0971-4693/94 Euro 10.00
Allelopathy Journal 35 (1): 109-128 (2015) *International Allelopathy Foundation* 2015
Tables: 4, Figs : 8

Effects of seasonal dynamics of phenolics in oak forest on truffles (*Tuber macrosporum* Vitt.)

L. DJURDJEVIĆ*, G. GAJIĆ, S. JARIĆ, O. KOSTIĆ, M. PAVLOVIĆ, P. PAVLOVIĆ and M. MITROVIĆ

Department of Ecology Institute for Biological Research 'Siniša Stanković', University of Belgrade, Bulevar Despota Stefana 142, 11060 Belgrade, Serbia
E. Mail: kalac@ibiss.bg.ac.rs

(Received in revised form: November 19, 2014)

ABSTRACT

We studies the seasonal dynamics of phenolics in leaves, litter and soil of the dominant host tree oak (*Quercus robur* L.) growing with ectomycorrhizal fungi truffles (*T. macrosporum*) in oak forest (*Fraxino angustifoliae-Quercetum roboris* Jov. et Tomić 1979) near River Danube, near Belgrade. In litter, the highest content of free phenolics was in April (primordial growth period of truffles) and bound phenolics in August (truffles ripening- start of harvest). Due to intensive decomposition of forest litter during the vegetative growth period, free phenolic acids increased and the bound phenolic acids decreased. There was reduction in ratio of bound cinnamic to benzoic acids, it indicated the microbial degradation of lignin and the transformation of cinnamic derivatives into benzoic acid derivatives. In the top soil layer, where the majority of truffle fruit bodies were found (28.31 kg/ha/year), the free phenolics (direct influence on truffle growth and development) contents were up to 58.36 µg/g. As the mycelia and fruit bodies of truffle grow in phenolic-rich forest soil, hence, we assumed that the truffle is well-adapted to high phenolics content.

Keywords: Disturbed ecosystems, Ectomycorrhizal fungi, host plant metabolites, phenolic acids, *Quercus robur*, rhizosphere soil, truffles, *Tuber macrosporum.*

INTRODUCTION

Truffles (*Tuber* sp.) are underground ectomycorrhizal fungi that are closely associated with numerous tree species. The most frequent host plants for truffles are trees and shrubs from genuses *Quercus, Populus, Fraxinus, Carpinus, Coryllus, Fagus* and *Rosa*. Truffles biology, taxonomy, morphology, anatomy, biogeography, mycorrhization, biochemistry, genetics, ecology, life cycle, cultivation etc. had been extensively studied (7,9,12-15,18,26,30,31,34,47,48, 50,51,53,57,58, 62,64,68). However, the effects of host plant metabolites (phenolics) on truffles group of ectomycorrhizal fungi have not been studied. Phenolics are present in all plant organs, both as free forms and forms bound with other compounds, such as lignin [the common compound in tree species (40)] and polysaccharides of cell walls (124). Phenolics play major role in plant-plant, plant-soil-microbial and plant-pathogens interactions (17,22,24,67).

*Correspondence author

Phenolics are added to soil through leaching (foliage and stem), leaf litter, roots exudates, glandulose trichomes and microbial degradation of dead plant remains (mostly lignin). Due to the abundance, the dominant trees in forest ecosystems are of great allelopathic importance (19,21,32,40,66). Phenolic compounds inhibits some fungi (35), but some fungi grow in phenolic solutions, using the phenolic compounds as source of carbon and energy (49). Phenolics exerts fungal-species and genus-specific effects on their pre-symbiotic growth (spore germination, hyphal length, hyphal branching, formation of auxiliary cells and secondary spores of mycorrhizal fungi) and play role as signalling molecules in the symbiotic relationships between the woody plants and ectomycorrhizal fungi (27,39,61).

Figure 1. The *Fraxino angustifoliae-Quercetum roboris* forest with old pedunculate oak trees and fruiting bodies of *T. macrosporum,* found by specially trained dogs. The fruit bodies were found in the surface soil layer (0-5 cm depth).

Truffle (*T. macrosporum* Vitt.) are common species in Serbia, in hilly terrains, alluvial and moist soils near streams (55) (Figure 1). The hosts of *T. macrosporum* in Serbia are *Quercus robur* L., *Populus nigra* L. and *Populus alba* L. (51). To study the allelopathic interference of donors (host plants) and acceptors of phenolics (ectomycorrhizal fungi), the total phenolics and phenolic acids contents in litter and rhizosphere soil of pedunculate oak forest (*Fraxino angustifoliae-Quercetum roboris*) with autumnal truffle (*Tuber macrosporum* Vitt.) was analyzed monthly (March to October, 2012).

MATERIALS AND METHODS

I. Plant community

The forest *Fraxino angustifoliae-Quercetum roboris* Jov. et Tomić 1979 is located near the River Danube ($44^0$49'14" N, $20^0$27'44" E, 70,75 m.s.l.), near Belgrade. The forest has developed on temporary waterlogged soil in disturbed ecosystem [degraded

by excessive timber cutting, then *Populus nigra* and *P. alba* invaded the habitats of pedunculate oak (*Quercus robur*)]. Pedunculate oak almost exclusively forms the highest floor of forest. There are also few plants of *Fraxinus angustifolia* Vahl. and *Populus alba* in this floor. *F. angustifolia* dominates in shrub and lower tree floor. There was greatest diversity of herbaceous species in forest floor and the most abundant species were: *Stellaria media* (L.)Vill., *Cerastium glomerata* Thuill., *Taraxacum officinale* Webb., *Urtica dioica* L., *Galium aparine* L., *Cardamine pratensis* L., *Lamium purpureum* L., *Fraxinus angustifolia, Lythrum salicaria* L. and *Glechoma hederacea* L (Figure 8).

II. Collection of plant material, litter and soil samples

The samples of freshly fallen autumn leaves, litter and soil from *Fraxino angustifoliae-Quercetum roboris* forest with truffle (*T. macrosporum*) were collected monthly (March to October) at various stages of truffle growth (Table 1).

Table 1. The life cycle of *T. macrosporum* during the vegetation period (March to October)

Month	Growth Phase
March	Apearance of primordia
April	Growth of primordia
May	Initial development of fruiting bodies
June	Rapid truffle growth begins
July	Truffles begin to ripen –first mature specimens
August	Truffles continue to ripen – harvesting begins
September	Truffles continue to ripen--and are harvested
October	Harvesting continues

**Source:* Unpublished data of Dr. M. Milenković.

Soil samples were collected monthly (March to October) from the 3- transects (each 20 m wide and 50 m long) in study forest. After the removal of partially decomposed litter, soil samples were collected from the surface layer (up to 5 cm depth) including the 'fermentation' (Of) and the organo-mineral 'humic' (Ah) horizons (6 x 0.5 kg). After the removal of visible plant remains, the soil was dried at room temperature, ground and passed through a 2 mm mesh screen ($n = 6$).

III. Litter bag experiment

Three transects (each 20 m wide and 50 m long) were made in study forest: (i). Forest along the sandy levee next to River Tamiš, (ii). Central part of forest and (iii) Far edge of forest. In beginning of October 2012, freshly fallen leaves were collected on plastic nets randomly distributed in forest in all 3-transects. Fifty g leaf litter (without adhering material) was dried at 60^0C to a constant weight and enclosed in plastic net (mesh size 1 mm) bags (20 x 20 cm). Sixteen bags were randomly placed on the forest floor in each transect in the beginning of October 2012. For the seasonal analysis of phenolic compounds, two bags were taken out from each transect (n 2 x 3 =6) every month (March to October, 2013). The litter from the bags after removing the soil and roots, was dried at room temperature, ground and passed through a 2 mm mesh screen. In the beginning of October 2012, two samples of fallen leaves were collected randomly from each transect as starting material for analysis of total phenolics and phenolic acids, (3 transects and two

samples from each: 6 x 0.5 kg; n=6). The leaves were dried at room temperature, ground and passed through a 2 mm mesh screen.

IV. Truffle fruit bodies yields

The fruiting bodies of *T. macrosporum* were sampled during August 2012 and in January and February 2013. Sporocarps were found with specially trained dogs, who smells the volatile allelochemicals released from the mature tubers (sporocarps) of truffles. After removing the soil, the fruiting bodies were weighed. Their average weight was 10-50 g, while bigger ones (50-100 g) were rare. The fruit bodies yield of *T. macrosporum* in this forest was 28.31 kg/ha/year.

