frontiers | Frontiers in Agronomy

ORIGINAL RESEARCH
published: 17 May 2022
doi: 10.3389/fagro.2022.868573

Detection of Foreign Material in Soybean (*Glycine max*) Grain

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The United States (US) is ranked second in the world in exporting soybean with Louisiana ranked 17th for exporting agricultural products, including soybean. Importing countries maintain high standards for the level of foreign material (FM) accepted in soybean grain shipments. While it has been identified that changes are needed, there is limited research on what comprises the FM, specifically weed seeds, in these samples. The objective of the study was to determine what proportion of the FM consists of weed seeds and to quantify those seeds in Louisiana grain elevators and in Mississippi River Valley (MRV) barges bound for Louisiana. Fifty soybean samples were taken from barges traveling down the Mississippi River Valley (MRV) as well as 56 total samples from Louisiana grain elevators. Each sample was weighed, and each fraction of the FM was separated, weighed, and counted to determine the exact proportion of each fraction. Twelve different weed seeds were found within the FM samples. The amount of weed seeds found was not consistent based on where the sample was retrieved, grain elevator or MRV barge, however the total FM was <1% for all MRV barge samples. A better understanding of the FM in soybean grain samples will allow the midsouthern US to know more accurately which weed seeds are found within grain bound for exportation.

Keywords: international trade, weed seeds, soybean, foreign material (FM), grain

OPEN ACCESS

Edited by:
Bhagirath Singh Chauhan, The University of Queensland, Australia

Reviewed by:
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Specialty section:
This article was submitted to Weed Management, a section of the journal Frontiers in Agronomy

Received: *02 February 2022*
Accepted: *04 April 2022*
Published: *17 May 2022*

Citation:
Schwartz-Lazaro LM, LaBiche G and Copes JT (2022) Detection of Foreign Material in Soybean (Glycine max) Grain. Front. Agron. 4:868573. doi: 10.3389/fagro.2022.868573

INTRODUCTION

Weeds are a major factor in yield loss across the major cropping systems. Weed seeds that are present at harvest can reenter the soil seedbank by being spread out of the rear of the combine (Shergill et al., 2020) or can contaminate harvested grain (Owen and Powles, 2020). Contaminated grain samples, whether from weed seeds or other materials, can cause for the grain to be devalued or rejected. Weed seed dispersal can be caused by humans, machinery (i.e., movement of equipment from field to field or via being sold), livestock movement, and planting contaminated grain (Humburg et al., 2009; Hogan and Phollips, 2011; Owen and Powles, 2020). An additional pathway in which weed seed can be introduced into a system is through grain imports (Shimono et al., 2010, 2015; Wilson et al., 2016). However, as grain samples move from farm to port, the associated risks of foreign material (FM) are reduced (Wilson et al., 2016). Wilson et al. (2016) identified six points, or events, along the pathway that have relevance for weed risk, including crop-weed associations at the point of origin, farming practices, grain handling practices, transport and storage, import requirements, and end use of grain in the country of destination. Each event can be used to reduce risks of FM.

The level of FM, or weed seeds specifically, can be reduced initially through overall knowledge of crop production and harvesting practices. Crop production practices such as the use of tillage, planting date, row spacing, crop variety, and so forth can influence the crops' ability to outcompete weeds (Swanton and Weise, 1991). Additional weed management decisions include starting with weed free fields and utilizing chemical weed management where appropriate (Norsworthy et al., 2012). Chemical weed control options vary by crop and can be very effective in controlling weeds, however with the rise of herbicide resistant (HR) weeds (Heap, 2021) the amount of weed seeds present at harvest has increased leading to increased contaminated grain shipments (Shimono et al., 2010). Additionally, volunteer crops can be problematic at harvest (Shimono and Konuma, 2008). Combine setup is another critical component to ensure a reduction of weed seeds present within the grain. Ensuring that the combine fans and sieves, as well as air flow throughout the combine is properly set up prior to harvest can reduce weed seeds within the grain.

