A Comparison of the Cattail Pollen (*Typha angustifolia* L.) Adhesion on Different Fabrics

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Abstract

As pollen has been produce vastly and it can be recovered from a variety of sources because of its resistance to destroy. Moreover, it can be preserved for many years without particularizing storage, pollens can be identified to a specific plant type, site, region, or country of their origin. It can adhere to various surfaces and can be attached tightly. The pollen has a unique characteristic which suitable for identification similar to a pollen print. which brings to assume or predict place or environment around the crime scene. Pollen can be used to link to victims, suspects, and objects related to the crime scene or reconstruct situations to identify the location of pollen.

The reference pollen in this study is a cattail pollen (*Typha angustifolia* L.) because it is a plant that can be seen all over Thailand, currently widespread throughout the world. This grass usually grows covered up tightly in areas that are overgrown and dirty place and it can produce much pollen thus conceal the murder.

The objectives of this research were to compare the adhesive efficacy of the cattail pollen on different fabrics and study palynological characteristic of cattail pollen. This study could be used to consider the part of supportive document in forensic investigation and help depicting how to use the Hirox® program in analyzing the result.

The population of cattail pollen grains used in this experiment was collected in the area of Phitsanulok province. Three types of fabrics including cotton, denim and polyester were dust with pollen grains and left undisturbed for four different periods of time (1 day, 3 days, 5 days and 7 days) before studying the adhesive capability and palynological characteristic of pollen. Scanning electron Microscope was used in characteristic study, while the pollen counting was performed on the light microscope with the help of the Hirox® software and the Hirox® microscope in RGB counting mode. All data were statistically analyzed by One-Way ANOVA, which analyzing the differentiation of the pollen quantity on different fabrics. The result revealed a statistically significant difference at the $p < 0.05$ level between groups of fabrics. The quantity of pollen grains adhering to the fabrics showed the highest value on the polyester, followed by the denim, whereas the cotton found lowest amount.

Keywords: Pollen, Forensic science, Biological evidence, Microscope, *Typha angustifolia* L.

Introduction

In Thailand, there are plenty of criminal and violent cases. In order to bring the offender for punishment, the evidence must be gathered to prove the offense. The scientific evidence is credible and acceptable in the judicial process and society. Forensic scientists perform both physical and chemical analyses on physical evidence obtained by crime scene investigators and law enforcement officials at the crime scene. The scientific experts used microscopic examination techniques, complex instruments, scientific principles, and reference literature to analyze evidence to identify both class and individual characteristics.

Nowadays, information on the forensic process has opened widely. Therefore, the offender received various information. Resulting in the offender being more careful to make criminal offenses such as wearing protective gloves, detecting fingerprints, incineration of evidence, etc. Forensic scientist has to find traces that the offender left unintentionally or accidentally brought along to solving the case that has occurred.

The botanical evidence is a type of evidence that offenders are often careless. Offenders also neglect to eliminate but is not yet prevalent in the selection for verification. This field lacking experts and technical information. In which the botanical evidence can be both true evidence and supporting evidence, such as the disappearance case of Holly Wells and Jessica Chapman. The pollen found in the shoes of the suspect matches the pollen found at the scene. Rape case in Auckland New Zealand, been able to accurately identify the area of rape from pollen (Mildenhall, 2006). The murder in New York by Henry Lee Lucas, the case after the Bosnian War as well as moving the body to another location are examples. The objective of the palynological and associated soil analyses was to determine the environmental profile of the original burial sites and to try and find a connection with the secondary sites where different environmental profiles existed (Kukes, 2015)., and the investigation of an execution-style murder. This case shows that the season of pollen spread is relevant in forensic cases. Pollen analysis of the victim's clothing and comparator samples from the scene recovered significant numbers of silver beech pollen grains. The murder and subsequent discovery of the body three months later took place well outside the flowering season of silver beech, yet that was the dominant pollen type on the clothes of the victim, and although the defendant had only been at the scene a few hours. (Brown, 2006).

Pollen has many different advantages from general evidence such as pollen size is very small, between 7-200 microns (20-60 μm average). Humans cannot see with the naked eye. Therefore, criminals cannot tell that they have pollen attached to them from the scene

One of the problems focuses on what the courts and law enforcement personnel deem to be the reliability of using pollen and spore data as forensic evidence. Because each plant species produces a unique pollen grain, the morphological differences among each species are often very minute. The analysis of most forensic pollen and spore studies relies on the use of light microscopy, but at this level, the precision needed for species identification is usually not possible. This level of added precision can often be resolved for individual pollen types, and even for conducting total pollen analyses of a sample with the added resolution gained by using a scanning electron microscope (SEM); (Jones & Bryant, 2007).

