BIOLOGIA PLANTARUM 36 (2): 221-228, 1994

The effect of gamma irradiation on growth characteristics and arbutin content of bearberry (*Arctostaphylos uva-ursi*, cv. Arbuta)

M. SOVOVÁ*, J. OPATRNÁ** and L. JAHODÁŘ*

*Department of Pharmaceutical Botany and Ecology, Faculty of Pharmacy, Charles University, 501 65 Hradec Králové, Czech Republic**
*Research Institute for Plant Production, Drnovská 507, 161 06 Praha 6, Czech Republic***

Abstract

Between 1986 and 1989 we studied the influence of γ-irradiation (2.5 - 80 Gy) on growth processes and the content of arbutin glycoside. Bearberry [*Arctostaphylos uva-ursi* (L.) Sprengel] shows polycyclic characteristics of growth; the vegetation period is divided by summer dormancy (June) into periods of spring and summer growth. As plants age the summer dormancy gets longer and the period of summer growth is shorter. Irradiation with a dose of 80 Gy was lethal and a dose of 60 Gy damaged plants so much that they were not able to grow in the first spring after irradiation. Significant growth stimulation (both in the height of plants and in branching) was shown only in the second year after irradiation (2.5 - 60 Gy). In the fourth year the growth in all irradiated variants was weaker than in the control. Doses of 2.5 and 5 Gy did not influence the content of arbutin significantly; higher doses of irradiation changed the dynamics of production and decomposition which is connected with growth changes.

Introduction

Bearberry [*Arctostaphylos uva-ursi* (L.) Sprengel] is an evergreen shrub of 0.25 to 1 m height with plagiotropic and orthotropic branches which, in contact with the ground, produce roots easily and in that way form sizeable bushes. Growth analysis of bearberry growing under Canadian conditions (Saskatchewan) was done by Remphrey *et al.* 1983a,b. The authors studied separately the growth of three kinds of sprouts: dominant (D), subdominant (SD) and nondominant (ND). From the point of view of the growth architecture, D sprouts have a colonizing function, they usually grow in a plagiotropic way and have 30 - 60 leaves. SD sprouts are shorter and erect. They cover the area taken by D sprouts and they usually grow from the axillary buds

---

Received 19 September 1992, *accepted* 5 October 1993.

of D sprouts. The average number of leaves is 14. ND sprouts are short with several leaves. They arise from the buds on SD or on older branches.

In one-year growth cycle, periods of intensive growth alternate with periods of dormancy (Remphrey and Steeves 1984). Leaves in a dormant winter bud initiated emerge on a spring, D sprouts forming some additional leaves. Few of these are formed on SD. The most active growth lasts about 5 weeks. In blooming plants the floral sites differentiate in a terminal bud, in vegetative sprouts a dormant bud is formed. The second, summer growth activity is not regular. It occurs usually after a period of rain. Short sprouts with several leaves are formed.

From the pharmaceutical point of view the main effective substance of the bearberry plant is a phenolic glycoside arbutin that is effective as an antimicrobial agent in diseases of the urinary tract. Cultivar Arbuta (Musil *et al.* 1984) has been selected in Czechoslovakia. It has a high content of arbutin in the leaves. It differs from the standard type in stunted growth, the distribution and number of trichomes on a leaf blade and in absence of blooming (Leifertová *et al.* 1980). The aim of this paper is to characterize growth processes and their changes in plants exposed to ionizing irradiation. We anticipated that some of the lower doses could positively affect the growth of leaves without reducing yields of the main product - arbutin.

Materials and methods

Spontaneously rooted sprouts of *Arctostaphylos uva-ursi* (L.) Sprengel cv. Arbuta came from an experimental field in the town of Klášterec nad Orlicí, Czech Republic. In the spring of 1985 they were brought to the garden of medicinal plants at the Faculty of Pharmacy in Hradec Králové and after two months of acclimatization irradiated with ^{60}Co - doses of 2.5, 5, 10, 20, 40, 60, 80 Gy (*Chizotron Chirana*, distance of 1 m, dosing speed 9.93 m s^{-1}). In each treatment there were 12 plants which were planted into beds after irradiation. We began to evaluate results of irradiation on 10 selected annual shoots in the spring of 1986 and finished it in 1989. Annual shoots were evaluated every year at the beginning and after finishing their spring growth and after finishing their summer growth period.

