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FIRST REPORT OF RUST CAUSED BY *PUCCINIA EMACULATA* ON CULTIVATED SWITCHGRASS IN MISSISSIPPI

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INTRODUCTION

Switchgrass (*Panicum virgatum* L.), initially cultivated in the early 1980s as a forage crop in Mississippi, has received increased attention with the establishment of perennial grasses as biomass feedstock crops. Beginning in July 2010, and annually since, symptoms of brown to black foliar spotting with margins of chlorosis and signs of abundant uredinia production were observed on plots of 3 year-old 'Alamo' and 9 year-old 'EG1101' switchgrass, established at the Leveck Animal Research Facility in Starkville, Mississippi (Figures. 1, 2).

Figure 1. *Puccinia emaculata*-infected Alamo switchgrass (right) showing symptoms of rust compared to healthy, asymptomatic switchgrass (left).

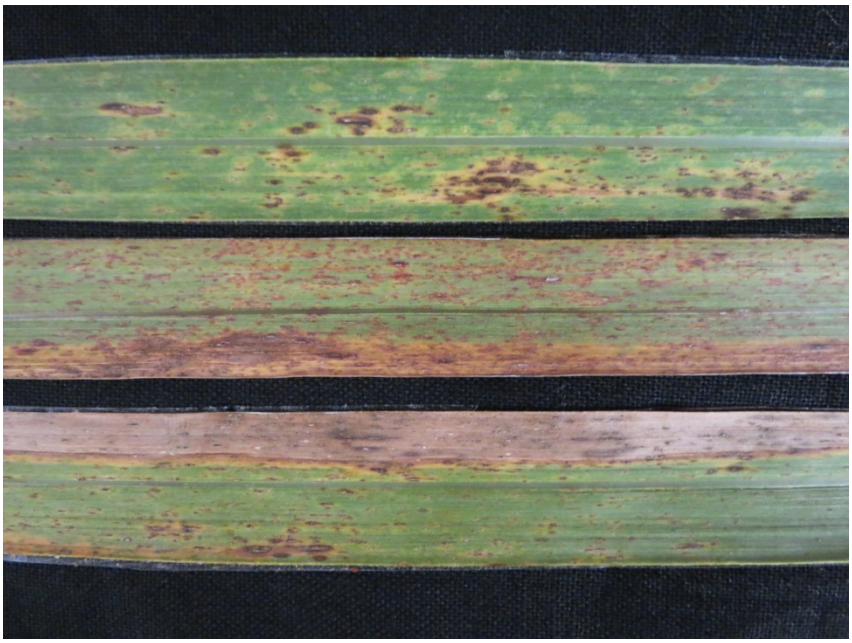

Figure 2. Foliar symptoms of *Puccinia emaculata*-infected switchgrass exhibiting brown to black spots, chlorotic margins as well as signs of uredinia production.

Uredinia were epiphyllous, amphigenous to mostly adaxial and yellowish orange to mostly cinnamon. Urediniospores were globose, echinulate, 24.9 ± 2.7 µm in diameter, with 3 equatorial pores and 1.5 to 2.0 µm thick cinnamon walls. Teliospore production was observed in late summer. Telia were epiphyllous, amphigenous to adaxial, and dark brown to black. Teliospores were dark brown, two-celled, oblong to ellipsoid, 34.3 ± 2.5 µm long with an apical cell width of 17.6 ± 3.9 µm and a basal cell width of 13.6 ± 1.7 µm. Pedicels were light brown to colorless and 20.9 ± 8.4 µm long (Fig. 3).

Urediniospore and teliospore morphology were consistent with that described for *Puccinia emaculata* Schw. (1). Pathogenicity of urediniospores collected from infected leaves was evaluated on 14 to 28 day-old Alamo switchgrass established from surface disinfested seed. Fresh urediniospores were suspended in sterile water with 0.01% Tween 20 (20 mg spores/ L), and ten pots of seedlings (~20-30 per pot) were inoculated with a mist bottle until runoff. Five more pots were misted with sterile water with 0.01% Tween 20 as controls. Plants were placed in a dark mist chamber at 27°C for 16 to 24 hours then transferred to a growth chamber at 27°C with a 12-h photoperiod for symptom development. Chlorosis and sporulation similar to that in the field were observed on inoculated plants 14 days post inoculation (Figures 4, 5).

Figure 3. Two-celled *Puccinia emaculata* telia observed on field-grown Alamo switchgrass in late summer in Mississippi.

Figure 4. Chlorosis and sporulation induced on switchgrass following inoculation using urediniospores of *Puccinia emaculata* collected from field-grown plants.

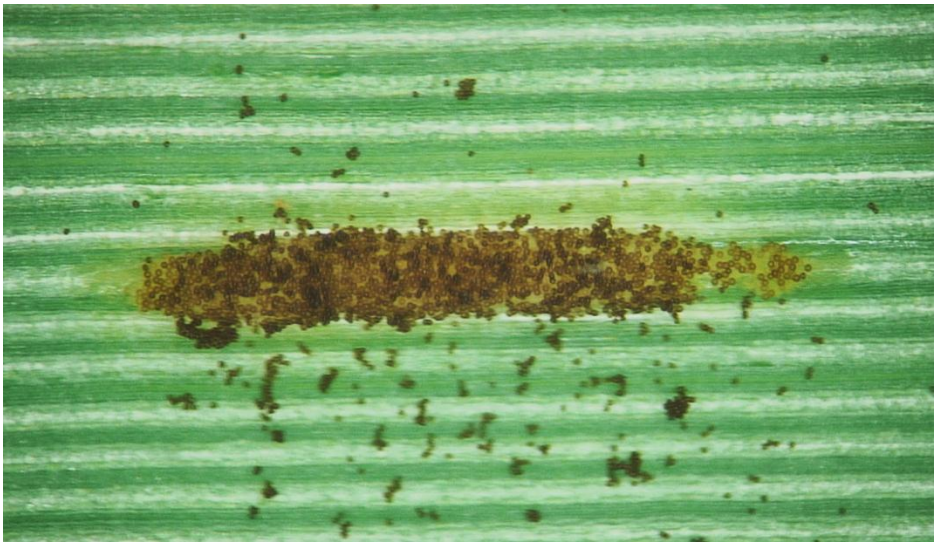

Figure 5. Uredinia production of *Puccinia emaculata* from inoculated switchgrass in pathogenicity evaluations.

No symptoms were observed on the control plants. DNA was extracted from urediniospores collected from field infected and inoculated 'Alamo' plants and primers Rust1 (2) and PuccF (5'-CAATGGATCTCTAGGCTCTC-3', B. Olson, *unpublished data*) were used to amplify and sequence 1,200-bp PCR products containing partial sequences of the nuclear ribosomal internal transcribed spacer (ITS) region and large subunit (LSU). Sequences from both spore collections were identical (GenBank Accession No.

KC515382) and exhibited 98% identity with ITS-LSU sequences from *P. emaculata* (GenBank Accession No. EU915294) collected from 'Falcon' switchgrass in Tennessee (3). A previous report identified microsatellite loci from *P. emaculata* isolates collected from switchgrass in Mississippi (4); and a specimen of *P. emaculata* (BPI 064583), deposited in 1897 in the USDA-ARS Systematic Mycology and Microbiology Laboratory Specimen Database, was collected from native switchgrass in Mississippi (5). However, this is the first report of rust on switchgrass cultivars grown as biomass feedstocks, and includes descriptions of the disease and identification of the pathogen utilizing morphology, molecular analysis, and pathogenicity assays. Switchgrass rust has been reported in the neighboring states of Arkansas (6) and Tennessee (3). This report broadens the known distribution of rust caused by *P. emaculata* which could cause greater economic losses as the biofuel industry expands throughout the southeastern United States.

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SCANNING ELECTRON MICROSCOPE ANALYSIS OF *COPTOTERMES FORMOSANUS* AND *RETICULITERMES VIRGINICUS* ALATE ABDOMEN (ISOPTERA: RHINOTERMITIDAE)

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INTRODUCTION

A handful of publications describe the termite abdomen, all from observations by light microscopy (Geyer, 1951; Matsuda, 1976; Tuxen, 1970; Belyaeva, 2006). Scanning electron microscopy could reveal morphological features of termite abdomen not visible with a light microscope. The objective of this study was to analyze scanning electron micrographs of abdomens of two subterranean termite species, *Reticulitermes virginicus* Banks and *Coptotermes formosanus* Shiraki.

