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Original article

Dimensional and genetic characterization of the last oriental plane trees (*Platanus orientalis* L.) of historical sites in Lazio (central Italy)

Mario Ciaffi [a], Anna Maria Vettraino [a], Enrica Alicandri [a], Antonio Tomao [a,b], Francesca Adducci [a], Elena Kuzminsky [a,*], Mariagrazia Agrimi [a]

[a] *Department for Innovation in Biological, Agro-food and Forest systems (DIBAF). University of Tuscia, Via S. Camillo de Lellis, snc, I-01100 Viterbo, Italy*
[b] *Council for Agricultural Research and Economics, Research Centre for Forestry and Wood, Viale S. Margherita, 80, 52100 Arezzo, Italy*

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ABSTRACT

In central Italy, *Platanus orientalis* L. specimens characterize many gardens in urban and suburban villas. In this research, centuries-old oriental plane trees were studied in different historical sites of Lazio according to the COVE (COnservation of VEteran trees) multidisciplinary model. Historical sources, morphological and dendrometric aspects, crown quality, genetic traits of each specimen, as well as their susceptibility to the canker stain disease, were investigated. *Platanus orientalis* was clearly distinguished from *P. occidentalis* and their hybrid *P. acerifolia* through molecular tools. UPGMA analysis based on SSR and ISSR molecular markers clustered the plane trees in different sub-groups, probably according to the different sites of sampling. These findings, supported by historical and morphological data collected in the considered sites, showed that almost all the trees studied are a small remnant of those planted in the period running from the second half of XVI century to the first decades of XVII century. Plant health conditions diverge within and among the sites inspected. Past-prolonged severe pruning treatments of trees located near the main monuments affected their growth, causing faster and premature senescence. However, the management of historical gardens has so far led to an efficient prevention of *Ceratocystis platani* introduction, spread and establishment. This study highlights the importance of a multidisciplinary approach to interpret the present status of the ancient tree asset within historical sites and let the past become a lesson for the future in a broader scenario of conservation and management of cultural heritage.

1. Introduction

Platanus orientalis L., commonly named oriental plane tree (OPT), is a tertiary flora relict species spreading from the Caucasus to the Himalaya as up to the Mediterranean; in southern Italy, there are small patches of forest stands (Campania, Calabria and eastern Sicily) (Rinaldi et al., 2019), while Rosati et al. (2015) consider this species as an archaeophyte.

During the Roman era, in the Italian landscape the OPT was widely cultivated as an ornamental tree of particular value in urban and periurban context. Neglected during the medieval period, when the life was marked by difficulties and calamities including famine, plague, and war, OPT was reintroduced in the Italian peninsula only in the XIV century (Di Bérenger, 1965), making this species one of the protagonists of gardens within Italian villas until the XVII century. In this period, its fortune mirrored the widespread use during classical Greek and Roman times, once again for their beauty and shadow. In addition, for the owners, OPT represented a real status symbol (Grimal, 1990; Schievenin, 2014; Tosco, 2018). It is worth mentioning that in several historical sites OPTs were planted according to geometric patterns in harmony with the architectural elements, as Pliny the Elder already recommended in his Naturalis historia (Tosco, 2018). Today, due to various events, in some sites those trees no longer exist (*i.e.*, Horti Farnesiani in Rome and Palazzo Giardino in Parma) (Cogotti, 2018; Mambriani, *in verbis*). Nevertheless, ancient specimens within monumental complexes, set up between the XVI to the XVII century, are still present in Lazio region. Probably, the pedoclimatic characteristics of these areas enabled this hygrophilous and fast-growing species to reach majestic shapes (Rix and Fay, 2017). Today the conservation of ancient or rare germplasms is shared as a pivotal challenge for cultural assets (Abbate et al., 2020; Mattioni et al., 2020; Petruccelli et al., 2021). No specific management protocols have been designed for veteran trees in these historical sites.

* Corresponding author.
E-mail addresses: ciaffi@unitus.it (M. Ciaffi), vettrain@unitus.it (A.M. Vettraino), enrica.alicandri@unitus.it (E. Alicandri), antonio.tomao@unitus.it (A. Tomao), francesca.adducci@studenti.unitus.it (F. Adducci), elkuz@unitus.it (E. Kuzminsky), agrimi@unitus.it (M. Agrimi).

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The lack of guidelines for maintenance in historical gardens may lead to discontinuous or ineffective technical interventions, thus increasing the probability of tree failure and the risk of damage to people and monuments. Unsuitable management practices or pests and diseases could compromise the stability and health of plane trees, including canker stain caused by *Ceratocystis platani* (Walter) Engelbr. & T.C. Harr. (former *Ceratocystis fimbriata* f. sp. *platani*). This pathogen, after its initial introduction to Italy during the World War II, probably through package materials, was reported in France, Albania, Armenia, Greece, Switzerland and Turkey (EPPO, 2020). It causes wilt, cankers and eventually the death of its hosts, with great economic, social and environmental impacts. In addition, in the last three centuries, *P. acerifolia* (Aiton) Willd, the hybrid species between *P. orientalis* and *P. occidentalis* often replaced *P. orientalis*. This hybrid is widely appreciated as an urban ornamental tree for its big crown, its resilience to biotic and abiotic stresses and the efficacy in removing small particulate pollutants (Swoczyna et al., 2015; Selmi et al., 2016). For the above characteristics, *P. acerifolia* was introduced also in some Italian villas, changing the original design, also in terms of genetic constitution of the plant material. Detecting the presence of *P. acerifolia* is difficult because of its wide phenotypic variability. In fact, this species is often misidentified with the parental ones, and particularly with *P. orientalis*. Therefore, the genetic characterization of plant material of historical sites could also provide useful information to the managers; this in order to respect the original project by replanting plane trees with the original genotypes if necessary.

Recently, a methodology named COVE (COnservation of VEteran trees) was developed to propose an interdisciplinary protocol for the management and conservation of veteran trees within historical gardens (Ciaffi et al., 2018). The aim of the above mentioned study was to extend the use of COVE to the historical sites of Rome and to the surrounding areas of the town; this in order to increase knowledge and provide information useful to sustain their management.

