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ZINC-65 DISTRIBUTION AND RADIOSENSITIVITY OF GROWTH AND Zn^{65} UPTAKE IN RED OAK SEEDLINGS*

G. N. BROWN† and F. G. TAYLOR, Jr.
Radiation Ecology Section, Health Physics Division,
Oak Ridge National Laboratory, Oak Ridge, Tennessee, U.S.A.

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Abstract—External fast neutron irradiation of red oak seedlings (*Quercus rubra* L.) inhibited primary and secondary stem growth, root growth, and new leaf development. An initial stimulation of Zn^{65} uptake was observed following irradiation, but ceased to be significant 22 days post irradiation. Retardation of Zn^{65} uptake was found several weeks following exposure of seedlings to 1000 rad. Physiological explanations for growth inhibition and both stimulation and inhibition of Zn^{65} uptake are discussed.

At the end of a growing season, Zn^{65} was most highly concentrated in secondary roots, followed by primary roots, stems and leaves in decreasing order: 91, 7·7, 0·83 and 0·42 per cent of the total.

Résumé—Une irradiation par les neutrons rapides de plantules de chêne rouge (*Quercus rubra* L.) inhibe la croissance caulinaire primaire et secondaire, la croissance radiculaire et le developpement de nouvelles feuilles. Une stimulation initiale de la prise de Zn^{65} a été observée après irradiation. Elle cesse cependant après 22 jours. On a constante un retard de l'absorption du Zn^{65} quelques semaine après irradiation des plantules par 1000 rads. On donne des explications physiologiques de l'inhibition de croissance ainsi que de l'inhibition de stimulation de l'absorbtion de Zn^{65}.

A la fin de la saison, la plus forte concentration de Zn^{65} se rencontre dans les racines secondaires. Ensuite viennent les racies primaires, les tiges et les feuilles par ordre decroissant: 91, 7.7, 0.83 et 0.42% du total.

Zusammenfassung—Äussere Bestrahlung von Keimlingen der roten Eiche (*Quercus rubra* L.) mit schnellen Neutronen hemmte das Wachstum der Primär- und Seitensprosse, der Primär- und Seitenwurzeln und die Entwicklung von neuem Laub. Nach der Bestrahlung wurde zunächst eine Stimulierung der Aufnahme von Zn^{65} beobachtet, die aber 22 Tage nach der Bestrahlung nicht mehr von Bedeutung war. Eine Verzögerung der Zn^{65}-Aufnahme zeigte sich einige Wochen nachdem die Keimlinge einer Strahlung von 1000 rad ausgesetzt waren. Es werden physiologische Ursachen der Wachstumshemmung sowie Stimulierung und Hemmung der Zn^{65}-Aufnahme diskutiert.

Am Ende einer Vegetationsperiode ergab sich folgende Verteilung der Gesamtmenge von Zn^{65}: bei Nebenwurzeln der äusserst hohe Wert von 91%, bei Primärwurzeln 7,7%, bei Sprossen 0,83% und bei Blättern 0,42%.

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† Present address: School of Forestry, University of Missouri, Columbia, Missouri, U.S.A.

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INTRODUCTION

PHYSIOLOGICAL ecology tends to give separate attention to isotope movement in plants and to radiation effects on plants, without considering how ionizing radiation might modify the uptake and distribution pattern of nuclides.[1,2,7,8] In this and most other tracer studies, activity levels are intentionally kept low to avoid changes of normal processes due to internal dose. In the present study external radiation was added as a treatment to show its effects on growth and Zn^{65} uptake in red oak seedlings (*Quercus rubra* L.).

Zinc is of interest as a metabolically essential nutrient which is less commonly studied than the major elements in forestry and ecological experiments. It also happens to be a neutron activation product which could furnish a significant contribution to environmental radioactivity following thermonuclear detonations.

Zinc-65 distribution was evaluated throughout non-irradiated red oak seedlings to determine normal points of internal accumulation and re-cycling mechanisms.

