

Allelopathic effects and weed suppressive ability of cover crops

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ABSTRACT

Field and laboratory experiments were conducted to investigate the weed suppressing effects of cover crops in single and mixed cultivation. Weed densities in the field experiments ranged from 0 to 267 plants/m² with *Chenopodium album* L., *Matricaria chamomilla* L., *Stellaria media* (L.) Vill. as predominant weeds. It was found that mustard (*Sinapis alba* L.), fodder radish (*Raphanus sativus* var. *niger* J. Kern) and spring vetch (*Vicia sativa* L.) suppressed weeds by 60% and cover crop mixtures controlled weeds by 66% during the fallow period at three experimental locations in 2013, 2014 and 2015. The biochemical effect of the same cover crops/mixtures on weed growth was analysed in laboratory experiments. Aqueous cover crop extracts were applied on weeds and analysed using LC/MS/MS. Mean germination time, germination rate and root length of weeds were determined. Extracts prolonged the germination time by 54% compared to the control with only water. In all cases, inhibitory effects on germination rate and root length were measured. Weed density in the field was found to be correlated with the root length in the germination tests. Our work reveals that biochemical effects play a major role in weed suppression of cover crops.

Keywords: allelopathy; erosion; root growth; competition; inter cropping

Cover crops have several beneficial effects on agricultural fields. They reduce wind and water erosion (De Baets et al. 2011), increase biological soil activity (Mendes et al. 1999) and prevent leaching of nutrients (Teasdale 1996). Therefore, cover crops play an important role in conservation agricultural systems (Triplett and Dick 2008). Furthermore, cover crops have suppressed up to 90% of weed species and volunteer crops emerging after harvest of cereal crops (Brust et al. 2014, Jabran et al. 2015). One reason for the reduction of weeds by cover crops is the intensive competition for light, water, space and nutrients (Bezuidenhout et al. 2012). Another reason might be release of different allelopathic substances from cover crops and crop residues that can also suppress weed growth (Farooq et al. 2011). Different plant species are able to synthesize allelopathic substances in leaves, fruits, roots or seeds (Radosevich et al. 1997). A variety of chemicals, such as phenolics, flavonoids or terpenoids are known to possess allelopathic properties (Macías et al.

2007). Such compounds may be released by cover crops in the soil via leachates or root exudates, or by decomposition of plant biomass such as mulch (Bonanomi et al. 2006). Few studies demonstrate the inhibitory effects of different cover crops on weed growth in field and laboratory experiments (Jabran et al. 2015). In several studies, only the overall effect of weed suppression was measured, which is a combined effect of competition and allelopathy. The aim of this study was to separately analyse competitive and allelopathic effects of cover crops on weed species.

The objectives of this study were to analyse the weed suppressive effects of cover crops and cover crop mixtures in the field, if cover crop mixtures compared to single-cultivation result in better weed control efficacy and biomass yield, and which biochemical effects those cover crops and cover crop mixtures have on weed germination. This would enable us to separate the competitive and biochemical effects on weed species.

Table 1. Detailed information of the four field experiments

	Bad Sassendorf 2013	Heidfeldhof 2014	Ihinger Hof 2014	Ihinger Hof 2015
Mean max./min. temperature (°C)	18.5/2.4	19.9/0	19.3/0.2	19.3/0.2
Mean annual rainfall (mm)	768	659	790	790
Rainfall during the experimental period (mm)	197	252	329	196
Soil	loessic loam	deep loam, subsoil clay	silty loam	deep loam, subsoil clay
pH/organic carbon (%)	7.1/1.33	5.9/1.22	6.3/2.9	7.0/1.22
Mineral nitrogen content (N _{min}) (kg/ha)	38	18.2	36.8	10.2
Harvest of cereals (date)	24 th June	3 th August	17 th August	28 th July
Soil preparation	stubble cultivator (5 cm)	moulboard plough (20 cm)	stubble cultivator (4 cm)	stubble cultivator (4 cm) + deep cultivation (20 cm)
Soil preparation date	2 nd August	5 th August	15 th August	29 th July; 19 th August
Cover crop sowing date	23 th August	12 th August	20 th August	21 th August
Cover crop sowing depth (cm)	3	2	2	2
Cover crop inter-row distances (cm)	12.5	16	16	16