V. Extraction of phenolics from plant litter and the fallen leaves of dominant trees

Both phenolic acids and total phenolics were extracted from dry litter material (6 x 2 g evry month) with 30 ml of distilled water for 24 h at room temperature. The water extract was adjusted to pH 2.0 with 2N HCl and phenolics were transferred to ethyl acetate (3 x 30 ml). The mixture was evaporated to dryness and the residue dissolved in 4mL of 80% (v/v) methanol (free phenolics) and used for HPLC analysis or stored at -20 ^{0}C. Bound phenolics were prepared by boiling the residue that remained after the first procedure in 2N HCl for 60 min and transferring to ethyl acetate (3 x 30 ml). Total phenolics and phenolic acids (free and bound forms) were extracted from the freshly fallen autumnal leaves of dominant trees (starting material) in the same way as for litter, from 6 x 2 g of dry plant material (n=6).

VI. Extraction of soil phenolics

Free forms of phenolics were extracted from 6 x 30 g of dried soil samples collected every month in distilled water at room temperature and shaken for 24 h (n = 6). The water extract was adjusted to pH 2.0 with 2N HCl and transferred to ethyl acetate (3 x 30 ml). The mixture was evaporated to dryness and the residue was dissolved in 4 mL of 80% (v/v) methanol (free phenolics) and used for HPLC analysis or stored at -20^{0}C. Residual soil was treated with 15 ml of 2N NaOH and after boiling for 24 h, the bound phenols were determined (36). The quantity of total phenols (free and bound forms) in litter and soil was detected spectrophotometrically, using the Folin-Ciocalteau's reagent (23). The calibration curve was prepared based on different concentrations of ferulic acid.

VII. Determination of phenolic acids by HPLC

Phenolic acids were detected between 210 and 360 nm using a Hewlett Packard diode array detector (HP 1100 HPLC system). Separation was achieved with a Nucleosil 100-5 C_{18} column; 5 μm, 4.0x250mm (Agilent Technologies, USA). A step-gradient of acetonitrile in water was used: 15% acetonitrile (5 min, gradient), 30% acetonitrile (20 min, gradient), 40% acetonitrile (25 min, gradient), 60% acetonitrile (30 min, gradient), 60% acetonitrile (35 min, gradient), and 100% acetonitrile (45 min, isocratic). In order to avoid the tailing of the phenolic acids, 0.05% *o*-phosphoric acid was added to the solvents. The flow rate was 1ml/min. The injection volume was 5μl for each of the 6 samples of freshly fallen autumnal leaves and for each of the 6 samples of litter and soil taken each month. The identity of the phenolic acids was determined by the comparison of retention times and the maximum of absorption of known peaks with pure standards. *p*-

Hydroxybenzoic and syringic acids (Acros organics, USA), and ferulic, vanillic and *p*-coumaric acids (Serva, Germany) were used as phenolic standards. Units of phenolic acids were expressed in micrograms per gram of freshly fallen leaves, plant litter and soil dry weight (23).

Statistical analyses

The statistical evaluation of differences in the total content of phenolics and the composition of phenolic acids in plant material, litter samples and soil samples was performed with ANOVA tests. The data was processed using the Statistica 6.0 for Windows statistical package. Relationships were considered as significant at $p < 0.05$.

RESULTS AND DISCUSSION

Phenolics in freshly fallen leaves of dominant trees

In freshly fallen autumnal leaves of dominant tree species were collected as starting material in the beginning of October 2012, the total free and bound phenols contents were equal (12,405.23 and 12,506.27 μg/g). Five phenolic acids (*p*-coumaric, ferulic, *p*-hydroxybenzoic, vanillic and syringic) were found. Two of them are derivatives of cinnamic acid: ferulic (4-hydroxy-3-methoxy-cinnamic acid) and *p*-coumaric acid (trans-4-hydroxy-cinnamic acid), while 3-compounds were derivatives of benzoic acid: *p*-hydroxybenzoic acid, vanillic acid (4-hydroxy-3-methoxy-benzoic acid) and syringic acid (3.5-dimetoxy-4-hydroxy-benzoic acid. The bound forms (*p*-coumaric, ferulic, *p*-hydroxybenzoic and vanillic) dominated ($p < 0.001$) than free phenolic acids. The amount of free syringic acid was 9.4 times higher than bound forms (Table 2, Figure 2). The free forms (were 6- times more) and bound forms (were 4- times more) of derivatives of benzoic acid were more than cinnamic acid, due to high contents of bound vanillic and free syringic acids. Phenolic acids in leaves of dominant trees gathered as starting material account only for small percentage of the total phenols [1.48% free forms and 2.30% bound forms].

Table 2. Total phenolics and phenolic acids content (μg/g) in freshly fallen leaves of dominant trees gathered as starting material

	Free	Bound	Total
Total phenolics	12405.23	12506.27 ^{ns}	24991.51
p-Coumaric acid	12.01	25.24 ***	37.25
Ferulic acid	15.36	33.09 ***	48.45
p-Hydroxybenzoic acid	-	4.33 ***	4.33
Vanillic acid	21.1	210.46 ***	230.5
Syringic acid	135.49 ***	14.46	149.96

*** Significantly different at 0.001, ns-not significant, by t-test ($n = 6$).

Our these results about the content of total free and bound phenolics in freshly fallen leaves of dominant trees in forest with *T. macrosporum* are in accord with previous findings for different tree species, where the phenolics content varied between 2.94-77.00

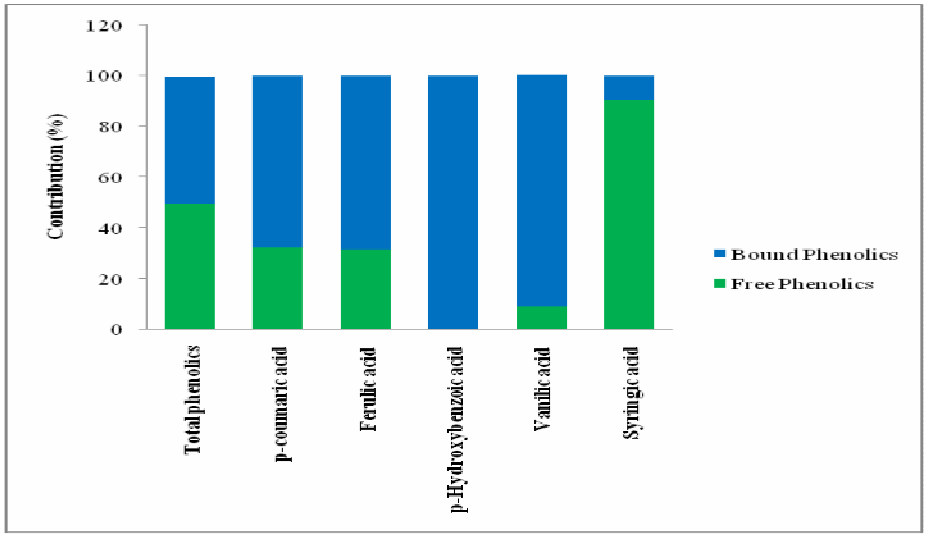

Figure 2. Total phenolics and phenolic acids contribution (%) in freshly fallen leaves of dominant trees gathered as starting material.

mg/g (20,38). The dominant plant species, especially trees leaves are the main source of phenolics and phenolic acids in forest ecosystems. Thus after leaf fall, they enter the litter and are added to soil (19,20,21). For example, the fallen leaves of the oaks *Q. conferta* and *Q. cerris* contains 44.16 mg/g and 42.86 mg/g of total phenolics (22).

In our study, in freshly fallen leaves of dominant trees in community with *T. macrosporum*, derivatives of benzoic acid dominated than derivatives of cinammic acid, both in their free and bound forms, due to the high contents of free syringic acid and bound forms of vanillic acid in their tissues. Contrarily, in freshly fallen leaves of *Q. conferta* and *Q. cerris*, derivatives of cinammic acid predominate (22). Phenolic acids contents in the leaves of dominant trees in the forest with *T. macrosporum*, collected as starting material, both in their free and bound forms were: 0.1-1.1% and 0.03-1.7%, respectively, of total phenolics. It is a similar to fallen leaves of other tree species (19,20,21,22).

Seasonal dynamics of total free, bound phenolics and phenolic acids

(I). Forest litter

The litter was mainly partially decomposed leaves of dominant trees (*Q. robur, F. angustifolia* and *P. alba*) (Fig. 3). The highest concentrations of free phenolics were found at the beginning of vegetative growth (April), afterwards they decreased and remain constant until the end of vegetative period (October). Contrarily, the contents of bound phenolics in litter decreased at the beginning of vegetative growth (April), and reached peak during the summer (August) then decreased by the end of vegetative period. It was found that when the level of free phenolics in litter increased, the bound forms decreased and *vice-versa*.

