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Variability of *Juniperus communis* subsp. *communis* based on phenolics accumulation and glucosidase activity

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Abstract: Four populations of *J. communis* L. subsp. *communis* of natural localities in forested regions of the Bory Tucholskie (Poland) were analysed in respect of phenolic compounds concentration and β-glucosidase activity. The variability noted in needles was confirmed by adaptation changes; water-logged and poor insolated localities (deciduous mixed fresh forest, dmff – no 3 and coniferous mixed bog forest, cmbf – no 4) stimulated the phenolics accumulation and enzyme activity. On the contrary, the level of phenolics in unripe berry-like cones was almost two fold lower under disadvantageous juniper growth and fructification conditions, whereas the mature berries from poor vigour plants were characterized by higher concentrations of these metabolites. Obtained results show that morphological variation of juniper population was accompanied by variability in phenolic metabolism.

Additional key words: juniper needles, berry-like cones, environmental variability

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Introduction

The genus *Juniperus* L. comprises about 70 species widespread from the arctic zone to the mountains of tropical countries (Seneta and Dolatowski 2000). In Poland, the following two species occur in the wild: *Juniperus communis* L. (*J. communis* L. subsp. *communis* and *J. communis* L. subsp. *alpina* (Sm.) Èelak.) and *Juniperus sabina* L. (Mirek et al. 1995; Seneta and Dolatowski 2000).

J. communis L. subsp. *communis* is a common species widespread throughout the country in the lowlands and lower mountain localities. It grows mainly in the undergrowth of pine and spruce forests (sporadically in deciduous forests) or on their edges, but also in open areas on dry meadows, among agricultural fields, or in heaths. The ecological scale of occurrence of *J. communis* L. subsp. *communis* is very wide but the best conditions for its development are found in coniferous forests. The only limiting factor of its occurrence is an excess of water. It is a light-demanding species but its soil requirements are low (Boratyńska and Boratyński 1978). *J. communis* L. subsp. *communis* was listed among the most common and important woody species colonizing abandoned farmlands (Faliński 1980).

The species is considered to be very variable and is found in different profile forms from prostrate and shrubby forms through pyramidal and columnar forms to arborescent ones (Bobiński 1974; Seneta

1981; Bugała 1991). Numerous distinguished varieties and profile forms are widely used in verdure.

Grześkowiak and Bednorz (2002) in the study on morphological variability of *J. communis* L. subsp. *communis* showed the background of environmental conditions on the wholesomeness and general plant shape, as well as its 'fructification'. Moreover, juniper is known as a plant rich in secondary substances (Muras 1987). Biflavones, diterpenes, phenol glucosides, and recently neolignan and flavonoid glycosides from *J. occidentalis* and *J. communis* var. *depressa* needles have been isolated and characterized (Ilyas and Ilyas 1990; San Feliciano *et al*. 1991; Nakanishi *et al*. 2004). Synthesis and accumulation of chemicals which belong to phenolic compounds have been described as a consequence of several environmental stresses including drought, low nutrient supply, intensive irradiation, pollutants, *etc* (Zobel and Nighswander 1990, Bennett and Wallsgrove 1994, Karolewski and Giertych 1995, Bussotti *et al*. 1998). Phenolics are present in varying quantition and forms and they have several functions.

The objective of this work was to examine β-glucosidase, an enzyme involved in the metabolism of phenol glycosides, and the concentration of total phenolics of juniper needles and berry-like cones in respect to environmental variability.

Material and methods

Four *J. communis* L. subsp. *communis* populations from natural localities in the Bory Tucholskie (forested region in the north of Poland), similarly as in previous paper (Grześkowiak and Bednorz 2002), were investigated (Table 1). Plant material was collected at the beginning of June for enzyme activity and in September for phenols accumulation from four shrubs of each locality treated as replications. Weighed portions of needles sampled from one-year-old and perennial shoots and of unripe (green) and mature (navy blue) berry-like cones were frozen in liquid nitrogen and stored for several days at –20°C.

Extraction and assay of β-glucosidase were done according to Nichols et al. (1980). Frozen leaf tissues (200 mg) were ground in a chilled mortar using 0.1 M sodium-phosphate buffer (3 ml) containing insoluble polyethylene glycol (20 mg). The homogenates were centrifuged at 15.000 g for 20 min. Assays were run at 35°C using 4-nitrophenyl-b-D-glucopyranoside as the substrate. The reaction was terminated with 0.2 N NaOH and read at 405 nm. Proteins were estimated by the Bradford method (1976) using BSA as standard. Activity was expressed as nmoles of p-nitrophenol (p-NP) per hour per fresh weight of needles and as specific units.

