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Level of Catechin, Myricetin, Quercetin and Isoquercitrin in Buckwheat (*Fagopyrum esculentum* Moench), Changes of Their Levels during Vegetation and Their Effect on The Growth of Selected Weeds

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Buckwheat is well-known as a crop rich in flavonoids, however, attention has usually only been paid to the main flavonoid rutin as an important natural antioxidant or as a possible allelopathic compound. Therefore, some of the other constituents found within individual plant parts of buckwheat (isoquercitrin, quercetin, catechin, and myricetin), as well as changes of their level during the growing season, were determined by HPLC analysis. The effects of these compounds on plant growth were proved on seven plant species. In buckwheat, isoquercitrin represented the largest component of the selected compounds. The strongest inhibitive effects on the growth of those selected plants were produced by catechin. Quercetin and isoquercitrin had weak inhibitive effects. Myricetin did not show any influence on plant growth. Hence we suppose that myricetin, isoquercetin and quercetin do not have important function in allelopathy of buckwheat. Buckwheat as row material for functional foods could be a significant source of another antioxidant, isoquercitrin.

KEYWORDS: Allelopathy; buckwheat; catechin; developmental stage; quercetin 3-*O*-*β*-D-glucoside; myricetin; quercetin; weed

INTRODUCTION

Flavonoids have attracted attention, primarily as natural antioxidants, for the prevention of diseases of advanced-age such as atherosclerosis and cancer, as well as coronary and heart diseases. However, flavonoids are necessary for fertility and normal pollen development; they influence auxin transport and play an important function in the interactions between plants and other organisms. Flavonoids are one of many allelopathic agents that plants produce in order to reduce competition (*1*). Common buckwheat (*Fagopyrum esculentum* Moench, family Polygonaceae) is one of the plants rich in flavonoids, and also one of the crops with allelopathic potential (*2, 3*). Buckwheat is known for its high rutin (quercetin 3-rutinoside) level, but also contains other flavonoids, such as quercetin, hyperoside (quercetin 3-*O*-*β*-D-galactoside), quercitrin (quercetin 3-*O*-α-L-rhamnoside), epicatechin, orientin, vitexin, isovitexin, and isoorientin (*4*−*6*).

Rutin, quercetin, quercitrin, and kaempferol have been suggested as possible allelopathic compounds released by plant roots (*7*). Rutin exudation by the roots of buckwheat was established by Kalinova et al. (*8*). Additionally, due to its established phytotoxicity, rutin has been suggested as one of the possible allelopathic compounds in buckwheat (*3*). However, the source of these allelopathic compounds is not only from the root exudates but also from crop residues, which could play a more important role in agriculture utilization through applications such as mulch, green manure, or incorporation as plant pellets into the soil. Quercetin and isoquercitrin are precursors in the biosynthesis of rutin which is probably formed by the 3-glycosylation of quercetin following the rhamnosylation of isoquercitrin (*9*). Therefore, quercetin and isoquercitrin (quercetin 3-*O*-*β*-D-glucoside) could arise from rutin by the decomposition of buckwheat plant parts in the soil. As a result, they can contribute to or decrease the allelopathic effect of buckwheat residues. The amount of isoquercitrin in different plant parts of common buckwheat had not previously been determined. Catechin, another of the flavonoids, was suggested as a possible allelopathic compound of *Centaurea maculosa* (*10, 11*). Catechin was also identified in the green parts of buckwheat by Iqbal et al. (*12*). Myricetin had not previously been identified in common buckwheat.

The aim of this work was to determine the level of selected flavonoids (quercetin, isoquercitrin, myricetin and catechin) in different parts of buckwheat, their level changes during development, and their effect on the growth of selected weeds.

