analysis. This paper reports the results of research only. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture. The US Department of Agriculture is an equal opportunity provider and employer.

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Survey of *Trogoderma* species (Coleoptera: Dermestidae) Associated with International Trade of Dried Distiller's Grains and Solubles in the USA

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Abstract

Dried distiller's grains and solubles, DDGS, is a valuable commodity with substantial international trade. Vietnam discovered an infestation of *Trogoderma inclusum*, an actionable quarantine pest, in DDGS from the USA in 2012.

All subsequent shipments to Vietnam were required to be fumigated. A shipment to Vietnam from the USA 2015 was then discovered with *T. variabile*. We surveyed the presence and activity of *T. inclusum* and *T. variabile* at locations in the USA that provide DDGSs for shipment to Vietnam. Seven facilities in four states that either produced DDGSs or that facilitated bulk shipments were studied. Pheromone traps were deployed at each location and monitored for several weeks. *T. variaible* was trapped at all seven sites while *T. inclusum* was trapped at just five of these. *T. variabile* were captured in nearly every trapping period and at higher numbers than *T. inclusum* at five locations, while two locations captured more *T. inclusum* than *T. variabile*. Spatial variation seemed to occur within each site, but there was no common pattern among facilities. Substantial numbers of beetles were caught in the outdoor sticky flight traps for most locations, except for relatively low flight trap numbers at locations 1, 4 and 6. The results show that *T. variabile* and *T. inclusum* are commonly associated with DDGSs produced in the USA, that these beetles could infest product being shipped overseas, and provide information that can be used to develop risk assessment and pest management programs for the future.

Keywords: Coleoptera, Dermestidae, DDGS, Vietnam, quarantine.

Introduction

The United States Grains Council (USGC) learned in late 2012 that the Vietnamese government's Plant Protection Department (PPD) had discovered an infestation of the larger cabinet beetle, *Trogoderma inclusum*, an "actionable" quarantine pest for Vietnam, in a shipment of Dried Distillers Grains and Solubles, DDGS, from the US (USGC 2012). The Vietnam PPD required the infested shipment be fumigated and then re-exported. The PPD also required that all DDGS shipments from the US to Vietnam be fumigated before delivery from that time forward. The US DDGS industry complied with the required fumigation on all subsequent shipments. No infested shipments were reported in the subsequent three years, until a shipment of 12 containers of DDGS from Norfolk, VA on September 17, 2015 was inspected in Vietnam at arrival on October 27, 2015 and found to be infested with live warehouse beetles, *Trogoderma variabile*, a close relative to *T. inclusum*. It is presumed that this shipment had been fumigated at the time of export, as required by agreement. Assuming that fumigation was performed on the commodity before leaving the US, the infestation could have occurred via one of two ways: the fumigation was not entirely effective in completely disinfesting the shipment, or that infestation occurred after the Norfolk fumigation, but before the delivery in Vietnam six weeks later.

Both *T. variabile* and *T. inclusum* are stored grain insect pests that are commonly found in the US and around the world as part of a complex of many pest species that infest post-harvest agriculture products (Aitken 1975). Commodities infested by these species incude cereal grains, ground or milled grain products, nuts, dried fruits and numerous value-added food products (Hagstrum and Subramanyam 2009). *T. variabile*, the more common of the two, is reported in the scientific literature to occur in Vietnam. To our knowledge, *T. inclusum* has not been reported to exist in Vietnam, though it is reported in the entomology literature as occurring in Thailand. The Vietnam PPD considers *T. inclusum* to be an exotic pest subject to quarantine regulations that would involve inspection followed by some action if discovered. Quarantine action for introduction of *T. inclusum could include* disinfestation of arriving shipments via fumigation, return of infested commodity to the source country, or destruction of an infested shipment. All life stages (egg, larva, pupa and adult) of both beetle species can be effectively killed by properly fumigating with an effective gas such as phosphine or methyl bromide. Both species occur in the US, and it is likely that these species could feed on and reproduce in DDGS, but we have not found published reports of these species infesting DDGS. In any case, we know that both species are common in the US and in many other countries, and that these pests can probably infest DDGS and travel with shipments from the US to any of our trading partners.

