



Allelopathic influence of common buckwheat root residues on selected weed species

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Received: 27 August 2018 / Revised: 27 December 2018 / Accepted: 7 May 2019 / Published online: 11 May 2019
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Abstract

This study was conducted to determine if common buckwheat (*Fagopyrum esculentum* Moench) root residues (BRR) in soil are effective in limiting the growth and metabolic responses of barnyard grass (*Echinochloa crus-galli* (L.) P. Beauv.), wind grass (*Apera spica-venti* L.), cleavers (*Galium aparine* L.), and tiny vetch (*Vicia hirsuta* L.). After removal of above ground parts, BRR of 14-day-old buckwheat plants remained in soil for an additional 7 days when the weeds were seeded. After 30 days of growth, biomass of above ground parts of the weeds as well as free and bound phenolic acids and flavonoids were determined. Antioxidant capacity and peroxidase activity were measured in barnyard grass and cleavers. The biomass of 30-day-old plants of barnyard grass and cleavers grown in bare soil was approximately 5- and 3.5-fold higher, respectively, than in plants grown in the presence of BRR. BRR did not affect the biomass of wind grass and tiny vetch plants, but activate the antioxidant response, increase in peroxidase activity, and the content of phenolic compounds in weed tissues, which indicates an adaptation to the stressful environmental conditions.

Keywords *Fagopyrum esculentum* · Allelopathy · Growth · Phenolic acids · Flavonoids

Introduction

The influence of common buckwheat (*Fagopyrum esculentum* Moench) on other plants has been known for hundreds of years (Tominaga and Uezu 1995; Kalinova et al. 2005; Golisz et al. 2007b, reviewed by Falquet et al. 2015). Buckwheat tissues contain numerous compounds with potential allelopathic properties (Iqbal et al. 2002; Kalinova et al. 2007; Golisz et al. 2007a). It is generally believed that

various phenolic acids and flavonoids are responsible for the weed suppressive activity of buckwheat (Kalinova et al. 2005; Golisz et al. 2007b; Kalinova and Vrchotova 2009). In addition, it has been reported that the suppression of some weeds is caused by the light competition of rapidly growing buckwheat plants (Bicksler and Masiunas 2009; Björkman and Shail 2013).

Studies on the allelopathic properties of plants are often carried out using aqueous, alcoholic, or other extracts for germination and/or the growth of seedlings of crop plants as well as weeds (Golisz et al. 2007b). Buckwheat extracts enhanced the content of phenolic compounds and flavonoids in tomato and maize tissues, which may be related to activation of the defense mechanism to stressful conditions (Szwed et al. 2014). There are other compounds in buckwheat that may have allelopathic efficiency (Kalinova et al. 2007, 2011; Szwed et al. 2014). It is known that the presence of buckwheat residues in soil can suppress various weeds (Tsuzuki and Dong 2003; Xuan and Tsuzuki 2004; Kato-Noguchi et al. 2007; Kumar et al. 2008, 2009).

Data available in the literature indicate that the accumulation of reactive oxygen species (ROS) in plant cells is under the influence of many allelopathic compounds present in the environment (Pergo and Ishii-Iwamoto 2011; Kaur

Communicated by A. Gniazdowska-Piekarska.

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s11738-019-2885-y>) contains supplementary material, which is available to authorized users.

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et al. 2012; Chi et al. 2013). Research conducted by Kaur et al. (2012) showed that essential oils produced by redstem wormwood (*Artemisia scoparia* Waldst. & Kitam.) resulted in the generation of ROS in the roots of 5-day wheat seedlings, increased lipid peroxidation, loss of membrane permeability, and death of root cells. Activation of antioxidant mechanisms was found to be under the influence of phenolic compounds and flavonoids (Pergo and Ishii-Iwamoto 2011; Mahmood et al. 2013; Ahuja et al. 2015).

