The Ginkgo, the Most Ancient Living Tree

The resistance of *Ginkgo biloba* L. to pests accounts in part for the longevity of this species.

Randolph T. Major

The maidenhair or ginkgo tree, *Ginkgo biloba* L., has probably existed on earth longer than any other tree that is now living (*1*). Darwin called it "a living fossil" (*2*).

The ginkgo tree (Figs. 1 and 2) is the sole surviving species of the group of plants known as Ginkgoales, whose ancestry has been traced back more than 200 million years (*3*). Apparently the group of trees to which *Ginkgo biloba* belongs evolved from ancient seed ferns during Permian times. Trees of the genus *Ginkgo* have existed, apparently, since the late Triassic or earliest Jurassic period (*4*). During the Tertiary and Quaternary periods great upheavals of the earth, followed later by an ice age, destroyed the ginkgo trees and other Ginkgoales in most of the world. The destruction was least in east Asia, and probably all of the ginkgo trees known today came from this part of the world. It is doubtful, however, whether a natural stand of ginkgo trees is to be found anywhere in the world today. Fortunately for the tree, its recent history has been extended by man's appreciation of its beauty and usefulness as a shade tree. Also, its seeds are prized as a food and as a medicine in some parts of the world. Its wood has been used not only for firewood but also for making chessmen and fine oriental lacquerware (*3*).

One wonders why this plant has persisted unchanged over so many millions of years. Obviously it must have been eminently suited to the environments in which it found itself, for otherwise it would have died out or would have changed.

The author is a professor of chemistry at the University of Virginia, Charlottesville.

Today we believe that evolution occurs largely through mutations of the genetic material deoxyribonucleic acid. These changes may be brought about by chemical or by physical means (*5*). X-rays are particularly effective in producing mutations in genes. Certainly radiation from cosmic rays and from radioactive substances in the soil produce mutations (*6*). It seemed possible, then, that the chromosomes and genes of *Ginkgo biloba* might be particularly resistant to mutation by radiation. However, this is probably not the case. A. H. Sparrow of the Brookhaven National Laboratory has found that this plant is as sensitive to radiation as are other gymnosperms which have large chromosomes and nuclei (*7*). But, while both natural radiation and chemicals are undoubtedly capable of causing mutations of the genes of ginkgo trees, the importance of these changes is kept to a minimum by the rather long time span from one generation to the next. These trees do not begin to reproduce until they are more than 20 years old (*3*), but they continue to reproduce up to an age of over 1000 years (*1*). As a result, they have very much less chance of changing their characteristics than most other plants have, for most plants have a shorter reproductive period.

The ginkgo tree is reputed to be completely resistant to all serious pests and diseases and to have a high tolerance of city smoke and industrial fumes (*3*). Since it seemed that such resistance and tolerance might be at least part of the explanation for the longevity of the ginkgo, my associates and I investigated these qualities of the trees.

Resistance to Insects

Insects are among the most serious of plant pests. However, the ginkgo tree is reported to be free from insect infestation. In fact, the leaves are used in China and Japan as bookmarks which are supposed to keep silverfish and larvae of other insects away from books. Also, the wood is highly valued for the manufacture of insect-proof cabinets (*8*). However, Hartzell and Wilcoxon found that acetone-and-water extracts of leaves of the ginkgo tree had no lethal effect on mosquito larvae, such as similar extracts of the leaves of a number of other trees had (*9*).

S. D. Beck of the University of Wisconsin found that alcoholic extracts of ground whole roots of *Ginkgo biloba* strongly inhibited the growth of the larvae of the European corn borer, *Pyrausta mubilalis*. Similar extracts of stems of *G. biloba* inhibited the growth of these larvae very little. The European corn borer is highly polyphagous (*10, 11*). Larvae of this borer fed and grew on a diet of ginkgo leaves. On the other hand, the addition of an aqueous extract of ginkgo leaves to a basal, purified diet fed corn-borer larvae inhibited the growth of the larvae. When the ginkgo extract was in such amount that each gram of diet contained the extract of 2 grams of ginkgo leaves, inhibition of growth was 98 percent and mortality was approximately 90 percent within an 8-day experimental period (*10*). Ginkgo leaves are quite acid. It is possible that some or most of the above-described inhibition of growth may be due to this acidity. Beck has found that malic acid and oxalic acid are both toxic to corn-borer larvae, and that each inhibits feeding (*10*).

