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MACROBOTANIC DATA IMPLEMENTING FORENSIC GEOLOGY INVESTIGATIONS

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Abstract. The contribution of botany to the analysis of geological traces should not be neglected in forensic investigations. Small plant traces, especially if widespread in quantity, easily adhere to the subjects involved in criminal events. However, the identification of highly fragmentated plant structures often relies to skilled experts. The present paper deals with the morphological descriptions of thorns and similar plant appendices produced by the plants thriving in the area under investigation in a court case regarding the disappearance of two subjects in a rural area of the countryside. Comparative observations between unknown and known samples, related to victims and crime scene respectively, made it possible to identify traces found on the victims as plants thriving in the area under investigations. Basing on the punctual distribution of individual plants in the investigated territory, the results of the comparative analyses allowed to reconstruct the victims' path in the hours immediately preceding their death. Consequently, these data provided valuable information for implementing the geological investigation carried out for the judicial authority.

1. Introduction

Forensic Sciences are defined as the application of science for the resolution of judicial cases (Picozzi and Intini 2009; Saferstein 2017). Their purpose is to determinate the probative value of the crime scene and scene of events, and related physical evidence. Science, therefore, assumes an important and unique role in the justice system, a role that refers to the ability of the forensic expert to provide accurate and objective information on the facts that occurred in the past during the fulfillment of an event.

Forensic Sciences include various disciplines useful for the resolution of judicial cases; they are used in forensics to reconstruct a historical, unknown fact, starting from known facts, that may be documented through the use of scientific laws and/or technical-scientific methods (Picozzi and Intini 2009; Saferstein 2017; Somma 2022, 2023a,b,c).

A high number of disciplines are useful in Forensic Sciences, mostly including legal medicine and forensic veterinary medicine (toxicology, serology, molecular medicine, pathology, dentistry), physical anthropology, psychology/psychiatry/sociology, physics, chemistry, geology, biology, archaeology, entomology, informatics, image and video forensics, among many others. All disciplines can give informations that have to be integrated in a multi- and trans-disciplinary approach.

In forensics, comparative analyses are frequently employed, aimed to compare a sample of unknown origin (reference or questioned sample) with a sample of known origin (control sample), by using the same analytical procedures, in order to determine whether or not they have a common origin (Prychid, Rudall, and Gregory 2003; Coyle *et al.* 2005a; Ward *et al.* 2005; Virtanen, Korpelainen, and Kostamo 2007; Aquila *et al.* 2014; Cofré González and Valdivia Moraga 2014; Wiltshire *et al.* 2015; Šumberová and Ducháček 2017; Ochando *et al.* 2018; Song, Yang, and Choi 2020; Pramod, Kumar, and Nasir 2021).

In Forensic Botany and Geology, the main goal of the comparisons is associating (or excluding) traces of inorganic and organic elements of unknown origin collected on bodies, victim's and suspect's belongings, motor vehicles, and work instruments, with (or from) soils/sediments of known origin collected on the crime scenes, suspect' homes, work sites, or other sites of investigative interest (Ruffell and McKinley 2008; Di Maggio *et al.* 2013; Somma 2023a). The forensic comparison is performed between the combinations of properties, selected from the known and unknown samples for comparison, that best represent the characteristics of the material under investigation. The question of which and how many properties should be selected obviously depend on the type of materials examined, if inorganic (minerals, rocks, fossils), anthropogenic (bricks, concrete, glass, plastic, fibres, paper), or organic (animal and vegetal remains).

The principle on which forensic scientists rely is that, as a result of the interaction between two objects or bodies, the contact inevitably results in a transfer of physical traces (Locard's principle). These traces, inorganic or organic (animal or vegetal), allow the connection between the two objects or bodies that have interacted and, consequently, of a person (victim or suspect) with the scene of the events. Therefore, geological and soil evidence should better be examined by a team of forensic geologists and botanists to obtain as much information as possible from such traces (Somma *et al.* 2023c).

