

USDA APHIS PPQ CPHST FY2019 NSHAPP Progress Report

Project Title: Pilot Biosecurity Framework for Imported Seeds

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Project Objective:

To develop a comprehensive biosecurity framework for imported seeds to prevent the introduction of seed borne and seed transmitted pathogens, as well as other pests of phytosanitary concern. The original objective of this project was to develop a program for testing imported cucurbit seeds for CGMMV. This effort focused on recognition through accreditation standards of seed companies that already have quality management systems in place for addressing pest, pathogen, and phytosanitary management, provision of guidance to seed importers that do not have quality management systems in place, and establishment of a seed health testing network. As the program developed a desire to recognize seed company's efforts in pest risk mitigation through a systems approach became a new area of focus. The objective has shifted to developing a framework for recognition of different risk mitigation strategies employed by seed companies around the world, in an effort to produce CGMMV free cucurbit seed.

Accomplishments:

Report of Imported Seed Lots:

A program of testing imported CGMMV was established and data continues to be collected. Seed companies, seed brokers and trade groups were targeted in an effort to encompass a wide range of cucurbit seed imports. Groups participating in the pilot program signed a compliance agreement drafted by APHIS. The agreement utilized state seed regulatory officials in getting agreements signed and ensuring awareness between local officials and participants. Testing for CGMMV is conducted by following the internationally recognized standard method, ISTA 7-026. Participants agreed to use this method to test all melon, watermelon and cucumber lots imported into the United States. To date, over 31,000 seed lots have been tested and reported under the program, data is shown in Table 1. Throughout the duration of the project, some companies included data on imported lots of squash and pumpkin (*Cucurbita spp.*) which were not required under the compliance agreement, see Table 2. These lots were included in the final count as it has been demonstrated that some *Cucurbita spp.* are affected by CGMMV. Additionally, at least 2 companies included data on lots that were produced in the US even though the target of the program was on imported seed lots. These data were also included in the final dataset. The number of lots of *Cucurbita spp.* and for lots produced in the US, only represent a small portion of the number of lots of these types. This data is not representative of

the number of seed lots of *Cucurbita spp.* being imported or of the number of cucurbit lots being produced in the US and is included here for completeness.

Shortly after participants began reporting data on seed lots, an agreement was reached to allow testing of small lots of seed at 5% of the total lot. This percentage was based on an infection unit theory that was presented at the ASTA Flower and Vegetable Conference in January 2017. Small lots were defined as lots being smaller than 20,000 seeds. It was decided that standard testing of 2000 seeds would require 10% or more of the total lot which is a large percentage of highly valuable seed. The total data set does include data on small lots and participants were not required to specify the size of lots being tested so the percentage of small lots in this dataset is unknown. There have been positive detections on small lots including a large percent of the total positive detections of Spain origin seed. In the fall of 2019, a shipment of breeder material was imported from Spain containing 347 small lots of seed. Of these, 82 lots tested positive for CGMMV. These lots were all produced in close proximity, by one breeder. This data skews the dataset a bit and the risk of CGMMV infected commercial lots from Spain is likely not as high as is reflected in this data. It does highlight the risk of breeding material and attention should be given to sampling schemes and testing protocols specific to small lots of seed.

Seed Origin	Positive Lots	Total Lots
Australia	0	1
Brazil	0	818
Canada	0	1
Chile	0	13536
China	24	1681
Ecuador	0	41
France	0	390
Guatemala	0	3821
India	15	763
Israel	7	121
Italy	0	521
Japan	0	11
Kenya	0	3
Latvia	0	1
Mexico	0	1047
Netherlands	0	3540
Peru	0	2395
South Africa	0	32
Spain	91	880
Taiwan	0	16
Tanzania	0	197
Thailand	2	1238
United States	4	674
Unknown	0	46
Vietnam	1	8
Total	145	31782

The dataset demonstrates that over 42% of all cucurbit seed imports are coming from Chile. Considering all of South America, well over 60% of all cucurbit seed imports into the US originate in these countries. Currently, CGMMV has only been reported in isolated incidents in Brazil and is not widespread (CABI). If the virus were to become endemic in South America, the risk of importing cucurbit seed infected with CGMMV into the US would be much higher. Throughout the duration of this project, there has been continued discussion with ASTA and the NPPO of Chile (SAG) about the importance of keeping this area free to CGMMV. SAG is currently surveying cucurbits produced in the country ensure that any introductions of the virus are quickly detected and mitigated.

Table 1. Total number of cucurbit lots reported under the NSHAPP program between Jan. 2016 and Oct. 2019 including lots produced in the US and *Cucurbita spp.* that were not required to be reported in the compliance agreement.

Seed Species	Positives	Total Lots
Cucumber	108	6318
<i>Cucurbita spp.</i>	0	913
Melon	16	12558
Watermelon	21	11993
Total	145	31782

Table 2. Total number of lots reported by species and the number of positives for each species.

