Chemical ecology and conservation biological control

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Abstract

Elucidating the chemical ecology of natural enemies, herbivores and host plants is important in the development of effective and successful integrated pest management (IPM) strategies where abundance and distribution of natural enemies could be manipulated by semiochemicals for improved conservation biological control (CBC). In response to attack by herbivores, plants produce semiochemicals called Herbivore-Induced Plant Volatiles (HIPVs) which act to repel pests and attract their natural enemies. Damaged, and in some cases, intact plants may also produce volatile signals that warn other plants of impending attack. Some of these intact plants are used as intercrops in ‘push–pull’ strategies; cropping systems based on stimulo-deterrent principle, where the target crop is intercropped with herbivore repellent plants (push) while attractant plants (pull) are planted around this intercrop. The intercrop, in addition to repelling the herbivores, attracts and conserves natural enemies thereby ensuring continued suppression of the pests. This natural delivery of semiochemicals for CBC is currently being exploited by smallholder farmers in eastern Africa in the management of cereal stem borers in maize and sorghum. Synthetic HIPVs also have the potential to effectively recruit natural enemies, thereby improving CBC as has been demonstrated in a series of field experiments in vineyards and hop yards in the Pacific Northwest of the United States. Potentially, plants could be ‘turned on’ by synthetic HIPV signals, and therefore become sources of natural enemy-recruiting volatiles. With the rapid development of plant molecular biology, modification of secondary plant metabolism is also possible which could allow appropriate semiochemicals to be generated by plants at certain growth stages. By identifying the promoter sequences associated with external plant signals that induce biochemical pathways, plant defense genes could be ‘switched on’ prior to insect attack. We review recent research on ‘push–pull’ strategies and synthetic HIPVs in recruitment of beneficial arthropods and warding off pest attack.

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1. Introduction

Attraction of insects to plants and other host organisms involves detection of specific semiochemicals (natural signal chemicals mediating changes in behavior and development) or specific ratios of these semiochemicals (Pickett et al., 2006). Plants colonized and damaged by herbivorous insects produce a group of volatile organic compounds (VOCs) often referred to as Herbivore-Induced Plant Volatiles (HIPVs), which may include semiochemicals that act as repellents for herbivorous pests and as attractants for organisms antagonistic to these pests, such as predators and parasitoids. In the dual-purpose role, these signals indicate that the plant is already infested and, therefore, less suitable as a host but, on the other hand, they may increase foraging by predators and parasitoids (Pickett et al., 2006).

It is widely accepted that plants respond to attack by specific herbivore species through their induced direct and indirect defenses (Karban and Baldwin, 1997; Lou et al., 2006). In direct defenses, the chemicals target the herbivore resulting in its retarded development or death (Lou and Baldwin, 2003), whereas in indirect defenses, the chemicals (i.e., HIPVs) increase herbivore mortality through the recruitment of parasitoids and predators (Thaler, 1999;
Kessler and Baldwin, 2001). Studies on the mechanisms leading to the production of HIPVs have revealed the role of herbivore-specific elicitors (Mattiacci et al., 1995; Alborn et al., 1997; Halitschke et al., 2001). These elicitors can activate various signaling pathways in the plant resulting in accumulation or release of defensive chemicals (Kessler and Baldwin, 2002). Additionally, we have observed that there are also intact plants that naturally produce similar VOCs without any herbivore damage (Khan et al., 1997a). Biological control agents (natural enemies) use a range of these VOCs to locate their prey.

Conservation biological control (CBC) is an approach that seeks to preserve the resident natural enemy populations in a cropping setting and enhance their abundance and activity, particularly through cultural techniques. Exploitation of chemical ecology in this approach involves incorporating practices that attract these natural enemies into the cropping system while providing suitable nutrient sources within the system. The latter involves provision of sources of nectar and other carbohydrates such as honeydew that enhance the longevity and fecundity of natural enemies thereby maximizing their impact on pest populations. Alternatively, refuges in the form of unsprayed plants or provision of alternative hosts that feed on deliberately sown and managed grasses, trap crops, windbreaks or hedgerows can significantly enhance and maintain natural enemies in and around crops.

CBC research in many cropping systems is frequently focused on improving the reliability of this strategy to suppress pests. Strengthening the natural enemy community both in terms of population density and species diversity is the aim of much of this research (Cardinale et al., 2003). Inevitably there are two aspects of this problem that need to be addressed: (1) attraction of beneficial arthropods to the crop during early cropping phases and (2) maintenance of these populations throughout the life of the crop. Manipulation of on-farm habitats to improve the attractiveness of crop ecosystems to beneficial arthropods is a major area of current research (Landis et al., 2000). This strategy is based on the idea that providing more and better resources (e.g., nectar, refugia) will allow larger populations of beneficial arthropods to reside in and near crops. Numerous examples of the potential and practicality of such approaches are available (Landis et al., 2000; Midega and Khan, 2003; Gurr et al., 2004; Midega et al., 2006; Koji et al., 2007). One of the most practical of these approaches is the ‘push–pull’ strategy (Cook et al., 2007). ‘Push–pull’ is a novel approach in integrated pest management (IPM) which uses a repellent intercrop and an attractive trap plant. Insect pests are repelled or deterred away (push) from the main crop and are simultaneously attracted to a trap crop (pull). The ‘push–pull’ strategy uses a combination of behavior-modifying stimuli to manipulate the distribution and abundance of insect pests and/or natural enemies (Khan et al., 1997a,b; Cook et al., 2007; Hassanali et al., 2008). Accordingly, we discuss how chemical ecology is exploited in conservation of natural enemies using Herbivore-Induced Plant Volatiles and ‘push–pull’ strategies.

