

ALLELOPATHY OF *PAEONIA OFFICINALIS* L. 1753 SSP. *BANATICA* (ROCHEL) SOÓ 1945, A PANNONIAN ENDEMIC AND RELICT SPECIES

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Abstract - *Paeonia officinalis* L. 1753 ssp. *banatica* (Rochel) Soó 1945 represents a Pannonian endemic and relict plant species. As an endangered, disappearing species, it is protected according to IUCN and included into the Red Book of the Flora of Serbia. In the Deliblato Sands, in a community of English oak (*Quercus robur*), only a single population of this peony consisting of 74 individuals with a reduced reproduction capacity occurs. Since this could be the consequence of negative allelopathic influence of dominant species of tree, shrub and herbaceous plant layer, we performed allelopathic studies that included qualitative and quantitative analyses of phenolic acids and total phenolics both in the litter and soil of this community. Surface soil layer under *Paeonia officinalis* L. 1753 ssp. *banatica* was found to contain 34.36 $\mu\text{g g}^{-1}$ and 564.42 $\mu\text{g g}^{-1}$ of free and total bound phenols, respectively. A deeper soil layer (10-20 cm) contained much lower amount of free (only 4.44 $\mu\text{g g}^{-1}$) and 571.73 $\mu\text{g g}^{-1}$ of total bound phenol compounds. In the surface soil layer only three free phenolic acids (*p*-coumaric, *p*-hydroxybenzoic and vanillic acid) in minute amounts (0.57-1.69 $\mu\text{g g}^{-1}$) were detected. In the deeper soil layer *p*-coumaric acid was absent. Soil covered with *Paeonia officinalis* ssp. *banatica* contains five phenolic acids in bound form, *p*-coumaric and vanillic acid being the most abundant (10.22-30.70 $\mu\text{g g}^{-1}$). These forms are evenly distributed in the surface and deeper soil layer.

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INTRODUCTION

Paeonia officinalis L. 1753 ssp. *banatica* (Rochel) Soó 1945 represents a Pannonian endemic and relict plant species. It has a limited range of distribution extending to Hungarian Mecsek Mountain, Roumanian part of the Banat and the Deliblato Sands. In the Deliblato Sands, only one locality - Flamunda and only a single population of this peony in an English oak forest were referred to in the available literature (Čol i ć and B r o z 1969). Because of that, this species has been protected and included into the Red Book of the Flora of Serbia. The population consists of a critical number of individuals (74) with a reduced reproduction capacity. Until 1994, only about 20% of individuals were generative with an uncertain seed reproduction. In 1994, the population was transilluminated and as a result, in

1995 the number of generative individuals was doubled and juvenile germinative sprouts appeared (juvenile 22%, juvenile germinative 49% and generative 30%) (Š t o j š i ć 1995; Š t o j š i ć *et al.* 1995). We hypothesized that such a small number of individuals and a reduced reproduction capacity could be the consequence of negative allelopathic influence of dominant plant species from the layer of trees, shrubs and herbaceous plants. This prompted us to perform qualitative and quantitative analyses of phenolic acids and total phenolic compounds both in the litter and the soil under the paeony. R i c e (1974) mentioned 15 groups of compounds, most of them being secondary metabolites including phenolics as significant for allelopathy, due to their inhibitory action. Because of their universal

distribution, high abundance and an important role in plant life, phenolics were the most extensively studied group of compounds interesting from allelopathic point of view. Accumulation of phenolic phytotoxins in soil under dominant plant species within phytocoenoses, leads to the inhibition of seed germination, seedling growth, water, phosphorus and calcium uptake, as well as of photosynthesis. This results in a decreased number of individuals within a community, *i.e.* complete elimination of some plant species (Bate-Smith 1969; Lodhi and Rice 1971; Chou and Muller 1972; Rice 1974, 1979; Lodhi 1976, 1978; Whitehead *et al.* 1983; Kuiters and Denneman 1987; Lyu and Blum 1990; Djurdjević 1991; Li *et al.* 1992 a,b; Djurdjević *et al.* 1997 a,b).

