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TECHNICAL NOTE

CRIMINALISTICS

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Environmental Pollen Trapped by Tobacco Leaf as Indicators of the Provenance of Counterfeit Cigarette Products: A Preliminary Investigation and Test of Concept

ABSTRACT: The global trade in counterfeit tobacco products is increasingly taking market share from legal brands in many parts of the developed world, with attendant adverse economic, health, criminal, and other societal impacts. Knowing the geographical source is central to developing new strategies for curbing this illicit trade, and here, the potential of environmental pollen extracted from manufactured cigarettes is examined. Two samples representing U.S. and Chinese brands were investigated for their pollen content. Results indicate that tobacco leaf very efficiently captures environmental pollen (about 1800 and 12,600 grains per cigarette, respectively) with no detectable self-contamination by the tobacco plant. In both cases, the flora is typical of open space environments, but pollen type counts indicate very different distributions of species. This preliminary investigation indicates that palynology has the potential to constrain geographical source(s) of tobacco, particularly if regionally localized species can be recognized among the pollen.

KEYWORDS: forensic science, pollen, palynology, tobacco, provenance, counterfeit, cigarette

Identifying the source of tobacco is becoming increasingly important in support of efforts to stifle the supply of counterfeit cigarettes that are gaining a large market share in parts of Europe and North America. Here, we test the concept that pollen may provide a signature of the tobacco cultivation environment that could be used in conjunction with chemical and other indicators to identify the sources of major seizures made by customs authorities.

Tobacco smoking is the cause of death of about half of its habitual users (1). The fraction of the population that smokes is rapidly increasing in the developing world, and smoking is now predicted to claim about a billion lives over the 21st century (2). The World Health Organization's Framework Convention on Tobacco Control that entered into force in 2005 and which has hitherto attracted 160 national signatories identifies a range of measures aimed at reducing the enormous toll on human health caused by smoking (3). One of the most effective control measures is tobacco product taxation. Cost is the greatest single influence on an individual's decision to quit, and every 10% increase in tobacco taxes results in about 4% reduction in consumption (4). Counterfeit products undermine this fiscal policy aimed at supporting a public health objective by reducing the financial incentive to quit (5). Minors often start smoking, because they are diverted to illicit sources when they are refused cigarettes at legal outlets. There is also evidence that counterfeit cigarettes emit higher levels of some toxins, including major carcinogens, compared with their genuine equivalents (6,7). As well as these health impacts, the counterfeit trade has other societal effects including close association with organized crime and the use of funds to support terrorist activities (8). The abuse of trademarks (brand names) by counterfeiters involves manufacturers in financial losses and deceives the customers (9). For these reasons, as well as the obvious loss of substantial taxation revenues, governments in the developed world are actively seeking effective strategies for combating the supply of counterfeits and limiting their market penetration (10). This has led to alliances between governments and cigarette manufacturers, as exemplified by the EU-Philip Morris anti-contraband and anti-counterfeiting agreement (11).

Tobacco products are legal in almost all countries, yet there is a rapidly growing trade in illicit products that has been estimated at over 10% of the global cigarette market, depriving governments worldwide of annual revenues of U.S. $40–50 billion (5). Attempts to reduce this illicit supply have led to the development of overt and covert indicators for tracking the movement of legal brands (5). This approach has already had a significant impact in the United Kingdom (8). However, success in reducing the quantities of legal cigarettes that have been illegally diverted to evade taxes (contraband genuine product) appears to be having an unintended consequence with the complementary increase in counterfeit product. For example in 2001–2002, 15% of all U.K. customs seizures of illicit cigarettes were counterfeit, but by 2006–2007, this had risen to 70% (10,12), while in the U.S.A. seizures of counterfeit products exceeded those of genuine brands by 2003 (13).

Counterfeit cigarettes in the European and North American markets are thought to originate principally in China, but other countries in SE Asia, the Balkans, Ukraine, Russia, Paraguay, and the Middle East have been implicated, whereas some are manufactured in the final destination country (8,9). The presence of robust evidence of geographical origin may encourage jurisdictions to combat the counterfeiters at source, and for this reason, there is need for

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simple, accurate, and consistent methods to constrain origin. Tobacco leaf is naturally variable, and this makes it potentially sensitive to geographical source, although it is recognized that blending of tobacco will mix and dilute any such indicators of source. In this regard, it is helpful that many cheap counterfeits are made from unblended tobaccos or blends from the same broad geographical region.

The aim of this paper is to carry out a preliminary investigation into the proposition that pollen representative of its local environment is trapped on the growing tobacco leaf and remains attached to the leaf throughout the production process, so that it may be recovered and used to constrain the geographical location of the tobacco used in producing these cigarettes. Palynology has been shown in many other areas of forensic science to provide robust indicators of locality (14), and this contribution seeks to establish whether it might have any potential for sourcing counterfeit tobaccos. Success in this aim would provide criminal and customs authorities with a new forensic tool that could potentially match seized counterfeit products to the geographic origins of the tobacco.