METHODS AND MATERIALS

Three-year-old red oak seedlings (*Quercus rubra* L.) were planted in aerated, polyethylene tubs (7 in. dia. $\times$ 6 in. high) containing 5 in. of top-soil from an Emory series. After transplanting, the plants were allowed to establish themselves for one year prior to treatments. Groups of plants (13 per treatment) received 500 and 1000 rad fast neutrons (tissue dose in air) delivered over a period of 8 min after the period of initial spring growth and greatest radiosensitivity. Immediately following irradiation, plants in the two treatments, plus a control group of plants, were tagged with Zn^{65} by injecting a total of 50 μc per tub. The isotope was administered in 5 injections of 10 μc each at equal distances from the seedlings in a pentagonal pattern, evenly distributed over a soil depth of 3 in. to surface. Soil increments, sub-divided into 1-in. layers and radioassayed, showed that the Zn^{65} was evenly distributed throughout the top 3 in. of soil. Time of irradiation and inoculation was selected so that the effects on uptake and translocation would not be masked by leaf initiation and rapid stem elongation.

Stem growth was measured from the time of irradiation throughout the growing season according to stem class (primary, secondary and tertiary). From the time of irradiation throughout the growing season, leaf disks of equal area (200 mm^2) were taken from mature leaves in a manner that would not introduce error due to accumulation of isotope near major veins.[9] Disks were oven dried for 24 hr at 100°C, weights were determined, and the disks were assayed for Zn^{65} (Packard Auto-Gamma Spectrometer, Series 140A). Background activity was determined from radioassay of disks collected from non-irradiated and non-inoculated seedlings. To ascertain the rainout of Zn^{65} from senescing leaves, blotter papers were fitted over the tubs and allowed to remain throughout leaf senescence accompanied by rainfall. Blotter papers were then assayed for Zn^{65}.

Internal distribution of Zn^{65} was determined by removing all soil from a non-irradiated plant's roots and subdividing the plant into leaves, stems and roots prior to radioassay.

RESULTS AND DISCUSSION

Primary stem growth responded to fast neutron radiation as illustrated in Fig. 1. Significant retardation of growth was observed in both groups of irradiated plants. Plants receiving 1000 rads exhibited significant growth retardation compared to control plants from 25 days following irradiation until the end of the growing season, while plants receiving 500 rad did not show significant retardation compared to controls until 52 days following irradiation. GUNCKEL and SPARROW[3] have reviewed the literature reporting stem growth inhibition resulting from ionizing radiation.

Secondary stem growth was much more erratic than primary stem growth. The control plants averaged $30{\cdot}62 \pm 7{\cdot}33$ mm, plants receiving 500 rad averaged $6{\cdot}31 \pm 2{\cdot}42$ mm, and plants receiving 1000 rad averaged $4{\cdot}33 \pm 1{\cdot}00$ mm between the time of irradiation and the end of the growing season. Since secondary stem growth did not demonstrate a normal distribution, WILCOXON's ranking test[15] was used to analyse the data. Plants receiving either level of radiation produced significantly lower secondary stem growth than control plants (5% level),

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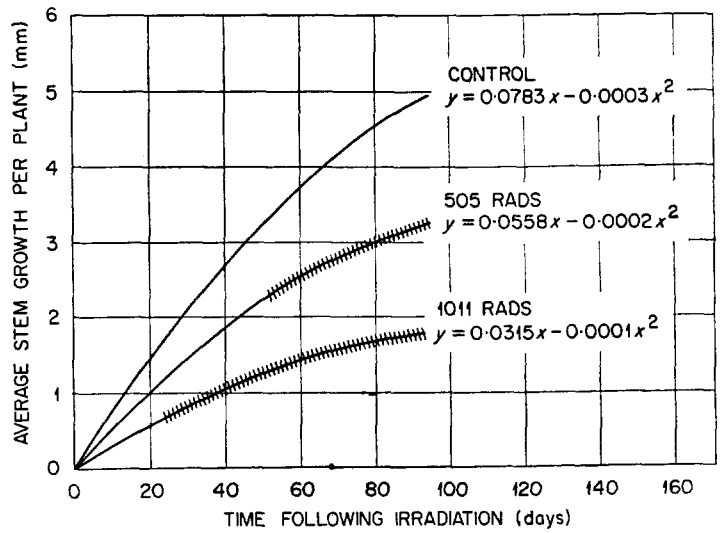

FIG. 1. Primary stem growth of red oak seedlings following fast neutron irradiation. The curves give the best fits to their respective data points. /////// indicates significant difference from control (5 per cent level).

but did not differ between the two levels of radiation. New leaf development was also too erratic to compare irradiated plants with controls. None of the thirteen plants receiving 1000 rad and only one of the thirteen receiving 500 rad developed new leaves following irradiation, but five of the thirteen control plants developed between four and six new leaves per plant. Dry weights of leaf disks collected from mature leaves increased throughout the growing season but did not differ between control plants and irradiated plants. Root systems of plants removed from the soil at the end of the growing season showed root growth differences comparable to stem growth differences between control and irradiated plants. Root systems of all plants were distributed throughout the entire soil media. GUNCKEL and SPARROW[3] have reviewed literature discussing responses of leaf and root growth to ionzing radiation.

