Abstract: Insecticide resistant pests become increasingly difficult to control in current day agriculture. Due to environmental and health concerns, the insecticide portfolio to combat agricultural pests is gradually decreasing. It is therefore crucial to make rational decisions on insecticide use to assure effective resistance management. However, resistance monitoring programs that inform on pest susceptibility and resistance are not yet common practice in agriculture. Molecular markers of resistance that are turned into convenient diagnostic tools are urgently needed and will only increase in importance. This review investigates which factors determine the strength, diagnostic value and success of a diagnostic marker, and in which cases recent technical advances might provide new opportunities for decision making in an operational meaningful way.
Significance and interpretation of molecular diagnostics for insecticide resistance management of agricultural pests

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Abstract

Insecticide resistant pests become increasingly difficult to control in current day agriculture. Due to environmental and health concerns, the insecticide portfolio to combat agricultural pests is gradually decreasing. It is therefore crucial to make rational decisions on insecticide use to assure effective resistance management. However, resistance monitoring programs that inform on pest susceptibility and resistance are not yet common practice in agriculture. Molecular markers of resistance that are turned into convenient diagnostic tools are urgently needed and will only increase in importance. This review investigates which factors determine the strength, diagnostic value and success of a diagnostic marker, and in which cases recent technical advances might provide new opportunities for decision making in an operational meaningful way.
Introduction

The control of pests that attack our crops is one of the major challenges and costs in agriculture today, as production losses due to arthropod pests continue to grow and reach up to 20% of the total crop yield [1,2]. The development of insecticide resistance becomes an increasingly important problem: more than 500 insect and mite species are now resistant to at least some of insecticides used for their control [3,4]. In addition, the availability of new crop protection chemistry becomes more and more challenging due to the increasing costs for discovery, development and registration, in part driven by public concerns on environmental safety and human health [5-9]. This probably results in company decisions not to develop new chemistry if projected revenues are minor, for example when compounds target only specific pests in minor crops.

Giving the rising resistance problem and pressure on pesticide portfolio, it is very important to make rational decisions on insecticide use [10]. While a few key mosquito species are controlled by a limited number of insecticide classes, and exposure is mainly via residual contact of sprays and treated bed nets [11], the options in agriculture are more complex. Several classes of insecticides with different modes of action and different arthropod exposure routes (direct or residual contact, ingestion by chewing, sucking, rasping ...) are applied in multitude in different cropping systems, giving more options for pesticide choice and insecticide resistance management (IRM).

Diagnostic tools that monitor susceptibility in pest populations could play a crucial role in the choice of chemicals, as they allow to manage or avoid incidence and spread of resistance. Diagnostic bioassays have since long been developed for several agricultural pests and disease vectors. While extensive monitoring programs are an integral component of mosquito control programs [12], this is much less the case for the numerous agricultural pests on the diversity of crops. Crop pest monitoring programs seem limited to some of the key pests in major crops like corn, soybean, rice and cotton, yet primarily as a result of spontaneous research programs or industry driven activities for managing and launching their new products, but more rarely as systematic country level activities [13,14].

Bioassays are often employed for resistance monitoring [10], however their feasibility in high throughput depends on whether insects can be easily collected, stored, and grown in the lab, and equally, whether the host plant is easily cultivated in the lab or artificial diets for pests are available. For example, resistance screens for Bt toxins in key lepidopteran pests have been largely profited from the ability to mix these toxins with artificial diets [15].
As increasing number of molecular markers for resistance are being identified, high throughput fast and accurate molecular diagnostic platforms could be used to overcome the need for time-consuming bioassays. However, using this data in making decisions on insecticide use is the next challenge, because of the potential limited predictive value of the markers and/or lack of clearly established links with operational impact.

This review investigates to what extent, and in which cases, molecular diagnostics can be reliably used to manage resistance and inform decisions on insecticide use in time and space in the field. As this subject has been recently reviewed for vectors of human disease [11,16], we focus here on agricultural pests.

