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Plant volatile-mediated signalling and its application in agriculture: successes and Challenges

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Summary

Key words: agriculture, companion plants, genetic modification (GM), induced defence, plant communication, plant volatile-mediated signalling, push–pull, semiochemicals.

The mediation of volatile secondary metabolites in signalling between plants and other organisms has long been seen as presenting opportunities for sustainable crop protection. Initially, exploitation of interactions between plants and other organisms, particularly insect pests, foundered because of difficulties in delivering, sustainably, the signal systems for crop protection. We now have mounting and, in some cases, clear practical evidence for successful delivery by companion cropping or next-generation genetic modification (GM). At the same time, the type of plant signalling being exploited has expanded to signalling from plants to organisms antagonistic to pests, and to plant stress-induced, or primed, plant-to-plant signalling for defence and growth stimulation.

I. Introduction

Volatile secondary metabolites of plants can provide signals acting as recognition cues for detection and colonization by other organisms, most obviously insects. Where crops are concerned, these insects join pathogens and weeds as major constraints to food production. In modern agriculture, such constraints are managed by a range of synthetic and largely eradicant pesticides. Apart from the often rapid development of resistance in the pest, pathogen or weed, these are registered for use in ways avoiding risk to human

and environmental health (Pickett, 2013). However, for more sustainable pest management than seasonal, and often multi-seasonal, deployment of pesticides, crop resistance to pests, delivered via the seed, will be essential (Baulcombe, 2009).

Crop breeding programmes, now advanced by new molecular techniques, have provided evidence for the value of seed-delivered pest management. Evidence for the value of a wider range of genetic pest resistance than provided by the often closely related crop varieties used in breeding programmes is demonstrated by *Bacillus thuringiensis* (Bt) crops. These express, by genetic modification

(GM), genes for proteins based on the sequences of insect endotoxins from *B. thuringiensis*, with associated increases in sustainability (Lu et al., 2012). Advances in the molecular biology of plant secondary metabolite regulation and biosynthesis now render such compounds targets for exploitation by GM in agriculture. In choosing specific pest targets, particularly insects, plant volatile-mediated signalling offers a further advantage in that the modes of action do not involve toxic mechanisms, which can negatively impact public perception. This is specifically a consideration with insects which, although a class of Arthropods, are animals, and the sites of action for many insecticides are common not only to both pest and beneficial insects, but also to vertebrate animals, including human beings. For insects, the signalling receptor systems are essentially similar to human olfactory recognition but, although the signal compounds involved can be detected by human olfaction, this detection is very seldom as sensitive, and usually without a specific signalling role. In plants and pathogens, although there are no peripheral sensory nervous systems as for animals, the volatile signals are detected by sophisticated systems (see Section VI). Thus, by exploiting natural plant genetic diversity in breeding, by the use of companion plants and by creating new GM plants with modified volatile-mediated signalling systems, we are set to exploit this approach to reducing pest-, disease- and weed-related constraints in agriculture.

Plant signalling via volatile secondary metabolites allows recognition not only of hosts, but also of nonhosts. These may, by being taxonomically different from host plants, have features such as toxic metabolites to which a potential pest is poorly adapted, or unadapted. Host plants rapidly become nonhosts during feeding or as a consequence of other developmental stresses, and this results in signalling to successive invaders that the initial host is no longer appropriate. The realization that taxonomically based nonhost signalling could be related to damage stress signalling by colonized hosts (Nottingham et al., 1991) was an important development in working towards strategies for use of volatile-mediated signalling in agriculture. The further appreciation that this latter type of volatile signal could also induce, or prime, plant defence (Baldwin & Schultz, 1983; Baldwin et al., 2006) adds considerable power to developing new plant control strategies by providing plant-to-plant signals as a means to switch on defence genes in a companion crop. These can be identified from the natural diversity of plant species or utilized from a GM plant engineered specifically for this purpose.

Elicitors from plant-attacking organisms are now being characterized generally as small-molecular-weight lipophilic secondary metabolites, after the pioneering identification of volicitin from the regurgitant material of an herbivorous caterpillar (Alborn et al., 1997). Other elicitors with novel structures, but similar physico-chemical properties, have been identified (Alborn et al., 2007) from different insect taxa and again require, as for volicitin, damage by the herbivore for the compound to elicit defence responses in the plant. The type of defence elicited could be direct (Oliver et al., 2000; Scholz et al., 2015), or produced as indirect volatile defence signals in which compounds repellent to the herbivore, but attractive to organisms antagonistic to the herbivore (e.g. para-sitoids), are elicited (Alborn et al., 2007; Scholz et al., 2015). The natural elicitors of defence offer opportunities for nonconstitutive

defence gene expression and can thereby be linked to pest presence, in contrast to the prophylactic treatments using constitutive gene expression currently deployed in GM crops.

Here, we review the above aspects, particularly in terms of application to agriculture, of volatile-mediated signalling in crops and wild plants. We then describe the successful push–pull system for managing lepidopterous stemborer pests of cereals in sub-Saharan Africa by delivery of signals using companion cropping. Challenges will then be discussed for delivery to industrial agriculture of pest management signalling, specifically by GM.

II. Plant volatile-mediated host signalling

This is an extensively studied area and offers a great range of tools for trapping pest insects. However, crops themselves are highly effective competitors to artificial delivery of these signals. The signals can relate to the volatile chemistry of plants generally. Specificity can be exhibited by mixtures of ubiquitous signals (Bruce & Pickett, 2011), which include oxidation products, for example (E)-2-hexenal and (Z)-3-hexen-1-ol from the lipoxygenase pathway, and their derivatives, for example (Z)-3-hexenyl acetate. It has been possible, using gas chromatography-coupled electrophysiology with the insect antenna (either electroantennogram or single neuron recording) (Pickett et al., 1998, 2012), to identify and quantify complex mixtures which, in the laboratory, mimic the natural signal (Webster et al., 2008a). For example, the signal used by the black bean aphid, *Aphis fabae*, in detecting its host, the bean *Vicia faba*, incorporates a 16-component mixture of ubiquitous plant volatiles (Webster et al., 2008b) (Table 1). However, such a mixture would be extremely difficult to deliver artificially. Usually, individual components are detected by specific olfactory neurons (Blight et al., 1989; Hansson et al., 1999). For example, aphid detection of (E)-2-hexenal is by a neuron that barely responds to closely related compounds from the same biosynthetic pathway (Pickett et al., 1998). This allows recognition between components of a mixture released from a point source, as opposed to from diverse points (Baker et al., 1998; Baker, 2009; Bruce & Pickett, 2011).

For artificial delivery, although a range of slow-release formulations and devices are available (Bruce et al., 2007; Bakry et al., 2015), components of a mixture having different volatilities present practical problems, currently solved only by using separate release substrates and thereby separate release points for the individual components. Opportunities exist for exploiting Raoult's law, as shown by Heath et al. (1986), which states that the partial vapour pressure of each compound of an ideal mixture of liquids is equal to the vapour pressure of the pure component, multiplied by its mole fraction in the mixture. This presents a completely novel opportunity for delivery of complex mixtures while maintaining an essential ratio of release between compounds of widely differing volatilities, and protection of intellectual property (IP) for new developments is being considered. However, delivery of competitive, and even superior, host signals from companion plants is a reality, and is the goal for the 'pull' plants in the push–pull or stimulo-deterrant diversionary system, originally proposed by Miller & Cowles (1990) and discussed in detail in Section VII.