2. Materials and methods

2.1. Selection of veteran oriental plane trees

For this purpose, we used different sources consulting historical archives and documents, such as the Doria Pamphili Archives, the monastic Archives of Grottaferrata Abbey, the State Archives of Rome and Modena, and the digital copies of the books of two trustees of Villa Borghese: Jacopo Manilli (1650) and Domenico Montelatici (1700). We also considered reference studies on the history of garden architecture from XVI to XVII centuries in Lazio, such as D'Onofrio (1963); Guarrera (1999); Campitelli (2003); Santese (2005); Guerrieri Borsoi (2016), and Cogotti (2018). In addition, we referred to the census card of monumental trees of the province of Rome (https://www.cittametropolitanaroma.it). Based on these sources, four sites were selected: Villa d'Este (Est), Villa Borghese (Bor), Villa Aldobrandini (Ald), and San Nilo Avenue (SNA) with nearby surroundings in Grottaferrata (Gr). At first, these plane trees were repeatedly inspected to verify the plant status, collecting leaves and bark samples for morphological characterization. The three majestic century-old plane trees of SNA in Gr, identified in the list of monumental trees of Rome province as *Platanus hybrida* Brot and labelled as RM0146/147/148, were included in this study, together with other two veteran plane specimens of Gr. This was due to their leaf morphological traits ascribable to *P. orientalis*.

2.2. Taxonomic identification and genetic variability of veteran oriental plane trees by molecular analysis

2.2.1. Sample collection

For molecular analyses, three fully expanded leaves per tree were collected in spring 2019 from the following 48 plane trees (Table 1): 29 trees from Ald, Bor and Est in the province of Rome, as well as five trees along the SNA and surrounding gardens in Gr, two OPTs from Villa Lante (VL) and Palazzo Farnese (PF) in the province of Viterbo, and 12 accessions from an INRA collection, representing the three *Platanus* species, *P. orientalis*, *P. occidentalis* and *P.* acerifolia. Moreover, regarding VL and PF, only one genotype per site was considered, due to a substantial genetic homogeneity among all the 23 OPTs from VL and the two OPTs from PF (Ciaffi et al., 2018). Once collected, leaves were immediately frozen in liquid nitrogen and stored at −80 °C until DNA extraction.

Table 1
Species, individual code, site of collection and origins of the plane trees used in the genetic analyses.

Species	IC	Acronym	Site Collection	Geographic origin
P. orientalis L.	1-3	PorGr1-3	Grottaferrata (Italy)	Unknown
	4-17	PorAld1-14	Frascati (Italy)	Unknown
	18-28	PorBor1-11	Roma (Italy)	Unknown
	29-32	PorEst1-4	Tivoli (Italy)	Unknown
	33	PorVL	Bagnaia (Italy)	Unknown
	34	PorPF	Caprarola (Italy)	Unknown
	35	PorPB	INRA Avignon (France)	Macedonia - Greece
	36	PorSE2	INRA Avignon (France)	Samos - Greece
	37	PorCRE 1	INRA Avignon (France)	Xania (Crete) - Greece
	38	PorHAM	INRA Avignon (Fance)	Hamadan - Iran
P. acerifolia (Aiton) Willd.	39	PacGr4	Grottaferrata (Italy)	Unknown
	40	PacGr5	Grottaferrata (Italy)	Unknown
	41	PacBU	INRA Avignon (France)	Paris - France
	42	PacMUT	INRA Avignon (France)	Montpellier - France
	43	PacGB	INRA Avignon (France)	by Santamour (1972)*
	44	PacPB	INRA Avignon (Frane)	by Panetsos (1984)*
P. occidentalis L.	45	PocILL1	INRA Avignon (France)	Morgan County (USA)
	46	PocPB	INRA Avignon (France)	Athens (USA)
	47	PocLAB	INRA Avignon (France)	Stoneville (USA)
	48	PocM11	INRA Avignon (France)	Sikeston (USA)

* (See Ciaffi et al., 2018).

2.2.2. DNA extraction

Total genomic DNA was extracted from 200 mg of frozen tissue, using NucleoSpin® Plant II kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's instructions. All DNA samples were stored at −20 °C until use.

2.2.3. LEAFY gene analysis

For the identification of specific alleles of the *LEAFY* gene primer pair Un.For/Un.Rev was used to amplify both *P. orientalis* (about 700 bp) and *P. occidentalis* (about 800 bp) genomes, while primer pair Occ.For/Occ.Rev was used to assess the presence of a product of about 400 bp specific for *P. occidentalis* genome (Pilotti et al., 2009). PCR reactions and analysis of the amplification products were performed according to Ciaffi et al. (2018). PCR products obtained with the primer pair Un.For/Un.Rev from the three "monumental trees" along SNA in Gr and from two plants chosen as representative of OPTs from Ald, Bor and Est were cloned into the pGEMT easy plasmid vector (Promega). The products were sequenced using an ABI Prism DyeTerminator sequencing kit (PE Applied Biosystem). The specificity of the sequenced amplified fragments was checked by a BLAST search in the NCBI database.

2.2.4. Molecular markers analysis

The genetic variability among the 36 OPTs from the six sites of Lazio region and their genetic relationships with 12 representative *P. orientalis*, *P. occidentalis* and *P. acerifolia* accessions (Table 1) were analyzed by Simple Sequence Repeat (SSR) and Inter Simple Sequence Repeat (ISSR) markers (Tables S1 and S2).

SSR primer pairs were multiplexed, labelling their forward primer with FAM, TAMRA or JOE (Eurofin Genomics). PCR amplifications were performed according to Rinaldi et al. (2019). Amplified SSR products were genotyped using ABI PRISM 3500 sequencer and sized in accordance with GeneScan LI500Liz standard using Gene Mapper 4.0 software.

For the ISSRs, PCR reactions and the analysis of amplification products were performed according to Ciaffi et al. (2018).