We were contracted by the USGC in mid-2016 to assess the presence of *T. inclusum* and *T. variabile* in representative supply-chain contexts of DDGS production and commerce in the midwestern USA. Information on the occurrence and relative abundance of the target insects can be used to estimate the risk of infestation at DDGS facilities and then infer how that risk could lead to these pests being carried in shipments to Vietnam. It is hoped that the USGC and other trade or agricultural product

organizations could use such insect risk information to develop better pest prevention and mitigation practices for the DDGS industry. Specific objectives for us were:

- Select and engage DDGS companies in the north-central Midwest of the USA, including both ethanol plants and trans-loading facilities, to participate in the project.
- Make site visits to each of the cooperating companies to conduct a thorough inspection, interview key personnel, deploy insect traps for *T. variabile* and *T. inclusum*, and develop plans for continued trapping.
- Analyze all traps from each facility for the presence and numbers of the target species, with specific attention to relative numbers of insects trapped over time throughout the trapping season, and among specific trapping sites at each company.

Materials and Methods

Participating companies were in our geographic area of interest, whcih was the corn-growing region of the US at sites located in the states of Illinois, Indiana, Iowa and Missouri. These sites included five ethanol plants, numbered 1-5 in Table 1 below, and two trans-loading facilities, numbers 6 and 7. On-site visits were made to participating facilities during May, June, July and August of 2016. All facilities we studied were using corn as the grain to be distilled into ethanol and the manufacturing procedures at the ethanol plants were similar. Briefly, grain was delivered, stored, mashed with water, yeast and additives for fermentation, the ethanol separated and purified form the fermentation product distillation after which ethanol was prepared for delivery and the DDGS were dried, cooled and loaded for delivery. DDGS trans-loading facilities had a simpler lay-out compared to ethanol plants. The only activities at trans-loaders was to receive recently processed DDGS from ethanol plants and then load shipping containers for movement across the US, including to export terminals for shipment overseas.

Traps were deployed at four indoor locations and two outdoor locations at each of the ethanol and trans-load facilities in this study. We used traps baited with the synthetic pheromone attractant that is used by both *T. variabile* and *T. inclusum*. The lure is synthetic mimic of the female-produced sex pheromone that attracts males in nature. Two different traps were used: one known as the "Dome Trap" (Figure 1) for walking insects, and the other a "Storgard II" sticky trap (Figure 2) for flying insects. A single Dome trap was placed at each of four indoor locations such as fermentation, distillation, loading and one or more spots in the flat storage. A sticky trap was hung at about 2 m off the ground outdoors at the farthest east and west borders of each facility. Traps were deployed during the initial site visit to each of the cooperating facilities. One individual at each company was then responsible for collecting the traps after a two-week period, shipping the traps back to us at KSU, and then deploy a new set of traps sent by us for use at the same locations for another two weeks. Our trapping system therefore allowed for detection of the target species of beetles at four indoor and two outdoor locations at each of our study sites, and we had two or more trapping periods throughout the season to assess any change in insect populations or activity over time.

Fig. 1 The Dome trap (left) used for trapping crawling *Trogoderma* adults inside ethanol and trans-load facilities. Dome traps were placed at the bottoms of pillars or at floor-wall junctions at four places indoors (second and third from left) and then returned to the laboratory for processing (right).

Fig. 2 Storgard II sticky trap hung on a fence near the periphery of a research site (left). Beetles fly to the red rubber stopper that is slowly releasing the synthetic female sex pheromone, and then are stuck on the sticky trapping surface inside the trap (second from left). Adult *Trogoderma* are found in the trap (second from right), removed and cleaned in solvent prior to being identified to species and counted (right).

Results

There was a range of trapping periods across cooperators based on the dates we began trapping at a given facility and also due to time availability of cooperators to help with the project. Therefore, the number of trapping periods ranged from 2 trapping periods at facility 6, to 7 trapping periods at both locations 1 and 2.

Initial trap captures revealed numbers of beetles in traps ranging from no beetles upwards to over 100 in a two-week period. We soon realized that there were more than two species represented in traps at all the locations. Some insects that do not use the same pheromone as the lure used in our traps may still responded to the trap and be captured. Once we separated all members of the genus *Trogoderma* from others, we then gave special attention to accurate identification methods published by earlier researchers for these species to become proficient in the identification. Characters related to color of the elytra and diagnostic morphological features of the eyes, were critical for identification (Figure 3).

