# INTRODUCTION TO NUCLEAR TECHNIQUES IN AGRONOMY AND PLANT BIOLOGY

P. B. VOSE

# *Introduction to Nuclear Techniques in Agronomy and Plant Biology*

by

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PERGAMON PRESS

OXFORD ● NEW YORK ● TORONTO ● SYDNEY ● PARIS ● FRANKFURT

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|---|---|
| U.K. | Pergamon Press Ltd., Headington Hill Hall, Oxford OX3 0BW, England |
| U.S.A. | Pergamon Press Inc., Maxwell House, Fairview Park, Elmsford, New York 10523, U.S.A. |
| CANADA | Pergamon of Canada, Suite 104, 150 Consumers Road, Willowdale, Ontario M2J1P9, Canada |
| AUSTRALIA | Pergamon Press (Aust.) Pty. Ltd., P.O. Box 544, Potts Point, N.S.W.2011, Australia |
| FRANCE | Pergamon Press SARL, 24 rue des Ecoles, 75240 Paris, Cedex 05, France |
| FEDERAL REPUBLIC OF GERMANY | Pergamon Press GmbH, 6242 Kronberg-Taunus, Hammerweg 6, Federal Republic of Germany |

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First edition 1980

**British Library Cataloguing in Publication Data**

Vose, Peter B
Introduction to nuclear techniques in agronomy and plant biology.—(Pergamon international library).
1. Agricultural research 2. Nuclear physics
3. Botany
I. Title
630'.7'2 S540.N/ 79-40778

ISBN 0-08-024924-8 (Hard cover)
ISBN 0-08-024923-X (Flexicover)

Printed in the United States of America

# *Preface*

NUCLEAR techniques have undergone great development during the past twenty years, and methods which at best were experimental have now become routine. Nowhere is this more true than in the field of agriculture and biology, where Cyril Comar published his classic as long ago as 1955.

Probably the author of any textbook primarily dealing with well-established facts requires to justify himself! However, I have had the feeling that existing texts did not do justice to the subject, and/or were unnecessarily theoretical. It is very easy to make a text concerned with nuclear techniques difficult and complex, while hiding the essential fact that the principles and methods are usually quite straightforward. It is not so easy to estimate what to leave out as being unnecessary for a basic understanding, and I hope that in attempting to keep the text straightforward I have not missed out anything essential.

The text material has arisen in various ways: some had been previously written for other purposes e.g. lectures on various occasions, about half has been especially written. As the book is not a "committee book" obviously the author knows some parts less well than others, but I hope that specialists will not be too unhappy at my treatment of their subjects. It need hardly be said that the references are not meant to be exhaustive. They have been chosen to illustrate a point, to amplify and to show the scope of techniques. In many cases, others could have equally well been chosen.

Hardly any scientific textbook stands alone: it is complementary to other texts. For those instructors organizing a laboratory class in soil-plant relations and seeking ideas for class experiments then the IAEA Tech. Rept. Series No. 171 (IAEA, 1976) can be recommended, while plant breeders will find the FAO/IAEA *Manual on Mutation Breeding*, 2nd Ed. (IAEA, 1977) an invaluable sourcebook.

I am happy to acknowledge my debt to past and present colleagues from whom I have learned much. However, I have had a little difficulty with specific references, due to the material having been put together over quite a long period, so if any people feel that their work has not been properly acknowledged I trust that they will accept my apologies. I should particularly like to thank Dona Diva Athié for invaluable help with the manuscript.

# *Acknowledgments*

THE following individuals and companies are sincerely thanked for supplying illustrations and permission to publish:

