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POLLEN FROM THE EXOSKELETONS OF STABLE FLIES, *STOMOXYS CALCITRANS* (LINNAEUS 1758), IN GAINESVILLE, FLORIDA, U.S.A.

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Abstract

Stable flies are an important pest of humans and livestock. Despite being blood feeders, they also visit flowers to eat nectar. Stable flies with pollen adhering to their exoskeletons were collected at the University of Florida Horse Teaching Unit and taken to the Paleobotany and Palynology Laboratory at the Florida Museum of Natural History for processing and identification. The pollen on all the specimens was identified as Carolina willow, *Salix caroliniana* Michaux 1803. This small shrub or tree is found throughout Florida in wetland areas. This study demonstrates the utility of pollen analysis in interdisciplinary studies.

Key words: Stable flies; pollen; *Salix caroliniana*; *Stomoxys calcitrans*; Florida.

INTRODUCTION

Stable flies, *Stomoxys calcitrans* (Linnaeus 1758), are hematophagous, pestiferous flies that are a major pest of humans and livestock in the United States and many other regions (Hogsette and Farkas, 2000; Broce et al., 2005). Although both sexes feed on blood, they are known to visit flowers and eat nectar (Jones et al., 1985). A list of plants visited by stable flies in Florida has been compiled by observation and identification of pollen adherent to the flies (Tseng et al., 1983). If the source of the pollen could be identified, the habits of the flies would be better defined, thereby adding to the information on stable fly dispersal and feeding habits (Hogsette and Ruff, 1985; Hogsette et al., 1987). Stable flies are not pollen feeders because their mouth parts are unsuitable (Jones et al., 1985). However, these flies visit plants for a nectar source and, in doing so, become contaminated with pollen. An understanding of the foraging and nectar-feeding habits of insects is important for several reasons. Pollen identification may be useful in the determination of feeding habits, migration patterns, migration routes, and origins (Jones and Jones, 2001). Migration patterns and routes are important because corridors of movement may be predicted, and origins of dispersal can be identified.

MATERIAL AND METHODS

During a March, 2008 study of stable flies at the Horse Teaching Unit (HTU), University of Florida, Gainesville (Alachua County), Florida, stable flies with lateral and ventral surfaces covered with a bright yellow pollen-like substance were captured on sticky traps. Several of these flies were brought to the Paleobotany and Palynology Laboratory at the Florida Museum of Natural History for investigation and possible identification of the pollen.

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Initially, a single fly was used as a test to determine suitable processing methods that would produce satisfactory recovery of the pollen adhering to the body of the flies. A single fly, bearing pollen grains, was placed into a 4 ml vial. A small amount of 100% ethanol was added to the vial and gently shaken. This effectively freed the pollen grains from the fly body and hairs. The liquid was then poured off into a 15 ml centrifuge tube and processed using standard palynological procedures for modern pollen (Traverse, 2007). Twelve additional samples of pollen washed from the bodies of flies with 100% ethanol were then processed. An optical check, using a dissecting microscope, of the flies made before the ethanol rinse, indicated very little pollen was present in these twelve samples. Because of this, the samples were transferred to 1.5 ml caped, conical, plastic centrifuge tubes (Eppendorf™ microcentrifuge tubes), and washed once with distilled water to remove most of the ethanol. A single wash in glacial acetic acid removed the water in preparation for acetolysis. The samples were then treated with about 1.0 ml of acetolysis mixture (acetic anhydride and concentrated sulfuric acid in a 9:1 ratio), and heated in a water bath for 10–12 minutes at about 95˚C. Following acetolysis, the samples were centrifuged, decanted, washed once in glacial acetic acid, and washed using distilled water. The residues were retained in the Eppendorf tubes for slide preparation. Extreme care was taken at this stage, because the residues appeared clear, suggesting that little or no pollen was recovered.

The microscope slides were prepared using glycerin jelly as the mounting medium. The coverslips were sealed with clear nail polish. All slides and remaining residues are stored at the Paleobotany and Palynology Laboratory, Florida Museum of Natural History, Gainesville, Florida, U.S.A. Photographs of the pollen grains were made using a Nikon Coolpix 4500™ camera mounted on a Leitz Dialux 20™ transmitted light research microscope. The specimens are located by stage coordinates marking an x and y axis location for the Leitz Dialux 20 microscope. Pollen used for comparison with the pollen adhering to the fly bodies was collected from the herbarium at the Florida Museum of Natural History (FLAS), and processed using the techniques outlined by Traverse (2007) for modern pollen. Species of *Salix* were used as a comparison with the pollen attached to the bodies of the flies. Measurements were made on 10–15 grains at x400 magnification. Table 1 gives the herbarium sheet data for the specimens examined, and representative specimens are illustrated in Plate 1.

