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The Use of Green-Stained Wood Caused by the Fungus *Chlorociboria* in Intarsia[1] Masterpieces from the 15th Century

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Summary

Green-stained wood from one of the intarsia panels of the Gubbio *studiolo*, (Italy, 15th century, in the collection of The Metropolitan Museum of Art, New York) and an intarsia panel made by Fra Giovanni da Verona, (Italy, around 1500, in the collection of the Historical Museum of Amsterdam), were sectioned and examined by light, scanning and transmission electron microscopy and energy dispersive X-ray microanalyses. Sections of green-stained wood from the intarsia panels contained evidence of fungal hyphae in vessels, fibers and parenchyma cells. Colored deposits that were yellowish-orange or green were found to be extensive in ray parenchyma cells and in vessels and fibers adjacent to the ray parenchyma. The staining pattern within the wood was not uniformly distributed but concentrated in areas where fungal hyphae were present. The woods were identified as *Populus* sp. No fungi were evident in samples obtained from other woods (*Quercus* sp. and *Juglans* sp.) adjacent to the green-colored wood within the artwork. X-ray microanalyses of the green wood showed no evidence of high concentrations of metal ions that would be present if inorganic dyes were used. Transmission electron microscopy showed the ultrastructural distribution of fungal hyphae and pigmented substances within the woody cells as well as an erosion of the secondary wall layers in some cells. A comparative study of recently collected green-stained wood caused by the fungus *Chlorociboria* (syn. *Chlorosplenium*), showed the micromorphology of fungal colonization and pigment deposition was identical to the patterns observed in the green wood of the 500 year old intarsia panels.

Introduction

The intarsia workers of Renaissance Italy were masters in selecting the woods that they needed for their extraordinary *trompe l'oeil* images. Every subtle range of wood-grain and color was utilized with great skill in order to create the delicately composed works of art (Puerari 1967). Their typical 'palette' would be compiled from locally available woods ranging from the very light *Euonymus* sp. through the middle tones of *Juglans* sp. to the deepest blacks of *Quercus* sp. (actually buried oak that was recovered from bog environments). The use of these woods can be identified from the actual intarsia works and is supported by documentary evidence published by, e.g., Puerari 1967, Haines 1983, and Rognini 1985. A unique place in this 'palette' is taken by a green-colored wood. The unusual greenish-blue or verdigris coloration of this wood is not a natural color associated with wood from any known tree species. Wherever it appears in the intarsia panels it is used with great refinement, as in the rendering of natural scenery with hills, trees and floral leaves. The color was used for book covers and book locks. It simulates fabric, and in very small pieces it was used in imitation of porphyry stone. Although the green-colored wood was not used by very many intarsia workers, a fair amount of it was used by Fra Giovanni da Verona (1457/58–1525) suggesting that he had ready access to it. The most masterly use of the green wood was by the workshop of Giuliano da Maiano (1432–1491) whose intarsia work in the New Sacristy of the Cathedral in Florence, was completed in 1469 (Haines 1983). Purchases of the green-colored wood do not appear on the published listings for the New Sacristy, nor for Fra Giovanni da Verona's choir and Sacristy of Santa Maria in Organo, Verona. This leads us to believe that the green-colored woods were perhaps obtained by the intarsia workers themselves.

The knowledge of the intarsia technique travelled from Italy across the Alps and with it the practice of using green wood for inlays. Augsburg in South Germany, for example, was well known in the 16th century for the manufacture, trade, and use of stained intarsia veneers. In 1593 the wood workers guild issued a decree that the sale of stained wood desired by Dukes and gentlemen is permitted within Augsburg and also within a six mile radius (Hellwag 1924). It was

[1] The word intarsia comes from the italian 'Tarsia' which means inlay. It is the technique in which one material is inlaid into a matrix of another material, commonly wood. This technique of decorating wooden surfaces with inlay became very popular in Renaissance Italy, and celebrated its "golden age" towards the end of the 15th century (Ferretti 1982). Intarsia is cut with chisels and knives (the fretsaw was introduced in the middle of the 16th century); the inlaid wood having a typical thickness of approximately 5 mm.

always assumed that the green-stained wood was a result of artificial staining techniques, but the Augsburg intarsia work exhibits the same visual characteristics associated with green stain caused by the fungus, *Chlorociboria*.

This investigation was done to ascertain the nature of the green-stained wood by examining samples of Italian intarsia masterpieces of the 15th century.

