AN ABSTRACT OF THE THESIS OF

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Title: Radiation-induced Mutations in Sweet Cherry (*Prunus avium* L.) cvs Napoleon and Bing

Abstract approved: _____

Experiments were conducted using gamma-irradiation to determine radiosensitivities of main and accessory buds, to increase the proportion of mutant tissue, and to determine the type of damage and mode of recovery in irradiated shoot apices of sweet cherry cvs Napoleon and Bing. Survival, growth, and the types of mutations on V1 (primary) shoots and V2 plants were observed. LD_{50} values, based on survival of forced buds were about 5kR for both acute and fractionated irradiation in air, 5.5kR for acute exposure in water, and 6kR for fractionated dose in water. 0.39-0.69 accessory buds/site on non-irradiated 'Napoleon' had forced after 30 days in the glasshouse. In the 'Bing' field experiment with main buds, the LD_{50} for both acute and fractionated irradiation in air was 3.5kR. In water, the LD_{50} was 5kR for acute treatment and 6.5kR for fractionated dose. In the irradiated 'Bing' accessory bud field experiment, bud-growth was influenced by: a) irradiation dosage, b) bud maturity as related to position on the scion, and c) vigor of scions as represented by diameter.

The overall mutation frequency in 'Napoleon' V2 shoots derived from main buds was

7.5%: 0.04% growth-reduced mutants, 0.4% total leaf mutants, and 7.1% partial leaf mutants. The V2 trees derived from the lower buds had the highest mutation frequencies, for both overall mutations (9.7%) and for partial leaf mutants (9.6%) but a slightly lower frequency of total leaf mutants (0.3%). By contrast, higher buds had fewer overall mutations (6.6%), fewer partial leaf mutants (6.1%), but a slightly higher frequency of total leaf mutants (0.5%). The overall mutation frequency in 'Bing' V2 shoots was 7.3%: 0.34% were growth-reduced mutants, 2.3% were total leaf mutants, and 4.7% were partial leaf mutants. The height/diameter/number of nodes (H/D/N) ratios of all the V2 growth-reduced mutants and 8 of the V3 were 2 standard deviations below that of control plants. For efficient recovery of growth-reduced mutants we recommend propagating buds 11-30 on V1 shoots derived from irradiated main buds.

Of 3307 V2 shoots derived from irradiation of accessory buds of 'Bing' in 1983, the overall mutation frequency was 2.7% and 5 (0.15%) growth-reduced mutants were identified. Of 2765 V2 shoots derived from irradiation of accessory buds in 1984, the overall mutation frequency was 3.3%: 15 (0.54%) were growth-reduced mutants, 28 (1.0%) were total leaf mutants, and 48 (1.7%) were partial leaf mutants. A larger mutant sector was present in V1 derived from accessory buds than those from main buds as revealed by the higher number of total leaf mutant and growth-reduced mutant repeats in V2 families of the former. To improve isolation of growth-reduced mutants, propagate buds 2-33 on V1 shoots derived from irradiated accessory buds.

The type of irradiation damage in the apical meristem was both random and localised. Recovery of irradiated apices was via flank meristem, central meristem, or leaf primordia and axillary meristem. There was evidence of radiosensitivity gradient.

Radiation-induced Mutations in Sweet Cherry, (*Prunus avium* L.) cvs Napoleon and Bing

by

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Typed by Shaharudin Saamin

To Mazli

Esqandar

Dianelly

and my parents

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" How did I get here? Somebody pushed me. Somebody must have set me off in this direction and clusters of other hands must have touched themselves to the controls at various times...."

Joseph Heller

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PREFACE

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Chapter 1

INTRODUCTION

The sweet cherry, *Prunus avium* L. is the largest temperate zone orchard fruit tree, which often makes harvest difficult. Compact trees are advantageous because of their precocity, efficiency of harvesting, and thicker canopies which shield the fruit from sun and rain (Lane, 1977). Lapins (1963,1975) used X-rays to induce compact mutants in 'Lambert' and 'Stella' cherry. Other workers have also used ionizing irradiation to induce growth-reduced habit in cherry (Donini, 1976; Thompson, 1979), in apple (Decourtye, 1970; Visser et al., 1971; Campbell and Lacey, 1973; Zagaja and Przybyla, 1973; Ikeda and Nishida, 1982). The irradiation program in the Department of Horticulture initiated 10 years ago has the primary aim of induction of useful compact mutants in 'Napoleon' (Royal Ann) and 'Bing', the major commercial cultivars in Western USA.

The dosage which induces the highest frequency of mutations and the method of isolating the mutant sector are the two major factors that influence the successful mutation induction in fruit trees. The most efficient method to recover the mutants in subsequent propagation depends on plant factors such as the level of organization of axillary meristems, the type of damage and recovery in the apical meristem following irradiation, and the availability of accessory buds. Mutation is a single cell event and successful irradiation of a multicellular meristem will normally result in chimeral plants. Manipulation of irradiation doses can cause substantial cell elimination leading to a reduction of cells

initials and increased size of mutant sector (Gaul, 1961). In cherry, dormant accessory buds are in various ontogenetic stages and all have fewer meristematic cells than the main buds. They appear to be a suitable target for irradiation and recovery of whole mutants (Lapins, 1971; Katagiri and Lapins, 1974).

This gamma irradiation study on 'Napoleon' and 'Bing' cherry comprises 4 experiments. The main objective is to induce compact mutants by irradiating dormant lateral and accessory buds. Other objectives include: I) to compare various irradiation methods for increased mutation induction, II) to determine the distribution of mutations with regard to bud positions on the V1 shoot which help in efficient recovery of mutants, and III) to study the type of irradiation damage and mode of recovery in lateral main buds.

Chapter 2

REVIEW OF LITERATURE

INTRODUCTION

Knowledge of the laws of mutation will probably lead to the artificial production of mutations at will, and thus the creation of completely new properties in plants and animals.

HUGO DE VRIES, 1901

Mutation is said to occur when there is a heritable structural change in the genetic material. Mutations are random events (Brewbaker, 1964) and all show pleiotropic side effects that are usually detrimental. Mutations provide the basic variability (Lawrence, 1968; Garber, 1972; Simmonds, 1979) required for plant improvement by breeding. Induced and spontaneous mutations can invoke similar spectrum of genetic changes (Briggs and Knowles, 1967). Mutation induction provides a unique complementary tool to conventional cross breeding.

The concept of producing artificial mutations and utilizing them for breeding cultivated plants was first suggested and advanced in 1901 by de Vries. In 1922, Alberto Pirovino in 1922 in Italy made attempts to modify plants by using X-rays and ultraviolet light (Spencer, 1964). It was Muller (1927) who proved that the mutation rate in *Drosophila* could be increased markedly by the use of X-rays, and this was followed by Stadler (1928a, b) who also found the same to be true for barley and maize. Soon after these important discoveries Nilsson-Ehle and Gustafsson in Sweden began experiments in mutation breeding in barley using X-rays. After World WarII numerous mutagenic agents, both physical and chemical, became available for use in mutation breeding of seed and vegetatively propagated crops

leading to 485 released or approved cultivars as shown in Table I (from Gottschalk and Wolff, 1983).

Table 1. Released or approved varieties of seed and vegetatively propagated crops derived through mutation induction (after Gottschalk and Wolff, 1983)

Mutagens	Vegetatively propagated crops		Seed propagated crops		All crops	
	No.	%	No.	%	No.	%
X rays	125	51.0	87	36.3	212	43.7
Gamma rays	103	42.1	94	39.2	197	40.6
^{32}P beta rays	-	-	2	0.8	2	0.4
Neutrons	9	3.7	29	12.1	38	7.9
Combination of different rays	5	2.0	2	0.8	7	1.4
Total physical mutagens	242	98.8	214	89.2	456	94.0
Chemical mutagens	2	0.8	23	9.6	25	5.2
Combination of physical and chemical mutagens	1	0.4	3	1.2	4	0.8
Grand total	245	100.0	240	100.0	485	100.0

MUTATION BREEDING IN FRUIT IMPROVEMENT

Induced mutation breeding in fruit trees (apple) began in the early 30s by Murneek and Stadler using X-rays (Bishop, 1967). Others, such as Granhall (1953) and Bishop (1967), used X-rays for mutation induction in apple. Some of the released or approved fruit cultivars developed through various mutation breeding programs worlwide as of 1985 are shown in Table II (from Micke et al, 1985).

Fruit tree species are highly heterozygous and often polyploids which complicate the

inheritance of traits. Conventional hybridization results in wide segregation in the progeny which necessitates the use of very large populations to recover the desired recombinant. The principal advantage of mutation breeding in vegetatively propagated plants is that it provides an efficient approach to improve an existing outstanding cultivar which is deficient in one or few traits (Broertjes, 1968). Gene mutations are usually recessive (Allard, 1960) and more likely to be expressed in a heterozygous genetic background. Although selection efficiency for polygenic traits is low, the mutation frequency is high (Brock, 1972). In obligate apomicts or seed-sterile plants, mutation breeding is the only means to artificially increase genetic variation (MacKey, 1961; Hanna and Burton, 1981).

Mutation breeding is a useful complementary tool in certain aspects of conventional breeding (Hough et al., 1964; Bishop, 1967; Sigurbjornsson, 1977; Swaminathan and Driscoll, 1977; Lapins, 1983; Konzak, 1984). It can be used for the following purposes:

1) to induce a new characteristic, such as compact growth habit, self-compatibility, disease resistance, fruit skin color, or ripening time, if such trait is not readily available in the genepool;

2) to use the induced mutant for further hybridization;

3) to break linkages with undesirable characteristics or pleiotropic effects;

4) to uncover and homogenize existing chimeras and make mutants stable;

5) to reduce incompatibility in wide crosses;

6) to induce transitory sexuality in apomicts; and

7) to induce haploids.

Some general considerations which need to be addressed before embarking on a mutation breeding program include: 1) choice of mutagen; 2) dosage; 3) plant part and ontogeny for irradiation; 4) availability of one or few meristematic cells that could give rise to whole mutants; 5) methods of propagation to maximize isolation of mutant sectors; and 6) to stabilize chimeras (Hough, 1965; Donini, 1976; Broertjes, 1977).

Table 2. Fruit cultivars derived from mutation induction or the use of induced mutants in cross breeding (after Micke et al., 1985)

Cultivar	Place and date of release	Kind and date of mutagenic treatment [treated variety]	Main improved attributes
Citrus sp.			
Star Ruby	USA, 1970	Thermal neutrons 1959, [Hudson]	Red flesh like parent variety, but almost seedless (0-9 seeds instead of 40-60)
Eriobotrya japonica Lindl.			
Shiro-mogi	Japan, 1981	Gamma rays 20kR seeds, [Mogi]	Large fruit size, good taste
Ficus carica L.			
Bol (Abundant)	USSR, 1979	Gamma rays 5-7kR, seeds	
Malus pumila Mill.			
Belrene	France, 1970	EMS 1%, growing shoots 1961, [Reine des reinettes]	Earlier maturing, more colored and bigger fruit, yield somewhat reduced
Blackjoin BA 2 520	France, 1970	Gamma rays 5kR dormant trees, 1963 [Jonathan Blackjoin]	Improved and more regular red colored fruit
Lysgolden (Goldenir)	France, 1970	Gamma rays 5kR dormant trees, 1963 [Golden Delicious]	Fruit free of russetting, yield somewhat reduced
McIntosh 8F-2-32	Canada, 1970	Gamma rays, shoots [McIntosh]	Improved skin color, resistance to Podosphaera leucotricha and Venturia inaequalis
Olea europia L.			
Briscola	Italy, 1981	Gamma rays 4kR rooted cuttings, 1968 [Ascolana tenera]	50% growth-reduced, precocious, easy harvest, male sterile
Prunus armeniaca L.			
Early Blenheim	Canada, 1970	Thermal neutrons shoots [Blenheim]	Matures one week earlier than parent, annual yielding, self-incompatibility
Prunus avium L.			
Compact Lambert	Canada, 1964	X-rays 4kR, scions, 1958, [Lambert]	Compact growth, precocious, heavy cropping, fruit size slightly reduced
Compact Stella 35B-11	Canada, 1974	X-rays 4kR, scions, 1964, [Stella][a]	Compact growth, otherwise like Stella
Lapins	Canada, 1983	Van x Stella selected 1971	Larger firmer fruit, resembles Lambert, ripens 2 days later, upright growth habit, self-fertile, more productive than most cvs

Table 2 Continued

Stella	Canada, 1968	Lambert x John Innes Seedling 2420 (self-fertile mutant by X-ray treatment of pollen	First good quality self-fertile sweet cherry
Sunburst	Canada, 1983	Van x Stella selected 1971	Fruit resembles Stella, very large and resists rain splitting, not as firm as Bing or Van, matures with Van, good growth habit, self fertile very productive
Prunus cerasus L.			
Karlik Samorodka	USSR, 1979	Gamma rays, buds	Dwarf type
Plodorodnaya	USSR, 1977	X-rays	Parhenocarpic
Prunus persica L.			
Magnif 135	Argentina, 1968	Gamma rays chronic 1962-63, [Magnif 43]	Bigger fruit with deeper skin color, 7 days earlier maturity
Plovdiv 6	Bulgaria, 1981	Halle x Dupnishka Gamma rays, 1966 pollen of [Dupnishka]	Higher yield, large fruit, good quality, middle early flowering
Punica granatum L.			
Khyrda	USSR, 1979	Gamma rays 5-7kR, seeds	Dwarf
Ribes sp.			
Westra	FRG, 1968	X-rays 1.5kR, 1949 [Westwick Choice]	Strong erect habit

[ ] treated variety, line, clone...
a (underlined) mutant used in the cross

MUTATION INDUCTION

Many agents have the ability to break the chromosome, alter the chemical makeup of DNA, or otherwise induce heritable changes. Mutagens commonly have the capacity of inducing cell death, either immediately or through delayed or mitotic death effective at the time of cell division. Mutagens have had different effects; some seem to affect certain loci of the chromosomes more than others (Russel, 1963). Mutagens are of two types, physical and chemical.

Physical mutagens

The irradiation energy from physical mutagens induces chemical reactions within the plant tissue, resulting in structural changes in the DNA. These changes, if maintained in DNA replications, are manifested as mutations. The characteristics of the different types of ionizing radiations are briefly summarized in Table III. In the process of penetration, high energy

Table 3. Types of radiation and their characteristics (after Sparrow, 1961)

Types of radiation	Source	Descriptions	Penetration into plant tissue of av. density
Ultra-violet	8 W Hg arc lamp	Electromagnetic radiation	Very limited (Brewbaker et al. 1965)
X-rays	X-ray machine	Electromagnetic radiation	Few mm to many cm
Gamma rays	Radioisotopes (^{60}Co, ^{137}Cs) and nuclear reactors	Electromagnetic radiation	Many cm
Neutrons (fast, slow, and thermal)	Nuclear reactors (piles) or accelerators	Uncharged particles slightly heavier than proton; not observable except through its interaction with nuclei in the material it traverses	Many cm
Beta particles, fast electrons, or cathode rays	Radioisotopes or accelerators	Electrons (+ or -) ionize much less densely than alpha particles	Several mm
Alpha particles	Radioisotopes	He nuclei, ionize very densely	Fraction of mm
Protons or deutrons	Nuclear reactors	H nuclei	Many cm

irradiation produces ions by colliding with atoms and releasing electrons, which in turn collide with other atoms releasing further electrons, etc. The change in electron number transforms a stable atom or molecule into the reactive ionic state. Thus, along the track of the high-energy ray, a track of ions is formed which can initiate various chemical reactions. Energy transfer is higher with increases in the density of ionizations which is expressed in

LET (linear energy transfer), which is the energy dissipated per unit length along the tracks of the ionizing particles. Gamma, X and beta rays cause sparse ionizations along the track of the ionizing particle (electron). When the ionizing particle consists of an atomic nucleus, alpha rays, or recoil nuclei knocked out by fast neutrons, the ionizations occur more densely (Ahnstrom, 1977). This will vary from 8 ionizations/μ in high energy beta and gamma radiation, X-rays, neutrons radiation, and alpha radiation, to 130,000 ionizations/μ for atomic rays. One ionization event is sufficient to inactivate a dry molecule of DNA or of an enzyme (Lea, 1955).

Radiation dosages have been measured in various units. Roentgen (r) is a unit of exposure and not a unit of absorption. In water or in tissue, approximately two ionizations are produced by 1r/μ^3. The rad (radiation-absorbed dose) is a unit of absorbed dose, and equals 100erg/g. For X-rays, 1rad is equal in energy to 1.08r in water.

Ionizing radiation can cause gene mutation, chromosome, chromatid, or subchromatid aberrations, changes in chromosome number, inhibition of cell division, induction of mitotic activity, death of nuclei or cells, partial or complete sterility, retardation or stimulation in growth rate, and the induction of abnormal growth. Neutrons are more effective mutagens than X or gamma rays in most crops (Bender, 1970), including apple (Bishop, 1959). In fruit crops, the majority of changes induced are probably chromosomal (Broertjes and Van Harten, 1978) and mainly deletions (Nybom and Koch, 1964).

Ultraviolet radiation leads to excitation of electrons, but not ionization. It has low penetrating power and its use in plant material is limited to pollen grains. Compared to X-rays, ultraviolet radiation produces more gene mutations relative to chromosome aberrations. Deficiencies induced by ultraviolet radiation are terminal but deficiencies induced by X-rays are interstitial and associated with chromosome breaks.

Chemical mutagens

Chemical mutagens include alkylating agents, urethane, alkaloids, peroxides, formaldehyde, substances related to nucleic acid, and nitrous acid (Auerbach, 1961). They are commonly used in mutation induction of seed propagated plants. They react via direct chemical action within chromosomes and cause more gene than chromosome mutations. Difficulties in incorporating the chemical into bud meristem render them ineffective for vegetatively propagated plants (Bowen, 1965; Nybom, 1961). The more frequently used chemical mutagens are:

diethyl sulphate (dES);

ethyl methane sulphonate (EMS) and methyl methane sulphonate MMS);

isopropyl methane sulphonate (iPMS);

azide;

colchicine;

ethylenimine (EI);

N-nitroso-N-ethylurea (NEU);

N-nitroso-N-methylurea (NMU);

1,4-bisdiazoacetylbutane.

EMS is a more powerful mutagen than X-rays and induces about 5 times as many chlorophyll mutations in barley as do X-rays (Gaul, 1963). In fruit crops, EMS has been applied on dormant buds of blackcurrant (Nybom and Koch, 1964), growing shoots of apple with successful development of the improved cv Belrene, and protoplast of orange (Vardi et al., 1975). Colchicine has been applied on shoot tips of apricot (Lapins, 1975), grapes (Dermen, 1954; Dermen and Scott, 1962) to induce polyploidy, aneuploidy, and morphological variations.

Genetic damage

The quantitative relationships of dose of sparsely ionizing radiation and response vary for different mutational events. Usually, depending upon the division stage at which cells are irradiated, chromosome aberrations may include either one or both chromatids. Irradiation at early interphase, before DNA synthesis has begun, usually causes breaks which appear later as having occurred when the chromosome was as yet unreplicated (chromosome breaks). Breaks induced in the interphase period after DNA synthesis has begun usually appear separately in each of the two chromatids of a chromosome (chromatid breaks)(Figure I). Dose-response patterns are essentially linear for both terminal deletions, as shown in Figure I, as well as for intragenic changes. The linearity of dose responses suggests that when a certain number of ionizations occur within a certain critical volume, a mutation occurs. This quantitative relationship gave rise to the target theory (Lea, 1955; Chu et al., 1961). The target theory assumes the gene to be the target, and mutation to be the result of a "hit" in the gene by a number of ionizations sufficient to alter it. Lea (1955) calculated that a minimum of 20 ionizations is necessary within a target volume to break the chromosome. A schematic representation of alterations in DNA caused by radiation is shown in Figure II.