2. Exploitation of chemical ecology in CBC: an overview

Predators and parasitoids, like their prey and hosts, often use sex or aggregation pheromones to bring the sexes together for mating. In this developing research area, the identification, synthesis and use of pheromones to manipulate populations of natural enemies in pest management are potentially important (Aldrich, 1999; Wermelinger, 2004). One of the few examples of commercialization of a predator pheromone to enhance biological control is the aggregation pheromone of the spined soldier bug, Podisus maculiventris (Say). Spined Soldier Bug Attractors™ (Sterling International, Veradale, WA, USA) are mainly targeted at the home garden market, but this approach has also been used to suppress Colorado potato beetle, Leptinotarsa decemlineata Say, populations in potato fields (Aldrich, 1999). The use of semiochemical attractants (e.g., host/prey-derived chemicals) to increase recruitment and retention of beneficial arthropods in crop ecosystems is another area of opportunity for enhancement of CBC. Kean et al. (2003) identified ‘spatial attraction’ of natural enemies as the best way of enhancing CBC. Their results suggested an almost linear relationship between natural enemy attraction and prey equilibrium.

Although the exploitation of semiochemical attractants in pest management to date is limited, research on semiochemicals and the natural enemies of herbivores has expanded greatly in recent years (Pickett et al., 2006). Suggestions for possible utilization of these chemicals in pest management include (1) enhancing searching efficiency of natural enemies (Lewis and Martin, 1990), (2) bringing natural enemies into search mode (Gross, 1981), and (3) making novel or artificial host–prey species acceptable in a mass rearing program (Vinson, 1986). A good example of enhanced searching efficiency is manipulation of the green lacewing, Chrysoperla carnea (Stephens), through the use of tryptophan (Hagen et al., 1976). Tryptophan is present in honeydew (a sugary by-product of feeding by aphids, scale insects, etc.) and spraying honeydew in crops can attract chrysopid lacewings (McEwen et al., 1994). Planting molasses grass, Melinis minutiflora Beauv., between the rows of maize significantly increases the parasitism of maize stemborer larvae by Cotesia sesamiae (Cameron) (Khan et al., 1997a) and is being used as a strategy to control stemborers in eastern Africa (Khan et al., 2001 and this review). More recently, 2-phenylethanol, a volatile emitted from some plant species, was also found to be attractive to lacewings, particularly female C. carnea, indicating that this compound may be an ovispositional stimulant (Zhu et al., 1999). This volatile is also attractive to some lady beetle species and a predator lure based on this compound has recently been developed and marketed as Benallure® (MSTRS Technologies, Ames, IA, USA).
3. Herbivore-Induced Plant Volatiles (HIPVs)

3.1. The potential of synthetic HIPVs as tools for recruitment of beneficial insects to agroecosystems: field experiments in the Pacific Northwest USA

The science of HIPVs represents one area of chemical ecology research that has great potential for developing effective and practical semiochemical-based strategies for manipulating natural enemy populations in crop pest management. In essence, plants attacked by herbivores emit specific chemical signals. These are the ‘words’ of a complex language used to ‘warn’ other plants of impending attack and to recruit predatory/parasitic arthropods for ‘bodyguard’ services. Such plant ‘bodyguards’ respond to the language of plants in distress and benefit from the ‘bodyguard’ services. Such plant ‘bodyguards’ respond to the language of plants in distress and benefit from the ‘bodyguard’ services. Such plant ‘bodyguards’ respond to the language of plants in distress and benefit from the ‘bodyguard’ services. Such plant ‘bodyguards’ respond to the language of plants in distress and benefit from the ‘bodyguard’ services.