Materials and Methods

The green-stained wood from the intarsia masterpieces that were examined in this investigation were from the Gubbio *studiolo* in the collection of the Metropolitan Museum of Art, New York, Rogers Fund (accession number 39.153), and from a panel in the collection of the Historical Museum of Amsterdam, (accession number OKA 8707 1AB). The Gubbio *studiolo* is a small room that was built for Federico da Montefeltro's Ducal palace in Gubbio, Italy, at the end of the 15th century. It has an irregular groundplan of about 125 square feet and consists of intarsia wall panelling which extends from a tiled floor to a hight of 7.4 feet. The intarsia panels create the illusion of an interior with open cupboards containing, among other things, books, musical instruments, arms and armor, and in the center panel the emblem of the order of the Garter. A set of probably seven panel paintings were believed to have been mounted above the intarsia panels. At approximately 16 feet high was a gilded and polychrome painted coffered ceiling supported by a cornice (Remmington 1941; Cheles 1986). Although the maker of the intarsia panels is unknown, the style and quality of the work suggest a Florentine workshop.

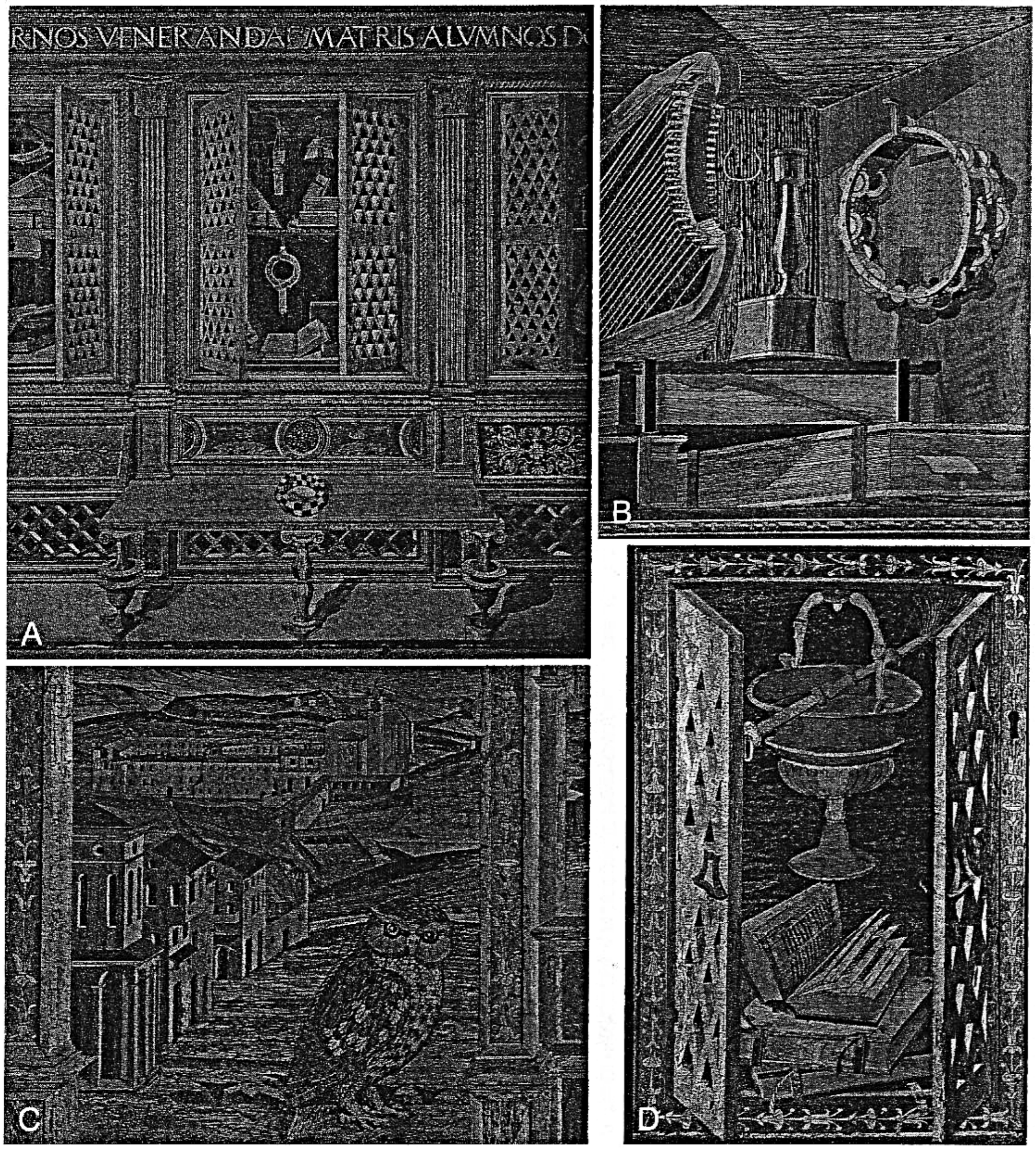


Fig. 1. Examples of intarsia from the 15th and early 16th century with green-stained wood. **A.** Center panel from the long wall of the Gubbio *studiolo*, in The Metropolitan Museum of Art, Rogers Fund, New York. **B.** Panel from the upper right corner of the long wall of the Gubbio *studiolo* shown in Fig. A. The green-stained wood is present in the book cover. Other samples were taken from surrounding dark colored woods (*Quercus* sp. and *Juglans* sp.) for comparison of ultrastructure and X-ray microanalysis to the green-stained. **C.** Detail of an intarsia panel from the choir stalls of Monte Oliveto Maggiore by Fra Giovanni da Verona showing extensive use of green-stained wood in the landscape. Reprinted with the kind permission from Soprintendenza per i Beni Artistici E Storici, Siena. **D.** Intarsia panel, by Fra Giovanni da Verona, in the Historical Museum of Amsterdam, with green stain located in the book cover and book locks, courtesy of The Historical Museum of Amsterdam.

The intarsia panel in the Historical Museum of Amsterdam is undocumented, but the style and 'hand' are so similar to Fra Giovanni da Verona's work that the panel is thought to be by him (Wilmering, unpublished data). Its provenance is only partly known but by analyzing the linear perspective in the panel it is clear that it must have been a lower panel in perhaps a lectern or a cabinet (Brizzi 1989, pp. 138, 139; Rognini 1985, pp. 104).

During the conservation treatments at the Metropolitan Museum of Art, New York and the Historical Museum of Amsterdam, small segments of green-stained wood were removed from the intarsia panels.

