



## Tansley review

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

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## Summary

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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, flourished 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 physicochemical 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. parasitoids), 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 electroantennography 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-deterrent 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)- $\beta$ -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 & Gershenzon, 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 C<sub>11</sub> and C<sub>16</sub> 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, C<sub>15</sub> (sesquiterpene) and C<sub>20</sub> (diterpene), are ubiquitous signals for host plants no longer valuable as hosts as a consequence of prior damage (Tholl *et al.*, 2011).

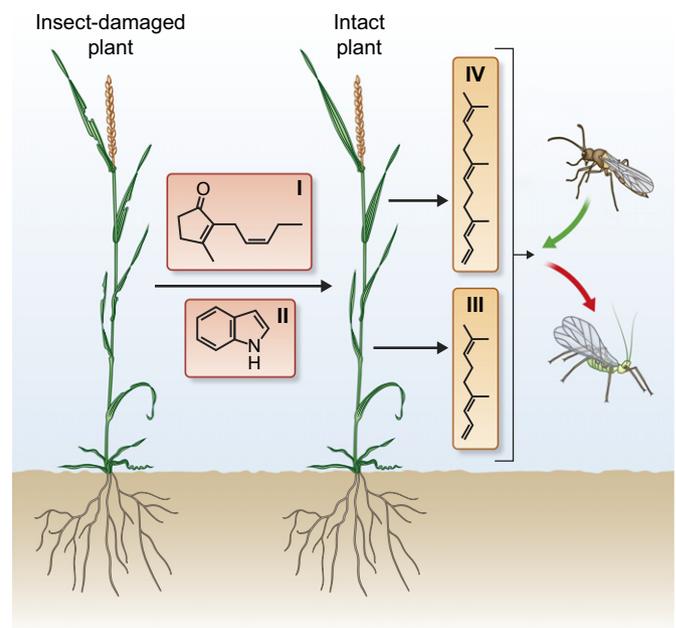
In certain ecological situations, the isoprenoid oxidation products such as 6-methyl-5-hepten-2-one can signal to higher trophic levels, for example the aphid parasitoid *Aphidius ervi*, indicating the presence of its host, the pea aphid, *Acyrtosiphon*

*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 tetranorterpene 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 tetranorterpene (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.  $\beta$ -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*-jasmonate, 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*-jasmonate as an emulsifiable concentrate (Bruce *et al.*, 2003). In laboratory experiments, similarly encouraging results were obtained for *cis*-jasmonate-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*-jasmonate-induced indirect defence, in each case, involved signalling with volatile oxidation products of the isoprenoid pathway, including the tetranorterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) and TMTT. Extensive commercial field trials have been made with *cis*-jasmonate 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*-jasmonate 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*-jasmonate signalling using a sentinel plant concept (Birkett & Pickett, 2014). A sensitive sentinel plant emitting *cis*-jasmonate, 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, Francesco 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 & Azcón-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 Peña *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*)-(1*R*,9*S*)-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 (Köllner *et al.*, 2008; Turlings *et al.*, 2012).

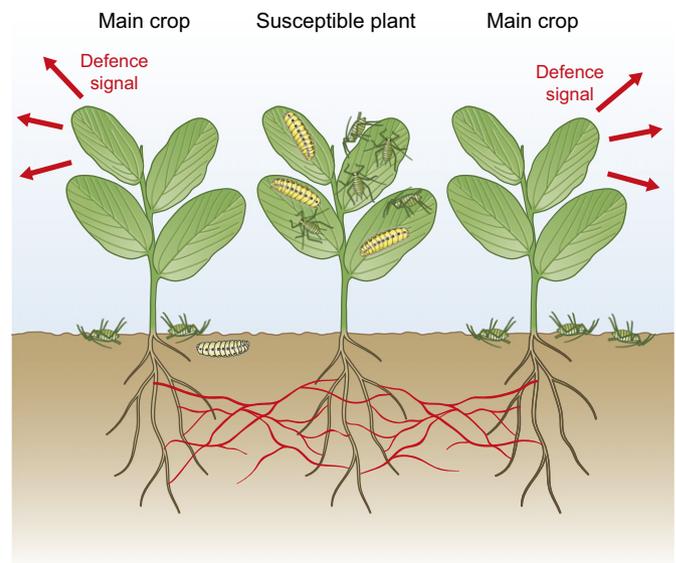
Other types of volatile-mediated signalling in the rhizosphere are being studied, but the technology required for such work needs further improvement. For example, a recent demonstration of volatile-mediated signalling involving sesquiterpenes from ectomycorrhizal fungi influencing root architecture 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 (Sánchez-López *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.

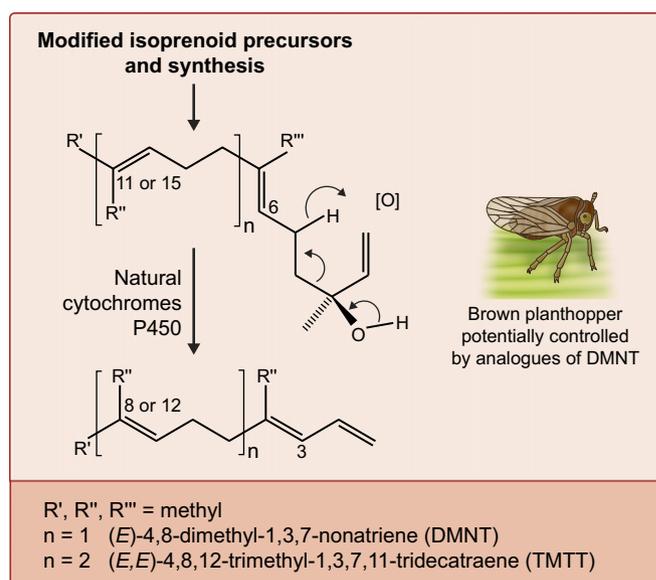
## VI. Plant volatile-mediated signal transduction

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  $\alpha/\beta$ -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  $\alpha/\beta$ -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-2*H*-furo(2,3-*c*)-pyran-2-one (karriginolide-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 karriginolide, it is clear that molecular recognition involves the  $\alpha/\beta$  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-hexenylvicinoside 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,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 *planta* 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', 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 tetranorterpene DMNT and TMTT (Fig. 3).