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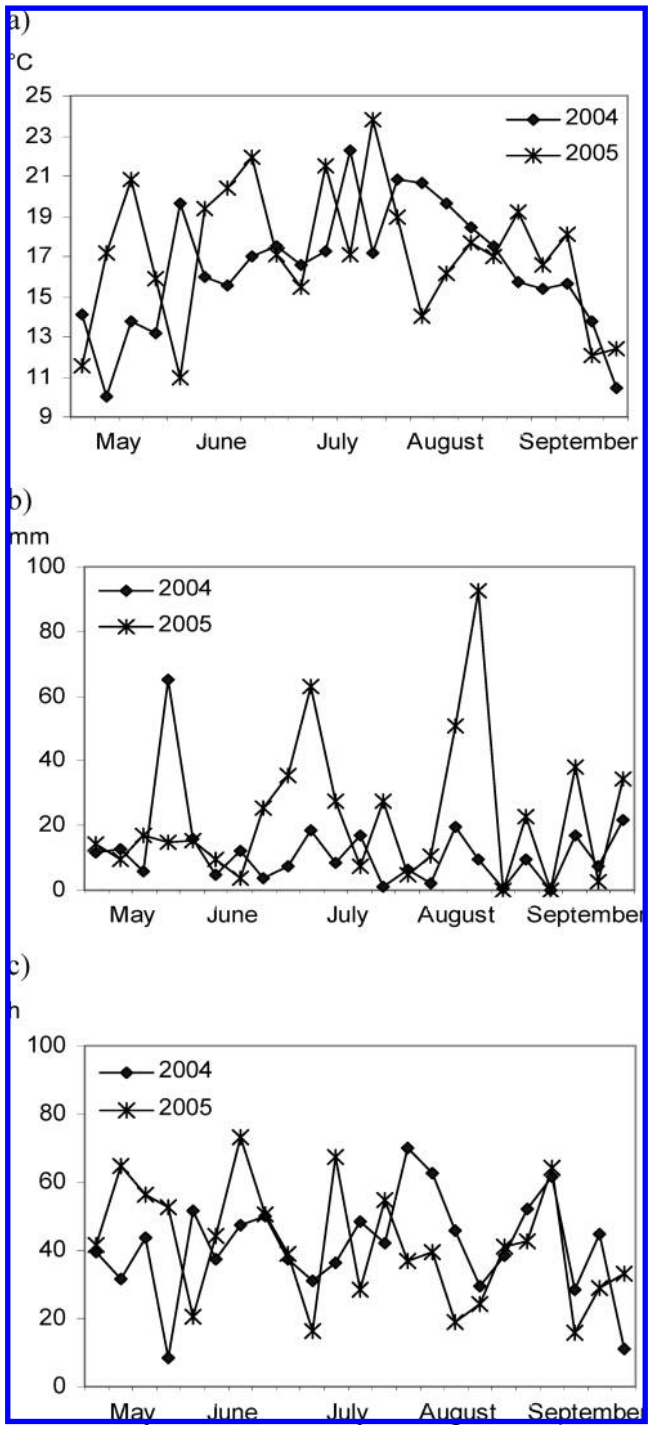

Figure 1. Development of (**a**) main daily temperature; (**b**) precipitation; and (**c**) hours of sunshine during the growing period in 2004 and 2005.

MATERIALS AND METHODS

Materials. Common buckwheat *Fagopyrum esculentum* Moench (varieties Pyra, Emka, Krupinka) was grown on plots (each 12.5 m^2) in Ceske Budejovice (48° 57′ 42″, 14° 28′ 05″, 380 m elevation, sandy-loam soil, pH 5.6). Achenes were sown in 12.5 cm wide rows, using a seed drill for precise drilling with a density of 200 plants/m^2 on 14th May 2004 and 25th May 2005. The total precipitation, hours of sunshine, and mean temperature are given in **Figure 1**.

Plants (25 in number) were sampled three times during the growing period: at the beginning of branching (20th and 23rd June), at full flowering (20th July and 15th July), and then at harvest time (3rd and 19th September). The plants were divided into roots, stems, leaves, and inflorescences, after which they were lyophilized, ground in a MM200 laboratory mill (Retsch, Germany), and then stored in closed containers within a freezer (−18 °C). Number of replicates was at least three.

Table 1. HPLC-DAD Features of Flavonoids

compound	RT,[a] min	LOD,[b] μg/mL	LOQ,[c] μg/mL
myricetin	36.0	0.3	1.018
catechin	10.66	0.08	0.25
quercetin	46.75	0.22	0.72
isoquercitrin	28.53	0.15	0.49

[a] Retention time at 220 nm. [b] Detection limits. [c] Limits of quantification.

