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# Plant defenses

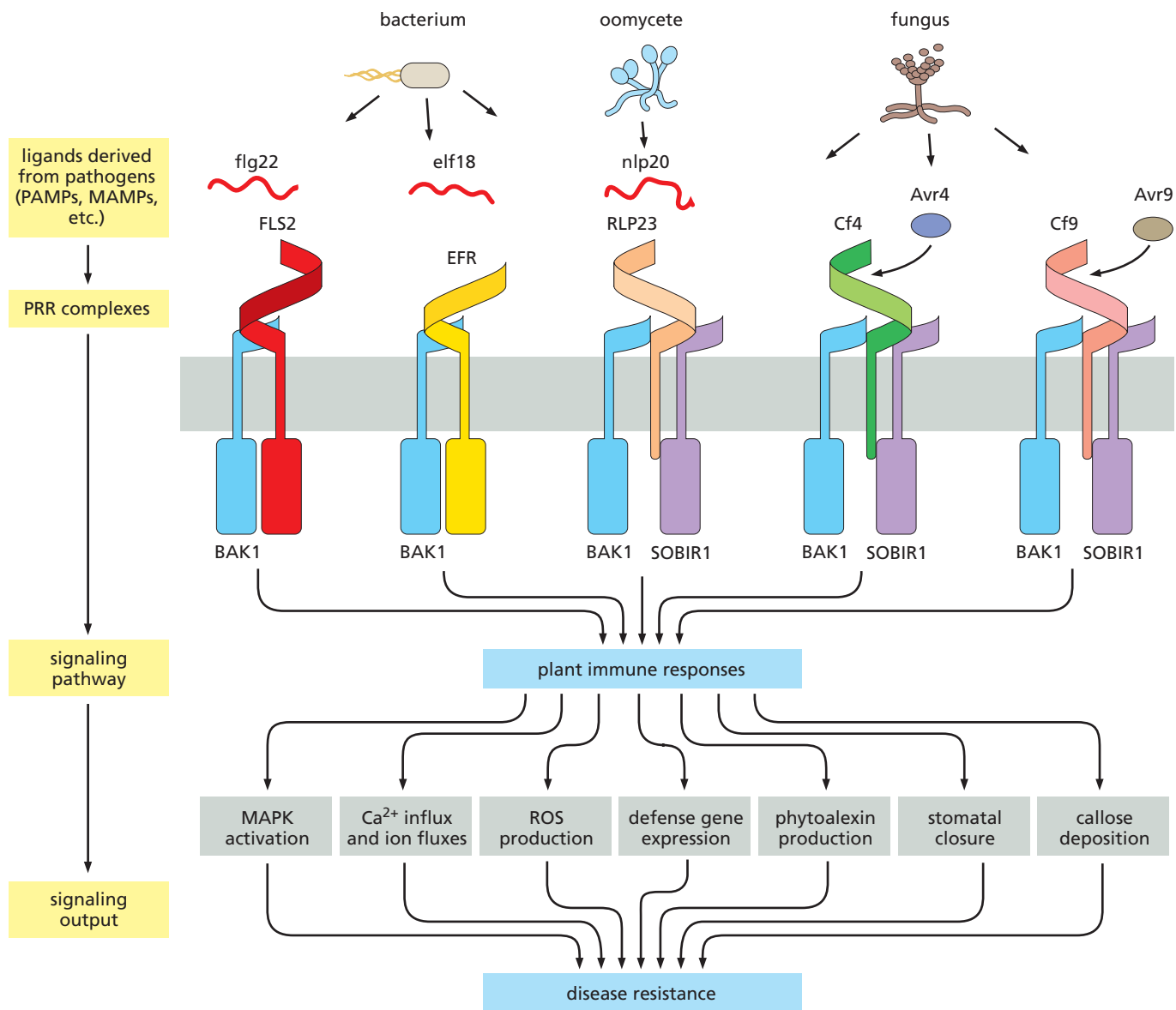
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# 15

