

Is growing buckwheat allelopathic?

Ist wachsender Buchweizen allelopathisch?

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Abstract

The growth repressive effect of common buckwheat (*Fagopyrum esculentum*) on redroot pigweed (*Amaranthus retroflexus*) was studied by separating resource competition from root interactions between the two plant species in a pot trial in the phytotron. In order to verify this result in situ field trials were performed. A strong repression of redroot pigweed growth by buckwheat could be observed independently of shading. However, soil both from the field and phytotron trials in which buckwheat had been growing didn't have an effect on redroot pigweed and lettuce (*Lactuca sativa*) growth. Assuming that allelopathic compounds are present in the soil solution supplementary experiments were conducted. Lettuce root length was measured after exposing seeds to different "buckwheat soil" extracts. Moreover, buckwheat and lettuce developed at the same time next to each other in petri dishes. In none of the experiments an influence on lettuce and redroot pigweed development could be observed. We conclude that there are either no allelopathic molecules in the soil solution (not soluble in water) or that they are rapidly degraded. The observed growth inhibiting effect seems to be due to a long term and constant exposure of small quantities of allelopathic molecules. However, it is also possible that growth repression of redroot pigweed by buckwheat is not due to allelopathy.

Keywords: Allelopathy, *Amaranthus retroflexus*, *Fagopyrum esculentum*, resource competition, root interactions, weed suppression

Zusammenfassung

Echter Buchweizen (*Fagopyrum esculentum*) unterdrückt das Wachstum von Zurückgebogenem Amarant (*Amaranthus retroflexus*). Dieser Effekt wurde in einem Topfversuch im Phytotron untersucht, indem die Konkurrenz um Ressourcen von den Wurzelinteraktionen zwischen den beiden Pflanzenarten getrennt wurde. Um dieses Ergebnis zu verifizieren, wurden in situ-Feldversuche durchgeführt. Eine starke, von der Beschattung unabhängige, Wachstumsunterdrückung von Amarant durch Buchweizen konnte beobachtet werden. Allerdings hatte Erde, in der Buchweizen gewachsen war, sowohl aus dem Feldversuch als auch aus einem Phytotron-Versuch, keinen Einfluss auf das Wachstum von Amarant und Salat (*Lactuca sativa*). Unter der Annahme, dass allelopathische Verbindungen in der Bodenlösung vorhanden sind, wurden zusätzliche Experimente durchgeführt: Salatsamen wurden verschiedenen Extrakten von „Buchweizen-Erde“ ausgesetzt um anschließend die Länge der Salatwurzeln zu messen. Ausserdem wurden Buchweizen und Salat gleichzeitig nebeneinander in Petrischalen angezogen. In keinem der Versuche konnte ein Effekt auf die Entwicklung von Amarant und Salat beobachtet werden. Wir schliessen daraus, dass in der Bodenlösung entweder keine (wasserlöslichen) allelopathischen Verbindungen sind oder, dass sie rasch abgebaut werden. Der beobachtete wachstumshemmende Effekt scheint durch das Vorhandensein konstant kleiner Mengen an allelopathischen Verbindungen, die über einen langen Zeitraum hinweg vorhanden sind, hervorgerufen zu werden. Allerdings ist es auch möglich, dass die Wachstumsunterdrückung von Amarant durch Buchweizen nicht auf allelopathische Effekte beruht.

Stichwörter: Allelopathie, *Amaranthus retroflexus*, *Fagopyrum esculentum*, Unkrautunterdrückung, Wettbewerb um Ressourcen, Wurzelinteraktionen

Introduction

It is largely known that growing stands of common buckwheat (*Fagopyrum esculentum*) successfully suppress weeds in the field (TOMINAGA and UEZU, 1995; CREAMER and BALDWIN, 2000; KALINOVA, 2004; TSCHUY et al., 2014; FALQUET et al., 2015). However, so far it is not clear what this suppression is due to (FALQUET et al., 2015). It has been proposed that allelopathy plays an important role (GOLISZ et al., 2007; KALINOVA et al., 2007; KATO-NOGUCHI et al., 2007; TIN et al., 2009). Allelopathy has been defined as any direct or indirect harmful or beneficial effect by one plant on another through production of chemical compounds that escape into the environment (RICE,

