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AIRBORNE STARCH GRANULES AS A POTENTIAL CONTAMINATION SOURCE AT ARCHAEOLOGICAL SITES

Andrew R. Laurence, Alston V. Thoms, Vaughn M. Bryant and Cassandra McDonough

*Well-known allergy literature attests to a presence of airborne starch granules from human and natural activities and illustrates that starch granules within pollen grains from starch-rich plants are released when pollen grains rupture in mid-air during thunderstorms. This study reports on starch granules extracted from Texas air samples and ruptured pollen grains from seven ethnographically important geophyte species, as well as maize (*Zea mays *L.). Starch granules from pollen grains are compared to those in storage organs of these plants. Results confirm that storage-like starch granules are airborne and that starch granules inside pollen can be indistinguishable from starch granules in the respective storage organs.*

Key words: *airborne starch, artifact analysis, starch granule analysis, starch rain*

*En publicaciones muy conocidas sobre las alergias se ha demostrado la presencia de gránulos de almidón transportados por el aire que derivan de actividades humanas o de origen natural. Estas publicaciones también demuestran que durante las tempestades se liberan al aire gránulos de almidón provenientes de granos de polen de plantas ricas en almidón. En este estudio se presentan datos sobre gránulos de almidón de muestras de aire de Texas y de granos de polen roto de siete especies de geófitos de gran importancia etnobotánica y de maíz (*Zea mays *L.). Los gránulos de almidón provenientes de granos de polen se compararon con los de los órganos de almacenamiento de esas mismas plantas. Los resultados confirman que los gránulos de almidón provenientes de los órganos de almacenamiento son transportados por el aire y que los gránulos de almidón del interior del polen son indistinguibles de los de los sus respectivos órganos de almacenamiento.*

Introduction

Starch granule analysis is an important tool used by archaeologists to obtain evidence of plant utilization. What has made starch granule analysis so valuable is the concept that the presence of starch granules on artifacts can provide direct evidence of plant utilization. This idea is based on findings that residues from starch-rich plants adhere to artifacts during cooking and food-processing activities (Fullagar 2006; Mercader 2009; Torrence 2006; Zarrillo et al. 2008). While studies confirm that starch granules from cooked or processed plants adhere to artifacts as a result of cooking and processing activities, there is evidence that starch rain could contaminate artifacts prior to burial or during or after excavation (Loy and Barton 2006).

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The issue of airborne starch contamination in archaeological studies has been mentioned by numerous researchers. Barton and Matthews (2006) noted the possibility that airborne starch deposited during excavations can affect interpretations of ''fossil'' starch granules from archaeological materials. To identify possible contamination of artifacts, Loy and Barton (2006) suggested leaving microscope slides out in the field to test for airborne starch deposited from industrial activity. Archaeologists have also tested for airborne starch contamination by leaving microscope slides exposed to the air in research laboratories and curation facilities (Loy and Barton 2006; Parr 2002; Zarrillo and Kooyman 2006). Nugent (2006) recovered unmodified and damaged airborne starch granules in a curation facility and a lab, which averaged 12.6 µm and 18.4 µm in size, respectively. Similarly, Williamson (2006) identified contamination within the lab from starch granules that originated from a nearby flour mill. Although the potential for modern airborne starch granules, particularly from industrial products has been recognized, we are not aware of any studies published on this subject specifically.

Research presented here addresses the presence of airborne starch granules, the role starch-rich pollen grains play in the generation of starch rain, and the potential impact on the archaeological record. To investigate the presence of airborne starch granules, air samples were taken from three ecological zones in Texas. In addition, pollen grains from seven wild, starch-rich Texas geophyte species were ruptured, and the recovered starch granules were compared to starch granules from the plants' underground storage organs (USO's). We also ruptured pollen grains from *Zea mays* L. (maize) and compared the starch therein to the starch granules recovered from ripened kernels.

Background

Transient and storage starch granules are the two main types found in starch-rich plant species (Gott et al. 2006). Starch-rich plants include domesticated species such as maize and Irish potatoes (*Solanum tuberosum* L.) as well as wild geophytes, including false garlic bulbs (*Nothoscordum bivalve* (L.) Britton) and winecup tubers (*Callirhoe involucrata* Torr. & A. Gray.), which are known either ethnographically or archaeologically to have been used as food (Havard 1895; Moerman 1998:304-305; Thoms 2008). Transient starch granules are generally described as small (~1 µm in size), although they may be as large as 7 µm. These generally non-diagnostic, temporary grains are formed within the chloroplasts of cells (Buléon et al. 1998; Haslam 2004) during the day when energy is produced and are broken down at night for use in other parts of the plant or transferred to storage organs (Raven et al.1999).

Storage starch granules are located in storage organs (roots, seeds, and fruits) of starch-rich plants and are usually described as being comparatively large, typically > 5 µm (Gott et al. 2006). Significant size and morphological variation has been reported for starch granules recovered from an individual plant, as well as among members of the same species (Delcour and Hoseney 2010:23-25; Hoseney 1994:40; McDonough et al. 2000; Reichert 1913; Rooney and Suhendro 2001; Serna-Saldivar 2010:109). For instance, Kent (1975) reported that starch granules recovered from maize seeds can be anywhere from 2 to 30 µm in diameter with spherical, angular, or polygonal morphologies. Starch granule variation within an individual seed has been argued to be the result of the starch granules location within a storage organ (Kent 1975). With regard to the archaeological record, transient starch granules are rarely studied because they are considered to be non-diagnostic (Haslam 2004). Storage starch granules, however, are used as direct evidence for ancient plant utilization because they are more readily identified to family, genus, or species (Gott et al. 2006; Haslam 2004).