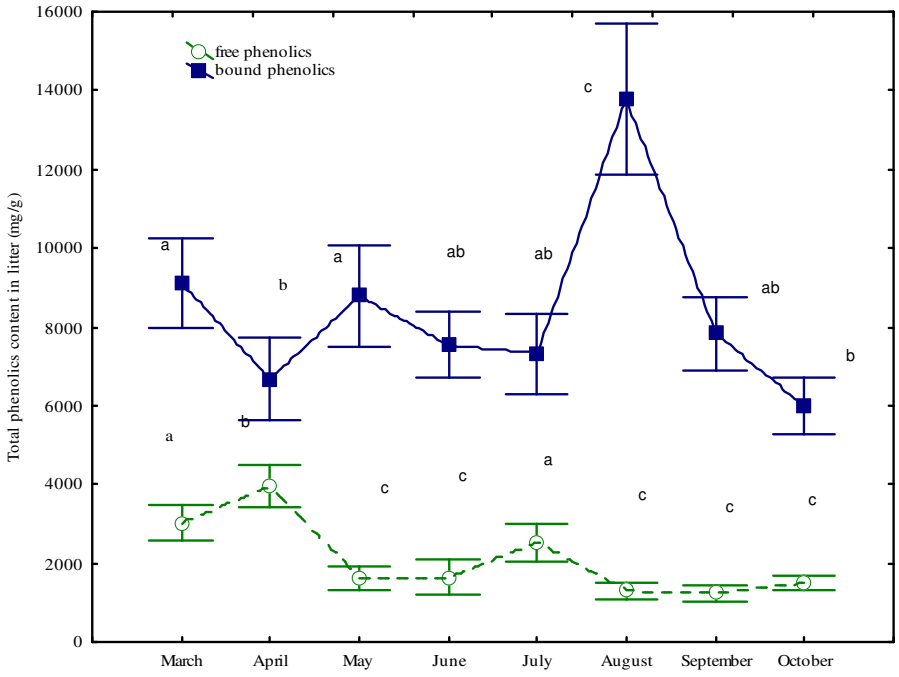

Figure 3. The seasonal dynamics of total free and bound phenolics in litter during the experimental period (from March to October). The different letters indicate significant differences between the concentrations of phenolics at $p < 0.05$ (ANOVA), n=6 for each month.

The loss of total free phenolics in partially decomposed litter at the beginning of vegetative season (April 2013) was 75.64% and 89.54% in August and October 2013, respectively. While the loss of total bound phenolics in partially decomposed litter between beginning of October 2012 and March 2013 was 26.80%. However, at the end of vegetative season (October 2013), this loss was 47.88%. In litter during the vegetative period, the amount of free phenolic acids was between 11.50 and 90.11µg/g. *p*-Coumaric, ferulic and vanillic acids reached their highest concentrations during spring (April-May), while *p*-hydroxybenzoic and syringic acids peaked in summer (June-July). The levels of *p*-coumaric and vanillic acids increased again during September and October. After a sudden decrease at start of vegetative growth (April), the level of bound forms of phenolic acids (*p*-coumaric, ferulic, *p*-hydroxibenzoic and vanillic) in litter did not change till July. All bound phenolic acids in litter, except *p*-hydroxibenzoic, reached their highest contents in August and decreased thereafter (Fig. 4). The content of bound phenolic acids during vegetative growth was always greater than free forms.

The seasonal dynamics of free and bound phenolic acids expressed as percentage of total free and bound phenols in partially decomposed litter is shown (Fig. 5). The free phenolic acids (%) in litter increased from March (1.59%) to May (12.29%) of the total free phenols. After decrease in June (7.47%), their percentage increased to 16.14% in October. At the beginning of vegetative growth, bound phenolic acids were 4.68% of total bound phenols and decreased to 3.41% in August during the most vegetative growth

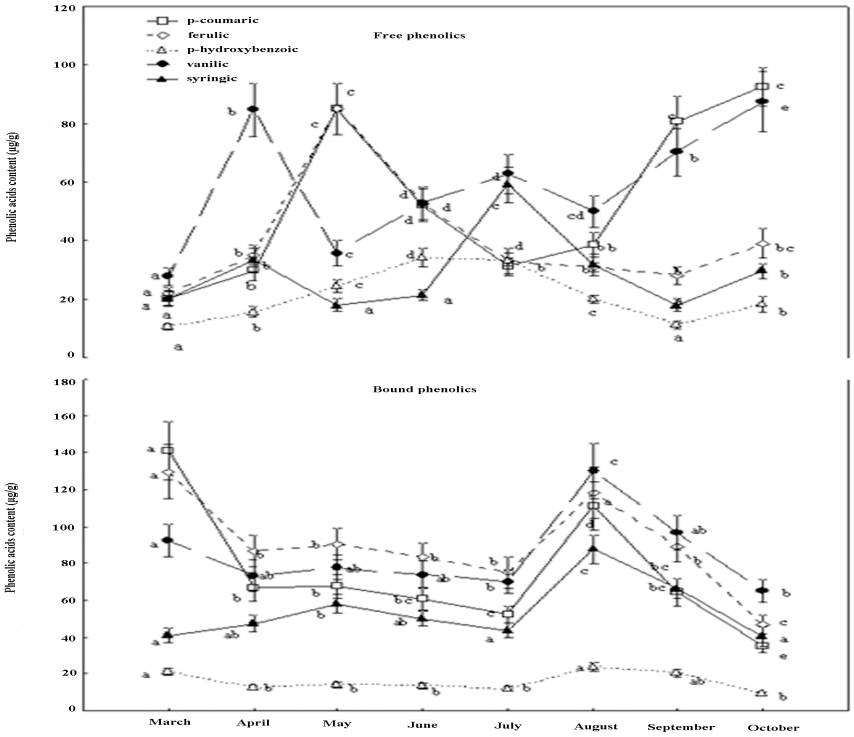

Figure 4. The seasonal dynamics of free and bound phenolic acids in litter during the experimental period (March to October). The different letters indicate significant differences between concentrations of each phenolic acid at $p < 0.05$ (ANOVA), n=6 for each month.

season, the dynamics of bound phenolic acids as percentage of total bound phenols followed the opposite trend to free forms i.e. when the percentage of free forms increased, bound forms decreased. The seasonal changes in the free phenolic acids were more pronounced (1.59% at the beginning of vegetative growth (March) up to 16.14% at the end maturity (October) than bound forms (4.68% at start of vegetative growth decreased to 3.29% at the end).

The of total free phenolics content in litter was 3- times lower than in fallen leaves, due to leaching of water-soluble phenolics by precipitation and the microbial decomposition of dead plant remains. Owing to leaching of free phenols, insoluble and less degradable compounds (such as lignin, cellulose and hemicelluloses), predominate in the litter during the later stages of decay (42,56). This explains the decrease in the amount of total free phenols in litter than in freshly fallen leaves, with severe loss of 75.64% at the beginning of vegetation in March and 89.54% in August. The loss of total bound phenols is almost half of free phenols, due to their slower microbial decomposition. Similar results were obtained for the litter of *Pinus contorta* Douglas ex Loudon, *Acacia longifolia* (Andrews.)Willd., *Alnus glutinosa* (L.) Gaertn, where insoluble and less degradable

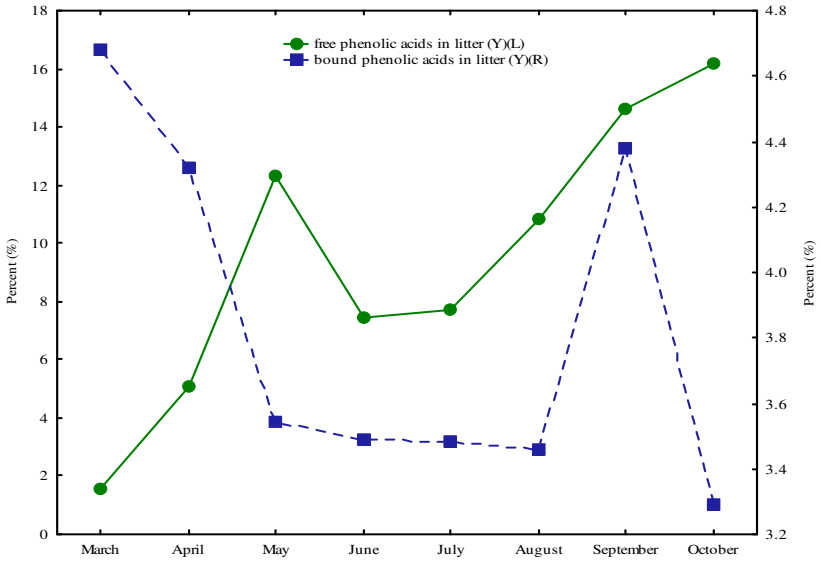

Figure 5. The free and bound phenolic acids contents in litter as percentage (%) of total phenols (free and bound).