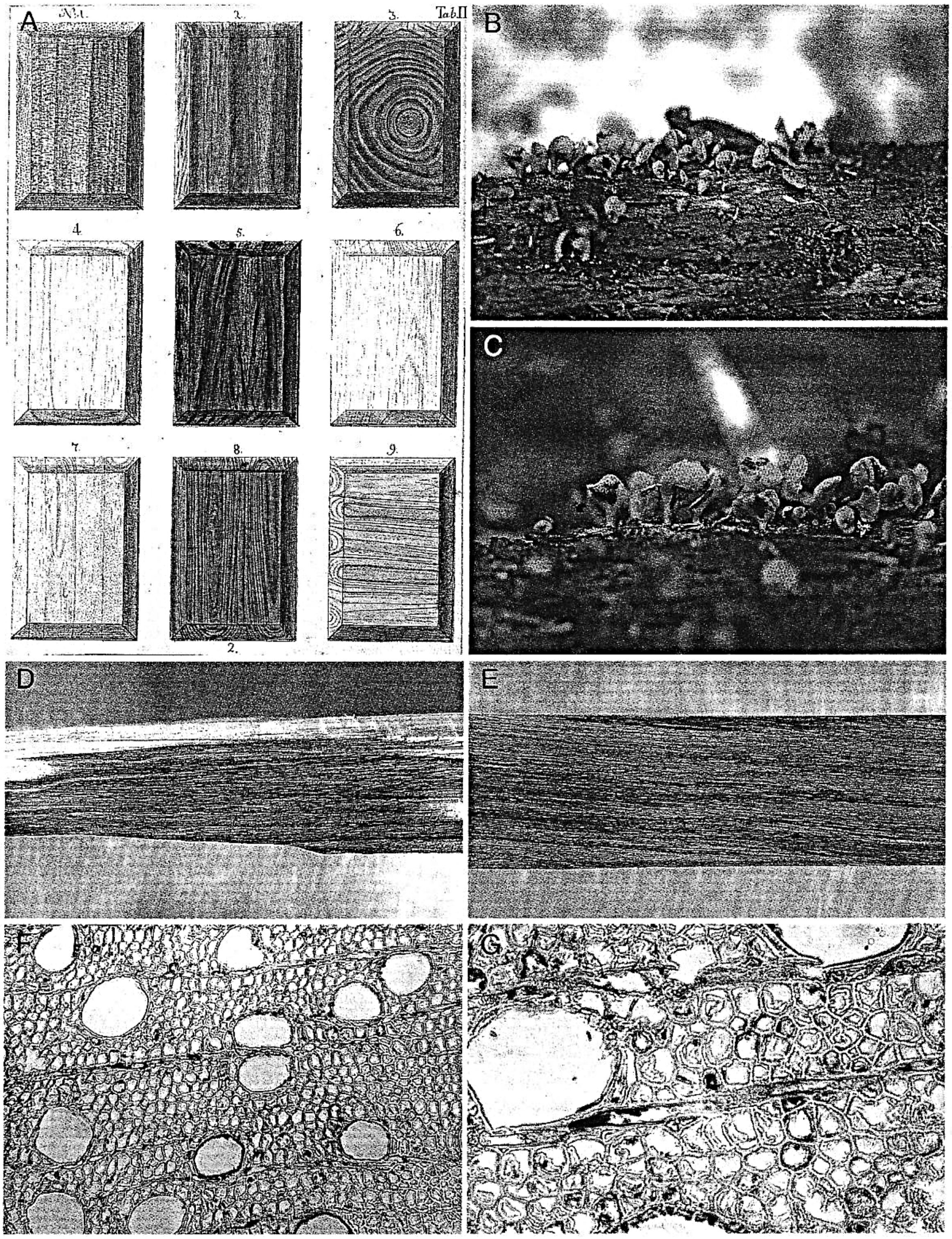


Fig. 2. A. Photograph of a hand colored print showing a variety of naturally colored wood blocks from a German book published in 1773. A block of green-stained *Populus* wood is located in the Center. This figure is used courtesy of Smithsonian Institution Libraries, Cooper-Hewitt Branch, New York.) **B.** and **C.** Greenish-blue fruiting bodies of *Chlorociboria* on the surface of wood from *Populus* sp. collected from the forest. **D.** and **E.** Green stain within wood from dead *Populus* sp. trees recently collected from the forest. **F.** and **G.** Light micrographs of transverse sections made from green-stained wood in the Gubbio *studiolo* (see Figs. 1A and B). A combination of dark green and yellowish-orange pigments are located in ray parenchyma cells and in some fibers and vessels. Extensive coloration is found within the ray parenchyma cells.

Sections from each sample were prepared for light, scanning and transmission electron microscopy. Wood segments used for light microscopy were infiltrated with water, frozen at −20°C in a Cryo cut freezing microtome and sectioned. Sections were mounted and observed without histological staining. Other sections were mounted for scanning electron microscopy or X-ray microanalyses using previously published procedures (Adaskaveg *et al.* 1991; Blanchette *et al.* 1987, 1988, 1991). Additional samples were fixed, dehydrated and embedded in Quetol resin for transmission electron microscopy. Sections were prepared and examined as previously described (Abad *et al.* 1988).

