OVERVIEW OF INSECT RESISTANCE MONITORING FOR INSECTICIDES: FACTORS IMPACTING THE DESIGN AND IMPLEMENTATION OF RESISTANCE MONITORING PROGRAM

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IRAC

Insecticide Resistance Action Committee

CONTENTS

Background

The development of insecticide (and acaricide) resistance in insect/mite pests is an evolutionary process in response to the selection pressure imposed by use of insecticides to manage pest populations. The first insect resistance case was documented in the US to an inorganic insecticide (sulfur-lime) as early as in 1914. Currently, for almost every class of insecticides, e.g., cyclodienes, organophosphates, carbamates, pyrethroids and insect growth regulators, and relatively newer classes of neonicotinoids and diamides, resistance cases have been documented. Insect genetics and biology, in combination with pest management practices (e.g., intensive or improper use of a given insecticide), are the main factors driving the rapid evolution of resistance. In simple terms, individual susceptibility to insecticides varies within insect populations, and selection pressure caused by utilizing these products allows less susceptible individuals (carrying resistance genes) to survive and pass the resistance trait on to their offspring. Over time, the proportion of resistant individuals in a population increases as susceptible ones are eliminated by the insecticide. Ultimately, resistant individuals become common enough that the insecticide loses its efficacy leading to control failures. Insecticide resistance development can be delayed by implementing Integrated Pest Management (IPM) and Insecticide Resistance Management (IRM) strategies. Although in some parts of the world assessing insecticide resistance risk and implementing resistance management strategies may be required by Regulatory Authorities, it is important that the industry coordinates efforts to prevent or delay the development of resistance.

Resistance monitoring is an important element of IRM plans for insecticides (as well as for genetically modified crops – *Bt* crops). Monitoring results can provide an early warning of resistance evolution, advance the understanding of factors that drive resistance evolution, document the effectiveness of IRM strategies, and provide relevant information to guide implementation of effective pest management practices. Early detection of resistance in a target pest population can facilitate early interventions and extend product life (durability), thereby benefiting growers and agricultural production systems. Additionally, resistance monitoring can identify field-evolved resistance locally prior to broader spread and guide better management practices in the affected and non-affected areas.

The goals of this document are to: define the scope of proactive and reactive insecticide resistance monitoring strategies; describe the major factors influencing the design of a resistance monitoring program; and discuss issues in implementing effective monitoring programs (both sensitivity and cost).

Scope of Insecticide Resistance Monitoring Programs - Proactive and Reactive

A proactive monitoring program is intended to detect early signs of resistance development in response to the use of a given product, which would allow for evaluation and if necessary, change of management practices that could increase product durability and or limit the spread of the resistance. On the other hand, a reactive monitoring program focuses on determining whether resistance evolution is responsible for compromised field performance, followed by the implementation of a suitable mitigation strategy.

Proactive Monitoring Programs

Proactive resistance monitoring measures changes in insect susceptibility (at a population level) to a given active insecticidal ingredient (a.i.) or tracks performance of a product over time at a given location before performance failure occurs. Therefore, proactive monitoring programs involve systematic testing of field collected populations (preferably using IRAC approved methods) (Table 1) and/or systematic field surveys of product performance (Table 2). A proactive monitoring program consists of two parts: establishing baseline susceptibility or product performance baseline, and subsequent systematic monitoring of insect susceptibility (resistance) or product performance with comparison to the baseline data. Establishing baseline susceptibility involves measuring the initial variability in sensitivity of a given insect population to an a.i. or determining the field performance of an a.i. prior to large scale exposure of target insect field populations (i.e., commercialization in major crop production areas). After baseline establishment, the susceptibility of field populations is systematically monitored using methods in line with those used for establishing the baseline and or insecticide performance (i.e., efficacy) is evaluated over time in regions of high use to identify deviations from the baseline.

Reactive Monitoring Programs

Reactive resistance monitoring relies on detection and report of reduced efficacy of an insecticide in the field (Table 2). Control failure or unexpected damage reports by growers, crop consultants and extension advisors can be collected and investigated. The reporting and documentation of these cases enable the identification of potential resistant populations of the target pest and can trigger remedial actions (e.g., altering product use patterns, best management practices, and recommending additional pest management tools). Ideally, insects are sampled from the area with a control failure and tested using an appropriate

bioassay method to confirm resistance by comparing to previously established sensitivity baselines. The confirmation of resistance may lead to additional management and/or mitigation measures at the regional level.