The literature appears to indicate that this is the first attempt to investigate environmental pollen in tobacco products. At the outset, it was unclear whether tobacco extracted from cigarettes would be suitable as it might be so heavily contaminated with its own pollen (self-contamination) that any signal from other plants would be swamped. However, two factors encouraged this investigation. First, the surface of the tobacco leaf has a dense coverage of trichomes forming a mat of fine hairs that efficiently trap atmospheric particles (15). Most of these trichomes also secrete a viscous terpene-rich exudate that renders the leaf surface sticky, effectively retaining trapped particles (16). Second, the standard agronomic practices of topping and suckering, involving respectively the physical or chemical removal of buds before flowering to encourage growth (17), significantly reduce the potential for self-contamination with pollen.

TABLE 1—*Pollen counts for U.S. reference cigarette 1R5F and commercial Chinese cigarette brand Hongtashan.*

"Brand"	1R5F	Hongtashan
No. of cigarettes	5	5
Mass of tobacco (grams)	2.6	3.3
Lycopodium* count	47	250
Pollen per gram[†]	23,420	2400
Pollen per cigarette[†]	12,177	1586
Pollen count (includes U)	254	176
Asteraceae	181 (71.3%)	11 (6.3%)
Artemisia	4 (1.6%)	2 (1.1%)
Poaceae <40 microns	17 (6.7%)	53 (30.1%)
Poaceae[‡] Cereals	6 (2.4%)	16 (9.1%)
Poaceae (>40 microns)		5 (2.8%)
Chenopodiaceae	16 (6.3%)	8 (4.5%)
Caryophyllaceae	5 (2.0%)	1 (0.6%)
Cheno.[‡]/Caryoph.[§]	5 (2.0%)	5 (2.8%)
Pinus	5 (2.0%)	4 (2.3%)
Myrtaceae[‡]	1 (0.4%)	14 (8.0%)
Lactuceae	1 (0.4%)	
Brassicaceae	1 (0.4%)	
Quercus	1 (0.4%)	
Plantaginaceae	1 (0.4%)	
Apiaceae		2 (1.1%)
Cyperaceae		5 (2.8%)
Dodonaea[‡]		18 (10.2%)
Unidentified (U)[¶]	10 (3.9%)	32 (18.2%)
Unidentifiable[¶]	17	64

*Marker spores added to sample in known quantity, tabulated figures represent numbers recovered.

[†]Pollen concentration calculated as pollen count × added Lycopodium (11,267 ± 370) divided by Lycopodium count.

[‡]Indicates strong similarity but some uncertainty in the formal identification.

[§]Ambiguity reflects the lack of reference material for non-European flora.

[¶]See text for distinction between unidentified and unidentifiable pollen; the latter has not been included when calculating percentages.

Methods

Two cigarette products were selected to represent geographic extremes. 1R5F is a blend of U.S. tobacco leaf that is used internationally as a reference standard for cigarette emissions testing (18). It is taken to be representative of ultra-low tar brands in the United States. The sample used in this study was obtained from the producers at the University of Kentucky in 2001. Hongtashan is the biggest-selling Chinese brand (second globally only to Marlboro in terms of sales) and is manufactured in Yunnan province from local tobacco, Yunnan having the largest tobacco production in China (19). The sample used in this study was purchased from an official government outlet in Beijing in November 2006.

For each brand, five cigarettes were chosen randomly from one pack of 20. The filters were removed, and the cigarette papers were cut open to release the tobacco. The amount of tobacco obtained for each brand was weighed. A standard pollen preparation involving NaOH, sieving, and acetolysis (acetylation) (20,21) was used to concentrate palynomorphs. One tablet containing a known number of marker grains of *Lycopodium clavatum* (11,267 ± 370) was added to each sample, so that an estimate of absolute pollen content could be made for the given sample mass (22,23). Samples were dried using alcohols and mounted in silicone fluid on sealed slides for microscopic examination. These preparations were carried out in a filtered air environment to prevent contamination by local pollen sources.

A number of contiguous traverses were counted for each sample. Pollen was identified where possible and the positions of all grains were noted, so that further attempts at identification could be made, and pictures taken of unidentified types and other examples. Pollen types are listed in Table 1, mainly under family names. There were significant numbers of unidentified grains, particularly in the Hongtashan sample, as our expertise and reference samples are primarily in European floras. Unidentified pollen that we think have a good chance of future identification are included in the pollen total. Pollen listed as "Unidentifiable" is characterized by very poor preservation status (i.e., the grains are extremely folded, broken, or corroded) or they lack sufficient distinctive features to allow accurate identification. A small number of grains within this category may not be pollen. The "Unidentifiable" numbers have not been included in the total pollen count or the percentage values as is standard practice in pollen studies.