The adhesion of pollen in materials or objects depends on the sculptural type of the exine and the particular sticky components present on this external layer (pollenkitt); there are also electrostatic charges which can reduce pollen adhesion by electrostatically repelling pollen from the anther, such as the van der Waals forces which include mechanical interlocking (Bowker & Crenshaw, 2007; Vaknin, Gan-Mor, Bechar, Ronen, & Eisikowitch, 2000).

Therefore, pollen of plants can be used as an evidence for the trial. These factors lead to a comparative study of the adhesion efficiency of pollen on different surfaces. To provide supporting information for further forensic work. Although, forensic palynology has been a part of forensic science that has been instrumental in fighting for over 50 years (Mildenhall, 2008), The field of palynology will be successfully implemented in crime in other countries. But it has not been widely used in civil or criminal cases in Thailand.

The reference pollen in this study is a *Typha angustifolia* L. because it is a grass that can be found all over the world. The plants are commonly found growing in shallow freshwater areas. Cattails shed a great deal of pollen. Buried seeds and pollen can survive for long periods of time. Dry grass is a good source for pollen. In forensic palynology ever use grass pollen for investigation such as homicide case of a baby and the forensic potential of pollen in dry grass in Austria (Weber & Ulrich, 2016). Period time for conduct this study is 7 days

that represent the time for wash off clothes in daily routine and type of fabrics in this experiment is commonly used for producing generally clothes including cotton, polyester, and denim

Methods and Materials

The research was investigated the pollen adhesion on different fabrics which cover *Typha angustifolia* L, commonly known as narrow-leaf cattail, is a marginal, semi-aquatic, herbaceous perennial, native to northern and central Asia, North America, Europe, Africa and invasive worldwide. Cattail can be found in the following habitats for example marshes, ditches, fens, pond, and lake margins, floating bog mats, roadside ditches, irrigation canals, and backwater areas of rivers and streams (Finkelstein, 2003). Pollen in this study were collected from Phitsanulok, Thailand. The experiments were performed at forensic laboratory in the Royal Police Cadet Academy and laboratory in the Dermscan Asia.

During the flowering season (September-November), Pollen were collected directly from cattail flower by gently tapping on the white paper. Pollen will be fallen on the paper, then separated the pollen from contaminated objects such as dried leaves, stick or dust. Pollen was storage at room temperature and humidity 40-80% in clean zip lock bags until used.

Scanning electron microscope (SEM) was used for studying the characteristics of pollen. One centimeter of cutting carbon tape was placed on the stub and then spreading the pollen onto the tape. All images were adjusted for desired size and clarity. The pollen's images captured were compared with the database in order to identify species. The second test was conducted on imprinted samples of the three types of fabrics. The study simulates a natural adhesion of known pollen onto fabrics, using a direct contact followed by a tapping.

For fabrics preparation, the cotton from a T-shirt, denim from jeans and polyester from the skirt. Cutting it to 4x4 cm, 4 pieces per fabric. The pollen were weigh and divided it into 9 petri dishes. A 4x4 cm sponge was used to print the pollen from each petri dish and move it onto each fabric by placing a sponge once a time. This experimental protocol is trying to mimic a natural attaching of known pollen onto the material, using a direct contact followed by gentle placing. Later, the blower was used to blow the excess pollen for spreading to prevent clumping five times on each side (totally 4 sides) was supplied after pollen application. Three types of fabrics (3 repetitions/ fabric) were counted at day 1, 3, 5 and 7, respectively after pollen application, seven days is enough for someone who wash the cloth resulting the pollen was rinsing off according to the daily routine at room temperature, humidity 40-80% by each sample was separated by plastic partition in order to prevent pollen contamination. SEM was generated (1 repetition/ fabric). Pollen on fabrics were imaging by microscope Hirox® KH-8700, MXG-2016Z lens, 100x magnification, and counting by Hirox® software (Figure 1).

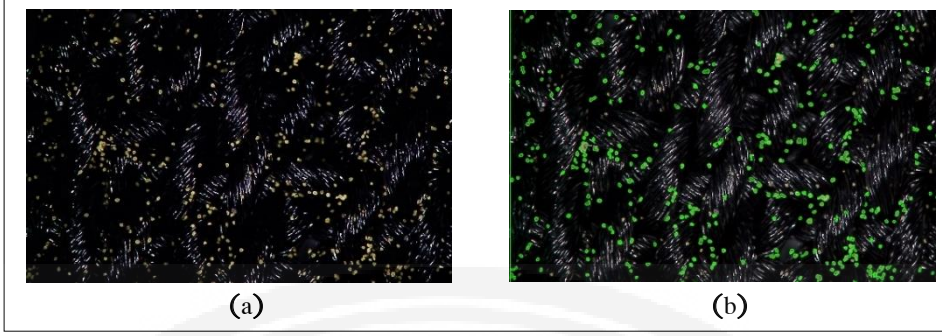

Figure 1 Before (a) After (b) Pollen on polyester at day 5 counting by Hirox ® software
Yellow objects are pollen (a) Green marks are areas of counting pollen from the software (b)

