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Evaluation of pests affecting maize imported across Malawi borders

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Abstract

Cross-border trade is one of the major factors that puts maize at risk as it creates pathways for spread of different maize pests in Malawi. This study was carried out to identify pests associated with imported maize in order to improve phytosanitary measures and reduce the introduction of pests. Random sampling technique was used to collect imported maize at selected borders and commercial cities with the aim of identifying pests present during importation. There were no significant differences (p>0.05) in the population of insect pests before and after incubation in all the surveyed districts. Significant differences were observed after incubation for larger grain borer (LGB) and common maize weevil. The highest percentage infestation (32%) of living LGB was recorded in Karonga and the least in Mzuzu (p<0.05). On the other hand, for maize weevil, the highest percentage infestation (20%) was recorded in Nkhatabay while the least was observed in Lilongwe district (p<0.05). Common fungal pathogen species isolated included *Fusarium* (70%), *Aspergillus* (29%) and *Penicillium* (1%). The highest percentage of kernel infection with *Fusarium* sp. was recorded in Dedza and Lilongwe while for *Aspergillus* sp. were in Mzuzu. The *Aspergillus* sp. isolated were *A. niger* (29%), *A. flavus* (22%) and *A. parasiticus* (5%) while *Fusarium* sp. isolated was *Fusarium verticilloides.* The study provided the status and causes of storage losses by various pests on maize. It is recommended that the phytosanitary system present at various Malawi borders where maize is imported should be strengthened. This can be achieved through human and infrastructure capacity building, strict compliance with importation regulations and improved funding of organizations mandated to ensure phytosanitary compliance.

Key words: *Aspergillus sp.,* consignment, food security, grain storage, random sampling, sanitary and phytosanitary measures.

Introduction

Pests cause significant crop losses worldwide, especially in maize and are barriers to the achievement of global food security and poverty reduction (Reynolds *et al.,* 2015). According to Jimma *et al*., (2016), more than sixty diseases and a number species of insects affect maize worldwide. Global trade and exchange have been identified as major contributors to the dispersal of many pests into different regions of the world where they previously did not exist (Sundstrom *et al*., 2014). Larger grain borer is a known destructive pest that is causing major post-harvest losses in maize in many African countries (Tefera *et al*., 2011). According to Murayama *et al*., (2017), Malawi loses about 40% due to LGB. Other common storage insect pests that affect maize grain and are regulated pests include common maize weevil (*Sitophilus zeamays*) grain moth (*Sitotroga cerealella*) and flour beetle (*Tribolium confusum*) (Murayama *et al*., 2017).

Fungal pathogens are among the causal factors of sixty diseases that affect maize. Ranked second after insects, fungi are among the principal factors that lead to deterioration, poor quality and subsequently, yield loss on farmer's maize across the maize value chain (Suleiman *et al.,* 2015). Common storage fungal pathogens include, *Aspergillus* sp.*, Fusarium* sp. and *Penicillium* sp. which are the most predominant species that attack maize (Odhiambo *et al*., 2013). *Aspergillus, Fusarium, Penicillium* species are known to produce mycotoxins and toxic metabolites. According to Tsedaley and Adugna (2016), these metabolites reduce the quality and viability of maize seed. These fungal pathogens also cause significant plant diseases such as seed rot, seedling blight, Bipolaris leaf spot and Cuvularia leaf spot (Tsedaley and Adugna, 2016).

According to Odhiambo *et al.* (2013), fungal pathogens in maize do not only reduce maize yield but also affect germination, storage and quality, with a potential of affecting trade, human and animal life. Health problems may arise through consuming mycotoxin-contaminated produce (Odhiambo *et al*., 2013). Fungi associated with grain contamination at storage may cause seed deterioration and affect the germination of seed when sown

(Lizárraga-Paulín *et al*., 2013). Development of fungi can be affected by moisture content of the maize, temperature, storage time and degree of fungal contamination prior to storage. Some insects and mites whenever present in the maize grain, facilitate fungal dissemination (Odhiambo *et al.,* 2015). To build a strong phytosanitary system, it is important to know and have a list of pests that can be a useful tool for decision-making and planning of management programs to protect the integrity of a country's resources. The main objective of this study was thus to identify pests that affect maize imported across the borders of Malawi.