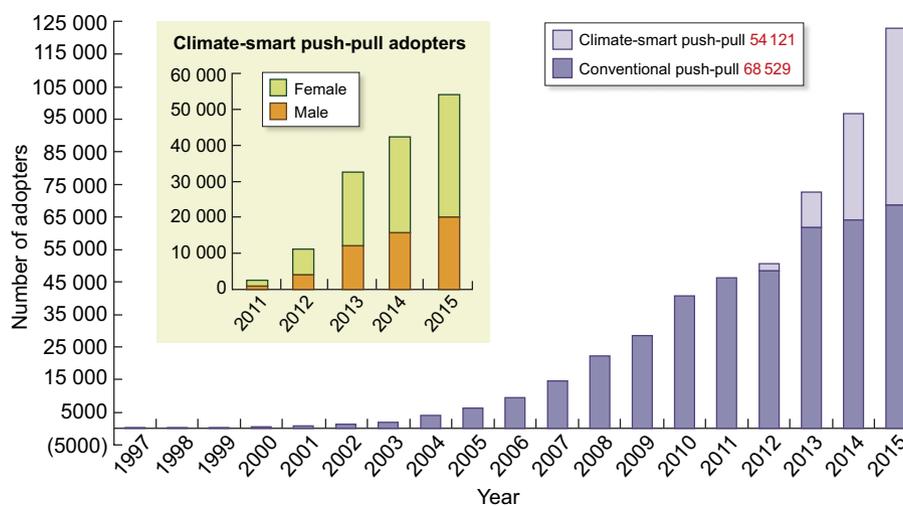
## VII. Success and lessons from exploiting plant volatile-mediated signalling by companion cropping: push–pull

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 stem-borers. 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 (Mutiyambai *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 tetranorterpene DMNT and TMTT, for the parasitoid *C. sesamiae*. There is, in such experiments, always the possibility that, rather than a signal from a

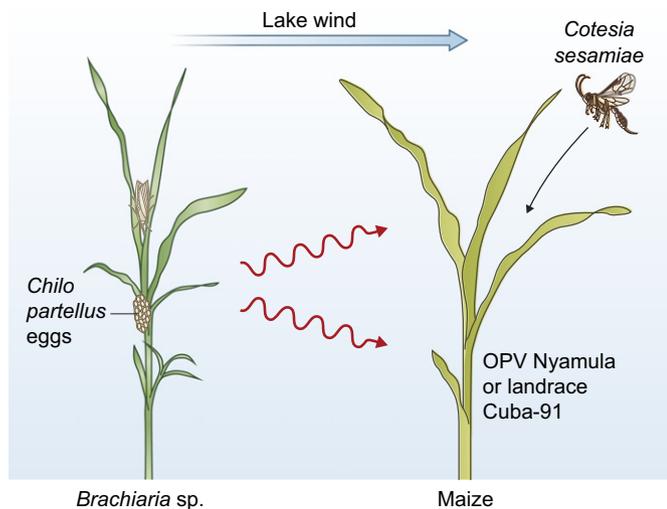
damaged or otherwise stressed plant being received and causing secondary signalling, the original signal volatiles from the damaged plant could be absorbed and then re-emitted. However, neither of two physically similar hybrid maize varieties, Western Seed Hybrid 505 and Powani hybrid, produced the secondary 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*-jasmone 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 *Cotesia 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*)- $\beta$ -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*-jasmonate 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*)- $\beta$ -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 extrafloral 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.

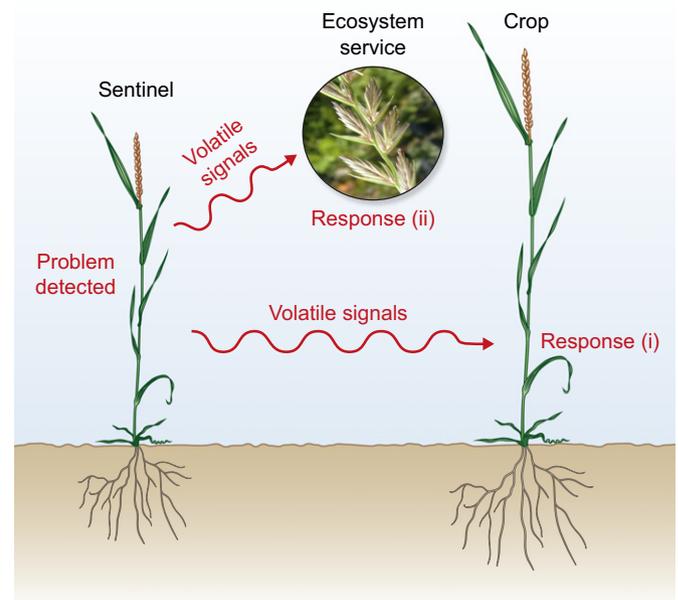
Thus, we have a largely unappreciated but rational way by which to overcome resistance and to identify these new signals. The approach is the same as for the original signal and is by bioassay-guided fractionation. Electrophysiology coupled with gas chromatography, applied to the insects developing resistance, 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 (Niehuis *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).

## Acknowledgements

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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 (Höller *et al.*, 1994).

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## References

- Agelopoulos N, Birkett MA, Hick AJ, Hooper AM, Pickett JA, Pow EM, Smart LE, Smiley DWM, Wadhams LJ, Woodcock CM. 1999. Exploiting semiochemicals in insect control. *Pesticide Science* 55: 225–235.