2.3. Dendrometric survey

This survey considered the following 58 veteran OPTs classified through molecular tools from the six different sites of Lazio (Fig. 1): 23 from VL, 2 from PF, 11 from Bor, 3 from Est, 16 from Ald (4 in the Secret Garden and 12 in the Water Teather), and 3 from Gr (Figs. S1 and S2). During the summer 2019, the specimens were georeferenced and their dendrometric traits were measured using the portable computerized station Field-Map - Antelope model® (IFER-MMS Ltd, Prague, Czech Republic). In particular, the following data were collected: i) diameter at breast height (DBH; cm), ii) total height (m), iii) crown base height (m), iv) crown projection area (m^2). From these basic data, additional indicators were derived to describe crown volume (obtained by multiplying the crown projection area by the crown height) and tree shape, here measured by the ipsodiametric coefficient (calculated as the ratio between total height and DBH, both expressed in metres). A qualitative judgment about the aging and health status of the tree crowns was also recorded using the five categories proposed by Drenou (2001): young crown, adult crown, senescent crown, beginning of mortality, and dead crown.

The tree volume and biomass were not estimated, since most of OPTs are characterized by large wood decays and have been recently pruned.

2.4. Susceptibility to Ceratocystis platani

Two different isolates of *C. platani* collected in Caserta (AT-Cp) and Viterbo (VT2) and maintained at the Mycological Collection (DIBAF, University of Tuscia, Italy) were used in this experiment. The susceptibility test was done according to Vettraino et al. (2011), lightly modified. Briefly, OPT twigs of approximately 4–9 mm diameter were sterilized (70 % ethanol) and, after removing the bark, inoculated with a plug of the pathogen taken from the margin of a freshly growing culture on MEA (Malt Extract Agar). Lesions were wrapped with moist gauze and sealed with Parafilm M® for preventing contaminations. Control treatments were inoculated by applying sterile MEA discs. Five replicate treatments were set up in a randomized block design for each of tested tree and *C. platani* isolates. After inoculation, shoots were incubated for 7 days at 21 °C and 100 % relative humidity. At the end of the experiment, the length of bark necrosis was measured on each shoot. Koch's postulates were fulfilled by isolation from infected tissue.

2.5. Data analysis

For the analysis of SSR and ISSR markers, a single matrix was created by converting the size of the amplification fragments of the SSR markers into 1/0 values (presence/absence). The genetic distances for phylogenetic relationships among the different genotypes were estimated using the coefficient of Nei (1973). The obtained distances matrix was used to construct a phylogenetic tree using the UPGMA (unweighted pair-group method with arithmetic averages) clustering method in MEGAX software (Kumar et al., 2018). The reliability of the tree topology was assessed *via* bootstrapping over 1000 replicates using the PAUP* 4.0 software (Wilgenbusch and Swofford, 2003).

A Bayesian-based clustering method was also applied on multi-locus

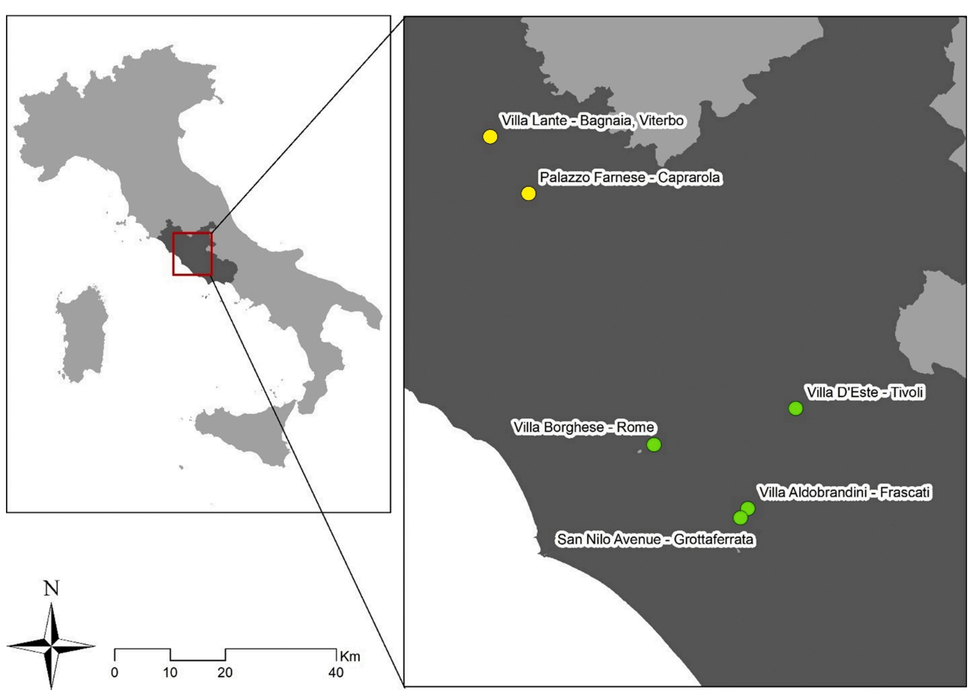

Fig. 1. Map of the historical sites (in green) of Lazio (central Italy) where plane trees have been sampled during the study. In yellow the two historical sites considered by Ciaffi et al. (2018). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

SSR and ISSR data using STRUCTURE v. 2.3.4 software (Pritchard et al., 2000) to infer genetic structure by defining the numbers of clusters in the dataset, assign the individuals to each of the identified cluster and identify admixture individuals. The number of inferred groups was evaluated at values of K ranging from 1 to 10, and for each K, ten runs with a length of burn-in period of 50,000 followed by 500,000 Markov Chain Monte Carlo (MCMC) replicates were performed. The most likely number of clusters (K) was determined using the ΔK method, as well as by examining the plateau of the Ln Pr(X/K) and considering the value of the standard deviation of Ln Pr(K) (Evanno et al., 2005).

Dendrometric data were analyzed by a hierarchical cluster analysis (Ward's method). The category proposed by Drenou (2001) used for the evaluation of the tree vitality of all the surveyed OPTs were summarized at cluster level by a conditioned frequency analysis. Data were analyzed using the R software (version 6.3).

Data of susceptibility to *C. platani* were submitted to one-way ANOVA, and the means were compared by using Tukey's pairwise tests at a significance level of 0.05.

3. Results

3.1. Historical information and survey

Plane trees were mapped and measured in four historical sites located in the province of Rome: Est, Bor, Ald, and Gr (Fig. 1, green dots). Other two sites were also included in this study: VL and PF in the province of Viterbo (Fig. 1, yellow dots). Guarrera (1999) and Frommel (2005) have already reported descriptions of the last two sites.