Samples of other wood used in the same intarsia panel of the Gubbio *studiolo* located immediately adjacent to the green wood were also obtained and examined. In addition, samples of *Populus* sp., *Alnus* sp., and *Fraxinus* sp. with a green stain caused by the fungus *Chlorociboria* were collected from forests in Minnesota, New York and Great Britain. This fungal stained wood also was used for microscopy studies outlined above.

Literature from as early as the 18th century was reviewed in this study to ascertain the early knowledge of naturally occurring green-stained woods.

Results

The intarsia panels of the 15th century, with their extraordinary images, were created by using a variety of different wood colors (Figs. 1A to D). The green wood was used imaginatively by different craftsmen over many centuries (Figs. 1A, C, and D). The earliest reference to naturally occurring green-stained wood is in a German book published in 1773 containing hand-colored prints of various types of wood collected as unusual objects of nature and displayed in "curiosity cabinets". Several wood species are shown with varying natural colorations as well as a block of *Populus* sp. and *Salix* sp. wood with green stain (Fig. 2A). These woods are described as "*Populus* or *Salix* (e sylva virdis) that developed a green color because they were in the earth for a long time" (Sepp 1773). A drawing of one block shows the intense green coloration of the wood (Fig. 2A).

In various temperate forests of Europe and North America, a green stain can be found within dead trees caused by the fungus *Chlorociboria* (Dixon 1974, 1975) (Syn. *Chlorosplenium*) (Figs. 2B to E). It is common in *Populus* wood causing a greenish-blue stain (Figs. 2D and E), and produces a similarly colored fruiting body on the surface of the wood (Figs. 2B and C).

