2004 BELTWIDE COTTON CONFERENCES, SAN ANTONIO, TX – JANUARY 5-9

THE USE OF POLLEN TO DETERMINE STINK BUG DISPERSAL

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Abstract

Southern green stink bugs [(*Nezara viridula* (L.)] are occasional insect pests in cotton and other agricultural crops. Damage to the plant from stink bug feeding includes the loss of plant fluids and the deformation and abortion of seed and fruiting structures. Although stink bugs are known to move from host to host, little is known about their dispersal between cropping systems. Pollen analyses are an effective tool in determining long and short distance migration and dispersal. Since stink bugs feed on plant parts including flowers and fruits, it is likely that they can become contaminated with pollen. Adult stink bugs were collected in Burleson Co., TX and examined for pollen. Pollen and spores were found in light microscopy analyses but not in scanning electron analyses. Seventeen pollen taxa and three spore taxa were found in the stinkbugs including pollen from Asteraceae, cotton (*Gossypium hirsutum* C. Linnaeus), corn (*Zea mays* C. Linnaeus), and false honeysuckle (*Gaura* sp.). In laboratory tests when fed 50% sugar-water containing *Lycopodium clavatum* C. Linnaeus spores, 100% of the stinkbugs contained spores for up to seven days. The presence of pollen and the longevity of the *Lycopodium* spores indicate that pollen analyses can be used to determine dispersal. However, future research is needed to correlate the pollen recovered from stink bugs and surrounding habitats and to field test the use of *Lycopodium* as an artificial maker for stink bug dispersal.

Introduction

The southern green stink bug [(*Nezara viridula* (L.)] is a cosmopolitan stink bug that occurs in the United States from Virginia to Florida and west to Texas and Oklahoma (McPherson et al. 1994). In early spring, adult stink bugs leave overwintering sites and begin feeding and reproducing. They are attracted to plants with growing shoots and developing fruits and seeds (McPherson and McPherson 2000). Over 30 dicotyledonous families and several monocotyledonous families are damaged by this insect pest (Panizzi 2000, Panizzi et al. 2000, Todd 1989). "Preference" for a particular feeding host plant changes with the host plant's phonological development. As fruits and seeds mature, plants become less attractive and stink bugs disperse to other plants.

Although the damage from stink bugs is usually confined to fruiting structures, stink bugs may feed on and damage other plant parts including the stems, fruits, seeds, and flowers (Barbour et al. 1988; Panizzi 1997; Riley et al. 1997). Damage to the plant from stink bug feeding includes the loss of plant fluids, the injection of destructive digestive enzymes, the deformation and abortion of seed and fruit structures, attack by pathogenic and decay organisms, and delayed plant maturation (Jones and Caprio 1994; McPherson and McPherson 2000; Ragsdale et al. 1979).

Hollis (2000) ranked the stink bug complex as the fifth most costly pest in the Cotton Belt during 1999. Damage to cotton from stink bugs includes discoloration of the lint, loss of young bolls, and reduction in yield (Barbour et al. 1988; Bundy et al. 1998). One of the first indications of internal boll damage is the presence of a feeding stylet sheath (McPherson and McPherson 2000). Turnipseed et al. (1995) found three species of stink bugs damaging cotton in South Carolina. This damage occurred on both Bt and non-Bt cotton.

Southern green stink bugs come in close contact to the flowers of a plant when they are looking for and feeding on the young fruits. Thus, pollen from the flowers can be dislodged and fall on the stink bugs and into floral and extra-floral nectaries. Therefore, pollen may be a natural marker and an effective tool in determining long- and short-distance migration and dispersal of stink bugs.

The purpose of this research was to answer three questions. First, can pollen be found in or on stink bugs? Second, because stink bugs feed on the plant's phloem, will they feed on a sugar-water solution? Third, can sugar-water spiked with *Lycopodium clavatum* C. Linnaeus spores be used as an artificial marking technique for stink bugs?

Methods

Pollen Analyses

Southern green stink bugs were collected in Burleson Co., TX, from 17-26 September 2002 and frozen. For scanning electron microscope analyses, 25 stink bugs were removed from the freezer and thawed. Because the exoskeleton of the stink bugs contained secretions from the scent glands, the insects were dried in an oven at 250˚ for 3 days. Once dried, they were mounted on scanning electron microscope stubs, coated with 400 Å of gold palladium and examined for pollen.

