather than just occurring at the site of attack, changes in calcium could also be seen in neighbouring leaves within just a few minutes of insect feeding. How these changes in calcium in neighbouring leaves are brought about is not yet known, although the researchers reckon that there might be a link with electrical signals.

## Whispers in the wind

To most people, the idea that plants might communicate with one another must seem very far-fetched. Certainly, this was very much the case even among plant scientists in the 1980s. To suggest this to fellow researchers at that time would have been akin to wading into crocodile-infested waters; at

the very least, it would invite controversy. Enter David Rhoades, a young researcher at the University of Washington in Seattle, who was studying defensive chemistry in trees. During his experiments in the late 1970s, he observed that caterpillars on sitka willows and red alders grew poorly when neighbouring trees were already infested with caterpillars. He had expected that the caterpillars on undamaged trees would perform better than those on damaged trees, so the result was a surprise. Since there was no physical contact between the damaged and undamaged trees, Rhoades suggested that perhaps damaged leaves produced a volatile signal that was detected by the undamaged neighbours. He published these findings in 1983,[30] and just a few months later, his findings were confirmed by Ian Baldwin, a twenty-five-year-old research assistant at Dartmouth College in New Hampshire, who was working with Jack Schultz. They had placed tree seedlings with damaged leaves in an airtight chamber and passed air from this chamber into another airtight chamber containing undamaged plants. As a control, undamaged plants received air from a chamber containing no plants. The results were clear—tree seedlings receiving air from the chamber containing plants with damaged leaves possessed greater levels of defensive phenolic compounds, leading Baldwin and Schultz to conclude that volatile signals had been released from the damaged leaves and were subsequently perceived by the undamaged plants.[31] When these data were published, the popular press went into overdrive and so was born the idea of 'talking trees'.

The media might have loved the idea of talking trees, but the scientific community was harder to convince. The experiments conducted by Rhoades and by Baldwin and Schultz were criticized because they had inadequate controls or because alternative explanations had not been considered. To make matters worse, Rhoades found it difficult to repeat his observations. The criticisms and scepticism effectively put a halt to work on plant volatile communication and Rhoades found it difficult to get funding for his research. He abandoned it to concentrate on teaching and eventually gave up science altogether.[32] Just a few years later, the tide began to change and the area of volatile plant communication started to gain

credibility. As we saw earlier in this chapter, in 1990 Ted Farmer and Bud Ryan at Washington State University showed that volatile methyl jasmonate could trigger defences in tomato leaves. In the subsequent twenty-five years, an increasing flow of publications (150 papers in 2013 alone) has demonstrated the existence of volatile plant communication, and, like the phenomenon of induced resistance, what was once thought to be fanciful or mistaken is now considered an established scientific fact.

Most of the early studies on volatile communication between plants were conducted under controlled conditions in the laboratory. These studies demonstrated that volatiles released from damaged plants elicited responses in neighbouring plants. One significant study, conducted by workers at USDA in Gainesville, Florida, took things a bit further and showed that maize plants that had been exposed to volatiles from neighbouring plants produced substantially more jasmonic acid and volatiles when they were subsequently damaged.[33] This is called priming and rather than triggering defences directly, it places the plant on alert. When the plant is subsequently attacked, it responds with an intense and rapid activation of defences. Priming is rather like the boy scout of plant defence, where being prepared is everything. It is a clever move by plants, since defences are only activated upon attack, thereby ensuring that energy and resources are not wasted.

But was the release and perception of volatile compounds really relevant to plants growing, as Ian Baldwin and his colleagues put it, in the rough and tumble of the natural environment? Some researchers had started to examine volatile communication in the field and one of them, Richard Karban, working at the University of California at Davis, demonstrated that wild tobacco plants (*Nicotiana attenuata*) suffered less damage from grasshoppers when their neighbours were damaged sagebrush (*Artemesia tridentata*) plants.[34] André Kessler and colleagues, working with Baldwin, subsequently took things a bit further. They used two plants in their study, sagebrush, which releases a range of biologically active volatiles when damaged, and wild tobacco, a plant much used in work on plant defence. Among the volatiles released by damaged sagebrush is methyl jasmonate,

although it is released at concentrations that are not sufficient to trigger defences under field conditions. How could plant-to-plant signalling work in nature if the volatiles are not released in sufficient quantities? Experiments were set up, both in the lab and in the field, using sagebrush plants that were damaged by clipping to release volatiles, and tobacco plants, in which defence responses were measured. What Kessler and his colleagues found was that rather than directly triggering defences in tobacco plants, the volatiles released from sagebrush were priming the plants' defences—production of defensive proteinase inhibitors was greatly accelerated when the primed plants were attacked and this, in turn, led to less caterpillar damage and a high mortality rate among the caterpillars.[35]

If volatile emission from plants is increased following insect attack, what happens when plants are attacked by pathogens? Studies conducted on lima bean plants (*Phaseolus lunatus*) at a field site in Mexico found that when plants were located close to neighbours infected with the bacterial pathogen *Pseudomonas syringae*, they developed a greatly increased resistance to this pathogen. It seemed that volatile compounds were emitted from the infected lima bean plants, and these were perceived by neighbouring plants, which then became primed to defend themselves against a possible bacterial onslaught.[36] Research published in 2015 by workers in South Africa showed that rust infection of wheat led to the release of volatiles and when these were picked up by neighbouring wheat plants, defences were activated leading to enhanced resistance to rust infection.[37]

## Recruiting carnivorous bodyguards

If volatiles emitted from attacked plants can be perceived by other plants, it seems reasonable to suggest that volatiles can also be detected by insects. Indeed, volatile compounds are commonly used by insect herbivores to choose host plants.[38] Olfactory cues can also be perceived by the enemies of herbivorous insects—insect predators and parasitoids. The latter could be straight out of a horror movie, living on or in their insect host,

ultimately killing it. These enemies take advantage of the fact that plants under attack by hungry insects emit a cocktail of volatiles, which acts like a gaseous advertisement, allowing them to locate their prey. Towards the end of the 1980s, Marcel Dicke and his colleagues in the Netherlands found that plants infested by spider mites emit volatile compounds that attract predatory mites,[39] and in 1990, Ted Turlings and co-workers in the USA demonstrated that female parasitic wasps used volatiles emitted from attacked maize plants to locate their prey—lepidopterous caterpillars.[40] Plants under attack start releasing volatiles quickly and insect enemies are equally speedy in arriving on the scene. When caterpillars of the large white butterfly, *Pieris brassicae*, start chomping on Brussels sprout leaves, the parasitoid wasp *Cotesia glomerata* arrives within the hour. What happens next is like a scene from the film *Alien*, for the wasps lay their eggs in the caterpillars by stabbing the poor creatures with their needle-like ovipositors (Plate 19). The eggs then hatch and the resulting larvae develop within the hapless caterpillars, emerging from their still-living hosts some twenty days later. The caterpillars die soon afterwards.

The composition of the volatile cocktail emitted by a plant varies depending on the species of insect attacking it; volatile composition also varies with plant species, so the same insect feeding on different plant species is likely to induce different blends of volatiles. Even different varieties of the same plant can release different volatile blends. A good example is cucumber, where attractiveness to predatory mites is dependent on variety. The most attractive variety, which seduced twice as many predators as its least attractive compatriot, emitted a volatile blend which differed qualitatively from its less attractive fellows. Here, the composition of the volatile cocktail was more important in attracting predatory mites than the quantity of the volatile emission.[41] The two-spotted spider mite, *Tetranychus urticae*, is a common insect pest, with a host range of more than 900 different plant species. One of its enemies is the predatory mite, *Phytoseiulus persimilis*, which is blind and relies on smell to locate its prey. By using volatile cues, this predator can distinguish between plants being attacked by its prey and plants that are safe and well. So far, so good. But

hang on a minute—if its prey can feed on nearly 1,000 different plant species, each of which is likely to emit a different volatile blend, isn't this likely to completely confuse the predator? Apparently not, since the predatory *P. persimilis* can alter its olfactory response in the light of experience. Researchers reared the predatory mites on different species of plants attacked by its two-spotted prey and discovered that the predators developed a preference for the volatiles released by the plant on which they were being reared. In fact, feeding for more than 24 hours in the presence of a volatile cocktail induced a preference for that particular odoriferous blend. So the predatory mites can learn from experience, which is a valuable attribute when one's prey can feed on so many different hosts. But surely not all odours coming into its olfactory range are worth following up? Is it possible that the predators are attracted to specific compounds in the volatile cocktail, giving them the ability to identify them in volatile blends they had not experienced previously? Apparently not, since the predatory mites seem to possess a limited ability to identify individual components in a volatile blend. Rather, they appear to learn to respond to the volatile cocktail as a whole.[42]

With the proverb 'attack is the best form of defence' in mind, plants have taken heed and have gone one step further—if you are going to attack, get in early. For plants, slaying the newborn is too late; instead, they prefer to slay the unborn, or to be more precise, they prefer their allies to dispatch the unborn. Plants can detect when an insect herbivore lays eggs on its leaves, leading to the release of volatiles, which in turn attract insect enemies. This was demonstrated by Torsten Meiners and Monika Hilker in 1997, when they found that deposition of eggs on elm leaves by the elm leaf beetle induced the release of volatiles which attracted *Oomyzus gallerucae*, a parasitoid with a taste for the eggs of the elm leaf beetle.[43] Hilker and co-workers later found that egg deposition on needles of Scots pine by the pine sawfly, *Diprion pini*, induced the release of volatiles which attracted the wasp *Chrysonotomyia ruforum*, a specialist which feasts on the eggs of the unfortunate sawfly. Whereas many such specialist parasitoids respond innately to cues from their host, this wasp needs to learn from experience,

but once it has its first whiff of the egg-induced aroma, it's all systems go. This wasp is also a very choosy diner, since it is not attracted to volatiles released by pine sawfly larvae feeding on pine needles—this diner prefers an exclusively egg-based diet.[44] In some cases, not only do the volatiles attract enemies, they also repel insects looking to lay eggs. On the wild crucifer, *Brassica nigra*, oviposition by the cabbage butterfly *Pieris brassicae* attracts parasitic wasps, but also repels pregnant *P. brassicae* females from laying their eggs on that plant. This seems sensible in that it avoids competition once the eggs hatch, but it also benefits the plant, since there will be fewer larvae to feed on the leaves.[45]

## Plant galls and defence

As a boy growing up in rural Trinidad, I was intrigued by the strange growths that appeared on the leaves and stems of many plants. They looked almost out of place—that they should not be there, these weird-shaped protuberances. It was not until much later—when I came to university in the UK—that I discovered that many of these odd lumps and bumps on plants were galls, many of which were caused by insects. A great many kinds of insect cause galls, but the real experts are gall midges and cynipid wasps. The tiny cynipid wasp, *Biorhiza pallida*, is responsible for a familiar gall, the oak apple, which forms on oak leaves. In fact, there are hundreds of species of oak gall wasp and they are responsible for an astonishing variety of galls on oak trees. How do such tiny insects cause these growths, and what happens to plant defence in all of this?

Gall-inducing insects exert particularly profound effects on their hosts, resulting in, among other changes, the most exquisite modifications of plant shape. These insects are expert at getting the plant to build them accommodation, providing them with shelter, protection from enemies, and a ready supply of food. In some cases, gall-inducing insects get the plant to secrete sugary honeydew, attracting ants which act as six-legged

bodyguards, protecting the galls from predation. Gall-inducing insects need more than external protection—they also require protection from their host. This need not be a problem, since in many cases they can suppress the plant's defences and even manipulate them for their own benefit.[46]

The tephritid fly, *Eurosta solidaginis*, induces galls on the goldenrod plant, *Solidago altissima* (Plates 4 and 5). When goldenrod plants are attacked by caterpillars of the generalist herbivore *Heliothis virescens*, volatiles are released, which would attract predators and parasitoids. Incredibly however, if the plant is attacked by the gall-inducing fly first, not only is there no volatile release by the plant, but the ability of the plant to produce volatiles when attacked by caterpillars of *H. virescens* is suppressed. So, by manipulating the plant's ability to produce volatiles, the gall-inducing fly avoids being eaten by predators, but as an unfortunate consequence for the plant, it also shields the ever-hungry caterpillars from its enemies.[47] But not all gall-inducing insects suppress volatile release by their hosts. In fact, some make use of the plant's volatile cry for help to ward off their own enemies. The aphid *Slavum wertheimae* induces galls on wild pistachio trees and in the process, the plant releases a volatile bouquet of terpenes. Researchers found that this volatile alarm call acted as a deterrent to goats, which browse on leaves of these trees—a clear case of getting your victim to protect you.[48]

The gall wasp *Antistrophus rufus* causes the formation of rather inconspicuous galls on flowering stems of the prairie plants *Silphium laciniatum* and *S. terebinthinaceum*. Larvae of the wasp feed within the galls and eventually adult wasps emerge from dead plant stems. Adult males emerge before the females and must then find females with which to mate. This is no easy task however, since the females are hidden within a mass of dead plant stems and the males have just days to live. To make matters worse, the males need to locate the females in stems of the plant species in which they were born. In other words, if the male emerged from eggs within the stems of *S. laciniatum*, it must find its mate in stems of that species. That's the sort of pressure that can put a real damper on performance, but these

wasps aren't put off easily. The time-limited paramours walk along dead stems, drumming their antennae on the surface and eventually get themselves into position over sites from which females will emerge. But how on earth do the males find the right spot among the matrix of dead and desiccated plant stems? John Tooker and colleagues found that they use a volatile bouquet released by the plant to locate the females and impressively, they can distinguish between volatiles from the two plant species because they contain different proportions of three monoterpenes. What is even more impressive is that the wasps can alter the ratios of these monoterpenes, providing the sex-starved males with a volatile satellite navigation system, pinpointing the location of the waiting females.[49]

## Enlisting the help of feathered friends

Insect predators are not alone in finding their prey by homing in on olfactory cues from plants under attack. Insectivorous birds are also attracted to trees infested with insects. Birds could potentially see insect larvae on leaves or the damage inflicted to infested leaves, but research in Finland demonstrated that birds were attracted to insect-infested trees without seeing either the larvae or the damaged leaves. This suggests that the birds were using volatile cues emitted by the infested trees, although the mechanism of attraction remained unproven.[50] Subsequent studies in the Netherlands set out to determine whether the attraction was smell. The researchers found that great tits could not only discriminate between trees infested with caterpillars and uninfested trees, without seeing the insects or the damage they inflicted, but they did so by smell. Infested and uninfested trees produced different volatile blends and this was used by the birds to home in on their meal. Insectivorous birds such as great tits feed their young on lepidopteran larvae, which are available for a short period. The ability to detect volatiles emitted by trees infested with their prey is of clear benefit to the birds. There is also a benefit to the plant, since the removal of ever-hungry caterpillars will reduce damage and plant death.[51]

## The benefits of talking to oneself

There seems little doubt that volatiles emitted from plants under attack can alert neighbours of impending danger. In nature, however, just how effective is such inter-plant communication likely to be? This question is not trivial, especially when it was shown that plants located just 20 cm away from wounded plants show limited responsiveness to the neighbouring alarm call. If volatiles only travel a short distance, what leaves are they likely to affect? Martin Heil and Rosa Adame-Álvarez at CINVESTAV in Mexico decided to examine the distance over which volatile signals from lima bean moved, under natural conditions. They found that neighbouring plants received and responded to volatile emissions at a maximum distance of 50 cm from the emitting plant. In a natural setting, this meant that more than 80% of leaves within that radius were other leaves on the emitting plant. In other words, under natural conditions, volatiles released by lima bean under attack are likely to be perceived by other leaves on the same plant. In their words 'short signalling distances make plant communication a soliloquy'.[52]

If volatile signal transmission is only really effective over relatively short distances, could it be that volatile release by attacked plants fulfils an altogether less altruistic role? Alarm signals can move within the plant via its vascular system, but will only reach those leaves or branches with a direct connection to the attacked leaf. Leaves with no direct vascular connection to the attacked leaf will receive no alarm signal. Viewed from this perspective, it seems plausible that volatile emission from wounded leaves might act to alert other leaves on the same plant which will not receive a vascular signal. Christopher Frost and colleagues in the USA examined this possibility using hybrid poplar. They found that volatiles released by leaves under attack by larvae of the Gypsy moth (*Lymantria dispar*) primed defences in adjacent leaves with little or no connection to the afflicted leaves.[53] In the same year this work was published (2007), Martin Heil and Juan Carlos Silva Bueno reported the results of experiments they had conducted on lima bean. This plant has extrafloral nectaries at the base

of its leaves, and when the plant is attacked, they secrete a nectar that attracts predatory arthropods. They found that volatiles released following leaf damage led to increased production of extrafloral nectar in other leaves on the same plant, as well as in leaves on neighbouring plants.[54]

## Dealing with multiple attackers

In nature, plants have to fend off different types of attacker, each with a different approach to getting into the plant and gaining access to the food locked within its cells. For a sessile organism, unable to run away or punch its way out of trouble, this requires coordinating alarm signals and establishing priorities. What type of attacker is it? What's the best way of countering this particular offensive? Earlier in this chapter we saw that two molecules, salicylic acid and jasmonic acid, are the major players orchestrating the plant's defences, the former involved in tackling pathogens such as powdery mildews and rusts, which are biotrophs and need to keep the host cells alive, and the latter directing defence against pathogens which kill plant cells (necrotrophs), as well as herbivorous insects. This seems straightforward enough, and relatively simple. But life is never *that* straightforward or simple. And indeed, this two-pathway system for coordinating defences is influenced by a range of other molecules—plant hormones such as ethylene, auxin, cytokinin, and abscisic acid—that help in orchestrating the defensive response. As if these various signalling pathways and modulators weren't enough, there are antagonistic and synergistic interactions between them, known as hormonal crosstalk, which helps the plant to fine-tune its response to the attacker.[55]

Plants, like the rest of us, can't do everything. Resources are limited and, under attack, must be used effectively. This means mounting the most appropriate defence and being able to provide the energy and resources to do so. Crosstalk between the salicylic acid and jasmonic acid pathways provides the plant with a powerful tool to prioritize one pathway over another, depending on the nature of the attacker.

## Getting the better of defences

In the tit-for-tat world of plant defence, where over time, plants and herbivores try to outmanoeuvre each other in an attempt to gain the upper hand, it should come as no surprise that insect herbivores would attempt to subvert the plant's ability to produce volatiles. This is exactly what researchers at the University of California, Davis, found when they studied the interaction between *Arabidopsis* and caterpillars of the large white butterfly and the beet armyworm. These chewing insects were able to selectively suppress the plant's production of green leafy volatiles. Perhaps unsurprisingly, the researchers found that the caterpillars preferred to eat leaves that had not been primed for defensive action by the volatiles. For the caterpillars, a major advantage of suppressing volatile emission by the plant would be the freedom to feed without the unwanted attention of predators and parasitoids.[56]

# 4

# Weapons of war

Being sessile puts you at a big disadvantage when it comes to dealing with your enemies, because you can't run away. In a hostile world, with a great many things wanting to eat you, and handicapped by your immobility, an effective defence is essential. Plants, it must be said, don't do things by halves. Skimping on defence is not the plant way. Couple this with many millions of years of dealing with enemies and the result is a combination of fortifications and weapons guaranteed to impress anyone with an interest in warfare. Essentially, plant defence has two components, one spatial and the other temporal. In their review of defence in conifers, Franceschi and colleagues suggest that the spatial component of defence can be compared to the defences of a medieval castle, made up of inner and outer walls and battlements surrounded by a moat.[1] This analogy works particularly well for trees, but can also be applied to non-woody plants. The temporal component of this defensive system represents the production of defences following attack, as well as during plant development, such as when it produces new leaves or roots. This plant defensive system can be surprisingly durable. Among the oldest living organisms on the planet are conifers, some of which can live for up to 4,000 years. During such an incredibly long life, these trees will have had to endure countless attacks by pathogens and pests. Their survival is testament to the incredible effectiveness of plant defences. I want to begin our journey through the defensive system of plants by looking at it as an attacker would face it. Although the first defence an attacker is likely to face is structural, the plant's

fortifications can conceal hidden stashes of chemicals, designed to inflict severe injury on the attacker. Right from the start, the attacker faces a fearsome combination of fortifications and chemical weapons.

## Let's get physical

All aerial plant surfaces are covered with a waxy layer known as the cuticle. When plants first started to colonize the land, some 450 million years ago, they were faced with a whole raft of challenges imposed by their new environment. These terrestrial pioneers needed to deal with desiccation, a massive problem when one's previous existence was aquatic, as well as temperature extremes and exposure to ultraviolet (UV) radiation. The plant response to these challenges, and especially the need to retain water as it grew in its new terrestrial environment, was the development of a hydrophobic surface layer, the cuticle. Plant cuticles are very effective at reducing water loss from aerial surfaces (transpiration), allowing them to control water movement using stomata. But there is more to the cuticle than preventing excessive water loss. The cuticle is the first point of contact between a plant and many microbes and insects and, indeed, waxes in the cuticle are important cues for the development of various fungi on the leaf surface. Likewise, differences in cuticular waxes can be used by specialist insects when choosing suitable sites for feeding or oviposition. As important as the cuticle might be to the development of pathogenic fungi or insect behaviour, it is still a barrier, lying between the attacker and the nutrition within the leaf.

Although some pathogens enter leaves via stomata or wounds, many pathogenic fungi must breach the cuticle in order to gain access to the plant cells lying below.[2] We have already seen that some fungi simply blast their way through the cuticle using hydrostatic pressure, while others soften the cuticle prior to forcing their way through. The problem with the latter approach is that it releases components of the cuticle, which can be recognized by the plant as a sign that it is under attack. As we've

seen previously, such recognition of the 'damaged self' will elicit defence responses.

For many insects, getting a meal means being able to get a good grip on the plant surface. This is not as easy as it might appear, because waxes in the cuticle can determine the ability of the insect's toes (tarsae) to adhere to the surface. This means that waxy leaves can be less infested with insects than glossy leaves, while some plants operate what is known as the 'greasy pole syndrome', where flower stalks are covered in waxy material, making it difficult for insects to reach the flower itself.[3]

Now, let's get back to fungi for a minute and specifically to the fungus that has just managed to make its way through the cuticle. It is closer now to the nutriment within the cells—almost there in fact. Well, actually, things have just got a whole lot worse for the microbial robber, because it now has to tackle the cell wall.

The plant cell wall is truly remarkable, providing structure to the plant body, as well as protection against the stresses imposed by the environment. As we saw in Chapter 2, it is composed mainly of cellulose, a long chain of linked sugar (glucose, to be precise) molecules that gives wood its remarkable strength and, incidentally, forms much of what we call dietary fibre. This cellulose is bundled into fibres called microfibrils, which are cross-linked with other carbohydrates called hemicelluloses, providing great strength to the structure. All of this is embedded in a gel-like matrix of pectins, the stuff used by cooks and jam-makers to thicken jams and jellies. This impressive, but weird-sounding structure, is the primary cell wall, and yes, you've guessed it, if there is a primary cell wall, there should also be a secondary cell wall. Many plant cells form this secondary structure once the cell has stopped growing. These secondary cell walls are frequently impregnated with lignin, a polymer composed of phenolic compounds, which provides great rigidity to the cell wall. All this talk of strength and rigidity has hopefully made you think of a structure that would be very difficult to breach. Indeed, lignified cell walls are highly impermeable to pathogens. But since many plants become diseased, it would appear that some pathogens can get through the cell wall. As was

mentioned briefly in Chapter 2, many pathogens have evolved the means of breaking through the cell wall, and they do so using a cocktail of enzymes, capable of breaking down the individual components, softening the structure, and allowing the pathogen to grow through into the cell itself.[4] The enzyme cocktail used by pathogens to breach plant cell walls includes cellulases, hemicellulases, and pectinases. Hemicellulases such as xylanase, degrade the linear backbone of xylan, the major hemicellulose in cell walls, while pectinases such as polygalacturonase attack the pectic backbone of the cell wall, which is comprised of homogalacturonan. In newly formed pectin, the homogalacturonan is protected from the ravages of pathogen pectinases by the addition of methyl ($CH_3$) groups—they are said to be methyl-esterified. However, a component of the enzyme cocktail produced by many pathogens is pectin methylesterase, which removes the methyl groups from homogalacturonan, thereby enabling the pathogen to break it down. It seems curious therefore that *Arabidopsis* plants attacked by pathogens such as the fungus *Alternaria brassicicola* should increase activity of their own pectin methylesterases, since this would allow the pathogen to attack and break down homogalacturonan in the cell wall. A possible explanation for this counterintuitive behaviour is that by increasing pectin methylesterase activity, the plant is stimulating the release of cell wall fragments (i.e. DAMPs, thereby activating defences and halting further pathogen progress).[5] The importance of pectin methylesterases to the ability of some pathogens to infect their hosts was demonstrated by Vincenzo Lionetti and co-workers. Pectin methylesterases can be inhibited by specific proteins and in the plant these are used to regulate the activity of the enzymes. The researchers overexpressed the genes coding for two of these inhibitors in *Arabidopsis* and found that the ability of the fungus *Botrytis cinerea* to infect the plant was greatly reduced.[6]

On the face of it, a plant faced with a cocktail of enzymes capable of causing such serious damage to their cell walls might seem defenceless. On the contrary, plants can counter this enzymatic onslaught by producing proteins capable of inhibiting the component enzymes, including xylanase inhibitors and polygalacturonase inhibitor proteins (PGIPs). PGIPs are

located in the plant cell wall and can limit fungal invasion. For example, transgenic tomato plants overexpressing a PGIP gene from pear, and hence exhibiting accumulation of PGIP, displayed reduced colonization of the grey mould fungus *Botrytis cinerea*.[7] Reducing fungal ingress through the cell wall is not the only function of PGIPs.[8] When fungal polygalacturonases attack the pectin in the plant cell wall, they release cell wall fragments that are capable of acting as DAMPs and triggering defences. Continued action of the fungal polygalacturonases reduces these cell wall fragments to a size too small to trigger defence. Although the plant's PGIPs inhibit the fungal polygalacturonases, the inhibition is not complete and a small amount of fungal enzyme activity remains. This residual fungal polygalacturonase activity is sufficient to release some cell wall fragments but is not enough to break them down into fragments too small to act as DAMPs. So the plant gets two defensive functions from one protein.

Fungal pathogens are not alone in producing enzymes capable of degrading cell walls. Two can play that game, for plants also produce enzymes with cell wall degrading ability. These include chitinases and glucanases, which attack the chitin and glucan components of fungal cell walls, respectively. Since plants do not contain chitin, it was proposed that the various chitinases present in plants function to protect them against fungal invasion by degrading fungal cell walls. In fact, it seems that chitinase and glucanase are a double act, working together to break down cell walls of invading fungal pathogens.[9] Such attacks on fungal cell walls will release cell wall fragments and, as you might expect, these can act as DAMPs, triggering plant defences. It will come as no surprise to learn that fungal pathogens have evolved the wherewithal to counter the plant's defensive enzymes. Researchers in the Netherlands found that the tomato leaf mould fungus, *Cladosporium fulvum*, produces a protein which binds to its own cell walls where it protects the fungus against the action of tomato chitinases as it invades the plant.[10] As if to prove the adage that it is unwise to put all your eggs into one basket—or in this case to rely exclusively on one strategy to deal with the host's chitinases—this fungus was subsequently shown to produce a protein capable of binding to any fragments of

chitin liberated by plant chitinase.[11] This sequestering of the chitin fragments effectively prevented their use in triggering plant defences. Yet other mechanisms exist to deal with plant chitinase. The fungus *Fusarium verticillioides* produces an enzyme called fungalysin, which cleaves and inactivates the defensive chitinases produced by its plant hosts. The plant response to this fungal manoeuvre, discovered by researchers in Russia and the USA, is to produce molecules capable of inhibiting fungalysin. These molecules, known as wheat antimicrobial peptides since they were first found in wheat, inhibit hyphal growth of the fungus.[12]

## Structural reinforcements

Targeting the pathogen's cell wall-degrading enzymes and putting them out of action is one approach to tackling microbial invaders. Another is to add structural reinforcements to the cell wall, making it harder for the pathogen to breach the barrier. This is the equivalent of repairing any breaches of the castle walls as soon as they occur. In the midst of a sustained assault on a fortified castle, one can imagine that carrying out such repairs would be almost impossible. But many plants do just this, producing structures called papillae (see Plate 6) at sites of attempted pathogen penetration.[13] These defensive structures, also known as cell wall appositions, were first discovered by the renowned German surgeon, botanist, mycologist, and one of the founding fathers of plant pathology, Anton de Bary, in 1863.[14] Some 32 years later, they were found to contain callose, a plant polysaccharide formed of linked glucose molecules.[15] We now know that papillae can contain additional constituents, including phenolics, lignin, hydrogen peroxide, and the enzyme peroxidase. This is very handy, since phenolics and lignin are toxic, lignin can add great structural strength in its own right, and peroxidase can use hydrogen peroxide to cross-link phenolic compounds and proteins, thereby reinforcing papillae. Formation of papillae occurs early in the plant's defence response and is thought to slow down pathogen ingress, allowing time

for additional defences to be deployed. This all sounds very neat and tidy, but in fact the role of papillae in plant defence is still debated. After all, papillae can be found at sites of successful defence by the plant, as well as at sites of successful pathogen penetration. To make matters worse, mutants of *Arabidopsis* that lack a gene for making callose and so are unable to deposit it at sites of fungal attack, display enhanced resistance to powdery mildews.[16] But all is not lost. It appears that although callose deposition is not required for penetration-based defence against powdery mildews adapted to colonize *Arabidopsis*, it is required for effective defence against non-adapted species of powdery mildews. Indeed, over-expression of a gene for callose synthesis in *Arabidopsis* led not only to increased and early deposition of callose at sites of attempted pathogen penetration, but also to complete resistance against both adapted and non-adapted powdery mildews.[17] It is thought that cell wall-degrading enzymes produced by fungi can gain access to the plant cell wall via very small pores in the wall, called nanopores. Dennis Eggert and his colleagues in Germany used a technique called localization microscopy to obtain the precise localization of single fibrils of callose in *Arabidopsis* under attack by the adapted powdery mildew fungus *Golovinomyces cichoracearum*. They discovered that in *Arabidopsis* resistant to powdery mildew, callose produced by the plant cells seals these cell wall nanopores and also forms a layer on the surface of the cell wall, preventing entry of the cell wall degrading enzymes of the fungus (see Plate 7).[18]

Callose deposition can also act as an effective barrier against certain types of insect attacker. The brown planthopper, *Nilaparvata lugens*, feeds on leaves of rice plants by using its piercing mouthparts, known as stylets, to force its way into the leaf. Its target is the vascular system of the plant, specifically the phloem. A plant's vascular system consists of two major components, the xylem vessels responsible for transporting water and mineral nutrients from the root to the shoot, and the phloem, responsible for moving sugars formed in leaves as a result of photosynthesis, to all other parts of the plant. Essentially, the phloem consists of two types of cell: sieve elements and companion cells. Most plant cells are not islands,

isolated from one another, but rather have very thin cytoplasmic connections between them, known as plasmodesmata. These connections allow for the movement of molecules between neighbouring cells. As sieve elements mature, the plasmodesmata in the cell walls adjacent to other sieve elements widen to form sieve plates. Mature sieve elements stack on top of each other, and because of the sieve plates between them, form long vessels capable of conducting sugars and other materials. In case you were wondering how companion cells fit into all of this, they are connected to sieve elements by plasmodesmata, providing a route for movement of sugars into the sieve elements from the rest of the leaf. Now, back to the brown planthopper. Once its stylet has entered the leaf and found the phloem, it can start ingesting phloem sap containing sugars and other organic compounds, such as amino acids. If a leaf is infested with a large number of these planthoppers, a great deal of sugar can find its way out of the plant and into the six-legged robber. But all is not lost, because if the plant is resistant to the planthopper, genes responsible for callose synthesis are activated, resulting in deposition of callose on sieve plates at the point of stylet insertion. This is remarkably effective and prevents the insect ingesting phloem sap. In susceptible plants, on the other hand, the insect is clearly one step ahead in the evolutionary arms race, because its feeding activates glucanases in the plant, leading to the breakdown of the deposited callose. This allows the planthopper on a susceptible plant to continue feeding unimpeded.[19]

No journey through the structural aspects of plant defence would be complete without spending some time on lignin. It is a major component of secondary plant cell walls where it cross-links cellulose microfibrils, providing a rigid and impermeable structure. Unlike lower plants such as mosses and liverworts, which are unable to synthesize lignin, the presence of lignin in higher plants means that they have the strength and rigidity to grow tall, to form stems and branches capable of bearing flowers and fruits, and to form xylem vessels capable of transporting water up the stems of even the very tallest trees.[20]

Lignin is a complex polymer, formed of sub-units called monolignols. These monolignols are synthesized in the cell and then transported to the cell wall, where they are polymerized to form lignin. Plants are experts at making the most of their resources and it should come as no surprise therefore that, in addition to its other functions, lignin is also used in defence. Many plants respond to attack by insects and pathogens by depositing lignin and lignin-like materials at the site of attack. Lignin not only provides a structural barrier against attack, but its presence in the cell wall can limit degradation of polysaccharides by cell wall-degrading enzymes, and can reduce the diffusion of toxins from the pathogen to the plant and of nutrients from the plant cell to the invading pathogen. If lignin biosynthesis is disrupted, the ability of the plant to resist pathogen attack can be compromised. So when expression of phenylalanine ammonia lyase, an enzyme involved in the synthesis of lignin building blocks, was suppressed in tobacco, resistance to the fungal pathogen *Cercospora nicotianae* was reduced.[21] In a similar vein, researchers in Saskatoon in Canada found that silencing genes responsible for monolignol biosynthesis in wheat increased susceptibility to powdery mildew.[22] The cell wall is clearly very important to plants and maintaining its integrity is crucial. An indication of just how important cell wall integrity is to plants was provided by researchers from the John Innes Centre in Norwich in the UK. They discovered that *Arabidopsis* plants compromised in their ability to produce cellulose activated lignin synthesis and defence responses, suggesting that plants can monitor the integrity of their cell walls and respond accordingly.[23]

Insects in search of a good meal can find their dining experience affected by lignin. The toughness of plant tissues has long been thought to provide an important defence against insect herbivores and the presence of lignin would greatly increase the biomechanical strength of this defence. In order to get a meal, larvae of the tobacco stem weevil, *Trichobaris mucorea*, burrow into the stems of *Nicotiana attenuata* and feed on the pith. Researchers at the Max Planck Institute for Chemical Ecology in Jena, Germany, produced plants in which lignin synthesis was disrupted and found that the stem

weevil larvae took advantage of the softer stems of the lignin-deficient plants.[24] In fact, insect herbivores, in general, seem to prefer plants with less lignin. Subsequent experiments by researchers from this lab demonstrated that in plants with greatly reduced lignin contents, and which developed weak, soft stems, lignin precursors were diverted into production of defensive compounds (phenolamides). These chemicals were produced both developmentally, as the plant grew, and especially so following insect attack. Clearly, faced with the prospect of a greatly weakened structural defence, alternative defensive arrangements become a priority, highlighting the importance of lignin as a defence against insect herbivores.[25]

For some of us, the mention of cork summons up visions of opening a bottle of good red wine, but as unlikely as it might seem, there is more to cork than wine. Cork is formed from a layer of cells in the cortex of stems known as the cork cambium. When cells in the cork cambium divide, they give rise to parenchyma cells to the inside and thick-walled cork cells to the outside. These cork cells become invested with the waxy, waterproofing substance suberin, and when the cells reach maturity, they die. Cork cambium and the cells that derive from it are known collectively as the periderm, the most famous of which is that of the Cork Oak, *Quercus suber*. These were the cells observed by Robert Hooke using an early microscope and published in his book *Micrographia* in 1667.[26] So what has cork got to do with plant defence? Well, faced with pathogen attack, some plants produce several layers of cork cells just beyond the site of attack. A good example is the formation of cork layers in potato tubers following infection with *Rhizoctonia solani*, the cause of stem canker and black scurf. These cork layers not only halt the progress of the pathogen, they also block the flow of nutrients and water to the invader, which essentially starves it to death.[27] So next time you remove the cork from your bottle of wine and pour yourself a well-deserved drink, just spare a thought for those unfortunate organisms for whom cork spells the end.

For some plants, it is not enough to block ingress of the attacker. Since a pathogen can only inflict damage if it is in contact with the plant, why not expel the invader? If this seems rather far-fetched, think again, for leaves of

plants such as ornamental cherry do exactly this. Following attack by certain types of pathogen, leaves of cherry trees dissolve the glue (known as the middle lamella) binding adjoining cells together and by doing this in a circle of cells surrounding the site of attack, can effectively cut the pathogen off from the surrounding leaf tissue. The central area of leaf containing the pathogen dies, shrivels up and drops out. Although this results in a hole in the leaf, that is preferable to a rampant pathogen, which would end up inflicting considerably more damage.[27]

For some pathogens, the final destination is the vascular system of the plant, specifically the xylem vessels. These pathogens, the vascular wilts, include the fungus causing Dutch Elm disease, *Ophiostoma novo-ulmi*, the killer of millions of elm trees in Europe and North America. Once it enters the xylem vessels, the water moving through the vessels can spread the fungus to other stems and leaves. This places the plant in great danger, since large areas of the aerial parts of the plant can become infected. This seems like game, set, and match to the pathogen. But a defensive solution is at hand. Xylem vessels have structures known as pits in their walls. These are essentially unlignified areas of the vessel walls that allow movement of water between adjacent xylem vessels. In plants that are resistant to a vascular wilt pathogen, the presence of the invader in a vessel triggers parenchyma cells surrounding the vessel to protrude their protoplast into the lumen of the vessel. This structure is called a tylose and the formation of several of these in a xylem vessel can block the flow of water, and as a consequence, the spread of the pathogen. This can be remarkably effective, especially if tylose production occurs quickly enough ahead of the invading pathogen.[27]

## Chemical warfare

Structural defences will keep many attackers at bay, but they won't keep them all out. When you can't run away from hungry diners intent on putting you on their menu, relying on one type of defence is asking for

trouble. This is where chemicals really come into their own and things begin to get rather gruesome. Imagine, after much struggle and bloodshed getting through the outer walls of a well-fortified castle, that instead of facing the enemy on solid ground, you suddenly find yourself falling into an inner moat filled with corrosive, deadly chemicals. This is pretty much what many attackers face when attempting to invade plant tissues. In fact, on some plants, the attacker will need to face chemicals while still on the plant surface. Onions with coloured scales—red and brown onions for example—contain phenolic compounds that leach into water droplets on the surface of the onion. The onion smudge fungus, *Colletotrichum circinans*, needs water for its spores to germinate, but instead of giving life, the chemicals in the water droplets kill the pathogen.[28] This must be the equivalent of having boiling oil poured over you as you start to clamber up the castle wall.