The sections of wood from the Gubbio *studiolo* and the Fra Giovanni da Verona intarsia panels, examined

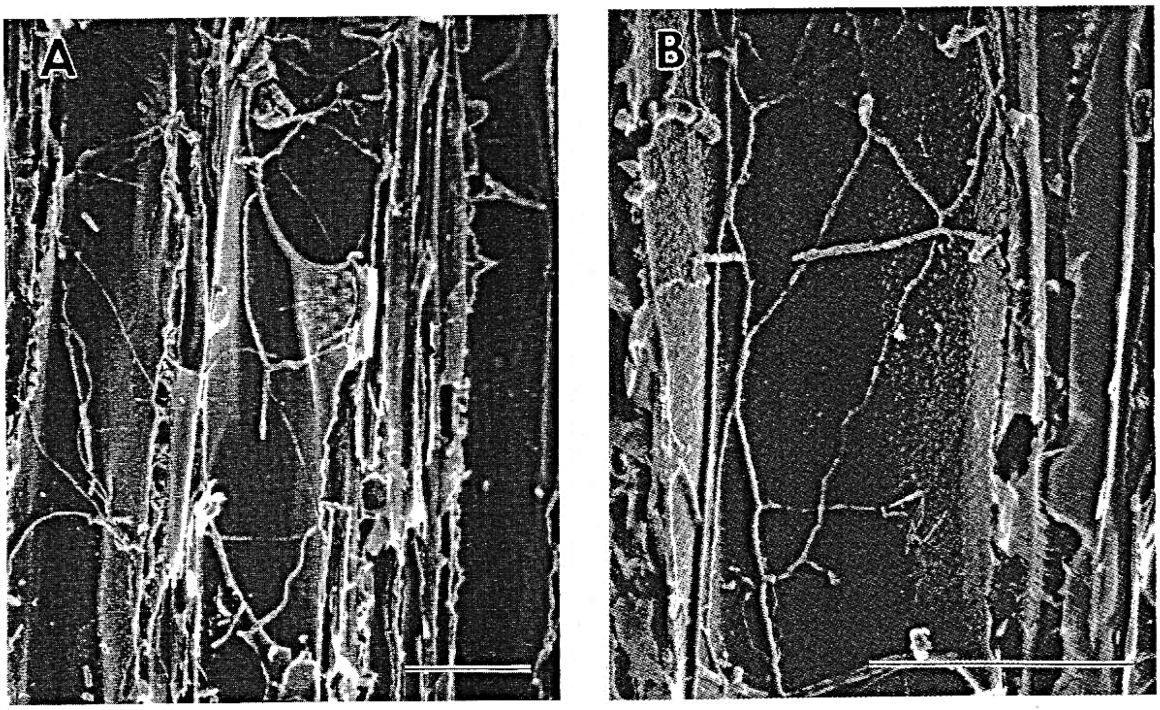
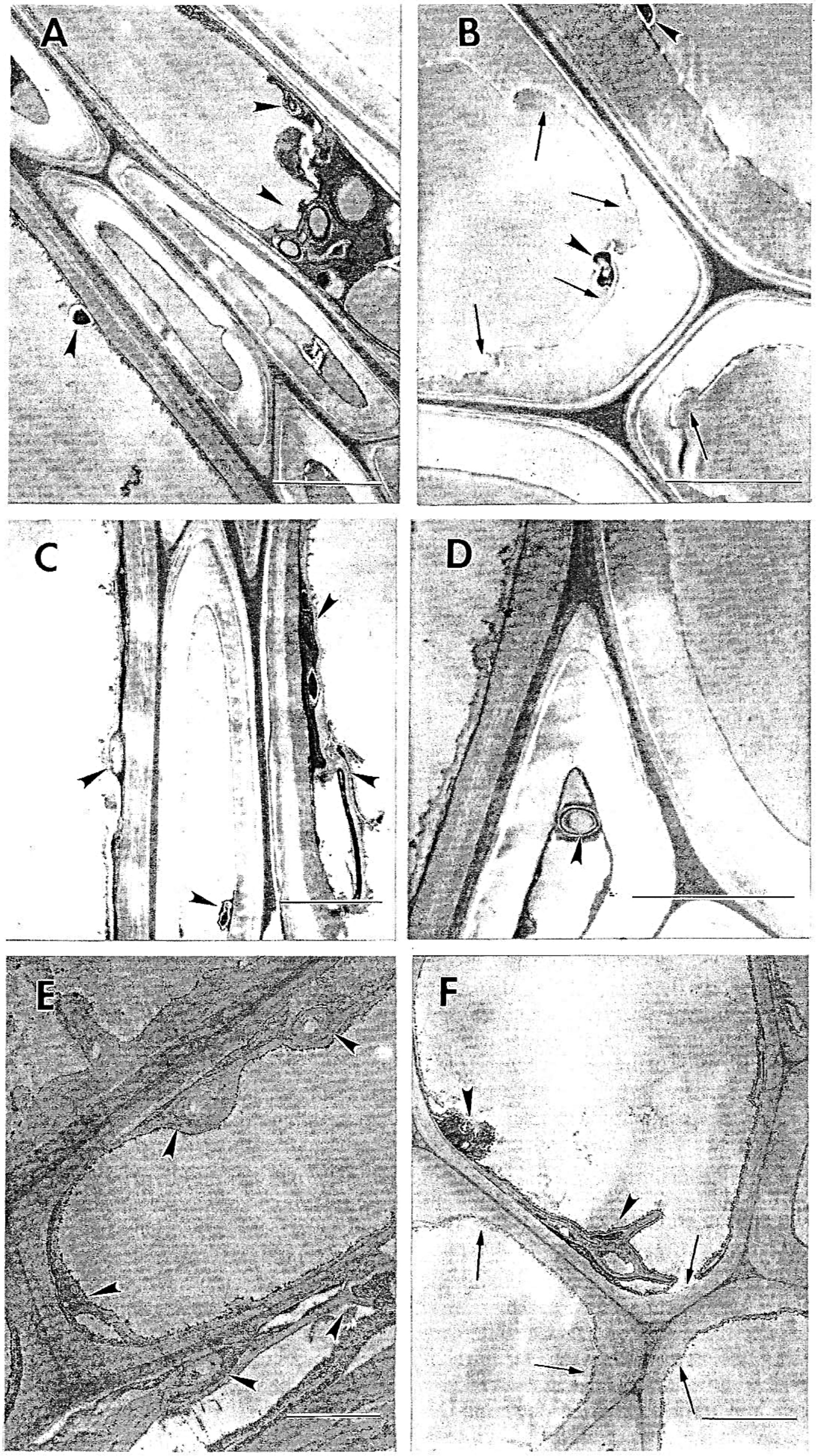