The various compounds present in buckwheat plants are responsible for the allelopathic properties: flavonoids, phenolic acids, fatty acids, and alkaloids. Golisz et al. (2007b) found a high content of flavonoids and phenolic acids in Polish buckwheat cultivars, and they believe that rutin is the main allelopathic compound of these cultivars. Above ground tissues of buckwheat also contain gallic acid which caused a stronger inhibiting effect on the growth of dicotyledonous species than of grass species (Iqbal et al. 2003; Kato-Noguchi et al. 2007). In addition to flavonoids and phenolic acids, 4-hydroxyacetophenone was also identified in buckwheat root exudates and in extracts of soil after buckwheat cultivation (Kalinova et al. 2007). Tsuzuki and Dong (2003) identified ferulic, caffeic, and chlorogenic acids, as well as fatty acids among the allelochemicals in buckwheat. In other studies, eugenol, coniferyl alcohol, and 3,4,5-trimethoxyphenol were identified in buckwheat stems, leaves, and roots (Kalinova et al. 2011). In the above ground parts of buckwheat, Iqbal et al. (2002) found the alkaloids fagomine, 4-piperidone, and 2-piperidinomethanol which showed a strong inhibitory effect on lettuce growth. Despite many studies, there is no conclusive evidence of which compounds are directly responsible for the phenomenon of allelopathy in buckwheat. Probably, the whole set of compounds present in buckwheat tissues is involved in this phenomenon, along with microorganisms present in the soil (Gfeller et al. 2018). Moreover, Kumar et al. (2008) suggested that the mechanisms by which buckwheat residue can suppress weeds may include biological control of fungal pathogens and decreased nitrogen availability.

Some barnyard grass biotypes have evolved cross resistance to some herbicides, including imazamox, imazethapyr, and penoxsulam (Riar et al. 2013). Wind grass (loose silky bentgrass) (*Apera spica-venti* L.) is an important weed in Europe with an increasing number of herbicide-resistant populations (Babineau et al. 2017). Therefore, it is important to identify alternative methods to combat this weed. Tominaga and Uezu (1995) showed that the inhibition of plant growth by extracts from the soil in which buckwheat grew inhibited root elongation of barnyard grass and purslane (*Portulaca oleracea* L.), but had no effect on the growth of *Galinsoga ciliata* (Raf.) S.F. Blake.

Common buckwheat can alter its root exudation after weed recognition and suppresses its growth, as was found

for *Amaranthus retroflexus* (Gfeller et al. 2018). Kumar et al. (2008) reported that buckwheat residues in soil suppress Powell amaranth (*Amaranthus powellii* S. Wats.), shepherd's-purse (*Capsella bursa-pastoris* L.), and corn chamomile (*Anthemis arvensis* L.).

In our preliminary screening tests, whole buckwheat plant residues as well as the root residues alone affected germination, growth, and biomass of several weed species. These preliminary tests revealed a much stronger allelopathic effect of the buckwheat roots alone without the aerial parts. Among the economically important weeds tested, two species, barnyard grass, and cleavers were particularly sensitive to buckwheat root residues in the soil, and two other weed species, wind grass and tiny vetch, were resistant. It has been hypothesized that the remains of buckwheat in the soil will have a lower impact on the content of antioxidants in the tissues of species that are not resistant to these residues than in resistant ones.

The aim of our study was to identify links between some metabolic indicators including the concentration of phenolic acids and flavonoids, peroxidase activity, and antioxidant potential and the differential tolerance of four weed species to buckwheat root residues that remain in the soil. These indicators are related to the scope of oxidative stress in plants.

Materials and methods

Plant material and growth conditions

We examined monocotyledonous and dicotyledonous weed species that occur in Europe and the USA and have strong competitive interactions. The monocotyledonous weeds studied were barnyard grass [*Echinochloa crus-galli* (L.) P. Beauv.; EPPO code ECHCG] and wind grass (*A. spica-venti* L.; EPPO code APESV), and the dicotyledonous weeds were cleavers (bedstraw and catchweed) (*Galium aparine* L.; EPPO code GALAP) and tiny vetch (*Vicia hirsuta* L.; EPPO code VICH). Weed seeds were collected from plants found in fields of cereals and root crops near Radzyń Podlaski (Eastern Poland). Breaking of seed dormancy in the case of barnyard grass and cleavers was performed by a 2-week wet stratification. The vetch seeds were scarified in concentrated sulphuric acid for 30 min and then rinsed five times with distilled water. After that, the seeds were sterilized for 2 min in 70% ethyl alcohol and then for 10 min in 5% sodium hypochlorite with a drop of detergent. Subsequently, the seeds were rinsed once in 0.01 N HCl and three times with distilled each time water, within 10 min. Next, the seeds were stored at room temperature in the dark and dry conditions until used in this study.