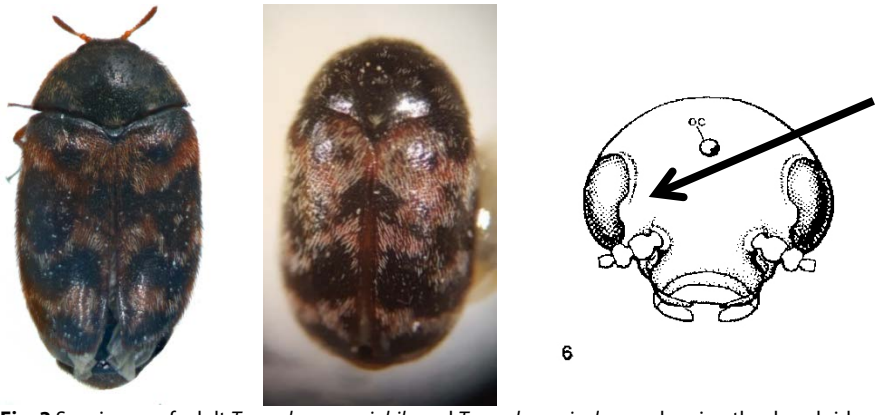

Fig. 3 Specimens of adult *Trogoderma variabile* and *Trogoderma inclusum* showing the dorsal sides. The species are very similar, but are separated by the black and brown bi-color appearance of the wing covers over which thin hairs were distributed in *T. variaible* (left) vs *T. inclusum* (middle) with just a black color under the white hairs. The best diagnostic character is the "notch" on the interior margin of the eye in *T. inclusum* shown at the arrow, and the lack of that notch, so that the margin of the eye is uniform and complete, in *T. variabile*. See ISPM (2016) for details.

Table 1 reports the numbers of adult beetles of both target species trapped at the indoor and outdoor locations for each of our seven study sites during the summer and fall of 2016. We have combined the numbers of beetles captured in the four indoor traps at each location, and these numbers are present in bold text in Table 1. Dome traps at all of our seven sites captured T. variaible,

while *T. inclusum* was trapped at five out of the seven sites, with none trapped at sites 4 and 7. Interestingly there were no *Trogoderma* trapped in our outdoor traps at locations 4 and 7, which suggests that these two species were in low or undetectable population levels at these places. *T. variabile* were captured during nearly every trapping period and at higher numbers than was *T. inclusum* at locations 1, 2, 4, 6 and 7. Traps at locations 3 and 5 captured more *T. inclusum* than *T. variabile*. Although Table 1 reports the sum of beetles from the four indoor traps in each time period, we found some trap to trap variation within and between facilities. For example, the trap in the fermentation area of site 1 consistently caught more *T. variabile* than did other locations at that facility. For the two trans-load sites that had Dome traps near the four corners of the flat storage, one corner seemed to consistently capture more beetles than any other. Spatial variation seemed to occur within each site, but there was no clear similarity between companies regarding which part of a facility had more beetles than another. Substantial numbers of beetles were caught in the outdoor sticky flight traps for most locations, except for relatively low flight trap numbers at locations 1, 4 and 6.

Tab. 1 Average numbers of adult *T. variabile* and *T. inclusum* captured per week in the indoor dome traps (sum of four trap), Inside, and in the two outside sticky flight traps to the west and east of the buildings, Out-W and Out-E, over a given number of trapping weeks at numbered ethanol plants and trans-loading facilities during the Summer-Autumn of 2016.

