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RADIATION-INDUCED ANATOMICAL MODIFICATIONS IN FOREST TREES

J. P. WITHERSPOON AND F. G. TAYLOR, JR.

Radiation Ecology Section, Health Physics Division,
Oak Ridge National Laboratory, Oak Ridge, Tennessee

ABSTRACT

Aberrant stem and foilage samples were collected from several forest tree species irradiated by fast neutron or ^{60}Co gamma sources. Common gross morphological abnormalities such as fused stems, alterations of size and shape of leaves and necrosis in meristematic regions were examined microscopically.

These frequently-reported changes in plant growth habit were described by effect of radiation on internal structure. Probable effects of these tissue changes on the physiological function of organs are discussed. Of particular significance were anomalies in vascular tissues of foilage which could effect the transport of both mineral and food substances.

INTRODUCTION

Modifications of growth and development of organs of higher plants may be induced by exposure to appropriate doses of ionizing radiation. These modifications are due to damage to cytological, genetical or physiological processes in cells and tissues. These responses have been reported previously for many higher plants exposed to X-rays (Johnson 1936), gamma-rays (Gunckel, *et al.* 1953; Gunckel and Sparrow 1954), and fast neutrons (Witherspoon 1965). Moreover several review articles have treated this subject, particularly in the case of gamma radiation (Sparrow and Pond 1956, Gunckel 1957, Gunckel and Sparrow 1961) in some detail. Morphological effects of irradiation in plants were summarized by Gunckel and Sparrow (1961) who also discussed the variables affecting structural responses.

There is little specific information about physiological or metabolic disturbances in plants which have undergone changes in organ structure. How functionally efficient, for example, are the thickened, chlorotic leaves resulting from irradiation of buds? Certainly an assessment of the ecological effects of ionizing radiation would be influenced by the ability of aberrant plant organs to manufacture and transport necessary materials.

Anatomical analyses of foilage and stem abnormalities should offer leads to likely functional significances of these radiation-induced structural changes. This report describes some of the abnormal organ structures in irradiated forest tree species; Virginia pine *(Pinus virginiana* Mill), sassafras *(Sassafras albidum* Nutt.), *Sequoia gigantea,* ash *(Fraxinus* sp.), sumac *(Rhus* sp.), dogwood *(Cornus florida* L.), persimmon *(Diospyros virginiaia* L.), red maple *(Acer rubrum* L.), tuliptree *(Liriodendron tulipifera* L.); emphasizing anatomical changes that may be functional.

MATERIALS AND METHODS

Since initial operations in 1963 of the unshielded Health Physics Research Reactor at Oak Ridge National Laboratory, portions of contiguous forest have been exposed to discontinuous, low-level, fast neutron radiation. Studies on the effects of this radiation in terms of mortality, growth and gross morphological changes have been reported (Witherspoon 1965). Additional laboratory studies (Witherspoon 1967), on radiosensitivity of forest tree species to acute radiation exposures have been performed. In both field and laboratory studies irradiated plant materials were collected for routine microscopic examination. Major structural abnormalities in foilage and wood of tree species have been cataloged according to type of radiation, mode of radiation delivery and dose.

Preparation of tissues for anatomical study followed standard botanical techniques. Shoot apices and leaf materials were killed and fixed in Craf III, dehydrated and infiltrated with a standard tertiarybutyl alcohol series and embedded in Paraplast. Woody material was killed and fixed in FAA. Sections were cut 10 to 25 microns thick and stained with safranin and fast green.

RESULTS AND DISCUSSION

Meristems. The relative radiosensitivity of meristems was recognized early, and reasons for their radiosensitivity were reviewed by Gunckel (1957) and Sax and Schairer (1963). Loss of apical dominance after chronic irradiation with X or gamma-rays has been frequently observed in both gymosperms and angiosperms (Sax and Schairer 1963); less frequently after chronic fast neutron irradiation (Witherspoon 1965).

Differential radiosensitivity of zones within shoot meristems have been described by Pratt *et al.* (1959) for angiosperms, and by Miksche *et al.* (1962) for yew. After exposure to ionizing radiation, both inhibition of mitotic processes and induced physiological changes may be manifested in stem mortality or stem growth inhibition.

Apical meristems of leader shoots of Virginia pine *(Pinus virginiana* Mill) were exposed to lethal, acute doses of fast neutron radiation (100 and 300 rads), and were collected two weeks later. Damage is described using the zonal termnology of Sacher (1954).