HPLC Analysis. The plant sample (0.25 g dry weight (DW)) was extracted with 3 mL of 90% methanol for 30 min at room temperature, filtered through a MN GF-1 glass filter (Macherey-Nagel, Germany) and centrifuged following the procedure described by Kalinova et al. (*6*). An HP 1050 HPLC (Hewlett-Packard, USA) was equipped with a G1315B DAD detector (Agilent) and 150 mm × 2 mm i.d., 3 μm, Luna C18 (2), column (Phenomenex, USA). The mobile phase consisted of solvent A (5% acetonitrile + 0.1% *o*-phosphoric acid) and solvent B (80% acetonitrile + 0.1% *o*-phosphoric acid) with the following gradient: 5%−35% B in 55 min, 35%−60% B in 65 min. The flow rate was 0.25 mL/min. Flavonoids were identified by comparison the retention time and UV−vis absorbance profile of sample data to standards that were obtained from Sigma-Aldrich Co. (USA). The injection volume of standards and samples was 5 μL. The spectra were recorded in the range from 190 to 600 nm. Flavonoids were quantified at 220 nm by using linear calibration curves of standards (six different concentrations, correlation coefficient >0.993). Retention time (RT), detection limits (LOD) and limits of quantification (LOQ) of individual compounds are given in **Table 1**.

Preparation of Flavonoids for Bioassays. Commercially obtained standards of quercetin, (+)-catechin, isoquercitrin and myricetin were proved, according to the method described by Kalinova et al. (*8*). Each compound was diluted in 90% methanol, and was applied onto filter paper in a Petri dish. Treatment with methanol alone was used as the control. Afterward, the methanol was evaporated, and 3 mL of distilled water was added to the filter paper. The selected concentrations ranged from 10 to 0.001 mM of each compound in water.

Bioassays. Thirty seeds of each of the species (white mustard, *Sinapis alba L.*; yarrow, *Achillea millefolium* L.; ribwort plantain, *Plantago lanceolata* L.; Dutch clover, *Trifolium repens* L.; perennial ryegrass, *Lolium perenne* L.; barnyard grass, *Echinochloa crus-galli* (L) P.B.; and redroot pigweed, *Amaranthus retroflexus* L.) were sown on the filter paper with an aqueous extract of the flavonoid in a Petri dish. All Petri dishes were enclosed with parafilm and were placed in the dark at 22 ± 2 °C. After 72 h incubation, the lengths of the roots and hypocotyl were measured. There were at least three replicated plates for each of the concentrations.

Data Analysis. The following equation was used for calculation of the percentage influence of a compound: Percentage influence (%) = (length of hypocotyl or root of a plant treated with the tested compound − length of hypocotyl or root of a plant treated with distilled water as the control) × 100/control.

Statistical analyses were performed by using Statistica 6.0 software. Data were evaluated using analysis of variance (two-way model: stage × year) for each compound, with a subsequent Tukey HSD test. The effective concentration required for 50% inhibition (EC_{50}) was established, based on the fitted regression equations.

RESULTS AND DISCUSSION

The Flavonoid Level. According to Datta et al. (*13*), flavonoids are important as chemotaxonomic markers in the genus *Polygonum*, where glycosylation at C-3 of the quercetin moiety is the most common feature; this was confirmed by isolation of isoquercitrin from *P. stagninum*. We found isoquercitrin in *Fagopyrum esculentum* Moench (previously called *Polygonum fagopyrum* L). However, isoquercitrin was also identified in other representatives of the family Polygonaceae,

Table 2. Level of Catechin, Quercetin and Isoquercitrin in Different Plant Parts during the Growth Period in 2004 and 2005 (Mean of Three Varieties ± SD)

	catechin (μg/g DW)		quercetin (μg/g DW)		isoquercitrin (μg/g DW)	
plant parts	2004	2005	2004	2005	2004	2005
			Branching			
leaves	28.2 ± 16.3	nf[a]	27.5 ± 10.7	25.5 ± 5.5	795.4 ± 130.4	454.0 ± 30.4
stems	nf	nf	24.1 ± 7.0	11.1 ± 1.2	1286.8 ± 141.3	942.5 ± 151.2
roots	455.9 ± 56.4	229.1 ± 36.6	nf	nf	726.4 ± 100.2	404.1 ± 77.2
			Flowering			
leaves	94.0 ± 8.2	188.7 ± 41.9	46.4 ± 12.0	37.6 ± 9.4	1547.2 ± 149.0	1256.6 ± 83.3
stems	nf	nf	8.1 ± 1.1	7.7 ± 0.2	1990.2 ± 196.7	1188.0 ± 23.8
flowers	616.4 ± 109.8	198.8 ± 54.5	58.1 ± 15.8	122.1 ± 31.2	1520.9 ± 124.0	1883.1 ± 216.9
roots	252.2 ± 20.5	181.2 ± 22.0	nf	nf	744.7 ± 68.0	441.0 ± 17.2
			Harvest			
leaves	49.1 ± 7.7	89.4 ± 17.2	90.8 ± 9.9	832.9 ± 102.2	1283.4 ± 251.4	2648.7 ± 89.5
stems	nf	nf	10.9 ± 2.0	12.6 ± 1.7	2040.6 ± 201.3	1576.6 ± 167.5
roots	360.8 ± 39.4	115.4 ± 6.0	nf	nf	566.7 ± 74.0	402.3 ± 31.9

