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Glyptostrobus europaeus (Brongn.) Heer in Arkansas

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ABSTRACT

Glyptostrobus europaeus (Brongn.) Heer, a fossil gymnosperm is found at a few locations in central North America, in deposits of the Eocene. This work cites previously reported localities and reports a new site at Hooker, Arkansas. This deposit appears to be the most abundant for specimens of **G. europaeus** in the Eocene of North America. History of the Hooker site, characteristics of the living species **G. pensilis** (Abel) Koch and **G. europaeus** is presented. World-wide distribution of both species through time, is discussed. Specimens of many parts of the life cycle of **G. europaeus** are recorded and illustrated.

Glyptostrobus europaeus is a fossil gymnosperm belonging to the Coniferales, the Taxodiaceae and is closely related to the genera **Taxodium** and **Cryptomeria**.

There are one hundred and twenty papers published in which the fossil species of **Glyptostrobus** is described, according to this author's review. The greater percentage of these is on worldwide distribution and paleoecology. About two percent of the publications deal with the taxonomy and morphology of the species.

Florin (1963) in his monograph on the distribution of Conifer and Taxad genera of both past and present species, states that numerous finds have been made in the Tertiary of continental Europe from the Eocene to the Pliocene. He also states that in western North America, **Glyptostrobus** ranged from Montana to southern Alaska in the Paleocene; from Idaho and Montana through Oregon it was widespread, even to Nevada and northern California in the Eocene and Oligocene. He does not take note of any of the recorded finds that were made in the past as far as the Wilcox group of central and eastern North America are concerned. Berry (1916) records twig impressions from the Holly Springs sand formation at Oxford, Mississippi. Berry (1930) also records twig impressions from the same formation at LaGrange, Fayette County, Tennessee and from the same formation in Chester County, Tennessee.

Another site, the Brandon Lignite of Vermont, was interpreted by Berry (1919) as early Tertiary and probably Eocene from megafossil evidence. Later authors however questioned his interpretation. Traverse and Barghoorn (1953), through a pollen analysis of the Brandon lignites, did obtain **Glyptostrobus** pollen and found their pollen samples contained tropical, subtropical and warm temperate species; this is typical of other Eocene deposits during Wilcox times.

The Hooker, Arkansas deposit is an outcropping of the lower Eocene, namely Wilcox formation. All known Wilcox sites in Arkansas are of the upper part of this

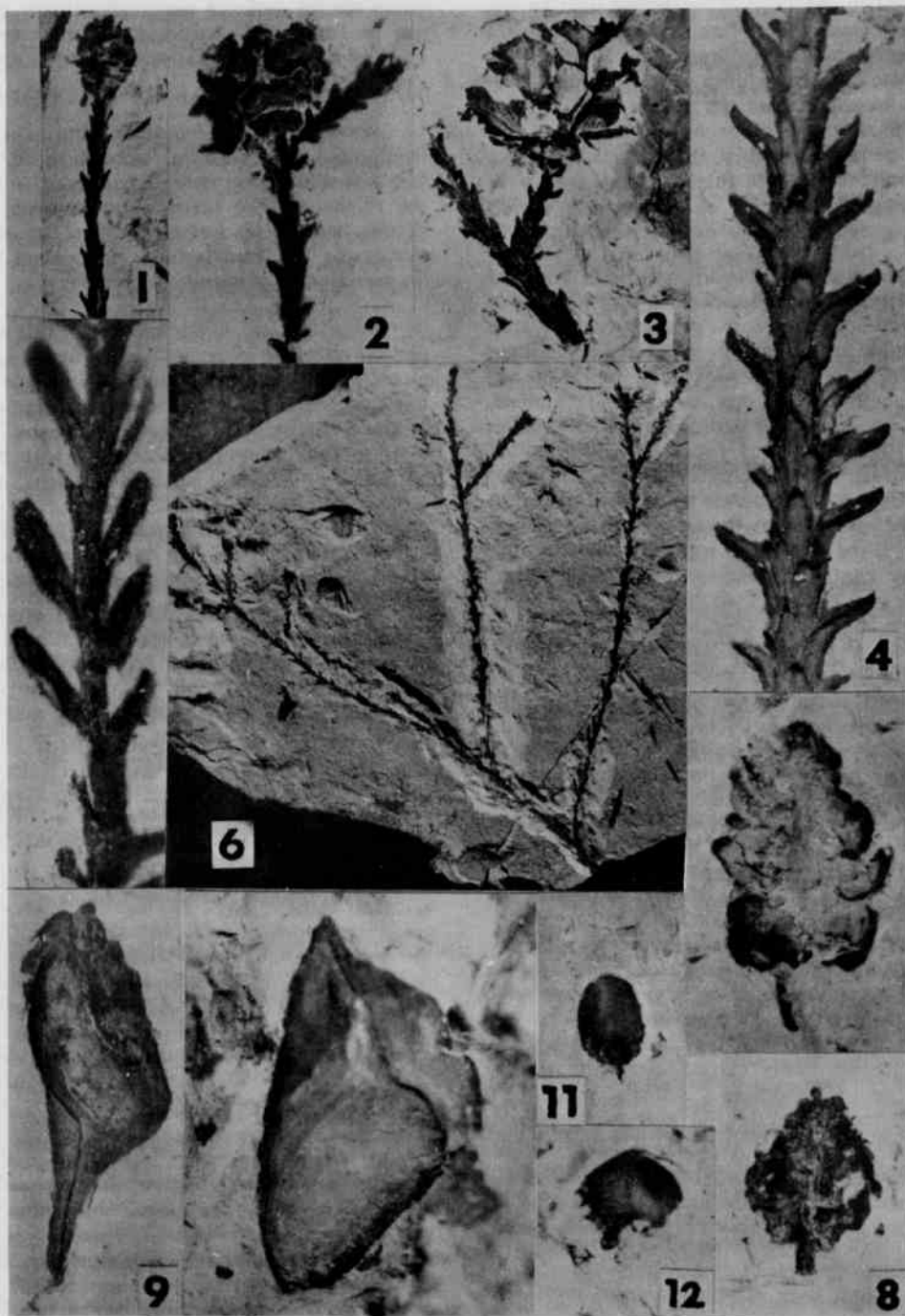
formation and are usually referred to as the Grenada formation. Indications are that during this period, an already warming trend was taking place toward Claiborne and Jacksonian times, or the Middle and Late Eocene.