1984). Trying to answer the question whether phytotoxic root exudates are implicated in weed suppression by buckwheat, we developed a method allowing to separate resource competition from growth repressive root interactions (FALQUET et al., 2014). As a model weed we used redroot pigweed (*Amaranthus retroflexus*) as we had observed reduced growth in buckwheat stands in agricultural fields. We therefore also studied the weed suppressive ability of buckwheat in a field trial. Moreover we used different methods commonly used in the field of allelopathy research like the co-cultivation of the potentially allelopathic plant (donor) and the targeted plant species (receiver) in petri-dishes (KALINOVA et al., 2005; KATO-NOGUCHI et al., 2007; CIARKA et al., 2009) experiments with soil in which buckwheat had been growing (RIMANDO and DUKE, 2003; CIARKA et al., 2009; SADAQA et al., 2010) as well as different methods of soil extraction (KALINOVA et al., 2007; JANKOWSKI et al., 2014; OTUSANYA et al., 2014).

Materials and Methods

Separation of resource competition from growth repressing root interactions

The effects of light competition and growth repressive root interactions between buckwheat and redroot pigweed were studied separately in pot trials where both species were grown together according to FALQUET et al. (2014). Vertical nets (\pm NET= net) between the two plant species were used to prevent shading on pigweed and impermeable plastic barriers (\pm P = plastic) inhibited root contact. Four different conditions were tested: A = -NET, -P; B = +NET, -P; C = -NET, +P and D = +NET, +P. Water and nutrient supply were kept constant throughout the experiment. Pigweed dry weight (DW) was determined 28 days after sowing (DAS) (Fig. 1).

Field trials

To study weed suppression by buckwheat in the field, buckwheat was sown on the 7th of August 2014 at a density of 75 kg/ha in four blocks of 24m². In addition eight blocks of the same size were left with bare soil. Within each block pigweed was sown on the same day into the inter-rows of buckwheat and on bare soil respectively. To prevent light competition from buckwheat, nets (NET+/NET-) were placed within certain inter-rows 11 DAS. 28 and 55 DAS pigweed plants were harvested in all conditions prior to determination of DW (Fig. 2). For a detailed description of the field trial conditions and all other methods described below please refer to GFELLER and WIRTH (2015).

Experiments with soil in which buckwheat had been growing

In the above mentioned 2014 field trial soil samples were taken 11, 26 and 42 DAS within the buckwheat stands and on bare soil. Subsequently lettuce and redroot pigweed were grown in this soil in the phytotron to determine DW after 15 and 21 days (d) respectively (Fig. 3).

In a second experiment soil was taken from pots in which buckwheat had been cultivated for 10 d in the phytotron. 50 g of this soil was used to cover lettuce and redroot pigweed seeds in petri dishes. As a control bare soil was used. After 7 d lettuce and pigweed root length was determined (Fig. 4).

Experiments with soil extracts

Flex rhizon samplers (Rhizosphere Research Products, The Netherlands) which allow collecting soil pore water, were introduced into pots in the phytotron. Three conditions were tested: pots with buckwheat, pots with buckwheat and pigweed and bare soil. 29 DAS soil water samples were taken and 6 ml were transferred into petri dishes. Subsequently 10 lettuce seeds were sown and root length was determined after 5 d of growth in the climate chamber under light conditions (Fig. 5).

In a second independent experiment the same three conditions were tested. This time soil from the pots was extracted with boiling water after 20 days of plant growth. Subsequently 6 ml of extract were used to moisten blotting paper in petri dishes prior to sowing of 10 lettuce seeds per petri dish. In addition tap and double distilled (dd) water were used as control. Lettuce root length was measured after 8 d of growth in the dark in the climate chamber (Fig. 6).