compounds prevailed during the later stages of decomposition, whereas extractable phenolics and tannins were lost rapidly from the leaves by leaching (59). In our study, free forms of five phenolic acids were only 1.48% of the total free phenolics in the fallen leaves collected as starting material, which is in accord with our previous findings (20,21,22). During the vegetative growth period, with increase in the microbial decomposition of leaf litter, the free phenolic acids as percentage of total free phenolics were 16.14%. The content of free phenolics increased in spring (April), due to the environmental conditions (increased temperature and moisture). Favourable environmental conditions increase the activity of microbial decomposers of organic matter. The curves of seasonal levels of free and bound phenolics have opposite trends, because when the levels of bound phenols decrease, those of free phenols increase, which points to intensive microbial activity during the whole vegetation period. It slows down only during the period of drought which usually occurs in June and July. Furthermore, based on the dynamics of free phenolic acids contents, it can be asserted that intensive ligninolytic activity of microorganisms in litter takes place during the whole vegetative period. Its intensity decreased in summer dur to higher air and soil temperature and low moisture level in litter and soil. The ligninolytic activity is also confirmed by the fact that the percentage of free phenolic acids increases suddenly at beginning of vegetative period, when conditions for the microbial activity are more favourable and that the percentage is highest between September and October (up to 16.14% of total free phenolics). In general, the level of bound forms of phenolics in litter is always several times higher than free forms, as their incorporation into microbial organic mass occurs as well as the creation of resistant humus material (40,49). The major part of structural units of soil humus appears during the degradation of lignin and other phenolic compounds of plant origin. The important components for the synthesis of humic acids are different phenolic compounds

from plant lignin and flavonoids. Some phenols, such as phenolic acids and phenolic polymers, are synthesized by the microorganisms themselves (both *Macromycetes* and *Micromycetes*) (40).

In the pedunculate oak forest, the ectomycorrhizal fungus *T. macrosporum*, the intensively decomposed the leaf litter during the whole vegetation period. This is evident from the reduction in the levels of total free and bound phenols in litter than their concentrations in freshly fallen leaves (at the beginning of and during the vegetative season). This can also be seen based on the seasonal dynamics of free and bound phenolic acids in partially decomposed litter. Phenolic acids as products of microbial degradation of lignin can serve as reliable indicators of the activities of microorganisms during the vegetative growth. Fungi play an important role in leaf litter and forest floor material decomposition by decomposing the lignocellulose matrix, other organisms are only rarely able to decompose litter. Lignin decomposition involves numerous chemical reactions, which indicates that a set of extracellular enzymes is responsible for the fungal decomposition of lignin (16,44). The partial mineralisation of lignin or dehydrogenative polymers of lignin monomers has been demonstrated for some ectomycorrhizal fungi (10,28,55,65). This indicates the ability to produce lignin peroxidase, laccase and manganese peroxidase activities. Polyphenol oxidases are produced by range of ectomycorrhizal and ericoid mycorrhizal fungi (10). Fungal phenol oxidizing enzymes of ectomycorrhizas (laccases, peroxidases and tyrosinases) can contribute substantially to the humification processes in soil (33). Due to cellulolytic, hemicellulolytic, pectinolytic and ligninolytic activities, the ericoid mycorrhizal endophyte (*Hymenoscyphus ericae*) can decompose the components of plant cell wall, facilitating access to mineral nutrients sequestered within the moribund plant cells walls. Ericoid mycorrhizal fungi are capable of greater phenolic degradation than most ECM species and their degradative ability is associated with production of extracellular phenol-oxidizing enzyme, tyrosinase (4,11).

The highest content of free phenolics in litter of pedunculate oak forest was at the beginning of vegetative growth, while the highest content of bound forms was during the summer. It was contrary to our earlier findings on the seasonal dynamics of phenols and microorganisms in partially decomposed sessile oak litter. The level of free phenolics (was several times higher than bound forms) peaked in July and was much lower at the beginning (April) and at the end of vegetative period (October). At the same time, the abundance of ammonifiers and humifiers was at peak during spring and autumn, when free phenolics in litter were at low level, which indicates the significant regulatory role of phenolics in the population structure of soil microorganisms (19). Osono and Takeda (56) investigated the decomposition of lignin, holocellulose, polyphenols and soluble carbohydrates in relation to nitrogen (N) dynamics in the tree leaf litter. They found negative correlation between the litter mass loss rate and lignin content and positive correlation between the content of polyphenols and soluble carbohydrates. This was further supported by findings of Baar and de Vries (1) that the removal of litter and humus layers increased the number of ectomycorrhizal types (*Laccaria bicolor*, *Paxillus involutus* and *Rhizopogon luteolus*) on Scots pine seedlings, while the addition of organic material led to their decrease.

(II). Forest soil

Dynamics of phenolics contents and Truffles growth: The amount of free phenolics in soil decreased from March (74.06 µg/g, when primordia appeared) to May (38.0µg/g), when the development of truffle fruit bodies began (Fig. 6). Thereafter it stagnated until August (truffles continue to ripen), and then increased till end of vegetation season in October (93.62 µg/g) i.e harvest of fruit bodies). However, the dynamics of bound phenols in soil, showed the opposite trend to free forms, with their levels increasing till June (1,496.00µg/g, 38.95 times higher than free phenols, when truffles grow rapidly); then decreased till September (truffles continue to ripen and harvested) and at end of vegetative growth and harvest of truffles increased to peak (1,528.0 µg/g). Such dynamics of free and bound forms of phenols indicate the constant microbial activity in soil during the vegetative season.

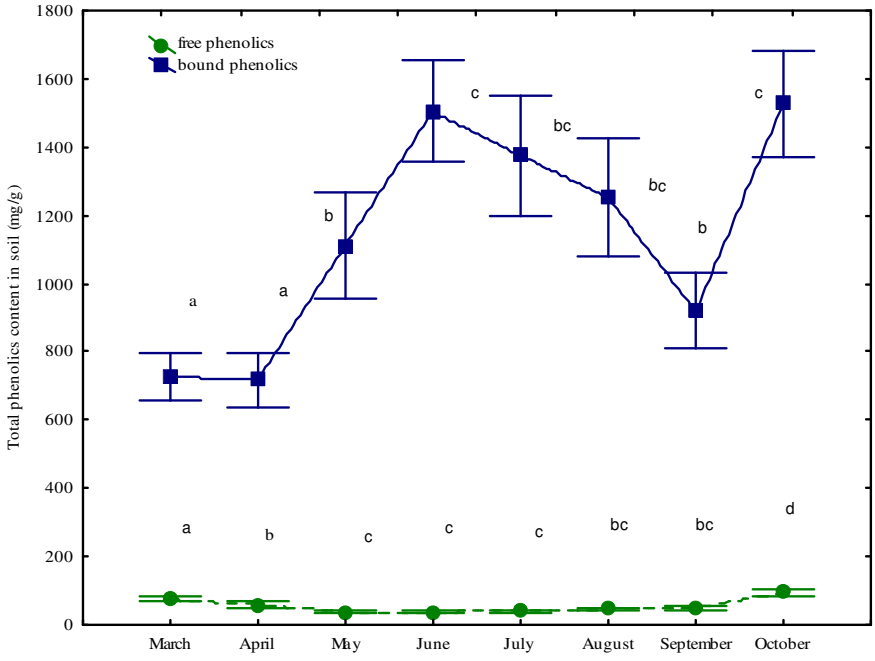

Figure 6. The seasonal dynamics of total free and bound phenolics in soil during the experimental period (March to October). The different letters indicate significant differences between the concentrations of phenolics at p <0.05 (ANOVA), n=6 for each month.