Recently, synthetic versions of HIPVs have been used to either attract predators and parasitoids or to induce plants to produce their own HIPVs in field experiments. Thus, synthetic jasmonic acid applied directly to crop plants elicited production of HIPVs and increased parasitism of caterpillar pests (Thaler, 1999). Kessler and Baldwin (2001) showed that synthetic HIPVs incorporated in lanolin paste applied near eggs of a moth increased predation by a predatory bug. The first demonstration of the potential of synthetic HIPV as direct field attractants for beneficial insects was provided by James (2003a,b,c). These studies showed attraction of a number of insect species and families to methyl salicylate (MeSA) and (Z)-3-hexenyl acetate (HA) in a Washington hop yard. Insects attracted to MeSA included the green lacewing, Chrysopa nigricornis Burmeister (Chrysopidae), the bigeyed bug, Geocoris pallens Stal. (Geocoridae), the mite-eating ladybeetle, Steathor punctum picipes (Casey) (Coccinellidae) and species of hoverflies (Syrphidae). Three beneficial species were attracted to HA, a predatory mirid bug, Deraeocoris brevis Uhler, a minute pirate bug, Orius tristicolor (White) and S. punctum picipes. Subsequent synthetic HIPV/trapping studies revealed at least 13 species or families of beneficial insects responded to one or more synthetic HIPVs. Thirteen HIPVs attracted one or more species/family of beneficial insect (summarized in Table 1 and in James, 2005).

Evidence for recruitment and retention of beneficial insects in grapes and hops in experiments in 2003 using controlled-release dispensers of MeSA was presented in James and Price (2004). In a replicated experiment conducted in a grape vineyard, sticky cards in blocks baited with MeSA captured significantly greater numbers of five species of predatory insects (C. nigricornis, Hemerobius sp., D. brevis, S. punctum picipes, O. tristicolor) than sticky cards in unbaited blocks. Four insect families (Syrphidae, Braconidae, Empididae, Sarcophagidae) were also significantly more abundant in the MeSA-baited blocks, as indicated by sticky card captures. Canopy shake samples and sticky card monitoring conducted in a MeSA-baited, unsprayed hop yard indicated development and maintenance of a beneficial arthropod population that was nearly four times greater than that present in an unbaited reference yard. Four times as many S. punctum picipes and six...
times as many *O. tristicolor* were sampled in the MeSA yard. Similar contrasts in abundance of these predators and others were apparent when compared with levels recorded in the yard in previous years. The large population of predatory insects in the MeSA-baited hop yard was associated with a dramatic reduction in spider mite and aphid numbers, the major arthropod pests of hops, in late June and sub-economic populations were maintained for the rest of the season. Further data on the effects of MeSA dispensers in enhancing CBC in hops and grapes were provided in James et al. (2005). The evidence presented in these papers and James and Price (2004) shows that the use of controlled-release MeSA in crops can potentially increase recruitment and residency of populations of certain beneficial insects improving CBC. Lower deployment rates of MeSA dispensers (180/ha) appear to recruit/retain larger populations of beneficial insects in hops than higher rates (444–556/ha) (Figs. 1 and 2).

Field data for a possible plant-signaling function of MeSA and HA in eliciting indirect defense responses in plants was obtained in a more recent study. The abundance of some carnivorous and parasitic insects was significantly increased near hop and grape plants sprayed with botanical oil pesticides containing MeSA and HA (James, 2008). It seems likely that the plants were ‘signaled’ by MeSA, HA or other plant-derived compounds to emit a blend of volatiles to recruit ‘bodyguards’. Exposure to MeSA and HA was likely interpreted by the plants as evidence of pest attack against nearby plants and a warning to defend themselves. Charleston et al. (2006) showed application of a botanical extract made from the syringa tree, *Melia azedarach* L. to cabbage plants, increased plant volatile emission and attracted the wasp parasitoid, *Cotesia plutellae* (Kurdjumov). The ‘signaling’ compound in *M. azedarach* was not identified. These results provide further evidence of elicitor roles for MeSA and HA with optimism that these compounds can be used in crop protection programs to improve CBC.

The use of botanical oil-based pesticides in combination with HIPVs like MeSA and HA, as a strategy for alerting plants to a herbivore threat and inducing natural defenses, may have great potential as a crop protection strategy. In three field experiments conducted on hops and grapes, plants sprayed with canola oil or peppermint/rosemary oil pesticides formulated with small concentrations of the HIPVs, MeSA or HA, attracted significantly greater numbers of some predatory and parasitic insect species than unsprayed plants (James, 2008). Hop plant cultivar
strongly influenced the results obtained in this study, suggesting that botanical oil/HIPV induced plant volatile emissions varied qualitatively and/or quantitatively according to cultivar, as has been demonstrated for other plant species (Gouinguene et al., 2001; Lou et al., 2006). These botanical oil/HIPV field experiments demonstrate the use of oil-based pesticides in combination with plant signals like MeSA and HA, as a potential strategy for alerting plants to a herbivore threat and inducing natural defenses. The hypothesis that plants may respond to airborne HIPV or sprays incorporating HIPVs, by emitting blends of volatiles tailored to recruit natural enemies specific to herbivore pests of the emitting plant, has been backed up by recently obtained laboratory data. Hop plants exposed to gaseous MeSA emit a different profile of volatiles than nonexposed plants (D.G. James and V. Hebert, unpublished). Work is underway to qualify and quantify these observations and will be reported in due course. Herbivore-induced plant signals may also function as ‘primers’, alerting nearby plants to a potential herbivore or pathogen threat, but without invoking a full defense mechanism or strategy (Engleberth et al., 2004). Primed plants respond quicker to herbivore or pathogen attacks than unprimed plants. Artificially ‘signaled’ plants might also invoke direct biochemical defense mechanisms to decrease their palatability to herbivores and increase their resistance to pathogens (Arimura et al., 2000; Shulaev et al., 1997). Currently, the use of controlled-release dispensers of MeSA is being extensively field-tested in hop yards and vineyards in Washington State and as indicated above, is showing considerable potential in increasing resident populations of beneficial insects, suppressing pest populations and improving biological control (James, 2003a,b; James and Price, 2004; James et al., 2005). The use of MeSA in botanical oil pesticides may provide an alternative method of delivering the same result.