Phenolic compounds feature heavily in plant defence and none more so than chlorogenic acid. It was first discovered in coffee in 1932 and although it is usually referred to as a single compound, there are in fact a range of chlorogenic acids in coffee, each formed by the addition of different groups, caffeic acid, for example, to quinic acid. Chlorogenic acids are the most abundant phenolic compounds in coffee and they are responsible for much of its bitter taste.[29] These phenolics also have powerful antioxidant properties, a fact not lost on medical researchers worldwide. Indeed, coffee has been reported to lower the risk of cardiovascular disease, to help protect against gout, tooth decay, gallstones and type-2 diabetes.[30] To offset these protective effects, it is worth noting that coffee also contains the carcinogens 4-methylimidazole and acrylamide.[31] It seems that within every silver lining there is a dark cloud.

So what about chlorogenic acid and plant defence? It certainly has antimicrobial properties and has been associated with resistance of, for example, potato to the common scab pathogen, *Streptomyces scabies*. But chlorogenic acid is no one-act wonder. When oxidized in the plant to its corresponding quinone, it is then able to inactivate enzymes, including those used by pathogens as they attempt to gain access to plant cells.

Chlorogenic acid itself has been shown to inhibit the production of cutinase by the brown rot fungus *Monilinia fructicola*, which would seriously hamper its ability to get through the plant cuticle.[32] Indeed, high levels of chlorogenic acid in the epidermal tissues of peach have been suggested to be responsible for the resistance of some peach varieties to this pathogen. Just in case inhibiting fungal cutinase does not halt fungal progress, chlorogenic acid can also inhibit polygalacturonase production by the brown rot fungus, thereby impairing its chances of breaching the plant cell wall. Yet another string in the chlorogenic acid bow comes in the form of its ability to inhibit the synthesis of fungal toxins. Some phytopathogenic fungi produce toxins as a means of facilitating their establishment in plant tissue. One such pathogen is the fungus *Alternaria alternata*, which produces the toxin alternariol. Chlorogenic acid was found to suppress its production by this fungus and importantly, levels of chlorogenic acid were significantly greater in tomato varieties known to be resistant to the fungus, compared to susceptible varieties.[33]

## It pays to be hairy

Insect attackers can also face a combination of structural and chemical defence on the leaf surface. The leaf surfaces of many plants are covered with densely packed fine hairs or spines known as trichomes.[34] These epidermal protuberances are morphologically very diverse, ranging from simple, single-cell projections to complex, multicellular structures with specialized secretory cells. Essentially, they come in two forms, non-glandular and glandular, the former acting as a physical obstacle to insect movement of plant surfaces and the latter releasing various forms of chemical repellents. If non-glandular trichomes are long enough, they can prevent the insect from reaching the leaf surface. The proboscis of the leafhopper *Empoasca fabae* is between 0.2 and 0.4 mm long and although it might be able to penetrate the leaf, the presence of trichomes on hairy soybean leaves prevents it from reaching the nutrients within the vascular

system. Non-glandular trichomes on some plants are rather more elaborate. Those found on leaves and stems of *Mentzelia pumila*, known as the desert blazing star and found in the western USA and north-west Mexico, are hooked.[35] In fact, this plant produces three types of trichome, all fearsome looking. The most common bears a crown of recurved barbs at the tip and occasionally has similar barbs on the shaft of the trichome, giving the appearance of a grappling iron (Plate 8).

A second type of trichome is similar in having recurved barbs at the tip, but also has recurved barbs along the length of the shaft. The third type has a pointed tip and barbs along the length of the trichome which curve upwards. This seems like the leaf surface from hell. An insect would need to be very hungry, determined, and skilful to tackle such a leaf. During a field study in Arizona in 1991, Thomas Eisner and colleagues observed a range of insects stuck on leaves and stems of these plants, all of which were trapped by the trichomes. At first sight, this seems like a pretty effective defence—after all, insects trapped on such a 'sticky' leaf surface are unable to damage the plant. But all is not as it appears because an aphid species, *Macrosiphum mentzeliae*, manages to avoid entrapment by the trichomes and can feed happily on the plant. The aphids appear to achieve this remarkable feat by tiptoeing through the thorns! To make matters worse for the plant, a coccinellid beetle, *Hippodamia convergens*, which preys on these aphids and is therefore an ally of the plant, is trapped and incapacitated by the trichomes. In this case, any adaptive benefit provided by the triumvirate of trichomes appears to be offset by a cost.[35]

## Double trouble—hairy and toxic

If you are thinking that having a trichome-covered, sticky plant surface is a mixed blessing, think again. A great many plants produce trichomes and indeed, almost 30% of vascular plants produce glandular trichomes—the ones that release chemicals. Moreover, quite a number of arthropods commonly found on these trichome-producing plants (such as assassin

bugs, stilt bugs, and green lynx spiders) can move around on sticky plant surfaces without becoming stuck. These bugs consume both living and dead prey trapped on sticky plants, and are clearly adapted to tackling such surfaces. It seems therefore that the benefits of attracting such sticky plant-adapted predators by producing glue-secreting and hooked trichomes might well outweigh the costs of excluding non-adapted predators.

Plants and herbivores have been battling it out for a very long time and it seems likely that for much of this time plants have been using trichomes as an anti-herbivore defence. Indeed, there is evidence for trichomes from the Late Carboniferous, some 300 million years ago. Researchers in Münster in Germany and Kansas in the USA found several types of trichome on fronds and tendrils of the seed fern *Blanzyopteris praedentata*, which, based on their morphology, might have functioned as deterrents against insect herbivores.[36] Glandular trichomes were found on most parts of the foliage and tendrils and appeared to possess a touch-sensitive mechanism that opened a secretory cell when touched. These trichomes appeared functionally similar to 'explosive' trichomes found on certain members of the Curcurbitaceae and Solanaceae around today and which, when touched by an insect, release a sticky exudate which promptly sticks to the insect's legs, impeding its movement.

As impressive as Velcro-like and sticky plant surfaces might be, there is another side to trichomes, for many produce chemical cocktails that pack a deadly punch. Such trichomes comprise several different types of cell, generally a basal cell in the epidermal cell layer, one or more stalk cells, and secretory cells at the apex, where the chemicals are made. Glandular trichomes of some plants, such as tobacco, secrete oils or resins containing terpenes, while those in members of the Lamiaceae, such as mint, are covered with a thick cuticle and accumulate volatile terpenes in a cavity beneath the cuticle. One member of this plant family, the wonderfully named *Colquhounia seguinii*, was recently found to contain three new terpenes, all of which deterred feeding by generalist insects and one of which was seventeen times more effective than commercially available neem oil, which itself contains the insecticidal terpene azadirachtin.[37]

The herbaceous perennial plant *Tanacetum cinerariifolium*, also known as pyrethrum, is used to produce a group of potent insecticidal compounds known as pyrethrins. These pyrethrins occur throughout the aerial parts of the plant, but are concentrated in the dry fruits (known as achenes because they contain a single seed) which are densely covered with glandular trichomes. Interestingly, seedlings of these plants do not have trichomes and cannot produce pyrethrins themselves. So how do seedlings of pyrethrum plants defend themselves? The answer illustrates exactly why studying plants is such a fantastic job, full of surprises. Researchers in the Netherlands found that the building blocks for making pyrethrins are transported from the trichomes to the pericarp of the seed, where they are converted into pyrethrins. As the seed matures, the pyrethrins are absorbed by the embryo and during seed germination, the pyrethrins stored in the embryo move into the tissues of the young seedlings. Hey presto! Instant defence against insect herbivores and fungal pathogens. It seems that parent plants really do take care of their young.[38]

As you might have guessed by now, terpenes are common constituents of glandular trichomes, although other classes of compound are manufactured and stored. The alkaloid nicotine is a minor component of the glandular trichomes of tobacco, *Nicotiana attenuata*, while O-acyl sugars, viscous liquids consisting of aliphatic acids of different lengths combined with sucrose, are the most abundant chemicals in the glandular trichomes of Solanaceous plants. Many of these acyl sugars are effective defences against insects ranging from aphids and white flies through to spider mites. You might be forgiven for thinking that insects would avoid glandular trichomes at all costs. Some Lepidopteran herbivores actually feed on trichomes and for those feeding on *N. attenuata*, the trichomes provide their first meal. And what a meal, because it provides far more than nourishment. Larvae of *Manduca sexta* feeding on trichomes of *N. attenuata* ingest the acyl sugars which are hydrolysed to volatile compounds, imparting the larvae and their frass with a very distinct odour. Unfortunately for the larvae, this gives away their presence to a ground-hunting ant, which loves nothing better than a larval meal.[39]

For humans, mealtime is not usually considered to be dangerous, although it depends on who is cooking, I guess. For insect herbivores, meals can be fraught with danger. Leaves of the ornamental pelargonium, *Pelargonium x hortorum*, have glandular trichomes which produce a chemical cocktail that includes quisqualic acid, a neurotoxin, and phenolic acids known as anacardic acids. The latter are also found in the shells of cashew nuts (*Anacardium occidentale*) and are reported to possess antibacterial activity and to be effective against tooth abscesses. Quisqualic acid is also found in flower petals of *P. x hortorum* and Japanese beetles feeding on flowers of this plant often become paralysed after consuming just a couple of petals.[40] *P. x hortorum* leaves produce two types of glandular trichome, short and tall, the latter producing anacardic acids and exudates from these trichomes are toxic to mites and small insects. The anacardic acids are also sticky and hapless aphids and mites, finding themselves on leaves of this plant, become stuck on the toxic goo and die.

## Plants that sting

Most of us are familiar with stinging nettles. Even brushing an exposed hand, arm, or leg against a stinging nettle causes sharp pain and irritation to the affected area. Stinging hairs on the European nettle, *Urtica dioica*, are composed of a multicellular pedestal surmounted by an elongated stinging cell. These often have a bulbous end which breaks off when touched, revealing a sharp point, rather like the tip of a hypodermic syringe. The English scientist and polymath Robert Hooke first reported stinging hairs on *Urtica* spp. in 1665,[26] and in 1849, formic acid was proposed as the major chemical irritant.[41] Since then, a number of other chemicals have been proposed as the causative agents of irritation resulting from *Urtica* stings, including histamine, acetylcholine, and serotonin. Research published in 2006 involving characterization of chemicals from stinging hairs of *U. thunbergiana* revealed that the chemicals responsible for the long-lasting pain caused by this nettle are oxalic and tartaric acids. These stings did

contain formic acid, histamine, and serotonin, but their concentrations were too small to induce significant pain.[42]

In his excellent book on leaf defence,[43] Ted Farmer suggests that the four plant families that produce stings (Euphorbiaceae, Urticaceae, Losaceae, and Boraginaceae) are relatively modern in evolutionary terms. He suggests that this is consistent with stings being particularly effective against herbivores of a relatively recent evolutionary origin (i.e. vertebrates). Stings don't appear to be particularly effective against invertebrates, but there is much evidence to suggest their effectiveness against mammalian herbivores. Misaki Iwamoto and colleagues at Nara Women's University in Japan studied herbivory of Japanese nettles, *U. thunbergiana*, in Nara Park where sika deer have been protected for 1,200 years.[44] Nettles in this park produce many more stinging hairs than nettles from areas without deer. The researchers found that the Indian Red Admiral butterfly showed no egg-laying or feeding preference for hairy or almost-hairless nettles, whereas deer browsed almost-hairless nettles more heavily than their hairy counterparts. Earlier work using sheep and rabbits found much the same—these herbivores preferred to munch on nettles with a lower density of stings on their leaves than the more common higher sting-density nettle. As you might expect, there are always exceptions and some mammalian herbivores can deal with stings. Mountain gorillas eat nettles and yet avoid getting stung. They achieve this by rolling up the leaves so the stings face inwards and get crushed before the gorillas start eating.[45] I'm not sure I would be brave enough to try that trick.

## Let's get really physical

Something else I don't care to try is eating leaves on a plant bearing huge thorns. Yet this is exactly what some animals do. In fact, many animals, including giraffes, feed on thorny plants. Acacias are the preferred food of giraffes and form most of their diet when available, despite the fact that they possess formidable thorns. Looking at a giraffe browsing the branches

of an acacia tree, one is struck by how skilfully they avoid the thorns, using their tongue to draw leaves towards the front lower teeth, which act as a comb, stripping branches against the giraffe's toothless upper palate. It might seem that thorns on acacia trees provide no defence at all against giraffe browsing. But there is good experimental evidence that thorns do defend acacias against giraffe herbivory. Researchers working on the Athi plains south-east of Nairobi in Kenya found that removing thorns from *Acacia seyal* plants greatly increased herbivory by wild, free-ranging giraffes, compared to plants with a full complement of thorns. Branches within reach of the giraffes subsequently produced a greater density of longer thorns than did higher branches out of the giraffes' reach.[46]

The range of spiky things on plants includes not just thorns, but also spines and prickles. Thorns tend to be woody, sharp-pointed branches, while spines are defined as sharp-pointed petioles, midribs, veins, or stipules, although the two terms are often used synonymously. They can be derived from an entire organ, as in the thorns in most cacti, which are modified leaves, and the thorns on acacias, which are modified stipules (outgrowths usually borne in pairs at the base of the leaf stalk or petiole). In contrast, prickles refer to any sharp-pointed outgrowth from the epidermis or cortex of a plant organ, such as the prickles on the stems of roses. Although some of these structures undoubtedly have other functions, such as climbing aids in rattans, or filtering out ultraviolet radiation in cacti, most have evolved as a defence against herbivores—and vertebrate herbivores in particular. It might appear rather simplistic, but there is a synchrony between the occurrence of spiny plants and the presence of large herbivores, as in the savannahs of Africa. Ted Farmer suggests another way of looking at this—in parts of the world with few large vertebrate herbivores prior to the arrival of man, there are few native spiny plants. The example used by Farmer is of New Caledonia, where, of the roughly 3,000 species of vascular plants, only twenty-three species have anything approaching spines that could be used in defence. Vertebrate herbivores such as rodents and other ground-dwelling mammals are absent from the flora and indeed there are only nine species of mammal on the island, all of

which are bats. Rodents are important herbivores of palms, feasting on the young shoots and ripening fruits and the thorns and prickles on many palms are likely to act as a defence against these mammalian herbivores. There are thirty-two species of palm on New Caledonia, none of which are spiny, which, in view of the lack of vertebrate herbivores, is perhaps not surprising.[43]

## Defence can be tough

One way to avoid being eaten is to be tough. Plants might seem like soft targets for herbivores, but some are too tough even for the most determined vegetarians. Leaves of palm trees are a good example. The young shoots and ripening fruits might be a nice snack for rodents, but mature leaves are a different prospect. They are fibrous and very tough. This is because they contain fibre cells (or sclerenchyma), which are heavily lignified and pretty much indigestible. The primary role of fibre cells is to provide mechanical strength for the plant and they are not usually considered as a plant defence. However, because of the toughness they impart to plant leaves, possession of fibre cells can be a very useful deterrent to herbivory.

Some plants don't just rely on inner strength—they obtain it from external sources. Many plants take up minerals from the soil and deposit them in leaves and stems. One of the minerals taken up and accumulated, especially by grasses, sedges, and horsetails, is silica. Plants take it up from the soil as silicic acid and deposit it primarily in solid bodies called phytoliths in vacuoles and epidermal cell walls, as well as in leaf hairs, trichomes, and spines. These structures were named by the German naturalist Christian Gottfried Ehrenberg in 1835, who called them Phytolitharia or 'plant stones'.[47] Charles Darwin identified a number of these structures in dust blown on to the deck of HMS *Beagle* when the ship was off the coast of the Cape Verde Islands in 1833. He sent the samples to Ehrenberg who identified more than thirty types of phytolith.[48] Phytoliths can be beautiful

structures, which are extremely hard-wearing, and because different plants make distinctive types of phytoliths, they can be used to identify plant remains in ancient deposits. By examining phytoliths in dinosaur dung (coprolites), researchers discovered that titanosaurid sauropods living in India 65–71 million years ago, ate grasses.[49] This is important, because it not only provides evidence that vegetarian dinosaurs ate more than conifers, cycads, and ferns, it also demonstrates that grasses originated and had already diversified during the Cretaceous Period. It appears that the dinosaurs were generalist herbivores, eating a wide range of plant material, since the researchers also found phytoliths from a range of non-grass angiosperms in the dinosaur coprolites.

The presence of silica must make plant tissues a really tough meal. In fact, there is clear evidence that high levels of silica in plants can act as an effective defence against both invertebrate and vertebrate herbivores, many of which are deterred by the abrasiveness of silica-containing tissues. Silica in plant tissues can abrade teeth and a link has been suggested between the evolution of continuously growing teeth in rodents, for example, and a diet of grass, which is rich in silica.[50] But silica does more than wear down teeth. Eating a diet enriched in this mineral can reduce the growth rate and digestive efficiency of both insect and mammalian herbivores. Researchers at the University of York fed larvae of the insect herbivore *Spodoptera exempta* a silica-rich diet and found that the efficiency with which the larvae converted their meal into body mass was reduced. Amazingly, these effects occurred even if the larvae were fed their silica-rich diet for a short period, although the negative effects on the insect increased the longer they were kept on the diet. Eating this abrasive diet increased mandible wear, an effect which happened very quickly and further reduced feeding efficiency and growth of the larvae. As if all this were not bad enough, these effects were not reversible, even if the insects were switched to a silica-free diet. This is truly remarkable, because failure of insect herbivores to adapt to a silica-based defence will have major implications for fitness.[51]

Other minerals accumulate in plant tissues, the most common of these being calcium. This accumulates in plant tissue usually as calcium oxalate,

often in the form of star-shaped crystals called raphides. More than 215 plant families are known to accumulate calcium oxalate and one of its proposed functions is as an anti-herbivore defence. One particularly detailed study by researchers in the USA and Norway examined calcium oxalate crystals in the secondary phloem of forty-six conifer species, characterizing their distribution in relation to defence against bark beetles. They found large differences in crystal deposition between Pinaceae and non-Pinaceae conifers. Calcium oxalate accumulated in both conifer types, but the greatest accumulation occurred in the non-Pinaceae conifers. Whereas members of the Pinaceae accumulated crystals within cells, the non-Pinaceae members accumulated them extracellularly, with crystals embedded in and enveloped by cell wall material. The researchers reckoned that an individual bark beetle attempting to feed on one of the non-Pinaceae conifers (e.g. Taxus, Podocarpus, Cupressus) would encounter so many sheets of calcium oxalate crystals in successive cell walls as it tried to bore through the phloem and cambium that progress would be very difficult. This slowing down of beetle progress would allow deployment of stored phenolic compounds and synthesis of defensive resin, making life for the invader very hard indeed.[52]

The idea that calcium oxalate crystals act as a defence against herbivores has been around for a long time. This hypothesis was tested in 2006 when Kenneth Korth and his colleagues used mutants of the legume *Medicago truncatula* compromised in their ability to accumulate calcium oxalate crystals to study effects on the beet armyworm, *Spodoptera exigua*. The calcium oxalate-rich wild type plants did not appeal to the herbivorous larvae, which much preferred their calcium-deficient relatives. When given no choice but the crystal-laden legume, growth of the larvae suffered and their mortality increased. The calcium oxalate crystals seemed to wear down the larval mandibles and to interfere with the ability of the larvae to convert ingested plant material into biomass.[53]

Just landing on a leaf can be risky for an attacker and, as we've seen, trying to enter the plant's tissues is fraught with danger. Without venturing very far into the plant, the attacker can encounter a formidable array of

physical and chemical defences. Some of these are ready for action prior to the attack, while others are deployed once the attack has begun. But this is just a taster of what's in store for any attacker because the plant has yet to reveal its full chemical arsenal. Sometimes, the cost of getting something to eat is very high indeed.

# 5

# Deadly chemistry

Plants are brilliant chemists. Using the energy of sunlight to turn carbon dioxide and water into carbohydrate is so impressive that it deserves a Nobel Prize. In fact, the American chemist Melvin Calvin won the Nobel Prize in Chemistry in 1961 for revealing how plants are able to achieve this remarkable feat. But plants are no one-trick ponies. Their chemical wizardry is responsible for the bewildering array of compounds that plants use to defend themselves against attack.

Chemical weapons are a very important part of a plant's defensive armoury. Before they can be deployed, the weapons must be produced and then stored safely. Some plants store part of their chemical arsenal under pressure, only to be deployed upon attack. This involves two different types of plant structure—secretory canals called lacticifers and secretory or resin ducts.[1] Lacticifers are specialized cells which produce and store latex, a water-soluble emulsion containing highly polymerized terpenes as well as proteins. Some lacticifers consist of a single cell several centimetres long, which may be branched or unbranched. These are known as non-articulated lacticifers and can be found in spurges (*Euphorbia* spp.), milkweeds (*Asclepias* spp.), and *Cannabis* spp. They are also found in *Antiaris* spp., the latex of which contains the cardenolide (a cardiac glycoside) toxin antiarin, used as a poison for arrow tips in hunting. The other group of lacticifers consists of a file of elongated cells that can extend some considerable distance and which may also be branched or unbranched. In some plant species, the chains of cells can connect laterally to form a net-like

structure. These are articulated lacticifers, found in, for example, the para rubber tree (*Hevea brasiliensis*), poppies (*Papaver* spp.), and the humble lettuce (*Lactuca sativa*).[2]

When a lacticifer is damaged, by insect feeding for example, latex oozes out and upon exposure to air, becomes sticky. The hapless insect becomes stuck, but worse is to come, because this glue can be powerfully toxic. The cardenolides in milkweed latex are potent toxins which inhibit $Na^+/K^+$-ATPases, the cation pump responsible for transporting $Na^+$ out of the cell and $K^+$ into it, thereby maintaining membrane potentials. This is bad news for insects not adapted to feeding on these plants, but can also be unwelcome news for insects specialized to feed on milkweeds, such as the monarch butterfly, *Danaus plexippus*.[3] Most developmental stages of this herbivore can deal with these cardenolides, but early instar stages sometimes fare less well. Although they try to avoid contact with the toxic goo by cutting small trenches through the leaves, their heads and mouthparts can become covered in latex, which they imbibe as they try vigorously to clean themselves. The larvae become cataleptic, although whether this is the result of the cardenolides or other toxic constituents of the latex is unclear. Nevertheless, the unfortunate larvae, in their cataleptic state, can become mired in the goo or can fall off the plant, becoming a meal for ever-hungry predators.[4]

Whereas lacticifers are living cells, resin ducts or secretory canals are essentially intercellular spaces which develop either through separation or breakdown of cells.[1] These spaces can connect and ramify to form a complex system of canals in the plant. Resin producing plant families include the Burseraceae, which houses two well-known trees, frankincense (*Boswellia*) and myrrh (*Commiphora*). In several species of *Bursera* the resin in these ducts is under sufficient pressure that rupturing them can squirt the contents many tens of centimetres, blasting insects off the leaf.[5] Other resin-producing plants include conifers, which manufacture oleoresins. There is evidence that these were made by early gymnosperms more than 300 million years ago, before the emergence of conifers. The oleoresins in some conifers, such as those belonging to the genera *Abies*, *Cedrus*, and

*Tsuga*, accumulate in sac-like structures called resin blisters, whereas those produced by *Pinus*, *Picea*, *Larix*, and *Pseudotsuga* accumulate in resin ducts. When the tissues surrounding these blisters and ducts are damaged, by insect feeding for example, the resin is exuded and on exposure to air eventually becomes solid. This provides a physical and chemical barrier to insect feeding.[6]

As hinted at earlier, some insects are adapted to feeding on latex and resin-producing plants and have developed the wherewithal to deal with these defences. Larvae of the monarch butterfly, which are milkweed specialists, have evolved $Na^+/K^+$-ATPases which are insensitive to cardenolides, although, as we have already seen, early stage larvae can be affected and there can also be an impact on later instars, which can suffer reduced growth. From the insect perspective, perhaps the best approach is to avoid or at least minimize contact with the latex or resin. This is exactly what many insects try to do. Resin ducts and lactifers typically follow the vascular bundles in the plant and by severing the leaf veins or cutting a trench across them, the insect ruptures the secretory canals, thereby reducing the outflow of resin or latex beyond the cuts.[7] Larvae of the monarch butterfly, for example, chew a trench or furrow in the leaf midrib, releasing the lactifer contents, allowing the larvae to feed beyond the cut site, where there is little latex flow. But perhaps the best way of avoiding contact with the toxic gloop from the secretory canals is to get someone else to do the dangerous job of cutting or trenching the leaf veins. This is exactly what males of the cerambycid beetle (*Tetraopes femoratus*) do—they let the females do the dirty work. Males of this beetle prefer to feed on milkweed leaves previously fed on by females, where the leaf midrib has already been cut, draining the latex and making the leaf edible. Although this might appear to be chauvinistic behaviour by the males, as is often the case, appearances might be deceptive. Males of this beetle are smaller than females, which might make it difficult for them to grasp the leaf midrib and cut the vein and because their mandibles are smaller, they are likely to become more easily glued together than the females' mandibles. Incredibly, mating occurred more frequently on these disarmed

leaves proving what a potent combination danger and sex is. Clearly, the life of a female cerambycid beetle is not dull.[8]

## Plant poisons

Cardenolides are not just toxic to insects, for as hinted at above, they can be used as a poison for arrow tips used in hunting. Two of the most well-known cardenolides, digoxin and digitoxin, are used therapeutically for treating cardiac failure. However, poisonings with these cardenolides occur and require urgent hospital treatment. Probably because symptoms of severe toxicity may not occur for up to twenty-four hours for digoxin and five days for digotoxin, as many as 20% of cases can end up as fatalities.[9] Other cardenolides have also been used for poisoning, including those present in yellow oleander (*Thevetia peruviana*), pink or white oleander (*Nerium oleander*), and the sea mango tree (*Cerbera manghas*). Seeds of the sea mango tree, which contain the cardenolide cerberin, were used for centuries in Madagascar as an ordeal poison. It was believed that illnesses, death, and natural catastrophes were the work of witches and the method used to 'prove' that one was not a witch was to take the poison ordeal. It is estimated that this ritual was responsible for the death of 3,000 people per year in central Madagascar, home of the Hovas people. Incredibly, more than 6,000 people were reported to have died in just one poison ordeal. The use of poison rituals was banned in Madagascar in 1861 by King Radama II, although it is suspected that the practice may have survived in remote parts of the island.[10]

In Kerala in southern India, 537 cases of poisoning attributed to the sea mango tree were reported in an eleven-year period from 1989 to 1999. Apparently, to commit suicide, the white fleshy kernel of the seed is consumed as part of a sweet, while for homicide, a few kernels are mixed with chillies to disguise the bitter taste of the poison. Having ingested the poison, intentionally or not, death comes after some three to six hours. In parts of India and Sri Lanka, yellow oleander is used as a means of

self-harm, with tens of thousands of poisoning cases occurring each year.[11] Although there is an antidote (one used to treat digoxin poisoning), it is expensive and not readily available in the areas where these poisonings are common.

## Smoking kills

As most smokers know, nicotine is extremely addictive. It binds to acetylcholine receptors and when it does so at certain nerve–nerve synapses in the brain, it stimulates the nerve cells to fire off an electrical impulse. This causes it to act as a stimulant, but its addictive properties arise because it stimulates nerve cells in the reward pathways in the brain.[12] Nicotine is also toxic and especially so to insect herbivores. Insects adapted to nicotine-producing plants have evolved resistance to the alkaloid, but non-adapted insects feeding on tobacco plants fare badly. The tobacco hornworm, *Manduca sexta*, is, as its name suggests, a tobacco specialist. Even so, its growth is slowed when feeding on a high-nicotine diet, whereas the same diet would kill non-adapted insects. By silencing a gene involved in the synthesis of nicotine, researchers in Jena, Germany, produced coyote tobacco plants (*Nicotiana attenuata*) containing 95% less nicotine than non-manipulated plants. When given a choice between these essentially nicotine-free plants and unaltered plants, larvae of both the tobacco hornworm and the non-adapted beetle *Diabrotica undecimpunctata*, preferred the former. The low-nicotine plants were attacked by herbivores more frequently and suffered three times more damage from insects than plants with their full nicotine complement.[13]

Tobacco hornworm larvae can not only tolerate levels of nicotine that would kill non-adapted herbivorous insects, they are also able to co-opt their diet-acquired nicotine for their own defence. Wolf spiders are major nocturnal predators of insects in the native habitat of the coyote tobacco plant, the Great Basin Desert in Utah. They tend to be put off tobacco hornworm larvae because of the nicotine they ingest when feeding on the

tobacco plants. Hornworm larvae become a much more attractive proposition however, if they are fed on nicotine-deficient tobacco plants. But the nicotine-ingesting larvae don't need to be eaten for wolf spiders to be put off dining on them, because the larvae have an unusual, but effective means of keeping the spiders at bay: bad breath. The larvae pass ingested nicotine from their midgut to the haemolymph (its blood) from whence it can be exhaled through their spiracles during spider attack. According to the researchers who conducted this work, the wolf spiders are deterred by nicotine-rich halitosis.[14]

Nicotine can also be found in flowers of the coyote tobacco plant, with highest concentrations at the base of the corolla, which surrounds the ovary. This also happens to be where floral nectar is typically found. The primary function of floral nectar is to attract and reward pollinators. However, floral nectar of many plants, including coyote tobacco, contain toxic compounds, which deter unwanted visitors to the flowers, including nectar robbers and nectar thieves. The former pierce flowers to extract nectar instead of entering them, while the latter tend to visit flowers as do pollinators, but transfer little pollen as a result of a mismatch with the morphology of the flower. Consuming nicotine-containing nectar can also change the behaviour of pollinators. Hummingbirds are major pollinators and they tend to visit more flowers per plant if that plant produces nicotine. Why should they continue to visit flowers containing repellent nicotine-laden nectar? In the coyote tobacco plant, nicotine levels in flowers are highly variable, even among flowers in the same inflorescence. It was suggested that this variability altered hummingbird behaviour, resulting in their visiting many more flowers in search of those containing low levels of nicotine. In turn, this altered hummingbird behaviour increased outcrossing rates in the tobacco plants. Amazingly, it seems that by manipulating their own chemistry, plants can alter the behaviour of pollinators in order to increase their reproductive success.[15]

Plants can be full of nasty surprises for those intent on receiving without giving back. Palestinian sunbirds are common pollinators of tree tobacco (*Nicotiana glauca*), but they can also be found robbing plants of floral nectar

by piercing the base of the corolla and helping themselves. In the long run, crime never pays and the sunbird robbers will suffer the consequences. Researchers found that nectar robbing by these sunbirds led to an immediate increase in the concentration of anabasine, an alkaloid with greater potency than nicotine. It seems that by robbing the plants of floral nectar, the sunbirds are ingesting much greater amounts of toxic alkaloids than they would be if they obtained the nectar by legitimate means.[16]

## Steroidal nightmares

Ecdysteroids are steroidal hormones present in all classes of arthropods, in which they regulate aspects of development, metamorphosis, and reproduction. Insects cannot make the steroid nucleus in any quantity and in order to synthesize steroidal hormones, such as ecdysone, the moulting hormone, they must obtain cholesterol or sitosterol from their diet. Interestingly, analogues of ecdysteroids can be found in plants. They are known as phytoecdysteroids and are found in more than one hundred plant families. Why plants possess phytoecdysteroids is still debated, although, because they can mimic the activity of moulting hormones in insects, a role in plant defence has been suggested.[17] Plants tend to possess a cocktail of phytoecdysteriods and although they can be found throughout the plant, there is some evidence that the highest concentrations occur in those parts of the plant which are most important for survival, either of the plant or of the species into the next generation (seeds, for example).

Not all insects are affected by phytoecdysteroids, but many are, and the effects on the unlucky herbivore can be gruesome enough to seem like they have come straight out of a horror movie. Larvae of the silkworm, *Bombyx mori*, fed ecdysteroids were unable to remove the old cuticle during moulting, with fatal consequences, while larvae of the pink bollworm, *Pectinophora gossypiella*, developed three heads. In the latter case, three heads were not better than one, since they masked the insect's mouthparts and it starved to death.[18] When phytoecdysteroids were fed to larvae of the Indian

meal moth, *Plodia interpunctella*, not only was moulting and development disrupted, some larvae became cannibalistic.[19] So much for the healthy vegetarian option.

## Cabbages with attitude

Getting children to eat vegetables is a battle all parents face. Although they might consider carrots and peas, cabbage and Brussels sprouts are treated with disgust. Mind you, Brussels sprouts are hardly a popular vegetable among parents! It seems the pungent aroma and bitter taste of these brassicas is very much an acquired taste. These characteristics of brassicas are imparted by sulphur-containing compounds called glucosinolates, which, together with their breakdown products, are known as mustard oils. If present in sufficient amounts, as in wild brassicas, glucosinolates can be toxic to animals, causing a range of symptoms, including severe gastro-enteritis. In vegetable brassicas, they are present in smaller amounts but they are still toxic to many insects.

In brassica tissues, glucosinolates are kept in separate cells from the enzyme responsible for breaking them down, myrosinase. When an insect starts chomping on a brassica leaf, this cellular compartmentation is broken down, bringing the glucosinolates and myrosinase into contact, releasing isothiocyanates and nitriles.[20] The combination of glucosinolates and breakdown products is unpalatable and toxic to many generalist insects and to several specialists that live on non-brassica crops. Larvae of the cotton bollworm, a generalist lepidopteran, avoid feeding on the mid-vein and periphery of rosette leaves of *Arabidopsis* and feed instead on the inner lamina of the leaves (see Plate 9). When researchers examined the mechanisms underlying this behaviour, they discovered that the major glucosinolates of *Arabidopsis* were more abundant in the tissues of the midvein and leaf periphery than the inner lamina. This avoidance of glucosinolate hot spots in the leaf is hardly surprising considering that these compounds can kill larvae of susceptible insects.[21]

Despite their toxicity, glucosinolates and isothiocyanates do not deter all insects. The brassica specialist *Plutella xylostella* prevents the action of myrosinase on glucosinolates, thereby avoiding production of the breakdown products,[22] while larvae of the small white butterfly, *Pieris rapae*, possess a gut protein which redirects glucosinolate breakdown towards the formation of less-damaging nitriles, which are then excreted in the faeces.[23] Some insects go further and co-opt the mustard oil bomb for their own use. Specialist brassica feeders such as the cabbage aphid, *Brevicoryne brassicae*, sequester glucosinolates from their host plants and avoid generating toxic breakdown products by compartmentalizing myrosinase into crystalline microbodies. Any predator wanting a quick snack of cabbage aphid will disrupt this compartmentalization, inadvertently deploying the mustard oil bomb and putting the predator right off any further snacking.[24] One particularly enterprising herbivore, the flea beetle *Phyllotreta striolata*, not only selectively accumulates glucosinolates from its host, it has evolved its own myrosinase.[25]

It is difficult to imagine that substances that can repel and even kill insects can be used by other insects as attractants, acting as stimulants of feeding and oviposition. However, this is exactly what happens with glucosinolates. The major glucosinolate in cabbage is sinigrin and when it is hydrolysed by myrosinase, its mustard oil allyl isothiocyanate is formed. This breakdown product also repels insects, but is not so repellent to humans, since it is the active principle in the much-favoured table condiment, mustard. Sinigrin is lethal to many insects and yet it is a positive feeding stimulus to the cabbage butterfly, *Pieris brassicae*, and the cabbage aphid, *Brevicoryne brassicae*. Its importance as a feeding stimulant to these insects is highlighted by experiments showing that if larvae of the cabbage butterfly, raised on their usual diet of cabbage leaves, are transferred to a diet lacking sinigrin, they refuse to eat and eventually die. Cabbage aphids are also attracted to their host plants by the presence of sinigrin and if, on alighting on a plant and inserting their stylets to start feeding, they fail to detect sinigrin, they quickly fly off in search of a sinigrin-containing host.[26] This glucosinolate is also an oviposition stimulant and adult female

cabbage butterflies can be tricked into laying their eggs on filter paper providing it has been laced with sinigrin. In case you are wondering whether insects need to start feeding before they encounter glucosinolates that we assume would be within the leaf, the answer is that glucosinolates are also found on the leaf surface. Using highly sensitive mass spectrometry, researchers in Germany and the USA found glucosinolates on the leaf surfaces of *Arabidopsis*, at concentrations sufficient to attract specialist lepidopteran feeders. Interestingly, the second most abundant of the glucosinolates detected on leaf surfaces was present just in trace quantities within the leaf. The researchers suggest that, from the perspective of the plant, there must be benefits to having glucosinolates on the leaf surface to offset their use as attractants to specialist insects.[27] Given their toxicity, they could act as a first line of defence, deterring non-specialist insects and pathogens.

Some plants seem to be one step ahead of the game. Unlike most of its fellow brassicas, plants of the genus *Barbarea* contain both glucosinolates and saponins. The latter are triterpenoid compounds that are antimicrobial and also act as feeding deterrents against insects. The diamondback moth, *Plutella xylostella*, is attracted to its host plants by virtue of their glucosinolate fingerprint. Given a choice, these insects prefer to lay their eggs on young leaves on *Barbarea* plants, likely because these leaves contain high concentrations of glucosinolates, which attract the moths. This, however, is a fatal attraction, since the larvae that emerge from these eggs encounter an abundance of saponins when they start to feed and survival rates are low. The researchers who conducted this work speculated that in *Barbarea* plants, glucosinolates might have been a first line of defence, which was overcome by the moth. The plant response to this defence defeat was to produce saponins as a second line of defence, putting it, for the time being, one step ahead of the moth.[28]

Some plant tissues are meant to be eaten. Fleshy fruits attract animals which discard the seeds once they have consumed the pulp, thereby facilitating seed dispersal. However, some fruits contain toxic compounds, including fruit of the desert plant, *Ochradenus baccatus*, which contains

glucosinolates. Fruits of this plant have a unique compartmentation of the glucosinolates from the breakdown enzyme myrosinase, for the glucosinolates are found in the pulp while the enzyme is found in the seeds. Various rodents eat *Ochradenus* fruit, including *Acomys russatus*, which is a seed predator and eats both pulp and seeds, and *A. cahirinus*, a seed disperser, which eats the pulp but expels the seeds. The bitter taste and toxicity of intact glucosinolates are increased considerably by the breakdown products, so rodents eating both pulp and seeds face a dining nightmare. Unsurprisingly, the house mouse, *Mus musculus*, which does not usually encounter *Ochradenus* fruit, is put off by the taste of the glucosinolates in the pulp. The seed predator, *A. russatus*, has a low sensitivity to the taste of the fruit and is not put off by the bitter taste of the glucosinolates. In addition, it has the means of dealing with the glucosinolates and their breakdown products in its gut, ensuring that it suffers little in the way of toxicity. Since the seed disperser *A. cahirinus* does not consume the seeds, it does not face the full onslaught of the breakdown products, which is just as well, since it does not have the wherewithal to deal with the toxins. The mustard oil bomb mechanism seems effective in protecting seeds against most rodent consumers, apart from those which have evolved the means to cope with the bitter taste and the toxins.[29]

## Chemical weapons made to order

Sometime around 1911, the French botanist Noël Bernard observed that tubers of two orchid species, *Orchis morio* and *Loroglossum hircinum* (= *Himantoglossum hircinum*) developed resistance to further fungal infection provided they had already been infected by the fungus *Rhizoctonia repens*. He found that 'even a relatively limited infection of the plant (say one root out of twelve on *Himantoglossum hircinum*) is sufficient for the orchid's tubers to acquire fungicidal capacity'. He placed infected orchid tuber tissues on agar and discovered that the growth of fungi subsequently added to the agar was inhibited. This, reasoned Bernard, suggested that the infected orchid tuber

tissue produced a diffusible inhibitor of fungal growth. These observations were published in a paper in 1911, submitted to the journal *Annales des Sciences Naturelles, Botanique* by Bernard's wife, following his untimely death at just 37 years of age.[30] The diffusible compounds observed by Bernard were not identified until much later, but in the meantime, two German researchers working on late blight of potato made a similar discovery. In some classic experiments in 1940, Müller and Börger found that potato tubers previously inoculated with an avirulent (unable to cause disease) race of *Phytophthora infestans* were protected from the disease if they were subsequently inoculated with a virulent (disease-causing) race of the pathogen.[31] They then painstakingly cut away the tuber tissue reacting to the avirulent race and found that the underlying potato tissue was still resistant, not just to the virulent race, but to other pathogens as well. Müller and Börger suggested that the potato tubers had accumulated a defence compound and named it phytoalexin (from the Greek phyton = plant, and alexin = protecting substance). It took more than twenty years before the first phytoalexin was isolated and characterized. In 1999, it was estimated that more than 300 phytoalexins had been identified from some 900 plant species representing forty plant families. Further phytoalexins have been discovered since then, and all can be grouped according to their structures and their biosynthetic pathways. For example, sulphur-containing indole phytoalexins are produced mainly by brassicas such as cabbage, sesquiterpene phytoalexins by potato and other members of the Solanaceae, and isoflavanoid phytoalexins by legumes belonging to the Papilionoideae subfamily, such as the garden pea. Having said that, some plants produce several related and unrelated phytoalexins, a good example being rice, which produces sixteen different phytoalexins.[32]

Plants make and accumulate a great many compounds that possess antimicrobial properties, but what distinguishes phytoalexins from the rest is that they are only made and accumulated following attack. Those antimicrobial compounds already present in the plant before attack, or ones made after attack but only using pre-existing constituents, are called phytoanticipins.