As for vegetal traces, experts of both systematics and anatomy of plants are required. Plant systematics is a broad discipline including the study of evolutionary relationships between plant species and their taxonomic identification (Bates, Anderson, and R. D. Lee 1997; Gerola *et al.* 2006; Tripodi 2006; Judd *et al.* 2019; Balouet *et al.* 2021). Typically, taxonomic identification is the first approach in forensic analysis of plant evidence (Bhatia *et al.* 1973; Bock and Norris 1997; Caccianiga and Compostella 2009; Biswas, Gupta, and Talapatra 2016; Bock and Norris 2016b,c). It goes in concert with the anatomy of plants, which can serve both to aid in the identification of species as well as directly to verify the physical correspondences between traces (Dilcher 2001; Coyle *et al.* 2005b; Aquila *et al.* 2014; Bezerra, Sant Anna Cavalcante, and Abreu Lima 2020; Caccianiga *et al.* 2021a). Vascular plants are combinations of various structures, such as roots, stems, leaves, flowers, fruits, and seeds, which have unique morphologies and anatomical structures, albeit often fragmented or microscopic (Bock and Norris 1997; Hall and Stern 2012; Evert and Eichhorn 2013; Bock and Norris 2016a).

In forensics, the contribution of systematic botany to the analysis of forensic soils, in which the plant component may be easily present (*e.g.*, as fragments of plants, pollen, microalgae including diatoms, mycological fragments, *etc.*), should not be neglected (Bhatia *et al.* 1973; Picozzi and Intini 2009; Ezegbogu 2021). The soil is a complex system where, together with inorganic and anthropogenic components, the organic component is generally very abundant (Hopfensperger 2007; Hall 2012b). The animal component shows the occurrence of almost exclusively invertebrates (both adults and all developmental stages of insects, nematodes, arachnids, molluscs, and annelids), but also fragments of exoskeletons, excrements, hair, and feathers of *vertebrata* (Hall 1988). The vegetal component is made up of small plant traces, especially if widespread in quantity, as in the case of small-sized seeds or fruits, pollen, spores, and thorns. These materials may easily adhere and transfer to the clothing or footwear of the crime actors (Ladd and H. C. Lee 2005; Bojňanský and Farkašová 2007; Caccianiga *et al.* 2021b; Kasprzyk 2023) and consequently may acquire determinant significance in the investigations. However, due to the high fragmentation of plant structures, their taxonomic identity often relies only to skilled experts.

The present paper deals with a Forensic Botany investigation, implementing a Forensic Geology casework, carried out on the vegetal traces found on the corpses of two subjects disappeared in a rural area a few years ago. These traces were compared with plants thriving in the area of finding of the corpses and vegetal component of the soils present there. Among the identified traces of plant remains, hundreds of thorns were found on the victims' belongings. Thorny appendages are pointed structures mostly developed by plants as a defense against herbivores (Abdelahad *et al.* 2014). They are generally sclerified by the apposition of lignin, suberin, and polyphenolic metabolites, which give them high resistance to both physical-chemical and microbial attacks (Di Stefano *et al.* 2012). Such peculiar characteristics together their aptitude to easily transfer from the sites to the people, make thorns excellent forensic vegetal traces as they may resist to degradation and are detectable after time. Thorns collected in the geological and soil traces found in the victims were identified on the base of morphological characters and compared with similar plant appendices produced by the species present on the site of finding in the countryside. These botanical data, evaluated together the geological evidence, were used in order to track the victims' presence in the crime scene and their possible walking route.

2. Case Study

Two individuals, disappeared from their home, were suspected to be kidnapped. Their corpses (victims 1 and 2) were found in the countryside not too far from the last sighting site (Somma *et al.* 2023c). Evidence of inorganic and organic traces were found on the victims. The judicial authority asked to one of the authors (R.S.) to ascertain if the victims walked or did not on the site of finding.

3. Materials and Methods

Forensic Botany deals with principles, techniques, and methods of the botany, in order to assist law enforcements, judicial authority, and lawyers for the solution of criminal cases (Saferstein 2017; Morabito and Somma 2023). Fragments or individuals of terrestrial plants, algae, or fungi may be investigated with the aim to link items or the crime actors to the crime scene, the scene of events, or sites of investigative interest (Somma *et al.* 2023c). Analogously, Forensic Geology (also known as Geoforensics and Forensic Geosciences) applies principles, techniques, and methods of the earth sciences for solving criminal cases (Murray 2004a).

