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Pilot Study

# Detection of Pollen Adherence on Different Fabrics Using Digital Microscopy: A Pilot Study with Bougainvillea Glabra and Parthenium Hysterophorus

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**ABSTRACT**

**Background:** Pollen transferred to clothing can link a person or object to an outdoor scene, but its evidential value depends on how well different fabrics retain grains after disturbance.
**Objective:** To document the adherence of pollen from Bougainvillea glabra and Parthenium hysterophorus on four commonly used fabrics (cotton, terry-cotton, crepe and georgette) and to describe the fraction retained after mechanical disturbance by vortexing.
**Methods:** Fabric squares (5 × 5 cm) were imaged with a Miscope digital microscope (Zarbeco LLC; 100×) before and after pollen application. Pollen was dusted directly from flowers onto the fabrics, and grains were counted manually, segment by segment. The samples were then vortexed (5 min, 2800 rpm) and recounted under identical settings. Three replicates were used per species–fabric combination. Retention was expressed as the percentage of grains remaining after vortexing, with exact (Clopper–Pearson) 95% confidence intervals.
**Results:** A total of 163 grains were counted before vortexing (57 B. glabra; 106 P. hysterophorus). Overall retention was 68.4% (95% CI 54.8–80.1) for B. glabra and 76.4% (95% CI 67.2–84.1) for P. hysterophorus. For B. glabra, retention was 100% on crepe and georgette (n = 8 and 4 grains, respectively), 64.1% on terry-cotton and 33.3% on cotton. For P. hysterophorus, retention was highest on cotton (92.6%), followed by georgette (81.8%), terry-cotton (76.3%) and crepe (60.0%). Terry-cotton carried the most B. glabra grains before vortexing (39).
**Conclusions:** In this pilot study, the ranking of fabrics by retention differed between the two species, which suggests, as a hypothesis for further testing, that pollen type and fabric properties interact. The small counts and the absence of replicate-level data preclude formal statistical comparison.

**Keywords:** Forensic Palynology, Pollen Retention, Trace Evidence, Fabrics

## INTRODUCTION

Pollen grains represent the male gametophytic stage of seed plants and possess distinctive structural characteristics that facilitate their identification and survival under varying environmental conditions. Each pollen grain consists of an outer exine, primarily composed of sporopollenin, and an inner intine containing cellulose and pectic substances. Variations in pollen size, shape, surface ornamentation and structural features provide important taxonomic information and make pollen useful in forensic investigations. [1] Palynology is the scientific study of pollen grains, spores and other microscopic organic particles. In forensic science, it has emerged as a valuable tool for establishing associations between individuals, objects and geographical locations.

Based on Locard's exchange principle, microscopic materials, including pollen, may be transferred whenever two surfaces come into contact. Owing to their environmental distribution, morphological diversity and ability to persist on different substrates, pollen grains can provide useful trace evidence for reconstructing events and investigating potential links between crime scenes and individuals. [2,3] The application of forensic palynology has been demonstrated in several criminal investigations, including murder, sexual assault and burglary cases. In the Baby Doe investigation, pollen recovered from clothing and a blanket provided geographical information that assisted investigators in narrowing down the possible location associated with the case. [4] Similarly, the presence of substantial quantities of Hypericum pollen on clothing was interpreted as evidence of direct contact with vegetation rather than incidental airborne deposition in a burglary investigation. [5] In another criminal investigation, the combined identification of Coprosma pollen and fungal spores helped establish a connection with a suspected crime scene. [6] Pollen analysis has also been used to examine the reliability of suspects' alibis by comparing trace botanical evidence with the environmental characteristics of particular locations. [7] The reliability of forensic pollen examination depends considerably on appropriate sample collection, preservation, laboratory processing and identification. Various sampling techniques have been described, including vacuum collection, scraping, brushing, scrubbing and adhesive tape lifting from clothing, footwear, vehicles and human remains. [8] Adhesive tape lifting is particularly useful for textile examination because it allows direct sampling of selected fabric surfaces and facilitates the recovery of microscopic particles. [9] Compared with vacuum-based collection, tape lifting can provide effective contact with the sampled substrate, making it a practical approach for recovering trace pollen from clothing and other materials. [10]