For phenolic compound determinations frozen leaves or berries (200 mg) were homogenized in 3 ml 80% methanol and additionally washed with 2 ml of methanol. Combined liquids were centrifuged (30 min, 12.000 g) and the supernatants were evaporated in a speed vacuum concentrator (Heto Lab Equipment A/S, Denmark) to give aqueous suspensions. These suspensions were extracted with 2 × 5 ml of ethyl acetate (30 min on a Vortex shaker) and the combined ethyl fractions were evaporated to dryness. Residues were dissolved in 1 ml of 80% methanol and water diluted before analysis. The phenolics were measured by Folin-Ciocalten's reagent (Sigma) according to Johnson and Schaal (1957) and expressed as coumaric acid equivalent.

All results were subjected to Anova statistical analysis and the Tukey's HSD test using the Statistica 6 software.

Results

Activity of β-glucosidase in juniper leaves was approx. two fold higher in young needles, but those with completed growth than in 2–3 year old needles (Figs 1 and 2). Such dependencies were found both in terms of fresh weight (Fig. 1) and specific activity (Fig. 2). Taking into consideration positions of plant growth a differentiation of activity was found in young leaves; in locality no 3 and no 4 plants exhibited higher activity than in remaining localities, at the same time these tissues contained more protein, thus specific activity was lower. In 2–3 year old leaves glucosidase activity was very stable, and apart from locality no 1 with a two fold higher activity, it remained similar.

Phenolic compound contents were analyzed in leaves (Fig. 3) and in berry-like cones (Fig. 4). In nee-

Table 1. Location and occurrence conditions of *J. communis* L. subsp. *communis* populations; Fc. – forest compartment, cmff – coniferous mixed fresh forest, cff – coniferous fresh forest, dmff – deciduous mixed fresh forest, cmbf – coniferous mixed bog forest

No of population (sample)	Location (north of Poland – Bory Tucholskie)	Forest habitat type	Soil subtype	Forest community
1	Kamienna Karczma Forest-range, Fc. 115 f	cmff	Podzolized rusty soils	*Peucedano-Pinetum*
2	Leśna Huta Forest-range, Fc. 254 f	cff	Podzolized rusty soils	*Peucedano-Pinetum*
3	Trzechowo Forest-range, Fc. 130 Fb	dmff	Podzolized rusty soils	*Querco roboris-Pinetum*
4	Trzechowo Forest-range, Fc. 130 Fgx	cmbf	Transitory peat soils	*Vaccinio uliginosi-Pinetum*