Fig. 3. Scanning electron micrographs of green-stained wood from the intarsia panel by Fra Giovanni da Verona, The Historical Museum of Amsterdam. **A.** Section showing vessel elements with fungal hyphae within cell lumina. **B.** Remnants of fungal hyphae are extensive within the cells of the green-stained wood. Bar = 50 μm.

Fig. 4. Transmission electron micrographs of transverse sections from green-stained wood in an intarsia panel from the Gubbio *studiolo* (15th century) in The Metropolitan Museum of Art, New York (**A** and **B**), from the intarsia panel by Fra Giovanni da Verona in The Historical Museum of Amsterdam (**C** and **D**) and from recently collected wood from the forest (**E** and **F**). **A.** Hyphae within the vessels, fibers and ray parenchyma cells can be seen (arrowheads) with pigmented substances accumulated around hyphae within a ray parenchyma cell. **B.** Hyphae (arrowheads) within fibers with an erosion of the cell wall is apparent (arrows). The secondary wall is eroded in some areas with degradation occurring in the wall from the lumen toward the middle lamella. **C.**. Hyphae (arrowheads) and pigmented substances within various wood cells. **D.** Hypha (arrowhead) and pigmented substances located in cells with relatively intact cell walls. **E.** Hyphae and pigmented substances (arrowheads) occurring in ray parenchyma cells and fibers. Many hyphae found within this recently collected green-stained wood appeared to be alive with active cytoplasm. **F.** Hyphae and pigmented substances (arrowheads) in fibers with secondary walls showing an erosion type of degradation. Bar = 5 μm.


A
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by light microscopy, showed that the stain was composed of green and yellow-orange deposits. These deposits were most frequently observed within ray parenchyma cells and in cells adjacent to the rays (Figs. 2F and G). Staining was not uniform throughout the wood. Some cells, such as the ray parenchyma cells and adjacent contact cells, were occluded with the stain while others remained void of any occlusions. Vessels contained sparse deposits that were associated with contact areas of ray parenchyma cells. The woods were identified as a *Populus* species. Scanning electron microscopy of radial sections showed the presence of fungal hyphae within cells of the 500 year old green-stained wood (Figs. 3A and B). Fungal hyphae were evident within vessels, fibers and parenchyma cells associated with the green stain. Samples of other inlaid woods, *Quercus* sp. and *Juglans* sp., located immediately next to the pieces of green-stained wood of the same panel (Figs. 1A and B) from the Gubbio *studiolo* were also examined. These sections showed no evidence of fungal hyphae in any of the cells examined (micrographs not shown).