Factors Impacting Implementation of Resistance Monitoring Programs

Defining the goals of a resistance monitoring program is key to selecting the level of proactivity and sensitivity needed for resistance monitoring (Roush and Miller 1986, Sumerford et al. 2013). A common understanding and alignment on these goals should be built among key stakeholders such as pesticide manufacturers, growers, regulatory authorities, government officials, and public-sector scientists. Resistance monitoring programs should be tailored to address local reality and needs. The decision-making process to implement a resistance monitoring program (proactive or reactive) requires an examination of factors that include appropriate level of investment, existing regulatory requirements, infrastructure and technical capacity, biology and ecology of target pests, level of control of the pest by the product, and the status of resistance (Table 3). These factors can be placed into three groups: 1. **The appropriate level of investment,** reflecting the ability to adjust resistance management strategies based on monitoring results; 2. **The quality of infrastructure and technical capacity available;** and 3. **The nature of resistance being monitored,** which reflects product efficacy, insect biology and the genetic basis of resistance.

Appropriate Level of Investment

Proactive resistance monitoring programs are generally more labor and resource intensive and require greater levels of investment than reactive programs. Therefore, it is important to consider the value of the products to growers because the return on investment is a primary consideration when designing a monitoring program. Moreover, the ability to change resistance management practices in response to monitoring results is central in setting the appropriate level of investment. When evaluating the appropriate level of investment and considering the methodology to be used, the spatial and temporal intensity of sampling and testing are also important. These should be based on the magnitude of the resistance risk (intensity of resistance), as well as pest biology and ecology (e.g., number of generations per year, reproductive rate, and dispersal propensity- spread of resistance). In some jurisdictions, regulatory requirements may define the scope of resistance monitoring activity and methods to be used. Where regulatory requirements differ from the most effective

monitoring approach, it is important to outreach to local regulators and academics to explain and demonstrate the basis for adjustments.

Infrastructure and Technical Capacity Available

The infrastructure quality and technical capacity available is an important factor to consider in designing a resistance monitoring program. The requirement needed to implement resistance monitoring programs for insecticides may be less demanding than for transgenic crops (*Bt* crop), but in many cases, monitoring traits and insecticides can be combined, allowing multiple uses of insects sampled and/or fields being monitored. Most IRAC approved bioassay methods are relatively simple and can be conducted under basic laboratory settings. However, capacity to sample field populations and insect rearing are essential and need to be examined prior to implementing a monitoring program based on laboratory bioassays.

Nature of the resistance being monitored

The pest biology, the genetic basis of resistance (e.g., resistance mechanism, number of genes, number of alleles, functional dominance), the mode of action of the product, and the level of control of the pest by a given product can all influence the rate of resistance evolution and capacity to detect it. Most insecticides provide high levels of control of susceptible individuals in a population but may allow less susceptible (i.e., resistant) individuals to survive and pass that genetic trait to their offspring. If resistance is governed by recessive genes (i.e., heterozygotes are controlled at the same high level as susceptible individuals), the rate of increase in resistance alleles in an insect population is expected to be exponential, whereby a period of small changes in frequency ("lag phase") is followed by a rapid increase. Recessive inheritance makes the early detection of small changes in allele frequency difficult because only homozygous resistant individuals can survive a high diagnostic-dose (Roush and Miller 1986). Once the frequency of these resistance alleles is sufficiently high for a diagnostic-dose assay to detect them, they are likely to have entered the rapid increase phase and resistance may appear abruptly in the field. Therefore, it is beneficial to understand the genetic basis of resistance when making decisions on establishing a proactive monitoring program and the testing strategy to be used. In the case of products providing intermediate levels of control of the pest, resistance genes that only provide moderate increases in fitness may be governed by non-recessive alleles, which would

lead to a steady evolution of resistance over time that is relatively simpler to detect in a monitoring program (Beeman 1983).

Implementation of Resistance Monitoring Programs

The first decision to be made is whether the resistance monitoring program to be implemented will be proactive or reactive. If the decision is to implement a proactive resistance monitoring program, then the second question to be answered is whether it will be laboratory-based, field-based, or a combination of both. In the case of a laboratory-based proactive program, the fundamental elements to be considered are: 1) define bioassay methodology; 2) establish baseline susceptibility for major target pests ideally prior to commercial deployment of the product in each region; and 3) systematically monitor susceptibility of field populations (full dose-response study or using diagnostic/ discriminating dose) and assess deviations from the baseline (resistance ratio) using appropriate methods.