3.1.1. Site 1 - Villa d'Este (Tivoli)

Villa d'Este (XVI century, 1550-1572), declared a World Heritage Site by UNESCO, is located 30 km NE of Rome on the northern slopes of the Tiburtini Mountains. Here Cardinal Ippolito II d'Este revived the splendor of the courts of Ferrara, Rome and Fontainebleau and renewed the magnificence of Villa Adriana. This Villa was almost completed when the Cardinal died in 1572. Since 1930, Est has been restored and became a public property (https://www.levillae.com/i-luoghi/villa-deste/). In Est the OPT garden originally included 54 specimens, planted in a period of about three years between 1563 and 1565 (Guarrera, 1999). Currently, only four OPTs are present near the Oval Fountain, three in front of the fountain (Fig. S1A) and one younger OPT in the neighbouring garden. The genetic characterization involved all the four specimens (PorEst 1-4, Table 1) while the younger specimen was excluded from the dendrometric survey due to its significantly reduced dimension with respect to the other specimens of the garden (DBH 80 cm).

3.1.2. Site 2 - Villa Borghese (Rome)

In a period ranging from 1606 to 1633, Cardinal Scipione Borghese, extending a family property placed in the current area of Piazza di Siena, conceived Villa Borghese Pinciana. The architects Flaminio Ponzio and Giovanni Vasanzio, flanked by the gardener Domenico Savini da Montepulciano, built the first layout of this villa dotted with stunning buildings, beautiful fountains, sculptures, and attractions, all surrounded by gardens filled with rare and exotic plants and flowers (Campitelli, 2003). An increasing and relevant number of OPTs were planted in two sectors of the park, named *Primo Ricinto* and *Terzo Ricinto*. Indeed, Manilli (1650) and Montelatici (1700) reported 64 and almost 100 plane trees, respectively, shading squares, fountains and the lake (Peschiera). The gardens were deeply transformed in the late XVIII century, becoming a public park in 1903. The 11 veteran specimens, which today we can admire at Bor gardens, belong to the original layout of the *Terzo Ricinto*, in a place now called Plane tree valley. There is a lack of information regarding the planting date of these remnant individuals, but there is a consensus that the original planting design was established around 1620 (Campitelli, *in verbis*). In this study, we considered all these 11 specimens for both dendrometric and genetic analyses (Fig. S2B) (PorBor1-11, Table 1).

3.1.3. Site 3 - Villa Aldobrandini (Frascati)

Also known as Villa Belvedere (late XVI century - early XVII century), Ald is about 30 km SE from Rome. In 1598, Cardinal Pietro Aldobrandini entrusted its construction to the architects Giacomo Della Porta, Carlo Maderno and Giovanni Fontana. Consisting in a big building surrounded by terraces with parks and gardens, fountains, and attractions including the majestic Water Teather, Ald was almost completed on the death of the Cardinal in 1621 (http://www.visitcastelliromani.it). In 1683, Giovanni Battista Pamphili acquired it, while in 1760 it became the possession of the Borghese family. Later, in 1837 Ald returned to the Aldobrandini family who still owns it today. At present, it is possible to admire 21 fascinating centuries-old OPTs in two distinct gardens placed at the same level as the Water Teather (WT). The first is the garden of the WT, designed to delight and impress visitors with buildings of fine architecture and water tricks, while the second is the Secret Garden, a place for the pleasure of the owners only. Gian Battista Agucchi, trustee of the villa, in a text of 1611, transcribed by D'Onofrio (1963), reported that two gardens with OPTs were being planted in the garden at the level of the WT, although there is no data on the number of OPTs. However, in the Aldobrandini family, it is handed down that only the OPTs of the WT garden belong to the original layout. In order to study the relationship between the WT and the SG trees, currently consisting in 12 and 9 specimens, respectively, the genetic analyses included all the 12 WT plants (PorAld 1-12, Table 1) and only 2 SG plants (PorAld 13-14, Table 1), while all the 12 WT plants and PorAld 13-16 were considered in the dendrometric analyses (Fig. S1C).

3.1.4. Site 4 - San Nilo Avenue (Grottaferrata)

The history of Grottaferrata (Gr), a small village near Frascati, is strongly linked to the presence of the San Nilo Abbey, founded in 1004 by San Nilo from Rossano. The village of Gr developed along the two main access roads to the Abbey, the "Stradone" and the "Olmata" (elm tree row), corresponding respectively to the current *Corso del Popolo* and San Nilo Avenue (SNA). Plane trees gradually replaced the elm trees of the SNA, although historical sources did not provide information on the number of specimens of the original planting (Guerrieri Borsoi, *in verbis*). From the site of Gr, for the genetic analyses, the three monumental trees along SNA (PorGr 1-3, Table 1) and one specimen from a close public garden (Pac Gr 4 in Table 1) were selected; in addition, another specimen of plane tree was collected from the courtyard of the Abbey (Pac Gr5, Table 1). Conversely, the dimensional analyses were carried out only on the three monumental OPTs growing along SNA (PorGr 1-3 in Fig. S1B).

3.2. Taxonomic identification

Amplification of the *LEAFY* gene with Un.For./Un.Rev primers (Fig. 2A) showed the presence of a single product of about 700 bp characteristic of *P. orientalis* for PorGr 1-3 (lanes 1-3, Fig. 2A), for all the plane trees from Ald (PorAld 1-14, lanes 4-17), Bor (PorBor 1-11, lanes 18-28), Est (PorEst1-4, lanes 29-32), and for the two reference plane trees from VL and PF (lanes 33 and 34, respectively). On the other hand, the amplification of DNA from the same plane trees using Occ.For/Occ.Rev primers did not result in any amplification product (lanes 1-34, Fig. 2B). The sequences of PCR products with Un.For./Un.Rev primers from PorGr 1-3 and from the two plants chosen as representative of the plane trees from each of three historical villas in the Rome province (Ald, Bor and Est) showed a high level of homology (sequence identities comprised between 95–100 %) with *LEAFY P. orientalis* sequences deposited in NCBI databases (*e.g.* AY706059.1, AY706058.1 and AY706060.1). These findings clearly suggested that both the plane trees from the three villas in the province of Rome and the three "monumental trees" from the SNA of Gr belong to *P. orientalis*.

