Industry Perspectives on Insect Resistance Monitoring for Transgenic Insect-Protected Crops: Factors Impacting the Design and Implementation of Resistance Monitoring Program for Insect Control Traits

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Contents

Background

Resistance monitoring is an important element of Insect Resistance Management (IRM) plans for transgenic, insect-protected crops. The structure and goals of insect resistance monitoring plans have varied since the early efforts to monitor resistance to insecticides to the relatively recent introduction of plants expressing insecticidal proteins of *Bacillus thuringiensis* (*Bt* crops). Resistance monitoring can be used to advance understanding of the factors that drive resistance evolution, to document the effectiveness of IRM strategies, to provide an early warning of the initial evolution of resistance, or to provide real-time information that can be used to select pest management tools. An important goal of

resistance monitoring should be to identify the evolution of resistance in a target insect pest population early enough that management interventions can be initiated to extend product life (durability), benefiting growers and agricultural production systems. Additionally, identification of field-evolved resistance in a local area prior to spreading can indicate that management practices should be changed in other areas.

Despite efforts to delay the onset of resistance to *Bt* crops, several cases of field-evolved resistance have been reported (Van Rensburg 2007; Storer et al. 2010; Farias et al. 2014; Dhurua et al. 2011; Gassmann et al. 2011; Chandrasena et al. 2018). In all these events, resistance was initially detected at the field level, after the investigation of unexpected target pest injuries were observed on the *Bt* plants. Although in same cases lab-based monitoring activities were implemented, results did not indicate clear, biologically significant, and actionable changes in the susceptibility of tested populations prior to observations of injury to the *Bt* crops. Moreover, the actions taken generally were to hasten the switch from single *Bt* protein products to *Bt* pyramids (plants containing more than one *Bt* protein active against the same target pest but with differences in mode (site) of action). Such a switch is a response to decreased product performance rather than to detection of resistance in monitoring programs. This may be reflective of the low sensitivity of the monitoring programs (e.g., methods and protocols) to changes in the frequency of resistant individuals, associated and amplified by a wide variation in infrastructure, technical capacity, pest biology and nature of resistance seen globally. On the other hand, more precise methods to tracking resistance alleles while still at low frequency may be used to investigate the effectiveness of current IRM strategies and indicate whether adjustments are warranted. However, a gene-based monitoring approach would still require the confirmation of the biological relevance (e.g., assessment of impact to product performance) of any change detected in the bioassay. This complex and diverse set of circumstances indicates the need to review how technical elements and logistical factors are used to determine the goals, design, and implementation of resistance monitoring strategies and subsequently revise general recommendations. The goals of this document are to: 1) define the spectrum of proactive and reactive monitoring strategies; 2) describe the major factors influencing the planning of a resistance monitoring strategy for insect control traits, and 3) discuss implementation of cost-effective monitoring plans.

Proactive and Reactive Insect Resistance Monitoring Programs

Different approaches to resistance monitoring can be arrayed along a spectrum from proactivity to reactivity (Table 1). A proactive resistance monitoring program is intended to detect shifts in resistance allele frequencies in response to the use of a transgenic crop and or early signs of changes in technology performance allowing for timely application of management practices that increase product durability by managing changes in resistance

frequency or limit the spread of it. Conversely, a reactive monitoring program encompasses the development of a system to identify changes in product field performance and determine whether resistance evolution is responsible (Table 1).

Proactive Insect Resistance Monitoring Programs

There are several methods typically used to measure the susceptibility of an insect collection for proactive monitoring (Table 1). These vary in the level of change they can detect and therefore in the degree of proactivity. "Genotypic assays" represent a sophisticated and sensitive set of methods used to estimate the frequency of resistance alleles in natural field populations (e.g., F2 screens, F1 screens and molecular assays described below). "Phenotypic assays" using feeding tests where field-collected populations are challenged with purified or semi-pure proteins in artificial diet and or testing field collected insects against plants expressing the *Bt* proteins are less sensitive to changes in allele frequencies. Systematic monitoring of field performance using sentinel plots (Venette et al. 2000) or surveys of commercial fields for damage plants can be used to monitor for suspected cases of insect resistance that are already occurring and identify geographic areas with highest risk (Matten et al. 2004).