MATERIAL AND METHODS

Field study. Four cover crop experiments were carried out at three different locations from 2013 to 2015 (Table 1). All four experiments were designed as a randomized complete block design with four replicates. The plot size varied from 30 to 40 m² over locations with a plot width of 3 m. In 2013, the experiment was carried out in Bad Sassendorf (BS) (51.33°N, 8.13°E, 107 m a.s.l.), in 2014 at Heidfeldhof (HD) (48.71°N, 9.19°E, 370 m a.s.l.) and Ihinger Hof (IHO) (48.74°N, 8.92°E, 478 m a.s.l.). The experiment at IHO was also repeated in 2015.

Cover crops were sown in rows with a distance of 12–16 cm and a depth of 2 cm (Table 2) with a common drilling machine for each farm. No additional fertilizer was applied. Experiments included six different cover crop treatments (Table 2). A control plot with no cover crop was also included. Weed density was counted twice (5 and 9 weeks after sowing (WAS)) by using a frame (0.1 m²) at three randomly selected positions within each plot. Dried cover crop shoot-biomass was measured in an area of 0.1 m² at five randomly selected positions at 7 and 12 WAS. BS had 4 treatments and was measured at 12 WAS at four randomly

selected positions. Afterwards, cover crop biomass was projected on 1 m². Plants were washed and dried at 90°C for a period of two days.

Laboratory study. Germination tests with aqueous cover crop extracts were conducted to evaluate the biochemical effect of cover crops on weeds. The same cover crops and cultivars were tested as those used in the field trials. Germination and root length of the weed species *Chenopodium album* L., *Matricaria chamomilla* L., *Stellaria media* (L.) Vill. and *Veronica persica* Poir. were tested. These species were also predominant in the field trials. Cover crops were sown separately in six 5 L pots each, containing a soil mixture of 50% compost, 25% loam and 25% sand. The amount of seeds per pot was calculated using recommended sowing rates. Pots were irrigated daily and fertilized with 2 g N-P-K (14-7-17) every two weeks. Above ground biomass and roots were harvested after 10 weeks, representing the same development stage of the cover crops within the field. Plant material was washed with water and then chopped (Robert Bosch GmbH, AXT Rapid 2200, Gerlingen, Germany) and crushed into powder using liquid nitrogen. Deionized water was added to reach a concentration of 0.125 g fresh plant matter per mL H₂O. This concentration was chosen

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Table 2. Weight proportion, sowing rate and 1000-seed weight of the evaluated cover crop treatments at three different locations (Bad Sassendorf, Ihinger Hof, Heidfeldhof)

Treatment	Cover crop	Weight proportion (%)	Sowing rate (kg/ha)	1000-seed weight (g)
1	untreated control	–	–	–
2	<i>Sinapis alba</i> L.	100	15–18	5.5
3	<i>Raphanus sativus</i> var. <i>niger</i> J. Kern	100	8	21
4	<i>Vicia sativa</i> L.	100	85–120	70
5	mixture 1 (M1)	–	37	–
	<i>Vicia sativa</i> L.	43	–	70
	<i>Avena strigosa</i> Schreb.	22.5	–	16
	<i>Raphanus sativus</i> L.	25.5	–	5.5
	<i>Trifolium alexandrinum</i> L.	6	–	3
	<i>Guizotia abyssinica</i> (L.f.) Cass.	3	–	2.5
	mixture 2 (M2)	–	40–42	–
6	<i>Vicia sativa</i> L.	25	–	70
	<i>Pisum sativum</i> L.	24	–	160
	<i>Lupinus angustifolius</i> L.	18	–	150
	<i>Avena strigosa</i> Schreb.	13	–	16
	<i>Trifolium alexandrinum</i> L.	10	–	3
	<i>Phacelia tanacetifolia</i> Benth.	6	–	2
	<i>Guizotia abyssinica</i> (L.f.) Cass.	4	–	2.5