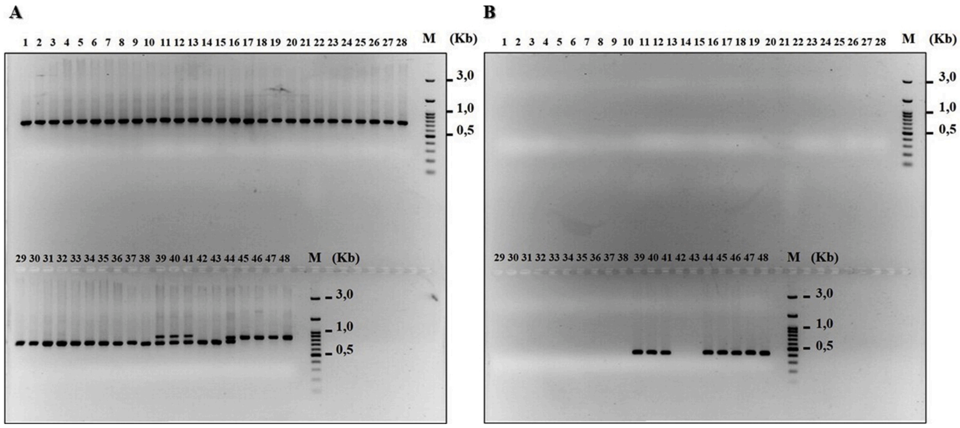

Fig. 2. Agarose gel electrophoresis of PCR products by primer pairs Un.For/Un.Rev (A) and Occ.For / Occ.Rev (B) of the 48 plane trees analysed in this study.The numbers on each lane indicates the different plane trees as reported in Table 1.

On the other hand, the two other plane trees of Gr (PacGr 4 and PacGr 5) were identified as belonging to the hybrid species *P. acerifolia*, because of the presence of DNA fragments from both *P. orientalis* and *P. occidentalis* genomes with Un.For./Un.Rev primers (lanes 39 and 40, Fig. 2A), and the detection of the specific *P. occidentalis* product of about 400 bp with Occ.For/Occ.Rev primers (lanes 39 and 40, Fig. 2B).

The goodness of the molecular approach used for the taxonomic identification was confirmed by the results from the *P. orientalis*, *P. occidentalis* and *P. acerifolia* accessions used as references (lanes 35-48, Fig. 2A and B). Indeed, the amplification of the *LEAFY* gene allowed us to differentiate most of the accessions belonging to the three species, with the exceptions of *P. acerifolia* accessions PacMUT and PacGB, in which only the *P. orientalis* DNA fragments was observed (lanes 42 and 43, Fig. 2A and B), confirming data from literature (Pilotti et al., 2009; Ciaffi et al., 2018).

3.3. Genetic relationship and structure of the analysed Platanus genotypes

The UPGMA dendrogram based on the Nei's coefficient (1973) using both SSR and ISSR markers clustered the *Platanus* genotypes into three well-supported major clades (bootstrap support value higher than 90 %) corresponding to *P. orientalis, P. acerifolia and P. occidentalis* groups (Fig. 3A).

Confirming results from LEAFY gene analysis, the OPTs from the villas of Rome and Viterbo provinces, as well as the three monumental trees of SNA (PorGr 1-3), clustered together with the INRA *P. orientalis* accessions, while the two other plane specimens from Gr (PacGr4 and PacGr5) were included in the group of the INRA *P. acerifolia* accessions (Fig. 3A).

Based on the Nei's genetic distances, the large clade containing all the OPTs could be further subdivided into six subclusters, generally related to the provenance of the genetic material (Fig. 3A). Starting from the top of the dendrogram, the first subcluster included the three OPTs located in front of the Oval fountain of Est (PorEst 1-3), which were genetically distinct from the younger OPT (PorEst 4). The second subcluster contained 10 of the 11 OPTs from Bor (PorBor 1-3 and PorBor 5-11) and one OPT from Ald (PorAld 14). Interestingly, apart from PorBor4, which was clearly distinguished from the remaining 10 OPTs of Bor, these latter showed a remarkable genetic similarity, with five sharing identical SSR and ISSR profiles (PorBor 2 and PorBor 5-8), and the others differing only for the presence/absence of one or two ISSR fragments (PorBor 1, PorBor 3 and PorBor 9-11). The third and fourth subclusters included the 3 specimens classified as monumental trees from Gr (PorGr 1-3) and 13 of the 14 OPTs from Ald, indicating a likely common origin of these OPTs. As previously highlighted for Bor, a remarkable genetic similarity was also found for several specimens from Ald, with six trees sharing identical SSR and ISSR profiles (PorAld 6-10

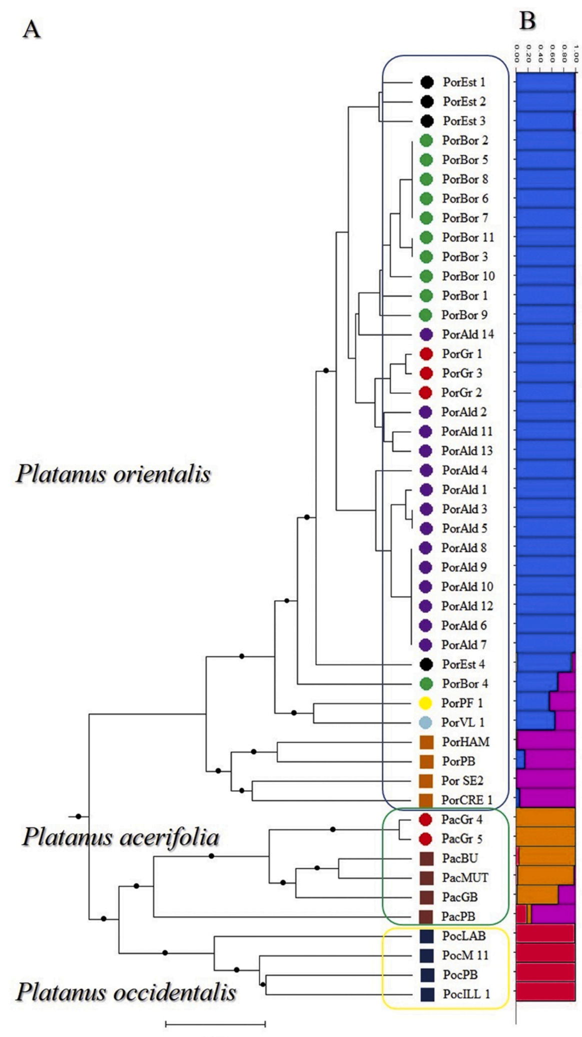

Fig. 3. Clustering of 48 *Platanus* genotypes reported in Table 1. A) UPGMA dendrogram based on Nei's genetic distances. Branches indicated with dots represent bootstrap support more than 80 % (1000 repetitions). B) Structure bar plot of average proportions of membership (Q) for the same genotypes for K = 4 (in light-blue, pink, orange and red). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