Experiments with germinating buckwheat seeds

Six pre-germinated buckwheat seeds together with 10 lettuce seeds were placed on blotting paper in petri dishes. The control treatment consisted of lettuce only. 15 ml of tap water were added to the petri dishes before placing them in a phytotron under light and dark conditions. 3 DAS lettuce root length was determined (Fig. 7).

Results and discussion

Buckwheat grows fast and quickly develops a dense canopy that prevents weeds from receiving enough light. Therefore it has been proposed that weed suppression by growing buckwheat is mainly due to the competition for light (BICKSLER and MASIUNAS, 2009). In our own field trial in 2013 shading played a decisive role in pigweed growth suppression by buckwheat (TSCHUY et al., 2014). The importance of light competition in pigweed growth suppression by buckwheat could be confirmed in the phytotron. When pigweed was grown under controlled conditions without light competition (+ NET) and in the absence of root interactions with buckwheat (+P), it developed very well (Fig. 1, condition D). However, when the buckwheat canopy was present (-NET) and roots of the two species interacted (-P) pigweed growth was significantly repressed by 89% (Fig. 1, condition A). This strong growth repression could partly be explained by shading (absence and presence of the net (\pm NET)): B versus A -68% and D versus C -52% and partly by root interactions (absence and presence of the plastic barrier (\pm P)): C versus A -77% and D versus B -65% growth repression (Fig. 1). This experiment shows that growth repression of pigweed by buckwheat is not only due to shading, but that root interactions between the two species also play an important role (FALQUET et al., 2014). As nutrient supply was kept constant at a high level throughout the experiment we suppose that nutrient competition didn't play a decisive role.

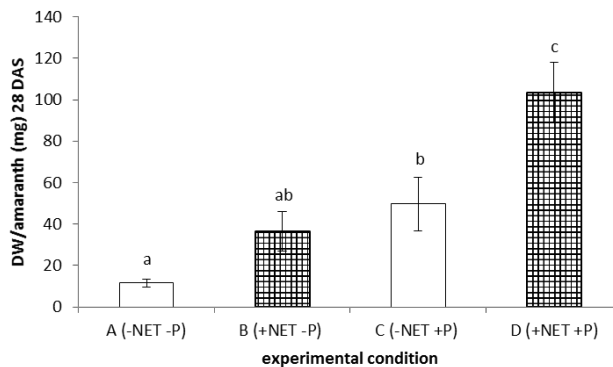


Fig. 1 Pigweed growth suppression by buckwheat is due both to shading and root interactions of the two species. Values are means \pm SE of 10 replicates. The different letters above the columns indicate significant differences ($P < 0.05$) according to Bonferroni adjusted p-values.

Abb. 1 Die Wachstumsunterdrückung von Amaranth durch Buchweizen ist sowohl auf die Beschattung als auch auf Wurzelinteraktionen zwischen den beiden Pflanzenarten zurückzuführen.

The growth repressing effect of buckwheat on pigweed could be confirmed in the field trial in 2014. 28 and 55 DAS pigweed plants within the buckwheat canopy (NET-) were 63 and 89%

smaller than control plants on bare soil. However, pigweed growth in the absence of light competition (NET+) was also strongly suppressed by the presence of buckwheat (-55% and -89%) indicating that under the environmental conditions of 2014 the growth repressing effect of buckwheat on pigweed was strong and independent of shading (Fig. 2). Due to a cold weather period in August 2014 buckwheat developed very slowly and couldn't establish a very dense canopy. Light competition by shading was therefore much smaller than in a similar field trial in 2013 (Tschuy et al., 2014). However, shading by the buckwheat canopy was present, but didn't have an influence on pigweed growth. This result confirms that besides shading growth repressive effects in the root zone play an important role for the observed effect. Our working hypothesis is that weed suppression by buckwheat is partly due to allelopathic root interactions.

