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Influence of walnut trees (*Juglans regia* L.) on soil urease activity

(Short communication)

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Abstract. Soil urease is an enzyme catalyzing the hydrolysis of urea. The activity of the enzyme influences the content of ammonia ions in soil and thereby the fertility of the soil. 5-hydroxy-1,4-naphthalenedione (juglone) is a compound which inhibits urease. The compound is released by trees of the *Juglandaceae* family (walnut trees) and is present in their leaves, roots, husks and fruits. The aim of the study was to determine the effect of walnut trees on the activity of soil urease. Urease activity was determined in the soil beneath the tree crown and beyond.

It has been shown that soil urease activity is higher in the area outside of the tree crown than beneath it. Less active urease slows down the natural nitrogen cycle resulting in decreased soil fertility. The lower soil fertility slows the growth of plants. This shows non direct influence of juglone on the growth of plants in the vicinity of walnut trees.

The greatest difference in urease activity beneath and beyond the crown was observed near the oldest tree. This indicates that the development of the crown and root system increases the amount of secreted juglone.

Soil active acidity and exchange acidity were measured. The study did not show a significant correlation between soil urease activity and soil acidity in the observed pH range.

Keywords: walnut tree, urease, juglone, soil.

INTRODUCTION

Walnut trees (*Juglans regia* L.) are an economically important tree species, highly valued for its timber and edible nuts. This species grows well in virtually all parts of the world with a temperate climate (McGranahan, Leslie, 1991) from the Carpathian mountains through the middle east and into the Himalayas. The world's leading producer of walnuts is China (1.9 mln t) followed by the USA (0.57 mln t), Iran (0.35 mln t) and Turkey (0.21 mln t). In Europe, the main producers are Ukraine (0.11 mln t), Romania (0.04 mln t), France (0.04 mln t) and Italy (0.01 mln t). The production in Poland in 2017 was 3718 t (FAOSTAT, 2017).

The northern edge of the area where walnut trees are habitually found in Europe runs through Poland. The tree commonly grows in south-east Poland (Lenda et al., 2012). Walnut orchards make up a significant part of the fruit orchards in Poland. The total area of walnut orchards in 2014 was 2.8 thousand hectares (GUS, 2015). Apart from cultivated and controlled plantations of walnut trees there is an increasing number of trees which grow in the wild. Walnut trees are recognized as a potentially invasive species because of their fast growth and allelopathic abilities (Lenda, Skórka, 2009; Lenda et al., 2012). Structural changes in Polish agricultural economy (transition from socialist economy to free-market economy) caused many areas to be abandoned. These areas are covered with wild flora including walnuts trees. The expansion of the species is a result of the habit of native birds of the corvidae family (e.g. jackdaws, rooks) to transport nuts and hide them in the soil. Lenda et al. (2012) surveyed area of south of Poland. The number of individual walnut trees outside of the orchards, in the studied area was found to be 5 to 1099 units per hectare. Assuming that abandoned fields cover 20% of the studied area the increasing number of wild growing walnut trees significantly increases the territory which is influenced by this species.

The negative allelopathic effect of trees of the *Juglandaceae* family on other plants is well documented (Appleton et al., 2009; Ercisli et al., 2005). The family name is related to juglone (5-hydroxy-1,4-naphthalenedione). The compound can be isolated from the leaves, roots, husks and bark of the *Juglandaceae* tree. Well known, and rich in juglone content, is the walnut tree (*Juglans regia* L.). The

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content of juglone in green husks is in the range 20.56–42.78 mg/100 g fresh mass (FM) and in leaves 5.42–22.82 mg/100 g FM (Cosmulescu et al., 2011).

Juglone has varying effects on plants, including an inhibition of seed germination and plant growth (Babula et al., 2014; Rudnicka et al., 2014), a reduction in the chlorophyll content (Terzi et al., 2003) as well as inhibition of photosynthesis (Hejl et al., 1993; Jose, Gillespie, 1998). The compound is known as an inhibitor of several enzymes e.g. thioredoxin reductase, dihydroorotate dehydrogenase, parvulins (Cenas et al., 2004; Knecht et al., 2000; Henning et al., 1998) as well as urease (Kot et al., 2010). Juglone was found to act as a strong, time and concentration dependent inactivator of urease.

Urease (urea amidohydrolase, EC 3.5.1.5) is an ubiquitous enzyme catalyzing the hydrolysis of urea:

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{urease}} 2NH_3 + CO_2.$$

The enzyme activity facilitates nitrogen circulation in the environment. The enzyme has been found in plants, algae, fungi and bacteria (Mobley, Hausinger, 1989; Sirko, Brodzik, 2000). These organisms are a source of the soil urease. Urease present in soil appears as intracellular urease present in living cells of organisms producing the enzyme, extracellular urease and urease immobilized by soil colloids. All these forms of urease create the soil urease activity (Qin et al., 2010).