Starch granules within pollen grains of starch-rich plants, often described as storage starch, provide energy for growth of the pollen tube (Baker and Baker 1979; Grayum 1985). There are several ways in which starch in pollen can be liberated. Especially common is the rupturing of pollen grains during thunderstorms by means of osmotic shock from electrical charging and thunder (Suphioglu et al. 1992; Taylor et al. 2007). Starch can be expulsed through a fracture or through an aperture in the pollen grain and this can occur on the ground or in mid-air (El-Ghazaly et al. 1996; Taylor and Jonsson 2004; Taylor et al. 2007). Pollen grains from wind-pollinated species in particular (e.g., birch, maize and other grasses) are prone to rupture due to their size or thin walls (exines). Some wind-pollinated grains lack pollen tubes, and are designed to rupture enabling genetic material to complete fertilization (Wodehouse 1935:351). Whether starch-rich pollen grains rupture or lose material through their apertures in mid-air or on the ground, their starch granules are released directly or recycled back into the atmosphere after deposition.

Most studies of airborne pollen starch focus on wind-pollinated species (e.g., Behrendt and Becker 2001; Burge and Rogers 2000; D'Amato 2002; D'Amato et al. 2002; D'Amato et al. 2007; Erpenbeck et al. 2005; Levetin and Van de Water 2001; Motta et al. 2006; Schäppi et al. 1997; Schäppi, Taylor, Pain et al. 1999; Schäppi Taylor, Staff et al. 1999; Suphioglu et al. 1992). Several studies, however, reveal that starch granules are also present in insect-pollinated pollen grains and can become airborne given the right atmospheric conditions. When pollen grains on anthers of many insect-pollinated plants are no longer viable, the pollen dries and their sticky surface lipids evaporate or desiccate. This dehydrated pollen is often liberated when the flower is shaken by touch or wind (Lewis and Vinay 1979). Foraging insects also can dislodge pollen from the anthers, thereby allowing it to become airborne (Solomon 1984). In particular, high winds allow these liberated pollen grains to enter the atmosphere where they can rupture and release starch (Lewis and Vinay 1979; Solomon 1984). Small numbers of insect-pollinated pollen grains have been recovered from air samples (Erkara 2008; Keynan et al. 1991; Lewis et al. 1983). In North America, for example, pollen from insect-pollinated species in the Liliaceae, Malvaceae, and Asteraceae families has been recovered from airborne samples under various environmental conditions (Lewis and Vinay 1979).

Natural starch rain from wind- and insect-pollinated pollen grains contributes to respiratory allergies (Behrendt and Becker 2001; Lewis and Vinay 1979; Schäppi et al. 1997; Taylor et al. 2007). Some of the most common allergen-related pollen grains are from genera in the grass and birch families. Among the well-cited examples are ryegrass (*Lolium perenne* L.) and birch (*Betula verrucosa* Roth.) with an average of 700 and 400 small (~0.6-2.5 μm) starch granules per pollen grain, respectively (Suphioglu et al. 1992; Schäppi et al. 1997; Schäppi, Taylor, Staff et al. 1999). Grote et al. (2000, 2003) provide TEM (transmission electron microscopy) micrographs of starch granules embedded within the cytoplasm of ryegrass and birch pollen, among other species. These data suggest that a great deal of pollen starch of the same general size as starch in storage organs could be released into the atmosphere when pollen grains rupture. This assertion is supported by the collection of over 100,000 starch granules per m^3 of air in Melbourne, Australia, during the grass pollination season (Schäppi, Taylor, Pain et al. 1999; Schäppi, Taylor, Staff et al. 1999).

Starch granules can also become airborne due to human activities. The use of latex gloves powdered with corn starch is the primary source of airborne starch granules found in hospitals and medical buildings (Dave et al. 1999; Newsom and M. Shaw 1997; Newsom and P. Shaw 1997; Swanson and Ramalingam 2002). Similarly, starch granules from flour are often airborne within bakeries and are attributed to a condition known as Baker's Asthma (Laurière et al. 2008). Studies investigating the air downwind of industrial plants housing flour have also found starch granules that originated from these buildings (Anto et al. 1993). One of the authors (McDonough) has observed this phenomenon, noting starch granules collecting on cars parked near feed mills. This observation is consistent with the occurrence of dust explosions in food-processing facilities when high concentrations of starch granules, suspended in the air, ignite when a heat source is present (Amyotte and Eckhoff 2010). Since the above literature review suggests that starch granules are airborne, three locations in Texas were sampled for airborne starch granules as part of ongoing archaeological and atmospheric science investigations.

Study Area

College Station (Figure 1) is located in the Post Oak Savannah of southeast Texas (Ecosystem Science and Management, Texas A&M University [ESSM, TAMU] 2005). Annual rainfall is 900-1000 mm (34.4 - 39.4 in), and occurs predominantly in the spring and autumn (ESSM, TAMU 2005). Post oak (*Quercus stellata* Wangenh.) and blackjack oak (*Q. marilandica* Münchh.) predominate, while juniper (*Juniperus*), elm (*Ulmus*), hackberry (*Celtis*), and hickory (*Carya*) are common. The grassland component is dominated by little bluestem grass (*Schizachyrium scoparium* (Michx.) Nash) (ESSM, TAMU 2005). Soils tend to be fine sandy or silt loam.

Fort Hood (Figure 1) is located within the Lampasas Cut Plain (juniper-oak savannah) in central Texas. This region is characterized by an average annual rainfall of 826 mm (32.5 in), most of which occurs in the late spring and early fall (Kibler 2004). Uplands are dominated by various species of oak (*Quercus*), juniper (*Juniperus*), and mesquite (*Prosopis*) while intermediate surfaces are composed of open grasslands dominated by little bluestem grass (Schizachyrium scoparium) and Indian grass (*Sorghastrum nutans* (L.) Nash) (Anderson et al. 2005; Kibler 2004). Soils tend to be clay loams although patches of sandy loam also occur.