MATERIAL AND METHODS

Description of the Deliblato Sands - The Deliblato Sands represent a wast, 35 km long and about 20 km wide region of Southern Banat extending between the Danube and Carpathian Mountains. Sand masses are forming the dunes in the direction north-west - south-east with wider or narrower depressions between them. South-eastern part of the Deliblato Sands is low (altitude 70-100 m a.s.l.), while north-eastern one is higher (altitude 193 m a.s.l.). The climate is characteristic for semi-steppe and differs from that in surrounding regions. May and June, *i.e.* the end of vernal period and early summer are characterized by abundant rainfalls. Average water precipitate makes some 633 mm. The least amounts of the rainfalls are recorded at the end of summer and beginning of autumn, *i.e.* during July, August and September. Extreme variations in temperature, not only during the year but also during the day are the most significant climate characteristic of the Deliblato Sands.

The substrate consisting of eolic drifts represents a genetic or young genetic soil. The substrate made of sand of neutral pH is composed of aluminosilicates, quartz, calcium carbonate and a poor mixture of clay and humous matters. Quartz grains are resistant while aluminosilicates are susceptible to a rapid decomposition. The presence of calcium carbonate acts supporting pedogenetic processes. The soil as a substrate for vegetation is very young and different regarding its fertility (Drakulić 1969).

In vegetation of the Deliblato Sands, fragments of autochthonous forests and shrubs are succeeded by groups of sand and meadow-steppe vegetation. In the sand, as a genetically young structureless soil, a gradual development of vegetation is expressed from barely for-

med pioneer communities up to complex phytocoenoses of herbaceous plants and forest trees. Based on the studies of plant cover of this region, sand, steppe, swamp and forest type of vegetation with the corresponding associations and subassociations can be distinguished (Stjepanović - Veselić 1979).

Description of the habitat - The habitat examined throughout the present study occurs in the central part of the Deliblato Sands. It represents a forest clearing (50 m in diameter) closed from all sides, formed by clearing of underwood with *Rhamnus cathartica* as dominant species and individual over 100-year-old trees of English oak (*Quercus robur*). At the edges, old English oak (*Quercus robur* 1.1) and Austrian pine (*Pinus nigra* 1.1) 18-20 m high trees (30-50 cm in diameter) occur. Tree layer consists of: *Populus tremula* 3.3, *Populus alba* 1.1 and *Quercus robur* +. Shrub layer is very dense and includes the following species: *Populus tremula* 3.3, *Populus alba* 2.2, *Rhamnus cathartica* 4.3, *Ligustrum vulgare* 3.3, *Viburnum lantana* 1.2, *Cornus sanguinea* 2.2, *Crataegus monogyna* 2.2, *Agrimonia eupatoria* 2.1, *Quercus robur* 1.1, *Origanum vulgare* 1.1, *Berberis vulgaris* 1.2, *Sambucus nigra* 1.2, *Melilotus officinale* 1.1, *Erigeron canadensis* 1.1, *Sonchus arvensis* +, *Cirsium lanceolatum* +, *etc.* Herbaceous plant layer is composed of 51 species including: *Erigeron canadensis* 2.2, *Rhamnus cathartica* 2.2, *Calamagrostis epigeios* 3.3, *Clinopodium vulgare* 2.2, *Crataegus monogyna* 2.2, *Dactylis glomerata* 2.2, *Melica transilvanica* 1.2, *Paeonia officinalis ssp. banatica* 1.2, *Agrimonia eupatoria* 1.1, *Rubus caesius* 1.1, *Reseda lutea* 1.1, *Robinia pseudoacacia* 1.1, *Calamintha officinalis* 1.1, *Iris variegata* 1.2, *Verbascum nigrum* 1.1, *Geranium sanguineum* 1.1, *Campanula trachelium* 1.1, *Lotus corniculatus* 1.1, *Euphorbia cyparissias* 1.1, *Prunus tenella* 1.1, *Fragaria vesca* 1.1, *Linaria vulgaris* +, *Galium aparine* +, *Achillea millefolium* +, *Potentilla tormentilla* +, *Geum urbanum* +, *Knautia arvensis* +, *Hypericum perforatum* +, *Tussilago farfara* +, *Torilis arvensis* +, *Melandryum album* +, *Lappa maior*, *etc.*

Phenolic compounds were analyzed in soil samples taken from the surface (0-10 cm) and from the depth of 10-20 cm, as well as in the samples of partially decomposed litter. After drying, root parts from soil samples were removed and the samples sieved. Partially decomposed litter consisted of leaves and twigs of trembling poplar, hawthorn, white poplar, English oak, herbaceous plants, *etc.* After drying and grinding, the samples were sieved (pore size 0.5 mm) and kept in a refrigerator until the analyses.

