Plants fight back

by STEPHEN DAY

When a pest or disease attacks a plant, a slow-motion battle begins. Within minutes of the attack, plant cells on the front line switch on defence-related genes that make those cells more toxic to assailants and also physically stronger – more difficult for a disease to penetrate or a herbivore to chew. During the next few days, similar changes occur throughout the plant and boost resistance even in undamaged leaves, shoots and roots. Now researchers are discovering how warning signals in the plant coordinate these responses, and how manipulating these signals could induce crops to fight pests and diseases more vigorously.

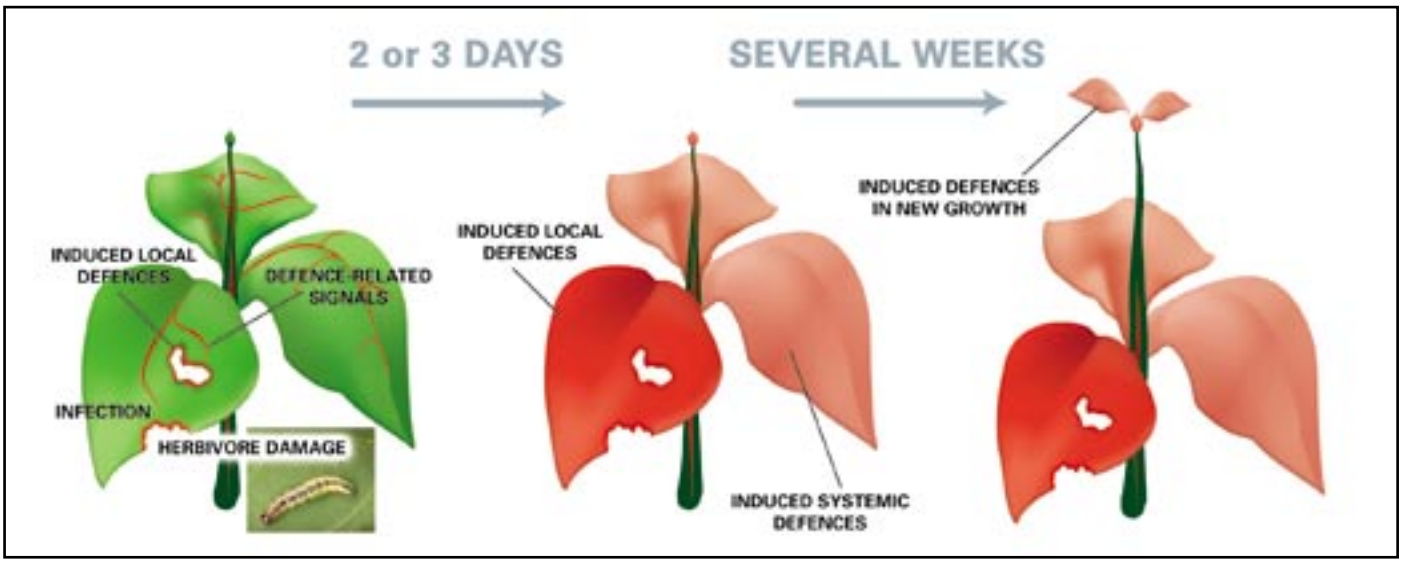


Figure 1. Herbivores and pathogens induce additional defences

a) Within minutes or hours of herbivore damage or pathogen infection, cells near the affected site turn on defence-related genes and produce compounds that boost both chemical and physical resistance – indicated by the red colouring. At the same time, signalling molecules warning of the attack are transported to the rest of the plant.

b) Over the next two or three days, cells in undamaged sections of the plant respond to the warning signals by switching on defence-related genes and boosting their own resistance. This 'systemic' response is not as great as that near the damage site.

c) Increased resistance to pathogens and/or herbivores is maintained for the next several weeks and extends to newly formed leaves, shoots and roots.

1. INTRODUCTION

For both herbivores and disease-causing micro-organisms **(pathogens)**, plants represent rich, stationary sources of nutrients. Therefore, to survive, plants need defences. Bark, thorns, spines and hairs give physical protection and combinations of anti-microbial, toxic and/or unpleasant-tasting compounds provide a chemical shield. (The hot spice in chilli peppers, for example, deters herbivores; while the minty compound in peppermint – menthol – is toxic to many micro-organisms.)