When ginkgo leaves are damaged in the presence of oxygen, 2-hexenal is produced (*12*). Bevan, Birch, and Casewell have reported that this aldehyde is an insect repellent (*13*). Apparently the concentration of 2-hexenal produced by ginkgo leaves is insufficient to repel European-corn-borer larvae. On the other hand we have found in our laboratory at the University of Virginia that Japanese beetles normally will die of starvation rather than eat ginkgo leaves. However, the beetles eat fresh cherry leaves coated with juice from pressed ginkgo leaves almost as readily as they eat uncoated leaves. Moreover, the beetles will eat ginkgo leaves if the leaves are coated with juice from pressed cherry leaves, or coated with beetle attractants such as eugenol or valeric acid (*14*).

From the studies reported above it appears that the roots of *Ginkgo biloba* contain a substance which is toxic to some insects at least. The stem of the tree also is slightly toxic. Further, the leaves contain a small amount of a substance (or substances) which is toxic to some insects but very slightly so, if at all, to others. The high acidity of the leaves may account for some of this effect. The production of 2-hexenal by the leaves when they are damaged may account, at least partially, for the resistance of the leaves to the ravages of some insects.

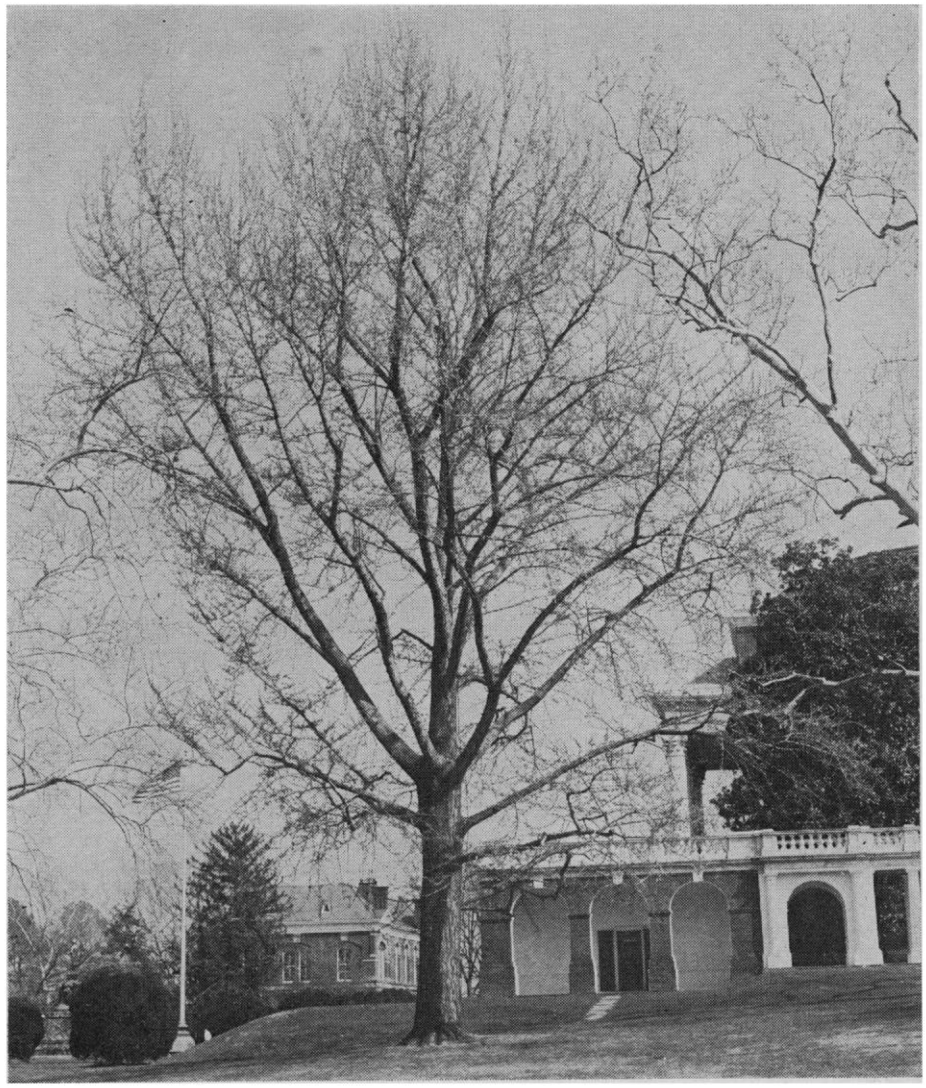


Fig. 1. Ginkgo tree in the spring on the grounds of the University of Virginia.