For light microscopy analyses, 60 stink bugs were thawed and chemically dissolved to remove the insect tissue but not the pollen. Once dissolved, the pollen residue was rinsed several times with distilled water and once with 95% ETOH. Glycerin was added and the pollen residue of each stink bug was placed into a vial and left on a hot block at 25˚ C for 24 hours. After the ETOH was evaporated, one drop of each stink bug's pollen residue was placed onto a glass slide and examined for pollen.

Sugar-Water Feeding and Lycopodium Marking

Stink bugs were collected over a 5-day period, put into a cage and fed green beans. Once there over 150 stink bugs remained alive for three days, 140 were removed, placed into a separate cage, and starved for two days. A 50% sugar-water (V/V) solution was prepared and 10 dissolved and rinsed *Lycopodium clavatum* tablets (each containing 11,500 ± 500 spores) were added to the sugar-water solution. A 50% sugar-water ratio was used because the solution needed to be sweet enough to attract the stink bugs, but not so thick that the solution might not go through the stylets. The sugar-water-spore solution was put into a small specimen lid and placed into the cage. Stink bugs were allowed to feed freely on the solution. After 24 hours, the sugar-water-spore solution was removed. Stink bugs were sacrificed at 0, 24, 48, 72, 96, 144, and 166 hours after the sugar-water solution was removed. Stink bugs were dissected and examined for the presence of the spores.

Another 140 stink bugs were tested with a 5% sugar-water (v/v) solution. A 5% sugar-water solution was chosen as a minimum amount of sugar that could be added. Ten dissolved and rinsed *Lycopodium clavatum* tablets (each containing 11,500 ± 500 spores) were added to the sugar-water solution. These stink bugs were starved for 1 week prior to the feeding of the sugar-water solution. As in the experiment using 50% sugar water, the stink bugs were sacrificed at 0, 24, 48, 72, 96, 144, and 166 hours.

Results and Discussion

Pollen Analyses

No pollen or spores were found on the 25 stink bugs that were examined with SEM. This may be due in part to the drying of the stink bugs so that they could be viewed with SEM. The lack of external pollen and spores also may be due to secretions of stink bugs that may have removed external pollen and spores.

Pollen was found in 52 (87%) of the 60 stink bug residues that examined with LM (Table 1). Nearly 150 pollen grains were found in the samples (Table 1). Seventeen pollen and three fungal spore taxa were found in the LM samples. Pollen from both entomophilous (insect pollinated) and anemophilous (wind pollinated) taxa was found in the samples.

Entomophilous taxa found in the samples included beggar's ticks (*Bidens* sp.), cotton (*Gossypium hirsutum* C. Linnaeus), false honeysuckle (*Gaura* sp.), squash (*Cucurbita maxima* A. Duchesne), and watermelon [*Citrullus lanatus* (C. Thungerg) J. Matsumura & R. Nakai] (Table 2). All of these are listed as host or possible host plants by McPherson and McPherson (2000). Anemophilous taxa found in the samples included Poaceae (grass family), corn (*Zea mays* C. Linnaeus), and Cheno-Ams (Chenopodiaceae and *Amaranthus*) (Table 2). Pollen from anemophilous taxa is usually considered as contaminants; however, approximately 40 species of grasses (including corn), 10 species of Chenopodiaceae, and 3 species of Amaranthaceae, all anemophilous, are stink bug host plants (McPherson and McPherson 2000).

Sugar-Water Feeding and Lycopodium Marking

Several times stink bugs were seen going into and coming out of the small lid containing the sugar-spore-water. In the laboratory tests, 100% of the stink bugs fed the 50% sugar-water-spore solution contained spores (Table 3). Stink bugs retained the spores for at least 7 days. Fifteen pollen taxa were found in the dissected stink bugs. Starving the stink bugs for two days was not enough to clear out any previous pollen or spores.