These physical and chemical defences are in place before any attack begins and are often enough to keep assailants at bay. When they fail, however, damaged plants don't surrender—they fight back. Wounded and diseased plants activate extra physical and chemical defences, not only at the damaged site but also in healthy tissue (see Figure 1). As a result, the whole plant becomes more resistant to subsequent assaults.

Now researchers are discovering how plants detect attacks by herbivores and pathogens. They have isolated chemical warning signals produced by damaged cells that trigger defences in other parts of the plant. They are even learning how to turn on these extra defences *before* an attack, to 'vaccinate' crops against the onslaught of pests and diseases.

QUESTION 1

Over the centuries, farmers have selected the least bitter, most succulent of their fruits and vegetables as the source of seeds for the next year. Why has this resulted in many crops being more susceptible to pests and diseases than their wild relatives?

QUESTION 2

The waxy cuticle that covers the shoot not only reduces evaporation but also keeps most microorganisms outside the plant. Some pathogens penetrate the cuticle by a combination of physical force and cuticle-digesting enzymes. How could pathogens without this ability reach the inner tissues of the shoot?

2. DEAD STOP FOR DISEASE

Organisms that prey on plants range in size from elephants to viruses and can be roughly divided into two groups: those that parasitise plants and those that eat them. Most microbial pathogens – fungi, bacteria and viruses – are parasites: they survive by

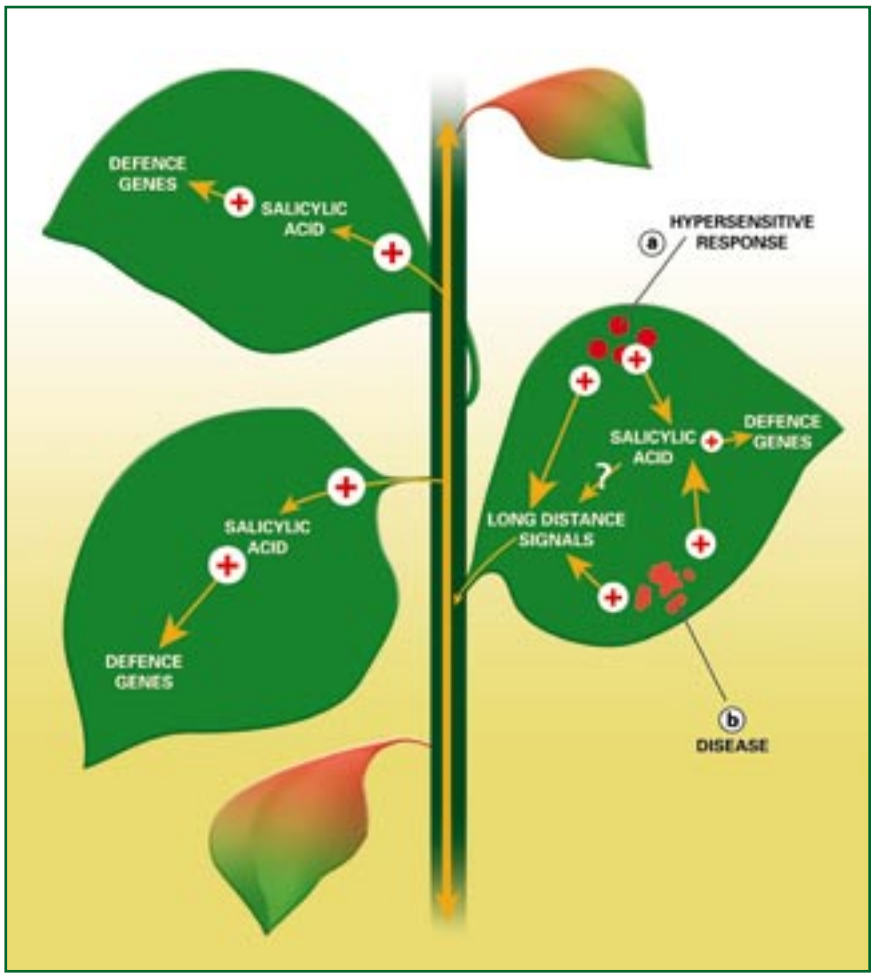


Figure 2. Systemic acquired resistance

Infection by pathogens often induces systemic acquired resistance (SAR). Infection-related cell death: resulting either from the hypersensitive response (a), or from disease (b), promotes the synthesis of salicylic acid in the infected leaf. In turn, salicylic acid promotes the activity of defence related genes. The infected leaf exports long-distance signals that induce salicylic acid synthesis – and consquently defence-related gene expression – in the rest of the plant. The nature of these signals is unknown, but they may include salicylic acid itself, which is exported from infected leaves.

siphoning nutrients from a living host. In contrast, most pests are herbivores: they obtain their nutrients by consuming plant tissues.