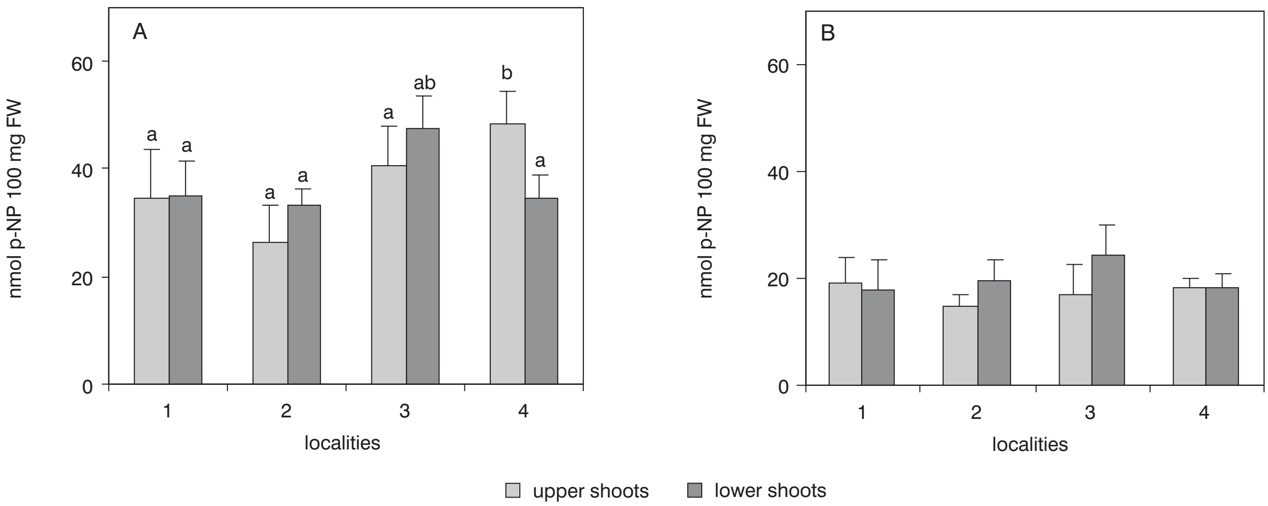

Fig. 1. β-glucosidase activity of four populations of *J. communis* L. subsp. *communis* of one year (A) and 2–3 years old (B) needles of upper and lower shoots, expressed per fresh weight; localities: 1 – coniferous mixed fresh forest (cmff) and 2 – coniferous fresh forest (cff), sunny positions; 3 – deciduous mixed fresh forest (dmff) and 4 – coniferous mixed bog forest (cmbf), poor vigor shrubs

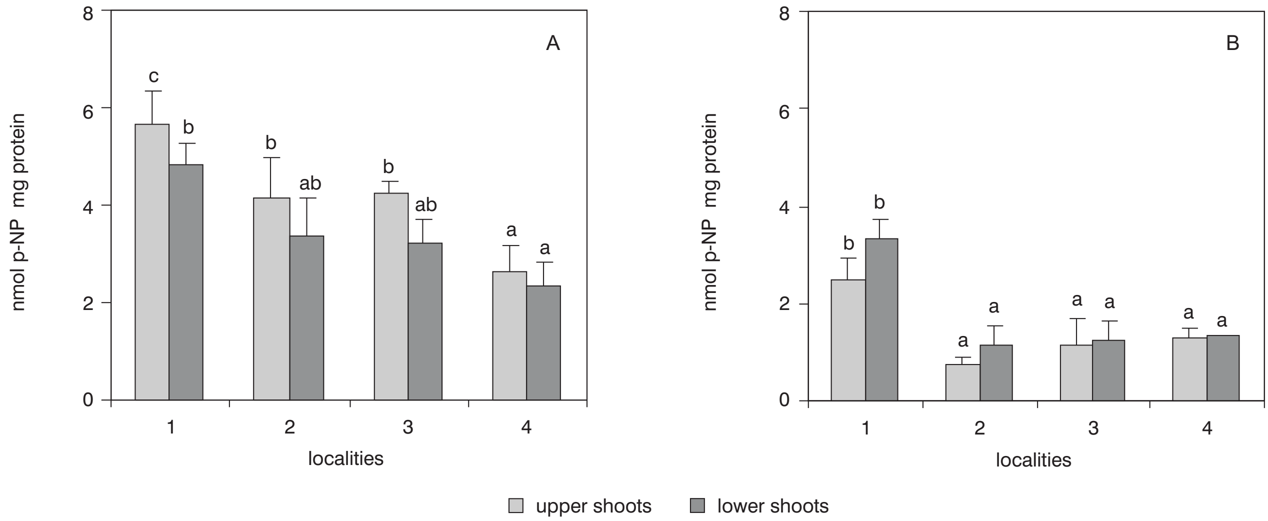

Fig. 2. β-glucosidase specific activity of four populations of *J. communis* L. subsp. *communis* of one year (A) and 2–3 years old (B) needles of upper and lower shoots; localities: 1 to 4 as on fig. 1

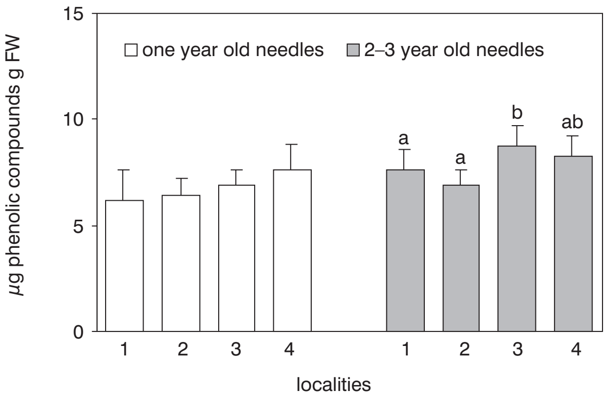

Fig. 3. Phenolic compounds concentration of four populations of *J. communis* L. subsp. *communis* needles; localities: 1 – coniferous mixed fresh forest (cmff) and 2 – coniferous fresh forest (cff), sunny positions; 3 – deciduous mixed fresh forest (dmff) and 4 – coniferous mixed bog forest (cmbf), poor vigor shrubs