Analyses of phenolic compounds

a) *Analysis of phenolic compounds in litter* - Free forms of phenolics and phenolic acids were extracted from dried litter (4 g) in boiling 80% ethanol and ethylacetate. The bound phenol forms were extracted in ethylacetate after pretreatment procedure involving 60 min boiling of dried material in 2N HCl (M i j d l a *et al.* 1975).

b) *Analysis of phenols in soil* - Free forms of phenolics were extracted for 24 h from the samples containing 30 g of dried soil with boiling ethylacetate (3x50 mL). Residual soil was treated with 15 mL of 2N NaOH and after boiling for 24 h the bound phenols were determined (H e n n e q u i n and J u s t e 1967; K a t a s e 1981 a, b). The quantity of total phenolics (free and bound forms) in soil samples was detected spectrophotometrically, using the Folin-Ciocalteus reagent (F e l d m a n and H a n k s 1968). The calibration curve was constructed with different concentrations of ferulic acid.

Qualitative and quantitative analyses of phenolic acids (PA) were performed employing ascending two-dimensional paper chromatography. This method provides a satisfactory separation of five PA, including two derivatives of cinnamic acid (ferulic or 4-hydroxy-3-methoxy cinnamic acid and *p*-coumaric or *trans*-4-hydroxycinnamic acid) and three derivatives of benzoic acid (*p*-hydroxybenzoic acid, vanillic or 4-hydroxy-3-methoxy benzoic acid and syringic or 3,5-dimethoxy-4-hydroxybenzoic acid). These components were separated using two solvent systems: 1. isopropanol : ethylacetate : NH_4OH : H_2O (30 : 50 : 1 : 19) and 2. 2% acetic acid. Dried chromatograms were sprayed with *p*-nitroaniline and 20% Na_2CO_3 and developed chromatographic spots were eluted with 45% ethanol. The quantity of PA was determined spectrophotometrically (a Shimadzu UV-160 spectrophotometer), measuring the absorbance of each acid at a characteristic wavelength (M i j d l a *et al.* 1975). All analyses were performed in triplicates in three independent experiments.

RESULTS AND DISCUSSION

As shown in Fig. 1, surface soil layer under *Paeonia officinalis* ssp. *banatica* was found to contain 34.36 $\mu\text{g g}^{-1}$ free and 564.42 $\mu\text{g g}^{-1}$ total bound phenolic compounds. A deeper soil layer (10-20 cm) contained significantly lower amount of free phenolics (only 4.44 $\mu\text{g g}^{-1}$) and 571.73 $\mu\text{g g}^{-1}$ of total bound phenolic compounds, what is almost equal to their concentration in the surface layer. In the soil samples taken from the surface layer, only three free phenolic acids (*p*-coumaric, *p*-hydroxybenzoic and vanillic acid) in small amounts (0.57-1.69 $\mu\text{g g}^{-1}$) were detected. Soil samples taken from the deeper layer contained no *p*-coumaric acid (Fig. 2).

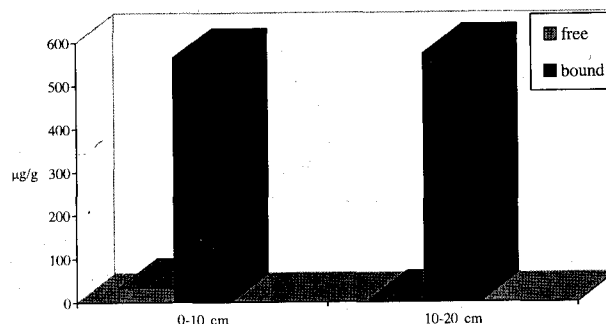


Fig. 1. Content of total phenolic compounds in the soil under *Paeonia officinalis* ssp. *banatica* ($\mu\text{g g}^{-1}$).