Taking into consideration the amount of seed being produced in South America, the breakdown of seedlots imported by month shows a peak in the spring, Figure 1. Many lots are coming in from Chile and other South American countries at the end of the season in the southern hemisphere. Positive detections, not including the 82 breeder positives in Oct. 2019, tend to occur in the winter months as shown in Figure 2. Since all of the infected seeds came from the northern hemisphere, these highlight when that seed is moving into the US and being tested.

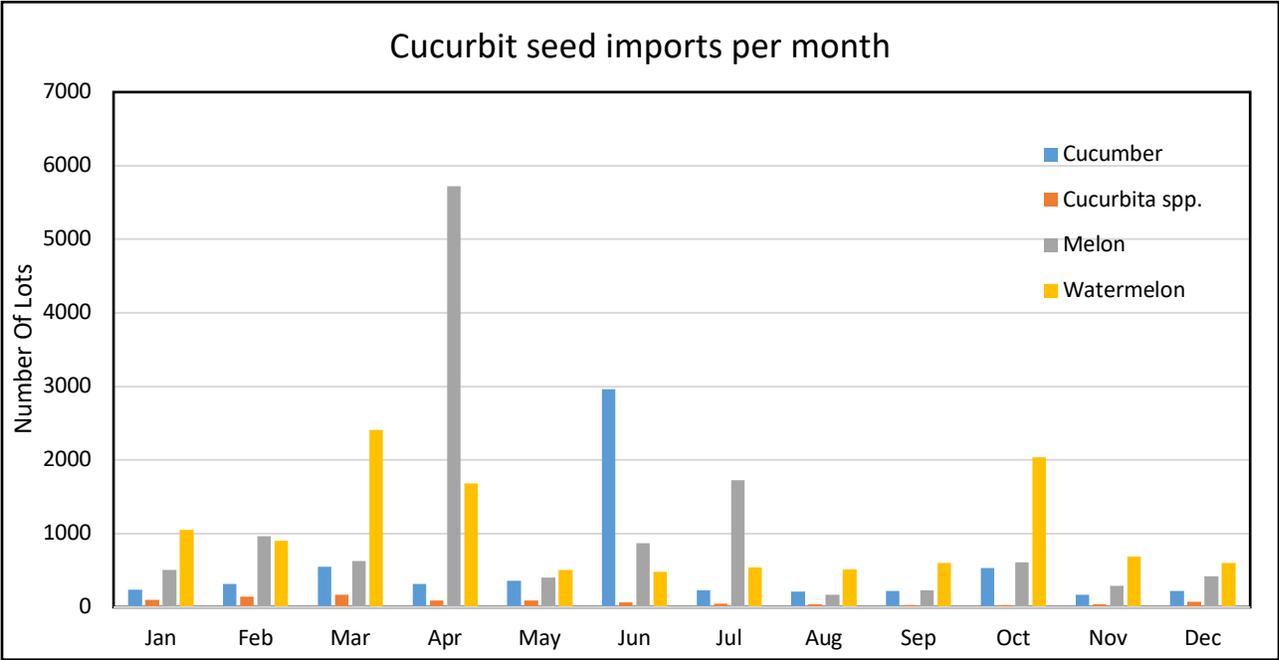


Figure 1. The total reported seed lot data by month reported for all seed types.

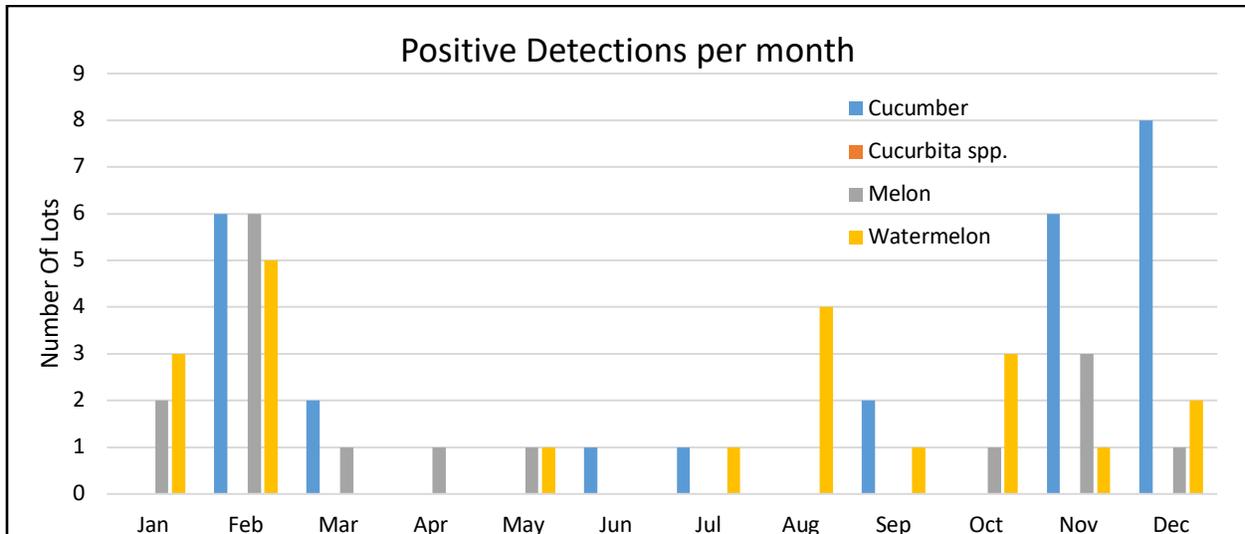


Figure 2. The total seed lot positives by month reported for all seed types.