The dose responses for certain types of mutational events following irradiation are not linear. The production of intercalary deletions requires two breaks in the same chromatid. Similarly, translocations require the production of two independent breaks. The induction of these two independent events is nearly proportional to $(dose)^{3/2}$, and the dose response is nonlinear. The reduction in the expected $(dose)^2$ appears to result from the restitution of many breaks.

Densely ionizing irradiations produce deletions in direct proportion to dose. With these radiations the two breaks involved are not produced independently but may result from the passage through the tissue of a single particle. This also implies that only those breaks that

are close together can participate in an exchange; estimates of this critical distance range from 1-0.1μ.

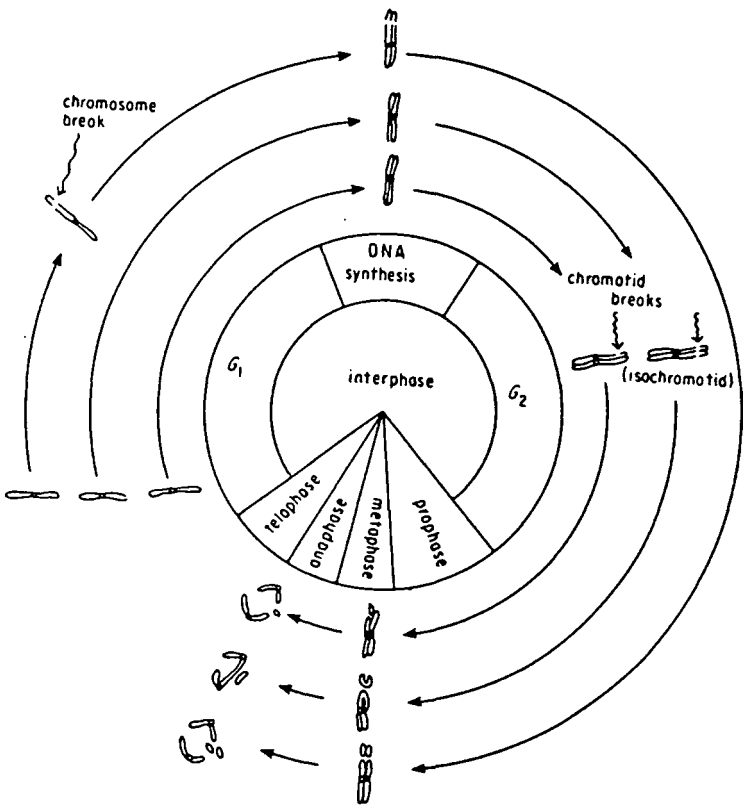

Figure 1. Types of terminal deletions produced by single breaks at the one-strand (G1) or two strand (G2) stages during a mitotic cycle (after Whittinghill, 1965).

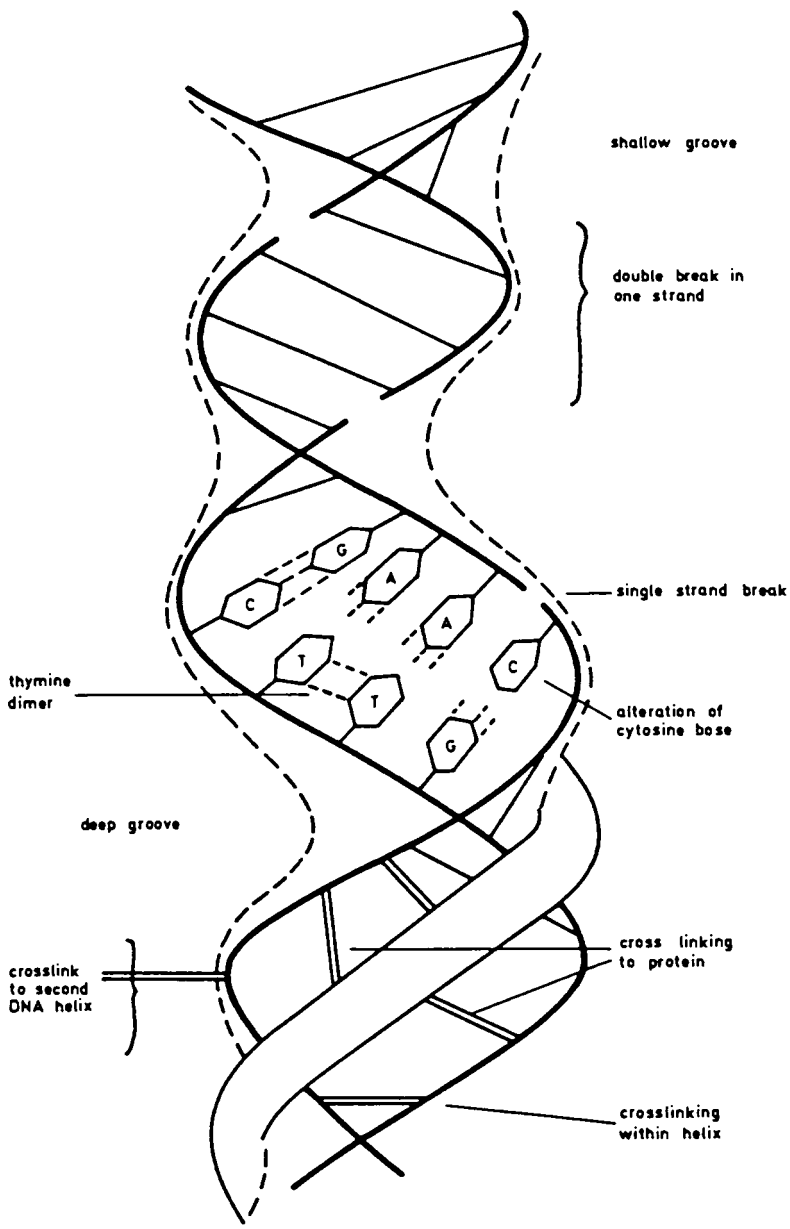

Figure 2. Schematic representation of alterations in DNA caused by radiation (after Ahnstrom, 1977)

Radiosensitivity

However, the target theory alone is not sufficient to explain radiation effects in higher plants. The differences in response, or radiosensitivity, for different radiation treatments have a complex basis, including such genetic factors as interphase chromosome volume (Sparrow et al., 1964) in which ploidy is a factor (Yamakawa and Sparrow, 1965), heterozygosity of gene loci (Broertjes and Van Harten, 1978) as well as such biological factors as metabolic and meristematic activity, tissue hydration, dormancy and physiological stage and exposure to such environmental factors as oxygen and temperature during radiation treatment (Conger et al., 1977; Konzak et al., 1961; Nilan et al., 1965). Donini (1975), found greater tolerance of fruit tree buds in nitrogen than in oxygen or air, particularly at higher doses. Oxygen interacts with radiation-induced free radicals and increases the frequency of chromosome aberrations and biological damage (Konzak et al., 1970). Higher temperatures during irradiation increased mutations of the S gene (Lewis and Crowe, 1954), but generally the effects of irradiation are enhanced by low temperatures. Another factor to consider is the time available for repair of chromosomal damage. Cells of active meristems are usually more sensitive than those of dormant tissues. In dormant meristems the cells are usually in G1 although they can also be in G2 (D'Amato, 1977). Of the four stages usually recognized, (M, G1, S and G2) G2 is the most sensitive to sparsely ionizing radiation. Woody plants are more sensitive to radiation injury than are herbaceous plants. Diploids are more sensitive than corresponding tetraploids. Figure III illustrates some of the possible interactions that may take place during intermediary stages between the irradiation and the appearance of aberrations.

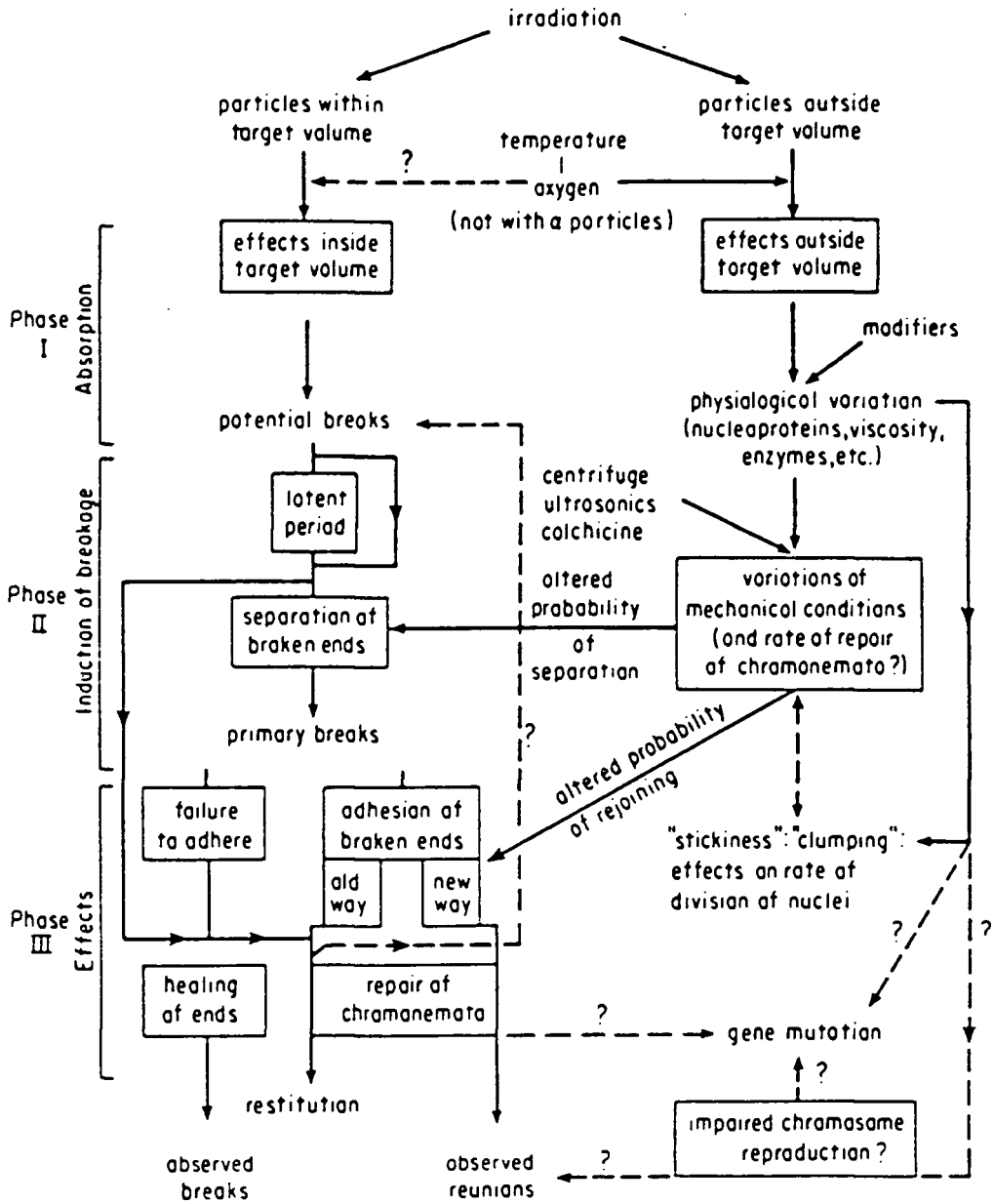

Figure 3. Different possible pathways and interactions causing genetic damage in organisms as a result of irradiation (after Wagner and Mitchell, 1964).

Irradiation methodology

The amount of mutagen applied/unit time (dose rate) affects both mutation frequencies and spectra. Dose rate is usually expressed as roentgen (r) or rad (R)/minute. Genetic changes that show linear response to dose are not affected by dose rate. Whereas those genetic changes that show a curvilinear relation to dose are affected by dose rate. Thus, high intensity radiations are more effective in producing chromosomal mutations. Nishiyama et al. (1964, 1966) observed that somatic mutaton frequency in *Avena* increased at higher dose rate.

In acute exposures, dose rates used in fruit crops may range from 10 - 1000R/min. The treatment lasts a few minutes to several hours. In chronic irradiation, doses may range from 10 - 100R/day and exposures are continued over weeks, months, or years. A low but effective rate is 10R/day, a working level about 25-50R/day. In semiacute treatment, the dose is less than 1R/min (Lapins, 1983). Acute doses have been found to be more effective than chronic doses. Nishida (1973) reported that a semiacute dose of 1kR/day was more effective than the lower doses of 23-50R/day. Techniques that have been used infrequently are fractionated doses and recurrent irradiation.

The dose required to produce 50% lethality (LD_{50}) has been applied in many irradiation programs in fruit trees (Lapins, 1965; Donini, 1975; Bishop, 1959; Lapins et al., 1969). For sweet cherry, *Prunus avium* L. the LD_{50} for dormant buds is in the range of 4-4.5kR of X-ray or gamma irradiation (Donini, 1975; Thompson, 1979). Differences in LD_{50}'s for comparable plant material could be caused by the dose rate used or by the stage of rest of dormant buds.

The1983 dose profile of ^{60}Co radiation chamber at the Oregon State University is shown in Appendix I. The ^{60}Co source was installed in January 1976. The 12.7cm diameter high flux chamber is convenient for acute treatment of dormant scions while the low flux chamber protects the bases of scions against severe irradiation damage and consequent success in

grafting. However, differential dose rates along the perimeter and centerline of the chamber result in variation in target dose.

PLANT MATERIAL FOR MUTAGENESIS

Mutation is a random single cell event. In order that a mutation can manifest itself in a whole plant, the target material for mutagen treatment must be meristematic cells. Shoot tip meristems in leaf buds or in seeds are typical targets for treatment. Ovules, pollen, and microspores have been used to a lesser extent. Pollen mother cells have been treated successfully to induce mutations in the self-incompatibility gene of sweet cherry (Lewis and Crowe, 1954).

Mutations in somatic tissues are usually induced by treating leaf buds with mutagens. Leaf buds on scions, stem cuttings, rhizomes, tubers, or bulbs can be used, or adventitious buds may be induced to develop, immediately following mutagenic treatment, on root cuttings, leaves, petioles, pedicels, or other somatic tissue.

Organization of the shoot apex

Active meristems of dicotyledonous plants typically include an apical dome of tissue around which are arranged in a clearly defined order a series of primordia of increasing age with increasing distance from the apex. The structure of the apex is generally described in terms of tunica-corpus concept formulated by Schmidt in 1924 (Esau, 1965; Hara, 1973; Broertjes and van Harten, 1978); i.e. consisting of 2 zones of cells, namely a tunica consisting of a low number of layers (2-3 usually) overlying a corpus mass of cells. The tunica layers have been termed as germlayers LI and LII and the corpus as germlayer LIII (Satina et al., 1940; Satina and Blakeslee, 1941). Cells of the tunica are charaterized by anticlinal divisions so that each layer has a reasonable degree of independence and continuity, although periclinal divisions can occur as observed in LI of grapes (Thompson and Olmo,

1963). In the corpus cell division is not oriented in any particular plane. Cells of both zones may be involved in the development of a leaf primordium depending on the number of cell layers in the tunica. If the tunica consists of a single layer, the periclinal divisions that lead to the formation of a leaf primordium occur in the corpus. If the tunica is multilayered, however, the periclinal divisions may occur in either the inner layers of the tunica alone or the tunica and corpus combined. Axillary buds occur in the axils of leaves and give rise to branches. Developmentally, the axillary bud is initiated after the primordium of the leaf whose axil subtends it; therefore, the axillary bud is more closely related ontogenetically to the leaf above it than to the leaf whose axil subtends it. Adventitious buds generally arise on pedicels or leaves but not in roots from a single cell of the outermost histogenic layer.

The growth pattern within a vegetative apex is a dynamic system in which considerable cell movement occurs . Some have suggested that most growth occurs as a result of divisions in highly meristematic cells, the apical initial cells, which retain their position at the tip of the apex (Gifford and Corson, 1971). Others have suggested that there are no specialized apical initials; rather, whenever a cell is in a particular position in the meristem it functions as an initial.

Evidence regarding the role of apical cells has been provided by studies of chimeras (Stewart and Dermen, 1970). Because of the occurrence of large sectors of mutant tissues they concluded that there are 1-3 central initial cells actively dividing and responsible ultimately for the growth of vegetative and flowering shoots.

Although the apical initials have the crucial role in shoot growth they divide only at a slow rate and most divisions occur in active regions away from the tip, on the flanks of the apex where the leaf primordia arise. The low rates of division of the apical cells was the basis of the theory that there was a quiescent center in apical meristems; but the central zone has been found to be metabolically (West and Gunckel, 1968) and mitotically active (Tepfer,

1960). Stewart and Dermen (1970) concluded that a cell proximal to the initials can give rise to cells making up 3-5 nodes of growth. One division per 12 days would be adequate to maintain growth in the species of *Ligustrum* that they studied.

The number of initial cells in the shoot apex may differ with the species and possibly to some extent with varieties, perhaps depending on the degree of apical dominance expressed. Moh (1961) for example, concluded that the shoot apex of the *Coffea* embryo is controlled by a single initial cell because of high frequency of non-chimeric mutants.

Tissue and cellular origin of mutations

With vegetatively propagated plants, mutations originate as chimeras in one or more of the 3 (LI, LII, LIII) histogenic layers of shoot apices, or from adventitious buds which develop from one or a small number of initial cells. Mutations only in LIII which contribute predominantly to corpus tissue may not be detectable even if they affect the whole of the LIII tissue. Those only in LII (sub-epidermal layer) may not be expressed until they pass through a sexual stage, and those only in LI will be expressed immediately in the phenotype but not in the genotype or germ line tissues. A mutation occurring in only one layer is a periclinal chimera if it affects entire LI, LII, or LIII layer, or a mericlinal chimera if only part of a tissue layer is involved. A sectorial chimera affects a segment of all tissue layers, while the solid mutant or homohistant, affects all tissue layers. Both periclinal and mericlinal mutants are common when multicellular meristematic buds are irradiated. Even when a single progenitor cell is treated, the subsequent cell lineage might be chimeric because (1) the mutation is induced in a chromatid at G2, (2) a reverse mutation (repair) may occur in one of the daughter cells or later on in the course of development, and (3) the mutation may involve a cytoplasmic organelle that segregates in mitosis.

Following irradiation treatments severe enough to kill some but not all of the cells of a

shoot apex reorganization of apical meristems occurs. For example, a heavily damaged LII may be replaced by LIII. Thus the breeding behavior of a mutant can be different from that of the original cultivar.

The survival and fate of a mutated cell during organogenesis from a shoot apex depends on a large number of factors which include (Davies, 1983; Konzak, 1984):

1. Position of the cell within the meristem. If an affected cell is in the center of an actively dividing primordium any slight competitive disadvantage is likely to be more significant than if it is in a position where cells are dividing more slowly. Mutations occurring in cells of leaf initials, for example, will be present as a small patch of mutant tissue on that leaf only. Mutations induced in apical initial cells often produce mutated sectors. If the mutated initial cell is stabily positioned in the shoot apex its derivative will form longitudinal mutated sectors (sectorial chimeras). With sectorial chimeras later-formed axillary buds will occur partially or completely within the mutated tissue.

2. Extent of the chromosomal damage. It is most likely that a diploid cell with anything other than gene mutations or small deletions will be at a disadvantage with normal cells.

3. Total number of surviving cells. The mutant cell has increased chance of survival when many of the meristematic cells are injured or killed following irradiation.

Methods to reduce or overcome chimerism and to isolate mutations

1. It is generally observed that there are more whole or solid mutants after higher doses. Very severe damage to the bud apex may result in the regeneration of the apex from a single or few cells and, eventually, in a whole-shoot mutation (Lapins and Hough, 1970).