- Al-Babili S, Bouwmeester HJ. 2015. Strigolactones, a novel carotenoid-derived plant hormone. *Annual Review of Plant Biology* 66: 161–186.
- Alborn HT, Hansen TV, Jones TH, Bennett DC, Tumlinson JH, Schmelz EA, Teal PE. 2007. Disulfoxy fatty acids from the American bird grasshopper *Schistocerca americana*, elicitors of plant volatiles. *Proceedings of the National Academy of Sciences, USA* 104: 12976–12981.
- Alborn HT, Turlings TCJ, Jones TH, Stenhagen G, Loughrin JH, Tumlinson JH. 1997. An elicitor of plant volatiles from beet armyworm oral secretion. *Science* 276: 945–949.
- Alleman RK, Birkett MA, Miller D, Pickett JA. 2016. BBSRC research grant no. BB/N012526/1 Novel semiochemicals for crop protection through synthetic biology. [WWW document] URL <http://www.bbsrc.ac.uk/research/grants-search/AwardDetails?FundingReference=BB/N012526/1> [accessed 15 February 2016].
- Babikova Z, Gilbert L, Bruce TJA, Birkett MA, Caulfield JC, Woodcock CM, Pickett JA, Johnson D. 2013a. Underground signals carried through fungal networks warn neighbouring plants of aphid attack. *Ecology Letters* 16: 835–843.
- Babikova Z, Johnson D, Bruce TJA, Pickett JA, Gilbert L. 2013b. How rapid is aphid-induced signal transfer between plants via common mycelial networks? *Communicative & Integrative Biology* 6: 325904.
- Bacelli I, Mauch-Mani B. 2015.  $\beta$ -aminobutyric acid priming of plant defense: the role of ABA and other hormones. *Plant Molecular Biology* 91: 703–711.
- Baker T. 2009. Representations of odor plume flux are accentuated deep within the moth brain. *Journal of Biology* 8: 16.
- Baker TC, Fadamiro HY, Cosse AA. 1998. Moth uses fine tuning for odour resolution. *Nature* 393: 530.
- Bakry AM, Abbas S, Ali B, Majeed H, Abouelwafa MY, Mousa A, Liang L. 2015. Microencapsulation of oils: a comprehensive review of benefits, techniques, and applications. *Comprehensive Reviews in Food Science and Food Safety* 15: 1541–4337.
- Baldwin IT, Halitschke R, Paschold A, von Dahl CC, Preston CA. 2006. Volatile signalling in plant-plant interactions: “Talking trees” in the genomics era. *Science* 311: 812–815.
- Baldwin IT, Schultz JC. 1983. Rapid changes in tree leaf chemistry induced by damage: evidence for communication between plants. *Science* 221: 227–279.
- Balmer A, Pastor V, Gamir J, Flors V, Mauch-Mani B. 2015. The ‘prime-ome’: towards a holistic approach to priming. *Trends in Plant Science* 20: 443–452.
- Barto EK, Hilker M, Muller F, Mohny BK, Weidenhamer JD, Rillig MC. 2011. The fungal fast lane: common mycorrhizal networks extend bioactive zones of allelochemicals in soils. *PLoS ONE* 6: e27195.
- Barto EK, Weidenhamer JD, Cipollini D, Rillig MC. 2012. Fungal superhighways: do common mycorrhizal networks enhance below ground communication? *Trends in Plant Science* 17: 633–637.
- Bateman GL, Nicholls PH, Chamberlain K. 1990. The effectiveness of eleven sterol biosynthesis-inhibiting fungicides against the take-all fungus, *Gaeumannomyces graminis* var. *tritici*, in relation to their physical properties. *Pesticide Science* 29: 109–122.
- Baulcombe D. 2009. Reaping the benefits: Science and the sustainable intensification of global agriculture. The Royal Society Policy Report. [WWW document] URL <https://royalsocietypublishing.org/topics-policy/publications/2009/reaping-benefits/> [accessed 31 October 2016].
- Beale MH, Birkett MA, Bruce TJA, Chamberlain K, Field LM, Huttly AK, Martin JL, Parker R, Phillips AL, Pickett JA *et al.* 2006. Aphid alarm pheromone produced by transgenic plants affects aphid and parasitoid behaviour. *Proceedings of the National Academy of Sciences, USA* 103: 10509–10513.
- Birkett MA, Bruce TJA, Martin JL, Smart LE, Oakley J, Wadhams LJ. 2004. Responses of female orange wheat blossom midge, *Sitodiplosis mosellana*, to wheat panicle volatiles. *Journal of Chemical Ecology* 30: 1319–1328.
- Birkett MA, Campbell CAM, Chamberlain K, Guerrieri E, Hick AJ, Martin JL, Matthes M, Napier JA, Pettersson J, Pickett JA *et al.* 2000. New roles for *cis*-jasmone as an insect semiochemical and in plant defence. *Proceedings of the National Academy of Sciences, USA* 97: 9329–9334.
- Birkett MA, Chamberlain K, Hooper AM, Pickett JA. 2001. Does allelopathy offer real promise for practical weed management and for explaining rhizosphere interactions involving higher plants? *Plant and Soil* 232: 31–39.
- Birkett MA, Pickett JA. 2014. Prospects of genetic engineering for robust insect resistance. *Current Opinion in Plant Biology* 19: 59–67.