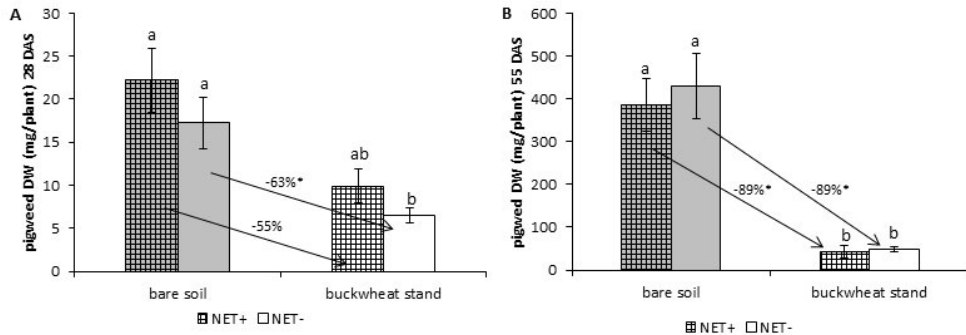


Fig. 2 Growth repression of pigweed by buckwheat in the 2014 field trial. (A) 28 DAS, (B) 55 DAS. Values are means \pm SE of 8 replicates for buckwheat and 16 for bare soil. Means not sharing the same letter are significantly different (Tukey's HSD, $p < 0.05$).

Abb. 2 Wachstumsunterdrückung von *Amarant* durch *Buchweizen* im Feldversuch 2014. (A) 28 DAS (28 Tage nach Saat), (B) 55 DAS (55 Tage nach Saat).

In order to know whether phytotoxic compounds in the soil of the buckwheat stands were responsible for the observed growth repression, lettuce and pigweed seeds were sown on soil samples from buckwheat and control plots with bare soil obtained at three different dates from the field trial in 2014. No effect of the "buckwheat soil" on lettuce and pigweed DW could be observed after 15 and 21 d of growth respectively (Fig. 3). This indicates that possibly present allelopathic molecules were not sufficiently concentrated or not persistent enough to provoke growth repression in our trial conditions. According to KALINOVA et al. (2005 and 2007) soil from a buckwheat stand had a growth repressing effect on lettuce root growth. We therefore also tested the effect of "buckwheat soil" obtained from a pot trial in the phytotron on root length of lettuce and pigweed. Again no difference could be observed (Fig. 4). We conclude that methods using "buckwheat soil" are not suitable for the study of allelopathic buckwheat root exudates. However, the obtained results might also be due to the fact that different soil types influence the activity of an allelopathic compound (HIRADATE et al., 2010).

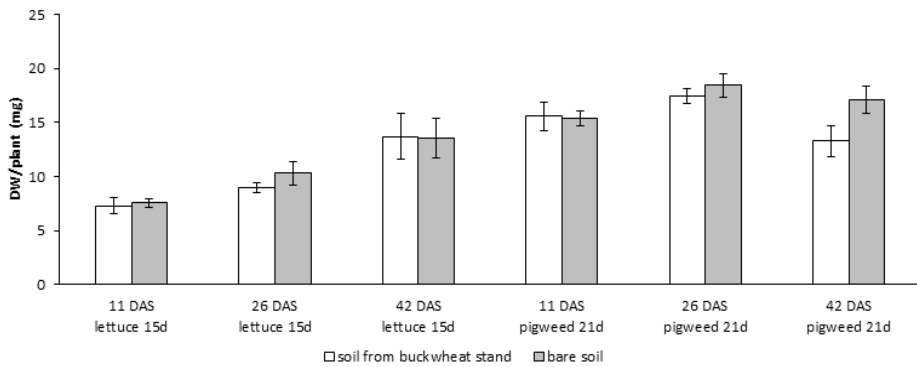


Fig. 3 No effect of soil from a buckwheat stand in the field on lettuce and pigweed growth. Values are means \pm SE of 12 replicates for buckwheat and 24 for bare soil. There were no statistically significant differences in DW between the samples from a buckwheat stand and from bare soil at the different dates (non-parametric Dunnett's test, $p < 0.05$).

Abb. 3 Felderde, in der Buchweizen gewachsen ist, hat keinen Einfluss auf das Wachstum von Salat und Amarant.