For effective defence, less can be more. The key to good defence is to get in quick—speed is king. In some classic and elegant experiments, John Bailey and Brian Deverall demonstrated that in a variety of French bean resistant to the fungal pathogen responsible for anthracnose, *Colletotrichum lindemuthianum*, it took less than one-third of the phytoalexin phaseollin to stop infection, compared to a susceptible variety. The key here was the accumulation of the phaseollin by eighty hours following pathogen attack, before the fungus had got its act together; in the susceptible variety, three times as much phytoalexin had accumulated by 160 hours following attack, but to no avail, because phaseollin accumulation did not start until some 120 hours after attack, by which time the pathogen had completed the infection process.[33] Sometimes you can actually see this happening—well, providing you have a high-powered light microscope. Sorghum produces two phytoalexins—apigeninidin and luteolinidin—which are red- and orange-coloured. When sorghum leaves are attacked by *Colletotrichum sublineolum*, these phytoalexins are synthesized in the cytoplasm of the epidermal cells, where they accumulate in colourless vesicles or inclusion bodies. These vesicles migrate to the site of attack, accumulate, and the phytoalexins within develop their red-orange colour, before finally being released to do their job. Here, as with the studies on French bean, the phytoalexins accumulated rapidly in the resistant plants and much more slowly in the susceptible variety.[34]

Just because a compound accumulates in the right place, at the right time, and in the right amount, does not prove that it is responsible for halting pathogen progress. What is required is the ability to manipulate the production of the compound in the plant and to determine whether this has any effect on pathogen infection. Maize produces a number of phytoalexins, one of which is the terpenoid compound zealexin. Formation of zealexin involves two enzymes, terpene synthase 6 and terpene synthase 11, both of which are highly induced in maize plants under attack by the smut fungus, *Ustilago maydis*. Inhibiting the activities of these enzymes by silencing the genes responsible for making them increased susceptibility of the

plants to the smut fungus, indicating that this phytoalexin plays a role in the defence of the maize plant against this pathogen.[35]

We normally associate serotonin with neurotransmission in mammals, where it plays a major role in mood control. To quote Frances Ashcroft in her brilliant book *The Spark of Life*,[36] 'happiness and despair are the two faces of the neurotransmitter serotonin'. Like many people, I have seen rather too much of the latter face of serotonin, but now it appears that there is another side to the (un)happiness hormone. Yes, you've guessed it—serotonin is found in plants—in forty-two different species in fact. What it does in plants is not well understood, but so far it has been reported to have roles in senescence, flowering, and plant defence. Lauren Du Fall and Peter Solomon, working in Canberra, conducted a comprehensive search for metabolites in wheat plants treated with an effector from the pathogen *Stagnospora nodorum*.[37] Known as metabolite profiling, this process revealed the accumulation of serotonin, which Du Fall and Solomon subsequently discovered is a powerful inhibitor of sporulation in this fungus. Serotonin also accumulated in wheat plants attacked by the fungus, although its levels were considerably lower than those obtained following treatment with the effector. This suggests that *S. nodorum* manages to suppress serotonin accumulation as part of the plant's defence responses. These workers proposed that because serotonin is a low molecular weight metabolite, which is synthesized by the plant following fungal attack, it should be classified as a phytoalexin. One way of increasing serotonin levels in humans and cheering us up is by vigorous exercise. Perhaps another way to lift our mood is to discover a new phytoalexin, although this option will only be available to a select few.

## Dying to save you

In Cambridge at the beginning of the 1900s, the British botanist and pioneer of what eventually came to be known as physiological plant

pathology, Professor Harry Marshall Ward, was studying the relationship between brome grasses and the brown rust *Puccinia dispersa* (synonym *Puccinia triticina*). He described and discussed these studies in a typically thorough paper in 1902,[38] where, in some interactions between the plant and the fungus, he observed:

> The tissues turn yellow and then brown or black, rapidly shrivelling as if corroded. This is due to the actual death of the cells and withering of the tissues at the infected spots, and at first I thought it must be owing to some other fungus having got in. It is so in some cases, but in the majority of those considered it appears to be due, rather, to the infecting tubes and hyphae being too destructive to adapt themselves to the host-tissues, and must be regarded as a sign of failure of infection, because the Uredo-mycelium is unable to advance in the dead area, and of course no pustules are developed.

In the last research paper he wrote before his tragically early death in 1906 at the age of just 52, Harry extended these observations to interactions between wheat and yellow leaf rust, *Puccinia glumarum*.[39] He used lines of wheat bred by Rowland Harry Biffen of the School of Agriculture at the University of Cambridge, which were susceptible or resistant to yellow leaf rust. Marshall Ward found that following inoculation of a resistant variety with the fungus, the hyphae began to shrivel and lose vitality after four to six days, as they attempted to penetrate the wheat leaf cells. Interestingly, plant cells surrounding the fungal hyphae began to degenerate, losing both their nuclei and chloroplasts. Harry's conclusion was that as a result of their excessively vigorous attack, the fungal hyphae had killed the host cells, thereby starving themselves to death. A few years later, in 1915, the 31-year-old American plant pathologist Elvin Charles Stakman, based at the University of Minnesota, reported the results of his work on the interactions between various cereal crops and the black stem rust fungus, *Puccinia graminis*.[40] He found that when plants which were practically immune (resistant) to the black stem rust fungus were inoculated, a limited number of plant cells were killed rapidly, after which the fungus seemed unable to develop further. He noted that in such interactions, the host plant was

hypersensitive to the fungus and called the phenomenon 'hypersensitiveness'. The phenomenon later became known as the hypersensitive response (HR) when it became apparent that this form of cell death was generally associated with resistance to many pathogens. Research since the late 1990s has suggested that the cell death triggered by pathogen attack can be dissociated from defence mechanisms and as a result, the term hypersensitive response is used today to describe both the arsenal of defences plants unleash during an incompatible interaction and the apparent suicide of plant cells in response to attack, which is known as hypersensitive cell death (HCD). Hypersensitive cell death is, in fact, a type of programmed cell death, in which plant cells under attack, and sometimes cells immediately surrounding them, orchestrate their own death. When a plant is attacked by an avirulent pathogen, or a non-adapted pathogen, there is rapid recognition of the assault and a HR is launched, often culminating in hypersensitive cell death.

As we saw in Chapter 1, a biotrophic pathogen requires living host tissue to survive and if it is to do so, it must avoid killing host cells. It stands to reason therefore that the rapid death of host plant cells upon attempted penetration (i.e. hypersensitive cell death) by a biotroph, such as a rust fungus, will lead to the demise of the invader (Plate 20). However, although hypersensitive cell death is likely to be an effective defence against biotrophic pathogens, it seems unlikely to be much use against necrotrophs, whose *modus operandi* is to kill host cells as quickly as possible and then feast on the dead tissues. Some particularly enterprising necrotrophs, such as *Botrytis cinerea*, actually stimulate plant cells to undergo hypersensitive cell death ahead of their advancing hyphae in order to pave the way for the rapid colonization of the plant tissue. But what about hemibiotrophs, those pathogens which start off their parasitic career as a biotroph but then switch to the dark side by developing a necrotrophic habit? One might expect that providing death of the plant cells occurred while the pathogen was in its biotrophic phase, hypersensitive cell death would be an effective defence. Indeed, it might well be effective against such pathogens, but stopping invasion might have more to do with other defences rather

than death of the host cells. *Arabidopsis* develops a hypersensitive response when challenged with a fungal pathogen not adapted to growing on it, such as the hemibiotroph *Colletotrichum gloeosporoides*. However, as demonstrated by Japanese and Polish researchers in 2014, although the hypersensitive response is effective in stopping the pathogen, this is not the result of hypersensitive cell death, but of other defences deployed during the attempted invasion.[41] Indeed, a similar situation was reported for *Arabidopsis* interacting with another hemibiotroph, *C. higginsianum*, where progress of the invading pathogen was halted as it attempted to breach the cell wall and establish its initial biotrophic hyphae within the epidermal cells. Here too, the resistance observed was not associated with hypersensitive cell death.[42] In fact, cell death is only part of any hypersensitive response, since the dead cells often contain high concentrations of antimicrobial compounds. These chemicals are made both by the attacked cells before they die and by the surrounding, living cells, and creates a hostile environment for pathogens. Death of the plant cells under attack also prevents any toxins or effector molecules secreted by the invading pathogen from moving beyond the localized graveyard of self-sacrificed plant cells.

Exactly how plant cells are killed during hypersensitive cell death is still a matter of controversy and debate. The cells could die as a result of the defence responses triggered during the hypersensitive response or they might die as a result of processes totally unrelated to the accumulation of toxic metabolites. In the latter case, it could be that cell death is a form of programmed cell death. This is a highly regulated process, orchestrated by the dying cell, often with some help from neighbouring cells. Programmed cell death is an important part of plant development, occurring, for example, in the formation of xylem vessels. It is also important in animals,[43] where it falls into three classes: *apoptosis*, where enzymes called caspases break down key components of the cell; *autophagy* (from the Greek 'to eat oneself'), where targeted constituents of the cell are engulfed by a membrane and degraded by a lysosome; and *necrosis*, a form of traumatic cell death resulting from injury to the cell. As I write this in July 2015,

our understanding falls some way short of being able to paint a clear picture of the mechanisms responsible for hypersensitive cell death in plant–pathogen interactions.

## Silencing the enemy

In 1928, it was reported that in tobacco where the lower leaves exhibited severe symptoms of infection by the *Tobacco ringspot virus*, the upper, younger leaves not only showed no symptoms, they also became resistant to subsequent infection by the same virus.[44] The mechanism responsible for this effect remained a mystery for more than sixty years. Viruses multiply by hijacking the plant's genetic machinery, getting it to replicate the viral nucleic acid, which, for most viruses, is RNA. During the replication process, viral RNA accumulates and this is the starting point for what has become known as RNA silencing.

In the early 1990s, David Baulcombe and his colleagues at the Sainsbury Lab in Norwich in the UK had been working on resistance to virus infection in plants.[45] They wondered whether, by inserting into plants, genes constructed to contain all or part of a virus gene, they might be able to immunize plants against virus infection. Their idea was that the once inside the plant cells, the constructed gene would be expressed and the resulting protein might disrupt the replication cycle of the virus. The experiment worked, in that some of the plants generated were resistant to virus infection. However, they also obtained a strange result. They had expected that the constructed gene would be highly expressed in the plants showing resistance to virus infection. Instead, the gene was expressed in plants which were susceptible to the virus. In other words, the result was exactly the opposite of what would be expected from such an experiment. What struck them from their experiments was that the virus resistance they obtained was highly specific for strains of the virus most similar to the constructed gene. Moreover, this gene conferred resistance even if the RNA was not translated into protein. This led Baulcombe and his co-workers to

speculate that an antisense RNA might determine the specificity in this 'RNA-silencing' mechanism. What they needed was evidence to support their hypothesis and so they set about to look for this hypothetical antisense RNA. To do this, they set up screens to identify genes coding for any proteins necessary for this RNA to exert an effect.

Andrew Hamilton had joined Baulcombe's research group and started looking for the predicted antisense RNA. Hamilton was using a procedure known as gel electrophoresis, which separates macromolecules such as DNA, RNA, and proteins according to their size and charge. Samples to be analysed are placed at the bottom of the gel and an electric current applied. Negatively charged nucleic acid molecules move through the gel, with shorter molecules moving faster and migrating further up the gel than larger molecules. According to Baulcombe, Hamilton was not having much luck detecting the predicted RNA. One evening, because he had to rush off to play football, Hamilton stopped his gel electrophoresis early, before it had finished its full run. When Hamilton returned to the lab the next day and looked at the gel, the predicted RNA molecules were there. Previously, the small RNA molecules had simply run off the top of the gels and were therefore not detected. Thanks to football, small interfering RNAs had been discovered.

Baulcombe and his colleagues wondered whether they had stumbled across a process used naturally by plants to protect themselves against virus infection. They found subsequently that RNA silencing is normally induced in plants attacked by a virus and if the RNA-silencing machinery is disabled, plants become hyper-susceptible to virus infection. They eventually discovered that viral nucleic acid codes for proteins capable of suppressing RNA silencing in the plant. Clearly, nature had got there first![45]

So how exactly does RNA silencing work? First, virus double-stranded RNA is set upon by plant enzymes known as dicer-like proteins. These enzymes cut the viral double-stranded DNA into specific fragments of between twenty-one and twenty-four nucleotides in length—the small interfering RNAs on Hamilton's gels. The two strands of the small interfering

RNA are separated and one of the strands becomes incorporated into what has become known as the RNA-induced silencing complex. This complex contains two enzymes with important roles—one enzyme is required for binding the RNA strand, while the other, a nuclease, is capable of degrading RNA. Using the RNA strand as a template, the complex recognizes and binds viral RNA molecules containing the complementary nucleotide sequence. The virus RNA molecules are then degraded by the nuclease, thereby suppressing the accumulation of virus RNA in the host plant.

RNA silencing is a potent defence mechanism that is effective even against rapidly replicating viruses.[46] Because the complex is targeted by small interfering RNAs derived from double-stranded virus RNA, it is specific for viral RNA and the host plant's RNAs are not affected. But it gets even better. There is a mobile silencing signal that can move with the virus or ahead of it. This means that the virus cannot escape RNA silencing by moving between the plant's cells or in its phloem.

As have already seen, many plant viruses code for proteins capable of suppressing RNA silencing, thereby allowing the virus to replicate within the plant's cells. A suppressor from the Tomato bushy stunt virus binds directly to the short double-stranded RNA molecules preventing them from being incorporated into the RNA-induced silencing complex. In another example, a suppressor from the *Turnip mosaic virus* disrupts RNA silencing by interfering with the function of the dicer-like enzyme.

Small interfering RNAs are not only involved in virus resistance. In 2006, researchers at the University of California campuses at Riverside and Berkeley demonstrated the involvement of a small interfering RNA in the resistance of *Arabidopsis* to the bacterium *Pseudomonas syringae*.[47] Another type of small RNA molecule, microRNA, has also been found to be involved in regulating plant defence. For example, in 2010, Chinese researchers demonstrated that *Arabidopsis* produces a number of microRNAs which are required for resistance to bacteria.[48] Many other examples have been reported of the involvement of small RNA molecules in plant defence and as you might expect, in the suppression of host defences by attackers. Indeed, there is increasing evidence for the transfer of such

molecules between different organisms, involved in regulating many aspects of development and responses to the environment.

## The rhythm of defence is a wonderful thing

It has often been said that in this life, timing is everything. In fact, many processes in animals, plants and fungi are controlled in a 24-hour cycle known as a circadian rhythm. These rhythms are important in determining, for example, the sleeping and feeding patterns of all animals, including humans. The first of these rhythms to be discovered were the movements made by leaves of the sensitive plant, *Mimosa pudica*, which opened and closed at a particular time each day. We now know that circadian rhythms are not a response of the plant to changes in light or temperature in its environment, since they continue when plants are moved to complete darkness and unchanging environmental conditions. Instead, plants possess an internal system capable of measuring 24-hour intervals in order to generate these rhythms. Interestingly, a circadian rhythm can show a peak at any time over the 24-hour cycle. So, for example, some genes are expressed at dawn, others in the middle of the day, and others in the evening.

In 2012, research undertaken in Xinian Dong's lab at Duke University in the USA found that a number of genes involved in plant defence were controlled by the circadian clock in *Arabidopsis*. On closer examination, the expression of these genes was found to be greatest at dawn and expression occurred even in the absence of pathogen attack. But why should this be so? It turns out that one of the plant's pathogens, the downy mildew *Hyaloperonospora arabidopsidis*, produces its spores during the night and by having a defensive system that switches on at dawn, the plant is able to anticipate an attack from the pathogen.[49] In a similar vein, in work published in 2015 by Robert Ingle and co-workers, *Arabidopsis* was found to be least susceptible to infection by the fungus *Botrytis cinerea* at dawn.[50] This situation changed as the day progressed however, with the plant becoming

more susceptible as the day wore on. Here, plants were at their most susceptible just a couple of hours after dark. The results from both of these studies suggest that plants anticipate an increased likelihood of attack at dawn. Interestingly, in other research, the virulence of *B. cinerea* was found to be regulated by a circadian clock, with the fungus least able to infect plants at dawn.[51] Ingle and colleagues suggested that perhaps *B. cinerea* uses its own circadian clock to align its attack strategy with times when the plant is least resistant.

If plant defence is regulated by a circadian clock, then altering the functioning of the clock should affect plant defence responses. Indeed, work by Chong Zhang and colleagues demonstrated that disrupting the functioning of two key components of the circadian clock in *Arabidopsis* severely compromised its resistance to pathogen attack.[52] These workers used the pathogenic bacterium *Pseudomonas syringae* in their studies. As we've seen previously, this bacterium enters the plant via natural openings such as stomata on the leaf surface. Stomata close at night and the workers found that during this period, plants rely on stomatal closure, rather than other types of defence, to prevent pathogen entry into leaves. Indeed, at night expression of non-stomatal forms of defence is low. In contrast, during the day, when stomata are open, plants need other forms of defence and not surprisingly, expression of these defences is higher during daytime. It seems that plants rely on different defences to respond to pathogen attacks at different times of the day (and night).

Surely if plant defence against pathogens is in tune with circadian rhythms, it stands to reason that defence against herbivore attack should be similarly affected. It is known that the expression of wound-inducible genes in plants follow a circadian pattern and Danielle Goodspeed and colleagues at Rice University in Houston, Texas, wondered this might enable plants to anticipate herbivore attack through a cyclical activation of defences. They decided to examine this by studying herbivory of *Arabidopsis* by caterpillars of the cabbage looper, *Trichoplusia ni*.[53] Their findings indicated that herbivory by the caterpillars and accumulation of jasmonic acid, which mediates anti-herbivore defences, follows a circadian pattern,

peaking during the day. This suggests that the plant is geared up to maximize its defence when the caterpillars are likely to be feeding. Goodspeed and her co-workers tested this by rearing caterpillars under a day/night regime that shifted their circadian clocks by twelve hours. Caterpillars placed on plants whose circadian clocks had not been altered were therefore feeding when plant defences were low. As a result, the caterpillars ate their fill unhindered by plant defences and grew rapidly.

It seems that Sammy Davis Junior got it right—the rhythm of life certainly does have a powerful beat.

# 6

# A little help from your friends

Plants are at the bottom of the food chain and it certainly seems that everything wants to eat them. We have seen, however, that plants are perfectly capable of defending themselves, which is just as well given their inability to flee at the first sign of danger. But no matter how self-reliant you are, life can be made a great deal easier with a little help from others. Plants are no exception and over the course of their long evolutionary history, they have forged symbiotic relationships with many different organisms. The word symbiosis is derived from Ancient Greek and means simply 'living together'. If both partners benefit from living together, the symbiotic relationship is mutualistic, whereas if one partner in the relationship gains at the expense of the other, the relationship is parasitic. In this life, you don't get something for nothing (unless you're a parasite), and in the mutualistic relationships of plants with other organisms, what they bring to the joint table is food. They might also provide shelter and a place to live, but their ability to photosynthesize means that they can provide their symbiotic partners with carbohydrates and other organic foodstuffs. In turn, the various partners that plants have shacked up with provide benefits ranging from greater access to nutrients in the soil to protection from parasites and predators. Some of the plants' partners actually live within the plant and in order to do so, must find a way of dealing with their host's surveillance and defence systems.

## Plants and fungi can live together peacefully

It seems likely that vascular plants evolved from a species of charophyte or green alga which became semi-aquatic and began to colonize the land during the Ordovician Period, 488–443 million years ago.[1] These early colonists would have encountered a harsh environment, with barren land covered with poor soils containing no organic matter but plenty of mineral nutrients. At about the same time, aquatic fungi were also starting to move on to the land. These early fungi would have been at a great disadvantage compared to their green neighbours, being unable to photosynthesize. Rather than parasitizing the algae to obtain the sugars they could not make themselves, the two colonists formed a mutualistic symbiosis, the algal partner providing carbohydrates and the fungal partner providing inorganic nutrients thanks to its ability to extract and assimilate nutrients from the still-poor soil. Such a relationship, possibly representing an early lichen, would have provided a selective advantage over non-symbiotic early colonists, thereby facilitating the development of more complex tissues. It is 150 years since the Swiss botanist Simon Schwendener demonstrated that lichens are composite organisms consisting of two partners. Now it appears that we need to think again, because research published recently in the journal *Science* reveals that many lichens have three partners. The previously undetected partner in this ménage à trois—hiding in plain sight since scientists began to study lichens microscopically in the 1860s—turns out to be a basidiomycete yeast. And the surprises don't end there, because these fungi belong to an entirely new group, separated from their closest known relatives by 200 million years.[2] Fittingly for research on symbiosis, and in common with most research today, this ground-breaking work was carried out by a team of researchers including members from the USA, Canada, Austria, and Sweden.

Plants and fungi clearly have a long history of working together and perhaps the best known of these relationships is that involving plant roots and certain fungi. This mutualistic symbiosis is called a mycorrhiza (from the Greek for 'fungus' and 'roots') and as with a lichen, the plant

partner provides sugars while the fungal partner provides greater access to soil nutrients, especially phosphate.[3] There are several types of mycorrhizal association, but the two major types are ectomycorrhizae and endomycorrhizae. In the former, also known as sheathing mycorrhizae, the fungus surrounds the root, although fungal hyphae also penetrate the root and grow between its outer cells (the cortex) (see Plates 21 and 22). Endomycorrhizal fungi grow predominantly within the root and actually penetrate its cells, forming a structure called an arbuscule within the plant cell—hence the more commonly used name for this type of association—arbuscular mycorrhiza. The arbuscule, which resembles a cauliflower or broccoli floret, is greatly branched, providing a large surface area for uptake of sugars and other foodstuffs from the host cell (see Plate 10). The most amazing thing about the arbuscule is the fact that it never ruptures the plasma membrane of the plant cell. The arbuscule actually resides within the plant cell, surrounded by its greatly invaginated plasma membrane. This is both an intimate and a sophisticated relationship. It is also ancient. Evidence indicates that arbuscular mycorrhizal-like fungi originated between 462 and 363 million years ago, placing them within the period that plants colonized the land. Fossil evidence suggests that primitive plants were associated with fungi closely resembling modern arbuscular fungi in the early Devonian Period, 410–360 million years ago. In fact, it is generally accepted that the ability of early vascular plants to colonize the land was dependent upon their association with these fungi.

## Forming a relationship

The previous paragraphs make the establishment of the mutualism between early plants and fungi sound easy. In truth, even in ancient times, any fungus attempting to enter a plant root, despite its good intentions, would have to deal with the plant's defences. Obviously the early arbuscular mycorrhizal fungi managed this feat and their descendants now have some 450 million

years of experience in dealing with plant defences. So how *do* mycorrhizal fungi deal with plant defences?

Initially, symbiotic microbes are recognized by the plant as alien organisms and so they must manipulate the plant's immune system in order to establish a mutualistic relationship. As we saw previously, plants perceive two kinds of elicitor molecule during their initial encounter with a pathogenic fungus—those derived from the pathogen itself and those generated as a result of damage inflicted during the attack. The latter elicitors are generated as the fungus attempts to get through plant cell wall using a cocktail of hydrolytic enzymes. Interestingly, ectomycorrhizal fungi lack such enzymes and as a result, they do not produce damage-induced elicitors that would trigger an immune response. The plant will still detect those elicitors associated with the mycorrhizal fungus itself, but right at the very start of the interaction, fewer elicitors are available for the plant to detect. This suggests that plant defences will be triggered as the mycorrhizal fungus starts its interaction with its prospective host and this is precisely what happens. During the early stages of the interaction of an arbuscular mycorrhizal fungus with the plant, genes associated with defences are activated, but are subsequently suppressed by the mycorrhizal fungus.[4]

Of course, the most favourable outcome for any organism keen on establishing a relationship with a plant is to avoid recognition in the first place. Pathogens try to achieve that using molecules called effectors, which act by blocking perception of elicitors by the plant. There is growing evidence that mycorrhizal fungi try to do the same thing. When the complete genome sequence for the ectomycorrhizal fungus *Laccaria bicolor* was published in 2008, researchers reported that one particular gene, *MYCORRHIZAL iNDUCED SMALL SECRETED PROTEIN 7 (MiSSP7)*, was the most highly up-regulated gene in the symbiosis of the fungus with plant roots. This gene was subsequently found to encode an effector protein which proved to be indispensable for the establishment of the mutualistic symbiosis between the fungus and host roots. It transpires that the protein is secreted by the ectomycorrhizal fungus following detection of signals from plant roots, whereupon the protein is imported

into plant cells via endocytosis (a process by which molecules are transported into a cell by engulfing them). Once in the cell, it is transported to the nucleus where it begins its job of altering the expression of genes involved in establishing the symbiosis.[5]

Discovery of the effector in *Laccaria bicolor* was greatly facilitated by the availability of the complete genome sequence. Sequencing the genome of this fungus was made easier because it was possible to grow it in culture, away from the plant. In this way, sufficient fungal biomass could be grown for analysis. Working with arbuscular mycorrhizal fungi is very different, for here the fungus is biotrophic and cannot be grown away from its host. This makes the job of obtaining sufficient fungal material for analysis very difficult. Unsurprisingly therefore, a complete genome sequence for an arbuscular mycorrhizal fungus such as *Glomus intraradices*[6] is not available. This did not deter researchers from the Botanical Institute in Karlsruhe in Germany, who set about to determine whether effectors are also produced by this fungus. They found that *Glomus intraradices* does indeed produce an effector protein (SP7) capable of short-circuiting the plant defence programme. The way SP7 achieves this is remarkable. The researchers found that the effector interacts with a special protein in the plant, known as a transcription factor, which is highly induced when the plant is under attack by a pathogen. Once induced, the transcription factor activates the expression of various defence genes. When *G. intraradices* interacted with the plant root, the transcription factor was induced during the early stages, transiently and at a low level. It appeared that full induction of the transcription factor was prevented by its interaction with the effector SP7 in the plant's nucleus. The scientists wondered whether SP7 could be a universal effector, capable of promoting the biotrophic status of a fungus within a plant cell. To test this idea, they expressed SP7 in an aggressive plant pathogenic fungus, *Magnaporthe grisea*, which causes rice blast. Amazingly, the rice blast fungus expressing the effector caused considerably less disease than the unaltered pathogen. It seems therefore that SP7 is not only an effector protein that aids the establishment of the arbuscular mycorrhizal symbiosis, but might also be a universal effector, capable of reducing host defence

responses and promoting the ability of a fungus to form a biotrophic relationship with its host plant.[7] This use of effector proteins by mycorrhizal fungi, with its striking similarity to the strategy used by pathogens, has led some researchers to suggest that mutualistic fungi might be living in 'pretend harmony' with their hosts.

Pretend harmony or not, the fact remains that mycorrhizal fungi *do* set up a mutualistic relationship with their hosts and in order to do this, they need to both suppress defences *and* start a molecular dialogue that allows them to establish a fully functioning symbiosis. The dialogue begins outside the root, in the soil, where strigolactones, plant hormones secreted by plant roots (of which more later), are detected by the arbuscular mycorrhizal fungus, stimulating its growth. In turn, the mycorrhizal fungus produces diffusible molecules called 'Myc factors', which are perceived by the plant, triggering a reprogramming of the plant's genes and the expression of symbiosis genes.

## Bacterial allies

While most plants get their nitrogen by taking up soluble nitrate or ammonium from the soil, some plants have their nitrogen supplied by their own in-house bacterial friends. Legumes, for example, form a symbiotic association with bacteria capable of the remarkable feat of fixing atmospheric nitrogen and converting it into organic forms of nitrogen. Much of this nitrogen, in the form of amino acids, amides, and other types of organic nitrogen, is passed to the host plant and in return, the bacterial residents get the carbohydrates they cannot make. The bacteria responsible for nitrogen fixation include *Rhizobium* and its relative *Bradyrhizobium*, known collectively as rhizobia. They only fix nitrogen when they have formed a mutualistic association with their legume host and are comfortably accommodated within a special structure called a nodule, which develops on the plant roots. The establishment of the symbiosis, a process known as nodulation, is akin to allowing someone to move in to one of the

rooms in your house—there needs to be bit of identity checking just to make sure you're not inviting a criminal in to your home. The microbial identity-check starts when the bacteria in the soil perceive chemical signals, usually flavonoids, which diffuse from the roots of their prospective host. The bacterial response to this chemical invitation is to produce their own signal molecules, known as Nod factors, which interact with the root hairs on the host root, causing them to curl at their tips. From these kinks in the root hairs, tubular ingrowths from the cell wall, known as infection threads, are formed (see Plate 11). Infection threads containing the bacteria grow from cell to cell through the root, stimulating cell divisions which lead eventually to the development of a nodule. Infection threads enter cells in the developing nodule and release bacteria encased within a membrane derived from the host plasma membrane. Once within the nodule cells, the rhizobia bacteria differentiate into much larger bacteriods, capable of fixing nitrogen.[1] This is the sort of house-sharing friend you want—someone who can pay handsomely for services rendered.

The fact that rhizobia can form this intimate relationship with the host root is testament to their ability to deal with the plant's defences. We've already seen that plants detect elicitors produced by pathogens, thereby setting in motion a cascade of events leading to a defence response. One of the most-studied elicitors produced by bacterial pathogens is flagellin, a structural protein in the flagellum (a whip-like structure used by certain bacteria to allow movement). Flagellin from plant pathogenic bacteria is a potent elicitor of plant defences, with its immunogenic properties residing in a specific portion of the molecule, which is highly conserved. Researchers were able to produce a synthetic version of this portion of the flagellin molecule, which they named Flg22, and found that it was a powerful activator of defences in a range of plants. Treatment of the legume *Lotus japonicus* with Flg22 not only triggered defences, it also inhibited rhizobia infection and nodulation. But not all flagellin is the same. So, flagellin from the symbiotic bacterium *Sinorhizobium meliloti*, for example, is sufficiently different to be incapable of eliciting defences.[8]

Failure of legume roots to recognize flagellin from symbiotic rhizobia does not mean that defences are not activated. In *Lotus japonicus*, just as we saw with mycorrhizal fungi, the legume initially recognizes its symbiotic partner as a potential threat, since defence-related genes are induced. However, as with mycorrhizal fungi, these defence genes are subsequently down-regulated, suggesting that the symbiotic bacteria have evolved the wherewithal to actively suppress host defences. In fact, work carried out by Nicolas Maunoury and colleagues, working in France and Hungary, found that in the interaction between the legume *Medicago truncatula* and the symbiotic *Sinorhizobium meliloti*, gene expression was reprogrammed (transcriptional reprogramming) in two waves. In the first wave, genes involved in defence were repressed, while in the second wave, genes involved in nodulation were activated.[9]

We have seen that recognition of signals produced by the legume root (usually flavonoids) induces the rhizobia to synthesize Nod factors, which in turn trigger the development of nodules on the appropriate species of host plant. Nod factors are lipochitooligosaccharides (LCOs)—basically, they comprise a backbone of chitin on to which various functional groups (fatty acids and acetyl groups, for example) have been added. It was always assumed that plants that do not form symbioses with rhizobia do not recognize and respond to Nod factors. However, work by Yan Liang and associates based in Gary Stacey's lab at the University of Missouri demonstrated that Nod factors could partially suppress defences.[10] Moreover, the defence suppression occurred in both legumes and non-legumes, including *Arabidopsis*, which, as a member of the Brassicaceae, cannot form a mycorrhizal association. The establishment of both mycorrhizal and rhizobial symbioses depends, at least in part, on a common set of plant genes and both depend on the recognition of LCOs—'Myc factors' for mycorrhizal symbiosis and Nod factors for legume–rhizobia symbiosis. Since mycorrhizal associations are ancient, having evolved some 450 million years ago, it is assumed that the ability of plants to recognize LCOs evolved first in this symbiosis and was co-opted later by legumes to support the more recently (~ 60 million years ago) evolved rhizobial symbiosis. It is possible, though,

that the ability to recognize LCOs is more ancient than the first appearance of the mycorrhizal symbiosis, perhaps evolving first in plant–pathogen interactions, before being adapted for a symbiotic role at a later stage.

## Preventing greed in a mutualistic symbiosis

In any long-lasting relationship, trust is important. But just in case the mycorrhizal fungus or nitrogen-fixing bacterium oversteps the mark and gets greedy, the plant can step in to return the interaction to sustainable levels. Once the symbiosis has become established, the plant can regulate the amount of fungal proliferation or nodulation, preventing excessive removal of carbon. This phenomenon is known as autoregulation and can be demonstrated using split-root experiments. So, if one half of a plant root is already colonized by an arbuscular mycorrhizal fungus, colonization of the other half of the root is suppressed. Similarly, if one half of a legume root is already infected by rhizobia, nodule development on the other root half is inhibited. Research suggests that short peptides produced in the root during mycorrhizal establishment or *Rhizobium* infection and nodulation are transported to the shoot where they are perceived, leading to the generation of a shoot-derived inhibitor. This inhibitor (as yet unidentified, although there are several candidates) is then transported to the root, where it suppresses further mycorrhizal colonization or nodulation.[4]

## Microbial protectors

There can be more to friendship than getting on well together. Apparently, friendship can be good for your health. The relationship between plant roots and mycorrhizal fungi has long been known to confer various benefits on the host, the most widely reported of which is increased access to phosphate in the soil. It seems however that the benefits don't end there.

Since the late 1970s, evidence has been accumulating that mycorrhizas can be good for a plant's health, in particular by protecting them against attackers. Various hypotheses were put forward to explain these protective effects, including improvement in the plant's nutritional status and changes in the population of soil microbes capable of antagonizing pathogens. In the mid-1990s, researchers studying the effects of mycorrhizal colonization of tomato roots on infection by the pathogen *Phytophthora parasitica*, noticed something unusual. They found that not only was pathogen infection reduced in mycorrhizal roots compared to roots of non-mycorrhizal plants, proliferation of the pathogen was reduced in both mycorrhizal and non-mycorrhizal parts of the root.[11] In subsequent work, the researchers, based in Dijon and Granada, used a split-root system to study these effects further. They separated the root system of an intact tomato plant in two, and placed one half of the root into soil with no mycorrhizal inoculum and the other half into soil containing inoculum of the arbuscular mycorrhizal fungus, *Glomus mosseae*. As before, they found that both mycorrhizal and non-mycorrhizal parts of the root exhibited less pathogen infection and development and in both cases this was associated with increased defences. Root cells containing arbuscules of the mycorrhizal fungus were immune to the pathogen and resisted the pathogen by reinforcing the cell wall with callose at the site of attack. In the non-mycorrhizal half of the root, root cell walls were also strengthened and any pathogen hyphae attempting to penetrate the root cells were quickly encased in a callose-rich cement. These studies provided clear evidence that mycorrhizal colonization of tomato protected the roots against pathogen infection by activating both localized and systemic induced resistance.[12]

Putting up a fight when you are attacked is all well and good, but if you live in a hostile world, it's useful to be prepared for the next assault. As we saw earlier, plants that have been attacked can put their defences on alert, enabling them to react quickly to subsequent attacks. This is the phenomenon known as priming and has been demonstrated for tomato plants attacked by nematodes and insects.[13,14] Having a mycorrhizal buddy is clearly good for your health.

Plant roots exude substances known as strigolactones into the soil. These carotenoid-derived compounds act as germination signals for seeds of parasitic plants such as *Striga* and *Orobanche*. For a long time, researchers were puzzled by this conundrum—why would plants produce and exude a signal which promotes infection by a parasite? The answer came in 2005, when Japanese researchers found that strigolactones induce branching in hyphae of arbuscular mycorrhizal fungi and promote colonization of the root.[15] Since arbuscular mycorrhizal fungi were around at least 200 million years before the appearance of parasitic plants, it would appear that the parasites have hijacked a signalling mechanism used in mycorrhizal symbiosis for their own ends.[16] To return to the here and now, once the mycorrhizal symbiosis has been established, production and release of strigolactones is greatly reduced. This might be responsible for the protective effects of mycorrhizal colonization against parasitic plants, suggesting a possible use of arbuscular mycorrhizal fungi in controlling parasitic plants, especially where more conventional approaches have failed.

## Establishing a network of friends

Unless we bury our heads in the sand, we don't tend to see plant roots. But because they are out of sight does not mean they are not important. We inhabit an Emerald Planet,[17] where leaves perform the wondrous process of photosynthesis, but out of the light, in the darkness of the soil, roots perform functions without which the greenery above would perish. They anchor plants in the soil, take up water and nutrients to supply the rest of the plant, and they can store carbohydrates for use during hard times. Roots must therefore be able to defend themselves against attack by a multitude of soil-dwelling ne'er-do-wells. It might be surprising to learn that roots are proactive in their defensive duties, capable of shaping the community of microbes that inhabit the area around them—a region known as the rhizosphere. Roots achieve this influence over soil microbes by releasing a variety of biologically active compounds into the

rhizosphere. The various constituents of these root exudates can attract, stimulate, repel, inhibit, and even kill microbes. We have already come across some of the microbes attracted to the rhizosphere—mycorrhizal fungi and nitrogen-fixing bacteria.

Among the bacteria that thrive in the rhizosphere are plant growth-promoting rhizobacteria, which do exactly as their name suggests. One of the ways these bacteria enhance plant growth is by suppressing pathogens and other deleterious microbes in the soil. But some strains of these bacteria also have another string to their bow—they stimulate the plant's ability to defend itself.

