AACCI Molecular Markers Technical Committee and ILSI Brasil Host Workshop on Sampling and Detection for Seed Production

The AACC International Molecular Markers Technical Committee joined forces with ILSI Brasil May 20–21, 2014, to deliver a workshop in Sao Paolo, Brazil, on sampling and detection for seed production. The goal of the workshop was to improve the understanding of sampling and sampling statistics among government inspectors in Brazil and to provide a better understanding of the technical difficulties inherent in measuring genetically modified organisms (GMOs) in grain and seed. Key messages included the inability to test to zero, the fact that low thresholds increase sample size and testing costs, and the fact that replicate samples at low thresholds will not necessarily give the same results. These are key issues that if not properly understood can lead to misinterpretation of results and regulatory problems with seeds and field trials.

AACCI traveling team in Sao Paulo (left to right): Anne Bridges, Randal Giroux, Ray Shillito, Marcus Vinicius Segurado Coelho, Doug Miller, and Tim Perez.

The traveling workshop team included AACCI members Ray Shillito (AACCI Molecular Markers Technical Committee chair from Bayer CropScience), Anne Bridges (AACCI Approved Methods chair), and Randal Giroux (Cargill), who were joined by Doug Miller (Illinois Crop Improvement) and Tim Perez (Monsanto) to deliver the program. In the final weeks of preparation we called on a number of our AACCI staff and AOCS colleagues, who all rose to the occasion and helped get the technical side of the program ready.

Our key customer was the Department of Plant Inspection of the Ministry of Agriculture, Livestock, and Supply (Ministério da Agricultura, Pecuária e Abastecimento [MAPA]), members of which attended in full force. Attendees from across Brazil travelled to Sao Paolo, and other ILSI Brasil and ACCCI members were invited to participate in the two day program. A total of just over 100 attendees filled the conference room, leaving no vacant seats. The program was delivered in English, with simultaneous translation into Portuguese.

The program was presented in four sections. The technical presentations in the first section were designed to bring us all to the same place, covering the status of row crops grown in Brazil, which are genetically modified for insect resistance and herbicide tolerance. The session was opened by Marcus Vinicius Segurado Coelho (head of the MAPA inspection agency, CBio/SDA/MAPA) and both AACCI and ILSI Brasil members, who welcomed the participants. The previous ILSI workshop (2011) covering GMO test methods was summarized for those who had not attended. The presentations then included an overview of the seed industry in Brazil, a discussion of the wide range of modern plant breeding techniques available in the tool box, including GMOs as just one option, and the status of GMO approvals for production in Brazil.

In the next section we discussed the supply chain, introducing the topic of sampling with a discussion of the grain and oil seed supply chain. The movement of commodity crops through the supply chain leaves very few recovery options if there is "noncompliant material" introduced at the beginning of the chain. Similarly, it is critically important to maintain the quality of the product as it moves through the supply chain.

Sampling approaches evolve as the product moves through the chain. To emphasize this, each of the different points of sampling in the chain were described by a different presenter. For example, sampling of crops in the field has different challenges depending on the crop, timing, and size of the operation. Mapping a growing field for sampling is quite different from collecting samples in a continuous stream of beans or grain seeds (which is more similar to sampling grains or beans in the supply chain). Because we couldn't demonstrate this on site, the presenters used many pictures to emphasize the differences. Finally, sampling for laboratory testing, which is often overlooked even though it is equally critical to obtaining reliable test results that represent the bulk load, was discussed to close out the session.

On the second day of the workshop, the conference room was full, ready, and waiting. The morning section included discussions on the validation of test methods and the need for refer-

Workshop participants from government, industry, and academia. Sessions were supported with simultaneous translation.

Participants enjoy the opportunity to network and discuss workshop topics during a coffee break.

ence materials. We described the different types of reference materials and where they can be obtained for GMO analysis. These materials are very important in any testing program, and understanding their limits and alternative options that can be used as substitutes (with qualifications) are key. We used certified reference materials available from AOCS to prepare some of the samples for the practical testing session later in the day. The session ended with a presentation on the availability of testing resources in Brazil given by Nilson César Castanheira Guimarães (Laboratório Nacional Agropecuário, MAPA, Brazil).