Previous investigations have demonstrated that pollen retention varies according to fabric composition, surface characteristics, environmental exposure and mechanical disturbance. Bull et al. examined the transfer and persistence of trace particulates on different clothing materials, highlighting the importance of substrate characteristics in forensic evidence recovery. [11] Rowell reported that pollen persistence was influenced by environmental contact and subsequent washing conditions. [12] Boi investigated pollen attachment to commonly encountered materials, including paper, cotton, polyester and denim, demonstrating differences in particle retention among substrates. [13] Furthermore, Webb et al. established that the intensity of physical wear significantly influenced the quantity of pollen retained on clothing and evaluated the effectiveness of different laboratory retrieval procedures. [14] Research involving Typha angustifolia pollen also demonstrated variation in adhesion across textile materials, with polyester showing greater pollen retention than cotton under the investigated experimental conditions. [15] Additional studies have confirmed that pollen may remain detectable on human skin following handwashing and that plant-derived pollen DNA can persist on cotton fabrics, supporting the potential value of pollen evidence in forensic investigations. [16,17] Although conventional microscopic examination remains important for pollen identification, advanced analytical approaches such as scanning electron microscopy and molecular DNA-based techniques can provide additional morphological and genetic information. However, these methods may involve higher operational costs, specialised equipment and considerable laboratory expertise. [18] Consequently, digital microscopy represents a potentially accessible approach for preliminary examination, documentation and comparative assessment of pollen grains recovered from textile surfaces. Two plant species commonly encountered in Indian outdoor environments were selected for the present investigation: Parthenium hysterophorus L. and Bougainvillea glabra Choisy. Parthenium hysterophorus, commonly known as carrot grass, is an invasive member of the family Asteraceae. It produces small flower heads containing relatively small, wind-dispersed pollen grains characterised by echinate or spiny surface ornamentation. Previous studies have reported pollen dimensions of approximately 14.5–17.5 µm, making its morphological characteristics relevant for microscopic examination. [19,20] Bougainvillea glabra, belonging to the family Nyctaginaceae, is a widely cultivated ornamental plant commonly found in tropical and subtropical environments. Its pollen grains are comparatively larger, generally tricolpate, and have been reported to exhibit variable pollen viability and fertility characteristics. [21–23] These differences in pollen morphology provide an opportunity to investigate whether pollen characteristics influence attachment and retention on different textile substrates.

## MATERIALS AND METHODS

**Study design:** This was a laboratory pilot study with

a qualitative component (microscopic documentation) and a descriptive quantitative component (manual counting). Two pollen species were applied to four fabric types, with three replicates per species–fabric combination. Each sample was imaged before and after vortexing. All experiments were carried out at Kristu Jayanti, Bengaluru, India.

**Plant material and fabrics:** Plants were photographed in situ before collection to record their condition and growth setting (isolated and flower-dense areas). Ten to fifteen flowers of each species (B. glabra, purple; P. hysterophorus, white) were collected in an open field at North Kothanur, Bengaluru, Karnataka, India, between 16 and 20 January 2024, using scissors, gloves, masks and paper bags to limit contamination and exposure. The species were identified using standard floras. Flowers were stored individually in separate druggist-folded envelopes to prevent cross-contamination. Cotton (yellow), terry-cotton, georgette and crepe fabrics were purchased from commercial shops and cut into 5 × 5 cm squares for standardisation.

**Baseline imaging and pollen application:** Before pollen application, each fabric square was imaged with a digital microscope to establish a baseline and to confirm the absence of pre-existing particles that could be confused with pollen. Flowers of each species were then gently dusted onto the fabric surface to ensure direct contact between pollen and fabric, using the same procedure for all samples.

**Microscopy and counting:** Samples were examined with a Miscope digital microscope (Zarbeco LLC) at 100×. The instrument was moved systematically from one edge of the fabric to the other in an up-and-down pattern across five segments of each fabric square, and an image was captured for each segment. Pollen grains were counted manually from the images, segment by segment.