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The normal structure of the apical meristem is depicted in Fig. 1(a). Subtending the apical initial cell zone (apical summit) are the more vacuolated cells of the central mother cell zone. Files of elongating cells below the mother cells indicate the pith meristem, while cells of both flanks comprise the peripheral tissue zone. Apices from plants receiving 100 rads (Fig. 1(b, c) revealed early necrosis of cells in the apical initial and central mother cell zone. Complete absence of an apical initial zone and necrosis of cells in the central mother cell zone were evident in plants receiving 300 rads, Fig. 1(d). Cells and cell components of controls had greater affinity for stains than those of irradiated apices. Necrosis of apical initial cells is critical since these precursor cells mitotically produce all the cells of the shoot apex (Sacher 1954). Damage to cells of the peripheral tissue zone might be manifest as anomalous cortex, epidermis, vascular tissues, or lateral appendages. Damage to apical initials alter central mother cells derived from them and could subsequently affect differentiation of the rib meristem, or the inner layers of the peripheral tissue zone.

Tissue damage that has progressed to the extent shown in Fig. 1(d) leads to loss of the growing tip. Exposure to lower-level acute doses (Fig. 1(b) or low-level chronic radiation may not produce irreparable damage to all cells, thus allowing regeneration.

Stems. The common responses of stems to irradiation include dichotomy, fasciation, localized swelling, fusion, and formation of adventitious organs. The external aspects of these effects have been described for many species of plants (Gunckel and Sparrow 1961). More information is available for herbaceous than for woody species. Thresholds of radiation necessary to produce these effects differ among species, and some species have a greater tendency to exhibit a particular kind of response. For example, fusion of an axillary meristem with the apical meristem occurs frequently in sassafras saplings (*Sassafras albidum* Nutt.) near the Health Physics Research Reactor. There are cases in which