The study was conducted in air-conditioned plant growth room and focused on the impact of soil containing buckwheat root residues (BRR) on the growth of the weed species by measuring the mean weight of the plant (biomass). Common buckwheat (*F. esculentum* Moench, cv. Hruszowska) seeds were sown in four containers (80 × 15 × 15 cm; length × width × depth) filled with commercial horticultural soil (6 kg; 18 dm³, HIT-TORF, pH 6.48; 6.9 g N kg⁻¹; 0.65 g P kg⁻¹, Poland). Temperature in the air-conditioned plant growth room was maintained at 24 ± 2 °C during days (16 h) and 18 ± 2 °C during nights (8 h). PAR (ca. 100 μmol m⁻² s⁻¹) was provided by high-pressure sodium lamps (400 W, Plantaster, Osram, Germany). The containers with growing buckwheat were watered every morning. After 14 days of buckwheat plant growth, the aboveground parts of buckwheat plants were removed, but the roots remained in the soil for an additional 7 days when weed seeds were sown (100–120 per container). The initial root biomass in soil was 193 ± 32 g in one container. To soil of another group of containers, whole buckwheat plants were added after they have been cut into small parts. The biomass of whole plants in one container was, on average, 521 ± 44 g. Each weed species was seeded in different sets of two containers for each treatment after 7-day decomposition of buckwheat in soil. The control soil containers without buckwheat plants and roots were incubated under identical conditions.

Assay of biomass, antioxidant capacity, and peroxidase activity

The mean biomass per plant of above ground parts of weeds was determined 30 days after sowing. Each combination (control, soil with remnants of buckwheat) contained two containers in which weed seeds were sown. The biomass of one plant was determined by dividing the weight of all plants by their number. The biomass measurements were made in four replications, with one replicate being the average mass of aboveground parts of one plant growing in one half of each container.

The antioxidant capacity and peroxidase activity also were determined in fresh tissues of barnyard grass and cleavers. The total antioxidant capacity was measured using the DPPH[•] radical reduction method (1,1-diphenyl-2-picrylhydrazyl, Sigma), according to which its quenching is associated with a change in the color of the solution from dark violet to yellow (Brand-Williams et al. 1995).

Peroxidase activity was determined according to the method described by Velikova et al. (2000). This method is based on the oxidation of guaiacol by hydrogen peroxide, resulting in the formation of a red–brown color, whose concentration was measured spectrophotometrically.

The degree of plasma membrane damage, expressed as leakage of electrolytes, was determined by the method

described by Uddin et al. (2014) using a conductivity meter (HACH conductivity/TDS meter 44600-00, Poland).

Determination of free and bound phenolic acids and flavonoids

Plant samples were freeze-dried (Christ Alpha 1–2 LD+, Germany), and pulverized tissues were analyzed by HPLC–MS/MS for the concentration of various forms of phenolic acids and flavonoids (free, esters, and glycosides). The profile and content of phenolic acids and flavonoids were determined according to the modified method of Wiczkowski et al. (2016). Briefly, a crude extract was obtained from 0.2-g freeze-dried plant samples by extraction using thermomixer with mixture of methanol, water, and formic acid 80:19.9:0.1 (v/v). The extraction was repeated five times, and the obtained crude extracts were collected. Free forms of phenolic acids and flavonoids were isolated with diethyl ether after adjusting the initial extract to pH 2 with 6 M HCl. Esters were hydrolyzed in nitrogen atmosphere for 4 h at room temperature with 4 M NaOH. Subsequently, glycosides were hydrolyzed in the residues with 6 M HCl for 1 h at 100 °C. After adjusting to pH 2, free forms of phenolics released from glycosides and esters were extracted with diethyl ether. The all extractions were carried out in triplicates by use of sonication and centrifugation, and the obtained ether extracts were evaporated to dryness under stream of nitrogen at 35 °C. The phenolics, both free and released form bound forms, were dissolved in 80% methanol, and centrifuged and subjected to HPLC–MS/MS analysis. Aliquots of extracts were injected into an HPLC system equipped with an HALO C₁₈ column (2.7 μm particles, 0.5 × 50 mm, Eksigent, USA) at 45 °C at a flow rate of 15 μL min⁻¹. The elution solvents were A (water/formic acid; 99.05/0.95; v/v) and B (acetonitrile/formic acid, 99.05/0.95, v/v). The gradient was used as follows: 5% B for 0.1 min, 5–90% B in 1.9 min, 90% B for 0.5 min, 90–5% B in 0.2 min, and 5% B for 0.3 min. For HPLC–MS/MS analysis, a QTRAP 5500 ion trap mass spectrometer (AB SCIEX, USA) was used. Optimal ESI-MS/MS conditions including nitrogen curtain gas, collision gas, ion spray source voltage, temperature, nebulizer gas, and turbo gas were as follows: 25 L min⁻¹, 9 L min⁻¹, –4500 V, 350 °C, 35 L min⁻¹, and 30 L min⁻¹, respectively. Qualitative and quantitative analyses were conducted in the negative mode by multiple reaction monitoring (MRM) of selected ions in the first quadrupole (Q1) and third quadrupole (Q3). The transitions for ferulic acid, *p*-coumaric acid, chlorogenic acid, caffeic acid, sinapic acid, vanillic and *iso*-vanillic acid, protocatechuic acid, syringic acid, luteolin, apigenin, kaempferol, and quercetin were 193.1/134.1, 163.1/119.1, 353.2/191.1, 179.1/135.1, 223.1/164.1, 167.1/108.0, 153.0/109.0, 197.0/182.0, 285.1–133.1, 269.0–151.1, 285.0/93.0, and 301.1/179.1