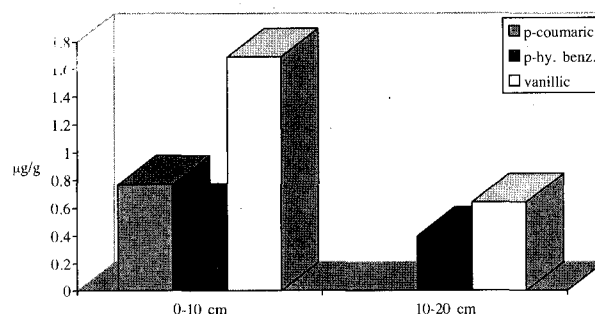


Fig. 2. Amount of free phenolic acids in the soil under *Paeonia officinalis* ssp. *banatica* ($\mu\text{g g}^{-1}$).

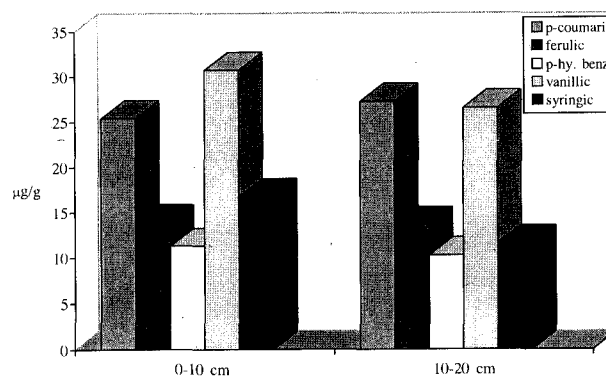


Fig. 3. Content of bound forms of phenolic acids in the soil under *Paeonia officinalis* ssp. *banatica* ($\mu\text{g g}^{-1}$).

Soil under the peony contained five phenolic acids as bound forms, *p*-coumaric and vanillic acid being the most abundant ($10.22\text{--}30.70 \mu\text{g g}^{-1}$). The amount of bound forms of phenolic acids in the surface layer was about equal to that found in the deeper soil layer, *i.e.* they were evenly distributed (Fig. 3). K u i t e r s and D e n n e m a n (1987) studied phenolic compounds in podzolic sandy soil under *Fagus sylvatica* in Netherlands and found that bound phenolic forms occurring in higher amounts ($70\text{--}80 \mu\text{g g}^{-1}$) than free forms ($40\text{--}57 \mu\text{g g}^{-1}$) accumulate during autumnal and winter period. These data are in accordance with our earlier results demonstrating that surface soil layer on Avala Mt. under community *Orno-Quercetum virgilianae* with *Lithospermum purpureocoeruleum* as a dominant species contains $63.22\text{--}143.57 \mu\text{g g}^{-1}$ of free phenolic forms and $266.67\text{--}565.27 \mu\text{g g}^{-1}$ of bound ones (D j u r d j e v i ć *et al.* 1998).

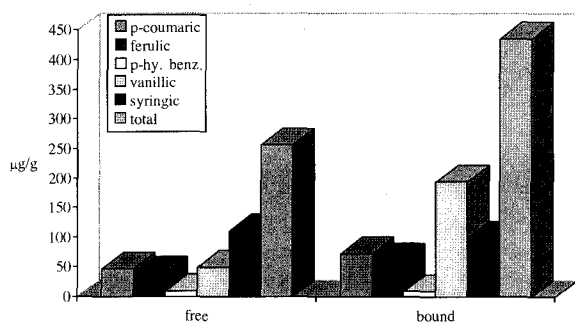


Fig. 4. Amount of free and bound forms of phenolic acids in forest litter ($\mu\text{g g}^{-1}$).

In the litter consisting of partially decomposed leaves and twigs of trembling poplar, hawthorn, white poplar, English oak, *etc.* 10.13 mg g^{-1} of free and 14.09 mg g^{-1} of total bound phenolic compounds were found. It contained five phenolic acids with dominance of bound forms. *p*-Coumaric, vanillic and syringic acid were the most abundant ($61.78\text{--}194.49 \mu\text{g g}^{-1}$) (Fig. 4). Forest litter under different dominant tree species was reported to contain five phenolic acids and several phenolic aldehydes formed by lignin degradation and total amount of phenolic compounds ranged from $10.6\text{--}20.8 \text{ mg g}^{-1}$ (K ö g e l and B o c h t e r 1985; K ö g e l 1986; D j u r d j e v i ć and O b e r a n 1995, 1998; D j u r d j e v i ć *et al.* 1999).