MATERIAL AND METHODS

Reticulitermes virginicus alates were collected from a swarm in Oktibbeha County, Mississippi, and *C. formosanus* alates from light traps in Pearl River County, Mississippi. Alates were identified to species using a picture key (Messenger, 2002) and preserved in 70% alcohol.

In preparation for electron microscopy, female specimens were removed from alcohol and air dried for about 20 minutes prior to dissection of the subgenital plate (sternite 7) with fine needles (Roboz Surgical Instruments, Gaithersburg, MD) and dissecting scissors (Fine Science Tools, Inc., Foster City, CA). Legs were removed to minimize electron microscope charging. Dissected female and intact male specimens were transferred for 15 minutes each to 95% and then 100% ethanol, with one change for each concentration. This was followed by soaking in 25%, 50%, and 75% hexamethyldisilazane (HMDS): straight ethanol for 15 minutes, and then transfer to 100% HMDS for 15 minutes with one change. After air drying in a fume hood, specimens were mounted on stubs with carbon paste, sputter coated, and viewed with a Zeiss EVO-40VXP environmental electron microscope.

RESULTS

Surface structures of alate abdomens were easily discernable in scanning electron micrographs (Figures 1-6). Numerous papillae (about 1 µm width, surrounded by a base approximately 5-10 µm diameter) are apparent from female abdomens with the subgenital plate removed, mostly on and around the ventral valves (Figures 2-4). The gonopore region of undissected males was conspicuous just below the styli, but the aedeagus was not visible (Figure 5). Cerci appear similar for both sexes and between species (Figure 6). The spermathecal opening above the ventral valves (Belyaeva and Dovgobrod, 2006) was not visible due either to damage during dissections or incomplete removal of subgenital plates.

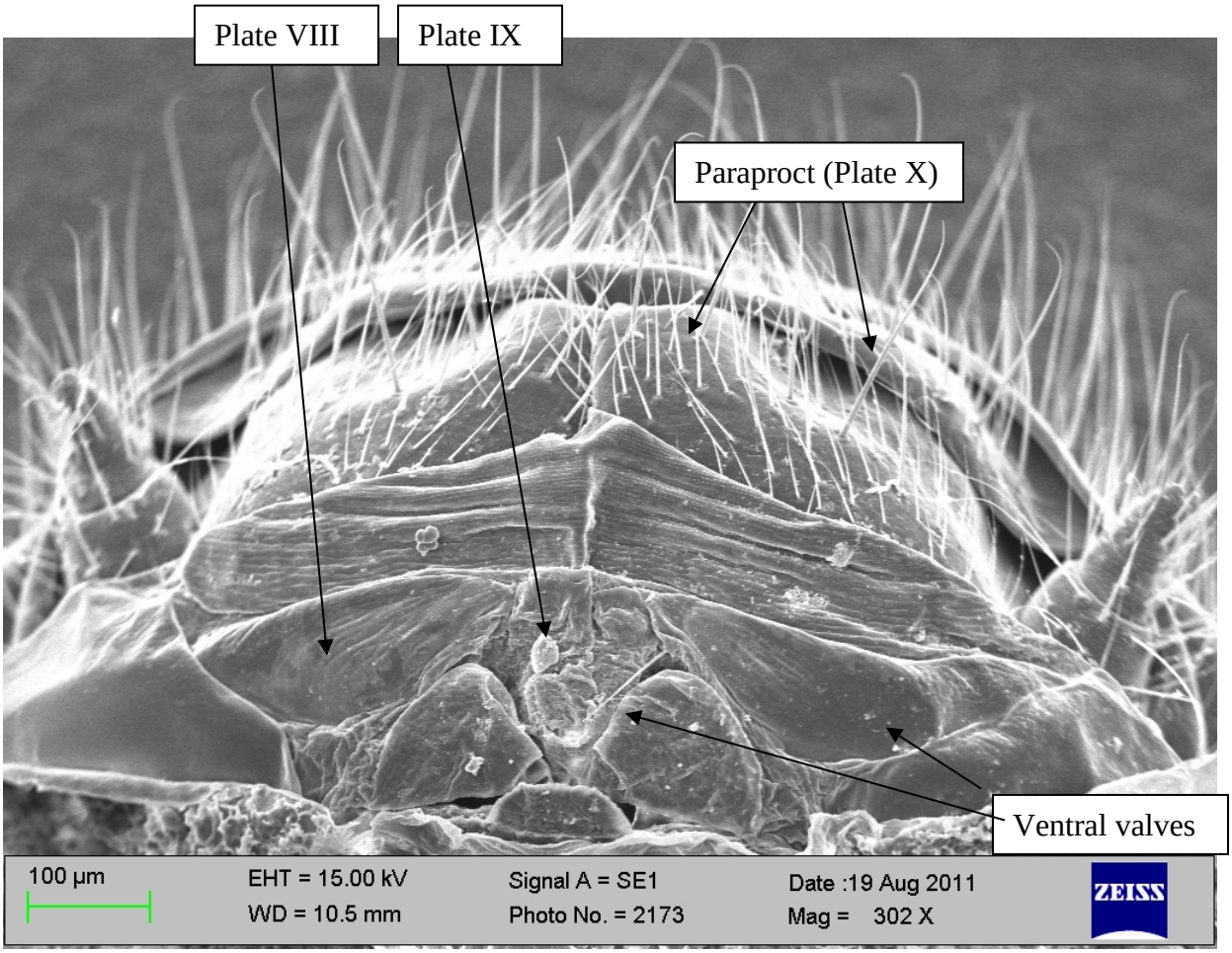


Figure 1: Female *C. formosanus* abdomen with subgenital plate removed

DISCUSSION

Abdominal structures generally appeared similar between species. Styli of males (Figure 5) and cerci for both sexes (Figures 1, 6) were larger for *C. formosanus*, but otherwise resembled those of *R. virginicus*.

Subterranean termites of the genus *Reticulitermes* are very similar in appearance. For *Reticulitermes* in the southeastern United States, slightly different morphology of alate heads is useful for species identification (Messenger, 2002). However, the key was published before formal recognition of an additional species in the region, *R. malletei* Clement, and diagnostic morphological features will need to be compared with that of the new species to determine whether the key is accurate.

Structures of female alate abdomens are distinctive between termite families and species (Belyaeva and Dovgobrod, 2006) and therefore provide a definitive means for species identification. Since scanning electron microscopy allows for clear observation below 1 µm (Figure 3), it would be useful in determining whether even very minute differences exist between species in surface morphology, as well as for providing new details of morphological features not visible by light microscopy.