Faced with these very different styles of attack, plants have evolved distinct but overlapping defence systems, one primarily triggered by pathogens and one usually induced by herbivores. This section considers what is probably a plant's most effective weapon against pathogens – cell suicide.

When a plant cell detects an infecting pathogen, that cell and often its immediate neighbours synthesize a range of anti-microbial toxins and wall-strengthening compounds (for example, adding lignin to the cellulose wall). At the same time, these cells trigger their own deaths. The result is a barely visible brown speck, a poisonous prison that fences the pathogen away from living tissue. Around the dead zone, cells reinforce the barrier by producing additional toxins; strengthening their walls; and synthesizing enzymes to degrade the walls of invading fungi and bacteria.

Collectively this is called the **hypersensitive response**. It is triggered within minutes of an infection and can block attacks not only from fungi, bacteria and viruses but also from disease-causing nematodes and even some aphids—both of which feed off living cells. In general, if a pathogen triggers the hypersensitive response then the infection fails. 'It's usually strongly correlated with resistance,' says Jonathan Jones of the Sainsbury Laboratory, Norwich.

Research in the 1940s showed that the ability of plants to recognize infecting pathogens and so trigger the hypersensitive response depends on **resistance genes *(R genes)***. Ever since, R genes have been the keystone of programmes to breed disease-resistance into crops. 'The beauty of resistance genes,' says Jones, 'is that the plant makes new defences precisely in the cells that are being attacked.' In this way, the plant expends as few resources as possible in defeating the infection.

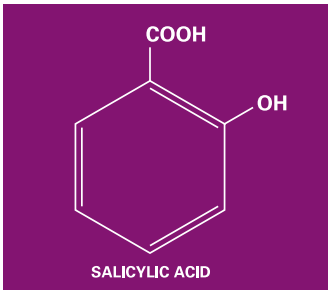
Unfortunately, R genes have a problem. Normally, each R gene only allows a plant to detect some races of a pathogen while other races escape notice (see ***The discovery of R genes***). Therefore the shield that R genes provide has gaps. Pathogen strains that avoid detection by all the R genes in a particular variety can infect without triggering the hypersensitive response. The result is that plant breeders are on a treadmill. Every time they create a variety with the correct R genes to defeat the major strains of a disease, a new strain is likely to arise that avoids these R genes, forcing farmers to rely on chemical control. In practice, this means that disease-resistant varieties rarely remain resistant for more than ten years, and sometimes lose their resistance within two or three years.

QUESTION 3

The human immune system identifies pathogens using antibodies. These either circulate by themselves in the blood or are carried by white blood cells. In contrast, all living cells in higher plants appear to express R genes and so can recognise pathogens. What feature of plant cells would prevent a human-like immune system from functioning in plants?

QUESTION 4

In wild populations, individual plants of the same species carry different combinations of R genes. On farms, however, each field contains a single crop variety—a 'monoculture' of a genetically similar plants, each with same combination of R genes. Why does this make crops more susceptible to disease than wild populations?



3. STATE OF ALERT

Looking for alternatives to R gene-based resistance, researchers are studying other ways in which plants combat pathogens, in particular, they are analysing events away from the infection site.

Biologists have shown that after a pathogen triggers the hypersensitive response, tissue near the infection site sends warning signals throughout the plant. These signals induce cells in completely healthy leaves, shoots and roots to switch on defence-related genes and so boost both their physical and chemical resistance to disease. In addition, the whole plant becomes more responsive to subsequent attacks, for example triggering the hypersensitive response more readily when detecting pathogens.

These plant-wide defences result in **systemic acquired resistance (SAR)** and protect the plant against a broad range of diseases for the next several weeks (see Figure 2). For example, infecting a tobacco plant with a fungal pathogen induces increased resistance not only to that pathogen, but also to other species of fungi and to disease-causing bacteria and viruses. It is as if catching chicken pox could immunise you against the 'flu. Furthermore, SAR can be induced by pathogens that infect without triggering the hypersensitive response. In this case, the plant reacts to the damage a successful infection causes, for example fragments of breached cell walls and molecules leaking from diseased cells.