During 1994 both the trees and the shrubs were cleared from the stand. This led to an intensified illumination and a decreased negative allelopathic influence of trees and shrubs on the peony. As a result, the

number of generative peony individuals was doubled and juvenile germinative seedlings appeared (S t o j š i ć *et al.* 1995). However, during the time, sprouts were developed from tree and shrub stumps, and in the stand with the population of *Paeonia officinalis ssp. banatica*, the process of restoration of English oak phytocoenosis is going on. Phytocoenological examination shows that shrub and herbaceous plant layer with over 60 plant species is well developed and they can express a negative effect on the increase of the peony population. The results of experimental phytocoenological studies in the forest of Hungarian oak and Turkey oak on Avala Mt. confirmed that *Lonicera caprifolium* as a dominant species in the shrub and herbaceous plant layer expressed a negative effect on some herbaceous plants and tree seedlings, as well as on actinomycetes and nitrifying microorganisms (M i š i ć *et al.* 1978, 1979; D i n i ć *et al.* 1982, 1983). It has been also reported that the removal of dominant species from shrub and herbaceous plant layer leads to an increased number of individuals and aboveground mass of species previously present in small number and inhibited (C h o u and M u l l e r 1972; M i š i ć *et al.* 1978, 1979; D i n i ć *et al.* 1982, 1983; D j u r d j e v i ć 1989). In this way allelopathic influence and competition of a dominant species for basic ecological factors within a phytocoenosis gets eliminated and as a result, expansion of a suppressed and minor plant species occurs.

Negative allelopathic influence of trees and shrubs on herbaceous plant species is well documented and it was demonstrated that phenolic compounds play an important role in this process. Phenolic phytotoxins occurring in all organs of dominant tree species, especially in the leaves of *Quercus borealis*, *Q. alba*, *Platanus occidentalis* and *Celtis occidentalis*, express a negative influence on herbaceous plant species within a phytocoenosis. It has been reported that fallen down leaves of these tree species contain several phenolic acids, scopoline and scopoletine (N a i b and R i c e 1971; L o d h i 1976, 1978).

In the community of English oak with the peony examined throughout the present study, herbaceous plant layer with 51 species is well developed. Tissues of different herbaceous species also contain phenolic compounds (total phenolics up to 10.98 mg g^{-1}) and free and bound forms of phenolic acids (up to $499.71 \mu\text{g g}^{-1}$) (W h i t e h e a d *et al.* 1983; D j u r d j e v i ć *et al.* 1997a) that can express inhibitory effects on other plant species (L i *et al.* 1992 a,b).

Ecological conditions in the Deliblato Sands are rather harsh (frosts, strong winds, drought, deficit of soil moisture, shortage of humus and mineral matters).

In addition, one should take into account permanent negative effects of anthropological activities on ecosystem as a whole. Under such circumstances, phenolic compounds of trees and shrubs as an additional unfavourable factor can be critical either for elimination or a decrease in the number of sensitive species individuals. This is well documented by a relatively high number of protected plant species (Čolić and Broz 1969; Šainović 1987). Vegetative period of *Paeonia officinalis* ssp. *banatica* is short and from this point of view it has no advantages in relation to woody and shrub species with a much longer vegetative period. Because of an extremely low number of individuals within the population of the peony, and thus a low number of the seeds, it was impossible to examine the influence of phenolic compounds on both seed germination and seedling growth. Such studies would make allelopathic investigations of this species complete.

The data from the available literature demonstrate that besides endemic and relict species, the other plants are also endangered in the presence of dominant plant species in all phytocoenoses, i.e. it can be claimed that this is a universal phenomenon. However, our responsibility for the survival of *Paeonia officinalis* ssp. *banatica* is extremely large because the population consists at present of some 74 individuals, what is a critical number and the possibilities for biological propagation are at a minimum. This rare endemic and relict subspecies is under a strict protection as a natural rarity.