Results

Pollen characteristics

Identification of the pollen species and characteristic by using the Australasian Pollen and Spore Atlas *Typha angustifolia* L. is perennial herbaceous plant. The plant leaves are flat, very narrow and 1.5–2 m tall. The stalk that are about as tall as the leaves; stalks are topped with brown fluffy, sausage-shape flowering heads. The plants have sturdy, rhizomatous roots. Pollen size average is 20–40 microns (Figure 2).

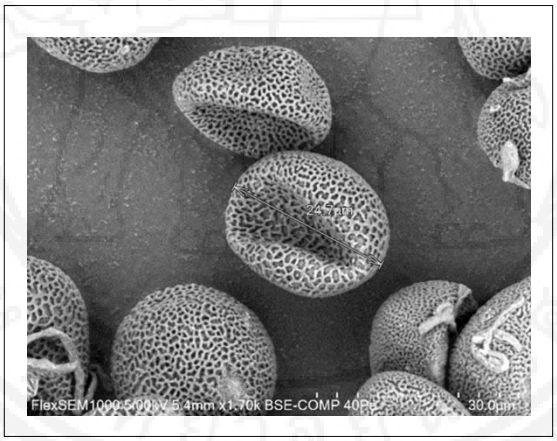

Figure 2 Pollen imaging by SEM at magnification 1700x

Pollen adhesion

After day 1, 3, 5 and 7 respectively, the pollen quantity was analyzed per fabric. Non- pollen attaches not found pollen in all kinetics. For pollen attaches found an average of pollen is; cotton (358, 290, 194 and 135 grains/FOV), denim (635, 472, 374 and 281 grains/FOV), and polyester (382, 302, 280 and 203 grains/FOV). The quantity of pollen adhesion on the fabrics shows the lowest value for the cotton, followed by denim. Polyester has the highest quantity, (Table 1). On all fabrics show the decreasing trend of pollen quantity which decreases significantly different at the p 0.05 level on different kinetics and fabrics (Table 2).

Table 1 Comparison $\bar{x}$ and S.D. of pollen quantity on each fabric at day 1, 3, 5 and 7 respectively

Kinetics	Cotton		Denim		Polyester	
	$\bar{x}$	S.D.	$\bar{x}$	S.D.	$\bar{x}$	S.D.
Day 1	358.33	18.877	381.67	41.284	635.33	43.890
Day 3	290.00	47.571	302.00	65.506	472.33	18.824
Day 5	194.33	15.822	279.67	60.011	374.33	16.862
Day 7	135.33	20.257	203.00	26.514	281.00	7.211

This table shows the quantity of pollen decrease continuously following time in all types of fabrics. At all kinetics, Polyester has the highest number of pollens and the lowest number of pollens is denim.

Table 2 Pollen quantity compare between fabric at each kinetics

	Kinetics	**Mean**	**±**	**SEM**	**p**
Cotton and Polyester	D1	277.000	±	29.766	0.000*
	D3	182.333	±	182.333	0.003*
	D5	180.000	±	30.317	0.001*
	D7	145.667	±	16.092	0.000*
	Kinetics	**Mean**	**±**	**SEM**	**p**
Denim and Cotton	D1	23.333	±	29.766	0.463
	D3	12.000	±	39.181	0.770
	D5	85.333	±	30.317	0.031*
	D7	67.667	±	16.092	0.006*
	Kinetics	**Mean**	**±**	**SEM**	**p**
Polyester and Denim	D1	253.667	±	29.766	0.000*
	D3	170.333	±	39.181	0.005*
	D5	94.667	±	30.317	0.021*
	D7	78.000	±	16.092	0.003*

*. The mean difference is significant at the 0.05 level.

Under the study conditions, after first pollen attaches. The result presents a statistically significant difference at the $p < 0.05$ level between groups of fabrics.