Stink bugs were not seen going into or coming out of the 5% sugar-water-spore solution nor were *Lycopodium* spores found in these stink bugs. *Lycopodium* spores were found in only one dissected stink bug (Table 4). Three spores and an Asteraceae pollen grain were found in this individual.

Conclusion

Pollen was found in the LM analyses of stink bugs but not in the SEM analyses. The physics and optics of SEM prevent the observation of oily samples; therefore all samples must be dry. It is possible that by using an environmental scanning electron microscope (ESEM), stink bugs can be observed without being dried.

The majority of the recovered pollen was from plants listed as stink bug host plants (McPherson and McPherson 2000). This indicates that pollen analyses can be a valuable tool in determining stink bug dispersal.

Stink bugs did feed on a 50% sugar-water solution but not on a 5% solution. The presence and longevity of the *Lycopodium clavatum* spores in these insect pests make the spores a possible artificial marker.

Little is known about southern green stink bug dispersal between cropping systems. It is known that southern green stink bug "preferences" for a particular plant changes with the maturation of the plant. Plants that are most "attractive" are those that are developing fruits and seeds.

The use of pollen as a natural marker and *Lycopodium* spores as an artificial marker to aid in determining stink bug dispersal is promising. However, future research is needed. ESEM studies need to be conducted to determine if electron microscopy is a viable alternative to light microscopy. The taxa of pollen recovered from stink bugs needs to be compared to surrounding trap and crop habitats to fully assess the use of pollen for stink bug dispersal. Finally, the use of *Lycopodium* as an artificial maker for stink bug dispersal needs to be evaluated in field tests.

Acknowledgements

I appreciate the efforts of Drs. Juan D. Lopez, Jr. and Ab Latheef for collecting the stink bugs. I am indebted to Ester F. Wilson for her tireless dissections and processing efforts. I thank Drs. Cynthia Sheffield and Vaughn M. Bryant, Jr. for their editorial comments and help.

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Table 1. Stink bugs examined for pollen and the number of stink bugs containing pollen.

Date	Number of stink bugs examined	Number of stink bugs with pollen	Number of pollen grains	Number of pollen taxa
17 Sept. 2002	10	10	33	8
19 Sept. 2002	10	10	38	8
20 Sept. 2002	10	10	15	4
23 Sept. 2002	10	5	5	2
24 Sept. 2002	10	10	24	7
26 Sept. 2002	10	7	32	7
Total	60	52	147	17

Table 2. Pollen taxa, common name, and the number of pollen grains per taxon found in the stink bug samples.

Taxa	Common name	Pollen grains
Asteraceae spp.	sunflower family	15
Asteraceae, *Bidens* sp.	beggar's ticks	2
Caprifoliaceae, *Viburnum* sp.	viburnum	4
Cheno-Am	goosefoot family and amaranthus	49
Cucurbitaceae, *Citrullus lanatus*	watermelon	1
Cucurbitaceae, *Cucurbita maxima*	squash	3
Euphorbiaceae, *Croton* sp.	croton	4
Fabaceae spp.	bean family	19
Malvaceae, *Gossypium hirsutum*	cotton	2
Onagraceae, *Gaura* sp.	false honeysuckle	1
Poaceae	grass family	4
Poaceae, *Zea mays*	corn	12
Scrophulariaceae	figwort family	5
Unknowns		26

Table 3. Stink bugs adults fed 50% sugar-water solution spiked with *Lycopodium clavatum* spores and the number of pollen taxa found in the samples.

Hour	Number of stink bugs	Number of stink bugs with spores	Number of spores	Number of pollen taxa
0	20	20	56	2
24	20	20	159	3
48	20	20	130	7
72	20	20	220	3
96	20	20	332	5
144	20	20	447	2
168	20	20	146	1
Total	140	140	1,490	15

Table 4. Stink bug adults fed 5% sugar-water solution spiked with *Lycopodium clavatum* spores and the number of pollen taxa found in the samples.

Hour	Number of stink bugs	Number of stink bugs with spores	Number of spores counted	Number of pollen taxa
0	20	0	0	0
24	20	0	0	0
48	20	1	3	1
72	20	0	0	0
96	20	0	0	0
144	20	0	0	0
168	20	0	0	0
Total	140	1	3	1