Evidence that some of these rhizobacteria could induce resistance to pathogens came in the form of three studies published in 1991. In one of these studies, researchers at the University of Utrecht in the Netherlands used carnation plants and the Fusarium wilt pathogen *Fusarium oxysporum* f. sp. *dianthi*. A suspension of the rhizobacteria was poured on to roots of carnation cuttings and one week later, stems were inoculated with the pathogen. The results were clear—plants treated with the rhizobacteria had a significantly lower incidence of Fusarium wilt.[18] Another of the 1991 studies was conducted by workers in the Department of Plant Pathology at Auburn University in the USA, using the host-pathogen system much favoured by Joe Kuć—cucumber and the fungus *Colletotrichum orbiculare*. They screened ninety-four strains of plant growth-promoting rhizobacteria for their ability to elicit induced systemic resistance in cucumber to the leaf-infecting fungus and found six that provided very effective disease control.[19] For the disease suppression obtained with rhizobacteria to be the result of induced systemic resistance, there needed to be clear evidence that it was plant-mediated and extended to parts of the plant not in contact with the bacteria. Studies on a variety of plants demonstrated that not only were the rhizobacteria not recoverable from sites of pathogen challenge, lipopolysaccharides extracted from the rhizobacteria were able to elicit induced systemic resistance, thereby ruling out protective effects arising from bacterial metabolism. In some elegant experiments using cucumber

and the vascular-wilt pathogen *Fusarium oxysporum* f. sp. *cucumerinum*, the researchers at Auburn University used a bioluminescent rhizobacterial strain to monitor movement of the bacteria within the plant. They applied this to one half of a split-root system and inoculated the other half of the root with the vascular wilt pathogen. The luminescent rhizobacteria protected the plant against the vascular wilt, although it did not move from its application site on the root.[20]

It turns out that the resistance induced by these bacteria is mediated by the signalling molecules jasmonic acid and ethylene. This means that the resistance is effective against attackers sensitive to defences dependent on these two hormones (i.e. necrotrophic pathogens and insect herbivores). Plants that associate with these rhizobacteria are primed. In other words, their defences are put on alert and are only deployed once the plant is attacked.[21]

What has become clear over the past decade or so, is the importance of the soil microbial environment in shaping how plants respond, not just to attackers, but to stress in general.[21] The influence of the soil microbial environment was highlighted by research published in 2015 by Ian Baldwin's group in Jena. They had been using the same field for fifteen years for experiments on *Nicotiana attenuata*. Some seven years into their experiments, they began to notice increasing numbers of plant deaths due to root-borne pathogens. They had inadvertently created the problem faced by farmers and growers who grow the same crop on land continuously—a lethal build-up of the soil pathogen population. They set out to find an approach to tackling the problem and found that a mixture of native bacteria reduced disease incidence and plant mortality significantly. Interestingly, five members of this bacterial consortium were essential for the disease reducing effects to occur, but they were only effective together, not separately. As the researchers pointed out, 'a plant's opportunistic mutualistic associations with soil microbes have the potential to increase the resilience of crops'.[22]

## Hidden helpers

Tall fescue grass had long been used as a cool season forage crop before reports began to emerge in the 1940s of health problems in livestock fed on this plant. Cattle given hay made from tall fescue grass showed signs of lameness in winter, sometimes leading to loss of the affected foot, giving rise to the name 'fescue foot'. Subsequently, researchers found that extracts of tall fescue grass obtained from a farm where cattle were exhibiting signs of lameness possessed vasoconstrictive properties. In fact, symptoms of fescue foot were similar to those observed with ergot poisoning, resulting from infection of rye by the fungal pathogen *Claviceps purpurea*.[23] This fungus produces structures called sclerotia (ergots) on cereal heads where grains should form and end up being harvested along with normal rye grains. The sclerotia are produced by the fungus as a survival structure, to help protect it over the winter months. They are packed full of alkaloids as a sort of chemical protection as they lie in the soil waiting for spring. Some of these chemicals, such as ergotamine, are powerful vasoconstrictors, preventing blood flow to tissues and starving them of oxygen in the process. The problem arises when people or animals eat rye grain contaminated with ergots. The ergot fungus causes a disease, known as holy fire or St Anthony's fire, which was a scourge in the Middle Ages, responsible for the deaths of more than 50,000 people in southern France alone in the period between 990 and 1130. Symptoms of this frightening disease included hallucinations and a feeling of burning skin or insects crawling under the skin. In severe cases, extremities became gangrenous, often resulting in the loss of hands and feet. This dreadful affliction was so frequent that a religious order, the Hospitallers of St Anthony was founded in France in 1095 to help care for victims during their painful suffering. The connection between ergots and the disease was not made until 1670, by a French physician, Dr Thuillier. However, farmers remained unconvinced of this connection for another couple of hundred years and eventually, in 1853, the mycologist Louis Rene Tulasne finally determined that ergots were produced by a fungus and not by the rye plant.[24,25]

Although the symptoms of fescue foot suggested that a toxin similar to that produced by the ergot fungus (the alkaloid ergotamine) might be involved, it was not until 1977 that an endophytic fungus was found to be the culprit[26] and a couple of years later that alkaloid production was found to be responsible for the symptoms. We now know that fescue foot is caused by the endophytic fungus *Neotyphodium coenophialum*, which produces ergopeptine alkaloids. The symptoms of fescue foot appear to be caused by the most abundant of these alkaloids, ergovaline.[27]

The curious thing about fescue foot is that the fungus responsible for producing the toxic alkaloids is an endophyte. In other words, this is a fungus that lives within the plant, but unlike the ergot fungus, it is not pathogenic. Fungal endophytes are a ubiquitous component of terrestrial plant communities, with every plant species examined to date harbouring them within their tissues. Among the best studied are those inhabiting the aerial tissues of temperate grasses and includes the tall fescue endophyte, *Neotyphodium coenophialum*.[27] Hyphae of these fungi grow between the cells in the aerial parts of the plant, including the inflorescences and seeds, without causing symptoms. Because it can grow into host seeds, the fungus can be transmitted from mother plant to offspring. This is known as vertical transmission. These endophytes are associated with a range of benefits to the host plant, including reduced herbivory and systemic resistance against pathogens, known collectively as defensive mutualism. Fungal endophytes also inhabit the foliage of woody plants, but these appear to be horizontally transmitted—fungal propagules germinate on the surface of the foliage and enter the plant either by penetrating the cuticle or via stomatal pores. Unlike their vertically transmitted counterparts in grasses, where single fungal genotypes will typically infect individual plants, endophytes associated with woody plants can be highly diverse within individual host plants. This is especially true in tropical forests, where up to twenty different fungal species can coexist within an individual leaf. Because of the close resemblance of many endophytes of woody plants to pathogens, it was thought that the chances of them being involved in a defensive mutualism with their host plants were slim. However, research by Elizabeth

Arnold and colleagues discovered that foliar fungal endophytes of the cocoa tree provided protection against *Phytophthora* infections. Interestingly, young leaves of the cocoa tree lack endophytes initially, but they accumulate endophytes as they mature.[28]

Endophytes appear to provide protection against pathogens by various mechanisms, including the production of toxic compounds and inducing systemic resistance. However, in the yew tree, they provide protection against pathogens by a completely novel mechanism. Yew is well known as the source of the anti-cancer drug taxol, a diterpenoid compound with antimicrobial activity. In fact, taxol is produced not just by the yew but also by its fungal endophytes. It has long been a mystery why both the host and the endophytes should produce the same toxic compound. Some fascinating research, published in 2015, set out to unravel this mystery. Yew trees form branches from buds that lie underneath the bark. This results in cracking of the bark, providing a ready access point into the tree for pathogens and one that is not easy to defend. You might well wonder why the plant which can produce taxol does not use it to wipe out invaders. The problem is that taxol inhibits cell division and so releasing it near buds would stop their growth. What to do? Well, this is where giving endophytes a home pays off. Researchers found that, in response to attack by wood-decaying fungi, the fungal endophyte increases its synthesis of taxol. However, in order to protect plant cells from the toxic taxol, the fungus sequesters it in hydrophobic bodies. These missiles laden with fungicidal taxol are then released by exocytosis (the export of material out of a cell in vesicles) in response to fungal attack, targeting pathogen entry points, such as cracks in the bark. The taxol-laden bodies coalesce, providing a toxic seal across the potential access point. The authors of the research suggest that yew might have recruited these taxol-producing endophytes to act as mobile, autonomous, vascular-sentinels, similar to the role provided by immunity cells in animals.[29]

The anti-herbivore effects of the fungal grass endophytes are attributable, in part, to alkaloids produced by some strains of the fungi—ergot alkaloids, indole-diterpenes, lolines, and peramine. Ergot alkaloids include lysergic

acid and ergopeptines and are toxic to vertebrates and invertebrates. They affect the central and peripheral nervous systems of vertebrates and can act as agonists or antagonists of dopamine, serotonin, and adrenergic receptors. Lysergic acid and its derivatives are responsible for the psychedelic effects observed in mammals, while the ergopeptines such as ergotamine induce vasoconstrictive effects as we saw above for St Anthony's fire caused by the ergot fungus. Indole-diterpenes include lolitrem B, which is the main causative agent of ryegrass staggers, a disorder affecting livestock grazing on endophyte-infected perennial ryegrass.[27] Peramine was identified in extracts of perennial ryegrass infected with the endophyte *Neotyphodium lolii* and acts as an insect deterrent. When research was undertaken in an effort to eliminate the endophyte from ryegrass as a means of preventing staggers in New Zealand sheep, researchers discovered that the resulting endophyte-free plants were too badly damaged by the Argentine stem weevil to be used in practice. Subsequently, strains of the endophyte were identified which lacked the lolitrem alkaloids but still produced peramine. Commercial cultivars containing these endophyte strains do not cause staggers in sheep and as a result have been introduced into commercial practice.[27]

The protection conferred upon grasses by their fungal endophytes is only partly attributable to the production of toxic alkaloids. Work carried out at Rutgers University in New Jersey, USA, by Karen Ambrose and coworkers found that fungal endophytes of grasses belonging to the genus *Epichloë* possess an insect toxin gene. This gene is similar to a gene with the intriguing name *makes caterpillars floppy (mcf).* The *mcf* gene is produced by a bacterium, *Photorhabdus luminescens,* which inhabits the gut of insect-invading nematodes. When the nematodes invade a caterpillar, the bacteria are released into the hapless insect's bloodstream where they start producing toxins that kill the unfortunate caterpillar within a mere twenty-four hours. The toxins are produced by the *mcf* gene and as the gene's name implies, they make the caterpillar go floppy before its demise. Ambrose and her colleagues discovered that the *mcf* gene ended up in the fungal endophytes by horizontal gene transfer from a bacterium which was either

present in the soil or was associated with a plant host. They estimated that the gene transfer occurred sometime between 7.2 and 58.8 million years ago—as the authors point out, dating of fungal evolution is difficult because of the very poor fossil record. Nevertheless, it seems that at least in grasses infected with these endophytes, toxins produced by *mcf* genes may play a role, together with alkaloids, in conferring protection against insects.[30]

Leaf-cutting ants are one of the most important causes of leaf damage and loss in Neotropical regions. These ants maintain an obligate symbiosis with a fungus, which digests the leaf material collected by the ants, thereby providing food for the ants and their offspring. But the ants are fussy about the leaves they collect. It seems that on their leaf-cutting forays, they prefer harvesting leaves with lower densities of endophytes. When they *do* harvest leaves with high endophyte loads, they take considerably longer to do so—45% longer, in fact. This might reflect the greater toughness of leaves with high densities of endophytes, since leaves of cocoa trees, for example, with high endophyte loads contain more lignin and cellulose than those with lower levels of endophytes. The fact that ants prefer to cut leaf material with low endophyte loads suggests that the fungal endophytes might be exacting a cost on the ants or their fungal colonies. Experiments conducted by Sunshine Van Bael and colleagues suggested that leaves with high endophyte loads limit productivity of the young fungal colonies, especially those with few, inexperienced worker ants. During the early stages of colony growth, there are few worker ants available to collect and clean leaf material. This is when incipient colonies are most likely to fail in the field. Van Bael and her co-workers suggested that endophytes are functionally analogous to constitutive defences of plants, slowing down the growth rates of ant colonies, thereby leading to greater mortality among fledgling colonies.[31] In subsequent work, Tobin Hammer, working with Van Bael, found that beetles fed a diet of endophyte-rich plants were nine times more likely to suffer predation by ants. Why this should be so is not known, but increased predation of the herbivorous beetle would result in less feeding on the plant, reducing plant damage and loss. As the authors suggest, the

endophytes could be providing an indirect, enemy-mediated defensive service to plants.[32]

## Making your mind up—endophyte or pathogen?

In some species of the fungal endophyte *Epichloë* the onset of flowering in the grass host triggers a big change, for both fungus and plant. The start of flowering causes the fungus to start its sexual cycle, in which it changes from being asexual but mutualistic, to being sexual but pathogenic. In its pathogenic sexual state, hyphae proliferate over the flag leaf surrounding the inflorescence, preventing its emergence and giving rise to the phenomenon known as 'choke'.[33] This is clearly Jekyll and Hyde behaviour and begs the question—are these *Epichloë* endophytes really mutualistic symbionts or pathogens whose growth is modulated by the host plant?

The association between the grass host and its endophytes is a highly controlled affair. In this relationship host defences are suppressed, fungal growth is strictly controlled and production by the fungus of any chemical which might trigger a plant defence response is inhibited. Maintaining such a relationship requires a complex interplay of both plant and fungal genes in order to either promote mutualism or enable/prevent the transition to pathogenesis. It is interesting then that when an *Epichloë* mutant, disrupted in its ability to use a stress-activated signal, was inoculated into perennial ryegrass, the once-mutualistic microbe became a pathogenic monster.[34] Work in this area is in its infancy and there is much to discover, but one thing seems certain, a helpful ally can quickly become a dangerous enemy.

As a final word on endophytes, one might be tempted to speculate on which came first, the endophyte or the pathogen. Well, research published in 2014 revealed that the fungus *Harpophora oryzae*, an endophyte of rice, and a relative of the pathogen *Magnaporthe oryzae*, evolved from a pathogenic ancestor. It seems that the initial split of *H. oryzae* from its pathogenic relatives occurred some 67 million years ago, corresponding well with

the origin of the first grass families, 55–77 million years ago. *H. oryzae* finally split from its pathogenic relations 15 million years ago, correlating with the divergence of barley, wheat and oats, which occurred 13–25 million years ago. It seems possible that the differentiation among these different fungi occurred in response to the divergence of their respective host plants.[35]

## Ants to the rescue

The English mining engineer Thomas Belt travelled to Nicaragua in 1868 to supervise the operations of a gold-mining company. He was an amateur naturalist and during his four years in Nicaragua, when he wasn't overseeing the mines, he collected birds, butterflies, and beetles, and made some important natural history observations. He noticed that ants belonging to the genus *Pseudomyrmex* (see Plate 23) inhabited the conspicuously swollen hollow thorns of the bull-horn acacia. What's more, the ants responded vigorously to any intrusion, seeing off large herbivores as well as leafcutter ants. He also noted that, situated at the leaf bases of the *Acacia*, were extrafloral nectaries and small yellow fruit-like bodies (Beltian bodies) which the ants took back to their nests. He found 'honey-secreting glands' in other plants too and noted that ants attracted to the nectar provided protection to the plants. He concluded that the ants 'are really kept by the *Acacia* as a standing army'. Belt was the first naturalist to observe this interaction and his view that the ants provided protection in return for a reward has been firmly proved.[36]

The interaction between *Acacia* and *Pseudomyrmex* ants has been studied in detail since Belt's observations. The importance of the protection provided by the ants to the well-being and survival of the *Acacia* was demonstrated clearly in a series of studies carried out in the 1960s in Mexico by a young graduate student, Daniel Janzen.[37] He found that *Acacia* shrubs and trees lacking the ants suffered far greater damage from insect herbivory than plants harbouring the ants. In occupied trees, the ants drove off invading insects, killing most of them. This treatment was not reserved

for insects—plants too suffered the wrath of the *Pseudomyrmex* protectors. Alien plants within 40 cm of the inhabited tree or shrub were literally chewed to bits and left for dead. This aggressive protection of plants by their ant inhabitants has long been used as a means of controlling pests. In China, for example, artificial ants' nests have been used for hundreds of years to control pests in *Citrus* plantations, while in some tropical countries ants' nests are taken into plantations of cocoa and other crops, presumably to provide protection against herbivorous insects.

Plants that form these obligate, symbiotic mutualisms with ants are known as myrmecophytes and tropical plants of more than one hundred genera provide an abode for specialized ant colonies, usually providing them with food. These ants are completely dependent on their host plant and they exhibit an extensive repertoire of defence and cleaning behaviour. They tackle insects and their eggs, other plants that might pose a threat, and in some cases, plant pathogens.[38] Protection against pathogens has not received as much attention as warding off herbivores, but Martin Heil and colleagues found that the ant-plant *Macaranga* could be infected with fungal pathogens when its mutualistic ant, a species of *Crematogaster*, was absent, but not if ant colonies were present.[39] Work published in 2014 found that mutualistic ants can also provide protection against bacterial plant pathogens and that part of this protection might be the result of bacteria associated with the ants' legs—a sort of biological control, with the biocontrol agent delivered by the ant.[40]

The ferocious protection provided by ants can even ward off large vertebrates. Adrian Barnett and colleagues studied the protection provided by colonies of the ant *Pseudomyrmex viduus* living in the leguminous tree *Macrolobium acaciifolium* in Jaú National Park in Amazonas State in Brazil. The seeds of this tree are an important part of the diet of vertebrate herbivores, including the golden-backed uacari, a medium-sized primate, Northern Amazonian red squirrels, and various parrots and macaws. The researchers found that trees inhabited by ants suffered considerably less seed predation than trees with no ant protectors. But the presence of mutualistic ants does not guarantee protection against primates. Ants of

the genus *Crematogaster* associate with various species of *Codonanthe*, small creeping vines found in Brazil, Central America, and the West Indies. These ants build a nest among the roots of the vines and provide protection against various herbivores. The golden-backed uacari eats the leaves and flowers of the vine, but manages to avoid the ants by removing a short trailing section of vine and taking it to an ant-free perch to begin its meal.[41]

In a mutualistic relationship, both partners must pull their weight and the mutualism between plants and ants is no exception. Mutualistic relationships are open to exploitation by partners that simply don't do enough. In a plant–ant mutualism, has the plant any control over the suitability of its partner? If so, how does it select a partner that will deliver the goods? Martin Heil at CINVESTAV in Mexico set out to answer these questions. In Mesoamerica, *Acacia* species that provide generous rewards are inhabited predominantly by defending mutualistic ants. In contrast, *Acacia* plants that are stingy with their rewards tend to be defended by exploiters—ants that take but don't defend. Heil monitored the development of newly founded ant colonies on high-reward and low-reward *Acacias* for seven months to determine whether reward production correlates with preferred maintenance of defending ants on the respective host plants. He found that the diversity of ants decreased more quickly on high-reward compared to low-reward hosts, with mutualistic ants most likely to dominate the more generous *Acacias*. It seems that the more generous provision of nectar by the high-reward plants shifted the competitive balance between the mutualistic, defending ants and the non-defending, parasitic ants. *Acacias* appear to be able to screen their potential ant partners without needing information on their quality or identity—a sort of competition-based screening. The idea is that since mutualistic ants are more adapted than their parasitic counterparts to make use of the plants' food rewards, and increasing the rate at which the reward (e.g. extrafloral nectar) is provided increases the aggressiveness of the ants, increasing the provision of the food reward would favour mutualistic ants over their parasitic comrades.[42]

In a plant–ant mutualism that works well, it is in the plant's best interests to keep hold of the ant partner and avoid exploitation of the relationship. In *Acacia*, their obligate ant partner, *Pseudomyrmex*, feeds only on the sucrose-free nectar produced by the plant. Generalist insects that would exploit this mutualism are not attracted to the sucrose-deficient nectar. But why should *Pseudomyrmex* feed only on the *Acacia's* sucrose-free nectar? It seems that the enzyme invertase, responsible for cleaving sucrose into glucose and fructose, and which is present in the ant's gut, is inhibited by chitinase, which is present in the extrafloral nectar produced by the *Acacia*. When young worker ants ingest their first meal of nectar, their gut invertase is inhibited, forcing them to continue feeding on the extrafloral nectar, since they cannot digest any other food. The plant is clearly manipulating the digestive capacity of the ant in order to increase its dependence on the plant's food rewards.[43]

There is no doubt that having an army of ants at one's beck and call is an effective way of keeping herbivores at bay. However, the presence of ants, no matter how ferocious, does not put off all herbivores. Take the sap-sucking bug, *Piezogaster reclusus*. This seemingly foolhardy bug specializes on bull-horn *Acacias*, despite the presence of the plants' attendant ants. It seems the trick is to use chemical camouflage. Chemicals present in the cuticle of the bugs fool the ants, which allow the bugs to feed on the *Acacia* undisturbed. However, this chemical mimicry seems to be colony-specific, since transferring individual bugs between ant colonies led to the bug being attacked.[44] For some herbivores, the solution is more straightforward. Workers of *Pseudomyrmex nigropilosus*, a parasitic ant that steals food from ant-defended *Acacia* trees, walks away from trouble. The fact is it can walk 2.6 times faster than the ants protecting the *Acacia* proving that walking is not just good for you—it can save your life.[45]

Not all relationships between plants and ants are obligate and indeed, it is more common for plants and ants to have a flexible arrangement. The sort of flexible protection provided by ants could be useful for plants that have what is known as a nursery pollination system. Here, larvae of the pollinator develop in the plants' flowers. However, this creates a problem in

terms of defence, since arming the flowers with defences is likely to affect the pollinator larvae. What is required is a form of defence that leaves the pollinator larvae unharmed, but is still effective against herbivores and parasites. This is where ants come in handy and indeed various plants make use of these six-legged peripatetic protectors. Fig trees are pollinated by wasps and Yuccas by moths and in both cases the presence of ants reduces the number of parasites and increases the number of pollinators emerging from fruits. Charlotte Jandér, working at the Smithsonian Tropical Research Institute in Panama (she is now based at Harvard University), studied the mutualism between fig trees and their pollinating wasps. She found that the presence of ants reduced herbivory of figs, reduced numbers of parasitic wasps, and led to fewer abortions of developing figs. This resulted in more pollinators and more seeds in fig trees protected by ants.[46]

Going it alone in a hostile world is not easy, but having friends, or at least others to share the burdens, can make all the difference. Over a long period of evolutionary history, plants have established collaborations that provide both support for their growth and development, and defence against their enemies. We've seen that most plants have such a collaboration with mycorrhizal fungi, so it seems rather strange that members of the Brassica family appear to have jettisoned their mycorrhizal allies and lost the ability to form a relationship with their fungal friends. But appearances can be deceptive. Roots of the model Brassica, *Arabidopsis*, are now known to be colonized by many different species of bacteria and it has been suggested that the plant might be using interactions with some of these bacteria to provide a different type of collaborative relationship.[47] Watch this space—it seems that there is much still to be discovered about symbioses in plants.

# 7

# The never-ending struggle

It is a sobering thought that all living organisms on our planet are a potential source of food. Being able to move enables an organism both to search for food and to flee from predators. However, even if you can run away from predators, you might still get caught and so some sort of defence would be useful. Self-defence assumes much greater importance if you are unable to move. It is likely that defences against parasitism and predation evolved early during the evolution of life. Freshwater green algae, the likely ancestors of land plants, bristle with chemical defences, and so it seems reasonable to assume that plants were already well equipped to defend themselves against attackers when they first moved on to land more than 470 million years ago. Conrad Labandeira and colleagues provided evidence for an extensive repertoire of herbivory on a liverwort carried out by arthropods in the Middle Devonian Period, some 388 million years ago. This represents the earliest occurrence of external foliage-feeding and galling in the fossil record. What's particularly interesting is their discovery of oil body cells in the fossil liverwort, similar to those present in modern liverworts containing terpenoid compounds. These structures are thought to represent a defence against herbivores and indeed, modern liverworts that concentrate terpenoids in oil glands are toxic to slugs and leafcutter ants. Labandeira and his co-workers found that the suspected oil bodies were concentrated along the perimeter of the thallus of the fossil liverwort, suggesting a role in deterring margin-feeding herbivores.[1]

## Putting a price on defence

Equipping an arsenal of defences requires a major commitment by an organism, since it depends on a suitable level of investment. This is because producing defences requires both energy and building materials, the latter predominantly in the form of carbon and nitrogen. Photosynthesis can usually supply enough carbon to synthesize defensive compounds such as terpenoids, but providing sufficient nitrogen to make alkaloids is usually more difficult because nitrogen uptake by plants is limited. It has been estimated that whereas it takes 2.6 grams of photosynthetically produced carbon to make terpenoids, double that amount is required to manufacture alkaloids.[2] Defence is clearly an expensive business. But plants also require these resources to grow and develop and produce offspring, and so they are faced with a difficult choice—grow or defend.[3] It seems reasonable at this point to ask whether there is any evidence that plant defence actually diverts energy and resources away from growth and reproduction, in other words, whether they incur allocation costs. Many studies have failed to find such costs, but some have and in these cases the costs were large. If resistance is costly, one needs to be sure that the defences will be used (i.e. that herbivore attack is likely to occur). Large costs are a strong selective disadvantage to resistance when herbivores are not present. After all, what's the point of investing so much hard-earned energy and resources to provide defence against an enemy that might never appear on the scene? However, things look rather different when herbivores do appear and in some studies where the costs of resistance were high, the benefits outweighed the costs in the presence of herbivores.[4]

Resistance can sometimes be costly in other ways too. Amassing a strong defensive capability can inadvertently keep out friends as well as enemies. These ecological costs include effects on mutualists such as mycorrhizal fungi and pollinators which, despite the benefits they provide to their hosts, can be adversely affected by their partner's defences.[5]

## Seeing patterns in plant defence

Biologists have long been intrigued by just how well defended plants are against attacks by herbivores. Many careers have been spent trying to understand and predict how and why plant defences vary and this ongoing quest has led to, and been guided by, a number of hypotheses. Many of these hypotheses assume that defence is costly to the plant.

As we have seen, mounting a defence against attack requires production of chemicals and erection of structural barriers which places a constraint on the plant because it diverts energy and resources away from growth and development. Herein lies the dilemma faced by plants—to grow or defend. This was the title of the classic 1992 paper by Daniel Herms and William Mattson, who formulated the growth-differentiation balance hypothesis.[3] This hypothesis is based on the premise that there is a trade-off between growth and differentiation processes (which includes defence) in plants. It predicts that rapidly growing plants will have low levels of defensive chemicals because making new leaves means there is little carbohydrate left over to manufacture expensive chemicals. Of course plants don't just face attacks from herbivores and pathogens, they also face stiff competition from other plants. So the Herms and Mattson model takes account of both competition between plants and herbivory. Ultimately, the evolutionary outcome of the interactions is mediated by the availability of resources. Being able to grow quickly can be important for plants trying to get ahead of their neighbours and so competition between plants selects for growth. In contrast, herbivore attack selects for allocation of resources to production of defensive chemicals, giving rise to differences in the life history strategies adopted by plants. For example, plant species living in environments where competition from other plants is more important than herbivore attack are likely to possess adaptations that optimize growth with minimal investment in defence. This would involve using inducible defences which are only produced when the plant is attacked and defence compounds that are active at low concentrations.

Hypotheses are made to be tested and the growth-differentiation balance hypothesis has been tested in numerous studies. In one such study, published in 2014, Daniel Ballhorn, together with several colleagues, tested the hypothesis using lima bean plants,[6] which uses cyanogenesis as a defence against herbivores. This chemical defence sounds brutal, involving the release of hydrogen cyanide from cyanide-containing precursors in response to damage. Since the cyanide-containing precursors contain nitrogen, they are considered to be more expensive for the plant to make than carbon-based defences. Intuitively, mounting a cyanogenesis-based defence would limit the amount of resources available for growth and reproduction. In their study, Ballhorn and his co-workers used lima bean plants with quantitatively different levels of the cyanogenesis defence—high and low cyanogenic genotypes—in competition with each other, and in the presence or absence of herbivory by the Mexican bean beetle, *Epilachna varivestis*. They found that the well-defended, high-cyanogenic plants produced less biomass and fewer seeds than their more poorly defended, low-cyanogenic counterparts when they were grown in the absence of herbivores. This suggests that producing an effective cyanogenic defence is indeed expensive for the plant, hence the reduced growth and seed production. Although the high-cyanogenic plants were able to fend off the herbivorous beetle, they competed poorly with their plant neighbours. In contrast, the low-cyanogenic plants were poorly defended but were better equipped to tolerate inter-plant competition. These results provide clear support for the growth-differentiation balance hypothesis. Indeed, it's possible that intense competition between plants might act as a selective force favouring low expression of expensive, constitutive defences such as cyanogenesis. Interestingly, these plants also possess a high level of inducible defences, which, because they are only produced when required, are less costly to the plant. On the other hand, high cyanogenesis might represent a selective advantage when plants are constantly exposed to greater herbivore pressure.

The Herms and Mattson model suggests that plants which evolve under conditions where resources are abundant and competition from other

plants is intense should delay any investment in defences until the demands of growth are met. However, various species of fast-growing plants (some species of poplar are a good example) maintain large concentrations of defensive phenolic compounds from spring into summer, when demands for growth are great. Some species even have their highest concentrations of defensive phenolics in young, developing leaves. Rather than supporting the Herms and Mattson model, these data support what is known as the optimal defence hypothesis. This proposes that plants with an evolutionary history of high herbivory will prioritize the production of defensive compounds at the expense of growth. According to this hypothesis, young leaves will be well-defended since they are of greater value to the plant—they have a lifetime of photosynthesis and carbon production ahead of them compared to older leaves, whose potential has been largely realized. The hypothesis also proposes that such tissues are likely to be highly vulnerable to herbivores. In accord with this hypothesis, researchers found that in the brown mustard plant, *Brassica juncea*, glucosinolate-based defences were highest in cotyledons (first leaves emerging from a germinating seed) during periods when they were critical for plant growth and fitness.[7] Reproductive tissues are also of considerable value to plants and can be at great risk of attack. One would expect, therefore, that these structures would be well-defended. This was found to be the case in wild parsnip, where reproductive structures contained high levels of defensive furanocoumarins, whereas roots, which were at less risk of herbivore attack, contained low levels of the chemical defences.[8]

## Plant invasions and defences

In 1958, the English ecologist Charles Elton published *The Ecology of Invasions by Animals and Plants* in which he expressed his concerns related to the ecological consequences of the movement of species.[9] In Elton's words 'we are living in a period of the world's history when the mingling of thousands of kinds of organisms from different parts of the world is setting up terrific

dislocations in nature'. Biological invasions have increased dramatically since Elton's comments, attributed to human activities such as global trade and transport, extending the range of distribution of many species to new geographical areas. These invasions have an economic impact, but also represent a major threat to biodiversity and natural ecosystems. However, surviving and eventually flourishing in a new environment is not easy and many alien introductions fail. In fact, only a small fraction of alien species become successfully established. Success depends upon competing effectively with native species or occupying empty ecological niches. One factor that might help alien species is the likelihood that in their new environment, their natural enemies will not be present. This forms the basis of the enemy release hypothesis, the foundation of which was laid by Elton in 1958, and it is one of the explanations often considered for the success of invasive species. The idea is that following their introduction to a new geographical region, plants experience a reduction in attack by the natural enemies with which they have co-evolved. Liberated from their natural enemies, the interlopers suffer less damage by herbivores and parasites compared to neighbouring, native plants and as a result, they increase in size and fecundity.[10]

The downside to all this is that the absence of natural enemies reduces selection for resistance against them. With no natural enemies around, why go to the trouble and cost of maintaining defences? Instead, why not put the energy and resources into growing, increasing in size and improving one's ability to compete against neighbouring plants? This forms the basis of the evolution of increased competitive ability hypothesis.[11] The enemy release and evolution of competitive ability hypotheses are not mutually exclusive and could be viewed as linked—freedom from natural enemies and the relaxation of defences enabling plants to invest their resources in improving their competitive ability. Studies conducted using the fast-growing annual, *Arabidopsis thaliana*, have demonstrated that exclusion of herbivores can quickly lead to the relaxation of defences. A study of Canada goldenrod plants by researchers from Cornell University in the USA found that plants from long-term experimental plots from which herbivorous

beetles had been excluded evolved a reduced level of constitutive defence.[12] This work also found that freeing plants from herbivory can lead to the evolution of increased competitive ability against other plant species. Moreover, this increased competitive ability appeared to be due to the production of chemicals which were toxic to a competitor grass, *Poa pratensis*. Not only does this study provide direct evidence that release from herbivory can cause rapid evolution of increased plant competitive ability, it also provides evidence for a newer hypothesis—the novel weapons hypothesis. This proposes that some highly invasive plants become dominant because they possess novel chemicals to which their new, native neighbours have not been previously exposed. The result is that neighbouring natives are particularly badly affected and the invasive newcomer becomes the competitor king.

## Shifting defences

Although various studies have provided evidence in support of the evolution of increased competitive ability hypothesis, not all data collected have been supportive. So greater plant performance has been observed in some species of invasive plants but not in others, while the greater susceptibility of invasive species to herbivory, as predicted by the hypothesis, has often not been found. As some researchers pointed out however, most of the previous studies had not considered the differences between specialist and generalist herbivores. As we saw in Chapters 4 and 5, plants are equipped with a huge arsenal of defences. Those used against herbivores can be divided into two types, quantitative and qualitative. The former, including defences such as trichomes and tannins, are expensive to produce, but have the benefit of being effective against both specialist and generalist herbivores. Qualitative defences, on the other hand, are cheaper to make and include chemicals such as alkaloids and glucosinolates. These act against generalist herbivores but specialists often become adapted to them. When a plant invades a new geographical region, because their specialist herbivore

enemies are absent, they can shift the manufacture of defences from the more costly quantitative defences to the cheaper qualitative defences. This saving in energy and resources can be put to good use in increasing the plant's competitive ability. The evolutionary shift from quantitative defence to qualitative defence by plants invading a new geographical area is known as the shifting defence hypothesis. This was tested by researchers from Leiden University in the Netherlands, who conducted experiments on Tansy ragwort (*Senecio jacobaea*), a noxious invasive weed of wide global distribution. This plant is native to Eurasia, where it is attacked by more than seventy herbivores, including the Cinnabar moth. They found that although invasive populations of the plant from North America, Australia and New Zealand allocated more resources to increasing competitive ability by increasing growth and reproduction, the shift from defence towards competitive ability was only partial. In these invasive plants, defence against generalist herbivores increased, while those used against specialist herbivores decreased. This suggests that the absence of specialist herbivores leads to the evolution of lower protection against specialists and increased competitive ability, while at the same time shifting protection towards generalist herbivores.[13]

## To be seen is to be eaten

In the mid-1970s, Paul Feeny, an Englishman who spent his career at Cornell University in the USA, observed that plants that were easily visible or highly apparent to herbivores tended to have different chemistry than plants which were less apparent. He suggested that the kind of chemical defence a plant has against herbivores and pathogens depends on how easily the plant can be discovered by its enemies. Plants that are not easily detected by herbivores are less likely to be attacked and suffer damage and so don't need a huge defence arsenal. Feeny called this the plant apparency hypothesis and when it was originally proposed, it was linked to the life history of the plant.[14] So large perennial plants such as trees, which are in

the same place year after year, are easily found by herbivores, whereas plants that appear on the scene following a disturbance (fire, for example) are not always there and so will be less apparent to herbivores and not so easily found. The idea is that less apparent plants would invest their resources in qualitative defences, such as glucosinolates, alkaloids, and cardenolides—small molecules which are relatively cheap to make, although they are readily overcome by specialist herbivores. In contrast, more apparent, easy-to-find plants would invest in quantitative defences—mechanisms robust enough to deal with more frequent and consistent attack. This would require considerable investment since such defences are expensive to manufacture and a lot of the defences would need to be produced. Oak trees are typical apparent plants, due to their lifespan and size. But size isn't everything because despite being large, they represent poor quality food for herbivores, since they have low levels of nitrogen in their leaves. They are, however, well-defended, with tough leaves containing high concentrations of tannins, which tend to be effective against both generalist and specialist herbivores.

Conclusive evidence that the plant apparency hypothesis can predict the types of defences that plants employ to defend themselves against herbivores is hard to find. Perhaps this should come as no surprise since, almost by definition, apparency is confounded with plant life history—comparing trees with herbaceous plants, for example. Where good evidence does exist in support of the plant apparency hypothesis, it is for its importance in influencing the likelihood of herbivore attack. Plants might become less apparent to herbivores if they grow intermingled with other species and as a result might suffer less attack and damage. This is known as associational resistance. Bastien Castagneyrol and colleagues conducted a study at a site south of Bordeaux which is part of the Observatoire Régional de la Phénologie (ORPHEE) experiment.[15] Set up in 2008, the experimental plantation contains nearly 26,000 trees of five native species—European birch, pedunculate oak, Pyrenean oak, holm oak, and maritime pine. The researchers assessed insect herbivory on saplings of pedunculate oak growing in stands together with the four other tree species. They found that the

abundance of leaf miners on the saplings decreased with increasing tree diversity and that levels of infestation decreased with decreasing tree apparency. The oak saplings became less apparent to the leaf miners when they were mixed with neighbouring trees that were taller than them. Other studies have also shown that host trees are less infested with insect herbivores when concealed by neighbouring, non-host trees. This might be because the presence of non-host plants disrupts visual or olfactory cues used by insects to locate their hosts.

The likelihood of herbivore attack, or the rate at which plants encounter herbivores, can be affected by the physical environment. One could predict, for example, that plants growing in areas with sparse vegetation are more likely to be found by herbivores compared to plants growing in vegetation-covered areas and so should be well defended. There is evidence to support the view that the frequency with which plants encounter herbivores shapes defensive capability. For example, plants growing on islands with low herbivore or parasite densities tend to reduce their investment in defence. Johan Stenberg and colleagues studied evolutionary interactions between plants and their herbivores using populations of meadowsweet (*Filipendula ulmaria*) on six islands in the Gulf of Bothnia in Sweden.[16] These islands represent a gradient of increasing temporal coexistence between the plant and two specialist leaf beetles, *Galerucella tenella* and *Altica engstroemi*. They found that defences such as condensed tannins and phenolics were positively correlated with island age. In other words, these defences increased in the plants following herbivore colonization of an island and continued to increase as the length of time of coexistence of the plant and the beetles increased.