In the growth analysis we followed the number and length of sprouts and the way of branching in individual growth periods. We evaluated the number of branches growing only in the spring growth period, only in the summer period and in both growth periods. The total growth was characterized by the number of short branches (up to 5 cm), medium branches (5 - 9 cm) and long branches (more than 9 cm).

For arbutin determination we took leaves from the middle part of the annual shoot in 4 phases of development: in the periods of spring growth, summer dormancy, summer growth and at the beginning of winter dormancy. The content of arbutin was determined by spectrophotometry (Jahodář *et al.* 1986) at 286 nm on a *Specord UV-VIS* apparatus with digital converter *TEC-1* (*C. Zeiss*, Jena).

Results and discussion

We found that the growth of cv. Arbuta differs from the growth of Canadian bearberry as described by Remphrey *et al.* (1983a,b) and Remphrey and Steeves (1984a,b). Cv. Arbuta has a regular seasonal growth period, summer dormancy and a long summer period of intensive growth (June - October). The spring growth period always started, irrespective of meteorological conditions of individual periods of vegetation in 1986 - 1989, in the first 10 d of April and lasted 40 - 60 d (Fig. 1). The following summer dormancy was shortest in one-year-old plants (11 d), in the following years it extended up to 32 d in four-year old plants. We never observed symptoms of blossom differentiation during that time. This dormancy was followed by intensive summer growth which forms long colonizing annual shoots very often with roots. The period of summer growth was shortened in connection with the aging of the plants. While in the one-year-old plants it lasted 117 d, it was 63 d in the four-year old ones. Remphrey *et al.* 1983a,b and Remphrey and Steeves (1984a,b) described differentiation of bloosom bases in Canadian bearberry plants in the period of summer dormancy - these blossom buds do not grow further and they bloom in the following spring. Buds that remained vegetative during summer dormancy grow in the summer period rather exceptionally, if there is very wet weather.

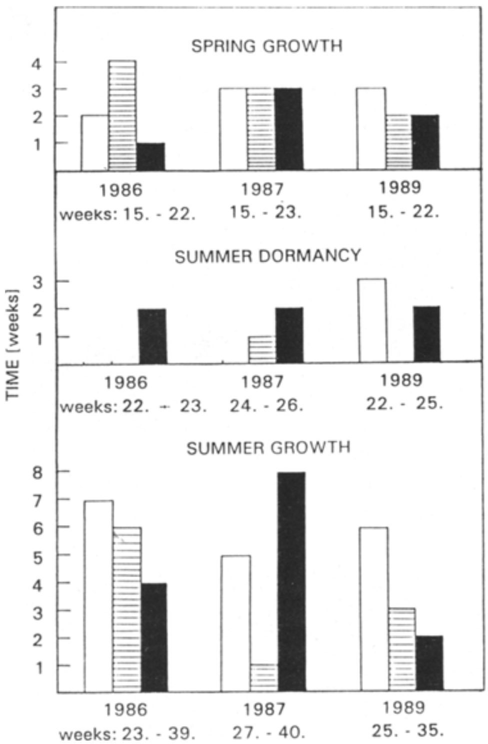

Fig. 1. Length of growth periods according to the amount of rainfall (*hatched columns* - normal rainfall, *open columns* - less than 75 % of normal, *full columns* - more than 125 % of normal)

We followed the influence of γ-irradiation on the growth of plants in the first, second and fourth years after exposure to 2.5, 5, 10, 20, 40, 60 and 80 Gy. With respect to the fact that the intensity of growth of the plant is reduced significantly with the increasing age of the plant (Hlupá 1987), and also unequal meteorological conditions (Fig. 1) can influence the growth, we judged the influence of the irradiation only in comparison to non-irradiated control plants of the same year. The growth of selected annual shoots was characterized by the total length of growth (Fig. 2) and by the number of newly arisen branches (Fig. 3), then by percentage of short, medium and long branch representation (Fig. 4). Polycyclic growth was expressed by the number of branches growing only in spring, both in spring and in summer, and only in summer (Fig. 5, 6).