Proactive Resistance Monitoring
Laboratory-based Insecticide Resistance Monitoring

1. Approaches and methods

Several approaches and methods (ordered below from high to low in terms of sensitivity and cost) can be used in resistance monitoring programs:

Genotypic assays

In the category of genotypic assays, F2 or F1 screens can be used to estimate the frequency of resistance alleles. The F2 screen is an effective method for detecting rare, recessive resistance alleles (Andow and Alstad 1998). However, it is labor intensive and insect rearing requirements can be expensive. This approach requires the pair mating of field collected insects (field parents), and the sibling-mating of the F1 progeny (inbred family lines) to produce the F2 progeny to be screened (bioassayed) for the presence of resistance alleles using a discriminating concentration of an insecticide (Bird et al. 2017), ideally utilizing a method approved by IRAC (Table 1). Compared to the F2 screening procedure, F1 screens are simpler, but require the creation of a field-relevant, single-gene resistant strain and – as with F2 screens – is of value where resistance is largely recessive. The F1 screen involves pair mating of field collected insects with resistant strain insects. The F1 offspring of these pairings are bioassayed using a discriminating concentration of an insecticide to screen for

resistance alleles. Given the resource needs, and the fact that most insecticide resistance is not recessive in nature, such assays will have limited application in insecticide resistance monitoring programs.

Molecular assays

Molecular assays may detect resistant alleles at low frequencies at an early stage of resistance evolution. Molecular screening tools can be used if field-relevant resistance alleles have already been characterized. For example, after resistance has developed and the responsible genetic mutation(s) have been identified in one region, molecular tools can be developed to detect resistance due to the same mutation(s) in other regions. Such approaches have become increasingly feasible and cost-effective with the advance of molecular technologies. Molecular methods permit field-collected insects to be preserved and tested, obviate complexities of rearing pests, and greatly increase the efficiency of detecting specific resistance-conferring mutations. However, because they are specific to known resistance alleles, reliance on molecular monitoring may result in not detecting resistance conferred by other alleles or genes. Thus, molecular monitoring methods typically should be complemented with phenotypic assay-based monitoring efforts.

Phenotypic assays

This type of assays is commonly used for assessing susceptibility baseline and selecting a diagnostic concentration. The susceptibility level (normally represented by the 50% lethal dose or concentration: LD_{50} or LC_{50}) of an insect population is measured through concentration-response studies in a laboratory under defined conditions. It is essential to standardize the methodology used to quantify insecticide susceptibility for a given pest species to ensure quality comparisons over time and space. IRAC has a set of approved methods (Table 1) available on its website and recommends using these methods for resistance monitoring programs so that better comparisons can be achieved for data collected cross labs and geographical regions.

Table 1. List of IRAC approved dose-response testing methods (xx 2021)

ID Number	Pest Species	Applicable Modes of Action	Comments
001	*Myzus persicae*	1A, 1B	Dip method for all growth stages, video available
019	*Acyrthosiphon pisum* *Aphis fabae* *A. glycines* *A. gossypii* *A. nasturtii* *Aulacorthum solani* *Macrosiphum euphorbiae* *Metopolophium dirhodum* *Myzus persicae* *Nasonovia ribisnigri* *Sitobion avenae*	1A, 1B, 3A , 4A, 9B, 12A, 23, 29	Dip method for adults and nymphs, video available
023	*Myzus persicae*	28	Feeding method for nymphs
024	*Aphis gossypii*	28	Feeding method for adults and nymphs
002	*Psyllids spp.*	1B	Dip method for all growth stages
003	*Tetranychus urticae* *Panonychus ulmi*	10A	Dip method for eggs only

004	*Tetranychus urticae* *Panonychus ulmi* *P. citri*	1A, 12B, 12C	Dip method for adults
012	*Panonychus ulmi*	21A	Petri-dish method for adults
013			Dip method for adults
005	*Nephotettix cincticeps* *Nilaparvata lugens*	1A, 1b, 4A, 9B, 16, 23,	Dip method for adults or nymphs, video available
006	*Tribolium castaneum*	1B	Filter paper method for all growth stages
007	*Helicoverpa zea* *Heliothis virescens* *Pseudoplusia includens*	1A, 1B, 2A, 3A, 7A 15, 18	Dip method for adults and larvae
020	*Helicoverpa zea* *Heliothis virescens* *Spodoptera eridania* *S. exigua* *S. frugiperda* *S. littoralis* *S. litura*	28	Diet method for larvae
008	*Bemisia tabaci*	19	Dip method for adults
015	*Bemisia tabaci*	1B, 3A, 4A, 9B, 29	Dip method for adults

