Heliyon 6 (2020) e03386

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

When scientists become detectives: investigating systematic tree poisoning in a protected cove



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ARTICLE INFO

Keywords: Dendroecology Dendrogeochemistry Tree wounding Quantitative wood anatomy Acer pseudoplatanus Juglans regia Applied ecology Flora Environmental assessment Environmental health Environmental toxicology Environmental science

ABSTRACT

The systematic killing of trees is usually aimed at eradicating pests or alien plant species susceptible to harm existing natural ecosystems. In some cases, trees may become the subject of dispute between neighbors, which sometimes ends in tree death after months or years of dispute. In this paper, we analyze a case of clandestine tree killing and look into ways through which evidence left by delinquents can be analyzed a posteriori with state-ofthe-art approaches. The investigation presented here looks at a series of old-growth trees that were supposedly poisoned inside a protected, nineteenth century grove in Switzerland. After the sudden, unexplained death of several old Black poplar (Populus nigra) trees along the main alley in fall 2015 and their subsequent removal, the dying of five additional, neighboring Sycamore maple (Acer pseudoplatanus) and English walnut (Juglans regia) trees in 2016 promptly triggered a suite of criminal investigations at the property. During an initial inspection, a large number of boreholes was found in the root plates of the dying trees. We present findings obtained from treering, wood anatomical and dendrogeochemical investigations performed on root, stem and leave material from the assumedly poisoned trees and show that massive amounts of chemical elements - supposedly in the form organic pesticides with high Al, As, Fe, Cr, Ni contents, aluminum phosphides or glyphosate-based pesticides were injected into 36 boreholes drilled into the roots around September 2016. Results obtained in this study are currently used in criminal investigations, and are a nice example of how scientific detectives can help their "real World" colleagues in identifying delinquents.

1. Introduction

The systematic killing of trees and shrubs is often aimed at eradicating pests or any kind of alien plant species from an environment where they are expected to harm existing, natural ecosystems (Crowley et al., 2017; McAlpine et al., 2016). Tree and shrub removal can be realized by physically cutting the undesired species (Hulme, 2015; Muzika, 2017), or by removing the bark completely around its circumference, a method called "ring barking" or "girdling" (Choi et al., 2010; Merceron et al., 2016; Noel, 1970). Immediately after ring barking, most trees have sufficient carbohydrate reserves in root cells to maintain an active cell metabolism and thus root growth. However, these reserves are gradually consumed, such that growth will cease and root cells will start to starve from a lack of carbohydrate (Salisbury and Ross, 1992; Taiz and Zeiger, 2002). Water and nutrient uptake is then affected and the tree starts to shed foliage, and finally, the plant above the zone of ring-barking dies, which may result in the death of the whole plant. Other, more sophisticated treatments include the specific application of herbicides to the trunk, bark or foliage (Conover et al., 2016; Hata et al., 2016; Sher et al., 2018). A direct pathway is, however, needed for herbicide to enter the plant's vascular tissue. Such pathways can, for instance, be achieved by making a series of downward cuts through the bark around the entire circumference of the tree trunk.

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https://doi.org/10.1016/j.heliyon.2020.e03386

Received 8 July 2019; Received in revised form 15 November 2019; Accepted 4 February 2020

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Trees are also a frequent subject of dispute between neighbors (Doskow and Guillen, 2017). Tree disputes vary in character and complexity because of ecological and interpersonal factors. For a tree owner, a tree might provide shade, privacy, support for wildlife and amenity to their property. However, for a neighbor, the same tree may become a cause for concern if it is likely to damage their property or cause harm to its occupants. Tree disputes may thus occur due to (i) falling leaves, pollen and fruits becoming a nuisance, (ii) tree encroachment over boundary lines, (iii) an entire tree falling across boundary lines onto neighboring land or (iv) simply because a tree blocks views and/or obscures a property. A quick internet search points to numerous cases in which trees have died for unknown reasons after months or years of dispute. In the past, copper nails have often been used to kill trees as they allow a combining the negative effects that physical, chemical, and biological disturbances have on tree vitality (Dujesiefken et al., 2005b, 2005a; Dujesiefken and Rhaesa, 1999), and as they do not necessarily leave clear evidence on the principle or single cause of death. More recently, several cases have been reported where herbicides, pesticides or other poisonous substances have been employed to illegally kill vascular plants. Interestingly, however, and while a large body of literature exists on poisoning of people and animals by trees (e.g., Ferreiro et al., 2010; Guha, 2011), we are currently unaware of any papers addressing the clandestine killing of trees and ways through which evidence left by the delinquents can be analyzed a posteriori with state-of-the-art approaches.