At harvest, critical factors contributing to weed contamination levels include timing (i.e., timely harvest, weather conditions, crop vs. weed height, weed maturity, and combine settings (Forcella et al., 1996; Davis, 2008; Shimono and Konuma, 2008). Weed seeds, above the header cutting height, are likely to be harvested with the grain. Early maturing weed species (i.e., grass spp), in soybean (*Glycine max* L. Merr), shed a large amount of their seeds prior to harvest. Other species, such as pigweed species (*Amaranthus* spp.), morningglory species (*Ipomea* spp.), and ragweed species (*Ambrosia* spp.), retain high amounts of seed from crop maturity through delayed harvest (four weeks after maturity) (Schwartz-Lazaro et al., 2021a,b). Although weed species and weed densities vary from farm to farm and by cropping system, best management practices (BMPs) can reduce the number of weeds and consequently weed seeds that have escaped season long management.

From the farm level, harvested grain typically moves through a series of elevators on its way to export, where it is cleaned and graded to determine its market value. Grain grading examines both quality (e.g., minimum test weight, heat damaged kernels) and cleanliness (e.g., percent FM) are specified for each grade of a given crop, with the highest grade representing the highest quality. Foreign material, specifically in soybean grain, is defined as all matter that passes through a 0.318 cm round-hole sieve, and all matter other than soybean remaining in the sieved sample after sieving [Federal Grain Inspection Service (FGIS), 2009]. This material can include other crop seed, cracked or broken crop seed, weed seed, soil particles, rocks, stems, leaf debris, etc. The percentage of allowable FM in grain shipments varies based on the grade of the grain and often a maximum limit of specific weed seeds, which varies by species and export country, in the FM are imposed in grain exports [Federal Grain Inspection Service (FGIS), 2009].

In the US, Louisiana is ranked 17th for being one of the main agricultural product exporters, 51% of which is soybean, and the US is ranked second for exporting soybean specifically, with China being the largest importer (Kennedy, 2018; USDA APHIS, 2020). Some countries that import high quantities of US soybean maintain strict standards for FM in soybean grain shipments specifically to limit the amount of weed seeds, potentially HR, that may enter that country [United States Department of Agriculture Animal and Plant Health Inspection Service Plant Protection and Quarantine (USDA APHIS), 2017]. For example, China has specifically identified 36 weed species of concern that make up 80% of intercepted weed seeds. Four weed species of are particular concern: ragweed species, common cocklebur (*Xanthium strumarium* L.), johnsongrass [*Sorghum halepense* (L.) Pers.], and pigweed species (USDA APHIS, 2020). While these concerns have begun to be addressed, there has been limited research on what consists of the FM, specifically weed seeds, in these samples from farm to export (Schwartz-Lazaro et al., 2019). The objective of the study was to determine what proportion of the FM consists of weed seeds and to quantify those seeds in Louisiana grain elevators and in Mississippi River Valley (MRV) barges bound for Louisiana.

MATERIALS AND METHODS

Data Collection

In 2018 and 2019, soybean grain samples, 25 samples in 2018 and 31 samples in 2019 for a total of 56 samples, were collected from five Louisiana grain elevators across Louisiana (**Figure 1**). Additionally in 2019, 50 samples were collected from 12 total barges traveling down the Mississippi River Valley (MRV) to Louisiana ports located in New Orleans, LA. Grain samples were collected by probing grain hoppers a total of four times The sample was further divided into four parts at the grain elevator: 40% was retained for test weight, 30% for grain moisture, 20% for grain quality, and 10% to assess FM. The FM fraction that was retained by the grain elevator was the sample that we assessed further. A similar method was conducted for the MRV barge samples. Each sample was weighed on arrival. The FM samples were weighed and then separated into fractions though use of a series of sieves with pore size ranging from 0.318 cm round to 0.035 cm opening (355 microns). Material not passing through each sieve size was separated into the following categories: whole soybean seed, broken soybean seed, soil, rocks, leaf debris or stems, weed seed by species, and other crop seed by species. Soil, rocks, and leaves and steams were pooled into a single category further known as "debris." Each category was subsequently weighted, and the number of seeds were counted in the applicable categories. Weed seeds were identified by visual inspection under a microscope. A subsample of the seeds were grown out in the greenhouse for confirmation. Soil was the only substance remaining after use of the smallest sieve size and was weighed.

