Plants fight back
Teachers' Guide

Summary

Plants attacked by herbivores or pathogens respond by synthesising additional physical and chemical defences and so increasing their resistance to subsequent assaults. The induced defences are initiated close to the damaged site but within a few days spread to encompass the whole plant and so boost resistance even in healthy tissue. This booklet discusses the mechanisms responsible for induced resistance and its potential use for crop protection.
The booklet also includes two boxes. ***The discovery of R genes*** leads students through the genetic experiments that defined the role of R genes in allowing plants to recognize pathogens. ***Plants that call for help*** briefly discusses how the synthesis and release of volatile compounds by plants attracts predators of pests.

Assumed knowledge

This booklet is directed at post-16 students studying A-level biology or Scottish Advanced Highers. It may also be useful to those studying a range of other F.E. or H.E. courses. The text assumes a basic knowledge of the structure of plants and plant cells. Box 1 and the questions therein require an understanding of Mendelian genetics, including the relationship between dominant and recessive alleles of a gene.
The booklet introduces several terms that may be unfamiliar to students: these are given first time in bold text and defined in the glossary.

Answers to questions

1. By selecting plants that were most pleasant to eat, farmers gradually bred out chemical and physical defences against pests and pathogens.

2. Pathogens that cannot penetrate the cuticle enter the shoot through stomata or wounds (viruses are often injected directly into the plant on the mouthparts of aphids).

3. Antibodies and white blood cells can move through the body to guard against pathogens. A specialised immune system such as this is impossible in plants because the cell wall renders plant cells immobile. Therefore, every plant cell must be able to detect the full range of potential pathogens.

4. Monocultures are particularly susceptible to disease because a pathogen that can infect one plant will be able to infect the whole field, leading to an epidemic. In contrast, each strain of a pathogen can normally only infect a subset of the plants in a wild population.

5. The girdling experiments indicate that defence-related signals pass out of, and into leaves in the vascular tissue. The ability of signals to move up and down the shoot suggests that such signals travel in the phloem.

6. Because plants watered with jasmonic acid direct more nutrients and energy into defence, they have fewer resources left to produce seeds, therefore such plants set fewer seeds than untreated plants in pest-free areas [experiment (a)]. In moderately infested areas, however, treated plants incur significantly less pest damage than their untreated neighbours and consequently are able to set more seed [experiment (b)]. In heavily infested areas, increased resistance in treated plants is overwhelmed by the number of pests and so does not give them significant protection [experiment (c)]. These results suggests that the benefits of inducing resistance in crop plants must be weighed against the cost of such resistance to the plant; and also that induced resistance cannot be relied on to combat severe pest attacks.
Reference: Baldwin, I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. Proc. Natl. Acad. Sci. USA, vol. 95, pp. 8113-8118.

7. A simple two-by-two Punnett square shows that a quarter of the F2 had the genotype *LL,* half had the genotype *Ll,* and a quarter had the genotype *ll.* Because resistance conferred by *L* is dominant, the genotypes *LL* and *Ll* are resistant, i.e. three quarters of the population.

8. Because the parents are homozygous for different alleles at both genes, all the F1 are doubly heterozygous, i.e. *Ll, Nn.* Since resistance is dominant, these plants are resistant to both race 22 and race 24.

9. As described in the text, the Bison variety has the genotype *ll, nn*; i.e. it is homozygous recessive at both genes. Bison cannot therefore detect either AVR^L or AVR^N in rust and so would be susceptible to the new strain that carried both *AVR* genes.

10. If predators are repeatedly drawn to plants that are not infested with their prey, the learned association between the scent and the presence of prey will break down - a case of crying 'Wolf!'