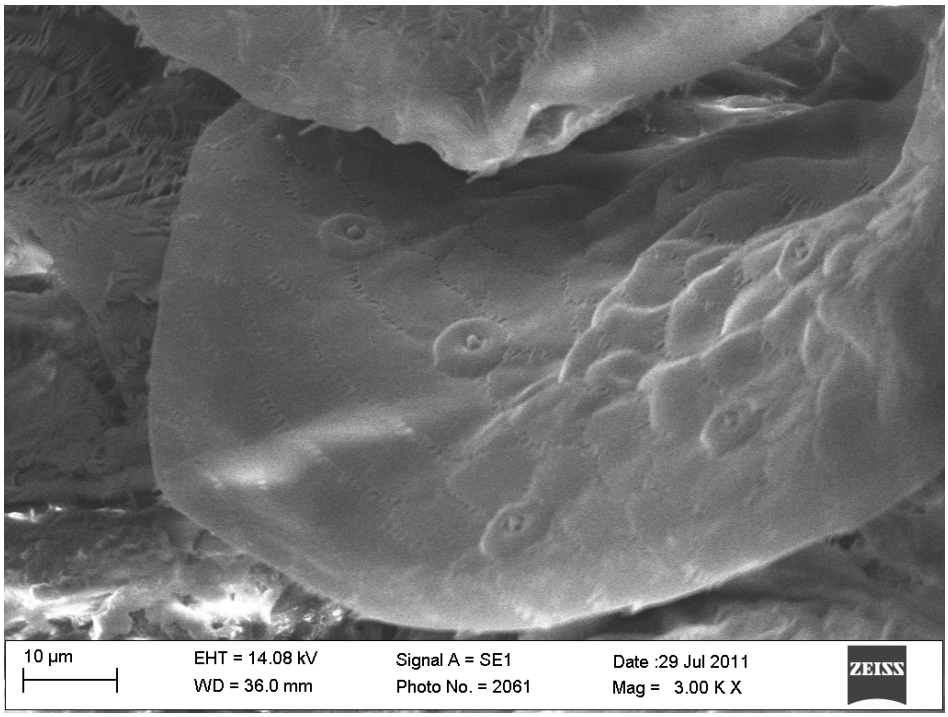


Figure 2: Ventral valve of female *R. virginicus*

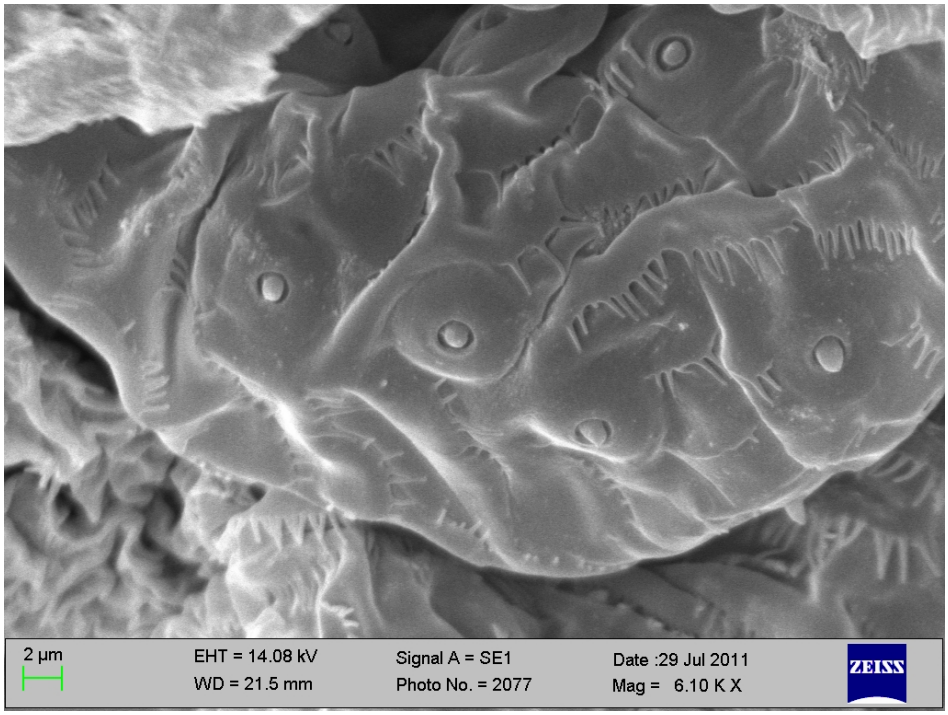


Figure 3: Detail of papillae and other structures on *R. virginicus* ventral valve

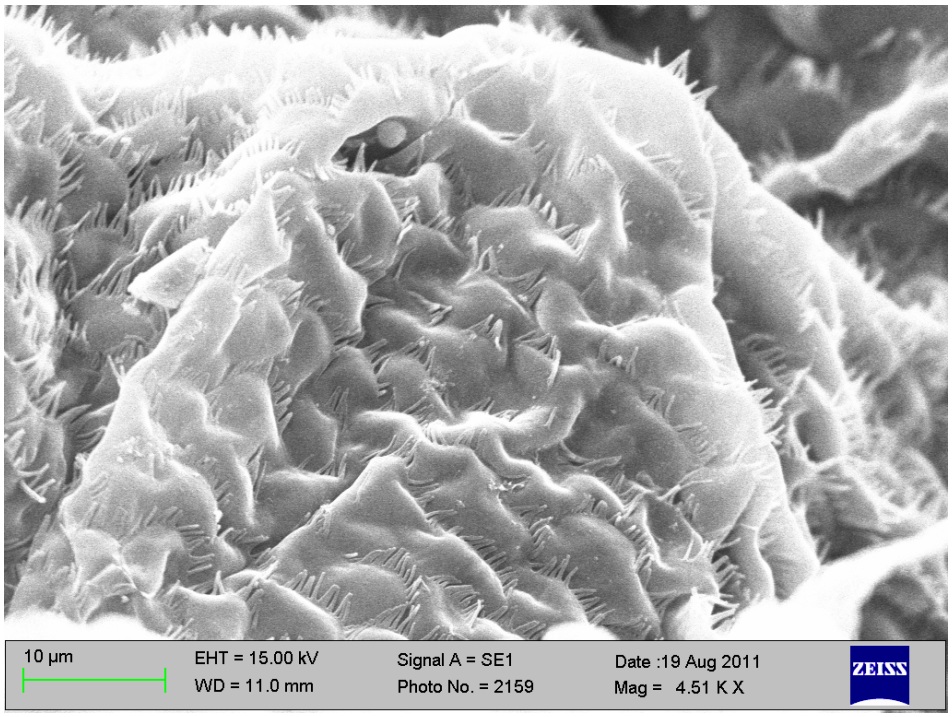


Figure 4: Detail from center area of female *C. formosanus* abdomen with subgenital plate removed

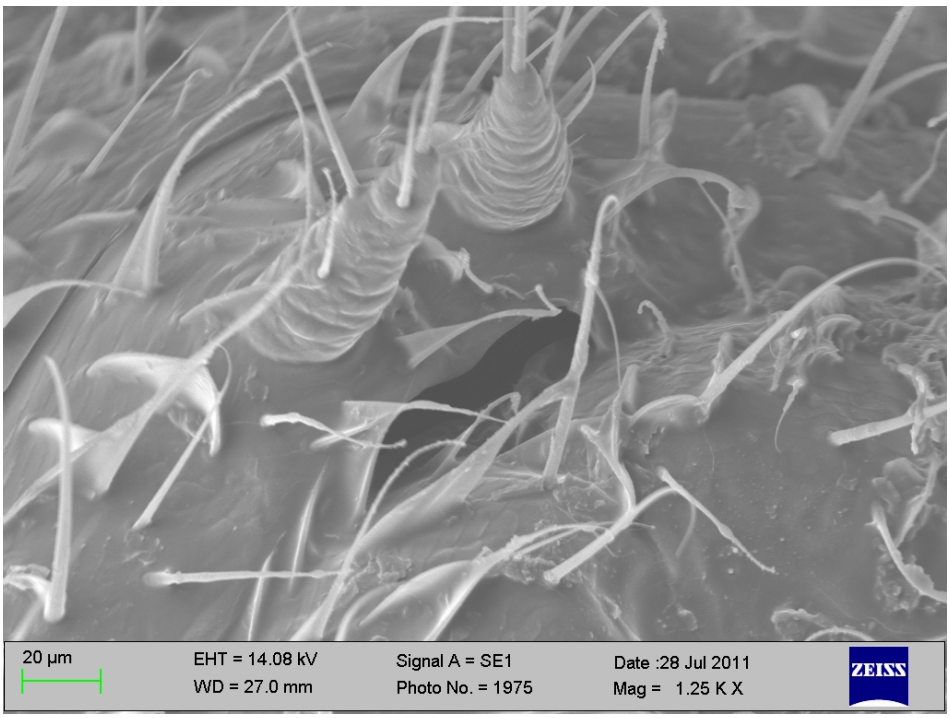
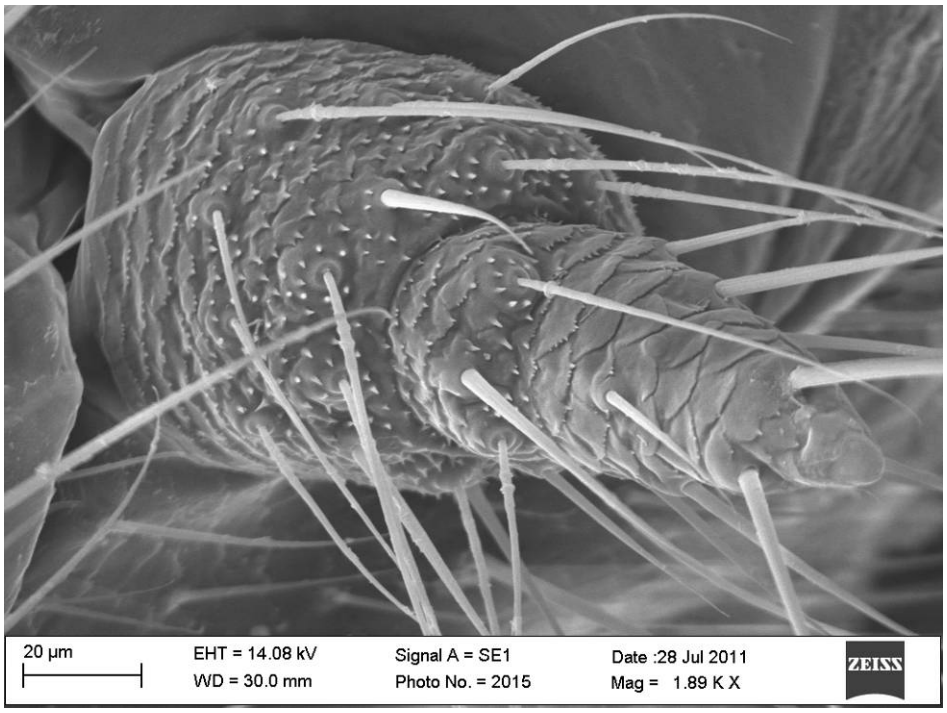