## Availability of resources

Paul Feeny's idea that long-lived or apparent species invested more heavily in defences compared to short-lived or non-apparent species was accepted by many researchers. However, some considered that the underlying

mechanisms might be explained differently. One researcher who thought so was Phyllis Coley, then based at the University of Utah in the USA. She had conducted work in the lowland rain forest on Barro Colorado Island in Panama in the late 1970s through to the early 1980s. Her objective was to test Feeny's apparency hypothesis in a tropical rainforest with its high species diversity. Coley classified some trees as unapparent since they were short-lived and grew only in gaps in the forest that allowed light to enter. Other trees were considered apparent because, in addition to being long-lived, they were shade-tolerant and so could grow both in gaps and in the understorey of the forest. She found that, as predicted by the plant apparency hypothesis, unapparent trees invested fewer resources in defence. However, these trees were still damaged and suffered six times more herbivory than the shade-tolerant, apparent trees. Coley also found that there was no difference in the variability of herbivore damage for the gap- and shade-growing trees, even though it should have been greater for the gap-growing trees given that some would escape discovery while others would be found and attacked. It seemed that plant apparency could not explain the differences in the defence strategies adopted by the different trees. What Coley found instead was a strong correlation between the amount of resources a tree invested in defence and the level of damage it suffered. She also obtained a strong negative correlation between the growth rate of a species and its investment in defence. So perhaps the rate at which a plant grows might be important in determining the costs and benefits of defence?[17]

Coley's results from Panamanian rainforests were remarkably similar to those obtained around the same time by John Bryant, Stuart Chapin, and David Klein working at the University of Alaska.[18] They studied the browsing of twigs by hares in a boreal forest and found strong correlations between plant growth rate and both the investment made in defence by the plants and damage by the hares. Spurred on by these results, in 1985, Coley, Bryant, and Chapin proposed that a plant's investment in defences was not the result of differences among species in apparency, but to differences in the cost/benefit ratio of those defences. In their paper in

the journal *Science*,[19] they suggested that when resources are limited, plants with inherently slow growth rates are favoured over those with faster ones. The idea is that for fast-growing plants, investing in defence is risky, because re-allocating resources from photosynthesizing leaves towards defence would have a much greater negative effect on them than on slow growers. Fast-growing plants can afford to invest less in defence because they can replace damaged leaves quickly. This hypothesis also postulates that pressure from herbivores is a characteristic of the environment and not the apparency of a plant species. So even if the risk of being attacked is the same across different plant species, selection would favour different levels of defence in species exhibiting inherently different growth rates. The reasoning underlying this is that the inherent growth rate of a plant determines both the cost of defending itself and the impact of attack on its growth and fitness.

## Controlling defence is a balancing act

It is well known that the evolutionary response of plants to herbivore attack is influenced greatly by selective pressures in the environment, one of which is nutrient availability. When resources are in good supply, there is usually selection for characteristics that enable plants to grow rapidly. But if an environmental resource dwindles and begins to limit plant growth, this will affect the way plants respond to herbivore attack. Just a few paragraphs ago, we came across the work of John Bryant and his colleagues on mammalian herbivory in boreal forests. In their 1983 paper,[18] they found strong correlations between plant growth and its investment in defence. But the focus of their paper was broader than that, for they were interested in exploring the constraints the environment poses on plant defence against herbivory. They found that woody plants adapted to growing in environments with little in the way of resources have inherently slow growth rates that constrain their ability to replace tissue lost through herbivory via new growth. To deal with herbivory, such plants have

evolved chemical defences that are present throughout their lives. In contrast, plants adapted to environments where resources are abundant grow rapidly, enabling them to grow beyond the reach of browsing mammals and replace lost tissue quickly. Because of this, these plants tend to be chemically defended only in their juvenile period of growth. Bryant and his colleagues suggested that because nutrients such as nitrogen are more limiting than light in boreal forests, the slow-growing trees found in such forests use defences that are carbon-based (phenolics and terpenes, for example) rather than nitrogen-based. On the other hand, in habitats that are rich in nutrients, nitrogen-based defences such as alkaloids and cyanogenic glycosides assume greater importance. This forms the basis of the carbon–nutrient balance hypothesis. It postulates that the balance between carbon and nitrogen in the plant, which is determined by the availability of resources, exerts direct control over the production of defensive chemicals. In turn, this allocation affects the palatability of the plant to herbivores and its anti-herbivore defences.

Many studies have examined this hypothesis and there is much experimental and correlative evidence to support it. But a great many studies have failed to confirm the predictions of the hypothesis. Still, as Nancy Stamp pointed out in her aptly named 2003 review 'the quagmire of plant defence hypotheses', the carbon–nutrient balance hypothesis predicts that plant species can have some combination of fixed and flexible allocation to defence.[20] This can vary from a completely fixed allocation to a completely flexible allocation.

## Evolution of plant defence—where do beneficial microbes fit in?

As we saw in Chapter 6, the vast majority of plants form mutualistic associations with bacterial and fungal partners. These partnerships are nutritionally based and can exert profound changes in the way resources are allocated in host plants. It won't have escaped your notice that none of

the plant defence hypotheses we have looked at have mentioned mutualistic associations. Mind you, are you surprised? Trying to get to grips with these hypotheses is like trying to extricate oneself from quicksand, so including an additional layer of complexity on the hypotheses would be like your rescuer throwing you a bag of bricks rather than a lifeline. This difficulty did not deter Rachel Vannette and Mark Hunter who, in 2011, proposed the resource exchange model of plant defence.[21] This proposes that the costs and benefits of mutualistic associations will influence the plant's resource status and importantly, how these resources are allocated to growth and defence. So, for example, when nutrient exchange between the plant and its mutualistic partner is optimal, plant growth and defence are maximized. Vannette and Hunter tested their model by growing milkweed with two species of arbuscular mycorrhizal fungi, *Scutellospora pellucida* and *Glomus etunicatum*. They found that increasing colonization of milkweed roots by *Scutellospora* increased both plant growth and defences (latex exudation and production of cardenolides), thereby supporting their defence model. However, root colonization by *Glomus* led to a decline in plant growth and latex exudation. This suggests that the increasing carbon cost to the plant of having this particular mycorrhizal association outweighed any nutritional benefits provided by the fungal partner. Nevertheless, because both plant growth and defence were decreased in this interaction, it seems that plant growth and defence are coupled, as predicted by the model. We might be tempted to think that mutualistic associations are always beneficial to the plant, but this is clearly not the case. So in a mycorrhizal association where the demand by the fungus for carbohydrate is great, something has to give and this might turn out to be resource-hungry defence.

## Looking for patterns in the evolution of plant defence

Picture the scene—the coffee room in the Department of Biological Sciences at Stanford University in the early to mid-1960s. The biologist Paul

Ehrlich mentions to his colleague Peter Raven that it seems strange that the butterflies he was studying fed on plants of two plant families—the Plantaginaceae and the Scrophulariaceae. Raven replies that this is not at all strange, and so starts daily discussions centred around patterns of food plant use in butterflies. As can happen occasionally in departmental coffee rooms, where interesting discussions can develop into ideas that demand to be explored, the two biologists began to search the literature for information on plants fed on by butterflies and the characteristics those plants had in common. Before long they realized that secondary compounds present in the plants played a major role in the butterfly–plant interactions. From these beginnings emerged their classic paper 'Butterflies and plants: a study in co-evolution' published in the journal *Evolution* in 1964.[22] Ehrlich and Raven used their co-evolutionary framework to better understand the observation that insect herbivores usually have narrow host preferences and that closely related insects feed on closely related plants. They suggested that such assemblages arise when a plant evolves a novel means of escaping from its predators allowing it to live in an enemy-free space. In time, this novel plant lineage can diversify or radiate, but in so doing creates a new niche for herbivores. Eventually, some of the insect herbivores will evolve the means to overcome the novel plant trait—a counter-adaptation—enabling them to feed on the plant and ultimately, other related plants.

A good example of a novel plant defence leading to radiation or diversification is that of plant latex and resin canals. These have evolved repeatedly and are highly convergent in flowering plants, occurring in 10% of all plant species. Brian Farrell, David Dussourd, and Charles Mitter of the University of Maryland in the USA figured that resin canals might be expected to allow plant radiation to occur, creating what they called 'an adaptive zone' with little herbivore attack. They compared the diversities of plant lineages that possess independently evolved resin canals with their sister groups. They found that plant clades with latex and resin canals were significantly more species-rich than sister clades lacking resin canals, providing some evidence that evolution of particular defences coincides with

adaptive radiation[23]—the process whereby organisms diversify rapidly from an ancestral species into a number of new forms, especially when an environmental change makes new resources available and opening up new ecological niches.

The pace at which evolution occurs is important in describing the diversification of life. It is also central to the concept of adaptive radiation. Some researchers have argued that a characteristic of adaptive radiation is an initially high rate of trait evolution which slows down with time. Some of the more recent models of adaptive radiation, constructed to aid understanding of the process, predict that most changes in traits should occur early in the diversification of species. As the number of species increases, the rate at which changes in traits occurs would begin to decline. Researchers decided to test the enemy-driven adaptive radiation prediction of Ehrlich and Raven by examining trait evolution in fifty-one species of North American milkweeds.[24] They studied seven traits in the milkweeds, ranging from seed size to cardenolides and latex. They found early bursts of evolution for two traits, latex production and seed mass. Their study also showed that species-rich milkweed lineages underwent a proportionately greater decline in latex and cardenolides compared to species-poor lineages and, moreover, the rate at which these changes occurred was most rapid early in the radiation. These results were interpreted to mean that reduced investment in defensive traits accelerated diversification early in the adaptive radiation of milkweeds.

Once a novel plant defence has been overcome by the evolution of a counter-adaptation by an insect herbivore, Ehrlich and Raven suggested only the evolution of an additional, novel, and more powerful defence would allow that plant lineage to continue to diverge. Good evidence for this prediction comes from work undertaken by Scott Armbruster who studied the ecology and evolution of relationships among a group of vines belonging to the genus *Dalechampia*.[25] It appeared that multiple systems of defence evolved in this genus. The first defensive system to appear on the scene was the deployment of triterpene resins to provide protection for flowers. In fact, this was a 'pre-adaptation', which allowed the evolution of

the resin-based, pollinator-reward system. So pollination of flowers in this genus by resin-collecting bees originated as a defence. Once this resin defence system of the flowers was lost by conversion to a pollinator-reward system, a sequence of defence innovations followed over time. This included deployment of resin to protect developing ovaries and seeds and use of sharp, detaching trichomes on enveloping sepals to defend developing seeds. So at least one pollinator-reward system originated by modification of a defence, and several defence systems arose through modification of pollinator and advertisement systems.

## Some things are just too complicated to repeat

A quick glance at the biochemical pathways responsible for producing the various defences used by plants is all it takes to appreciate their complexity. Some researchers have argued that because of this complexity, these pathways probably only evolved once, or perhaps just a few times. Thereafter, the pathway might have been modified within a plant clade during evolution, although such modifications need not have made the pathway more complex. Looking at the different classes of defence chemicals in plants reveals a striking fact—particular classes of defensive chemicals tend to dominate certain plant families. This is called phylogenetic conservatism, good examples of which are cardenolides in the dogbane family and glucosinolates in the brassicas. Other impressive examples of phylogenetic conservatism include the defensive chemicals that occur in three plant families: quinolizidine alkaloids and non-protein amino acids in Fabaceae, steroidal alkaloids in Solanaceae and iridoids and essential oils in Lamiaceae. The distribution of these compounds is almost mutually exclusive in these families. Exceptions do exist and so a certain class of chemical might be absent in a particular plant family, but present in all neighbouring and ancestral taxa, and vice versa. Tropane alkaloids are defensive chemicals with a widespread distribution in the Solanaceae. Although these chemicals are highly conserved among some tribes within this family (e.g. Datureae)

and occur in most taxa, they occur only sporadically in other more distantly related tribes (e.g. Physaleae).

Although the co-evolutionary theory of plant–insect interactions predicts a close correlation between plant relatedness and defences, relatively few studies have tested it. Indeed, when the prediction has been tested, the correlations are not always close. This is certainly the case in work carried out by Judith Becerra on species of *Bursera*, common trees in the dry forests of Mexico.[26] She found only a weak relationship between plant relatedness and chemical defences among the different species. Results such as these suggest divergent selection on defences used against herbivores, meaning that closely related species would not necessarily have similar defences. It is argued that this should make it more difficult for herbivorous insects to track plant hosts during the course of evolution, so reducing herbivore pressure on plants. It seems that in the tropics, there is increasing evidence for the dissimilarity in plant defences between close relatives. It could be that divergent selection on defences by insect herbivores might be necessary for closely related plant species to co-exist in tropical forests. Some authors suggest that this could potentially explain the remarkably high local diversity of such forests.

## Why are there so many defence chemicals?

Having read Chapter 5, you will be only too aware of the great diversity of chemical defences available to plants. Why there should be such a diversity of defensive chemicals has long exercised evolutionary biologists. Some workers have suggested that selection favours plant lineages with a broad capability to make these compounds because such plants are more likely to come up with novel chemicals with sufficient toxicity to keep attackers at bay. In other words, in order to protect themselves and their progeny against attackers, plants must continually churn out novel variants of defensive chemicals. Michael Speed and colleagues, based at the Universities of Liverpool, St Andrews, and York in the UK, decided to construct a

theoretical model to examine the evolution of defensive chemicals in a plant and the evolution of resistance to these chemicals in insect herbivores.[27] They based their model on the interaction between wild parsnip and its nemesis, the parsnip webworm. Wild parsnip defends itself against webworm attack using up to five different kinds of furanocoumarin. It is known that following the introduction of wild parsnip into North America in the nineteenth century, an arms race developed, with increasing plant toxicity followed by increased ability of the webworm to detoxify the newly developed chemical variant. According to Speed and his colleagues, the fact that wild parsnip has several defensive chemicals at its disposal is important. A model published in 2012 predicted that increasing the number of defensive traits involved in a co-evolutionary interaction increases the likelihood that the victim (the plant) will escape its exploiter (the insect herbivore).[28] According to this model, the victim only needs to beat the exploiter at one trait to survive, whereas the exploiter needs to overcome all of the victim's defences to succeed. Interestingly, when Speed and his co-workers ran their newly constructed model, it showed that co-evolution maintains toxin diversity in plant populations. It seems that increasing the numbers of defensive chemicals increases plant fitness and lowers the likelihood of the plant becoming extinct. In turn, this can increase the variability of individual defence chemicals across generations. In short, this theoretical model suggests that co-evolution can explain the incredible diversity of defensive chemicals in plants.

## What about the genes during co-evolution?

When dealing with co-evolution between plants and insect herbivores it is all too easy to talk about defence traits but to completely ignore the fact that these traits are the result of gene expression. The co-evolutionary process can last for tens of millions of years and it is not unreasonable to wonder what happens to defence genes during such prolonged periods. Plant–herbivore co-evolution can be looked at from two angles. The

step-wise model proposes that the evolution of a particular defence in plants is driven by herbivores. The herbivores subsequently manage to overcome the defence and in response, the plant evolves a new defence trait. As we've already seen, this process can go on and on in a seemingly never-ending arms race. Assuming that each defence trait in plants is controlled by a different set of genes, adaptive substitutions in genes for a particular defence only take place during the relatively short period during which that defence trait evolves. The other way of looking at plant–herbivore co-evolution is the so-called gene-for-gene model. Here, the same defence genes might undergo adaptive substitutions for long periods of time. One example of this model is *R*-genes (resistance genes) in plants, which can diversify under selection pressure from avirulence genes in pathogens. This game of genetic tit-for-tat could go on indefinitely, but the continual duplication and loss of *R*-genes suggests that individual genes do not undergo evolutionary changes for long periods. Because most studies have examined selection on families of genes, trying to determine the duration of selection on individual genes has proved difficult. Mark Rausher and Jie Huang of Duke University in the USA decided to take on this challenge by examining patterns of selection on the plant defence gene *threonine deaminase*. This gene performs a 'housekeeping' function in most organisms—it encodes a protein which catalyses the first step in converting the amino acid threonine to isoleucine. In two species in the Solanaceae family however, the gene is involved in defence against insect herbivores. Tomato has two copies of this gene with different functions. One copy of the gene maintains the housekeeping function, while the other copy has evolved a defensive function against lepidopteran larvae. Wild tobacco also possesses the gene with the defensive function, although it is not clear whether this gene was duplicated from the housekeeping variant or whether it evolved independently. Rausher and Huang showed that a single copy of the *threonine deaminase* gene was duplicated two or three times near the base of the Solanaceae phylogenetic tree. One copy of the gene retains the housekeeping function, while the other copy evolved

defensive functions. A detailed study of the Solanaceae phylogenetic tree indicated that the gene underwent adaptive substitutions for a period of between 30 and 50 million years. The researchers suggest the most likely explanation for this extended period of evolutionary changes to this gene is fluctuating herbivore abundance. So, during periods of low herbivore abundance, selection pressure to maintain the defensive function of the gene is relaxed, allowing the accumulation of mutations with slightly deleterious effects, whereas when herbivore abundance is high, mutations with beneficial effects are favoured. Whatever the mechanism(s) underlying these evolutionary changes in the *threonine deaminase* gene, it seems that it has been evolving adaptively for long periods of time. The results also provide evidence that co-evolution can operate on a single gene over long periods of geological time.[29]

Let us return to *R*-genes for a minute. They exhibit a remarkable ability to diversify under selective pressure from pathogens—as new *avirulence (avr)* genes appear in pathogen populations, providing the pathogen with the ability to avoid plant recognition, host plants come up with new *R*-genes allowing them once again to detect the pathogen and activate defences. How can *R*-genes evolve so rapidly? Well, it seems likely that there is selection for a high level of polymorphism of the genes (i.e. many variants of the gene exist within a plant population). Polymorphism of *R*-genes can be simple or complex. In the former case, there is a single copy of the gene and polymorphism arises because many different alleles of the gene (an allele is an alternative form of the same gene) exist in the plant population. For example, flax has ten alleles of an *R*-gene that confers resistance to rust, while barley has some thirty alleles of a gene conferring resistance to powdery mildew. Complex polymorphism of *R*-genes involves clusters of *R*-genes giving rise to multigene families. Genes that are closely related lie adjacent to one another on the chromosome and an individual plant might have several slightly different copies of a particular *R*-gene. These can arise by duplication or recombination (exchange of genetic material either between multiple chromosomes or between different regions of the same chromosome), for example.

Co-evolution of plants and pathogens is often thought of as evolving rapidly, implying a quick turnover of *R*- and *avr*-genes. But this is not always the case. Some *R*-gene polymorphisms can be very long-lived. In *Arabidopsis*, the *R*-gene *RPM1* is a single-copy gene which is present in resistant ecotypes of the plant but is absent from susceptible ecotypes, which have the *rpm1* allele of the gene. It appears that the *rpm1* allele was created by deletion of a functional *RPM1* gene and has been maintained for the past 10 million years. This is a long time for a plant to keep what is effectively a resistance gene that has been 'overcome' by pathogens. The arms race model of plant defence predicts that overcome or defeated *r* alleles are replaced by new *R* alleles, with the defeated and obsolete alleles removed from the plant population. Instead, some workers proposed a 'trench warfare' model for the evolution of *RPM1*, in which both functional and defeated alleles are long-lived, although their frequencies in the gene pool would fluctuate over time. But why should a defeated *r* gene be maintained for such a long time? A possible explanation is that the functional *R*-gene is disadvantageous to the plant when the pathogen is not present. In fact, field experiments in which there was no obvious pathogen pressure revealed that having the *R*-gene imposed a cost to the plant in terms of reducing seed production. There is evidence that in *Arabidopsis*, duplicated *R*-genes have frequently been deleted during the evolution of its genome, which suggests that superfluous *R*-genes might carry a cost.

## Plant defence—war without end

If too many cooks can spoil the broth, then too many plant defence theories can certainly give you a serious headache. But while you reach for the paracetamol, just think for a minute why the evolution of plant defence should be so complicated. There are many thousands of plants, even more attackers of various types, all interacting in hugely different environments and habitats. Defences that work against an attacker in one

environment might not be effective in a different environment. How do you defend adequately against insects and pathogens? What do you do with all these blasted defences if there is no attacker around? What a shame you can't just get up and run away—that would reduce the need for all this defensive paraphernalia. The fact is, however, that against all the odds, plants have got it sussed. They have evolved the means of detecting their attacker, identifying it and deploying the most appropriate combination of defences to send it packing. If there is more than one attacker, they can 'decide' which attacker should be a priority. They also have the wherewithal to allow in beneficial organisms while at the same time making mincemeat of the want-something-for-nothing brigade. It really should come as no surprise that studying all this would be at the same time incredibly satisfying and headache-inducing. We can get rid of a headache. For plants, it is a war without end, because as long as there are plants, there will be things that want to eat them.

# 8

# Martial arts for plants

Despite the fact that just about everything wants to eat plants, they are still around. Not only are they still here, they are flourishing, exhibiting a remarkable ability to cope with whatever is thrown at them. In large part, this is due to their defensive armoury, which is bristling with surveillance, detection, and defence systems. So why would plants need help to defend themselves? In natural systems, plants can certainly hold their own against attackers. The problems arise when plants are put into huge fields with thousands upon thousands of their genetically identical compatriots. Vast areas of mono-cropping provide ideal conditions for pathogens and insect herbivores to multiply and spread. Conditions such as these are a central part of modern agriculture in many parts of the world and they stack the odds against plants. This is when plants need help, because without it, diseases and pests can wreak havoc, destroying crops and livelihoods, with far-reaching social and economic consequences.

The first example that springs to mind is the Irish potato famine of the mid-nineteenth century—when a microbe, the late blight pathogen *Phytophthora infestans*, destroyed potato crops, leading to the death of at least 1 million people in Ireland and the emigration of some 1.5 million more. Blight was responsible for a further famine in 1916 when 700,000 people died in Germany because they could not protect their potato crops; apparently, copper was needed for ammunition, leaving nothing for fungicide production.[1] Then there is coffee rust (see Plate 24), which reduced coffee production in what was Ceylon by 95% in a twenty-year period from

1870. Coffee plantations, not to mention livelihoods, were destroyed, and eventually production was switched to tea.[1] Not too long after that came black stem rust of wheat. In 1916, 200 million bushels of wheat were lost to this pathogen in the USA, with another 100 million bushels lost in Canada. This was economically devastating and to make matters worse, occurred during the First World War, when wheat was needed in great quantities to supply the Allied nations and their soldiers.

There is a disease which has caused as much economic and human misery as potato blight, but which does not get much of a mention, even in plant pathology textbooks. Brown spot of rice is caused by the fungus *Bipolaris oryzae* and it was responsible for an epidemic in the province of Bengal in north-eastern India in 1943. In most years, this disease did not cause significant yield losses. However, in 1942, the monsoon season was prolonged and the rain that should have stopped in early September continued through into November. Crops became heavily infected and the pathogen spread rapidly, causing yield losses of between 70% and 90%. With rice in short supply, prices rocketed and many Indians could not afford to buy their staple food. More than 2 million people died of starvation or related illnesses.[1]

But all of this was long time ago. Surely this sort of thing can't happen in the twenty-first century? Think again. Incredibly, all of these diseases are still major problems. Potato blight, for example, still causes huge damage globally, with epidemics in Bangladesh and western India in 2009 and 2010 leading to crop losses of more than 35%. In fact, annual losses in developing countries, where fungicides are often not available to farmers and growers, has been estimated by the Food and Agriculture Organization at $3 billion. What's worse is that we now have a few other pathogens to add to the blacklist of devastating plant diseases. High on the list is Fusarium head blight which affects wheat and barley. The United States Department of Agriculture (USDA) ranks this as the worst disease to hit the USA since the rust epidemics of the 1950s. According to the USDA, farmers have lost more than $3 billion as a result of Fusarium head blight since 1990.[2] In addition to destroying grain and reducing yields, there is a more sinister

side to this disease. The fungus causing the disease, *Fusarium graminearum*, produces mycotoxins that pose a serious threat to the health of humans and domestic animals. The major toxin produced by this fungus is deoxynivalenol, sometimes called vomitoxin because of its effects on the digestive system of pigs and other monogastric animals. Humans unlucky enough to eat flour contaminated with this toxin exhibit symptoms of nausea, fever, headaches, and vomiting.

Insects cause their fair share of crop destruction too. Take the Western corn rootworm, *Diabrotica virgifera virgifera*. This beetle is native to North America and is the major pest of maize in the US corn belt, estimated to cost farmers at least $1 billion every year in lost yield and treatments to try and control it.[2] Over the years, it has evolved resistance to various control measures, including chemicals and crop rotations. Its detection in Europe in recent times makes it a pest of global significance. But in terms of outwitting all of our attempts at control the master must be the Colorado potato beetle, *Leptinotarsa decemlineata*, which in the space of fifty years has managed to overcome fifty-two different classes of chemical—including cyanide!

Pest and disease problems don't just affect crop plants. Plants in natural settings, wild plants, plants in parks and gardens can all be affected. Dutch elm disease is one of many examples I could use. The disease is caused by a fungus, *Ophiostoma novo-ulmi* and spread by an insect, various species of elm bark beetle. Introduced into the USA in the 1920s by furniture makers who used imported European elm, it killed more than 40 million American elms and many millions of trees in Europe. The disease reached New Zealand in 1989, and was eventually eradicated, although a new outbreak occurred in Auckland in 2015.[3] More recent times saw the appearance of sudden oak death, caused by the pathogen *Phytophthora ramorum*. This microbe infects not just oak—it has a host range of more than one hundred plant species. It has been responsible for the deaths of millions upon millions of trees and shrubs in North America and Europe and in the ten-year period until 2008, is estimated to have killed more than one million trees in coastal Californian forests.[4] Also responsible for the destruction of huge numbers of trees is

the mountain pine beetle, *Dendroctonus ponderosae*. Native to the forests of western North America from Mexico to British Columbia, it has destroyed more than 44 million acres of pine forest in British Columbia alone since 1998. The death of huge numbers of pines has had some serious consequences. In Yellowstone National Park, for example, bears and birds have lost a rich source of food, while falling trees have led to the closure of camps and sparked wildfires by toppling power cables. Underlying all of this death and destruction is climate change, since rising temperatures and increasing drought have resulted in stressed trees. In a stressed state, the trees are unable to resist beetle attacks and they succumb to invasion.[2]

These examples are just a few of the many cases where disease and pest outbreaks have resulted in profound changes to our agricultural and natural systems. And rather than being a thing of the past, disease and pest outbreaks are very much a feature of the present and our future. So plants do need our help after all and there is much we can do to help them better resist the multifarious attackers they will encounter.

## It's all about breeding

Agriculture would probably have been a short-lived affair were it not for the natural genetic resistance of plants. Without the innate ability of plants to fend off attacks from pathogens and pests, we could not have begun their systematic cultivation. In those early days, already armed with the knowledge of which plants were safe to eat and which were likely to put an end to one's brief existence on the planet, the emphasis would have been on selecting plants that were nutritious and yielded well. Later, because plants had been taken from their natural environment and were grown as single stands of the same species, diseases and pests would have become troublesome. Thereafter, the early farmers would have started to select plants that showed few signs of disease and seemed to produce a reasonable yield, in spite of being afflicted.

Although plant diseases were mentioned by the Greek philosopher Democritus around 470 BC, it was some 170 years later that another Greek philosopher, Theophrastus, made plants a subject of systematic study. In fact, he wrote two books on plants, 'The Nature of Plants' and 'Reasons of Vegetable Growth' and although plant diseases were a small part of his work, he did recognize differences in disease susceptibility among crop cultivars. Because nobody at that time could explain what caused plant diseases, it was believed that they were the result of the wrath of God. As a result, avoiding plant diseases depended on pleasing the gods. One god who needed to be placated was Robigo, the goddess of blight, red rust, or mildew. According to the Roman author, naturalist, and natural philosopher Pliny the Elder, the second King of Rome, Numa Pompilus, instituted the festival of Robigalia to celebrate the honour of Robigo (or Robigus, since there is some uncertainty regarding the gender of this agricultural deity). The festival was held on 25 April, the time when when crops were most vulnerable to disease, and included sacrifices of a young red dog and sheep and offerings of incense and wine.[1]

It took the best part of 2,000 years before the first documentary evidence concerning plant resistance appeared. As early as 1788, early maturing cultivars of wheat were being grown in the United States to avoid infestation by the Hessian fly, *Mayetiola destructor*. Four years later, resistance to this insect pest was identified in the wheat cultivar Underhill in New York.[5] However, it was not until the rediscovery of Mendel's laws of heredity by the English scientist William Bateson in 1900 that breeding plants for resistance to pests and diseases was undertaken. One of the people credited with laying the foundations of plant breeding is Rowland Biffen, a Cambridge graduate and student of Harry Marshall Ward. Returning from an expedition to study rubber production in Central America, Brazil, and the West Indies in 1897, he decided to focus his efforts on agriculture. Biffen joined the University of Cambridge as a lecturer in the newly created Department of Agriculture in 1899 and was an early recruit to Mendelian genetics. Prompted by Ward, Biffen began studying resistance of wheat to yellow rust.[6] He began experiments in which he crossed the resistant

cultivar Rivet with the susceptible cultivar Red King and found that in the second (F2) generation the ratio of susceptible to resistant progeny was very close to the 3:1 ratio predicted by Mendel's laws if the character under study was controlled by a single gene. This demonstration that immunity to yellow rust in wheat is controlled by a single recessive gene was crucially important in the development of genetics.[7] The significance of this discovery was not appreciated at the time and was met with considerable scepticism—but more of this later. In any event, Biffen's star was in the ascendancy. In 1908, a chair in Agricultural Botany was created for him at Cambridge and in 1910 came his first triumph—the release of a new rust-resistant wheat variety called 'Little Joss', which was widely grown in Britain for the next forty years. 'Little Joss' was considered a landmark of modern plant breeding. In fact, the interest generated by this wheat variety prompted the creation of the Plant Breeding Institute in Cambridge (as part of the University's School of Agriculture) and Biffen was appointed as its first director. Biffen maintained that 'plant breeding was a game of chance played between man and plants, the chances seemingly in favour of the plants'. His research prompted agriculturalists to search for genes for disease resistance in wheat and other crops. Today, the development of crop cultivars with resistance to pathogens and pests is an important part of modern agriculture.

## How to breed for resistance

According to the late, great Eddie Cochran there are three steps to heaven. As it happens, there are also three steps to producing a resistant plant cultivar. Step 1, you obtain a source of genetic resistance. Step 2, you get hold of a means of identifying and selecting the resistance. Step 3, you find a way to introduce the resistance into a plant to produce a new cultivar that is commercially acceptable. Now that sure seems like heaven to plant breeders. In fact, for a plant breeder, getting to heaven is dependent on genetic variability. If the crop is outbreeding, it might already exhibit

significant genetic variation. However, if the crop is devoid of the requisite variation, it must be sought from alternative sources. This is where the plant breeder is likely to turn to a germplasm collection. Such a collection is likely to include wild species, weeds, and landraces. Before the development of modern agriculture, seed stocks of crop plants were maintained by farmers. This resulted in thousands of genetically distinct cultivars, adapted to local environmental conditions—landraces. As you might imagine, there was considerable genetic heterogeneity in landraces, but much of this has been lost during the development of modern crop varieties. As farmers and early plant breeders selected plants for particular characteristics, fewer and fewer plants were used to start new populations. Gradually, the genetically heterogeneous landraces were transformed into pure-breeding, homozygous lines[8] (homozygous individuals possess two identical alleles at a particular locus on a chromosome).

A plant breeder looking for new sources of resistance to a pathogen or pest might search in its centre of diversity. Here, the pathogen or pest is endemic and the plant and attacker are likely to have co-evolved. The potato originates from the Andes of South America, where there are several related species. Since this region is also thought to be the centre of origin of the late blight pathogen, it seems sensible to search for new sources of resistance to blight in this region. A wild relative of the potato, *Solanum demissum*, was discovered to be a useful source of resistance to late blight and, in the 1940s and 1950s, this resistance was bred into a number of commercial varieties. Four major genes for blight resistance were found and used to produce a range of new potato varieties, with each variety containing one of these major genes. Unfortunately, this new resistance was overcome by the blight pathogen within just a few years. Herein lies a major problem with using resistance based on one gene. When used in a crop that is grown in large areas, it can quickly be overrun by the rapidly evolving pathogen.[9] Over twenty major resistance genes against potato blight have been identified from wild relatives of potato, but as we will see shortly, ideas about how to use these genes to generate new resistant potato varieties are changing.[10]

Another means of obtaining novel resistance genes is mutation breeding. This is based on the use of treatments that cause mutations, such as $\gamma$-irradiation and chemicals, including ethyl methyl sulfonate, which induce alterations in DNA. The problem with this approach is that it is random, sometimes yielding mutants exhibiting a desirable property or function, such as disease resistance, and at other times giving rise to mutations with a loss of function. An example of mutation breeding that has been highly successful is the development of durable resistance to the powdery mildew fungus in barley. The first barley mutant exhibiting resistance to powdery mildew was induced by X-rays in a German variety in 1942 and many other mutants were subsequently generated. Some of these mutants were found to possess independently induced mutant genes in one chromosome locus and were given the designation *mlo*. Until the mid-1970s, all the known *mlo* genes conferring resistance to powdery mildew were the result of induced mutations. Excellent resistance to powdery mildew had been found in some accessions arising from barley seed samples collected in Ethiopia by German expeditions in 1937 and 1938. It was later discovered that this outstanding resistance was due to *mlo* genes, which had spontaneously occurred in the Ethiopian barley. Resistance conferred by *mlo* genes has been used widely across Europe without any evidence of the resistance being overcome by the fungus.[11]

Once a suitable source of resistance has been found, it must then be introduced into the chosen crop species. How this is achieved depends on the reproductive system of the crop plant and at this point, a distinction must be made between self-pollinating species and those that are largely cross-pollinating. Populations of self-pollinating plants possess little, if any, heterozygosity (heterozygous individuals possess two different alleles of a gene at a particular locus on a chromosome), and tend to consist of many closely related homozygous lines. In this case, since individual plants are fully homozygous, the aim of the plant breeder is to produce a pure line of homozygous plants. In contrast, all plants in a population of outcrossing species are highly heterozygous. If such plants are inbred, plant vigour will deteriorate, a phenomenon known as inbreeding depression. For

outbreeding crop species, the plant breeder will aim to maintain heterozygosity, or to restore it at the final stage of the breeding process.

One method used by plant breeders and which is applicable to both inbreeding and outbreeding crop species, is mass selection. This involves growing a large number of plants and swamping them with a pathogen or pest, thereby ensuring that only the most resistant plants survive. These can then be bulked up to grow the next generation. The idea that the progenies of the best individual plants are likely to be superior to the progeny of a random sample of a plant population was first proposed by the French botanist Louis de Vilmorin in 1856. The method has the advantage of requiring little in the way of technology. However, it has the disadvantage that although it is good at selecting for disease or pest resistance, this might not be associated with other agronomic traits the breeder might be looking for, such as high quality. There is also a limit to the usefulness of simply applying selection to pre-existing plant diversity.[12] For plant breeding to be truly creative and useful the breeder needs to shuffle genes that confer desirable traits—and that involves more effort and resources.

The majority of commercially released cultivars of self-pollinating crop species were produced using a method known as pedigree breeding. This involves selecting individual plants in the second and subsequent generations, allowing a precise pedigree of each line to be traced through the breeding programme. The aim is to produce a set of lines combining the best characteristics of both parents. It is based on complementation of traits and so is an efficient method for breeding for traits such as disease or pest resistance. It appeals to plant breeders as it allows them to produce better cultivars by assembling, in the same plant, desirable traits from different plants.[12]

If the breeder has identified a gene for resistance and wishes to transfer it into a susceptible variety that has many other, desirable characteristics, such as high yield, a particularly useful method to use is backcrossing. The idea is quite simple: the plant bearing the resistance gene is crossed with the susceptible variety to be improved and the resulting progeny are crossed back to the susceptible variety. This backcrossing is repeated until the

offspring have 99% + of the desirable genes as well as the gene for resistance.[12]

There are cases where the resistance gene to be transferred is tightly linked with a particular morphological trait (flower colour, for example). In the breeding process, the resistance and the morphological trait are transferred together to the progeny. So if plant selection is made on the basis of the presence of the morphological trait, the selected plants will also be disease resistant. In plant breeding, such morphological traits are known as markers. The problem is that they are uncommon and they can come with a serious downside—they might have a negative effect on the plant's performance. It would be far better to use a marker based on the plant's genotype rather than its phenotype and, indeed, this is possible using genetic or molecular markers. A molecular marker is a sequence of nucleotides located near the gene of interest and because of the close proximity of the two, they tend to stay together as each new generation of plants is produced. This is known as genetic linkage. A perfect molecular marker would show complete association with the desired gene, providing the breeder with confirmation of the presence of the desired gene.

## Resistance comes in many forms

It's all well and good knowing *how* to breed for resistance, but what kind of resistance are we aiming for? After all, with resistance, as with much in life, one size does not fit all. There are different types of resistance, each with its own characteristics, ability to control attackers, and longevity.

We saw earlier that although Rowland Biffen had demonstrated that immunity to yellow rust in wheat was caused by a single gene, there was considerable scepticism surrounding his work. Critics said that there was little point in breeding new cultivars if pathogens could change host, which was a widely held view at the time. In 1894 the Swedish mycologist and plant pathologist Jakob Eriksson had proposed that within one species

of a fungal pathogen there could exist a number of morphologically similar forms, each growing on a different species of host plant. This turned out to be correct, but Eriksson also proposed that these different forms were not fixed. According to what was known as Eriksson's 'Bridging Theory', the form of stem rust that usually attacked wheat might adapt to attack barley and subsequently develop the ability to attack rye. Various scientists, including Harry Marshall Ward and Ernest Stanley Salmon, obtained results that supported the theory, but others did not. Eventually, nearly twenty years later, the 'Bridging Theory' was finally destroyed by the thorough work of the brilliant young Elvin Charles Stakman working at the University of Minnesota.[6] Stakman undertook his undergraduate and postgraduate work at Minnesota and then stayed on to work at the university—an association that lasted some seventy-five years. In his PhD studies, he demonstrated that the rust species *Puccinia graminis* exists in several different forms, each attacking a different host (e.g. wheat, or oats, or rye). These were known as *formae speciales* (f.sp.), so, for example, the rust attacking wheat is *Puccinia graminis* f.sp. *tritici*, while that attacking oat is *P. graminis* f.sp. *avenae*. Stakman subsequently demonstrated that within each *forma* there can exist a large number of 'physiologic races' each with the ability to attack some cultivars of the host but not others.[13]

Getting back to Biffen's work on yellow rust of wheat, it is easy to see why sceptics, thinking that fungal pathogens could easily change host, saw little point in breeding new disease-resistant cultivars of wheat. But Biffen published his work in 1907, stimulating others to study the inheritance of resistance in different plant–pathogen interactions. Interest in this area increased enormously following Stakman's demolition of 'Bridging Theory'.

Thanks to the work of Stakman and other, later researchers, we now know that some plant cultivars are resistant to some races of a pathogen, but susceptible to other races of the same pathogen. This is called race-specific resistance and because it tends to be controlled by one gene (in some cases a few genes), it is also known as monogenic resistance.