Panax Nucleonics, Redhill, U.K. (Figs. 4.1, 4.7, 4.10, 10.3), Beckman Instruments Inc., Irvine, Calif., U.S.A. (Fig. 4.10), Mullard Ltd., London, U.K. (Fig. 4.5), Nuclear Enterprises Ltd., Reading, U.K. (Fig. 4.9), Rank Hilger Ltd., Margate, U.K. (Fig. 9.2), EG & G Ortec, Oak Ridge, Tenn. U.S.A. (Fig. 9.3), Takashi Muraoka, CENA, Piracicaba, Brasil for autoradiographs (Figs. 10.3, 10.4 and 10.5), Dr. D. M. Silva, CENA, Piracicaba, Brasil (Fig. 10.6), Prof. Dr. W. Kühn, Institut für Strahlenbotanik, Munich, FDR (Fig. 13.5), Dr. Y. Watanabe, Institute of Radiation Breeding, Ohmiya-machi, Ibaragi, Japan (Fig. 15.1), Dr. B. Sigurbjörnsson, Agricultural Research Institute, Reykjavik, Iceland (Fig. 15.3), Drs. M. L. Petersen and J. N. Rutger, University of California, Davis, U.S.A. (Fig. 15.5), Dr. A. Tulmann Neto, CENA, Piracicaba, Brasil (Fig. 15.6), Dr. C. Broertjes, ITAL, Wageningen, Netherlands (Fig. 15.7). Other photographs are by the author, but I should like to thank Dr. V. F. Nascimento Filho (Figs. 3.1, 3.2, 5.10), and Dr. K. Reichardt (Fig. 14.13) of CENA, for their assistance.

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CHAPTER 1

# *The Nature of Isotopes and Radiation*

## THE ATOM

All matter is composed of atoms. An atom has a structure resembling the solar system, consisting of a positively charged *nucleus*, occupying little space but containing nearly the whole mass of the atom, while around the nucleus revolve the negatively charged planetary *electrons*. The diameter of the atom is about $10^{-8}$ cm or 1 Ångstrom Unit (*Å*), while the diameter of its nucleus is about $10^{-12}$ cm. The nucleus consists of *protons* (symbol *Z*), particles having a positive charge, and *neutrons* (symbol *N*) without any charge but with a mass nearly that of a proton. The proton is identical to the nucleus of the hydrogen atom.

The electrons, which are 1/1840 the mass of a proton, are arranged in a series of orbits and balance the positive nuclear charge due to the protons, thus giving a neutral atom. We speak of the orbits of the electrons being arranged in *shells*, and we identify them as *K, L, M, N, O* and *P* shells, from the innermost orbit outwards. There is only one electron in each orbit but there is more than one orbit in each shell, *K* containing 2 orbits, *L* containing 8 orbits, *M* with 18 and *N* with 32 orbits. If an electron is within its own orbit it is not radiating energy, but if an external force acts on it the electron jumps into another orbit with the liberation of a quantum of energy. The lowest energy orbits are the inner ones and these are the most stable. An electron may pass into successive orbits each nearer the nucleus, losing energy at each jump until it achieves the smallest possible orbit, when the atom is in the normal state. It will be seen that an atom consists largely of empty space, its overall size being determined by the outermost orbit.

In the neutral atom the charge on the nucleus, the *atomic number* (symbol $Z$ = number of protons), always equals the extranuclear electrons. The extranuclear electrons determine the chemical properties of an element, and therefore an element may be defined as a substance composed of atoms with the same net positive charge on the nucleus, i.e. having the same atomic number. The number of protons in the nucleus is characteristic of a particular element, though the atoms of an element need not necessarily have the same number of neutrons in the nucleus. The sum of the protons and neutrons is known as the *mass number* (symbol *M*) and corresponds to the atomic weight of the element. The term *nuclide* is a general expression describing a species of atom as characterized by the number of protons and neutrons in its nucleus. Atoms of an element which have a different number of neutrons, $N$, but the same number of protons, $Z$, that is they are nuclides having the same atomic number but with a different mass number, are called *isotopes*.

The relationship of neutrons and protons in the constitution of isotopes is well illustrated by the simplest case of the isotopes of hydrogen. There is common *hydrogen* with one proton, but no neutrons; *deuterium*, or heavy hydrogen with one proton and one neutron; and *tritium*, a radioactive form of hydrogen with one proton and two neutrons. Thus these nuclides have the same number of protons but a different number of neutrons. Having the same number of protons they naturally have the same number of extranuclear electrons, and are therefore isotopes having the same chemical properties. The nucleus of the deuterium atom is known as a deuteron and is an important particle in certain reactions (Chapter 2). Figure 1.1 illustrates the classical example of the isotopes of hydrogen and their comparison with helium, while Fig. 1.2 contrasts the structure of some of the isotopes of carbon and nitrogen.