Soil enzymes are natural mediators and catalysts of biochemical soil processes, such as decomposition of organic matter, humus formation and decomposition, production of mineral nutrients, nitrogen fixation, as well as the circulation of carbon, nitrogen and other basic elements (Błońska et al., 2017). Therefore, the activity of soil enzymes is a good indicator of the metabolic potential, fertility and quality of soils.

The aim of the study was to show the influence of walnut trees (*Juglans regia* L.) on the activity of soil urease. The results of laboratory tests on the influence of juglone on urease have already been published (Kot et al., 2010). However, there is a lack of data related to field studies examining the effect of walnut trees on natural soil urease. Considering the area of walnut orchards in Poland, recognition of its influence on soil quality seems to be an environmentally important issue.

MATERIALS AND METHODS

Five walnut trees growing in the park in Krakow (Southern Poland) were chosen for the study. The park is situated at some distance from urban development which reduced the impact of anthropogenic influence on the soil and trees. Each tree had grown at a considerable distance from other trees. The soil samples were collected in autumn from two areas: beneath and beyond the crown of the trees (distance equal to double the radius of the tree crown). The fact that trees loose leaves in autumn facilitates infiltration of substances from those leaves into the soil. For each tree sixteen soil samples were collected: eight beneath and eight beyond the crown. The points of soil sampling were distributed radially around the tree (Figure 1). The samples collected beyond the tree were considered as references. Two facts were taken into account when the reference area was chosen: the area had to be free of walnut leaves and the kind of the soil had to be the same as under the tree. The characteristics of the walnut trees are listed in Table 1. A soil sampler stick was used to collect soil from a depth of 0–30 cm. The soil was dried in air and sifted through a 2 mm sieve.

Determination of urease activity

A 5.00 g sample of soil was mixed with 5.00 ml of 200 mM phosphate buffer pH = 7.0, 1.00 ml of 100 mM EDTA and 2.50 ml of 0.72 mM urea. The mixture was incubated for 24 h at 37 ^{o}C. Afterwards, 15.00 ml of 1 M KCl solution

Table 1. Characteristics of the walnut trees. The walnut tree number corresponds to the sample number.

Walnut tree number	Age [year]	Trunk diameter [cm]	Crown diameter [m]	Fruits
1	9	15	3.2	-
2	11	14	3.5	-
3	20	23	5.5	+
4	26	27	7.5	+
5	31	32	8.7	+

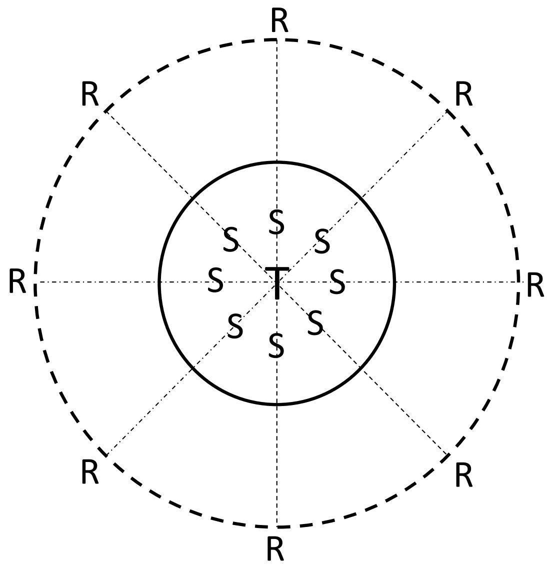

Figure 1. Location of the points of soil sampling. Letters relate to: T – walnut tree, S – soil samples beneath the crown of the tree, R– references soil samples beyond the crown of the tree. Constant line indicates the range of the walnut tree crown, dotted line indicates the double radius from the walnut tree trunk.

in 0.01 mM HCl were added to stop the enzyme activity and the mixture was shaken for 30 min. Then, the mixture was filtered and 1.00 ml of the filtrate was drawn for ammonia determination. In a separate experiment the natural ammonia present in the soil and in the urea solution was determined.

Determination of ammonia

Ammonia was determined by spectrophotometric, phenol--hypochlorite method (Weatherburn, 1967). Spectrophotometer Marcel Media was used for absorbance recording. The absorbance was registered at 630 nm. The measurements were performed at ambient temperature.