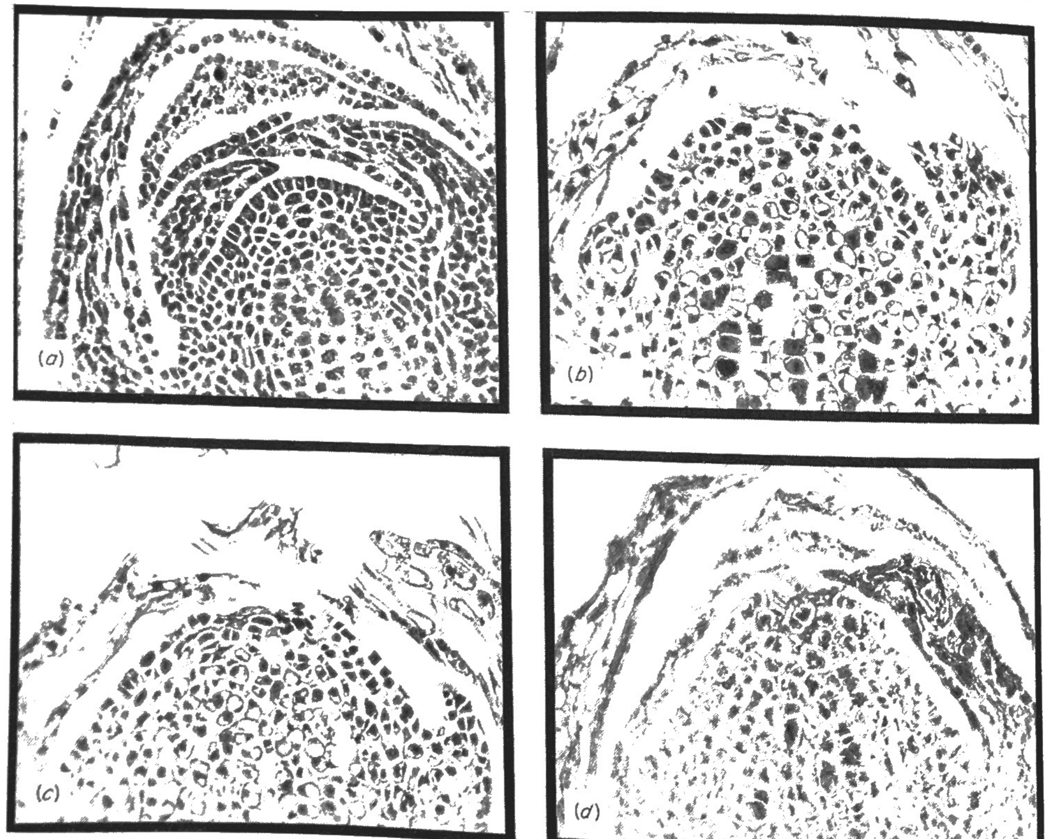

Figure 1. Median longitudinal sections of shoot apex in Virginia pine following fast neutron irradiation.
(a) Meristem of control.
(b and c) Necrosis of apical initial cells (100 rads).
(c) Absence of apical initial zone and necrosis of central mother cells (300 rads).

an axillary meristem was initiated but failed to develop independently from the apical meristem (Fig. 2). The resultant stem appears to be two stems fused longitudinally, and represents a situation in which the newly initiated axillary bud (spring 1965) failed to be "left behind" and developed concurrently with the apical meristem throughout that growing season, and finally produced a separate branch in the spring of 1966. Such fused stems can be considered morphologically single stems since there is continuity of pith, xylem, cambium and cortex (Fig. 3) along the entire internode. Similar stem fusions have been observed in dogwood and white oak following comparable fast neutron radiation exposures of 500 to 1000 rads per year delivered discontinuously (Witherspoon 1965).

A more common response of stems to irradiation is fasciation. Stem growth is inhibited in proportion to increasing dose rate or total dose above a particular threshold value. When shortened stems are accompanied by multiple, adventitious, axillary buds producing irregular leaves or multiple shoots, a fasciated or clustered appearance results. While these fasciated structures may represent an increase in biomass, individual organ size is much reduced, and organs are often aberrant in shape and color. Fasciation may increase surface area and may effect both photosynthetic and transpiration rates, positively or negatively.

Leaf Responses. Leaves of irradiated plants are often aberrant in color, form or texture. These changes may result from chromosome breakage, or from disruptions of normal auxin or mineral metabolism (Gunckel and Sparrow 1961). A very common response of chronically irradiated plants is twisting, thickening, puckering, fusion, or; most commonly; a simple reduction of the blades. The fact that leaf blades are so frequently altered by ionizing radiation indicates that terminal and marginal leaf meristems have greater radioresistance than plate rib meristems (Gunckel and Sparrow 1961). Why should this be so? Presently there seems to be no explanation for this differential radiosensitivity.

In a study of foliar tissue of giant *Sequoia* seedlings exposed to acute gamma radiation, needles (44 days postirradiation) were generally gnarled and swollen on plants receiving total radiation doses of 697 and 1119 rads. While mesophyll cells appeared to be necrotic and somewhat larger than controls, the greatest effect was evident in the vascular bundle. The anomalous vascular tissues were compared to controls. Extensive development of transfusion tissue, reduction in number of tracheids per xylem row, and absence of xylem parenchyma cells were observed (Fig. 4). It is probable that vascular tissues which have suffered this degree of disorientation and loss of tissue integrity would transport less efficiently than normal tissues. Conversely, "twin" resin ducts were found (Fig. 5). This condition has also been induced by mechanical founding as well as by irradiation (Thomson and Sifton 1925). This duplication of secretory ducts might result in increased resin output or an altered function

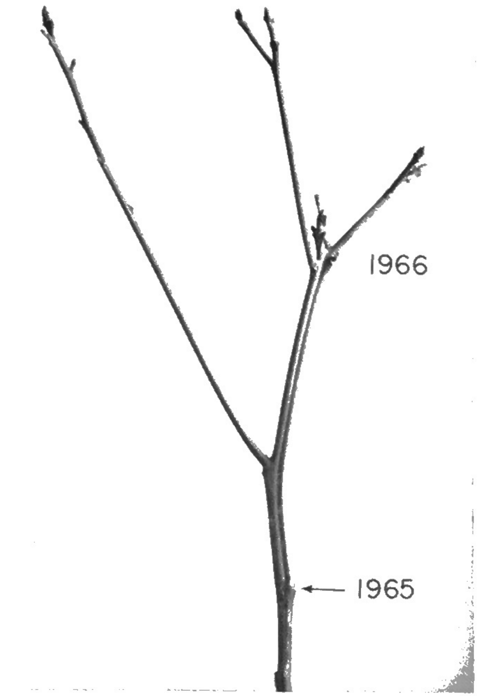

Figure 2. Stem fusion in sassafras following chronic exposure (2.5 rads per day for 1 year) to fast neutron radiation. Arrow denotes site of axillary meristem initiation (1965 node); note the groove formed by the concurrent development of the apical and axillary meristem resulting in fused internodes between 1965 and 1966.