- Blight MM, Pickett JA, Wadhams LJ, Woodcock CM. 1989. Antennal responses of *Ceutorhynchus assimilis* and *Psylliodes chrysocephala* to volatiles from oilseed rape. *Aspects of Applied Biology* 23: 329–334.
- Blight MM, Smart LE. 1999. Influence of visual cues and the isothiocyanate lures on capture of the pollen beetle *Meligethes aeneus* in field traps. *Journal of Chemical Ecology* 25: 1501–1516.
- Box JIB, Prince D, Pitino M, Maffei ME, Win J, Hogenhout SA. 2010. A functional genomics approach identified candidate effectors from the aphid species *Myzus persicae* (green peach aphid). *PLoS Genetics* 6: e1001216.
- Brillada C, Nishihara M, Shimoda T, Garms S, Boland W, Maffei ME, Arimura G. 2013. Metabolic engineering of the C<sub>16</sub> homoterpene TMTT in *Lotus japonicus* through overexpression of (*E*, *E*)-geranylinalool synthase attracts generalist and specialist predators in different manners. *New Phytologist* 200: 1200–1211.
- Bruce TJA, Aradottir GI, Smart LE, Martin JL, Caulfield JC, Doherty A, Sparks CA, Woodcock CM, Birkett MA, Napier JA *et al.* 2015. The first crop plant genetically engineered to release an insect pheromone for defence. *Scientific Reports* 5: 118–125.
- Bruce TJA, Hooper AM, Ireland LA, Jones O, Martin JL, Smart LE, Oakley J, Wadhams LJ. 2007. Field trapping trials of pheromone baited traps for orange wheat blossom midge, *Sitodiplosis mosellana*. *Pest Management Science* 63: 49–56.
- Bruce TJA, Martin JL, Pickett JA, Pye BJ, Smart LE, Wadhams LJ. 2003. *cis*-Jasmone treatment induces resistance in wheat plants against the grain aphid, *Sitobion avenae* (Fabricius) (Homoptera: Aphididae). *Pest Management Science* 59: 1031–1036.
- Bruce TJA, Martin JL, Smart LE, Pickett JA. 2011. Development of a semiochemical baited monitoring trap for bean seed beetle, *Bruchus rufimanus*. *Pest Management Science* 67: 1303–1308.
- Bruce TJA, Matthes MC, Chamberlain K, Woodcock CM, Mohib A, Webster B, Smart LE, Birkett MA, Pickett JA, Napier JA. 2008. *cis*-Jasmone induces *Arabidopsis* genes that affect the chemical ecology of multitrophic interactions with aphids and their parasitoids. *Proceedings of the National Academy of Sciences, USA* 105: 4553–4558.
- Bruce TJA, Midega CAO, Birkett MA, Pickett JA, Khan ZR. 2010. Is quality more important than quantity? Insect behavioural responses to changes in a volatile blend after stemborer oviposition on an African grass. *Biology Letters* 6: 314–317.
- Bruce TJA, Pickett JA. 2011. Perception of plant volatile blends by herbivorous insects – finding the right mix. *Phytochemistry* 72: 1605–1611.
- Bucek A, Matouskova P, Vogel H, Sebesta P, Jahn I, Weißflog J, Svatos A, Pichova I. 2015. Evolution of moth sex pheromone composition by a single amino acid substitution in a fatty acid desaturase. *Proceedings of the National Academy of Sciences, USA* 112: 12586–12591.
- Chamberlain K, Bateman GL, Nicholls PH. 1991. Volatile analogues of penconazole and their activity against the take-all fungus, *Gaeumannomyces graminis* var. *tritici*. *Pesticide Science* 31: 185–196.
- Chamberlain K, Evans AA, Bromilow RH. 1996. 1-Octanol/water partition coefficient (Kow) and pKa for ionisable pesticides measured by a pH-metric method. *Pest Management Science* 47: 265–271.
- Chamberlain K, Guerrieri E, Pennachio F, Pettersson J, Pickett JA, Poppy GM, Powell W, Wadhams LJ, Woodcock CM. 2001. Can aphid-induced plant signals be transmitted aerially and through the rhizosphere? *Biochemical Systematics and Ecology* 29: 1063–1074.
- Chamberlain K, Khan ZR, Pickett JA, Tshova T, Wadhams LJ. 2006. Diel periodicity in the production of green leaf volatiles by wild and cultivated host plants of stemborer moths, *Chilo partellus* and *Busseola fusca*. *Journal of Chemical Ecology* 32: 565–577.
- Cook SM, Rasmussen HB, Birkett MA, Murray DA, Pye BJ, Watts NP, Williams IH. 2007. Behavioural and chemical ecology underlying the success of turnip rape (*Brassica rapa*) trap crops in protecting oilseed rape (*Brassica napus*) from the pollen beetle (*Meligethes aeneus*). *Arthropod-Plant Interactions* 1: 57–67.
- Cook SM, Smart LE, Martin JL, Murray DA, Watts NP, Williams IH. 2006. Exploitation of host plant preferences in pest management strategies for oilseed rape (*Brassica napus*). *Entomologia Experimentalis et Applicata* 119: 221–229.
- Cosme M, Ramireddy E, Franken P, Schmülling T, Wurst S. 2016. Shoot- and root-borne cytokinin influences arbuscular mycorrhizal symbiosis. *Mycorrhiza* 26: 1–12.
- Dabrowska P, Shabab M, Brandt W, Vogel H, Boland W. 2011. Isomerization of the phytohormone precursor 12-oxophytodienoic acid (OPDA) in the insect gut:

- a mechanistic and computational study. *Journal of Biological Chemistry* 286: 22348–22354.
- Deep DS, Rose HS. 2014. Study on the external morphology of the eggs of maize borer *Chilo partellus* (swinhoe). *Journal of Entomology and Zoology Studies* 2: 187–189.
- Degenhardt J, Hiltbold I, Kollner TG, Frey M, Gierl A, Gershenzon J, Hibbard BE, Ellersieck MR, Turlings TCJ. 2009. Restoring a maize root signal that attracts insect-killing nematodes to control a major pest. *Proceedings of the National Academy of Sciences, USA* 106: 17606.
- Dewhurst SY, Birkett MA, Loza-Reyes E, Martin JL, Pye BJ, Smart LE, Hardie J, Pickett JA. 2012. Activation of defence in sweet pepper, *Capsicum annuum*, by *cis*-jasmonate, and its impact on aphid and aphid parasitoid behaviour. *Pest Management Science* 68: 1419–1429.
- Ding BJ, Carraher C, Löfstedt C. 2016b. Sequence variation determining stereochemistry of a  $\Delta 11$  desaturase active in moth sex pheromone biosynthesis. *Insect Biochemistry and Molecular Biology* 74: 68–75.
- Ding BJ, Hofvander P, Wang HL, Durrett TP, Stymne S, Lofstedt C. 2014. A plant factory for moth pheromone production. *Nature Communications* 5: 3353.
- Ding BJ, Lager I, Bansal S, Durrett TP, Stymne S, Löfstedt C. 2016a. The yeast ATF1 acetyltransferase efficiently acetylates insect pheromone alcohols: implications for the biological production of moth pheromones. *Lipids* 51: 469–475.
- Ditengou FA, Muller A, Rosenkranz M, Felten J, Lasok H, von Doorn MM, Legue V, Palme K, Schnitzler JP, Polle A. 2015. Volatile signalling by sesquiterpenes from ectomycorrhizal fungi reprogrammes root architecture. *Nature Communications* 6: 6279.
- Du Y, Poppy GM, Powell W, Pickett JA, Wadhams LJ, Woodcock CM. 1998. Identification of semiochemicals released during aphid feeding that attract parasitoid *Aphidius ervi*. *Journal of Chemical Ecology* 24: 1355–1368.
- Eigenbrode SD, Nichloas A, Birch E, Lindzey S, Meadow R, Snyder WE. 2016. A mechanistic framework to improve understanding and applications of push-pull systems in pest management. *Journal of Applied Ecology* 53: 202–212.
- Engelberth J, Alborn HT, Schmelz EA, Tumlinson JH. 2004. Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences, USA* 101: 1781–1785.
- Erb M, Veyrat N, Robert CAM, Xu H, Frey M, Ton J, Turlings TCJ. 2015. Indole is an essential herbivore-induced volatile priming signal in maize. *Nature Communications* 6: 6273.
- Galis I, Gaquerel E, Pandey SP, Baldwin IT. 2009. Molecular mechanisms underlying plant memory in JA-mediated defence responses. *Plant, Cell & Environment* 32: 617–627.
- Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, James AA. 2015. Highly efficient Cas9-mediated gene drive from population modification of the malaria vector mosquito *Anopheles stephensi*. *Proceedings of the National Academy of Sciences, USA* 112: R6736–E6743.
- Gibson RW, Pickett JA. 1983. Wild potato repels aphids by release of aphid alarm pheromone. *Nature* 302: 608–609.
- Guo Y, Zheng Z, La Clair JJ, Chory J, Noel JP. 2013. Smoke-derived karrikin perception by the  $\alpha/\beta$  hydrolase KA12 from *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* 110: 8284–8289.
- Haas UJ, Grimm C. 2013. Patent no. WO 2013/037719 A1. [WWW document] URL <http://www.google.com/patents/WO2013037719A1?cl=zh> [accessed 21 March 13].
- Haas UJ, Grimm C, James JR. 2013. Patent no. WO 2013/037758 A1. [WWW document] URL <http://www.google.co.uk/patents/WO2013037758A1?cl=und> [accessed 21 March 13].
- Halkier BA, Gershenzon J. 2006. Biology and biochemistry of glucosinolates. *Annual Review of Plant Biology* 57: 303–333.
- Hansson BS, Larsson MC, Leal WS. 1999. Green leaf volatile-detecting olfactory receptor neurones display very high sensitivity and specificity in a scarab beetle. *Physiological Entomology* 24: 121–126.
- Hardie J, Isaacs R, Pickett JA, Wadhams LJ, Woodcock CM. 1994. Methyl salicylate and (-)-(1*R*,5*S*)-myrtenal are plant-derived repellents for black bean aphid, *Aphis fabae* Scop. (Homoptera: Aphididae). *Journal of Chemical Ecology* 20: 2847–2855.
- Harper AL, Trick M, Higgins J, Fraser F, Clissold L, Wells R, Hattori C, Werner P, Bancroft I. 2012. Associative transcriptomics of traits in the polyploid crop species *Brassica napus*. *Nature Biotechnology* 30: 798–802.
- Heath RR, Teal PEA, Tumlinson JH, Mengekko LJ. 1986. Prediction of release ratios of multicomponent pheromones from rubber septa. *Journal of Chemical Ecology* 12: 2133–2143.
- Hegde M, Nobre Oliveira J, da Costa JG, Bleicher E, Goulart Santana AE. 2012. Aphid antixenosis in cotton is activated by the natural plant activator *cis*-jasmonate. *Phytochemistry* 78: 81–88.
- Heil M, Adame-Alvarez RM. 2010. Short signalling distances make plant communications a soliloquy. *Biology Letters* 6: 843–845.
- Heil M, Ton J. 2008. Long distance signalling in plant defences. *Trends in Plant Science* 13: 264–272.
- Hilker M, Meiners T. 2006. Early herbivore alert: insect eggs induce plant defence. *Journal of Chemical Ecology* 32: 1379–1397.
- Hinton HE. 1981. *Biology of the insect egg, vol I–III*. Oxford, UK: Pergamon Press.
- Höller C, Micha SG, Schulz S, Francke W, Pickett JA. 1994. Enemy-induced dispersal in a parasitic wasp. *Experientia* 50: 182–185.
- Johnson D, Gilbert L. 2014. Interplant signalling through hyphal networks. *New Phytologist* 205: 1448–1453.
- Jutsum AR, Gordon RFS, Ruscoe CNE. 1986. Proceedings of British crop protection conference. *Pest Disease* 1: 97.
- Kappers IF, Hoogerbrugge H, Bouwmeester HJ, Dicke M. 2011. Variation in herbivory-induced volatiles among cucumber (*Cucumis sativus* L.) varieties has consequences for the attraction of carnivorous natural enemies. *Journal of Chemical Ecology* 37: 150–160.
- Khan ZR, Ampong-Nyarko K, Chiliswa P, Hassanali A, Kimani S, Lwande W, Overholt WA, Pickett JA, Smart LE, Wadhams LJ *et al.* 1997a. Intercropping increases parasitism of pests. *Nature* 388: 631–632.
- Khan ZR, Chiliswa P, Ampong Nyarko K, Smart LE, Polaszek A, Wandera J, Mulaa MA. 1997b. Utilisation of wild gramineous plants for management of cereal stem borers in Africa. *Insect Science and its Application* 17: 143–150.
- Khan ZR, Midega CAO, Hassanali A, Pickett JA, Wadhams LJ, Wanjoya A. 2006a. Management of witchweed, *Striga hermonthica*, and stem borers in sorghum, *Sorghum bicolor*, through intercropping with greenleaf desmodium, *Desmodium intortum*. *International Journal of Pest Management* 52: 297–302.
- Khan ZR, Midega CAO, Hutter NJ, Wilkins RM, Wadhams LJ. 2006b. Assessment of the potential of Napier grass *Pennisetum purpureum* Schumacher varieties as trap plants for management of *Chilo partellus* (Swinhoe) (Lepidoptera: Crambidae). *Entomologia Experimentalis et Applicata* 119: 15–22.
- Khan ZR, Midega CAO, Pittchar JO, Murage AW, Birkett MA, Bruce TJA, Pickett JA. 2014. Achieving food security for one million sub-Saharan African poor through push–pull innovation by 2020. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 369: 20120284.
- Khan ZR, Midega CAO, Wadhams LJ, Pickett JA, Mumuni A. 2007. Evaluation of Napier grass (*Pennisetum purpureum*) varieties for use as trap plants for the management of African stem borer (*Buseola fusca*) in a push–pull strategy. *Entomologia Experimentalis et Applicata* 124: 201–211.
- Kim G, LeBlanc ML, Wafula EK, dePamphilis CW, Westwood JH. 2014. Genomic-scale exchange of mRNA between a parasitic plant and its hosts. *Science* 345: 808–811.
- Köllner TG, Held M, Lenk C, Hiltbold I, Turlings TCJ, Gershenzon J, Degenhardt J. 2008. A maize (*E*)- $\beta$ -caryophyllene synthase implicated in indirect defense responses against herbivores is not expressed in most American maize varieties. *The Plant Cell* 20: 482–494.
- Koutroumpa FA, Monsempe C, Francois MC, Royer C, De Cian A, Concordet JP, Jacquin-Joly E. 2016. Heritable genome editing with CRISPR/Cas9 induced anosmia in a crop pest moth. *Scientific Reports* 6: 29620.
- Lamb A, Green R, Bateman I, Broadmeadow M, Bruce TJA, Burney J, Carey P, Chadwick D, Crane E, Field R *et al.* 2016. The potential for land sparing to offset greenhouse gas emissions from agriculture. *Nature Climate Change* 6: 488–492.
- de Lange ES, Farnier K, Gaudillat B, Turlings TCJ. 2016. Comparing the attraction of two parasitoids to herbivore-induced volatiles of maize and its wild ancestors, the teosintes. *Chemoecology* 26: 33–44.
- Lechner E, Achard P, Vansiri A, Potuschak T, Genschik P. 2006. F-box proteins everywhere. *Current Opinions in Plant Biology* 9: 631–638.