2. Treatment of specific developmental stages where single or few cells exist that contribute to the development of the shoot or progeny can be used. Accessory buds which remain dormant if the main bud is intact consist of fewer cells than the main leaf bud.. Elimination of the main bud may force accessory buds to develop into shoots, as shown in

sweet cherry (Katagiri and Lapins, 1974) and grape (Donini, 1975). Similarly, axillary buds which generally have a less-developed shoot meristem terminal buds, can be induced to develop following mutagenic treatment by selective pruning or application of growth regulators. For seed propagated crops, the immature or mature pollen can be treated and used in making controlled pollinations, the fertilized egg can be treated prior to the first zygotic division, and the embryo can be treated during early organogenesis prior to or at the time of formation of the embryonic shoot apex.

3. Use of adventitious bud technique as described by Broertjes and van Harten (1978). Adventitious buds, which normally develop from single cells, are forced to develop from mutagen treated leaf cuttings (includes petiole base, leaf blade, midrib, etc.), exposed roots, stem cuttings, and specialized structures (rhizomes, stolons, bulbs, corms, storage roots, etc.). Roots of self-rooted cultivars of apple have been forced to induce adventitious bud and shoot formation from few cells. Adventitious shoots from disbudded and irradiated tubers have given practically chimera-free mutants under certain conditions (Van Harten et al., 1972). In *Citrus* sp., adventitious buds develop on the wound callus of leaf cuttings and decapitated young seedlings (Spiegel-Roy and Kochba, 1973).

Efficient adventitious bud techniques have not been developed for propagating many of the woody plant species. Consequently, methods such as disbudding, cutting back, grafting,budding, or rooting of cuttings of tissues taken from first year shoots even if inadequate, are the target materials used. Donini (1975) has illustrated effective proceedures for isolating mutations of grape and fruit trees, as cherry, apple, and pear. Similar methods are applicable to apricot and peach (Lapins, 1973; Visser, 1973).

4. *In vitro* cell and tissue culture offers the means to induce mutations in single cells which eventually regenerate into whole plants. Tissue cultures of sugar cane (Heinz, 1973), protoplast culture of potato (Shepard et al., 1980; Wenzel et al., 1979) appear to have special

promise for mutation breeding. Another approach is to treat the growing shoot tip of a plant with a mutagen and then propagate via the axillary-bud-proliferation technique to recover the mutants.

USEFUL MUTANTS IN FRUIT CROPS

Spontaneous mutants in fruit crops of apple, citrus, peach, and grape have been exploited commercially. Induced mutations further increase variability and refine many fruit cultivars in traits that are lacking. Some of the useful traits that have been induced and found in released or approved cultivars include (also refer to Table II):

Fruit color, fruit setting, flesh characteristics and seedlessness

In stone fruits, the only fruit character mutants described to date have been fuzzlessness (Donini, 1976), and larger fruit with deeper red skin color in peach, *Prunus persica*. The latter mutant was isolated after gamma field irradiation of the cv. Magnif 43 and introduced as cv. Magnif 135 in Argentina. In apple, Lapins et al (1969) and Lapins (1973) described an induced mutant of McIntosh with a brighter red skin and flesh color, apparently resulting from a reduction in chlorophyll content of the fruit. This mutant was introduced in 1970. Decourtye and Lantin (1981) have marketed in France a russett-free mutant 'Lysgolden' induced from 'Golden Delicious'. They also induced the deeply colored, larger fruited mutant 'Belrene', and an improved and more regularly red colored mutant 'Blackjoin BA2-520'.

The self-fruitful sweet cherry cv. Stella and its compact mutant, Compact Stella obtained their self compatibility from a radiation induced self fertility mutant isolated by Lewis and Crowe (1954). The induced self compatibility allele is now extensively used in sweet cherry breeding (Lapins, 1973).

In *Citrus* , a nearly seedless mutant grapefruit 'Star Ruby' was induced by exposure of the apomictic seeds from a deep red flesh sport to thermal neutrons.

Plant structure and form

Numerous compact mutants in cherries were induced by Lapins (1973), Zagaja and Przybyla (1973), and Donini (1976); a compact apricot (*Prunus armeniaca* L.) was induced by Lapins (1973). Similar mutants also have been induced in apples by other workers (Lapins, 1973; Zagaja and Przybyla, 1973; Visser, 1973). Compact mutants also have been induced in pears (Visser, 1973).

Physiological traits

In fruit trees early ripening and annual bearing are important traits because earlier marketing often provides a higher economic return. Thus, several early maturing mutants of apple, peach, cherry, and apricot mutants have been selected (Lapins, 1973; Broertjes and Van Harten, 1978).

Disease resistance

Disease resistance appears to be readily induced in apple. Lapins (1973) induced variants of McIntosh apple with resistance to powdery mildew (*Podosphaera leucotrica)* and to apple scab (*Venturia inequalis*) one of which has been released (Broertjes and Van Harten,1978; Sigurbjornsson and Micke, 1973). Similarly, Campbell and Wilson (1977) report variation in mildew infection of Cox Pippin apple mutant clones.

IDENTIFICATION AND EVALUATION OF MUTANTS

Mutations that are visibly expressed in the phenotypes such as leaf morphology, branching habit or growth reduced forms can be easily identified in the first year while traits such as yield, precocity or fruit quality require many years to characterize. Some types of mutations can be identified based on correlation of traits. Plants with small leaves of abnormal

texture almost invariably bear small, abnormal fruit. Chlorophyll deficient foliage is usually associated with low fertility. The schedule for propagation and selection of irradiated sweet cherry leading to release of 'Compact Lambert' is shown in Table [4].

Table [4]. Development of 'Compact Lambert' sweet cherry via mutation induction (Lapins, 1963; 1973).

Year		Propagation and selection schedule
1	1958	Several 100 scions of cv. Lambert irradiated with 4kR of X-rays with the object of inducing a semi-dwarf growth to reduce harvesting costs, which amount to 50% of production cost. Irradiated scions grafted on root stocks in the greenhouse.
2-3	1959-60	Lower 5 buds of V1 propagated individually to produce V2 nursery trees.
4-5	1961-62	First observations and selection for growth and other traits (dark foliage, sturdy growth and short internodes).
6-7	1963-64	Trees with internodes below average repropagated, planted in orchards, and reselected.
8-9	1965-71	Second test-orchard planted, V3 generation evaluated for economic merits in comparison with parent.
10	1972	Compact types released to commercial growers as 'Compact Lambert' and used in cross breeding. Height of tree only 1/5 of a normal tree on a vigorous rootstock. Comes into bearing very early and bears very heavily, but fruit ripens about 5 days later and is slightly smaller.

Chapter 3

COMPARATIVE RADIOSENSITIVITIES OF MAIN AND ACCESSORY BUDS OF SWEET CHERRY (*PRUNUS AVIUM* L.) WITH VARIOUS IRRADIATION METHODS

Summary. For determination of LD50 dormant main buds of 'Napoleon' exposed to 4kR to 12kR of acute and fractionated gamma rays were forced in the glasshouse. Forcing of unirradiated and irradiated acceesory buds was also done in the glasshouse. For the field experiment 'Bing' main and accessory buds on dormant scions were irradiated and then grafted. Survival and growth of primary (V1) shoots were observed. LD50 values were about 5kR for both acute and fractionated irradiation in air, 5.5kR for acute exposure in water, and 6kR for fractionated dose in water. In the 'Bing' field experiment with main buds, the LD50 for both acute and fractionated irradiation in air was 3.5kR. In water, the LD50 was 5kR for acute treatment and 6.5kR for fractionated dose. The V1 shoots showed 52% frequency of leaf aberrations, and 33% bifurcations and/or fasciations. In the irradiated accessory bud field experiment, bud-growth was influenced by: a) irradiation dosage, b) bud maturity as related to position on the scion, and c) vigor of scions as represented by diameter. With all treatments, the number of buds that grew per potential bud site decreased from the base towards the tip, decreased with increasing dosage, and decreased with acute as compared to fractionation. Sixteen percent of the V1 shoots derived from accessory bud irradiation had aberrant leaves, 2 were total leaf mutants, and 2 had shorter internodes.

Key words. Irradiation, main, accessory, LD50, aberrations

Comparative radiosensitivities of main and accessory buds of sweet cherry (*Prunus avium* L.) with various irradiation methods

Introduction

Exposure of dormant scions to acute and semi-acute gamma rays in air (Lapins 1965, Decourtye, 1970; Poll,1974; Thompson,1979; Ikeda and Nishida, 1982) or in water (Campbell and Lacey , 1973; Lacey and Campbell 1977) has induced beneficial mutations in deciduous fruit trees. Donini (1974, 1982) reported that reirradiation of V1 shoots resulted in increased mutation frequency in sweet cherry, *Prunus avium* L. Reirradiation also enhanced mutation rates in mulberry (Fujita and Nakajima, 1973). Irradiation at higher doses caused substantial cell elimination which led to a reduction of the number of initial cells and an increased size of mutated sectors (Gaul, 1961; Lacey and Campbell, 1979) and increasesd mutation frequency (Lapins,1973, Zagaja et al.,1982). Lapins (1971), and Katagiri and Lapins (1974) reported that accessory buds of sweet cherry had fewer cells than the apical meristems of main lateral buds. In comparison to more developed main buds, accessory buds gave a higher proportion of total mutants in cherry (Lapins, 1971), pear (Nishida, 1973) and a higher frequency of growth-reduced mutants in apple (Zagaja and Przybyla, 1973).

Although the mutation rate increases at higher doses, recovery of mutants may be lower due to exessive cell injury and death. Therefore doses approximating LD_{50} (amount of energy required to produce 50% lethality) have been found to be most effective for

mutation induction. Irradiation of dormant sweet cherry buds with acute gamma or X-rays at LD50 of 4-4.5kR has been successful in inducing mutations, including compact growth mutants (Lapins, 1963, 1971; Donini, 1976; Thompson, 1979). In apple, compact mutants have been induced at LD30 (Visser, 1973) and LD50 (Lacey and Campbell, 1982). To determine radiosensitivity and LD50, irradiated buds were forced in the greenhouse (De Vries et al., 1970; Donini, 1974).

The experiments in this irradiation study of 'Napoleon' and 'Bing' sweet cherry (*Prunus avium* L.) were to: 1) to determine LD50's for main and accessory buds with acute and fractionated irradiation in air and in water, 2) to determine if irradiation of accessory buds would result in larger mutant sectors in V1 shoots than irradiation of main buds, and 3) to identify the most appropriate developmental stage of accessory buds for successful forcing for mutation induction.

Materials and Methods

Forcing of 'Napoleon' main and accessory buds, 1983 and 1984

For determination of the LD50 of 'Napoleon', lateral main buds were forced in the glasshouse in 1983. Dormant scions were collected in mid-January at the Lewis Brown Horticulture Farm, Oregon State University. The scionwood was stored at 0°C before irradiation in early March. Fifteen 20cm scions per treatment were placed with basal end up` in the high-flux irradiation chamber with a dose rate of 670rad/min and exposures of

4-12kR, acute or fractionated gamma rays, in air or in water at the Radiation Center ^{60}Co facility. Dose fractionation consisted of 1-2kR exposures at 12hr intervals. Following irradiation, both treated and control scions were forced in the glasshouse by submerging the basal 4cm in water. The water was replaced twice a week and 1cm of the submerged ends were cut back weekly. Growth and survival of the top 3-5 lateral buds on each scion were recorded weekly for 1 month.

Forcing of unirradiated 'Napoleon' accessory buds in the glasshouse was also carried out in March, 1983. All main lateral buds on 18-20cm scions were excised by making V cuts at their bases, leaving 2 potential accessory bud sites at each node. Lanolin was applied on the cut surfaces, the basal 4cm of the scions was immersed in water, and the containers were maintained in the glasshouse at 20°C. The number of accessory buds that developed shoots was recorded 4wks later. The experiment was repeated in March, 1984 with controls and also with accessory buds that had been exposed to 3, 4, 5 or 6kR acute gamma rays in water.

Irradiation of 'Bing' main (1983) and accessory buds (1984) for the field experiment

For the 'Bing' main bud irradiation in 1983, dormant, one year old scions were obtained from a commercial nursery in mid-January and stored at 0°C until irradiation on April 16th. The irradiation methodology and range of exposures (3-10.5kR) was the same as that for the glasshouse experiment. The scions were then grafted into limbs of Mazzard seedling rootstocks. The number of primary or VI shoots, length of shoots, and leaf and growth aberrations on shoots were recorded in August.

For the 'Bing' accessory bud irradiation in 1984, dormant scions were obtained

a commercial nursery in late January. Also, V1 shoots derived from both irradiated main and accessory buds of 'Bing' were collected in late February. The scions were held at 0°C until irradiation on April 20th with 3kR or 4kR fractionated doses in water. All main buds were excised and both treated and controls were then grafted in the field. Bud survival, length of shoots and leaf, and growth aberrations were recorded 9wks after grafting. In order to determine the effect of bud position on accessory bud development another group of scions was cut into three 17cm sections (minus the immature 10cm tip) labelled terminal (A), mid (B), and basal (C) and exposed to 3kR or 4kR acute in air or fractionated in water. Bud survival and diameter of scions of V1 shoots were recorded in mid-September.

The Chi square test and SPSSX (Statistical Packages for Social Sciences) were used to compare the various treatments.

Results

Determination of LD50 for forced 'Napoleon' main buds, 1983

The average bud survival for irradiated 4-6kR 'Napoleon' scions forced in the glasshouse, was significantly higher for exposures in water (61%) than in air (49%), but there was no significant difference between fractionated (58%) and acute (53%) irradiation (Table I). LD50 values, based on survival of forced buds at the end of the 3rd week were about 5.0kR for both acute and fractionated irradiation in air, 5.5kR for acute exposure in water, and 6kR for fractionated dose in water. Six kR was the highest dosage

of any treatment which resulted in viable shoots at 1mo. Even though attempts were made to force some buds from higher dosages (fractionated in both air and water, and acute in water), shoots failed to grow.

Forcing of unirradiated and irradiated 'Napoleon' accessory buds in the glasshouse, 1983 and 1984

In 1983, unirradiated 'Napoleon' scions had an average of 0.39 accessory buds forced per site after 30 days in the glasshouse (Table II). Of 75 nodes, 52% had single, and 13% had double accessory buds forced. In the remaining 35% bud sites no accessory buds forced. In 1984 controls, 0.69 accessory buds had forced persite in the glasshouse. A single accessory shoot grew at 43% of the nodes, double accessory buds developed at 47% of the nodes, and at the remaining 10% no accessory buds forced. The LD_{50} for forced accessory buds (i.e. in terms of 50% of the buds that forced in controls) following acute gamma irradiation in water was approximately 3.5kR.

Irradiation of 'Bing' main buds: field experiment 1983

The LD_{50} for both acute and fractionated irradiation in air was approximately 3.5kR (Table III). In water, the LD_{50} was 5kR for acute treatment and 6.5kR for fractionated dose. The main buds tolerated higher exposures in water than in air, and higher for fractionated than for acute irradiation. Of 691 buds on irradiated scions that showed initial growth, only 408 (59%) developed into shoots, the rest either formed rosettes or did not survive. With increasing doses there was decreasing bud survival; acute treatments caused a more abrupt decline than fractionated.

Growth of V1 shoots from irradiated main buds was similar to that described by Pratt (1968) and Thompson (1979). The lower 10 leaves from irradiated buds exhibited radiation damage; the first 4-5 leaves were spaced closer, smaller, with irregular margins and small chlorotic spots whereas the next 3-5 leaves were larger and tended to be asymmetrical. Above the 10th node some leaves were aberrant or vestigial leaves. Some aberrant leaves represent mutations while others apparently were caused by irradiation damage to the apical region. Shoots with disrupted phyllotaxy were frequently observed. The frequency of leaf aberrancies above the 10th node in V1 shoots of 'Bing' ranged from 25-67% depending on the dosage with the average frequency at 52% (Table IV). The aberrant leaf frequency ranged from 51 to 55% following exposures in air or water with acute or fractionated doses. No V1 shoots from irradiated main buds had all aberrant leaves; i.e. there were no total leaf mutants. There was 3 to 72% reduction in length of V1 shoots from irradiations. For all irradiation treatments, 33 or 8% of the shoots were bifurcated or fasciated. The frequency of bifurcations ranged from 4% (fractionated exposures in air) to 11% (acute doses in air). The frequency of rosettes was lowest (20%) for fractionated treatment in air, and highest (43%) for fractionated exposures in water. Of the total 396 irradiated nodes with surviving shoots, 6 (2%) had 2-3 multiple shoots arising at the bases of the nodes. No bifurcations, rosettes or multiple shoots were observed in controls.

Irradiation of 'Bing' accessory buds: field experiment 1984

In the 1984 irradiated 'Bing' accessory bud field experiment, bud growth 2mo after grafting was influenced by: a) irradiation dosage, b) bud maturity as related to position on

the scion, and c) vigor of scions as represented by diameter. With all irradiated treatments, the number of buds that grew per potential bud site decreased from the base towards the tip, decreased with increasing dosage, and decreased with acute as compared to fractionation (Tables V and VI). In controls, the number of forced buds per site was 0.19 in (A), 0.37 in (B) and 0.48 in (C). With 3kR fractionated irradiation in water the number of buds forced decreased to 0.1 in (A), 0.28 in (B) and 0.43 in (C), and with 3kR acute irradiation in air, the number per site decreased further to 0 in (A), 0.06 in (B), 0.33 in (C). At the higher acute dosage, 4kR, virtually no accessory buds forced whereas with this same dosage fractionated there were 0.09 in (A), 0.18 in (B), and 0.32 in (C).

Since frequencies of accessory shoots with at least one aberrant leaf were not significantly different for the irradiated 'Bing' standard and reirradiated (V_1 accessory, and V_1 main), and with 3kR and 4kR fractionated doses in water, the data was pooled (Table V). Of 540 V_1 shoots derived from accessory buds, 84 (16%) had 1-30 aberrant leaves. The frequency of stem bifurcations in accessory buds was not related to irradiation dosage; there were 17% at 3kR , 11% at 4kR and 14% for control. The frequency of rosettes were similar in control (22%) and at 3kR (21%), but was less at 4kR (12%).

In V_1 shoots from irradiated buds had 4 (0.74%) total mutants, 2 with shorter internodes and 2 with total leaf mutants. Multiple shoots (3-5) regenerated on15% of the irradiated nodes as compared to 4% at control nodes.

Discussion

The greater tolerance of apical meristems to higher irradiation dosage in water than in air indicates that water slightly buffered tissues against radiation damage. Water provides a more even dose distribution by preventing "surface build-up" effect of gamma radiation (Lacey, 1976; Lacey and Campbell, 1979). "Direct hits" from exposures in air are more damaging to the cells.

With irradiation in water the LD50's were the same for forced buds in the glasshouse and for grafted trees in the field whereas for irradiation in air the LD50's in the glasshouse were 1.5kR higher than in the field. The difference in LD50's for irradiation in air under the two environments reflects the length of time elapsed before evaluation. In the glasshouse buds were evaluated at the 3rd week and in the field evaluation was at the 9th week. In the field, although some buds pushed initially they failed to continue growing.

In both the glasshouse and field experiments the difference in radiosensitivities of main and accessory buds was reflected in lower LD50 for the latter. Katagiri and Lapins (1974) reported that accessory buds of 'Bing' cherry were more radiosensitive than the apical meristem of main buds because they are less advanced ontogenetically and, thus, have fewer meristematic cells. Because there are fewer apical initials in accessory buds, and less intrasomatic competition, there should be a greater chance of recovery of total shoot mutants. Four (2 leaf mutants and 2 compacts) V1 accessory shoots were total mutants, but there were none in shoots derived from main buds.