[a] Not found.

e.g. *Rheum nobile* (*14*). Nevertheless, probably one of the highest amounts of isoquercitrin (about 9.4 mg/g) was contained in the flowers of St. John's wort (*Hypericum perforatum* L.) (*15*).

In our study, isoquercitrin was present in buckwheat at the greatest level of all the selected compounds. The amount of isoquercitrin was higher than the epicatechin level (*6*), and thus this compound is probably the flavonoid most represented in common buckwheat, after rutin. This is important because, according to Hopia and Heinonen (*16*), isoquercitrin, quercetin, and myricetin have higher antioxidant activities than rutin.

We identified isoquercitrin in all common buckwheat plant parts (**Table 2**). According to Suzuki et al. (*9*), isoquercitrin is also contained within buckwheat achenes, with a maximal level (0.85 μg/g of tissue) on the 25th day after pollination.

Our results indicate that isoquercitrin is dominant in buckwheat stems. If we compare individual plant parts at the flowering stage, isoquercitrin concentration decreased in the following order: flowers > stems > leaves > roots. The isoquercitrin level changed significantly in both stems and leaves during the growing period (**Figure 2**). In the stems, the level increased gradually. Double the level of isoquercitrin was found in leaves at the flowering stage, in comparison to the level at the beginning of branching. After flowering, the isoquercitrin level in the leaves, on average, continued to increase until harvest; but it was influenced by the weather in the given year. Either the isoquercitrin accumulation decreased (in 2004) or increased (in 2005). In our previous study, a similar tendency was observed with the rutin level in the leaves of common buckwheat (*6*). Changes in the roots were not significant, and isoquercitrin stayed at the same level.

Differences in the weather conditions for each year significantly influenced the level of isoquercitrin in all parts, except the flowers, where the influence was not significant. Unambiguously higher level in the stems, leaves (except leaves at harvest time), and roots was established in 2004. During 2004 there was a precipitation deficit, compared to 2005 (316 vs 551 mm), especially in the period from the 15th June to 20th July, and again in August (**Figure 1**). A smaller quantity of precipitation was also connected partly with higher daily temperatures and more hours of sunshine. This period included the time of buckwheat sampling at the stage of branching and flowering. According to Saffan (*17*), the isoquercitrin level in two-week-old peanut seedlings doubled with increasing temperature (22−40 °C).

In the present study, genotypes did not show significant differences in the isoquercitrin level, except the stems, where the Pyra variety contained a significantly lower level of isoquercitrin than did the two other varieties.

We found a positive correlation between the rutin level (data not shown) (*6*) and the isoquercitrin level in leaves (0.5053, $P \leq 0.05$), as well as in the roots (0.6674, $P \leq 0.01$). These results agree with the findings of Suzuki et al. (*9*) that isoquercitrin (a product of 3-*O*-glucosyltransferase) is an intermediate for rutin biosynthesis in the plant tissues.

Catechin, a compound typical particularly for tea, has also been identified in plants from the family Polygonaceae. According to Liu et al. (*18*), rhizomes of *Polygonum bistorta* contained about 0.53% and *P. paleaceum* about 0.14% of catechin. The presence of catechin was also confirmed in *Rheum ribes* L. (*19*).