Transmission electron micrographs of sections from the green-stained wood showed hyphae and electron-dense deposits in cells that appeared unaltered and also in cells where wall degradation was evident (Figs. 4A to D). In cells with hyphae, an erosion of the secondary wall was evident (Fig. 4B). The secondary wall appeared to be eroded from the cell lumen toward the middle lamella. This type of degradation is best described as an early stage of white rot or Type 2 degradation (erosion of the secondary wall) caused by soft rot fungi.

Wood from green-stained wood, colonized by *Chlorociboria*, was collected from dead trees within various forests and sectioned. A comparison of this naturally stained wood was made with the green-stained wood from the intarsia panels. Micromorphological and ultrastructural observations showed the stained wood from the forest to have similar characteristics to the wood in the intarsia panels (Figs. 4E and F). The green stain from the dead forest trees had both green and yellowish-orange deposits associated with ray parenchyma cells and other cells adjacent to the parenchyma. Fungal hyphae were associated with the deposits and were evident in all cell types. An erosion type of cell wall degradation also was observed (Fig. 4E) as well as electron dense pigmented substances within nondegraded cells (Fig. 4F).

Energy dispersive X-ray microanalyses of the green wood from the Gubbio *studiolo* and the Fra Giovanni da Verona panels did not show elevated concentrations of copper, iron or other metal ions that would be expected if the wood had been stained with an inorganic stain to achieve the green color. Moderate levels of calcium, potassium and sulfur were observed. The green-stained wood from the forest showed elevated concentrations of calcium and silicon. Wood samples (*Quercus* sp. and *Juglans* sp.) taken from areas in the same Gubbio *studiolo* panel near the green-stained wood showed moderate concentrations of calcium, potassium, sulfur and iron.

Discussion

The results reported in this paper demonstrate that the green wood typically used in the intarsia panels of the 15th century, by some of the finest artists of this type of work, was caused by a fungus. The patterns of staining and morphology of wood colonization are identical to deciduous wood colonized by *Chlorociboria aeruginosa* or other closely related species. The micromorphological and ultrastructural information conclusively shows that the green-stained wood of *Populus* sp. was not produced by prepared organic or inorganic stains applied by the craftsmen. Instead, the distribution of the stain, intimate association of the stain with fungal hyphae, and ultrastructural characteristics of colonization indicate that the stain was produced by a fungus. An important aspect of the fungal stained wood is the extensive distribution of colored substances within the ray parenchyma cells and not in the vessels. Artificial stains infiltrated into the wood would not have this unique spatial characteristic. The presence of fungal hyphae also were associated with all green-colored samples from the intarsia panels examined. The patterns of colonization observed are similar to staining patterns produced by many other stain fungi that impart various grey, blue, brown, yellow or red colorations in deciduous and coniferous woods (Ballard *et al.* 1982; Bauch *et al.* 1991; Boyce 1961; Eriksson *et al.* 1990; Zink and Fengel 1988). The stain fungi grow prolifically within ray parenchyma cells and colored substances may be found primarily intracellularly in the fungal hyphae or produced intra- and extracellularly (Bauch *et al.* 1991; Zink and Fengel 1989; 1990).

The colored compound that produces the green color was first identified by crude extractions made in 1868 and called xylindein (Rommier 1868 cited in Blackburn *et al.* 1965). This naphthaquinone pigment was further characterized more recently by Blackburn *et al.* (1965). It has been shown by these investigators that several different forms of the compound exist in xylindein. The various derivatives of xylindein range in color from yellow to green. The yellowish-orange substances observed within ray parenchyma cells and in some fibers and some vessels (Figs. 2F and G) are undoubtedly altered forms of xylindein. Apparently, the various forms of xylindein exist within different cell types of the wood possibly resulting from differences in pH or other factors that induce or inhibit various chemical reactions. The presence of both yellowish-orange and blue-green pigmented substances within the wood cells appears responsible for the brilliant verdigris coloration observed macroscopically within the stained wood.