Of the 5- free phenolic acids (*p*-coumaric, ferulic, *p*-hydroxibenzoic, vanillic and syringic), the vanillic, *p*-hydroxibenzoic and syringic acids were found in soil during the vegetative season, while, ferulic and *p*-coumaric acid were detected only at end of vegetative growth (Fig. 7). The levels of *p*-hydroxybenzoic acid in March was low (0.66 µg/g) and of vanillic acid in October was also low (11.08µg/g). Vanillic and syringic acid

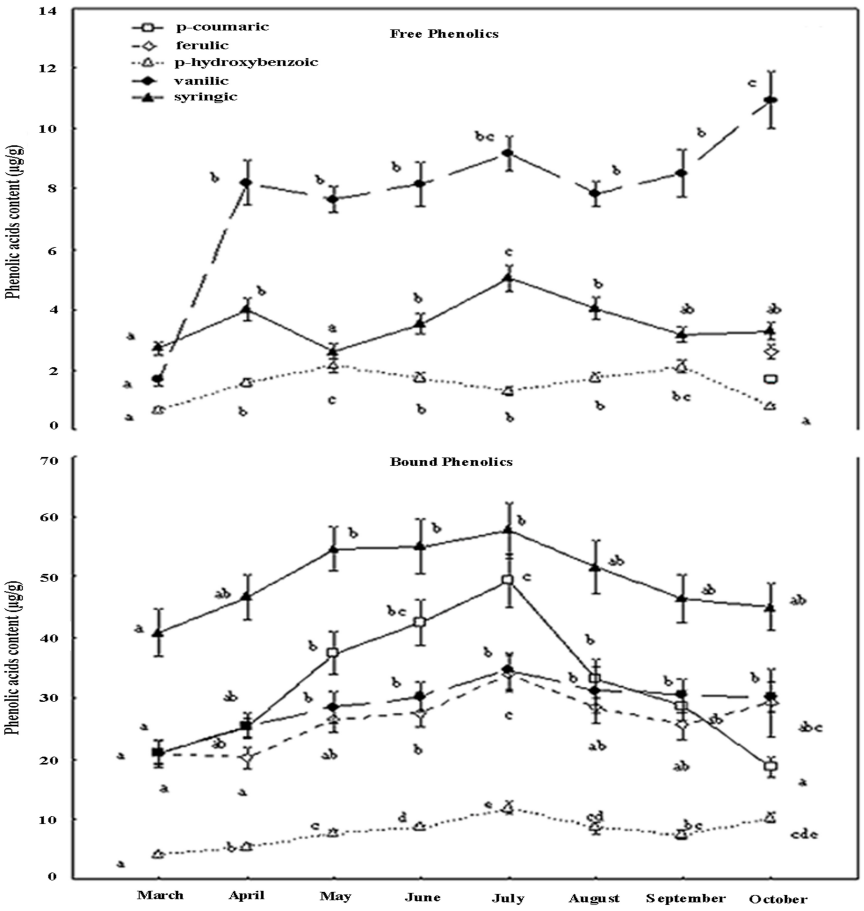

Figure 7. The seasonal dynamics of free and bound phenolic acids in soil from March to October. The different letters indicate significant differences between concentrations of each phenolic acid during the experiment at $p<0.05$ (ANOVA), n=6 for each month.

peaked twice, in April (8.17 µg/g of vanillic acid and 4.07 µg/g of syringic acid) and July (9.15 µg/g of vanillic acid and 4.99 µg/g of syringic acid), when the truffles begin to ripen. The seasonal dynamics of bound phenolic acids (Fig. 7), showed that their concentrations increased from March, reaching a peak in July, afterwards their concentrations decreased or stagnated till end of vegetative season in October. Syringic and *p*-coumaric acids predominated, while *p*-hydroxibenzoic acid was in least quantity. During the vegetative growth, the levels of bound forms of phenolic acids was several times higher than free forms (bound syringic acid was 20.8 times higher than free forms).

The free phenolic acids contents in soil as percentage of total free phenols increases from the beginning of vegetative growth (March: 6.83%) to June (38.45%), then decreased to 20.92% at end of vegetative growth phase in October. While in the vegetative growth period, the level of bound phenolic acids as percentage of total bound phenols

reached maximum twice [in April (17.03%, when primordia grow) and in September (16.00%)]. Similar to fall in percentage of free phenolic acids in soil, the percentage of bound forms also decreased (May-August). The percentage of free phenolic acids in soil was always greater than bound forms (Fig. 8).

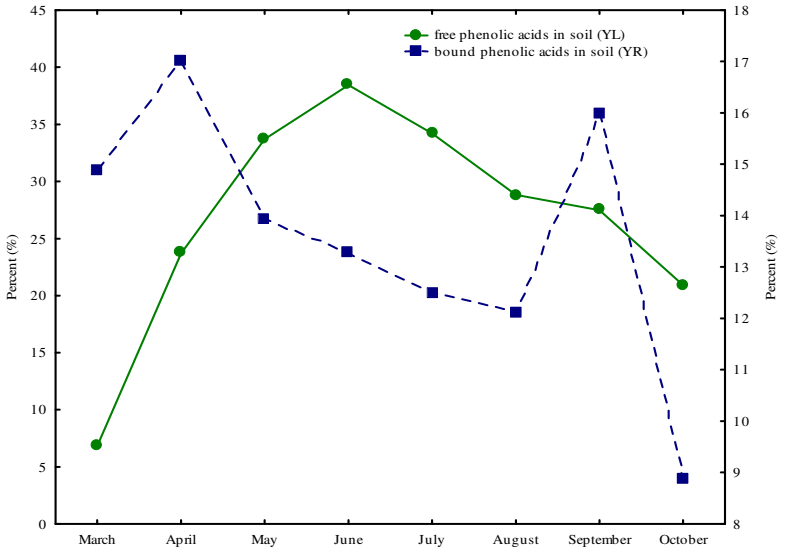

Figure 8. The free and bound phenolic acids contents in soil as a percentage (%) of total free and bound phenolics.

In our study, the amount of free phenols in the soil decreased during the spring and summer. On the other hand, the amount of bound phenols increased so that their levels were up to twice as high as at the beginning of vegetation (March-April). Soil solution concentrations of phenolics are product of the inputs of phenolic acids (e.g., leaching of plant materials, microbial activity, root secretions and exudations and root cell autolysis), losses of phenolic acids (e.g., sorption by soil particles, microbial utilization, uptake by roots) and soil water content. For example, Kuiters and Denneman (42) studied the monthly fluctuations in the free and bound phenolics content in highly podzolizic sandy soil in beech forest and found a distinct seasonal pattern at the site - phenolics reached their highest level in late winter (January), then the concentration fell to its former level during January- March. Authors argued that the decrease in phenolic acids in spring was a result of increased microbial activity due to high soil temperature and soil's adsorption of organic matter during humification. Similarly, in our study the high concentrations of bound phenolics between May and September can also be attributed to increased microbial activity due to the high soil temperature (which leads to transformation of free forms of phenolics into bound forms and their adsorption to soil particles during humification). According to our previous research, the seasonal dynamics of phenolics in soil depends on the type of forest phytocoenosis and the dominant species in it. The highest content of total phenolics occurs in summer in a sessile oak forest (19); it falls in spring, but increases

during summer and autumn in a forest of black ash and Virgilius oak (20); and it falls between March and May in the rhizosphere soil of the vernal ephemeroid *Arum maculatum* in a beech and linden forest (25).

The contents of total bioavailable phenolics [in different oak forest soils in Serbia, which directly effects the plants and microorganisms, (particularly *T. macrosporum*, found exclusively in pedunculate oak forests)] in the Virgilius oak forest were 143.60 µg/g (20) and 74.06 µg/g in the inundate pedunculate oak forest (21). Compared to our results, Kuiters and Dennenman (42) detected significantly higher levels of free phenolics (155 µg/g) in the humic layer of soil beneath *Quercus robur*. However, Lodhi (45,46) found considerably higher content of bound phenolic acids in the soil developed below *Fraxinus pensilvanica* Marsh*, Quercus macrocarpa* Michx. and *Celtis laevigata* Willd.

In general, the abundance of genera of ectomycorrhiza (ECM) was higher in the organic soil layers than the mineral soil horizon. While *Cenococcum geophilum* preferred the organic soil layers probably due to potentially higher ligninolytic capacity than other ectomycorrhizal fungi (2), *Lactarius* spp., *Tomentella* spp. and *Tuber* spp. were generally most abundant in the mineral soil horizons (4). The fruiting bodies of *T. macrosporum* are generally found in the the humic surface soil layer (0-5cm), with 6.60% humus, 3.83% C and 0.35% N (21), which is contrary to the findings of Baier et al. (2) for *Tuber* spp., found in the mineral soil horizons in a Norway spruce (*Picea abies* [L.] Karst.) plantation on a mountainous dolomitic site (1,050 m above sea level) in the Bavarian Limestone Alps. The greatest proportion of fruiting bodies of *T. macrosporum* was found in 0-5cm soil layer (Figure 8), nearly 30 kg/ha/year (21). This can be attributed to the specific conditions which prevail in the floodplain forest with *T. macrosporum*. During November-beginning of March, the soil is under water and in remaining months, the level of ground water is high, hence, the root system of most host trees of truffles is found in surface layer of soil. The transformation of organic matter is characterized by alternating aerobic and anaerobic phases. Due to the prolonged period of aerobic transformation of organic matter, the humus has mullic properties and the C:N ratio indicates that the mineralization process is slowed (21).