**Resistance mechanisms and molecular markers**

The development of resistance is an evolutionary phenomenon of which the mechanisms are most often described in terms of toxicodynamic and toxicokinetic changes in the physiology and biochemistry of resistant strains. This includes changes in penetration, activation, metabolism, transport and excretion for toxicokinetic mechanisms (any changes that alters the amount of toxin that reaches the target-site), and changes to the pesticide target-site (structural changes, knock-out, amplification) for toxicodynamic mechanisms [17,18]. Although this physiological classification is useful in describing the resistance phenotype that results from genetic changes, and sometimes allows specific field interventions such as the use of synergists in metabolic resistance, the actual type of genetic change (mutation) is more relevant to precisely understand the evolution and spread of resistance genes in populations. In addition, it largely determines whether accurate and sensitive molecular diagnostic markers can be feasibly developed. For example, a simple point mutation in a target-site is much more easily turned into a DNA-based marker than increased expression of a metabolic resistance gene. In the latter case, it is much more likely to develop a diagnostic marker based on RNA or protein abundance, than a marker based on the actual mutation, as cis and especially trans acting mutations regulating gene expression have remained elusive for most cases of metabolic resistance in most pests. A few studies in mosquitoes are now providing DNA markers for metabolic resistance [19,20], while recently developed mapping tools, such as NGS-based bulked segregant analysis [21], might facilitate the identification of QTL markers for major agricultural pests [22-24].

**Factors affecting the strength and diagnostic value of a molecular marker**

One of the issues to consider when developing a diagnostic marker, is the breath of its geographical applicability. As outlined above, pests can develop resistance by multiple mechanisms, and whether
different populations of a certain species develop resistance with similar mechanisms is not always clear. For some target-site resistance cases, it is known that similar, if not identical mutations evolve in different populations of the same species, and even among species. For example, resistance to pyrethroids has been associated with kdr and super kdr mutations at domain II of the voltage-gated sodium channel in at least 50 different arthropod species [18]. More recent examples include the G4946E mutation in the ryanodine receptor, conferring resistance against diamides, which has been reported in four different lepidopteran species, including Plutella xylostella populations spread across 3 continents [25-28], while alterations in the ABCC2 or ABCC3 gene, strongly associated with Cry1-toxin resistance, have been identified in seven different lepidopteran species [29]. Furthermore, mutations at identical position in chitin synthase 1 (chs1), conferring resistance against benzoylureas, buprofezin and etoxazole, have been reported in three different arthropod species, both insects and mites (F. occidentalis, P. xylostella and the spider mite Tetranychus urticae) [30-32]. T. urticae is one of the rare examples where the frequency of a whole panel of different target-site mutations has been investigated worldwide (Table 1 and e.g. [33]), revealing the presence of identical mutations often across continents. The global presence of these and other target-site mutations might be related to functional constraints in pesticide targets, which have been suggested to be considerably high, probably promoting the success of a few amino acid substitution that are constraint-free [34]. Nevertheless, even if conserved target-site mutations are present in populations in broad geographical context, their relative importance in the resistant phenotype needs to be sufficiently high to reliably predict resistance and serve as diagnostic marker. For T. urticae, the phenotypic strength of the most common mutations has been determined by repeated back-crossing and marker assisted selection, which is feasible for this species with short generation time. This revealed that in most cases, the presence of the mutation explained the larger part, if not the complete phenotype, suggesting that target-site mutations are a very good predictor of resistance levels in this species [35-37]. In addition, the dominance and fitness cost of certain resistance mutations was determined [31,36,38,39], which further increases the value of a certain molecular marker for IRM [38,40-42]. As introgression of a marker is not feasible for most insect species (however, see [43,44] for exceptions), gene editing in Drosophila and/or pest species have also been a very useful tool for validating and measuring the role and effect of certain mutations in resistance against insecticides [30,45-53].

The interpretation of metabolic resistance in the context of developing molecular markers is even more complex. This is especially true for many serious pests that are polyphagous. It was previously shown that similar gene-expression responses evolved after both the development of pesticide resistance as adaptation to a new host [54,55]. The ‘pre-adaptation syndrome’, as discussed by Dermauw and
colleagues [54], confounds the potential interpretation of some of the key players in metabolic resistance, as candidate metabolic resistance genes might be overexpressed both in relation to pesticide detoxification as well as upon host plant exposure. In addition, although recombinant expression flowed by metabolism assays, reverse genetics by RNAi, or ectopic overexpression have provided different levels of validation for the involvement of detoxification genes in the resistance phenotype, finding appropriate markers has been even more challenging. The complexity of metabolic resistance is also determined by the target marker: while in some insects, such as the pollen beetle Meligethes aeneus a single P450 (CYP6BQ23) seems to be primarily responsible for pyrethroid resistance [56] indicating a single RNA/protein marker, in others, such as Helicoverpa armigera several members of the lepidopteran-specific CYP6AE subfamily can metabolize esfenvalerate [57], moving the target marker at the P450 subfamily level. Nevertheless, successful diagnostic assays for P450 based resistance have been developed in some cases, such as the polyphagous white fly Bemisia tabaci ([58], Figure 1A), which clearly indicates that this needs to be evaluated case by case.