Reactive Insect Resistance Monitoring Programs

Reactive resistance monitoring relies on reports from growers, crop consultants, industry representatives in the field or extension entomologists of any potential reduced efficacy in the field (Table 1). Such reports can be used to support the identification of those areas with suspected resistance and provide recommendations of effective remedial actions to growers (e.g., Best Management Practices) while additional testing to confirm resistance is being carried out.

Whether pursuing a proactive or a reactive insect resistance monitoring program, the detection of putatively resistant individuals in the field or a laboratory is not equivalent to proving field-evolved resistance. Further analyses are needed to demonstrate that genetic change in pest susceptibility correlate consistently with the use of an insecticidal compound. Nevertheless, such results from monitoring programs can be used to trigger changes in management practices before any confirmatory steps are completed.

Factors Impacting the Implementation of a Resistance Monitoring Program for Insect Control Traits

The central question in designing resistance monitoring programs is how early the evolution of resistance can be detected. The nature of resistance impacts the ability to detect it using any given method (Roush and Miller 1986). For instance, if the goal of the monitoring program is to detect small changes in resistance alleles, more proactive and sensitive approaches to monitoring are required. Alternatively, if the goal is to document field-evolved resistance associated with changes in field susceptibility of insect populations caused by exposure to *Bt* crops, then reactive approaches may be sufficient as outlined in Sumerford et al. 2013. A common understanding and alignment to the main goal of a resistance monitoring program should be built amongst key stakeholders such as growers, crop developers, business leadership, regulatory authorities, government officials, public-sector scientists, and others. Having this alignment will help to determine the level of proactivity and sensitivity needed for resistance monitoring.

The main challenge when designing a monitoring program is to identify practical effective monitoring tools that are proportionate to the benefits of resistance monitoring to the agricultural system. Resistance monitoring programs should be tailored to address local needs considering the local reality. Therefore, there is no single recommended approach or method to monitor resistance for all circumstances. The decision-making process to implement an insect resistance monitoring program (Proactive or Reactive) and the methods to use to assess and or document resistance, requires a diligent examination of the factors impacting the implementation of a resistance monitoring strategy to guide the design or the simplification of existing programs when needed. Additionally, if appropriate more than one approach to monitoring could be implemented, for instance to generate field-relevant information.

There are several elements impacting the selection and implementation of an approach and or methods of choice to monitor resistance (Table 2): 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. These elements can be grouped in three groups of factors: 1. The **appropriate level of investment** based on ability to adjust resistance management strategies grounded on monitoring results, 2. the quality of **infrastructure and technical capacity available** and 3. the **nature of resistance being monitored** resulting from the efficacy of trait and genetic basis of resistance. Defining the goal of the monitoring program in context to the expected nature of resistance is key to identify the level of proactivity and sensitivity needed for resistance monitoring.

Appropriate Level of Investment

The return on investment in resistance monitoring should be a primary consideration when designing a monitoring program. Proactive resistance monitoring programs are generally more labor and resource intensive than reactive programs (Table 2). Therefore, it is important to consider the value of the insect protection trait to growers and the ability to change resistance management practices in response to monitoring results. When evaluating the appropriate level of investment, both the spatial and temporal intensity of sampling are important considerations. These should be based on the magnitude of the resistance risk as well as pest biology and ecology (number of generations per year, reproductive rate, and dispersal propensity). In some jurisdictions, regulatory requirements define the resistance monitoring methods that should be pursued in a country limiting the options of methods of choice for alternatively monitoring resistance. In these situations, when regulatory requirements may differ from the most effective monitoring approach it would be important outreach to local regulators and academics to explain and demonstrate the basis for simplifications or adjustments to current monitoring programs.