based on preliminary germination tests. After agitation (24 h, 200 rpm) at room temperature (RT), extracts were centrifuged (4500 rpm, 10 min, RT) and poured into a Büchner funnel lined with nylon filter (1.2 µm). Untreated control was prepared with deionized water (0 mg/mL). Per petri dish (φ 60 mm), thirty weed seeds were placed on filter paper. Three mL of extract were applied per dish, representing the optimum extract volume. Afterwards, they were sealed (Parafilm M®, Neenah, USA) and stored in a climate chamber at 12 h/12 h day/night cycle with temperatures of 20°C/15°C for 10 days. Newly germinated seeds were measured daily for calculating mean germination time (MGT) after Ellis and Roberts (1980). Germination rate and root length were determined after a period of 10 days.

LC/MS/MS (Velos, Thermo Scientific, Waltham, USA) detection was conducted to identify several amines (allylamine, benzylamine, 4-hydroxybenzylamine, [3-(aminomethyl)indole] as specific degradation products of glucosinolates (sinigrin, glucosinalbin, glucobrassicin, glucotropaeolin) in *S. alba* and *R. sativus* (Petersen et al. 2001). A mixture of fresh plant material (4 g) and 15 mL NaOH was heated (20 min,

85°C) for the degradation of the glucosinolates to amines. The mixture was filtrated and steam distilled. The aqueous distillate was derivatized and subjected to LC/MS/MS analysis. Amines were converted in corresponding amounts of glucosinolates using the molar ratio coefficient (Petersen et al. 2001).

Statistical analysis. An analysis of variance (ANOVA) was performed in all data, using R version 3.0.2 (Vienna, Austria). Means were compared using the Tukey's-HSD test at 95% level of probability when the ANOVA *F*-test showed significant differences at 0.05 probability level.

RESULTS AND DISCUSSION

Weed suppression and biomass yield. Averaged over all locations, *S. alba*, *R. sativus* var. *niger*, *V. sativa* and both tested mixtures of cover crops (M1 and M2) reduced weed density by 57, 62, 62, 68 and 64%, respectively, compared to the untreated control 9 WAS (Figure 1).

At location BS, average weed reduction of all cover crop treatments was 18% compared to the untreated control. A significant difference was