## OM15.1 MEDICINAL AND AGRICULTURAL VALUE OF PLANT-PRODUCED DETERRENTS AND TOXINS

Many plant-produced toxins have very important uses in medicine in appropriate doses and formulations such as the anti-cancer agents taxol and vinblastine, the pain-relieving opiates codeine and morphine, and the antimalarial artemisinin. Other toxins have applications as pesticides, including the pyrethroids and azadirachtin. Many of the important bioactive compounds produced by plants are alkaloids, derived through complex biosynthetic pathways from amino acids including aspartic acid, lysine, ornithine, tyrosine, or tryptophan and present in extraordinary diversity in the plant kingdom. Bioactive alkaloids have powerful effects on animals, but they have not been implicated in resistance to microbes and are more likely to affect herbivores. Cocaine, caffeine, morphine, and nicotine are well-known plant alkaloids that are important because of their effects on human behavior, but many other alkaloids are also of economic significance. For example, quinine from the bark of the tree *Cinchona* was the original antimalarial drug: it is toxic to the apicomplexan *Plasmodium* that causes malaria. Other alkaloids are important because of their adverse effects on farm animals. Grazing animals are poisoned by eating alkaloid-containing plants in pastures, such as ragworts (*Senecio* spp., containing the alkaloid senecionine) and lupins (*Lupinus* spp., containing the alkaloid lupinine).

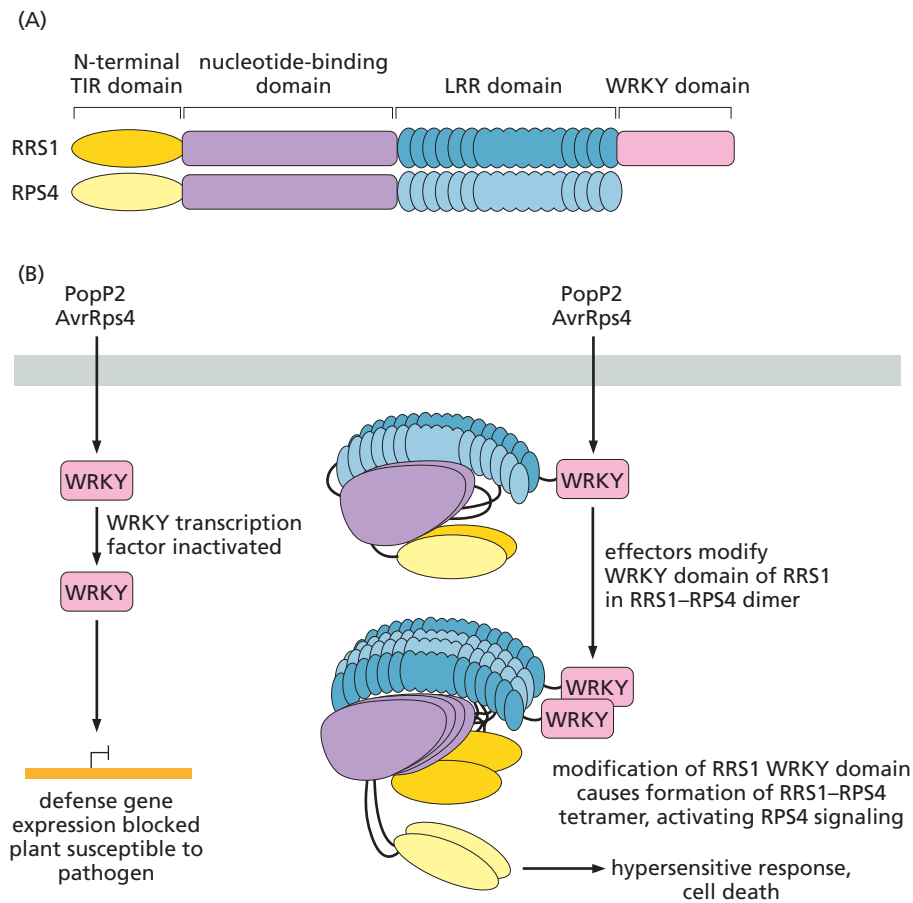
## OM15.2 EXAMPLES OF PRR COMPLEXES THAT RECOGNIZE DIVERSE LIGANDS



**Figure OM15.2-1** Examples of PRR complexes that recognize diverse ligands. The cartoon shows the similarity in structure of pattern recognition receptors responsible for recognition of organisms as diverse as bacteria, oomycetes, and fungi. The complexes shown here all contain a receptor-like kinase or receptor-like protein (RLK or RLP) responsible for binding the ligand, and the co-receptor BAK1. Some contain additional BAK1-like co-receptors (not shown). Where the receptor is an RLP, lacking a cytoplasmic receptor domain, the complex contains a receptor-like cytoplasmic kinase SOBIR1. When activated by ligand binding, the PRR complexes trigger signaling pathways that lead to many diverse outputs (*left panel*). These include rapid cellular responses and the expression of a very wide range of genes with products that bring about disease resistance (*lower panel*, described in detail in Sections 15.3 and 15.4). (Adapted from Ma X *et al.* (2016) *Trends Plant Sci* 21:1017–1033.)