SYSTEMATIC SECTION

Ten of the thirteen samples contained pollen in varying amounts. All the pollen recovered from the flies was identified as Carolina willow, *Salix caroliniana* Michaux 1803 (Salicaceae), and is described here.

Family SALICACEAE Mirbel 1815
Salix Linnaeus 1753
Salix caroliniana Michaux 1803
Plate 1, figs. 1–6

Description. Pollen grains single, tricolporate or tricolporoidate, prolate to mostly subprolate; colpi

Table 1. Data on *Salix* specimens examined for comparison with the pollen attached to the stable flies. The habit and habitat data are provided as recorded from the herbarium sheet.

Taxon	Accession Number	Collector's Data	Locality in Florida	Habit/Habitat
Salix caroliniana Michaux 1803	23761	C.L. Deam, number 64065	Highlands County	Tree, 3 m tall
Salix caroliniana Michaux 1803	23762	Godfrey and Kurz, number 54681	Holmes County	Small tree, floodplain
Salix floridana Chapman 1860	23763	W.A. Murrill, February 5th 1941	Lake County	Low hammock
Salix floridana Chapman 1860	23764	W.B. DeVall, April 5th 1942	Putnam County	Not available
Salix humilis Marshall 1785	23765	A.S. Jensen, March 4th 1981	Taylor County	Clumps 1–3 m tall
Salix eriocephala Michaux 1803	23766	R.K. Godfrey, number 79360a	Jackson County	Shrub up to 3 m tall

meridionally arranged, long, narrow, reaching to the poles; pores indistinct, appearing as a thinned area within the copal margins; surface reticulate, heterobrochate, reticulum finer at poles and along colpi margins, lumina maximum size 1.5 μm diameter, muri about 0.5 μm wide; exine 1.0–1.5 μm thick, two-layered, nexine about as thick as sexine; dimensions 21(22.8)24 x 17(19)21 μm; p/e = 1.2 subprolate (10 grains measured).

Remarks. It is of interest to note that cursory examination of the pollen, using a dissecting microscope, while still attached to the body of the flies appeared more prolate to perprolate in general shape. Once the pollen was transferred to centrifuge tubes and washed in water, the shape changed dramatically to subprolate. The take-up of water demonstrated the principle of harmoegathy, i.e. "…the process by which pollen grains and spores change shape to accommodate variations in the volume of the cytoplasm caused by hydration," (Punt et al., 1994).

RESULTS AND DISCUSSION

All the pollen recovered from the fly bodies is of *Salix caroliniana*. Of the 400 species of *Salix* (Mabberley, 1997), six species occur in Florida (Argus, 1986). These include *Salix babylonica* C. Linneaus 1753 (weeping willow), *Salix caroliniana* A. Michaux 1803 (Carolina willow), *Salix eriocephala* A. Michaux 1803 (Missouri river willow), *Salix floridana* Chapman 1860 (Florida willow), *Salix humilis* H. Marshall 1785 (dwarf willow), and *Salix nigra* H. Marshall 1785 (black willow). *Salix caroliniana, Salix floridana*, and *Salix humilis* occur in Alachua County, Florida (Wunderlin and Hansen, 2004).