- Lee S, Badieyan S, Bevan DR, Herde M, Gatz C, Tholl D. 2010. Herbivore-induced and floral homoterpene volatiles are biosynthesized by a single P450 enzyme (CYP82G1) in *Arabidopsis*. *Proceedings of the National Academy of Sciences, USA* 107: 21205–21210.
- Lu Y, Wu K, Jiang Y, Guo Y, Desneux N. 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. *Nature* 487: 362–365.
- Magara HJO, Midega CAO, Otieno SA, Ogot CKPO, Bruce TJA, Pickett JA, Khan ZR. 2015. Signal grass (*Brachiaria brizantha*) oviposited by stemborer (*Chilo partellus*) emits herbivore-induced plant volatiles that induce neighbouring local maize (*Zea mays*) varieties to recruit cereal stemborer larval parasitoid *Cotesia sesamiae*. *International Journal of Sciences: Basics and Applied Research* 19: 341–357.
- Martin N, Moore K, Musto CJ, Linn CE Jr. 2016. Flight tunnel response of male European corn borer moths to cross-specific mixtures of European and Asian corn borer sex pheromones: evidence supporting a critical stage in evolution of a new communication system. *Journal of Chemical Ecology* 42: 51–54.
- Matsui R, Amano N, Takahashi K, Matsuura H. 2015. Biosynthetic pathway of *cis*-jasmane. *II International Symposium on Pyrethrum*. 39 Kyoto, Japan.
- Matthes MC, Bruce TJA, Chamberlain K, Pickett JA, Napier JA. 2011. Emerging roles in plant defence for *cis*-jasmane-induced cytochrome P450 CYP81D11. *Plant Signalling & Behaviour* 6: 1–3.
- Matthes MC, Bruce TJA, Ton J, Verrier PJ, Pickett JA, Napier JA. 2010. The transcriptome of *cis*-jasmane-induced resistance in *Arabidopsis thaliana* and its role in indirect defence. *Planta* 232: 1163–1180.
- Midega CAO, Bruce TJA, Pickett JA, Pittchar JO, Murage A, Khan ZR. 2015. Climate-adapted companion cropping increases agricultural productivity in East Africa. *Field Crop Research* 180: 118–125.
- Midega CAO, Khan ZR, Amudavi DA, Pittchar JO, Pickett JA. 2010. Integrated management of *Striga hermonthica* and cereal stemborers in finger millet (*Eleusine coracana* (L.) Gaertn), through intercropping with *Desmodium intortum*. *International Journal Pest Management* 56: 145–151.
- Miller JR, Cowles RS. 1990. Stimulo-deterrent diversionary cropping: a concept and its possible application to onion maggot control. *Journal of Chemical Ecology* 16: 3197–3212.
- Moraes MCB, Sereno FTPS, Micheriff MFF, Pareja M, Laumann RA. 2009. Attraction of the stink bug egg parasitoid, *Telenomus podisi* (Hymenoptera: Scelionidae) to defence signals from soybean, *Glycine max* (Fabaceae), activated by treatment with *cis*-jasmane. *Entomologia Experimentalis et Applicata* 131: 178–188.
- Murage AW, Midega CAO, Pittchar JO, Pickett JA, Khan ZR. 2015. Determinants of adoption of climate-smart push-pull technology for enhanced food security through integrated pest management in eastern Africa. *Food Security* 7: 709–724.
- Mutyambai DM, Bruce TJA, Midega CAO, Woodcock CM, Caulfield JC, Van Den Berg J, Pickett JA, Khan ZR. 2015. responses of parasitoids to volatiles induced by *Chilo partellus* oviposition on Teosinte, a wild ancestor of maize. *Journal of Chemical Ecology* 41: 323–329.
- Niehuis O, Buellesbach J, Gibson JD, Pothmann D, Hanner C, Mutti NS, Judson AK, Gadau J, Ruther J, Schmitt T. 2013. Behavioural and genetic analyses of *Nasonia* shed light on the evolution of sex pheromones. *Nature* 494: 345–348.
- Nottingham SF, Hardie J, Dawson GW, Hick AJ, Pickett JA, Wadhams LJ, Woodcock CM. 1991. Behavioural and electrophysiological responses of aphids to host and nonhost plant volatiles. *Journal of Chemical Ecology* 17: 1231–1242.
- Oliver JE, Doss RP, Williamson RT, Carney JR, De Vilbiss ED. 2000. Bruchidae-mitogenic 3-(hydroxy-propanoyl) esters of long chain diols from weevils of the Bruchidae. *Tetrahedron* 56: 7633–7641.
- Oluwafemi S, Dewhurst SY, Veyrat N, Powers S, Bruce TJA, Caulfield JC, Pickett JA, Birkett MA. 2013. Priming of production in maize of volatile organic defence compounds by the natural plant activator *cis*-jasmane. *PLoS ONE* 8: e62299.
- Paschold A, Halitschke R, Baldwin IT. 2006. Using 'mute' plants to translate volatile signals. *Plant Journal* 45: 275–291.
- de la Peña E, Echeverría SR, van der Putten WH, Freitas H, Moens M. 2006. Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*. *New Phytologist* 169: 829–840.