Along a scion shoot, dormant accessory buds were at various stages of development

indicated by the decreasing percentage of buds forced from the base to the top. Lapins (1971) reported that 30% of the accessory bud sites in dormant buds of 'Bing' had developed bud initials with meristematic tissues ranging from 150-450μ in width. Katagiri and Lapins (1974) observed that removal of the main buds forced accessory buds to produce shoots, but did not stimulate accessory buds at sites where bud initials had not yet developed. As the stage of development is related to age of the node and vigor of the shoot, we obtained the highest percentage of forced buds from the basal portion of vigorous shoots.

Moderate irradiation damage to the promeristem of the shoot apex and recovery by flank meristem has been reported to cause bifurcated shoots (Lapins and Hough, 1970). We observed the highest frequency of bifurcations (18%) for acute 5.5kR irradiation in water. Lapins et al (1969) reported that for apple the highest frequency of bifurcations was 57% at 3.5kR and for peach it was18% at 5kR. Bishop (1967) observed up to 30% bifurcations in shoots of irradiated apple scions whereas the control had less than 1%. He suggested that the bifurcations were due to an upset in the polarity of meristematic divisions caused by the irradiation.

Severe damage to the apical meristem of main buds frequently caused rosetting (35% of all irradiated buds versus none in controls). In rosettes, only the preformed leaf primordia developed into highly aberrant tightly spaced leaves. A less frequent consequence of apical meristem damage is the formation of multiple shoots at a node (2% in main buds versus none in controls). Possibly this is due to forcing of lateral meristems.

The much higher frequency of multiple shoots at irradiated accessory bud sites (15%) as compared to main bud sites (2%) can be explained by the differential effect of

irradiation on the two developmental stages. Scattered cell damage, as reported by Pratt (1968), is not sufficient to totally disrupt the integrity of the more highly developed, multicellular, apical meristem of the main bud. However, such damage in the less developed accessory bud meristems could cause lesions between the relatively few cells, and thereby stimulate the organization of as many apices as there were lesions. The relatively few multiple shoots at non-irradiated accessory nodes (7%) may have been induced by physical damage, or shock, due to excision of the main bud. Comparable frequencies of bifurcations and rosettes in irradiated and non-irradiated buds suggests that these responses may have also been caused by the mechanical excision.

The very high frequency of V1 shoots with aberrant leaves (50% from irradiated main buds) as compared mutation frequencies reported by Lapins (1971) in V2 shoots of 'Bing' (12%) or by Thompson (1979) in V2 shoots of 'Napoleon' (8.8%) indicates that only about 20% of these V1 aberrancies are associated with a mutant axillary bud. This is consistent with Lapins' et al. (1969) observation that only 19.6% of aberrant leaves in V1 shoots of apple and peach were associated with mutations in V2 shoots. Lapins (1983) further explained that the primordia of leaves and those of secondary buds have parallel but, presumably, independent ontogeny and that relatively few of the cells associated with leaf initials become involved with secondary bud formation.

In conclusion the following considerations deserve emphasis:

1) Preliminary irradiation of buds and forcing them in the glasshouse provides reliable information on appropriate range of dosages for use in a large scale field experiment; 2) Accessory bud forcing is greater in the lower (older) part of vigorous scions; 3) As a

consequence the LD_{50} for accessory buds is also greatly influenced by the vigor or age of buds. For basal buds from vigorous scions the LD_{50}'s were approximately 1.5kR lower than that of main buds. 2.5-3kR irradiation in air and 4kR irradiation in water are appropriate doses for accessory buds on vigorous scions; 4) Radiosensitivity of buds is affected by irradiation technique. Fractionation of doses resulted in higher LD_{50}'s (by ≥1kR) than acute doses. The same effect is shown by irradiation of buds in water versus air; and 5) Irradiation of accessory buds results in slightly larger mutant sectors than irradiation of main buds evidenced by the occurrence of 4 (0.74%) total shoot mutants in accessory bud shoots versus none in main bud shoots, and by the greater number of aberrant leaves on shoots showing such aberrancies; i.e. 4.8 in accessory bud shoots and 3.4 in main bud shoots.

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Table 5. Survival and growth of irradiated main buds of 'Napoleon' forced for 21 days in the greenhouse,1983

Irradiation techniques	Dose (kR)	Potential no. of buds	Bud survival No.	%	Shoot length cm	%[a]
Control						
Dormant buds	0	139	136	98	3.6	100
Irradiated in air						
Acute	4	67	44	66	2.1	58
	5	73	39	53	1.4	39
	6	71	17	24	0.9	25
Fractionated	4	70	50	71	2.3	64
	6	71	22	31	2.1	58
	8	67	9	13	d	d
Irradiated in water						
Acute	4	67	55	75	2.5	69
	5	68	41	60	1.3	36
	6	62	25	40	1.2	33
	8	70	14	20	wd	wd
	10	67	17	25	wd	wd
	12	69	6	9	wd	wd
Fractionated	4	62	50	81	2.8	78
	6	64	30	47	1.9	53
	8	66	21	32	wd	wd
	10	70	9	13	wd	wd
	12	64	4	6	wd	wd
Subtotal						
In air	4-6	352	172	49b	1.8	49
In water	4-6	323	201	61c	1.9	54
Acute	4-6	408	221	53b	1.6	44
Fractionated	4-6	267	152	58b	2.3	63

a % of control; wd wilted or dead

b, c differ significantly at p=0.05 using Chi square test

Table 6. Number of accessory buds forced on unirradiated and irradiated dormant scions of 'Napoleon' after 30 days in the glasshouse, 1983 and 1984

Irradiation methods/(scion)	Dose (kR)	No. of Scions	Potential bud sites	Buds forced per site (% control)
1983				
Unirradiated	0	18	150	0.39
1984				
Unirradiated	0	15	140	0.69 (100)
Acute in water	3	14	142	0.53 (77)
	4	14	146	0.21 (30)
	5	14	128	0.10 (14)
	6	14	140	0.12 (17)

Table 7. Bud survival of 'Bing' V1 shoots derived from irradiated main buds: field experiment, 1983

Irradiation techniques/(kR)		No. of scions	Tot. no. of buds	Bud survival No. (%)[a]
Control	0	21	59	47 (100)
In air				
Acute	3	23	105	52 (63)
	4	46	175	32 (23)
	5	42	175	12 (9)
	6	23	99	0
Fractionated	4	23	99	29 (36)
	5	44	184	23 (16)
	6	49	185	16 (11)
	7	23	95	3 (4)
In water				
Acute	4.5	47	207	148 (90)
	5.5	47	178	38 (26)
	6.5	22	100	10 (13)
	8.5	23	100	1 (1)
Fractionated				
	4.5	23	94	76 (100)
	5.5	48	184	128 (88)
	6.5	46	203	75 (46)
	7.5	24	111	37 (41)
	10.5	23	91	11 (15)

Table 7 Continued

Subtotal				
In air	(3-6)	250	1022	164 (16)
In water	(4.5-6.5)	233	966	465 (48)
Acute	(3-6.5)	250	1039	292 (28)
Fractionated	(4-6.5)	233	949	347 (37)

a expressed as % of control

Table 8. Growth and development of 'Bing' V1 shoots derived from main buds: field experiment, 1983

Irradiation techniques/(kR)		No. V1 shoots	Av. length cm (%)[a]	Aberrant shoots No. (%)	Bifur-cations %	Rosettes[b] %
Control	0	12	76 (100)	0	0	0
In air						
Acute	3	33	57 (75)	14 (42)	12	35
	4	24	45 (60)	11 (46)	13	29
	5	4	21 (28)	1 (25)	0	60
	6	0	-	-	-	-
Fractionated	4	12	66 (87)	8 (67)	0	29
	5	17	68 (89)	10 (59)	6	23
	6	16	66 (87)	10 (63)	6	6
	7	2	-	1	-	1
In water						
Acute	4.5	83	70 (92)	50 (60)	2	39
	5.5	34	64 (84)	13 (38)	18	21
	6.5	7	58 (77)	3 (43)	14	22
	8.5	1	-	1	-	-
Fractionated						
	4.5	50	61 (80)	27 (54)	4	35
	5.5	62	72 (94)	37 60)	10	46
	6.5	37	73 (97)	17 (46)	5	46
	7.5	22	72 (94)	10 (45)	0	39
	10.5	5	51 (67)	1	2	7
Subtotal						
In air	(3-6)	106	54 (71)	54 (51)	8	30
In water	(4.5-6.5)	273	66 (87)	147 (54)	7	39
Acute	(3-6.5)	185	53 (70)	92 (50)	9	35
Fractionated	(4-6.5)	194	68 (89)	109 (56)	6	39

a % of control; b % based on (number of V1 shoots + number of rosettes)

Table 9. Leaf and growth aberrations on 'Bing' V1 accessory shoots following fractionated irradiation in water, 1984

Scion	Dose (kR)	No. of scions	No. of V1 shoots	Av. length (cm)	Aberrant shoots No. (%)	Bifurcations %	Rosette[a] %
1.'Bing' standard	0	10	25	68	0	16	19
	3	50	126	64	24 (19)	11	13
	4	50	91	64	14 (15)	10	7
Subtotal		100	217	64	38 (18)	11	10
2. V1 'Bing' accessory	0	10	31	56	0	16	24
	3	50	107	57	15 (14)	19	28
	4	47	87	55	12 (14)	13	19
Subtotal		97	194	56	27 (14)	16	24
3. V1 'Bing' main	0	9	31	71	0	10	23
	3	22	66	63	9 (14)	20d	21b
	4	49	63	72	10 (16)	11d	11b
Subtotal		71	129	68	19 (15)	16	16
All scion types	0	29	87	65	0	14d	22
	3	122	299	61	48 (16)	17d	21b
	4	146	241	64	36 (15)	11d	12c
Total		268	540	63	84 (16)	14	17

a as percentage of (number of surviving shoots + number of rosettes)
b, c, d different letters within column, different rows were significantly different at p=0.01 using Chi square test

Table 10. Forcing of irradiated 'Bing' accessory buds in relation to scion maturity (terminal, A; mid, B; basal, C), dosage, and irradiation technique: field experiment, 1984

Irradiation technique	Scion diameter (cm)	No. of buds forced (No. of buds forced per site) Dose 0kR	3kR	4kR
Acute in air	0.5-0.6 (A)	7 (0.19)	0	0
	0.61-0.80 (B)	30 (0.37)	9 (0.06)	1 (0.003)
	0.81-1.00 (C)	63 (0.48)	4 (0.33)	-
Fractionated in water	0.5-0.6 (A)	7 (0.19)	11 (0.10)	21 (0.09)
	0.61-0.80 (B)	30 (0.37)	193 (0.28)	166 (0.18)
	0.81-1.00 (C)	63 (0.48)	152 (0.43)	43 (0.32)

Chapter 4

METHOGOLOGY FOR RADIATION-INDUCED MUTATIONS IN MAIN LATERAL BUDS IN *PRUNUS AVIUM* L. CVS NAPOLEON AND BING

Summary. Dormant scions of 'Napoleon' were exposed to acute 5kR gamma irradiation in water. For 'Bing', the scions were exposed to acute or fractionated doses of 4 -12kR gamma rays, in air or in water. Leaf and growth-reduced mutants of V2 trees were identified. Of 5209 'Napoleon' V2 trees the overall mutation frequency was 7.5%: 0.04% growth-reduced mutants, 0.4% total leaf mutants, and 7.1% partial leaf mutants. The V2 trees derived from the basal buds had the highest mutation frequencies, for both overall mutations (9.7%) and for partial leaf mutants (9.6%) but a slightly lower frequency of total leaf mutants (0.3%). By contrast, upper buds had fewer overall mutations (6.6%), fewer partial leaf mutants (6.1%), but a slightly higher frequency of total leaf mutants (0.5%). Among 2942 'Bing' V2 trees (including all treatments) the overall mutation frequency was 7.3%: 0.34% growth-reduced mutants, 2.3% total leaf mutants, and 4.7% partial leaf mutants. The height/diameter/number of nodes (H/D/N) ratios of all the V2 growth-reduced mutants and 8 of the V3 were 2 standard deviations below that of control plants. The mutation frequencies for irradiation in air and for fractionated doses were respectively not significantly higher than for irradiation in water and for acute doses. For efficient recovery of growth-reduced mutants we recommend propagating only the upper buds on V1 shoots of sweet cherry.

Key words. Acute, fractionated, V2 trees, mutation frequency, growth-reduced.

Methodology for radiation-induced mutations in main lateral buds in *Prunus avium* L. cvs Napoleon and Bing

Introduction

Various biological and environmental factors affect radiation-induced mutation frequency and spectrum in plants. Such factors include cultivars (Decourtye, 1970; Visser et al, 1971; Lacey and Campbell, 1982; Zagaja et al, 1982) developmental stage of bud primordia (Merricle and Merricle, 1961; Sparrow et al, 1961), age and size of buds (Lapins et al, 1969; Lapins, 1973; Katagiri and Lapins, 1974), number of bud primordia (Pratt, 1968), position of buds on irradiated shoot (Lapins and Hough, 1970; Thompson, 1979; Lapins, 1971), dose and dose rate (Nishida, 1973; Sparrow et al, 1961; Donini, 1982), and environmental conditions before, during and after treatment (Conger et al, 1977; Broertjes and van Harten, 1978; Sparrow and Woodwell, 1962).

In sweet cherry, *Prunus avium* L., tolerance to higher dosage was observed when dormant scions were irradiated in water (Thompson, 1983). Campbell and Lacey (1973), Lacey and Campbell (1977; 1979;1982) irradiated apple scions in water and suggested that water probably provides a more even dose distribution. Use of chronic and recurrent irradiation techniques have resulted in increased dose tolerance, higher mutation frequency, and broader mutation spectrum in vegetatively propagated plants (Nakajima, 1973; Fujita and Nakajima, 1973; Fujita and Wada, 1982). In apple, desirable mutants were obtained mostly from chronic treatment while acute treatment resulted in pollen sterility in most cases (Ikeda and Nishida, 1982). On the contrary, Sparrow et al (1961)

reported that chronic exposures or dose fractionation reduced somatic mutations.

Smaller trees are desirable because they can be planted at higher density and orchard operations are facilitated. Lapins (1973, 1975), Donini (1976, 1982), and Thompson (1979) had success in getting radiation-induced compact mutants in sweet cherry, and Decourtye (1970), Visser (1973), Visser et al, (1971), Zagaja and Przybyla (1973), Lacey and Campbell (1977, 1979) in apple. The objectives of this study were: 1) to manipulate irradiation techniques viz. acute and fractionated gamma irradiation in air and in water in order to increase mutation frequency and size of mutant sectors in 'Napoleon' ('Royal Ann') and 'Bing', the two major commercial cultivars in western USA; 2) to identify bud positions on the primary shoot which yield the highest frequency of whole plant mutants, including growth-reduced mutants, in order to optimize recovery.

Materials and Methods

I. Irradiation of 'Napoleon', 1982

Dormant scions of 'Napoleon' were obtained from a commercial nursery in February 1982, stored at 0°C, and irradiated April 19th and 20th. 15cm scions immersed in water were lowered with apical ends downwards into the high-flux radiation chamber where they were exposed to approximately 5kR acute ^{60}Co gamma rays at the Radiation Center, Oregon State University. The dose rate along the perimeter of the chamber ranged from 635-895rad/min. Dose rate of 895rad/min was used to determine exposure duration of 5min 36sec and target dose of 5kR. Following irradiation, scions were immediately grafted

onto Mazzard seedling rootstocks. In September, mature buds on primary, or V1[1], shoots were individually budded in sequence from base to apex onto seedling rootstocks. During the following summer the V2 trees were examined in mid-July and in mid-August, for partial and total leaf mutants and for growth-reduced mutations. Partial leaf mutants are defined as having one to most of the leaves aberrant or partially aberrant, and total leaf mutants are probably periclinal chimeras had all leaves aberrant. Compact growth habit and shorter plants that either stopped growth early or were slower growing are the two types of growth-reduced mutants identified. Compact mutants are noticeably shorter, have a thicker stem for their height, and have shorter internodes than normal. Plants approximating 13-16 nodes per 25cm midsection of the stem were categorized as semi-compacts, and those with 17 nodes or more as compacts. Trees that were shorter, or stopped growth early were also selected. Plant height (H), diameter at mid-point (D), and the number of nodes (N) in a 25cm mid-shoot section of potential growth-reduced mutants and of control trees were recorded in early September and used for ratios.
Growth-reduced mutants were propagated onto seedling rootstocks, using 10 buds/selection for verification in V3. Height, diameter, and number of nodes of V3 trees were recorded when trees were dormant. Late leafing V2 trees identified in early April 1984 were also repropagated for verification in V3. The Chi square test and Statistical Packages for Social Sciences (SPSSX, 1983) were used to analyse the data.

[1] Terminology used is that of Lapins (1965)

II. Irradiation of 'Bing', 1983

Dormant scionwood of 'Bing' was obtained from a commercial nursery in mid-February 1983 and stored at 0°C. 26cm scions, 25 or 50 scions per treatment were placed basal ends in the radiation chamber and exposed to acute or fractionated doses of 4-12kR gamma rays, in air or in water in mid-April. A mean dose of 671rad/min (average of 786rad/min at the perimeter and 557rad/min at the centerline) was used to determine approximate target exposures in the high flux chamber. Dose fractionation consisted of 1-2.5 kR exposures at 12hr intervals. Irradiated and control scions were grafted immediately. Buds from vigorous V1 shoots were patch or T-budded, 2 to a rootstock on Sept. 14th. The basal 5-6 nodes of the cutback V1 shoot were allowed to grow and produce V2 shoots "in place". Leaf and growth-reduced mutants of budded (B) and cutback (C) V2 trees were identified in July 1984. Tree height, diameter, and number of nodes were measured as for 'Napoleon'. Potential growth-reduced mutants were repropagated, 10 buddings/selection, in mid-September. V2 trees identified as having late bud-break on April 9th, 1985 were also repropagated for verification in V3.

Results

I. Induced mutations in 'Napoleon' following irradiation in water

In a population of 5209 surviving V2 trees the overall mutation frequency was 7.5%. There were 2 (0.04%) growth-reduced mutants, 22 (0.4%) total leaf mutants, and 367 (7.1%) partial leaf mutants.

Growth-reduced mutants.

The degree of growth reduction was expressed as percentages of control for both height and H/D/N ratios. Six possible growth-reduced mutants ranging from 79-88% of normal height were identified (Table I). Two of these, compact 13-1 and semi-compact 7-14 were 79% and 86% of normal height and their H/D/N ratios were 51% and 79% of normal respectively. They were verified to be growth-reduced in the V3 generation with 77% and 89% height respectively of control. No reversions to normal were noted. The other 4 possible growth-reduced V2 selections appeared to be normal in the V3. The lower height of all V2 trees as compared to the corresponding V3 trees, was because the former were double-budded on rootstocks whereas the latter were single-budded.

Partial and total leaf mutants

Leaf mutations in V2 trees were similar to those reported previously in cherry (Lapins, 1971; Thompson, 1979); they included aberrations of leaf size, shape, thickness, texture, serrations on margins, and chlorophyll content. Partial mutant leaves are usually assymmetrical and, because they contain mixtures of varying proportions of mutant and non-mutant tissues , are more difficult to characterize. There were 1 to 35 aberrant leaves on partial mutant trees, with a mean of 4.4 and a standard deviation of 4.2.