Biologists now believe that a core component of SAR is a signalling molecule called **salicylic acid** (a close relative of aspirin, which is acetylsalicylic acid). In the hours following a pathogen attack, salicylic acid is synthesised near the infection site and then later in all parts of the plant. Spraying uninfected plants with salicylic acid activates defence genes and induces SAR. Similarly, tobacco plants can be made permanently disease-resistant by genetically modifying them to synthesise salicylic acid constantly. In contrast, plants modified to destroy any salicylic acid they produce never mount SAR and so are more susceptible to disease.

These results suggest that salicylic acid acts as a 'master switch' that induces plant cells to switch on defence-related genes and increase their immunity to pathogens. There is now a commercial equivalent to salicylic acid: a synthetic analogue called 'acibenzolar-S-methyl (ASM)' that allows farmers to induce SAR in their crops before a pathogen attacks. Unlike conventional chemical control, inducing SAR relies on the plant to defend itself and so results vary. 'It is clearly a biological process and not the same thing as spraying with a fungicide,' says Steven Beer of Cornell University. 'There is variation among plants, and also with different growing situations, and with plant age.'

According to Jones, a key factor in the success of this approach is the availability of nitrogen, which plants require to synthesise proteins. 'These anti-microbial processes require the production of a lot of proteins,' he says. 'In nitrogen-limited conditions, if you add ASM you actually inhibit growth.'

Beer himself led a group of researchers who discovered another compound that farmers can now use to induce SAR—a protein called 'harpin'. Harpin is produced by bacteria that cause the disease 'fireblight' in apple and pear trees. Beer and his colleagues showed that harpin triggers the hypersensitive response in plants resistant to fireblight. Spraying crops with harpin 'fools' plants that they have recognised a pathogen, inducing them to synthesize salicylic acid and mobilise extra defences.

QUESTION 5

Researchers carried out experiments on cucumber seedlings to investigate how warning signals that induce SAR are transported from infected leaves to the rest of the plant. They found that:

a) If the vascular tissue in a leaf petiole is completely cut ('girdled') before that leaf is infected with a pathogen, then the leaf acquires increased resistance but the rest of the plant does not.

b) If the petiole of a leaf is girdled and then a different leaf is infected, all leaves apart from the girdled leaf acquire increased resistance.

c) When a leaf is infected, resistance is boosted in leaves both above and below that leaf on the shoot.

From the results, deduce in what tissue warning signals are most likely to be transported. Give reasons for your answer.