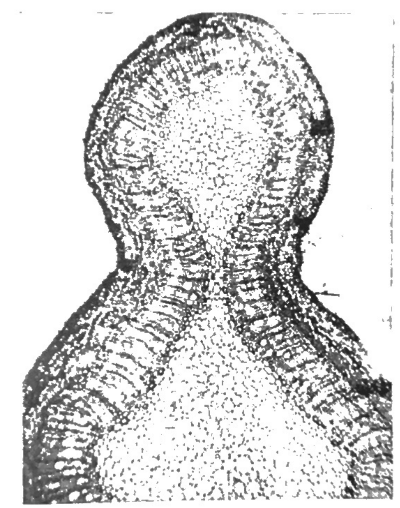

Figure 3. Cross section of fused sassafras stems illustrating continuity in pith, xylem, cambium and cortex.

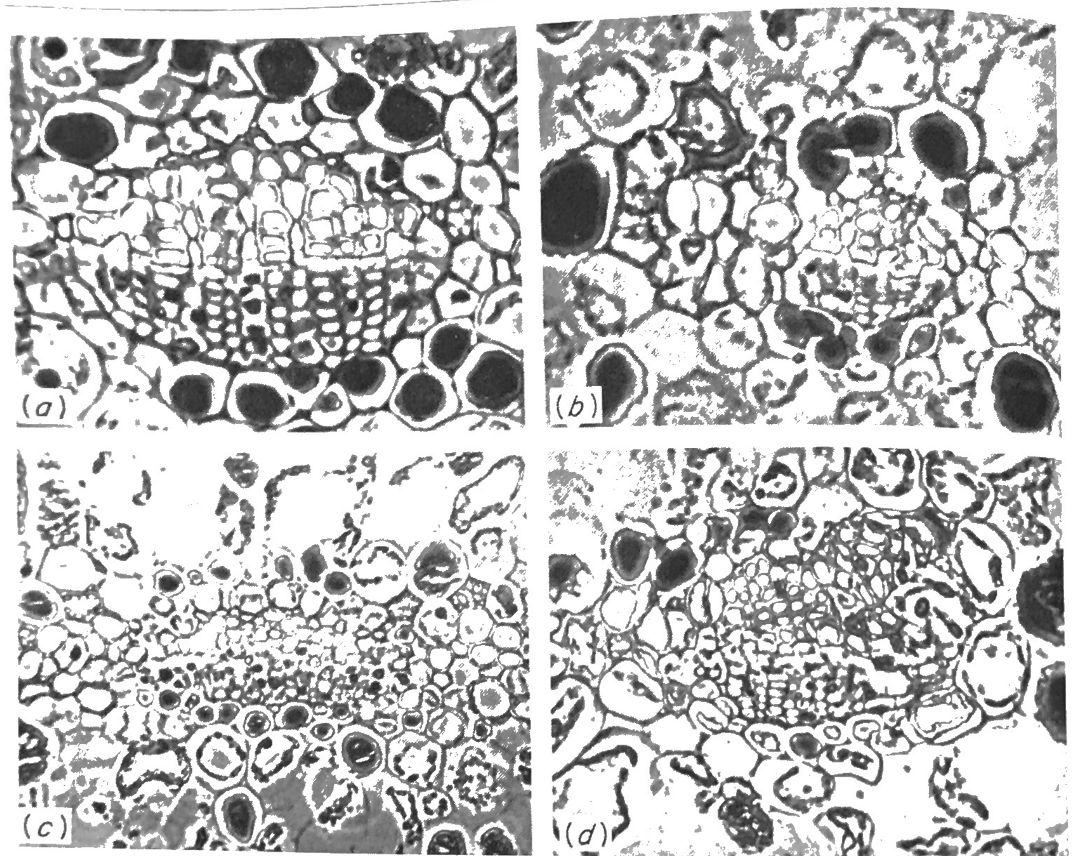

Figure 4. Anomalous vascular tissues in foliage of giant Sequoia seedlings following acute gamma irradiation at 44 days postirradiation, Xylem is adaxial.
(a) Control, xylem cells in tabular rows, interspaced with xylem parenchyma. Phloem cells also in orderly rows.
(b) 346 rads, increased volume of transfusion tissues to the left of the vascular strand.
(c) 697 rads, reduction in number of tracheids per xylem row, and extensive development of transfusion tissue.
(d) 1119 rads, absence of xylem parenchyma cells between xylem rows, and uneven thickening of some individual tracheids.

which probably is not important to subsequent plant growth.