Table 1 Plant volatile-mediated signals from intact bean plants, *Vicia faba*, used in host location by the black bean aphid, *Aphis fabae*

Compound	Biosynthesis	Compound	Biosynthesis
(E)-2-Hexenal	Fatty acid derived	Benzaldehyde	Via phenylalanine ammonia lyase activity
1-Hexanol	Fatty acid derived	Methyl salicylate	Via phenylalanine ammonia lyase activity
(Z)-3-Hexen-1-ol	Fatty acid derived	6-Methyl-5-hepten-2-one	Isoprenoidal
Octanal	Fatty acid derived	(R)-Linalool	Isoprenoidal
(Z)-3-Hexen-1-yl acetate	Fatty acid derived	(E)-(1R,9S)-Caryophyllene	Isoprenoidal
Decanal	Fatty acid derived	(E)-b-Farnesene	Isoprenoidal
Undecanal	Fatty acid derived	(S)-Germacrene D	Isoprenoidal
		(E,E)-4,8,12-Trimethyl-1,3,7,11-tridecatetraene (TMTT)	Isoprenoidal

This diverse list of volatile compounds, with one additional unknown, comprises the entire signal for host recognition. Within the biosynthetic groups, which indicate the link between primary plant metabolism and these secondary metabolites, the compounds are given in order of decreasing volatility. TMTT is mostly found as a plant volatile signal relating to stress but here, in the context of the other signal components, it is expressed constitutively at a low level as a component of the overall attractive mixture (Webster et al., 2008b).

Highly attractive trap plants, on which eggs are laid but larvae cannot develop, also have the advantage of facilitating destruction of the attracted herbivore, for example by the leaf and stem material being fed to farm animals. Alternatively, traps designed to kill the herbivore are required and, although insecticides can be deployed, mechanical entrapment by surfactant-treated water or adhesives is preferred (Bruce et al., 2007, 2011).

Volatile-mediated signalling associated with specific host taxa can provide signals delivered more simply than for attractive mixtures. Such signals can relate to toxicants to which specialist herbivores have become evolutionarily adapted. Thus, volatile organic isothiocyanates can be used by herbivorous insects specializing on plant families of the order Brassicales, which contain toxic glucosinolates releasing isothiocyanates by catabolism within the plant (Halkier & Gershenson, 2006). Although specific olfactory neurons respond to the organic isothiocyanates, these compounds can be further discriminated by neurons in antennae of, for example, the cabbage seed weevil, *Ceutorhynchus assimilis*, responding to specific structural types of organic isothiocyanates (Blight et al., 1989). These signals can be used competitively in brassicaceous crops by incorporation into lures slowly releasing the compounds to attract pests into traps (Smart et al., 1996; Blight & Smart, 1999). However, intrinsic toxicity and instability of the organic isothiocyanates present problems and so, again, companion cropping has been studied as an option (Cook et al., 2006, 2007). Further examples of taxonomically based signalling beyond the brassicaceous glucosinolate/isothiocyanate system and the more widespread cyanogenic glycoside systems exist, but there are relatively few that are understood ecologically, compared with the number of plants defended by highly toxic secondary metabolites. It is currently assumed that recognition of hosts relies mainly on mixture recognition, but it may be that we have as yet been unable to recognize the associated specific volatile signals. Thus, a profitable approach to this problem may lie in the study of the molecular basis of insect olfactory recognition. As the insect olfactory system is linked to the motor neuronal responses of behaviour via the central nervous system, learning also plays an important role (Webster et al., 2013) in natural molecular structure recognition by olfaction. This latter aspect of signalling represents another unique feature for insects and animals generally, as

opposed to signalling in other kingdoms, including fungi and plants, which is not always appreciated when developing new strategies for agriculture.

III. Plant volatile-mediated nonhost signalling

From an evolutionary standpoint, nonhost signalling is largely advantageous to an organism attacking a plant. Thus, when plants are attacked, they can signal to other organisms, particularly herbivorous insects, which would not derive value from a host already colonized because, as well as competition for host resources, there could be cannibalism of eggs or early-stage larvae by larger, conspecific larvae. Nonetheless, the plants can benefit by recruitment of organisms antagonistic to those at the herbivorous, or second trophic, level. The nature of such chemical signalling can be as for the original host recognition, but can also involve perturbation of mixture composition by the production of higher concentrations of certain components, or overall increased release, as a consequence of oxidation reactions associated with plant tissue damage. Indeed, perturbation of mixtures by increased amounts of components of host recognition mixtures, for example the isoprenoid oxidation product 6-methyl-5-hepten-2-one in the background of wheat (*Triticum aestivum*) flower volatiles, causes repellency of the orange wheat blossom midge, *Sitodiplosis mosellana* (Birkett et al., 2004). Single compounds originating from damage-related oxidation, such as (E)-2-hexenal, can act as individual compounds in the recognition mixture for *V. faba*, but cause repellency of *A. fabae* when presented alone (Webster et al., 2010). Other isoprenoid oxidation products such as the so-called homoterpenes (more correctly termed tetranorterpenes), comprising C₁₁ and C₁₆ isoprenoids [e.g. (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT)](Table 1), derived by oxidation of the tertiary alcohols relating to higher isoprenoidal homologues, that is, C₁₅ (sesquiterpene) and C₂₀ (diterpene), are ubiquitous signals for host plants no longer valuable as hosts as a consequence of prior damage (Tholl et al., 2011).

In certain ecological situations, the isoprenoid oxidation products such as 6-methyl-5-hepten-2-one can signal to higher trophic levels, for example the aphid parasitoid *Aphidius ervi*, indicating the presence of its host, the pea aphid, *Acyrthosiphon*

pisum (Du et al., 1998). Thus, *A. ervi*, when parasitizing aphids feeding on fabaceous plants, has a potential host range that includes *A. fabae* and the vetch aphid, *Megoura viciae*. However, this particular signal allows recognition of its specific host, *A. pisum*, which causes the plant to produce the signal. Studies on aphid elicitors of defence are progressing (Box et al., 2010; Pitino & Hogenhout, 2013; Zust & Agrawal, 2016), but such specificity has not yet been explained although, unlike other insect elicitors previously identified, they are characterized as aphid-derived effector proteins. The tetraterpenes are also ubiquitous in signalling foraging behaviour in a wide range of predators and parasitoids (Tholl et al., 2011). These compounds are highly volatile and unstable, but can be exploited by release from ‘push’ plants in the push–pull system, which is discussed later in Section VII. Also under investigation is engineering of genes for biosynthesis of their precursors as sesquiterpene and diterpene secondary alcohols, and for the oxidative production of the tetraterpenes (Lee et al., 2010; Brillada et al., 2013; Birkett & Pickett, 2014), potentially for exploitation by GM in answer to the challenge of targeting nonpheromonal signals for plant protection (see Section VIII).

The type of nonhost signalling considered previously can also include taxonomically specific signals because of the similarity, from an evolutionary standpoint, of nonhosts appearing as such through herbivore damage and via taxonomy to which the herbivore has not adapted. Indeed, although the plant volatile methyl salicylate was studied primarily as a plant stress-related signal (Shulaev et al., 1997; Agelopoulos et al., 1999), we had observed previously that it can indicate a plant as being a nonhost, as defined by taxonomy. This phenomenon was reported originally in host-alternating aphid species where a seasonal host, for example the winter or primary host, has nonhost characteristics for aphids searching for the summer or secondary host (Hardie et al., 1994; Pettersson et al., 1994). *cis*-Jasmone, formally related to the plant hormone jasmonic acid, was discovered initially as a host signal from *Ribis nigrum*, the winter host of the lettuce aphid, *Nasonovia ribis-nigri*, and is also responsible for its repellency from the summer host, lettuce, *Lactuca sativa* (Birkett et al., 2000). *cis*-Jasmone was subsequently shown to act generally as a repellent of insect herbivores and as a recruiting signal for higher trophic level antagonists, such as ladybirds and parasitoids of other insect species having a taxonomically diverse host range.