Materials and Methods

Sample collection and experimental design and layout

The maize grain samples were collected from the Northern, Central and Southern regions of Malawi, bordering entry points (Dedza, Mchinji, Nkhatabay, Songwe, Muloza and Chiponde as well as the major cities in the regions (Mzuzu, Lilongwe and Blantyre). A total of forty-five maize samples were collected five times from each site. Sampling was done randomly and replicated four times. Complete Randomized Design (CRD) was used in the laboratory to identify live pests and fungal pathogens.

Identification of insect pests in maize samples and other grain contaminants

Four replications of one hundred kernels from each sample was used in the identification of insect pests and other grain contaminants following the method described by Sserumaga *et al.,* 2015. The samples were incubated in glass jars which had previously been cleaned and disinfected with 3% sodium hypochlorite. Using a magnifying glass, data on damaged kernels at incubation, number of kernels with holes, number of kernels with moulds, number of rotten kernels and total number of grain contaminants was collected from each batch of 100 kernels. Data on living pests such as LGB, common maize weevil, grain moth and flour beetle were also taken. This included the total number of living pests, total number of dead pests and total number of all available pests. After incubating the samples for 30 days at room temperature, data was collected with the same set of parameters afore-mentioned being taken. Percentage infection of damaged

kernels was calculated as follows (Sserumaga *et al.,* 2015):

$$\text{Damaged Kernel \%} = \frac{\text{No. of damaged kernel}}{\text{Total number of kernel}} \text{x}100$$

Determination of *Aspergillus* species and other fungal pathogens in the maize kernels

This procedure involved the use of light plastic sandwich boxes (1,850 ml) which were initially sterilized in 99.9% ethanol and allowed to dry. One hundred kernels collected from each district was replicated thrice. The kernels were surface sterilized in sodium hypochlorite at a concentration of 2.5% for three minutes and rinsed in sterile distilled water in three consecutive petri dishes. The kernels were transferred into sandwich boxes lined with three moistened absorbent paper towel sheets and sterilized in UV light for 15 minutes (Marcos, 2015). The boxes were covered with plastic lids and incubated for 24 hours. To suppress kernel germination, the boxes were kept in a deep freezer (-20^{0}C) for 6 hours. Later the sandwich boxes were incubated at room temperature (25±1^{0}C) for a period of 7 days as described by Tripathi, 2018. Percentage infection of the kernels was assessed as described by Sserumaga *et al*. (2015). The incidence of each fungus species was calculated as follows:

$$\text{Infected Kernels (\%)} = \frac{\text{No. of kernels infected by particular spp.}}{\text{Total number of infected kernels}} \text{X}100$$

Fungal identification was based on macro morphological characteristics such as surface of the colonies, texture and micro morphological characteristics like conidia head, shape and vesicle as used by Adithiya *et al.,* 2017. Fungal growth colonies on the kernels were visualized using stereo-binocular microscope and identified to genus level.

Determination of Colony Forming Units (CFU) by *Aspergillus* and other fungal species in the maize kernels

The serial dilution technique was used to determine the colony forming units (CFUs) of fungal species in the maize kernels (Marcos, 2015). A total of 250 maize kernels were surface sterilized with sodium hypochlorite and rinsed using sterile distilled water thrice. The 250 maize kernels were blended into

fine powder. Ten grams of the milled powder was suspended into 90ml distilled water and shaken for 30 minutes using a mechanical shaker. Suspension of 1ml was transferred into 9ml of distilled water, vortexed and diluted into subsequent 9ml up to 10^{-4} dilution. Dilutions of 10^{-2}, 10^{-3} and 10^{-4} were plated in selective molten potato dextrose agar (PDA) media, gently swirled, mixed and incubated at 37^0 C for 5-7 days as described by Jarvis *et al.*, 1986. Each sample was replicated three times and growth was observed using Jenko dissecting microscope at x2-x10 magnification (Sibakwe *et al.*, 2017).

Colony Forming Units (CFU) was calculated using the formula (Metzger *et al.*, 2003):

$CFU/g = A*10^n / V$)

Where A = Number of colonies

10^n = Level of dilution at which counting was carried out

V = Volume of inoculation

Data analysis

The data collected from assessed maize samples was subjected to Analysis of Variance (ANOVA) using GenStat software package (version 18.2) with locations, treatments and samples as factors and measurements as variables. Means were separated using Tukey's Protected Least Significant Difference (LSD) test at 5% level of significance.