(m/z), respectively. Every compound was quantified based on the HPLC–MS/MS peak area at the appropriate MRM according to the corresponding linear calibration curves (0.01 – $0.5 \mu\text{g mL}^{-1}$).

Statistical analyses

Two-way analysis of variance (ANOVA) and Tukey's post hoc test were used to check the significance of differences in the biomass of aboveground parts of weed species, and chemical analyses of phenolic acids, flavonoids (free, and bound), antioxidant activity, and peroxidase activity. Treatment effects were regarded as statistically significant at $P < 0.05$. The phenolic compounds, total antioxidant capacity, peroxidase activity, and leakage of electrolytes were analyzed in triplicate. Biomass measurements were made in four replications, with one replicate being the average mass of aboveground parts of one plant growing in one half of container. Calculations were performed using the Statistica 12 software package (StatSoft, Poland).

Results

Buckwheat root residues (BRR) present in the soil inhibited growth of barnyard grass and cleavers, no effect was observed in wind grass, and the mean weight (biomass) of tiny vetch plants increased by BRR in soil (Fig. 1). Inhibition of growth in two species was evident, because, in control treatments, the biomass of 30-day-old plants of barnyard grass and cleavers were approximately 5- and 3.5-fold higher, respectively, than in these plants grown in the presence of BRR. However, the addition of entire plants to the soil (roots and aboveground parts) did not significantly affect the biomass of the three weed species studied. In the case of wind grass, even growth stimulation was recorded, but this

result is uncertain due to the poor germination of the seeds of this species.

In the examined weed species, the presence of *trans*-cinnamic acid (t-CA) and its derivatives (*p*-coumaric acid, synaptic, caffeic, ferulic, and chlorogenic) and benzoic acid derivatives (protocatechuic, syringic, vanillic, and *iso*-vanillic) have been demonstrated (Table 1; Supplemental Tables S1, S2, S3). The quantitatively dominate acid was t-CA, the concentration of which ranged from 2100 to $7700 \mu\text{g g}^{-1}$ of dry matter (Supplemental Table S1). This acid was present in free and bound forms as conjugated esters and glycosides. Relative to the control treatment, the presence of BRR resulted in a twofold increase in the total concentration and formed in cleavers and wind grass. In cleavers and in wind grass, respectively, a significant increase in the concentration of t-CA glycosides was found 8 and 28 times higher than those in control plants.

Among the *trans*-cinnamic acid derivatives, the highest concentration in the leaves of the evaluated species was caffeic acid, and the lowest concentration was sinapic acid (Supplemental Tables S2 and S3). The influence of BRR on accumulation of caffeic acid and sinapic acid was ambiguous and depended on the plant species and the acid being evaluated. However, the concentration of their glycosides increased in most species, but to a lesser extent than glycosides of *trans*-cinnamic acid (Supplemental Table S1). The BRR in the soil caused an increase in the total content of phenolic acids in the leaves of all weed species studied (Table 2). The content of free phenolic acids was increased by BRR in three of the four weed species, with the exception of tiny vetch. In the case of glycosides of phenolic acids, a particularly large effect of BRR occurred in the leaves of cleavers and wind grass. The presence of BRR increased glycoside content 17-fold in cleavers and 27-fold in wind grass. The impact of the BRR led to an increase in the content of benzoic acid derivatives in the leaves of barnyard grass and cleavers (Table 2; Supplemental Tables S2 and S3). In the