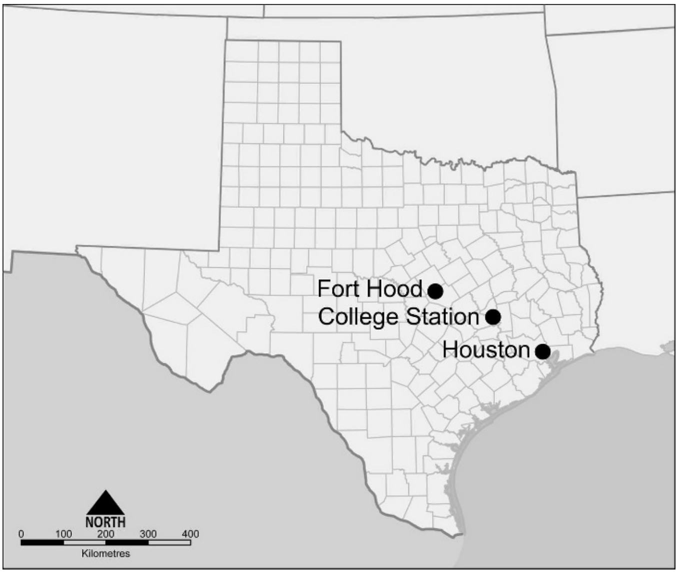

Figure 1. Sampling locations in Texas.

Houston (Figure 1) is located within the transition zone of the Coastal Plains and Piney-woods of Texas. The Coastal Plains, located along the Gulf of Mexico, are characterized as a natural grass prairie dominated with Indian grass, switchgrass (*Panicum virgatum* L.), and big bluestem grass (*Andropogon gerardii* Vitman). Hardwood trees, predominantly live oak (*Quercus virginiana* Mill.), grow along the rivers and streams (Multiple Land Resource Area Report [MLRA] 2006). The Piney-woods, located along the Texas-Louisiana border, is predominantly composed of pine and hardwood species. Pine species, particularly shortleaf (*Pinus echinata* Mill.), longleaf (*Pinus palustris* Mill.), and loblolly (*Pinus taeda* L.), dominate the uplands whereas hardwood species dominate the bottomlands (Texas Parks and Wildlife Department [TPWD] 2007). The methods used to collect air samples from each of these regions are described in the next section.

Materials and Methods

Collection of Air Samples

To assess the presence of airborne starch granules, several experiments were designed and conducted both outdoors and in laboratory settings. The first of these entailed analysis of a sample collected by a PIXE air-streaker sample, provided by the Department of Atmospheric Science at Texas A&M University. The sample was taken in a mixed land-use area north of downtown Houston, Texas.

The air sample taken by the PIXE air streaker was collected approximately 2 m above the surface of the ground. From March 21 through March 25, 2009, it sampled one liter of air every 25 minutes by capturing and then ''shooting'' air at an aluminum disk, which is used to collect air particles. Because the sample was intended to study only small atmospheric aerosols, a 12 µm filter prevented larger airborne particles, such as pollen grains, from entering. Upon completion of the sampling, the aluminum disk was removed using powder-free latex gloves and immediately sealed in a sterile plastic bag and was kept frozen until our analysis in the lab.

To remove atmospheric particles, the disk was placed in a 1000 ml beaker filled with distilled water and allowed to soak for five minutes. The water was then mixed by stirring the beaker with the disk still in it. The disk was rinsed with distilled water, and all of the water was centrifuged to concentrate the particles. The supernate (water and plant debris) was decanted, and the remaining material was mounted on a slide for analysis. Permount was used as the mounting medium to curate the slides as a permanent record. There remains disagreement among researchers as to whether Permount masks certain morphological characteristics of starch granules. Because our only objective of this study was to assess the potential for airborne starch contamination and not starch granule identification, we believe the use of Permount as a mounting medium was warranted.

Additionally, three experiments were conducted at or near the Texas A&M University's main campus in College Station to test the air for starch rain. One of these entailed collecting five air samples in sterile 9 cm petri dishes placed on an exterior window ledge on the third floor of the Anthropology Building, 8 m above ground level in November 2009. A small amount of distilled water was added to form a thin layer in the bottom of each dish, and the dishes were placed on the ledge for 2.25, 6, 22.5, 24, and 48 hours, respectively. When the petri dishes were collected, the water was pipetted into sterile 15 ml test tubes. Samples were centrifuged, the supernate decanted, and the remaining material was placed directly on slides for analysis using Permount as the mounting medium.

Another sample was collected in the College Station area from a local city park in November 2009. Here a sterile tub (31.4 cm × 39 cm) containing enough distilled water to cover the bottom was placed on a table 1 m above the ground and left undisturbed for 48 hours. In another experiment, a similar sterilized tub with distilled water was left for one hour beside a well-travelled inter-campus road lined with live oak trees. Mature acorns, many of which were well-crushed, covered portions of the road and adjacent sidewalk. From both tubs, the water and deposited materials were concentrated into sterile 50 ml test tubes, which were centrifuged, and then decanted. The remaining material was mounted on slides for analysis with Permount.

Finally, air samples were also taken from a grassland setting and a wooded area at Fort Hood, located near Killeen, Texas. The air at Fort Hood was tested in June 2010 by leaving five sterile 9 cm petri dishes each with a thin layer of distilled water at ground level for 2, 7, 8 (2 samples), and 8.5 hours. Three of the air samples were taken in a grassland area and two of the samples were taken in a wooded area. They were collected and processed in the same way as the College Station air samples.

Collection of Starch in Pollen

Pollen grains processed for starch came from *Zea mays* (domesticated maize) and seven wild Texas geophyte species: *Claytonia virginica* L. (eastern spring-beauty), *Callirhoe involucrata* (winecup), *Cooperia drummondii* Herbert (rain lily), *Liatris mucronata* DC. (narrow-leaf gay feather), *Hypoxis hirsuta* (L.) Coville (yellow star-grass), *Nothoscordum bivalve* (false garlic), and *Habranthus tubispathus* (L'Hér.) Traub (copper lily). Among the wild species, *Callirhoe involucrate*, *Claytonia virginica*, *Cooperia drummondii*, the genus *Liatris* and *Nothoscordum bivalve* have been identified archaeologically or are known ethnographically to have been utilized as food by Native American populations in Texas and the vicinity (Havard 1895; Moerman 1998:304-305; Thoms 2008). Except for *Liatris mucronata,* which was collected at Fort Hood, Texas, the rest of the geophyte plant samples were collected near College Station, Texas (Figure 1). Pollen from maize was collected when the plant was nearing maturation from a field in the Blackland Prairie midway between College Station and Fort Hood.

