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New directions for improving crop resistance to insects by breeding for egg induced defence

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Plant defence responses to insect oviposition, including tritrophic interactions with natural enemies of herbivores, have rarely been targeted in crop breeding programmes. Emission of herbivore induced plant volatiles (HIPVs) that attract natural enemies early on at the egg-laying stage of herbivore attack could provide timely biological control of pests and deter subsequent oviposition. This is needed in an agroecological context where the third trophic level often does not keep pace with the growth rate of pests. Our very recent data, using maize as an example, show that herbivore egg induced volatile emission is very rare in commercial hybrids but common in farmer selected landraces. Strategies for crop genetic improvement to enhance such responses to insect attack are considered.

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Introduction

Wild plants have evolved intricate defence mechanisms against insect herbivores [1–3] over a period of 400 million years [4]. These depend partly on primary recognition of the insect mediated by specific herbivore-derived molecules, termed herbivore-associated molecular patterns or HAMPs [5,6,7]. Induced defence occurs after recognition of insect attack and includes ‘indirect defence’ entailing the emission of herbivore induced plant volatiles (HIPVs) that allow them to attract parasitoid and predator insects that are tuned in to these signals and use them when foraging for prey insects [8,9].

Several studies have shown that plants are able to even detect insect egg deposition (the earliest stage of herbivory) and respond by activating direct and indirect defences early before larvae hatch and cause damage by feeding (reviewed by [10]). This ‘early herbivore alert’

reduces the lag period for arrival of natural enemies. Parasitism of eggs by egg parasitoids reduces larval emergence and performance. Furthermore, emission of volatile cues attractive to larval parasitoids following egg deposition means that they are recruited in anticipation of larval emergence and parasitism can start before the larvae can cause much damage to the plant. Parasitoids are under selection pressure to respond to such cues and even distinguish between mechanical damage and those induced by the presence of their hosts as this enhances their foraging efficiency eventually improving their ecological fitness. Attraction of parasitoid wasps can improve biological control in open field environments, as already shown with intercrops [11]. Such indirect defence has perhaps been neglected as a potential trait for breeding increased crop resistance to insect pests. Breeding may have favoured ‘bottom-up’ rather than ‘top-down’ insect resistance [9]. In this paper we discuss genetic variation in crop responses to insect attack and strategies for crop genetic improvement to enhance such traits. To illustrate the concept we use our recent studies in maize with the stemborer *Chilo partellus* and its parasitoid *Cotesia sesamiae* because we have now found that insect egg induced volatile emission does exist in commercial hybrids meaning that there is a prospect of introgressing this trait into improved varieties.

Fighting insects like a wild plant?

The impact of crop domestication on responses to insect attack was considered at the 15th Symposium on Insect–Plant Interactions (August 17–22, 2014; University of Neuchâtel, Switzerland). Plants subjected to artificial selection may have lost defence traits, used for protection against insects in nature, especially if crops are selected for yield in a pesticide treated background. Conversely, insect resistance may increase if breeders purposely select for insect resistance, although such breeding is often empirical and without characterisation of the underpinning mechanism. Palmgren *et al.* [12] hypothesised that traits allowing plants to withstand adverse environmental conditions have been lost while selecting for traits that made plants easier to harvest and/or resulted in higher yield. de Lange *et al.* [13] reviewed resistance to biotic stress in teosintes (wild maize species) which appear to have greater resistance against a number of pests than cultivated maize.

Wild plants or less domesticated landraces are a promising source of traits that could enable plants to withstand insect attack and other stressful conditions. Strategies

are required to identify resistance traits and introgress them into domesticated crop germplasm. Next generation sequencing (NGS) technologies being used to generate whole genome sequences for a wide range of crop species, when combined with precise phenotyping methods, can provide powerful and rapid tools for identifying the genetic basis of agriculturally important traits and for predicting the breeding value of individuals in a plant breeding population [14]. These approaches will greatly facilitate the identification of useful traits. However the phenotyping is often more time consuming than the genotyping [15].