IV. Plant volatile-mediated signalling between plants via air

Related to nonhost signalling, plant-to-plant signalling is mostly considered as stress-related signalling from one plant to another of the same species, that is, phytopheromones, although other taxa can be responsive to the phytopheromones of particular species (Fig. 1). As a consequence of observing the wider role of *cis*-jasmone in signalling to higher trophic levels (Birkett et al., 2000), thereby recruiting insects attacking herbivores, further studies demonstrated a role in inducing plant defence, initially in *V. faba* and then in *Arabidopsis thaliana*, and crop plants including cereals (Bruce et al., 2008; Pickett et al., 2012). *cis*-Jasmone, although related to

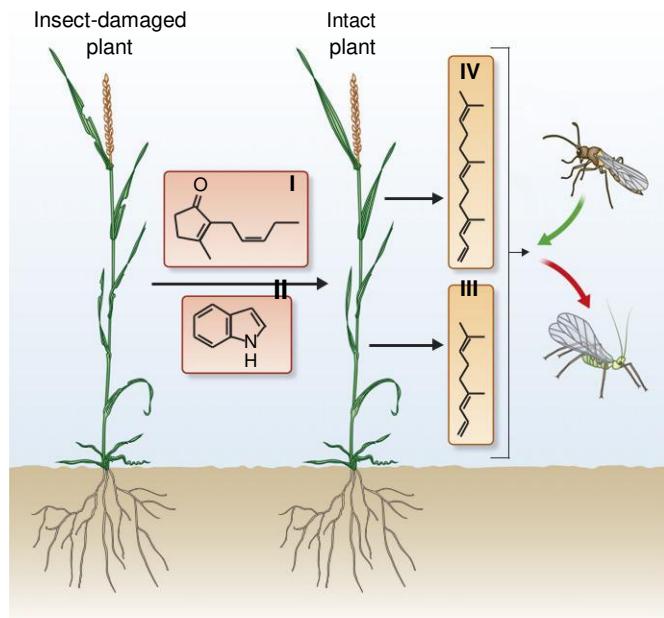


Fig. 1 Plants damaged by herbivore feeding, or plants imitating attacked plants, release stress-related signals such as (I) *cis*-jasmone (Bruce et al., 2008; Pickett et al., 2012) and (II) indole (Erb et al., 2015), which are selectively detected by intact plants. Indirect defence is then induced by the release of stress-related signals such as (III) (E,E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (IV) (E,E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT). Compounds III and IV are components of signals acting alone or in combination with other volatile stress-related compounds normally produced directly from damaged plants to repel herbivores, for example pests, and to attract predators or parasitoids that attack the herbivores. Although compounds comprising plant insect signalling can be released by plants constitutively, it is the raised concentration induced by damage in the contextual background of other constitutive signals that most often determines the defence role of these signals. Therefore, to make a crop plant repellent to pests and attractive to foraging beneficial insects such as parasitoids, it is possible merely to increase the release of even just one of the stress-related compounds, such as DMNT or TMTT. This presents an economy when exploiting plant volatile-mediated signalling by genetic modification (GM) (Birkett & Pickett, 2014).

jasmonic acid, signals differently (Matthes et al., 2010, 2011) and is probably produced, rather than from jasmonate via oxidative decarboxylation, via isomerization of 12-oxophytodienoic acid (Dabrowska et al., 2011), for which further evidence is emerging (Matsui et al., 2015). Nonetheless, *cis*-jasmone is volatile by merit of having lost the carboxylic acid group, whereas methyl jasmonate is volatile as a consequence of esterification (Birkett et al., 2000). This is analogous to the creation of the volatile, and thereby external, stress signal methyl salicylate by esterification of the plant stress hormone salicylate (Agelopoulos et al., 1999).

cis-Jasmone is capable of inducing defence in many plant species without the deleterious effects associated with methyl jasmonate and other jasmonates. *b*-Aminobutyric acid (BABA) is also known to prime plants (Bacelli & Mauch-Mani, 2015), although this can cause conflicting phytotoxic effects. Nonetheless, priming is a crucially important aspect of defence and in-depth studies with such tools are leading to a more exploitable understanding of this phenomenon (Balmer et al., 2015). Jasmonates can prime plants for defence, but the results can be erratic (Smart et al., 2013).

Although there are many underpinning issues, the molecular mechanisms by which a memory effect of jasmonate-mediated defence responses is obtained have been elucidated (Galis et al., 2009). For cis-jasmone, the priming effect can be potentially valuable, for example against leaf hoppers, such as *Cicadulina storeyi*, a vector of maize streak virus (Oluwafemi et al., 2013).

A number of other plant stress-related volatiles can also show induction of defence and priming, including lipoxygenase pathway products (Engelberth et al., 2004). Indole, a more recently identified stress-related volatile plant priming signal (Erb et al., 2015), shows considerable promise for practical development and also has a potential role in direct defence against herbivory (Veyrat et al., 2016). Successful experimental field trials in wheat against the grain aphid, *Sitobion avenae*, showed long-term protection after defensive genes were switched on by an electrostatically sprayed aqueous formulation of cis-jasmone as an emulsifiable concentrate (Bruce et al., 2003). In laboratory experiments, similarly encouraging results were obtained for cis-jasmone-induced attraction of the egg parasitoid, *Telenomus podisi*, of soybean stink bug pests (Moraes et al., 2009), antixenosis against the cotton aphid, *Aphis gossypii* (Hegde et al., 2012), and increased parasitoid behaviour against the aphid *Aulacorthum solani* on sweet pepper *Capsicum annuum* in the glasshouse (Dewhurst et al., 2012). The cis-jasmone-induced indirect defence, in each case, involved signalling with volatile oxidation products of the isoprenoid pathway, including the tetranorterpenes (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and TMTT. Extensive commercial field trials have been made with cis-jasmone as a defence elicitor on a range of crops around the world, but the results, although sometimes excellent, are too erratic for further development. As a consequence, growth stimulant effects were noticed, demonstrating a new role for cis-jasmone as a volatile plant-derived signal. Further patents were commercially filed, including for use in growth stimulation and greening in amenity turf (Skillman et al., 2011; Haas & Grimm, 2013; Haas et al., 2013). Such turf is a mixture of grasses, including the annual meadow or blue grass, *Poa annua*, which are largely unimproved genetically and are generally the same as wild-type species. We believe it is likely that there are wild-type traits promoting responses to natural elicitors, and that this relates to the more erratic nature of herbivory-induced defence volatiles found across commercial varieties (Kappers et al., 2011).

Because grasses are closely related to commercial cereal crops, understanding this phenomenon in grasses may give a lead to further genetic improvement of such crops. Also, the role of wild-type grasslands in providing ecosystem services, including habitats for beneficial insects, and in mitigating climate change by carbon sequestration (Lamb et al., 2016), could perhaps be exploited further via cis-jasmone signalling using a sentinel plant concept (Birkett & Pickett, 2014). A sensitive sentinel plant emitting cis-jasmone, when appropriate conditions appear, could switch on growth when nutrients, water and sunlight are not limiting in the main wild grassland stand or crop of related grasses. Nonetheless, more will need to be understood, particularly with regard to the transcriptional responses of these volatile plant-derived signals, before we can fully realize their potential (Paschold et al., 2006). However, new evidence of the potential regulation of

phytohormonal regulators such as cytokinins via bioactive responses to stress may show a putative mechanism for exploiting regulators such as cis-zeatin-type cytokinins (Schafer et al., 2015), using plant volatile-mediated signalling.

V. Plant volatile-mediated signalling between plants through soil

As long ago as 2001 (Birkett et al., 2001; Chamberlain et al., 2001) we, together with Emilio Guerrieri (Consiglio Nazionale delle Ricerche, Fransesco Pennacchio, University of Basilicata, Italy) and Guy Poppy (University of Southampton, UK) reported that, when plants were damaged by aphid feeding, signals passed through the natural rhizosphere to neighbouring undamaged plants, resulting in induced defence which included volatile-mediated signalling, negatively to aphids and positively to aphid parasitoids. This was also demonstrated when the plants were grown hydroponically and the signal remained in the aqueous medium after the damaged plant was removed, with the signal acting on an intact replacement plant. However, in spite of the convenience of being able to explore this phenomenon in an aqueous medium, we have not yet completed chemical characterization of the rhizosphere-signalling system, although we have characterized the resulting volatile-mediated signalling to insects at the two trophic levels.