and PorAld 12) and others differing only for the presence/absence of one or two ISSR fragments (PorAld 1, 3 and 5). At the bottom of the dendrogram, the fifth subcluster included the two representative OPTs from the gardens of VL and PF in the province of Viterbo, which are clearly distinct from a genetic point of view from the group of OPTs from the villas of Rome province (bootstrap support value higher than 80 %). Finally, the last subcluster included the four OPTs accessions from INRA used as references.

Bayesian analysis revealed that the *Platanus* genotypes could be represented by two (K = 2) or four (K = 4) genotypic groups (Fig. S3A). According to the separation obtained through simulation models, for K = 2, the two groups comprised the gene pools from the *P. orientalis* genotypes (red) and both *P. acerifolia* and *P. occidentalis* accessions (blue), with five *P. orientalis* genotypes (33–38 in Table 1) of "mixed origin" having the membership probabilities Q < 80 % for both clusters (Fig. S3C). However, subdivision into a larger number of groups (K = 4), which in particular considers the taxonomic classification of the different genotypes analyzed (Fig. 3B), was well supported by the results obtained with the clustering analysis based on the UPGMA method (Fig. 3A). The analysis of the standard deviations of probabilities obtained in different simulations also helps to define the number of groups and their genotype structure (Evanno et al., 2005). In Fig. S3B, the separation of genotypes into four groups (K = 4) coincided with the one of the lowest values of standard deviation (1.293746) of probability. Hence, four groups represent better the genetic structure of the studied genotypes. Considering K = 4 (Fig. 3B), the four groups encompassed plane genotypes as follows. The first (light blue labelled) included OPTs from villas in Rome province. The second (pink labelled) the four OPT accessions from INRA, with PorVL and PorPF, the two representative OPTs from Viterbo province, which could be considered of "mixed origin", having the membership probabilities Q < 80 % for both clusters, confirming the results of the UPGMA clustering analysis (Fig. 3A). The third (orange labelled) comprised the two *P. acerifolia* genotypes from Gr (PacGr4 and PacGr5) and two of the four *P. acerifolia* accessions from INRA (PacBU and PacMUT), while the fourth, labelled in red, included the four INRA *P. occidentalis* accessions. Significant admixture levels involving the different *P. orientalis* and *P. occidentalis* gene pools were detected in the two INRA *P. acerifolia* accessions, PacGB and PacPB, likely indicating their recent hybrid origin.

3.4. Dendrometric survey

The main dendrometric attributes are reported in Table 2. The mean values for DBH and height of all the studied OPTs were 132.44 cm and 17.5 m, respectively. More than 80 % of the surveyed plane trees had a DBH greater than 100 cm. The larger trees have been found in Bor and PF, where mean DBH exceeded 170 cm and total height was higher than 18 m. Concerning total height, trees at the third level of VL (3 L) and in the WT of Ald never exceeded 15 m and showed the smaller crowns, being crown projection areas and crown volumes always lower than 70 m^2 and 500 m^3, respectively. These results are likely due to continuous pruning aimed at reducing branch failure risk, which is a very relevant issue to be considered in the management of veteran trees, especially in densely frequented areas (Tomao et al., 2015). On the other hand, trees not subjected to recent severe pruning (like those in Bor and Gr) showed well-developed crowns with a volume greater than 4500 m^3. In Fig. S4 a view of the plane trees and their spatial context in the four sites studied is reported.

Cluster analysis performed on dendrometric attributes identified three different groups (Fig. 4). Cluster 1 had the largest number of units (35), while the second one was the smallest, counting only six trees. The third cluster, which included an intermediate number of trees from several sites, was characterized by the highest variability having a higher dissimilarity distance if compared to the others.

The dendrometric attributes of the three groups are reported in Table 3. Cluster 1 included OPTs characterized by lower DBH and height, and less expanded crowns. Data referred to all the sites considered with the exclusion of GR and PF. Cluster 2 comprised trees with intermediate values of dendrometric attributes, while cluster 3 included the biggest trees, in terms of DBH, height and crown size. Then, according to the categories proposed by Drenou (2001), each cluster was described in terms of tree vitality (Table 4). Trees in cluster 1 showed lower vitality and compromised crown health status (beginning of mortality) than trees in clusters 2 and 3. Indeed, dead trees were assigned only to cluster 1. Some examples of hollow trees with evident dead wood are reported in Fig. S5. In cluster 3, the vitality status is generally better than in the other two, with all the trees classified in the category "adult crown", suggesting that less intensive pruning and management can result in a larger and healthier crown.

3.5. Susceptibility to *Ceratocystis platani*

Most of the trees in the study areas presented few or no twigs with the morphological and physiological characteristics required by the protocol above described. Thus, susceptibility tests were performed on five plants located in Ald (PorAld4 and PorAld16), Bor (PorBor4 and PorBor5) and Est (PorEst1). In all cases, the twigs inoculated with the pathogen showed at the inoculation points significantly higher lesions

Table 2
Mean dendrometric attributes of plane trees surveyed in the historical sites. Values in brackets represent standard deviation.