Figure 5: Male *R. virginicus* abdomen showing styli and opening to gonopore



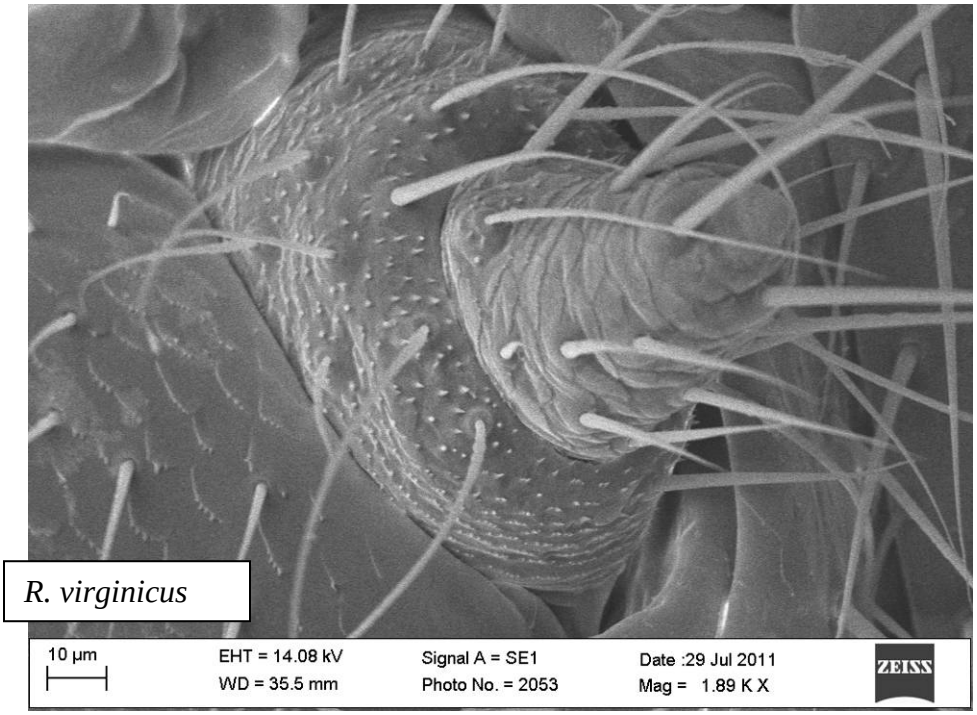


Figure 6: Cerci of female *C. formosanus* and *R. virginicus*

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LIPID, GLYCOGEN, PROTEINS, URIC ACID, AND BODY WATER IN TERMITES AND OTHER INSECTS

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INTRODUCTION

Like other insects, termites store fat and carbohydrate in certain types of cells. Uric acid, the main waste product of protein digestion for terrestrial insects, also accumulates in specialized fat body cells of termites as well as the closely related cockroach. Uric acid can be utilized as a nutrient by termites and cockroaches following digestion by uricolytic bacteria harbored in the hindgut or fat body. These bacterial symbionts convert uric acid, which contains four nitrogen atoms, to ammonia that is used for protein synthesis by the insect host. Body water must be maintained within a narrow limit for an insect's survival. Subterranean termites must remain in humid, enclosed areas, or will quickly lose body water and die. This literature review covers food molecules, uric acid, and body water involved in insect nutrition, metabolism, and digestion, with termites given emphasis.

Uric Acid

Uric acid is probably synthesized in insect fat body (Candy 1985) and is the primary metabolic waste product of most insects (Chapman, 1998; Nation 2002). The uricolytic pathway is the main route for uric acid production in insects, starting with the digestion of proteins (Nation 2002). The pathway also occurs in mammals and birds (Downer 1982). Less common is the nucleolytic pathway, with uric acid precursors of nucleic acids, purines, or pyrimidines rather than proteins (Downer 1982).

Some insects store uric acid in specialized fat body cells called urocytes (Chapman 1998). Stored uric acid has been described as precipitated spherules of potassium or sodium urates (Mullins 1979). Insects that store uric acid include tobacco hornworm larvae, larval *Chrysopa* lacewings, and adult mosquitoes (Chapman 1998).

Termites and cockroaches store uric acid at all life stages. In laboratory culture these insects have been reported to store the molecule in large quantites (Mullins and Cochran 1975; Potrikus and Breznak 1980a; Arquette et al. 2006). American cockroaches fed a high protein diet accumulated up to 31% uric acid (dry weight) (Mullins and Cochran 1975). Workers of eastern subterranean termites (*Reticulitermes flavipes*) measured 45% uric acid (dry weight) after 18 months in a laboratory, compared to 1% at the time of field collection (Potrikus and Breznak 1980a). However, the degree of uric acid accumulation in captivity varies widely by colony (Arquette et al. 2006).

In contrast to numerous studies demonstrating uric acid mobilization from fat body of cockroaches (Cochran 1975), only limited study has been performed in this area with termites (Breznak 2000). There is controversy as to whether uric acid accumulation in termite fat body is permanent or temporary. One theory holds that uric acid in fat body is transported via the Malpighian tubules to the hindgut, where it undergoes anaerobic digestion by uricolytic bacteria (Breznak 2000). Three species of uricolytic bacteria have been identified from *R. flavipes* hindgut, each converting uric acid to carbon dioxide, ammonia, and acetate (Breznak 1982 and 2000). Ammonia is subsequently used for manufacturing proteins in termite fat body (Potrikus and Breznak 1981; Breznak 2000). Uricolytic bacteria are present in sufficient numbers to provide the termite with significant dietary nitrogen (Potrikus and Breznak 1981). Most uric acid reaching the hindgut is digested by uricolytic bacteria, with little excreted in feces (Potrikus and Breznak 1980b). Feces from two additional subterranean termite species, *Coptotermes formosanus* and *Reticulitermes virginicus*, also contain little or no uric acid (Arquette and Rodriguez 2012).

A second theory submits termites permanently excrete uric acid to fat body, with nitrogen for amino acid synthesis predominantly provided by nitrogen-fixing spirochetes in the hindgut rather than uricolytic bacteria (Slaytor and

Chappell 1994). From this view, uricolytic bacteria obtain uric acid from cannibalized termites (Slaytor and Chappell 1994; Breznak 2000). High uric acid accumulation in termite fat body in captivity may result from loss of nitrogen fixing ability, which has consistently been reported to occur soon after field collection (Slaytor 2000), as well as from digestion of cellular proteins during starvation (Slaytor and Chappell 1994).

Limited investigations have considered whether uric acid is involved in termite metabolism (Breznak 2000). However, for the closely related cockroach, the storage, mobilization, and metabolism of uric acid has been well documented (Cochran 1975). One of these studies (Mullins and Cochran, 1975) reported the body weight of female American cockroaches (*Periplaneta americana*) increased to 30% uric acid (dry weight) when the insects were fed a high protein diet, but levels dropped when starved (Mullins and Cochran 1975). Another study found that removal of blattabacteria, which are mutualistic uricolytic bacteria harbored in cockroach fat body, resulted in smaller and slower-maturing insects with diminished fecundity (Brooks and Richards 1956). Fat body of cockroaches with blattabacteria removed contained twentyfold higher levels of uric acid than control insects. Transplanting normal cockroach fat body into nymphs lacking blattabacteria caused the growth rate to increase. Blattabacteria-free regions of fat body appeared white due to accumulated uric acid, while transplanted fat body containing blattabacteria was translucent from lack of uric acid (Brooks and Richards 1956).