Fig. 1 Biomass (mean weight of plant aboveground parts \pm SD) of 30-day-old plants of four weed species grown in the soil with common buckwheat residues and without such residues (control). (1) Barnyard grass [*E. crus-galli* (L.) P. Beauv.]; (2) cleavers (*G. aparine* L.); (3) wind grass (*A. spica-venti* L.); (4) tiny vetch (*V. hirsuta* L.). Results with the same letter were not significantly different at $P < 0.05$ (post hoc Tukey's test)

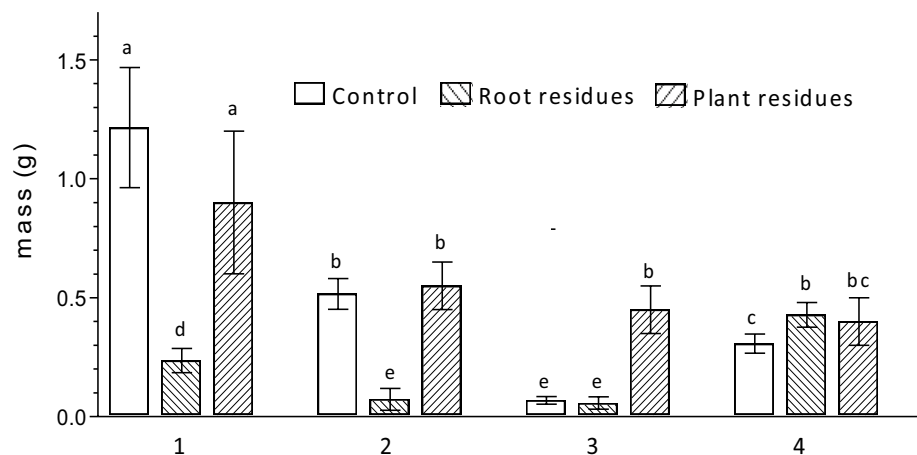


Table 1 Concentration of total free and bound phenolic acids in the leaves of four weed species grown in the control soil and soil-contained buckwheat root residues (BRR)

Treatment	Barnyard grass (<i>E. crus-galli</i> (L.) P. Beauv.)	Cleavers (<i>G. aparine</i> L.)	Wind grass (<i>A. spica-venti</i> L.)	Tiny vetch (<i>V. hirsuta</i> L.)
Total free phenolic acids (F)				
Control	102.2 ± 4.1 ^{b,†}	1624 ± 76 ^b	1375 ± 80 ^b	32.70 ± 0.20 ^a
BRR	222.9 ± 8.9 ^a	3129 ± 91 ^a	2191 ± 26 ^a	31.56 ± 0.82 ^a
Total phenolic acid esters (E)				
Control	2165 ± 20 ^b	2553 ± 100 ^a	2172 ± 82 ^a	109.7 ± 3.5 ^b
BRR	2596 ± 128 ^a	2614 ± 63 ^a	1586 ± 8.2 ^b	130.6 ± 2.8 ^a
Total phenolic acid glycosides (G)				
Control	145.3 ± 3.3 ^a	155.2 ± 4.3 ^b	138.0 ± 1.8 ^b	2454 ± 34 ^b
BRR	103.0 ± 4.0 ^b	2712 ± 87 ^a	3771 ± 245 ^a	3471 ± 9.1 ^a
Total phenolic acids (F+E+G)				
Control	2413 ± 27 ^b	4332 ± 172 ^b	3685 ± 40 ^b	2596 ± 38 ^b
BRR	2922 ± 133 ^a	8455 ± 242 ^a	7549 ± 211 ^a	3633 ± 11 ^a

[†]All data are presented as the mean ± SD. Results with the same letter were not significantly different at $P < 0.05$ (post hoc Tukey's test). Comparisons were made between control and buckwheat root residues treatments (BRR) within each combination of total free and bound phenolic acids and within each weed species

Table 2 Concentration of total *trans*-cinnamic acid, *trans*-cinnamic acid derivatives, benzoic acid derivatives, and flavonoids ($\mu\text{g g}^{-1}$ dry weight) in the leaves of four weed species grown in the control soil and soil-contained buckwheat root residues (BRR)