X-ray microanalyses of the intarsia wood samples confirmed the results that the stain was not due to the application of inorganic stains. Large concentrations of metal ions were not found. In 1431 Jehan le Begue copied a recipe from the notes of Johannes Alcherius for staining wood green for which brass filings were essential. Johannes Alcherius had travelled to Italy at the very beginning of the 15th century where he collected workshop recipes on painting (Merrifield 1967). The X-ray microanalyses would have detected the use of such a stain by confirming the presence of copper. The moderate concentrations of calcium, potassium, and sulfur observed in the green wood and surrounding areas could have been from residues of previous restoration treatments or could also be from the wood itself. Concentration of iron, found in the wood adjacent to the green stain (*Quercus* sp. and *Juglans* sp.) were not observed in the green-stained wood. The use of organic green stains also have to be ruled out. These stains are very fugitive and would certainly have lost their color.

The extent of decay that can occur in wood by *Chlorociboria* is not fully known. *In vitro* studies to determine the capacity of this fungus to degrade wood have not been completed. The results presented here suggests that the fungus may cause an erosion of the secondary wall layers. This form of decay appears similar to the nonselective decay caused by some white rot Basidiomycetes or the Type 2 (erosion) form of soft rot produced by many Ascomycetes (Blanchette *et al.* 1990; Eriksson *et al.* 1990; Nilsson 1985). Eroded cell walls were evident in the samples from field collected wood and the intarsia panels suggesting that the green stain fungus is the causal agent. It also may be possible for other wood destroying fungi to colonize wood concomitant with the green-staining fungus. Dead *Populus* trees or cut logs in forest ecosystems could have a variety of fungi colonizing the wood allowing incipient stages of decay to progress into the green-stained wood.

The brilliant green color of wood colonized by *Chlorociboria* is very attractive and our results prove that this wood has been used in Italy as early as the 15th century. Various green woods used in other Italian and northern European intarsia and marquetry work also exhibit the visual characteristics associated with fungal-stained green wood. The green wood was utilized for inlaying extensively by many craftsmen but within the boundaries of availability and well-kept workshop secrecy. Since hardwoods degrade rapidly in forest conditions that are conducive to green stain development, the green-stained wood was available in smaller pieces and was therefore only suitable for inlay.

One of the earliest references to this wood, discussed by Blackburn *et al.* (1965), was in 1728 when a green stain of a vine prop was discussed at the Parisian Academic des Sciences (Tulasne and Tulasne 1865). Another important early reference to green-stained wood can be found in Icones Lignorum published in 1773 that showed a segment of *Populus* wood with a green stain (Fig. 2A). A hand colored print of this wood published in the book reveals the mottled nature of the staining pattern and accumulation of colored material within the wood cells.

It also has been reported by Berkeley in 1860, that some wood used by craftmen in Tunbridge Wells, England for Tunbridge ware was from green-stained wood that apparently was caused by the fungus, *Chlorociboria* (Berkeley 1860; Dixon 1975). Pinto (1970) and Austen (1989) state that the vivid green-colored wood used in cube patterns and for leaves of floral bandings in Tunbridge ware was largely obtained from fallen branches of oak, birch and other timbers, which were in an early stage of "decay". Pinto also mentions that the "decay" could only be used in the early stage of fungal attack. Two Tunbridge ware makers, Edmund Nye (1797–1863) and Thomas Barton (1819–1903), were attracted to the use of this green wood and a report on Barton's exhibits at the 1864 Tunbridge Wells Industrial Exhibition seemed to imply that this color was a recent introduction in Tunbridge ware.

The use of green-stained wood by various intarsia workers from different regions of Italy during the 15th and 16th centuries suggests that a fairly readily available source of green-stained wood existed. Craftsmen working throughout the centuries had excellent knowledge of natural wood colors, including naturally occurring wood stains. It may have been likely for these craftsmen to have found sufficient quantities of green-stained wood for their frequent use by chance collections in surrounding forests. It is an intriguing thought whether the knowledge existed during the 15th century of methods to inoculate cut logs of hardwoods by contact with infected logs or mycelia of the fungus, or whether the cut logs were just left in the forests to promote the occurrence of the green stain. This type of knowledge would be very advanced for the time since it was not until the mid to late 19th century that fungi were identified to be the cause of wood stains and decays (Hartig 1878). Although one can only speculate about the possible ways the green-stained wood was procured, it is evident that at the very least they had a keen understanding of the progressive stages of the green-staining, and that the wood was selected at an appropriate stage showing optimum staining characteristics but without appreciable loss of wood integrity.

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