Statistical Analyses

There was no significant difference between years for the Louisiana grain elevators, so data were pooled. Data were analyzed with a two-way ANOVA using the PROC MIXED procedure in SAS 9.4 (SAS Institute Inc., Cary, NC). Significance was assessed at $P < 0.05$. A first first-order autoregressive covariance structure [type = ar(1) in PROC MIXED] was used in the model because it returned the lowest Akaike's Information Criteria (AIC) fit statistic compared with unstructured or compound symmetry. Means separation of significant interactions and, when appropriate, main effects, were based on least square means (LSMeans) tests.

Abbreviations: FM, foreign material; *Glycine max* (L.) Merr, soybean.

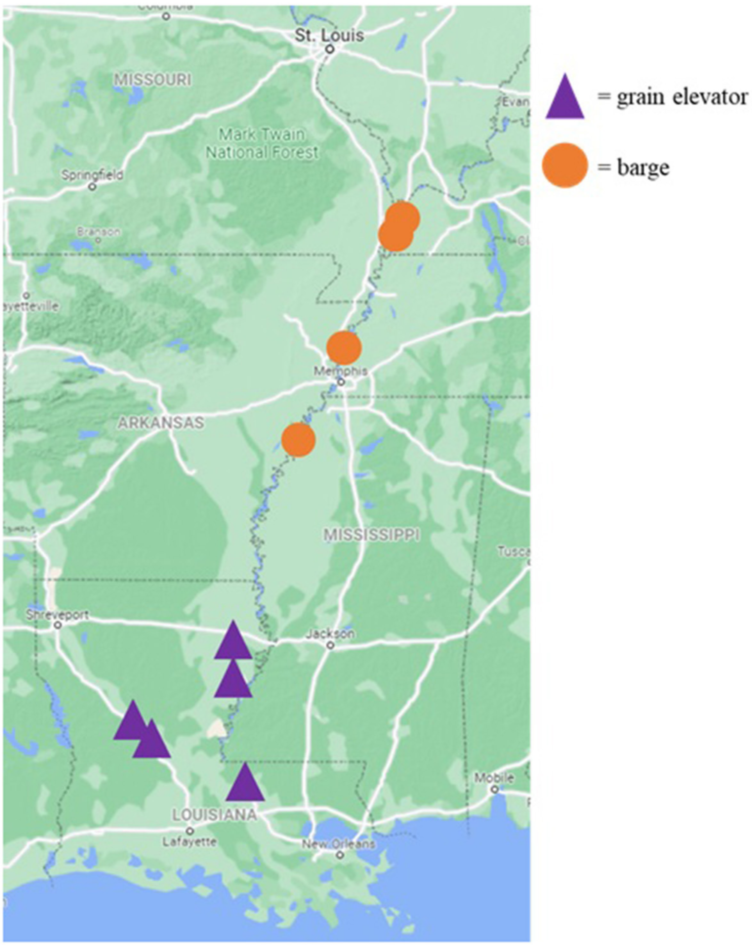

FIGURE 1 | Map of approximate locations of where grain elevator (purple triangles) and Mississippi River Valley barge (orange circles) samples were collected from.

RESULTS AND DISCUSSION

Louisiana grain elevator samples ranged in total weight from 180 to 220 g, whereas MRV samples ranged from 200 to 2,524 g. All grain samples contained FM. The range in sample size was due to the proportion of the graded sample that we received. The MRV barges are larger than individual trucks of grain that the Louisiana grain elevators received.

The amount of FM was not consistent between grain elevators (1.1 to 9.3%) and barge (1.5 to 69.9%) samples. The debris component made up most of the FM (**Table 1**). While proportions of FM from the grain elevator were over the 1% FM threshold that is in place for agricultural imports into some countries, whereas the MRV barge samples were within the US Department of Agriculture's Plant Protection and Quarantine phytosanitary regulations (between 1 and 2% FM) range. This shift is likely due to seed cleaning and blending that occurs across various stages of the grain shipments. Conventional seed cleaning includes the use of aspirators, screens, gravity tables, and other separators to remove debris and weed seeds from the crop based on size, shape, or weight (Wilson et al., 2016). Cleaning removes FM and may be done on-farm, at local grain elevators, or when grain is received at feed mills or processing plants (Wilson et al., 2016). Blending involves balancing the costs of cleaning grain against the market value and blending high- and low-FM grain to produce the desired FM level (Lin, 1996).