Auditing of Participants:

During 2017, in an effort to audit the testing being done by entities under the compliance agreement, we asked that entities send a duplicate sample of the last lot they tested under the program to Iowa State University Seed Lab for testing. Ideally, audits would be completed to ensure that all imported seed lots are being tested and reported in addition to ensuring that the agreed upon method is being completed correctly. This allowed ISU to test the lots according to ISTA 7-026 to verify the results. However, issues were raised with industry groups about what would happen if discrepant results had been discovered. In order to avoid these issues, it has been determined that sending out refereed samples was a better way to audit the testing being done in participating labs. The previous drawback to this was the inability of entities in California to receive permits for sending infected seed. Working with the California Department of Food and Ag and the USDA, permitting issues have been resolved and all participants have permits to receive CGMMV infected seed. A panel of seed samples was prepared, containing melon, cucumber and watermelon seed lots with varying levels of infection as well as negative seed samples. The seed panels were shipped near the end of April, 2018. The data from these samples was collected during the first week of June, 2018. All participants completed this proficiency test successfully and all positive samples were detected by all participants. There were 2 labs who provided evidence that they completed the CGMMV proficiency test under the NAL program which we accepted in place of our panel. We feel that this provided sufficient evidence that participants and labs in the program are completing the testing for CGMMV as expected.

Treated Seed Study:

Treated seed also became an issue of concern, as the ISTA method is not validated on treated seed. In order to facilitate testing of a majority of imported cucurbit lots a plan to validate a method for treated seed was developed. Four active ingredients that are commonly applied to cucurbit seed were obtained. The active ingredients are metalaxyl (Alligance), Thiram 42S, Maxim 4FS and Farmore. Farmore is a preformulated product with a blue colorant added. Metalaxyl, thiram and maxim was used to treat seed with and without a colorant added. Green

and red colorants were used. ISTA 7-026 method was used with treated seed. This was done on seed of melon, watermelon and squash with varying levels of infection. While a full analysis of the data is still in progress, preliminary results indicate that it is possible to test treated cucurbit seed using this method. Further experiments are planned as some of the “negative” seed lots had one or two positive results out of twenty. We need to determine if this is due to actual infection of the seed or some sort of cross reaction with the seed or treatment. Even with the possible false negatives, we did have positive results as expected from all samples indicating that there were no false negatives in the data. The data represents 88 different seed, seed treatment and colorant combinations and over 3,500 ELISA wells!

Systems Approach:

As additional declarations for pests on seed moving between different countries increase, becoming more difficult to meet, international phytosanitary discussions have centered around utilizing a systems approach to manage the risk of seed transmittable diseases. In considering how to move forward with the pilot program, we felt it would be useful to begin to implement a systems approach addressing CGMMV in imported cucurbit seed. The ultimate goal of this work is to align with the Regulatory Framework for Seed Health (ReFreSH) that USDA-APHIS is developing.

During February and March of 2019, discussions about how to manage and mitigate the risk of CGMMV in California production areas have occurred as a result of continued detections of positive seed after harvest. During these calls and face to face meetings we have been able to provide information that can be utilized on the risk of imported cucurbit seed. This data allows industry and regulatory agencies to determine if import regulations are needed and if so, how they can be most effectively applied. This also emphasizes the need for a variety of strategies for reducing the risk of disease on imported seed, including a systems approach for import of high value and limited quantity breed materials. The small lots system approach that is being drafted as part of NSHAPP will help meet this need.

During 2019, work has focused on helping to finish the documents for ReFreSH. In an effort to have a pilot project that is most aligned with ReFreSH in the future, it is essential for our documents and foundation to be as similar as possible. To this end, finishing the ReFreSH standard and working on the manual need to be in a near final form as we finalize the parallel NSHAPP program. In early May of this year, a face to face meeting was held with the group of industry and USDA-APHIS staff tasked with working on the ReFreSH manual. In working on this, we have been able to make progress on NSHAPP as we see what terminology and content is used in these documents. Work continues to share information about this effort so that we can find participants for the pilot project and to inform trading partners about how this program is progressing and will possibly operate in the future.

For more information on participating in this program or efforts to develop a systems approach for small lots of cucurbit seed, please see our website: <https://seedhealth.org/nshapp/>. Inquiries can also be made to Dr. Gary Munkvold at munkvold@iastate.edu to Dr. Ed Podleckis at Edward.v.podleckis@usda.gov.