Botanic investigation accomplished for this casework was devoted to the study of species producing thorns, spines, prickles, and other pointed structures (Gerola *et al.* 2006), hereinafter referred to as "thorny plants", given their ease of transferring by contact to footwear, clothing, and bodies. With regard to the descriptions of these thorny plants, it was chosen, for the sake of simplicity of reading, to use the generic term "thorns" to indicate pointed plant structures, although aware that the use of the term "prickle" should have been differentiated for the spiny epidermal structures of *Rosa* and *Rubus*, “thorns” strictly for stem-derived structures, and "spines" for other structures derived from leaves, petioles or stipules (Crang, Lyons-Sobaski, and Wise 2018).

Plant evidence was searched, identified, sampled, and analyzed first of all in the geological and soil traces found on the victims (Morabito and Somma 2023; Somma *et al.* 2023c). Control samples were collected from the soils and the plants growing in the scene of events. Plants were examined in the study territory, firstly with a remote sensing approach observing aerial imagery (Ruffell and McKinley 2008; Donnelly *et al.* 2021; Spoto, Somma, and Crea 2021; Somma *et al.* 2023c; Spoto, Barone, and Somma 2023) and secondly carrying out several botanic surveys during judicial inspections. Plant samples were photographed *in situ* and any character considered useful for the investigations, such as the morphology of living plants and the appearance of the sampling site, was annotated. Significative plant fragments, in the purpose of taxonomic identifications, were then collected and preserved simply by pressing and drying. Collection sites of both plants and soils were georeferenced with GPS and sampling activities were carried out and repeated in a time interval of one year.

The vegetal component found in the soils and in the geological traces was separated from the inorganic one. It, analysed by forensic botanists, resulted to be composed of seeds, fruits, leaves, stems, roots, rhizomes, flowers, thorns (included prickles and spines), in entire forms or fragments of them. Thorny elements fixed in the shoes were extracted by means of dental and surgical instrumentation. A comprehensive description of thorny plant as they appeared *in situ* as well as of their thorny elements extracted from the victims’ belongings was made, and explanatory pictures were captured, both *in situ* and in the laboratory under the stereoscopic microscope.

In the laboratory, for each collected thorny plant and thorny element (hundreds of samples), morphological analyses and taxonomic identifications were performed, based on: i) macroscopic observation, ii) stereomicroscopic analysis (Figure 1), and iii) extensive biometric analysis. In particular, the following different characters of the thorns were observed and recorded: i) morphology, ii) size, iii) color. Each element was manually analyzed and measured under stereoscopic microscope (Stereo Discovery V20 with camera Zeiss) with the aid of a software dedicated to image analysis, and SEM-EDS system (Scanning Electron Microscope - QUANTA FEG 450 model - FEI, operating in low vacuum, chamber pressure of 50 Pa, at 20.00 kV with AMETEK EDS system and workstation).

For the taxonomic identification and the nomenclature of species, reference was made to the analytical keys and to the treatment contained in Pignatti (2017-2019), and to the web resources available on *Acta Plantarum* (2007), continuously updated.

Geological investigation accomplished for this casework was carried out on the inorganic traces found on the bodies and their belongings in laboratory and on the inorganic component of the soils collected *in situ*. Forensic geologists accomplished sedimentological, morphoscopic, morphometric, and mineralogical analyses by means of the stereoscopic microscope and the SEM-EDS system previously reported.

4. Results

4.1. Forensic Botany. The investigated site fell within an area of temperate-warm Mediterranean maquis vegetation, dominated by evergreen *sclerophyllous* species, widespread from the sea level to around 1,000 m of altitude, and associated with other species with a prevalent Steno- and Euri-Mediterranean distribution. The Mediterranean maquis is a typical plant formation of the coastal areas of the Mediterranean Sea and of a few other regions with a similar climate (L. A. Gianguzzi 2007; L. Gianguzzi, Papini, and Cusimano 2016).

In the investigated area, four zones with different botanical characteristics were distinguished.