In common buckwheat, we found the highest catechin level in the flowers, a lesser amount in the roots, and the least in the leaves (**Figure 2**). According to Danila et al. (*20*), a low concentration of catechin is also contained in buckwheat achenes (3.3 mg /100 g of buckwheat flour). We did not detect this compound in the stems. However, Golisz et al. (*3*) determined 0.03 mg/g DW of catechin in the stems of *Fagopyrum esculentum* at the full-flowering stage. This could result from varietal differences. In this study, we did not confirm varietal differences in the catechin level in any parts of buckwheat plant. Since Golisz et al. (*3*) also found a higher catechin level in the leaves (0.22 mg/g DW), and in inflorescences (0.42 mg/g DW) at the full-flowering stage, another possible reason for this divergence might be different environmental conditions than in our study such as more hours of sunshine, less precipitation, as well as higher main daily temperatures.

The catechin in buckwheat leaves changed significantly during the growing period reaching its maximum at the flowering stage. Conversely, Das and Griffiths (*21*) described an accumulation trend in the leaves of *Uncaria gambir* Roxb. (an increase from 5% to 9.4%, of fresh weight). The decrease of catechin in those leaves sampled before the harvest, in both years, could result from the presence of leaves of different ages, due to the indeterminate growth of common buckwheat. In the roots, the changes caused by crop development were not significant.

The concentration of catechin in roots and flowers was dependent upon the environmental conditions of that year, with higher level in 2004. Differences between years in the catechin level in leaves were not significant ($P > 0.05$). In our experiment, 2004 was a drier year than 2005; therefore, our results agree with the findings of Kirakosyan et al. (*22*) that water-deficit stress for 10 days increased the productivity of catechin about five times in hawthorn species. The flavonoid level is often

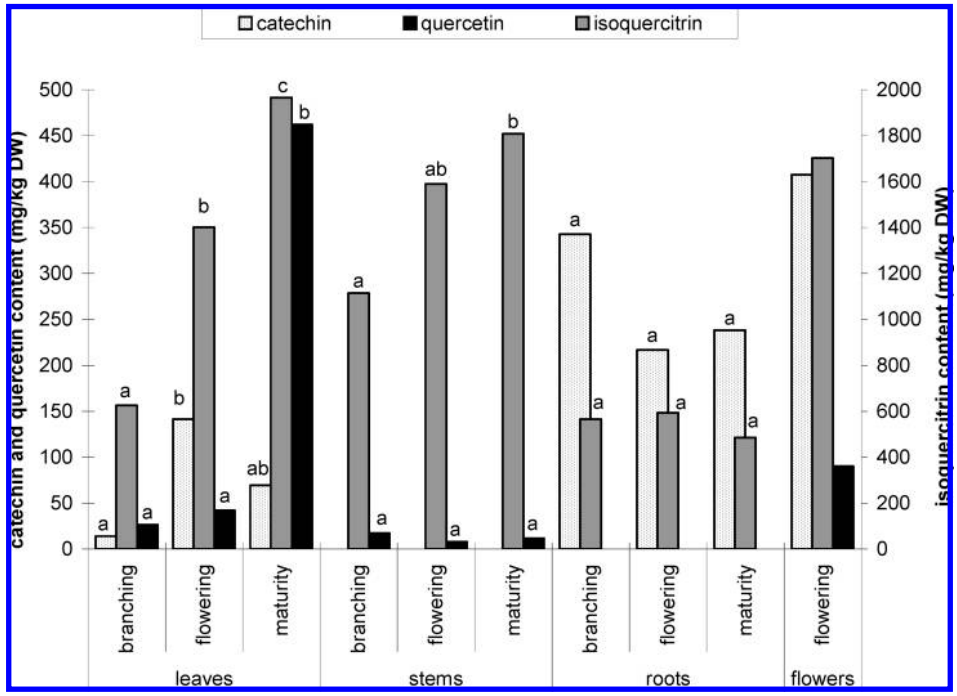

Figure 2. Mean level of catechin, quercetin and isoquercitrin in common buckwheat and their changes during the growing period (a, b, c: differences among the developmental stages specially for each plant part and compound).

influenced by UV radiation, but in the case of catechin, Zheng et al. (*23*) observed only a low influence of UV-B irradiation upon the accumulation of foliar catechins in tea (*Camellia sinensis* L.).

The catechin level in the roots correlated positively with the rutin (0.6965, $P \leq 0.01$) and quercetin levels (0.6365, $P \leq 0.01$) in these parts. The reason for this correlation could be caused by the same precursor, taxifolin (dihydroquercetin), which is the substrate for the biosynthesis of quercetin and subsequently for rutin on the one hand, and for the biosynthesis of catechin through leucocyanidin on the other hand. Secondary plant metabolites are the most stress-related organic compounds. Catechin is found in root exudates of many plants, e.g. spotted knapweed (*12*) and probably buckwheat, as well. Therefore, the changes of catechin levels in roots could be the plant response not only to abiotic factors but also to biotic stimuli, such as the presence of weeds.