## OM15.3 AN IMMUNE RECEPTOR THAT CONSISTS OF TWO NLR PROTEINS ENCODED BY ADJACENT GENES

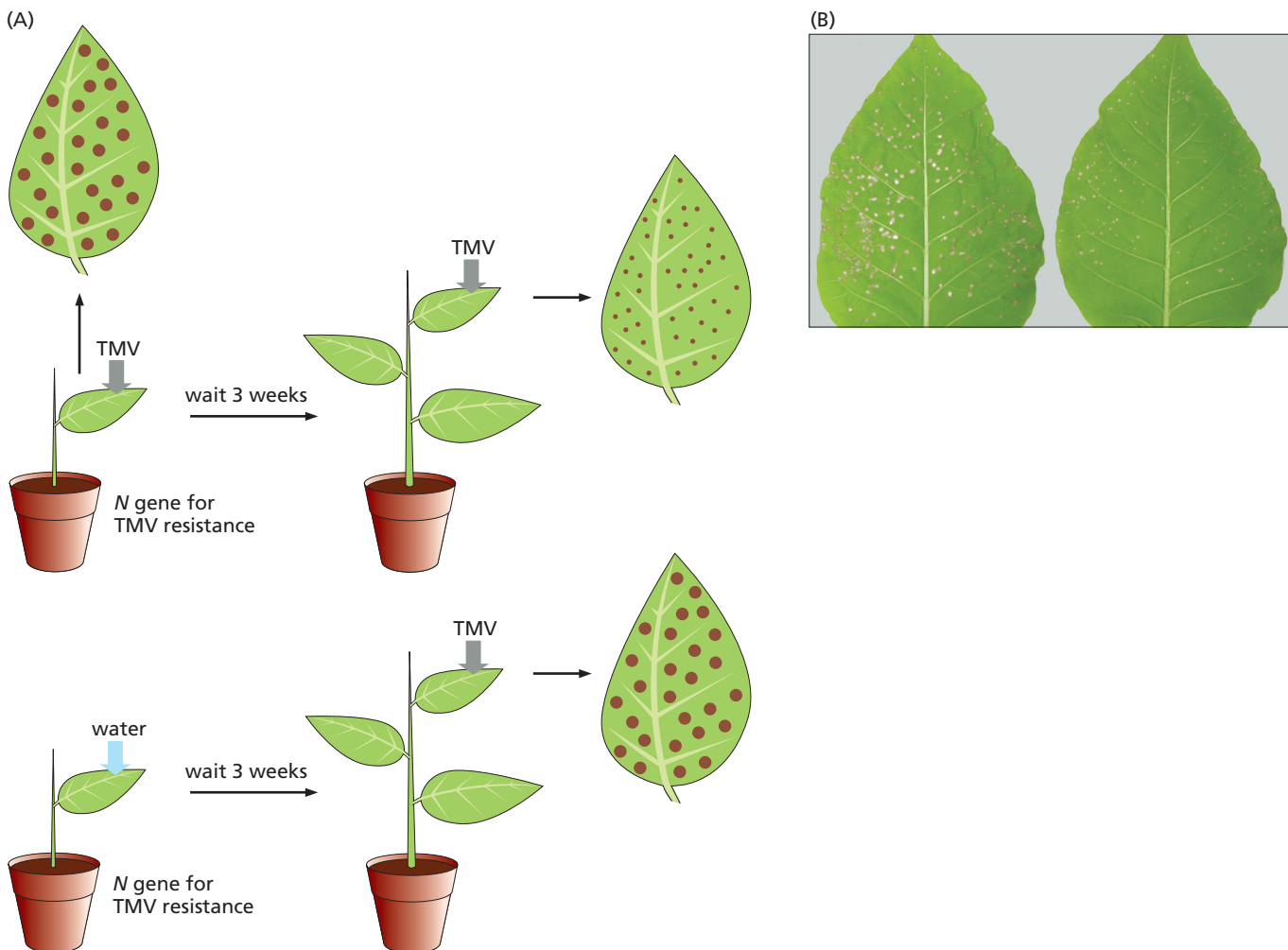
Although most *R* genes reside at single loci at which allelic variation determines effector recognition, some *R* gene loci carry two linked *NLR* genes, both required for function. For example, Arabidopsis *RPS4* and *RRS1* are adjacent genes encoding two TIR-NLR proteins, both of which are required for effector recognition and defense activation. The *RPS4*–*RRS1* complex recognizes AvrRps4 from *Pseudomonas syringae* and PopP2, an acetyltransferase from *Ralstonia solanacearum* that causes bacterial wilt. Both effectors inhibit WRKY transcription factors that play crucial roles in plant defense. The *RRS1* component of the *RPS4*–*RRS1* complex carries a C-terminal WRKY transcription factor domain that both AvrRps4 and PopP2 can modify. The modifications are recognized by the complex as a signal to activate *RPS4*-dependent defense (Figure OM15.3-1). Surveys of plant genome databases reveal many other domains integrated into NLR proteins that are also likely to be homologs of (and decoys for) domains in host proteins that are authentic effector targets.