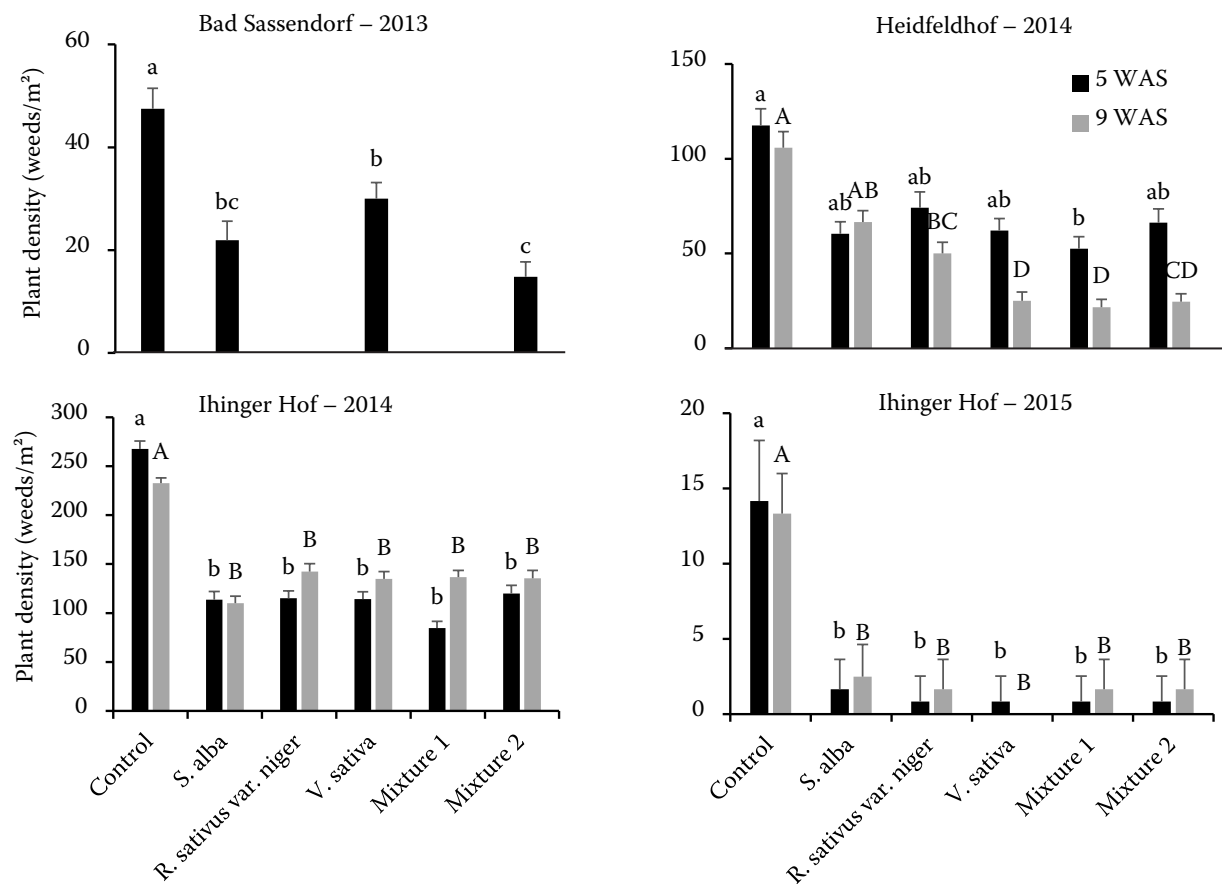


Figure 1. Weed density in cover crops counted 5 and 9 weeks after sowing (WAS) at 3 locations. Means plus standard error with identical letters within graph do not differ significantly based on the Tukey's-HSD test ($P \leq 0.05$). 5 (small letters) and 9 (capital letters) WAS were separately grouped

observed for M2, compared to the other cover crop treatments.

At location IHO in 2014, cover crops reduced weed density by 59% 5 WAS and 43% 9 WAS. Lowest weed density was observed at plots with *S. alba* with 110 plants/m² at 9 WAS. *S. alba* is well known for effective weed suppression within the field (Brust et al. 2014). Furthermore, the single-cultivation of *R. sativus* var. *niger*, *V. sativa*, M1 and M2 achieved a significant decrease of weed population compared to the untreated control. Similar results were observed during autumn period in the study of Lawley et al. (2011). Five WAS differences of the weed suppressive ability can be observed between treatments. Higher weed population was observed in treatment M2. *R. sativus* var. *niger*, included in treatments 3 and 5, had a faster emergence compared to all the other plants but decelerated after the 6 WAS. That might have resulted in less resource competition with the

weeds. *V. sativa* resulted in a smaller leaf area than the rest, resulting in higher weed densities especially at 9 WAS. In 2015, no differences in regard to weed control were observed over all cover crop treatments. This was contributed to drought in that specific year. Due to water shortage weeds were possibly unable to emerge. At location HD, cover crops reduced weed density by 47% (5 WAS) and 64% (9 WAS) compared to the untreated control. At 9 WAS, the lowest weed density was observed in M1, but the differences compared to the treatments *V. sativa* and M2 were insignificant. At location HD, 5 WAS weed density on *S. alba* treatment, was similar, even slightly smaller than the rest of the cover crop treatments. Yet, at 9 WAS it was higher compared to the other treatments. *S. alba* growth was reduced due to the appearance of *Athalia rosae* L. (turnip sawfly) (Hymenoptera, Tenthredinidae) in this location. That reduced the efficacy of *S. alba* on weeds.