Uptake of Zn^{65} from the soil, translocation, and accumulation in mature leaves responded to fast neutron irradiation as illustrated in Fig. 2. Plants receiving either level of radiation showed an early stimulation in Zn^{65} uptake as compared to control plants. Between 18 and 72 days following irradiation, differences in uptake and accumulation were not significant. From 72 days until the end of the growing season, plants receiving 1000 rad accumulated significantly less Zn^{65} in the leaves than control plants, while plants receiving 500 rad did not differ from controls throughout the remainder of the growing season.

VASIL'EV and RYBALKA[14] observed a slight enhancement of mineral uptake in wheat seedlings receiving 3000 and 5000 R of X-irradiation, and suggested that a more rapid movement of protoplasm following moderate radiation doses might temporarily enhance mineral uptake. BARBER and NEARY[1] observed an increase in uptake of non-exchangeable Rb by *Chlorella* immediately after exposure to X-irradiation, and proposed that the stimulation resulted from oxidation of sulfhydryl groups. TANADA[12] found that excised mung bean roots exposed to u.v. radiation showed temporary stimulation of rubidium uptake, providing that Mg and Ca were absent from the growth medium. He suggested that radiation-induced unfolding of coiled molecules such as ribonucleoprotein located near the cytoplasmic surface of root epidermal cells might temporarily make cationic binding sites more available for cation uptake. Excitation of any active carrier conceivably could temporarily stimulate mineral uptake.

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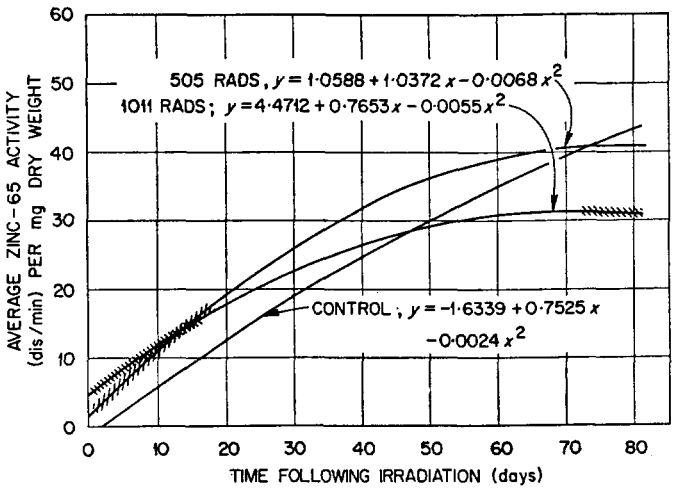

FIG. 2. Zinz-65 uptake and accumulation in mature leaves of red oak seedlings following fast neutron irradiation. The curves give the best fits to their respective data points. /////// indicates significant difference from control (5 per cent level).

Several investigators differ in findings and interpretations concerning inhibition of mineral uptake by roots of irradiated plants.[2,7,8,14] RUSSELL *et al.*[8] suggested that radiation damage to root tips could inhibit mineral uptake by the root tip cells. TANADA[12] proposed a "one-hit" inactivation of mineral binding sites such as those available on ribonucleoprotein near the cytoplasmic surface of root epidermal cells. HODGSON[4] demonstrated a reduction in translocation of P^{32} to meristematic tissue following X-irradiation, possibly a result of damage to terminal buds and meristematic regions which serve as major sinks for mineral translocation. In sequoia seedlings, TAYLOR[13] observed radiation damage to vascular tissue, which could inhibit mineral translocation and hence accumulation in leaves. BARBER *et al.*[2] observed little effect of ionizing radiation on uptake of readily exchangeable minerals. VASIL'EV and RYBALKA[14] also observed little effect of ionizing radiation on mineral uptake by wheat seedlings in some instances, and suggested that vacuolized cells are the only root cells which absorb minerals and that such cells are not as radiosensitive as are root meristematic cells.