This paper therefore aims at investigating a series of old-growth trees that were supposedly poisoned inside a heavily protected, nineteenth century grove in Switzerland (Figure 1), with the assumed goal to enhance visibility of scenic landscapes from a series of residential homes. After the unexplained death of several old Black poplar (*Populus nigra*) trees along the main alley in fall 2015 and their subsequent removal, the dying of five additional neighboring Sycamore maple (*Acer pseudoplatanus*) and English walnut (*Juglans regia*) trees in autumn 2016 promptly



Figure 1. Overview of the locations of *Acer pseudoplatanus* and *Juglans regia* trees poisoned in 2016 (red stars) in a 19th century grove in Switzerland. Note that in 2015, several *Populus nigra* trees (black stars) died abruptly and were since removed from the site by the property owners. Trees sampled for comparison (i.e. undisturbed reference trees without any boreholes in their root plates or any other signs of anthropogenic disturbances) are marked with C.

triggered a suite of criminal investigations at the property. During a first inspection, a large number of boreholes was found in the root plates of the dying trees. This unexpected finding prompted a request from the owners to assist detective investigations with scientific analyses. In this context, we were asked to: (i) assess tree vitality before the criminal act and the timing of tree poisoning; (ii) quantify the amount and spread of potentially harmful substances in the root, stem and leave systems; and to (iii) infer possible hypotheses for tree poisoning. The paper is organized along these three research questions. For each of them, we provide the scientific basis used before the relevant findings are presented. The wider implications of this work are discussed briefly in a separate chapter. Unlike other papers, and to protect anonymity of potential suspects, we do not provide a study site description as criminal investigations are still ongoing at the time of paper writing.

2. Field inspection and sampling design

Field inspection and sampling of affected trees took place in November 2016 and after first signs of dieback were reported in three *A. pseudoplatanus* (T1, T2, T5) and two *J. regia* (T3–T4) trees (Figure 2). All trees showed drill holes in the exposed roots with a constant diameter of 4 mm and drill depths ranging from 3–5 cm. The number of holes per tree varied between 4 (T5) and 10 (T3), for a total of 36 detectable holes. The varying depths correspond roughly with the root radius, such that damage was limited mostly from the bark to the root pith, but not beyond.

After the mapping of boreholes, we extracted the liquid contained in 15 voids with disposable pipettes (Figure 3) and stored it in small containers with 10% supra-pure nitric acid at 4 °C. Further material was then sampled from the root, stem and leave systems as follows: five wedges were cut from the affected roots with a saw to obtain contaminated bark and root wood samples for dendrogeochemical analyses. We also selected 10 increment cores at breast height (*c*. 130 cm above ground) to analyze the spread of chemical components from the roots to the stem, before adding five leaves from each tree to the sample collection. As with the liquid, all samples were stored at 4 °C. In addition to dendrogeochemical analyses, the root wedges and increment cores were also used to determine growth conditions of the five trees prior to the assumed poisoning and for the intra-seasonal dating of the criminal action.

3. Tree vitality and the timing of tree poisoning

Symptoms of declining tree health and dieback can be caused by a wide range of factors other than poisoning, such as changes in climate (Choat et al., 2018), environmental conditions (Fazan et al., 2017; Madrigal-González et al., 2017), mechanical injury (Stoffel et al., 2005; Stoffel and Bollschweiler, 2008; Stoffel and Klinkmüller, 2013), nutrient deficiency (Gessler et al., 2017), defoliating insect attacks (Das et al., 2016; Saulnier et al., 2017; Panzavolta et al., 2018), nematodes (Calvão et al., 2019; Eisenback et al., 2015), fungi (Crous and Wingfield, 2018; Schmidt, 2006), or viruses (Agrios, 2004). Usually, whenever a vascular plant is experiencing stress due to one or several of these factors, it will become less resilient and thereby more susceptible to disease (Anderegg et al., 2015; Camarero et al., 2015; Navarro-Cerrillo et al., 2019). In the case of drought, Choat et al. (2018) and McDowell et al. (2008) thus demonstrated that partial disruption of water transport and the regulation of water loss from plants lead to increased mortality through the depletion of carbohydrate reserves used in respiration and to increased vulnerability to pests and pathogens. Before confirming the assumed fatal effect of poisoning, we therefore analyzed tree growth rates in the three A. pseudoplatanus and two J. regia trees. This was done by measuring annual growth rings - with a stereomicroscope coupled to a Lintab measuring device - and visually comparing the resulting growth curves to those of undisturbed reference trees (marked with a C in Figure 1). As shown in Figure 4a, the reference trees and the five dying trees show very similar interannual variations and any sign of declining tree growth is