Laboratory Space

Two laboratories in the Department of Anthropology at Texas A&M University were used in these experiments. Both labs were tested for airborne contamination as were materials used during various stages of starch granule analysis. To test for possible starch contamination, sterilized petri dishes with a thin layer of distilled water were placed in both labs for two consecutive periods of 48 hours. The water was pipetted out of the petri dishes into 15 ml test tubes and centrifuged to concentrate any trapped material. After the supernate was decanted, the remaining material was mounted on a slide with Permount for analysis.

Laboratory supplies tested for starch granules included unused sterile 15 ml and 50 ml test tubes, microscope slides, coverslips, zinc bromide, 400 ml and 1000 ml beakers, pipettes, sonicating toothbrush heads, powder-free gloves, one-dram vials, Permount, and distilled water. Slides and coverslips from open and unopened boxes were examined for starch using a polarized-light microscope. Once it was determined that the distilled water was free of starch, it was used in testing microscope slides and coverslips.

To assess whether starch granules trapped in human hair might be a source of contamination, a few strands of hair from two volunteers and from the author (Laurence) who conducted this portion of the experiment were rinsed with distilled water and centrifuged to concentrate any particles attached to the hair. The supernatant was decanted, and all remaining material was mounted on a slide for analysis using Permount. Given that many hair products, including conditioners and hair gel, use starch as a thickening agent, samples of shampoos and conditioners used by the individuals were placed, without mounting medium, directly on slides and analyzed.

Processing of Pollen Samples for Starch

Sonication was used to rupture the pollen from each species. This method consisted of removing the anthers or stamen of each flowering plant and placing these parts in a 15 ml test tube with 6 ml of distilled water. Each test tube was vortexed to release pollen from the anthers. The stamen and anthers were removed from the sample, and the pollen was sonicated from 30 to 120 seconds in a Delta 5 Sonicator, to ensure the pollen of each species ruptured sufficiently. The anthers were not tested to determine if starch was adhering to their surfaces, which could potentially contribute starch to the samples. The test tubes were then centrifuged for three minutes at 2300 RPM to concentrate the material, and then the supernate was decanted. Because most researchers agree that all morphological characteristics of starch are visible when mounted in water, all remaining material was mounted in water on slides for analysis. In many cases, multiple attempts with the sonicator were required before the pollen would rupture. To maximize the chance of rupturing the pollen, hundreds of pollen grains per sample were sonicated. Finally, starch has been reported to survive the acetolysis procedure, a technique used in pollen studies to remove cellulose (Shogren and Biswas 2006; Tomasik and Schilling 2004). To assess the validity of this, stamens of a false garlic flower were processed using acetolysis for 10 minutes at a temperature of 80° C, which has been deemed safe for recovery of both starch and pollen (Shogren and Biswas 2006; Tomasik and Schilling 2004).

Microscopic Analysis

Mounted slides were observed under polarized, quarter-wavelength, brightfield illumination (i.e., light microscopy [LM]) using a Nikon Optishot petrographic microscope. A few mounted samples were observed under differential interference contrast (DIC) using an Olympus BX51 microscope. Starch granules recovered from the pollen of each species were compared to the starch reference collection from storage organs of each species on file at the Texas A&M University Archaeological-Ecology Laboratory. As such, pollen samples were derived from one set of plants, while another set of the same species from the same localities was used to create the storage-organ starch reference collection. Two sets of starch reference samples from each species are on file – one set is mounted in water and the other in Permount. All comparisons between starch recovered from pollen and the storage organs of each species were made with slides mounted in water. Reference slides mounted in Permount were made for curation purposes. All polarized and brightfield micrographs of pollen and starch were taken at 400× magnification, where as those under DIC were taken at 800× magnification with oil immersion.

Several samples were also observed under a scanning electron microscope (SEM). They were prepared by either smearing starch from storage organs onto a carbon coated aluminum stub or placing a drop of material on a stub after sonication of pollen, which was then allowed to dehydrate. All samples were vapor-coated with iodine-potassium-iodide (IKI) solution so as to allow better observation of starch with backscatter electrons (BSE). Importantly, IKI solution only reacts with and coats starch granules (Gott et al. 2006). After the samples were vapor-coated with IKI, they were coated with carbon to prevent charging of the samples during SEM observation. Observing starch granules under BSE confirms that the observed objects are starch granules, since the higher atomic mass of iodine, from the IKI solution, relative to the carbon background produces more backscatter electrons, thereby making the starch granules appear white against a black background (Petersen et al. 1983).

Results

Air Samples

All air sampling devices from Houston, College Station, and Fort Hood yielded starch granules (Table 1). They also captured pollen grains but only

Table 1. Number of starch granules, clusters, and micro-material found in each air sample.

Sample	Number of isolated starch granules	Number of starch granule clusters	Number of plant micro-materials with starch	Total number of starch granules	Time (hr.) exposed
Houston	5	0	0	5	96
Col. Sta. Window 1	8	0	1	9	2.25
Col. Sta. Window 2	17	1	1	19	22.5
Col. Sta. Window 3	4	0	0	4	24
Col. Sta. Window 4	16	0	0	16	48.5
Col. Sta. Window 5	20	0	0	20	6
Col. Sta. Park	21	1	0	22	48
Col. Sta. Roadside	> 1,000	0	0	> 1,000	1
Fort Hood Sample 1	0	0	0	0	2
Fort Hood Sample 2	3	0	0	3	8.5
Fort Hood Sample 3	1	0	0	1	7
Fort Hood Sample 4	> 100	0	0	> 100	8
Fort Hood Sample 5	2	0	0	2	8

starch data are reported here. Three categories of starch granules were identified: (1) isolated starch granules, (2) clusters of starch granules, and (3) starch granules packed within plant material. Isolated grains occurred most commonly (Figure 2).