The first one was an extended reforestation area, where shrub species predominated, such as Spanish broom (*Spartium junceum* L.), lentisk (*Pistacia lentiscus* L.), strawberry tree (*Arbutus unedo* L.), prickly pear [*Opuntia ficus-indica* (L.) Miller], spiny broom [*Cytisus infestus* (C. Presl) Guss.], with tree species, such as olive (*Olea europaea* L.), cork oak (*Quercus suber* L.), holm oak (*Quercus ilex* L.), pear tree (*Pyrus communis* L.), oleander (*Nerium oleander* L.), and exotic *pittosporum* [*Pittosporum tobira* (Thunb.) W.T. Aiton]. In addition, there were some thorny lianas, such as the evergreen rose (*Rosa sempervirens* L.), the bramble (*Rubus ulmifolius* Schott), the sarsaparilla (*Smilax aspera* L.), together with a population of wild reed (*Arundo plinii* Turra).

A second area was characterized by the meadow produced by intense anthropic pasturage, where non-grazed thorny grasses predominated, such as *Galactites tomentosus* Moench, *Cynara cardunculus* L. subsp. *cardunculus*, and thistles belonging to various genera (*Carduus* sp., *Sylibum* sp., *Carlina* sp., *Cirsium* sp., *etc.*), rare scattered shrubs of wild plum (*Prunus spinosa* L.), shrubs of hawthorn (*Crataegus monogyna* Jacq.), abundant wild pear (*Pyrus spinosa* Forssk.), and rare presence of sarsaparilla (*Smilax aspera* L.).

The third and fourth areas were peculiar plant formations influenced by the anthropic action. An area dominated by cork oak (*Quercus suber* L.) graded into a shrub formation with Mediterranean maquis, with a continuum of intermediate structures (maquis forest) or degraded forms. In the cork forest (*Quercus suber* L.), more or less tall shrubs and lianas occurred, which in some places made it impenetrable (forest maquis), especially in the passage towards the Mediterranean maquis. The shrub species associated with the cork oak were lentisk (*Pistacia lentiscus* L.), spiny broom [*Cytisus infestus* (C. Presl) Guss.], *phillyrea* (*Phillyrea latifolia* L.). Low shrubs were Montpellier cistus (*Cistus monspeliensis* L.) and butcher's broom (*Ruscus aculeatus* L.). Sarsaparilla (*Smilax aspera* L.) predominated among thorny lianas, while evergreen roses (*Rosa sempervirens* L.) and brambles (*Rubus ulmifolius* Schott) were less common. In the cork forest widespread tree heath plants (*Erica arborea* L.) also occurred. In some open areas, wooded meadow (*dehesas*) where thorny species occurred, such as wild pear (*Pyrus spinosa* Forssk.), hawthorn (*Crataegus monogyna* Jacq.), and wild plum (*Prunus spinosa* L.) were present.

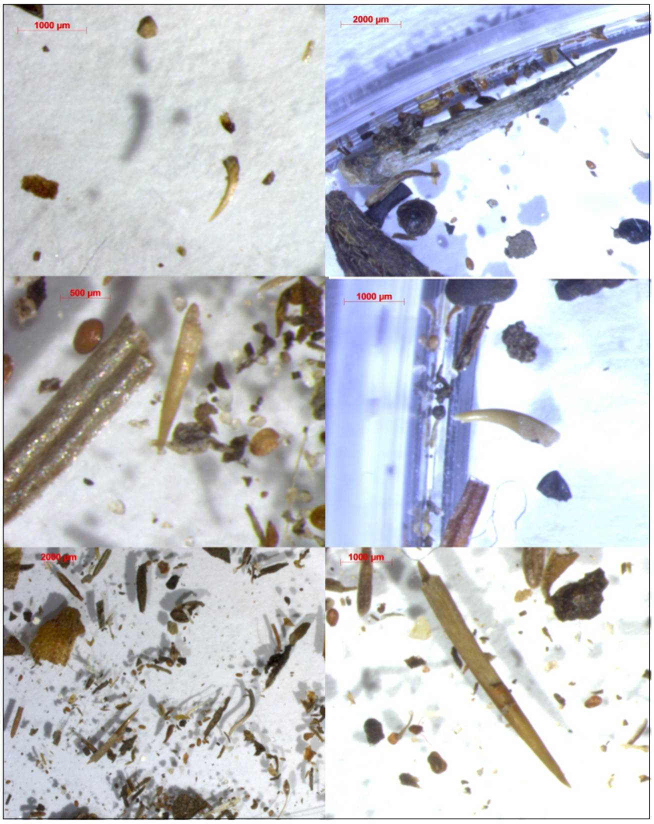

FIGURE 1. Vegetal component including thorny elements taken from the soil traces found inside the shoes of victim 1.