After a coffee break, the program focused on the sampling process and creating sampling plans using Seed Calc (developed by ISTA), a Microsoft Excel spreadsheet application for designing and interpreting sampling protocols. Ray Shillito and Tim Perez first took us through a basic understanding of probability sampling and some theoretical applications. With the basics in hand, we moved on to a practical demonstration involving the "Sampling Game." The game used role playing (exporter, importer, and regulator) to demonstrate the results of sampling a bulk load (large bowl) of beans to which a few (low level) colored beans were added. This led to the samples withdrawn by participants sometimes containing a colored bean and sometimes not.

The result of seeing different positive and negative results in each sampler's tray quickly caught the audience's attention and emphasized the message of probability theory, the identification of practical levels for detection (the impossible zero), and the importance of use of different protocols for different situations. Tim explained the "surprising importance" of negative results when multiple subsamples are used to analyze a lot. This demonstration very effectively emphasized that multiple samples from the same lot may not yield the same results when the level of the target (in this case, colored beans) is low.

It was great to see the willingness of all the participants to put this demonstration into practical application. Rounding out the program was a hands-on session that included examples of sampling low-level contamination in bulk materials. Participants took turns grinding samples and using lateral flow strips to test samples of GMO cotton seed (LibertyLink provided by Bayer CropScience). To simulate material testing RoundUp Ready soy (a Monsanto product), a certified reference material that can be purchased from AOCS, was added at a low level to preground non-GMO soybeans. The lateral flow strips used in this session were provided by Envirologix Inc.

By this stage in the workshop program, even the coffee-break discussions were on topic, and the final question-and-answer session provided an opportunity for the workshop team to reinforce the key messages and to fill in gaps in the presentations. It also gave local experts opportunities to ask more challenging questions and, for some, a chance to work one-on-one with Tim Perez on using the Seed Calc software (free to download from www.seedtest.org).

It wasn't all work for the workshop team, however. We enjoyed great hospitality from the local organizers, and the ILSI president and other representatives of the ILSI Brasil committee invited us for a typical Brazilian dinner at Dalva & Dito, where we were greeted by celebrity chef Alex Atala.

The presence of the workshop team in South America allowed us to accept an invitation from the Department of Agriculture in Peru, SENASA (Servicio Nacional de Sanidad Agraria del Perú), to present a short form of the program focused on managing GMOs in sowing seed imports and, in particular, LLP (low-level presence). Peru imports more than 50% of its row-crop seeds from Brazil and Argentina, where GMOs are common. Correct sampling and testing protocols are critical to prevent disruption of trade in seeds that are important for farming in Peru. The SENASA department responsible for plant inspection and seed testing organized this workshop to increase their understanding of the issues and make sure they had considered all the options.

ILSI Brasil hosts welcoming dinner at Dalva & Dito. Left to right: Aldo Baccarin, Doug Miller, Mariela Weingarten Berezovsky, Chef Alex Atala, and Cristhiane Bothona.

The workshop group consisted of 45 inspectors and management, and the one day workshop was incorporated into a week-long training program for their plant inspectors. We focused on the importance of good protocols, practical limitations, and the cost of short cuts or mistakes in the implementation of protocols. A key message was the importance of representative sampling, variability between duplicate or multiple samples when testing at low levels, and the strengths and weaknesses of quick tests such as lateral flow strips. Again, we had the opportunity to present the "Sample Game," using role playing with sampling beans, and to use ground RoundUp Ready soybean materials to demonstrate the use of lateral flow strips.

The team enjoyed an introduction to Peruvian local food specialties courtesy of SENASA and also found excellent Peruvian restaurants close to our hotel. Lima is recognized as the trending food capital of South America, and we took advantage of the options in the short time we were there.