A relevant question for the strength of a marker is also: in how many cases the resistance is caused by the mechanisms under investigation (alone). We need to recognize that the resistance is often polygenic and consists (in many instances) of “major” genes and “minor” genes, and potentially different evolutionary solutions have been selected in different populations. The predictive value of a marker can therefore only be validated in combinations with bioassays in a certain geographical region at a certain time. A validated molecular marker can be subsequently used alone for resistance monitoring but should be used in conjunction with bioassays at certain time intervals, in case new mechanisms evolve.

Methods in molecular diagnostics

The majority of molecular diagnostics used for monitoring insecticide resistance in agricultural pests (described in Table 2 [16,59-64]) are based on nucleic acid detection (DNA and/or RNA). Simple/low-tech versions of PCR- (AS-PCR, PCR-RFLP) and isothermal LAMP are used to detect the presence of known mutations or differentially expressed genes (targeted analysis). In these cases, mutant allelic frequency (MAF) is calculated through screening of several individuals. Sequencing based methods (Sanger, pyrosequencing, next generation sequencing) also allow for the unbiased analysis of the whole genes or transcriptomes revealing potential new SNPs. Improved/High-tech versions of PCR-based methods (rtPASA/SYBR Green qPCR, TaqMan qPCR, ddPCR, lyophilized pellets, LabDisk) and sequencing (Nanopore, NGS transcriptome analysis) allow quantification of MAF within the analyzed sample; thus, samples or populations can be pooled beforehand. More importantly, the exact same technologies can be used for
assessing gene expression levels at the RNA level in the same samples used for target-site mutation quantification and thus yield important information regarding metabolic resistance [65,66].

At the protein level, most technologies have been developed to monitor metabolic resistance, with the exception of few target enzyme assays. This is achieved either by assessing the enzymatic activity (cytochrome P450 monooxygenases, glutathione-S-transferases, carboxyl/choline esterases) via general or more specific substrates, or the quantification of protein expression levels via specific antibodies [67].

Today, target-site mutations are usually assayed by Sanger sequencing for small sample sizes and TaqMan qPCR for higher throughput needs in which case the cost per sample drops significantly. Metabolic resistance is most frequently determined at the mRNA level by singleplex SYBR Green RT-qPCR at relatively low cost and high throughput. Finally, in situations where large sample screening is required for known resistance mutations, including searching for low frequency/rare mutations, Droplet Digital PCR (ddPCR) could be a valuable tool [66]. It can be used to assess MAF in bulk samples with a detection limit of at least 1 mutated individual in a pool of 1000. The same pooled sample can also be used quantify the number of metabolic gene transcripts with very high accuracy, when working with RNA/cDNA templates. Current ddPCR cost may be too high, but prices are expected to drop for already available and new platforms.

Conclusions and future perspectives

Due to concerns on environmental safety and human health, the portfolio of synthetic insecticides is gradually diminishing. To prevail the efficacy of current and future insecticides, the development and application of molecular markers for evidence based IRM will become more crucial. Although resistance monitoring is not common practice yet, this will surely change when the efficacy of a particular insecticide becomes even more crucial in a context where alternative crop protection strategies will rely on a ‘last resort’ chemical intervention. Robust molecular markers are of great value for IRM. However, in many cases, such strong markers are not available/known and more correlation studies between resistance and molecular markers alone or in combination across geographical regions should be performed, to validate the strength and value of a marker in place and time. Furthermore, while in the past the development of molecular markers was focused on functional markers (e.g. target-site resistance mutation), hypothesis-free approaches (e.g. QTL mapping) and third generation sequencing technologies might generate markers regardless of underlying mechanisms. This will become more and more feasible with the advent of high-quality genome sequences for many if not most pests. Last, distribution of marker-based resistance information in an operationally meaningful way, is challenging but will remain crucial. The development
of modern interactive databases and ICT platforms that support such decision making, need to be further
developed and implemented.

Acknowledgments

We apologize in advance to our many colleagues for the inspiring articles we did not have space to feature.

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Annotated references

*Vontas and Mavridis 2019 (ref 16)

A critical review on the true value of molecular diagnostic tools for tracking insecticide resistance in mosquito vectors.

**Riga et al. 2017 (ref 35)

Using marker-assisted inbreeding, a large number of target-site resistance mutations was introgressed in a susceptible genetic background of T. urticae. This allowed to assess the phenotypic strength of a single resistance mutation, not confounded by additional resistance mechanisms, and partly determines its diagnostic value. It also allowed to determine associated fitness costs in a follow-up study, see Bajda et al. (ref 38)

Zuo et al. 2017 (ref 45)

The use of CRISPR-Cas9 gene editing to validate (the strength of) a SNP marker, G4946E conferring diamide resistance, in a lepidopteran pest species. The mutation was previously validated in the genetic model organism Drosophila by Douris et al. (ref 50)

*Dermauw et al. 2013 (ref 54)
Adaptation to host plants and pesticides select for similar responses in the polyphagous mite *Tetranychus urticae*, potentially confounding the predictive value of a metabolic marker such as overexpression of a detoxification gene.