Treatment	Barnyard grass (<i>E. crus-galli</i> (L.) P. Beauv.)	Cleavers (<i>G. aparine</i> L.)	Wind grass (<i>A. spica-venti</i> L.)	Tiny vetch (<i>V. hirsuta</i> L.)
Total <i>trans</i> -cinnamic acid				
Control	2130 ± 35 ^a	3805 ± 174 ^b	3549 ± 5.8 ^b	2354 ± 35 ^b
BRR	2430 ± 131 ^a	7737 ± 237 ^a	7393 ± 209 ^a	2923 ± 8.3 ^a
Total <i>trans</i> -cinnamic acid derivatives				
Control	92.4 ± 2.1 ^a	215.1 ± 3.9 ^b	70.4 ± 0.7 ^a	81.1 ± 1.5 ^b
BRR	88.9 ± 1.0 ^a	372.2 ± 2.0 ^a	62.0 ± 0.1 ^b	240.9 ± 0.3 ^a
Total benzoic acid derivatives				
Control	190.6 ± 5.7 ^{b,†}	312.1 ± 6.1 ^b	66.5 ± 1.1 ^b	161.6 ± 4.4 ^b
BRR	403.6 ± 3.8 ^a	346.0 ± 2.2 ^a	94.8 ± 2.2 ^a	469.2 ± 3.3 ^a
Total flavonoids				
Control	7.0 ± 0.1 ^b	6.6 ± 0.1 ^b	7.9 ± 0.1 ^b	389.3 ± 11.0 ^b
BRR	10.4 ± 0.1 ^a	17.5 ± 0.0 ^a	14.5 ± 0.5 ^a	563.0 ± 15.0 ^a

[†]All data are presented as the mean ± SD. Results with the same letter were not significantly different at $P < 0.05$ (post hoc Tukey's test). Comparisons were made between control and buckwheat root residues treatments (BRR) within total benzoic acid derivatives or within total *trans*-cinnamic acid derivatives and within each weed species

tissues of wind grass, a decrease in the concentration of t-CA derivatives was observed, and in barnyard grass, it reached a concentration close to the control.

In the leaves of the evaluated weed species, the presence of free and bound forms of quercetin, kaempferol, apigenin, and luteolin has been demonstrated (Supplemental Table S4). The concentration of flavonoids was low, compared to the content of phenolic acids, and did not exceed $5 \mu\text{g g}^{-1}$ of dry matter. The exception was tiny vetch leaves in which the tissue concentration of apigenin exceeded $380 \mu\text{g g}^{-1}$. Under the influence of BRR in the soil, an increase in the concentration of flavonoid glycosides occurred in tissues of all species (Supplemental

Table S4). While the quercetin concentration increased in tissues of barnyard grass and cleavers, the quercetin concentration decreased in leaves of tiny vetch and remained unchanged in leaves of wind grass.

Inhibition of the biomass accumulation of weed species in the presence of BRR in the soil was associated with an increase in the concentration of *trans*-cinnamic acid and total phenolic acids in leaves as confirmed by Pearson's correlation coefficients (r). Statistical analysis of the data showed a negative significant correlation between the biomass of seedlings of the all examined weed species and content of *trans*-cinnamic acid ($r = -0.555$; $P = 0.05$),

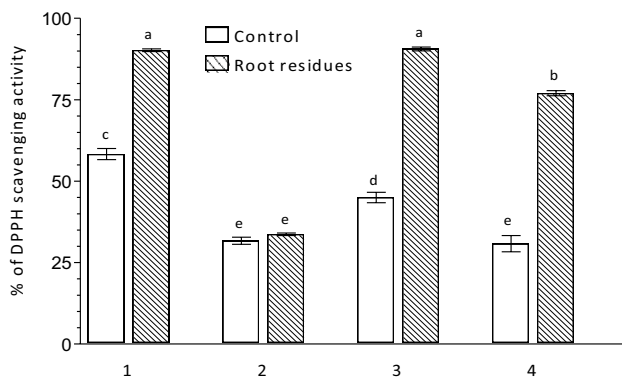


Fig. 2 Antioxidant activity in tissues of 30-day-old plants of weed species grown in the soil with common buckwheat root residues and without such residues (control). (1) and (2) Barnyard grass [*E. crus-galli* (L.) P. Beauv.] leaves and epicotyls, respectively; and cleavers (*G. aparine* L.) leaves (3) and hypocotyls (4), respectively. Results with the same letter were not significantly different at $P < 0.05$ (post hoc Tukey's test)

as well as between the biomass and total phenolic acids ($r = -0.546$; $P = 0.05$).