This type of resistance is highly effective and usually operates by triggering a hypersensitive reaction to the invading pathogen. Its weakness lies in the fact that because it is controlled by a single gene, it can easily be overcome by the development of new physiological races of the pathogen. This is what happens when a new, disease-resistant crop variety is released onto the market, is widely grown, and then within a few years, becomes susceptible to the pathogen. This is also true for interactions between plants and parasitic plants. Resistance to broomrape, which is based on single, dominant genes, was introduced into sunflower from wild relatives nearly fifty years ago and has proved very effective. However, widespread use of the resistant sunflower cultivars has led to the appearance of new races of broomrape and a loss of resistance.[14]

In insect–plant interactions, monogenic resistance is usually known as biotype-specific resistance and can be overcome by insects developing new biotypes. This can take as little as three generations and can lead to breakdown of resistance in a new cultivar even before it is grown widely.

Monogenic resistance is of the all-or-nothing type. But there is another type of resistance which could be described as little but wide-ranging. All plants have some resistance to each of the pathogens that usually attack them. This is known as partial resistance and although the level of resistance it provides is lower than that provided by monogenic resistance, it tends to be effective against most races of an attacker. This type of resistance is controlled by many genes, hence the alternative name, polygenic resistance. Because many genes are involved, it is difficult for pathogens to overcome and so the resistance it provides is long-lasting or durable. In plant–insect interactions, where this resistance is known as biotype nonspecific, it might not be any more durable than biotype-specific resistance, especially if the mechanism underlying the resistance is based on the concentration of a single compound. If, on the other hand, the resistance is based on multiple mechanisms, the risk of it being overcome by the insect is considerably reduced.

The term partial resistance was first used by researchers from the Netherlands in 1975 to describe the resistance exhibited by barley to brown rust, where, despite being susceptible to the pathogen, the rate of development of pathogen epidemics was reduced. The researchers found that the resistance was governed by up to seven genes, acting additively. The fungus simply did not perform well on these plants and took longer to produce fewer spores than if they had been growing on fully susceptible plants.[15]

The problem with partial resistance is that it still allows pathogens to infect and colonize the plant, albeit at a reduced level. This might not be enough for farmers to manage disease sufficiently and in addition it is more difficult to breed for than monogenic resistance. This, coupled with the fact that monogenic resistance can break down quickly, led plant pathologists to search for more durable forms of resistance. They were (and still are) on the lookout for resistance that continues to provide disease control even after exposure to the pathogen for a prolonged period. What is important here is that the resistance remains effective for a long time. We have already come across a good example—*mlo* resistance in barley to powdery mildew. The *mlo* gene was introduced into new cultivars in 1979 and still gives good control of powdery mildew in Europe. It works by reinforcement of the cell wall at the site of attempted penetration by the fungus (papilla formation, as we saw in Chapter 4). This is difficult for the fungus to overcome and probably accounts for the longevity of the resistance.

If monogenic resistance is so easily overcome by pathogens, might it be possible to introduce several resistance genes into a plant, making life much more difficult for the pathogen? This has been tried and found to work. It is achieved by repeatedly crossing plant lines with different resistance genes until multiple resistance genes are present in a single cultivar. This is known as pyramiding of resistance genes and should, in theory, be durable. Although it is very unlikely that a pathogen could overcome multiple resistance genes, there is the possibility that pyramiding could select for a super race of pathogen possessing just such an ability. That does not bear thinking about.

## Mixing things up—making life difficult for the attacker

Protecting crops is a difficult and risky business. On the plus side, cultivars with excellent resistance to various pathogens might be available. On the downside, growing those cultivars over large areas brings with it the high risk of the pathogens overcoming the resistance, especially if the resistance is monogenic. The useful life of a cultivar can be cut short if it is not used sensibly. So how might farmers and growers use cultivars with monogenic resistance sensibly?

Since the problem lies with the genetic homogeneity of our cropping systems, some researchers have proposed that we increase genetic diversity. Theoretically, this should decrease disease in the short term and increase the durability of disease resistance in the long term. One idea put forward nearly seventy years ago was the use of multilines. These are a set of cultivars that differ in just one gene—a gene for disease resistance, for example—otherwise they are genetically identical. Being genetically identical, their seed can be mixed and the different lines grown together to create a plant population containing several different resistance genes. So instead of a pathogen being presented with a continuous sea of susceptible plants, it is faced with a patchwork of different resistances, making it difficult to overcome any single resistance. This concept was initially put forward by Neal Jensen in 1952 and Norman Borlaug and John Gibler in 1953 and has been used successfully.[16,17] The first multiline variety in wheat was released in Colombia to control stripe rust and has also been used to control this disease in the US Pacific Northwest. Despite its success, in practice it takes considerable time and effort to breed multilines and they might end up being based on relatively few resistance genes.

An alternative approach, but one which is simpler to put into practice than multilines is the use of mixtures. The idea here is to mix together seed of several genetically distinct cultivars and grow them as a single crop. Each cultivar in the mixture would contain one or more different resistance genes. Mixtures have been shown to control disease and research by Adrian

Newton at the James Hutton Institute in Dundee, UK, has shown that disease control improves as the number of cultivars in the mixture increases.[18] Achieving good disease control depends on getting the correct mixture of cultivars and this, in turn, will depend on the pathogen being targeted. Get it right and very high levels of disease control are possible. In one very large experiment, where more than 3,000 hectares of a mixture of susceptible and resistant rice cultivars was grown, rice blast was reduced by more than 90% and yield was increased by a similar amount. The mixtures concept is sound enough, but growers have been reluctant to adopt it in practice. The problem lies with the market for the crop and the demand for a product of a particular quality. Since a mixture contains several different cultivars, each with its own characteristics, getting a product of uniform quality might be difficult.

## Immunizing plants—it's no joke

We know that when plants are attacked, they can alert other parts of the plant, allowing those tissues to prepare for the onslaught. The signals responsible for providing the advanced notice of attack depend on the type of attacker. So if the invader is a biotrophic pathogen such as a powdery mildew fungus or a virus, or a piercing/sucking insect such as an aphid, salicylic acid is an important part of the signalling cascade. If the plant is under attack by a necrotrophic pathogen or a chewing insect, jasmonic acid, usually in combination with ethylene, is involved in sounding the alarm. However, there is no need for the plant to be attacked to sound the alarm; this can be done by applying the signals themselves to the plant. It doesn't even have to be the signal molecules—compounds that mimic the signals would do the job, as would a range of other compounds, some natural, others synthetic.[19]

Searching for compounds that can raise the alarm and trigger the plant's defences has preoccupied researchers in academia and industry for several years and many compounds have been discovered that can do just that.

Increasingly, the search has focused on agents that can 'prime' the plant. Such agents would not trigger the plant's defences directly, but rather would put defences on alert, ready to be put into action rapidly and intensely if an attack does materialize. It is easy to see why priming is important. What's the point in triggering plant defences if any attack never comes? Far better to get everything ready so a defence can be mounted only when required. This saves on energy and resources and from the farmer's perspective, this is great, since the saved energy and resources would go into yield.[19]

The search for agents capable of activating plant defences has led to a new generation of crop protection agents. Yet the first activator of plant defences to be released on the market was only found to operate in this way after it started to be used in practice. In the mid-1970s, a chemical called probenazole was introduced for the control of blast disease on rice in Japan. Made by the Japanese company Meiji Seika Kaisha Ltd, it was marketed as Oryzemate® and was used for some time before it was suspected of triggering plant defences. It was only later, with increased understanding of induced resistance in plants, that probenazole was finally proven to activate defences and its mode of action was fully elucidated. Probenazole has been in continual use for more than forty years and so far there have been no reports of the rice blast pathogen developing insensitivity to it.[20,21]

Crop protection agents that worked by activating the plant's defences was a new departure for agrochemical companies. Their usual approach was to use mass screens set up to highlight compounds that showed activity against as wide a range of pathogens as possible. They would determine whether the compounds—fungicides for example—exerted a protective effect, that is, whether they could prevent the fungal pathogen infecting the plant, whether they had curative activity (i.e. were they capable of stopping a fungus that had already started to colonize the plant), or whether it exhibited anti-sporulant activity, which is self-explanatory. Identifying compounds capable of activating the plant's defences required a different approach and initially screens were used that were based on those

employed by the pioneer of induced resistance research, Joe Kuć, who worked predominantly on cucumber and tobacco. The agrochemical company Ciba-Geigy, later to become Syngenta, used cucumber for the initial mass screens. The test chemicals were applied to a small area of leaf and the whole plant was then inoculated with the anthracnose fungus. The researchers would then compare the pattern of protection provided with reference plants, in which resistance had been induced by inoculating a leaf with a pathogen and waiting a day or two before inoculating the whole plant with anthracnose (i.e. mimicking the induction of resistance as it would occur naturally). This initial screen identified two groups of compounds with resistance inducing abilities—isonicotinic acid derivatives and benzothiadiazoles. The most promising compounds from these groups were then subjected to more searching tests, aimed at discovering the range of pathogens protected against, whether they exhibited any direct action on the pathogen, and the effects on the plant itself. Compounds getting through these tests were then subjected to even further investigation—including field testing—before decisions were made about possible commercial development. Eventually, following extensive field testing, one compound was selected in the early 1990s. The compound, given the internal code CGA 245704, was the benzothiadiazole compound acibenzolar-S-methyl, an analogue of the signalling compound salicylic acid. It activates systemic acquired resistance in plants and is active against a broad range of plant diseases. It was introduced in Europe and other parts of the world as Bion® and in the USA as Actigard®.[22]

These novel crop protection agents have a number of characteristics in common. Unlike a fungicide, which can kill a fungal pathogen and as a result provide complete disease control, plant defence activators rarely provide complete disease control. Usually, they reduce infection and spread by the pathogen and, as a result, levels of disease control can vary enormously. Also, because these activators work via the plant, factors in the environment that affect the plant can influence its ability to induce resistance. And because different plants respond differently to changes in the environment, the effects of the resistance activators vary from one plant

species to the next. All of this leads to variability in disease control, which tends to worry farmers and growers. Nevertheless, some of the activators have been integrated successfully into crop protection practice where they provide much-needed disease control. This is especially true in situations where particular pathogens have become insensitive to fungicides and also in cases where resistant crop varieties have lost their resistance to certain pathogens. Here, incorporating a resistance activator into a crop protection programme could help to prolong the useful life of both fungicides and resistant crop varieties.

## Airborne defence

Jasmine is a popular woody climber with a delicate, intoxicating scent. So much so that oil from jasmine flowers is widely used in both women's and men's fragrances. Apart from making you smell good, splashing on a bit of jasmine-containing perfume might also be good for you. According to German researchers jasmine fragrance alters responses to the neurotransmitter GABA ($\gamma$-aminobutyric acid) making the smell of jasmine as good as valium at calming the nerves.[23] One of the components of this fragrance, and of the volatiles emitted from various flowers, is *cis*-jasmone, a compound now known to play a role in plant defence.

A group of researchers led by scientists at Rothamsted Research Station in Harpenden in the UK was studying interactions between plants and aphids and were particularly interested in the host-alternating behaviour of the blackcurrant-lettuce aphid, *Nasonovia ribis-nigri*. This aphid colonizes plants in the Asteraceae, such as lettuce, in the summer, but moves on to plants belonging to the Saxifragaceae, such as blackcurrant, in the winter. So, blackcurrant plants are attractive to morphs of the aphid produced in the autumn, but is avoided by morphs produced in the spring and summer, which prefer their summer host, lettuce. Michael Birkett and his colleagues were looking for compounds produced by blackcurrant that might be responsible for this aphid behaviour. They detected *cis*-jasmone in the

volatiles emitted by blackcurrant and found that it was directly repellent to the blackcurrant-lettuce aphid, as well as another aphid, the damson-hop aphid. But the surprises did not end there, because they found that *cis*-jasmone attracted aphid enemies, specifically the seven-spotted ladybird and an aphid parasitoid. This ability to provide defence against herbivorous insects extended beyond wheat. Later work showed that soybean treated with *cis*-jasmone attracted a parasitoid that feeds on eggs of the stink bug, a pest of soybean, while cotton plants treated with *cis*-jasmone were repellent to the cotton aphid.[24]

The ability of the naturally occurring *cis*-jasmone to induce defences against herbivorous insects and attract their natural enemies suggested potential uses of the compound in crop protection. One approach being examined by the Rothamsted researchers and their collaborators is the selection and breeding of crop cultivars that release *cis*-jasmone following herbivore damage, for example. The idea here is that when such plants are attacked, the *cis*-jasmone released would trigger the release of volatiles from neighbouring plants, speeding up their defensive reactions and attracting herbivore enemies.[25] Another exciting possibility being investigated by this group is the development of sentinel plants. Because *cis*-jasmone is known to up-regulate a number of defence genes, the researchers hit upon the idea of fusing a luciferase gene to the promoter sequences of the defence genes. Because expression of the luciferase gene results in light emission, when *cis*-jasmone is sprayed onto the plants containing these fused genes, they would be activated, resulting in light emission. Plants with this light-emitting ability, if planted in crops, would act as sentinels, warning of insect attack or disease development.[26]

It is well established now that plants under attack by insect herbivores release a blend of volatile compounds that attracts enemies of the herbivore. However, it appears that this ability might have been inadvertently bred out of many of our modern crop cultivars. The Rothamsted scientists, together with colleagues working in Kenya, found that when the stem borer moth *Chilo partellus* deposited eggs on leaves of landrace cultivars of maize, volatiles were emitted that attracted two types of parasitic

wasp—one that feeds on eggs and the other on the larvae that emerge from the eggs. However, this sophisticated defence strategy was not present in commercial hybrid maize cultivars, suggesting that it had become lost during breeding. Nevertheless, its presence in landraces would provide material for use in breeding programmes aimed at reintroducing the defence in new cultivars.[27]

In a farmer's field, the release of volatiles to attract enemies of the attacking pest can occur a bit too late to prevent some damage and yield losses to the crop. A plant that has evolved a trait that might prove useful here is an African forage grass, *Brachiaria brizantha*. This grass responds to oviposition by the stemborer moth by suppressing the main volatile used by the insect in finding its way to the grass to lay its eggs. This makes the grass invisible to female stemborer moths and prevents further egg-laying on the grass. What's even better is that although this host-location volatile is suppressed, the other volatile components are increased, making the volatile blend more attractive to parasitic wasps that feed on the young larvae.[28] The search is on to identify and develop cereal cultivars that exhibit similar traits, since it would be useful in protecting crops against insect pests.

The idea that plant volatile emissions might be manipulated to help crop plants tackle pests is not new. Specific volatiles have been genetically altered and shown, in laboratory tests, to increase attraction of predators and parasitoids of insect pests. Similar results have also been obtained in carefully controlled field studies. One such study used the interaction between maize and a major pest, the western corn rootworm. When roots of maize are attacked by this pest, its roots emit $\beta$-caryophyllene, a below-ground volatile signal that attracts nematodes that arrive on the scene to devour the voracious pest. Sadly, most North American maize cultivars have lost the ability to emit this volatile signal and so are unable to recruit the help of their nematode ally. Researchers restored this ability to a maize line by transforming it with the gene responsible for its production. The restored maize line was planted into a field infested with the corn-worm, but unlike their unrestored comrades, which suffered huge damage

to their roots, the altered maize escaped with little damage.[29] Similar results were obtained with rice, where genetically altering volatile emissions greatly increased control of the brown planthopper, a major pest of rice. Demonstrations such as these open up possibilities for protecting crops by genetically manipulating their ability to attract predators and parasitoids. To do this effectively, however, requires a clear knowledge of which particular components of a plant's volatile emissions should be altered.

## Companions in arms

In sub-Saharan Africa, cereals such as maize, sorghum, finger millet, and rice are the most important food and cash crops for millions of rural farming families. The production of these crops is, however, severely constrained by insect pests such as cereal stemborers and by the parasitic plant *Striga*. Attacks by either of these pests can completely destroy a crop.

The host range of the cereal stemborers is wide and includes many wild species. The presence of these wild hosts maintains populations of the stemborers when the cultivated crops are not being grown, but they also harbour natural enemies of these pests. In fact, reports suggest that wild hosts can act as a buffer against stemborer attack on cultivated crops, which suggests that they play a natural role as trap plants. This was the background to the start of collaborative research between the UK and Kenya aimed at developing a sustainable approach to managing cereal stem borers involving what is known as 'push-pull' technology.[30]

In 1990, James Miller and Richard Cowles of Michigan State University in the USA published a paper on a concept called 'stimulo-deterrent' diversion. They were looking for ways to control onion fly (*Delia antiqua*). The idea was to manipulate the ovipositional behaviour of the onion fly by treating onion seedlings with chemical deterrents while simultaneously providing deeply planted onion sets on which the pest prefers to lay its eggs. Basically, the main crop is protected by negative cues that reduce

infestation by the pest, providing the 'push' effect. This could be done by, for example, growing a plant able to provide such cues, a companion crop, between the rows of the main crop. The volatile cues emitted by the companion crop would attract enemies of the pest. For the 'pull' effect, a trap crop would be grown, say for example around the perimeter of the main crop. The trap plants would attract the pest to lay their eggs and if this egg-laying activity could trigger the plant's defences, even better. The overall effect would be to greatly reduce the pest population on the main crop.[31]

Because smallholder farmers in developing countries have traditionally used companion crops to augment staple crops such as cereals, a starting point for using 'push-pull' technology was already in place. Field trials in Kenya showed that this approach to protecting crops works. It involves intercropping cereal crops with a forage legume such as *Desmodium* and planting Napier grass (*Pennisetum purpureum*) as a border crop. *Desmodium* repels stemborer moths (push) and attracts their enemies, while Napier grass attracts them (pull). *Desmodium* more than earns its keep since it also suppresses the devastating parasitic plant *Striga*. 'Push-pull' technology has been so successful that the plan is to disseminate it to 1 million farm households in sub-Saharan Africa by 2020.[32]

## Genetic engineering to protect crops

Although plants can be treated with chemicals—either naturally derived or synthetic—to enhance their ability to defend themselves against attack or to attract allies to help them in that fight, there seems little doubt that having a crop plant that can go it alone and defend itself is a sensible approach to crop protection. Traditional plant breeding is the approach most often used to generate new crop cultivars with disease or pest resistance. Another approach is to use genetic engineering to produce new crop cultivars with specifically enhanced defensive capability. Genetic engineering approaches to producing plants with enhanced resistance have

been around for some time. The first plant gene to be successfully transferred into another plant species, thereby enhancing its resistance to insect attack, was the cowpea trypsin inhibitor.[33] Our understanding of the molecular basis of interactions between plants and pathogens has increased enormously since the late 1980s. This increased understanding has, in turn, allowed researchers to develop increasingly sophisticated approaches to enhancing disease resistance in crops. This can be illustrated by looking at work on the genetic modification of potato to increase its resistance to the ever-present threat, potato blight.

As we saw in Chapter 2, plants need to recognize the presence of an attacking pathogen in order to activate its defences. This recognition ability is provided by resistance genes in the plant. For an attacking pathogen to get past the defences, it must stop them being triggered and to do this, it produces molecules known as effectors, which prevent the resistance genes from doing their job. The interaction between potato and the potato blight pathogen is no exception. Resistance genes in the potato give it the ability to recognize specific effectors produced by the pathogen. If it recognizes the effector, defences are activated and the pathogen is stopped. Of course, as time passes, the pathogen can evolve new effector molecules capable of blocking the ability of the resistance genes to detect its sneaky ingress.

In breeding a new potato variety for resistance to blight, breeders would typically introduce one new resistance gene (from a wild relative of potato) at a time. As you can imagine, this takes time and much effort and always ends in the pathogen overcoming the new resistance gene by evolving a new effector. For the plant breeders, not to mention the farmers, this must be soul destroying. You can understand therefore, why plant breeders are not keen to introduce single resistance genes into new potato varieties. However, as Jonathan Jones and his colleagues in Norwich and Dundee have pointed out,[34] recent advances in our understanding of the interactions between potato and the blight pathogen have improved the prospects for using single resistance genes. The problem in the past has been that breeders have been unable to choose resistance genes capable of

recognizing the effectors that are absolutely essential for the pathogen to invade the plant. However, this has now changed. It is now possible to confirm that each transferred resistance gene is doing its job of recognizing its cognate effector and to check that defence has been activated. The potential exists to insert a stack of resistance genes into a particular cultivar, keeping all of its favoured agronomic characteristics but with the added advantage of resistance to potato blight. Ultimately, it might be possible to introduce resistance genes into the plant, giving it the ability to recognize all of the blight's indispensable effectors. In this way, it should be possible to produce potato varieties with resistance that the blight pathogen will find very difficult to overcome.

Soybean is one of the world's major crops and is one of the main sources of vegetable oil and plant protein worldwide. Pathogens are no respecters of the importance of crops to humans and this is certainly true of the Asian soybean rust, *Phakopsora pachyrhizi*. This fungus is one of soybean's most damaging pathogens, capable of causing yield losses of up to 80%, with infection levels as low as 0.05% affecting crop yields. It was first reported in Japan in 1902 and was confined to Asia and Australia until 1997 when it was discovered in Uganda. Over the next four years it spread to Zimbabwe and South Africa and then, in 2001, it was reported in Paraguay. It took just another year before it was present in Brazil and northern Argentina and by 2003 it was present in most soybean producing areas of Brazil. The seemingly unstoppable rust was found for the first time in the USA, in Louisiana, in November 2004 and quickly spread to other southern states. The pathogen is spread by the wind and it is reckoned that it entered the southern USA from Colombia courtesy of Hurricane Ivan. No commercial cultivars are fully resistant to the rust and this, together with its ability to spread rapidly and devastate soybean crops, led to Asian soybean rust being considered a possible weapon of bioterrorism, along with the bacterium responsible for anthrax (*Bacillus anthracis*) and the viruses causing the haemorrhagic fevers—Ebola, Marburg, Lassa, and yellow fever. Asian soybean rust is clearly a pathogen to be taken seriously. Unfortunately, controlling it is challenging, to put it mildly. Resistance bred into commercial

soybean cultivars was overcome rapidly by newly evolved strains of the rust, meaning that routine applications of fungicide are required on a huge scale. In 2002, in Brazil alone, the costs incurred in attempting to control the rust was estimated at around $2 billion per year.[35]

So what can be done to improve genetic resistance in soybean to this devastating pathogen? With no suitable resistance available in the plant, researchers decided to look elsewhere—in other legumes, to be precise. One legume they examined was pigeon pea, a close relative of soybean, known to be a host for Asian soybean rust. A consortium of researchers, led by Peter van Esse and Jonathan Jones in Norwich and Sérgio Brommonschenkel in Viçosa in Brazil, discovered a gene in pigeon pea that codes for an immune receptor able to recognize Asian soybean rust. The researchers transferred this gene from pigeon pea to soybean, enabling the latter to recognize the invading rust, leading to rapid triggering of defences and full resistance to the attacker. This work has considerable significance, since the Leguminosae is a large family with some 700 genera and 20,000 species, providing access to a huge pool of resistance genes, some of which might be useful in generating soybean cultivars able to fend off this very damaging pathogen.[36]

## Silencing the genes

Unlike most viruses that infect animal cells, and which have genomes made of DNA, most plant-infecting viruses have an RNA genome. As we saw in Chapter 5, double-stranded RNA produced during replication of the virus can be targeted by the cells' RNA interference machinery, slicing it up, thereby preventing the genetic instructions it contains being translated into protein. In effect, the viral RNA is silenced and the plant is protected from the damaging effects of the virus.

We can also make use of this RNA silencing to control pathogens and pests, by targeting genes important for growth, development, and survival

of the attacker.[37] So, for example, if double-stranded RNA of the attacker gene could be expressed in the host plant, its RNA silencing machinery should generate small interfering RNA molecules which would, by becoming part of the RNA-induced silencing complex, target and degrade the attacker RNA. This approach was used by researchers to target an effector gene produced by the barley powdery mildew fungus. Double-stranded RNA molecules targeting RNA transcripts of this fungal effector gene led to reduced development of the fungus on the plant.

So what about the prospects for using this approach to control pathogens and pests in practice in the real world? In 2013, Brazilian workers reported the characterization of the first commercial transgenic cultivar of dry bean (*Phaseolus vulgaris*) that is immune to *Bean golden mosaic virus*. This virus is a major constraint to bean production in Latin America and research was undertaken to determine whether RNA silencing might provide a means of controlling the pathogen.[38] Research published by these workers in 2007 demonstrated that by silencing a gene required for virus replication, bean lines could be generated which were highly resistant to the virus.[39]

Using RNA silencing to provide effective control of pathogens and pests requires identification of suitable targets in the attacking organism. Genomes of many fungal pathogens and other organisms have been sequenced and many more are currently being worked on. These will provide researchers with an arsenal of candidate genes to design and test constructs to use in silencing. We know that RNA silencing works and can provide control of pathogens and pests, but how should we deploy this new technology in practice? If we want to ensure that pathogens and pests don't rapidly overcome the newly developed resistance, should we use plants expressing this type of resistance in rotation? Should we use it in combination with classically bred resistant varieties? As with every new technology and approach to crop protection, there is much to be considered before it can be used wisely in practice.

## CRISPR plant defence

Like us, bacteria don't like getting a virus infection. But rather than popping pills (which just tackle the symptoms of the infection), bacteria have come up with a cunning means of dealing with future attacks by the viral thugs—they destroy its genome, and since the virus genome contains the genetic information necessary for it to replicate, the virus is doomed. The way it works is ingenious. If the bacterium is attacked by a virus it has not encountered previously, it copies sections of the virus DNA and 'stores' it in its genome, providing a sort of genetic memory of the virus. The next time the bacterium encounters the virus, it uses an enzyme to chop up any DNA sequences of the virus matching the stored sequence. Examining the genome of a bacterium reveals many such stored sequences derived from previous virus attacks. These are known as Clustered Regularly Interspaced Short Palindromic Repeats, which hardly trips off the tongue and so is referred to by its acronym, CRISPR.[40]

CRISPR comes under the umbrella of 'genome editing' and allows researchers to make precise changes at specific locations in the genome. This new technology could be used to insert or replace specific genes or to disrupt their function, providing a powerful new tool with which to better understand plants. CRISPR technology has already been used experimentally to enhance resistance in rice to the blast fungus,[41] and to enhance resistance to the *Tomato yellow leaf curl virus*, a DNA virus responsible for severe crop losses in sub-Saharan Africa.[42]

CRISPR is a very new technology and as such the risks and benefits of its use need to be examined carefully. As with any new crop protection technology its potential use must be considered in the context of other methods available for controlling pathogens and pests of concern. It is important that such evaluations are carried out rigorously because in order to protect our crops we need all the weapons at our disposal.

## Genetic engineering and crop protection—where to next?

The introduction of transgenic crops into the marketplace has been increasing at a remarkable rate. Transgenic soybean, maize, cotton, and oilseed rape now occupy almost one-third of the total area of these crops globally. This might seem surprising given the problems encountered with the introduction of genetically engineered crops.

There is concern, as there is with traditional plant breeding, that introduction of just one gene into a crop plant risks the usual problem of being rapidly overrun by the pathogen or insect. There are options available to tackle such concerns, including releasing new cultivars sequentially, pyramiding or stacking resistance genes, and gene rotation, where one gene is alternated with another. If a resistance gene is expressed constitutively in the plant, considerable selection pressure is exerted on the pest or pathogen to evolve the means of overcoming it. However, if the gene is targeted to a particular part of the plant—the leaves, or flowers, for example—it might only be switched on at certain stages of the plant's growth. This would reduce selection pressure on the pathogen.

There are also concerns relating to possible environmental side effects of using genetically engineered plants. These include effects on non-target organisms such as pollinators, and the possibility of outcrossing of the transgene and its transfer into wild relatives. Considerable effort is being expended in this area to try to better understand the environmental consequences of using genetically engineered crops. The costs required to provide the data necessary for any genetically engineered crop cultivar to get through the regulatory process is prohibitively high. This means that only new cultivars with the potential to recoup the huge investment made by biotechnology companies are likely to make it to the market. That is a shame, since genetic engineering approaches offer the potential to provide durable resistance while reducing the environmental impact of agriculture.

# Epilogue

Five years have passed since the last major battle with the enemy. The attackers had been vanquished, but the defenders knew, as sure as night follows day, that the enemy would be back, better prepared than the last time. The intervening period has not exactly been peaceful—the enemy continued to probe the defences from time to time, but the defenders were always able to beat them off. And so they waited. Waited for the next push—the one where, because they had come up with some new way to fool the defenders, the fight would determine their fate for a long time to come.

Eventually the attack came and this time, the defender's surveillance systems did not pick up the approaching enemy. Having beaten the early warning system, the enemy entered the fortress unseen. They continued to infiltrate the fortress and still, days after the initial assault, they were not detected. Soon however, the sheer numbers of invaders could no longer remain undetected and then all hell broke loose. The defenders began a ferocious attempt to kill and maim the invaders, using up huge amounts of resources in an attempt to rid themselves of their mortal foes. But try as they might to immobilize them, there were simply too many. Weakened by the brutality of the attack and with little energy left to continue the fight, they succumbed. The enemy had won and now every fortress in the land would be at huge risk. Their plan to build identical fortresses throughout the land was now going to backfire, since having figured out how to breach the defences, they would all be easy prey for the enemy. Their only hope

was the few fortresses that had not adhered to the plan and had decided to go their own way, trying out newer detection systems. Yes, that was their only hope. But it would take time. And until then, they would have to endure the awfulness of life where everything you produce is taken away from you, leaving you with too little for yourself and your young ones. Many would not survive, death would be everywhere.

And so it goes on, the unending war between plants and attackers, the inexorable cycle of victory and defeat. That is the natural way. But there is a new force to be reckoned with. It is difficult to know what to make of this interloper. They use plants, but in a way not seen before. They take them out of their natural surroundings, they grow lots of them together, making them easy prey for attackers. On the other hand, they try to protect them from attack. It's confusing—are these interlopers a force for good or for bad? The jury is out.

# GLOSSARY

**Alkaloid** A naturally occurring chemical compound containing basic nitrogen atoms. Alkaloids are found primarily in plants and include the stimulants caffeine and nicotine, and the toxic compound atropine.

**Allele** An alternative form of the same gene. These are located at the same position, or locus, on a chromosome. Many organisms are diploid, possessing two alleles at each genetic locus, with one allele inherited from each parent. An allele can be dominant or recessive.

**Allelopathy** The chemical inhibition of one species by another. In essence, it is a form of chemical competition, with the 'inhibitory' chemical released into the environment e.g. the soil, where it affects the growth and development of neighbouring plants.

**Allocation cost** The cost associated with diverting energy and resources away from plant growth and other processes towards defence.

**Arbuscule** Structure formed by certain types of mycorrhizal fungus within cells in a plant root. The structure never ruptures the plasma membrane of the root cell and so remains outside it. The arbuscule provides a large surface area of contact between the plant cell and the mycorrhizal fungus, allowing exchange of nutrients.

**Arthropod** An invertebrate animal possessing an exoskeleton, a segmented body and jointed appendages. Arthropods include insects, spiders, and crustaceans.

**ATP** Adenosine triphosphate. This is nature's energy store and is the chemical currency used by all cells.

**ATPase** Enzyme that catalyses the breakdown of ATP thereby releasing the energy required to drive cellular processes. Some ATPases are found in membranes and move solutes across the membrane against a concentration gradient (e.g. Na+/K+-ATPase, which pumps sodium [$Na^+$] out of cells while pumping potassium [$K^+$] into cells, both against their concentration gradients).

**Avirulent** Term used to describe a pathogen which is able to penetrate a plant but which has insignificant effects on its functioning i.e. it is unable to cause disease.

**Biotroph** An organism that can only live and reproduce on another living organism. A biotroph is completely dependent on its host for sustenance. Examples include powdery mildew and rust fungi.

**Chitinase** An enzyme that degrades chitin, which is a component of the cell walls of fungi and the exoskeleton of insects.

**Circadian rhythm** A roughly 24-hour cycle in the physiological processes of organisms. Although generated endogenously, they can be modulated by external factors such as temperature and light.

**Co-evolution** Term used to describe cases where two, sometimes more, species reciprocally affect each other's evolution. Thus, an evolutionary change in the ability of a plant to defend itself might affect the ability of a herbivore to eat the plant; in turn, this might affect the evolution of the plant, thereby affecting the evolution of the herbivore, and so on.

**Convergent evolution** The process whereby organisms that are not closely related evolve similar traits independently of each other as a result of needing to adapt to similar environments or ecological niches.

**DNA** Deoxyribonucleic acid. DNA is the molecule that carries the genetic information in all cellular forms of life and some viruses. It is a polynucleotide (i.e. it consists of long chains of nucleotides). The ability of DNA to store and transmit information lies in the fact that it consists of two polynucleotide strands that twist around each other to form a double-stranded helix.

**Ecological costs** These arise from the negative effects of resistance on the interaction of a plant with its abiotic or biotic environment and which affect the plant's fitness. For example, resistance mechanisms in the plant that are effective in tackling its enemies might also deter its friends, such as pollinating insects or mycorrhizal fungi.

**Effector** A molecule used by a plant pathogen to aid infection of specific plant species. For example, effectors of biotrophic pathogens include proteins which are capable of suppressing the plant's immune responses.

**Elicitor** A molecule capable of inducing a plant defence response.

**Endophyte** An organism, usually a bacterium or fungus, that lives within a plant for at least part of its life cycle without causing apparent harm. Some fungal endophytes, such as those interacting with certain grasses, produce compounds which are toxic to invertebrate and vertebrate herbivores.

**Enzyme** A protein that acts as a biological catalyst, helping complex reactions to take place within cells.

**Eukaryote** A life form comprised of one or more complex cells, each containing organelles including a nucleus, cytoskeleton, mitochondrion and, in plants, a chloroplast.

**Fitness** Used to describe how good an organism is at leaving offspring in the next generation. It is relative and so an individual organism is said to be more fit than another if it produces more offspring throughout its life.

**Flagellin** A structural protein that is the main component of the flagella of bacteria. It is recognized by the plant's immune system and is a powerful elicitor of defence responses.

**Gene expression** The process by which genetic instructions are used to produce gene products, usually proteins. These proteins are used to perform essential functions in cells. Genes are comprised of DNA, which carries the genetic blueprint used to make all the proteins in a cell. Every gene contains a particular set of instructions that code for a specific protein.

**Glucanase** Enzyme that breaks down glucan, a polysaccharide comprising several molecules of glucose.

**Haustorium** A structure produced by certain types of parasitic fungus (e.g. powdery mildews and rusts) which enters plant tissue but does not rupture the membrane of the plant cell, thereby keeping the cell alive. The fungus uses it to draw nutrients from the plant cell. Parasitic plants also produce a haustorium, but this is different in structure. It is produced by the root of the parasitic plant, entering the plant and forming linkages with the plant's vascular system (xylem or phloem). The parasitic plant uses it to obtain water and nutrients from its host.

**Hemibiotroph** Usually a fungal pathogen which starts off its parasitic life as a biotroph, but subsequently switches to a necrotrophic lifestyle. During the biotrophic phase, damage to the plant is minimal, but following the switch to its necrotrophic phase, plant cells and tissues are damaged and killed.

**Heterozygous** Refers to individuals possessing two different alleles (e.g. A and a) of a gene at a particular locus on a chromosome. The allele 'A' is dominant, while allele 'a' is recessive. This means that in the heterozygous individual 'Aa', the dominant characteristic 'A' is shown, while the recessive characteristic 'a' is not observed.

**Homozygous** Refers to individuals possessing two identical alleles at a particular locus on a chromosome. A recessive characteristic will only be shown if an individual is homozygous for the recessive allele i.e. it possesses the alleles 'aa' (see the heterozygous example in the previous entry).

**Hypha** The long, branching filamentous structure of a fungus or Oomycete, providing their main means of vegetative growth. Unicellular fungi such as yeasts do not produce hyphae. Fungal or Oomycete colonies consist of a mass of hyphae known as the mycelium.

**Immune** Term used, in plant pathology, to describe a plant that is able to completely prevent penetration by a microbe. Although the majority of interactions between plants and microbes are likely to be of this type, it can be difficult to establish whether a plant is immune to a particular pathogen, since some pathogens are able to penetrate the plant and exist, for varying periods, without causing symptoms.

**Lacticifer** A type of secretory cell found in the leaves and/or stems of plants. There are two types of lacticifer, articulated and non-articulated, the former comprising a series of cells that are joined together and which can extend considerable distances, and the latter consisting of one elongated cell that can be tens of centimetres long. Secondary metabolites (e.g. latex) are produced and stored in the cells of the lacticifer.

**Landrace** A local variety of a domesticated plant (or animal) species with a long history of local cultivation and which has become well-adapted to local environmental conditions. Landraces are genetically diverse, but this has arisen not through plant breeding, but via continued regeneration of seed by farmers.

**Mutualism** An association between organisms of two different species in which each partner benefits. Examples of mutualisms include the partnership between legumes (e.g. peas or beans)

and nitrogen-fixing bacteria, and the association between roots of many plants and mycorrhizal fungi.

**Mycelium** The mass of branching, thread-like hyphae forming a colony of a fungus or Oomycete.

**Mycorrhiza** An association between plant roots and certain types of fungi in which both partners benefit. The plant provides carbohydrates for the fungus, while the fungus provides increased access to soil nutrients such as phosphate.

**Necrotroph** A parasitic organism such as a fungus or bacterium, that kills plant tissue as it grows and feeds off the dead plant material. The fungal pathogen *Botrytis cinerea* is an example of a nectrotroph.

**Parallel evolution** The evolutionary process by which two or more related but separate species, living in the same environment, develop similar adaptations for survival.

**Parasitoid** A parasitic insect that lives in or on its host, an insect or other arthropod, and eventually kills it. For example, parasitoid wasps lay their eggs on or in other insects and once the egg hatches, the emerging larvae eat the host alive before emerging as an adult.

**Pathogen** A microorganism such as a fungus, bacterium or virus, that disrupts the functioning of its host and causes disease.

**Peptide** A compound of two or more amino acids in which a carboxyl group of one is united with an amino group of another.

**Phloem** The vascular tissue in plants responsible for transporting carbohydrate (sucrose) from source tissues (leaves) to sink tissues such as roots and developing flowers.

**Photosynthesis** Process by which solar energy is converted into chemical energy. It is carried out by algae, plants and certain bacteria and involves the conversion of carbon dioxide and water into carbohydrate (glucose) using energy from sunlight, which is captured by chlorophyll. During this reaction, oxygen is also formed. This is known as oxygenic photosynthesis. In non-oxygenic photosynthesis, which is carried out by certain types of bacteria (e.g. purple bacteria and green sulphur bacteria), the electrons required for the process are provided, not by water, but by other compounds, such as hydrogen sulphide. In this case, solid sulphur is produced as a by-product instead of oxygen.

**Plasmodesmata** Small channels that directly connect the cytoplasm of neighbouring plant cells to each other, thereby establishing living bridges between the cells.

**Prokaryote** Organism in which the cellular structure is defined largely by the absence of a DNA-containing nucleus and other organelles. Bacteria are prokaryotes.

**Protein** A large molecule composed of polymers of amino acids joined together by peptide bonds.

**Recombination** Refers to the exchange of genetic material either between multiple chromosomes or between different regions of the same chromosome.

**Resistance** Term used to describe the ability of a plant to restrict the growth and development of an attacker. Unlike immunity, resistance is not all-or-nothing and in practice, plants can exhibit a range of responses. For example, high levels of resistance curb

can development of the attacker to such an extent that no symptoms are produced, while in plants expressing low levels of resistance, the attacker might be able to develop and disrupt plant function, thereby producing symptoms.

