# Effects of aqueous above-ground biomass extracts of cover crops on germination and seedlings of maize

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## Einfluss von Extrakten aus oberirdischen Zwischenfruchtrückständen auf die Keimung und die Keimlinge von Mais

## 1 Introduction

Along wheat and rice, maize is one of the most valuable field crops due to its high productivity and various uses in human and animal nutrition, as well as in industrial processes (Liebereі and Reisdorff, 2007). The traditional maize cropping system, in which ploughing is carried out in autumn and the field is left unplanted in winter, arises enviromental concerns over erosion, runoff and leaching of nutrients (for example, nitrogen). Consequently, planting catch and cover crops, alongside with reduced tillage is recommended (Rüegg et al., 1998). Additionally, growing autumn-sown cover crops can increase yield and N content in the subsequently grown maize (Kramberger et al., 2009).

In reduced tillage systems, cover crops' residues are incorporated at shallow depths or left on the soil surface (Morris et al., 2009). Such decomposing residues have been reported to inhibit the growth of weeds and subsequent crops (Alsaadawi, 2001), due to allelopathy – a direct or indirect harmful or beneficial effect of one plant on another through


### Zusammenfassung

Allopathische Effekte von Zwischenfruchtrückständen in der obersten Bodenschicht können die Etablierung und somit den Ertrag von Kulturpflanzenbeständen beeinflussen. Gerade bei Mais (*Zea mays*) ist eine gute Bestandesetablierung aufgrund der weiten Pflanzenabstände und der fehlenden Bestockung von Bedeutung. Deshalb wurde der Einfluss von Rückständen der Zwischenfrüchte Ackerbohne (*Vicia faba*), Weißer Senf (*Sinapis alba*), Phacelia (*Phacelia tanacetifolia*), Sommerhafer (*Avena sativa*) und Sonnenblume (*Helianthus annuus*) bei Extrakt-Konzentrationen von 1 %, 5 % und 10 % auf die Keimungsindices und die Keimlingseigenschaften von Mais in einem Laborversuch getestet. Die Gesamtkeimung und die Keimungsgeschwindigkeit nahmen generell mit höheren Extrakt-Konzentrationen ab, wobei die nachteilige Wirkung am geringsten bei den Extrakten von Weißem Senf und Phacelia und am höchsten bei jenen von Ackerbohne war. Das Wachstum der Keimlingswurzeln war generell stärker durch die Extrakte beeinträchtigt als das Wachstum des Keimlingssprosses, vermutlich aufgrund des intensiveren Kontaktes der Wurzeln mit den Extrakten.


**Schlagwörter:** Ackerbohne, Weißer Senf, Phacelia, Sommerhafer, Sonnenblume.


### Summary

Allelopathic effects of cover crop residues in the upper soil layer may affect crop establishment and thus final yield; this is of special concern for non-tillering and wide-spaced crops like maize (*Zea mays*) in reduced tillage systems. Therefore, aqueous extracts of above-ground field collected biomass of the cover crops faba bean (*Vicia faba*), white mustard (*Sinapis alba*), phacelia (*Phacelia tanacetifolia*), spring oat (*Avena sativa*) and sunflower (*Helianthus annuus*) at concentrations of 1 %, 5 % and 10 % were tested for their influence on germination indices and seedling characteristics of maize under laboratory conditions. Total germination and germination speed was generally impaired with increasing extract concentration; the detrimental effect was smallest with mustard and phacelia and largest with faba bean extracts. Seedling root growth was generally more strongly affected by extracts than shoot growth presumably due to the more intensive contact of roots with the extracts.


**Key words:** Faba bean, white mustard, phacelia, spring oat, sunflower.

chemical compounds which are released to the environment (RICE, 1984). In agriculture, allelopathy plays a major role in weed control, crop protection and crop establishment (CHON and NELSON, 2010). For example, the incorporation of sunflower residues resulted in effective weed suppression in broad beans, therefore allowing the reduction of synthetic herbicides (ALSAADAWI et al., 2011). The emergence of a high proportion of the sown seed is essential for an optimum crop stand establishment of the non-tillering and wide-spaced crop maize to avoid gaps in the crop stand and thereby yield reduction (TEKRONY et al., 1989).

Laboratory experiments are a first step in understanding crop residue influences on the germination and early growth of crops. The aim of this study is to assess the effects of water extracts of above-ground residue biomass of different cover crops on germination and early shoot and root maize growth.