More recently, together with David Johnson's group (University of Aberdeen, UK) and others, we have shown that an extremely powerful signalling system exists where aphid-damaged plants are connected by mycelial networks of arbuscular mycorrhizal fungi. Thus, bean plants, *V. faba*, rendered repellent to the pea aphid, *A. pisum*, and attractive to its parasitoid, *A. ervi*, by aphid feeding, transferred these properties to intact plants when connected via a shared mycorrhizal fungal network (Babikova et al., 2013a). Other potential connections were mechanically obstructed, leaving the mycelial network shown unambiguously to be responsible. There was no suggestion that plant volatile-mediated signals affecting the insect behaviour were translocated in the system. The systemic fungal signals travelling between plants through the rhizosphere by means of the fungal network will be difficult to capture for characterization, although molecular biological approaches may facilitate these further studies. It is evident that this signalling moves within the rhizosphere between plants relatively rapidly, that is, starting within 24 h from initial insect infestation (Babikova et al., 2013b), which implies a clear developmental benefit to the plants receiving the signal (Heil & Ton, 2008; Heil & Adame-Alvarez, 2010). The role of fungal networks in this general context is an expanding area of study (Pozo & Azcon-Aguilar, 2007; Song et al., 2010; Cosme et al., 2016), particularly in connection with induction of resistance to root pathogens (Whipps, 2004) and nematodes (de la Pena~ et al., 2006). The potential for direct transmission of soil allelochemicals via mycorrhizal networks has been considered (Barto et al., 2011) and these could potentially induce the volatile-mediated signalling by the signal-receiving plant. Although the likelihood of chemically mediated signalling in this system is widely acknowledged, it is suggested that electrical signalling may enable transmissions over relatively long distances (Johnson & Gilbert, 2014).

Surprisingly, transmission of volatile lipophilic compounds through the soil is relatively facile (Bateman et al., 1990; Chamberlain et al., 1991). This is not yet widely appreciated in the signalling literature. However, highly volatile pesticides such as tefluthrin (Jutsum et al., 1986) achieve valuable soil mobility against insect pests in the rhizosphere. These compounds are rendered volatile by incorporation of a high level of fluorine substitution which precludes molecular cohesion, as with perfluorocarbon polymers in ‘nonstick’ cooking and other devices. Turling’s group has dramatically demonstrated volatile-mediated rhizosphere signalling in which maize (*Zea mays*) plant roots, damaged by larvae of corn rootworm, *Diabrotica virgifera virgifera*, release the volatile sesquiterpene hydrocarbon (E)-(1R,9S)-caryophyllene to attract entomophagous nematodes (Rasmann et al., 2005). Thus, in a population density-dependent manner, a root-feeding herbivore uses an induced plant volatile as an aggregation cue (Robert et al., 2012a) and as a means to make host selection (Robert et al., 2012b). The value of this approach has been demonstrated in the field (Degenhardt et al., 2009), and approaches to raising the level of this type of volatile-mediated signalling in the rhizosphere are being explored by breeding and supplementing the entomophagous nematode population (K€ollner et al., 2008; Turlings et al., 2012).

Other types of volatile-mediated signalling in the rhizosphere are being studied, but the technology required for such work needs further improvement. For example, a recent demonstration of volatile-mediated signalling involving sesquiterpenes from ectomycorrhizal fungi influencing root architecture claims an isomer of thujopsene to be responsible (Ditengou et al., 2015). However, we notice that the work failed to characterize the compound by formally recognized analytical protocols, although an authentic sample of the thujopsene compound demonstrated the activity claimed.

It is suggested that volatile-mediated signalling in the rhizosphere can occur directly via common mycorrhizal networks between plants, providing a ‘network enhanced bioactive zone’ by which the volatile signals are preserved from soil degradation, and that this process enhances transmission between the plants (Barto et al., 2012). In that publication, the chemical signals are termed ‘infochemicals’, which is both technically and taxonomically unsatisfactory, as the term ‘semiochemical’ should be used where there is evidence of a signalling role. However, it is an interesting proposition. Further work would require studies targeting rhizosphere semiochemicals with narrow ranges of lipophilicity, for example, log/octanol/water coefficients (Chamberlain et al., 1996), to dissect, and test separately, various hypotheses relating to the physical properties of the semiochemicals by which they would influence the mode of transmission. This could then include a route via the internal cytoplasmic region of the hyphae and the air passage created as a hyphal cord interior. The subject of rhizosphere organisms using volatile signals in communicating with plants is rapidly expanding (Sanchez-Lopez et al., 2016). For example, the aerial volatile signal indole, referred to in Section IV (Erb et al., 2015), is also produced as a signal by rhizosphere organisms, thereby promoting root development by interfering with auxin signalling via the plant.

Another analogy between rhizosphere and terrestrial plant volatile-mediated signalling is that, just as plants linked by

arbuscular mycorrhizal fungi transfer messages between plants, causing stress-related signalling, so plants linked by parasitic plants such as the dodder, *Cuscuta pentagona*, may also signal between plants. In this case, mRNAs have been shown moving at high levels and in a bidirectional manner across the species (Kim et al., 2014). Thus, cues transmitted from the plants instigating plant signalling could be mediated by mRNAs. This is also recognized as being a general mechanism for communication between the kingdoms involving small RNA (sRNA)-mediated RNA interference (RNAi) (Weiberg et al., 2015). This could relate to the arbuscular mycorrhizal plant plant interactions that initiate indirect defence in unattacked plants (Babikova et al., 2013a).

Clearly, we need to work towards overcoming major challenges to using mycorrhizal signalling by further chemical and molecular characterization of the mechanisms by which volatile-mediated signalling is effected via rhizosphere signalling. However, in the meantime, it may be possible empirically to exploit, for example, common mycelial networks to induce defence in the main stand of crop plants after initial attack on sacrificial, susceptible sentinel plants grown as companion intercrops (Fig. 2). This could be attempted by choosing a fabaceous crop and establishing mycorrhizal connections to include susceptible plants, even comprising different species from the main crop, which would thereby perform the role of sentinel plants.

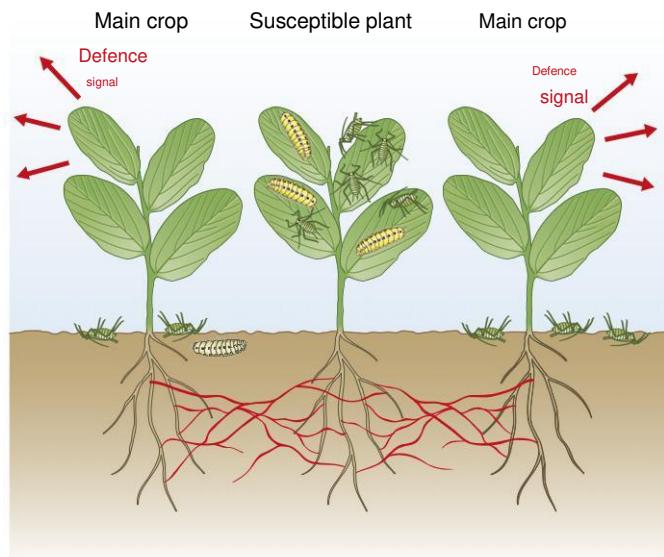


Fig. 2 Stress-related signals from damaged plants, for example elicited by herbivore attack, can pass through soil within the plant rhizosphere (Birkett et al., 2001; Chamberlain et al., 2001) and, more effectively, via shared arbuscular mycorrhizal fungal networks (see rhizosphere connections in red) to intact plants (Babikova et al., 2013a,b). These cause induction of volatile defence signals repelling herbivores and attracting parasitoids to attack the herbivores. This opens up the possibility of using susceptible plants within the main crop so that, when attacked, susceptible plants signal via mycorrhizal rhizosphere connections to the main crop. This would then mount defence when needed, rather than suffering the metabolic cost of constitutive defence normally provided by resistant crop plants.