Determination of soil pH

Soil pH was measured using routine method: ISO 10390:2005 Soil quality – Determination of pH. pH was measured using a glass electrode in a 1:5 (volume fraction) suspension of soil in water (pH_{H2O}) and in 1 M potassium chloride solution (pH_{KCl}).

Statistical analysis

All experiments were performed in triplicate and the results are presented as a mean±standard deviation of the mean. Pearson's correlation was applied for assessment of the correlation between variables.

RESULTS AND DISCUSSION

The activity of soil urease was determined in two areas: beneath and beyond the crown of the tree. Ponder and Tadros (1985) proved that juglone concentrations declined with soil depth and distance from the walnut tree. The amount of juglone in soil was halved at twice the distance from the walnut tree trunk, from 2.78 µg/g soil at 0.9 m to 1.36 µg/g soil at 1.8 m. Therefore, a distance of twice the crown radius was chosen as a reference area surrounding the walnut tree.

The obtained results of soil urease activity are presented in Table 2 and Figure 2. It was shown that for all samples studied the activity of urease in soil outside of the range of the crown was higher than the activity of urease in the soil beneath the crown of the walnut tree. The decrease of the activity was in the range of 5% to 36%. The lowest change was observed for young, fruitless trees (samples 1 and 2), twice higher change showed for the mature, fruitful trees (samples 3 and 4). The highest decrease of urease activity was noticed for the oldest tree (sample 5).

The soil active acidity and exchange acidity were measured. Soil pH_{H2O} varied in the range of 6.04–7.40 (in the area beneath the crown) (Table 3). The optimum pH for urease activity is in the range of 6–8 (Fidaleo and Lavecchia, 2003). Pearson's coefficient R was used for assessment of correlation between urease activity and pH. It was shown that R-squared was below 0.5 which indicated a very weak correlation between variables. Therefore, the influence of pH in the aforementioned range on urease activity can be neglected.

Our previous laboratory research proved that juglone acted as a strong, time and concentration dependent inactivator of urease. The field studies examining the effect of walnut trees (the source of juglone) on natural soil urease also showed a decrease in enzyme activity in the vicinity of the tree. Less active urease slows the natural nitrogen cycle resulting in decreased soil fertility. The consequence of this

Table 2. Activity of the soil urease in the vicinity of walnut trees: beneath and beyond the crown. All values are presented as mean ± SD for eight replicates. The sample numer corresponds to the walnut tree number.

Sample number	Urease activity [µg N-NH_3/g/24 h]		Decrease of urease activity [%]
	beyond the crown	beneath the crown	
1	482 ± 12	458 ± 28	5
2	475 ± 10	439 ± 21	8
3	435 ± 31	374 ± 20	14
4	571 ± 22	486 ± 14	15
5	672 ± 35	428 ± 10	36

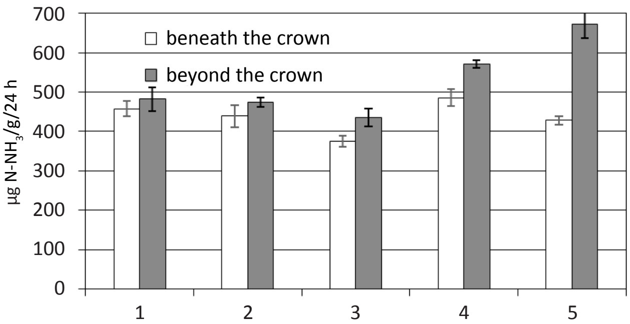

Figure 2. Activity of the soil urease in the vicinity of walnut trees: beneath and beyond the crown. Each numbers represents the respective walnut tree number (see Table 1). All values are presented as mean ± SD for eight replicates.

Table 3. Acidity of the soil in the vicinity of walnut trees: beneath and beyond the crown.

Sample number	Acidy of soil			
	beneath the crown		beyond the crown	
	pH_{KCl}	pH_{H2O}	pH_{KCl}	pH_{H2O}
1	6.71	7.33	6.60	7.24
2	5.86	6.42	6.03	6.54
3	6.00	6.34	6.04	6.99
4	6.62	7.40	6.41	7.10
5	5.56	6.04	5.95	6.75

fact is a non direct influence of juglone on the growth of plants in the area surrounding walnut trees.

CONCLUSIONS

1. The activity of soil urease is affected by the proximity of walnut trees.
2. Proximity to walnut trees decreases the activity of soil urease.
3. The greatest difference in urease activity beneath and beyond the crown of the walnut tree was observed for the oldest walnut tree. This indicates that the development of the tree increases influence on urease.
4. This study did not show correlation between soil urease activity and soil pH, nor active acidity neither exchange acidity.

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