Six species of trees were subjected to discontinuous, low-level fast neutron radiation. Reductions in leaflet number observed in ash (Fig. 6, a) and sumac (b), unilateral lamina (c, left) in dogwood, and reduced lamina in persimmon (d, left) and red maple (f, right) all illustrate anomalies resulting in decreased surface. The bifid lamina in dogwood (Fig. 6, c right) occurred with much less frequency than the other aberrant types. Generally, leaves of all these species were wrinkled, chlorotic and puckered with irregular margins. These types of foilage can appear spontaneously in nature as a result of modifications of developmental processes by insects or environmental factors such as frost. However, the natural frequency would be expected to be low compared to 50 to 75 percent frequencies observed in some irradiated trees (Witherspoon 1965).

Sections of irradiated yellow poplar (*Liriodendron tulipifera* L.) leaves are presented in cross sections as representative of damage (Fig. 7). Wrinkling or wavy leaf surfaces appeared to be the result of necrosis among epidermal cells (b, c) while puckering was the result of enlarged mesophyll cells in the region of the palisade tissue (d). The vascular strand in the region of the mid rib (c) was all but obliterated by pressures from surrounding tissues. Irradiated tissues appeared to have a greater affinity for stains than did controls. Inter-cellular air spaces were absent from irradiated tissues.

Structural anomalies such as these might easily influence photosynthesis, transpiration, and the exchange of

