ANALYSES OF BOLL WEEVILS CAPTURED NEAR HAY BALES

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Abstract

Boll weevils re-infesting cotton fields in Louisiana were often correlated with the movement of large hay bales in the area. Boll weevils were collected in pheromone traps that were placed downwind from hay bales that were in varying stages of decay in numbers ranging from 10 to greater than 30. Boll weevils were collected in the Uvalde District of the Boll Weevil Eradication Foundation in Texas. To indicate possible refuge of the weevils near or in hay bales, the weevils were examined for pollen grains, fungal spores and phytoliths (plant cells). Over all 103 pollen grains from 38 different taxa were found in the samples. No one pollen type was found in all samples. More grass pollen grains (25 including 2 corn pollen grains) were found than any other taxon. Following the grasses were the "low spine" Asteraceae type with 14 grains, and then the Cheno-Ams and Celtis sp. (hackberry), both with 7 grains each. Cotton (Gossypium) pollen grains were found in three weevils. The diversity of pollen taxa is representative of the vegetation in the Uvalde District, Texas, which is in the South Texas Plains Vegetation Zone. Seven fungal spore types and 44 fungal spores were found in the samples and one type of fungal hypha. No single spore type occurred in all the samples. One Puccinia (wheat rust) and a Stemphylium spore were found in the samples. No grass phytoliths were seen in the unacetolyzed or acetolyzed samples. The pollen taxa present, the generalistic habit of the fungi and the lack of grass phytoliths, indicate that these weevils did not exist for long periods within or near the hay bales. However, additional research and a greater number of weevil samples are needed to determine if these techniques can be used to distinguish weevils associated with hay bales.

Introduction

The boll weevil, Anthonomus grandis Boheman, remains one of the most devastating insect pests of cotton, Gossypium hirsutum L., in the southern United States. Boll weevils (BW) are especially destructive to cotton because the adult females oviposit directly in developing flower buds (squares) and bolls.

Boll weevils are captured in pheromone traps that are extensively used to detect and monitor weevil populations (Sappington and Spurgeon 2000). Variations in conditions surrounding individual traps are accountable for considerable intertrap capture variation (Jones et al. 1992). Structures such as brush lines, ginning silos, hay bales, etc. influence the airflow and microclimate around traps and affect the number of BW captured in a trap (Sappington and Spurgeon 2000). Boll weevils re-infesting cotton fields in Louisiana were often correlated with the movement of large hay bales in the area.

The purpose of this research was to determine if standard pollen analyses could be used to establish if the weevils took refuge within or directly around hay bales. To indicate possible refuge of boll weevils in the bales, three criteria were assumed. First, the weevils should contain a high number of grass (Poaceae) pollen grains even though some hay is baled prior to the entire field being in flower. Second, because hay often easily molds, there should be a high number of fungal spores and/or hyphae (filaments). Third, if the BW took refuge or were living close to the hay, grass cells (phytoliths, Fig. 1) should be present.

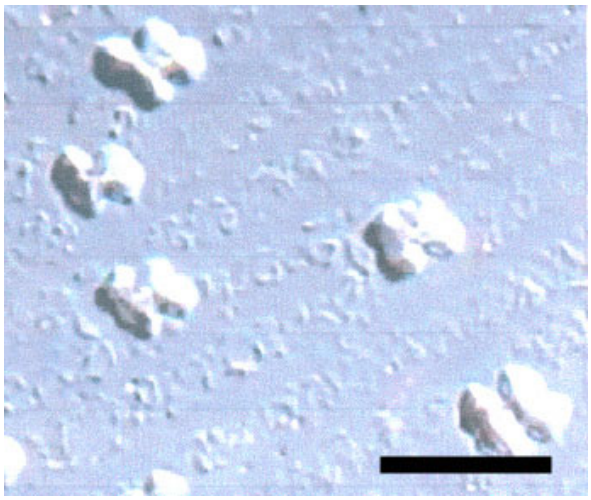

Figure 1. Grass phytoliths showing their typical shape. Bar = 20 µm.