3.2. Prospects for using HIPVs in CBC

The crop protection implications for using HIPVs as an aid to CBC are significant and exciting, and hopefully further field-based research in other crop systems will yield results comparable to those obtained in hops and grapes. Similar examples exist where use of synthetic HIPVs or ‘precursors’ have resulted in reduced pest populations and enhanced natural enemy abundance and activity

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Fig. 1. Mean (±SE) abundance and phenology of mite predators, Orius tristicolor (Hemiptera: Anthocoridae), Geocoris pallens (Hemiptera: Geocoridae), Deraeocoris brevis (Hemiptera: Miridae) and spider mites (Tetranychus urticae) in Washington State hop yards in 2004 baited with low (180 dispensers/ha) and high (516/556 dispensers/ha) deployment rates of methyl salicylate (MeSA) or left unbaited. Unbaited sites used insecticides/miticides to control pests, baited sites did not.
Treatment of agricultural crops with either controlled-release dispensers or botanical oil-based pesticides containing synthetic HIPVs to ‘turn on’ indirect plant defenses and enhance recruitment of natural enemies of pests, has the potential to be both effective and practical. However, numerous questions remain to be answered concerning aspects of the deployment and optimization of this strategy as a means of improving CBC, as well as possible undesirable ramifications. The ‘signaling’ hypothesis is at this stage the most plausible and reasonable explanation for the field successes seen in hops and grapes, but clearly requires validation, from laboratory...
experiments which are underway and beginning to yield data (D.G. James and V. Hebert, unpublished). Analysis of the volatiles emitted by various crop plants exposed to botanical and/or mineral oil pesticides with or without MeSA or HA and the response of natural enemies to these volatiles, will be most instructive. More studies are also required on the influence of plant cultivars on HIPV emissions and natural enemy attraction and other possible influencing factors like fungal infection, nutrient availability, etc. (Gouinguene and Turlings, 2002; Gouinguene et al., 2001). Research on the ‘strength’ of artificially applied ‘signals’ needed for priming plants to respond to herbivore threats, compared to the signals required for invocation of full defense, is also needed. Signaling crop plants to fully defend themselves when herbivores/pathogens are not present may result in unacceptable costs to the plant if it adversely affects harvest yield/quality etc. It could also lead to specialist natural enemies learning to associate HIPVs with an absence of prey. Generalist natural enemies would be less likely to develop a negative association, if at least some prey types were present. The research published on synthetic HIPVs and natural enemy attraction in hops and grapes cannot be extrapolated to provide likely outcomes for their use in other crops. Exposure of other crop types to MeSA and HA may result in emission of different HIPV blends and consequent attraction of different natural enemies. Thus, the strategy needs to be researched and tailored to individual crop types and agricultural environments. The diversity and abundance of natural enemy communities surrounding crop ecosystems is also likely to have a substantial impact on the success of synthetic HIPVs in drawing predators and parasitoids into crops. An HIPV strategy for increasing natural enemy recruitment and CBC is likely to work best in heterogenous agricultural landscapes that have crops and natural vegetation zones well integrated. In these landscapes, ‘reservoirs’ of natural enemy populations will likely be within ‘calling’ distance of crops. The HIPV approach to improving CBC is less likely to work in large, broad acre cropping systems (e.g., cotton, wheat) with considerable distances to natural vegetation and natural enemy-inhabiting areas. However, appropriate field experiments with synthetic HIPVs have not yet been reported for broad acre cropping systems. Retention of natural enemies in crops could be improved by providing nutritional resources in the form of nectar-bearing ground covers, an approach which dovetails nicely with natural enemy recruitment by HIPVs (Attract and Reward). An alternative approach to using synthetic HIPVs to directly or indirectly attract beneficial insects is to genetically modify crop plants to enhance or modify HIPV production. The first steps on this path have recently been taken with genes controlling production of specific HIPVs successfully inserted into plants not normally able to produce these HIPVs. Transformed plants were then demonstrated to be attractive to predatory mites or parasitic wasps (Kappers et al., 2005; Schnee et al., 2006). Production of crop varieties that have improved abilities to attract natural enemies of herbivores is still some way off, but is an area that is likely to receive much attention in the future. For most natural enemies, the key attractants remain to be identified, so considerably more basic research on HIPV production by crop plants attacked by specific herbivores, is still required.