**Mechanical disturbance:** After the first imaging, each sample was vortexed for 5 min at 2800 rpm (vortex shaker, 220–240 V AC, 50 Hz) to impose a defined mechanical disturbance. The same samples were then re-imaged with identical microscope settings, and the remaining grains were counted in the same five segments.

**Data analysis:** For each species–fabric combination, counts before and after vortexing were totalled over the five segments and the replicates. Retention (%) was calculated as (grains after ÷ grains before) × 100, and exact Clopper–Pearson 95% confidence intervals were computed for each proportion in Python (SciPy). These intervals treat grains as independent trials, whereas grains on the same fabric piece are clustered; they are therefore descriptive and probably narrower than the true uncertainty. No hypothesis tests comparing fabrics or species were performed, because the pooled counts do not allow the replicate-level variance to be estimated and several cells contain very few grains.

## RESULT

A total of 163 pollen grains were recorded across the four textile materials before vortexing, comprising 57 grains of Bougainvillea glabra and 106 grains of Parthenium hysterophorus (Table 1). Following vortexing at 2800 rpm for 5 minutes, 39 grains of B. glabra and 81 grains of P. hysterophorus remained on the respective fabrics. The overall retention percentages were 68.4% (95% CI: 54.8–80.1) for B. glabra and 76.4% (95% CI: 67.2–84.1) for P. hysterophorus. Although P. hysterophorus demonstrated a numerically higher retention percentage, the overlapping confidence intervals do not provide sufficient evidence of a difference between the two species.

**Table 1. Distribution and retention of B. glabra and P. hysterophorus pollen grains across different textile materials before and after vortexing (5 minutes at 2800 rpm).**

| Species | Fabric | Before | After | Retention (%) | 95 % CI (%) |
|---|---|---|---|---|---|
| B. glabra | Cotton | 6 | 2 | 33.3 | 4.3 – 77.7 |
| B. glabra | Terry-cotton | 39 | 25 | 64.1 | 47.2– 78.8 |
| B. glabra | Crepe | 8 | 8 | 100.0 | 63.1– 100 |
| B. glabra | Georgette | 4 | 4 | 100.0 | 39.8– 100 |
| B. glabra | All fabrics | 57 | 39 | 68.4 | 54.8– 80.1 |
| P. hysterophorus | Cotton | 27 | 25 | 92.6 | 75.7– 99.1 |

| | | | | | |
|---|---|---|---|---|---|
| P. hysterophorus | Terry-cotton | 38 | 29 | 76.3 | 59.8–88.6 |
| P. hysterophorus | Crepe | 30 | 18 | 60.0 | 40.6–77.3 |
| P. hysterophorus | Georgette | 11 | 9 | 81.8 | 48.2–97.7 |
| P. hysterophorus | All fabrics | 106 | 81 | 76.4 | 67.2–84.1 |

CI, confidence interval. Values represent pooled counts from five fabric segments and three replicates per species–fabric combination. Confidence intervals were calculated using the exact Clopper–Pearson method.

**Retention of Bougainvillea glabra pollen:** Among the four textile materials, terry-cotton exhibited the highest initial deposition of B. glabra pollen, accounting for 39 of the 57 recorded grains (68.4%). Comparatively, cotton, crepe and georgette showed substantially lower initial counts, with 6, 8 and 4 grains, respectively. Following vortexing, all eight grains deposited on crepe and all four grains on georgette remained detectable, corresponding to an observed retention of 100% for both materials. However, these estimates were based on relatively small initial counts. Terry-cotton retained 25 of 39 grains (64.1%; 95% CI: 47.2–78.8), whereas cotton exhibited the lowest retention, with only 2 of 6 grains remaining (33.3%; 95% CI: 4.3–77.7). Despite the complete retention observed on crepe and georgette, terry-cotton accounted for the largest absolute number of retained grains, representing 25 of the 39 grains recovered across all four fabrics.