Given the acidic substrate, composed of quartzarenites, other species, such as holm oak (*Quercus ilex* L.) and downy oak (*Quercus pubescens* Willd.), were rarely encountered. The Mediterranean maquis formed an impenetrable tangle of shrubs and small trees no taller than 4-5 m, almost exclusively evergreen and *sclerophyllous*, with a rich contingent of species, such as myrtle (*Myrtus communis* L.), lentisk (*Pistacia lentiscus* L.), spiny broom [*Cytisus infestus* (C Presl) Guss.], rhamnus (*Rhamnus alaternus* L.), strawberry tree (*Arbutus unedo* L.), Montpellier cistus (*Cistus monpeliensis* L.), *phillyrea* (*Phillyrea latifolia* L.), and wild olive (*Olea europaea* L. var. *sylvestris*); among the lianas there were sarsaparilla (*Smilax aspera* L.). In the small clearings inside the maquis there were blackthorns or wild plums (*Prunus spinosa* L.), hawthorns (*Crataegus monogyna* Jacq.), and wild pears (*Pyrus spinosa* Forssk).

The degraded area of the Mediterranean maquis was characterized by low plants with a floristic composition very similar to that previously described even if the relationships between the species changed. Cistus (*Cistus monspeliensis* L.) dominated the area, wild pear (*Pyrus spinosa* Forssk) was more widespread, while evergreen rose (*Rosa sempervirens* L.) and bramble (*Rubus ulmifolius* Schott) were present as very low shrubs. Moreover, being a degraded maquis, clearings, dominated by herbaceous species, also occurred, especially annuals (*therophytes*), such as thistle (*Galactites tomentosa* Moench) and various graminaceous plants, among which the most abundant was *Cynosurus echinatus* L. Rare plants of *Erica arborea* L. were present.

In summary, in the investigated area, eight main thorny species were identified: *Crataegus monogyna* Jacquin, *Prunus spinosa* Linnaeus, *Pyrus spinosa* Forsskål, *Rosa sempervirens* Linnaeus, *Rubus ulmifolius* Schott (*Rosaceae*), *Cytisus infestus* (C. Presl) Gussone (*Fabaceae*), *Smilax aspera* Linnaeus (*Smilacaceae*), and *Cynara cardunculus* Linnaeus (*Asteraceae*).

The vegetal traces, collected from the soil present in the belongings of victim 1, consisted of five thorny species (eighty-one thorns). *Rosa sempervirens* L., *Rubus ulmifolius* Schott, *Cynara cardunculus* L., *Cytisus infestus* (C. Presl) Gussone, *Smilax aspera* L., and fragments identified at the subfamily level as *Rosaceae Amygdaloideae* (Figure 1) were recognized.

The vegetal traces, collected from the soil present in the belongings of victim 2, consisted of three thorny species (sixteen thorns). *Cynara cardunculus* L., *Cytisus infestus* (C. Presl) Gussone, *Smilax aspera* L., and fragments identified as either *Rosa sempervirens* L. or *Rubus ulmifolius* Schott were recognized.

4.1.1. *Comparative analyses.* Comparative analyses of the vegetal trace found on the victims with that collected on the soils and plants of crime scene were particularly focused on thorny elements. The eighty-one and sixteen thorns found on victim 1 and 2, respectively, belonged to thorny plants recognized also in the investigated area.

4.2. Forensic Geology. Siliciclastic sands and silts made up of grains of quartz with hematite coatings and a minor amount of grey clay minerals were identified in the traces sampled on both the victims and their belongings. Peculiar grains of dolostones were also found on the shoes of victim 2. Analogous features were ascertained in the specimens of soil sampled on crime scene.