Quercetin is a naturally occurring flavonol found in many vegetables, e.g. onion and other plants. In the family Polygonaceae, quercetin was only detected in low concentrations. Iwashina et al. (*14*) characterized quercetin as one of the four minor compounds in *Rheum nobile*. Fabjan et al. (*5*) detected only traces of quercetin in the herb *Fagopyrum tataricum* Gaertn. while Peng et al. (*24*) found that common buckwheat flour contained about 20 μg /g of quercetin.

At the flowering stage, we found the highest amount of quercetin in inflorescences and conversely, the lowest quercetin concentration was established to be in the stems (**Table 2**). We did not detect quercetin in buckwheat roots. Correspondingly, the quercetin level determined in the parts of buckwheat, at the flowering stage, was lower in our study (by about 10×) than the values established by Golisz et al. (*3*) in *F. esculentum*.

The changes in the quercetin level during the growing season were only significant in the leaves. However, the significant difference of the leaves sampled before the harvest were influenced by a considerable increase of the quercetin level in the leaves at the harvest period in 2005. Consequently, the growth stage is not important for the quercetin level; and our results agree with the findings of Patil et al. (*25*) where they did not find important differences in the quercetin level at the different growth stages of onion.

According to Kirakosyan et al. (*21*), cold stress (4 °C) increased the quercetin level in *Crataegus* species by three times. In our study, as well, the cause of the increase of the quercetin level could be low temperatures before plant sampling (mean daily temperature 9.6 °C). In 2005, plants were sampled later (19th September) than in 2004 (3rd September); and immediately after three days with average daily temperatures about 5.5 °C lower than in the preceding days.

The influence of weather on the quercetin level was only significant in the leaves, where, with the exception of the harvest period, the quercetin level was higher in 2004, the drier and sunnier year. According to Kirakosyan et al. (*21*), drought for 10 days caused an increase in the level of quercetin in the leaves of hawthorn by seven times, on average. Quercetin can also increase in the plant as a response to UV-B radiation (*26*). Autooxidation could be another reason of lower level of quercetin. According to Takahama and Hirota (*27*), quercetin in onion (*Apium cepa*) was autooxidized to 3,4-dihydroxybenzoic acid and 2,4,6-trihydroxyphenylglyoxylic acid.

In our study, significant differences in quercetin accumulation were not found among buckwheat varieties. In contrast, Hofmann et al. (*26*) established varietal differences in the quercetin level among *T. repens* populations while Patil et al. (*25*) found only small differences among genotypes of onion. This study shows that weather and variety had only a weak, if any, influence on the quercetin level in buckwheat. Most likely, the site is a more important environmental factor in the determination of quercetin concentration as Patil et al. (*25*) found in the case of onion. We found a positive correlation between quercetin and rutin levels in the leaves (0.7502; $P \leq 0.001$), probably because the leaves are the location of the highest levels of rutin accumulation.

The highest myricetin concentration (about 1000 ppm) was probably that identified in the flowers of neem (*Azadirachta indica* A. Juss) and from the family Polygonaceae; the presence

Table 3. Influence of Different Concentrations (1−10000 μmol) of Isoquercitrin on Weed Growth

weed	plant part	sig[a]	eq	R^2	EC_{50}, μM
E. crus-galli	root	*	$y = -7.8933 \ln(x) + 39.966$	0.81	89120
	hypocotyl	**	$y = -5.9887 \ln(x) + 12.69$	0.88	35160
L. perenne	root	*	$y = -10.589 \ln(x) + 70.818$	0.85	90100
	hypocotyl	**	$y = -15.106 \ln(x) + 127.05$	0.80	33800
P. lanceolata	root	*	$y = -4.978 \ln(x) + 4{,}2944$	0.93	54550
	hypocotyl	*	$y = -24.253 \ln(x) + 163.67$	0.90	37145
S. alba	root	**	$y = -7.9362 \ln(x) + 18.927$	0.81	5914
	hypocotyl	**	$y = -5.9706 \ln(x) + 4.7263$	0.85	9565

[a] Significance of difference of compound from the control. $^{**}P < 0.01$, $^{*}P < 0.05$.