Materials and Methods

Boll weevils were collected in the Uvalde District of the Boll Weevil Eradication Foundation in the Texas Boll Weevil Eradication Foundation's South Texas Winter Garden Zone (Fig. 2) in pheromone traps that were in close proximity to hay bales. Five weevils were examined for pollen, fungal spores and hyphae, and phytoliths (PFP). The gut was removed and placed into a glass slide. A drop of a glycerin-stain mixture was placed over the gut, and it was covered with a cover slip. Light microscopy (LM) was used to examine the gut tissue.

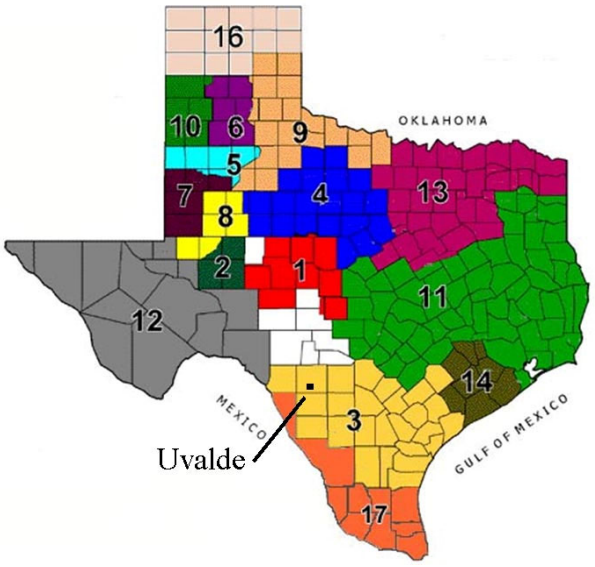

Figure 2. The Texas Boll Weevil Eradication Zones showing Uvlade, TX.
3 = the South Texas or Winter Garden Eradication Zone.

After the examination of the gut, it was returned to its original BW "body" and both the gut and body were acetolyzed for 10 minutes (Jones and Coppedge 1999). Glass slides were made from the residue. Samples were examined with LM.

Results and Discussion

Pollen

Only four pollen grains and a single fungal spore were found in all the unacetolyzed BW guts. One Cheno-Am (combination of Chenopodiaceae and Amaranthus pollen), one "low spine" Asteraceae (sunflower family), one grass (Poaceae), and one unknown Fabaceae (bean family) pollen grains were found. There were no fungal spores or phytoliths found in the guts. The main problem with examining a unacetolyzed gut is that the density of the gut tissue hides PFP. In addition, PFP diagnostic characteristics are obscured and exact identification is usually impossible (Fig. 3).

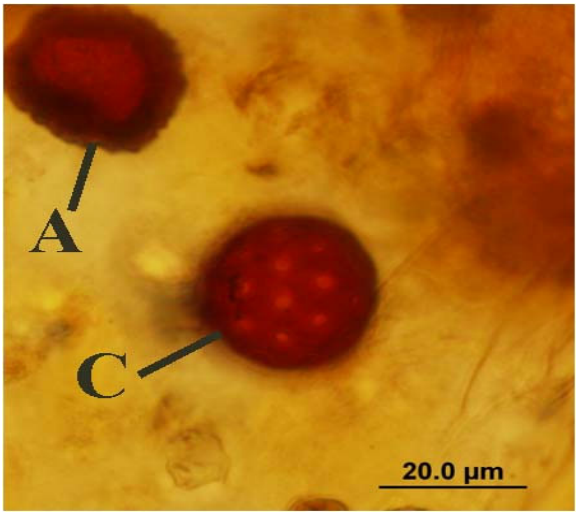

Figure 3. An unacetolyzed boll weevil gut showing two pollen grains. A = a low "spine" Asteraceae pollen grain and C = a Cheno-Am pollen grain.