Exploiting Herbivore-Induced Plant Volatiles to enhance the efficacy and reliability of CBC is a developing field, both exciting and rich in potential (Turlings and Ton, 2006). The substantial data and literature resource accruing from basic and primarily laboratory-based studies over the past two to three decades on HIPVs, is providing an excellent foundation and framework for applied and ecological studies in the field. The prospect of using either airborne or spray-applied HIPVs to stimulate emission of HIPV blends from crop plants which mimic those emitted when the plants are attacked by pests, is particularly appealing. Whether this translates to increased recruitment of important natural enemies and enhanced CBC of crop pests, is a question that must be answered for each crop and geographic situation.

4. The ‘push–pull’ strategy

The term ‘push–pull’ was first conceived as a strategy for insect control by Pyke et al. (1987) in Australia. They investigated the use of repellent and attractive stimuli, deployed in tandem, to manipulate the distribution of Helicoverpa spp. in cotton, thereby reducing reliance on insecticides, to which the moths were becoming resistant. The concept was later formalized and refined by Miller and Cowles (1990), who termed the strategy stimulodeterrent diversion while developing alternatives to insecticides for control of the onion maggot, Delia antiqua Meigen. They proposed to attract gravid females to onion culls and to protect the main crop with a combination of a feeding deterrent and a toxin. As a principle, the ‘push’ and ‘pull’ components should generally be nontoxic so that the strategy could be integrated with methods for population reduction, preferably biological control. ‘Push–pull’ strategies should maximize efficacy of behavior manipulating stimuli through the additive and synergistic effects of integrating their use. The strategy could be a useful tool for IPM programs reducing pesticide input so that biological control and especially CBC (Barbosa, 1998; Pickett and Bugg, 1998; Landis et al., 2000) can be ‘incorporated’ as an additional population-reducing component.

Several ‘push–pull’ strategies are currently under development in a process that includes formulation and laboratory/field testing (Cook et al., 2007). The most successful example of a ‘push–pull’ strategy, indeed the only one currently in practice being used by farmers, was developed in Africa by the International Centre of Insect Physiology and Ecology (ICIPE) and Rothamsted Research (Khan et al., 2001) for the control of cereal stemborers, Chilo partellus Swinhoe and Busetella fusca Fuller, on cereal crops (Fig. 4). This strategy was developed using technologies
appropriate to resource poor farmers and has shown a high adoption rate and spontaneous technology transfer by farmers, resulting in significant impact on food security through increased farm productivity in the region.

Plants that have been identified as effective in ‘push–pull’ tactics include Napier grass, *Pennisetum purpureum* Schumach, Sudan grass, *Sorghum vulgare sudanense* (Piper) Hitchc., *M. minutiflora*, silver leaf desmodium, *Desmodium uncinatum* Jacq., and green leaf desmodium, *D. intortum* (Miller) Urban. Napier grass and Sudan grass have shown potential for use as trap plants, whereas molasses grass and silverleaf desmodium repel ovipositing stemborer moths. These plants are of economic importance to farmers in eastern Africa as livestock fodder and have shown great potential in stemborer management in farmer participatory on-farm trials. The strategy does not use any chemical deterrents or toxins, but uses repellent plants to deter the pest from the main crop.

The trap plants used in this strategy produce higher amounts of attractive green leaf volatiles than maize and sorghum, particularly at the times when stemborer moths seek plants for oviposition. It was observed that the total quantities of volatiles collected hourly, over a 9-h period, from Napier grass and the blue thatching grass, *Hyparrhenia tamba* (Steud.) Stapf., showed an approximately hundredfold increase in the first hour of the scotophase (Chamberlain et al., 2006), a period at which stemborer moths seek plants for oviposition (Påts, 1991). Thereafter, the amount decreased rapidly to levels present during photophase. Although onset of scotophase also triggered an increase in quantities of volatiles collected from two cultivars of sorghum and two out of three cultivars of maize, these increases were less dramatic than in the two wild grasses, being only up to 10 times as much as in the last hour of photophase. Further analysis showed that up to 95% of the increase in volatiles at the onset of the scoto-
phase was due to just four compounds, the green leaf volatiles hexanal, \((E)\)-2-hexenal, \((Z)\)-3-hexen-1-ol, and \((Z)\)-3-hexen-1-yacetate, with the latter dominating the volatile profile (Chamberlain et al., 2006). Additional analysis of these volatile samples using coupled gas chromatography–electroantennography (GC–EAG) and gas chromatography–mass spectrometry revealed that the wild hosts produced higher levels of physiologically active compounds compared with either of the cultivated hosts (Birkett et al., 2006). These trap plants also have inherent ability of not allowing development of trapped stemborers (Khan et al., 2006, 2007). The strategy also attempts to exploit the natural enemies, particularly parasitoids and predators, in the farming system (Khan et al., 1997a,b; Midega and Khan, 2003; Midega, 2005; Midega et al., 2006).