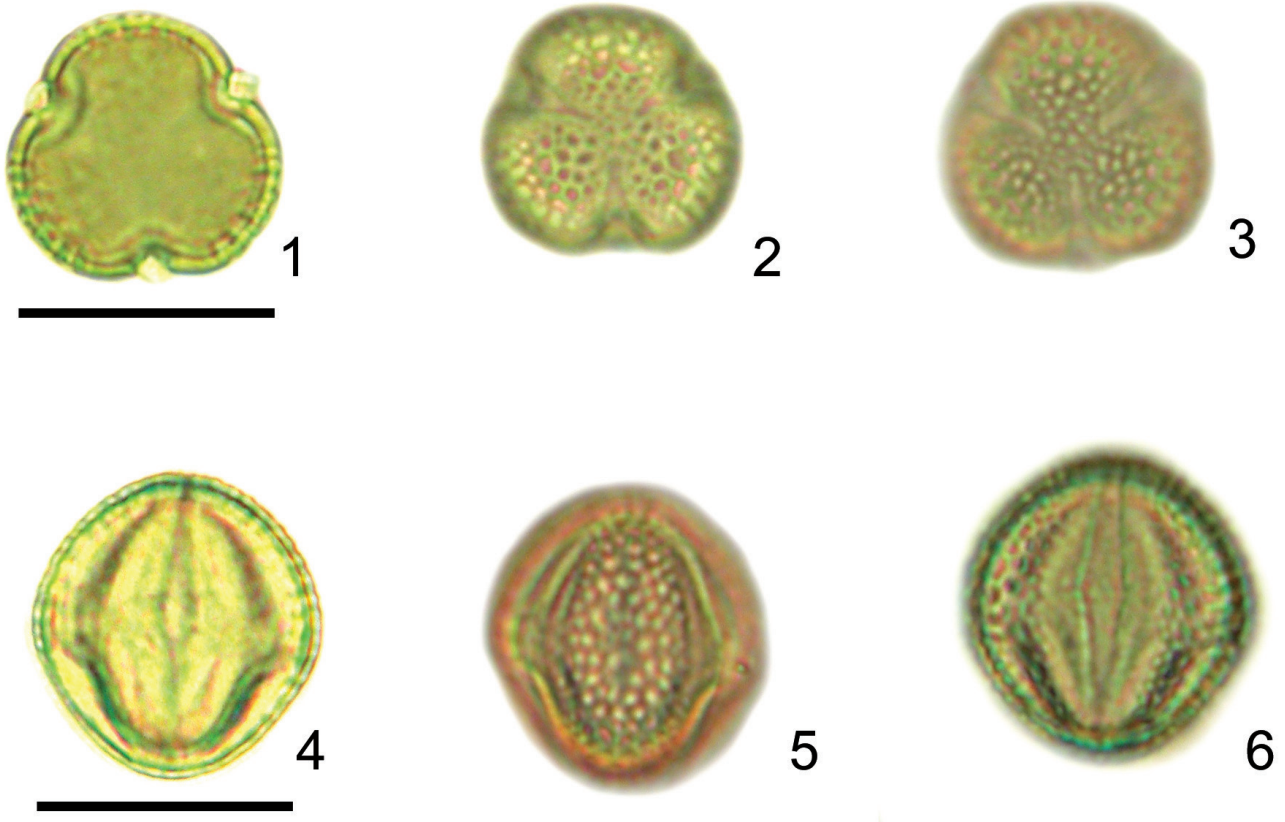

PLATE 1

Salix pollen recovered from stable fly body hairs, showing polar and equatorial views. Scale bar = 20 μm.

1–3 *Salix caroliniana*, polar view, PY01A 26.0x99.5, x 1500. Figure 2 illustrates the reticulate surface.

4–6 *Salix caroliniana*, equatorial view, PY01A 29.4x101.5, x 1500. Figure 6 shows the nature and length of the colpus.

Measurements for the three Alachua County species of *Salix*, based on a count of 10 grains for each species are:

Salix caroliniana 21(21.5)24 x 17(19)21 μm; p/e ratio = 1.13.

Salix floridana 26.5(28.6)31.5 x 21.5(23.7)26 μm; p/e ratio = 1.2

Salix humilis 26.5(28.7)31.5 x 19(22.2)26.5; p/e ratio = 1.3

Of these species, *Salix caroliniana* most closely compares with the pollen adhering to the body of the flies. In overall dimensions *Salix caroliniana* is nearer in polar and equatorial axis dimensions than the other two species, which tend to be somewhat larger. *Salix floridana* and *Salix humilis* are more prolate in shape, demonstrated by their polar axis to equatorial axis ratios (p/e), and in this regard are more similar to the pollen recovered from the flies. Additionally, the reticulum of *Salix caroliniana* compares well with the reticulum of the pollen grains collected from the fly bodies.

Based on maps from the "Atlas of Florida Vascular Plants" website (http://www.plantatlas.usf.edu/default.asp), *Salix caroliniana* is the most common species of *Salix* in Florida, and occurs throughout the state. It is very likely therefore that, based on the pollen features and the probability of occurrence, the pollen recovered from the bodies of the flies is that of *Salix caroliniana*. The Carolina willow (*Salix caroliniana*) is a small shrub or tree reaching about 6 m tall, commonly with several trunks. The leaves are green above with a lighter green to white-glaucous below, elongated to lanceolate, narrowing to a rather sharp point. The leaves bear fine serrations (teeth). The sexes are in separate flowers, which are numerous, small, and pale yellow. The species is distributed along streambanks and other wet places in the southeastern United States, reaching north to Illinois and east to Pennsylvania. The western limit is Kansas, Oklahoma, and Texas (http://plants.usda.gov/java/profile?symbol=SACA5).