HIPV emission is widespread in nature and lack of response to herbivory may be rare in wild plants [16]. It has been shown [17**] that HIPV-silenced *Nicotiana attenuata* plants had reduced fitness due to increased herbivory by *Manduca* spp. resulting in a twofold decrease in bud and flower production. Furthermore, Zakir *et al.* [18] found a significant reduction in oviposition by *Spodoptera littoralis* on undamaged plants adjacent to herbivore-damaged cotton plants under both field and laboratory conditions. Heil [9**] discussed use of HIPVs as tools in biocontrol and challenges faced in their application in agriculture. These include a lack of field studies, possible slower growth but increased damage by parasitised insects, attraction of hyperparasitoids or herbivores, lack of reward for attracted natural enemies and insufficient levels of natural enemies in the environment. These are important points that require further study, especially in outdoor field conditions and possible adjustment of agronomic practices, for example, conservation biocontrol to boost natural enemy populations and provide a suitable environmental context for the crops that 'cry for help'.

Plant responses to insect oviposition can be direct as well: *C. partellus* egg deposition on maize plants that release HIPVs has recently been shown to have direct effects on the herbivores themselves [19] such that plants already with eggs had lower subsequent oviposition. HIPVs are known to have repellent effects on gravid female moths [20]. Once the plant recognises it is being attacked other defences such as induced antibiotic or anti-nutritive substances are also produced by plants which are responding [21]. Together, direct and indirect defences slow down the growth rate of the herbivore. Direct defences do this by reducing settlement and suitability of the plant as a host. Indirect defences increase mortality of the herbivore thus leading to a slower population build-up. Zakir *et al.* [18] found direct effects on oviposition behaviour in *S. littoralis* and Geiselhardt *et al.* [22] demonstrated that *Pieris brassicae* oviposition on *Arabidopsis thaliana* reduced subsequent feeding and growth rates of the larvae. Furthermore, Fatourous *et al.* [23] found synergistic effects of direct and indirect defences on herbivore egg survival in a wild crucifer.

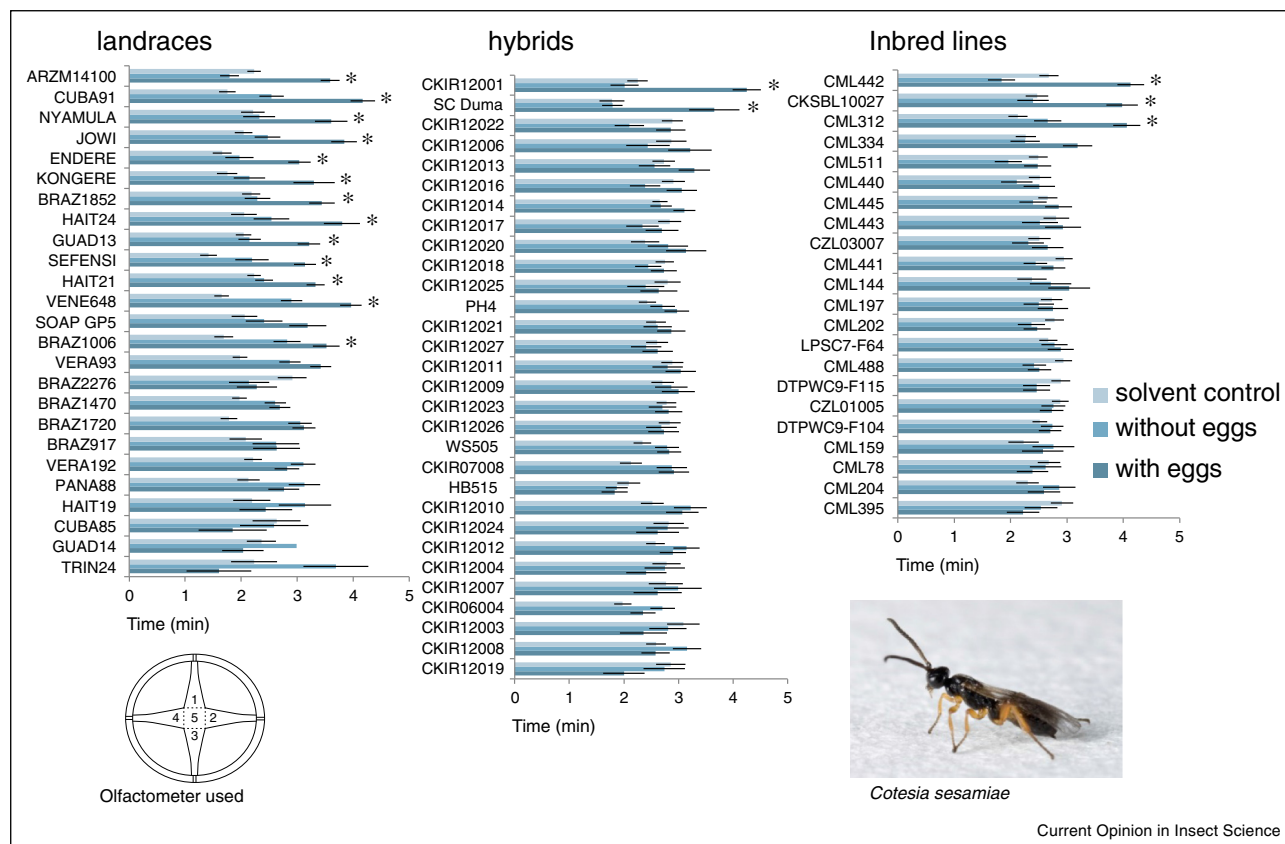
Egg induced defence against stemborer attack in maize