Background notes

Salicylic acid
Since ancient times, people have chewed the bark of willow *(Salix spp)* to relieve pain and fever. Hippocrates, for example, prescribed willow bark tea for the pain of childbirth. In 1838, salicylic acid was identified as the active ingredient of willow bark. Acetylsalicylic acid is a less toxic, synthetic derivative invented by Felix Hoffmann in 1897 – in part for his father who suffered arthritis but could not tolerate the stomach pains resulting from taking salicylic acid. Hoffmann worked for the pharmaceutical company Bayer, who gave the new drug the name 'aspirin' in 1899.
The importance of salicylic acid as a signalling molecule was discovered by R. F. White in 1979 when, allegedly at the suggestion of his mother, he injected aspirin into tobacco and showed that this protected against viral infection. Salicylic acid is now known to be both necessary and sufficient for the induction of systemic acquired resistance (SAR) against disease (see the text). Following an infection, salicylic acid is synthesised first in infected leaves and later throughout the plant. Salicylic acid is also exported by infected leaves and may therefore act as a long-range warning signal. However, such export cannot be detected until many hours after an infection has begun. Yet if a leaf is cut from the plant just six hours after that leaf is infected, the remainder of the plant still develops SAR. This and other experiments suggest that infected leaves export another, still unidentified warning signal.
The synthetic analogue of salicylic acid, acibenzolar-S-methyl, was identified from a screen of 40,000 compounds for their ability to induce disease resistance. It is sold under the trade names 'Actigard' (in the USA) and 'BION' (in Europe) by Syngenta (head office, Basel, Switzerland).

Ethene, jasmonic acid and methyl jasmonate.
These three signalling molecules are produced in response to wounding and especially grazing by herbivores. They induce defence-related genes in the plant, leading to an increase in resistance to herbivores. Ethene is also an important regulator of other aspects of plant growth and response to the environment, for example enhancing floral senescence and fruit ripening, and mediating responses to abiotic stress (such as drought or waterlogging) and mechanical stimuli (such as soil pressure, rubbing or wind).
Jasmonic acid may act as a long-range signal in the wound response and is transported from damaged leaves in the phloem (Figure 3). Ethene (a gas) and methyl jasmonate (a highly volatile scent) can diffuse from cell to cell through air spaces in the plant. In the lab, they can also diffuse from leaf to leaf and induce defence responses in neighbouring plants. It is not yet certain how significant this phenomenon is for plants growing in windier, outdoor conditions.
Methyl jasmonate is a major component of the scent of jasmine and honeysuckle flowers (and widely also used by the perfume industry). Methyl jamsonate is also an important part of the scent of some herbs, such as tarragon and wormwood *(Artemisia spp).* This may partly explain the success of these herbs as companion plants against pests. Researchers have shown that neighbouring plants respond to the methyl jasmonate released from these plants by activating their defences against pests.

The relationship between R proteins and AVR proteins.
R proteins (which are encoded by R genes, see box 1) allow cells to recognise pathogens that synthesise corresponding AVIRULENCE (AVR) proteins (see box 1) and so trigger the hypersensitive response. In the absence of the corresponding *R* gene in the host plant, pathogens carrying an *AVR* gene often cause more severe disease than pathogens that lack that gene, suggesting that undetected AVR proteins enhance the ability of the pathogen to cause disease.
The precise functions of R proteins are still largely unknown. Many *R* genes have now been sequenced and the majority of them encode proteins containing protein-to-protein binding domains. Despite this, there is little evidence for direct binding between R and AVR proteins. Instead it is likely that most R proteins detect AVR proteins indirectly. Some AVR proteins have similar amino acid sequences to protein digesting enzymes: R proteins may detect these by binding to the peptide fragments they produce. Other AVR proteins may weaken plant resistance by binding to other defense-related proteins in the host cell: R proteins may detect the resulting conformational changes in the affected plant proteins (see fig. 4).

Further information

For a review of plant defence against disease, see:
Dangle, J. L. & Jones, J.D.G. (2001) Plant pathogens and integrated defence responses to infection. *Nature* **vol. 411**, pp. 826-833.

Suggested practical work

Using *Penicillium* and apples, the SAPS website (see below) contains a demonstration of Koch's postulates for identifying the cause of a disease (Student Sheet 18). The practical also offers the opportunity to consider the role of both physical defences (the apple skin) and chemical defences (the phenolics that cause apple flesh to turn brown) in resisting infection.
A protocol to test whether wounding increases hair

production in bean leaves is given in: Wright, P.J. & Bonser, R. (1999) An investigation into induced plant defences. Journal of Biological Education vol. 33, pp. 217-219.

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