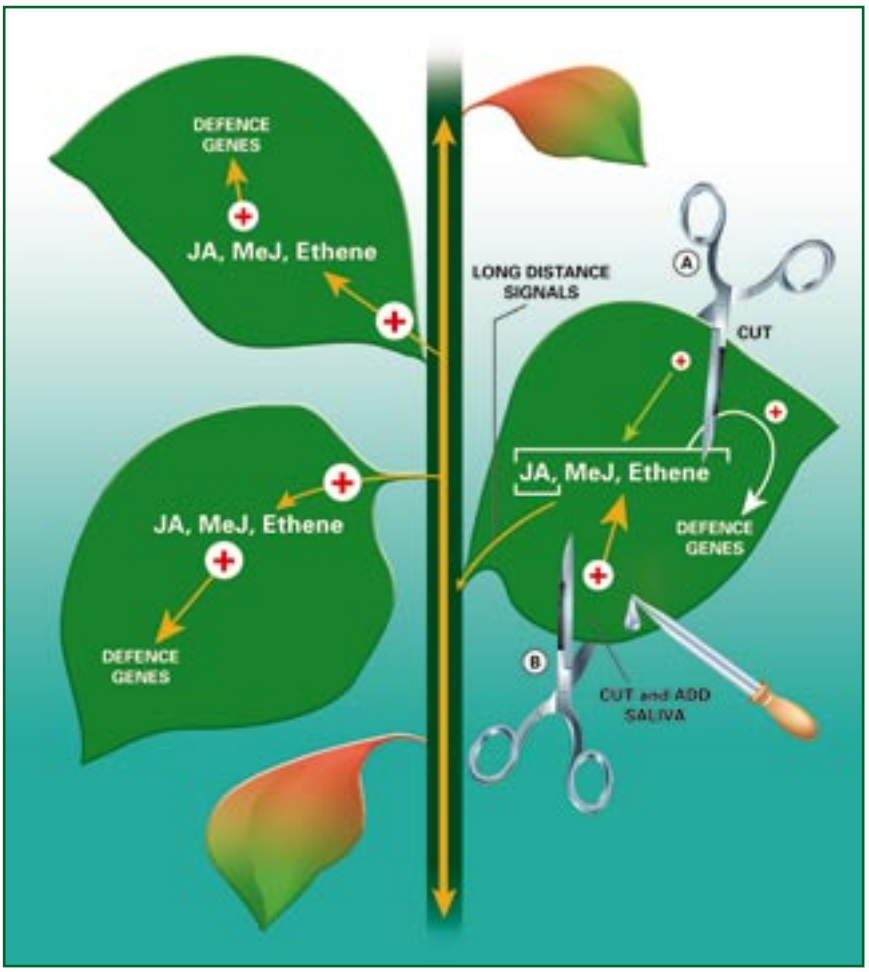


Figure 3. Wound responses

Wounds caused by herbivores induce a group of defences distinct from those that lead to systemic acquired resistance (see fig. 2). These defences can be induced by mechanically wounding the plant (a), but are greater if saliva is added to the cut (b). Such treatments promote the synthesis of jasmonic acid (JA), methyl jasmonate (MeJ) and ethene, which collectively activate defence related genes. The wounded leaf also exports long distance signals (which may include JA) to the rest of the plant. These signals induce JA, MeJ and ethene synthesis in undamaged tissues, leading systemic herbivore resistance.

4. BATTLE ON ANOTHER FRONT

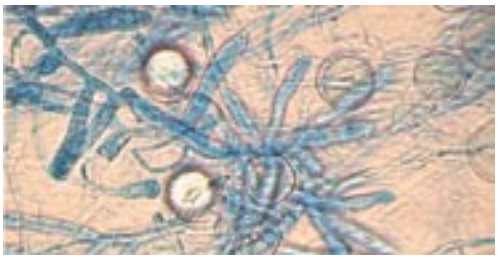
Just as plants respond to pathogens by mobilising extra defences, they deploy additional protection when attacked by pests—giving a vaccination-like effect similar to SAR but this time directed against herbivores. For example, some Californian grape growers inoculate their vines with a relatively mild pest called the Willamette spider mite to increase the resistance of the vines to the much more damaging Pacific mite.

In many cases the defences induced by pest attack—such as some toxins and wall strengthening substances – overlap with those induced by pathogens. However there are differences.

Depending on the species, pest-damaged plants may produce proteins that inhibit digestive enzymes in insects. They may also change how they grow. For example, grazing induces stinging nettles to produce leaves with more stings, and stimulates holly trees to grow leaves with more thickly packed spines. [Do damaged bean plants produce hairier leaves? See Suggested practical work in the Teachers' Guide.]

Simply attacking a plant with a pair of scissors can induce defences against herbivores. However the response is greater if saliva is added to the cut, suggesting that plants react to both physical damage and chemical clues about their attackers. Instead of salicylic acid (see above), plants damaged in this way—or attacked by real pests—synthesise the signalling molecules **jasmonic acid, methyl jasmonate** and **ethene**. All three molecules stimulate cells to switch on the defence-related genes responsible for increased resistance to herbivores *(See Figure 3)*.

Showing that what matters is the style of attack rather than the exact nature of the assailant, researchers have found that plants sometimes use their 'herbivore' defences when battling against disease. For example, plants deploy defences against 'pests' when infected by *Pythium*, the



fungus that causes overcrowded seedlings to rot at the base and 'damp off'. Whereas most pathogens parasitise living hosts (and so are called **biotrophs**); a few, like *Pythium*, survive by killing cells and producing enzymes to digest the remains (and so are called **necrotrophs**). In effect, necrotrophs 'eat' the plant—and the plant responds accordingly with 'anti-herbivore' defences. [To watch a necrotroph in action, try the Koch's Postulates practical. See Teachers' Guide.]

The fact that plants divide their resources between protection against biotrophs, and defence against herbivores and necrotrophs adds another potential trap for farmers wanting to induce resistance in their crops. Researchers have shown that spraying plants with salicylic acid to induce SAR can reduce the plants' ability to respond to herbivores. Similarly, spraying plants with jasmonic acid to induce herbivore defences can reduce the plants' ability to acquire SAR.

'If you crank up one, your run the risk of cranking down the other,' says Jones. 'Plants are always trading off between the various options.' Therefore, before farmers use techniques to induce resistance in their crops they must be sure that they are targetting the correct threat. It's no use having disease-free plants just to turn them into a meal for pests.