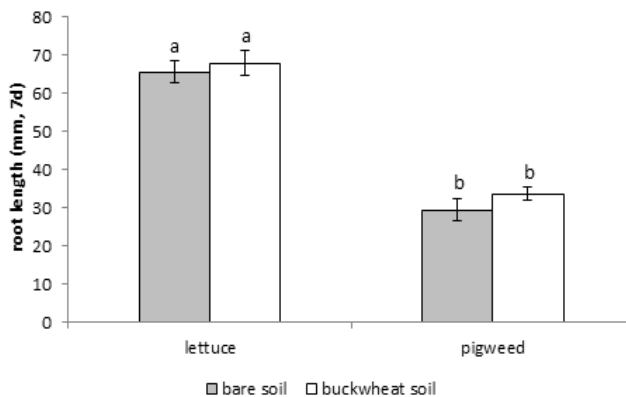


Fig. 4 No effect of soil in which buckwheat was grown in the phytotron on lettuce and pigweed growth. Values are means \pm SE of 60 replicates for lettuce and 30 for pigweed. Means not sharing the same letter are significantly different (ANOVA, $p < 0.05$).

Abb. 4 Topferde, in der Buchweizen in der Klimakammer gewachsen ist, hat keinen Einfluss auf das Wachstum von Salat und Amarant.

Another possibility is to test whether phytotoxic buckwheat compounds are present in the soil solution as indicated before (TOMINAGA and UEZU, 1995). Soil water samples obtained with flex rhizon samplers from pots in which buckwheat was growing with and without pigweed had no effect on lettuce root growth compared to control samples from bare soil (Fig. 5). This might be due to the fact that allelopathic molecules were present in very low concentrations, that they were rapidly degraded in the climate chamber during the lettuce growth test or that buckwheat phytotoxins are not soluble in water like other effective allelochemicals such as sorgoleone (DUKE, 2010; DUKE, 2015). In accordance to the protocol of KALINOVA et al. (2007) we tested whether soil

extraction with boiling water increased the presence of phytotoxic molecules as more or other compounds might be extracted by this method. Compared to the two control treatments with tap water and double distilled water, lettuce root length was significantly repressed by hot water extracts of "buckwheat soil" (Fig. 6). One could conclude that boiling water extracts from buckwheat soil contain growth repressing substances. However, when extracting bare soil the effect was even stronger. This result was observed in several independent experiments (results not shown). We cannot explain it. We conclude that water soil extraction is also inappropriate to study the allelopathic potential of buckwheat.

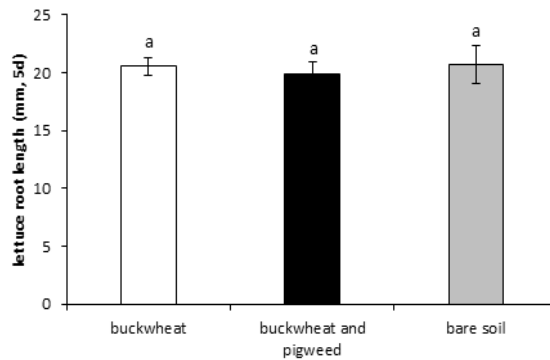


Fig. 5 No effect on lettuce root length growth of soil water extracts obtained with rhizon samplers from pots with buckwheat. Values are means \pm SE of 30 replicates and 10 for bare soil. There were no statistically significant differences (ANOVA, $p < 0.05$).

Abb. 5 Durch Rhizon-Sampler gewonnenes Bodenwasser aus Töpfen mit Buchweizen beeinflusst das Wurzelwachstum von Salat nicht.

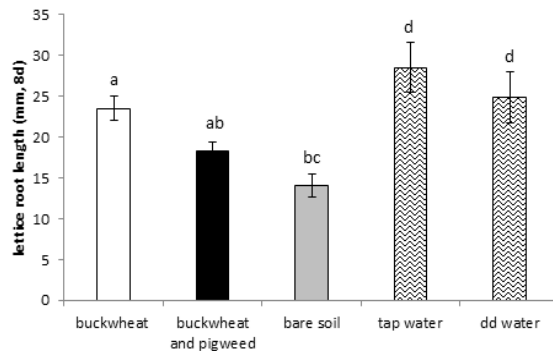


Fig. 6 No effect on lettuce root length growth of hot water soil extracts from soil in which buckwheat was cultivated. Values are means \pm SE of 60 replicates for soil with buckwheat, 20 for bare soil and 10 for water controls. Means not sharing the same letter are significantly different (rank based analysis of linear models, $p < 0.05$)

Abb. 6 Heißwasser-Bodenextrakte aus Töpfen in denen Buchweizen gewachsen ist beeinflussen das Wurzel Wachstum von Salat nicht.