VI. Plant volatile-mediated signal transduction

So far, we do not have a generic understanding of the signal transduction processes for volatile-mediated signalling to plants. Although the volatile small lipophilic molecules (SLMs) involved are often from structural groups related by their biosynthetic routes, it appears that the compounds are recognized as specific molecules, rather than there being generic ‘odour’ recognition (Birkett et al., 2000; Erb et al., 2015). This is analogous to animal and particularly insect olfaction, where specific molecular recognition is the normal process (Blight et al., 1989; Hansson et al., 1999), with apparent general recognition being only at very high stimulus concentrations. Clearly, lessons are to be learnt from hormone receptors and, not least, receptors for the strigolactones. These are carotenoid-derived plant hormones active externally in the rhizosphere, regulating development processes including plant, particularly root, architecture and availability of plant nutrients. In this system, recognition and response involve proteins described originally for both monocots and dicots as a/b-fold hydrolases, for example proteins such as D14 in rice (*Oryza sativa*), and generally leucine-rich-repeat F-box proteins discussed by Lechner et al. (2006), for example MAX2 referred to in *Arabidopsis* by Al-Babili & Bouwmeester (2015). The further transduction process is elaborated and reviewed, and the molecular recognition mechanism for the a/b-fold hydrolase type protein explained, by Seto & Yamaguchi (2014). Such developments have underpinned further structure function studies specifically targeting receptors in the parasitic weed genus *Striga*, which employ strigolactones in the rhizosphere for host location (Toh et al., 2015). Essential structural features of signalling strigolactones, involving the D-ring and its enzymatic detachment to give a hydroxybutenolide (Zwanenburg et al., 2016), relate to the volatile signal 3-methyl-2H-furo(2,3-c)-pyran-2-one (karrikinolide-1), which is released by the pyrolysis of plant tissue in wildfires and stimulates germination of the seeds of succession plants.

These hydroxybutenolide signals also have a structural analogy with volatile plant stress signals such as cis-jasmone. For this karrikinolide, it is clear that molecular recognition involves the a/b hydrolase proteins, including KA12 described in *Arabidopsis* by Guo et al. (2013). For cis-jasmone, the receptor system has not been elucidated. However, the up-regulation of specific genes by cis-jasmone in *Arabidopsis* may indicate involvement of, and specifically includes, an F-box protein gene (At2g4413036). Also up-regulated is a cytochrome p450, CYP81D11 (At3g28740), and this, by a protein interaction with cis-jasmone, could be the basis of recognition. Certainly, knockout plants interfering with the functionality of CYP81D11 are deficient in positive parasitoid responses found for the wild-type *Arabidopsis* (Matthes et al., 2010). A heptadeuterated cis-jasmone was synthesized (A. Hooper, unpublished), but appropriately labelled reaction products via the CYP81D11 could not be found (M. Birkett, unpublished). Volatile plant-derived signals can contribute directly to the signalling transduction process. For example, it has been shown that herbivore-damaged tomato (*Solanum lycopersicum*) plants release (Z)-3-hexenol. This is then taken up by intact plants and converted to (Z)-3-hexenylvicianoside which, via a mechanism

independent of jasmonates, negatively affects the performance of the common cutworm, *Spodoptera litura* (Sugimoto et al., 2014).

While we await definitive characterization of molecular recognition and transduction processes for volatile-mediated signalling to plants, we can use emerging synthetic biological approaches in the design of new signals and genes for their biosynthesis. For example, analogues of (S)-germacrene D, a potent stress related aphid repellent, cannot rationally be designed from docking studies with the associated olfactory proteins from the insects. However, a novel approach, in which nonnatural substrates of the plant synthase gene for (S)-germacrene D are fed to the enzyme, yields products that have sufficient similarity, in terms of the chemical space of the original ligand, that activity is rationally achieved (Touchet et al., 2015). The generality of the approach is now being

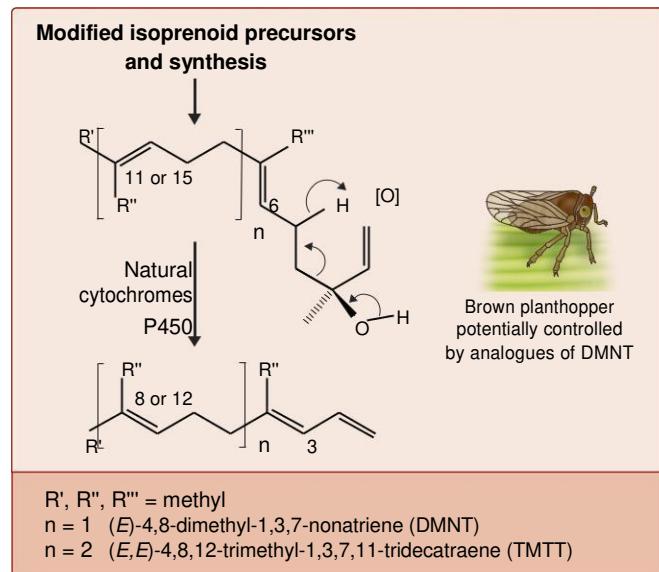


Fig. 3 Plant volatile-mediated signalling mostly relates to highly specific molecular recognition by the plant and, as a consequence, at higher trophic levels. Although some elements of volatile-mediated signal transduction have been elucidated, rational design of analogues of the natural signals is not yet possible. An alternative approach has been demonstrated, in which false substrates are fed to the final synthase enzyme for the signal and, where these substrates are converted, the signal analogue produced can have sufficient similarity to the chemical space of the natural signal for the analogue itself to be active (Touchet et al., 2015). This was demonstrated using the synthase gene for the plant stress-related signal (S)-germacrene D and highly active analogues were produced. Such an approach could be investigated for the unstable stress signals (E)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and (E)-4,8,12-trimethyl-1,3,7,11-tridecatetraene (TMTT), currently being developed against a rice pest, the brown planthopper, *Nilaparvata lugens*. Using natural cytochromes P450 in plants to exploit false substrate conversion as a criterion for producing active analogues, false substrates, for example where there is substitution into the methyl groups R^0 , R'' and R''' or cyclized analogues (e.g. between carbons 6 and 11 or 15), can be introduced into the isoprenoid precursor flux before the final signal synthesis. This process would exploit known biosynthetic routes to hormones with true homoterpene structures and other cyclic terpenes. Once active analogue signals are obtained, the natural cytochromes P450 used in this process could be mutated, as in the earlier example (Touchet et al., 2015), for greater efficiency in signal analogue production, taking into account structural differences in closely related cytochromes P450 (Bruce et al., 2008) for the process proposed.

explored with another plant-derived signal, epizingiberene, as a whitefly repellent (Allemann et al., 2016) and could be applied to other natural plant signals difficult to deploy directly, such as the tetranorterpenes DMNT and TMTT (Fig. 3).