Blattabacteria synthesize uricase and xanthine dehydrogenase (Wren and Cochran 1987; Breznak 2000) and are capable of breaking down uric acid to pyruvate *in vitro* (Donnellan and Kilby 1967). The pathway by which blattabacteria metabolize uric acid begins with the conversion of uric acid to allantoin, followed by allantoic acid, glyoxylate, glycerate, and finally pyruvate (Donnellan and Kilby 1967). Mycetocytes, which are specialized fat body cells harboring uricolytic bacteria, have been identified from only one termite species, *Mastotermes darwiniensis* (Bandi and Sacchi 2000). As all other termites only harbor uricolytic bacteria in the hindgut, stored uric acid would not be digested unless mobilized to the hindgut. Mobilization of uric acid from termite fat body remains controversial because this has not been directly demonstrated by experiment (Slaytor and Chappell 1994).

Proteins

The various classes of insect proteins include transport proteins, such as lipophorins in the hemolymph; regulatory proteins, including hormones of insects; defense proteins; and structural proteins, a variety of which are found in the cuticle (Neville 1975; Chapman 1998; Lenhninger et al. 2000). Over 100 kinds of proteins are known to occur in insect cuticle, functions of which include pigmentation and cuticular hardness (Hopkins and Kramer 1992; Chapman 1998).

Many insect proteins are mobile. For example, lipophorin is a conjugated protein that shuttles diacylglycerols via hemolymph (Chapman 1998). Urates may also be transported through hemolymph by proteins (Cochran 1975). Some proteins are transported to specific sites as required, such as cuticular proteins secreted from the epidermis during molting that originate in hemolymph (Reviewed in Neville 1975; Hopkins and Kramer 1992).

Although the core food of termites is lignocellulose, some protein is also obtained from the diet. Fungal tissue is a primary food source of subterranean termites (Waller and Curtis 2003) and is 20%-40% protein (dry weight). Other dietary sources of protein include dead protozoans obtained from proctodeal feeding, cannibalism and necrophagy, and coprophagy (Noirot and Noirot-Timothee 1969; Collins 1983; Hunt and Nalepa 1994; Nation 2002; Arquette et al. 2012). All termites produce proteolytic digestive enzymes (Collins 1983).

Insects predominantly synthesize amino acids in fat body (Chapman 1998). Synthesis of amino acids occurs at intermediate steps of glycolysis and the Krebs cycle (Lehninger et al., 2000). Lower termites such as the Formosan subterranean termite (*Coptotermes formosanus*) obtain essential amino acids synthesized by bacterial and protozoan symbionts (Mauldin et al. 1978). Intracellular bacterial symbionts of the German cockroach synthesize amino acids from urate precursors (McFarlane 1985). Amino acids are stored in hemolymph at 100-300 times the levels found in human blood, in excess of what is needed for protein synthesis (Chen 1985). In addition to comprising primary protein structure, amino acids also serve as precursor molecules. For example, glucogenic amino acids are converted to glucose during starvation (Lenhninger et al. 2000).

In aging adult insects, the rate of protein synthesis may decline due to an inability to repair or replace mitochondria (Sohal 1985). Therefore protein synthesis and subsequently enzyme activity is diminished (Sohal 1985; Brunk and Terman 2002).

The diet of termites is too low in nitrogen, amino acids, and protein for growth and egg development (Chen 1985). Nitrogen for adequate protein synthesis has been hypothesized to come from various sources. Ammonia produced from nitrogen fixation by bacterial symbionts is thought to provide termites with high enough levels of dietary nitrogen to compensate for the low nitrogen content of wood (Slaytor and Chappell 1994). Uricolytic

bacteria harbored in the paunch of subterranean termites may provide the insect with significant amounts of dietary nitrogen for protein production (Breznak 2000). Dead protozoan symbionts provide termites with dietary nitrogen (Breznak 1982; Collins 1983). Chitin contains a significant amount of nitrogen, about 7 percent by weight (Waller and LaFage, 1987). It is always associated with proteins in nature, and may be a source of nitrogen that termites can metabolize (Breznak 1982; Chapman 1998). Chitinase activity has been measured from *R. virginicus* workers (Arquette, 2011). This chitinase likely was from the gut, as the termites assayed had full gut contents, indicating they were not molting. When an insect molts its cuticle, digested chitin, and protein is contained in molting fluid, which is either re-absorbed through epidermal cells, or taken in through the mouth or anus (Neville 1975; Breznak 1982).

Glycogen

Glycogen is synthesized in insects by the addition of glucose molecules to a glucose primer, a process catalyzed by glycogen synthetase (Nation 2002). Insects store glycogen in fat body and epithelial gut cells (Nation 2002). Glucose cleaved from glycogen does not need to be transported, so can be used as an immediate fuel source (Candy 1985). Glycogen of insects is converted to glucose during periods of vigorous activity such as flight (Chapman, 1998) as well as during starvation (Wigglesworth 1942; Satake et al. 2000). Glycogen is also a source of trehalose for insects (Candy 1985). Glucose is cleaved from glycogen branches by the activated form of glycogen phosphorylase. Glycogen phosphorylase *b* is a stored, inactive form of the enzyme, and is activated to glycogen phosphorylase *a* to release glucose or phosphorylated glucose units from glycogen (Friedman 1985).

Gluconeogenesis, or glucose synthesis from non-carbohydrate precursors, occurs in starved insects (Candy 1985). Gluconeogenesis is essentially the reverse of glycolysis, except for three irreverable steps of glycolysis which are bypassed by alternate pathways (Candy 1985; Lenhninger et al. 2000). Amino acids are the main precursor molecules for gluconeogenesis. Other precursors are pyruvate, lactate, and glycerol (Candy 1985). The conversion of amino acids to carbohydrates by insects was first demonstrated in the *Aedes aegypti* mosquito, with glycogen levels shown to increase when mosquitoes are fed alanine or glutamine (Wigglesworth, 1942).

Glycogen levels of termites remain relatively steady for laboratory reared *R. flavipes* workers (Arquette et al. 2006). However, glycogen content varies widely for termites collected from different environments. Glycogen levels measured from crude extracts of *R. flavipes* workers collected from a home lawn on an Atlantic barrier island were 25-fold or more higher compared with colonies from forested locations (Arquette and Forscher, 2006). The reason for this difference is unknown.

Lipids

Lipids, mainly in the form of glycerides, provide insects with a major source of stored energy and metabolic water. At least 78% of insect lipids are triglycerides, with the proportion in fat body about 90% (Fast 1964; Beenakkers et al. 1985). Diglycerides in hemolymph are an important energy reserve for insects, comprising about 3% of the total lipid content (Bailey 1975; Beenakkers et al. 1985). Other lipids are integral components of waxes and other cuticular layers (Fast 1964; Nation 2002).

Insects obtain lipids from food and microbial symbionts (Chapman 1998) or synthesize it in fat body from carbohydrate or other precursor molecules (Bailey 1975). Acetyl CoA formed at the start of cellular respiration is used in long-chain fatty acid synthesis (Bailey 1975). Long-chain fatty acids and glycerols are esterified, forming triglycerides for storage in fat body (Bailey 1975). Lower termites also synthesize glycerides using acetate produced from fermentation by protozoan symbionts (Potrikus and Breznak 1981). Carter et al. (1972) reported that oleic acid makes up about 60% of the fatty acids of *R. flavipes* termites, while lineolic acid (18 carbon atoms) and palmitic acid (16 carbon atoms) each constitute about 10% of the total.

Insects cannot synthesize sterols (McFarlane 1985), and obtain it either from food or microbial symbionts of the gut (Chapman 1998). Clayton (1960) reported the production of a sterol, 22-dehydrocholesterol, from radiolabeled acetate fed to German cockroaches. He concluded this sterol was probably done by bacterial symbionts from an initial conversion of acetate to ergosterol, then to 22-dehydrocholesterol.

Most glycerides stored in fat body must be transported to other sites for oxidation (Candy 1985). Triglycerides are converted to diglycerides in fat body cells (trophocytes), which in turn are attached to lipophorins prior to entering hemolymph for transport (Bailey 1975). Lipophorins are reusable shuttles for transporting diglycerides in hemolymph to muscle where oxidation occurs (Beenakkers et al. 1985). When fatty acid chains of diglycerides are hydrolyzed, most of the remaining glycerol is transferred to hemolymph (Candy 1985).