Seeds of 14 different species contaminated the crop seed, with 12 being weed seeds. The most common weed species, as a proportion of the total cleaned grain samples, regardless of collection location, included: pigweed spp. (72%), morningglory spp. (31%), barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] (30%), johnsongrass (22%), ragweed spp. (14%), hemp sesbania (*Sesbania herbacea* L.) (8%), prickly sida (*Sida spinosa* L.) (8%), and sicklepod (*Senna obtusifolia* L.) (5%) (**Figure 2**). The remaining weed seeds were only found in the MRV barge samples (<1%). This is likely due to the fact that these weed species are not currently found within Louisiana. Further, the four species of concern (i.e., pigweed species, johnsongrass, common cocklebur, and ragweed species) that have been identified were found in each MRV barge sample. Only johnsongrass and pigweed species were found in the Louisiana grain elevator

TABLE 1 | Percentage (±SE) of the foreign material samples components for the Louisiana (LA) grain elevators (2018 and 2019 data pooled) and Mississippi River Valley (MRV) barge samples.

Sample	Mean % FM per grain sample	Composition of FM (% of total grain sample)		
		% debris of FM	% damaged soybean of FM	% weed seed of FM
LA grain elevator	2.86 ± 0.29	76.2 ± 0.58	13.6 ± 1.41	10.1 ± 0.07
MRV barge	1.30 ± 0.31	86.9 ± 0.32	6.95 ± 1.00	6.15 ± 0.03

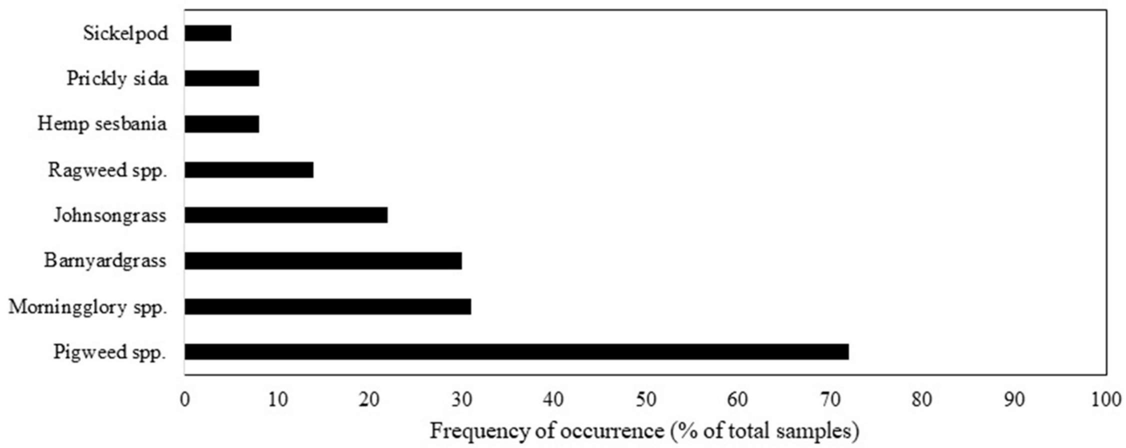

FIGURE 2 | Frequency of occurrence (% of the total grain samples) of common weed species.