QUESTION 6

Wild tobacco plants damaged by pests or watered with a suspension of methyl jasmonate increase the concentration of nicotine (a poison) in the shoot by around 50%. In such cases, nitrogen atoms in nicotine account for about 6% of the total nitrogen in the plant. Researchers found that:

a) In largely pest free areas (where all observed plants survived to maturity), tobacco treated with methyl jasmonate produced 17% fewer seeds than untreated tobacco.

b) In moderately infested areas (where an average of 80% of plants survived), treated plants produced 11% more seeds than control plants.

c) In highly infested areas (where only 19% of plants survived), the treatment caused no significant difference in seed production, which was very low.

Suggest an explanation for these results. What is their significance for attempts to induce herbivore resistance in crops?

5. CONCLUSIONS

Wild plants compete with each other for water, nutrients and light. Therefore, although each plant must protect itself from herbivores and pathogens, it must also avoid wasting resources fighting non-existent enemies. Consequently, plants have evolved both pre-made physical and chemical defences, and mechanisms that boost resistance only after an attack has begun.

Researchers are investigating whether artificially inducing plant defences before a pest or disease attacks will be useful in protecting crops. With millions of the same variety of plant growing together in a field, a crop represents an enormous opportunity to any pest or disease that is able to exploit it. Therefore, the risks of epidemics of pests or diseases are greater for crops than for wild plants. At the same time, farmers optimise growing conditions for their crops, giving them water, fertiliser and the correct spacing for ample light.

Under these circumstances, it may make sense to 'vaccinate' crops against pests and diseases by artificially inducing plant defences. However, this approach must be balanced against the cost of the defences to the crop – seen as a reduction in harvest – and the risk that in defending against one enemy, the plant will divert resources needed to battle another.