## 2 Material and Methods

### 2.1 Extract preparation

Above-ground residues of autumn-sown cover crops faba bean (*Vicia faba*) (FB), white mustard (*Sinapis alba*) (WM), phacelia (*Phacelia tanacetifolia*) (PH), spring oat (*Avena sativa*) (SO), sunflower (*Helianthus annuus*) (SF) were collected after winter on March 24$^{th}$, 2011 at the Experimental Farm of BOKU University in Groß-Enzersdorf (Austria). Residues were dried at 70 °C for 3d and grinded to fit into a 1 mm sieve. Residue powders were extracted with distilled water in a shaker at 30 rpm for 24 h at room temperature at a residue:extractant ratio of 1:100 (w/v) (1 %), 1:20 (w/v) (5 %) and 1:10 (w/v) (10 %). The extracts were sieved through a nylon mesh and then filtrated using round filters (type 11A, Roth GmbH+Co. KG, Germany); they were stored at 4 ºC and used within 1–2 days to avoid potential chemical alterations.

### 2.2 Experimental setup and data collection

Two experiments were conducted to assess germination properties and seedling characteristics. In both experiments, *Zea mays* seeds (cv. Chapalu) were used after surface-sterilization with NaClO.

#### *2.2.1 First experiment*

The germination experiment was conducted according to the pleated paper method (ISTA, 2008) using Grade 3014 Seed Testing Paper (Wenk LabTec GmbH, Germany). One-hundred seeds were placed in the boxes with four seeds in each of the 25 pleats. A paper strip underlying the pleated paper was used to ensure uniform moisture conditions. Germination progress was measured at 24 h intervals for 7 days. Seeds were regarded as germinated after a radical emergence of 2 mm.

#### *2.2.2 Second experiment*

The assessment of shoot and root length after 7 days was performed using the top of paper method (ISTA, 2008) with 15 seeds placed on top of two layers of Grade 0858 Seed Testing Paper (Wenk LabTec GmbH, Germany) in plastic boxes. The shoot:root ratio (S:R) was calculated based on the results.

Plastic boxes (18×13.5×6 cm) were watered respectively with 50 ml (Experiment 1) or 30 ml (Experiment 2) of extracts or distilled water for control. Moisture loss was minimized by closing the boxes. Experimental units were arranged completely randomized with four replications in a growth chamber at a constant temperature of 20 °C.

### 2.3 Calculation of germination indices

Following germination indices were calculated based on the germination counts: (1) total germination ($G_t$) (CHIAPUSIO et al., 1997), (2) mean germination time (MGT) (RANAL et al., 2009), (3) time to obtain 50 % germination ($T_{50}$) (COOLBEAR et al., 1984; modified by FAROOQ et al., 2005), (4) germination rate index (GRI) (MAGUIRE, 1962), (5) corrected germination rate index (CGRI) (HSU et al., 1985) and the (6) coefficient of variation of germination time ($CV_{Gt}$) (RANAL et al., 2009):

$$G_t = \frac{n_t \times 100}{n} \quad (1)$$

where $n_t$ is the number of total germinated seeds after 7 days and n is the number of seeds used.

$$MGT = \frac{\sum_{i=1}^{7} n_i \times t_i}{\sum_{i=1}^{7} n_i} \quad (2)$$

where $n_i$ is the number of newly germinated seeds at $t_i$ (days after the start of the experiment).

$$T_{50} = \frac{\left(\frac{n_t}{2} - n_i\right)(t_j - t_i)}{n_j - n_i} \quad (3)$$

where $n_t$ is the number of total germinated seeds and $n_j$ and $n_i$ are the cumulative number of seeds germinated by adjacent counts at times $t_j$ and $t_i$, respectively, when $n_i < n/2 < n_j$.

$$GRI = (G_1/1) + (G_2/2) + \ldots + (G_7/7) \quad (4)$$

where $G_1$, $G_2$, ... $G_7$ is the proportion of seeds which germinate on day 1, 2, ..., 7 following the set-up of the experiment and 1, 2, ..., 7 represents the corresponding day of germination. The GRI varies from 0 (no seeds have germinated) to 100 (all seeds germinate on the first day).

$$CGRI = \frac{GRI}{G_t} \times 100 \quad (5)$$

$$CV_{Gt} = \frac{s_{Gt}}{MGT} \times 100 \quad (6)$$

For calculating the $CV_{Gt}$, the variance of germination time ($s_{Gt}^2$) was calculated as follows:

$$s_{Gt}^2 = \frac{\sum_{i=1}^{7} n_i (t_i - MGT)^2}{\sum_{i=1}^{7} n_i - 1} \quad (7)$$

These indices are used to describe aspects of the germination process including germination capacity ($G_t$), time spent to germinate (MGT, $T_{50}$), germination speed (GRI, CGRI) and homogeneity ($CV_{Gt}$) (Ranal et al., 2009).