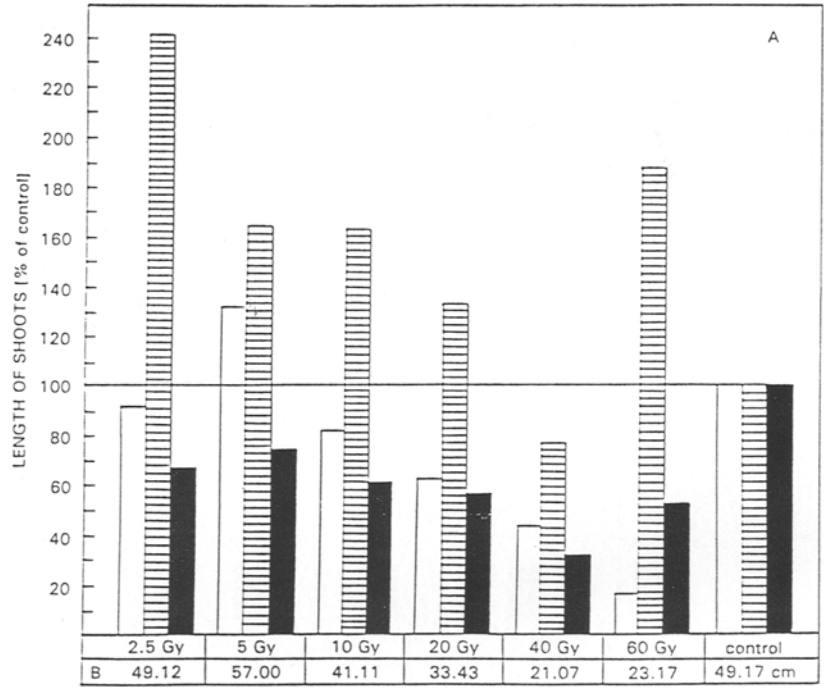

Fig. 2. *A* - Shoot length in irradiated plants with respect to control plants in year 1986 (*open columns*), 1987 (*hatched columns*) and 1989 (*full columns*). *B* - total length of shoots formed in three years.

An irradiation of 80 Gy is considered to be a lethal dose. Several weeks after irradiation the leaves got dark and 9 out of 12 plants died. The remaining plants did not grow either in the first or in the following years after irradiation and in the second winter period they died.

In the first year after irradiation (1986) plants exposed to lower doses of irradiation - 2.5 - 10 Gy - budded in spring one week earlier than the control plants. A stimulatory effect on the total length of the shoots was shown only after irradiation with 5 Gy (Fig. 2). The number of branches was also higher in plants irradiated by 2.5 and 10 Gy (Fig. 3). But with respect to the fact that the ratio of short branches also increased (Fig. 4), the effect of irradiation was shown only on the character of shrub growth, not on the total length of shoots. Doses of 20, 40 and 60 Gy inhibited growth. The seasonal character of the growth remained unchanged after irradiation with 40 Gy while the number of branches growing in both seasons was higher when compared to the control. Only the growth cycle of the plants exposed to irradiation of 60 Gy was damaged. They grew only in summer while their leaves had a dark red tinge (increased content of anthocyans). Damage to the apex was probably so great that new meristematic centres had to be constituted (Iqbal 1971, Gunckel 1982).