Results

There were no significant differences (p<0.05) in kernels infested by LGB in all the sampled districts before incubation. The highest percentage infestation (32%) of living LGB was observed in samples from Karonga followed by Nkhatabay and Blantyre districts. The least percentage infestation was recorded in samples from Mzuzu (5%), Dedza (7%) and Mangochi (3%). On the other hand, there were significant differences (p<0.05) for the total available living LGB in the maize samples after incubation across sampled districts. The highest percentage infestation (25%) was observed in Karonga district while the least was observed in Mulanje and Dedza districts (Table 1).

The population of common maize weevils did not differ significantly (p>0.05) among the districts before incubation but significantly differed after incubation. The highest

percentage infestation (20%) was observed in Nkhatabay followed by Karonga (19%) whilst the least was recorded from Lilongwe and Mzuzu districts (Table 2).

There was no significant variation in the population of grain moth and flour beetle before and after incubation in the different districts (Table 3 and Table 4). There were no significant differences (p>0.05) in the number of living, dead and total available grain moth in the imported maize kernels before and after incubation across all the districts. Similarly, there were no significant differences (p>0.05) in the number of living, dead and total available flour beetle in the imported maize kernels before and after incubation.

Table 1. Population of LGB infesting imported maize before and after incubation (Sample size: 100 grains)

	Before			After		
District	Live	Dead	Total	Live	Dead	Total
Blantyre	0.4	0.4	0.8	0.8ab	2.3	3.1ab
Dedza	0.0	0.0	0.0	0.1a	0.1	0.2a
Karonga	0.2	0.2	0.4	1.5b	3.1	4.6b
Lilongwe	0.0	0.2	0.2	0.5ab	1.9	2.4ab
Mangochi	0.3	0.1	0.4	0.1a	1.1	1.2ab
Mchinji	0.1	0.3	0.4	0.4ab	1.5	1.9ab
Mulanje	0.2	0.1	0.3	0.3ab	0.7	1.0a
Mzuzu	0.3	0.2	0.5	0.0a	2.1	2.1ab
Nkhatabay	0.5	0.3	0.8	1.00ab	0.9	2.0ab
Mean	0.22	0.2	0.42	0.52	1.52	2.06
CV%	310.9	399.6	331.1	202.8	161.2	138.2
LSD	0.5	0.5	0.7	0.8	1.9	2.1
F pr.	0.571	0.852	0.562	0.002	0.177	0.023

Means were separated by Tukey's Protected Least Significance Difference (LSD) at p=≤0.05, Means followed by same letter(s) within columns are not significantly different.

Table 2. Population of common maize weevil infesting imported maize before and after incubation (Sample size: 100 grains)

	Before			After		
District	Live	Dead	Total	Live	Dead	Total
Blantyre	0.0	0.0	0.0	2.4ab	0.3	2.7a
Dedza	0.5	0.3	0.8	1.7ab	0.5	2.2a
Karonga	1.5	0.4	1.9	3.6ab	0.7	4.3a
Lilongwe	0.5	0.1	0.6	0.7a	0.1	0.8a
Mangochi	0.7	0.1	0.8	1.9ab	0.3	2.2a
Mchinji	0.3	0.0	0.3	1.7ab	0.1	1.8a
Mulanje	0.6	0.1	0.7	1.7ab	0.1	1.8a
Mzuzu	0.6	0.2	0.8	1.1ab	0.1	1.2a
Nkhatabay	0.7	0.2	1.0	3.7b	0.3	4.1a
Mean	0.6	0.16	0.77	2.06	0.27	2.34
CV%	253.0	342.3	239.7	124.4	233.2	122.9
LSD	1.1	0.3	1.4	1.9	0.4	2.1
F pr.	0.375	0.534	0.294	0.020	0.277	0.025

Means were separated by Tukey's Protected Least Significance Difference (LSD) at $p=\leq 0.05$. Means followed by same letter(s) within columns are not significantly different.