Mutation frequency and bud position on V1 shoot

The frequency of mutations in V2 plants as related to bud position on the V1 shoot is given in Table II. A dormant bud of cherry has about 10 leaf primordia and foliage leaves

(Pratt, 1968; Lapins, 1971; Thompson, 1979). Thus, buds propagated from positions 6-10 were of pre-irradiation origin (designated as 'basal' buds), and buds 11 and higher were of post-irradiation origin ('upper' buds). The basal 4-5 buds on V1 shoots were too small or too congested to be propagated. The higher buds are combined into groups representing 4 successive 5-bud sections of the V1 shoot and a terminal 9-bud section (due to smaller number of buds per position). The basal buds had the highest mutation frequencies, for both overall mutations (9.7%) and for partial leaf mutants (9.6%) but a slightly lower frequency of total leaf mutants (0.3%). By contrast, upper buds had fewer overall mutations (6.6%), fewer partial leaf mutants (6.1%), but a slightly higher frequency of total leaf mutants (0.5%). With successively higher bud positions there was a decreasing frequency of both overall and partial leaf mutants. However, although numbers were small, the trend for total leaf mutants was in the opposite direction, i.e. increasing frequency with higher bud position. The 2 growth-reduced mutants, 13-1 and 7-14, were derived from bud positions 6 and 14, respectively, on the V1 shoot .

Distribution of mutant trees in V2 families

Identification of repeats of the same mutant within a family was relatively easy in the case of total mutants. However, for partial leaf mutants, identification of a similar mutant within a family or within a plant was often difficult due to mixtures of mutant and non-mutant tissues. Of the 425 V2 families with 3-39 surviving trees per family, 181 (43%) had no mutation and 130 (31%) had 1 tree with a mutation (partial or total). The remaining 114 (27%) families had 2-5 partial or total leaf mutant trees per family. Of the

234 V_2 families in which mutations were identified, only 21 (9%) possibly had the same mutation repeated in 2 or 3 partial mutant trees. There were no repeats within a family involving total leaf mutant plants. In 15 families with 2 or 3 plant repeats, the similar part mutant trees were derived from pairs of adjacent buds from positions 5-47 on the V_1 shoots. In the other 8 families with repeats, the V_2 trees were derived from bud positions separated by 1-7 nodes on the V_1 shoot.

II. Induced mutations in 'Bing' following various irradiation treatments

Among 2942 surviving V_2 trees (including all treatments) the overall mutation frequency was 7.3% (Table III). There were 10 (0.34%) growth-reduced mutants, 68 (2.3%) total leaf mutants, and 138 (4.7%) partial leaf mutants. Of the 217 mutations identified, 4.6% were growth-controlled, 31% were total leaf mutants and 64% were partial leaf mutants.

Growth-reduced mutants

A total of 11 growth-reduced mutants characterised by 43 to 84% of normal height were identified in the V_2. Height, diameter, and number of nodes of the V_2 and the V_3 are presented in Table IV. The growth-reduced trait of all mutants except 31-111 (which appeared normal) was also expressed in the V_3's as indicated by both their lower average height, and H/D/N ratios as compared to controls. The H/D/N ratios of all the V_2 mutants and 8 of the V_3 were 2 standard deviations below that of control plants. Three mutants, 31-137, 23-105, and 44-108 with V_3 heights ranging from30-51% and H/D/N ratios

35-56% of normal were promising compacts whereas 5 mutants, 16-58, 23-105, 32-19, 22-133, 24-121, and 14-22 with V3 heights ranging from 64-84% and H/D/N ratios 69-83% of normal were promising semi-compacts. Potential semi-compact mutant 22-133 which was 85% of control height in V2 (and 82% in V3) had a very low H/D/N ratio in V2 (43% of normal) but a considerably higher ratio in V3 (83% of normal). No reversions to normal were evident in V3 populations that showed growth-reduced habit.

Partial and total leaf mutants

Leaf aberrations on partial and total mutant trees were within the same spectra as in 'Napoleon'. The number of mutant leaves per partial mutant tree ranged from 1-21, with a mean of 6.9, and standard deviation of 5.2.

Mutation frequencies in budded (B) vs cutback (C) populations

There were 1667 budded (B) V2 trees and 1275 V2 shoots from cutback (C) V1's (Table III). An average of 5 (range 1-7) shoots grew from the cutback V1 shoots. Whereas the overall frequency of mutations was similar in C (7.8%) and B (7.3%), the relative proportions of the 3 types of mutants was significantly different in the 2 populations. The frequency of total mutants was higher in B (3.2%) than in C (1.2%) as was the frequency of growth-controlled mutants, 0.54% in B versus 0.08% in C. However, the frequency of partial leaf mutants was higher in C (6.3%) than in B (3.5%).

Effect of exposure to irradiation in air and in water on mutation frequencies

Irradiation in air produced higher but not significant frequencies of overall mutations, of partial leaf mutants, and of total leaf mutants than that in water. In air, the highest frequency of partial leaf mutants, total leaf mutants, growth-reduced mutants, and overall mutations was obtained with acute 3kR, and fractionated 4-5kR exposures (Table V). At higher dosages in air mutation rates declined. In water, there was not such a clear cut dosage-mutation relationship; the highest frequencies of total leaf mutants were obtained with 6.5kR and 7.5kR, whereas the highest frequencies of partial leaf mutants were seen for lower doses of 4.5 and 5.5kR. At higher exposures, acute in air (5kR and 6kR) and in water (8.5kR), and with fractionated dosage in water (10.5kR), there were very few or no surviving V1 shoots for repropagation.

Effect of acute vs fractionation of irradiation dosages on mutation frequencies

The overall mutation frequency for fractionated dosages was not significantly higher than for acute dosages (8.0% versus 7.0%)(TableV). Fractionation of dosages induced a higher but not significant frequency of partial leaf mutants than acute doses (5.6% versus 3.8%), and a similar frequency of total leaf mutants (2.1% versus 2.7%). Although numbers were small, there was a 2 fold increase in growth-reduced mutants with acute dosages (0.49% versus 0.25%).

Mutation frequency in relation to bud position on the V1 shoot

The frequency of mutations of 'Bing' V2 plants as related to bud position on V1 shoot

showed the same trend as for 'Napoleon' (Table VI). Basal buds had 5.9% partial and 1.6% total mutants whereas upper buds had 3.0% partial and 3.3% total mutants. With upper bud positions there was a clear cut decreasing trend in partials. Total leaf mutants seems to increase at least to bud positions 16-20 and, the highest frequency (3.8%) was at bud positions 31-39. Although numbers of growth-reduced mutants are small, the highest frequency occurred at bud positions 21-25.

Distribution of mutant trees in V2 families

Of the 184 V2 families in the B population, 101 (55%) had no mutation, 58 (32%) had only 1 mutant tree, and the remaining 25 (14%) families had 2-6 mutant trees per family. Of the 83 families in which mutations were identified, only 6 (7%) possibly had the same mutation repeated in 2 or more trees. Although low budding success (54%) undoubtedly broke some of the runs, there were no cases where all surviving members of a family had the same mutation. Thus, there were no whole family runs. In families with a single mutant tree, the tree was derived from bud positions 7-33 on the V1 shoots. Growth-reduced mutants were derived from bud positions 6, 8, 15, 20, 21, 25, and 28. In the 6 families with 2 similar mutant trees, all originated in the higher bud positions (12-25) on the V1 shoot. In the family with 5 different types of mutants, the trees originated from bud positions 8, 23, 25, 28, and 29; the first tree was a partial leaf mutant and the latter 4 were dissimilar total leaf mutants. In 3 of the 2-plant mutant repeats, the similar mutant trees were derived from adjacent pairs of bud positions 12-17 on the V1 shoot. In the other 3 repeats, the V2 trees were derived from bud positions 9-25 and were separated by 2-4

nodes on the V1 shoot. Of the 275 V2 families in the C population, only 6 (2%) families with 3-6 V2 shoots had repeats of 2-4 similar partial leaf mutants.

Discussion

Although we could increase dose tolerance of dormant buds of cherry by irradiation in water the overall mutation frequency was less than with irradiation in air at lower exposures. In water (acute), the overall mutation frequency of Napoleon cherry in this study was 7.5% whereas in air (acute), the rate reported by Thompson (1979) was 8.8%. In water (fractionated), the overall mutation frequency of 'Bing' in this study was 7.4% compared to 9.4% in air (fractionated). The trend of lower mutation rates for irradiation in water lends support to the hypothesis that water provide a more even dose distribution (Lacey, 1976; Lacey and Campbell, 1979) which buffers the meristematic cells against damage, but also against mutation.

Acute irradiation ('Bing') especially in air resulted in 4.2% total leaf mutants whereas fractionated irradiation resulted in 1.6% total leaf mutants. Acute irradiation in air probably gave a more direct hit and was high enough to result in lethality of many meristematic cells. A mutant cell, being surrounded by fewer non-mutant cells, has a greater chance of surviving intrasomatic competition and becoming expressed in a larger sector and, thus being identified as total leaf mutants. With fractionation, a low frequency of total leaf mutants (1.6%) was accompanied by a relatively high frequency of partial leaf mutants (7.5%). Exposures to 2-2.5kR in dose fractionation are insufficient to cause substantial meristematic cell elimination. There is probably scattered cell damage resulting

in small sectors which are recovered as partial leaf mutants. High frequency of total leaf mutants (4.5%) with acute exposures is accompanied by a relatively low frequency of partial leaf mutants (3.7%). This is expected because with a larger mutant sector there will be an increasing chance of recovery of total leaf mutants.

The decreasing mutation frequencies at higher exposures could be the result of high lethality of mutant cells or competitive disadvantage of affected cells in growth centers. Using moderate acute exposures of 3-4kR in air or 4.5 -5.5kR in water were the most successful for induction of total leaf mutants and growth-reduced mutants in both cultivars.

Higher mutation rates (consisting mainly of partials) occurred in plants derived from basal buds 6 to 10 in 'Napoleon' (Table II) and 2 to 10 in 'Bing' (Table VII). At higher bud positions there is a trend for decreasing mutation rates which was also reported in apple (Lapins and Hough, 1970). Others have also found higher mutation rates in those plant parts where axillary buds were already initiated at the time of irradiation (Bauer, 1957; Pratt, 1967; Lapins et al, 1969; Lapins, 1971; Thompson, 1979; Donini, 1982).

One needs to be able to locate the bud positions on the V1 shoot which yields the highest frequency of total leaf mutants for efficient recovery of stable and useful mutants. In 'Bing' the frequency of total mutants (total leaf mutants plus growth-reduced mutants) tends to increase with upper bud positions, at least to 21-25, and remains high to the uppermost positions propagated (31-39). The highest frequency of total leaf mutants (0.9%) for 'Napoleon' was at bud positions 31-39 as found in this study and in a previous report (Thompson, 1979), the highest frequency of total leaf mutants (3.2%) was also at the highest bud position. Lapins (1971) also observed in 'Bing' that the proportion of total

mutants was slightly higher from the upper buds than from the basal. Apparently, while the shoot is growing there has been sufficient time for the original mutant cell to divide and form larger sectors which occupy entire lateral bud meristems on V1 shoots. Increased recovery of total leaf mutants was also accompanied by higher frequency of growth-reduced mutants. To be easily identified, growth-reduced mutants need to be total or at least near total mutants. Higher frequency of growth-reduced mutants in 'Bing' than 'Napoleon' in this study was also related to higher frequency of total leaf mutants. Thus, high mutation rates *per se* may not be the best guide but the desired mutation type should be taken into account.

For efficient recovery of useful mutants we, and also previously Thompson (1979) recommend propagation of upper buds of V1 shoot of cherry rather than basal buds as recommended by Donini (1982).

There were differences between the two cvs in frequencies of mutant types, although overall mutation rates were similar with comparable dosages and irradiation techniques. In both our acute irradiation study and that of Thompson (1979), the frequency of total mutants and the proportion of mutants that are totals are lower in 'Napoleon' than in 'Bing', whereas the frequency of partial leaf mutants is higher in 'Napoleon' than in 'Bing'. Lapins (1971) also obtained a higher frequency of whole shoot mutants in 'Bing' (4.2%) as compared to Thompson's (1979) report 'Napoleon' (2.7%). Differences in stage of development of dormant buds at the time of irradiation, genetic constitution, and perhaps in apical organization and ontogeny of shoots are plausible explanations for the differential response of the two cultivars.

In apple, Campbell and Lacey (1973), and Lacey and Campbell (1979, 1982)

recovered up to 22% overall mutations for irradiation in water whereas 13% was the highest we obtained in Bing cherry (Table V). They also obtained runs of compact mutants in the V2 which indicates that all apical initials (at least in the histogenic layers involved with compact growth) in the V1 shoot are mutant and that these mutant initial cells remain in the critical position throughout the V1 shoot. This situation also enables preselection of total compact mutants in the V1 in apple, whereas this has not been possible in cherry. Decourtye (1970) recommended propagation of buds 8-12 whereas Lacey and Campbell (1979) recommended slightly higher buds 11-17. Pratt (1968) reported that irradiation damage in the apical meristem of cherry is random whereas in apple it has been reported to be localised in the promeristem with recovery from the peripheral meristem (Pratt, 1967; Lapins and Hough, 1970). Thus, V1 shoots in apple must have a stable, recovered apex whereas in cherry V1 shoots there is much instability. The mode of recovery in cherry has not been reported, and is currently under investigation. In our study and Thompson's (1979), mutant V2 trees were randomly distributed within families Thompson explained that this could be due to constant shifting of apical cell initials in the V1. The occurrence of predominantly longitudinal mutant sectors in V2 partial leaf mutants suggests that apical cell initials have become more stable in secondary shoots.

The 10 promising growth-reduced mutants in 'Bing' showed great variation in height, internode length, number of nodes, stem diameter, and H/D/N ratio (Table IV). This suggests that the dwarf trait is not monogenic. Variability lends to versatility in potential value of the growth-reduced mutants. Mutants that are 50-80% of control height may be more suitable for high density planting in orchards whereas those more compact ones may

be useful for home gardens. 'Compact Stella' is about half standard size and 'Compact Lambert' is one quarter the size of 'Lambert' (Lane, 1977).

From this study we conclude that : I) The highest frequency of total leaf mutants in 'Bing' was acute 3kR irradiation in air; whereas in 'Napoleon' it was 4.5kR acute irradiation in air. In both cultivars, although they tolerated higher dosages with water and fractionation there was a higher frequency of total leaf mutants with acute irradiation in air; II) Irradiation in water with both cultivars led to slight decrease in overall mutation frequency even with higher dosages, but especially a decreased frequency of totals and increased proportion of partials; III) In both 'Napoleon' and 'Bing' higher dosages not only decreased survival but also tend to decrease mutation frequency; IV) Fractionation in 'Bing' resulted in slightly higher overall mutation frequency but also a higher frequency of partial leaf mutants and lower frequency of total leaf mutants; V) The frequency of compact mutants in 'Bing' (0.34%) was higher than in 'Napoleon' (0.14%); VI) For efficient recovery of total leaf mutants we recommend propagating only the upper buds (above position 10) on V_1 shoots of sweet cherry. Basal buds have higher overall mutation frequency but lower frequency of total leaf mutants.

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Table 11. Height (H), diameter (D), and number of nodes (N) at 25cm stem midsection of potential V2 growth-reduced mutants of 'Napoleon' and their V3 generation.

Tree	V2 H (cm)	D (cm)	N	H/D/N[b] %[c]	V3[a] H (cm)	D (cm)	N	H/D/N %
Control								
Mean	126	1.09	11	10.5 (100)	187	1.38	12	11.7 (100)
Std. dev.	20	0.14	1.6	2.2	16	0.14	0.9	1.1
Growth-reduced								
13-1	100	1.02	18	5.4 (51)	144	1.16	12	10.7 (91)
5-48	111	1.06	15	7.0 (67)	195	1.46	11	11.8 (101)
8-10	111	1.23	12	7.5 (71)	212	1.45	10	14.2 (121)
4-85	100	1.01	12	8.2 (85)	175	1.22	11	13.6 (116)
7-14	108	1.09	12	8.3 (79)	166	1.17	14	9.9 (85)
4-2	110	1.07	12	8.5 (81)	170	1.28	11	12.1 (103)

[a] average of 2-10 V3 trees/family
[b] Height/Diameter/No. of nodes
[c] % of control

Table 12. The frequency of mutations in 'Napoleon' V2 trees as related to bud position on V1 shoot, 1983.

Bud position on V1 shoot	Total no. of plants	Partial mutants No.	%	Total mutants No.	%	Growth-reduced No.	%	All mutants No.	%
6-10	1551	144	9.6	5	0.3	1	0.06	150	9.7
11-15	1521	100	6.6	6	0.4	1	0.07	107	7.0
16-20	1194	73	6.1	6	0.5	0	0	79	6.6
21-25	617	35	5.7	3	0.5	0	0	39	6.3
26-30	210	11	5.2	1	0.5	0	0	12	5.7
31-39	116	4	3.5	1	0.9	0	0	5	4.3
Total	5209	367	7.1	22	0.4	2	0.04	391	7.5
Bud positions grouped according to pre- (basal) and post-irradiation (upper)									
6-10	1551	144	9.6	5	0.3	1	0.06	150	9.7a
11-39	3658	223	6.1	17	0.5	1	0.03	241	6.6b

a, b Significantly different at p=0.01 using Chi square test

Table 13. Frequencies of mutations in B (budded) and C (cutback) V2 populations of 'Bing' for the various treatments, 1984.

Population	V2 trees No.	Partial mutant No.	%	Total mutant No.	%	Growth-reduced No.	%	All mutants No.	%
C	1275	80	6.3a	15	1.2a	1	0.08	96	7.8a
B	1667	58	3.5b	53	3.2b	9	0.54	120	7.2a
Total	2942	138	4.7	68	2.3	10	0.34	216	7.3

a, b Significantly different within column and different rows at p=0.01 using Chi Square test

Table 14. Comparison of height (H), diameter (D), and number of nodes (N) in a 25cm midshoot section for potential growth-reduced mutants in V2 (1984) and V3 (1985) of 'Bing'

Tree	V2[a] H cm (%)[c]	D cm	N	H/D/N (%)[c]	V3[b] H cm (%)[c]	D cm	N	H/D/N (%)[c]
Control								
Mean	167 (100)	1.1	11	13.8 (100)	194 (100)	1.4	12	11.5 (100)
Std. dev.	23	0.2	1	1.7	13	0.1	1	1.4
Growth-reduced								
16-58	71 (43)	1.2	12	4.9 (36)	124 (64)	1.2	12	8.6 (75)
31-137	110 (66)	1.1	10	10.0 (72)	58 (30)	0.8	17	4.3 (37)
33-41	112 (67)	0.9	17	7.3 (53)	81 (42)	0.9	13	6.9 (60)
30-18	114 (68)	1.0	14	8.1 (59)	83 (43)	0.8	13	8.0 (70)
23-105	120 (72)	1.1	14	7.8 (57)	99 (51)	1.1	14	6.4 (56)
32-19	134 (80)	1.3	14	7.4 (54)	139 (72)	1.6	11	7.9 (69)
22-133	142 (85)	1.4	17	6.0 (43)	160 (82)	1.3	13	9.5 (83)
24-121	148 (89)	1.1	16	8.4 (61)	163 (84)	1.3	14	9.0 (78)
31-111	154 (92)	1.4	13	8.5 (62)	178 (92)	1.3	12	11.4 (99)
14-22	-	-	-	-	148 (76)	1.2	15	8.2 (71)
44-108	-	-	-	-	80 (41)	0.9	19	4.7 (41)

[a] budded on Mazzard seedling rootstocks
[b] budded on Colt rootstocks; data based on 3-16 surviving V3 trees
[c] % of control

Table 15. Mutation frequencies of partial and total leaf mutants, and growth-reduced mutants in 'Bing' (B and C populations combined) from the various irradiation techniques and doses.