**Retention of Parthenium hysterophorus pollen:** The initial distribution of P. hysterophorus pollen varied across the selected textile materials. Terry-cotton recorded the highest number of grains (38), followed by crepe (30), cotton (27) and georgette (11). After vortexing, cotton demonstrated the highest retention proportion, with 25 of 27 grains remaining (92.6%; 95% CI: 75.7–99.1). Georgette retained 9 of 11 grains (81.8%; 95% CI: 48.2–97.7), followed by terry-cotton with 29 of 38 grains (76.3%; 95% CI: 59.8–88.6). Crepe showed the lowest retention, with 18 of 30 grains remaining (60.0%; 95% CI: 40.6–77.3). The difference in retention between cotton and crepe was 32.6 percentage points. Although this finding indicates a potentially meaningful variation in pollen persistence between the two materials, the overlapping confidence intervals and limited sample counts do not establish a statistically significant difference.

**Comparative species-specific retention patterns:** A comparison of the two pollen species revealed contrasting retention patterns across the investigated textile materials (Table 1). Cotton exhibited the lowest retention of B. glabra pollen (33.3%) but the highest retention of P. hysterophorus pollen (92.6%). Conversely, crepe demonstrated complete retention of B. glabra pollen (100%), whereas its retention of P. hysterophorus pollen was comparatively lower (60.0%). Terry-cotton showed relatively consistent retention for both species, with values of 64.1% and 76.3%, respectively. Georgette demonstrated complete retention of the initially deposited B. glabra grains and 81.8% retention of P. hysterophorus grains. These observations indicate a possible relationship between pollen species and textile characteristics in determining particle persistence. Nevertheless, because of the limited grain counts and the absence of separately analysed replicate-level observations, the apparent species–fabric interaction should be interpreted as an exploratory finding requiring further investigation.

## DISCUSSION

**Principal findings:** The present experimental investigation demonstrated that pollen grains of both Bougainvillea glabra and Parthenium hysterophorus could be detected and documented on all four selected textile materials using digital microscopy. Following a standardised mechanical disturbance involving vortexing at 2800 rpm for five minutes, 68.4% of B. glabra grains and 76.4% of P. hysterophorus grains remained attached to the fabrics. These findings indicate that a considerable proportion of deposited pollen can persist on textile surfaces even after mechanical disturbance. Such persistence is relevant to forensic palynology, where the recovery of botanical trace materials from clothing and other personal objects may assist in establishing associations between individuals and particular environments. Previous investigations have similarly demonstrated that pollen and other microscopic particulates can remain associated with clothing under different environmental and physical conditions. [12,14]

**Influence of textile characteristics on pollen retention** One of the important observations was the variation in pollen retention among the four textile

materials. The retention pattern also differed between the two investigated plant species, suggesting that pollen persistence may depend on the combined characteristics of the pollen grains and the textile substrate. For B. glabra, terry-cotton demonstrated the greatest initial pollen deposition and the highest absolute number of retained grains. However, its proportional retention was lower than that observed on crepe and georgette. Cotton exhibited the lowest retention percentage for this species. This observation is broadly consistent with the findings of Vichitnark et al., who reported relatively low adhesion of Typha angustifolia pollen to cotton compared with other tested fabrics. [15] Nevertheless, differences in pollen species and experimental procedures should be considered when comparing these findings. P. hysterophorus exhibited its highest retention on cotton, while crepe demonstrated the lowest percentage. This contrasting behaviour suggests that the relationship between textile structure and pollen adherence may not be uniform across different plant species. Differences in pollen dimensions, surface ornamentation and interaction with individual textile fibres may contribute to these variations. The relatively smaller and echinate pollen grains of P. hysterophorus have been reported to measure approximately 14.5–17.5 µm, whereas B. glabra produces comparatively larger pollen grains, with reported dimensions ranging from approximately 26 to 50 µm. [19–21] It is possible that differences in grain dimensions and surface characteristics influence the extent to which pollen particles become lodged between fibres or remain attached to textile surfaces. However, these mechanisms were not directly investigated in the present study and should therefore be regarded as possible explanations rather than established findings. Another notable observation was the complete retention of B. glabra pollen on crepe and georgette. Although these results indicate that all initially detected grains remained following vortexing, the corresponding initial counts were only eight and four grains, respectively. Therefore, these percentages should not be interpreted as definitive evidence of superior retention. Initial pollen deposition and subsequent retention represent separate characteristics, and both should be considered when evaluating the forensic significance of a textile material.