Lipid content has been reported for termites maintained in laboratory culture and just after field collection (Arquette and Forschler, 2006; French et al., 1984; Carter et al., 1972). For *R. flavipes* workers, lipid content was measured between 2.3% and 6.7% from field

populations (Carter et al. 1972; Arquette and Forschler, 2006). Lipid levels fluctuate for some termite species in laboratory culture. Fat content of *Coptotermes acinaciformis* doubled after 2 weeks in a laboratory, then dropped to low levels by 6 weeks (French et al., 1984). Arquette et al. (2006) reported 2-3 fold higher fat levels for *R. flavipes* workers from separate colonies after 2 weeks in laboratory culture. Lipid content of workers from two colonies was maintained or increased under laboratory conditions, but those of a third colony eventually dropped to low levels. For *Mastotermes darwiniensis*, French et al. (1984) reported lipid content remained relatively steady over 4 weeks in a laboratory.

Decreasing levels of lipid for termites in laboratory culture appears to result from starvation, when stored lipids would be the main energy source of insects. For starved American cockroaches, lipids provide 66% of the insect's metabolic energy, compared to 22% from glycogen and 11% from protein (Fast 1964). Mauldin et al. (1977) reported *R. flavipes* measured 5.3% lipid (wet weight) shortly after field collection, then decreased to 1.3-1.4% after partial or total elimination of microbial symbionts, which in turn caused the termites to starve.

Body Water

Water has various physiological roles in insects. These include regulation of body temperature, providing a hydrostatic skeleton for body support, and transport of food molecules, hormones, respiratory gases, and excretory products (Hadley 1994). Insects obtain water from drinking, food, water vapor absorption, and metabolism (Hadley 1994) and lose water through the cuticle, spiracles, mouth, and anus (Edney 1977; Hadley 1994). Factors influencing water loss in insects include warm temperature, low humidity, air currents, molting, egg production, nutritional state, abrasion of the waterproofing wax layer of the cuticle, and unknown effects of aging (Becker 1969; Edney 1977; Hadley 1994).

Water homeostasis is the maintenance of proper cellular osmolarity (Hadley 1994; Nation 2002). Without physiological regulation of proper body water levels, too much water would result in an insect's cells swelling and rupturing, while excessive water lost results in precipitation of soluble metabolites, causing irreversible harm (Hadley 1994). To maintain optimal body water levels, an insect must take in enough water to offset the amount lost through respiration, transpiration, or excretion (Hadley 1994).

Insects absorb water vapor through the mouth and rectum (Edney 1977; Hadley 1994). The critical equilibrium humidity is the relative humidity at which absorption of water vapor and dessication are balanced (Hadley 1994). Critical equilibrium humidity differs between insect species, ranging from about 43% to near saturated humidity (Edney 1977 and Hadley 1994). While body mass usually decreases from water loss, some terrestrial arthropods still lose water at saturated humidity levels (Hadley 1994).

Metabolic water is an important source of water for many insects including termites (Lee and Wood 1971; Nation 2002). Metabolic water is produced by insects from oxidation of carbohydrates, lipids, and proteins from food or stored forms (Hadley 1994; Nation 2002).

Fats are a major source of metabolic water (Nation 2002). For example one molecule of palmatic acid (16 carbons) produces 108 water molecules when oxidized, while the breakdown of one glucose molecule nets four water molecules (Nation 2002). About 1.07 ml of water is produced per gram of metabolized fat (Hadley 1994). This is about twice the amount of metabolic water produced by weight from the metabolism of carbohydrates, and about 2.5 times the rate produced from the oxidation of protein to urea (Hadley 1994). However, fat requires about 2.5 times more oxygen for metabolism than glucose (Hadley 1994). Also, water is required for the synthesis of fats (Hadley 1994).

Cuticular lipids of the epicuticle provide insects with a barrier against body water loss (Chapman 1998). However, some water still diffuses through the cuticle even if heavily sclerotized, and exact sites of cuticular water loss is unknown (Hadley 1994). Cuticular transpiration increases when lipids in the epicuticle are disrupted from temperature, chemicals, adsorption by dusts, and mechanical disruption (Hadley 1994).

Spiracles are a major site of body water loss in terrestrial arthropods (Hadley 1994). Most insects reduce transpiration through spiracles with valves that open and close (Hadley 1994). The valves open in the presence of elevated CO_2 in the trachea, as well as by the action of motor neurons of the central nervous system (Hadley 1994). Metabolism increases with higher temperatures and activity levels, thus requiring spiraclular valves to stay open longer to exchange respiratory gases, and allowing more water to be lost (Chapman 1998). Specific amounts of water lost through spiracles are unknown for most insect species due to the difficulty in obtaining measurement (Hadley 1994).

Another main source of insect body water loss is urine. Some water is from urine is absorbed through the ileum and rectum prior to excretion (Collins 1969; Nation 2002).

Land arthropods are vulnerable to dessication, and usually stay in moist environments (Cloudsley-Thompson 1988). By burrowing into the soil arthropods avoid the dessicating environment above the surface (Hadley 1994).

Humidity in burrows comes from the surrounding soil or the tunnel's inhabitants (Hadley 1994). High humidity required for the survival of subterranean termites is maintained in the shelter tubes they construct (Lee and Wood 1971; Traniello and Leuthold 2000).

Hadley (1994) described a simple method of measuring insect body water content by obtaining the live weight followed by re-weighing after drying at around 60°C. The difference between live and dry weight is divided by the live weight to give the percent water content. Vacuum drying is another method for determining water content of arthropods (Hadley 1994). Most studies of the dessication tolerance of live insects measure water loss at 5% or less relative humidity (Hadley 1994). Sponsler and Appel (1990) reported lower survivable limits of dehydration for different *C. formosanus* and *R. flavipes* castes. *R. flavipes* workers placed in 0% relative humidity died when they lost 50% of their body water, which occurred after about 5 hours. *R. flavipes* lose water faster than other *Reticulitermes* species (Collins 1969).

While moist conditions are required for survival of subterranean termites, high humidity has been described as fatal to a drywood termite, *Cryptotermes brevis* (Walker) (Collins, 1969). When placed in 86% or higher relative humidity, this species swells up and dies due to an inability to transpire metabolically produced water to humid air (Collins 1969). There are three North American termite species that inhabit arid environments and cannot tolerate high humidity (Collins 1969).

Body water levels of termites have been considered as possible indicators of the vitality of termite populations (Arquette et al., 2006; French et al., 1984). French et al. (1984) did not regard body water levels as a reliable method for describing termite vitality. However Arquette et al. (2006) concluded *R. flavipes* workers are less healthy below a threshold of approximately 75% body water.

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EARLY EFFECTS OF POSS ON TISSUE HEALING USING A PIG MODEL

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ABSTRACT

Polyhedral oligomeric silsesquioxane or POSS chemicals have been used to prepare nanosized designed novel composites with a variety of potential applications. POSS is composed of a silicon–oxygen framework that can be easily functionalized with a variety of organic substituents. The purpose of this study was to determine if POSS can be used as a protectant barrier in wound care, and to determine with the response of the healing tissue towards the material. A pig skin wound model was used to test the POSS material over time. Biopsies were taken after 3 days to determine the short term response to the material which was compared with wounds which were not treated. The data shows that POSS was able to protect the skin against infection and did not interfere with the normal tissue healing response. The data suggests that the material may be serving as a barrier to protect against bacterial infection or may have antibacterial properties. More studies are needed to determine the longer term effects on wound healing.

INTRODUCTION

Up to the present, autologous skin grafts or flaps have been widely used for repairing skin and soft tissue defects. However, improved reconstruction poses greater donor site problems. To resolve these problems, skin substitutes have been developed using tissue engineering [1]. However, in order for skin substitutes to be applied to patients with full-thickness skin loss, the substitutes should function as an alternative to autologous skin, form an effective barrier against bacterial invasion, minimize inflammation and scar formation, improve fibrovascular tissue ingrowth, and have excellent reproducibility [2,3]. Polyhedral oligomeric silsesquioxane (POSS) with a distinctive nanocage structure consisting of an inner inorganic framework of silicon and oxygen atoms, and an outer shell of organic functional groups is one of the most promising nanomaterials for medical applications. Enhanced biocompatibility and physicochemical (material bulk and surface) properties have resulted in the development of a wide range of nanocomposite POSS copolymers for biomedical applications, such as the development of biomedical devices, drug delivery systems, dental applications, and , tissue engineering scaffolds. The purpose of our experiment was to determine the short term effects of applying POSS to a full thickness skin wound using the pig as a model.