HIPV-mediated indirect defences following larval feeding are well known in maize (e.g. [13*,24,25]). HIPV emission after oviposition of the stemborer *C. partellus* was recently discovered in certain maize landraces [26,27] but not in the commercial hybrids we examined initially. In the last two years, we screened for egg induced HIPV emission in a much wider range of maize germplasm (25 landraces, 30 hybrids and 22 inbred lines; Figure 1). This was to determine whether HIPV emission after stemborer oviposition could be found in improved, higher yielding maize varieties and to enable ongoing genome wide association studies (GWAS) [28] mapping single nucleotide polymorphisms (SNPs) linked to the egg induced indirect defence trait. We collected headspace samples of volatiles from plants with and without *C. partellus* eggs, analysing the volatiles by gas chromatography and exposed the parasitoid *C. sesamiae* to the volatiles in an olfactometer bioassay (Methods as in [26]). Plants were grown under insect free conditions; treated plants were caged overnight with five gravid naïve female stemborer moths and volatiles were then collected for 48 h. Egg hatch occurs five days after oviposition which meaning that the plants were only exposed to eggs and not to larvae.

We tested if gravid female *C. sesamiae* spent significantly more time in the olfactometer arms containing volatiles from plants exposed to egg deposition by *C. partellus* compared to arms with volatiles from unexposed plants or solvent control. Thirteen landraces were identified with the trait (Figure 1), whereas, out of 30 hybrid maize varieties tested, only two elicited significant attraction of female *C. sesamiae*. These were the CIMMYT line CKIR12001 and the commercial variety 'SC Duma 43', both of which strongly attracted parasitoids following oviposition ($F_{2,37} = 9.47$, $P = 0.0005$ and $F_{2,33} = 9.47$, $P < 0.001$, respectively). Compounds that were induced included (*Z*)-3-hexen-1-ol, limonene, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), methyl salicylate and (*E*)- β -farnesene. The parental inbred lines of CKIR12001 were then tested and all three of them elicited parasitoid attraction. Female *C. sesamiae* were significantly attracted to HIPVs from maize inbredlines CML 442 ($F_{2,37} = 21.84$; $P < 0.001$); CML 312 ($F_{2,37} = 16.60$, $P < 0.0001$) and CKSBL10027 ($F_{2,37} = 7.59$, $P = 0.0017$) exposed to *C. partellus* egg deposition, compared with volatiles from unexposed plants and blank controls (Figure 1). These could provide a genetic resource for introgressing the trait into high yielding maize lines to increase indirect defence against stemborers.

Headspace samples from plants with and without eggs were analysed by GC. Comparison of volatile profiles revealed a close correspondence between any egg induced changes in the volatile profile and attraction

Figure 1



Response of *Cotesia sesamiae*, a key parasitoid natural enemy of stemborers, to odours from maize plants with and without stemborer, *Chilo partellus*, eggs. Mean time spent (minutes \pm SE) is shown for each line. "*" indicates where there was a significant increase in time spent in the olfactometer region containing odours of plants with eggs. A diagram of the olfactometer and its regions is shown in the insert: region '1' had odours from plants with eggs, '3' odours from plants without eggs and regions '2' and '4' were solvent controls. Parasitoid responses were compared by ANOVA after converting the data into proportions and log-ratio transformation. Significant means were separated using Student–Newman–Keuls (SNK) test. An adult *C. sesamiae* is shown in the photo.