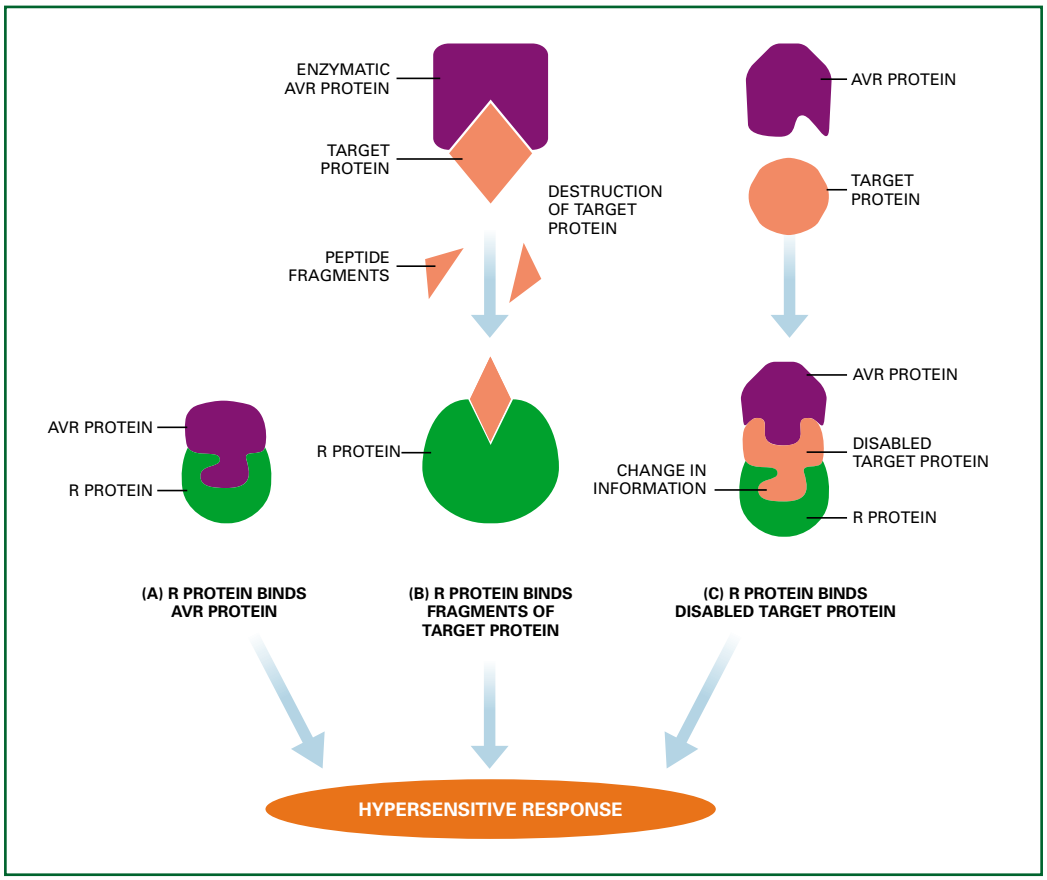


Figure 4. How cells recognize pathogens

The hypersensitive response (see Dead stop for disease) is triggered when an R protein in the plant cell detects an AVR protein synthesised by a pathogen. AVR proteins often form part of a pathogen's attack on the plant. They may be detected in the cell wall by R proteins in the plasma membrane, or inside the plant cell by cytoplasmic R proteins. *(a) R proteins may bind directly to AVR proteins. (b) R proteins may detect peptide fragments resulting from enzymatic attack by AVR proteins on target proteins. (c) R proteins may detect changes in the conformation of plant proteins that are targetted and disabled by AVR proteins.*

BOX 1

THE DISCOVERY OF R GENES

In the 1940s, the American geneticist Harold Flor carried out a series of experiments to investigate which genes determine whether flax plants resist rust disease (caused by the fungus *Melampsora lini*). Flor began by testing various races of rust against different varieties of flax. For example, he found that 'race 24' of rust could infect the Bison variety but not the Ottawa variety of flax. This was because Ottawa but not Bison plants reacted to this race by triggering the hypersensitive response (***see Dead stop for disease***). Flor crossed Bison with Ottawa and found that all the offspring (**the F1 generation**) were resistant to race 24. When he crossed F1 plants to each other, Flor found that three quarters of the next generation (the **F2**) were resistant to race 24 and one quarter were susceptible. He deduced that resistance and susceptibility to race 24 depended on a single gene, which he called *'L'*. Ottawa is homozygous for the dominant allele of this gene, which confers resistance, and so has the genotype *LL*. Bison is homozygous for the recessive allele, which confers susceptibility, and so has the genotype *ll*.

QUESTION 7

Flor's initial cross *(LL x ll)* generated only heterozygous, resistant plants with the genotype *Ll* (the F1). What were the genotypes of the F2, generated by the second cross, i.e. *Ll x Ll?* Why were three quarters of these plants resistant to race 24?

Flor found that resistance to other races of rust depended on other genes. For example, although Ottawa is resistant to race 24, it is susceptible to race 22. In contrast, the variety Bombay is susceptible to race 24 but resistant to race 22, whereas Bison is susceptible to both races. Resistance to race 22 depends on a gene that Flor named *'N'*, and again Flor found that resistance was dominant to susceptibility. Dominant alleles of N confer resistance to race 22, whereas recessive alleles confer susceptibility.

Corresponding to the patterns of resistance that Flor found: Ottawa has the genotype LL, nn; Bombay has the genotype was ll, NN; and Bison has the genotype ll, nn.

QUESTION 8

What is the genotype in terms of *L* and *N* of plants resulting from a cross between Ottawa and Bombay? Can either race 22 or race 24 infect these plants? Give an explanation for your answer.

Next Flor investigated the genes carried by different races of rust, for example to find the difference between race 22 and race 24. By crossing rust races, Flor identified genes in the fungus that caused *failure* of infection against particular varieties of flax. He called these genes 'avirulence' genes (meaning 'not-virulent', i.e. not infecting), or ***AVR genes.***

Genes in flax that are associated with resistance (such as L and N) have the collective name 'resistance genes' or ***R* genes**. Flor showed that particular fungus *AVR* genes in rust were responsible for the failure to infect flax plants that carried dominant alleles of particular *R* genes. So race 24 carries an *AVR* gene – called here 'AVR^{L}' – that renders it unable to infect flax carrying a dominant *L* allele (e.g. Ottawa). Similarly, race 22 carries 'AVR^{N}' and so cannot infect flax carrying a dominant *N* allele (e.g. Bombay). When Flor crossed race 24 and race 22 to create a rust strain that carried AVR^{L} and AVR^{N}, he found that the new strain could infect neither Ottawa nor Bombay (table 1).

TABLE 1	*R* allele in flax (Variety)	
AVR gene(s) in rust (Race)	*L* (Ottawa)	*N* (Bombay)
AVR^{N} (Race 22)	Diseased	Healthy
AVR^{L} (Race 24)	Healthy	Diseased
AVR^{N} & AVR^{L} (22 x 24)	Healthy	Healthy

QUESTION 9

Predict whether the new strain of rust, carrying both AVR^{L} and AVR^{N}, could infect the Bison variety? Give reasons for your answer.