Several authors claim that during germination and early development of buckwheat allelopathic compounds are exudated, causing growth suppression of lettuce radicle length (KALINOVA et al., 2005, KATO-NOGUCHI et al., 2007). We observed the contrary when lettuce and buckwheat were grown together in petri dishes on blotting paper. Both under light and dark conditions lettuce radicle length was stimulated by the presence of germinating buckwheat seeds (Fig. 7).

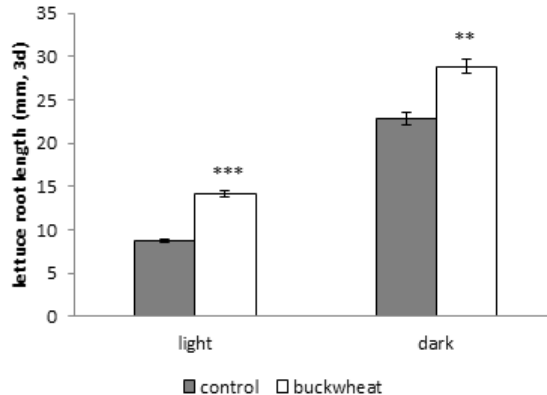


Fig. 7 No effect of germination buckwheat on lettuce root growth. Values are means \pm SE of 50 replicates. Star symbols above bars indicate that two-tailed paired student's test showed significant differences ($p < 0.05$).

Abb. 7 Keimende Buchweizen-Samen haben keinen Effekt auf das Wurzelwachstum von Salat.

For a more detailed description of our work please read the book chapter "Is growing buckwheat allelopathic?" (GFELLER and WIRTH, 2015). The corresponding pdf-file can be obtained from Judith Wirth.

Conclusion

With our work we could show that pigweed growth suppression is not only due to shading effects of the buckwheat canopy and that the effect of light competition is variable depending on the environmental conditions. However we could not prove that the observed effect is partly due to allelopathic buckwheat compounds. The obtained results can be explained by several hypothesis: buckwheat allelochemicals are not water soluble, they are rapidly degraded after root exudation or their concentration in the soil solution is very low. It is also possible that allelopathy doesn't play a role in pigweed growth suppression by buckwheat. However, we believe that the roots of the two species have to be in contact over a longer period of time in order to observe the effect. This might be due to very small quantities of allelochemicals that are constantly exudated by buckwheat roots leading to a cumulative effect.

References

- BICKSLER, A. J. and J.B. MASIUNAS, 2009: Canada Thistle (*Cirsium arvense*) Suppression with Buckwheat or Sudangrass Cover Crops and Mowing. *Weed Technol.* **23**, 556-563.
- CIARKA, D., H. GAWRONSKA, M. MALECKA and S.W. GAWRONSKI, 2009: Allelopathic potential of sunflower. II. Allelopathic activity of plants compounds released in environment. *Allelopathy J.* **23**, 243-254.
- CREAMER, N. G. and K.R. BALDWIN, 2000: An evaluation of summer cover crops for use in vegetable production systems in North Carolina. *Hortscience* **35**, 600-603.
- DUKE, S.O., 2010: Allelopathy: Current status of research and future of the discipline: A commentary. *Allelopathy J.* **25**, 17-30.
- DUKE, S.O., 2015: Proving allelopathy in crop-weed interactions. *Weed Sci.* **63**, 121-132.
- FALQUET, B., A. GFELLER, M. POURCELOT, F. TSCHUY and J. WIRTH, 2015: Weed Suppression by Common Buckwheat: A Review. *Environmental Control in Biology* **53**, 1-6.