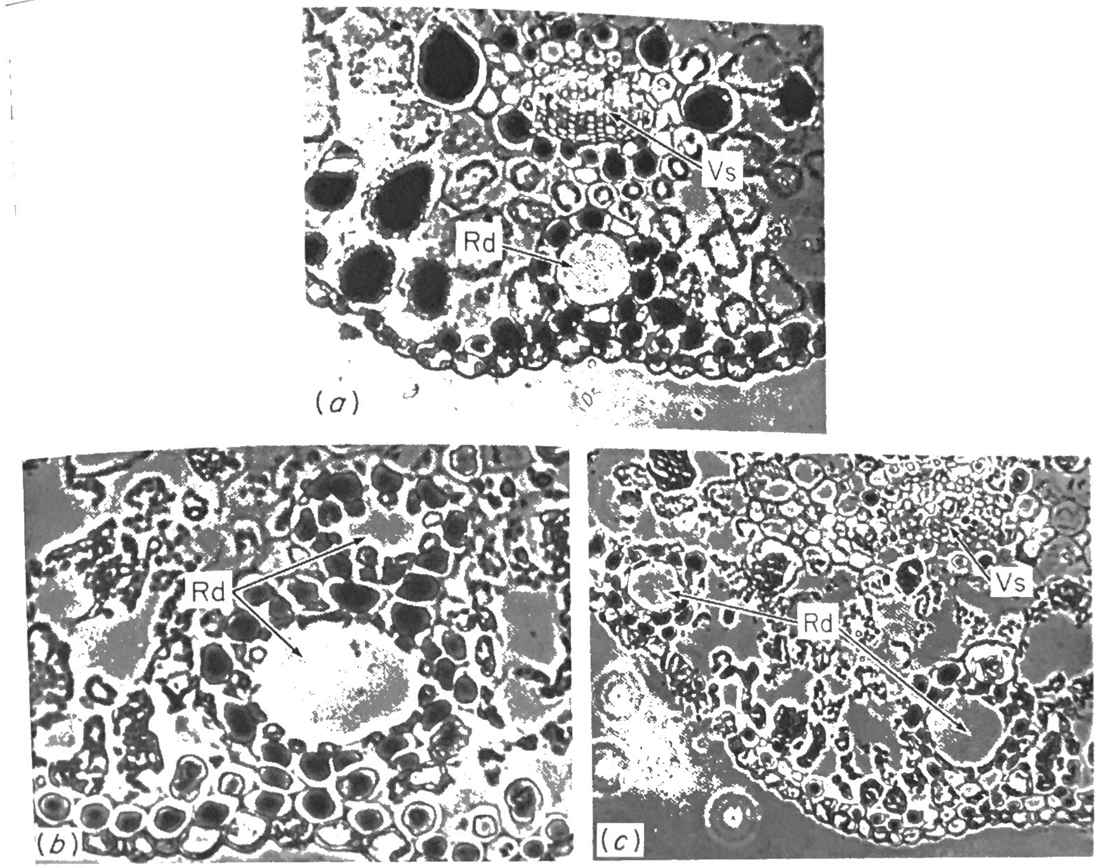

Figure 5. Cross sections through giant Sequoia leaves illustrating double or "twin" resin ducts (resin duct is abaxial).
(a) Control leaf depicting the normal location of the vascular strand (Vs) and resin duct (Rd).
(b) 697 rads, twin resin ducts.
(c) 697 rads, double resin ducts.

gases, thus affecting growth and longevity of the plant. Occurrence of anomalous vascular tissues in leaves has been offered as one explanation for differences in mineral uptake and translocation between control and irradiated plants (Brown and Taylor 1966). Results of a laboratory experiment with yellow poplar leaves tagged with ^{137}Cs by the authors showed that leaching losses from aberrant foliage exceeded those from controls by a factor of three over a 24-hour submersion period in distilled water.

Aberrant leaves produced in field or laboratory radiation studies usually remain functional, if at a reduced rate. They usually age earlier than normal leaves and fall earlier (McGinnis 1963).

To date, most of the physiological studies on structural changes in plants have been made to explain why or how plant organs respond to radiation (Gunckel 1957, Gunckel and Sparrow 1961). Interpreting the results of such growth alterations in terms of their significance for the organism or community is a necessary step in understanding the effects of radiation at ecological levels of organization.

Literature Cited

Brown, G. N. and F. G. Taylor, Jr., 1966. Zinc 65 distribution and radiosensitivity of growth and zinc 65 uptake in red oak seedlings. Radiation Botany 6, 519-524.

Gunckel, J. E., I. B. Morrow and E. Christensen, 1953. Vegetative and floral development of irradiated and non-irradiated plants of *Tradescantia paludosa*. American Journal of Botany 40, 317-332.

Gunckel, J. E. and A. H. Sparrow, 1954. Aberrant growth in plants induced by ionizing radiation. In Abnormal and Pathological Plant Growth. Brookhaven Symp. Biol 6, 252-279.

Figure 6. Leaves of six deciduous species illustrating puckering and thickening of lamina following discontinuous exposure to fast neutron irradiation for two years with a cumulative dose of 1200 rads.
Top row: left to right, white ash, winged sumac, dogwood.
Bottom row: left to right, persimmon, sassafras, red maple.

Gunckel, J. E. 1957. IV. The effects of ionizing radiation on plants: Morphological effects. Quarterly Review of Biology 32, 46-56.

Gunckel, J. E. and A. H. Sparrow, 1961. Ionizing radiations: biochemical, physiological and morphological aspects of their effects on plants. In *Encyclopedia of Plant Physiology* (W. Ruhland, ed.), Vol. XVI, Berlin, 556-611.

Johnson, E. L., 1936. Effects of X-rays upon green plants. In *Biological Effects of Radiation* (B. M. Duggar, ed.), McGraw-Hill Book Co., New York, London, 961-985.

McGinnis, J. T. 1963. Effects of radiation from an air-shielded reactor on forest litter production. In Radioecology (V. Schulty and A. W. Klement, eds.), Reinhold Publ. Corp., New York, 283-288.

Miksche, J. P., A. H. Sparrow, and A. F. Rogers, 1962. The effects of chronic gamma irradiation on the apical meristem and bud formation of *Taxus media*. Radiation Botany 2:125-129.

Pratt, C., J. Einset and Z. Mohammad. 1959. Radiation damage in apple shoot apices. American Journal of Botany 46, 537-544.

Sacher, J. A. 1954. Structure and seasonal activity of the shoot apices of *Pinus lambertiana* and *Pinus ponderosa*. American Journal of Botany 41, 749-759.

Sax, K. and L. Schairer, 1963. The effect of chronic gamma irradiation on apical dominance of trees. Radiation Botany 3, 283-285.