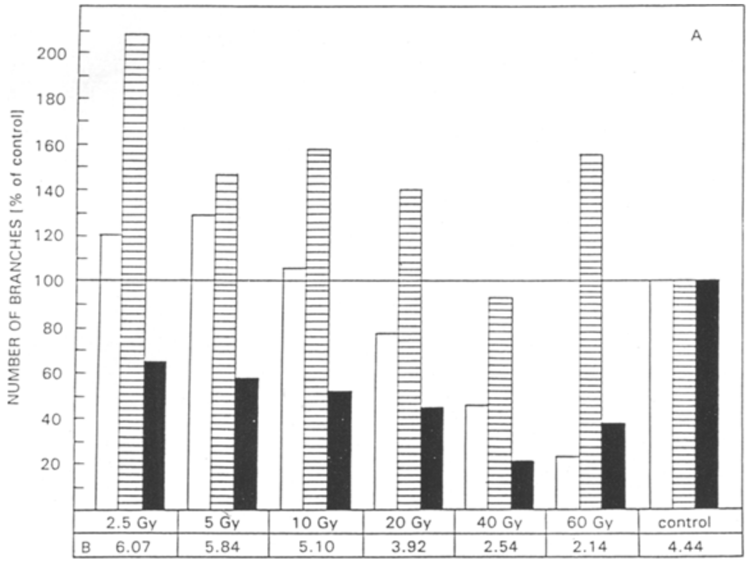

Fig. 3. *A*. Number of branches in irradiated plants expressed as a percentage of control plants in year 1986 (*open columns*), 1987 (*hatched columns*) and 1989 (*full columns*). *B*. The total number of branches formed in three years.

In the second year after irradiation a high stimulation of growth was shown at all irradiated variants with the exception of 40 Gy. Both the number of branches and the total length of shoots increased. The number of short branches was lowest in this year (Fig. 4). Plants exposed to higher doses of irradiation (2.5 - 10 Gy) had a significantly higher number of annual shoots growing in spring only. On the other hand, plants exposed to irradiation of 60 Gy had twice as many branches growing during the summer period than did the control plants. A climatically favourable vegetation period probably contributed to the growth (Fig. 1). It enabled restitution of growth in plants still retarded from a previous season.

In the fourth year after irradiation (1989) growth inhibition was shown at all irradiated variants - not only in the number of shoots but also in their total lengths. These changes could be due to faster aging of the irradiated plants compared to the control plants.

From the point of view of radiosensitivity the bearberry plant can be considered to belong among medium sensitive plants (Dugle 1984). Radiosensitivity is proportional to the size of volume of the nucleus in the interphase (Sparrow *et al.* 1986). It is also influenced by the presence of certain effective substances which can increase or decrease it. For example, many species from the family *Brassicaceae* are

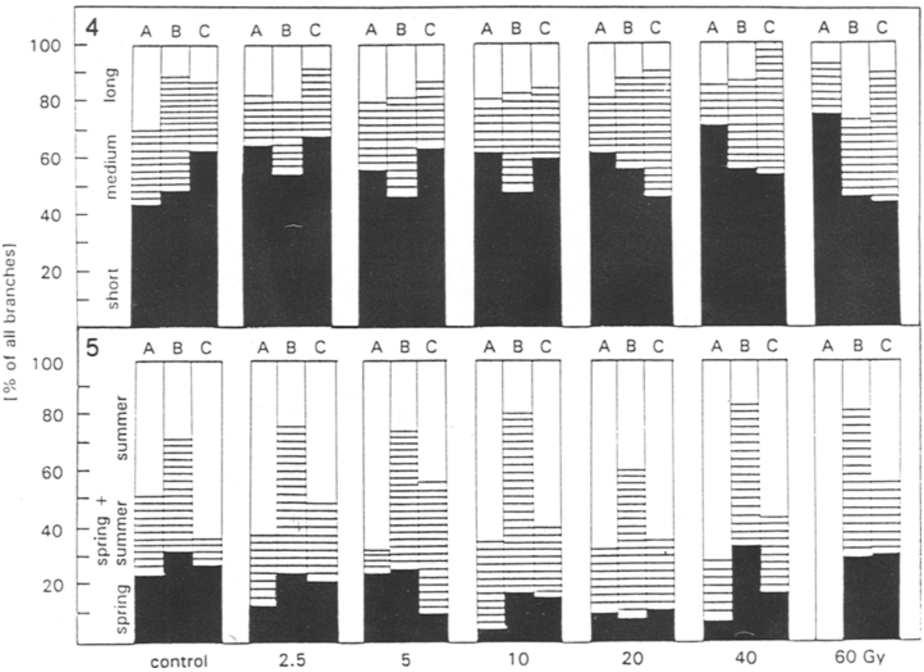

Fig. 4. Representation of short, medium and long branches in the total number of branches in the years 1986 (*A*) , 1987 (*B*) and 1989 (*C*). Branches longer than 9 cm (*open columns*), from 5 to 9 cm (*hatched columns*) and shorter than 5 cm (*full columns*).