### 2.4 Statistical analysis

Statistical analyses were performed using SAS version 9.2. The analysis of variance with subsequent comparisons of means was performed using the t-test on the significance level of $p<0.05$.

## 3 Results and discussion

Effects of different concentrations of each extract (including the control) on germination indices and seedling characteristics of maize are summarized in Fig. 1 and Fig. 2.

Total germination ($G_t$) was impaired with increasing extract concentrations of all cover crops compared to the control. The $G_t$ decreased more strongly with increasing extract concentrations of FB and SO, than with WM and PH with sunflower showing intermediate values (Fig. 1a).

On the individual extract level, a significant increase of the mean germination time (MGT) occurred for FB with increasing concentrations and for SF with a similar level of MGT with all three SF extract concentrations. MGT was also delayed by WM, PH and SO compared to the control (although statistically not significant) (Fig. 1b). Time to obtain 50 % germination ($T_{50}$) increased with increasing concentrations of FB, whereas the other extracts had no significant effect on $T_{50}$ (Fig. 1c). Differences between extracts and extract concentrations for MGT and $T_{50}$ were in a similar range for individual extracts and extracts concentrations.

The germination rate index (GRI) decreased gradually with increasing extract concentrations of FB, SO and SF. Also WM and PH extracts decreased GRI compared to control (not significant) (Fig. 1d). The corrected germination rate index (CGRI) decreased gradually with increasing extract concentrations of FB and SF and it was also impaired following the addition of MU, PH and SO compared to the control (not significant) (Fig. 1e). The variation of germination time ($CV_{Gt}$) slightly increased with higher extract concentrations of FB, WM and PH (not significant). SO and SF had a significantly higher $CV_{Gt}$ at a concentration of 5 % and 1 %, respectively, compared to the control.

The inhibition of germination after addition of several different cover crop extracts has already been reported for spring barley (Marcinkevičiene et al., 2013). Several factors are involved in the inhibition of germination by residue extracts, as shown for white mustard seed imbibed with sunflower leaf extracts: membrane deteriorations, enhanced $H_2O_2$ production, a disturbed hormonal balance between plant hormones, abscisic acid and ethylene in the seeds (Gniazdowska et al., 2007), induction of oxidative stress by phytotoxins within the extract in germinating seeds causing cellular damage (Oracz et al., 2007) and alterations in reserve mobilization and generation of energy in the catabolic phase of germination (Kupidłowska et al., 2006). Low concentrations of oat stubble were reported to even improve seed germination of cucumber, whereas higher concentrations had inhibitory effects (Wang et al., 2010). Germination indices were shown to vary between treated plants and are differently affected by several allelochemicals (Iftikhar Hussain et al., 2008). Germination speed was reported to