Some fungi may grow in media which have high concentrations (up to 5g/l) of orcinol, protocatechuic, *p*-hydroxybenzoic or vanillic acids, using these compounds as the only source of carbon and energy (49). However, Harrison (35) found that some fungi isolated from decomposed oak leaf litter are inhibited by the tannins of *Quercus petrea* and *Q. robur* leaves, which may therefore, be responsible for the lower microbial activity in decomposing oak leaf litter. Tannic acid and condensed tannins inhibit the enzymes β-glucosidase, amylase and cellulase, which are important for cellulose degradation (1). Douds *et al.* (27) reported that ferulic and caffeic acid depressed the growth of arbuscular mycorrhizal fungi, *Gigaspora gigantea* and *Gigaspora margarita*. Vanillic acid stimulated the hyphal growth and the branching of both fungi, and should increase the probability of contact between the fungus and host root. *p*-Hydroxyacetophenone, catechol, *p*-hydroxybenzoic and protocatechuic acids inhibited or stimulated the mycelia growth of two ectomycorrhizal fungi (*Cenococcum geophilum* and *Laccaria laccata)*, according to the kind of substance and its concentration. *L. laccata* was more affected than *C. geophilum* (8).

Stimulation or inhibition of growth of fungi by phenolic compounds are species dependent, Fries *et al.* (29) stated that soil application of *p*-coumaric acid,

p-hydroxybenzoic acid, or quercetin at 0.25mM stimulated the plant growth of *Trifolium repens* L. cv. Ladino and *Sorghum bicolor* L. and the colonization of roots by the AM fungus *Glomus intraradices*, whereas at 1.0mM, these phenolics were inhibitory to both growth and colonization. Contrary to this, Zeng and Mallik (69) established that an equimolar mixture of three phenolic acids (ferulic, o-coumaric, and o-hydroxyphenylacetic acid), commonly found in *Kalmia angustifolia*, had no negative effects on fungal growth at a concentration of 1mM. o-Hydroxyphenylacetic acid stimulates the growth of *Laccaria laccata*, *L. bicolor*, and *Paxillus involutus* at concentrations of 1mM. Some ectomycorrhizal fungi, such as *P. involutus* and *L. bicolor*, are able to degrade *Kalmia* phenolics and use the degraded products as a source of carbon to stimulate growth.

In the soil there is a mixture of different phenolic compounds and their stimulatory or inhibitory effect depends on the types of microorganisms and phenolic compounds, and their amounts. In summary, it can be said that phenolics exhibit fungal-species and genus-specific effects on pre-symbiotic growth (spore germination, hyphal length, hyphal branching and the formation of auxiliary cells and secondary spores of mycorrhizal fungi) and play a role as signalling molecules in symbiotic relationships between woody plants and ectomycorrhizal fungi (27,39,54,61,63).

Correlation between total phenolics and phenolic acids in litter and soil

Data on the correlation between total phenolics (free and bound forms) and phenolic acids (free and bound forms) in litter and soil (Table 3 and 4) confirmed negative correlation between (i). bound total phenolics in litter with free phenolic acids in litter and (ii). bound phenolic acids in litter and free phenolic acids in the soil. Bound phenolics in soil had negative correlation with free phenolic acids in litter, likewise, the bound phenolic acids in litter and free phenolic acids in soil ($p < 0.01$, 0.01 and 0.05 respectively). Furthermore, negative correlation between the free total phenolics in litter and bound phenolic acids in soil ($p < 0.05$) was found, but there was positive correlation between the bound total phenolics in litter and those in soil ($p < 0.01$). A positive correlation was found between the free phenolic acids in litter on the one hand, and bound phenolic acids in litter and free phenolic acids in soil on the other ($p < 0.01$). Bound phenolic acids in litter and free phenolic acids in soil correlate positively, as do free and bound phenolic acids in soil ($p < 0.05$). In addition, free benzoic acid in litter correlates positively with free cinnamic and free benzoic acid in soil, as well as with bound cinnamic and bound benzoic acid in litter. Free cinnamic acid in soil has a positive correlation with free benzoic acid in soil and bound cinnamic acids in both litter and soil. There was positive correlation between free benzoic acid in soil and bound cinnamic acids in both litter and soil ($p < 0.01$). Bound cinnamic acid in litter correlated positively with bound benzoic acids in litter ($p < 0.01$).
Our results showed positive correlation between the bound cinnamic acids in litter on the one hand and free and bound benzoic acids on the other. This confirmed that the microbial degradation of lignin occurs in litter as well as the transformation of derivatives of cinnamic acid into derivatives of benzoic acid. This is further supported by the fact that the ratio of cinnamic to benzoic acids (the bound forms of these acids) is constantly decreasing, which at the beginning of vegetative growth (March) was 1.74 and decreased to 0.72 at the end of vegetative period (October).

Table 3. Correlation coefficients between total phenolics and phenolic acids in litter and soil

	LFPh	LBPh	SFPh	SBPh	LFPA	LBPA	SFPA	SBPA
LFPh	1.00	0.17	0.19	0.36	-0.15	-0.29	-0.51	**-0.64***
LBPh		1.00	0.39	**0.84****	**-0.98****	**-0.95****	**-0.85****	-0.23
SFPh		.	1.00	0.58	-0.27	-0.40	-0.38	0.07
SBPh				1.00	**-0.80****	**-0.81****	**-0.76***	-0.14
LFPA					1.00	**0.88****	**0.88****	0.34
LBPA						1.00	**0.75***	0.12
SFPA							1.00	**0.68***
SBPA								1.00

*, ** Significantly different at 0.05 and 0.01, respectively, by t-test (n = 6). LFPh: Free total phenolics in litter; LBPh: Bound total phenolics in litter; SFPh: Free total phenolics in soil, SBPh: Bound total phenolics in soil; LFPA: Free phenolic acids in litter; LBPA: Bound phenolic acids in litter; SFPA: Free phenolic acids in soil; SBPA: Bound phenolic acids in soil.

Table 4. Correlation coefficients between various phenolic acids in litter and soil

	LFC	LFB	SFC	SFB	LBC	LBB	SBC	SBB
LFC	1.00	0.31	0.56	0.48	0.18	0.03	0.59	0.36
LFB		1.00	**0.81***	**0.80***	**0.95****	**0.95****	0.46	-0.42
SFC			1.00	**0.83****	**0.72***	0.59	**0.70***	0.01
SFB				1.00	**0.87****	0.63	**0.85****	0.19
LBC					1.00	**0.91****	0.58	-0.27
LBB						1.00	0.46	-0.46
SBC							1.00	0.21
SBB								1.00

*, ** Significantly different at 0.05 and 0.01, respectively, by t- test (n = 6). LFC : free cinnamic acids in litter, LFB: free benzoic acids in litter, SFC: free cinnamic acids in soil, SFB: free benzoic acids in soil, LBC: bound cinnamic acids in litter, LBB: bound benzoic acids in litter, SBC: bound cinnamic acids in soil, SBB: bound benzoic acids in soil.

CONCLUSIONS

We concluded that litter is the main source of phenols, which enter the soil after microbial degradation and the transformation of organic matter, primarily lignin. This was confirmed by the positive correlation between free phenolic acids in litter and those in soil, bound phenolic acids in litter and free phenolic acids in soil, and bound total phenolics in litter and those in soil. A significant part of decomposition and transformation of organic matter takes place in the litter, however the main part of metabolism of organic matter does not occur in litter but in the soil, as a more stable and favourable substrate for microbial activity. This was confirmed by the levels of free phenolic acids as a percentage of total free phenols, which was 1.48% in the leaves at starting material, 16.15% in litter and 38.45% in soil. This also applied to the level of bound phenolic acids as a percentage of total bound phenols, which was 2.30% in leaves, 4.68% in litter, and 17.03% in soil.

Our study showed that concentrations of phenolic acids in the litter and soil of the pedunculate oak forest with *T. macrosporum* were lower than inhibitory concentrations stated by other authors and were found in litter in the following ranges: free phenolic acids

(1.76×10^{-4} to 5.99×10^{-4}M) and bound forms (1.76×10^{-4} to 8.73×10^{-4}M). In soil, their concentrations were even lower, with free forms (1.06×10^{-5} to 6.58×10^{-5}M) and bound forms (1.60×10^{-4} to 2.98×10^{-4}M). The amount of total free phenols in the soil of pedunculate oak forest, which had a direct physiological effect on plants and microorganisms, decreased at the start of vegetative growth when primordia grow and during the vegetative growth period they were 38.00-50.35µg/g. These concentrations of free phenols also apply to the other phases in the development of *T. macrosporum* viz.; May (Development of fruiting bodies begins), June (Beginning of rapid growth of fruiting bodies), July (Truffles begin to ripen, August (Truffles ripen further), September (Truffles continue to ripen and were harvested).