Table 4. Influence of Different Concentrations (1−10000 μmol) of Quercetin on Weed Growth

weed	plant part	sig[a]	eq	R^2	EC_{50}, μM
E. crus-galli	root	*	$y = -11.72 \ln(x) + 91.217$	0.81	171500
	hypocotyl	*	$y = -19.183 \ln(x) + 179.39$	0.83	156000
L. perenne	root	**	$y = -10.683 \ln(x) + 9.9499$	0.85	273
	hypocotyl	*	$y = -23.951 \ln(x) + 74.815$	0.90	183
S. alba	root	**	$y = -5.1867 \ln(x) - 4.0054$	0.84	7050
	hypocotyl	**	$y = -5.6212 \ln(x) + 3.1663$	0.86	12813
T. repens	root	*	$y = -7.427 \ln(x) + 45.1$	0.98	364000
	hypocotyl	*	$y = -14.873 \ln(x) + 141.72$	0.90	396500

[a] Significance of difference of compound from the control. $^{**}P < 0.01$, $^{*}P < 0.05$.

of myricetin was also described in prostrate knotweed (*Polygonum aviculare* L.) (*28*). We detected myricetin in common buckwheat only in the stems at the stage of full flowering, at 6.15 μg/g DM, on average. In the other parts, this compound was below the limits of detection. We established a higher level of myricetin in 2004, as with the other flavonoids. Varietal differences could not be evaluated, because the myricetin was only found in the Emka variety.

The Effect of Flavonoids on the Growth of Selected Weeds. Data about the possible phytotoxicity of isoquercitrin, and its release into the soil, have not been published. Inderjit and Dakshini (*29*) detected a similar compound (quercitrin) from the natural weed-associated soils. In our bioassay, the growth of root and hypocotyl of *S. alba, L. perenne, E. crus-galli*, and *P. lanceolata* was significantly influenced by the presence of isoquercitrin. **Table 3** shows the estimated effective concentration for the 50% inhibition of their growth. *S. alba* was the plant most sensitive to isoquercitrin. The influence of isoquercitrin on the growth of *A. millefolium, A. retroflexus* and *T. repens* was not significant ($P > 0.05$). In the case of *T. repens*, we found only a stimulative effect with all of the used concentrations. Our results indicated that the effective concentrations of isoquercitrin are too high (they ranged from 6 to 90 mM) for a possible allelopathic effect on plants in field conditions.

The next flavonoid, quercetin, significantly influenced the growth of the following species (ordered from most to least sensitive): *L. perenne, S. alba, E. crus-galli* and *T. repens* (**Table 4**). The growth of *P. lanceolata, Achillea millefolium,* and *Amaranthus retroflexus* was not significantly influenced. The EC_{50} values ranged from 0.2 to 400 mM. At the bottom of this range, Nasir et al. (*30*) and Golisz et al. (*3*) found an EC_{50} value of about 0.5 and 3 mM for lettuce.

Parvez et al. (*31*) confirmed the inhibitive effects of quercetin on *Arabidopsis thaliana* plants. They observed both delayed growth and development, due to a decrease in photosynthesis and the reduced transpiration rate. We observed considerably different responses to quercetin among species. *T. repens* was among the least sensitive species. According to Mihailovic et al. (*32*), quercetin inhibits the nodulation of soy, given that an increasing quercetin concentration decreased the number of nodules; however, very low quercetin concentrations stimulated the number of nodules. Inderjit and Dakshini (*29*) suggested quercetin as the allelopathic compound of *Pluchaea lanceolata*.

In our previous study we concentrated on the root exudates of buckwheat; and there we also did not find this compound in either the soil or agar medium. Inderjit and Dakshini (*29*) also did not detect quercetin from weed-associated soils. This could have been caused by the very low concentrations. It confirms the findings of Narasimhan et al. (*33*), who found nanomole amounts of quercetin in the root tissue of *Arabidopsis*; whereas it was present in almost picomole amounts in the root exudates. Consequently, quercetin is probably not the main allelopathic compound; but it could be a product released in the soil during the decomposition of rutin. Each molecule of rutin would be hydrolyzed into a molecule of quercetin, rhamnose, and glucose. However, according to Golisz et al. (*3*) rutin has a higher specific allelopathic activity than quercetin.