Laboratory Space and Supplies

Analysis of petri dishes confirmed the presence of airborne starch granules in both labs, but none of the tested laboratory supplies, including the powder-free latex gloves, yielded starch granules. After 48 hours, the air sample in one lab contained 9 starch granules and the other lab's sample contained 7 granules, while after the second 48-hour period, 2 and 6 starch granules respectively were captured at these laboratories. Hair samples from all three individuals yielded starch granules. The first author's (Laurence) hair yielded 18 starch granules while the hair of volunteers' 1 and 2 hair yielded 30 and 12 starch granules, respectively. Starch granules representing several different types of grains were recovered from each person's hair. Of particular interest was the presence of damaged starch granules in two hair samples. Upon determining that hair acts as a ''starch trap,'' one of the authors (Laurence) stood over a sterilized 1000 ml beaker and ran his hand through his hair a few times to determine if his hair could be a source of contamination in the lab. An immediate examination revealed 22 isolated starch granules from different types of species and 11 clusters of starch granules in the beaker. Of the three individuals tested for starch granules in their hair, the conditioner used by volunteer one was the only hair product to contain starch granules.

Starch Encased in Pollen

Pollen grains from all seven species yielded starch granules, including a sample of false garlic pollen that was processed using acetolysis (Figure 3). In several cases, starch granules were observed within the pollen itself. They were determined to be inside the pollen grains (as opposed to above or below the

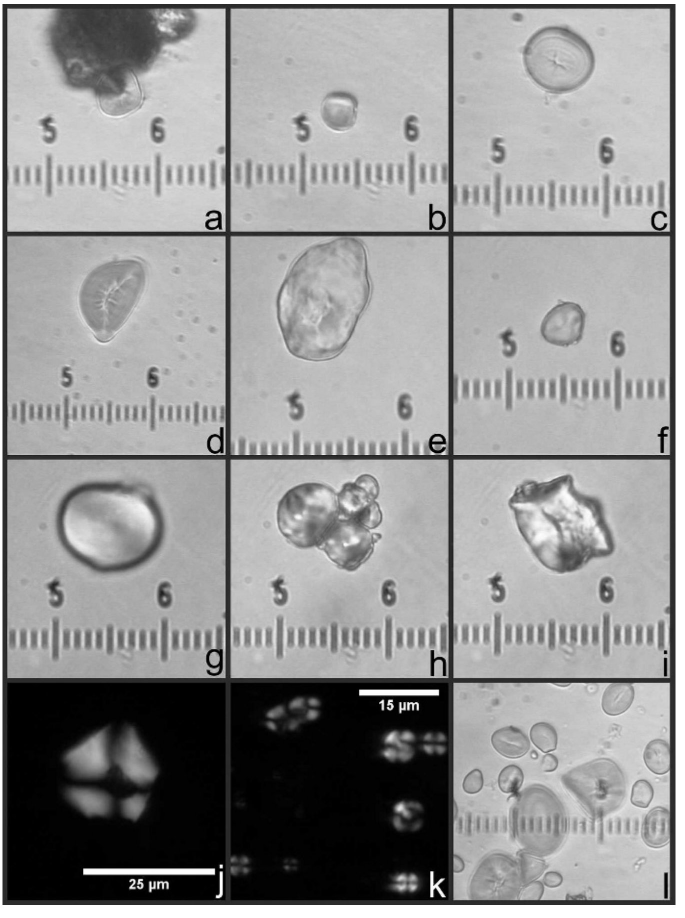

Figure 2. (a–b) Airborne starches from the PIXE air streaker, 2 m above ground in Houston, (c) petri dishes placed on a third story window ledge in College Station 8 m above the ground for 2 hours and 15 minutes, (d) 6 hours, (e) 24 hours, and (f) 48 hours, (g–i) a plastic tub 1 m above ground at a College Station park, (j–k) petri dishes at ground level at Fort Hood, and (l) a College Station road. Note the cluster of starch granules in micrograph h. Micrographs a–i and l are under brightfield illumination while micrographs j–k are under cross-polarized light. In micrographs a–i and l, each tick mark in the scale represents 2.5 μm.

pollen grains on the slide) because they rotated around the pollen grains' central axis.

Comparisons between starch granules recovered from the pollen grains and storage-organs measured considerable overlap in size in all cases. As shown by both the LM and SEM micrographs (Figures 3 and 4), morphologies of starch granules recovered from the pollen and storage organs were also very similar (Table 2). Significant variation in size and morphology noted in all species is consistent with the observations made by Delcour and Hoseney (2010:23–25), Hoseney (1994:40), Kent (1975), McDonough et al. (2000), Rooney and Suhendro (2001), and Serna-Saldivar (2010:109).

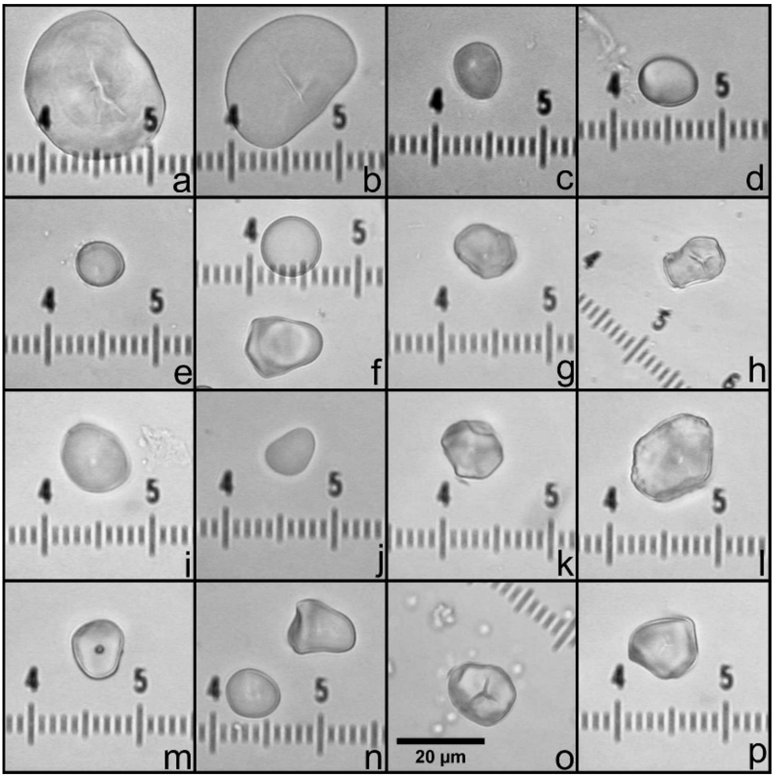

Figure 3. Starch granules from: (a) *Nothoscordum bivalve* pollen, (b) *Nothoscordum bivalve* bulb, (c) *Habranthus tubispathus* pollen, (d) *Habranthus tubispathus* bulb, (e) *Claytonia virginica* pollen, (f) *Claytonia virginica* bulb, (g) *Liatris mucronata* pollen, (h) *Liatris mucronata* rhizome, (i) *Cooperia drummondii* pollen, (j) *Cooperia drummondii* bulb, (k) *Callirhoe involucrata* pollen, (l) *Callirhoe involucrata* taproot, (m) *Hypoxis hirsuta* pollen, (n) *Hypoxis hirsute*, (o) *Zea mays* pollen, and (p) *Zea mays* seed. Note that the *Zea mays* starch granule in o is partially covered by a pollen grain. Each tick mark is 2.5 µm.