Resistance to Bacteria

Experiments carried out at the Boyce Thompson Institute for Plant Research in cooperation with our laboratory have shown that an acetone extract of macerated fresh ginkgo leaves stopped the growth of several bacteria which damage plants. The bacteria tested were *Erwinia amylovora, Escherichia coli, Pseudomonas phaseolicola, Xanthomonas phaseoli,* and *Bacillus pumilus*. However, additional experiments showed that antibacterial activity disappeared if the acetone extract, which was quite acid, was neutralized. Apparently the positive results obtained first were due to the acidity of the leaves (*15*).

Resistance to Viruses

J. W. Mitchell has found that substances obtained by means of crude alcoholic extraction of ground whole root of ginkgo trees, which was carried out in our laboratory, inhibited the production of symptoms by plants infected with southern bean mosaic virus and tobacco mosaic virus. The extracted substances in concentrations of 250 to 500 parts per million, in water, greatly suppressed the growth of these viruses. Extracts of the whole root were more effective than extracts of bark of the roots (*16*).

Resistance to City Fumes

As mentioned above, the ginkgo tree is reputed to have unusually high tolerance of city smoke and industrial fumes (*3*). However, experiments carried on in the Department of Plant Pathology of Rutgers University (*17*) showed that the ginkgo tree is no more resistant to sulfur dioxide and ozone than most other trees are. Sulfur dioxide and ozone are two of the most common phytotoxic compounds found as impurities in city air. This suggests that the ginkgo tree may be no more or no less sensitive to city smoke and fumes than other trees are.

Resistance to Fungi

Among the most damaging pests which attack trees are the fungi. However, the ginkgo tree is remarkably free from fungus attack. According to Pirone (*18*), the only leaf disease reported on this host is anthracnose caused by the fungus *Glomerella cingulata* (Stone) Sp. and Schrenk. The damage done by this fungus is negligible. Several wood-decay fungi have also been reported, but these are of rare occurrence.

2-Hexenal has been found in macerated leaves of the ginkgo tree. This aldehyde had an antifungal activity in concentrations sufficiently low to account for at least part of the resistance of ginkgo leaves to fungi (*12*). Schildknecht and Rauch have also reported the isolation of 2-hexenal from the leaves of a number of other plants, and have reported that it acts as a phytonicide when tested on certain Ciliata (*19*).

The unusual resistance of ginkgo leaves to fungi cannot be explained completely on the basis of the 2-hexenal content because other leaves, some of which are quite sensitive to fungi, also produce 2-hexenal when they are damaged (*20, 21*). However, the amount of 2-hexenal produced by ginkgo leaves is higher than that produced by the leaves of most other species tested. This may explain at least part of the greater resistance of ginkgo leaves to fungi (*22*). Nye and Spoehr (*21*) showed—and we have confirmed their finding (*22*)—that 2-hexenal is normally not present in detectable quantities in intact leaves but is formed by macerated leaves in the presence of oxygen. However, Schildknecht and Rauch found that leaves of the tree *Robinia pseudoacacia* would produce detectable quantities of 2-hexenal in 12 hours when the leaves were apparently growing normally on the tree (*23*). Nye and Spoehr suggested that the precursor of 2-hexenal might be 3-hexenal, oleic acid, or linolenic acid, or possibly all three of these substances (*21*). Recently, Drawert, Heimann, Emberger, and Tressl (*24*) reported that 2-hexenal is formed by the enzymatic oxidation of linolenic acid in fruit and leaves. This conclusion was based on their finding that, when homogenates of apples or leaves were oxidized in the air, their content of linolenic acid decreased as the content of 2-hexenal increased (*24*). This valuable finding does not conclusively prove that 2-hexenal is produced by the enzymatic oxidation of linolenic acid in macerated fruit or leaves. The simultaneous decrease in linolenic acid and increase in 2-hexenal may be coincidental. Additional proof of the occurrence of this reaction is needed. Toyama, Suzuki, Nakagami, and Yoshida have obtained 2-hexenal by the oxidation by air, in vitro, of the highly unsaturated acids of sardine oil. This required externally supplied heat (*25*). We have investigated the conversion of various C^{14}-labeled compounds, including linolenic acid, as possible precursors of 2-hexenal in ginkgo leaves (*26*).