## ISOTOPES

Isotopes are therefore slightly different forms of the same element, having the same chemical properties and characteristics, but each isotope having a slightly different atomic weight or mass number. It is this vital difference which enables us to make such good use of them, as a naturally occurring element either exists in the one isotope form, or if it exists in more than one form we know the characteristic properties. If therefore we take a minute amount of a rare isotope of an element we can use it as a *tracer* to follow the behaviour of much larger amounts of the common isotope of the same element.

We distinguish between the isotopes of an element by writing the mass number as a superscript alongside the symbol of the element, e.g. $^{12}C$ and $^{14}C$ for carbon, $^{31}P$ and $^{32}P$ for phosphorus, and $^{14}N$ and $^{15}N$ for nitrogen. Formerly, and still in quite common practice, the mass number was written as right superscript e.g. $C^{14}$, $P^{32}$, and $N^{15}$. Occasionally the atomic number, $Z$, and the number of neutrons, $N$, are also given with the symbol of the element, the former as a left subscript and the latter as a right subscript according to the general formula $^{M}_{Z}X_{N}$ where X is the chemical symbol, e.g. $^{11}_{6}C_{5}$ for carbon-11 and $^{60}_{27}Co_{33}$ for cobalt-60. Isotopes may be of two kinds, *radioactive* and *stable*.

A number of radioisotopes occur naturally in very small amounts, such as potassium-40, but the major contribution to biological research has come from radioisotopes artificially produced in nuclear reactors.

### Radioactive Isotopes

Radioisotopes are unstable, that is, they undergo spontaneous disintegration and give off atomic particles, as a stream of *radiation*, which can be of different types. The emission of atomic particles can be visualized as flashes of invisible "light". The atomic particles given off can be recorded by means of X-ray film or usually

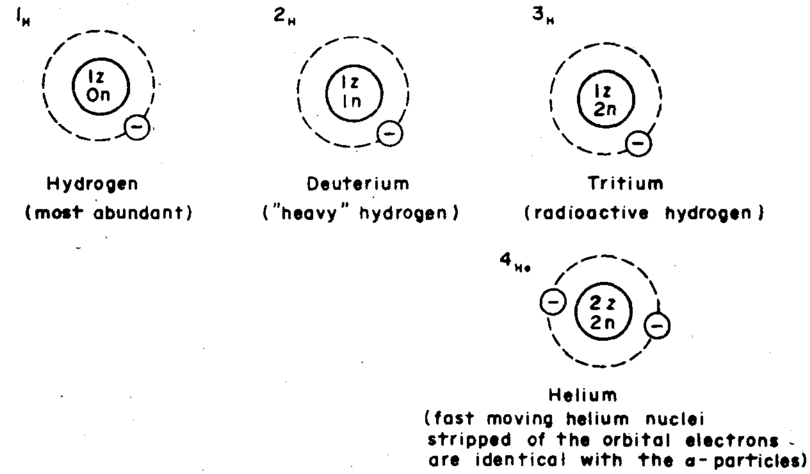


FIG. 1.1 The three hydrogen isotopes have the same number of extranuclear electrons balancing the positively charged protons of the nucleus, consequently they have the same chemical properties. Helium differs from $^{3}H$ in having 2 protons in the nucleus and hence is chemically different.

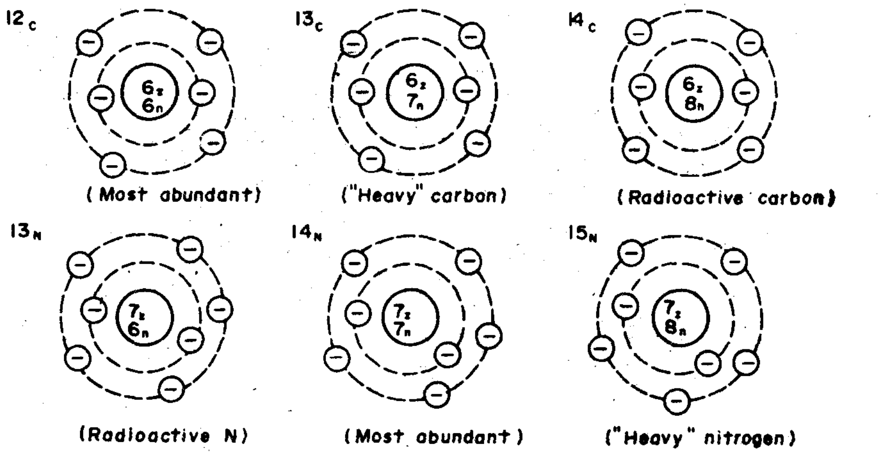


FIG. 1.2 Carbon and nitrogen differ by one proton in the nucleus and they have widely different chemical properties. Contrast the isotopes of both elements, which differ in the number of neutrons in the nucleus.