In the acetolyzed samples (gut and body), 103 pollen grains from 38 different taxa were found (Table 1). No one pollen type was found in every acetolyzed sample. The diversity of pollen taxa found in the samples is common for BW captured in the Winter Garden Region of TX (Jones and Coppedge 1996, 1999, 2001). The presence of *Typha* (cat tail), *Ulmus* (elm), *Celtis* (hackberry) and *Berchemia* (rattanvine) indicate that the BW had visited a riparian habitat.

Table 1. The identified pollen taxa and number of pollen grains per taxon.

Pollen Taxa	Number of grains
Anacardiaceae, *Toxicodendron* sp.	1
Asteraceae LS (3 taxa combined)	13
Cheno-Am (4 taxa)	7
Cupressaceae, *Juniperus* sp.	1
Fabaceae, unidentified	1
Fabaceae, *Acacia* sp.	1
Fabaceae, *Mimosa* sp.	1
Fabaceae, *Glycine max*	1
Malvaceae, *Gossypium hirsutum*.	3
Poaceae (4 taxa)	23
Poaceae, *Zea mays*	2
Polemoniaceae	1
Ranunculaceae	1
Rhamnaceae, *Berchemia*	5
Rhamnaceae, *Rhamnus*	1
Typhaceae, *Typha* sp.	5
Ulmaceae, *Celtis* sp.	7
Ulmaceae, *Ulmus* sp.	2

More grass pollen grains were found than any other taxon (Table 1). The majority of the grass pollen grains, 23, were small indicating that they were not cultivated grasses (Fig. 4). However, there were two corn, *Zea mays* L., pollen grains found in the samples.

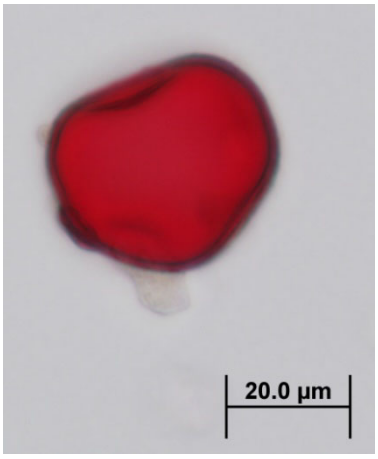

Figure 4. A grass (Poaceae) pollen grain found in the boll weevil samples.

Grass pollen grains found in BW are often higher and occur in more samples that other pollen taxa because they are easily recognizable to the family level and because of the number grass species and grass plants. The grass plant family (Poaceae) is the second largest plant family in Texas and is represented by 160 genera and 655 species (Jones et al. 1997). Grasses bloom year round and are found in all habitat types (Correll and Johnston 1970, Gould 1975).

In three locations of TX (including the Uvalde area), Jones and Coppedge (1999) found that grass pollen had the highest overall frequency of occurrence of any taxon in the BW samples and during May, they represented the second highest total number of pollen. Likewise, Hardee et al. (1999) found that grass pollen had the highest frequency of occurrence in BW captured during the summer and fall and the second highest relative frequency during the spring.

Following the grasses were the "low spine" Asteraceae with 14 grains, and then the Cheno-Ams and *Celtis* sp. (hackberry), each with seven grains. As with the grasses, Asteraceae, Cheno-Ams, and hackberry pollen are commonly found in the BW literature (Hardee et al. 1999, Jones and Coppedge 1999).

A single cotton (*Gossypium hirsutum* L,) (American upland cotton) pollen grain was found in three weevils (Fig. 5). One grain was whole indicating that cotton was foraged on within 24 hours prior to the death of the BW (Suh and Spurgeon 2001, Jones and Greenberg 2009). The other two cotton pollen grains were broken indicating that the BW had foraged on cotton 24 to 120 hours prior to death (Jones and Greenberg 2009).