Linolenic acid containing some C^{14} in each of its carbon atoms was added to ginkgo leaves, which were then finely ground. This mash was steam-distilled, and then the distillate was extracted with ether. The dimedon derivative of 2-hexenal could be obtained from the ether extract. The derivative was purified and crystallized by chromatographic procedures. From 0.4 to 1.2 percent of the radioactivity in the linolenic acid was recovered in the form of the 2-hexenal derivative. This showed that linolenic acid is converted into 2-hexenal in leaves, as suggested by Drawert, Heimann, Emberger, and Tressl (*24*). Linolenic acid itself has been found in ginkgo leaves by Tuzimoto (*27*). However, when C^{14}-labeled linolenic acid was added to water containing a little unlabeled 2-hexenal, the treatment outlined above, but without the use of leaves, also gave C^{14}-labeled 2-hexenal dimedon. The yields of 2-hexenal from linolenic acid were from 1.3 to 1.5 percent, whether the reaction was carried out in the dark or in the light. This indicates that the oxidation of linolenic acid in the air at room temperatures, regardless of whether leaves are present, yields 2-hexenal. The oxidation appears to be a normal chemical but nonphotochemical oxidation rather than an enzymatic one as Drawert, Heimann, Emberger, and Tressl (*24*) suggested.

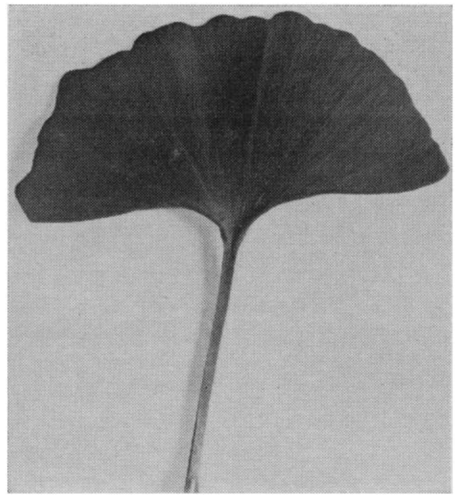


Fig. 2. Leaf of a ginkgo tree.

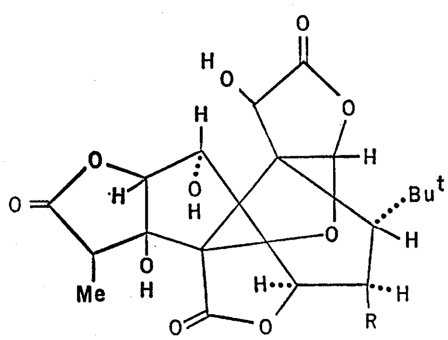
Due to the difficulty of explaining the resistance of ginkgo leaves to fungi solely on the basis of the amount of 2-hexenal produced when the leaves are damaged, my associates and I looked for other antifungal agents in the leaves. Steam distillation of ginkgo leaves in an atmosphere free of oxygen gave no 2-hexenal, but a small amount of an oil was obtained, amounting to about 10 milligrams of oil per 300 grams of leaves. This oil, in a concentration of 100 parts per million, inhibited the growth of the fungus *Monilinia fructicola.* It could, therefore, account for part of the antifungal activity of ginkgo leaves. The chemical nature of this oil was not determined, except by infrared analysis. The analysis indicated that the oil was largely a nonaromatic hydrocarbon possibly containing a carbonyl group.

Johnston and Sproston (*28*) found a waxy material in the cuticle of ginkgo leaves which reduced spore germination and inhibited germ-tube growth of certain fungi. They stated that they believe this wax may be responsible for at least part of the resistance of ginkgo leaves to fungi.

Adams and his associates found that the spores of various fungi on intact fresh ginkgo leaves will start to swell, forming appressoria, but that there is no penetration of the leaf epidermis, and that infection pegs are not formed. However, staining techniques showed that, while the leaves did not appear to be damaged, the chemical nature of the epidermal wall of the leaves was changed in the same way it is changed by mechanical damage to the leaf (*29*).