**RNA (Ribonucleic acid)** RNA is a linear molecule, similar in structure to DNA. It exists in a number of different forms, including ribosomal RNA, which is part of the ribosome and takes part in protein synthesis, and messenger RNA, which is copied from DNA. The genetic information transcribed from DNA on to the messenger RNA is then translated into protein by the ribosome.

**Stomata** Microscopic pores on the surfaces of leaves. Each stomatal opening is comprised of two cells, known as guard cells. In order for stomata to open, the guard cells swell up by taking in water and they close when the guard cells lose water. Stomata allow entry of carbon dioxide into the leaf for photosynthesis, but they also enable water to be lost from the leaf in a process known as transpiration.

**Susceptibility** Term used, together with resistance, to describe the ability of a plant to restrict the growth and development of an attacker. For each degree of resistance there is a corresponding level of susceptibility. For example, a plant exhibiting low levels of resistance to a pathogen, thereby allowing it to colonize its tissues and complete its life cycle, is said to be highly susceptible. Similarly, high levels of resistance are linked to low levels of susceptibility.

**Symbiosis** Term used to describe the close, often long-term interaction between two different species. There are different types of symbiotic interaction, including mutualism, in which both partners in the interaction derive benefit, commensalism, in which one partner enjoys a benefit while the other partner is not significantly affected, and parasitism, where one partner benefits at the expense of the other.

**Tolerance** Term used to describe the ability of a plant to grow, develop and complete its life cycle in spite of suffering substantial pathogen or pest attack.

**Transpiration** The evaporation of water from leaves. Transpiration occurs mostly through stomata—when they open to allow carbon dioxide into the leaf for photosynthesis, water is also lost to the atmosphere by evaporation. Leaves can also lose water through the cuticle, which is known as cuticular transpiration, although this is substantially less than water lost via open stomata. The amount of water lost through the cuticle depends on its thickness, so leaves with thick cuticles, such as cacti, lose very little water in this way.

**Trichome** A small hair or outgrowth from the epidermis of a plant. They are diverse in structure and function and include prickles and scales. Many are glandular, producing secretions, such as the essential oils produced by various plant families, e.g. mint.

**Virulent** Term used to describe a pathogen which exerts severe and harmful effects on its host.

**Xylem** The principal water conducting tissue of vascular plants. It transports water and dissolved ions from the roots to the aerial parts of the plant.

# REFERENCES

## Chapter 1


1. Gullan, P. J. and Cranston, P. S. (2014) *The Insects: An Outline of Entomology*. Oxford: Wiley-Blackwell.
2. Mora, C., Tittensor, D. P., Adl, S., Simpson, A. G. B., and Worm, B. (2011) How many species are there on earth and in the ocean? *PLoS Biology* 9(8): e1001127.
3. Heffer Link, V., Powelson, M. L., and Johnson K. B. (2002) Oomycetes. *The Plant Health Instructor.* DOI: 10.1094/PHI-I-2002-0225-01.
4. Money, N. P. (2014) *Microbiology: A Very Short Introduction.* Oxford: Oxford University Press.
5. Hanold, D. and Randles, J.W. (1991) Coconut cadang-cadang disease and its viroid agent. *Plant Disease* 75: 330–5.
6. Scott, P. (2008) *Physiology and Behaviour of Plants.* Chichester: John Wiley and Sons Ltd.


## Chapter 2


1. Darwin, C. (1880) *The Power of Movement in Plants.* London: John Murray.
2. Chehab, E. W., Eich, E., and Braam, J. (2009) Thigmomorphogenesis: a complex plant response to mechano-stimulation. *Journal of Experimental Botany* 60: 43–56.
3. Benikhlef, L., L'Haridon, F., Abou-Mansour, E., Serrano, M., Binda, M., Costa, A., Lehmann, S., and Métraux, J-P. (2013) Perception of soft mechanical stress in Arabidopsis leaves activates disease resistance. *BMC Plant Biology* 13: 133.
4. Bessire, M., Chassot, C., Jacquat, A-C., Humphry, M., Borel, S., Petétot, J.M-C., Métraux, J-P., and Nawrath, C. (2007) A permeable cuticle in Arabidopsis leads to a strong resistance to *Botrytis cinerea. The EMBO Journal* 26: 2158–68.
5. Kim, H., Ridenour, J. B., Dunkle, L. D., and Bluhm, B. H. (2011) Regulation of stomatal tropism and infection by light in *Cercospora zeae-maydis:* evidence for coordinated host/pathogen responses to photoperiod? *PLos Pathogens* 7(7): e1002113.
6. Collins, T. J., Moerschbacher, B., and Read, N. D. (2001) Synergistic induction of wheat stem rust appressoria by chemical and topographical signals. *Physiological and Molecular Plant Pathology* 58: 259–66.
7. Heil, M. and Land, W. G. (2014) Danger signals—damaged-self recognition across the tree of life. *Frontiers in Plant Science* 5: article 578.

8. Tanaka, K., Choi, J., Cao, Y., and Stacey, G. (2014) Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants. *Frontiers in Plant Science* 5: article 446.
9. Hann, C. T., Bequette, C. J., Dombrowski, J. E., and Stratmann, J. W. (2014) Methanol and ethanol modulate responses to danger- and microbe-associated molecular patterns. *Frontiers in Plant Science* 5: article 550.
10. Dixit, S., Upadhyay, S. K., Singh, H., Sidhu, O. P., Verma, P. C., and Chandrashekar, K. (2013) Enhanced methanol production in plants provides broad spectrum insect resistance. *PLoS One* 8(11): e79664.
11. Dodds, P. N. and Rathjen, J. P. (2010) Plant immunity: towards an integrated view of plant-pathogen interactions. *Nature Reviews Genetics* 11: 539–48.
12. Kleemann, J., Rincon-Rivera, L. J., Takahara, H., Neumann U., van Themaat, E. V. L., van der Does, H. C., Hacquard, S., Stüber, K., Will, I., Schmalenbach, E., and O'Connell, R. J. (2012) Sequential delivery of host-induced effectors by appressoria and intracellular hyphae of the phytopathogen *Colletotrichum higginsianum*. *PLoS Pathogens* 8(4): e1002643.
13. Navarro, L., Dunoyer, P., Jay, F., Arnold, B., Dharmasiri, N., Estelle, M., Voinnet, O., and Jones, J. D. G. (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signalling. *Science* 312: 436–39.
14. Navarro, L., Jay, F., Nomura, K., He, S. Y., and Voinnet, O. (2008) Suppression of the microRNA pathway by bacterial effector proteins. *Science* 321: 964–67.
15. Bardoel, B. W., van der Ent, S., Pel, M. J. C., Tommassen, J., Pieterse, C. M. J., van Kessel, K. P. M., and van Strijp, J. A. G. (2011) *Pseudomonas* evades immune recognition of flagellin in both mammals and plants. *PLoS Pathogens* 7(8): e1002206.
16. Van den Burg, H. A., Harrison, S. J., Joosten, M. H. A. J., Vervoort, J., and De Wit, P. J. G. M. (2006) *Cladosporium fulvum* Avr4 protects fungal cell walls against hydrolysis by plant chitinases accumulating during infection. *Molecular Plant-Microbe Interactions* 19: 1420–30.
17. Zamioudis, C. and Pieterse, C. M. J. (2012) Modulation of host immunity by beneficial microbes. *Molecular Plant-Microbe Interactions* 25: 139–50.
18. Jones, J. D. G. and Dangle, J. L. (2006) The plant immune system. *Nature* 444: 323–9.
19. Rivas, S. and Thomas, C. M. (2005) Molecular interactions between tomato and the leaf mold pathogen *Cladosporium fulvum*. *Annual Review of Phytopathology* 43: 395–436.
20. Wang, D. Y-C., Kumar, S., and Hedges, S. B. (1999) Divergence time estimates for the early history of animal phyla and the origin of plants, animals and fungi. *Proceedings of the Royal Society B* 266: 163–71.
21. Escalante-Pérez, M., Krol, E., Stange, A., Geiger, D., Al-Rasheid, K. A. S., Hause, B., Neher, E., and Hedrich, R. (2011) A special pair of phytohormones controls excitability, slow closure, and external stomach formation in the Venus flytrap. *Proceedings of the National Academy of Sciences of the USA* 108: 15492–7.

22. Böhm, J., Scherzer, S., Krol, E., von Meyer, K., Lorey, C., Mueller, T. D., Shabala, L., Monte, I., Solano, R., Al-Rasheid, K. A. S., Rennenberg, H., Shabala, S., Neher, E., and Hedrich, R. (2016) The Venus flytrap *Dionaea muscipula* counts prey-induced action potentials to induce sodium uptake. *Current Biology* 26: 286–95.
23. Peiffer, M., Tooker, J. F., Luthe, D. S., and Felton, G. W. (2009) Plants on early alert: glandular trichomes as sensors for insect herbivores. *New Phytologist* 184: 644–56.
24. Appel, H. M. and Cocroft, R. B. (2014) Plants respond to leaf vibrations caused by insect herbivore chewing. *Oecologia* 175: 1257–66.
25. Vadassery, J., Reichelt, M., and Mithöfer, A. (2012) Direct proof of ingested food regurgitation by *Spodoptera littoralis* caterpillars during feeding on *Arabidopsis. Journal of Chemical Ecology* 38: 865–72.
26. Heil, M. (2009) Damaged-self recognition in plant herbivore defence. *Trends in Plant Science* 14: 356–63.
27. Turlings, T. C. J., Alborn, H. T., Loughrin, J. H., and Tumlinson, J. H. (2000) Volicitin, an elicitor of maize volatiles in oral secretion of *Spodoptera exigua:* isolation and bioactivity. *Journal of Chemical Ecology:* 26: 189–202.
28. Schmelz, E. A., Carroll, M. J., LeClere, S., Phipps, S. M., Meredith, J., Chourey, P. S., Alborn, H. T., Teal, P. E. A. (2006) Fragments of ATP synthase mediate plant perception of insect attack. *Proceedings of the National Academy of Sciences of the USA* 103: 8894–99.
29. Chuang, W-P., Ray, S., Acevedo, F. E., Peiffer, M., Felton, G. W., and Luthe, D. S. (2014) Herbivore cues from the fall armyworm (*Spodoptera frugiperda*) larvae trigger direct defences in maize. *Molecular Plant-Microbe Interactions* 27: 461–70.
30. Wu, S., Peiffer, M., Luthe, D.S., and Felton, G.W. (2012) ATP hydrolyzing salivary enzymes of caterpillars suppress plant defences. *PLoS One* 7(7): e41947.
31. Banting, F. G. and Best, B. A. (1922) The internal secretion of the pancreas. *Journal of Laboratory and Clinical Medicine* 7: 251–66.
32. Green, T. R. and Ryan, C. A. (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defence mechanism against insects. *Science* 175: 776–7.
33. Ryan, C. A. and Pearce, G. (1998) Systemin: a polypeptide signal for plant defensive genes. *Annual Review of Cell and Developmental Biology* 14: 1–17.
34. McGurl, B., Pearce, G., Orozco-Cardenas, M., and Ryan, C. A. (1992) Structure, expression, and antisense inhibition of the systemin precursor gene. *Science* 255: 1570–3.
35. McGurl, B., Orozco-Cardenas, M., Pearce, G., and Ryan, C. A. (1994) Overexpression of the prosystemin gene in transgenic tomato plants generates a systemic signal that constitutively induces proteinase inhibitor synthesis. *Proceedings of the National Academy of Sciences of the USA* 91: 9799–9802.
36. Scheer, J. M. and Ryan, C. A. (2002) The systemin receptor SR160 from *Lycopersicon peruvianum* is a member of the LRR receptor kinase family. *Proceedings of the National Academy of Sciences of the USA* 99: 9585–90.
37. Albert, M. (2013) Peptides as triggers of plant defence. *Journal of Experimental Botany* 64: 5269–79.

38. Bartels, S., Lori, M., Mbengue, M., van Verk, M., Klauser, D., Hander, T., Böni, R., Robatzek, S., and Boller, T. (2013) The family of Peps and their precursors in *Arabidopsis:* differential expression and localisation but similar induction of pattern-triggered immune responses. *Journal of Experimental Botany* 64: 5309–21.
39. Hilker, M. and Meiners, T. (2006) Early herbivore alert: insect eggs induce plant defence. *Journal of Chemical Ecology* 32: 1379–97.
40. Reymond, P. (2013) Perception, signalling and molecular basis of oviposition-mediated plant responses. *Planta* 238: 247–58.

## Chapter 3

1. Conrath, U., Beckers, G. J. M., Flors, V., Garciá-Agustín, P., Jakab, G., Mauch, F., Newman, M-A., Pieterse, C. M. J., Poinssot, B., Pozo, M. J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, W., Zimmerli, L., and Mauch-Mani, B. (2006) Priming: getting ready for battle. *Molecular Plant-Microbe Interactions* 19: 1062–71.
2. Frost, C. J., Mescher, M. C., Carlson, J. E., and De Moraes, C. M. (2008) Plant defence priming against herbivores: getting ready for a different battle. *Plant Physiology* 146: 818–24.
3. Jeffreys, D. (2013) *Aspirin: the remarkable story of a wonder drug.* New York: Bloomsbury Publishing.
4. Stone, E. (1763) An account of the success of the bark of the willow in the cure of agues. In a letter to the Right Honourable George Earl of Macclesfield, President of the R.S. from the Rev. Mr Edmund Stone, of Chipping-Norton in Oxfordshire. *Philosophical Transactions of the Royal Society* 53: 195–200.
5. Beauverie, J. (1901) Essais d'immunisation des végétaux contre les maladies cryptogamiques. *Comptes Rendus des Séances de L'Académie des Sciences* 133: 107–10.
6. Ray, J. (1901) Sur les maladies cryptogamiques des végétaux. *Revue générale de Botanique* XIII: 145.
7. Ross, A. F. (1961) Systemic acquired resistance induced by localized virus infection in plants. *Virology* 14: 340–58.
8. Kuć, J. (1982) Induced immunity to plant disease. *BioScience* 32: 854–60.
9. Hammerschmidt, R. (2014) Introduction: definitions and some history. In: *Induced Resistance for Plant Defence: A Sustainable Approach to Crop Protection*, ed. D. R. Walters, A. C. Newton, and G. D. Lyon, 1–10. Oxford: Wiley-Blackwell.
10. Malamy, J., Carr, J. P., Klessig, D. F., and Raskin, I. (1990) Salicylic acid—a likely endogenous signal in the resistance response of tobacco to *Tobacco mosaic virus. Science* 250: 1002–4.
11. Métraux, J-P., Signer, H., Ryals, J., Ward, E., Wyss-Benz, M., Gaudin, J., Raschdorf, K., Schmid, E., Blum, W., and Inverardi, B. (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. *Science* 250: 1004–6.
12. Delaney, T. P., Uknes S., Vernooij, B., Friederich L., Weymann, K., Negrotto, D., Gaffney, T., Gur-Rella M., Kessmann, H., Ward, E., and Ryals, J. (1994) A central role of salicylic acid in plant disease resistance. *Science* 266: 1247–50.

13. Park, S. W., Kaimoyo, E., Kumar, D., Mosher, S., and Klessig, D. F. (2007) Methyl salicylate is a critical mobile signal for plant systemic acquired resistance. *Science* 318: 113–16.
14. Wiesner, J. (1892) *Die elementarstructur und das wachstum der lebenden substanz*. Vienna: Hölder.
15. English, J. Jr, Bonner, J., and Haagen-Smit, A. J. (1939) Structure and synthesis of a plant wound hormone. *Science* 90: 329.
16. Green, T. R. and Ryan, C. A. (1972) Wound-induced proteinase inhibitor in plant leaves: a possible defence mechanism against insects. *Science* 175: 776–7.
17. Farmer, E. E. and Ryan, C. A. (1990) Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences of the USA* 87: 7713–16.
18. Howe, G. A., Lightner, J., Browse, J., and Ryan, C. A. (1996) An octadecanoid pathway mutant (JL5) of tomato is compromised in signalling for defense against insect attack. *Plant Cell* 8: 2067–77.
19. McConn, M., Creelman, R. A., Bell, E., Mullet, J. E., and Browse, J. (1997) Jasmonate is essential for insect defense in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA* 94: 5473–7.
20. Mafli, A., Goudet, J., and Farmer, E. E. (2012) Plants and tortoises: mutations in the *Arabidopsis* jasmonate pathway increase feeding in a vertebrate herbivore. *Molecular Ecology* 21: 2534–41.
21. Koo, A. J. K. and Howe, G. A. (2009) The wound hormone jasmonate. *Phytochemistry* 70: 1571–80.
22. Farmer, E. E. (2014) *Leaf Defence.* Oxford: Oxford University Press.
23. Glauser, G., Dubugnon, L., Mousavi, S. A. R., Rudaz, S., Wolfender, J-L., and Fermer, E. E. (2009) Velocity estimates for signal propagation leading to systemic jasmonic acid accumulation in wounded *Arabidopsis*. *Journal of Biological Chemistry* 284: 34506–13.
24. Wildon, D. C., Thain, J. F., Minchin, P. E. H., Gubb, I. R., Reilly, A. J., Skipper, Y. D., Doherty, H. M., O'Donnell, P. J., and Bowles, D. J. (1992) Electrical signalling and systemic proteinase inhibitor induction in the wounded plant. *Nature* 360: 62–5.
25. Mousavi, S. A. R., Chauvin, A., Pascaud, F., Kellenberger, S., and Farmer, E. E. (2013) *GLUTAMATE RECEPTOR-LIKE* genes mediate leaf-to-leaf wound signalling. *Nature* 500: 422–6.
26. Lam, H. M., Chiu, J., Hsieh, M. H., Meisel, L., Oliveira, I. C., Shin, M., and Coruzzi, G. (1998) Glutamate-receptor genes in plants. *Nature* 396: 125–6.
27. Kang, S., Kim, H. B., Lee, H., Choi, J. Y., Heu, S., Oh, C. J., Kwon, S. I., and An, C. S. (2006) Overexpression in Arabidopsis of a plasma membrane-targeting glutamate receptor from small radish increases glutamate-mediated $Ca^{2+}$ influx and delays fungal infection. *Molecules and Cells* 21: 418–27.
28. Li, F., Wang, J., Ma, C., Zhao, Y., Wang, Y., Hasi, A., and Qi, Z. (2013) Glutamate receptor-like channel 3.3 is involved in mediating glutathione-triggered cytosolic calcium transients, transcriptional changes, and innate immunity responses in Arabidopsis. *Plant Physiology* 162: 1497–1509.

29. Kiep, V., Vadassery, J., Lattke, J., Maaß, J-P., Boland, W., Pieter, E., and Mithöfer, A. (2015) Systemic cytosolic $Ca^{2+}$ elevation is activated upon wounding and herbivory in *Arabidopsis*. *New Phytologist* 207: 996–1004.
30. Rhoades, D. F. (1983) Responses of alder and willow to attack by tent caterpillars and webworms: evidence for pheromonal sensitivity of willows. In: *Plant Resistance to Insects*, ed. P. A. Hedin, 55–68. Washington, DC: American Chemical Society.
31. Baldwin, I. T. and Schultz, J. C. (1983) Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science* 221: 277–9.
32. McGowan, K. (2013) How plants secretly talk to each other. Available at: <http://www.wired.com/2013/12/secret-language-of-plants/>, accessed 10 May 2016.
33. Engelberth, J., Alborn, H. T., Schmelz, E. A., and Tumlinson, J. H. (2004) Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences of the USA* 101: 1781–5.
34. Karban, R. (2001) Communication between sagebrush and wild tobacco in the field. *Biochemical Systematics and Ecology* 29: 995–1005.
35. Kessler, A., Halitschke, R., Diezel, C., and Baldwin, I. T. (2006) Priming of plant defense responses in nature by airborne signalling between *Artemisia tridentata* and *Nicotiana attenuata*. *Oecologia* 148: 280–92.
36. Yi, H-S., Heil, M., Adame-Álvarez, R. M., Ballhorn, D. J., and Ryu, C-M. (2009) Airborne induction and priming of plant defences against a bacterial pathogen. *Plant Physiology* 151: 2152–61.
37. Castelyn, H. D., Appelgryn, J. J., Mafa, M. S., Pretorius, Z. A., and Visser, B. (2015) Volatiles emitted by leaf rust infected wheat induce a defence response in exposed uninfected wheat seedlings. *Australasian Plant Pathology* 44: 245–54.
38. Schoonhoven, L. M., van Loon, J. J. A., and Dicke, M. (2005) *Insect–Plant Biology*. Oxford: Oxford University Press.
39. Dicke, M., Van Beek, T. A., Posthumus, M. A., Ben Dom, N., Van Bokhoven, H., and De Groot, A. (1990) Isolation and identification of volatile kairomone that affects acarine predator–prey interactions: involvement of host plant in its production. *Journal of Chemical Ecology* 16: 381–96.
40. Turlings, T. C. J., Tumlinson, J. H., and Lewis, W. J. (1990) Exploitation of herbivore-induced plant odors by host-seeking parasitic wasps. *Science* 250: 1251–3.
41. Kappers, I. F., Hoogerbrugge, H., Bouwmeester, H. J., and Dicke, M. (2011) Variation in herbivory-induced volatiles among cucumber (*Cucumis sativus* L.) varieties has consequences for the attraction of carnivorous natural enemies. *Journal of Chemical Ecology* 37: 150–60.
42. Van Wijk, M., De Bruijn, P. J. A., and Sabelis, M. W. (2008) Predatory mite attraction to herbivore-induced plant odors is not a consequence of attraction to individual herbivore-induced plant volatiles. *Journal of Chemical Ecology* 34: 791–803.
43. Meiners, T. and Hilker, M. (1997) Host location in *Oomyzus gallericae* (Hymenoptera: Eulophidae), an egg parasitoid of the elm leaf beetle *Xanthogaleruca luteola* (Coleoptera: Chrysomelidae). *Oecologia* 112: 87–93.

44. Hilker, M., Kobs, C., Varama, M., and Schrank, K. (2002) Insect egg deposition induces *Pinus sylvestris* to attract egg parasitoids. *The Journal of Experimental Biology* 205: 455–61.
45. Fatouros, N. E., Lucas-Barbosa, D., Weldegergis, B. T., Pashalidou, F. G., van Loon, J. J. A., Dicke, M., Harvey, J. A., Gols, R., and Huigens, M. E. (2012) Plant volatiles induced by herbivore egg deposition affect insects of different trophic levels. *PLoS One* 7(8): e43607.
46. Tooker, J. F. and De Moraes, C. M. (2008) Gall insects and indirect plant defences. *Plant Signalling and Behavior* 3: 503–4.
47. Tooker, J. F., Rohr, J. R., Abrahamson, W. G., and De Moraes, C. M. (2008) Gall insects can avoid and alter indirect plant defences. *New Phytologist* 178: 657–71.
48. Rostás, M., Maag, D., Ikegami, M., and Inbar, M. (2013) Gall volatiles defend aphids against a browsing mammal. *BMC Evolutionary Biology* 13: 193.
49. Tooker, J. F., Koenig, W. A., and Hanks, L. M. (2002) Altered host plant volatiles are proxies for sex pheromones in the gall wasp *Antistrophus rufus*. *Proceedings of the National Academy of Sciences of the USA* 99: 15486–91.
50. Mäntylä, E., Alessio, G. A., Blande, J. D., Heijari, J., Holopainen, J. K., Laaksonen, T., Piirtola, P., and Klemola, T. (2008) From plants to birds: higher avian predation rates in trees responding to insect herbivory. *PLoS One* 3(7): e2832.
51. Amo, L., Jansen, J. J., van Dam, N. M., Dicke, M., and Visser, M. E. (2013) Birds exploit herbivore-induced volatiles to locate herbivorous prey. *Ecology Letters* 16: 1348–55.
52. Heil, M. and Adame-Álvarez, R.M. (2010) Short signalling distances make plant communication a soliloquy. *Biology Letters* 6: 843–5.
53. Frost, C. J., Appel, H. M., Carlson, J. E., De Moraes, C. M., Mescher, M. C., and Schultz, J. C. (2007) Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecology Letters* 10: 490–8.
54. Heil, M. and Silva Bueno, J. C. (2007) Within-plant signalling by volatiles leads to induction and priming of an indirect plant defence in nature. *Proceedings of the National Academy of Sciences of the USA* 104: 5467–72.
55. Pieterse, C. M. J., Van der Does, D., Zamioudis, C., Leon-Reyes, A., and Van Wees, S. C. M. (2012) Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology* 28: 489–521.
56. Savchenko, T. and Dehesh, K. (2013) Insect herbivores selectively mute GLV production in plants. *Plant Signalling and Behavior* 8: e24136.

## Chapter 4

1. Franceschi, V. R., Krokene, P., Christiansen, E., and Krekling, T. (2005) Anatomical and chemical defences of conifer bark against bark beetles and other pests. *New Phytologist* 167: 353–76.
2. Agrios, G. N. (2005) *Plant Pathology*, 3rd edn. London: Elsevier Academic Press.

3. Whitney, H. M., Federle, W., and Glover, B. J. (2009) Grip and slip: mechanical interactions between insects and the epidermis of flowers and flower stalks. *Communicative and Integrative Biology* 2(6): 505–8.
4. Bellincampi, D., Cervone, F., and Lionetti, V. (2014) Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. *Frontiers in Plant Science* 5: article 228.
5. Bethke, G., Grundman, R. E., Sreekanta, S., Truman, W., Katagiri, F., and Glazebrook, J. (2014) *Arabidopsis* PECTIN METHYLESTERASEs contribute to immunity against *Pseudomonas syringae*. *Plant Physiology* 164: 1093–1107.
6. Lionetti, V., Raiola, A., Camardella, L., Giovane, A., Obel, N., Pauly, M., Favaron, F., Cervone, F., and Bellincampi, D. (2007) Overexpression of pectin methylesterase inhibitors in Arabidopsis restricts fungal infection by *Botrytis cinerea*. *Plant Physiology* 143: 1871–80.
7. Powell, A. L. T., van Kan, J., ten Have, A., Visser, J., Greve, L. C., Bennett, A. B., and Labavitch, J. M. (2000) Transgenic expression of pear PGIP in tomato limits fungal colonisation. *Molecular Plant-Microbe Interactions* 13: 942–50.
8. Federici, L., Di Metteo, A., Fernandez-Recio, J., Tsernoglou, D., and Cervone, F. (2006) Polygalacturonase inhibiting proteins: players in plant innate immunity? *Trends in Plant Science* 11: 65–70.
9. Mauch, F., Mauch-Mani, B., and Boller, T. (1988) Antifungal hydrolases in pea tissue. Part II. Inhibition of fungal growth by combinations of chitinase and β-1,3-glucanase. *Plant Physiology* 88: 936–42.
10. Westerink, N., Roth, R., Van den Burg, H. A., de Wit, P. J. G. M., and Joosten, M. H. A. J. (2002) The AVR4 elicitor protein of *Cladosporium fulvum* binds to fungal components with high affinity. *Molecular Plant-Microbe Interactions* 15: 1219–27.
11. van den Burg, H. A., Harrison, S. J., Joosten, M. H. A. J., Vervoort, J., and de Wit, P. J. G. M. (2006) *Cladosporium fulvum* Avr4 protects fungal cell walls against hydrolysis by plant chitinases accumulating during infection. *Molecular Plant-Microbe Interactions* 19: 1420–30.
12. Slavokhotova, A. A., Naumann, T. A., Price, N. P. J., Rogozhin, E. A., Andreev, Y. A., Vassilevski, A. A., and Odintsova, T. I. (2014) Novel mode of action of plant defense peptides—hevein-like antimicrobial peptides from wheat inhibit fungal metalloproteases. *FEBS Journal* 281: 4754–64.
13. Voigt, C. A. (2014) Callose-mediated resistance to pathogenic intruders in plant defense-related papillae. *Frontiers in Plant Science* 5: article 168.
14. De Bary, A. (1863) Recherches sur le dévelopement de quelques champignons parasites. *Annales des Sciences Naturelles; Botanique et biologie végétale* 20: 5–148.
15. Mangin, L. (1895) Recherches sur les Péronosporées. *Bulletin de la Societé d'histoire naturelle d'Autun* 8: 55–108.
16. Jacobs, A. K., Lipka, V., Burton, R. A., Panstruga, R., Strizhov, N., Schulze-Lefert, P., and Fincher, G. B. (2003) An Arabidopsis callose synthase, GSL5, is required for wound and papillary callose formation. *Plant Cell* 15: 2503–13.

17. Ellinger, D., Naumann, M., Falter, C., Zwikowics, C., Janrow, T., Manisseri, C., Somerville, S. C., and Voigt, C. A. (2013) Elevated early callose deposition results in complete penetration resistance to powdery mildew in *Arabidopsis*. *Plant Physiology* 161: 1433–44.
18. Eggert, D., Naumann, M., Reimer, R., and Voigt, C. A. (2014) Nanoscale glucan polymer network causes pathogen resistance. *Scientific Reports* 4: 4159.
19. Hao, P., Liu, C., Wang, Y., Chen, R., Tang, M., Du, B., Zhu, L., and He, G. (2008) Herbivore-induced callose deposition on the sieve plates of rice: an important mechanism for host resistance. *Plant Physiology* 146: 1810–20.
20. Smith, A. M., Coupland, G., Dolan, L., Harberd, N., Jones, J., Martin, C., Sablowski, R., and Amey, A. (2010) *Plant Biology*. New York: Garland Science.
21. Maher, E. A., Bate, N. J., Ni, W., Elkind, Y., Dixon, R. A., and Lamb, C. J. (1994) Increased disease susceptibility of transgenic tobacco plants with suppressed levels of preformed phenylpropanoid products. *Proceedings of the National Academy of Sciences of the USA* 91: 7802–6.
22. Bhuiyan, N. H., Selvaraj, G., Wei, Y., and King, J. (2009) Gene expression profiling and silencing reveal that monolignol biosynthesis plays a critical role in penetration defence in wheat against powdery mildew invasion. *Journal of Experimental Botany* 60: 509–21.
23. Caño-Delgado, A., Penfield, S., Smith, C., Catley, M., and Bevan, M. (2003) Reduced cellulose synthesis invokes lignification and defence responses in *Arabidopsis thaliana*. *The Plant Journal* 34: 351–62.
24. Diezel, C., Kessler, D., and Baldwin, I. T. (2011). Pithy protection: *Nicotiana attenuata's* jasmonic acid-mediated defences are required to resist stem-boring weevil larvae. *Plant Physiology* 155: 1936–46.
25. Gaquerel, E., Kotkar, H., Onkokesung, N., Galis, I., and Baldwin, I. T. (2013) Silencing an *N*-Acyltransferase-like involved in lignin biosynthesis in *Nicotiana attenuata* dramatically alters herbivory-induced phenolamide metabolism. *PLoS One* 8(5): e62336.
26. Hooke, R. (1665) *Micrographia: or, Some Physiological Descriptions of Minute Bodies Made by Magnifying Glasses.* London: J. Martyn and J. Allestry.
27. Walters, D. R. (2011) *Plant Defense: Warding Off Attack by Pathogens, Herbivores, and Parasitic Plants.* Oxford: Wiley-Blackwell.
28. Link, K. P. and Walker, J. C. (1933) The isolation of catechol from pigmented onion scales and its significance in relation to disease resistance in onions. *Journal of Biological Chemistry* 100: 379–83.
29. Farah, A. and Donangelo, C. M. (2006) Phenolic compounds in coffee. *Brazilian Journal of Plant Physiology* 18: 23–36.
30. Ranheim, T. and Halvorsen, B. (2005) Coffee consumption and human health: beneficial or detrimental? Mechanisms for effects of coffee consumption on different risk factors for cardiovascular disease and type 2 diabetes mellitus. *Molecular Nutrition and Food Research* 49: 274–84.
31. Davies, E. (2011) Chemistry in every cup. *Chemistry World* May: 36–9.

32. Bostock, R. M., Wilcox, S. M., Wang, G., and Adaskaveg, J. E. (1999) Suppression of *Monilinia fructicola* cutinase production by peach fruit surface phenolic acids. *Physiological and Molecular Plant Pathology* 54: 37–50.
33. Wojciechowska, E., Weinert, C. H., Egert, B., Trierweiler, B., Schmidt-Heydt, M., Horneburg, B., Graeff-Hönninger, S., Kulling, S. E., and Geisen, R. (2014) Chlorogenic acid, a metabolite identified by untargeted metabolome analysis in resistant tomatoes, inhibits the colonisation by *Alternaria alternata* by inhibiting alternariol biosynthesis. *European Journal of Plant Pathology* 139: 735–47.
34. Schoonhoven, L. M., van Loon, J. J. A., and Dicke, M. (2005) *Insect–Plant Biology*. Oxford: Oxford University Press.
35. Eisner, T., Eisner, M., and Hoebeke, E. R. (1998) When defense backfires: detrimental effect of a plant's protective trichomes on an insect beneficial to the plant. *Proceedings of the National Academy of Sciences of the USA* 95: 4410–14.
36. Krings, M., Kellogg, D. W., Kerp, H., and Taylor, T. N. (2003) Trichomes of the seed fern *Blanzyopteris praedentata:* implications for plant-insect interactions in the Late Carboniferous. *Botanical Journal of the Linnean Society* 141: 133–49.
37. Li, C-H., Liu, Y., Hua, J., Luo, S-H., and Li, S-H. (2014) Peltate glandular trichomes of *Colquhounia seguinii* harbour new defensive clerodane diterpenoids. *Journal of Integrative Plant Biology* 56: 928–40.
38. Ramirez, A. M., Stoopen, G., Menzel, T. R., Gols, R., Bouwmeester, H. J., Dicke, M., and Jongsma, M. A. (2012) Bidirectional secretions from glandular trichomes of Pyrethrum enable immunization of seedlings. *The Plant Cell* 24: 4252–65.
39. Weinhold, A. and Baldwin, I. T. (2011) Trichome-derived O-acyl sugars are a first meal for caterpillars that tags them for predation. *Proceedings of the National Academy of Sciences of the USA* 108: 7855–9.
40. Ranger, C. M., Winter, R. E., Singh, A. P., Reding, M. E., Frantz, J. M., Locke, J. C., and Krause, C. R. (2011) Rare excitatory amino acid from flowers of zonal geranium responsible for paralysing the Japanese beetle. *Proceedings of the National Academy of Sciences of the USA* 108: 1217–21.
41. Gorup-Besanez, E. F. (1849) Notiz über das vorkomen der Ameisensaure in den Brennesseln. *Journal für Praktische Chemie* 48: 191–2.
42. Fu, H. Y., Chen, S. J., Chen, R. F., Ding, W. H., Kuo-Huang, L. L., and Huang, R. N. (2006) Identification of oxalic acid and tartaric acid as major persistent pain-inducing toxins in the stinging hairs of the nettle, *Urtica thunbergiana. Annals of Botany* 98: 57–65.
43. Farmer, E. E. (2014) *Leaf Defence.* Oxford: Oxford University Press.
44. Iwamoto, M., Horikawa, C., Shikata, M., Wasaka, N., Kato, T., and Sato, H. (2014) Stinging hairs on the Japanese nettle *Urtica thunbergiana* have a defensive function against mammalian but not insect herbivores. *Ecological Research* 29: 455–62.
45. Tennie, C., Hedwig, D., Call, J., and Tomasello, M. (2008) An experimental study of nettle feeding in captive gorillas. *American Journal of Primatology* 70: 1–10.
46. Milewski, A. V., Young, T. P., and Madden, D. (1991) Thorns as induced defences—experimental evidence. *Oecologia* 86: 70–5.

47. Piperno, D. R. (2006) *Phytoliths: A Comprehensive Guide for Archaeologists and Paleoecologists.* Lanham, MD, New York, Toronto, and Oxford: AltaMira Press (Rowman & Littlefield).
48. Darwin, C. (1846) An account of the fine dust which often falls on vessels in the Atlantic Ocean. *Quarterly Journal of the Geological Society of London* 2: 26–30.
49. Prasad, V., Strömberg, C. A., Alimohammadian, H., and Sahni, A. (2005) Dinosaur coprolites and the early evolution of grasses and grazers. *Science* 18: 1177–80.
50. Vicari, M. and Bazely, D. R. (1993) Do grasses fight back? The case for antiherbivore defences. *Trends in Ecology and Evolution* 8: 137–41.
51. Massey, F. P. and Hartley, S. E. (2009) Physical defences wear you down: progressive and irreversible impacts of silica on insect herbivores. *Journal of Animal Ecology* 78: 281–91.
52. Hudgins, J. W., Krekling, T., and Franchesci, V. R. (2003) Distribution of calcium oxalate crystals in the secondary phloem of conifers: a constitutive defence mechanism? *New Phytologist* 159: 677–90.
53. Korth, K. L., Doege, S. J., Park, S-H., Goggin, F. L., Wang, Q., Gomez, S. K., Liu, G., Jia, L., and Nakata, P. A. (2006) *Medicago truncatula* mutants demonstrate the role of plant calcium oxalate crystals as an effective defense against chewing insects. *Plant Physiology* 141: 188–95.

## Chapter 5

1. Pickard, W. F. (2008) Lactifers and secretory ducts: two other tube systems in plants. *New Phytologist* 177: 877–88.
2. Hagel, J. M., Yeung, E. C., and Facchini, P. J. (2008) Got milk? The secret life of lactifers. *Trends in Plant Science* 13: 631–9.
3. Agrawal, A. A., Petschenka, G., Bingham, R. A., Weber, M. G., and Rasmann, S. (2012) Toxic cardenolides: chemical ecology and coevolution of specialised plant–herbivore interactions. *New Phytologist* 194: 28–45.
4. Zalucki, M. P., Brower, L. P., and Alonso-M, A. (2001) Detrimental effects of latex and cardiac glycosides on survival and growth of first-instar monarch butterfly larvae *Danaus plexippus* feeding on the sandhill milkweed *Asclepias humistrata*. *Ecological Entomology* 26: 212–24.
5. Becerra, J. X., Noge, K., and Venable, D. L. (2009) Macroevolutionary chemical escalation in an ancient plant–herbivore arms race. *Proceedings of the National Academy of Sciences of the USA* 106: 18062–6.
6. Lange, B. M. (2015) The evolution of plant secretory structures and emergence of terpenoid chemical diversity. *Annual Review of Plant Biology* 66: 139–59.
7. Dussourd, D. E. and Denno, D. F. (1991) Deactivation of plant defense: correspondence between insect behaviour and secretory canal architecture. *Ecology* 72: 1383–96.
8. Gontijo, L. M. (2013) Female beetles facilitate leaf feeding for males on toxic plants. *Ecological Entomology* 38: 272–7.