more conveniently and precisely by special electronic devices. The flash of energy in the form of an atomic particle enters a gas-filled Geiger-Müller tube or other type of detector, where it is converted into electrical energy and is registered by a counter.

## *Units*

The rate of spontaneous disintegration, or *decay*, of an isotope is used as an indication of the amount of radioactivity present, and from this derives the unit of radioactivity, the Curie (Ci). The Curie is defined as: *The amount of any radioactive material in which 3.7 × $10^{10}$ atoms disintegrate per second.* In experimental practice in biology, a Curie is quite a large amount of radioactivity and smaller fractions are usually referred to: e.g. the *millicurie* (mCi) which is 1/1000 of a Curie and the *microcurie* (μCi), equivalent to $10^{-6}$ Ci. Absolute activity, or disintegration rate, is expressed as disintegration per second (d.p.s.), or per minute (d.p.m.). These relationships are summarized in Table 1.1. It will be apparent that using the relationship lpCi = 2.22 d.p.m. or 1μCi = 37,000 d.p.s., activities expressed as "disintegrations" can readily be converted to Ci units.

TABLE 1.1
*The relationship of units of radioactivity to absolute disintegration rate*

| Decimal | Units of radioactivity | | Disintegration rate |
|---|---|---|---|
| 1 | 1 Ci | = 1 Curie | = 3.7 × $10^{10}$ d.p.s.<br>or 2.22 × $10^{12}$ d.p.m. |
| 1 × $10^{-3}$ | 1 mCi | = 1 millicurie | = 3.7 × $10^{7}$ d.p.s. |
| 1 × $10^{-6}$ | 1 μCi | = 1 microcurie | = 3.7 × $10^{4}$ d.p.s. |
| 1 × $10^{-9}$ | 1 nCi | = 1 nanocurie | = 3.7 × 10 d.p.s. |
| 1 × $10^{-12}$ | 1 pCi | = 1 picocurie | = 3.7 × $10^{-2}$ d.p.s.<br>or 2.22 d.p.m. |

The S.I. unit of radiation is the becquerel (Bq) based on the reciprocal second, as the physical dimension of activity is time to the power minus one ($s^{-1}$). There is some resistance to adopting the becquerel because of its inconvenient dimension: thus 1 Ci = 3.7 × $10^{10}$ Bq, or 1 μCi ≐ 37 kilo Bq, and 1 mCi = 37 mega Bq. The curie-related units are retained in this book.

## *Specific Activity*

In radioisotope experiments the absolute amount of radioactivity is seldom required and is therefore not measured, but comparative activity is recorded as pulses or counts per minute (c.p.m.) or as counts per second (c.p.s.). The counts from the "unknown" sample are then referred back to a "standard" of known composition and count rate. At this point we should understand the concept of *specific activity*. A radioisotope is most often accompanied by stable isotope, either in the initial preparation that is used for the experiment or when it is subsequently incorporated into biological material. Specific activity is then the amount of radioactivity per unit weight (or volume) of total element present, including both active and stable isotopes. Various expressions may be used, such as Ci/g, μCi/g, Ci/mole, μCi/ml, c.p.m./mg, etc.