In the course of the search for inhibitors of fungus growth in the leaves of *Ginkgo biloba,* we have isolated several hydroxy-lactones (*30*). We extracted powdered dry ginkgo leaves with acetone and then evaporated the acetone. The dark oily residue was extracted with a mixture of water and ether; the residue was discarded. The ether extract and additional ether extracts of the aqueous portion were combined and extracted with sodium bicarbonate. The bicarbonate solution was acidified and reextracted with ether. This ether extract was added to an ethyl acetate extract of the acidified solution. The semisolid material left after evaporation of the organic solvents was extracted with chloroform. The larger, chloroform-insoluble fraction was recrystallized from 95-percent ethanol. Two distinctly crystalline fractions were obtained from this material by countercurrent distribution in an apparatus built by H. O. Post (*31*), by the method of Hecker (*32*), with the following solvent system: acetone, 10 parts; carbon tetrachloride, 5 parts; water, 5 parts; methanol, 1 part. Analytical and infrared studies showed that we had two hydroxy-lactones, one having the formula $C_{20}H_{24}O_{10} \cdot 1\ H_2O$ and the other, present in smaller amount, having the formula $C_{20}H_{24}O_{11} \cdot 2\ H_2O$. In 1929 Furukawa reported (*33*) that he had isolated two hydroxy-lactones having the formulas $C_{11}H_{14}O_5 \cdot H_2O$ and $C_{11}H_{14}O_6 \cdot H_2O$. It appears that these were cruder forms of the lactones which we have isolated more recently. Quite recently K. Nakanishi and his associates have brilliantly established the complete structures of four hydroxy-lactones from the bark of the roots of *Ginkgo biloba* L. They reported that these lactones were also present in ginkgo leaves (*34*). Two of the lactones isolated by Nakanishi and his co-workers—namely, their ginkgolide B and ginkgolide C—seem to be identical with the two hydroxy-lactones that we have isolated from the leaves of the ginkgo tree. By a somewhat different process, Sakabe, Takada, and Okabe (*35*) have also isolated these lactones from ginkgo leaves.

On the basis of the findings of Nakanishi and his associates (*34*), we may conclude that these lactones have the following structures:



R = H in ginkgolide B; R = OH in ginkgolide C.

By a somewhat different procedure we have also isolated another hydroxy-lactone from the leaves of the ginkgo tree. This lactone has the empirical formula $C_{15}H_{18}O_8$. A comparison of the physical and chemical properties of this compound with those of the four lactones from ginkgo roots which were studied by Nakanishi and his associates has convinced Nakanishi and my associates and me that this C_{15} lactone is probably closely related to the C_{20} lactones. However, the complete structure of the lactone is unknown.

The antifungal activity of the three hydroxy-lactones isolated by us has been studied. None of them is significantly active against the fungus *Monilinia fructicola*.

Summary

Fossil records clearly demonstrate that the group of trees known as Ginkgoales, of which today *Ginkgo biloba* is the sole living member, date back over 200 million years. *Ginkgo bilboa* itself is one of the oldest of living plants (*4*). It appears that the longevity of this tree and its long reproductive period are at least partially responsible for the persistence of the species. The resistance of these trees to pests such as insects, bacteria, viruses, and fungi has been studied. It is believed that their unusual resistance to pests accounts in part for the longevity of the trees and also, in turn, for the longevity of the species.