016	*Trialeurodes vaporariorum*	1B, 3A, 4A, 7C, 9B, 16, 23	Dip method for nymphs and eggs, video available
009	*Leucoptera scitella* *Lithocolletis blancaedella*	15	Dip method for larvae and eggs
010	*Frankliniella occidentalis*	1B, 2A 3A, 5	Dip method for adults, video available
014		15	Dip method for larvae
011	*Meligethes aeneus*	3A	Vial method for adults, video available
021		4A	Vial method for adults, video available
025		1B	Vial method for adults, video available
027		22A	Vial method for adults, video available
017	*Cydia pomonella*	1B, 3A, 4A, 5, 6, 7A, 7B, 15, 18, 22A, 22B, 28, UN	Diet method for larvae
018	*Plutella xylostella*	1A, 1B, 2A, 2B, 3A, 5, 6, 15, 18, 22A, 22B, 28, UN	Dip method for larvae
022	*Tuta absoluta*	5, 22A, 28	Dip method for larvae, video available
026	*Musca domestica*	4A	Feeding method for adults
028	*Euschistus heros*	3A, 4A	Dip method for adults

029		3A, 4A	Topical method for adults
030		1A, 1B, 3A, 4A	Vial method for adults, video available
031	*Ceutorhynchus napi* *C. obstrictus* *C. pallidactylus* *Phyllotreta spp* *Psylliodes chrysocephala*	3A	Vial method for adults
032	*Diaphorina citri*	1A, 1B, 3A, 4A, 5, 6, 28	Dip method for nymphs
033	*Lygus Hesperus*	1A, 1B, 3A, 4A, 4B, 4C, 4D,	Dip method for adults and nymphs
101	*Diatraea grandiosella* *D. saccharalis* *Ostrinia nubilalis* *Sesamia inferens*	11A	Method for maize hybrids expressing Cry protein (Bt) traits

2. Establishing baseline susceptibility of insect populations

Generating "baseline" data to allow comparative analysis of susceptibility over time is critical for both proactive and reactive resistance monitoring programs. In some cases, historical baseline data may be available, but ideally baseline data should be generated prior to product launch or very early in market entry of an insecticide in a given region. The type of "baseline" data to be generated should reflect the approach and method of choice to be used to monitor resistance. For situations where monitoring programs will seek to identify population-level changes in pest susceptibility, the susceptibility of field collected insects to an insecticide should be assessed. This can be achieved by collecting insects from areas where product use is expected to be high and rearing them in a laboratory. Then the offspring (F1-F2) of the field collected insects are tested using a method ideally approved by IRAC (Table 1) to determine the average and variation in concentration/dose-response (LC_{50} or LD_{50}).

3. Systematic monitoring of susceptibility of field populations

Systematic monitoring of resistance of field populations (collecting populations from certain locations at a fixed time interval) should follow the same approach and method as used for establishing the baseline. For laboratory-based methods, use of diagnostic or discriminating concentration/dose can be implemented (Halliday and Burnham 1990) as a routine monitoring approach, with LC_{50}/LD_{50} assessment as needed. The main purpose is to collect and accumulate historical data, which enables assessment of susceptibility shifts from the baseline over time and evaluation of field resistance evolution.

Field-based Insecticide Resistance Monitoring

In the case of a field-based proactive resistance monitoring program, the fundamental elements to be considered are: 1) establish baseline of the efficacy of the insecticide ideally prior to commercial deployment of the product in each targeted region. It's necessary to define how to assess the performance (i.e., efficacy) of the insecticide against the target pests being monitored; 2) systematically monitor the field efficacy, ideally utilizing the same methodology used for establishing the field efficacy baseline.

1. Establishing field efficacy baseline

A field efficacy baseline (e.g., % control) can be established for a range of pest pressure levels of various targeted species, often over multiple growing seasons and across multiple locations. Field efficacy parameters should be relevant for tracking product performance over time after the product is launched to provide early indications of potential resistance. Documenting the field efficacy baseline sets product performance expectations under real-world field conditions. Moreover, field efficacy data enable analysis of the relationship between laboratory determined insect susceptibility (LC_{50}/LD_{50}) and product performance in the field.