Dose (kR)	No. of V1 shoots	No. of V2 shoots	Partial mutants No.	%	Total mutants No.	%	Growth-reduced No.	%	All mutants No.	%
In air										
Acute										
3	26	240	10	4.2	12	5.0	2	0.83	24	10.0
4	14	138	4	2.9	4	2.9	0	0	8	5.8
Fractionated										
4	16	200	20	10.0	3	1.5	0	0	23	11.5
5	12	121	12	9.9	3	2.5	1	0.83	16	13.2
6	9	122	5	4.1	1	0.8	0	0	6	4.9
7	2	49	0	0	1	2.0	0	0	1	2.0
Subtot.	79	870	51	5.9	24	2.8	3	0.34	78	9.0a

Table 15 Continued

In water										
Acute										
4.5	23	462	19	4.1	8	1.7	2	0.43	29	6.3
5.5	19	311	12	3.9	7	2.3	1	0.32	20	6.4
6.5	7	62	1	1.6	2	3.2	1	1.6	4	6.5
Fractionated										
4.5	13	257	15	5.8	3	1.2	1	0.39	19	7.4
5.5	13	322	24	7.5	7	2.2	1	0.31	32	9.9
6.5	16	298	7	2.4	9	3.0	0	0	14	4.7
7.5	15	247	8	3.2	7	2.8	1	0.40	16	6.5
Subtot.	106	1959	86	4.4	43	2.2	7	0.36	136	6.9a
Subtotal										
Acute	89	1213	46	3.8a	33	2.7a	6	0.49	85	7.1a
Frac.	96	1616	91	5.6a	34	2.1a	4	0.25	127	8.0a

a Similar letters within column and different rows indicate non- significant difference at p=0.01 using Chi Square test

Table 16. The frequency of mutations in 'Bing' V2 trees as related to bud positions on V1 shoots (all treatments).

Population	V1 bud position	No. of trees	Partial mutants		Total mutants		Growth-reduced		All mutants	
			No.	%	No.	%	No.	%	No.	%
C	2-6	1275	80	6.3	15	1.2	1	0.08	96	7.5
B	7-10	415	20	4.8	12	2.9	1	0.24	33	7.6
	11-15	501	20	4.0	14	2.8	2	0.40	36	7.2
	16-20	387	10	2.6	16	4.1	2	0.52	28	7.2
	21-25	223	6	2.7	7	3.1	3	1.35	16	7.2
	26-30	88	1	1.1	2	2.3	1	1.14	4	4.5
	31-39	53	1	1.9	2	3.8	0	0	3	5.7
Total		2942	138	4.7	68	2.3	10	0.34	216	7.3
Bud positions grouped according to basal and upper, or post-irradiation origin										
	2-10	1690	100	5.9	27	1.6	2	0.12	129	7.6a
	11-39	1252	38	3.0	41	3.3	8	0.6	87	6.9a

a Similar letters indicate non-significant difference at p=0.05 using Chi Square test

Chapter 5

RADIATION-INDUCED MUTATIONS FROM ACCESSORY BUDS OF SWEET CHERRY, *PRUNUS AVIUM* L.

Summary. Dormant scions of 'Bing' were exposed to 1-2.5kR gamma radiation. The main buds were excised and the scions grafted to allow the growth of accessory buds into primary shoots. The frequency and types of mutations on V2 populations are described. In a population of 3307 V2 shoots, the overall mutation frequency was 2.7%: 0.15% growth-reduced mutants were identified. The experiment was repeated using 3kR and 4kR fractionated doses in water. Differences in mutation frequency at 3kR and 4kR were not significant. Of 2765 surviving V2 shoots derived from irradiation of accessory buds of both standard and V1 shoots, the overall mutation frequency was 3.3%: 1.7% were partial leaf mutants, 1.0% were total leaf mutants, and 0.54% were growth-reduced mutants. For maximum mutation rate with adequate survival we suggest acute irradiation of accessory buds in air at dosages approximating LD50. A larger mutant sector was present in V1 shoots derived from accessory buds than those from main buds as revealed by the higher number of total mutant repeats in the families.

Key words. Accessory buds, fractionated, mutation, growth-reduced, mutant sector.

Radiation-induced mutations from accessory buds of sweet cherry ,*Prunus avium* L.

Introduction

Irradiation of a multicellular and histogenically layered plant meristem (Pratt,1963,1967; Tilney-Bassett, 1963) commonly results in mericlinal or periclinal chimeras, which can take several years and special techniques to identify and isolate the mutant tissue (Lacey and Campbell,1977; 1982; Visser, 1973). Application of higher irradiation doses causes substantial cell elimination which reduces the number of cell initials and results in increased size of mutated sectors (Gaul, 1961). To circumvent this problem of chimerism, the use of adventitious buds in some vegetatively propagated ornamentals has led to the induction of a very high proportion of total mutants (Broertjes, 1966, 1982; Broertjes and Van Harten, 1978 ; Van Harten, 1982; Doorenbos and Karper, 1975). However, this technique is not applicable for many deciduous fruit trees due to the inability to force adventitious bud growth.

In sweet cherry, *Prunus avium* L., recovery of total plant mutants in V_2 trees following irradiation of main buds is low, about 2-4% (Lapins, 1971; Thompson, 1979). By contrast, in apple, the frequency of total mutants in the V_2 can be as high as 15% (Lapins, 1965). Moreover, in apple preselection for compact mutants has been successful in the V_1 generation (Visser et al., 1971; Lacey and Campbell, 1979) which indicates that the entire apex in the V_1 shoot is mutant. By contrast, in cherry mutations occur sporadically on the

V1 shoot and preselection for compact mutants is not possible (Thompson, 1979). Accessory buds of cherry are in various stages of development and have fewer meristematic cells than the main lateral buds (Katagiri and Lapins, 1974). They can be readily induced to produce shoots by removing apical dominance of the main buds (Saamin and Thompson, 1986a). Accessory buds, in comparison to the main buds, resulted in larger mutant sectors in V1 and recovery of a higher proportion of total mutants in V2 (Lapins, 1971). In pear, a higher frequency of mutations was observed from irradiation of accessory buds (Nishida, 1973).

This irradiation experiment on accessory buds of 'Bing' cherry has three main objectives, viz: I) to increase the size of mutant sectors and thereby improve the efficiency of isolation of total mutants, particularly of the growth-reduced type; II) to compare the effects of irradiation dosages in air or in water on mutation frequency; and III) to study the distribution of mutations in relation to bud positions on the V1 shoot.

Materials and Methods

In 1983, dormant scionwood of 'Bing' was obtained from a commercial nursery in mid-February and stored at 0°C until irradiation on April 16th. The basal portions of 26cm scions (25 or 50 scions per treatment) were placed in the low-flux $^{Co}60$ irradiation chamber and exposed to 1-2.5kR acute or fractionated gamma-rays (dose rate of 145R/min) in air or in water. Following irradiation all main buds below the top 13cm of each scion (the top portion was used for a main bud irradiation study) were excised leaving the accessory buds intact. Lanolin was applied on the cut surfaces and the scions were grafted

in the nursery. In mid-September buds from vigorous V1 shoots were patch or T-budded, 2 to a rootstock. Because the basal 5-6 buds on the cutback V1 shoot were too congested to bud individually they were allowed to grow and produce V2 shoots "in place". Leaf and growth-reduced mutants of budded (B) and cutback (C) V2 trees were identified in July 1984. Two types of growth-reduced mutants were identified; compact growth habit (a noticeably shorter tree, thicker stem diameterfor its height, and shorter internodes than normal) and non-compact shorter plants that grew more slowly and/or stopped growth early are the two types of growth-reduced mutants identified. Plant height, diameter at mid-point, and the number of nodes in the 25cm mid-shoot section of potential growth-reduced mutants were recorded in early September. Growth-reduced mutants were then budded onto seedling rootstocks, 10 buddings/selection for verification in V3 the following summer. Height, diameter, and number of nodes of V3 trees were recorded when trees were dormant.

For accessory bud irradiation in 1984, dormant scionwood of 'Bing' was obtained from a commercial nursery in late January. Also, V1 shoots derived from both irradiated main and accessory buds of 'Bing' were collected in late February. The scions were held at 0°C until irradiation on April 20th, using 50 scions per treatment with 3kR or 4kR fractionated doses in water, and 3kR in air. Dose fractionation consisted of 1-1.5kR exposures at 12hr intervals. All main buds were excised and both treated and control scions were then grafted in the field. Because of the delayed growth of V1 shoots they were too immature to bud in the fall. Thus, they were grafted in early April the next year using 2 to 4 -bud scions. The 3 basal buds were too congested and not propagatable. Growth and leaf mutations on V2 trees were observed in July-August. Plant height, diameter, and number of nodes of potential

growth-reduced mutants and of control trees were recorded in early September. Each growth-reduced mutant was propagated onto 15 seedling rootstocks (5 buddings/selection replicated 3 times) for verification in V3. Height measurements were done in mid-July.

The Chi Square test and Statistical Packages for Social Sciences (SPSSX, 1983) were used to compare the various treatments.

Results

Irradiation of 'Bing' accessory buds, 1983

Of 1117 V2 shoots in the cutback (C) population, the overall mutation frequency was 4.8%: 42 (3.4%) were partial leaf mutants, 11 (1.0%) were total leaf mutants, and 1 (0.09%) was a growth-reduced mutant (Table I). As compared to exposures in water, exposures in air had markedly higher frequencies of overall mutations, partial leaf mutants, total leaf mutants, and also a higher proportion of all mutants that were totals. The spectrum of morphological leaf aberrations in partial and total leaf mutants was similar to that observed in main bud irradiation (Saamin and Thompson, 1986b). An average of four V2 shoots grew on stumps left after cutting the V1's used for repropagation.

Among 2207 surviving budded (B) V2 trees, the overall mutation frequency was 1.6%: 17 (0.8%) were partial leaf mutants, 14 (0.6%) were total leaf mutants, and 4 (0.18%) were growth-reduced mutants (Table I). As in the cutback population, there was a higher frequency of mutations for exposures in air than in water. The average frequencies of mutations for acute (1.2%) and fractionated (1.9%) dosages were not significantly

different. The overall mutation frequency was 2.7% for B and C populations combined (Table II). The frequencies of overall mutations, partial leaf mutants, and total leaf mutants were higher in the basal 2 groups of bud positions (2-6 and 7-10) than at upper bud positions. However, there was a striking difference in the relative proportion of partial leaf mutants and total leaf mutants in the B and C populations. In B 51% of all mutants identified were total leaf mutants whereas in C only 22% were total leaf mutants. Of the 138 budded V2 families, 28 (20%) had an average of 1.2 mutant[1] trees/family and 6 (4%) of the families had similar mutant repeats of 2-9 trees. Since the average bud survival for all treatments was only 55% the number of mutant trees/family was under-represented.

Among 3307 V2 shoots, initially 5 plants were identified as potentially growth-reduced with heights 52-75%, and H/D/N ratios 45-85% that of control. As the V3 heights ranged from 26-50% of control and the H/D/N ratios were 20-47% of control they were very compact (Table III). Selection 58-16 appears to be the most promising because in both V2 and V3 generations it was 50% of control height and about 45% H/D/N. V3 plants of the other selections were considerably more compact than the V2's, ranging from 26-34% H/D/N of control.

Irradiation of accessory buds of 'Bing', 1984

Since differences in mutation frequencies at 3kR and 4kR were not significant the two populations were combined. Of 2765 surviving V2 shoots derived from irradiation of accessory buds of V1 shoots, the overall mutation frequency was 3.3%: 1.7% were partial

[1] includes partial and total leaf mutants, and growth-reduced mutants

leaf mutants, 1.0% were total leaf mutants, and 0.54% were growth-reduced mutants. In this budded population, the proportion of all mutants that were total including growth-reduced mutants was 47%.

Two (13%) of the 15 growth-reduced mutants had been preselected in V1. The V2 growth-reduced mutants had heights 36-98% and H/D/N ratios 31-99% that of control. The V3's were 34-95% of control height and 7 mutants were less than 50%. In the V3 the growth-reduced mutants also showed marked variation in other traits such branching habit, erectness of growth, leaf color, thickness and size.

To determine the frequency of mutations in relation to bud position on V1 shoots, the V2 trees were grouped into six 3-bud groups (Table IV). The frequencies of partial leaf mutants, total leaf mutants, and overall mutations were higher for the 2 basal bud groups (7-15) than for bud groups at upper bud positions (19-45). With a few exceptions, with increasing bud positions on the V1 shoots the frequency of mutants decreased. Although the highest frequency of total mutants (including growth-reduced) was at bud positions 7-9, there was no decrease from the 13th to the 33rd position.

The size of mutant sector isolated is indicated by the proportion of mutant trees in each V2 family. In 1 (0.45%) of 222 V2 families, all 12 of the V2 plants were growth-reduced. Twenty-eight (12.6%) of the families had 2 or more total leaf mutant members, whereas 10 (4.5%) families had only 1 total leaf mutant. Eleven (5.0%) of the families had 2 or more partial leaf mutants and 25 (11.3%) families had 1 partial leaf mutant. Bud survival ranged from 50-90% depending on the vigor of the scions and the dosage. Bud survival affect the number of mutants/family.

Discussion

The lower LD_{50} of accessory buds as compared to main buds indicates that they are are less tolerant to irradiation (Saamin and Thompson, 1986a). Katagiri and Lapins (1974) also made a similar conclusion. This higher radiosensitivity is due to their less developed meristems. According to Lapins (1971) and Katagiri and Lapins (1974) 30% of the accessory buds had partially developed meristems or bud initials. Larger cell population, greater apical size during recovery, and faster rate of growth increase the radiotolerance of irradiated apices (Langenauer et al., 1973). As a consequence of the necessity of lower irradiation dosages, the mutation rate was lower in V_2's derived from accessory buds than in those from main buds (Saamin and Thompson, 1986b). High irradiation doses, within limits, increase mutation frequency in fruit trees (Nybom, 1970; Thompson, 1979; Zagaja et al., 1982; Lacey and Campbell, 1982) and other plant species (Wolff, 1960; Sparrow et al., 1961; Nishiyama et al., 1964; Leenhouts and Chadwick, 1974).

Although water and dose fractionation increase tolerance of meristematic tissues, as indicated by 50-60% survival of V_1 shoots following 4kR irradiation of accessory buds (Saamin and Thompson, 1986a), these treatments buffer against mutations. Sparrow et al. (1961) also reported that the yield of somatic mutations was reduced in many species by dose fractionation of acute exposures. Although the mutation frequency following irradiation in air at $LD \leq 25$ (1-1.5kR) was slightly lower than that of irradiation in water at $LD \geq 50$ (3-4kR), the proportion of total leaf mutants in the former was higher (61%) than in the

latter (47%). In main buds, fractionated irradiation in air at LD_{80} gave the highest mutation frequency (13.2%) in V_2's (Saamin and Thompson, 1986b). Thompson (1979) reported that the mutation rate at LD_{50} acute irradiation in air was 11.4%. As there was evidence of a very sharp decline in radiotolerance of accessory buds at dosages above LD_{50}, we suggest acute irradiation in air at $LD \leq 50$ (2.75-3kR). This may increase the mutation rates somewhat higher than those we obtained at LD_{25}. And irradiation at higher LD's (LD_{50}-LD_{80}) for main buds may increase the mutation rates in V_2 shoots derived from main buds.

In V_1 shoots derived from accessory buds, a higher frequency of leaf aberrations was also observed for the basal bud positions (Saamin and Thompson, 1986a). Whereas there were 33% leaf aberrations at the basal bud positions on the V_1, the frequency of mutations in V_2's derived from these basal buds was 4.5%. Thus, only 14% of the aberrant leaves in V_1 were associated with mutations in their corresponding axillary buds. Descendents of mutant meristematic cells in the less developed accessory buds contributed to a larger mutant sector along the basal portion of the V_1 shoot than did those in the more developed meristems of main buds. Thus, to improve recovery of total leaf mutants, including growth-reduced mutants, propagate buds 2-33 on V_1 shoots derived from accessory buds.

The variation in compactness among the growth-reduced mutants indicates that the trait is controlled by more than one gene. Changes in other traits commonly associated with compact growth such as thicker leaves could be due to epistatic or pleiotropic effects.

The average proportion of all mutants that were total including growth-reduced mutants was about the same in V_2's from the B populations of accessory shoots (49%) as in the B population of V_2's from main shoots (52%)(Saamin and Thompson, 1986b). Lapins

(1971) reported a similar proportion of total mutants (53%) from irradiation of accessory buds but a lower proportion (35%) from main buds. This discrepancy can be explained by the fact that Lapins propagated only buds 6-15 on the V1 shoots derived from main buds whereas we propagated all usable buds, up to position 45 and found that the frequency of total mutants increased with higher buds.

The size of the mutant sector in V1 can be inferred from the number and distribution of repeats, or runs of the same mutation within V2 families. The proportion of V2 families derived from accessory buds which had runs of total leaf mutants was13.3%, seven times higher than the proportion in families derived from main buds (Saamin and Thompson, 1986b). In one case, all the V2 plants in a family were growth-reduced mutants which indicated that they originated from either a periclinal chimera or a totally mutant shoot apex. Also, the proportion of families with 2 or more total mutants was double that of families with single partial leaf mutants. This further strengthened the hyphothesis that a larger mutant sector size was present in V1 shoots derived from accessory buds than in those from main buds. One important advantage of accessory buds is that identification and verification of total leaf mutants and growth-reduced mutants was much easier when they occurred in runs than when they occurred as single plants.

In summary, we conclude that: 1) Results indicate that acute irradiation of accessory buds in air at dosages approximating LD_{50} (2.75-3kR) may increase mutation rate with adequate survival; 2) A larger mutant sector can be induced in the meristem by irradiation of dormant accessory buds rather than main buds; 3) Recovery of growth-reduced mutants can be improved by propagation of buds 2-33 on the V1 shoots derived from irradiated accessory buds.