4.1. ‘Push–pull’ strategy and CBC: stemborer parasitoids

Throughout the development of the ‘push–pull’ strategy for cereal stemborers, exploitation of natural enemies has been attempted. The prospects for understanding and exploiting the interaction of hymenopteran parasitoids with their hosts have advanced rapidly, particularly with the discovery that semiochemicals released during herbivore damage can stimulate parasitoid foraging. We observed that planting \(M.\) \textit{minutiflora} between the rows of maize not only caused a dramatic reduction in stemborer infestation by acting as a repellent plant in the ‘push–pull’ strategy, but there was also a significant increase in parasitism of stemborer larvae by the indigenous parasitoid, \(C.\) \textit{sesamiae}, in the fields intercropped with \(M.\) \textit{minutiflora} (Khan et al., 1997a, 2000) (Fig. 5).

Volatile chemicals produced by \(M.\) \textit{minutiflora} repelled female stemborer moths but attracted foraging female \(C.\) \textit{sesamiae}. It was discovered, and further confirmed in subsequent behavioral studies, that the active compounds found specifically in \(M.\) \textit{minutiflora}, but not in the trap plants, comprised \((E)\)-ocimene, \((E)\)-4,8-dimethyl-1,3,7-nonatriene, \(\beta\)-caryophyllene, humulene and \(\alpha\)-terpinolene (Khan et al., 1997a, 2000; Pickett et al., 2006). The ocimene and nonatriene had already been encountered as semiochemicals produced during damage to plants by herbivorous insects (Turlings et al., 1990, 1995).

Subsequently, Khan et al. (1997a) established that the nonatriene was responsible for the increased parasitoid foraging in the intercropped plots. Indeed, when this compound was presented to \(C.\) \textit{sesamiae} in a Y-tube olfactometer at a level similar to that found in the volatiles from \(M.\) \textit{minutiflora}, it accounted for most of the attractiveness of the natural sample (Khan et al., 1997a, 2000). These data suggest that intact plants such as \(M.\) \textit{minutiflora} with an inherent ability to release such stimuli could be used in development of new crop protection strategies. However, local growth conditions of the plants influence chemical profiles of the volatiles produced by these plants. For example \(M.\) \textit{minutiflora}, which is adapted to cooler and drier high-altitude areas of Kenya, loses its attractiveness to \(C.\) \textit{sesamiae} when it is grown in hot and moist environments (Gohole et al., 2003). When the nonattractive molasses grass was grown in a cooler highland area, it gained back its attractiveness to \(C.\) \textit{sesamiae}, signifying the effects of regional climatic differences and/or growth conditions on volatile production and/or release by the plants. This finding demonstrates how the environment can influence \(M.\) \textit{minutiflora} to switch on and off the genes for production of nonatriene. This information could form a basis on which a decision could be made for different regions
Another group of plants used as repellent intercrops in ‘push–pull’ systems are in the genus *Desmodium*, such as *silverleaf, Desmodium uncinatum.* *Desmodium* spp. produce not only the nonatriene and ocimene, responsible for their repellency to stemborers, but also large amounts of \( \alpha \)-cedrene which was initially thought to be repellent to parasitic wasps (Khan et al., 2000). However, *D. uncinatum*, was found to produce enhanced amounts of the nonatriene and ocimene (particularly at its flowering stage) relative to other components such as \( \alpha \)-cedrene (Khan et al., 2000) and subsequent field studies have shown it leads to higher stemborer larval and pupal parasitoid activity when intercropped with maize (Midega, 2005). Studies in Kenya and South Africa showed that while mean rates of parasitism of *C. partellus* eggs did not significantly differ between the ‘push–pull’ and the maize monocrop plots, parasitism rates of larvae and pupae were significantly higher in the former at both sites. In a four-arm olfactometer study, attractiveness of *D. uncinatum* flowers to *C. partellus* larval parasitoids has been observed (Midega et al., unpublished data).

A careful selection of the plants to be used as trap plants in a ‘push–pull’ system is necessary to optimize CBC. Those plants/varieties that do not support any larval survival are not recommended as trap plants (Khan et al., 2006). Napier varieties like Bana grass (a hybrid of Napier grass and millet) are usually recommended since they support larval survival and development, albeit at a minimal level. Several species of grass infesting stemborers, which are not pests of maize, remain associated with trap plants and can play a useful ecological role by serving as alternate hosts for stemborer natural enemies during noncropping season (Khan et al., 1997b). Some of these stemborer species, for example those belonging to the genus *Poecinoma*, exploit wild grasses including Napier grass as their host plants (Khan et al., 1997b; Ndemah et al., 2001) and also serve as alternative hosts for parasitoids such as *C. sesamiae* (Khan et al., 1997b). Minimal larval survival is, therefore, a desirable trait in a ‘push–pull’ habitat management trap crop, as it is favorable for conservation of the parasitoids by providing continuous refugia to natural enemies as well as sources of nectar, pollen and alternate preys (Khan et al., 1997b, 2006).