			T. variabile			T. inclusum		
Site	Type	Weeks	Out-W	Inside	Out-E	Out-W	Inside	Out-E
1	Ethanol	14	1.5	10.9	1.1	0	0.1	0
2	Ethanol	14	48.5	71.4	21.1	0	0.2	0
3	Ethanol	14	125.4	4.9	20.5	87.5	18.1	10.2
4	Ethanol	6	5.2	100.3	10.0	0	0	0
5	Ethanol	10	21.5	24.6	44.0	67.8	53.9	131.2
6	Trans-Load	4	6.8	2.3	1.0	8.8	1.0	0
7	Trans-Load	10	44.6	12.4	19.0	0	0	0

Discussion

The research reported here clearly shows that the beetles *T. variabile* and *T. inclusum*, the two species that were intercepted in Vietnam with DDGS from the US, commonly occur at ethanol plants and trans-load facilities that handle and market DDGS. This result met the expectation we had at the outset. Both species are very common in the US and previous studies have found that both can be trapped in many geographic regions of the US. Although we have data showing the occurrence of these species, we cannot report the density or absolute abundance of these species at each site. Pheromone trapping is an indirect sampling method that can only detect presence vs absence of a pest, and the relative numbers across locations and over time. Insects per unit of commodity (e.g. per bushel of grain or hundred-weight of DDGS) or per square meter of space would require more thorough and laborious methods to directly sample the pest populations. During our visits to cooperator sites we collected spilled DDGS and found no insects of any kind upon sifting these samples at our lab. Our trapping work clearly showed differences in relative captures of the two species, and also within species and across locations in a plant. It appears that numbers trapped at a given location in a facility could point to a need for sanitation or pest control to clean or disinfest areas with high trap captures. Captures of *Trogoderma* beetles at our outside traps indicate that beetles can be both outdoors and indoors, while the source location of trapped beetles is not confirmed.

Despite both beetle species being common and widely distributed, the risk of DDGS infestation by these pests and the risk that such pests may be transported with infested product, should vary in predictable ways. Trapping shows these species are common and thus could infest a suitable grain product at most times and places when weather and other environmental conditions are good for insects. However, these beetles can infest and persist in DDGS in only a few cases. Corn delivered to a site could be infested after harvest and through transport and storage periods. The longer grain

is stored, the more likely that infestation will occur. However, before becoming DDGS the corn is mashed and cooked, a practice that will kill all insects. The fermentation and distillation processes are fully insecticidal, and the temperatures during DDGS drying are extreme, over 600 F. The cooling period lasts about 24 h and during the majority of that time the DDGS would be too hot for infestation. DDGS should be susceptible to insect infestation when it is cool and handled in the flat storage for the 1-2 days prior to being loaded and shipped. Trapping has shown that beetles can be at all locations mentioned here, but access to suitable new DDGS would be only at the flat storage and also at the loading out location. Trans-loading facilities have no heating practices that can kill insects, and our trapping study shows that the target beetles can be present, but the product does not stay long before it is loaded into a container and shipped out. We were fortunate to encounter a man from the US Grain Inspection Service at one of our trans-load facilities who was taking timed samples of DDGS while they were being loaded into a container. He said that the samples were to be sifted for insects back at his office, and he told us that he had never found any insects in any samples like these he has taken in the past. Even if infestation of cooled DDGS occurs commonly, a buildup of detectable numbers would require several weeks under suitable conditions for substantial reproduction and increases in pest populations to occur. After leaving a trans-loading facility the DDGS may reach their ultimate destination within one day, or after several days or weeks for domestic rail service, or weeks to months for international ocean-going shipment. It is these time periods after drying and cooling that DDGS can be at risk for infestation.

Fumigation is the most effective and practical means to treat a potentially infested commodity to eliminate actionable quarantine pests before the commodity arrives at its destination (Myers and Hagstrum 2012). None of the seven facilties studied reported fumigating DDGS prior to any internatiaonl shipments, and all had discontinued shipping product to Vietnam at the time of our work. We interviewed one fumigation company about their practices with containers of DDGS. We were told they had fumigated containers near an export terminal with phosphine gas for 24 hours, and then the containers were ventilated and transported locally for loading onto a barge or ship destined for export. In our opinion this practice would not be the most effective to ensure a good kill of pests and quarantine security for the product (Hagstrum and Subramanyam 2009). The time after ventilation and prior to loading on a ship represents a period of susceptibility to pest invasion into the recently fumigated product. Also, the 24-hour fumigation may not give the most effective kill due to the short exposure time. Some pest species and certain lifes stages can be relatively tolerant to phosphine and a longer fumigation may be recommended. Many other variables can affect the efficacy of a 24-hour phosphine fumigation of shipping containers.

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