References and Notes

1. H. J. Lutz, *Amer. Forests* **37**, 475 (1931).
2. H. Prideux-Brune, *J. Roy. Hort. Soc.* **72**, 446 (1947).
3. A. H. Franklin, *Virginia J. Sci.* **10**, 131 (1959).
4. C. A. Arnold, *An Introduction to Paleobotany* (McGraw-Hill, New York, 1947), p. 227.
5. G. W. Beadle, *Genetics and Modern Biology* (American Philosophical Society, Philadelphia, 1963).
6. C. Auerbach, *The Science of Genetics* (Harper, New York, 1961), p. 236.
7. A. H. Sparrow, private communication, 1962.
8. Hui-lin Li, *J. Amer. Hort. Soc.* **40**, 239 (1961).
9. A. Hartzell and F. Wilcoxon, *Contrib. Boyce Thompson Inst.* **12**, 127 (1941).
10. S. D. Beck, private communications, 1960, 1961.
11. ———, *Ann. Entomol. Soc. Amer.* **53**, 206 (1960).
12. R. T. Major, P. Marchini, T. Sproston, *J. Biol. Chem.* **235**, 3298 (1960).
13. C. W. Bevan, A. J. Birch, H. Casewell, *J. Chem. Soc.* **1961**, 488 (1961).
14. R. T. Major and H. J. Tietz, *J. Econ. Entomol.* **55**, 272 (1962).
15. G. L. McNew, managing director of the Boyce Thompson Institute for Plant Research, private communication, 1959.
16. J. W. Mitchell, U.S. Department of Agriculture, Agricultural Research Service, Crops Research Division, unpublished data.
17. Eileen Brennan, department of plant pathology, Rutgers University, personal communication.
18. P. P. Pirone, *Maintenance of Shade and Ornamental Trees* (Oxford Univ. Press, New York, 1941), p. 257.
19. H. Schildknecht and G. Rauch, *Z. Naturforsch.* **16b**, 301 (1961).
20. T. Curtius and H. Franzen, *Ann. Chem.* **390**, 89 (1912); *ibid.* **404**, 93 (1914).
21. W. Nye and H. A. Spoehr, *Arch. Biochem. Biophys.* **2**, 23 (1943).
22. R. T. Major, P. Marchini, A. J. Boulton, *J. Biol. Chem.* **238**, 1813 (1963). Later work in our laboratory shows that somewhat more 2-hexenal could be obtained from some of the leaves than we reported in the 1963 paper. Also later work has shown a somewhat closer correlation than was reported in the 1963 paper between analytical results for the gas-chromatographic method and the 2,4-dinitrophenylhydrazone method of analysis.
23. H. Schildknecht and G. Rauch, *Z. Naturforsch.* **16b**, 422 (1961).
24. F. Drawert, W. Heimann, R. Emberger, R. Tressl, *Ann. Chem.* **694**, 200 (1966).
25. Y. Toyama, K. Suzuki, T. Nakagami, K. Yoshida, *Mem. Fac. Eng. Nagoya Univ.* **9**, 125 (1957); *Chem. Abstr.* **53**, 741 (1959).
26. The experimental work was carried out by Drs. M. Alauddin, O. D. Collins, K. Jaeggi, and P. Marchini in our laboratories.
27. M. Tuzimoto, *J. Soc. Chem. Ind. Japan* **43**, suppl., 208 (1940); *Chem. Abstr.* **34**, 7974 (1940).
28. H. W. Johnston and T. Sproston, *Phytopathology* **55**, 225 (1965).
29. P. B. Adams, T. Sproston, H. Tietz, R. T. Major, *ibid.* **52**, 233 (1962).
30. The experimental work with these lactones was carried out in our laboratory by Drs. H. Tietz, P. Lasfargues, H. W. Schnabel, M. Alauddin, A. R. Patel, and K. Jaeggi.
31. H. O. Post, Scientific Instrument Co. Inc., Middle Village, New York.
32. E. Hecker, *Verteilungsverfahren in Laboratorium* (Verlag Chemie, Weinheim, Germany, 1955).
33. S. Furukawa, *Sci. Papers Inst. Phys. Chem. Res. Tokyo* **19**, 27 (1932); *ibid.* **21**, 273 (1933).
34. M. Maruyama, A. Terahara, Y. Itegaki, K. Nakanishi, *Tetrahedron Letters* **1967**, No. 4, 299 (1967); M. Maruyama, A. Terahara, Y. Nakadaira, M. C. Woods, K. Nakanishi, *ibid.*, p. 309; M. Maruyama, A. T. Terahara, Y. Nakadaira, M. C. Woods, Y. Takagi, K. Nakanishi, *ibid.*, p. 315; M. C. Woods, L. Muira, Y. Nakadaira, A. Terahara, M. Maruyama, K. Nakanishi, *ibid.*, p. 321; K. Nakanishi, *The Chemistry of Natural Products* (Butterworths, London, 1967), vol. 4, p. 81.
35. N. Sakabe, S. Takada, K. Okabe, *Chem. Commun.* **1967**, 259 (1967).

36. My associates and I are indebted to Mr. John L. Pratt for support of our research on *Ginkgo biloba* L. at the University of Virginia.