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Review Article

Analysis of plant soil seed banks and seed dispersal vectors: Its potential and limits for forensic investigations

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ABSTRACT

Plant seeds exhibit many species-specific traits, thus potentially being especially helpful for forensic investigations. Seeds of a broad range of plant species occur in soil seed banks of various habitats and may become attached in large quantities to moving objects. Although plant seeds are now routinely used as trace evidence in forensic practice, only scant information has been published on this topic in the scientific literature. Thus, the standard methods remain unknown to specialists in such botanical subjects as plant ecology and plant geography. These specialists, if made aware of the forensic uses of seeds, could help in development of new, more sophisticated approaches. We aim to bridge the gap between forensic analysts and botanists. Therefore, we explore the available literature and compare it with our own experiences to reveal both the potential and limits of soil seed bank and seed dispersal analysis in forensic investigations. We demonstrate that habitat-specific and thus relatively rare species are of the greatest forensic value. Overall species composition, in terms of species presence/absence and relative abundance can also provide important information. In particular, the ecological profiles of seeds found on any moving object can help us identify the types of environments through which the object had travelled. We discuss the applicability of this approach to various European environments, with the ability to compare seed samples with georeferenced vegetation databases being particularly promising for forensic investigations. We also explore the forensic limitations of soil seed bank and seed dispersal vector analyses.

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1. Introduction

Various plant parts can be useful as evidence [1,2]. The use of pollen has received particular attention regarding forensic approaches and criminal cases (e.g., [3–7]). Recently utilised methods also include the use of vascular plant, bryophyte, algal and fungal vegetative parts and/or spores, and seeds and/or fruits (e.g. [1,5,8–10]).

Although seeds are routinely used by forensic practitioners, few papers have been published on this topic. Thus, there is an overall lack of resources regarding the potential utility and limits of seeds as forensic evidence, with those available offering only very vague conclusions (see e.g. [11,12]). Moreover, the use of seeds and/or fruits has for the most part been reported only for those species having large seeds and/or fruits that are easily detectable without special equipment (see e.g., Mathewes [3] for *Osmorhiza berteroi* – fruits up to 2.5 cm, or Aquila et al. [13] for *Xanthium orientale* subsp. *italicum* – fruits between 2.2 and 2.8 cm). This could give the erroneous impression that only large seeds and/or fruits are suitable as forensic markers. However, many species possess seeds or fruits a few millimetres or less in size (for seed sizes of various species see, e.g., [14,15]), which can be quite useful for forensic purposes. These propagules may become attached to human or animal bodies, clothing, footwear, car wheels, or various tools [16–21] where, because of their small size, they are not readily detectable. However, because even such small seeds can be identified to the species level, and plant species differ in their ecology (not only in terms of habitat associations, but also other attributes, such as when they disperse their seeds), they can provide substantial forensic information. Additionally, because seeds are often present in organic detritus and soil, their occurrence can help identify the sources of such substrates found in the course of forensic investigations.

The presence of soil seed banks, which comprise the viable seeds (and one-seeded fruits) occurring in the soil, add to the potential usefulness of seeds as forensic tools. Both seeds that can long remain dormant in persistent seed banks and those that have shorter viability can be dispersed shorter or longer distances, depending upon seed and fruit characteristics and dispersal mode [22]. Seeds of some species, particularly those with persistent soil seed banks, can often even survive such stressful and changing conditions as those in human or animal digestive tracts, and may be well-adapted to various dispersal modes (cf. [20,21,23]).

Although various aspects of soil seed banks and seed dispersal have often been studied by biologists [18,24–26] and the resulting insights likely useful for forensic investigations, research specifically focused on forensic applications of seeds has been scarce. Thus, although it would be instructive to compare the forensic reliability of seeds and/or fruits relative to that of such plant materials as pollen and wood or other natural substances (e.g., soil, organic detritus) in realistic situations, such comparisons are rather exceptional (e.g. [6]). Moreover, even in studies that have included soil analysis, the seeds potentially contained in the soil have not been examined (see e.g. [27,28]). Many factors, e.g. size, shape and surface structure not only of seeds but also of the structures to which the seeds might become attached are known from an ecological point of view to significantly affect seed dispersal [18,22]. These factors should also influence the use and interpretation of seeds in forensic studies. However, ecology and forensic science explore different types of hypotheses and are practiced on different resolution scales, and thus the importance for each discipline of particular features of soil seed banks and seed dispersal processes is likely to differ as well.

In the present paper, we provide particular examples of plant species from various environments of temperate Europe and their potential value as forensic markers. Further, based on critical review of not only our own experiences with soil seed banks and seed dispersal but also of the relevant literature, we discuss both the potential and limitations of soil seed bank and seed dispersal analysis for forensic applications. We aim to: (1) encourage experimental testing that would contribute to development of more sophisticated methodological approaches for forensic purposes; (2) increase awareness of the utility of seeds as forensic markers in various situations, comparing their potential with that of pollens; and (3) bridge the gap between forensic analysts side and botanists.

2. How to find and identify seeds in soil

Three basic alternative approaches are employed in assessing the seeds contained in soils: the first two are wet sieving and flotation, both of which are followed by identification of seeds under stereoscopic microscope. The third is cultivation of soil samples and subsequent identification of emerged seedlings [18]. The advantages and disadvantages of each of these methods have been demonstrated independently by various researchers and discussed extensively (see e.g. [29–31]). In particular, flotation has been criticised for its inaccuracy [29], and therefore the two remaining approaches have been more widely used in recent soil seed bank analyses. Soil sieving and microscopic analysis of the material remaining on the sieves is best suited for forensic purposes. It is relatively cost-effective, and the time demand is comparable with that of pollen grain analysis, which is more widely known among forensic scientists. Another advantage is that this method is non-destructive and the extracted seeds may be maintained for many years in proper storage conditions (i.e., mainly dry storage in glass tubes). Moreover, this method enables detection not only of live but also dead seeds and their parts, which can also provide forensic information. However, combining this method with cultivation of either recovered seeds or of portions of the analysed soil samples may be required in specific cases, e.g. when seed viability should be tested in order to provide taphonomic information. Also, the combination of sieving with seed cultivation offers the possibility of using DNA analysis of emerged seedlings to confirm the accuracy of species identification based on seed morphology. Seeds extracted from the soil can be germinated under controlled laboratory conditions (e.g., in germination chambers) and with the help of chemicals that break dormancy, germination can be enhanced (e.g., [32]). This targeted procedure can shorten the total time necessary for sample analysis in comparison with commonly used greenhouse cultivation of large amounts of analysed soil.

3. Soil seed banks of temperate Europe—general traits and species composition

The soil seed bank to various extents reflects the vegetation present at the locality. The degree to which soil seed banks correspond to the vegetation present varies among habitats, vegetation types and individual localities (e.g., [33–35]). Thus, even localities covered with similar vegetation may exhibit large differences in soil seed banks, and soil seed banks of similar species composition may be found under different above-ground vegetation (e.g., [36–38]). Differences and similarities between soil seed banks and vegetation are usually related to particularities of the site development over time, e.g., land use or environmental changes. For instance, shade-tolerant species such as *Carex sylvatica*, *Moegringia trinervia* and *Scrophularia nodosa* are found in the soil seed banks of temperate mesophilous deciduous forests across Europe (e.g., [36,39,40]). Species such as *Juncus bufonius*, *Juncus articulatus*, *Gnaphalium uliginosum*, *Rorippa palustris* and *Ranunculus sceleratus*, on the other hand, are associated with