ACKNOWLEDGEMENTS

This work was supported by the Ministry of Education and Science, Serbia (Grant No 173018). We thank Dr. Miroljub Milenković, for his selfless assistance during field research and his expert help in describing the life cycle of *T. macrosporum*. The authors also thank Dr. Anka Dinić, a retired Senior Scientist, for her extensive help during field research of the *Fraxino angustifoliae-Quercetum roboris* forest with the autumnal truffle *Tuber macrosporum*.

REFERENCES

1. Baar, J. and de Vries, F.W. (1995). Effects of manipulation of litter and humus layers on ectomycorrhizal colonization potential in Scots pine stands of different age. *Mycorrhiza* **5**: 267-272.
2. Baier, R., Ingenhaag, J., Blaschke, H., Gottlein, A. and Agerer, R. (2006). Vertical distribution of an ectomycorrhizal community in upper soil horizons of a young Norway spruce (*Picea abies* L.) Karst.) stand of the Bavarian limestone Alps. *Mycorrhiza* **16:** 197-206.
3. Banach-Szot, M. (2007). Content of phenolic compounds in humus acids of forest soil. *Polish Journal of Soil Science* **40**: 67-77.
4. Bending, G.D. and Read, D.J. (1997). Lignin and soluble phenolic degradation by ectomycorrhizal and ericoid mycorrhizal fungi. *Mycological Research* **101**: 1348-1354.
5. Benoit, R.E. and Starkey, R.L. (1968). Enzyme inactivation as a factor in the inhibition of decomposition of organic matter by tannins. *Soil Science* **105:** 203-208.
6. Biocca, E., Palenzona, M., Ferrara, A., Nascetti, G., Mattiucci, S., D'Ambelio, S. and Balbo, T. (1988). Etudes preliminaires sur génétique (systems géne-enzyme) des espéces genere *Tuber*. II. Recherches sur les mycorhizes. C.R. 2 me Congr. Int. sur la truffe, Spoleto.
7. Blum, U. (1995). The value of model plant-microbe-soil systems for understanding processes associated with allelopathic interactions: one example. In: *Allelopathy: Organisms, Processes and Applications* (Eds., Inderjit, K.M.M. Dakshini, and F.A. Einhellig,) pp. 127-131. American Chemical Society, Washington, USA.
8. Boufalis, A., Pellissier, F. and Trosset, L. (1994). Responses of mycorrhizal fungi to allelopathy: *Cenococcum geophilum* and *Laccaria laccata* growth with phenolic acids. *Acta Botanica Gallica* **141:** 547-550.
9. Bradshaw, B.P. (2005). *Physiological Aspects of Corylus avellana Associated With French Black Truffle Fungus Tuber melanosporum and the Consequence For Commercial Production of Black Truffles in Western Australia*. Ph.D. Thesis. School of Biological Sciences and Biotechnology, Murdoch University Perth, Western Australia.
10. Burke, R.M. and Cairney, J.W.G. (2002). Laccases and other polyphenol oxidases in ecto- and ericoid mycorrhizal fungi. *Mycorrhiza* **12:** 105-116.

11. Cairney, J.W.G. and Burke, R.M. (1998). Extracellular enzyme activities of the ericoid mycorrhizal endophyte *Hymenoscyphus ericae* (Read) Korf & Kernan: Their likely roles in decomposition of dead plant tissue in soil. *Plant and Soil* **205:** 181-192.
12. Chatin, A.D. (1892). *La truffe*. Librairie J.B. Bailliére et fils, Paris.
13. Chevalier, G. (1974). Convergence des caracteres du peridium des ascocarpes et du manteau des mycorhizes chez quelques especes de Tuber. *Annals of Phytopathology* **5**: 317-318.
14. Chevalier, G. (1979). *Tuber aestivum* species. II. Ecology of truffles. *International Society for Mushroom Science* **10:** 977-993. Mushroom Science. Peterborough, England,
15. Chevalier. G. Riousset, and Dupre L., C. (1985). Europeannes truffle taxonomy. In: *Physiological Aspects of Genetic and mycorrhizae*. Proceedings, 1st Symppsium on mycorrhizae in Europe. pp 631-635.
16. Crawford, D.L. and Crawford, R.L. (1980). Microbial degradation of lignin. *Enzyme and Microbial Technology* **2**: 11-22.
17. Cvikrová, M., Malá, J., Hrubcová, M. and Eder, J. (2006). Soluble and cell wall-bound phenolics and lignin in *Ascocalyx abietina* infected Norway spruces. *Plant Science* **170**: 563-570.
18. Delmas, J. and Poitou, M. S. (1973). Contribution to the knowledge of the ecology of *Tuber melanosporum*: the truffle of Perigord. *Renders Accounts of the Academy of Agriculture*, France **18**: 1486-1494.
19. Djurdjević, L. and Oberan, V. Lj. (1998). Seasonal dynamics of phenolics and microorganisms in the plant litter of oak forest on Cer Mt. *Archives of Biological Sciences* (Belgrade) **50:** 231-236.
20. Djurdjević, L., Dinić, A., Kuzmanović, A. and Kalinić, M. (1998). Phenolic acids and total phenols in soil, litter and dominating plant species in community *Orno-Quercetum virgilianae* Gajić 1952. *Archives of Biological Sciences* (Belgrade) **50**: 21-28.
21. Djurdjević, L., Milenković, M., Pavlović, P. and Kostić, O. (1999). Allelopathic investigations in the *Fraxino angustifoliae-quercetum roboris* (Jov. et Tomić 1979) forest community with the autumnal truffle (*Tuber macrosporum* Vitt.). *Archives of Biological Sciences* (Belgrade) **51**: 27-33.
22. Djurdjević, L., Mitrović, M., Dinić, A., Pavlović, P., Bojović, S., Gajić, G. and Kostić, O. (2005). Allelopathic investigation of *Quercus conferta* and *Quercus cerris* domination in oak forest at Avala Mt. (Serbia). Proceedings 7th World Congress on Allelopathy, 21-26 August 2005, Charles Sturt University, Wagga Wagga, Australia. Web site www.regional.org.au/au/allelopathy, 28–31.
23. Djurdjević, L., Mitrović, M. and Pavlović, P. (2007). Methodology of Allelopathy Research: 2. Forest Ecosystems. *Allelopathy Journal* **20**: 79-102.
24. Djurdjević, L., Pavlović, P. and Mitrović, M. (2010). The effect of phenolic compounds on soil properties. In: *Soil Phenols*. (Eds., Muscolo, A., Sidari, M.) pp. 31-62. Nova Science Publishers, Inc. New York.
25. Djurdjević, L., Popović, Z., Mitrović, M., Pavlović, P., Jarić, S., Oberan, L. and Gajić, G. (2008). Dynamics of bioavailable rhizosphere soil phenolics and photosynthesis of *Arum maculatum* L. in a lime-beech forest. *Flora* **203**: 590-601.
26. Dominguez, J.A., Martin, A., Anriquez, A. and Albanesi, A. (2012). The combined effects of *Pseudomonas fluorescens* and *Tuber melanosporum* on the quality of *Pinus halepensis* seedlings. *Mycorrhiza* **22**: 429-436.
27. Douds, D.D., Nagahashi, G. and Abney, G.D. (1996). The differential effects of cell wall-associated phenolics, cell walls and cytosolic phenolics of host and non-host roots on the growth of two species of AM fungi. *New Phytologist* **133**: 289-294.
28. Entry, J.A., Donnelly, P.K. and Cromack, K. (1991). Influence of ectomycorrhizal mat soils on lignin and cellulose degradation. *Biology and Fertility of Soils* **11**: 75-78.
29. Fries, L.L.M., Pacovsky, R.S., Safir, G.R. and Siqueira, J.O. (1997). Plant growth and arbuscular mycorrhizal fungal colonization affected by exogenously applied phenolic compounds. *Journal of Chemical Ecology* **23**: 1755-1767.
30. Glamočlija, J., Vujičić, R. and Vukojević, J.B. (1997). Evidence of truffles in Serbia. *Mycotaxon* **65:** 211-222.
31. Glamočlija, J., Vujičić, R., Vukojević, J. and Milenković, M. (1996). Truffles in Yugoslavia. IUMS, *8th International Congres of Mycology Division*, Pp. 112. August 18-23, Jerusalem.
32. Grodzinsky, A.M., Golovko, E.A., Gorobets, S.A., Kostroma, E.J., Krupa, L.I., Prutenskaya, N.I., Seredjuk, L.S., Yurczak, L.D. and Šrol, T.S. (1987). *Experimental Allelopathy*. Naukova dumka, Kiev (Russian)
33. Günther, T.H., Perner, B., Gramss, G. (1998). Activities of phenol oxidizing enzymes of ectomycorrhizal fungi in axenic culture and in symbiosis with Scots pine (*Pinus sylvestris* L.). *Journal of Basic Microbiology* **38**: 197-206.