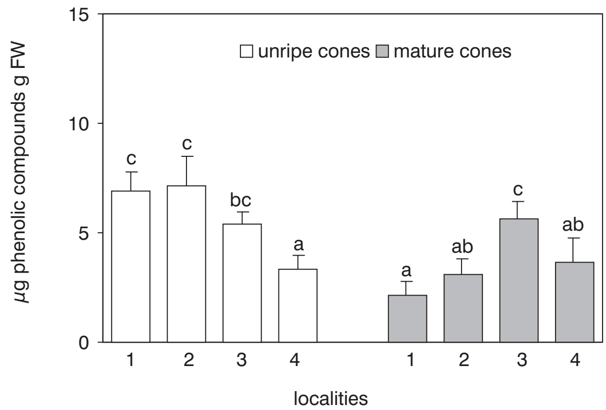

Fig. 4. Phenolic compounds concentration of four populations of *J. communis* L. subsp. *communis* berry-like cones; localities: 1 to 4 as on fig. 3

dles, both 1-year old, assayed in the second half of September, and in 2–3 year old the phenolic contents were similar and by approx. 10 to 25% higher in localities no 3 and 4 than in 1 and 2. A distinct differentiation between the juniper localities was found in the accumulation of phenolic compounds in berries; from 2 mg per 1 g fresh weight (locality no 1 in ripe and nave blue in colour) to approx. 7 mg in localities 1 and 2 in young cones. Phenolic contents were generally higher in unripe berries, and the highest were noted in localities on sunny position, i.e. no 1 and 2. In the phase of cone ripeness the phenolic contents in both these localities was reduced 2–3 times. The level of that compounds in cones of localities 3 and 4 was constant, i.e. independent from the age and ripeness of the organ.

Discussion

Variability of common juniper within forest sites of the Bory Tucholskie was the subject of previous biometric studies (Grześkowiak and Bednorz 2002). It was found that the most advantageous growth and development conditions were in the position of coniferous mixed fresh forest (no 1) and coniferous fresh forest (no 2). These localities were well-insolated, with barren and sandy soils, i.e. meeting the requirements of juniper. Plants under such conditions produced the highest amounts of berry-like cones, with large dimensions and the highest seed content. In localities of deciduous mixed fresh forest (no 3) and coniferous mixed bog forest (no 4) the vigour of *J. communis* subsp. *communis* shrubs was poor, berry-like cones were small and "fructification" much lower.

Investigations conducted in this study show that both differentiation in β-glucosidase activity and the content of phenolic compounds were dependent on plant growth conditions. Metabolic activity of leaves in localities 3 and 4 can indicate adaptation to environmental stresses. Inappropriate growth conditions, leading to poor vigour of plants had a stimulating effect on enzyme activity in young leaves. However, in 2-3 year old leaves the enzyme exhibited lower activity and it was similar in all the localities. Accumulation of phenolic compounds, higher in plants with poor vigour, was observed in young leaves and this level was also maintained in 2–3 year old needles. Bussotti et al. (1998) in leaves of beech trees (*Fagus sylvatica*) noted a marked increase of phenolics during the course of year, as the ecological conditions of the stand worsened. These substances have been identified mainly as tannins, which accumulate primarily in vacuoles and at a later stage they appear to be solubilized in the cytoplasm and retranslocated to the epidermal cells. In juniper needles, according to Nakanishi et al. (2004) and earlier in a study by Ilyas and Ilyas (1990) biflavones and phenol glucosides together with diterpene have been isolated. These compounds are well known as important chemotaxonomic markers, but are probably also important as ecophysiological and stress metabolites. Although as it was found by Nerg et al. (1994) qualitative differences in phenolic profiles and their concentration in *Pinus sylvestris* needles can be influenced by environmental stresses, these factors did not change the genetic regulatory system responsible for flavonoid biosynthesis (Oleszek et al. 2002). However, the relative concentrations of these compounds showed dependence on ecological conditions and moreover even on the origin of seeds.

Another aspect of the investigations was evaluation of the effect of habitat conditions on the accumulation of phenolic metabolites in berry-like cones. In these organs, in the phase of high sink activity preceding their ripening, accumulation of phenolic compounds was positively correlated with plant growth conditions. Good insolation, resulting in a high level of carbon skeletons, promoted the synthesis in phenolic compounds. However, the problem of a relatively high content of these compounds in berry-like cones of poor vigour plants, but only at the phase of their ripeness, would require further clarification.