To confirm the occurrence of oxidative stress, measurements of antioxidant potential through the rate of reduction of radical DPPH in tissues of BRR-sensitive species were conducted (Fig. 2). In the leaves and hypocotyls of cleavers under the influence of BRR, a more than twofold increase in the antioxidant potential was found. In the case of the barnyard grass leaves, this increase was over 34%, while, in the epicotyl of this species, the BRR did not affect the antioxidant potential.

During the assessment of BRR on the level of peroxidase activity, different reactions were observed in the tissues of both weed species (Fig. 3). The presence of BRR caused an increase in peroxidase activity in barnyard grass leaves and epicotyls. In the tissues of cleavers, this response was different, because BRR did not affect peroxidase activity in the leaves, whereas a decrease in peroxidase activity occurred in the hypocotyl tissues.

Discussion

Plant allelopathy is the process whereby organic compounds released from one species affect the growth and development of other plants. Mechanisms by which allelochemicals can suppress weeds directly may be accompanied by indirect effects due to control of fungal pathogens and decreased nitrogen availability (Kumar et al. 2009). Weed suppression can be achieved by changes in weed emergence, biomass, seed set, and seed bank (Liebman and Davis 2000).

The results of our research show the reduction of biomass of two weed species, barnyard grass and cleavers, under the

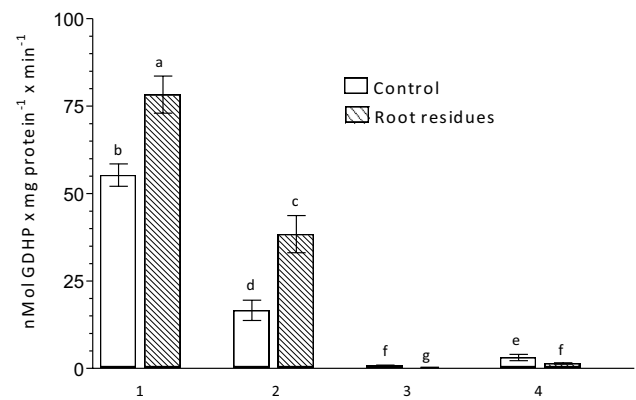


Fig. 3 Antioxidant activity in tissues of 30-day-old plants of weed species grown in the soil with common buckwheat root residues and without such residues (control). (1) and (2) Barnyard grass [*E. crus-galli* (L.) P. Beauv.] leaves and epicotyls, respectively; and cleavers (*G. aparine* L.) leaves (3) and hypocotyls (4), respectively. Results with the same letter were not significantly different at $P < 0.05$ (post hoc Tukey's test)

influence of buckwheat root residues (BRR), and the lack of that effect when residues of entire plants of buckwheat occurred in soil (Fig. 1). Such a phenomenon is reported for the first time, and its causes are unknown. Tominaga and Uezu (1995) previously noticed that the presence of buckwheat seedlings clearly inhibited the growth of barnyard grass growing next to it. In addition, Kumar et al. (2008) have shown that residues of whole buckwheat plants in the soil suppressed the growth of Powell amaranth (*A. powellii* S. Wats.), shepherd's-purse (*C. bursa-pastoris* L.), and corn chamomile (*A. arvensis* L.), and reported that the biomass of all three weeds was reduced in buckwheat residue compared to bare soil.

It is widely believed that allelochemicals influence the level of non-enzymatic antioxidants and increase the activity of enzymes (Ding et al. 2007; Pergo and Ishii-Iwamoto 2011; García-Sánchez et al. 2012; Ahuja et al. 2015). The major group of non-enzymatic antioxidants are polyphenolic compounds whose role is the removal of reactive oxygen species which affects many metabolic processes in cells (Cruz-Ortega et al. 2007). The results of our study demonstrated that the presence of BRR in the soil resulted in a significantly increased concentration of glycosides of phenolic acids and flavonoids in the leaves of the weeds (Tables 1, 2; Supplemental Tables 1–5). Among the phenolic acids, this phenomenon increased mainly the *trans*-cinnamic acid and benzoic acid derivatives and, to a lesser extent, the cinnamic acid derivatives. It is not clear what functions the glycoside forms of phenolics induce under the influence of these stress factors. According to Politycka (1996), the level of phenolic glycosides correlated inversely with membrane stability, which may indicate that a high content of phenolics may

promote the leakage of electrolytes. However, our studies did not confirm the occurrence of this phenomenon.