Infrastructure and Technical Capacity Available

The evaluation of the quality of the infrastructure and technical capacity available to accommodate resistance programs is also an important factor to be considered before committing to a monitoring approach and method (Table 2). For example, the typical feeding tests used in proactive, insect resistance monitoring programs require field-collected populations to be reared and then subsequent generations to be challenged either with purified or semi-pure proteins in artificial diet or with commercially relevant plant tissues expressing for example the *Bt* proteins of interest of the monitoring program. If the pest targeted by the resistance monitoring program can neither be sampled at the field level, or reared in a contained environment, then alternative methods should be explored. Systematically monitoring the efficacy of the *Bt* crops in the field or a reactive monitoring program based on a system to handle growers' complaints may be the only feasible approaches. If populations of the target pest species can be sampled and reared, other factors such as appropriateness of the investment to monitor resistance and likelihood of detecting resistance also need to be examined before committing to a monitoring approach and method(s).

Level of Control by Bt Crop and Nature of Resistance

The level of control of the pest impacted by the *Bt* crop, the genetic basis of resistance (e.g.; number of genes, number of alleles, functional dominance), and the intensity of the resistance trait together influence the ability to detect the alleles of interest (Table 2). For traits that provide intermediate levels of control of the pest, the presence of resistance mechanisms that provide moderate increases in fitness may be governed by non-recessive alleles that lead to a steady evolution of resistance over time. In these situations, the detection of incompletely dominant resistance can be more sensitive because a concentration of the insecticidal compound that discriminates heterozygous from homozygous resistant individuals in theory could be identified (Beeman 1983; Siegfried et al. 2007).

In the situation where *Bt* traits provide high levels of control of susceptible insects, resistance mechanisms must provide a large change in fitness on the transgenic crop for the insect to survive. Such resistance mechanisms tend to be governed by resistance alleles that are recessive (i.e. heterozygotes are also controlled at a high level). The rate of increase of these alleles in an insect population is expected to follow an exponential path, whereby a period of small changes in frequency ("lag phase") is followed by a rapid increase. Recessive inheritance makes the early detections of these small changes in allele frequency extremely difficult because many field-collected individuals are necessary to allow the pool of test insects (alleles) to contain homozygous resistant individuals that can survive a diagnostic-dose (Roush and Miller 1986). However, 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 in the field may appear abruptly. It's important, therefore, to understand the expected genetic basis of resistance when making decisions on establishing a proactive monitoring program and the testing strategy to be used.

Implementation of Resistance Monitoring Programs

There are two fundamental elements in a resistance monitoring program: 1) prior to significant deployment of the insect trait against the pest in the region (i.e.; ≤2% of product use), generate baseline information on the susceptibility of populations of target insect pests, the frequency of resistance alleles, and/or the field efficacy of a product containing insect traits; 2) monitor and assess departures from the baseline using appropriate monitoring methods.

Baseline susceptibility

Generating "baseline" data to allow comparations over time is important for proactive and reactive resistance monitoring programs. Ideally, baselines data should be generated prior to product launch or very early in the introduction of the *Bt* plant in the landscape. The type of "baseline" data to be generated should reflect the approach and method of choice to be used to monitor resistance.

Baseline susceptibility of insect populations

For situations where monitoring programs will seek to identify population-level changes in pest susceptibility, the susceptibility of field collected insects to the *Bt* proteins present in a crop being grown should be documented. 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 of the field collected insects are screened in feeding tests where insects are typically challenged with purified or semi-pure proteins in artificial diet to determine the average and variation in concentration-response. If assays with purified protein are used for the baselines, there should be an ability to continue production of the same quality of protein for the life of the monitoring program. Alternatively, if artificial diet methods are not available, insects can be tested against plants expressing the *Bt* proteins using plant tissue in a standardized laboratory bioassay or in whole plants in a greenhouse to determine average and variation in response to the levels of protein expressed in plants.

Baseline resistance allele frequency

For situations where monitoring programs will seek to identify changes in resistance allele frequency, the same protocol for genotypic monitoring (e.g., F2 screen, F1 screen or molecular assays described below) that will be used for monitoring should be followed to establish the spatial pattern of resistance alleles in areas where cultivation of the *Bt* crop is expected to be greatest.