Figure 2. The five Acer pseudoplatanus and Juglans regia trees started to show signs of dieback in autumn 2016 as a result of poisoning. The order of tree pictures corresponds to the numbering in Figure 1; trees T1, T2 and T5 are A. pseudoplatanus, T3 and T4 are J. regia.



Figure 3. (left) Close-up of one of the 36 boreholes identified in the five Acer pseudoplatanus and Juglans regia trees with discolored bark; (right) Extraction of the assumed poison from a borehole of T1.



Figure 4. (A) Changes in tree-ring width in the five poisoned trees (labeled T-01–T-05). The generally decreasing ring-width values are a typical sign of ageing trees (senescence); (B) Evolution of vessel numbers in the tree rings formed prior to the assumed poisoning in 2016 (values are given in the form of z-scores). Note that none of the five dying trees shows any sign of declining health in its ring or vessel patterns, not even in the 2016 growth ring.

clearly absent in the records prior to the poisoning. Differences in productivity between individual trees of course exist, but these are related to different basal areas of trees, differences in overall biomass, in soil conditions or presumably also to senescence effects (Vospernik et al., 2015). As other signs of external stress and/or evidence of mechanical injury (other than the recent boreholes) are clearly missing, we conclude that the abrupt decline in tree health and the sudden dieback are indeed related to the boreholes and the assumed poisoning.

Based on the analyses of tree-ring widths in the poisoned trees, first conclusions can be drawn on the timing of borehole infliction and poisoning. Because none of the trees shows a growth decline, not even in the 2016 growth ring, we may assume that the criminal act must have been committed at the end of summer or early fall 2016. To confirm this first-order hypothesis, we then analyzed (i) wood anatomical features in the dying trees and (ii) determined the moment of borehole infliction within the last ring (i.e. 2016) formed by the root.

To this end, root wedges were polished with increasingly finer sandpaper to facilitate the reading of rings and to determine the moment at which the holes have been drilled with high precision. In fact, trees start to overgrow injuries within days to a few weeks after wound infliction (Schneuwly et al., 2009), provided that wounding occurred during the growing season of trees (Stoffel and Hitz, 2008; Stoffel and Perret, 2006), which locally lasts from mid-May to early October (Kollas et al., 2014). As a result, any injury inflicted to a tree within the growing season can be dated with sub-annual, and sometimes even with monthly resolution (Arbellay et al., 2010a, 2010b; Schneuwly-Bollschweiler and Stoffel, 2012; Stoffel and Beniston, 2006).

The intra-annual position of the scars in all five roots analyzed points to a drilling that occurred at the very end of the growing season, presumably in September 2016. In fact, and while the tree started to compartmentalize the surfaces surrounding the borehole (Figure 5a) by forming barrier zones around the zone affected by the drilling and the



Figure 5. Examples of root wedges and bark pieces used to narrow down the intra-seasonal timing of borehole drilling and tree poisoning: (A). Drillhole in T4 (Juglans regia) with first signs of wound-induced discoloration and the occurrence of barrier zones (compartmentalization; in yellow). Evidence of discoloration stemming from a second hole in the same root can be found to the left; (B). Detail of a borehole in T1 (Acer pseudoplatanus) with xylem tissues and a limited evidence of callus tissue formation (white circle), pointing to injury very late in the growing season; (C) Desiccated phloem around the borehole in T5 (Acer pseudoplatanus), presumably induced by cambium damage during drill removal.

injection of a poisonous substance, the roots did barely have the time to overgrow the scar and to form chaotic callus tissue. As can be seen in Figure 5b, the drilling (and probably even more so the removal of the drill) has favored cambium damage beyond the outer limit of the borehole and a dissociation of the phloem (bark) from the xylem (wood). This dissociation can also be seen in Figure 5c, where the disruption of the cambium from the xylem and phloem has resulted in a marked desiccation of phloem fibers around the borehole. In trees T1, T5 (both *A. pseudoplatanus*) and T3 (*J. regia*), a tiny band of chaotic callus tissue has visibly formed at the contact of the wound with the intact cambium, whereas neither xylem nor phloem tissues were formed after wounding in trees T2 (*A. pseudoplatanus*) and T4 (*J. regia*).