There are a number of examples where increased parasitism of pests on a crop is linked with the presence of both primary and alternate prey (Powell, 1986). In field trials in Kenya, planting Sudan grass as a trap plant for stemborers, around maize fields increased efficiency of parasitoids. The presence of a higher population of stemborers on Sudan grass enabled natural enemies to colonize the crop plant in larger numbers than otherwise (Fig. 6). Dennis and Fry (1992) also reported the beneficial effects of field margin habitats in supporting greater arthropod diversity and in enhancing natural enemy populations within adjacent cereal fields in UK and Norway.

4.2. ‘Push–pull’ strategy and CBC: stemborer predators

CBC is an approach that can contribute to the realization of ecologically based production systems by increasing the levels of biocontrol provided by natural enemies (Bianchi and Van der Werf, 2004). While it is generally recognized that vegetational diversification of an agroecosystem often results in increases in abundance, diversity and efficacy of pests’ natural enemies (Root, 1973), the interactions among natural enemies, insect pests and the environment are often case-specific in such systems. We conducted a series of studies in Kenya and South Africa to assess the impact of the ‘push–pull’ strategy on stemborer predator abundance and efficacy, and whether the strategy could be combined with the *Bacillus thuringiensis* Berliner (Bt)-maize technology as part of an integrated

Fig. 6. Effect of Sudan grass as a trap plant on stemborer parasitization. Within a stemborer species, bars bearing different letters are significantly different at \( p = 0.05 \). Bars represent standard error of the means.
resistance management. Oviposition preference and predation rates of naturally infested stemborer eggs were assessed. Screen house-reared plants were infested with eggs, early-instar larvae, late-instar larvae and pupae of *C. partellus* in natural enemy exclusion studies. Disappearance of *C. partellus* eggs on control plants (those exposed to predators), attributable to predatory activity, was significantly higher in control than exclusion plants and similarly higher in ‘push–pull’ than monocrop plots in all cases. Recovery of early-instar larvae was generally low, similar between control and exclusion plants but significantly lower in ‘push–pull’ plots in two out of the three sites. Results showed enhanced predator abundance (Fig. 7) and activity on *C. partellus* eggs and early-instar larvae in the ‘push–pull’ system, signifying the value of this system in enhancing predator populations and efficacy (Midega and Khan, 2003) and a potential in resistance management through reduction of pest population getting exposed to the Bt toxin (Midega et al., 2006). The ‘push–pull’ effects combined with activity of these predators resulted in 50–125% reduction in stemborer populations in these plots relative to the maize monocrop, with concomitant grain yield increases of up to 90% (Midega et al., 2005). Similar levels of stemborer control and grain yield benefits have been achieved under farmer conditions in western Kenya (Khan et al., 2001, in press).

Additionally, creating field boundaries of Guinea grass, *Panicum maximum* Jacq., supports an abundance of stemborer egg and larval predators, particularly earwigs and spiders (Koji et al., 2007). Guinea grass, being an inferior host for stemborer larvae (Mohammed et al., 2004) only supports a small density of *C. partellus* larvae, indicating that the grass is a good agent of habitat management to selectively enhance arthropod predators of stemborers and act as a sink for the pest. Even though the grass stand harbors an abundant number of predators, the grass boundaries around maize fields often do not enhance predator populations within the crop field. This indicates that in order to enhance the biological control function of Guinea grass boundaries, creating a polyculture within the crop and early planting of the grass is desirable (Koji et al., 2007).

### 4.3. ‘Push–pull’ strategy and CBC-way forward

Natural enemies can make valuable contributions as a population-reducing component in ‘push–pull’ strategies. Elucidating the chemical ecology of predators and parasitoids and understanding their habitat requirements is important in the development of effective successful ‘push–pull’ strategies to manipulate their abundance and distribution for improved biocontrol. The importance of

![Fig. 7. Mean percentage stemborer predators recovered from experimental plots with *Bacillus thuringiensis* transgenic maize or nontransgenic maize in South Africa. Within a predator group, bars bearing different letters are significantly different at $p = 0.05$. Bars represent standard error of the means.](image-url)
population reduction by natural enemies in ‘push–pull’ strategies is likely to increase in the future as strategies for their behavioral manipulation are developed. With the discovery that intact *M. minutiflora* plants can imitate a damaged host plant, not only by repelling pests, but also attracting beneficial insects, we have a vision of using an intact plant to imitate a damaged plant and to induce defense in a crop plant itself. We are also reviewing prospects of using conventional breeding approaches to maximize and exploit these effects on stemborers and their natural enemies. These would involve transferring the systemic release of the nonatriene traits from *M. minutiflora* to maize itself. Because maize already produces nonatriene under insect attack, what we need is a maize plant that could produce the nonatriene by induction more effectively. The biochemistry of the nonatriene has already been investigated, and elegant work by Boland’s group at the new Max-Planck Institute for Chemical Ecology at Jena, Germany has demonstrated its generation by oxidative cleavage of the respective higher isoprenoidal homolog (E,E)-nerolidol (*Boland et al.*, 1998; *Piel et al.*, 1998). Its generation by intact *M. minutiflora* could facilitate the location and cloning of the genes involved, which could now be aided by the discovery that certain cultivars found in Africa do not cause increased parasitism and so may be deficient in the associated gene expression (*Chamberlain et al.*, 2000).