34. Hall, I.R., Zambonelli, A. and Primavera, F. (1998). Ectomycorrhizal fungi with edible fruiting bodies. 3. *Tuber magnatum, Tuberaceae*. *Economic Botany* **52**: 192-200.
35. Harrison, A.F. (1971). The inhibitory effects of oak leaf litter tannins on the growth of fungi, in relation to litter decomposition. *Soil Biology and Biochemistry* **3**: 167-172.
36. Hennequin, J. R. and Just, C. (1967). Presence of free phenolic acids in soil: study of their influence on germination and plant growth. *Annual Agronomy* **18**: 545-569.
37. Hyder, P.W., Fredrickson, E.L., Estell, R.E., Tellez, M. and Gibbens, R.P. (2002). Distribution and concentration of total phenolics, condensed tannins and nordihydroguaiaretic acid (NDGA) in creosotebush (*Larrea tridentata*). *Biochemical Systematics and Ecology* **30**: 905-912.
38. Ishikura N (1976). Seasonal changes in contents of phenolic compounds and sugar in *Rhus*, *Euonymus* and *Acer* leaves with special reference to anthocyanin formation in autumn. *Botanical Magazine* (Tokyo) **89**: 251-257.
39. Kikuchi, K., Matsushita, N., Suzuk, K. and Hogetsu, T. (2007). Flavonoid induce germination of basidiospores of the ectomycorrhizal fungus *Suillus bovinus*. *Mycorrhiza* **17**: 563-570.
40. Kögel-Knabner, I. (2002). The macromloecular organic composition of plant and microbial residues as inputs to soil organic matter. *Soil Biology and Biochemistry* **34**: 139-162.
41. Krings, U., Pilawa, S., Theobald, C. and Berger, R.G. (2001). Phenyl propenoic side chain degradation of ferulic acid by *Pycnoporus cinnabarinus* - elucidation of metabolic pathways using [5-2H]-ferulic acid. *Journal of Biotechnology* **85**: 305-314.
42. Kuiters, A.T. and Denneman, C.A.J. (1987). Water-soluble phenolic substances in soils under several coniferous and deciduous tree species. *Soil Biology and Biochemistry* **19**: 765-769.
43. Lapadatescu, C., Giniès, C., Quéré, J.L.L. and Bonnarme, P. (2000). Novel scheme for biosynthesis of Aryl metabolites from L-Phenylalanine in the fungus *Bjerkandera adusta*. *Applied and Environmental Microbiology* **66**: 1517-1522.
44. Lewis, G.N. and Yamamoto, E. (1990). Lignin: Occurrence, biogenesis and biodegradation. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**: 455- 496.
45. Lodhi, M.A.K. (1975). Soil-plant phytotoxicity and its possible significance in patterning of herbaceous vegetation in bottomland forest. *American Journal of Botany* **62**: 618-622.
46. Lodhi, M.A.K. (1978). Allelopathic effects of decaying litter of dominant trees and their associated soil in a lowland forest community. *American Journal of Botany* **65**: 340-344.
47. Mamoun, M. and Oliver, J.M. (1996). Receptivity of cloned hazels to artificial ectomycorrhizal infection by *Tuber melanosporum* and symbiotic competitors. *Mycorrhiza* **6**: 15-19.
48. Marjanović, Ž., Grebenc, T., Marković, M., Glišić, A. and Milenković, M. (2010). Ecological specificities and molecular diversity of truffles (genus *Tuber*) originating from mid-west of the Balkan Peninsula. *Sydowia* **62**: 67-87.
49. Martin, J.P. and Haider, K. 1971. Microbial activity in relation to soil humus formation. *Soil Science* **111**: 54-70.
50. Milenković, M., Glamočlija, J., Veljković, V. and Vukojević, J. (1992). Record of two *Tuber (T. aestivum* Vitt. and *T. melanosporum* Vitt.) species in Serbia. *Archives of Biological Sciences* (Belgrade) **44**: 223-228.
51. Milenković, M. and Marjanović, Ž. (1999). Current results on *Tuber spp*. research in Yugoslavia. Actes du V^{e} Congrès International. Scient et Culture de la Truffe (Paris), (4-6 mars 1999), pp. 218-222.
52. Mitrović, M., Jarić, S., Djurdjević, L., Karadžić, B., Gajić, G., Kostić, O., Oberan, Lj., Pavlović, D., Pavlović, M. and Pavlović, P. (2012). Allelopathic and environmental implications of plant phenolic compounds. *Allelopathy Journal* **29**: 177-198.
53. Mitrović, M., Milenković, M., Pavlović, P. and Djurdjević, L. (2006). Osmotic potential and water content in fruit bodies of white truffle (*Tuber magnatum* Pico) in two different forest soil in Serbia. In: *First Symposium on Belowground Fungi of Mediterranean Basin* (Ed. L. Khabar). Rabat, Morocco, 5-8 April 2004. pp 136-145.
54. Münzenberger, B., Hammer, E., Wray, V., Schauer, F., Schmidt, J. and Starck, D. (2003). Detoxification of ferulic acid by ectomicorrhizal fungi. *Mycorrhiza* **13**: 117-121.
55. Osono, T. (2007). Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecological Research* **22**: 955-974.
56. Osono, T. and Takeda, H. (2005). Decomposition of organic chemical components in relation to nitrogen dynamics in leaf litter of 14 tree species in a cool temperate forest. *Ecolical Research* **20**: 41-49.
57. Paolocci, F., Rubini, A., Riccioni, C. and Arcioni, S. (2006). Re-evaulation of the life cycle of *Tuber magnatum*. *Applied and Environmental Microbiology* **72**: 2390-2393.

58. Parguey-Leduc, A., Price, C. and Kulifaj, M. (1987). Morphology and structure of adult ascocarp of *Tuber melanosporum* Vitt. (Black Truffle of Perigord, Dyscomycetes). *Cryptogamia Mycologica* **8**: 173-202.
59. Pereira, A.P., Graca, M.A.S. and Molles, M. (1998). Leaf litter decomposition in relation to litter physico-chemical properties, fungal biomass, arthropod colonization, and geographical origin of plant species. *Pedobiologia* **42**: 316-327.
60. Rice, E.L. and Pancholy, S.K. (1973). Inhibition of nitrification by climax ecosystem. II. Additional evidence and possible role of tannins. *American Journal of Botany* **60**: 691-702.
61. Scervino, J.M., Ponce, M.A., Erra-Bassells, R. and Vierhelig, H. (2005). Flavonoids exhibit fungal species and genus specific effects on the presymbiotic growth of *Gigaspora* and *Glomus*. *Mycological Research* **109**: 789-794.
62. Sourzat, P. (2011). Black truffle cultivation and competing fungi. Proceedings, 7th *International Conference on Mushroom Biology and Mushroom Products* (ICMBMP7), Pp. 5211-5533.
63. Souto, C., Pellissier, F. and Chiapusio, G. (2000). Allelopathic effects of humus phenolics on growth and respiration of mycorrhizal fungi. *Journal of Chemical Ecology* **26**: 2015-2023.
64. Talou, T., Delmas, M. and Gaset, A. (1987). Principal constituents of black truffle (*Tuber melanosporum*) aroma. *Journal of Agricultural and Food Chemistry* **35**: 774-777.
65. Trojanowski, J., Haider, K. and Hütterman, A. (1984). Decomposition of ^{14}C-labelled lignin, holocellulose and lignocellulose by mycorrhizal fungi. *Archives of Microbiology* **139**: 202-206.
66. Whitehead, D.C., Dibb, H. and Hartley, R.D. (1983). Bound phenolic compounds in water extracts of soils, plant roots and leaf litter. *Soil Biology and Biochemistry* **15**: 133-136.
67. Witzell, J. and Martín, J.A. (2008). Phenolic metabolites in the resistance of northern forest trees to pathogens. Past experiences and future prospects. *Canadian Journal of Forest Research* **38**: 2711-2727.
68. Zambonelli, A. and Govi, G. (1983). Mycorrhization of *Quercus pubescens* with species of genus Tuber (*Tuber albidum*, *Tuber aestivum*, *Tuber magnatum*). *Micologia Italiana (Bologna) Edagricole* **12**: 17-22.
69. Zeng, R.S. and Mallik, A.U. (2006). Selected ectomycorrhizal fungi of black spruce (*Picea mariana*) can detoxify phenolic compounds of *Kalmia angustifolia. Journal of Chemical Ecology* **32**: 1473-1489.