**Figure OM15.3-1 Paired NLRs with an integrated decoy.** (A) Arabidopsis *RRS1* and *RPS4* are TIR-NLR proteins encoded by adjacent genes. *RRS1* carries an integrated WRKY transcription factor domain at its C-terminus: referred to as an integrated decoy domain. In the absence of a recognized effector, the two NLRs form a heterodimeric immune receptor. (B) The *RPS4*–*RRS1* immune receptor recognizes two bacterial effectors, AvrRps4 from *Pseudomonas syringae* and PopP2, an acetyltransferase from *Ralstonia solanacearum*. Both effectors inactivate WRKY transcription factors essential for full defense responses; Pop2 by acetylation, AvrRps4 by preventing binding to DNA (left). The mode of action of PopP2 is shown here. When PopP2 is secreted into host cells, it acetylates and thereby inactivates WRKY transcription factors. Both effectors can also modify the WRKY domain of *RRS1* (right). This brings about a conformational change that leads to heterotetramer formation and the activation of defense signaling via *RPS4*. Signaling culminates in a hypersensitive response and cell death. (From Cesari S *et al.* (2014) *Front Plant Sci* 5:606. Distributed under CC BY.)

## OM15.4 EVIDENCE FOR SYSTEMIC SPREAD OF RESISTANCE

Evidence for the systemic spread of resistance from a localized infection site comes from experiments with tobacco plants carrying the *N* resistance gene that encodes a TIR-NLR receptor. When a leaf is infected by a virulent (infectious) strain of tobacco mosaic virus (TMV), the spread of the virus through the leaf is accompanied by the development of chlorotic patches, giving leaves a mottled or mosaic green and yellowish appearance. Severe infections can result in necrosis and plant death. By contrast, inoculation with a strain of TMV to which the *N* gene confers resistance gives rise to a hypersensitive response (HR) in which groups of cells around the infection sites die, forming lesions on the leaf. Subsequent inoculation of other leaves on the same plant with the same or a different strain of TMV also results in cell death, but the lesions are much smaller than those on the first leaf inoculated. This indicates that the defense response is more effective in uninoculated leaves than in the inoculated leaf: the spread of the virus is restricted to fewer cells and its spread is halted more rapidly (**Figure OM15.4-1**). A signal from the first leaf to be infected must therefore have spread to other leaves, where it triggered increased resistance to viral infection. Tobacco plants in which systemic acquired resistance (SAR) has been triggered by TMV also show enhanced resistance to other diseases such as the oomycete blue mold (*Peronospora tabacina*).