**Nauen et al. 2015** (ref 58)

Reports a test kit based on an lateral flow test for the detection of CYP6CM1-based neonicotinoid resistance in white flies. The kit is as easy to use as a pregnancy test and is validated to provide a reliable estimate of resistance in populations across the globe.

*Bronzato et al. 2018* (ref 61)

The application of nanopore sequencing with the portable MinION variant as a tool for monitoring pathogens in plants and agricultural pests

*Zink et al. 2017* (ref 64)

One of the first studies documenting the application of ddPCR for monitoring molecular markers in pooled samples of agricultural pests.

**Figure Legends**

**Figure 1 - Current and future diagnostic assays**

(A) Test kit box based on lateral flow assay for the detection of CYP6CM-based neonicotinoid resistance in *B. tabaci*. Test line intensity provides reliable estimation of the presence and approximate levels of resistance. The major advantage of such a test is its user-friendly format allowing its application under field conditions without specialized equipment or training, and the quick availability of the test result within minutes. The test has been successfully validated against a number of neonicotinoid resistant *B. tabaci* strains and field populations around the globe [58]. (B) Droplet Digital PCR (ddPCR) and (C) Oxford Nanopore: two of the most promising technologies for future use in monitoring insecticide resistance in agricultural pests. Both can be used for pooled samples. Major additional advantages for ddPCR is that it can be used to accurately assay known mechanisms in large bulks of samples with high sensitivity and specificity. Additional advantages for Oxford Nanopore are the deep sequencing capabilities, the identification of potential novel mutations and the practicality of portable, “field-friendly” variants (MinION Nanopore).
Tables

Table 1 - Geographical distribution of major target-site resistance mutations across *T. urticae* populations

Table 2 - Current and future molecular diagnostic methods for assessing agricultural pest resistance

Supplementary Tables

Table S1 - Table 1 with references
No conflict of interest
Table 1 - Geographical distribution of major target-site resistance mutations across *T. urticae* populations. Additional references can be found in Table S1.

<table>
<thead>
<tr>
<th>target-site mutation</th>
<th>phenotypic strength</th>
<th>fitness cost</th>
<th>geographical distribution</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Europe</td>
</tr>
<tr>
<td>AChE</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G119S</td>
<td>n.i.</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>F331W/Y</td>
<td>n.i.</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>VGSC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M918L+F1534S</td>
<td>n.i.</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>F1534S+F1538I</td>
<td>n.i.</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>L1024V</td>
<td>strong</td>
<td>no</td>
<td>✓</td>
</tr>
<tr>
<td>F1538I</td>
<td>strong</td>
<td>n.i.</td>
<td>✓</td>
</tr>
<tr>
<td>CHS1</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>I1017F</td>
<td>strong</td>
<td>yes</td>
<td>✓</td>
</tr>
<tr>
<td>cybT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G132A</td>
<td>strong</td>
<td>yes</td>
<td>✓</td>
</tr>
<tr>
<td>G126S+A133T</td>
<td>strong</td>
<td>n.i.</td>
<td>✓</td>
</tr>
<tr>
<td>G126S+I136T</td>
<td>n.i.</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>G126S+S141F</td>
<td>strong</td>
<td>n.i.</td>
<td>✓</td>
</tr>
<tr>
<td>I260V+N326S</td>
<td>n.i.</td>
<td>-</td>
<td>✓</td>
</tr>
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<td>P262T</td>
<td>moderate</td>
<td>no</td>
<td>✓</td>
</tr>
<tr>
<td>GluCl1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G314D</td>
<td>weak</td>
<td>yes</td>
<td>✓</td>
</tr>
<tr>
<td>GluCl3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G326E</td>
<td>weak</td>
<td>yes</td>
<td>✓</td>
</tr>
<tr>
<td>PSST</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H92R</td>
<td>moderate</td>
<td>n.i.</td>
<td>✓</td>
</tr>
</tbody>
</table>

1 based on [35-37]: n.i., not investigated
2 based on [36,38]: n.i., not investigated; "no" should be interpreted as not detected under the conditions of [38]
3 mutation was detected in a lab strain
Table 2 - Current and future molecular diagnostic methods for assessing agricultural pest resistance