In order to improve conditions that would enable survival of this relict and endemic species of the Flora of Serbia, it would be necessary to remove all plant species which could in any way express negative effects from its immediate surrounding (distance of 3-4 m) (biochemical influence of phenolic compounds of dominant species, shade, competition for water, mineral matters and space). In this way, the peony in the forest clearing studied would be protected against excessive heat, loss of soil moisture and strong winds, as well as against inhibitory effects of phenolics liberated into the soil primarily from the litter of other species during the process of its decomposition.

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АЛЕЛОПАТИЈА БАНАТСКОГ БОЖУРА (*PAEONIA OFFICINALIS* L 1753 SSP. *BANATICA* (ROCHEL) SO6 1945), ПАНОНСКОГ ЕНДЕМА И РЕЛИКТА

Л. БУРЂЕВИЋ, АНКА ДИНИЋ¹, ВИДА СТОЈШИЋ², МИРОСЛАВА МИТРОВИЋ¹,
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Банатски божур (*Paenonia officinalis* L. 1753 ssp. *banatica* (Rochel) So6 1945) је панонски ендем и реликт. Као угрожена врста која ишчезава, према правилима IUCN заштићен је и увршћен у Црвену књигу флоре Србије. У Делиблатској Пешчари постоји само једна популација са 74 јединке у лужњаковој заједници (локалитет Фламунда) смањене репродуктивне способности, што је вероватно последица негативног аделопатског утицаја доминантних врста из спрата дрвећа, жбунова и зељастих биљака на банатски божур. Стога смо приступили аделопатским истраживањима која су обухватила анализу и мерење количине фенолних киселина и укупних фенола у стељи и земљишту ове заједнице. Резултати показују да површински слој земљишта под банатским божуром, садржи 34,36 $\mu\text{g g}^{-1}$ слободних и 564,42 $\mu\text{g g}^{-1}$ везаних укупних фенола. Дубљи слој земљишта (10-20 cm) садржи знатно мању количину слободних (само 4,44 $\mu\text{g g}^{-1}$) и 571,73 $\mu\text{g g}^{-1}$ укупних везаних фенола. У површинском слоју земљишта су детектоване само три слободне фенолне киселине (*p*-кумаринска, *p*-хидроксибензојска и ванилинска) у малим количинама (0,57-1,69 $\mu\text{g g}^{-1}$). Дубљи слој зељашта није садржао *p*-кумаринску киселину. Зељаште под банатским божуром садржи пет фенолних киселина у везаном стању, при чему највише има *p*-кумаринске и ванилинске киселине (10,22-30,70 $\mu\text{g g}^{-1}$). Не постоји разлика у количини везаних фенолних киселина у површинском

и дубљем зељашном слоју, пошто су равномерно распоређене.

У стељи која се састоји од делимично разложеног лишћа и гранчица трепетљике, глога, беле тополе, храста лужњака и др. измерено је 10,13 $\mu\text{g g}^{-1}$ слободних и 14,09 $\mu\text{g g}^{-1}$ везаних укупних фенола. Она садржи пет фенолних киселина, при чему преовлађују везани облици. Највише има *p*-кумаринске, ванилинске и сиригинске киселине (61,78-194,49 $\mu\text{g g}^{-1}$). Она садржи 5 фенолних киселина, при чему преовлађују везане форме. Највише има *p*-кумаринске, ванилинске и сиригинске (61,78-194,49 $\mu\text{g g}^{-1}$). Дискутована је могућа улога фенолних једињења доминантних врста биљака у фитоценози у смањењу бројности божура.

У лужњаковој заједници са банатским божуром, добро је развијен спрат жбунова и зељастих биљака (има више од 60 биљних врста), које могу негативно утицати на повећање популације божура. Као меру за побољшање услова за опстанак ове реликтне и ендемичне врсте наше Флоре, потребно је из непосредне близине популације банатског божура (на растојању од 3-4 m) уклањати све биљне врсте које на било који начин могу негативно утицати (биохемијски утицај фенолних једињења доминантних врста, засена, конкуренција за воду, минералне материје простор).