Wood anatomical features are increasingly used in tree-ring research, either to complement information contained in tree-ring width or to generate evidence on environmental changes (von Arx and Carrer, 2014; Wegner et al., 2013). In broadleaved tree species, vessels have been shown to record climatic (García-González and Fonti, 2008, 2006; Souto-Herrero et al., 2017) and hydrological (Kames et al., 2016) changes, often with seasonal resolution. In addition, a sharp reduction both in vessel number and diameter have been observed and thus employed to detect evidence of tree wounding (Arbellay et al., 2012a, 2012b; 2013; Tumajer et al., 2015). To assess potential changes in vessel parameters, we used a chisel to prepare small wood cubes (c. $0.5 \times 2 \times 2$ cm) from root segments showing the contact between the wound (borehole) and the intact wood. Wood samples were wetted to facilitate cutting before micro-cuts (15 µm) were prepared from the cubes using a sliding microtome. The cuts were prepared further by applying standard procedures, i.e. rinsing and staining with safranin, astrablue, and alcohol (from 50° up to absolute). The micro-cuts were then mounted on supports and fixed with Canada Balm, before they were dried in an oven for 48 h. Pictures were taken from the samples with a $25 \times$ magnification so as to allow quantitative wood anatomical analysis using WinCell. Vessel number and vessel size are reliable indicators of the hydraulic performance of a tree and consequently of its health. In the case of the poisoned trees, we do not observe any changes in vessel number (Figure 4b) or size, not even in the ring formed just prior to sampling, such that the assumption of a poisoning very late in the growing season 2016 can be underlined further.

4. Detection and quantification of anomalies chemical elements in trees

Exposure of trees to high concentrations of non-essential heavy metals, as well as to essential elements present in excess, has toxic effects and may thus cause physiological damage, such as cellular oxidative stress and tree viability (Rascio and Navari-Izzo, 2011). The effects depend on the nature and amount of metals accumulated in the tree

system as determined by the uptake, transport, and sequestration of the different elements (Burken et al., 2011). A number of studies has shown the ability of trees to take up and incorporate pollutants into their annual growth rings so that the accumulation of pollutants in tree rings may reflect to some degree the variation of pollutant concentrations in the environment at the time of tree-ring formation. The sequestered metals in different compartments of a tree system have been therefore used previously to assess past and present environmental pollution (Burken et al., 2011) or in the case of phyto-screening in environmental forensics (Balouet et al., 2009). Anomalies in the concentrations of chemical elements in the assumedly poisoned trees were analyzed in samples taken from the root, stem and leave systems of poisoned and reference trees. Sample preparation of root wood, root bark, tree wood, tree bark and leaves followed a procedure modified from the one described by Watmough and Hutchinson (2003, 2002). In brief, we dried samples at 60 °C during 72h, and then samples were dry ashed at 400 °C for 6h. Ashes were transferred into metal-free tubes and digested with concentrated HNO3 (Merck, suprapur) for 24h, filtered and diluted with MilliQ water for further analysis. This then allowed determination of trace elements of interest in root wood, root bark, tree wood, tree bark, leaves as well as the liquids contained in the boreholes from which samples were taken Aluminum (Al), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), zinc (Zn), arsenic (As), cadmium (Cd) and lead (Pb) were quantified. Results are given here according to the multi-element calibration (ICP VI, Merck KGaA) and Rh was used as an internal standard. The precision and accuracy of results were confirmed by repeated analysis of the certified reference material BCR®-482. Results were then compared in terms of differences between the poisoned and the control samples lacking evidence of boreholes or other signs of poisoning. In doing so we hypothesize that any possible unusual increase in concentrations in the poisoned trees of the elements listed above would be the result of exposure of the poisoned trees to alien substances, either in the form of major components, or as impurities. Given that all samples originate from trees of same area, growing on a surface of roughly 200 m², and more often even within a few meters from each other, environmental factor differences are considered as negligible in this study.

As tree poisoning was realized through boreholes in roots, highest element concentrations were found in the root system, and in particular in root bark. Figure 6 illustrates that the determined elements were present in significantly greater concentrations in the poisoned than in the reference trees, especially in the *A. pseudoplatanus* tree T2 where concentrations of *Al, Fe, Cr,* and *Ni* were 50–65 times higher than in control trees, and the increase of *As* was 88 times higher than normal for the study site. In the case of the other elements (*Co, Cu, Cd, Mn, Pb, Zn*) and trees (T1, T3-T5), the increase in the poisoned trees was unusually high as well by exceeding the concentrations found in the control trees by up to 20 times.