To explain his results, Flor proposed the **gene-for-gene hypothesis**, which states that resistance occurs whenever an *AVR* gene in rust matches a dominant allele of the corresponding *R* gene in flax. We now know that there are about 30 *R* genes distributed among flax varieties, each with a corresponding *AVR* gene in one or more races of rust. A similar situation occurs for all other plant-pathogen combinations in which the hypersensitive response is triggered.

Biologists now think that the proteins encoded by plant *R* genes – 'R proteins' – act as 'sentries' looking out for signs of a pathogen. *AVR* genes are responsible for the features of the pathogen that alert this sentry system. R proteins might bind directly to proteins encoded by AVR genes—'AVR proteins'—rather like antibodies binding to specific molecules on the surface of human pathogens. Alternatively, R proteins might detect AVR proteins indirectly (ways in which this could happen are shown in fig. 4).

As is often the case with dominant and recessive alleles, only dominant alleles of *R* genes encode functional versions of the respective R proteins. This allows a plant carrying a dominant allele to detect the corresponding AVR protein associated with a pathogen, and so trigger the hypersensitive response and resist infection. In contrast, plants homozygous for a recessive allele of an *R* gene do not make a functional R protein. Therefore such plants cannot detect the corresponding AVR protein, allowing the pathogen to infect without triggering the hypersensitive response.

BOX 2

PLANTS THAT CALL FOR HELP

Plants attacked by pests do more than boost their own defences. Wounded plants synthesise complex mixtures of volatile chemicals which then diffuse into the air. In effect, they change how they smell.

This response can be induced by spraying plants with jasmonic acid and is therefore a part of the overall defensive response to herbivores (see: **Battle on another front**). However, the volatile chemicals that wounded plants release do not harm pests directly, instead they are a call for help. For example, lima-bean plants attacked by spider mites release a blend of chemicals that attracts predatory mites. Maize and cotton plants eaten by caterpillars release scents that attract parasitic wasps (which lay eggs inside the caterpillars, turning them into a living food source for the wasp larvae).

Biologists are now investigating ways of making use of these cries for help. Researchers in California have shown that spraying tomato fields with jasmonic acid doubles the proportion of caterpillars on the plants that are attacked by parasitic wasps. It may also be possible to breed plants that will call for help more 'loudly'. For example, there is a wild relative of cotton that releases up to ten times the quantity of volatile compounds than do cultivated cotton plants after a pest attack.

QUESTION 10

In some cases, researchers have shown that predators must learn to associate the smell of a pest-damaged plant with the presence of their prey. If this is generally true, then spraying a field repeatedly with jasmonic acid to induce these scents and summon predators may not be an effective method of control. Explain why and give reasons for your answer.

Further reading

Plants talk, but are they deaf? M Dicke, A.A. Agrawal and J. Bruin (2003), Trends in Plant Science, 8, 403-405 (Available at http://www.botany.utoronto.ca/ResearchLabs/AgrawalLab/publications/papers/dicke%202003%20ts%20plants%20talk.pdf) *Accessed May 2004*.

GLOSSARY

Avirulence (AVR) gene	A gene carried by a pathogen that makes the pathogen detectable to host plants carrying the corresponding R gene, resulting in the hypersensitive response.
Biotroph	A pathogen that requires living host cells.
Ethene	The gas C_2H_4 – also called ethylene – which acts as plant signalling molecule. With jasmonic acid and methyl jasmonate it induces defences against herbivores.
F1	The first generation resulting from a genetic cross.
F2	The second generation, resulting from crosses among the F1.
Gene-for-gene hypothesis	The hypothesis that the hypersensitive response is triggered when there is a match between an AVR gene in a pathogen and an R gene in the infected plant.
Hypersensitive response	A very rapid response to infection in which one or a few host cells trigger their own death.
Jasmonic acid	A plant signalling molecule, with ethene and methyl jasmonate it induces defences against herbivores.
Methyl jasmonate	A volatile scent derived from jasmonic acid. With jasmonic acid and ethene it induces defences against herbivores.
Necrotroph	A pathogen that kills infected cells and uses their remains as its food source.
Pathogen	A disease-causing micro-organism.
Resistance (R) gene	A gene carried by a plant that allows it to detect a pathogen carrying a corresponding AVR gene, resulting in the hypersensitive response.
Salicylic acid	A signalling molecule synthesised after infection that is both necessary and sufficient to induce systemic acquired resistance.
Systemic acquired resistance (SAR)	A state of increased resistance to disease that develops throughout a plant after infection by a pathogen.