In general, BRR increases the level of phenolic acids and flavonoids to a greater extent in barnyard grass and cleavers and to a lesser extent in wind grass and tiny vetch (Tables 1, 2; Supplemental Tables 1–5). Increasing the concentration of phenolic acids and flavonoids in the leaves of weeds not sensitive to BRR indicates that these plants also may be subjected to oxidative stress caused by this factor. However, it does not inhibit their growth, probably because the defense mechanisms in wind grass and tiny vetch seem to be more effective than in barnyard grass and cleavers. Calculated negative correlation between the content of *trans*-cinnamic acid and plant biomass, as well as between the total phenolic acids and plant biomass suggests that the concentration of some polyphenols can be an indicator of the allelopathic influence of BRR on weed species. The increase in the content of phenolic compounds occurred both in the species of weeds sensitive to the remains of buckwheat roots as well as not being affected by it. This means that the allelopathic stress arises in the tissues of all species tested. However, various anti-stress defense mechanisms cause that growth is inhibited in some of them, while, in others, there was no such influence.

An important part of the allelopathic mechanism is the induction of oxidative stress and the disruption of the redox status in the tissues of acceptor plants (Cruz-Ortega et al. 2007). The results obtained in our study showed that the presence of BRR in the soil increased the total antioxidant capacity in the leaves of barnyard grass as well as leaves and hypocotyls of cleavers (Fig. 2). No difference was found compared to the control plants only in barnyard grass epicotyl tissues after 30 days of growth in the presence of BRR. According to Dudonné et al. (2009), the antioxidant activity of plant extracts correlated with the concentration of phenolic compounds. Our results confirmed that the increase in total antioxidant capacity was associated with an increased accumulation of polyphenolic compounds in tissues of the weeds growing in soil with BRR.

An important component of the enzymatic defense against reactive oxygen species (ROS) is peroxidases, which catalyze the decomposition reaction of hydrogen peroxide (Gechev et al. 2006). Increased peroxidase activity in plants growing in soil with BRR probably is related to the enhanced level of the ROS (Fig. 3). Higher peroxidase activity in barnyard grass growing in the soil containing BRR was confirmed in our study. However, this relationship was not observed in cleavers. A different reaction of both species to allelochemical stress may be associated with the activation of various defense mechanisms against oxidative stress. It is feasible; such distinct response is consequence of both, complex composition of BRR released into the soil and indirect role of microorganism taking part in allelochemicals decay

in the soil. The main reason for these differences may also be related to the unknown role of soil microorganisms (Kaur et al. 2009). The previous studies also pointed to the association of peroxidase activity with the presence of allelopathic compounds in the soil (Amist et al. 2014). Increased peroxidase activity resulting from solutions of pure phenolic compounds such *p*-hydroxybenzoic acid or coumarin has been reported (Ahrabi et al. 2011). Improving the efficiency of the mechanisms of neutralization of ROS by peroxidase may additionally be associated with an increase in the concentration of phenolic compounds which enhances the antioxidant capacity of plants (Grace and Logan 2000). According to Yamasaki et al. (1997), flavonoids can be an electron donor in these reactions.

Conclusions

The results presented here show inhibition of biomass accumulation of two weed species, barnyard grass, and cleavers, by the presence of buckwheat root residues in the soil. The observed inhibition of biomass accumulation was associated with some disturbances of secondary metabolism in tissues of weed species. Under conditions of allelochemical stress, activation of the antioxidant response, increase in peroxidase activity, and increase in the content of phenolic compounds in weed tissues were observed, which indicates an adaptation to the stressful environmental conditions. As stated here, the results reveal an allelopathic role of common buckwheat roots, and provide the basis for further studies enabling the use of common buckwheat residues for weed management.

Author contribution statement MS carried out plant experiments and basic analyses; WW and DS-N carried out LC–MS analyses of phenolic compounds; RLO and MH contributed to data analysis and preparing of manuscript. All authors read and approved the final manuscript.

Acknowledgements This work was supported in part by Ministry of Science and Higher Education (Poland) as a part of the statutory activities of the Faculty of Natural Sciences at the Siedlce University of Natural Sciences and Humanities (No. 13/91/S).

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