Discussion

The ability of pollen to adhere on the materials and is a factor in the criminal context in presenting in legal evidence (Rowell, 2009). It is also evidenced for linking crime scenes and victims or offenders. Pollen adhesion in clothing is useful if a person who committed a crime came in contact with pollen at the crime scene, especially plants actively releasing pollen. Almost all kinds of clothing become good pollen adhesion. This study shows a different type of adhesion, depending not only on the used species but also on the specific materials. The factor of pollen adhesion depends on pollen morphology such as exceptionally complex surface patterns, substance coating, electrostatic forces (Bowker & Crenshaw, 2007; Vaknin et al., 2000) and hydrophobic or hydrophilic of pollen surface (Lin, Lizarraga, Bottomley, & Meredith, 2015) or fabric, humidity and method of pollen adhesion is directly contact or air spread (Jantunen & Saarinen, 2011). There were researches that analyzes the

efficiency of pollen adhesion on different materials. A deep adhesion, especially in the fabrics and still has agglutination after being washed off (Mildenhall, 2006) From the results of this study, the majority of pollen was found on fabrics of polyester, denim and cotton, the amount decreases inversely with the increasing of time. The different results of the pollen adhesion on different fabrics can be used for forensic investigators. For example, forensic pollen samples were collected from the victim's shirt, pants, socks, and shoes. In addition, four separate samples of surface dirt were collected from the location where the body was found. The pollen types and percentages in each of the surface soil samples were similar. As a group, however, they did not exactly match the pollen "fingerprint" recovered from the various clothing items worn by the victim. The slight difference between the samples indicated that the victim may have lived some distance from where the body was found. Additional regional pollen studies produced one near-perfect pollen match with the victim's clothing. It suggested that the victim probably lived (and may have been murdered) far from where the body was found. This information helped police narrow the search for the victim's identity (Bryant, Mildenhall, & Jones, 1990). Fabric type was significantly related to pollen retention but interacted strongly with each plant species such that patterns were both complex and highly species- specific such as the efficiency of removing pollen with the standard washing protocol differed substantially according to plant species, fabric type, and the interaction between these factors (Webb, Brown, Toms, & Goodenough, 2018). The amount of pollen can help to reconstruct the situation in the crime scene and can be assumed a posture of the body of the victim before was murdered. For example, it may be important to know whether someone has hidden under a tree and has received a lot of pollen on their shoulders and less pollen elsewhere. It may be important to know whether clothing, worn by someone walking through a field, contains pollen that has become attached to the cuffs or base of their trousers (maybe just on one side), or higher up depending on the height of the vegetation. Plants do not need to be in flower at the time. It may also be important to know if someone had knelt, sat, or rolled on the ground, and pollen can be mapped on clothing to demonstrate this (Mildenhall, 2008). If they were not found other evidence, pollen is one choice for collecting when the time has passed over because pollen have the efficacy of adhesion on fabrics for a long time.

For the counting of pollen from images, microscope and software Hirox® are an easy-to-use. This method is suitable for counting large numbers of pollen. Due to the sizes of the pollen is small, microscope Hirox® is able to illustrate and analyses images at a magnification maximum of 100x. The software can analyze images easily and quickly with various functions. Although many images analyze may occasionally fail to detect pollen sometimes but the number of image analysis many times is consistent. The researcher can choose the type of analysis that is suitable for the object that needs to be studied.

Conclusion and Suggestions

The pollen grains analyzed show a capacity to adhere differently on fabrics, to become tightly adhesion, especially in the polyester, and even to continue to be attached after blowing and long- lasting until 7 days. Clothing may be a primary route of pollen collection that contributes to the contamination from the crime scene.

The experiment demonstrates more particularly that cotton, denim, and polyester fabrics are the especially main way for pollen transfer, for cattail pollen. The pollen evidence does not disappear over time; it stays

awaiting detection, becoming significant evidence. The differences in the variation of adhesion to materials can be responsible for a range of amounts of pollen being maintained.

Understanding the factors of errors counting by the software is important to decrease the occurrence of errors, such as low sharpness may affect counting because the image is unclear. Especially, the rim of the objects or the color of contaminated objects that similar to interest objects. The software will count that group of objects as one group. Before the study, the researcher should be pre-test or pre-analysis in order to check the accuracy of the analysis by software. This study was found that when interesting objects increase in the image, the efficiency of the software decrease. However, when the counting results show, we can know some obvious errors, such as number of pollens being lower than that of images observed by the visual evaluation.

Estimating too many colors or sizes can be an analytical error. Firstly, to reduce the risk of object size that is out of range should be selecting the correct range and suitable value according to the size of the species. Secondly, interesting objects should be spread. If each object is not clumps and has a clear boundary, it will make the counting more accurate. The tight adhesion may cause the software to analyze that group of objects into one group. Finally, the difference between background and object color (contrast) help the counting process easier. These reduce the process of image optimization.

Experimental results showed that the quantity of pollen change in each time had a significant effect but not include all factors which affect the efficacy of pollen adhesion. From this experiment, researchers suggest comparing this species with other species in which complex structure surface or other sizes, and suggest comparing different kinds of adhesion to substitute a situation in the crime scene.

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