VII. Success and lessons from exploiting plant volatile-mediated signalling by companion cropping: push–pull

The push–pull system has come to embody a platform for delivery of weed control, plant nutrition and forage for animal husbandry, in addition to pest management for smallholder cereal farmers in sub-Saharan Africa. However, it began as a companion cropping system against lepidopterous stemborer pests (Khan et al., 2014; Pickett et al., 2014). Initially, the cattle forage grass *Melinis minutiflora* provided a ‘push’ by repelling gravid stemborer moths, including the indigenous *Busseola fusca* and the exotic *Chilo partellus* from maize, with which it is grown as an intercrop. Although identified as having this role empirically, gas chromatography-coupled electroantennography (GC-EAG) and behavioural studies then demonstrated that the tetranorterpene DMNT was largely responsible for defending the maize with which *M. minutiflora* was intercropped. At the same time, it was discovered that this also resulted in substantially higher parasitism, for example by *Cotesia sesamiae*, of those stemborer larvae that were still able to infest the maize (Khan et al., 1997a). The ‘pull’ was provided by other cattle forage grasses, for example *Pennisetum purpureum* and *Sorghum vulgare sudanense*, that showed attractancy to gravid lepidopterous stemborers by release of high concentrations of ubiquitous plant volatile signals.

It has since been suggested that less agriculturally developed grasses such as *P. purpureum* and *Hyparrhenia rufa* release vastly more host recognition signals at the beginning of the scotophase than do cereal crop plants, contributing to the role of wild grasses as

superior hosts (Chamberlain et al., 2006). This offers a rare example, but one with growing exemplification (e.g. de Lange et al., 2016), of where there is an apparent evolutionary disadvantage for domesticated crop plants over related wild types. Nonetheless, the issue needs further study for wider exploitation. This also relates to earlier discussions on stress-related signalling to turf grasses, in which these less genetically improved species retain a greater signalling potential. A recent review of the mechanical framework of push–pull has made interesting observations on approaches to improve push–pull control of insects (Eigenbrode et al., 2016). It was suggested that this system, developed initially for pest management in sub-Saharan Africa, did not study short-range interventions between plants and insects. However, in the papers (Khan et al., 2006b, 2007), evidence is provided on the value of short-range attractancy by the trap crop *P. purpureum* to the stemborer moths *B. fusca* and *C. partellus*. Eigenbrode et al. (2016) propose various potential push–pull interventions but, for many, the signalling aspect is probably too weak to be effective and, also, the companion plants do not have value for farmers other than their role in crop protection.

In some regions where push–pull is practised, other intercrops have replaced *M. minutiflora*, for example forage legumes in the genus *Desmodium*, because, as well as controlling insect pests, plants in this genus specifically control parasitic weeds such as *Striga hermonthica*, in addition to fixing nitrogen within the system. This type of push–pull has been extended to many other cereal crops suffering damage by lepidopterous stemborers and parasitic weeds, including sorghum, *Sorghum bicolor* (Khan et al., 2006a), pearl millet, *Pennisetum glaucum*, finger millet, *Eleusine coracana* (Midega et al., 2010), and rain-fed rice, that is, NERICA (NEw RICE for Africa from *Oryza glaberrima* and *O. sativa*) (Pickett et al., 2010). New work on drought-tolerant companion crops for protecting sorghum, for example *Desmodium intortum* and the apomictic hybrid forage grass *Brachiaria Mulato II*, have been

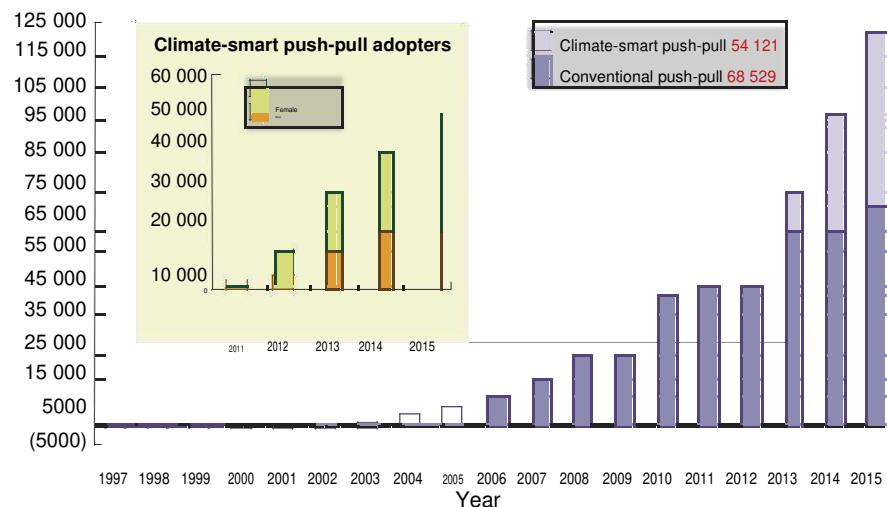


Fig. 4 Push–pull technology (Khan et al., 2014; Pickett et al., 2014) adoption rates have grown dramatically with the introduction of climate-smart (drought-tolerant) variants (Midega et al., 2015; Murage et al., 2015). From 2012, adoptions of the conventional push–pull levelled out, while the number of adopters of the climate-smart innovation grew exponentially. The rate of adoption of climate-smart push–pull by female farmers is significantly higher, and growing faster, than that by male farmers (inset), because of the technology’s labour-saving advantage. Women contribute most of the manual labour for weeding and for cut-and-carry fodder harvesting. The *Brachiaria* sp. used as trap and fodder crops in the climate-smart (Murage et al., 2015) push–pull system is easier to manage than the Napier grass, *Pennisetum purpureum*, used in the original push–pull.

developed to accommodate the aridification of cereal-growing regions as a consequence of climate change (Pickett et al., 2014; Midega et al., 2015; Murage et al., 2015). Take-up in smallholder farmsteads is over 120 000, with a considerably faster growth rate for climate-smart push–pull comprising drought-tolerant plants, and the increasing proportion of women 1.7 : 1 (Fig. 4) is showing that there is a preference for this technology by women farmers. Also, the clear economic value of the additional support for animal husbandry from cattle forage production provides an important economic driver for this push–pull system (Report of the Secretary-General of the United Nations, 2015). For Desmodium in the climate change-adapted push–pull system, there are clear and newly measured indications of carbon sequestration, prominent fixation of nitrogen and evidence of improved phosphorus availability in long-term studies on-farm (C. A. O. Midega, unpublished). This work also reports new, more highly drought-tolerant Desmodium species of African origin.

In terms of plant volatile-mediated signalling, new discoveries from maize crop plants offer further opportunities in agriculture. Smallholder cereal farmers benefiting from the push–pull system do not normally buy seasonal inputs of fertilizers, pesticides or seed. Although they, as a consequence, do not benefit from maize hybrid vigour, their self-saved seed, that is, seed from open-pollinated varieties (OPVs) such as Nymula and Jowi in western Kenya and the land races from which they have been locally adapted, show a signalling response directly to egg-laying by lepidopterous stemborers. Thus, the land race Cuba 91, and Nyamala and Jowi, bearing eggs laid by *C. partellus*, release volatile signals that recruit foraging by both egg parasitoids, for example *Trichogramma bournieri*, and larval parasitoids, for example *C. sesamiae* (Tamiru et al., 2011, 2012). This trait can be traced back to the maize ancestors, the teosintes (Mutyambai et al., 2015). This ‘smart’ trait is absent from most of the regionally commercially available, but unaffordable, hybrids. It is therefore now being investigated, both for exploitation in local breeding programmes and also, potentially, to sell, via IP protection in the interests of farmers from sub-Saharan Africa, to hybrid maize breeders in the North for invigoration of resistance to pests, and for insect control beyond Bt insect-resistant crops.