Since plants receiving 1000 rad differed significantly from control plants in Zn^{65} uptake beyond 72 days following irradiation in the present study, while plants receiving 500 rad did not differ, dose levels and time following irradiation may account for the inconsistencies in radiation effects on mineral uptake reported in the literature. The similarities between growth responses to radiation and later Zn^{65} uptake responses suggest that inhibition of mineral uptake might be a direct result of growth and developmental damage and an indirect result of radiation damage (Figs. 1 and 2). Such an indirect effect is plausible, since inhibition of Zn^{65} uptake does not occur until after significant growth inhibition resulting from radiation damage. Clearly, the initial stimulation of Zn^{65} uptake (Fig. 2) is not explained by general growth damage. Early stimulation of mineral uptake by radiation might conceivably produce toxic levels or relieve deficiency levels causing later growth retardation or stimulation. Neither Zn toxicity nor deficiency symptoms were observed in the irradiated red oak seedlings, however.

No evidence of rapid re-translocation of Zn^{65} out of mature leaves during senescence was found (Fig. 2). A literature review by RICE[6] concluded that Zn is not commonly re-translocated from older to younger leaves as the plant grows. SHAW *et al.*[10] also reported that Zn is not readily re-distributed in corn plants. Like-

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wise, WOOD and SIBLY[16] found that Zn was not re-translocated from leaves of oat plants during senescence nor during development of inflorescences and grain, but was supplied from roots and the soil medium to developing inflorescences and grain. In contrast, re-cycling of Zn from pea leaves was demonstrated by SUDIA and LINCK[11], but only during fruit development.

Rainout from leaves of red oak seedlings was unimportant since practically no Zn^{65} activity appeared on blotters placed under the seedlings during senescence. This lack of Zn^{65} leachability from red oak leaves is supported by WOOD's and SIBLY's[16] inability to remove Zn from macerated oat leaf material by dialysis.

Distribution and concentration of Zn^{65} in non-irradiated red oak seedlings is illustrated in Table 1. Roots had the highest concentration of Zn^{65}, with the secondary roots having a much higher concentration than the primary root. Stems had the next highest concentration of Zn^{65}, with leaves having the lowest. RICE[6] stated that Zn^{65} taken up by bean plants was found to be present in greatest quantities in the roots. On the other hand, MAKHONINA *et al.*[5] found Zn to be concentrated most in above ground portions of several plant species. WOOD and SIBLY[16] demonstrated continuous absorption of Zn throughout the life cycle of oat plants. Zinc-65 accumulation in leaves of non-irradiated red oak seedlings (Fig. 2) also suggests continuous absorption of Zn throughout the growing season. WOOD and SIBLY[16] further found 20–30 per cent of total plant Zn to be contained in developing leaves of oat plants. Only 0·42 per cent of total plant Zn^{65} was found in leaves of red oak seedlings (Table 1), but these leaves were mature at the time of Zn^{65} introduction to the soil medium, while leaves which developed after Zn^{65} introduction had much higher concentrations of Zn^{65}.

Since Zn^{65} uptake occurred throughout the growing season in red oak seedlings, and since Zn was not readily re-cycled or leached from the leaves, red oak seedlings serve as a temporary sink for this element. Annually, leaf fall following senescence would re-cycle the leaf Zn back to the litter and soil, but the major percentage in stems and roots would accumulate over many growing seasons. More turnover would occur from the death and decay of the woody plant, and especially release of ions found in roots.

The present study suggests that short-term stimulation and long-term reduction in Zn^{65} (or Zn) uptake occurs following acute exposure of red oak plants to relatively high levels of external radiation. The inhibition phenomenon may be a direct response to growth damage resulting from irradiation, and not a direct response to irradiation. However, the initial stimulation is more likely a direct response to radiation.

Table 1. Distribution of Zn^{65} in non-irradiated red oak seedlings following uptake from soil throughout one growing season

Plant component	Zn^{65} conc. (dis/min/mg dry wt.)	Total Zn^{65} per component (dis/min)	% total plant Zn^{65}
Leaves	3·561	5988	0·42
Stems	7·209	11,872	0·83
Primary roots	9·043	111,205	7·74
Secondary roots	221·930	1,307,614	91·02

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