A total of 100 starch granules from maize seeds and rain lily roots were counted and grouped according to size as were 100 starch granules recovered from pollen grains of both species (Figure 5). Rain lily and maize were chosen for this analysis for two reasons. First, maize represents a wind-pollinated species while rain lily represents an insect-pollinated species. Second, we were able to consistently rupture the pollen grains from both species in high frequencies, providing a representative sample of the size range of starch granules embedded in the pollen grains. Regarding the percentage of starch granules in each size class, there was considerable overlap in each species (Figure 5). Of the maize starch granules, 52% from the storage organs and 83% from pollen are 15 µm or smaller. Similarly, 60% of the starch granules from the rain lily bulb and 100% of the starch granules from pollen grains were 10 µm or smaller. Although larger rain lily grains did not show up in the first 100 count, several grains up to 20 µm in size were observed elsewhere on the slide. Both maize and rain lily had proportionally larger starch granules in their storage organs than in their pollen

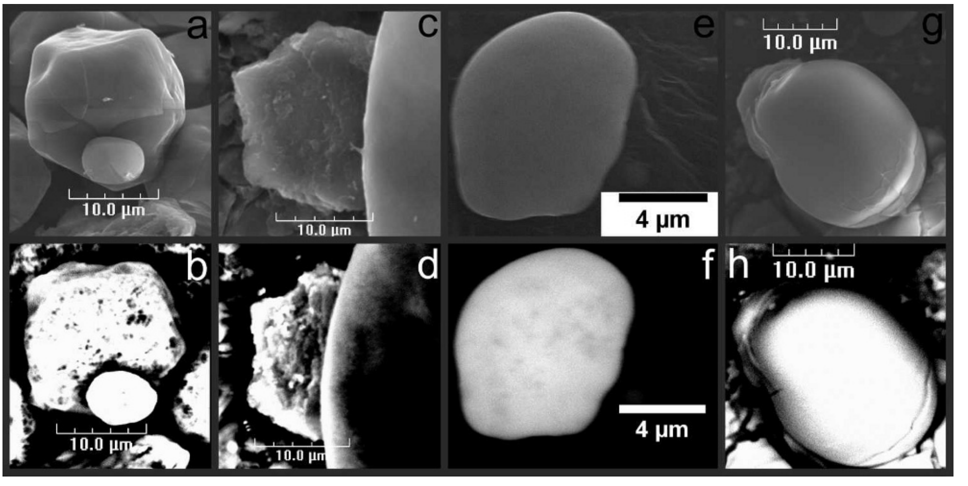

Figure 4. SEM micrographs of starch from: (a) *Zea mays* kernel under SE (secondary electrons), (b) *Zea mays* kernel under BSE (backscatter electrons), (c) *Zea mays* pollen under SE, (d) *Zea mays* pollen under BSE, (e) *Nothoscordum bivalve* bulb under SE, (f) *Nothoscordum bivalve* bulb under BSE, (g) *Nothoscordum bivalve* pollen under SE, and (h) *Nothoscordum bivalve* pollen under BSE. Note that the starch granule in micrographs a and b is overlain by a smaller starch granule and the *Zea mays* starch granule in c and d is partly covered by a pollen grain. The white edge of the pollen grain under BSE in d is due to the edge-effect (Kotera et al. 1990).

grains, but there was still substantial overlap in the size and morphology of starch granules between both species' pollen grains and storage organs (Figures 6 and 7).

Discussion

We note that our sample of plants' storage organs is small (n=8) and that our observations may not apply to all starch-rich taxa. Furthermore, we did not recover starch granules from pollen grains in the high densities reported by Suphioglu et al. (1992), Schäppi et al. (1997), and Schäppi, Taylor, Staff et al. (1999). Starch granules recovered from our samples, however, were larger than those reported from ryegrass and birch. Nonetheless, given that eight genera and five families are represented, and all analyzed species yielded storage-like starch granules in their pollen, we believe that two significant conclusions can be made from our experiments.

First, we report that storage-like starch granules are present in pollen grains of the investigated wind and insect-pollinated species. In many cases the starch granules in the pollen are virtually indistinguishable from those in respective storage organs. Second, we detected storage-like starch granules in the atmosphere that originate from natural and human activities. Therefore, an important finding of our research is that the mere presence of starch on an artifact cannot always be assumed to be a result of plant utilization. Barton (2007) also argued that the mere presence of organics on a tool is not enough to associate the organics with human actions. He suggested that only by understanding the

Table 2. Descriptions of starch granules found in storage organs and pollen grains following the terminology used by Gott et al. (2006).

