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Prospects of genetic engineering for robust insect resistance

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Secondary plant metabolites are potentially of great value for providing robust resistance in plants against insect pests. Such metabolites often comprise small lipophilic molecules (SLMs), and can be similar also in terms of activity to currently used insecticides, for example, the pyrethroids, neonicotinoids and butenolides, which provide more effective pest management than the resistance traits exploited by breeding. Crop plants mostly lack the SLMs that provide their wild ancestors with resistance to pests. However, resistance traits based on the biosynthesis of SLMs present promising new opportunities for crop resistance to pests. Advances in genetic engineering of secondary metabolite pathways that produce insecticidal compounds and, more recently, SLMs involved in plant colonisation and development, for example, insect pheromones, offer specific new approaches but which are more demanding than the genetic engineering approaches adopted so far. In addition, nature also offers various opportunities for exploiting induction or priming for resistance metabolite generation. Thus, use of non-constitutively expressed resistance traits delivered *via* the seed is a more sustainable approach than previously achieved, and could underpin development of perennial arable crops protected by sentinel plant technologies.

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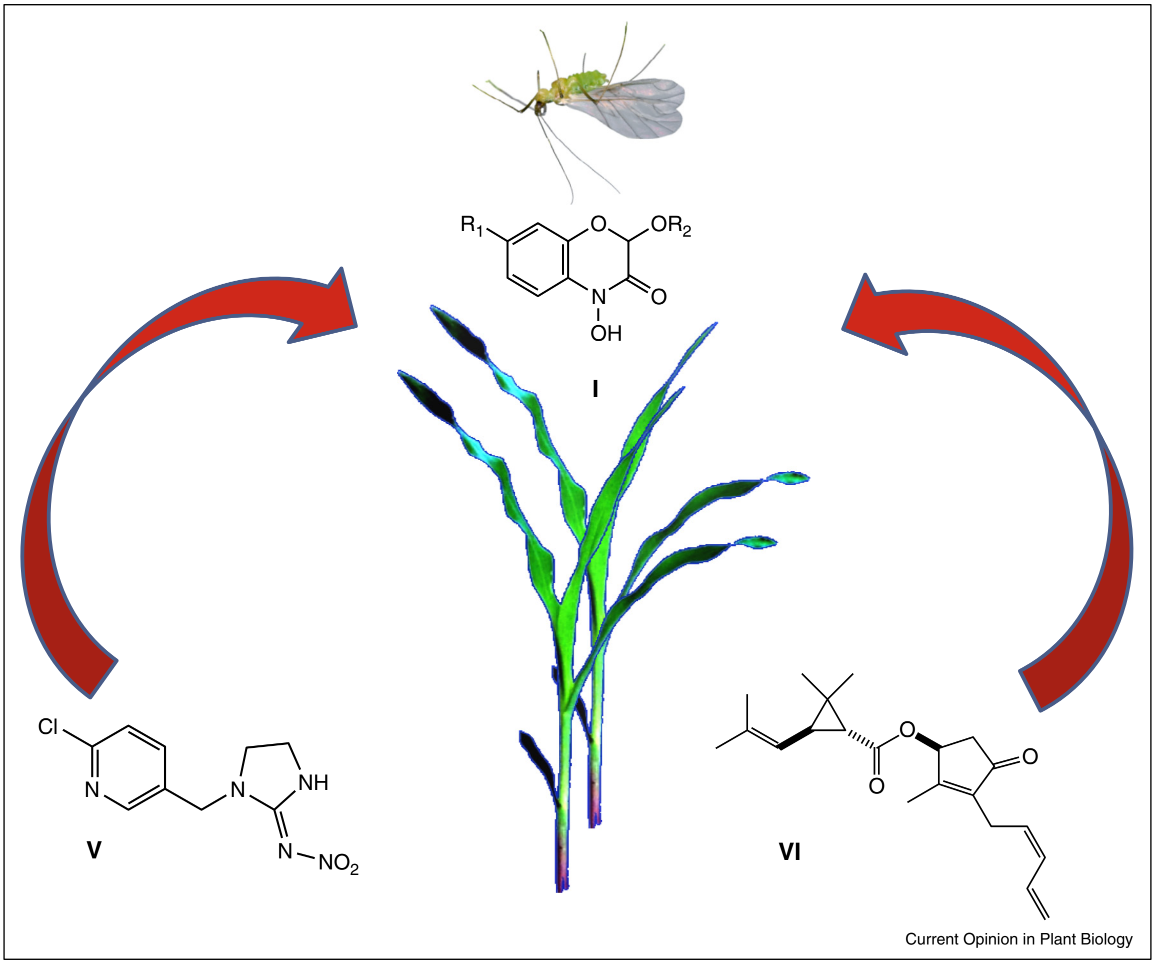
Introduction to the concept of creating robust insect resistance by genetic engineering of plant secondary metabolism