Table 3. Population of grain moth (*Sitotroga cerealella*) infesting imported maize kernels (Sample size:100 grains)

	Before			After		
District	Live	Dead	Total	Live	Dead	Total
Blantyre	0.3	0.1	0.4	0.7	0.3	1.0
Dedza	0.2	0.1	0.3	0.4	0.5	0.9
Karonga	0.1	0.0	0.1	0.5	0.1	0.6
Lilongwe	0.4	0.1	0.5	0.4	0.3	0.7
Mangochi	0.3	0.1	0.4	0.5	0.1	0.6
Mchinji	0.1	0.1	0.2	0.4	0.2	0.6
Mulanje	0.8	0.3	1.1	0.9	0.1	1.1
Mzuzu	0.1	0.1	0.2	0.1	0.1	0.2
Nkhatabay	0.5	0.1	0.6	0.5	0.1	0.6
Mean	0.3	0.5	0.4	0.5	0.2	0.7
CV%	264.4	378.3	313.3	268	289.6	252.4
LSD	0.6	0.3	0.8	0.9	0.4	1.3
F.pr	0.425	0.643	0.365	0.906	0.483	0.95

Means were separated by Tukey's Protected Least Significance Difference (LSD) at (p=≤0.05).

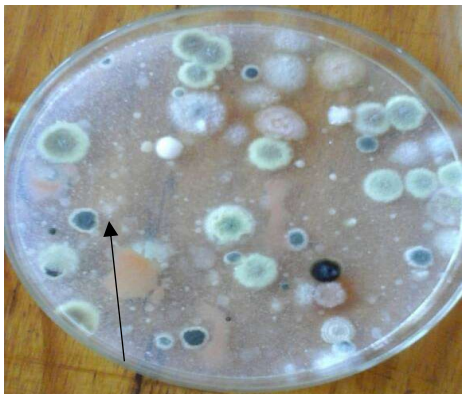
Table 4. Population of flour beetle (*Tribolium castaneum)* infesting imported maize kernels (Sample size: 100 grains)

	Before			After		
District	Live	Dead	Total	Live	Dead	Total
Blantyre	0.1	0.0	0.1	0.2	0.0	0.2
Dedza	0.6	0.2	0.5	0.9	0.0	0.9
Karonga	0.1	0.1	0.1	0.1	0.1	0.2
Lilongwe	0.2	0.0	0.2	0.5	0.0	0.5
Mangochi	0.1	0.0	0.1	0.0	0.0	0.0
Mchinji	0.2	0.0	0.2	0.1	0.0	0.1
Mulanje	0.1	0.0	0.1	0.0	0.0	0.0
Mzuzu	0.3	0.1	0.3	0.3	0.0	0.3
Nkhatabay	0.1	0.0	0.1	0.1	0.0	0.1
Mean	0.2	0.1	0.1	0.2	0.01	0.3
CV%	308.9	602.1	337.2	337.2	1161.9	336.1
LSD	0.4	0.2	0.4	0.6	0.1	0.6
F.pr	0.147	0.212	0.43	0.101	0.439	0.131

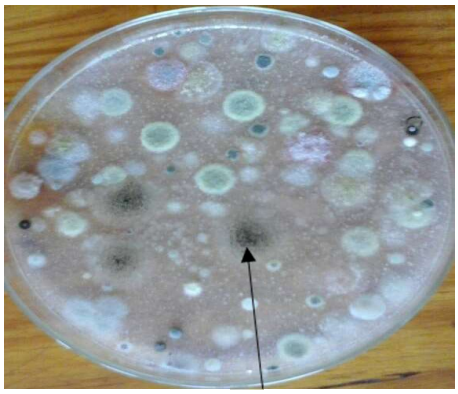
Means were separated by Tukey's Protected Least Significance Difference (LSD) at $p=\leq 0.05$.

Identified fungal pathogens

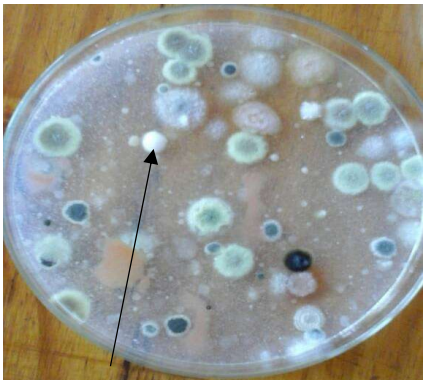
Mycological analysis of the maize samples showed a wide range of fungal pathogens that affected kernels from each district. Common fungal pathogens identified during direct plating and serial dilution included *Aspergillus* species (*Aspergillus flavus, Aspergillus niger, Aspergillus parasiticus*), *Fusarium* species and *Penicillium* species (Figure 1).