Table 5. Influence of Different Concentrations (1−5000 μmol) of Catechin on Weed Growth

weed	plant part	sig[a]	eq	R^2	EC_{50}, μM
A. millefolium	root	**	$y = -13.746 \ln(x) + 34.635$	0.80	472
	hypocotyl	**	$y = -18.393 \ln(x) + 65.27$	0.84	527
E. crus-galli	root	**	$y = -15.474 \ln(x) + 41.187$	0.91	362
	hypocotyl	**	$y = -13.12 \ln(x) + 15.562$	0.81	148
L. perenne	root	**	$y = -13.334 \ln(x) + 29.107$	0.80	377
	hypocotyl	**	$y = -15.536 \ln(x) + 24.373$	0.90	120
P. lanceolata	root	**	$y = -9.2391 \ln(x) - 13.012$	0.86	55
	hypocotyl	**	$y = -12.181 \ln(x) + 21.725$	0.84	360
S. alba	root	**	$y = -22.214 \ln(x) + 135.44$	0.90	445
	hypocotyl	**	$y = -23.511 \ln(x) + 116.57$	0.88	780
A. retroflexus	root	**	$y = -13.356 \ln(x) + 56.151$	0.83	2830
	hypocotyl	**	$y = -7.3179 \ln(x) + 16.866$	0.95	9296
T. repens	root	**	$y = -14.819 \ln(x) + 39.952$	0.85	433
	hypocotyl	**	$y = -11.919 \ln(x) + 1.1414$	0.98	169

[a] Significance of difference of compound from the control. $^{**}P < 0.01$.

Catechin had significant inhibitive effects on the growth of all selected plants (**Table 5**). The effective concentrations (from 0.05 to 0.5 mM) were similar with *A. millefolium, E. crus-galli, L. perenne, T. repens*, and *P. lanceolata*; the exception was *A. retroflexus*, which was not so sensitive to this compound. Iqbal et al. (*12*) found effective concentrations of catechin very close to ours, with a range from 0.2 to 0.5 mM for *Trifolium repens* and *Lolium multiflorum*. Golisz et al. (*3*) also established the effective concentrations for lettuce to be in this range (about 0.4 mM). These results indicate relatively low catechin selectivity on plant growth.

Iqbal et al. (*12*) suggested catechin as the possible allelochemical of buckwheat. Our results indicated that catechin, alone, is not the most important allelopathic compound of common buckwheat because the plant contains only low amounts of catechin in the above-ground biomass (leaves and stems). However, catechin could be playing an important function as a root exudate. In our previous study (*8*), we did not confirm the presence of catechin in root exudates of buckwheat seedlings; but it is probable that catechin is released during the later stages of buckwheat development. The question remains as to which catechin enantiomer is present in common buckwheat because Bais et al. (*10*) has found (−)-catechin to be more effective than (+)-catechin. According to Blair et al. (*34*), the activity of catechin can be quickly decreased in the soil due to degradation, especially in moist soil. The effectiveness of catechin is therefore very much dependent upon the field conditions.

From the evaluated compounds, myricetin was the least biologically active compound. Myricetin did not show a significant influence ($p > 0.05$) on the growth of any of the selected species. Conversely, Nasir et al. (*30*) found, under similar conditions, an EC_{50} for lettuce of about 0.5 mM. These inconsistencies could be due to the high sensitivity of lettuce to this compound. All applied compounds could be changed to other compounds by enzymatic or nonenzymatic reaction such as degradation of sugar moiety or oxidization. Therefore, it is necessary to verify the stability in future studies.

Our results indicate that buckwheat contains a significant amount of isoquercitrin that can participate in the potential antioxidant effects of buckwheat products, made from green parts of the plant. Catechin had the strongest inhibitive effect among the determined compounds; but due to the low level in buckwheat, its potential allelopathic function could be more important as a root exudate. Isoquercitrin and quercetin had weak inhibitive effects. Hence, we suppose that myricetin, isoquercetin and quercetin do not have important function in the allelopathic potential of buckwheat. Quercetin effects could be more significant in the case of the degradation of rutin into quercetin in the soil.

Supporting Information Available: HPLC-DAD chromatogram at 220 nm of the methanol extract of buckwheat stems (Emka variety, the year 2005). This material is available free of charge via the Internet at http://pubs.acs.org.

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