Some recent reports show that egg laying on plants by herbivores can induce defense in host plants (*Hilker et al.*, 2002; *Hilker and Meiners*, 2002). Our current research is focusing on enhancing the response of the plant to stemborer oviposition in terms of nonatriene production, so it could give an early defense against colonization of maize by stemborers. This is envisaged to alleviate the laborious intercropping approach. Furthermore, other opportunities have become available with the discovery of cultivars of desmodium which produce substantially more of the ocimene and nonatriene relative to the cyclic sesquiterpenes. These may prove to be more valuable in this crop protection program. Scientists at Rothamsted Research, UK, are investigating the cyclases and synthases by which sesquiterpene hydrocarbon semiochemicals are generated. This could provide means by which the genes for unwanted sesquiterpenes could be down-regulated in breeding programs. This will also provide the opportunity of generating isoprenoidal stress signals in intact plants which might potentiate parasitism, particularly if associated with external plant signals that could be used to ‘switch on’ these genes when necessary for crop protection purposes (*Chamberlain et al.*, 2000).

The trap plant surrounding maize crop influences the abundance of natural enemies that invade the field once a pest population is present. Just as many trap plants maintain a pest reservoir, they could also provide reservoirs of beneficial insects (*van Emden and Dąbrowski*, 1994). Therefore, as part of our continuing effort to manage cereal stemborers, we are working towards increased understanding of the associated arthropod species diversity with trap plants, which will help in selecting additional trap plants that would provide suitable habitat for natural enemies throughout the year.

The ‘push–pull’ strategy is currently the only effective method to control the stemborers in eastern Africa and is expanding rapidly. The method is based upon the exclusive use of semiochemicals which “push” stemborers away from the main crop, “pull” stemborers into trap crops and attract beneficial parasitoids. For sustainable food security and further poverty reduction, it is of key importance to reduce the risk of long-term failure of the strategy due to the evolution and invasion of stemborer and parasitoid strains with altered behavioral responses to the semiochemicals. There is considerable genetic variation underpinning the behavioral responses of insects to chemical cues, and laboratory experiments show that there is the potential of evolution in these traits resulting in a risk of failure of the control method. It is likely that some stemborers and parasitoids no longer respond to the cues from the repellent plants due to the associated fitness advantage. However, stemborer and parasitoid behavioral responses, or their interactions with the surrounding vegetation, may be in an evolutionary stable state even under the ‘push–pull’ cropping method. Our dataset, spanning more than 10 years, will be analyzed using novel computational and experimental tools to determine whether induced selection has, or is likely to occur in the target organisms.

5. Conclusions

This review provides two examples of how an understanding of chemical ecology of insects and plants can lead to development of novel strategies for improving conservation biological control of arthropod pests in agroecosystems. In the first example, the result of millennia of co-evolutionary ‘arms race’ between plants, herbivores and herbivore enemies, is being exploited by utilizing synthetic versions of chemical ‘words’ which make up intertrrophic ‘conversations’. Plants attacked by herbivores ‘scream’ for help from herbivore enemies and also alert and prime neighboring plants for impending attack. Parasitic and predatory arthropods recognize plant ‘screams’ as ‘dinner bells’ and move towards the plants to find the signaled food. Identifying the ‘screams’ and using synthetic versions of them, may allow us to control or manipulate the plant-arthropod dialogue in a crop, such that we can call in herbivore enemies earlier and more effectively. This has the potential to more effectively repel herbivores or increase predation/parasitism, thus improving conservation biological control. The field experiments thus far conducted in hop and grape agroecosystems show much promise for this approach. Evaluation is clearly warranted in other crop ecosystems.

In the second example, understanding the interactions of plants with insects and their natural enemies is demonstrated as yielding new ways of exploiting natural enemies
at the practical level. This may be delivered by application of natural plant activators or by trap cropping and intercropping regimes delivering a ‘push–pull’ system. Basic science, and particularly understanding the chemical ecology of plant-pest-natural enemy interactions, by combined analytical-chemical and behavioral studies, can lead through to real practical developments. These are already a reality but in many systems conventional pesticides are easier to deploy and can still be more effective. However, pesticide resistance continues to reduce the practical value of such agents and increased cost of maintaining registration can remove effective pesticidal agents from use. Resistance can develop against the approaches described here but by understanding the underpinning science, modifications can be made, whereas whole new toxophores must be developed to replace pesticides lost through resistance. There is also the benefit of exploiting natural processes rather than artificial interventions, which, besides offering advantages already given, could contribute further sustainability.

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References


volutinies in *Aphidius evis* Haliday (Hymenoptera: Braconidae). Biological Control 10, 159–165.