Species	Description of Starch in Storage Organ	Description of Starch in Pollen
Claytonia virginica	elongated to polyhedral shaped, presence of fissure radiating out of hilum, eccentric hilum, visible lamellae (visible under brightfield illumination)	elongated to polyhedral shaped, presence of fissure radiating out of hilum, eccentric hilum, visible lamellae
Callirhoe involucrata	elongated to polyhedral shaped granules, y-shaped fissures, eccentric hilum, visible lamellae	elongated to polyhedral shaped granules, y-shaped fissures, eccentric hilum, visible lamellae
Cooperia drummondii	elongated, polyhedral, lenticular and kidney-shaped granules, eccentric hilum, visible lamellae, compound and single granules (single granules are dominate)	elongated, polyhedral, lenticular and kidney-shaped granules, eccentric hilum, visible lamellae, compound and single granules (single granules are dominant)
Liatris mucronata	polyhedral shaped, visible hilum, eccentric hilum, y-shaped to single fissures, compound and single granules (single granules are dominant)	polyhedral shaped, visible hilum, eccentric hilum, y-shaped and single fissures
Hypoxis hirsuta	visible hilum, eccentric hilum, presence of fissures, elongated and polyhedral shaped, compound and single granules (single granules are dominant)	visible hilum, eccentric hilum presence of fissures, elongated and polyhedral shaped, compound and single granules (single granules are dominant)
Nothoscordum bivalve	visible lamellae, lenticular and kidney-shaped, visible hilum, eccentric hilum, various shapes of fissures	visible lamellae, lenticular and kidney-shaped, visible hilum, eccentric hilum, various shapes of fissures
Habranthus tubispathus	compound and single granules (single granules are dominant), elongated, lenticular, and kidney-shaped grains, visible hilum, x-shaped to single fissures, visible lamellae	compound and single grains (single granules are dominant), elongated, lenticular, and kidney-shaped granules, visible hilum, x-shaped to single fissures, visible lamellae
Zea mays	round, ovoid, and polyhedral shaped granules, visible hilum, centric to eccentric hilum, y-shaped fissures, visible lamellae (although very rare)	round and polyhedral shaped granules, visible hilum, centric to eccentric hilum, y-shaped fissures, visible lamellae (although very rare)

context of a tool can one interpret the organic material on that artifact. Organic residue studies should be used in conjunction with use-wear analysis and interpreted within an archaeological framework so that meaning can be derived from the results (Fullagar et al. 1996).

Barton et al. (1998) mentioned the potential for starch contamination in sediment containing artifacts. They argued that comparisons between starch granules recovered from used and unused surfaces on artifacts afford the analyst an indication as to the amount of potential starch contamination at a site. If the same types of starch granules are recovered from both used and unused surfaces then it is likely that starch granules are coming from contamination sources

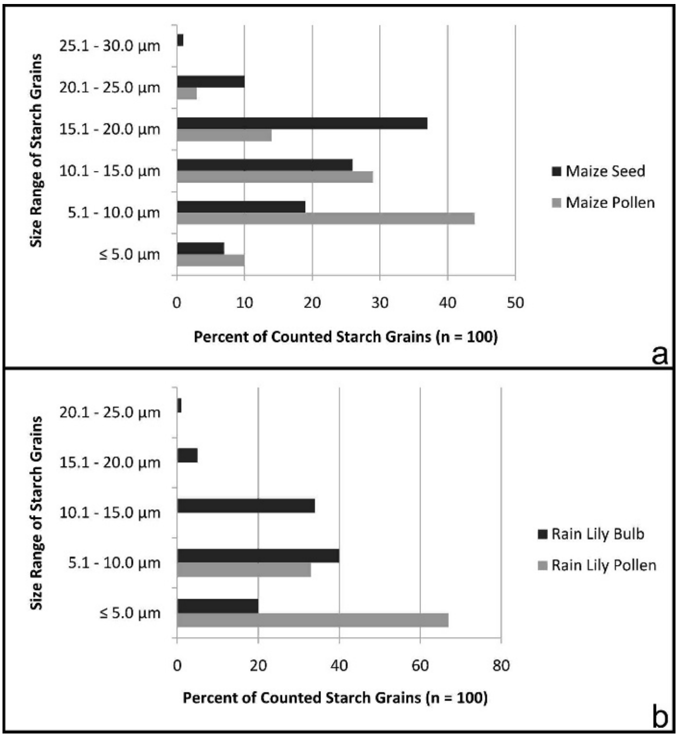

Figure 5. Comparison of size ranges between starch granules found in pollen grains and storage organs for (a) maize and (b) rain lily.

rather than from utilization. If, however, there are significant differences between the types of starch granules recovered from each surface, then the starch on the used surfaces probably resulted from utilization (Barton et al. 1998). However, we note here that starch granules become airborne as a result of plant processing (Laurière et al. 2008). Accordingly, it is likely that starch granules from processed plants could accumulate on the unused surfaces of artifacts. Quantitative analysis, in conjunction with use-wear analysis, may be able to determine if the starch on the unused surface is the result of starch granules liberated by the processed foods in the past. If so, higher concentrations should be found on the used surface. If concentrations are similar on various surfaces, then contamination may be from the environment at large. This principle can also be applied to airborne starch contamination at a site. Accordingly, if the same types of starch granules are recovered from many or all artifacts processed from different locations at a site, regardless of tool class, then the starch granules may come from contamination rather than use. Zarrillo and Kooyman (2006) found little or no starch when they tested for natural starch in the sediments at an archaeological site in Canada. They also tested one near-surface rock but found little or no starch. They attributed the absence to the actions of soil microbes, which are the primary consumers of natural starch (Haslam 2004; Zarrillo and Kooyman 2006).