Site	Subsite	DBH (cm)	Total height (m)	H/D	Crown base height (m)	Crown projection area (m^2)	Crown volume (m^3)
Villa Lante (VL)	1L	117.81 (32.62)	19.27 (4.33)	17.23 (4.71)	5.88 (1.55)	118.76 (86.58)	1763.93 (1518.57)
	2L	100.57 (10.40)	14.74 (9.33)	14.29 (8.32)	4.16 (0.83)	67.04 (73.32)	1086.1 (1580.26)
	3L	116.76 (15.36)	12.61 (2.91)	10.75 (1.69)	4.58 (1.39)	43.69 (27.36)	393.15 (296.01)
Villa Borghese (Bor)	B	178.91 (12.23)	21.84 (4.13)	12.3 (2.82)	4.34 (2.08)	296.01 (130.47)	5281.43 (2529.31)
Villa d'Este (Est)	E	142.67 (24.19)	19.57 (0.56)	13.93 (1.90)	4.5 (0.66)	132.73 (35.15)	2021.34 (633.23)
Palazzo Farnese (PF)	F	171 (2.83)	18 (0.57)	10.53 (0.5)	6.1 (0.57)	268 (97.44)	3244.32 (1462.74)
Grottaferrata (Gr)	G	163.66 (56.16)	22.7 (5.15)	14.96 (6.51)	4.83 (1.71)	255.91 (66.84)	4540.19 (1665.48)
Villa Aldobrandini (Ald)	SG	125.25 (15.44)	19.97 (2.12)	16.15 (2.64)	10.43 (2.06)	117.98 (49.45)	1163.62 (622.46)
	WT	112.58 (18.86)	13.56 (2.13)	12.42 (2.99)	6.81 (1.21)	68.95 (30.12)	490 (269.76)
Mean		132.44 (34.90)	17.5 (5.35)	13.62 (4.37)	5.65 (2.17)	139.81 (118.86)	2081.06 (2290.18)

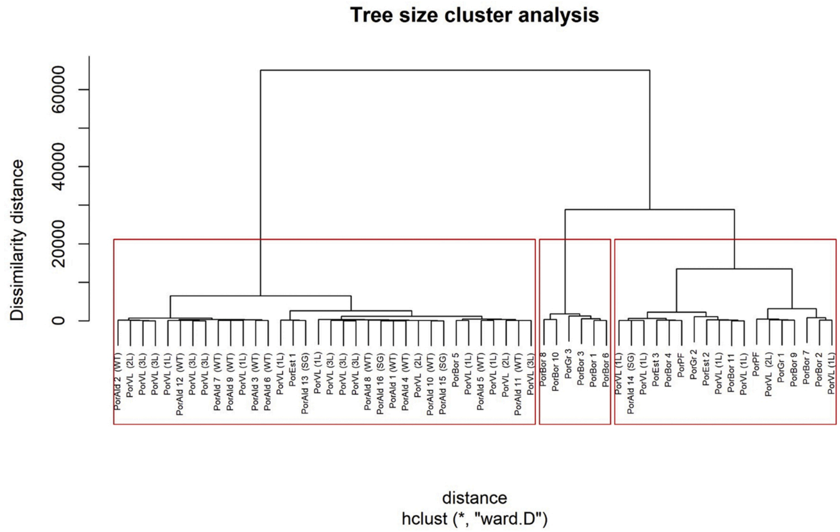

Fig. 4. Dendrogram resulting from tree size cluster analysis (different codes identify different subsites).

Table 3
Mean dendrometric attributes of plane trees belonging to the three identified clusters. Values in brackets represent standard deviation.

Clusters	DBH (cm)	Total height (m)	H/D	Crown base height (m)	Crown projection (m^2)	Crown volume (m^3)
1	113.5 (23.2)	14.4 (4.1)	13.14 (4.49)	6.0 (2.0)	62.9 (32.1)	575.3 (382.5)
2	155.6 (30.5)	21.3 (3.2)	14.33 (4.14)	5.6 (2.4)	210.5 (83.9)	3234.1 (1184.4)
3	173.8 (28.7)	24.0 (3.5)	14.33 (4.27)	3.8 (1.2)	376.6 (78.9)	7405.6 (630.4)

Table 4
Conditioned frequency analysis of the crown physiologic statuses of the trees belonging to the clusters.

Variable		Clusters 1	2	3	Mean value
Synthetic classification of crown physiologic status (Drenou, 2001)	Young crown	0	0	0	–
	Adult crown	40.0	83.3	100	74.4
	Senescent Crown	34.3	16.7		17.0
	Beginning of mortality	22.9			7.6
	Dead crown	2.8			1.0

compared to controls (ANOVA, $P < 0.05$). In particular, OPTs collected in Bor resulted to be the most susceptible with lesions 2.4 times longer than control, while plane trees of Ald and Est showed lesions 1.7 times greater than control (Fig. S6).

4. Discussion

Trees within cultural sites, important for their economic, cultural and landscape values, give a valuable benefit if the quality of their ecophysiological state is satisfactory and lasting. Nevertheless, their management is difficult and costly, even because objectives in monumental complexes can change over the time, based on environmental, economic and socio-cultural requests (Funsten et al., 2020). The results of this study, obtained from the application of the COVE model, confirm that the multidisciplinary approach is the appropriate tool for the evaluation of the capability of ancient trees to provide ecosystem services, including the cultural ones.

Based on historical data, the veteran OPTs considered in the present study should have been planted in a period ranging from the second half of XVI century to the first decades of XVII century and represent only a remnant amount of those of the original plantings (around 20 %). The healthy status of trees reflects the management strategies applied within the different sites. Most of the OPTs studied (Group 1 in Fig. 4) showed reduced dendrometric characteristics (Table 3) and lower vitality (Table 4) compared to the rest of the trees analysed (Groups 2 and 3 in Fig. 4). However, the dimensional values recorded and the relevance from the cultural and historical point of view make each OPT studied classifiable as a monumental tree according to the 39/2002 regional and 10/2013 national laws, respectively. Within the genus *Platanus*, *P. orientalis* is characterized by a prolonged longevity exceeding 500 years (Vigouroux, 2007). Therefore, it can be assumed that the OPTs under study are in a phase of natural senescence, which, in some cases, has been evidently accelerated by topping or pollarding practices, particularly evident in trees near architectural elements. Past severe pruning treatments, applied to reduce their crown size, were probably adopted to leave the architectures clearly visible to enjoy their view. For instance, some historical documents testified that princess Aldobrandini asked the gardener to prune the crown of the OPTs each year (Guerrieri Borsoi, 2016). For this, at Ald it is likely that the crowns of the OPTs of Water Theater (WT) garden were more severely pruned respect to those of the Secret Garden, due to its representative role. This management strategy might explain the lowest height values and less expanded crowns combined with a premature senescence of the OPTs planted in the WT garden of Ald and the OPTs placed in front of the Fountain of the Deluge in the third terrace of Villa Lante (VL).