Pesticides, and particularly insecticides, serve agriculture well, with the currently registered compounds presenting an extremely low risk to the environment and to human health, when used in crop protection under legally enforced protocols. However, seasonal application of pesticides to the crop is unsustainable in terms of the carbon footprint particularly associated with application. In addition, the readiness by which selection for insecticide resistance occurs *via* target site modification, often involving only one non-synonymous single nucleotide polymorphism (SNP), or *via* upregulation of a particular gene associated with metabolism [1], aggravates the problem of the inherent unsustainability of this type of intervention against pests. Breeding delivers resistance to crop plants *via* the seed and, by definition, is more sustainable, but has not produced sufficiently durable insect control for replacement of insecticides in mainstream food production. This is also true of weed control with regard to herbicides, but is less the case for pathogen control, although without current fungicides there would be unacceptable losses in food production [2]. Delivery of insecticides is extremely inefficient, with a very small percentage applied to the crop reaching the target insect and even less so the target site, but such compounds are robust in terms of stability and, provided delivery ensures sufficient bioavailability for effective toxic effects and does not lead to environmental contamination, selectivity is based mostly in unique aspects of the target insect physiology. Secondary plant metabolites can be small lipophilic molecules (SLMs) with similar physicochemical properties and toxicities to pesticides, and many have provided the lead structure or inspiration for synthetic insecticides, for example, pyrethrum for the pyrethroids [3], nicotine and other nicotinic acetylcholine receptor agonists for the neonicotinoids [4••] and stemofoline for the very recently introduced butenolides [4••]. Some insecticides are natural products, for example, spinosad, which comprises the natural spinosyns A and D. Thus, genes for the biosynthesis of the natural insecticidal SLMs are available in nature for exploitation by genetic engineering. The potential of doing this has been demonstrated by pioneering groups led originally by Gierl [5] and Moller [6], who established, respectively, the enzymology for the secondary plant metabolism that produces insecticidal SLMs, the hydroxamic acids [benzoxazinoids, e.g. DIMBOA (**I**), $R_1 = OCH_3$, $R_2 = H$] (Figure 1), and the precursors, the cyanogenic glucosides (**II**) and glucosinolates (**III**) for the toxicants hydrogen cyanide (HCN) and organic isothiocyanates (RNCS). Compound **I** can also be stored as a glucoside, which then also releases the toxic product upon tissue damage, for example, caused by insect feeding. Together with storage tissue location, *in situ* release can confer selectivity to insects. With the demonstration that non-producing crop plants can be genetically engineered in the laboratory to express these defence pathways [7] comes the prospect for developing this approach to insect control [8••]. There is further evidence from the long term success of engineering plants to produce proteins derived from *Bacillus thuringiensis* (Bt) [9•] against lepidopterous pests. Here, although not SLMs, the protein structures are unique in providing high levels of toxicity

Figure 1



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Naturally occurring insect toxicants (e.g. **I**) are small lipophilic molecules like commercial insecticides (e.g. **V**) or lead compounds (e.g. **VI**) for these.

against lepidopterous larvae. However, ingestion of the protein is required and certain gut conditions have to be present for activity. No similarly valuable related structures have become available. Although the enzymology for producing insecticidal secondary metabolites can be substantially more complicated than for Bt related proteins, it is evident that the discovery of metabolic gene clusters will provide less demanding routes for this type of genetic engineering [10••]. Non-target toxicity is dealt with by risk analysis before registration, but there are considerable concerns expressed in the media, particularly regarding neurophysiologically active insecticidal components but, also more importantly, the problem of rapid selection for resistance to toxicants where deployment is widespread. Both of these problems can be alleviated by targeting pheromones and other semiochemicals, another group of natural SLMs that affect insect behaviour and development. Here, much more complicated genetics are associated with the response by the pest to these compounds and so selection for resistance is more physiologically demanding for the pest. These SLMs, being highly specific signals that act at extremely low levels, are also intrinsically more benign than insecticidal SLMs. Indeed, the high volatility and chemical instability of many semiochemicals that contribute to their low risk also present problems of deployment, but such problems can be overcome by genetic engineering of plants for production on release, and this can indeed be achieved [11]. An alternative is to use genetically engineered plants as a factory for synthesis of pheromones otherwise only available by expressive chemical synthesis [12••]. Besides genes for pathways for the insect toxicants and semiochemicals being available for engineering insect resistance, there are phytopheromones that can induce or prime production so that the defence is only activated when the pest arrives, or the plant is primed to produce a greater activation when insect colonisation begins for which there are evolutionary trade-offs [13,14••]. Thus, the scene is set for robust non-constitutive insect resistance to be engineered into crop plants, opening opportunities for further advances in sustainable agriculture, including perennial arable crop protection by sentinel plant technology .