2. Systematically monitoring of field efficacy

Assessment of commercial product efficacy through methodical evaluation either of commercial fields or sentinel plots established for the purpose can provide direct observation of changes in efficacy. This approach can be useful when targeted to regions with a higher risk for resistance evolution. In the case of sentinel plots, it is important that the size and placement of the plots reflect the biology and ecology of the pest. Unsprayed plots are necessary in the experimental design to provide a measure of the pest population density to separate issues related to pest pressure from potential resistance issues. A positive aspect of such systematic field efficacy assessment is that the testing is done under field conditions

and under common management practices, including the use of best management practices. This approach can also allow targeted insect collections from fields showing control failures of an insecticide for laboratory bioassays to confirm resistance (a must-do step). Field efficacy baseline information of an insecticide is required to allow comparisons to cases of control failure.

3. Define the geographical range and intensity of commercial or sentinel field evaluation

It's important to ensure adherence to standardized methods for setting field efficacy baselines. An important step in deciding on a field-based proactive resistance monitoring program would be assessing the capacity available at the field level and therefore the scale of program needed. In case of detection of unexpected poor efficacy of an insecticide, it is recommended to implement plans to investigate and determine if resistance is the cause. A threshold should be selected for unexpected efficacy that would trigger a remedial action, including, if possible, a testing strategy of insects from these fields to confirm the resistance and recommend mitigation actions.

Reactive resistance monitoring

In the case of reactive resistance monitoring programs, it is necessary to design and implement systems for effectively collecting information on product efficacy based on farmers' experience. Similar to field-based proactive monitoring programs, control failures can be investigated to determine if resistance is the cause. A threshold for lack of control should be defined that triggers a remedial plan including, if possible, a testing strategy for insects from these fields to confirm resistance and recommend mitigation actions.

Remedial Action Plans

The value of resistance monitoring and management is that the monitoring activity enable triggers for remedial actions to ensure success of crop production. The triggers and actions will depend upon the proactivity and sensitivity of the resistance monitoring approach. Remedial action plans that are specific for a crop-pest system and fit growers' management practice should be developed to allow proper responses to cases of confirmed resistance. For example, with more proactive detection of emerging resistance, modifications to resistance management plans might be warranted so that they are better tailored to the properties of the resistance. With more reactive detection of resistance already established in fields, remedial actions generally focus on mitigation of the effects of the resistance through implementation of additional or alternative pest management tactics. In some cases, resistance confirmation can take additional cropping seasons and it may be appropriate to begin remediation programs while confirmation steps are ongoing.

Takeaways and Key Messages

Resistance to insecticides is a natural phenomenon stemming from the need to control pests in crop production. Establishing the goals of a program designed to monitor the evolution of insect resistance and implementing them properly are important. Approaches for monitoring pest resistance vary from proactively monitoring shifts in LC_{50}/LD_{50} and/or the frequency of resistance alleles in field-collected insect populations to reacting to reports from growers of any suspected control problems. In any case, establishing susceptibility and/or field efficacy baselines before commercialization are necessary. The appropriate level of investment, regulatory requirements, available infrastructure and technical capacity, and insect biology and the genetics of resistance should be considered in determining whether and how to monitor for insecticide resistance. Transparency, collaboration, and communication among stakeholders are all critical for effective and credible monitoring programs. Growers, researchers, regulators, and technology developers need a shared understanding of goals, methods, and interpretation of monitoring programs, as well as mitigation action plans.

Appendix: Glossary of terms used in this paper

There is inconsistency in the scientific literature in how key terms are used. For clarity, we define several key terms as used in the present paper.

Resistance: A genetically heritable reduction in the sensitivity of a target pest population to an insecticide that arises from exposure of the population to the insecticide in the field, with the potential to lead to control failure.

Population (a.k.a. general population, larger population): A group of actually or potentially interbreeding insects that are present in the same geographic area at the same time (this is the ecological definition of a population). A population can extend across multiple fields or larger area depending on the biology, particularly dispersal behavior, of the pest species.

Collection: The insects that are sampled from a population as part of a resistance monitoring program. A collection is representative of the insects present at the collection location. If the insects are not under active selection, their susceptibility is representative of the general population.

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