9. Roberts, D. M. and Buckley, N. (2009) Antidotes for acute cardenolide (cardiac glycoside) poisoning. *Cochrane Database of Systematic Reviews* 2006(4): Art. No. CD005490.
10. De Maleissye, J. (1991) *Histoire du poison*. Paris: F. Bourin.
11. Gaillard, Y., Krishnamoorthy, A., and Bevalot, F. (2004) *Cerbera odollam:* a 'suicide tree' and cause of death in the state of Kerala, India. *Journal of Ethnopharmacology* 95: 123–6.
12. Ashcroft, F. (2013) *The Spark of Life: Electricity in the Human Body*. London: Penguin.
13. Steppuhn, A., Gase, K., Krock, B., Halitschke, R., and Baldwin, I. T. (2004) Nicotine's defensive function in nature. *PLos Biology* 2(8): e217.
14. Kumar, P., Pandit, S. S., Steppuhn, A., and Baldwin, I. T. (2014) Natural history-driven, plant-mediated RNAi-based study reveals *CYP6B46*'s role in a nicotine-mediated antipredator herbivore defense. *Proceedings of the National Academy of Sciences of the USA* 111: 1245–52.
15. Kessler, D., Bhattacharya, S., Diezel, C., Rothe, E., Gase, K., Schöttner, M., and Baldwin, I. T. (2012) Unpredictability of nectar nicotine promotes outcrossing by hummingbirds in *Nicotiana attenuata*. *The Plant Journal* 71: 529–38.
16. Kaczorowski, R. L., Koplovich, A., Sporer, F., Wink, M., and Markman, S. (2014) Immediate effects of nectar robbing by Palestine sunbirds (*Nectarinia osea*) on nectar alkaloid concentrations in tree tobacco (*Nicotiana glauca*). *Journal of Chemical Ecology* 40: 325–30.
17. Speranza, A. (2010) Into the world of steroids: a biochemical 'keep in touch' in plants and animals. *Plant Signalling and Behavior* 5(8): 940–3.
18. Kubo, I., Klocke J. A., and Asano, S. (1983) Effects of ingested phytoecdysteroids on the growth and development of two lepidopterous larvae. *Journal of Insect Physiology* 29: 307–16.
19. Rharrabe, K., Sayah, F., and LaFont, R. (2010) Dietary effects of four phytoecdysteroids on growth and development of the Indian meal moth, *Plodia interpunctella*. *Journal of Insect Science* 10: 13.
20. Kissen, R., Rossiter, J. T., and Bones, A. M. (2009) The 'mustard oil bomb': not so easy to assemble?! Localization, expression and distribution of the components of the myrosinase enzyme system. *Phytochemistry Reviews* 8: 69–86.
21. Shroff, R., Vergara, F., Muck, A., Svatos, A., and Gershenzon, J. (2008) Nonuniform distribution of glucosinolates in *Arabidopsis thaliana* leaves has important consequences for plant defense. *Proceedings of the National Academy of Sciences of the USA* 105: 6196–6201.
22. Ratzka, A., Vogel, H., Kliebenstein, D. J., Mitchell-Olds, T., and Kroymann, J. (2002) Disarming the mustard oil bomb. *Proceedings of the National Academy of Sciences of the USA* 99: 11223–8.
23. Wittstock, U., Agerbirk, N., Stauber, E. J., Olsen, C. E., Hippler, M., Mitchell-Olds, T., Gershenzon, J., and Vogel, H. (2004) Successful herbivore attack due to metabolic diversion of a plant chemical defense. *Proceedings of the National Academy of Sciences of the USA* 101: 4859–64.

24. Bridges, M., Jones, A. M. E., Bones, A. M., Hodgson, C., Cole, R., Bartlet, E., Wallsgrove, R., Karapapa, V. K., Watts, N., and Rossiter, J. T. (2002) Spatial organisation of the glucosinolate-myrosinase system in *Brassica* specialist aphids is similar to that of the host plant. *Proceedings of the Royal Society of London B, Biological Sciences* 269: 187–91.
25. Beran, F., Pauchet, Y., Kunert, G., Reichelt, M., Wielsch, N., Vogel, H., Reinecke, A., Svatoš, A., Mewis, I., Schmid, D., Ramasamy, S., Ulrichs, C., Hansson, B. S., Gershenzon, J., and Heckel, D. G. (2014) *Phyllotreta striolata* flea beetles use host plant defense compounds to create their own glucosinolate-myrosinase system. *Proceedings of the National Academy of Sciences of the USA* 111: 7349–54.
26. Dethier, V. G. (1972) Chemical interactions between plants and insects. In: *Chemical Ecology*, ed. E. Sondheimer and J. B. Simeone, 83–102. New York: Academic Press.
27. Shroff, R., Schramm, K., Jeschke, V., Nemes, P., Vertes, A., Gershenzon, J., and Svatoš, A. (2015) Quantification of plant surface metabolites by matrix-assisted laser desorption-ionization mass spectrometry imaging: glucosinolates on *Arabidopsis thaliana* leaves. *The Plant Journal* 81: 961–72.
28. Badenes-Perez, F. R., Gershenzon, J., and Heckel, D. G. (2014) Insect attraction versus plant defense: young leaves high in glucosinolates stimulate oviposition by a specialist herbivore despite poor larval survival due to high saponin content. *PLoS One* 9(4): e95766.
29. Samuni-Blank, M., Izhaki, I., Gerchman, Y., Dearing, M. D., Karasov, W. H., Trabelcy, B., Edwards, T. M., and Arad, Z. (2014) Taste and physiological responses to glucosinolates: seed predator versus seed disperser. *PLoS One* 9(11): e112505.
30. Selosse, M-A., Boullard, B., and Richardson, D. (2011) Noël Bernard (1874–1911): orchids to symbiosis in a dozen years, one century ago. *Symbiosis* 54: 61–8.
31. Müller, K. O. and Borger, H. (1940) Experimentelle untersuchungen über phytophthorainfestans-resistanz der Kartoffel. *Arbeiten aus der Biologischen Reichanstalt für Land-und Forstwirtschaft* 23: 189–231.
32. Harborne, J. B. (1999) The comparative biochemistry of phytoalexin induction in plants. *Biochemical Systematics and Ecology* 27: 335–67.
33. Bailey, J. A. and Deverall, B. J. (1971) Formation and activity of phaseollin in the interaction between bean hypocotyls (*Phaseolus vulgaris*) and physiological races of *Colletotrichum lindemuthianum*. *Physiological Plant Pathology* 1: 435–49.
34. Lo, S. C. C., de Verdier, K., and Nicholson, R. L. (1999) Accumulation of 3-deoxyanthocyanidin phytoalexins and resistance to *Colletotrichum sublineolum* in sorghum. *Physiological and Molecular Plant Pathology* 55: 263–73.
35. Van der Linde, K. and Doehlemann, G. (2013) Utilizing virus-induced gene silencing for the functional characterisation of maize genes during infection with the fungal pathogen *Ustilago maydis*. *Methods in Molecular Biology* 975: 47–60.
36. Ashcroft, F. (2012) *The Spark of Life: Electricity in the Human Body*. London: Penguin.
37. Du Fall, L. A. and Solomon, P. S. (2011) Role of cereal secondary metabolites involved in mediating the outcome of plant-pathogen interactions. *Metabolites* 1: 64–78.

38. Ward, H. M. (1902) On the relations between host and parasite in the bromes and their brown rust, *Puccinia dispersa* (Erikss.). *Annals of Botany* 16: 233–315.
39. Ward, H. M. (1905) Recent researches on the parasitism of fungi. *Annals of Botany* 19: 1–54.
40. Stakman, E. C. (1915) Relation between *Puccinia graminis* and plants highly resistant to its attack. *Journal of Agricultural Research* 4: 193–9.
41. Hiruma, K., Fukunaga, S., Bednarek, P., Pislewska-Bednarek, M., Watanabe, S., Narusaka, Y., Shirasu, K., and Takano, Y. (2013) Glutathione and tryptophan metabolism are required for *Arabidopsis* immunity during the hypersensitive response to hemibiotrophs. *Proceedings of the National Academy of Sciences of the USA* 110: 9587–94.
42. Birker, D., Heidrich, K., Takahara, H., Narusaka, M., Deslandes, L., Narusaka, Y., Reymond, M., Parker, J. E., and O'Connell, R. (2009) A locus conferring resistance to *Colletotrichum higginsianum* is shared by four geographically distinct Arabidopsis accessions. *The Plant Journal* 60: 602–13.
43. Van Doorn, W. G. (2011) Classes of programmed cell death in plants, compared to those in animals. *Journal of Experimental Botany* 62: 4749–61.
44. Described in Smith, A. M., Coupland, G., Dolan, L., Harberd, N., Jones, J., Martin, C., Sablowski, R., and Amey, A. (2010) *Plant Biology*. New York: Garland Science, p. 555.
45. Baulcombe, D. (2015) RNA silencing in plants. *The Biochemist* 3(2): 10–13.
46. Padmanabhan, C., Zhang, X., and Jin, H. (2009) *Current Opinion in Plant Biology* 12: 465–72.
47. Katiyar-Agarwal, S., Morgan, R., Dahlbeck, D., Borsani, O., Villegas Jr, A., Zhu, J-K., Staskawicz, B. J., and Jin, H. (2006) A pathogen-inducible endogenous siRNA in plant immunity. *Proceedings of the National Academy of Sciences of the USA* 103: 18002–7.
48. Li, Y., Zhang, Q. Q., Zhang, J., Wu, L., Qi, Y., and Zhou, J-M. (2010) Identification of microRNAs involved in pathogen-associated molecular pattern-triggered plant innate immunity. *Plant Physiology* 152: 2222–31.
49. Wang, W., Barnaby, J. Y., Tada, Y., Li, H., Tör, M., Caldelari, D., Lee, D., Fu, X-D., and Dong, X. (2011) Timing of plant immune responses by a central circadian regulator. *Nature* 470: 110–15.
50. Ingle, R. A., Stoker, C., Stone, W., Adams, N., Smith, R., Grant, M., Carré, I., Roden, L. C., and Denby, K. J. (2015) Jasmonate signalling drives time-of-day differences in susceptibility of Arabidopsis to the fungal pathogen *Botrytis cinerea*. *The Plant Journal* 84: 937–48.
51. Hevia, M. A., Canessa, P., Müller-Esparza, H., and Larrondo, L. F. (2015) A circadian oscillator in the fungus *Botrytis cinerea* regulates virulence when infecting *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences of the USA* 112: 8744–9.
52. Zhang, C., Xie, Q., Anderson, R. G., Ng, G., Seitz, N. C., Peterson, T., McClung, C. R., McDowell, J. M., Kong, D., Kwak, J. M., and Lu, H. (2013) Crosstalk between the circadian clock and innate immunity in Arabidopsis. *PLoS Pathogens* 9(6): e1003370.

53. Goodspeed, D., Chehab, E. W., Min-Venditti, A., Braam, J., and Covington, M. F. (2012) *Arabidopsis* synchronizes jasmonate-mediated defense with insect circadian behaviour. *Proceedings of the National Academy of Sciences of the USA* 109: 4674–7.

## Chapter 6

1. Smith, A. M., Coupland, G., Dolan, L., Harberd, N., Jones, J., Martin, C., Sablowski, R., and Amey, A. (2010) *Plant Biology*. New York: Garland Science.
2. Spribille, T. et al. (2016) Basidiomycete yeasts in the cortex of ascomycete macrolichens. *Science* DOI: 10.1126/science.aaf8287.
3. Smith, S. E. and Read, D. (2008) *Mycorrhizal Symbiosis*, 3rd edn. London: Academic Press.
4. Zamioudis, C. and Pieterse, C. M. J. (2012) Modulation of host immunity by beneficial microbes. *Molecular Plant-Microbe Interactions* 25: 139–50.
5. Plett, J. M., Kemppainen, M., Kale, S. D., Kohler, A., Legué, V., Brun, A., Tyler, B. M., Pardo, A. G., and Martin, F. (2011) A secreted effector protein of *Laccaria bicolor* is required for symbiosis development. *Current Biology* 21: 1197–1203.
6. *Glomus intraradices* is now *Rhizophagus irregularis*.
7. Kloppholz, S., Kuhn, H., and Requena, N. (2011) A secreted fungal effector of *Glomus intraradices* promotes symbiotic biotrophy. *Current Biology* 21: 1204–9.
8. Lopez-Gomez, M., Sandal, N., Stougaard, J., and Boller, T. (2011) Interplay of flg22-induced defence responses and nodulation in *Lotus japonicas*. *Journal of Experimental Botany* 63: 393–401.
9. Maunoury, N., Redondo-Nieto, M., Bourcy, M., Van der Velde, W., Alunni, B., Laporte, P., Agier, N., Marisa, L., Vaubert, D., Delacroix, H., Duc, G., Ratet, P., Aggerbeck, L., Kondorosi, E., and Mergaert, P. (2010) Differentiation of symbiotic cells and endosymbionts in *Medicago truncatula* nodulation are coupled to two transcriptome switches. *PLoS One* 5: e9519.
10. Liang, Y., Cao, Y., Tanaka, K., Thibivilliers, S., Wan, J., Choi, J., Kang, C. H., and Stacey, G. (2013) Nonlegumes respond to rhizobial nod factors by suppressing the innate immune response. *Science* 341: 1384–7.
11. Cordier, C., Pozo, M. J., Barea, J. M., Gianinazzi, S., and Gianinazzi-Pearson, V. (1998) Cell defense responses associated with localized and systemic resistance to *Phytophthora parasitica* induced in tomato by an arbuscular mycorrhizal fungus. *Molecular Plant-Microbe Interactions* 11: 1017–28.
12. Pozo, M., Cordier, C., Dumas-Gaudot, E., Gianinazzi, S., Barea, J. M., and Azcón-Aguilar, C. (2002) Localized versus systemic effect of arbuscular mycorrhizal fungi on defence responses to *Phytophthora* infection in tomato plants. *Journal of Experimental Botany* 53: 525–34.
13. Vos, C., Schouteden, N., van Tuinen, D., Chatagnier, O., Elsen, A., De Waele, D., Panis, B., and Gianinazzi-Pearson, V. (2013) Mycorrhiza-induced resistance against the root-knot nematode *Meloidogyne incognita* involves priming of defense gene responses in tomato. *Soil Biology and Biochemistry* 60: 45–54.

14. Gange, A. C. (2001) Species-specific responses of a root- and shoot-feeding insect to arbuscular mycorrhizal colonization of its host plant. *New Phytologist* 150: 611–18.
15. Akiyama, K., Matsuzaki, K., and Hayashi, H. (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435: 824–7.
16. Cameron, D. D., Neal, A. L., van Wees, S. C. M., and Ton, J. (2013) Mycorrhiza-induced resistance: more than the sum of its parts? *Trends in Plant Science* 18: 539–45.
17. Beerling, D. (2007) *The Emerald Planet: How Plants Changed Earth's History*. Oxford: Oxford University Press.
18. Van Peer, R., Niemann, G. J., and Schippers, B. (1991) Induced resistance and phytoalexin accumulation in biological control of Fusarium wilt of carnation by *Pseudomonas* sp. Strain WCS417r. *Phytopathology* 81: 728–34.
19. Wei, G., Kloepper, J. W., and Tuzun, S. (1991) Induction of systemic resistance of cucumber to *Colletotrichum orbiculare* by select strains of plant growth-promoting rhizobacteria. *Phytopathology* 81: 1508–12.
20. Liu, L., Kloepper, J. W., and Tuzun, S. (1995) Induction of systemic resistance in cucumber against Fusarium wilt by plant growth-promoting rhizobacteria. *Phytopathology* 85: 695–8.
21. Pieterse, C. M. J., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. M., and Bakker, P. A. H. M. (2014) Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology* 52: 347–75.
22. Santhanam, R., Luu, V. T., Weinhold, A., Goldberg, J., Oh, Y., and Baldwin, I. T. (2015) Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. *Proceedings of the National Academy of Sciences of the USA* 112: E5013–E5020.
23. Guerre, P. (2015) Ergot alkaloids produced by endophytic fungi of the genus *Epichloë*. *Toxins* 7: 773–90.
24. Schumann, G. L. and D'Arcy, C. J. (2012) *Hungry Planet*. St. Paul, MN: APS Press.
25. Nicholson, P. (2015) The highs and lows of ergot. *Microbiology Today* 42 (1 February): 14–17.
26. Bacon, C. W., Porter, J. K., Robbins, J. D., and Luttrell, E. S. (1977) *Epichloë typhina* from toxic tall fescue grasses. *Applied and Environmental Microbiology* 34: 576–81.
27. Saikkonen, K., Gundel, P. E., and Helander, M. (2013) Chemical ecology mediated by fungal endophytes in grasses. *Journal of Chemical Ecology* 39: 962–8.
28. Arnold, A. E., Mejia, L. C., Kyllo, D., Rojas, E. I., Maynard, Z., Robbins, N., and Herre, E. A. (2003) Fungal endophytes limit pathogen damage in a tropical tree. *Proceedings of the National Academy of Sciences of the USA* 100: 15649–54.
29. Soliman, S. S. M., Greenwood, J. S., Bombarely, A., Mueller, L. A., Tsao, R., Mosser, D. D., and Raizada, M. N. (2015) An endophyte constructs fungicide-containing extracellular barriers for its host plant. *Current Biology* 25: 2570–6.
30. Ambrose, K. V., Koppenhöfer, A. M., and Belanger, F. C. (2014) Horizontal gene transfer of a bacterial insect toxin gene into the *Epichloë* fungal symbionts of grasses. *Scientific Reports* 4: 5562.

31. Van Bael, S. A., Estrada, C., Rehner, S. A., Santos, J. F., and Wcislo, W. T. (2012) Leaf endophyte load influences fungal garden development in leaf-cutting ants. *BMC Biology* 12: 23.
32. Hammer, T. J. and Van Bael S. A. (2015) An endophyte-rich diet increases ant predation on a specialist herbivorous insect. *Ecological Entomology* 40: 316–21.
33. Eaton, C. J., Cox, M. P., and Scott, B. (2011) What triggers grass endophytes to switch from mutualism to pathogenism? *Plant Science* 180: 190–5.
34. Eaton, C. J., Cox, M. P., Ambrose, B., Becker, M., Hesse, U., Schardl, C. L., and Scott, B. (2010) Disruption of signalling in a fungal-grass symbiosis leads to pathogenesis. *Plant Physiology* 153: 1780–94.
35. Xu, X-H., Su, Z-Z., Wang, C., Kubicek, C. P., Feng, X-X., Mao, L-J., Wang, Y-Y., Chen, C., and Zhang, C-L. (2014) The rice endophyte *Harpophora oryzae* genome reveals evolution from a pathogen to a mutualistic endophyte. *Scientific Reports* 4: 5783.
36. Belt, T. (1874) *The Naturalist in Nicaragua.* London: John Murray.
37. Janzen, D. H. (1967) Interaction of the bull's horn acacia (*Acacia cornigera* L.) with an ant inhabitant (*Pseudomyrmex ferruginea* F. Smith) in eastern Mexico. *University of Kansas Science Bulletin* 47: 315–558.
38. Heil, M. and McKey, D. (2003) Protective ant-plant interactions as model systems in ecological and evolutionary research. *Annual Review of Ecology, Evolution and Systematics* 34: 425–53.
39. Heil, M., Baumann, B., Andary, C., Linsenmair, K. E., and McKey, D. (2002) Extraction and quantification of 'condensed tannins' as valuable measure of plant anti-herbivore defence? Revisiting an old problem. *Naturwissenschaften* 89: 519–24.
40. González-Teuber, M., Kaltenpoth, M., and Boland, W. (2014) Mutualistic ants as an indirect defence against leaf pathogens. *New Phytologist* 202: 640–50.
41. Barnett, A. A., Almeida, T., Andrade, R., Boyle, S., Gonçalves de Lima, M., MacLarnon, A., Ross, C., Silva, W.S., Spironello, W. R., and Ronchi-Teles, B. (2015) Ants in their plants: *Pseudomyrmex* ants reduce primate, parrot and squirrel predation on *Macrolobium acaciifolium* (Fabaceae) seeds in Amazonian Brazil. *Biological Journal of the Linnean Society* 114: 260–73.
42. Heil, M. (2013) Let the best one stay: screening of ant defenders by *Acacia* host plants functions independently of partner choice of host sanctions. *Journal of Ecology* 101: 684–8.
43. Heil, M., Barajas-Barron, A., Orona-Tamayo, D., Wielsch, N., and Svatos, A. (2014) Partner manipulation stabilises a horizontally transmitted mutualism. *Ecology Letters* 17: 185–92.
44. Whitehead, S. R., Reid, E., Sapp, J., Poveda, K., Royer, A. M., Posto, A. L., and Kessler, A. (2014) A specialist herbivore uses chemical camouflage to overcome the defences of an ant-plant mutualism. *PLoS One* 9(7): e102604.
45. Amador-Vargas, S. (2012) Run, robber, run: parasitic acacia ants use speed and evasion to steal food from ant-defended trees. *Physiological Entomology* 37: 323–9.
46. Jandér, K. C. (2015) Indirect mutualism: ants protect fig seeds and pollen dispersers from parasites. *Ecological Entomology* 40: 500–10.

47. Venkateshwaran, M., Volkening, J. D., Sussman, M. R., and Ané, J-M. (2013) Symbiosis and the social network of higher plants. *Current Opinion in Plant Biology* 16: 118–27.

## Chapter 7

1. Labandeira, C. C., Tremblay, S. L., Bartowski, K. E., and Hernick, L. V. (2014) Middle Devonian liverwort herbivory and antiherbivore defence. *New Phytologist* 202: 247–58.
2. Gulmon, S. L. and Mooney, H. A. (1986) Costs of defence on plant productivity. In: *On the Economy of Plant Form and Function*, ed. T. J. Givnish, 681–98. Cambridge: Cambridge University Press.
3. Herms, D. A. and Mattson, W. J. (1992) The dilemma of plants: to grow or defend. *The Quarterly Review of Biology* 67: 283–335.
4. Strauss, S. Y., Rudgers, J. A., Lau, J. A., and Irwin, R. E. (2002) Direct and ecological costs of resistance to herbivory. *Trends in Ecology and Evolution* 17: 278–85.
5. Strauss, S. Y., Siemens, D. H., Decher, M. B., and Mitchell-Olds, T. (1999) Ecological costs of plant resistance to herbivores in the currency of pollination. *Evolution* 53: 1105–13.
6. Ballhorn, D. J., Godschalx, A. L., Smart, S. M., Kautz, S., and Schädler, M. (2014) Chemical defense lowers plant competitiveness. *Oecologia* 176: 811–24.
7. Wallace, S. K. and Eigenbrode, S. D. (2002) Changes in the glucosinolate-myrosinase defense system in *Brassica juncea* cotyledons during seedling development. *Journal of Chemical Ecology* 28: 243–56.
8. Zangerl, A. R. and Rutledge, C. E. (1996) The probability of attack and patterns of constitutive and induced defense: a test of optimal defense theory. *American Naturalist* 147: 599–608.
9. Elton, C. S. (1958) *The Ecology of Invasions by Animals and Plants.* London: Methuen.
10. Keane, R. M. and Crawley, M. J. (2002) Exotic plant invasions and the enemy release hypothesis. *Trends in Ecology and Evolution* 17: 164–70.
11. Blossey, B. and Nötzold, R. (1995) Evolution of increased competitive ability in invasive nonindigenous plants: a hypothesis. *Journal of Ecology* 83: 887–9.
12. Uesugi, A. and Kessler, A. (2013) Herbivore-exclusion drives the evolution of plant competitiveness via increased allelopathy. *New Phytologist* 198: 916–24.
13. Joshi, J. and Vrieling, K. (2005) The enemy release and EICA hypothesis revisited: incorporating the fundamental difference between specialist and generalist herbivores. *Ecology Letters* 8: 704–14.
14. Feeny, P. (1976) Plant apparency and chemical defense. In: *Recent Advances in Phytochemistry*, ed. J. W. Wallace and R. L. Mansell, 1–40. New York: Plenum Press.
15. Castagneyrol, B., Giffard, B., Péré, C., and Jactel, H. (2013) Plant apparency, an overlooked driver of associational resistance to insect herbivory. *Journal of Ecology* 101: 418–29.

16. Stenberg, J. A., Witzell, J., and Ericson, L. (2006) Tall herb herbivory resistance reflects historic exposure to leaf beetles in a boreal archipelago age-gradient. *Oecologia* 148: 414–25.
17. Coley, P. D. (1983) Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecological Monographs* 53: 209–33.
18. Bryant, J. P., Chapin, F. S. III., and Klein, D. R. (1983) Carbon nutrient balance of boreal plants in relation to vertebrate herbivory. *Oikos* 40: 357–68.
19. Coley, P. D., Bryant, J. P., and Chapin, F. S. III. (1985) Resource availability and plant antiherbivore defense. *Science* 230: 895–9.
20. Stamp, N. (2003) Out of the quagmire of plant defence hypotheses. *The Quarterly Review of Biology* 78: 23–55.
21. Vannette, R. L. and Hunter, M. D. (2011) Plant defence theory re-examined: non-linear expectations based on the costs and benefits of resource mutualisms. *Journal of Ecology* 99: 66–76.
22. Ehrlich, P. R. and Raven, P. H. (1964) Butterflies and plants: a study in coevolution. *Evolution* 18: 586–608.
23. Farrell, B. D., Dussourd, D. E., and Mitter, C. (1991) Escalation of plant defense: do latex and resin canals spur plant diversification? *American Naturalist* 138: 881–900.
24. Agrawal, A. A., Fishbein, M., Halitschke, R., Hastings, A. P., Robosky, D. L., and Rasmann, S. (2009) Evidence for adaptive radiation from a phylogenetic study of plant defences. *Proceedings of the National Academy of Sciences of the USA* 106: 18067–72.
25. Armbruster, W. S. (1997) Exadaptations link evolution of plant-herbivore and plant-pollinator interactions: a phylogenetic enquiry. *Ecology* 78: 1661–72.
26. Becerra, J. X. (1997) Insects on plants: macroevolutionary chemical trends in host use. *Science* 276: 253–6.
27. Speed, M. P., Fenton, A., Jones, M. G., Ruxton, G. D., and Brockhurst, M. A. (2015) Coevolution can explain defensive secondary metabolite diversity in plants. *New Phytologist* 208: 1251–63.
28. Gilman, R. T., Nuismer, S. L., and Jhwueng, D-C. (2012) Coevolution in multidimensional trait space favours escape from parasites and pathogens. *Nature* 483: 328–30.
29. Rausher, M. D. and Huang, J. (2015) Prolonged adaptive evolution of a defensive gene in the Solanaceae. *Molecular Biology and Evolution* 33: 143–51.

## Chapter 8

1. Schumann, G. L. and D'Arcy, C. J. (2012) *Hungry Planet.* St. Paul, MN: APS Press.
2. Information obtained from BBC News 'Science and Environment'. Available at: <http://www.bbc.co.uk/news/science-environment-15623490>, accessed 20 September 2016.
3. D'Arcy, C. J. (2000) Dutch elm disease. *The Plant Health Instructor.* doi: 10.1094/PHI-I-2000-0721-02.

4. Parke, J. L. and Lucas, S. (2008) Sudden oak death and ramorum blight. *The Plant Health Instructor.* doi: 10.1094/PHI-I-2008-0227-01.
5. Havens, J. N. (1992) Observations on the Hessian fly. *Society of Agriculture of New York* 1: 89–107.
6. Ayres, P. G. (2005) *Harry Marshall Ward and the Fungal Thread of Death.* St. Paul, MN: APS Press, 140–2.
7. Biffen, R. H. (1907) Studies on the inheritance of disease resistance. *Journal of Agricultural Science* 2: 109–28.
8. Wallwork, H. (2009) The use of host plant resistance in plant disease control. In: *Disease Control in Crops: Biological and Environmentally Friendly Approaches,* ed. D. R. Walters, 122–41. Oxford: Wiley-Blackwell.
9. Nowicki, M., Foolad, M. R., Nowakowska, M., and Kozik, E. U. (2012) Potato and tomato late blight caused by *Phytophthora infestans:* an overview of pathology and resistance breeding. *Plant Disease* 96: 4–17.
10. Kim, H-J., Lee, H-R., Jo, K-R., Mortazavian, S. M. M., Jan Huigen, D., Evenhuis, B., Kessel, G., Visser, R. G. F., Jacobsen, E., and Vossen, J. H. (2012) Broad spectrum late blight resistance in potato differential set plants Ma*R8* and Ma*R9* is conferred by multiple stacked genes. *Theoretical and Applied Genetics* 124: 923–35.
11. Jorgensen, J. H. (1992) Discovery, characterization and exploitation of *Mlo* powdery mildew resistance in barley. *Euphytica* 63: 141–52.
12. Breseghello, F. (2013) Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). *Journal of Agricultural and Food Chemistry* 61: 8277–86.
13. Christensen, C. M. (1992) *Elvin Charles Stakman.* Washington, D.C.: National Academy of Sciences.
14. Rispail, N., Dita, M-A., González-Verdejo, C., Perez-de-Luque, A., Castillejo, M-A., Prats, E., Román, B., Jorrín, J., and Rubiales, D. (2007) Plant resistance to parasitic plants: molecular approaches to an old foe. *New Phytologist* 173: 703–12.
15. Parlevliet, J. E. and Van Ommeren, A. (1975) Partial resistance of barley to leaf rust, *Puccinia hordei.* II. Relationship between field trials, micro plot test and latent period. *Euphytica* 24: 293–303.
16. Borlaug, N. E. and Gibler, J. W. (1953) The use of flexible composite wheat varieties to control the constantly changing stem rust pathogen. *Abstracts of the Annual Meetings of the American Society of Agronomy*. Dallas, TX: 81.
17. Jensen, N. F. (1952) Intra-varietal diversification in oat breeding. *Agronomy Journal* 44: 30–4.
18. Newton, A. C. (2009) Plant disease control through the use of variety mixtures. In: *Disease Control in Crops: Biological and Environmentally Friendly Approaches,* ed. D. R. Walters, 162–71. Oxford: Wiley-Blackwell.
19. Walters, D. R., Ratsep, J., and Havis, N. D. (2013) Controlling crop diseases using induced resistance: challenges for the future. *Journal of Experimental Botany* 64: 1263–80.
20. Watanabe, T., Igarashi, H., Matsumoto, K., Seki, S., Mase, S., Sekizawa, Y. (1977) The characteristics of probenazole (oryzemate) for the control of rice blast. *Journal of Pesticide Science* 2: 291–6.

21. Iwata, M., Suzuki, Y., Watanabe, T., Mase, S., and Sekizawa, Y. (1980) Effect of probenazole on the activities of enzymes related to the resistant reaction in the rice plant. *Annals of the Phytopathological Society of Japan* 46: 297–306.
22. Leadbeater, A. and Staub, T. (2014) Exploitation of induced resistance: a commercial perspective. In: *Induced Resistance for Plant Defense: A Sustainable Approach to Crop Protection*, ed. D. R. Walters, A. C. Newton, and G. D. Lyon, 300–15. Oxford: Wiley-Blackwell.
23. Sergeeva, O. A., Kletke, O., Kragler, A., Poppek, A., Fleischer, W., Schubring, S. R., Görg, B., Haas, H. L., Zhu, X-R., Lübbert, H., Gisselmann, G., and Hatt, H. (2010) Fragrant dioxane derivatives identify β1-subunit-containing $GABA_A$ receptors. *The Journal of Biological Chemistry* 285: 23985–93.
24. Birkett, M. A., Campbell, C. A. M., Chamberlain, K., Guerrieri, E., Hick, A. J., Martin, J. L., Matthes, M., Napier, J. A., Pettersson, J., Pickett, J. A., Poppy, G. M., Pow, E. M., Pye, B. J., Smart, L. E., Wadhams, L. J., and Woodcock, C. M. (2000) New roles for *cis*-jasmone as an insect semiochemical and in plant defense. *Proceedings of the National Academy of Sciences of the USA* 97: 9329–34.
25. Pickett, J. A., Birkett, M. A., Bruce, T. J. A., Chamberlain, K., Gordon-Weeks, R., Matthes, M. C., Napier, J. A., Smart, L. E., and Woodcock, C. M. (2007) Developments in aspects of ecological phytochemistry: the role of *cis*-jasmone in inducible defence systems in plants. *Phytochemistry* 68: 2937–45.
26. Pickett, J. A., Aradoťir, G. I., Birkett, M. A., Bruce, T. J. A., Hooper, A. M., Midega, C. A. O., Jones, H. D., Matthes, M. C., Napier, J. A., Pittchar, J. O., Smart, L. E., Woodcock, C. M., and Khan, Z. R. (2014) Delivering sustainable crop protection systems via the seed: exploiting natural constitutive and inducible defence pathways. *Philosophical Transactions of the Royal Society B* 369: 20120281.
27. Tamiru, A., Bruce, T. J. A., Woodcock, C. M., Caulfield, J. C., Midega, C. A. O., Ogol, C. K. P. O., Mayon, P., Birkett, M. A., Pickett, J. A., and Khan, Z. R. (2011) Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. *Ecology Letters* 14: 1075–83.
28. Bruce, T. J. A., Midega, C. A. O., Birkett, M. A., Pickett, J. A., and Khan, Z. R. (2010) Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. *Biology Letters* 6: 314–17.
29. Degenhardt, J., Hiltpold, I., Köllner, T. G., Frey, M., Gierl, A., Gershenzon, J., Hibbard, B. E., Ellersieck, M. R., and Turlings, T. C. J. (2009) Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. *Proceedings of the National Academy of Sciences of the USA* 106: 13213–18.
30. Pickett, J. A., Woodcock, C. M., Midega, C. A. O., and Khan, Z. R. (2014) Push-pull farming systems. *Current Opinion in Biotechnology* 26: 125–32.
31. Miller, J. R. and Cowles, R. S. (1990) Stimulo-deterrent diversionary cropping: a concept and its possible application to onion maggot control. *Journal of Chemical Ecology* 16: 3197–3212.

32. Khan, Z. R., Midega, C. A. O., Bruce, T. J. A., Hooper, A. M., and Pickett, J. A. (2010) Exploiting phytochemicals for developing a 'push-pull' crop protection strategy for cereal farmers in Africa. *Journal of Experimental Botany* 61: 4185–96.
33. Hilder, V. A., Gatehouse, A. M. R., Sheerman, S. E., Baker, R. F., and Boulter, D. (1987) A novel mechanism of insect resistance engineered into tobacco. *Nature* 330: 160–3.
34. Jones, J. D. G., Witek, K., Verweij, W., Jupe, F., Cooke, D., Dorling, S., Tomlinson, L., Smoker, M., Perkins, S., and Foster, S. (2014) Elevating crop disease resistance with cloned genes. *Philosophical Transactions of the Royal Society B* 369: 20130087.
35. Rupe, J. and Sconyers, L. (2008) Soybean rust. *The Plant Health Instructor.* DOI: 10.1094/PHI-I-2008-0401-01.
36. Kawashima, C. G. and 25 others. (2016) A pigeonpea gene confers resistance to Asian soybean rust in soybean. *Nature Biotechnology* 34: 661–5.
37. Nunes, C. C. and Dean, R. A. (2012) Host-induced gene silencing: a tool for understanding fungal host interaction and for developing novel disease control strategies. *Molecular Plant Pathology* 13: 519–29.
38. Aragão, F. J., Nogueira, E. O., Tinoco, M. L., and Faria, J. C. (2013) Molecular characterization of the first commercial transgenic common bean immune to Bean golden mosaic virus. *Journal of Biotechnology* 166: 42–50.
39. Bonfim, K., Faria, J. C., Nogueira, E. O. P. L., Mendes, E. A., and Aragão, F. J. L. (2007) RNAi-mediated resistance to *Bean golden mosaic virus* in genetically engineered common bean (*Phaseolus vulgaris*). *Molecular Plant-Microbe Interactions* 20: 717–26.
40. Doudna, J. A. and Charpentier, E. (2014) The new frontier of genome engineering with CRISPR-Cas9. *Science* 346: 1077. DOI: 10.1126/science.1258096.
41. Wang, F., Wang, C., Liu, P., Lei, C., Hao, W., Gao, Y., Liu, Y-G., and Zhao, K. (2016) Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene *OsERF922. PLoS One* 11(4): e0154027.
42. Ali, Z., Abulfaraj, A., Idris, A., Ali, S., Tashkandi, M., and Mahfouz, M. M. (2015) CRISPR/Cas9-mediated viral interference in plants. *Genome Biology* 16: 238. DOI: 10.1186/s13059-015-0799-6.

# INDEX

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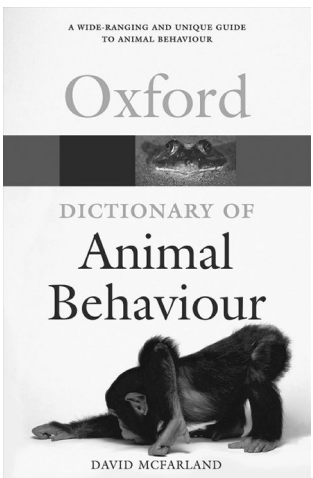
David McFarland

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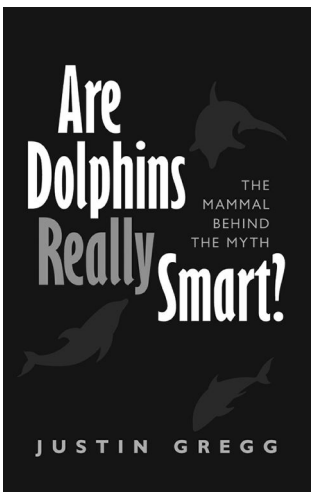
Justin Gregg

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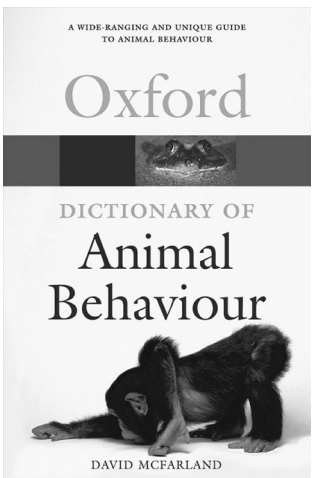


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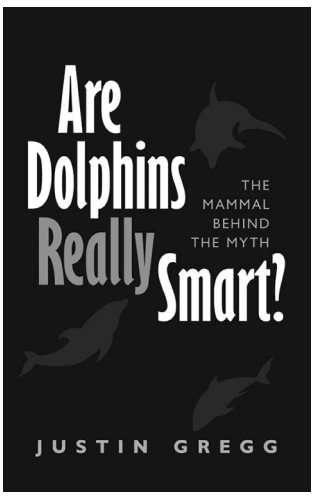


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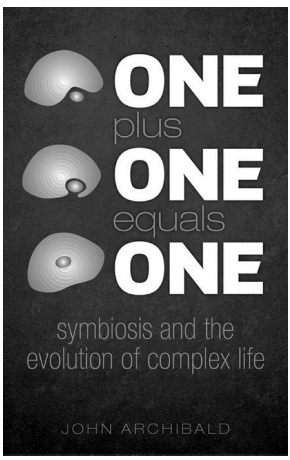


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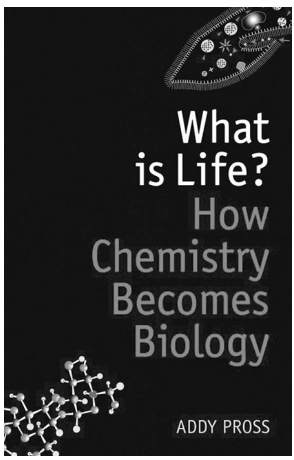


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