*Radioactive Decay and Half-life*

An important decay characteristic of a radioisotope is its *half-life*. The half-life of a radioisotope is defined as the time required for half of the radioactive atoms to undergo decay, or in other words for the radioisotope to "lose half its radioactivity". After the first half-life only half the original number of radioactive atoms remain; after the second half-life only a quarter remain; after the third only an eighth of the original

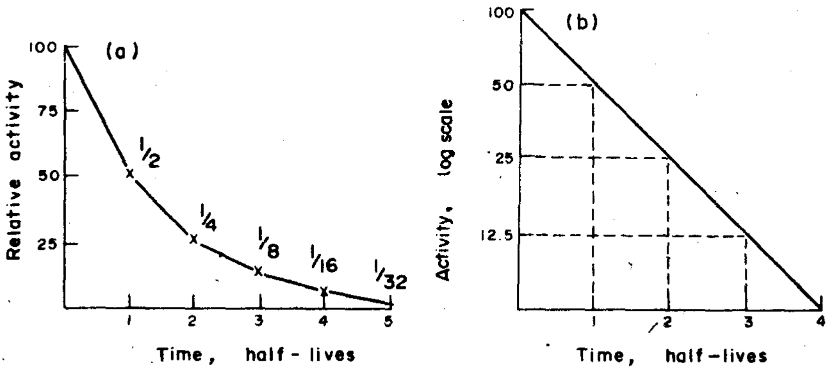


FIG. 1.3 Half-life of a radioisotope: the relationship of radioactivity to time, (a) linear plot (b) semi-log plot.

activity remains, and so on, as shown in Fig. 1.3(a). The half-life of an isotope may vary from seconds to hundreds of years, e.g. $^{11}C$ ($t_{1/2}$ = 20.4 minutes), $^{42}K$ ($t_{1/2}$ = 12.44 hours), $^{32}P$ ($t_{1/2}$ = 14 days), $^{14}C$ ($t_{1/2}$ = 5568 years). The rate of decay of any isotope is a basic property and cannot be altered by any treatment such as freezing or heating. Given the initial radioactivity of a preparation and the half-life of the isotope it is easy to determine graphically the activity at any subsequent time by plotting the decay curve: activity V time. If plotted on semi-log paper a straight line will be obtained due to the exponential nature of radioactive decay as shown in Fig. 1.3(b).

A more fundamental but often less convenient manner of expressing the decay characteristics of a radioisotope is by means of its *decay constant*, λ. The decay constant is the fraction of the number of atoms of a radioisotope which decay in unit time, and is expressed in terms of reciprocal time. It is established as follows from the fact that the number of disintegrations per unit of time is a constant fraction of the number of radioactive atoms present at that time:

The *activity*, $A^*$ of a substance is in effect its decay intensity, and this is proportional to the number of radioactive atoms which are present. Thus if $\frac{dN}{dt}$ is the disintegration rate, $N$ the number of radioactive atoms present at time $t$, and $\lambda$ is the decay constant, then

$$A^* = \frac{-dN}{dt} = \lambda N \qquad (1)$$

This equation is known as Rutherford's equation and the minus sign is used to indicate the decrease in the number of atoms with time.
Rearranging to obtain $\lambda$:

$$\lambda = -\frac{1}{N}\frac{dN}{dt} \qquad (2)$$

The decay constant is directly related to the half-life. If the differential equation (1) is integrated between the limits of $N_0$ and $N$, and $t_0$ and $t$, where $N_0$ and $t_0$ respectively represent the number of radioactive atoms present at zero time, then

$$\int_{N_0}^{N} \frac{dN}{N} = -\lambda \int_{t_0}^{t} dt \qquad (3)$$

and $$1n = \frac{N}{N_o} = -\lambda t \qquad (4)$$

giving, $$2.3 \log \frac{N}{N_0} = -\lambda t \qquad (5)$$

or in exponential form $$N = N_0 e^{-\lambda t} \qquad (6)$$

$e^{-\lambda t}$ is known as the *decay factor, f*.

The expressions decay constant and half life are readily convertible. From equation (6) it is apparent that the time required for half the original activity to decay is independent of the initial number of atoms. So if the time required for the original activity to decrease by a half is $t_{½}$ then:

$$½ N_0 = N_0 e^{-t_{½}} \qquad (7)$$

and $$\lambda t_{½} = 1n2 = 0.693 \qquad (8)$$

or $$\lambda = \frac{0.693}{t_{½}}, \quad \text{and } t_{½} = \frac{0.693}{\lambda} \qquad (9)$$

For convenience in practical tracer work, the half-life is mostly used, rather than the decay constant. It may be determined graphically, or alternatively if it is already

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