MATERIALS AND METHODS

Experimental Design: The goal of the first set of experiments is to test POSS for tissue compatibility. The first set of experiments evaluates the POSS material in partial thickness skin wounds and evaluates inflammation, re-epithelialization, cell migration from the lateral wound edges, presences of hair follicles, as well as for the formation of granulation tissue. For this model we made a total of 8 incisions per pig n= 2 pigs. A custom-made template with 1.5 x 1.5 cm squares separated by 2 cm was placed on the skin and the squares outlined with a surgical pen. The marked patches of skin were removed with a scalpel (#11 blade) and surgical scissors using aseptic technique and sterile instruments (All procedures were approved by the UMMC institutional animal care and use committee). Four wounds per pig will contain POSS formulation and four wounds per pig will contain saline. On day 3 representative wound per category were biopsied with a 4-mm punch situated so half of the punch was over the wound and half over adjacent tissue.

Bacterial Determination of the wounds:

Wounds were observed, swabbed, traced, and photographed at time 0, 1, and 3 days following surgery. Wounds were sampled with sterile swab and the swab was used to streak blood agar plates. The plates were placed in a 37ºC and observed for bacterial growth after 24 hours. The plates were photographed and data recorded.

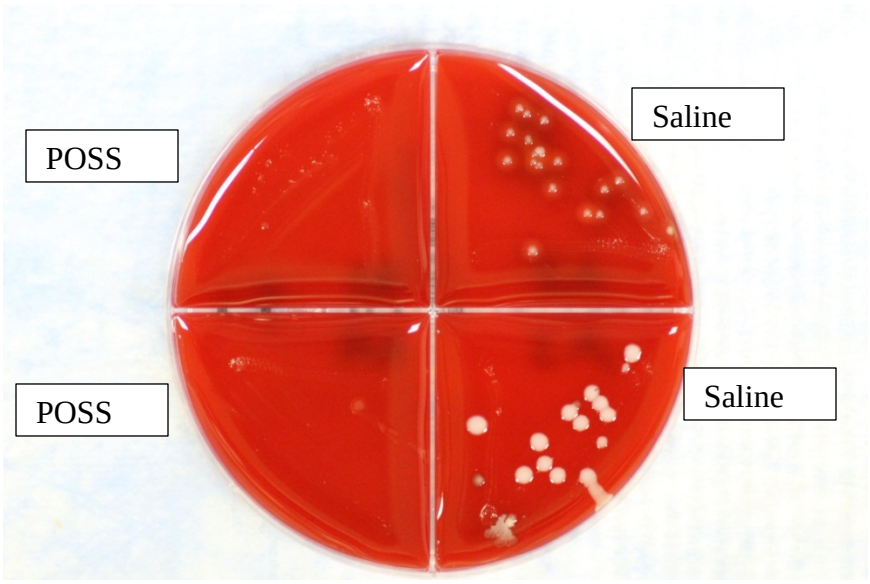
Histology:

Biopsied tissue was placed in 10% buffered formalin containing zinc and processed for routine paraffin embedding. Tissues were dehydrated using a series of increasing strengths of alcohol, cleared in xylene prior to embedding in paraffin. Section (5µm) were obtained using a microtome. The sections were placed on a glass slide and stained with hematoxylin and eosin to determine the presence of cells within the tissue at 3 days following surgery in the presence or absence of POSS.

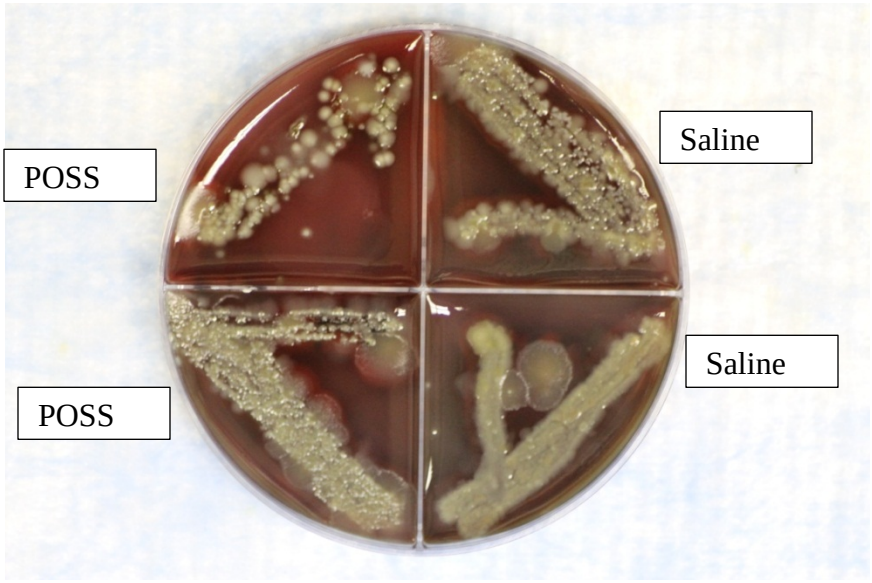
RESULTS

Bacteria

The POSS material was capable of protecting the skin from bacterial contamination within the first 24 hours compared to saline treatments. However, by 3 days there were no differences in the amount of bacteria within the POSS versus the saline treated skin wounds (Figures 1A and B).



Day 1

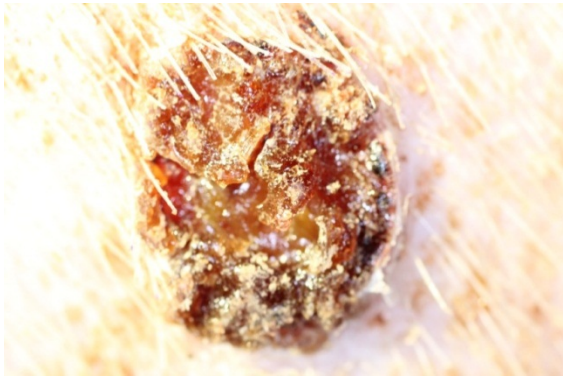


Day 3

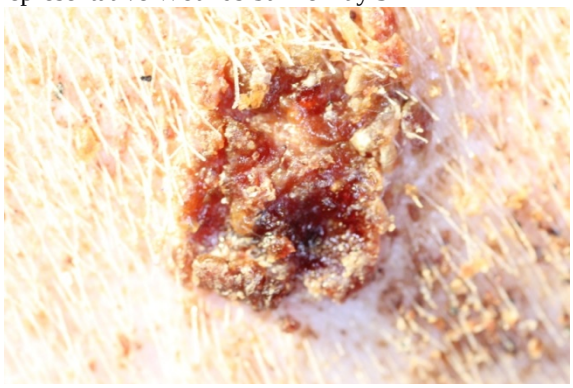
Figure 1A is a representative photomicrograph 24 hours after the administration of saline or POSS in an experimentally induced wound. Figure 1B is the same wound 3 days post wounding and application of POSS or saline.

Gross Morphology:

There were no differences in the gross morphology by three days between the samples (Figures 2 A and B)



Representative Wounds Saline Day 3



Representative wounds POSS Day 3

Figure 2A and B. Representative photomicrographs of wounds after 3 days of healing.

Digital imaging was used to determine the changes in the wound over-time and were measured and recorded. The wounds did not differ in size during the first three following treatment with either saline or POSS (Table 1).

Table 1: Digital Measurements of POSS and Saline treated wounds at days 1 and 3. The measurements were made using NIH Image J software.

Control Day 1 Digital Tracing (n=8 wounds)	Control Day 3 Digital Tracing (n= 8 wounds)
32.88	24.71
31.63	28.52
35.00	31.08
37.12	35.27
18.65	34.73
24.78	25.31
27.90	27.65
35.32	35.19

Histological Assessments of Biospy at 3 Days:

The wounds were biopsied at day 3 using a punch and collecting the later and middle aspects of the wound in order to obtain both normal and healing tissue for comparison. The Hematoxylin and Eosin staining of the wounds showed significant histological differences between the saline and POSS treatment groups (Figures 3 and 4). Saline treated wounds showed large presence of inflammatory cells and adipocytes within the medial aspects of the wounds and disorganized collagen formation, whereas, the POSS treated groups had presence of inflammatory cells as well as evidence of new vessel formation in the wound bed. The adjacent or lateral tissue in both control saline and POSS treated groups appeared in similar without evidence of an acute inflammatory response.