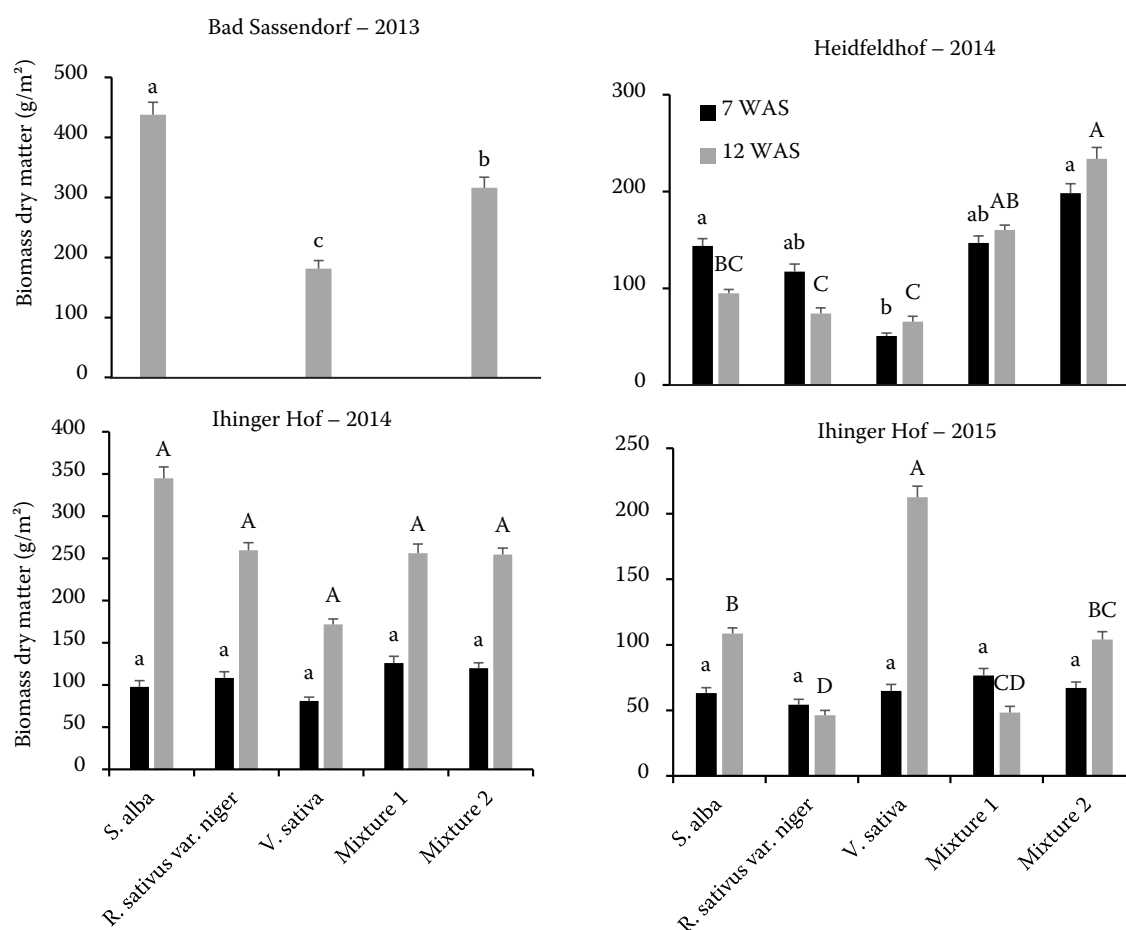


Figure 2. Average dry matter of cover crops harvested 7 and 12 weeks after sowing (WAS) over all 3 locations. Means plus standard error with identical letters within graph do not significantly differ based on the Tukey's *-HSD* test ($P \leq 0.05$). 7 (small letters) and 12 (capital letters) WAS were separately grouped

Additionally, the fast flowering of *S. alba* thinned out the canopy at the end of the vegetation period. Cover crop mixtures, due to their diversity, were able to react to unpredicted stress factors, in this case *A. rosae*. Therefore, mixtures can compensate potential deficiencies during the vegetation period, due to higher elasticity and the ability of recovery, as shown at this location. Nevertheless, if no stressors appear, single cover crop species were also successful in weed control as seen for *R. sativus var. niger* and *V. sativa*.