Salix pollen is remarkably stenopalynous, i.e. when examined using transmitted light, it shows little variation and close similarities between species. A more detailed examination of the pollen adhering to the bodies of the flies using scanning electron microscopy (SEM) may provide additional details that could confirm identification of the pollen to the species level. Bryant et al. (1991) found that SEM was better than transmitted light microscopy for pollen identification for corn earworm [*Helicoverpa zea* (Boddie 1850)] moths.

Salix pollen has previously been found on adult stable flies in west Florida (Tseng et al., 1983). The fact that stable flies from the HTU have also been visiting *Salix* flowers further confirms their tendency to utilize nectar even when animal and human hosts are available. It was intended to make inferences about the origins of the stable fly adults at the HTU based on the identification of the pollen found on the flies; however, this is not possible because there are numerous willows on the HTU property.

Stable fly adults demonstrably visit the flowers of *Salix caroliniana*, however, whether or not they pollinate these flowers requires additional research. Flies of all sizes are known to be pollinators (Borror et al., 1976), and the stable fly could be acting as such in its search for nectar. *Salix* is wind-pollinated to some degree, but insects also play a role. There is apparently no seasonal study of stable flies and the pollen they carry.

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References Cited

ARGUS, G.W.
1986 The genus *Salix* (*Salicaceae*) in the Southeastern United States, *Systematic Botany Monographs*, 9: 1–170.

BORROR, D.J., DE LONG, D.M., and TRIPLEHORN, C.A.
1976 *An introduction to the study of insects, 4th edition*. Holt, Rinehart, and Winston, New York, 852 p.

BROCE, A.B., HOGSETTE, J.A., and PAISLEY, S.
2005 Winter feeding sites of hay in round bales as major developmental sites of stable flies (Diptera: Muscidae) in pastures in spring and summer. *Journal of Economic Entomology*, 98: 2307–2312.

BRYANT, V.M. Jr., PENDLETON, M., MURRY, R.E., LINGREN, P.D., and RAULSTON, J.R.
1991 Techniques for studying pollen adhering to nectar-feeding corn earworm (Lepidoptera: Noctuidae) moths using scanning electron microscopy. *Journal of Economic Entomology*, 84: 237–240.

HOGSETTE, J.A., and FARKAS, R.
2000 Secretophagous and hematophagous higher Diptera, *In:* Papp, L., and Darvas, B. (eds.). *Contributions to a Manual of Palearctic Diptera, Volume 1 General and Applied Dipterology*. Science Herald, Budapest, 769–792.

HOGSETTE, J.A., and RUFF, J.P.
1985 Stable fly (Diptera: Muscidae) migration in Northwest Florida. *Environmental Entomology*, 14: 170–175.

JONES, C.J., HOGSETTE, J.A., PATTERSON, R.S., and MILNE, D.E.
1985 Effects of natural saccharide and pollen extract feeding on stable fly (Diptera: Muscidae) longevity. *Environmental Entomology*, 14: 223–227.

JONES, G.D., and JONES, S.D.
2001 The uses of pollen and its implications for entomology. *Neotropical Entomology,* 30: 342–350.

MABBERLEY, D.J.
1997 *The Plant-Book*. Cambridge University Press, Cambridge, 858 p.

PUNT, W., BLACKMORE, S., NILSON, S., and LE THOMAS, A.
1994 *Glossary of Pollen and Spore Terminology*. Laboratory of Palaeobotany and Palynology (LPP) Contribution Series No. 1, 71 p.

TRAVERSE, A.
2007 *Paleopalynology*. Second edition. Springer, The Netherlands, 813 p.

TSENG, J.M., JONES, C.J., and HOGSETTE, J.A.
1983 Nectar feeding and the stable fly, *Stomoxys calcitrans* L. (Diptera: Muscidae). *Journal of the Florida Anti-Mosquito Association*, 54: 40–41.

WUNDERLIN, R.P., and HANSEN, B.F.
2004 *Atlas of Florida Vascular Plants*. Florida Center for Community Design and Research. Institute for Systematic Botany, University of South Florida, Tampa. Available from: http://www.plantatlas.usf.edu/.