The Hooker deposit on which this work was done, was visited and observed by Professor R. E. Call in 1889; the account appears in the 1889 Report of the Arkansas Geologic Survey in 1891. He describes the site as composed of "Tertiary blue clay and imbedded in this clay near the summit of a hill, is a large stump of silicified wood in place as it grew, with all its roots still embedded and ramifying in every direction." He further states that "fifty feet away at a lower level, occurs another stump similarly disposed and near at hand are two or three silicified logs." Professor Call sent specimens of silicified wood to Dr. F. H. Knowlton, assistant Paleontologist of the U.S. Geological Survey, who named the wood **Cupressinoxylon calli** n. sp. in honor of the collector. This wood on microscopic examination had all the characteristics of **Glyptostroboxylon** sp. No. 1 described by Greguss (1967) in his work on the fossil woods of Hungary.

In the fall of 1948, Dr. Delzie Demaree and Dr. Dwight Moore accompanied the author to the site. The stump at the lower elevation to the east and the fossil logs had disappeared although there were numerous fragments of the silicified wood scattered about. The first stump, viewed by Professor Call was still standing. It was one meter and a half in diameter and approximately a meter high. The blue clay, described by Professor Call, was actually a shale, very hard and very fine-grained. In fact, it is the only deposit on Crowley's Ridge known to the author, that has the appearance of clay, is the same color as most Wilcox clays, and yet is a shale.

Pieces of loose shale were scattered over the site; partial impressions of angiosperm leaves and twig impressions that reminded one of cedar twigs, were evident. The so-called "cedar twigs" were **Glyptostrobus** twigs and led the author to study the deposit further. Since 1959, it has been excavated periodically. The farmer and owner of the land broke up the last re-

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Figures 1 - 12. 1. Young gynostobilus., X 2.32; 2. Mature unexpanded gynostobilus, X 2.32; 3. Mature expanded gynostobilus, X 2.32; 4. Cupressoid twig, X 5.25; 5. Young taxodioid twig, X 5.25; 6. Cupressoid twig with young androstrobili at apices, X .825; 7 - 8. Mature and young unexpanded androstrobili respectively, X 6.58; 9 - 10. Mature seeds, X 5.25; 11 - 12. Microsporophylls, X 6.58.

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maining stump into several pieces, and moved the re-assembled stump on to the lawn of his home next to the road. Most of our excavation has been at the exact spot where this last remaining stump stood. On closer inspection, the base of the stump described by Professor Call at the elevation was discovered and a third stump base smaller than the other two. Perhaps in the early part of the nineteenth century, three massive trunks stood on the summit of the hill; in situ, they grew with their roots in the geologic mud of a brackish marsh which now is the gray-blue shale of the deposit.

During this investigation, recovery has been made of over nine hundred catalogued specimens of twigs, both young and old; many seeds, both young and old that show great detail in all their characteristics; eighteen *Gynostrobilus* attached to their twigs and fourteen *Androstrobilus*. The shale matrix has hundreds of microsporophylls scattered through it and casts of their pollen sacs. Solid and hollow casts of young and old female gametophytes situated on ovuliferous scales and bracts, were found. When one *Gynostrobilus* was being prepared for cataloguing, a hollow gametophyte cast was accidentally ruptured. A fossil embryo was detected through the rupture in the cast-epidermis of the gametophyte. Investigation of three others yielded three more embryos cast in silica. Also a few samples of shale have been subjected to pollen analysis and *Glyptostrobus* pollen has been found to have been present. Most of the specimens are third-dimensional impressions either stained by brown ferric iron oxide or a blackish ferrous iron oxide. A white substance, sometimes amorphous in nature, or rather fibrous to crystalline in character, is found occupying these impressions. The female gametophyte casts from which the fossil embryos were obtained, were composed of this material. Until further chemical analysis can be made, the author tentatively suspects it to be a hydrous aluminum silicate.