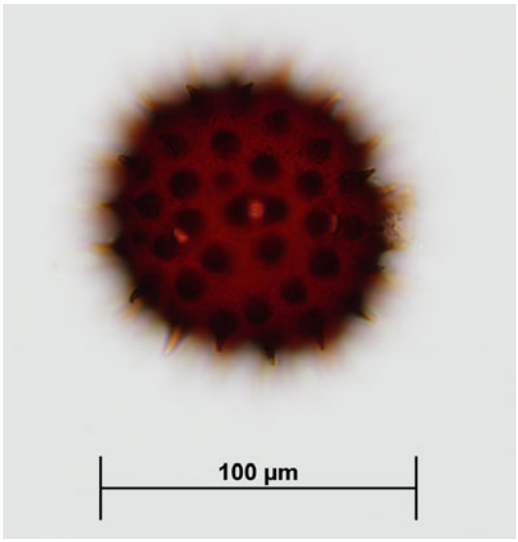

Figure 5. A whole *Gossypium hirsutum* (cotton) pollen found in one boll weevil sample.

The number of pollen taxa per BW ranged from six to 15 and the number of pollen grains from 11 to 30 (Table 2). The number of pollen grains per individual often depends on the type of pollen, the ornamentation or sculpturing of the outside layer of the pollen grain, the length of time the pollen grains were in or on the BW, and where the pollen grains were located (Cate and Skinner 1978, Jones et al. 2007, Jones and Greenberg, 2009, Suh and Spurgeon 2001).

Fungal Spores

Seven different fungal spore types and 44 fungal spores were found in the samples. No single spore type occurred in all the samples. Neither Claviceps nor Fusarium spores common in molding hay were found in the samples. Many of the spores found in these samples have been found in other insect pest samples including other weevils (Jones et al. 1992).

One *Stemphylium* sp. (Fig. 6) and one Puccinia sp. (wheat rust) spore were found in the samples. Neither of these spores is unique to hay, and both have been found in boll weevil samples from other locations.

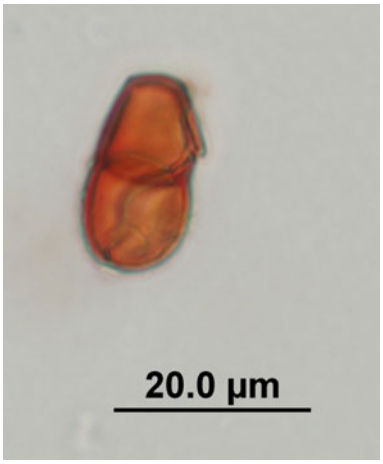

Figure 6. *Stemphylium* sp. fungal spore found in a boll weevil samples.

The problem with using fungi as a marker to determine if the weevils are taking refuge in or near hay is that most fungi are not restricted to only growing on and in hay. Most fungi grow on and in a variety of plants and animal, and in all kinds of decaying matter including dead BW remaining in pheromone traps for extended periods.

Phytoliths

Although phytoliths are not common in BW, they have been found (Jones, unpublished data). Phytoliths do not pick up the stain used for pollen analyses, but can be easily seen with LM because they are shiny and with phase contrast microscopy are whitish against a dark background. No grass phytoliths were found in the unacetolyzed or acetolyzed samples. However, some plant fibers were found in the samples. Upon re-examination of BW from other Texas locations, similar fibers were found, thus, they are not unique to hay.

Conclusion

Although more grass pollen was found than any other taxon, the number and taxa of fungal spores was low and there were no grass phytoliths. In addition, the fungi found in the samples were generalistic in nature and could have been attacking the BW after they died. More research and a larger sample size are needed to determine if pollen analyses can be used to distinguish BW captured in traps associated with hay bales. Future research should also include a sample of the inside and outside of the hay bales, and samples of the leaf litter and soil surrounding the bales. This would create a good reference to comparing PFP analyses. In addition, specific phytolith extraction techniques used for soil should be tested with BW to see if phytoliths could more easily be removed and identified.

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