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Table 17. Frequencies of mutations in cutback (C) and budded (B) V2 shoots derived from accessory buds of 'Bing' irradiated in 1983

Irradiation methods/Dose (kR)	No. V2 trees		Partial mutants (%)		Total mutants (%)		Growth reduced mutants (%)		All mutants (%)	
	C	B	C	B	C	B	C	B	C	B
Control	37	140	0	0	0		0	0	0	0
In air (1-1.5)	635	934	4.7a	1.0a	1.57	1.1	0.16	0.43	6.5a	2.5a
In water (1-2.5)	482	1273	2.5a	0.6a	0.21	0.3	0	0	2.7b	0.9b
Total	1117	2207	3.4	0.8	1.0	0.6	0.09	0.18	4.8	1.6

a, b Significantly different within column between rows at p=0.01 using Chi square test

Table 18. The frequency of mutations in 'Bing' V2 shoots derived from irradiated accessory buds as related to bud position on V1 shoots (1983 irradiation)

Population	V1 bud position	No. V2 trees	Partial mutants No. (%)	Total mutants No. (%)	Growth-reduced No. (%)	All mutants No. (%)
C	2-6	1117	42 (3.4)	11 (0.98)	1 (0.09)	54 (4.8)
B	7-10	477	8 (1.7)	8 (1.7)	0	16 (3.4)
	11-15	587	4 (0.7)	1 (0.2)	1 (0.17)	6 (1.0)
	16-20	515	5 (1.0)	2 (0.4)	1 (0.19)	8 (1.6)
	21-25	363	0	3 (0.8)	1 (0.28)	4 (1.1)
	26-30	177	0	0	1 (0.56)	1 (0.56)
	31-39	71	0	0	0	0
	Total	3307	59 (1.8)	25 (0.8)	5 (0.15)	89 (2.7)

Table 19. Comparison of height, diameter, number of nodes at 25cm midsection of stem of V2 potential growth-reduced 'Bing' mutants selected in 1984 and their V3 generation (1985)

Tree	V2 Height (cm)/(%)	Diameter (mm)	No. of nodes	H/D/N (%)	V3 Height (cm)/(%)	Diameter (mm)	No. of nodes	H/D/N %
Control								
Mean	170 (100)	1.1	11	14.4 (100)	194 (100)	1.4	12	11.5 (100
Std. dev.	22	0.1	1	1.1	13	0.1	1	1.4
Growth-reduced								
55-18	128 (75)	1.0	11	12.2 (85)	57 (29)	0.9	21	3.0 (26)
55-21	110 (65)	0.9	14	9.1 (63)	51 (26)	0.7	20	3.6 (31)
56-70	115 (68)	1.1	9	11.9 (83)	58 (30)	1.0	25	2.3 (20)
58-16	88 (52)	0.8	17	6.5 (45)	91 (50)	1.0	17	5.4 (47)
42-52	-	-	-	-	78 (40)	0.9	22	3.9 (34)

Table 20. The frequency of mutations in V2 trees of 'Bing' from irradiated accessory as related to bud position on V1 shoots (1984 irradiation)

V1 bud position	No. of V2 trees	Partial mutants No. (%)	Total mutants No. (%)	Growth-reduced No. (%)	All mutants No. (%)
7-9	587	12 (2.0)	8 (1.4)	5 (0.85)	25 (4.3)
13-15	631	15 (2.4)	7 (1.1)	2 (0.31)	24 (3.8)
19-21	593	8 (1.3)	5 (0.8)	3 (0.50)	16 (2.7)
25-27	475	8 (1.7)	4 (0.8)	3 (0.63)	15 (3.2)
31-33	298	3 (1.0)	3 (1.0)	2 (0.67)	8 (2.7)
37-45	181	2 (1.1)	1 (0.6)	0	3(1.7)
Total	2765	48 (1.7)	28 (1.0)	15 (0.54)	91 (3.3)

Chapter 6

GENERAL CONCLUSIONS

Our conclusions from these experiments on gamma-irradiated main and accessory buds of sweet cherry cvs Napoleon and Bing are:

Expt. I. Comparative radiosensitivities of main and accessory buds of sweet cherry (*Prunus avium* L.) with various irradiation methods

1. Preliminary irradiation of buds and forcing them in the glasshouse provides reliable information on appropriate range of dosages for use in a large scale field experiment.

2. Accessory bud forcing is greater in the basal (older) part of vigorous scions.

3. As a consequence the LD_{50} for accessory buds is also greatly influenced by the vigor or age of buds. For basal buds from vigorous scions the LD_{50}'s were approximately 1.5kR lower than that of main buds on vigorous scions. 2.5-3kR irradiation in air are appropriate doses for accessory buds on vigorous scions.

4. Radiosensitivity of buds is affected by irradiation technique. Fractionation of doses resulted in higher LD_{50}'s (by $\geq$1kR) than acute doses. The same effect is shown by irradiation of buds in water versus air.

5. Irradiation of accessory buds results in slightly larger mutant sectors than irradiation of main buds evidenced by the occurrence of 0.74% total shoot mutants in accessory buds versus none in main bud shoots, and by the greater number of aberrant leaves on shoots showing such aberrancies.

Expt. II. Methodology for radiation-induced mutations in main lateral buds in *Prunus avium* L. cvs Napoleon and Bing

1. The highest frequency of total leaf mutants in 'Bing' was acute 3kR irradiation in air; whereas in 'Napoleon' it was 4.5kR acute irradiation in air. In both cultivars, although they tolerated higher doses with water and fractionation there was a higher frequency of total leaf mutants with acute irradiation in air.
2. Irradiation in water with both cultivars led to slight decrease in overall mutation frequency even with higher doses, but especially a decreased frequency of total leaf mutants and increased proportion of partial leaf mutants.
3. In both 'Napoleon'and 'Bing' higher doses not only decreased survival but also tend to decrease mutation frequency.
4. Fractionation of doses in 'Bing' resulted in slightly higher overall mutation frequency but also a higher frequency of partial leaf mutants and lower frequency of total leaf mutants.
5. The frequency of growth-reduced mutants in 'Bing' (0.34%) was higher than in 'Napoleon' (0.14%).
6. For efficient recovery of growth-reduced mutants we recommend propagating only the upper buds (above position 10) on V1 shoots of cherry. Basal buds have higher overall mutation frequency but lower frequency of total leaf mutants.

Expt. III. Radiation-induced mutations from accessory buds of sweet cherry, *Prunus avium* L.

1. Results indicate that acute irradiation of accessory buds in air at dosages approximating

LD_{50} (2.75-3kR) may increase mutation rate with adequate survival.

2. A large mutant sector can be induced in the meristem by irradiation of dormant accessory buds rather than main buds.

3. Recovery of growth-reduced mutants can be improved by propagation of buds 2-33 on the V_1 shoots derived from irradiated accessory buds.

Expt. IV. Radiation damage and recovery in shoot apices of sweet cherry (*Prunus avium* L.)

1. Ionizing radiation inhibited scattered as well as localised cells in shoot apices of sweet cherry.

2. There was evidence of a radiosensitivity gradient in the shoot apex

3. Recovery from irradiation damage could be via one or more of the following modes : a) recovery by the flank meristem which formed a substitute meristem, b) the damaged cells of the apical meristem were crushed by the growth of adjacent cells and the healed meristem resumed growth, c) the apical meristem partially recovered but growth resumed via the leaf primordia/leaf foliages and axillary meristems.

4. Irradiation disturbed the integrity of the tunica layers.

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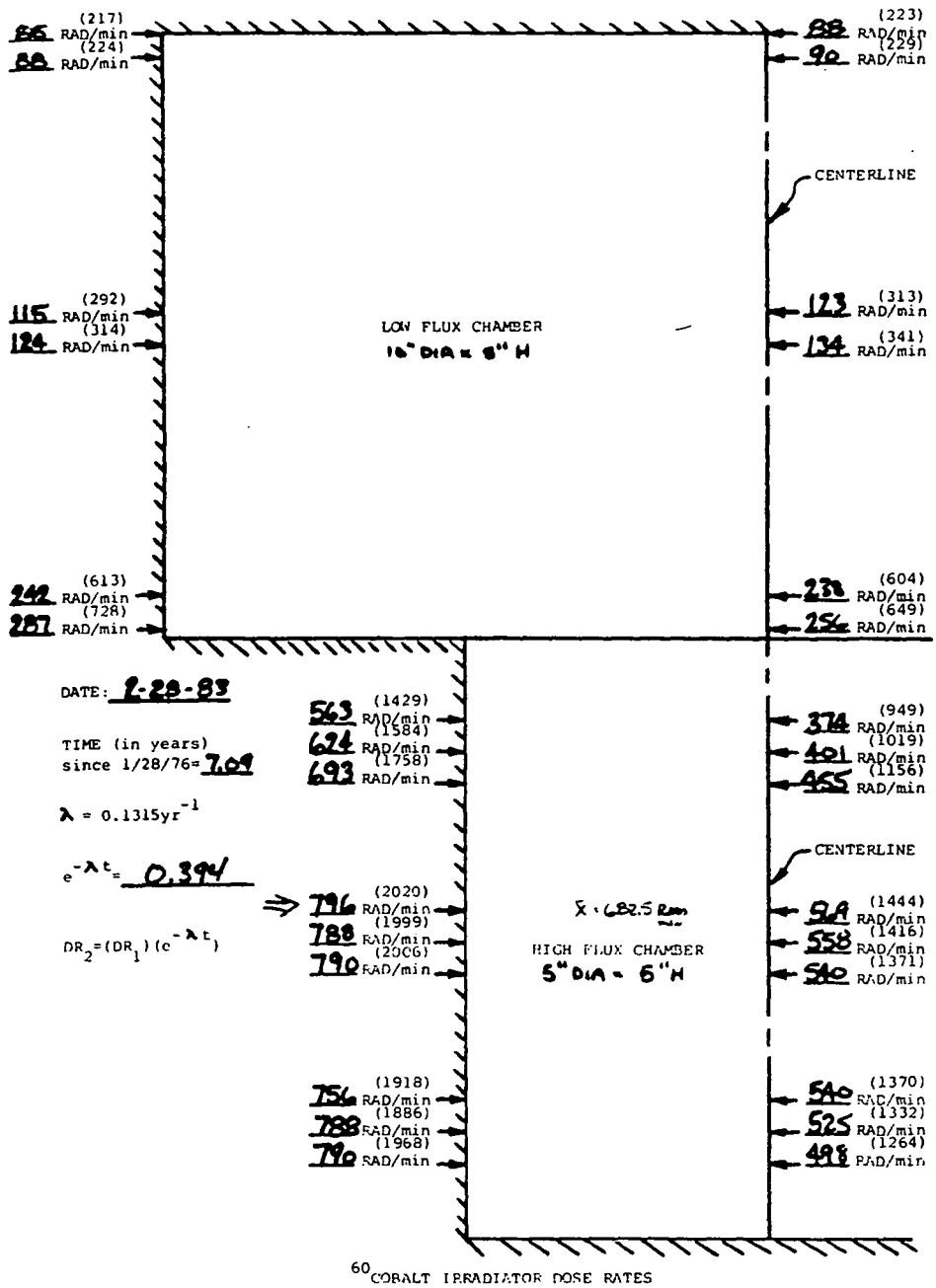
APPENDIX

Appendix 1. ^{60}Cobalt Irradiator Dose Rates



Appendix II. Irradiation methods and dose levels on dormant main buds of 'Napoleon' water-forced in the greenhouse, Mar. 4-Apr. 3, 1983

Irradiation method		Dose (kR)
Control		0
In air	Fractionated	2+2 (4)
		2+2+1 (5)
		2+2+2 (6)
		2+2+2+2 (8)
	Acute	4, 6, 8
In water	Fractionated	2+2 (4)
		2+2+1 (5)
		2+2+2 (6)
		2+2+2+2 (8)
		2+2+2+2+2 (10)
		2+2+2+2+2+2 (12)
	Acute	4, 5, 6, 8, 10, 12

Appendix III. Bud survival and growth and of 'Bing' V1 shoots derived from accessory bud irradiation: nursery experiment (1983).

Irradiation methods/(kR)	No. of scions	Potential bud sites	No. buds forced	Buds/site No. (%)[a]	No. V1 shoots	Av. length (cm) (%)[b]	AL[c] No. (%)	B[d] %	R[e] %
B. Grafted ('Bing')									
Control (0)					19	81 (100)	0	0	5
In air									
Acute (1-1.5)	134	530	119	0.22 (56)	110	53 (65)	29 (26)	5	15
Fractionated (1-1.5)	139	536	165	0.31 (79)	141	58 (72)	38 (27)	5	21
Subtotal	273	1066	284	0.27 (68)	251	56 (69)	67 (27)	5	19
In water									
Acute (1-2)	139	566	91	0.16 (41)	62	66 (81)	10 (16)	2	33
Fractionated (1-2.5)	164	734	186	0.25 (64)	110	64 (79)	12 (11)	6	43
Subtotal	303	1300	277	0.21 (55)	172	65 (80)	22 (13)	5	40
Subtotal									
Acute	273	1096	210	0.19 (49)	172	60 (74)	39 (23)	4	23
Fractionated	303	1270	351	0.28 (71)	251	61 (75)	50 (20)	6	33

a % of control; b % of control
c c aberrant leaves; d bifurcations; e rosettes; % based on total of rosettes and V1 shoots

Appendix IV. The number of aberrant leaves on 'Napoleon' V1 shoots derived from main bud irradiation (1983) and 'Bing' V1 shoots derived from accessory bud irradiation (1983 and 1984).

No. of aberrant leaves	1983 'Napoleon' No.	%	1983 'Bing' No.	%	1984 'Bing' No.	%	'Bing' 2yr total No.	%
0	190	46.5	333	78.7	705	87.1	1038	84.3
1	13	3.2	7	1.7	20	2.5	27	2.2
2	73	17.9	36	8.5	21	2.6	57	4.6
3	50	12.2	22	5.2	22	2.7	44	3.6
4	32	7.8	10	2.4	13	1.6	23	1.9
5	28	6.9	6	1.4	10	1.2	16	1.3
6	12	2.9	4	1.0	8	1.0	12	1.0
7	6	1.5	2	0.5	5	0.6	7	0.6
8	0	-	2	0.5	3	0.4	5	0.4
9	1	0.2	0	-	2	0.3	2	0.2
10	2	0.5	0	-	6	0.7	6	0.5
11	1	0.2	0	-	2	0.3	2	0.2
13	0	-	0	-	2	0.3	2	0.2
14	1	0.2	1	0.2	3	0.4	4	0.3
15-30	0	-	1	0.2	4	0.5	5	0.4
All (total mutant)	0	-	1	0.2	4	0.5	5	0.4
	409		423		809		1232	

Appendix V. Distribution of aberrant leaves on bud positions 11-40 on 'Bing' V1 shoots derived from accessory bud irradiation (fractionated 3kR and 4kR in water), 1984.

Bud position on V1 shoot	Aberrant leaves on 66 V1 shoots No. (%)
11-15	88 (33)
16-20	75 (28)
21-25	46 (17)
26-30	30 (11)
31-35	19 (7)
36-40	12 (4)
Total	270

Appendix VI. Frequency of repeats of similar aberrant leaves on 'Bing' V1 shoots derived from accessory bud irradiation, 1984

No. of similar aberrant leaves	Runs No.	%	No. of V1 shoots
2	46	68	32
3	9	13	7
4	7	10	6
5	2	3	2
6	1	1	1
7	2	3	2
9	1	1	1
	68		51

Appendix VII. Irradiation treatments of dormant 'Bing' scions, mid-April, 1983

Irradiation technique		Dose (kR)
A. In water	Fractionated	2+2.5 (4.5) 2+2+1.5 (5.5) 2+2+2.5 (6.5) 2+2+2+1.5 (7.5) 2+2+2+2+2.5 (10.5)
	Acute	4.5, 5.5, 6.5, 8.5
B. In air	Fractionated	2+2 (4) 2+2+1 (5) 2+2+2 (6) 2+2+2+1 (7)
	Acute	3, 4, 5, 6

Appendix VIII. The frequency of partial and total leaf mutant trees in V2 families of 'Napoleon' following gamma irradiation

	No. of families	No. of mutant trees/family	No. of different types of mutants 1	2	3	4	5
	181	0					
	130	1	130				
	71	2	10	61			
	25	3	1	5	19		
	14	4	0	0	3	11	
	4	5	0	0	1	1	2
Total	425						

Appendix IX. The frequency and distribution of mutant trees in V2 families (B) of 'Bing' following 3-7.5kR gamma irradiation.

No. of V2 families	Mutant trees per family	No. of different types of mutants 1	2	3	4	5
101	0					
58	1	58				
15	2	3	12			
6	3	0	1	5		
2	4	0	0	1	1	
1	5	0	0	0	0	1
1	6	0	0	0	0	1
184						

Appendix X. Frequencies of mutations in cutback (C) and budded (B) V2 shoots derived from irradiated accessory buds of 'Bing', 1983

Irradiation methods/Dose (kR)	No. V2 trees		Partial mutants (%)		Total mutants (%)		Growth reduced mutants (%)		All mutants (%)	
	C	B	C	B	C	B	C	B	C	B
Control	37	140	0	0	0		0	0	0	0
In air										
Acute (1-1.5)	262	425	4.6	0.5	0.76	1.1	0	0.23	5.3	1.8
Fractionated										
(1-1.5)	373	509	4.8	1.4	2.14	1.0	0.27	0.59	7.2	3.0
Total	635	934	4.7	1.0	1.57	1.1	0.16	0.43	6.5	2.5
In water										
Acute (1-2)	182	544	3.3	0.6	0	0.2	0	0	3.3	0.7
Fractionated										
(1-2.5)	300	729	2.0	0.7	0.33	0.4	0	0	2.33	1.1
Total	482	1273	2.5	0.6	0.21	0.3	0	0	2.7	0.9
Acute	444	969	4.1	0.5	0.45	0.6	0	0.10	4.5	1.2
Fractionated	673	1238	3.6	1.0	1.34	0.4	0.15	0.24	5.1	1.9
	1117	2207	3.4	0.8	0.98	0.6	0.09	0.18	4.8	1.6

Appendix XI. Frequencies of mutations in V2 trees 'Bing' and 'Napoleon' following 3-4kR gamma irradiation in water (except 3-A[a]) of accessory buds of standard and reirradiated scions, 1984

Scion	Dose (kR)	No. of V2 trees	Partial mutants No. (%)	Total mutants No. (%)	Growth-reduced No. (%)	All mutation: No. (%)
'Bing'						
Standard	Control (0)	72	0	0	0	0
	3-A[a]	203	4 (2.0)	2 (1.0)	0	6 (3.0)
	3	699	4 (0.6)	5 (0.7)	5 (0.72)	14 (2.0) b
	4	513	11 (2.1)	4 (0.8)	4(0.78)	19 (3.7) c
	Total	1415	19 (1.3)	11(0.8)	9(0.64)	39 (2.8)
Reirradiation (V1 scion)	Control	207	5 (2.4)	2 (1.0)	0	7 (3.4)
	3	754	24 (3.2)	13 (1.7)	6 (0.80)	43 (5.7)
	4	596	5 (0.8)	4 (0.7)	0	9 (1.5)
	Total	1350	29 (2.2)	17 (1.3)	6 (0.44)	52 (3.9)
Dosage	3kR	1656	32 (1.9) b	20 (1.2) b	11 (0.66) b	63 (3.8) b
	4kR	1109	16 (1.4) b	8 (0.7) b	4 (0.36) b	28 (2.5) b
	Total	2765	48 (1.7)	28 (1.0)	15 (0.54)	91 (3.3)
'Napoleon'						
Reirradiation (V2 scion)	Control	73	1 (1.4)	0	0	1 (1.4)
	3	780	31 (4.0)	8 (1.0)	3(0.38)	42 (5.4)
	4	644	17 (2.6)	3 (0.5)	4 (0.62)	24 (3.7)
	Total	1424	48 (3.4)	11 (0.8)	7 (0.49)	66 (4.6)

[a] acute irradiation in air

b, c Significantly different within column in different rows at p=0.05 using Chi Square test

Appendix XII. Frequencies of mutations in V2 trees of 'Bing' following 3kR and 4kR gamma irradiation in water of accessory buds, 1984

No. of V2 trees	Partial mutants No. (%)	Total mutants No. (%)	Growth-reduced No. (%)	All mutations No. (%
2765	48 (1.7)	28 (1.0)	15 (0.54)	91 (3.3)

Appendix XIII. The frequency and distribution of partial, total, and growth-reduced mutant trees in V2 families of 'Bing' and 'Napoleon' following gamma irradiation of accessory buds, 1984

	No. of families			No. of mutant	No. of mutant types[a]		
	'Bing' std.	'Bing' reirrad.(%)	'Napoleon' (%)	trees/family	1	2	3
	51 (62.2)	93 (64.5)	49 (54.4)	0	0		
	15 (18.3)	20 (13.9)	19 (21.1)	1	1		
	5 (6.1)	15 (10.4)	10 (11.1)	2	16	14	0
	8 (9.8)	6 (4.2)	6 (6.7)	3	14	3	4
	1 (1.2)	4 (2.8)	0	4	3	2	0
	0	1	2	5	2	1	0
	1	2	2	6	2	3	0
	0	2	0	7	2	0	0
	0	0	1	8	1	0	0
	1	0	0	9	1	0	0
	0	1	0	12	1	0	0
	0	0	1	21	1	0	0
Total	82	144	90				

Appendix XIV. Proportion of runs and mutant sector of total leaf mutants and growth-reduced mutants in V2 trees of 'Bing'