Figure 6. Fold-increase of eleven of the selected chemical elements in the root bark of poisoned trees. Results are given as ratios between elements measured in the five poisoned trees $[M]_T$ and the control trees $[M]_C$. Note that all chemical elements presented here show (much) higher concentrations in the poisoned than in the control trees. Concentrations were generally highest in tree T2, so the y-scale is different. All data – including for other parts of the trees analyzed – can be found in Supplementary Table S1.

Interestingly, concentrations were also high in stem bark, even if in much smaller concentrations (Supplementary Table S1). By contrast, weaker values were recorded in root wood, stem wood and in leaves, for which values were comparable, but with slightly different metal concentrations. These differences can be explained on the one hand by the timing of borehole drilling in the roots which occurred very late in the growing season (most likely in September 2016), meaning that virtually no time was left between the poisoning and the cessation of wood formation and nutrient transport from the root system to the stem and leave systems. In addition, the infliction of holes, or wounds in more general terms, leads to the formation of barrier zones, which may have hampered the transport of chemical substances through stem (xylem) tissues and leaves further. By contrast, the porous root bark (phloem) absorbed the poisonous substances more readily than did the xylem, possible allowing some transport of chemical elements through the phloem and assumedly to heights of *c*. 130 cm, where evidence of poisoning was clearly present in the stem bark. Interestingly, the liquids sampled in the boreholes did not yield results of high quality, because metal concentrations, unlike in the bark samples, were diluted heavily by rainfall on the one hand and/or no longer available in sufficient quantity due to evaporation. The values measured from liquids were thus much less reliable than those obtained from the phloem (and xylem) samples.

The limited number of samples analyzed here prevents any further interpretation or statistical analyses. As a first glance, however, one may realize that concentrations can vary strongly between trees, but that no pattern emerges between the number of boreholes, species and chemical element concentrations. At the same time, however, one may conclude that the unusually high concentrations of elements measured in all five trees suggest malign human interventions and massive use of poisonous substances.

The chemical elements found are commonly used in a large variety of products, and the substance that has been filled into the drill holes cannot be determined conclusively. Based on the composition of elements measured between the poisoned and control trees, the following options seem plausible: (i) sole addition of aluminum salts (Rout et al., 2001), provided that the other elements found are considered impurities; (ii) use of organic pesticides such as with high metal contents (Gimeno-García et al., 1996); (iii) use of aluminum or other metal phosphides as they commonly used in pesticides such as rodenticide, insecticide, or less likely, a fumigant such as those used for grain storage (Proudfoot, 2009); (iv) use of glyphosate-based pesticides in which heavy metals such as arsenic, chromium, cobalt, lead and nickel have been identified as contaminants in a majority of products (Defarge et al., 2018).

5. Final considerations

In this study, we investigated a series of old-growth *Acer pseudoplatanus* and *Juglans regia* trees that were supposedly poisoned inside a protected, nineteenth century grove in Switzerland. The study shown here was a true detective work and served criminal investigations that are still ongoing. We are unaware of any scientific papers addressing the clandestine killing of trees and ways through which evidence left by delinquents can be analyzed *a posteriori* with combined, state-of-the-art tree-ring, wood anatomy and dendrogeochemical approaches.

At the same time, and in view of the limited number of poisoned trees, the sample size presented in this study is too small for any kind of sophisticated statistical analyses. As such, this study has to be seen as a "best practice" example of how science can help criminal investigations rather than as a contribution to fundamental research. We conclude that the combination of different approaches used in tree and tree-ring sciences represent very valuable tools for criminal investigations, as they allow a narrowing-down of the timing of the poisoning and identification and spread of the harmful substances used in the root, stem and leave systems.

Declarations

Author contribution statement

Markus Stoffel: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Vera I Slaveykova: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Christophe Corona: Analyzed and interpreted the data.

Juan Antonio Ballesteros-Canovas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e03386.

Acknowledgements

The authors acknowledge financial support from the owners of the cove and are grateful for the opportunity they were given to work on such an unusual case. The authors also thank Associate Editor Tsair-Fuh Lin and two anonymous referees for their insightful comments and suggestions.

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