Baseline field efficacy of an insect control technology

For situations where monitoring programs will seek to identify changes in field efficacy of a *Bt* crop, the efficacy of the insect control traits in commercially relevant crops should be established before wide-spread deployment of the *Bt* crop in the landscape. The creation of such historical data is important to allow for comparisons to information generated in systematic evaluation of commercial *Bt* crops. Such data also enable a more complete analysis of the relationship between insect susceptibly and product performance. Baseline field efficacy can be established for a range of pest pressure levels using natural or artificial infestations, often over multiple growing seasons and across multiple locations. The set of evaluated parameters should be relevant for tracking product performance over time after the product is launched to provide early indications of potential resistance. Documenting the baseline efficacy of *Bt* traits supports the understanding of expected levels of damage to

the product under real-world conditions and establishes realistic expectations for product performance.

Assessment of resistance

Proactive Resistance Monitoring

Genotypic assays

F2 screens

The F2 screen is an effective method for detecting rare, recessive resistance alleles (Andow and Alstad 1998). However, it is labor intensive and rearing requirements can be expensive. This approach requires the pair mating of field collected insects (field parents), and the sibling-mating the F1 progeny (inbreds within family lines) to produce the F2 progeny to be screened (bioassayed) for the presence of resistance alleles (Andow and Alstad 1998; Matten et al. 2004; Huang et al. 2011). Success of this rearing approach appears to be species-dependent due to the potential for inbreeding depression and disease. It is important to keep track of the proportion of collected insects that result in successful bioassays when estimating the uncertainty in calculations of allele frequency. The screening for resistance alleles should be done when possible using plant material or a discriminating concentration of the *Bt* protein capable of distinguishing field-relevant resistant individuals from susceptible individuals. When using plant material to screen resistance alleles it is important that the *Bt* protein expression in the plants used is confirmed to be comparable to normal field expression, and that relevant plant tissues for which the insect species feed on are used, to reduce the probability of false positives (and negatives). Consistency in using plants in the same genetic background in the traited and control entries to provide plant material for the bioassays over time is desired. Although the F2 screen is considered ideal to detect rare resistance alleles in low frequencies, the sensitivity of the method is limited by the number of sibling families that can be obtained from a single collection (Siegfried et al. 2007). This method may not generate practical results if more than one recessive locus is necessary to confer the resistance to the *Bt* crop. The method may detect resistant alleles in one locus and not identify the others all in one family line. Therefore on-plant assays must confirm the relevance of the alleles detected in the F2 screen.

F1 screens

The F1 screen is a simplification of the F2 screen procedure and it requires the creation and access to a field-relevant and reasonably characterized single-gene resistant strain that the resistance within the strain is largely recessive. In the F1 screen, the resistant strain is

mated with field-collected insects. Likewise, this involves pair mating of field collected males and resistance strain virgin females. The F1 offspring of these pairings are bioassayed using plant material or a discriminating concentration of the *Bt* protein to screen for resistance alleles. The F1 screen method is expected to have a greater detection power than the F2 screen method, and to significantly reduce costs for detecting known resistance alleles (Yue et al. 2008). However, an F1 screen detects only resistance alleles at the same locus as the tested resistant strain, (although the actual resistance allele may differ) and does not detect recessive resistance at other loci. It therefore should be accompanied by phenotypic monitoring approaches.

Molecular assays

In addition to bioassays, molecular assays may detect resistant alleles at low frequencies at an early stage of resistance development. Molecular screening tools can be used only if field-relevant resistance alleles have already been characterized. For example, after resistance has developed in one region, molecular tools can be used to detect the same resistance in other regions. 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 genetic mutations. However, because it is specific to known resistance alleles, sole reliance on molecular monitoring may result in not detecting resistance conferred by other alleles or genes. Thus, molecular monitoring methods typically must be continually validated with bioassay-based monitoring efforts and should be accompanied by phenotypic monitoring approaches.