Scion	Dose (kR)	Proportion of mutant runs and mutant sectors					
		Total mutants runs			Growth-reduced runs		
		No.	%R[a]	%F[b]	No.	%R	%F
'Bing'							
Standard	3, 4	7	16.3	34.9	9	20.9	23.3
Reirradiation (V1 scion)	3, 4	9	16.1	37.2	7	12.5	31.5

a proportion of all mutants that were runs

b proportion of all trees in families that had mutant runs

Appendix XV. Number and proportion of V2 families with total mutants (includes growth-reduced) and partial leaf mutants

	Total mutants All V2 in family totals	≥ 2 tot. mutants per family	1 tot. mutant per family	Partial mutants 1 part. mutant per family	≥ 2 part. mutants per family
No. of families[a]	1	28	10	25	11
% of families	0.45	12.6	4.5	11.3	5.0

a Total number of families=222

Appendix XVI. Height, diameter, number of nodes at 25cm stem midsection of potential growth-reduced V2 trees of 'Bing' and 'Napoleon', 1984

Tree/ Treatment	Height (H) cm (%)	Diameter (D) cm	No. of nodes (N)	H/D/N (%)	Bud position on V1	V3 av. height (cm) (%)
Control (0kR)						
Bing						
Mean	118 (100)	0.8	13	11.3	-	74.2 (100)
Std. dev.	24	0.1	1			
Napoleon						82.5 (100)
Growth-reduced						
'Napoleon'; reirradiation						
1. 76-129d	48	0.7	14	4.9	13	21.3 (26)
76-129f	56	0.7	14	5.7	15	61.7 (75)
2. 76-130Ah	58	0.8	19	3.8	20	28.3 (34)
76-131k	71	0.9	17	4.6	29	42.4 (51)
3. 77-18l	109	0.9	10	12.1	30	60.5 (73)
4. 77-56a	107	0.8	9	14.9	7	61.2 (74)
5. 77-174k	73	0.7	17	6.1	30	32.7 (40)
77-177t	69	0.6	13	8.8	48	28.6 (39)
6. 77-235d	107	0.9	13	9.1	13	56.1 (68)
7. 78-179b	74	0.8	10	9.3	8	69.7 (84)

Appendix XVI Continued

'Bing'; reirradiation

1. 77-260c	90 (76)	0.7	13	8.8 (78)	20	52.7 (71)
2. 78-11a	57 (48)	0.6	14	6.3 (56)	7	48.5 (65)
78-11b	44 (37)	0.6	12	6.1 (54)	8	59.3 (80)
3. 78-11c	71 (60)	0.7	16	6.3 (56)	9	40.5 (55)
78-11d	72 (61)	0.8	17	5.3 (47)	10	33.6 (45)
78-11e	71 (60)	0.8	17	5.2 (46)	11	29.6 (40)
78-12f	68 (58)	0.8	18	4.7 (42)	15	42.7 (58)
78-12g	82 (69)	0.8	16	6.4 (57)	16	39.5 (53)
78-12h	84 (71)	0.9	16	5.8 (52)	17	38.4 (52)
78-12i	88 (75)	0.8	16	6.9 (61)	18	42.5 (57)
78-13j	69 (58)	0.8	18	4.8 (42)	22	41.4 (56)
78-13k	57 (48)	0.7	19	4.3 (38)	23	36.7 (49)
78-13m	51 (43)	0.7	18	4.0 (36)	25	65.1 (88)
4. 78-27f	94 (82)	0.7	11	11.2 (99)	26	64.4 (87)
5. 81-83a	97 (82)	0.8	17	7.1 (63)	7	49.1 (66)
81-84d	95 (81)	0.9	16	6.6 (58)	13	55.1 (74)
81-85i	102 (86)	1.0	16	6.4 (56)	21	59.1 (80)
81-86m	87 (74)	0.7	16	7.8 (69)	28	59.4 (80)
81-87o	100 (85)	0.8	16	7.8 (69)	34	52.2 (70)
81-87p	103 (87)	0.9	17	6.7 (60)	35	64.3 (87)
6. 81-196Bg	52 (44)	0.7	15	5.0 (44)	25	30.6 (41)
81-196Bh	56 (47)	0.7	16	5.0 (44)	26	27.9 (38)
81-196Bi	46 (39)	0.7	19	3.5 (31)	27	25.0 (34)

'Bing' standard

1. 79-104c	105 (89)	1.0	12	8.8 (77)	23	70.5 (95)
2. 79-119d	86 (73)	0.8	12	9.0 (79)	13	69.1 (93)
3. 79-248b	84 (71)	0.7	16	7.5 (66)	14	68.6 (92)
4. 80-26i	78 (66)	0.8	12	8.1 (72)	24	65.5 (88)
5. 80-245m	89 (75)	0.8	11	10.1 (89)	31	70.3 (95)
6. 81-14d	116 (98)	1.1	16	6.6 (58)	19	70.0 (94)
7. 81-18c	42 (36)	0.7	15	4.0 (35)	9	65.0 (88)
8. 81-253l	60 (50)	0.8	9	8.3 (74)	30	68.5 (92)
81-253m	62 (53)	0.7	9	9.8 (87)	31	33.2 (45)
9. 82-31Bb	116 (98)	0.8	16	9.1 (81)	8	50.6 (68)
82-31Bc	116 (98)	0.8	14	10.4 (92)	9	54.0 (73)
82-33Aj	114 (97)	0.8	15	9.5 (84)	22	53.4 (72)
82-33Bk	111 (94)	0.8	16	8.7 (77)	26	59.6 (80)
82-33Bl	115 (94)	0.9	14	9.1 (81)	27	63.9 (86)

Appendix XVII. Radiation damage and recovery in shoot apices of sweet cherry (*Prunus avium* L.)

Summary. Dormant scions of 'Bing' were exposed to fractionated 6kR gamma rays in air and then grafted. Irradiated and unirradiated main buds were sampled at 3 day intervals for one month. Buds were fixed in FAA, longitudinally sectioned, and stained with hematoxylin. Both random and localised cell damage were observed in irradiated apices. There was evidence of radiosensitivity gradient in the shoot apex. Recovery from irradiation damage was via flank meristem, central meristem, or leaf primordia and axillary meristems. The types of irradiation damage and modes of recovery were discussed in relation to shoot growth.

Key words. Gamma rays, damage, recovery, radiosensitivity, meristems.

Radiation damage and recovery in shoot apices of sweet cherry (*Prunus avium* L.)

Introduction

Manipulation of plant material for irradiation is dependent upon the growth pattern of the species concerned, and upon an understanding of the anatomy of the meristems irradiated and the ontogeny of the organs of interest (Hough, 1965). Studies on irradiation damage and regeneration of apical meristems provide further understanding on cellular organization that affect the recovery of mutations and their distribution in subsequent vegetative generations. Thus, appropriate irradiation technique and method of propagation can be used to reduce chimeras and to improve recovery of desired mutants. Patterns of irradiation damage in apical meristems of higher plants, fall into three categories; localized damage such as in apple (Pratt, 1963; Pratt et al., 1959), pear (Pratt, 1967), mulberry (Katagiri, 1973; 1976), *Capsicum annuum* L. (Iqbal, 1972), *Coleus blumei* (Crockett, 1968), *Picea glauca* (Moench) Voss (Cecich and Miksche, 1970), random damage as in sweet cherry, grape (Pratt, 1959; 1968), and *Parthenocissus tricuspidata* (Langenauer et al., 1973), or uniform as in wheat (Foard and Harber, 1961) and *Pinus lambertiana* (Fosket and Miksche, 1966).

Evidence of a radiosensitivity gradient within the shoot apex has been reported (Lawrence, 1968; Pratt, 1963; Cecich and Miksche, 1970; Iqbal, 1972; Pratt et al., 1959; Cordero and Gunckel, 1982). Features of radiation-damaged cells include darkly stained thickened cell walls, dense vacuolar inclusions, condensed nuclei, abscence of mitosis, and

collapsed cells (Pratt, 1959; 1968; Graham, 1972; Cordero and Gunckel, 1982).

Recovery from irradiation damage occurs in one or more ways; 1) direct healing of the shoot apex; 2) regeneration from the flank meristems; 3) axillary meristems taking over in shoot development (Pratt, 1963; Iqbal, 1970; Katagiri, 1976; 1973). The mode of recovery in irradiated shoot apices of main buds in sweet cherry has not been reported. This study is aimed at providing such information.

Materials and Methods

In 1984, dormant scionwood of 'Bing' was obtained from a commercial nursery in late January, stored at 0° until irradiation on May 1st to May 2nd. Ninety 26cm scions were placed with apical ends down in the radiation chamber and exposed to fractionated 6kR gamma rays in air at approximately 650rad/min. All irradiations were performed in the ^{60}Co facility of the Radiation Center, Oregon State University. Dose fractionation consisted of 2kR acute exposures at 12hr intervals. Both treated and control (unirradiated) scions were then grafted onto limbs of 2 year old Mazzard seedling rootstocks in the field within 2 days after irradiation. Six to nine 3-4 bud grafts from both irradiated and control were sampled starting from day zero (just before grafting) and at 3 day intervals for 1mo. Buds were excised and scales and outer developed leaves were trimmed off under a 10X dissecting scope. All buds were fixed in FAA, embedded in paraffin, sectioned longitudinally at 8μ, and stained in Delafield's hematoxylin (Johansen, 1940). A total of 163 growing buds were examined at 10 and 25 magnifications for cell damage and recovery. The width of the

apical dome, the number of recognizable histogenic layers, and the approximate number of cells in the LI layer (at median LS section) were recorded.

Results

Unirradiated shoot apices

Twenty two median longitudinal sections of unirradiated growing apices were examined under 25X magnification (Table I). Apices were typically dome-shaped with an average width of 0.56μ, 2-4 recognizable cell layers, and an average of 26 meristematic cells in the LI layer. Figure I shows the apical dome with a mound (leaf primordium). Cells were larger at the center of the dome and smaller at the leaf primodia or the axillary meristems of. Also, cells of the promeristem appeared to be morphologically similar. All the cells in LI and LII layers showed anticlinal divisions. Although the majority of the cells in LIII and deeper layers towards the corpus showed anticlinal divisions, periclinal divisions were also noted.

Irradiated shoot apices

A total of 141 median longitudinal sections of irradiated growing apices were examined under 25X magnification (Table II). The number of cell layers varied from 2-4, however, one apex with a single and one with 5 layers were observed during the recovery, 21 days after grafting. The average width of the apical dome was 0.76μ and the average number of meristematic cells in the LI layer was 28. Three periclinal cell divisions were observed in the LIII layer.

Irradiation damage to apical meristems was observed beginning day 3 after grafting (Table II). At day 3, 12 of 31 apices examined showed random cell damage in the histogenic layers, corpus and leaf primodia. Damaged cells were partially collapsed, had slightly thickened walls, and showed dark brown discoloration. Figure II shows an example of random cell damage in the shoot apex. Localised cell damage in the LI and LII histogenic layers, axillary meristems, corpus (in one apex), and leaf primordia was also observed in the apices. Localised cell damage was more frequent in the LI, and the first two cell layers in leaf primordia and axillary meristems. Usually part of the LI was affected and the damaged cells often extended to the adjacent mound or axillary meristem. Localised cell damage in the leaf primordium was more extensive than other affected apical regions. Nine apices showed no symptoms damage and 6 were doubtful cases.

Both random and localised cell damage became more evident 6 days after grafting. The damaged cells showed black discoloration and were more collapsed than before. Random cell damage in 7 of the apices was located in one or more of the following regions - in the histogenic layers, corpus, and leaf primordia. The domes of irradiated apices appeared to be more flattened and with slightly more irregular surfaces as compared to those of unirradiated ones. Of the 13 apices examined, 4 had 2 histogenic layers and the rest had 3 layers. The LI layer in two of the apices appeared to have sloughed off. Three of the irradiated apices showed no symptoms of damage.

At day 9, of 31 irradiated apices 13 showed random damage, 8 showed localised damage, 5 had no damage, and 5 were doubtful. The damaged cells were either fully collapsed and blackened (dead), highly vacoulated, or had discolored cytoplasm. There was a

larger number of random cells damaged in the LII and deeper histogenic layers and the corpus. Also, more collapsed cells were observed in the localised damaged areas and often a highly vacoulated cell layer was observed around the dead cells. Severely damaged leaf primordia adjacent to the dome were disintegrating and breaking off (Figure III). Both random and localised cell damage were observed in 5 apices. The domes of damaged apices were rather flattened. Collapse of damaged cells in the center of the original dome resulted in a two-dome feature observed in two of the apices (Figure IV).

Twelve days after grafting random or localised damage was observed in 14 out of 15 apices examined . The one exception was a doubtful case. Up to 4 layers of vacoulated or blackened, dead cells were observed in the localised damaged areas. In two of the apices, the central meristems were concave instead of dome-shaped because of the collapse and death of cells in the histogenic layers (Figure V). In these cases two mounds were observed adjacent to the depressed regions. Figure VI shows a layer of dead cells covering the shoot apex. In two cases dead cells covered more than half of the dome leaving part of the flank meristems undamaged (Figure VII). In apices with random cell damage the domes were flattened.

At 15 and 18 days after grafting the patterns of scattered and localised cell damage in irradiated apices were similar to those of day 12. Figure VIII shows a flattened apex with underlying dead cells due to irradiation damage.

By the end of the 3rd week after grafting buds that had extensive irradiation damage were dead and thus were not sampled. Buds that were growing showed areas of crushed dead cells at the surface or in the deeper cell layers of the apex. Recovery via both the flank and central meristems were observed. At day 24 , two of the apices examined had flattened

domes, 2 apices appeared normal, and 1 apex had dead cell remnants and encrustations on the surface layer. By day 27 the 3 apices examined had fully recovered from irradiation damage and cells were mitotically active (Figure IX).

Discussion

Both random and localised cell damage in the meristematic and elongating tissue of the apex of 'Bing' cherry were observed. Either one or both types of damage occurred on an apex. On the contrary, Pratt (1968) reported that ionizing radiation caused only random cell damage in the shoot apex of sweet cherry. Irradiated apices of lupine (*Lupinus albus* L.) showed both types of damage (Cordero and Gunckel, 1982).

There was evidence of radiosensitivity gradient in the shoot apex as indicated by the prevalence of localised cell damage in certain tissues. Radiosensitivity, in descending order was as follows: axillary meristem = leaf primordium > LI layer > deeper histogenic layers > corpus. The axillary meristems were highly radiosensitive because of fewer cells. The LI of two irradiated apices appear to be sloughed off because all cells were dead. During the recovery stage there was only one histogenic layer present in an apex. There was delay in appearance of irradiation-damaged cells in the corpus. This further indicates the higher sensitivity of the LI as compared to deeper meristematic tissue. Sagawa and Mehlquist (1957) reported in carnations that the first cell layer was most sensitive to radiation, and the same was observed in *Pinus rigida* (Bostrack and Sparrow, 1969). Pratt (1968) reported that the apical meristem was more inhibited than the axillary meristem but no

reference was made of a radiosensitivity gradient. A radiosensitivity gradient is more easily distinguished with localised type of cell damage than with random cell damage. Comparative radiosensitivity of different regions of the shoot apex varies with crops. In grapes, the LII layer was more susceptible to irradiation damage and the primordium initiating regions were more sensitive than mature apical tissues (Pratt, 1959). Vegetative shoot apices of irradiated *Coleus* shoots also showed more damage to the second tunica layer than to the first, and the deeper the layer or region within the meristem proper the greater the radiosensitivity (Crockett, 1968).

Recovery of the shoot apex from localised damage in the central meristem was evident 12 days after grafting. Mound (leaf primordia) became more pronounced in apices where the central zone became concave due to hypertrophied tunica cells. The flank meristem appeared to develop into a substitute dome albeit miniature if the localised damage covered more than one half of the apex. This also indicated that the central zone was more radiosensitive than the flank meristem, as similarly reported in *Capsicum annuum* L. (Iqbal, 1972), *Parthenocissus tricuspidata* (Langenaueret al., 1973), and mulberry (Katagiri, 1976). The formation of a substitute meristem by the cells lateral to the damaged apical meristem has also been described as a mode of recovery in irradiated apices of apple (Pratt, 1963; Lapins and Hough, 1970). Ball (1980) reported that regeneration from isolated portions of the shoot apex was greater from the flanks than from the centers.

The type of cell damage and recovery has a significant bearing on the distribution of mutations on V1 shoots. Random cell damage and in addition to constant shifting of meristematic cells may explain for the erratic distribution of mericlinal chimeras reported in cherry (Thompson, 1979; Saamin and Thompson, 1986a). In apples, where localised cell

damage occurs in the promeristem and recovery is from the flank meristem, isolation of periclinal chimeras on primary shoots is possible (Visser et al., 1971; Visser, 1973; Lacey and Campbell, 1977).

In less severely damaged apices the dome became flattened due to hypertrophied tunica cells. Resumption of growth of only the leaf primordia resulted in rosettes. Irradiation damage at the center of the dome resulted in the formation of a 2-dome apex which upon recovery may have developed into bifurcated/fasciated primary shoots.

The mode of recovery for random cell damage and localised cell damage in deeper meristematic tissue was by the growth of adjacent undamaged cells and resumption of growth of the central meristem, as was documented by Pratt in apple (1963).

Integrity of the LII layer was disturbed by the irradiation as indicated by the presence of periclinal cell divisions. Destruction of the outer apical layer could result in subsequent formation of a new meristem from the inner tissue. Disruption of histogenic layers causes the frequent changes in flower color in irradiated carnations (Sagawa and Mehlquist, 1957).

The acute irradiation dosage at 6KR in water was approximately at LD_{80} as indicated from a previous radiosensitivity study on dormant buds (Saamin and Thompson, 1986b). This explains the relatively few recovered apices.

Our conclusions from this experiment can be summarised as follows:

1. Ionizing radiation damaged scattered individual cells as well as localised groups of cells in shoot apices of sweet cherry;

2. There was evidence of a radiosensitivity gradient in the shoot apex.

3. Recovery from irradiation damage could be via one or more of the following modes:

a) recovery by the flank meristem which formed a substitue meristem, b) damaged cells of the apical meristem were crushed by the growth of adjacent undamaged cells and the healed meristem resumed growth, c) the apical meristem partially recovered but growth resumed via the leaf primordia and axillary meristems.

4. Irradiation disturbed the integrity of the tunica layers.

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Appendix

Table I. Features of longitudinal sections of unirradiated growing apices of 'Bing' at 25 magnifications

Day	No. of apices examined	No. of histogenic layers	Width of apical dome (μ)	No. of cells in LI
0	3	3-4	0.60	22
6	4	3-4	0.57	27
9	4	2-4	0.57	26
12	4	4	0.60	18
15	3	3	0.60	34
18	3	3	0.39	18
21	1	4	0.60	39

Appendix

Table II. Features, type of damage and mode of recovery of irradiated growing apices of 'Bing'

Day	No. of apices examined	No. of histogenic layers	Width of apical dome (μ)	No. of cells in LI	Type of damage and recovery
3	31	2-3	0.80	32	RD[a]=12; LD=4; ND=9; Doubtful=6
6	13	2-4	0.75	27	RD=7; LD=3; ND=3
9	31	2-4	0.90	34	RD=13; LD=8; ND=5 Doubtful=5
12	15	2-4	0.70	23	RD=6; LD=8; ND=8 Doubtful=1
15	10	2-4	0.65	33	RD=5; LD=2; Doubtful=3 Recovery: Flank meristems (3)
18	27	2-4	0.70	26	Recovery: Flank meristems (2)
21	6	1-4	0.95	22	Recovery: Flank meristem (1)
24	5	3	0.70	26	Recovery: Flattened dome (1)
27	3	3	0.70	29	Fully recovered apices. 1 with flattened dome

[a] RD=random damage; LD=localised damage; ND=no damage (Number indicates the number of apices involved)