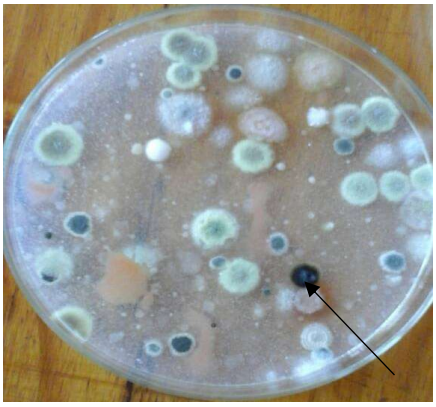
Aspergillus parasiticus



Aspergillus niger



Penicillium



Fusarium verticilloides

Figure 1. Different *Aspergillus* colonies and other fungal species isolated from maize observed at Biotechnology laboratory at Chitedze agricultural research station

The total kernel infection by different fungal pathogens varied significantly (p<0.05) across the districts (Table 5). Kernels with highest fungal contamination were from Mzuzu (15%) followed by Dedza (13%), while the least were from Nkhatabay (10%). Significant differences (p<0.05) of kernels infected with *A. flavus, A. niger* and *Fusarium* were observed across different districts. The highest percentage of kernels contaminated with *A. flavus* were from Mzuzu (26%) followed by Nkhatabay (22%) and Mchinji (18 %). The highest percentage of kernels contaminated by *Aspergillus niger* (22%) were from Mzuzu, followed by Mangochi (16%) and the least from Lilongwe (15%). The highest percentage of kernels contaminated with *Fusarium* were from Dedza and Lilongwe while Mchinji had the least (8%). However, no significant differences were noted in kernels contaminated with *A. parasiticus* and *Penicillium* species (Table 5).

Table 5. Percentage of kernels affected with different fungal species.

District	*A.flavus*	*A.niger*	*A.parasiticus*	*Fusarium*	*Penicillium*	Ttl of infect. Kernels
Blantyre	0.6a	20.2cd	0.2	45.3ab	0.2	66.56ab
Dedza	0.9a	16.9bcd	1.1	62.5b	0.1	81.57bc
Karonga	2.9abc	14.8bc	0.2	53.8ab	0.2	71.88b
Lilongwe	1.7ab	6.3ab	0. 9	61.5b	0.3	70.71ab
Mangochi	0.7a	25.8de	1.1	45.3ab	0.1	73.02b
Mchinji	0.4a	21.7cd	0.8	40.7a	0.1	63.69ab
Mulanje	1.3ab	23.5cd	0.9	47.9ab	0.2	73.78b
Mzuzu	4.2c	36.7e	0.3	54.7ab	0.0	95.89c
Nkhatabay	3.5bc	0.00a	0.4	46.1ab	0.0	49.96a
Mean	1.8	18.4	0.63	50.9	0.13	71.9
CV	121.9	51.5	142.0	34.1	323.9	121.9
LSD	1.580	6.861	0.687	12.55	0.331	1.580
F pr.	<.001	<.001	0.014	0.005	0.722	<.001

Treatments with different letters are significantly different @ p<0.05. Means were separated by Tukey's protected LSD -Least significance different @ 5% confidence.

The number of CFU that were observed during mycological analysis differed across the districts. Significant differences (p<0.05) were observed in the number of CFU of *A. niger*, *A. parasiticus* and *Fusarium* (Table 6). However, no significant differences were noted in the number of CFU of *A. flavus* and *Penicillium* species. The largest CFU of *A. flavus* were from Nkhatabay while the least were observed in Mchinji (24). Dedza had the largest number of CFU of *A. niger* and *A. parasiticus* (32%) while Nkhatabay (25%) and Mulanje (18%) had the least, respectively. The number of CFU of *Fusarium* species was higher in Karonga (24%) followed by Mchinji (15%) while the least were from Blantyre (10%) district. There were no significant differences in total CFU among the districts.