Fig. 5. Seasonal characteristics of the growth expressed in percentage of branches growing in in year 1986 (*A*) , 1987 (*B*) and 1989 (*C*). Only in spring (*full columns*), only in summer (*open columns*), both in spring and in summer (*hatched columns*).

radioresistant and their extracts containing sinapine have a radioprotective effect (Gu Ruiqi 1986). The higher production of anthocyans is considered to be a general reaction to the irradiation at higher doses of γ-radiation (Sparrow *et al.* 1986). This phenomenon was observed also in bearberry plants irradiated by doses of 60 and 80 Gy. Their leaves turned purple to various extents in the year of irradiation. The complex of anthocyans was isolated and so far we have proved that it consists of three components.

The content of arbutin in a plant had a characteristic course during growth (Leifertová *et al.* 1973), when phases with increased accumulation in leaf tissues alternated with those of increased metabolization (Fig. 7). It was mostly synthesized in the period of summer growth activity and plants entered into winter dormancy with high content of arbutin. During winter dormancy this content decreased a little, during spring growth of plants it decreased a lot and it reached its minimum at the beginning of summer dormancy. Glucose released from arbutin is probably used as a source of energy for growth processes. Thereafter its amount began to increase a little, later it increased more significantly. Higher doses of irradiation linked to growth inhibition to some extent reduced these changes of arbutin. It is also possible to suppose also an activation of the enzyme system catalyzing its metabolism. In the fourth year the course of arbutin degradation in irradiated plants (10 - 60 Gy) was still found to be different from the control plants. Lower doses of 2.5 and 5 Gy did not damage its physiological rhythm and its amount was decreased very little (Fig. 7).

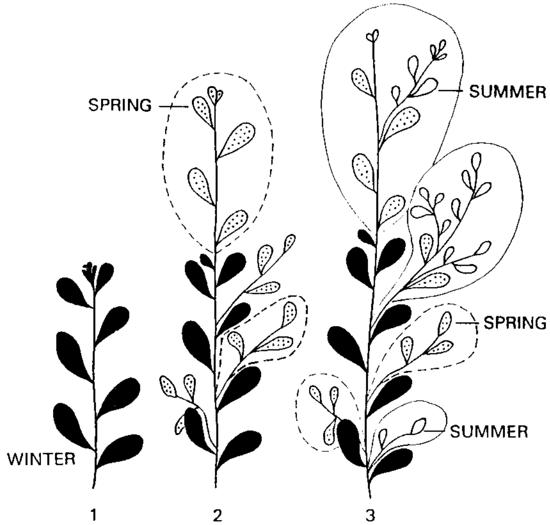

Fig. 6. Schematic diagrams showing the development of a shoot during a year.

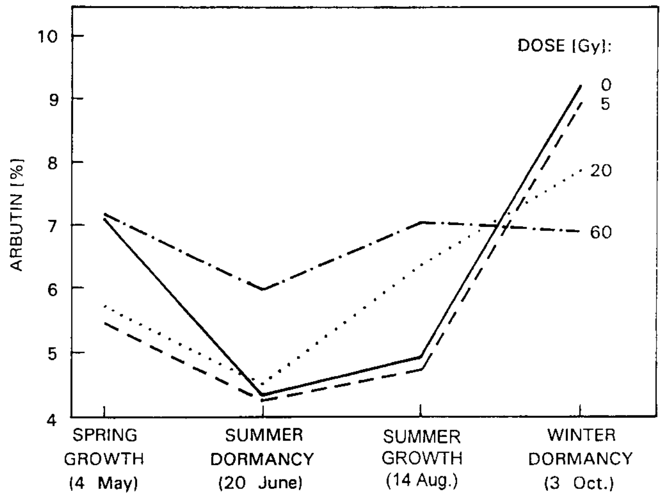

Fig. 7. The content of arbutin during growth cycles of the fourth year after irradiation (5, 20 and 60 Gy) and in control plants.