4.2.1. *Comparative analyses.* The results of the comparative analyses, related to the compositional and textural features of the inorganic component of the soil traces found on the victims and in the soil particles collected on crime scene, suggested an elevated degree of compatibility.

5. Discussion and Conclusion

In crimes occurring outdoor in the countryside, being vegetation widespread, the application of modern technologies for the lecture of the landscape, involving remote sensing (Ruffell and McKinley 2008; Di Maggio *et al.* 2013; Somma *et al.* 2018; Somma and Costa 2022, 2023) and laser scanner surveys are essential (Baldino *et al.* 2023; Somma *et al.* 2023a,b). Many are the forensic disciplines that may assist forensic botanists and geologists in such complex investigations (Lane *et al.* 1990; Ruffell and McKinley 2008; Hermanson and Wiedenhoeft 2011; Hall 2012a; Georgolopoulos, Parducci, and Drouzas 2016; Loppi 2019; Woodenberg *et al.* 2019; Lens *et al.* 2020; Jakoby *et al.* 2021; Spencer

2021; Yadavalli and Ehrhardt 2021; Somma *et al.* 2023c; Somma, Sutton, and Byrd 2023; Tagliabue *et al.* 2023).

In the field of Forensic Botany and Geology, a rich *plethora* of physical evidence can be significative, coming from the natural environment where the human being can interact during their passages. These evidence include geological and soil traces (Murray and Tedrow 1975; Marumo 2003; Murray 2004a,b; Ruffell and McKinley 2008; Magni and Di Maggio 2013; Somma 2023a; Somma *et al.* 2023d; Spoto 2023) containing fossils (Ruffell and McKinley 2008; Marra 2023; Marra, Di Silvestro, and Somma 2023; Somma and Maniscalco 2023), animal (Catts and Haskell 1990; Norris and Bock 2001; Hunter 2006; Wiltshire 2009; Gunn 2019; Cholewa, Bonar, and Kadej 2022; Byrd and Sutton 2023), and plant remains (Britton and Greeson 1987; Codd *et al.* 1999; Coyle *et al.* 2005b; Boyd 2006; Brown 2006; Craft, Owens, and Ashley 2007; Dev and Kasana 2007; Caccianiga, Bottacin, and Cattaneo 2012; Hard and Wallace 2012; Margiotta *et al.* 2015; Dourel *et al.* 2017; Brabazon *et al.* 2020; Caccianiga *et al.* 2021b; Morabito and Somma 2023) remains.

Comparative analyses of the association of the thorny plant remains extracted from the unknown samples allowed to identify the many thorns and thorn fragments found on clothes and footwear of both victims, as thorny plants thriving in specific sites of the area under investigations. Analogously, comparative analyses on the composition and texture of the inorganic grains associated with the vegetal component, allowed to link inorganic traces found on the bodies, clothes, and footwear of both victims to specific areas of the crime scene.

In the present investigation, the analysis of thorns in the plant component of the geological and soil traces was crucial. This investigation, based on the survey of the punctual distribution of individual plants and on the comparative examination of vegetal and geological remains found on the victims and on the sites, allowed to reconstruct the path followed by the two subjects in the hours immediately preceding their death. The detailed morphological description and the biometric data of the examined hundreds of thorns and thorny plants, together with the related photographic documentation, proved to be very useful in the identification of plant traces in the forensic case and, as a consequence, strongly implemented the geological investigation providing valuable and probative information to the judicial authorities.

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Author contributions

Conceptualization, M.M., F.M., R.S.; methodology, M.M., F.M., R.S.; software, M.M., F.M., R.S.; validation, M.M., R.S.; formal analysis, M.M., F.M.; investigation, M.M., F.M., R.S.; resources, M.M., F.M., R.S.; data curation, M.M., F.M., R.S.; writing original draft preparation, M.M., R.S.; writing review and editing, M.M., R.S.; visualization, M.M., R.S.; supervision, M.M., R.S. All authors have read and agreed to the published version of the manuscript.

Competing interests

The authors declare no conflict of interest.

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