Table 6. Number of CFU by different *Aspergillus* species and other fungal pathogens affecting the maize

District	*A.flavus*	*A.niger*	*A.parasiticus*	*Penicillium*	*Fusarium*	Total CFU
Blantyre	847bc	246ab	436ab	18.3	244.5a	1790
Dedza	104a	768c	1297b	23.3	313.5a	2506
Karonga	418ab	213ab	249ab	34.9	1454.7b	2370
Lilongwe	442ab	475bc	132ab	2.3	617.5ab	1670
Mangochi	692abc	321abc	175ab	15.7	501.6a	1705
Mchinji	101a	364abc	998ab	80.9	917.4ab	2461
Mulanje	415ab	464bc	61a	34.5	604ab	1579
Mzuzu	222a	367abc	151ab	84.9	551.0a	1377
Nkhatabay	1022c	0.0a	529ab	10.7	782.5ab	2344
Mean	473.7	357.6	447.6	33.9	665.2	1978
CV%	192.4	111.3	230.4	284.3	115.2	85.0
LSD	658.7	287.7	745.1	69.69	553.6	1214.6
F pr.	0.068	<.001	0.011	0.195	0.002	0.431

Treatments with different letters are significantly different @ $p<0.05$, Means followed by same letter(s) within columns are not significantly different. Means were separated by Tukey's protected LSD, CV = Coefficient variation, CFU Total: Total fungal colonies available in maize samples.

Discussion

Cross-border agriculture has been identified as one of the pathways that transmit pests across regions. The results of this study have provided key evidence that pests are transmitted into Malawi as maize is traded with other countries. The implication of these results suggests that imported maize grain is an important pathway in transmission of both regulated and non-regulated maize pests which affect quality and quantity of maize value chain.

During the study, A*spergillus* was found to be the most predominant fungal pathogen, corroborating findings by Tsedaley and Adugua (2016) who showed that the most predominant fungal species isolated from maize kernels belonged to the *Aspergillus* spp.

Ozay *et al*. (2008) and Nyasetia (2015) also noted that *Aspergillus* spp and other fungal pathogens had contaminated maize and hazel nuts kernels at storage. The presence of fungal pathogens such as *Aspergillus* is influenced by the percentage of the available factors. These include; damaged kernels, presence of foreign matter and impurities, presence of microorganisms, insects and mites, period of storage moisture content, the relative humidity, storage atmosphere and length of storage (Nyasetia, 2015; Ozay *et al*., 2008). Because of these conditions, the corn kernels lose mass, volume and strength and experience nutritional degradation, discoloration, development of unpleasant odors, heat and chemical changes. High *Aspergillus* population levels may also be attributed to late harvesting (Wanjiku, 2016).

During the study, it was observed that storage methods can increase the population of living pests. These storage facilities especially when unsealed, create a favorable environment for breeding and multiplication of pests. These results concur with Tefera *et al*., (2011a) who found that there was an increase of population of living pests due to storage environment. In addition, the study by Tefera *et al*., (2011) also suggested that temperatures between 27°C-32°C and 38°C increased the population of maize pests at storage. The current study however, contradicts with the findings of Bell (2014) who observed that population of insect pests decreased between 27°C-32°C and 38°C. According to Suleiman *et al*., (2015) storage of maize while relatively moist and warm may lead to rapid deterioration of the grains and promote the growth of microorganisms. In tropical and subtropical countries, a large portion of grain is harvested and stored under hot and humid conditions and most farmers lack proper knowledge, equipment and methods of drying grains (Matumba *et al*., 2015). Subsequently, maize being hygroscopic in nature, tends to absorb or release moisture (Matumba *et al*., 2015, Wanjiku, 2016) leading to increased maize moisture contents and increased deterioration. Given that the districts in which this study was carried out experience hot and humid conditions, this may have created conducive environment for the development of fungal pathogens.

Conclusion and recommendations

This study showed that cross-border trade is responsible for the spread of fungal pathogens and living pests associated with maize imported into Malawi. The detection of various living pests and pathogens before and after maize kernel incubation is a cause for concern because the pests affect maize across the value chain hence reducing the quantity and marketable quality of the produce. The results from this research should be considered in order to improve the effectiveness of Malawi's phytosanitary system. Imported maize should be sampled and analyzed for pests at borders before being released for consumption in various districts. Other imported materials posing the same threat should also be closely monitored.

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