At the first biomass yield, the highest output was observed at location HD, compared to the other locations. The mean value measured was 131 g/m² (Figure 2). For the cover crop treatments, M2 had the highest average, between locations (128 g/m²). For the second biomass yield, locations BS and IHO (2014) yielded the highest biomass (312 and 259 g/m²) and the most distinctive treatments were *S. alba* and M2 (246 and 227 g/m²). Brust

et al. (2014) reported similar cover crop yields. They presented an intensive and fast cover crop growth, both above and below ground. This fast cover crop development was the pre-requisite for high weed suppression. Therefore, the drought at IHO in 2015, which resulted in lower cover crop biomass, was expected to have a negative impact on the control efficacy. However, all treatments at IHO (2015) resulted in significant weed control. No correlation between biomass (g/m²) and weed density (plants/m²) over all three locations could be established (Figure 3). Our study suggests that either the combination of cover crop competition and shading effect due to dense canopy were already sufficient to lower weed density or as also suggested by Jabran et al. (2015), the biochemical effects play a stronger role in the overall weed suppression, than expected.

Germination tests with cover crop extracts. At concentrations of 125 mg/mL, germination

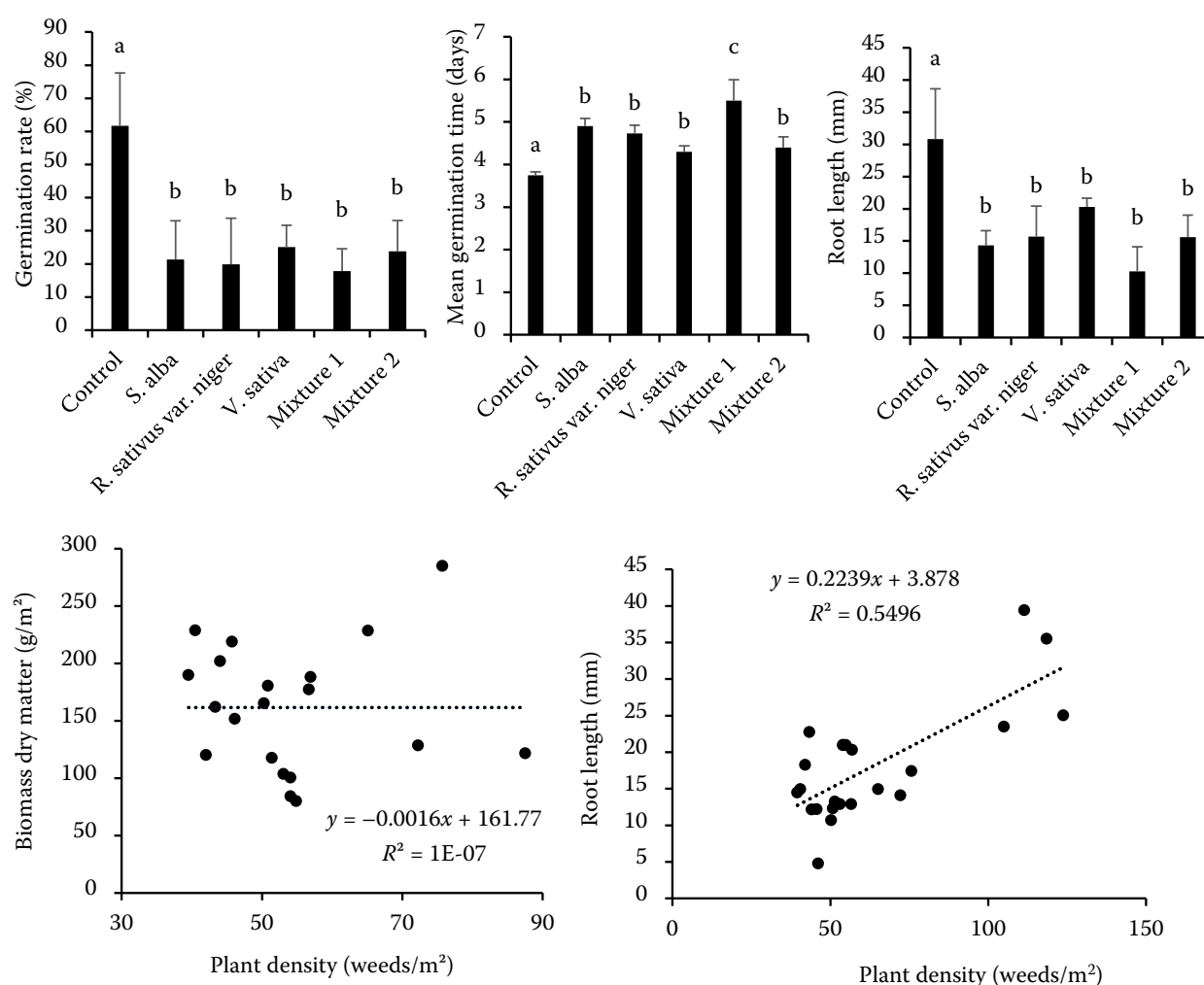


Figure 3. Germination rate, mean germination time and root length of all weeds treated with cover crop extracts (125 mg/mL) in germination tests and a correlation between root length in the laboratory test with weed density and between cover crop biomass with weed density in the field average over all three locations. Means with identical letters within graph do not differ significantly based on the Tukey's-HSD test ($P \leq 0.05$)

rate and root length of all weeds were reduced significantly over all cover crop extracts compared to the untreated control (Figure 3). Most inhibitory effects on germination rate and root length were observed for extracts of M1 (71% and 67%, respectively). Furthermore, MGT for all treatments was significantly different compared to the untreated control. No significant differences were measured amongst the treatments except for M1 in which germination was prolonged.