- Pettersson J, Pickett JA, Pye BJ, Quiroz A, Smart LE, Wadhams LJ, Woodcock CM. 1994. Winter host component reduces colonization by bird-cherry-oat aphid, *Rhopalosiphum padi* (L.) (Homoptera, Aphididae), and other aphids in cereal fields. *Journal of Chemical Ecology* 20: 2565–2574.
- Pickett JA. 1985. Production of behaviour-controlling chemicals by crop plants. *Philosophical Transactions of the Royal Society of London* 310: 235–239.
- Pickett JA. 2013. Food security: intensification of agriculture is essential, for which current tools must be defended and new sustainable technologies invented. *Food and Energy Security* 2: 167–173.
- Pickett JA. 2016. The essential need for GM crops. *Nature Plants* 2: 16078.
- Pickett JA, Aradottir GI, Birkett MA, Bruce TJA, Chamberlain K, Khan ZR, Midega CAO, Smart LE, Woodcock CM. 2012. Aspects of insect chemical ecology: exploitation of reception and detection as tools for deception of pests and beneficial insects. *Physiological Entomology* 37: 2–9.
- Pickett JA, Hamilton ML, Hooper AM, Khan ZR, Midega CAO. 2010. Companion cropping to manage parasitic plants. *Annual Review of Phytopathology* 48: 161–177.
- Pickett JA, Wadhams LJ, Woodcock CM. 1998. Insect supersense: mate and host location by insects as model systems for exploiting olfactory interactions. *Biochemist*: 8–13.
- Pickett JA, Woodcock CM, Midega CAO, Khan ZR. 2014. Push-pull farming systems. *Current Opinion in Biotechnology* 26: 125–132.
- Pitino M, Hogenhout SA. 2013. Aphid protein effectors promote aphid colonization in a plant species-specific manner. *Molecular Plant-Microbe Interactions* 26: 130–139.
- Pozo MJ, Azcón-Aguilar C. 2007. Unravelling mycorrhiza-induced resistance. *Current Opinion in Plant Biology* 10: 393–398.
- Rasmann S, Köllner TG, Degenhardt J, Hiltbold I, Toepfer S, Kuhlmann U, Gershenzon J, Turlings TCJ. 2005. Recruitment of entomopathogenic nematodes by insect-damaged maize roots. *Nature* 434: 732–737.
- Report of the Secretary-General of the United Nations. 2015. Agricultural technology for development. Page 11 [WWW document] URL [http://www.push-pull.net/UN\\_report\\_2015.pdf](http://www.push-pull.net/UN_report_2015.pdf) [accessed 26 October 2016].
- Robert CAM, Erb M, Duployer M, Zwahlen C, Doyen GR, Turlings TCJ. 2012b. Herbivore-induced plant volatiles mediate host selection by a root herbivore. *New Phytologist* 194: 1061–1069.
- Robert CAM, Erb M, Hibbard BE, French BW, Zwahlen C, Turlings TCJ. 2012a. A specialist root herbivore reduces plant resistance and uses an induced plant volatile to aggregate in a density-dependant manner. *Functional Ecology* 26: 1429–1440.
- Sánchez-López ÁM, Baslam M, De Diego N, Muñoz FJ, Bahaji A, Almagro G, Ricarte-Bermejo A, García-Gómez O, Li J, Humplík JF *et al.* 2016. Volatile compounds emitted by diverse phytopathogenic microorganisms promote plant growth and flowering through cytokinin action. *Plant, Cell & Environment*. doi: 10.1111/pce.12759.
- Schafer M, Brutting C, Meza-Canales ID, Grobkinsky DK, Vankova R, Baldwin IT, Meldau S. 2015. The role of *cis*-zeatin-type cytokinins in plant growth regulation and mediating responses to environmental interactions. *Journal of Experimental Botany* 66: 4873–4884.
- Scholz SS, Reichelt M, Mekonnen DW, Ludewig F, Mithöfer A. 2015. Insect herbivory-elicited GABA accumulation in plants is a wound-induced, direct, systemic, and jasmonate-independent defence response. *Frontiers in Plant Science* 6: 1128.
- Seto Y, Yamaguchi S. 2014. Strigolactone biosynthesis and perception. *Current Opinion in Plant Biology* 21: 1–6.
- Shulaev V, Silverman P, Raskin I. 1997. Airborne signalling by methyl salicylate in plant pathogen resistance. *Nature* 385: 718–719.
- Skillman SW, Grimm C, Johannes HU. 2011. Patent no. WO 2011/117272 A2. [WWW document] URL <http://www.google.com/patents/WO2011117272A2?cl=es> [accessed 29 November 2011].
- Smart LE, Blight MM, Hick AJ. 1996. Effect of visual cues and a mixture of isothiocyanates on trap capture of cabbage seed weevil, *Ceutorhynchus assimilis*. *Journal of Chemical Ecology* 23: 889–902.
- Smart LE, Martin JL, Limpalaër M, Bruce TJA, Pickett JA. 2013. Responses of herbivore and predatory mites to tomato plants exposed to jasmonic acid seed treatment. *Journal of Chemical Ecology* 39: 1297–1300.
- Song YY, Zeng RS, Xu JF, Li J, Shen X, Yihdego WG. 2010. Interplant communication of tomato plants through underground common mycorrhizal networks. *PLoS ONE* 5: e13324.