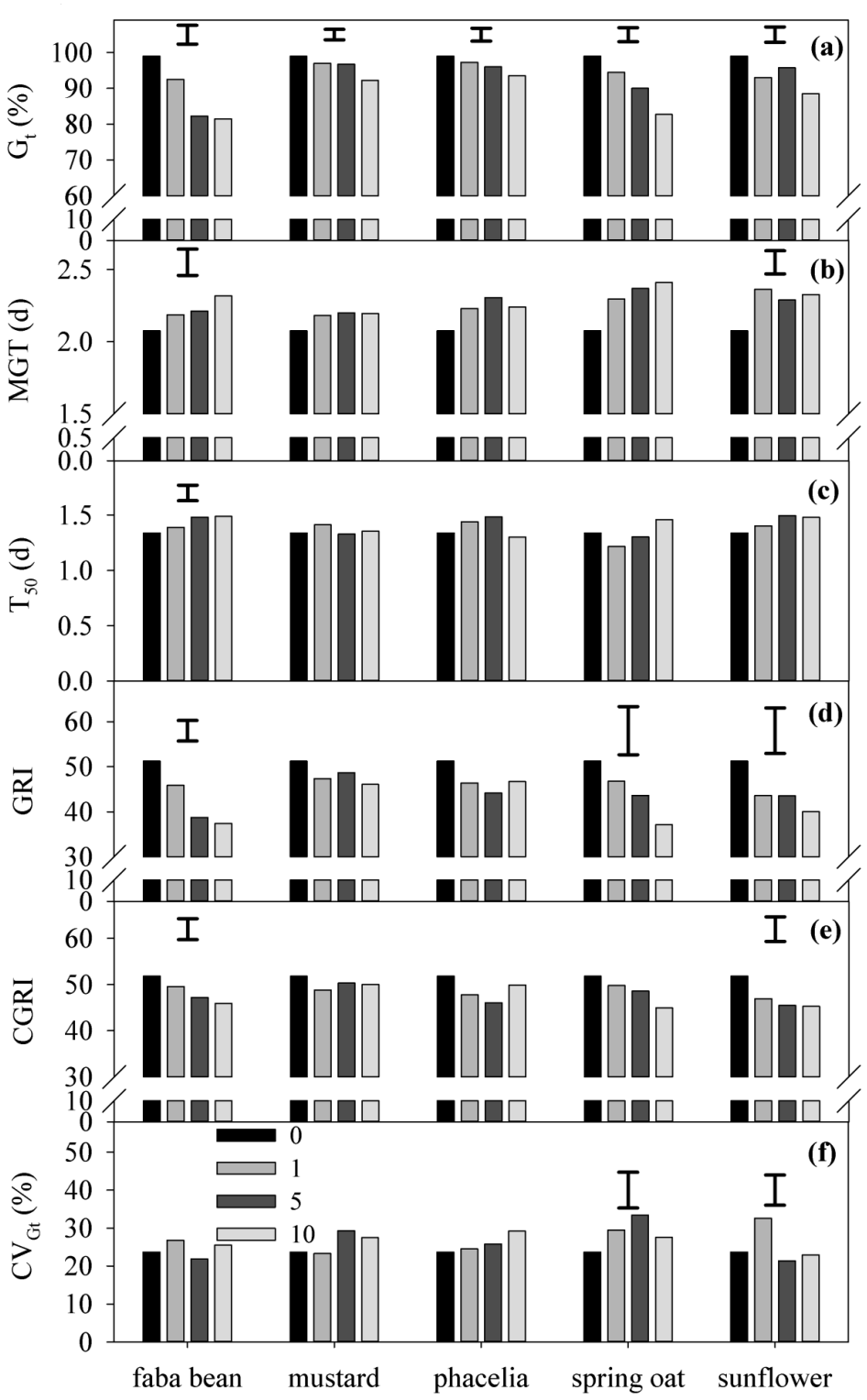


Figure 1: Total germination ($G_t$), mean germination time (MGT), time to obtain 50 % germination ($T_{50}$), germination rate index (GRI), corrected germination rate index (GRI), and coefficient of variation of germination time ($CV_{Gt}$) of maize as affected by different concentrations of above-ground biomass extracts of several cover crops. Error bars are LSD at $p<0.05$

Abbildung 1: Gesamtkeimung ($G_t$), mittlere Keimgeschwindigkeit (MGT), Zeit bis 50 % Keimung ($T_{50}$), Keimungsrate (GRI), korrigierte Keimungsrate (CGRI) und Variationskoeffizient ($CV_{Gt}$) von Mais in Abhängigkeit von verschiedenen Extrakt-Konzentrationen von oberirdischen Zwischenfruchtrückständen. Die Fehlerbalken zeigen die Grenzdifferenz ($p<0.05$)