observed in the olfactometer bioassays. Clear increases in levels of compounds such as DMNT, previously shown to be EAG-active and play a key role in parasitoid attraction [26], were found in the headspace samples collected from landraces with eggs that had elicited parasitoid attraction in the bioassays. Selected samples that were attractive in the bioassays were subjected to GC–EAG analysis. As in previous studies DMNT was one of the EAG-active compounds. Other EAG active compounds included 6-methyl-5-heptene-2-one, α -pinene, myrcene, (*R*)-linalool, limonene, methyl salicylate, (*E*)- β -farnesene and (*E*)-caryophyllene. Conversely, the vast majority of the maize hybrids did not show induction of behaviourally relevant compounds when plants were exposed to egg deposition and had near identical profiles for headspace samples collected from plants with and without eggs. These results suggest that it is possible to breed indirect defence against stemborers, which is induced by the earliest stage of herbivore attack, into higher yielding improved maize lines. DMNT, a key

compound known to attract *C. sesamiae* in a 'push–pull' companion cropping system [11], was elevated by egg deposition in all the maize varieties that exhibited this trait. This demonstrates the potential of exploiting maize plants possessing this trait in biological control of the stemborer pests.

There was a kind of reverse progression in which the indirect defence trait we are concerned with is common in the landraces screened but rare in the hybrid varieties. This is even more apparent if the wild ancestors of maize [13^{*}] are considered; we have recently investigated five teosinte species and found the indirect defence trait strongly expressed in four of them (Mutyambai *et al.*, in press). We also found the trait previously in signal grass [29] which is less domesticated than maize. Thus, there is a steady decline in prevalence of the trait going from wild ancestors to landraces to higher yielding hybrid varieties favoured by breeders. It is tempting to speculate that the conditions under which crops have been artificially

selected during the domestication process might have influenced the outcome, especially during recent decades when crops have often been bred in a pesticide treated background. Indeed, the one commercial hybrid in which the trait was found, SC Duma 43, appears to have been bred in an unusual way with selection for the ability withstand stresses such as drought to make it more suitable for the African smallholder market (*personal communication*, Joseph Mito, Seed Co, Kenya).

Crop genetic improvement to enhance responses to insect attack

Introgression lines of wild species into crop germplasm provide a powerful resource for bringing in the traits required [30]. However, it is often difficult to cross crops with wild relatives when they are distantly related and there is the problem of ‘linkage drag’ during which undesirable genes near the desired ones on the chromosome are brought across [30]. GM techniques could help solve this problem [12*,31,32*] but are not used as much as they could be by crop breeders due to marketing issues, influence of pressure groups and regulatory restrictions that make what could be an efficient process slow and expensive.

Given the political impediments to GM, conventional breeding is being focussed on by all but the largest seed companies because small companies cannot afford the costs of developing GM crops. Even large multinational companies such as BASF and Monsanto have decided to focus on conventional breeding for the European market although biotech crop development continues in other parts of the world. With marker assisted selection (MAS) the speed of conventional breeding can be improved [33] but it still remains a challenge to introgress beneficial traits from distant germplasm. The distinction between conventional and GM is increasingly unclear as conventional breeding allows random mutagenesis [31] while GM techniques, such as site directed mutagenesis, are becoming increasingly precise [34], not only for switching off genes but also for switching them on [35*]. Whatever the method of crop genetic improvement, there is considerable potential to increase protection of crops from insect attack by planting insect resistant seeds. We are currently doing GWAS in collaboration with ICRISAT and Cornell University in order to provide molecular markers for MAS of indirect defence traits in maize.

Conclusions

There is considerable potential to improve crop defences against insect attack by learning from nature and introgressing traits from crop wild relatives or landraces. For our maize example, the discovery of two hybrid varieties and three inbred parental lines that possess the indirect defence trait elicited by insect eggs in hybrid maize is promising. This demonstrates a real prospect of introgressing the trait into higher yielding hybrid maize varieties in

a reasonably short time. This could benefit small scale farmers in Africa who don't use pesticides to protect their crops but similar approaches are needed in other parts of the world where pesticide use is being restricted [36]. The current findings coupled with technological developments in crop genomics and genetics set the stage for plant breeding in which herbivore induced defences can be used to provide better crop resistance against insect attack.

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