27. Deutsche Arbeitsbesprechung über Fragen der Unkrautbiologie und -bekämpfung, 23.-25. Februar 2016 in Braunschweig

- FALQUET, B., D. ROUX, L. HENRIET, F. TSCHUY and J. WIRTH, 2014: Simple method to separate resource competition from allelopathic root interactions. *Allelopathy J.* **34**, 227-240.
- GFELLER, A. and J. WIRTH, 2015: 'Is growing buckwheat allelopathic?,' in J. E. Price (Ed.) *New Developments in Allelopathy Research*. Nova Science Publishers.
- GOLISZ, A., B. LATA, S.W. GAWRONSKI and Y. FUJII, 2007: Specific and total activities of the allelochemicals identified in buckwheat. *Weed Biol. Manag.* **7**, 164-171.
- HIRADATE, S., K. OHSE, A. FURUBAYASHI and Y. FUJII, 2010: Quantitative evaluation of allelopathic potentials in soils: Total activity approach. *Weed Sci.* **58**, 258-264.
- JANKOWSKI, K., J. JANKOWSKIA, G.A. CIEPIELA, J. SOSNOWSKI, B. WISNIEWSKA-KADZAJAN and A. MATSYURA, 2014: The initial growth and development of *poa pratensis* under the allelopathic influence of *taraxacum officinale*. *Journal of Ecological Engineering* **15**, 93-99.
- KALINOVA, J., 2004: 'Influence of Common Buckwheat on Growth of Other Plant Species,' 9th International Symposium on Buckwheat. Prague.
- KALINOVA, J., J. TRISKA and N. VRCHOTOVA, 2005: Biological activity of phenolic compounds present in buckwheat plants. *Allelopathy J.* **16**, 123-129.
- KALINOVA, J., N. VRCHOTOVA and J. TRISKA, 2007: Exudation of allelopathic substances in buckwheat (*Fagopyrum esculentum* Moench). *J. Agr. Food Chem.* **55**, 6453-6459.
- KATO-NOGUCHI, H., H. SUGIMOTO and M. YAMADA, 2007: Buckwheat Seedlings May Inhibit Other Plant Growth by Allelopathic Substances. *Environmental Control in Biology* **45**, 27-32.
- OTUSANYA, O.O., A.A. SOKAN-ADEAGA and O.J. ILORI, 2014: Allelopathic effect of the root exudates of *Tithonia diversifolia* on the germination, growth and chlorophyll accumulation of *Amaranthus dubius* L. and *Solanum melongena* L. *Research Journal of Botany* **9**, 13-23.
- RICE, E.L., 1984: *Allelopathy*, London: Academic Press Inc. (London) Ltd.
- RIMANDO, A.M. and S.O. DUKE, 2003: 'Studies on rice allelochemicals,' in C.W. SMITH, R. DILDAY, H. RICE (Ed.): *Origin, History, Technology and Production*. John Wiley and Sons, Inc., Hoboken, New Jersey.
- SADAQA, E.A., A.A. BAWAZIR and J.R. QASEM, 2010: Allelopathic activity of some common weeds species in onion fields. *Allelopathy J.* **26**, 175-184.
- TIN, W.W., T.H. HAYASHI, T. OTOMATSU, K. HIROSE, K. HASEGAWA and H. SHIGEMORI, 2009: Caprolactam, an Inhibitory Allelochemical Exuded from Germinating Buckwheat (*Fagopyrum esculentum*) Seeds. *Heterocycles* **78**, 1217-1222.
- TOMINAGA, T. and T. UEZU, 1995: Weed Suppresion by Buckwheat. *Current Advances in Buckwheat Research*, 693-697.
- TSCHUY, F., A. GFELLER, R. AZEVEDO, C. KHAMISSÉ, L. HENRIET and J. WIRTH, 2014: Unkrautunterdrückung durch Zwischenfrüchte: Analyse verschiedener Faktoren. *Agrarforschung Schweiz* **5**, 292-299.