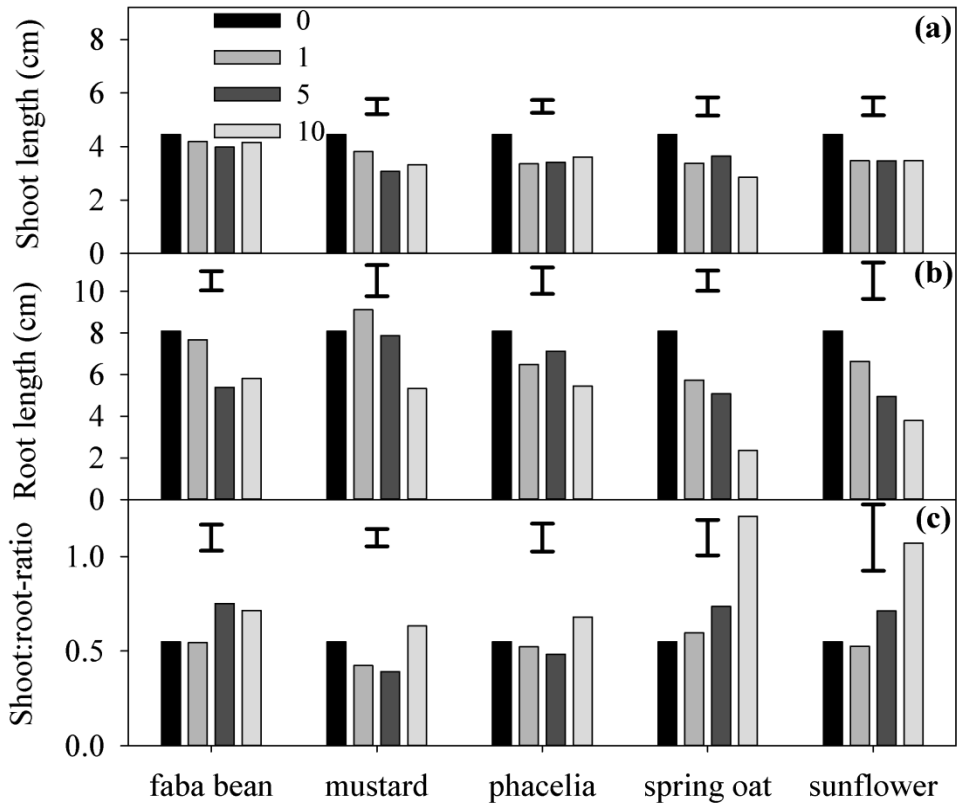


Figure 2: Seedling characteristics after 7 d as affected by different concentrations of above-ground biomass extracts of several cover crops. Error bars are LSD at p<0.05

Abbildung 2: Keimlingseigenschaften nach 7 Tagen in Abhängigkeit von verschiedenen Extrakt-Konzentrationen von oberirdischen Zwischenfruchtrückständen. Die Fehlerbalken zeigen die Grenzdifferenz (p<0.05)

be a more sensitive indicator of allelopathic effects than $G_t$ (AHMED and WARDLE, 1994). In any case, our results show similar trends of germination indices as affected by extract type for $G_t$ and for germination speed (GRI, CGRI) of maize. Among tested cover crop extracts, germination indices seem to be least impaired by extracts of WM and PH. Highest maize yields following these two crops among several tested cover crops were reported (PROCHAZKA and PROCHAZKOVA, 2012). Consequently, effects of extracts on germination may even influence the final yield.

Shoot length of maize seeds was significantly decreased by addition of cover crop extracts compared to the control (except for the FB where the slight decrease was not statistically significant). Yet no statistical differences occurred between extract concentrations of all extract. Thus, all three concentrations equally impaired shoot length. Shoot length was highest after addition of FB extracts compared to the other extracts (Fig. 2a).

Root length decreased most strongly with increasing extract concentrations of SO and SF. On the other hand, FB extracts had no effect at a concentration of 1 %, while 5 % and 10 % hampered root growth; extracts of WM and PH decreased root growth at 10 % only (Fig. 2b).

The shoot:root ratio was generally highest with extract concentrations of 10 % (except for FB where 5 % and 10 % had the highest S:R); the highest increase was observed following addition of SO and SF. WM extracts at 1 % and 5 % reduced the S:R compared to the control as just shoot and not root growth were reduced by these concentrations. Also PH extracts at 1 % and 5 % slightly impaired S:R (not significant) (Fig. 2c).

Results show that root growth was generally more strongly affected by extracts than shoot growth (except for 1 % and 5 % of WM). This might be due to more intense contact of roots with the extracts and consequently with inhibitory chemicals (JAFARIEHYAZDI and JAVIDFAR, 2011). Furthermore, shoot growth depends largely on cell expansion, which is relatively insensitive to allelochemicals; whereas root growth also requires cell proliferation, which is more affected by allelochemicals (NISHIDA et al., 2005). Changes

in the cellular ultrastructure of cucumber seedlings which were treated with allelochemicals indicated that the observed reduction of root elongation and lateral roots was caused by disruption of lipid metabolism and a reduction of protein synthesis (Burgos et al., 2004). Maize seedlings treated with shoot extracts of *Tithonia diversifolia* were reported to show a reduction of shoot and root growth, whereas the growth of plants of two weeks of age and older was enhanced. In conclusion, an extract can have both inhibitory and stimulatory effects on a plant depending on development stage (Oyerinde et al., 2009).

## Conclusion

Germination indices and seedling growth of maize were impaired with increasing concentrations of cover crop extracts. Germination indices were least affected by MU and PH and more strongly affected by FB, SO and SF extracts. Extract additions caused a higher reduction of root than shoot growth. Results of germination indices were not fully transferable to seedling growth; namely $G_t$ and GRI were most affected by FB extracts, but these were the only extracts that did not reduce shoot growth. The relevance of catch crop residues for germination and early growth of subsequently planted maize in the field should be further studied.

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