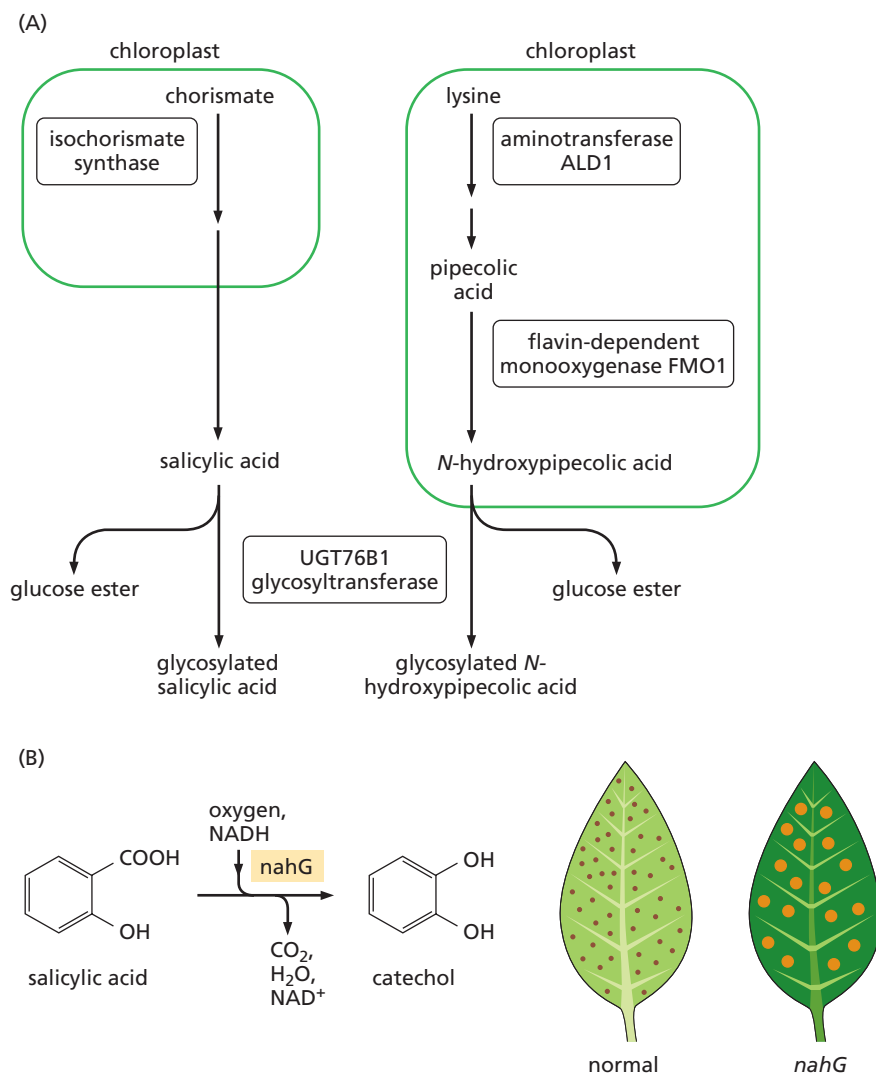
**Figure OM15.4-1 Systemic acquired resistance to tobacco mosaic virus (TMV) in a tobacco plant.** (A) When a plant carrying the *N* gene (an NLR gene that confers resistance to TMV) is inoculated with an avirulent strain of TMV, a hypersensitive response occurs on the inoculated leaf (*top*). This triggers systemic acquired resistance (SAR). Inoculation of a different leaf 3 weeks later produces a much more effective defense response, with less cell death. If, in a control experiment, the first inoculation is with water rather than virus (*bottom*), SAR is not triggered; inoculation of a different leaf 3 weeks later gives an unaltered defense response. (B) Leaves of a tobacco plant infected with TMV without (*left*) and with (*right*) a previous challenge. SAR is triggered only in the plant that was previously challenged; note the smaller lesions on this leaf. (B, courtesy of Sang-Wook Park and Dan Klessig.)

## OM15.5 SYNTHESIS AND FUNCTIONS IN SAR OF SALICYLIC ACID AND *N*-HYDROXYPIPECOLIC ACID

Plants possess two general routes for the biosynthesis of salicylic acid: the isochorismate synthase (ICS; present in chloroplasts) and the phenylalanine ammonia lyase pathways, both of which use chorismate as a precursor (chorismate is also the precursor of the aromatic acids: see Section 6.2). The relative contributions of these pathways to salicylic acid biosynthesis varies between plant species: the ICS pathway (Figure OM15.5-1A) is the more important in *Arabidopsis*, and the phenylalanine ammonia lyase is the more important in rice and the Solanaceae (potato, tomato, tobacco).

A requirement for salicylic acid was demonstrated by introducing into tobacco plants a bacterial gene (*nahG*) encoding an enzyme that degrades salicylic acid (Figure OM15.5-1B). Reduction in salicylic acid levels either by *nahG* expression or in an *ics1* mutant of tobacco (not shown) results in reduced SAR. In *N* tobacco plants expressing the bacterial *nahG* gene, inoculation with tobacco mosaic virus (TMV) results in much larger lesions than in wild-type plants. The virus multiplies throughout the plant, leading to a spreading necrosis as a weakened hypersensitive response is repeatedly triggered.