In agreement with literature, in this study molecular markers were confirmed to be useful tools for the genotyping of historical plantings (Hansen et al., 2014), particularly when there is a lack of information on the provenances of plant material (Salinero et al., 2020). All the 29 plane trees from the three historical villas of the province of Rome (Est, Bor and Ald) and those along the SNA in Grottaferrata (Gr) were identified as *P. orientalis* by *LEAFY* gene analysis (Fig. 2). Interestingly, the three SNA plane trees in Gr (PorGr 1-3) were previously recorded as *P. acerifolia* by the census cards of monumental trees (https://www.cittametropolitanaroma.it). Differently, the two other plane trees located near SNA (PacGr4 and PaGr5) appertained to the species *P. acerifolia,* indicating likely that after the original *P. orientalis* planting along SNA, several hybrid specimens were subsequently introduced in the same area.

These results were confirmed by SSR and ISSR markers, which allowed us to distinguish without ambiguity plane tree species, revealing significant variations among specimens and accessions of the three species (Fig. 3). The complexity of the genetic structure of the OPTs studied was recorded by the UPGMA analysis, which defined the presence of six subclusters, generally related to the site of sampling of the genetic material (Fig. 3A). These results support the hypothesis of different origin of the plants used for the establishment of the original planting of the historical sites located in Rome province compared to that of the plane trees within the Viterbo villas. At the same time, genotypes within the same site are characterized by a greater genetic uniformity, signifying that these plants are part of the original layout and likely have been obtained by vegetative propagation from the same mother plant. It is worth noting that the three monumental trees from SNA in Gr (PorGr 1-3) are strictly related from a genetic point of view to 13 of the 14 plane trees from Ald, supporting the hypothesis of a common origin. Concerning Villa d'Este, historical sources (Guarrera, 1999), dimensional measurements (Table 2) and molecular markers analysis (Fig. 3A) let us speculate that only the three OPTs located in front of the Oval Fountain (PorEst 1-3) belong to the original layout of the garden; the younger specimen PorEst4 was planted afterwards. Moreover, in this study the different historical process of the establishing of the gardens in the provinces of Viterbo (XVI century) and Rome (XVII century) has been confirmed by molecular markers analysis, which clearly grouped the two representative plants from Villa Lante (PorVL1) and Palazzo Farnese (PorPF1) in a cluster well distinguished from the group including OPTs from Rome province and the different INRA *P. orientalis* accessions used as references (Fig. 3).

Interestingly, no symptoms of *C. platani*, the causal agent of plane canker, was reported in the sites considered. Historical documents do not provide information about the possible presence and/or identification of pathogens in the past. It is likely that the continuous care of the gardens resulted in a fast removal of diseased trees or that the physical isolation of the gardens has so far protected the areas from the pathogen spread and establishment. Currently, historical gardens are much more exposed to pest invasion than in the past and if *C. platani* enters these sites, it could cause serious diseases. Pathogenicity tests showed susceptibility to the pathogen under artificial inoculations, confirming previous findings related to veteran plane trees (Ciaffi et al., 2018). Results obtained in the present study should be interpreted with caution because they refer to a low number of specimens, due to the absence of material to be tested; wider analysis could be carried out on vegetative propagated OPTs.

The renovation of historical gardens should consider that plants and seed trade are the main pathways by which pests can be introduced in new areas (Cleary et al., 2019) and Europe is particularly prone to pest invasion due to its variety of habitats and climates and the gaps in biosecurity (Eschen et al., 2015; Marzano et al., 2016; Vettraino and Santini, 2021). Moreover, the huge presence of pests in nurseries also represents a risk factor (Jung et al., 2016). Within this scenario, the use of micropropagated plane trees is highly recommended for the plantation of plants of cultural, economic or scientific interest such as plane trees. Recently, a specific culture medium was developed for an effective and easy micropropagation of ancient OPTs (Ciaffi et al., 2018; Kuzminsky et al., 2018).

5. Conclusions

Designed as *gardens of pleasure* for important families, in the XVI and XVII centuries, those historical gardens are today included among the most appreciated urban green areas, in some cases becoming public parks. In the last 50 years, they are increasingly gaining importance as a component of everyday people's quality of life. The managers of the monumental complexes now are facing, among other things, changes in social and cultural behavior and expectations, new climatic challenges, and possibility of exotic pest invasion. For all these reasons, the COVE model adopted in this study could be considered an efficient tool that may improve sustainable management, promoting strategies to minimize conflicts between the growing demand for natural resources within a cultural context and the need to conserve them. Starting from a detailed historical survey, the multidisciplinary approach provided by the COVE model allowed a clear taxonomic identification of the trees studied with interesting hypotheses on their genetic relationships and origin. Indeed, the source of plant material for the original plantings, even if still unknown, varies among the six villas studied in function of the period of construction and geographical location. It also highlighted the effects of past pruning on the current health status of the historical specimens, which could be further affected by canker stain. Vegetative propagation and *ex-situ* collection meet the need to prepare a strategy for the replacement of individuals, which are exhausting their life cycle.

A strict collaboration between historical sites keepers and urban forestry researchers could contribute to the improvement of the COVE model and its transferability to other contexts and other veteran species. The application of this model could allow, among other things, to make available to the community national and international databases for these unique gardens and sites, where history and nature meet in unique contexts, constituting a cultural heritage for humanity.

Data availability

Data will be made available on request.
The data that has been used is confidential.

Authors contributions

Conceptualization M.C., E.K. and M.A.; Data curation A.M.V., E.A., A.T. and F.A; Formal analysis A.M.V., E.A. and A.T.; Funding acquisition M.A.; Investigation A.M.V., E.A., A.T. and F.A.; Methodology A.M.V., E. A. and A.T.; Project administration M.C., A.M.V., E.K. and M.A.; Resources M.C., E.A., E.K.; Software E.A. and A.T.; Supervision M.C., A.M. V., E.K. and M.A.; Validation M.C., A.M.V., E.K. and M.A.; Visualization M.C., A.M.V., E.K. and M.A.; Roles/Writing - original draft M.C., E.K. and M.A.; Writing - review & editing M.C., A.M.V., E.A., A.T., E.K., F.A. and M.A.

Declaration of Competing Interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ufug.2022.127506.

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