The companion crops, being largely unimproved genetically, show associated highly prominent signalling properties. For example, the intercrop *M. minutiflora* releases signals that induce indirect secondary defence signalling in the appropriate varieties of neighbouring maize plants. When one of the first used drought-tolerant species of perimeter (trap) crops (i.e. the equivalent of the ‘pull’ plants in the original push–pull system), *Brachiaria brizantha* (which comprises one of the parents of *Brachiaria Mulato II*), is exposed to eggs of *C. partellus*, the emission of the normally major volatile (Z)-3-hexenyl acetate was substantially reduced, while release of certain minor components increased. These changes served to reduce herbivory but increased foraging by the parasitoid *C. sesamiae* (Bruce et al., 2010). *Brachiaria brizantha* was also found, when exposed to the pest *C. partellus*, to signal to the OPVs Nymula and Jowi, and the land race Cuba 91, causing these plants to release volatile attractant signals, including the tetrancoterpenes DMNT and TMTT, for the parasitoid *C. sesamiae*. There is, in such experiments, always the possibility that, rather than a signal from a

damaged or otherwise stressed plant being received and causing secondary signalling, the original signal volatiles from the damaged plant could be absorbed and then re-emitted. However, neither of two physically similar hybrid maize varieties, Western Seed Hybrid 505 and Powani hybrid, produced the secondary defence signalling observed for the nonhybrids. These studies were facilitated by placing damaged plants and controls upwind of intact receiver plants on the bank of Lake Victoria at Mbita Point (International Centre of Insect Physiology and Ecology (ICIPE)), Kenya, so that the regular afternoon onshore breeze would take the plant plant signal volatiles to the recipient plants (Magara et al., 2015) (Fig. 5). Further work on proof of the signal response in such recipient plants is in progress. However, the use of inactive recipient hybrid varieties as controls seems to provide conclusive results and could be used in overcoming, more widely, the challenge of determining causal effects in signalling systems.

The value of wild grasses noted in the *cis*-jasmine work is recommended for further investigation. Indeed, we originally surveyed sub-Saharan African wild grass diversity for traits from which we obtained the original push–pull companion crops (Khan et al., 1997b). We now propose to make a similar survey, but by searching for wild plants particularly effective at signalling, and in terms of both receiving and responding to such signals. It may be possible to enlist amateur or citizen botanists for this purpose and, in sub-Saharan Africa, local interest in village-based herbal cures, particularly among women, could be enlisted for this purpose, which would in turn be of advantage to local agriculture. This would be facilitated by the climate-adapted push–pull already being taken up by the thousands of farmsteads showing further gender bias towards women.

VIII. Exploitation of plant volatile-mediated signalling in agriculture by GM

Understanding the process of volatile-mediated defence signalling can facilitate breeding programmes, not only using marker-assisted molecular breeding, but also by identifying the functional, as well as regulatory, genes for biosynthesis of the signals. Where these signals are secondary metabolites, or are related to them, GM is an obvious and more direct route. Initially, the biosynthesis of insect pheromones led the field in terms of biosynthesis genes, and plants have been transformed to do this principally for production purposes. However, the wide range of identified attractant pheromones, for example lepidopterous sex pheromones (Ding et al., 2014, 2016a), offers considerable opportunities for development of trap or ‘pull’ companion plants.

Alarm pheromones potentially offer opportunities for negatively affecting pest colonization and, in the 1980s, we suggested such a role for the aphid alarm pheromone if released from crop plants by GM technologies (Gibson & Pickett, 1983; Pickett, 1985). By 2006, we had demonstrated the principle of this in *A. thaliana* against the aphid *Myzus persicae*, and also for increased foraging behaviour by the parasitoid wasp *Diaeretiella rapae* (Beale et al., 2006) which, together with *M. persicae*, is adapted to brassicaceous plant systems. In wheat, we then expressed, as synthetic genes, the synthase genes for production of the precursor farnesyl diphosphate

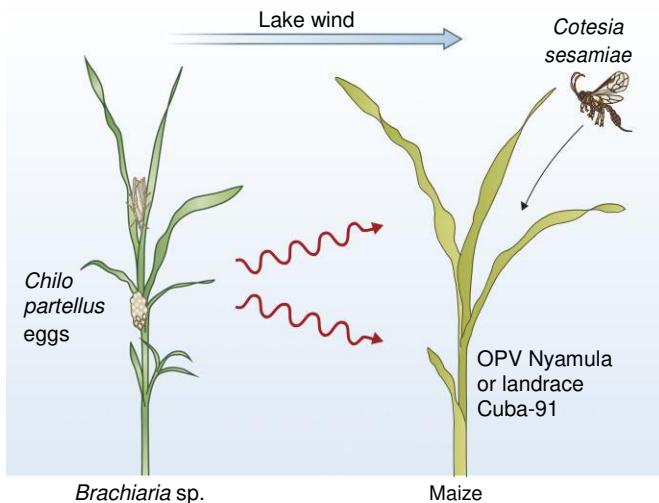


Fig. 5 Studies on plant signalling, at the International Centre of Insect Physiology and Ecology (ICIPE) Thomas Odhiambo Campus on the shores of Lake Victoria, Mbita Point, Kenya, prove that oviposition by the stemborer pest *Chilo partellus* on the signal grass *Brachiaria brizantha* induces defence in neighbouring maize plants, which are growing down-wind, i.e. further from the lake (Magara et al., 2015). Maize plants, not themselves exposed to stemborer eggs but exposed to *B. brizantha* bearing stemborer eggs, attracted the larval parasitoid *Cesamia sesamiae*, thus warding off further stemborer attack. Plants respond to attack by herbivores with the release of plant-mediated volatile signals. In return, natural enemies (predators and parasitoids) respond to these plant volatiles by foraging for their hosts. This tritrophic interaction leads to an 'indirect' plant defence that effectively recruits natural enemies. The extension of these studies indicates that oviposition by *C. partellus* on *B. brizantha* causes production of volatile signals that induce defence in smallholder farmers' own maize varieties (Nyamula and Jowi), and also a landrace maize from Latin America (Cuba 91), all of which attracted *C. sesamiae*, a parasitoid of *C. partellus*. In olfactometer bioassays, females of *C. sesamiae* were significantly more attracted to volatiles from the smallholder farmers' own maize varieties and the Latin America landrace maize when exposed to *B. brizantha* with *C. partellus* eggs than to volatiles from plants exposed to *B. brizantha* without *C. partellus* eggs. By contrast, hybrid maize did not show any induction of defence. These findings show promise for exploiting a highly sophisticated defence strategy in crop protection in smallholder crops, whereby parasitoids are recruited in advance, awaiting hatching of the eggs. When the eggs hatch, the larvae are attacked, stopping them from damaging the maize crop. This trait in

B. brizantha is now being tested further with other cereal crops, to assess the potential of its being used as a trap plant for developing new aspects of the push–pull system. OPV = open-pollinated variety.

and the pheromone (E)- β -farnesene, together with amino acid sequences for plastidial targeting. This was accomplished in 2012 in the contemporary elite wheat variety Cadenza and, with the single and double constructs, gave excellent repellency of cereal aphids and increased foraging by *A. ervi* in the laboratory. However, two spring sowings and a winter sowing, over 2012/2013, showed no evidence of aphid control or increased parasitism in the field (Bruce et al., 2015). The genetic engineering was highly successful, but constitutive expression may not be appropriate for a pheromone produced naturally as a short burst when aphids are attacked. We are therefore investigating new approaches to expression of the synthase genes, and thereby release of the pheromone in a manner more similar to that by aphids, by using plant defence elicitation, for example cis-jasmone signalling and aphid feeding-associated

induced effects. For the latter, a specific farnesyl diphosphate synthetic gene in wheat has been identified that is very rapidly up-regulated on aphid feeding (Zhang et al., 2015), and the promoter sequence for this gene is being investigated as a means of exploiting release of (E)- β -farnesene initiated by aphid feeding. The low levels of aphids and their parasitoids were also considered to be a problem in the field trials, so high parasitoid ecosystems will be targeted for future experiments.