Sparrow, A. H. and V. Pond, 1956. Some cytogenetic and morphogenetic effects of ionizing radiation on plants. In proceedings of A Conference on Radioactive Isotopes in Agriculture, Atomic Energy Commission Report Number TID-6512-139.

Thomson, R. G. and H. B. Sifton, 1925. Resin canals in the Canadian spruce *Picea canadensis* (Mill). B. S. P.—an anatomical study, especially in relation to traumatic effects and their bearing on phylogeny. Royal Society of London Philosophical Transactions. Series B. 214, 63-111.

Witherspoon, J. P. 1965. Radiation damage to forest surrounding an unshielded fast reactor. Health Physics 11, 1637-1642.

Witherspoon, J. P. 1967. Radiosensitivity of forest tree species to acute fast neutron radiation. Proc. Second National Symposium on Radioecology, Ann Arbor, Mich., 120-126.

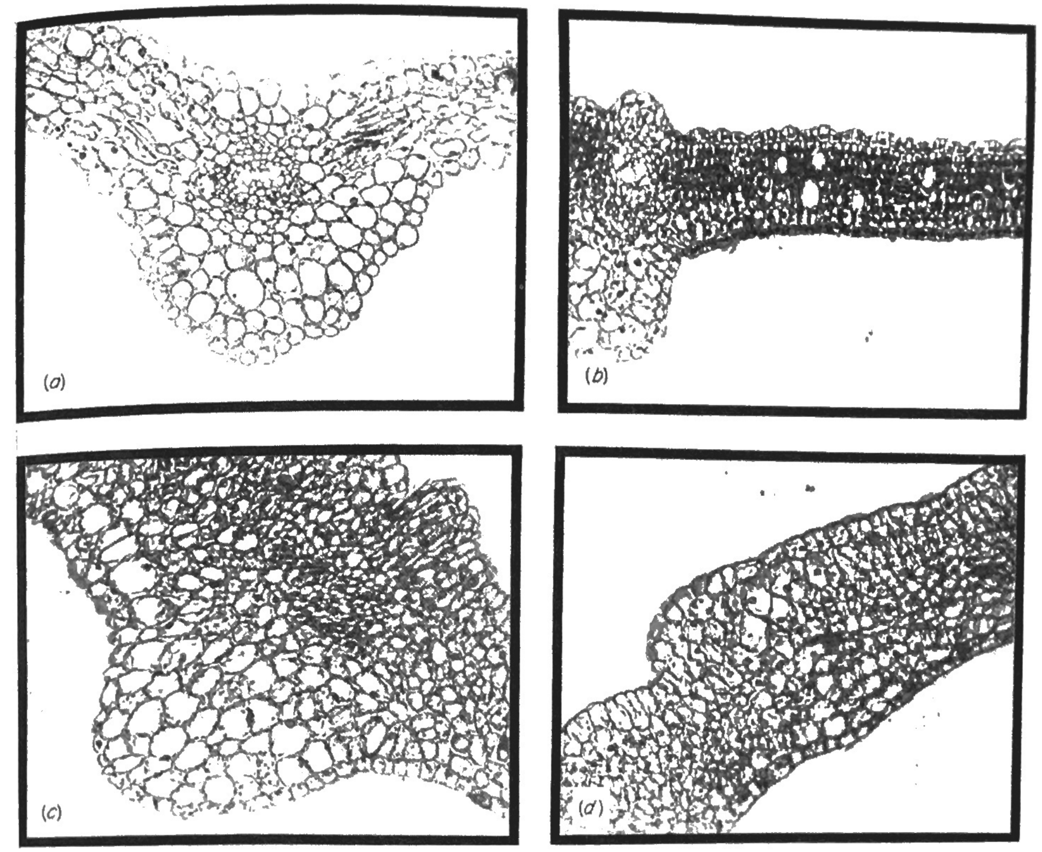

Figure 7. Internal structure of puckered and thickened lamina in yellow poplar seedlings following an acute gamma bud irradiation of 4500 rads.
(a) Control; epidermal cells uniform in size, vascular bundle, mesophyll and intercellular air spaces appear normal.
(b) irregular ridging of epidermal cells resulting in wrinkled lamina.
(c) vascular tissues nearly obliterated from pressures of surrounding cells.
(d) enlarged mesophyll cells resulting in a puckered appearance.