- Stenberg JA, Heil M, Ahman I, Björkman C. 2015. Optimizing crops for biocontrol of pests and disease. *Trends in Plant Science* 20: 698–712.
- Sugimoto K, Matsui K, Lijima Y, Akakabe Y, Muramoto S, Ozawa R, Uefune M, Sasaki R, Alamgir K, Akitake S *et al.* 2014. Intake and transformation to the glycoside of (*Z*)-3-hexenol from infested neighbors reveals a mode of plant odor reception and defense. *Proceedings of the National Academy of Sciences, USA* 111: 7144–7149.
- Tamiru A, Bruce TJA, Midega CAO, Woodcock CM, Birkett MA, Pickett JA, Khan ZR. 2012. Oviposition induced volatile emissions from African smallholder farmers' maize varieties. *Journal of Chemical Ecology* 38: 231–234.
- Tamiru A, Bruce TJA, Woodcock CM, Caulfield JC, Midega CAO, Ogot C, Mayon P, Birkett MA, Pickett JA, Khan ZR. 2011. Maize landraces recruit egg and larval parasitoids in response to egg deposition by a herbivore. *Ecology Letters* 14: 1075–1083.
- Tholl D, Sohrabi R, Huh J-H, Lee S. 2011. The biochemistry of homoterpenes – common constituents of floral and herbivore-induced plant volatile bouquets. *Phytochemistry* 72: 1635–1646.
- Toh S, Holbrook-Smith D, Stogios PJ, Onopriyenko O, Lumba S, Tsuchiya Y, Savchenko A, McCourt P. 2015. Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga*. *Science Magazine* 350: 203–207.
- Touchet S, Chamberlain K, Woodcock CM, Miller DJ, Birkett MA, Pickett JA, Allemann RK. 2015. Novel olfactory ligands via terpene synthases. *Chemical Communications* 51: 7550–7553.
- Turlings TCJ, Hiltbold I, Rasmann S. 2012. The importance of root-produced volatiles as foraging cues for entomopathogenic nematodes. *Plant and Soil* 358: 51–60.
- Unbehend M, Hanniger S, Meagher RL, Heckel DG, Groot AT. 2013. Pheromonal divergence between two strains of *Spodoptera frugiperda*. *Journal of Chemical Ecology* 39: 364–376.
- Veyrat N, Robert CAM, Turlings TCJ, Erb M. 2016. Herbivore intoxication as a potential primary function of an inducible volatile plant signal. *Journal of Ecology* 104: 591–600.
- Webster B, Bruce TJA, Dufour S, Birkemeyer C, Birkett MA, Hardie J, Pickett JA. 2008b. Identification of volatile compounds used in host location by the Black Bean Aphid, *Aphis fabae*. *Journal of Chemical Ecology* 34: 1153–1161.
- Webster B, Bruce TJA, Pickett JA, Hardie J. 2008a. Olfactory recognition of host plants in the absence of host-specific volatile compounds. *Communicative and Integrative Biology* 1: 167–169.
- Webster B, Bruce TJA, Pickett JA, Hardie J. 2010. Volatiles functioning as host cues in a blend become nonhost cues when presented alone to the black bean aphid. *Animal Behaviour* 79: 451–457.
- Webster B, Qvarfordt E, Olsson U, Glinwood R. 2013. Different roles for innate and learnt behavioural responses to odors in insect host location. *Behavioural Ecology* 24: 366–372.
- Weiberg A, Bellinger M, Jin H. 2015. Conversations between kingdoms: small RNAs. *Current Opinions in Biotechnology* 32: 207–215.
- Whipps JM. 2004. Prospects and limitations for mycorrhizas in biocontrol of root pathogens. *Canadian Journal of Botany* 82: 1198–1227.
- Zhang Y, Li ZX, Yu XD, Fan J, Pickett JA, Jones HD, Zhou JJ, Birkett MA, Caulfield JC, Napier JA *et al.* 2015. Molecular characterization of two isoforms of a farnesyl pyrophosphate synthase gene in wheat and their roles in sesquiterpene synthesis and inducible defence against aphid infestation. *New Phytologist* 206: 1101–1115.
- Zust T, Agrawal AA. 2016. Mechanisms and evolution of plant resistance to aphids. *Nature Plants* 2: 15206.
- Zwanenburg B, Pospíšil T, Zeljković SC. 2016. Strigolactones: new plant hormones in action. *Planta* 243: 1311–1326.



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