The high efficacy of extracts from *S. alba*, *R. sativus* var. *niger* and M1 in germination tests could arise due to the high amounts of phytotoxic and allelopathic substances in Brassicaceae plants. In *S. alba*, glucotropaeolin (45 mg/kg), glucosinabin (7.2 mg/kg) and glucobrassicin (< 0.1 mg/kg) were

detected. *R. sativus* var. *niger* contained lower rates of glucotropaeolin (7.3 mg/kg) and sinigrin (1.7 mg/kg). Glucosinolate hydrolysis products, like isothiocyanates can inhibit and delay germination (Haramoto and Gallandt 2004). The inhibitory ability of M1 might be attributed to synergistic effects of Brassicaceae species with other plants, which possess allelopathic traits (Jabran et al. 2015). Pérez and Ormeño-Nuñez (1991) suggest the presence of allelochemicals in *Avena* species and Liu et al. (2013) found similar results in *Trifolium* species. The presence of these species could have enhanced the inhibitory effects of M1 extract. Root length of the laboratory experiment was correlated with the weed density (plants/m²) of the field experiments ($R^2 = 0.55$). This correlation

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implies a high impact of phytotoxic substances in cover crops for effective weed suppression, along with the competitive factors, as presented by the studies of Altieri et al. (2011). Based on our results, allelopathic effects, triggered by cover crop cultivation, can reduce weed emergence.

In conclusion, weed suppression by cover crops is a result of competitive and biochemical effects. The correlation between field and laboratory studies suggests that the inhibition of weeds by active substances in several cover crop species plays an important role. Cover crops should be mixed to achieve high competition and biochemical suppression of weed growth.

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