<table>
<thead>
<tr>
<th>Methods</th>
<th>Category</th>
<th>Application</th>
<th>Pro (+) / Contra (-)</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Currently used molecular diagnostics</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS-PCR</td>
<td>‘Low-tech’ PCR-based</td>
<td>T, D</td>
<td>+ Applicable to basic laboratory settings</td>
<td>[59]</td>
</tr>
<tr>
<td>PCR-RFLP</td>
<td></td>
<td></td>
<td>+ Low-cost, simple</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Low specificity (AS-PCR)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Low throughput</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- High protocol run time</td>
<td></td>
</tr>
<tr>
<td>TaqMan</td>
<td>‘Hi-tech’ PCR-based</td>
<td>T, Q, M</td>
<td>+ High-throughput</td>
<td>[59,63]</td>
</tr>
<tr>
<td>HRM analysis</td>
<td></td>
<td></td>
<td>+ Easy protocol and result interpretation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- High capital cost (machine, equipment)</td>
<td></td>
</tr>
<tr>
<td>Direct sequencing/</td>
<td>PCR-Sequencing</td>
<td>T, D</td>
<td>+ Detection of unknown resistance mutations</td>
<td>[62]</td>
</tr>
<tr>
<td>Pyrosequencing</td>
<td></td>
<td></td>
<td>- No quantitative information</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- High capital and per reaction cost</td>
<td></td>
</tr>
<tr>
<td>LAMP</td>
<td>Isothermal amplification</td>
<td>T, D</td>
<td>+ No requirement for thermal cycler; Low cost</td>
<td>[60]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ Easy, rapid one-step protocol; “Naked-eye” result determination</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>+ Rugged, field-friendly variants can be developed</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- Complex and restrictive assay design</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- No quantitative information/ low specificity for SNPs</td>
<td></td>
</tr>
<tr>
<td><strong>Promising molecular diagnostics for future use</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct-in-lysate analysis</td>
<td>Multiplex direct Taqman (RT)</td>
<td>T, Q, M</td>
<td>+ Compatibility with most qPCR platforms</td>
<td>[16]</td>
</tr>
<tr>
<td>coupled with lyophilized pellets</td>
<td>qPCR</td>
<td></td>
<td>+ Fast, with minimum handling: all reagents in a single pellet</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>+ Multiplexing capability</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- High capital cost for qPCR machine</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>- Needs calibration for quantification</td>
<td></td>
</tr>
<tr>
<td>Droplet Digital PCR (ddPCR)</td>
<td>Third generation PCR</td>
<td>T, Q, M</td>
<td>+ Extremely accurate and sensitive</td>
<td>[64]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ Simplified analysis and experimental procedure</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>+ No calibration or controls needed for quantification</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- High capital and per-assay cost</td>
<td></td>
</tr>
<tr>
<td>Nanopore sequencing</td>
<td>Third generation sequencing</td>
<td>T, Q, M</td>
<td>+ Deep sequencing [RNA-, DNA-seq] capabilities</td>
<td>[61]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>+ High-throughput</td>
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<td></td>
<td></td>
<td></td>
<td>+ Identification of potential novel mutations</td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>+ Portable, “field-friendly” variants (MiniON Nanopore)</td>
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<td></td>
<td></td>
<td></td>
<td>- Requires complicated bioinformatic analysis</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>- High capital cost</td>
<td></td>
</tr>
</tbody>
</table>

T, D: Target-site, Detection of mutations; T, Q: Target-site, Quantification of mutation frequency (pooled samples); M: Metabolic resistance; AS: Allele Specific; HRM: High Resolution Melting; LAMP: Loop mediated isothermal amplification; RFLP: Restriction Fragment Length Polymorphism
Box 1 - Factors affecting the diagnostic value of a molecular marker for IRM

- Intensity of underlying resistance phenotype associated with the marker (how much is the phenotype determined by a single marker)
- Geographic distribution of the marker (on what scale do resistance mechanisms vary)
- Cross spectrum resistance predictive value of the marker
- Epistasis and how many resistance markers are required for diagnosis in each case.
- Untangle gene expression patterns associated with resistance and host plant (detoxification enzymes can be overexpressed after adaptation to pesticides and plant allelochemicals)
- Dominance and fitness cost of the resistance marker
- Robustness, accuracy and cost effectiveness of diagnostic assay to capture the marker
Resistance monitoring is not common practice in agriculture

Molecular markers can be a crucial tool in resistance management of agricultural pests

Strength and predictive value of a diagnostic marker depends on many factors

New technologies (MinION, ddPCR) will allow to determine mutation frequency at low levels
Supplementary Material
Table_S1.docx