Comparisons with the fossil specimens are now being made with the living monotypic species, *Glyptostrobus pensilis* (Abel) Koch. Today it has a very restricted distribution in the southeastern Chinese provinces of Fukien and Kwangtung. This species has had a stormy taxonomic past. It has been classified in the genus *Thuja* and in the genus *Taxodium*. Wodehouse (1935) called it a small shrub eight to ten feet high. Florin referred to it as a small tree. Metcalf (1937) states that it grows to a height of thirty-five feet, in the area around Foochow, China. The general appearance and habit of *Glyptostrobus* is very similar to that of *Taxodium*. The *Gynostrobilus* are pear-shaped and the foliage is polymorphic. Many genera in this family are dimorphic in foliage. Looking over specimens from both the Missouri Botanical Garden and the National Herbarium, cupressoid, taxodioid and cryptomeroid foliage was quite obvious. The cupressoid foliage is the most permanent; the other two types are deciduous.

The typical habitat of *Glyptostrobus* is on the borders of brackish swamps that are affected by the fall and

rise of tide. According to Metcalf, knees are evident in some places and are never prolifically produced as they are in *Taxodium*. Buttresses are also evident in this species but are more elevated and bulbous in character than in *Taxodium*.

Since the North American continent has been rising throughout the Tertiary, according to Chaney (1940), *Glyptostrobus* disappeared from our area at the end of the Pliocene. The distribution-patterns of not only *Glyptostrobus* but also several other gymnosperms and angiosperms has been greatly contracted by this influence on continental climates.

In conclusion, it appears, that the Hooker site is an exceedingly rich deposit for the fossil species of *Glyptostrobus*. It contains a predominance of plant fossils indicating the deposit was part of a non-alluvial, brackish swamp, essentially a settling basin with very little stream-type influent. Associated with the gymnosperm material, are several species of ferns; many species of angiosperm leaves and their fruits; two new Lower Eocene species of fossil mosses Wittlake (1968); nine species of insects and a few young marine oysters and clams in addition to one excellent specimen of a marine annelid.

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Enzymatic Evidence That Leucine From Tentoxin Is Levorotatory

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Introduction

Tentoxin is a highly specific, biologically active peptide from *Alternaria tenuis* Auct., a common seed and soil inhabiting fungus. Grossly, its biological activity consists solely of irreversibly blocking the development of chlorophyll in cotyledons of certain plants when applied to seeds during imbibition or to young seedlings prior to their emergence from the soil. Most dicots tested, with the exception of tomato and members of the cruciferae, have been found to be sensitive while most monocots tested were resistant with the exceptions of sorghum and crabgrass (1, 2, 6, 7).

Halloin and associates (4) have shown that tentoxin disrupts chloroplast development in sensitive species without noticeably affecting the ultrastructure of other organelles. They also demonstrated that it does not block the conversion of protochlorophyll to chlorophyll in either sensitive or insensitive species. In fact, they showed a slight but consistent increase in chlorophyll in resistant species.

The peptide was purified by Grable (3) from culture filtrates or mycelial mats of *Alternaria tenuis* grown on Richards solution supplemented with V-8 juice. Structural determinations (7) have revealed it to be a cyclic tetrapeptide consisting of leucine, N-methylalanine, glycine and N-methyldehydrophenylalanine. The sequence of these amino acids and the stereoisomerism of all but glycine must be established to complete the determination of the primary structure of this peptide.

This study was undertaken to determine which optical antipode of leucine is present in tentoxin.

(1) Undergraduate Research Participant and Professor of Plant Pathology, respectively.

Materials and Methods

Hydrolysis of the peptide

The crystalline toxin was hydrolyzed in 6 normal HCl at 110°C for 24 hours according to the procedure of Spackman (5). After removal of the HCl in vacuo the hydrolyzate was taken up in 10% 2-propanol for immediate spotting of preparative thin layer plates.

Separation of Leucine from the Hydrolyzate.

Leucine was separated from the hydrolyzate by preparative thin layer chromatography on 2mm thick plates of Silica gel PF (Merck) with a propanol: water (80:36) solvent in one dimension. Leucine was located on the developed chromatogram by reacting one edge of the plate with ninhydrin. The surface of the plate containing leucine (unreacted) was scraped into a Millipore filter and the leucine was leached from it with distilled water. The concentration of leucine recovered was determined on an aliquot from this solution using a standard colorimetric procedure.

Enzymatic Oxidation of Leucine

Snake venom L amino acid oxidase was used in a Warburg manometric procedure as outlined by Wellner and Meister (9) to determine the susceptibility of tentoxin-leucine to this enzyme. The concentration of tentoxin-leucine was 12 millimoles per ml. The enzyme was obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio and was used at a concentration of 100 mg/ml. At this enzyme level oxygen uptake with L leucine was 3.6 microliters per minute. Commercially prepared D and L leucine were used as controls. Three replications were employed.

Results

L amino acid oxidase oxidized leucine from tentoxin