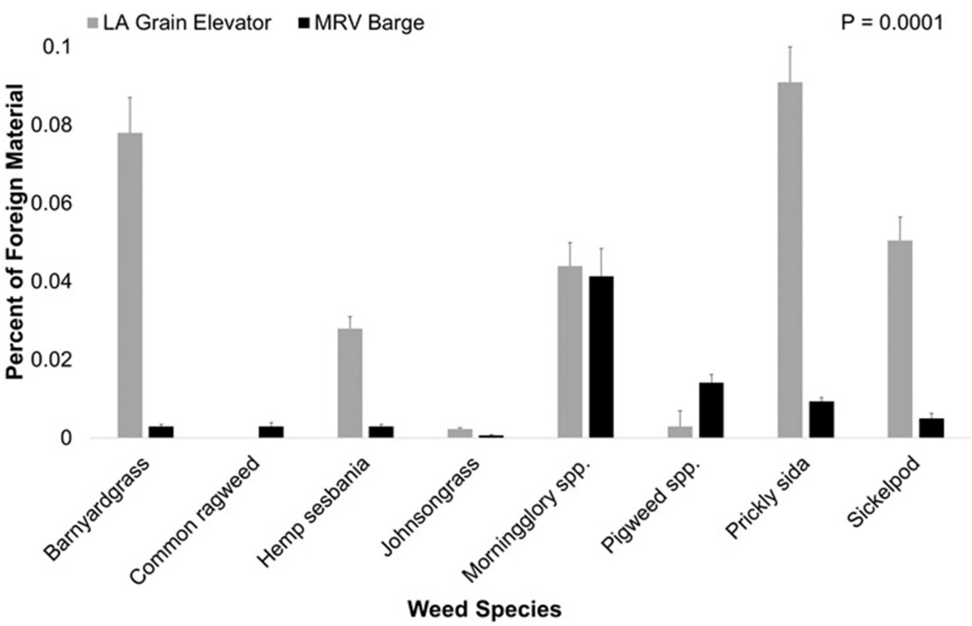

FIGURE 3 | Occurrence of weed seed in grain samples, at local Louisiana grain elevators and at various Mississippi River (MRV) barges, expressed as a percentage of the weight of foreign material.

samples as the other two are not typically seen in Louisiana cropping systems. These weed species all retain their seeds through soybean harvest (Schwartz-Lazaro et al., 2021a,b) and have some level of herbicide resistance across the midsouthern US (Heap, 2021). Corn (*Zea mays* L.) (11%) and rice (*Oryza sativa* L.) (7%) seeds were the crop seeds found in the grain samples.

High levels of FM were detected, but there were relatively low levels of each weed species (<0.5%). A highly significant interaction ($P = 0.0001$) between the number of weed seeds found in wither the grain elevators or on the MRV barges (**Figure 3**). Weed seeds made up 0.1 to 2.2% and 0.02 to 0.2% of the total FM, for the grain elevator and MRV barge samples, respectively. Prickly sida (0.09%) and barnyardgrass (0.07%) were the two most common weed seeds found in Louisiana grain elevators, whereas morningglory spp (0.04%) and pigweed spp (0.02%) were the most found weed seeds on the MRV barge samples. Pigweed spp (<0.01%) were one of the lowest numbers of weed seeds found in the Louisiana grain elevator samples. While overall percentages are relatively low (all <1% of the total FM), the total number of seeds found within these samples varied by species. For example, previous research has shown pigweed spp (i.e., Palmer amaranth (*Amaranthus palmeri* S. Watson) and common waterhemp [*Amaranthus tuberculatus* (Moq.) Sauer]) to produce an average of 31,000 seeds plant^{-1} and barnyardgrass to produce an average of 5,000 seeds plant^{-1} (Dalley et al., 2004; Jha et al., 2008; Schwartz-Lazaro et al., 2017a). This would translate to about 620 (0.25 g) and 350 (6.3 g) seeds or Palmer amaranth and waterhemp, respectively, within the FM of the Louisiana grain elevator samples (180 to 220 g samples) (seed weights per 100 seeds found in Schwartz-Lazaro et al., 2017b). The number of potential HR weed seeds is much larger than expected from the percentage of FM, which can be problematic.

Practical Implications

Weed seed contamination in grain shipments can pose a risk to export markets. Shimono et al. (2015) found that grain delivered to ports in Japan from Australia, Canada, and the US contained HR weed seed. The rise in HR weeds, especially in soybean, poses an economic threat to soybean grain exports and brings questions on where grain is sourced to limit the spread of weed seeds. However, adhering to BMPs from the beginning of the growing season and making sure that equipment (i.e. planter, sprayer, combine) is properly calibrated, especially at harvest, will likely limit weeds from escaping season long management and entering the grain. In the end, all management tactics that prevent weed seeds from returning to the soil seedbank reduce the yearly aboveground weed density and maintains the sustainability of farming systems in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

LS-L and GL developed the protocol, collected data, and processed the data. LS-L analyzed the data. All authors contributed to the article and approved the submitted version.

ACKNOWLEDGMENTS

We would like to thank JC for helping to obtain samples from Louisiana grain elevators. In addition, Rachel Porter, Katie Patterson, and Brooklyn Hampton for helping to process the foreign material samples. The authors would also like to thank the cooperating Louisiana grain elevators and The Russell Marine Group for providing the barge samples.

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