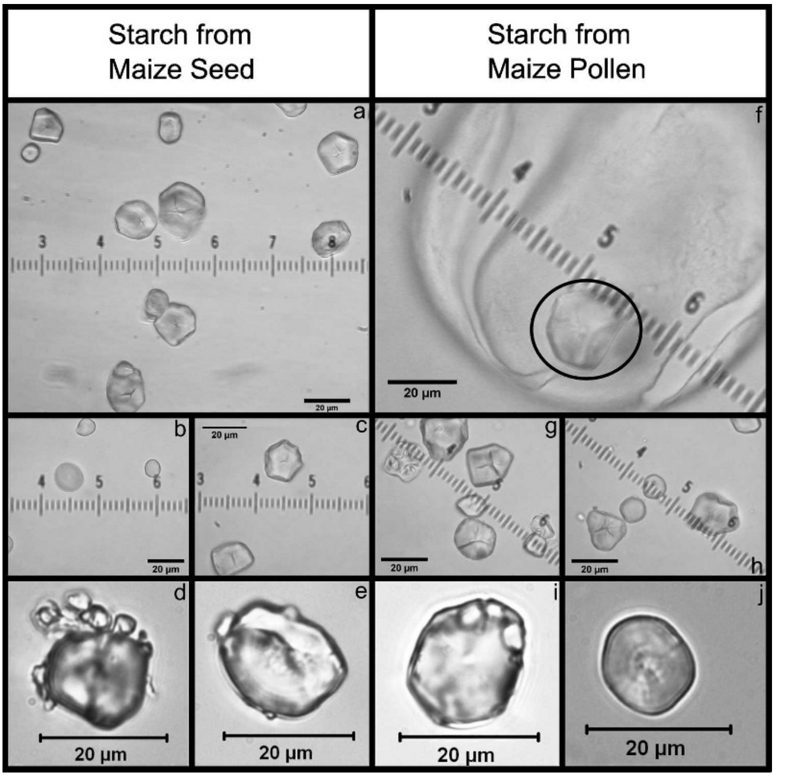

Figure 6. Starch granules from (a–e) maize seeds and (f–h) maize pollen grains. Micrographs a–c and f–h were taken under brightfield illumination at 400× magnification while micrographs d–e and i–j are under DIC at 800× magnification. Note the presence of visible lamellae and y-shaped fissures in starch granules from both maize seeds and pollen grains under DIC. The starch granule in f is embedded in a pollen grain.

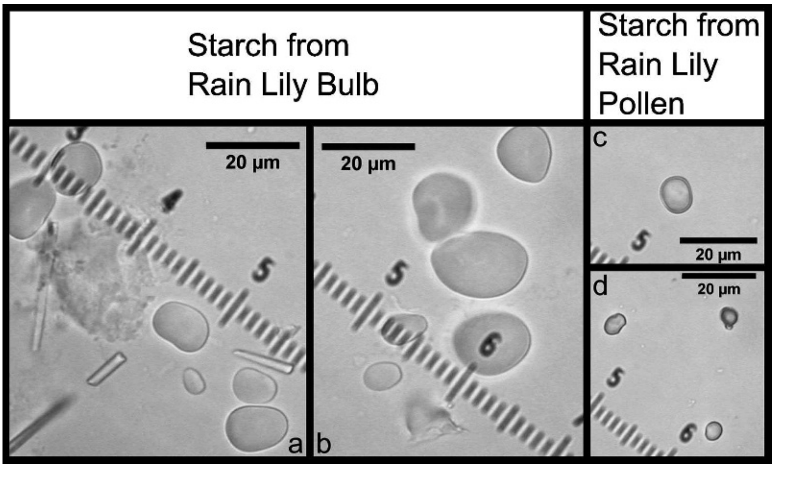

Figure 7. Rain lily starch granules from (a–b) bulbs and (c–d) pollen grains. Micrographs were taken under brightfield illumination at 400× magnification.

Based on the proven presence and potential for airborne starch contamination, we recommend the following protocol for the study of starch granules from archaeological contexts. Air samples should be taken during excavations to understand and control for modern starch rain that could affect the interpretation of the archaeological record at a given site. If commercial air sampling devices are not available, air samples can be taken by installing sterilized sealable containers with distilled water and leaving them exposed to the atmosphere during excavation. These air samples should then be analyzed in the laboratory with care to avoid post-recovery contamination. An understanding of local pollen records is also advisable.

There is also a need for further research. Given that some archaeological samples are known to have been contaminated by the starch of modern domesticates, the extent to which industrial sources such as mills and grain silos can contaminate archaeological samples also needs to be addressed (Barton and Matthews 2006; Loy and Barton 2006; Williamson 2006). Studies also need to be conducted to determine how far starch granules potentially travel once airborne.

If starch granules accumulate on artifacts and in features by means of starch rain, then starch granules from non-utilitarian species should also be recovered. Quantitative analysis is likely to be useful in determining which starch granules are the results of human behavior and which starch granules are the results of airborne contamination, given that there are higher concentrations of starch granules in individual storage organs than there are in pollen grains. Other things being equal and provided adequate preservation, the dominant starch granules on a given tool are most likely to result from use. Furthermore, comparatively high concentrations of modified and damaged starch granules on artifacts may suggest past human activity, although damaged starch granules have also been reported in regional air samples, in our labs, and in tests from the lab used by Nugent (2006). Non-cultural rocks from the site should also be tested, and the recovered starch assemblage should be compared to that recovered from artifacts (Barton et al. 1998; Zarrillo and Kooyman 2006).

Finally, steps need to be taken to reduce contamination in the lab. Artifacts should be gently washed or rinsed with distilled water prior to processing. This will remove loosely adhering starch granules on an artifact's surface where contamination is most likely to accumulate. Experiments conducted at the Archaeological-Ecology Lab, Texas A&M University, demonstrate that microcracks in the surfaces of artifacts provide protected settings for starch granules, even if the artifacts are washed. This technique is particularly useful for removing modern contamination if artifacts were collected in the field without using protocols to control for contamination. Analysts should also wear protective surgical-type caps to prevent contamination from their hair. As a further safeguard, starch removal and processing should be conducted on a clean-bench with a 1 μm air filter to prevent possible contamination from the lab. Artifacts should never be taken out of their sealed plastic bags and exposed to the atmosphere unless they are under the protected setting of a clean-bench or a similar device that removes or limits airborne contamination.

Conclusion

The present study provides confirmation that starch granules can become airborne, and reveals that starch granules from pollen may be indistinguishable from starch granules from storage organs. As starch-rich pollen grains rupture and starch-rich plants decompose, granules are released into the atmosphere and can be recycled from the ground surface by winds, creating a natural starch rain. Similarly, starch granules can be released into the atmosphere through human activities. Artifacts that were and are exposed to the atmosphere can collect natural and human-induced starch rain. This process clearly demonstrates how the accumulation of starch granules on artifacts and in features might occur even though the item never came into contact with starch-rich plants during their use-lives. Recognizing the presence of starch rain is only the first step. Future studies are required to understand the nature and distribution of starch rain and the impact starch rains could have on archaeological interpretations. With a greater understanding of starch rain, we will be better positioned to control for contamination of archaeological specimens that otherwise may lead to inaccurate interpretations of ancient food use.

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