Summing up, our studies showed that adverse environmental conditions promote accumulation of specific metabolites not only in juniper needles, but also, even to a higher degree, in berry-like cones, i.e. sink organs. Morphological variation in the four juniper population (Grześkowiak and Bednorz 2002) was accompanied by variability in phenolic metabolism.

References

Bennett R.N., Wallsgrove R.M. 1994. Secondary metabolites in plant defense mechanism. New Phytologist 127: 617–633.

Bobiński J. 1974. Jałowiec pospolity i jego rola w lesie. PWRiL, Warszawa.

Boratyńska K., Boratyński A. 1978. *Juniperus communis* L – Jałowiec pospolity. In: Atlas rozmieszczenia drzew i krzewów w Polsce. K. Browicz (ed.) Warszawa–Poznań.

Bradford M. 1976. A rapid and sensitive method for quantitation of microgram quanities of protein using the principle of protein-dye binding. Analytical Biochemistry 72: 248–254.

Bugała W. 1991. Drzewa i krzewy dla terenów zieleni. PWRiL, Warszawa.

Bussotti F., Gravano E., Grossoni P., Tani C. 1998. Occurrence of tannins in leaves of beech trees (*Fagus sylvatica*) along an ecological gradient, detected by histochemical and ultrastructural analyses. New Phytologist 138: 469–479.

Faliński J. B. 1980. Changes in the sex and age-ratio in populations of pioneer dioecious woody species (*Juniperus, Populus, Salix*) in connection with the course of vegetation succesion in abandoned farmlands. Ekologia Polska 28: 327–365.

Grześkowiak M., Bednorz L. 2002. Zmienność morfologiczna szyszkojagód jałowca pospolitego *Juniperus communis* L. subsp. *communis* w Nadleśnictwie Kaliska (Bory Tucholskie). Roczniki Akademii Rolniczej w Poznaniu 347, Seria Botanika 5: 71–78.

Ilyas M., Ilyas N. 1990. Biflavones from leaves of *Juniperus communis* and a survey of on biflavones of the *Juniperus* genus. Ghana Journal of Chemistry 1: 143–147.

Johnson G., Schaal L.A. 1957. Accumulation of phenolic substances and ascorbic acid in potato tuber tissue upon injury and their possible role in disease resistance. American Potato Journal 34: 200–209.

Karolewski P., Giertych M.J. 1995. Changes in the level of phenols during needle development in Scots-pine populations in a control and polluted environment. European Journal of Forest Pathology 25: 297–306.

Lamer-Zarawska E. 1980. Phytochemical studies on flavonoids and other compounds of juniper fruits. Polish Journal of Chemistry 54: 213–219.

Mirek Z., Piękoś-Mirkowa H., Zając A., Zając M. 1995. Flowering plants and pteridophytes of Poland. A checklist. Institute of Botany, Polish Academy Sciences. eds. Kraków.

Nakanishi T., Iida N., Inatomi Y., Murat H., Nada A., Murata J., Lang A.F., Iinuma M., Tanaka T. 2004. Neolignan and flavonoid glycosides in *Juniperus communis* var. *depressa*. Phytochemistry 65: 207–213.

Nerg A., Kainulainen P., Vuorinen M., Hanso M., Holopainen J.K., Kurkela T. 1994. Seasonal and geographical variation of terpenes, resin acids and total phenolics in nursery grown seedlings of Scots pine (*Pinus silvestris* L.). New Phytologist 128: 703–713.

Nichols E.J., Beckman J.M., Hadwiger L.A. 1980. Glycosidic enzyme activity in pea tissues and pea-*Fusarium* solani interactions. Plant Physiology 66: 199–204.

Oleszek W., Stochmal A., Karolewski P., Simonet A.M., Macias F.A., Tava A. 2002. Flavonoids from *Pinus sylvestris* needles and their variation in trees of different origin grown for nearly a century at the same area. Biochemical Systematics and Ecology 30: 1011–1022.

San Feliciano A., Caballero Reneta., Del Rey B., Sancho I. 1991. Diterpene acids from *Juniperus communis* var. *hemisphaerica*. Phytochemistry 30: 3134–3136.

Seneta W. 1981. Drzewa i krzewy iglaste. PWN, Warszawa.

Seneta W., Dolatowski J. 2000. Dendrologia. PWN, Warszawa.

Zobel A., Nighswander J.E. 1990. Accumulation of phenolic compounds in the necrotic areas of Austrian and red pine needles due to salt spray. Annals of Botany 66: 629–640.