*N*-hydroxy-pipecolic acid is synthesized from lysine in the chloroplast (see Figure OM15.5-1A). Evidence for its importance in SAR comes from studies of mutants lacking enzymes on the biosynthetic pathway (*ald1* and *fmo1* mutants) that are deficient in SAR. Exogenously supplied *N*-hydroxy-pipecolic acid is sufficient to induce SAR in wild-type



**Figure OM15.5-1 Salicylic acid and *N*-hydroxy-pipecolic acid metabolism and function in SAR.** (A) The defense hormones salicylic acid and *N*-hydroxy-pipecolic acid are synthesized by independent biochemical pathways. Their levels are modulated independently by formation of inactive glucose esters, and also coordinately by the action of a single glycosyltransferase that converts both to inactive glycosides. (B) (Left) Salicylic acid degradation catalyzed by the bacterial enzyme *nahG*. Expression of *nahG* in transgenic plants results in lines with differing levels of salicylic acid; this material has been a valuable tool in understanding the role of salicylic acid in SAR. (Right) Cartoon of the upper leaves of TMV-infected plants, 7 days after infection of a lower leaf. The untransformed plant carrying the *N* gene displays a hypersensitive response in the upper leaves, indicative of a full-functioning SAR. The transformed plant expressing *nahG* – hence having lower levels of salicylic acid – has failed to develop SAR in the upper leaves and has large and spreading lesions.

plants and also rescues the SAR defects of *ald1* and *fmo1* mutants. Moreover, when the Arabidopsis *N*-hydroxypipelicolic acid biosynthetic genes *ALDI*, *SARD4*, and *FMO1* are transiently co-expressed in tomato leaflets, *N*-hydroxypipelicolic acid accumulates in the absence of pathogen attack, and SAR is induced in distant, untreated leaves.

Levels of *N*-hydroxypipelicolic acid and salicylic acid are modulated independently through formation of their respective inactive glucose esters, and their levels are also coordinated by a single, pathogen-induced glycosyltransferase (UGT76B1) that converts both metabolites to their inactive glycosides.

## OM15.6 RNA SILENCING DEMONSTRATED BY SILENCING OF GFP IN TOBACCO

Under light of an appropriate wavelength, transgenic plants expressing green fluorescent protein (GFP) fluoresce. If they are subsequently infected with a modified form of potato virus X that also carries the *GFP* gene, silencing of the *GFP* transgene spreads (as seen by the loss of fluorescence) with the virus. (This phenomenon can also be induced when a second *GFP* gene is introduced by other methods, such as by infiltration of leaves with *Agrobacterium* containing a T-DNA that carries the gene, as shown in **Figure OM15.6-1**.) Silencing occurs because RNA-induced silencing complexes (RISCs) primed with GFP-encoding viral RNA are degrading the messenger RNA (mRNA) produced from the *GFP* gene introduced into the plant genome. If the virus does not carry the *GFP* gene, no silencing of GFP takes place and the plant remains fluorescent. Modified viruses can also be used to silence the plant's own genes, a method known as **virus-induced gene silencing (VIGS)**. If the virus contains part of the sequence of a plant gene, mRNA from that plant gene will be targeted for degradation when the virus is present. This is a valuable method for experimentally reducing or eliminating expression of specific plant genes. (See also Section 2.3.)



**Figure OM15.6-1 Silencing of GFP expression in tobacco by infiltration with *Agrobacterium* carrying a second *GFP* gene.** Transgenic tobacco plants expressing a gene that encodes green fluorescent protein (GFP) are fluorescent (yellow-green) under light of appropriate wavelength (the red color is due to the natural fluorescence of chlorophyll). Infiltration of a leaf (*lower arrow*) with *Agrobacterium* carrying the *GFP* gene triggers the silencing of the *GFP* transgene present in the plant. The mature leaf above the infiltrated leaf (leaf 1) continues to fluoresce because GFP protein was present in this leaf before infiltration. Upper leaves that were still expanding at the time of infiltration are partly silenced (leaves 2 and 3), and a very young leaf (leaf 4) is completely silenced. (From Voinnet O *et al.* (1998) *Cell* 95:177–187. With permission from Elsevier, courtesy of David Baulcombe.)