Phenotypic assays

Diet-based testing

There are two approaches to diet-based testing to monitor resistance: concentration-response bioassays measure susceptibility of a population; and discriminating or diagnostic concentration bioassays measure the frequency of potentially resistant individuals. Concentration-response bioassays are more appropriate for documenting resistance that has reached high levels, but not effective to detect small changes in resistance allele frequency (Halliday and Burnham 1990, Siegfried et al. 2007). Discriminating or diagnostic concentration bioassays can be used to estimate the frequency of putative resistant individuals. This technique is useful because all individuals are tested at an appropriate and informative concentration and reasonable sample sizes can be used to detect resistance at moderately low frequencies. Beyond determining mortality of tested insects, it can be used to measure sublethal effects of a less-than-high-dose trait. However, to detect resistant individuals when resistance alleles are rare (frequencies $<10^{-2}$) and recessive, using the diagnostic dose methodology would require extremely large sample

sizes for collection, rearing and bioassays. The diagnostic or discriminating concentrations doses chosen to be used for resistance monitoring should be indicative of genetic changes in case of field-relevant selection of resistance individual (alleles)and therefore in general "low" doses should be avoided.

Plant-based bioassays

Plant-based bioassays provide relevant exposure to a plant-produced insecticidal agent and therefore can be useful in understanding field-relevant performance of insect collections and insects with resistance identified through other approaches. As with diet bioassays, plant-based bioassays can measure the mean fitness of an insect collection on the plant material. In situations where insects normally show a very uniform response, such as mortality within a fixed time, these bioassays can be used to estimate the frequency of individuals that do not show the expected response. As with diet-based bioassays, plant-based bioassays can measure sublethal effects of a less-than-high-dose trait. Plant-based bioassays can also characterize the potential effects of any putative resistance using measures of the amount of plant material (e.g. leaf area) consumed. These bioassays can use excised plant material (replaced with fresh material at regular intervals) or whole plants. Plant-based bioassays, particularly whole-plant bioassays, provide the opportunity to investigate whether putative resistant insects can complete their development on the plant, which will determine whether a putative resistance allele can persist and spread through a population under selection by the transgenic crop. Moving from diet to plant tissue to whole plant increases field-relevance of the resistance trait detected. However, this progression also may increase complexity, variability, and resource costs.

Systematic field survey technologies' performance at the field level

Assessment of commercial product efficacy through methodical evaluation either of available commercial fields or of sentinel plots established for the purpose can provide direct observation of changes in plant efficacy. This approach can be useful when targeted to regions with a higher risk for resistance development. 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. Non-*Bt* plant 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. This is particularly important for non-high dose *Bt* crops that may withstand more injury in situations with high pressure. A typical positive aspect of systemically assessing the field efficacy of products is that the testing is done on the product under field conditions and under common management practices including the use of other best management practices. This approach can also allow targeted collections of field insect populations from fields showing unexpected levels of injury for resistance confirmation in laboratory bioassays. The expected damage (and survivorship) due to a target species can vary under a broad range of conditions (such as pest pressure,

hybrids/varieties being cropped and agronomic practices). Thus, baseline information on the expected level of efficacy of an insect control technology, and therefore expected damage due to a target species, needs to be available to allow comparisons to a suspicious case of unexpected damage. Insect collections from fields with unexpected damage must be tested with standard bioassays to distinguish a suspicious case of unexpected damage from cases of resistance development.

Reactive Resistance Monitoring

Field Surveillance

This approach is predicated upon effective processes to investigate reports from growers, crop consultants and extension advisors of unexpected injury by target pests in commercial fields. Such reports should be investigated to confirm that injury to the *Bt* crop was caused by the target pest and exceeds what is expected based on baseline efficacy and pest population pressure. This process of reporting and documentation supports the identification of regions with potentially resistant populations and should be used to trigger recommendations for additional pest management measures by growers, regardless of whether resistance is confirmed. The additional pest management should be pre-defined if possible, and a system established to track their recommendation, implementation, and effectiveness at the grower level.

Investigations into reports of unexpected pest injury should include sampling of the pest population, if possible, for additional testing using a diet bioassay or plant bioassay to confirm whether the population is resistant. The testing strategy should establish heritable basis for the resistance to the *Bt* crop that allows the production of reproductive adults. Confirmation of resistance may lead to additional management or mitigation measures at the regional level.

Remedial Action Plans

To be useful for managing pests and resistance, resistance monitoring should be associated with triggers for remedial actions. 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, and fit the growers' management system ought to be developed and available to allow proper responses to cases of suspect or confirmed resistance. The response to a case of suspected insect resistance might be different for findings in proactive monitoring programs that are laboratory-based or rely on systematic field surveys of product performance and reacting to grower's complaints. 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 established in the 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 the confirmation steps are continuing.