It may be that pheromones derived from insects present wider problems for exploitation in crop plants by GM, and therefore stress-related plant volatile-mediated signalling is being targeted. Thus, as the value of tetranorterpenes has been clearly demonstrated in agriculture by companion cropping approaches (see Section VII), these now provide specific targets for new GM crops (Bruce et al., 2008; Lee et al., 2010; Matthes et al., 2010, 2011). The synthetic biological approach embodied in Fig. 3 would also provide novel synthetic genes for producing improved analogues.

In delivering approaches to exploiting plant volatile-mediated signalling by GM, insect-derived elicitors of plant defence signalling will also be crucial (see Section IV). Egg-associated elicitors would be particularly valuable (Hilker & Meiners, 2006), because such materials require little or no leaf tissue damage (Hinton, 1981) and so could be applied externally to crop plants. Although morphological studies on eggs of *C. partellus* have been reported, no indication of the induction of secondary defence was revealed (Deep & Rose, 2014). However, egg elicitors for *C. partellus* have now been tentatively identified and synthetic material is under bioassay in Kenya at Mbita Point (ICIPE). Next-generation sequencing (NGS) of plants, and particularly RNA sequencing (RNA-Seq) for investigating the signal generation and recognition, for example, of the egg-elicited process in plants, offer new and generic opportunities for identification of these elicitors.

The value of NGS-based associative transcriptomics of traits is recognized as a valuable tool (Harper et al., 2012) but, with the use of defence elicitors to create specific differences in the transcriptome, RNA-Seq quickly picks up the candidate genes for defence. The promoter sequences for defence genes, such as those for biosynthesis of plant volatile-mediated signalling, will also be valuable in switching on defence genes in GM plants, particularly as this switching can be readily linked to pest presence rather than being constitutively expressed, as are current GM insect resistance genes. Possibly, further engineering of induced or primed signalling gene expression could be effected by highly targeted gene-editing techniques such as CRISPR-Cas9 which, together with the associated gene drive, are proving in the laboratory to be extremely powerful tools with haematophagous insects (Gantz et al., 2015) and, more recently, with phytophagous insects in relation to plant volatile signalling (Koutroumpa et al., 2016).

Whether exploitation of plant volatile-mediated signalling is by GM or other means, a question always raised is: what of resistance by the pest or beneficial organism? The answer must always be that, where signals are deployed to the disadvantage of an organism, that is, protecting a valuable food source from herbivory or offering false signals to beneficial insects looking for prey, then resistance will develop. Indeed, there must be a large element of falseness in exploiting biological control by plant volatile-mediated signalling,

and recently a strong argument has been made for offering extra-floral nectar to mitigate the falseness of signalling, including to parasitoids and predators (Stenberg et al., 2015). For natural plant signals, we would need only to identify a new, naturally occurring signal. This is unlike the situation with pesticides where an entirely new toxophore would have to be developed, with a new mode of action to overcome resistance by target site modification, or a new type of chemical structure to deal with metabolic resistance. Without dramatic evolutionary changes, requiring at least speciation, the organisms developing resistance would need a signal with a new molecular structure but having the same evolutionary role.

Thus, we have a largely unappreciated but rational way by which to overcome resistance and to identify these new signals. The approach is the same as for the original signal and is by bioassay-guided fractionation. Electrophysiology coupled with gas chromatography, applied to the insects developing resistance, quickly points to new signal compounds, which are then identified with chemical analytical spectroscopy in conjunction with chemical synthesis. The plant genes to be used in the new resistance-defeating GM plants would follow the route also followed for identifying those for the original signal, and the compounds would be closely related. Although this may be considered to be optimistically speculative, evidence from animal signalling via pheromones shows that, under evolutionary pressure or merely species isolation, the biosynthesis and receptor molecular recognition systems change in synchrony during the selection process (Nieuwoudt et al., 2013; Unbehend et al., 2013; Martin et al., 2016). Already, we can see the mechanism by which new pheromonal components can be generated during evolution (Bucek et al., 2015; Ding et al., 2016b). Nonetheless, this process of overcoming resistance should be preserved for essential use by deployment of the plant-derived signals in integrated systems such as push–pull, and not using GM as the sole pest management tool.

IX. Conclusions

Considerable advances have been made in the engineering of plant secondary metabolism in crop plants by GM. Although this approach has only recently been applied to metabolites comprising plant volatile-mediated signals, already, by more conventional technologies, evidence has been provided that these agents show promise for the future in crop protection. Eventually, many aspects of plant volatile-mediated signalling may be delivered, without seasonal treatment, by sentinel plants which are problem-sensitive and which, after experiencing a threat or even an opportunity, signal to the main perennial crops. These then mount a response which could be enhanced by GM (Birkett & Pickett, 2014). This also presents a novel opportunity to increase the value of plants offering ecosystem services, as suggested in Fig. 6, as a way forward, beyond the sentinel concept expressed previously (Birkett & Pickett, 2014; Pickett, 2016).

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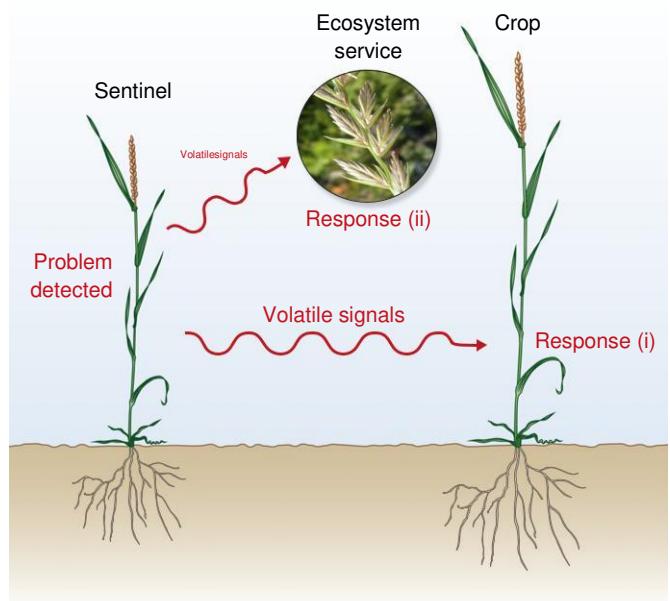


Fig. 6 In the original sentinel plant concept, susceptible plants respond to pest invasion, pathogen development and weed competition via promoter sequences from genes up-regulated in the early stages of such antagonism being used to express genes for highly chromophoric metabolites (e.g. anthocyanins), production thereby creating an optically readable warning for the onset of attack. This was then developed as a target for plant volatile-mediated signalling, whereby the susceptible plant would signal to the main crop to induce defence (Response (i)) (Birkett & Pickett, 2014; Pickett, 2016), as an alternative to potentially more metabolically expensive constitutive resistance. The susceptible plant could also be chosen or engineered to respond to opportunities, for example relating to photosynthesis, nutrients and water, to aid exploitation by the main crop. Ecosystem services are needed in the move towards more sustainable agriculture and can provide ways even of mitigating the carbon footprint of intensive agriculture production (Lamb et al., 2016), and could contribute to other types of ecosystem service. This could involve providing the parasitoid population from wild grasses exploited as conservation biological control, for example in the push–pull developed for sub-Saharan African cereal production (Khan et al., 2014; Pickett et al., 2014). As well as wild grasses retaining more of the ability to signal and respond to volatile signals compared with improved or hybrid crops, studying wild grasses could provide new genetic tools for exploitation of such signals and could open up the possibility of managing ecosystem services based on grasses (Response (ii)). Thus, ryegrass, *Lolium perenne*, a major host for the bird-cherry-oat aphid, *Rhopalosiphum padi*, the vector for barley yellow dwarf virus, an important economic constraint for wheat production, could be used to create large populations of parasitoids in conjunction with cereal production. The sentinel technology would be used to drive the parasitoids out of the ecosystem service provider and the parasitoids would then be captured by foraging signals from the crop. Such parasitoid dispersal signals are known from a higher trophic level interaction with hyperparasitoids (Holler et al., 1994).

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