Results and Discussion

Pollen counts are presented in Table 1. Examples of individual grains may be viewed at http://www.st-andrews.ac.uk/~wes/research/pollenimages. The only tree types positively identified are *Pinus* (pine) and *Quercus* (oak). However, Myrtaceae pollen was noted in both samples. Myrtaceae is a family of trees, shrubs, and creepers, which includes Eucalyptus and is found mainly in the Pacific region, Australasia, and Tropical America but also in China. All other identified types are herbaceous and can be linked to cultivation. The Asteraceae family (daisies) that includes *Artemisia* (mugworts) and Lactuceae (lettuce sub-family) encompasses many weed species as do the Chenopodiaceae (goosefoots) and Caryophyllaceae (pinks).

The pollen signature in the 1R5F (U.S.) tobacco sample is dominated by over 70% Asteraceae, most of which are the same species. There is also a strong signal (9%) from Poaceae (grasses) that may include some cereals. The strongest signal in the Hongtashan (China) tobacco pollen is from Poaceae at over 40%; this includes some cereal pollen and some very large Poaceae grains, which may be amenable to more specific identification. The Hongtashan tobacco also includes 10% of a grain tentatively identified as *Dodonaea* (hopseed, hopbush).

Total pollen numbers are very different between the two tobacco samples. 1R5F yields nearly an order of magnitude more pollen mass than the Hongtashan sample and about eight times more pollen grains per cigarette. It may be relevant that this difference correlates closely with chemical indicators of a dust component (silicate minerals), and thus different degrees of retention of airborne particulates by the leaf surface may be an important determinant. Numbers of "Unidentified" and "Unidentifiable" pollen were higher in the Chinese sample, but few of the unnamed grains were repeats. Unidentified pollen was compared to pollen type material collected from garden tobacco plants and to published images of *Nicotiana tabacum* (24) and other *Nicotiana* species on various Web sites, but no matches were found. The reason for the apparent lack of *Nicotiana* pollen probably reflects cultivation practices already described.

This preliminary investigation indicates that tobacco very efficiently traps and retains pollen from its cultivation environment with many if not all grains surviving processing. Large quantities of pollen are easily recovered even from individual cigarettes. Two samples of tobacco-trapped pollen from different continents have markedly different total pollen as well as distributions of pollen type counts, although both are strongly indicative of cultivation in open ground with few trees. These differences in pollen types suggest that type distribution may vary with location, and the preliminary results are sufficiently encouraging to warrant efforts at characterizing pollen in tobacco from major tobacco-growing regions. It will be important to characterize pollen extracted from tobacco grown in known localities and compare them with local flora. It is not known whether the tobacco plant exerts any selectivity with respect to the pollen it retains, and it is important to establish the degree to which the tobacco sample reflects the local environment. On the larger scale, it is anticipated that pollen from different continents will provide very different signals in terms of flora species and their relative abundances. In this respect, it is noteworthy that the top 10 global producers of tobacco are located on five different continents (4). It is also likely that individual pollen types may be identified that are restricted to particular continents and even smaller geographic regions within those continents. Finding such marker pollen types would substantially improve the robustness of the method. Turning this finding into a useful forensic tool will require the expertise of regional pollen specialists in different parts of the world, and to this end, we have placed images of the pollen types found in these samples on the Web site http://www.st-andrews.ac.uk/~wes/research/pollenimages.

We conclude from this investigation that pollen extracted from cigarettes has the potential to provide useful information on geographical source. It is recognized that different species and varieties of tobacco may have different capacities to retain pollen. Furthermore, different cultivation practices, storage, treatment, and manufacture of the product postharvesting may also influence the types of pollen to which the product is exposed and/or retention characteristics. These factors need further investigation. The method also has a major limitation in that the blending of tobacco will mix the geographical signals provided by pollen. This applies particularly to genuine commercial brands in developed countries. Counterfeiters in China tend to source their tobacco from local tobacco farmers who produce more than their state allocated quotas (9), and blending is less likely to be a problem. Elsewhere, factories tend to source their materials directly from the same suppliers as those supplying legitimate manufacturers, and sometimes these legitimate manufacturers engage in euphemistically termed "production overruns" for diversion to the illicit market (9). In these cases, matching pollen type distributions should help identify such diversion, and possibly even the perpetrator, as long as the pollen type distribution is known for the legitimate brand. Either way, pollen type distribution in tobacco will be practically impossible to replicate artificially thus offering a robust fingerprint of the origin(s) and subsequent history of its products.

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