**Forensic significance and methodological considerations:** The findings provide preliminary experimental evidence that textile composition may influence the persistence of pollen particles following mechanical disturbance. In forensic investigations, this variation may affect the quantity and composition of botanical trace evidence recovered from clothing, footwear and other fabric-based materials. The results also suggest that the absence or reduced recovery of a particular pollen type from a garment should not automatically be interpreted as evidence of a lack of environmental contact. Differences in textile structure and pollen characteristics may influence particle retention and subsequent recovery. Digital microscopy offered a relatively accessible and economical method for observing and documenting pollen grains directly on textile surfaces. Compared with advanced analytical techniques such as scanning electron microscopy and molecular identification, digital microscopy may provide a practical preliminary approach for examining pollen distribution and conducting basic comparative assessments. [18] However, its identification capabilities are dependent on image resolution, morphological clarity and the observer's expertise. Although the present findings demonstrate the feasibility of digital microscopic examination, further confirmation using reference pollen slides, higher-resolution microscopy or molecular techniques would strengthen species-level identification. In addition, the experimental vortexing procedure provides a controlled model of mechanical disturbance but cannot fully reproduce the complex conditions encountered during actual forensic contact and evidence collection.

**Study limitations and future research directions:** Several limitations should be considered when interpreting the findings. First, the overall number of pollen grains was relatively small, with unequal initial counts across the different species–fabric combinations. This limited the precision of individual retention estimates, particularly where only a few grains were initially recorded. Second, the analysis was based on pooled observations rather than separately reported replicate-level measurements. Consequently, variation between experimental replicates could not be assessed, and the calculated confidence intervals do not account for potential clustering within replicates. Third, the initial pollen load applied to each textile material was not quantitatively standardised. Therefore, differences in initial deposition and subsequent retention cannot be completely separated. Additionally, detailed textile characteristics, including fibre composition, weave pattern, surface roughness and thread density, were not systematically measured. The investigation also considered only one type of mechanical disturbance and a limited number of pollen species. Other factors, including environmental exposure, duration of contact, repeated handling, abrasion, washing and

natural wear, may substantially influence pollen persistence. Furthermore, manual microscopic examination may introduce observer-related variability, particularly when distinguishing pollen grains from textile fibres and other particulate materials. Future investigations should incorporate larger sample sizes, controlled pollen application procedures and independently recorded replicate-level observations. Detailed characterisation of textile properties would also help clarify the relationship between fabric structure and pollen retention. Statistical analysis using appropriate mixed-effects models could further investigate species-specific differences and interactions between pollen type, textile material and mechanical disturbance. Additional examination using scanning electron microscopy and validated pollen identification methods would improve morphological confirmation and analytical reliability.

## CONCLUSION

The present pilot investigation demonstrated the adherence and persistence of Bougainvillea glabra and Parthenium hysterophorus pollen grains on cotton, terry-cotton, crepe and georgette fabrics following standardised mechanical disturbance. Overall retention was 68.4% for B. glabra and 76.4% for P. hysterophorus. Variations in retention across the textile materials suggested a possible species-dependent relationship between pollen characteristics and fabric properties. However, the limited grain counts and absence of replicate-level statistical analysis restrict definitive comparisons.

## DECLARATIONS

**Ethics Approval:** Ethical approval was not required for this study because it did not involve human participants, human biological specimens or experimental animals.

**Funding:** The study was not received any external or internal financial support.

**Conflict of Interest:** The authors declare that they have no competing interests.

**Data Availability:** The data supporting the findings of this study are available from the corresponding author upon reasonable request.

**Author Contributions:** All author(s) contributed substantially to the conception and design of the study, sample collection, laboratory analysis, data interpretation, manuscript preparation, and final approval of the manuscript.

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