Control saline lateral aspect Control saline medial aspect Control saline medial aspect

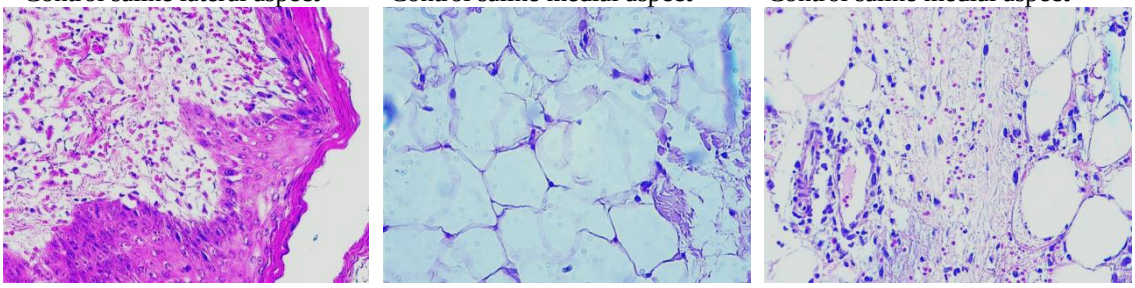


Figure 3. Biopsy results from representative saline treated wounds 3 days post wounding

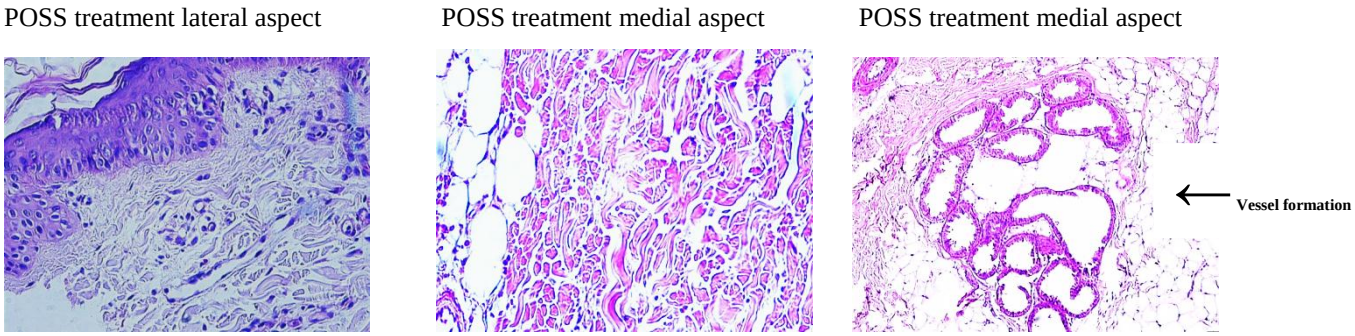


Figure 4. Biopsy results from representative POSS treated wounds 3 days post wounding

DISCUSSION

Dermal substitutes should be physically strong and easy to handle, endure contraction through durability, continue to attach viable fibroblasts during the whole wound healing process, enable the reconstruction of the dermal lattice with fibroblasts, and should endure hydrolysis during their presence in the wound until they are finally absorbed after complete wound healing [2,5].

In this study, the results were significantly different between the experimental group and control within the first 72 hours after wounding. The control group showed a more rapid wound healing than the POSS group, suggesting that POSS scaffolds have a strong affinity to cellular components of the surrounding soft tissue and blood vessels. The results support the idea that it is conceivable that POSS may inhibit inflammatory reactions and may promote wound healing by reducing biodegradation caused by proteolytic enzymes, which increases physical stability and induces the ready migration of cells from the surrounding tissue into the POSS. In addition, POSS can be used as a temporary protection membrane and can promote wound healing.

CONCLUSIONS

In conclusion, additional studies with POSS technology can be used to incorporate POSS with antibiotics as a potential dermal protectant that can be applied to an open wound to preserve the integrity of the tissue, maintain the moisture content, and increase wound healing.

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__ History and Philosphy of Sciences
__ Math., Computer Sci and Statistics
__ Marine and Atmospheric Sciences
__ Physics and Engineering
__ Psychology and Social Sciences
__ Science Education
__ Zoology and Entomology

Complete either the Membership/Pre-Registration form if you plan to attend and present at the meeting if you do not plan to attend the meeting please complete the membership form

MEMBERSHIP/ PRE-REGISTRATION INFORMATION
New ___ Renewal___
Mr. Ms. Dr._____
Address _____
City, State, Zip _____
School or Firm _____
Telephone_____ **Email**_____

PLEASE INDICATE DIVISION YOU WISH TO BE AFFILIATED _____
Before January 15, 2013…….Regular Member/Pre-Registraion $80 Student Member/ Pre-registration $40
After January 15, 2013…….Regular Member/Registraion $105 Student Member/ Pre-registration $50

MEMBERSHIP INFORMATION
New ___ Renewal___
Mr. Ms. Dr._____
Address _____
City, State, Zip _____
School or Firm _____
Telephone_____ **Email**_____

PLEASE INDICATE DIVISION YOU WISH TO BE AFFILIATED _____
Regular Member $25 Student Member $10 Life Member $250
Educational Member $150 Corporate Patron $1000 Corporate Donor $500

CHECKLIST
Please complete the following:
__ Enclose title of abstract (even if abstract has been submitted electronically)
__ Complete membership/registration form (this form)
__ Enclose the following payments (Make checks payable to Mississippi Academy of Sciences)
__ $25 per abstract
__ $80 regular membership/pre-registration fee OR $40 student membership/pre-registration fee (2014 membership must be paid for abstract to be accepted)
__ You must supply a check #_____ or P.O. #_____ (or indicate Pay Pal confirmation) _____

MISSISSIPPI ACADEMY OF SCIENCES—ABSTRACT INSTRUCTIONS
PLEASE READ ALL INSTRUCTIONS BEFORE YOU SUBMIT YOUR ABSTRACT ON-LINE

- Your paper may be presented orally or as a poster. Oral presentations are generally 15 minutes. The speaker should limit the presentation to 10-12 minutes to allow time for discussion; longer presentations should be limited accordingly. Instructions for poster presentations are linked here.
- Enclose a personal check, money order, institutional check, or purchase order for $25 publication charge for each abstract to be published, payable to the Mississippi Academy of Sciences. The publication charge will be refunded if the abstract is not accepted.
- The presenting author must be a member of the Academy at the time the paper/poster is presented. Payment for membership of one author must be sent for the abstract to be accepted.
- Attendance and participation at all sessions requires payment of registration.
- Note that three separate fees are associated with submitting and presenting a paper at the annual meeting of the Mississippi Academy of Sciences.
    1. An abstract fee is assessed to defray the cost of publishing abstracts and
    2. a membership fee is assessed to defray the costs of running the Academy.
    3. Preregistration payment ($25 regular; $10 student) may accompany the abstract, or you may elect to pay this fee before February 1, or pay full registration fees at the meeting.
- Abstracts may **only** be submitted on line via a link through the MAS website.The appropriate abstract fees can be paid via Paypal or sent via mail to Barbara Holmes at the Academy address.
- **Late abstracts will be accepted with a $10 late fee during November increased to $25 after that. Late abstracts will be accepted only if there is room in the appropriate division. They will be published in the April issue of the MAS JOURNAL.**
- Submit your appropriate fees NO LATER THAN NOVEMBER 1, 2013.

Ms. Barbara Holmes
Mississippi Academy of Sciences
Post Office Box 55907
Jackson, MS 39296-5907

GUIDELINES FOR POSTER PRESENTATIONS

- The Academy provides poster backboards. Each backboard is 34" high by 5' wide. Mount the poster on the board assigned to you by your Division Chairperson. Please do not draw, write, or use adhesive material on the boards. You must provide your own thumb tacks.
- Lettering for your poster title should be at least 1" high and follow the format for your abstract. Lettering for your poster text should be at least 3/8" high.
- Posters should be on display during the entire day during which their divisional poster session is scheduled. They must be removed at the end of that day.
- Authors must be present with their poster to discuss their work at the time indicated in the program.