References

Cordero, R.E., Gunckel, J.E.: The effects of acute and chronic gamma irradiation on *Lupinus albus* L. - I. Effects of acute irradiation on the vegetative shoot apex and general morphology. - Environ. exp. Bot. **22**: 105-126, 1982.

Dugle, J.R., Maych, K.R.: Responses of 56 naturally-growing shrub taxa to chronic gamma irradiation. - Environ. exp. Bot. **24**: 267-276, 1984.

Hlupá, V.: [Growth and Development of Plants of *Arctostaphylos uva-ursi* (L.) Spr. The Effect of Ionizing Irradiation.] - Thesis. Faculty of Pharmacy, Charles University, Hradec Králové 1987. [In Czech.]

Iqbal, J.: Recovery from cellular damage in vegetative shoot apices of *Capsicum annuum* L. after gamma irradiation. - Radiat. Bot. **10**: 337- 343, 1970.

Jahodář, L., Sovová, M., Klemera, P., Frýblová, Z.: The response of *Arctostaphylos uva-ursi* cv. Arbuta to ionizing irradiation. - Pharmazie **41**: 523-524, 1986.

Kak, S.N., Kaul, B.L.: Radiation induced mutations in *Mentha citrata*. - Indian Parfum. **32**: 173-175, 1988.

Leifertová, I., Hubík, J., Kudrnáčová, J., Dvořák, S.: Evaluation of phenolic substances of *Arctostaphylos uva-ursi* (L.) Spr. - Česk. Farm. **22**: 450-453, 1973.

Leifertová, I., Jahodář, L., Musil, P., Dvořák, S., Lisá, M., Němcová, T.: [Research and Breeding of *Arctostaphylos uva-ursi*.] - Thesis. Faculty of Pharmacy, Charles University, Hradec Králové 1980. [In Czech.]

Musil, P., Dvořák, S., Leifertová, I., Lisá, M., Jahodář, L.: A contribution to vegetative propagation of *Arctostaphylos uva-ursi* (L.) Spr. - Folia pharm. Univ. carol. prag. **7**: 16-26, 1984.

Remphrey, W.R., Steeves, T.A., Neal, B.B.: The morphology and growth of *Arctostaphylos uva-ursi* (bearberry): An architectural analysis. - Can. J. Bot. **61**: 2430-2450, 1983a.

Remphrey, W.R., Neal, B.R., Steeves, T.A.: The morphology and growth of *Arctostaphylos uva-ursi* (bearberry): An architectural model simulating colonizing growth. - Can. J. Bot. **61**: 2451-2458, 1983b.

Remphrey, W.R., Steeves, T.A.: Shoot ontogeny in *Arctostaphylos uva-ursi* (bearberry): The annual cycle of apical activity. - Can. J. Bot. **62**: 1925-1931, 1984a.

Remphrey, W.R., Steeves, T.A.: Shoot ontogeny in *Arctostaphylos uva-ursi* (bearberry): Origin and early development of lateral vegetative and floral buds. - Can. J. Bot. **62**: 1933-1939, 1984b.

Riov, J., Monselise, S.P., Kahan, R.S.: Effect of radiation on phenylalanine ammonialyase activity and accumulation of phenolic compounds in citrus fruit peel. - Radiat. Bot. **8**: 463-466, 1968.

Riqui, G.: The radiation protective effect of sinapine distributed in cruciferous plants on germinating seeds of barley and wheat. - Sci. sin. (Ser. B) **29**: 721-732, 1986.

Sparrow, A.H., Furuya, M., Schwemmer, S.S.: Effects of X- and gamma radiation on anthocyanin content in leaves of *Rumex* and other plant genera. - Radiat. Bot. **8**: 7-16, 1968.

Sparrow, A.H., Rogers, A.F., Schwemmer, S.S.: Radiosensitivity studies with woody plants. - I. Acute gamma irradiation survival data for 28 species and predictions for 190 species. - Radiat. Bot. **8**: 149-186, 1968.

Communicated by T. GICHNER