Takeaways and Key Messages

Resistance is a natural expectation stemming from the societal need to control key crop pests. Establishing goals of a program designed to monitor the evolution of insect resistance is critical to support a more informative definition of the approach and methods to be pursued. A wide range of approaches for monitoring insect resistance vary from monitoring the frequency of resistance alleles in field-collected insect populations to reacting to reports from growers of control problems. A single approach to resistance monitoring is likely not to fit the diversity of local needs and realities. The local situation and how it influences decisions based on the factors impacting the selection of approaches and methods to monitor insect resistance should be assessed. The appropriate level of investment, regulatory requirements, infrastructure, and technical capacity available, trait efficacy, and the genetic basis of resistance should be used to determine the resistance monitoring approach. Transparency, collaboration, and communication among stakeholders are all critical for credibility of the monitoring program and to respond collectively to changes in product performance. 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 confusion and inconsistency in the public literature in how many key terms are used. For clarity, we define several key terms as used in the present paper.

Resistance: A genetically heritable change in a target pest *population* that arises from exposure of the population to the transgenic insect protection trait in the field and reduces the sensitivity of the population to the trait.

Field-relevant resistance: *Resistance* that increases the fitness (survival and reproduction) of the insect population when developing on the transgenic insect protected crop. Field-

relevant resistance reduces or has the potential to reduce the ability of the trait to provide protection of the crop.

Population (a.k.a. general population, larger population): A group of actually or potentially interbreeding organisms that are present in the same geographic area at the same time (this is the ecological definition of a population). A population therefore extends across multiple fields or counties 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 group of insects present at the location of the collection. If the insects are not under active selection, their susceptibility is representative of the general population.

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Table 1. Approaches, methods and central technical components to assess resistance in proactive and reactive monitoring programs.

	Method Sensitivity Cost High				Method Sensitivity Cost Low	
	Proactive Monitoring Program					Reactive Monitoring Program
	Genotypic Assay		Phenotypic Assay		Systematic Field-level Survey of Technologies' Performance	
Baseline	Molecular	F2/F1 screen	Diet-based	Plant-based		Field Surveillance
Laboratory-based Field-based	• Geographical sampling of field populations – plan for a risk-based sampling focusing on areas with high risk for resistance evolution • Mating strategy of insects sampled from fields - examine capacity available and investment to mass mate insects versus forming single pairs • Execution of bioassays – ensure adherence to standardized methods and high quality of data being generated				• Define the geographical range of fields to be evaluated and intensity of observations - ensure adherence to standardize methods and high quality of data being generated • Assess the capacity available at the field level and therefore the scale of program • Outline threshold of unexpected damage; remedial plans, testing strategy of insects from fields with unexpected damage and mitigation actions if necessary	• Implementation of a system to effectively collect information from growers experiencing unexpected damage to transgenic technologies • Outline threshold of unexpected damage; remedial plans, testing strategy of insects from fields with unexpected damage and mitigation actions if necessary

Table 2. Grouping of major factors impacting the design of a resistance monitoring strategy for insect control traits.

Appropriate Level of Investment	Infrastructure and Technical Capacity Available	Level of Control by *Bt* Crop and Nature of Resistance
• What is the ability to change resistance management practices based on indications of monitoring programs? • What is the size of the business and therefore what level of investment is justifiable? • What are the regulatory monitoring requirements? • Are the appropriate primary target pests defined to be the objectives of monitoring?	• Can the target insect pest be artificially reared? • Is the testing capacity available? (e.g., laboratory, greenhouse, etc.) • Is infrastructure to properly assess and store testing material (e.g., proteins and seeds) available? • Is technical expertise for implementing a testing strategy available?	• Based on the expected product efficacy, are the genetics of resistance favorable to proactively detecting resistance? • Is the target pest biology and ecology favorable to proactively detecting resistance? • What is the expected level of intensity of the resistance trait (e.g. 10,000-fold vs. 50-fold)? • What is the status and geographical spread of resistance?