Assessing the biofumigation potential of Brassicaceae *cultivars* to *Verticillium dahliae*

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Introduction

Biofumigation is worldwide discussed as an alternative strategy to control *Verticillium dahliae*. The method is defined as incorporation of brassicaceous green manures containing glucosinolates (GSLs). These compounds are hydrolyzed in soil to isothiocyanates (ITCs), which have a toxic effect on microsclerotia (Fig. 4). The concentration of the glucosinolates varies within and between species of Brassicaceae, and consequently the concentration and type of isothiocyanates evolved also varies. Additionally ITCs differ in their toxicity to *Verticillium*. Testing the biofumigation potential of a large scale of *Brassica* genotypes in field trials is very extensive. Also it is difficult to provide reproducible results because of many influencing soil factors. Therefore an efficient lab bioassay was developed to assess the biofumigation potential of different genotypes under standardized optimal conditions and to compare the effect with their GSL profile. The aim was to select the best ones for further field trails.

Material and methods

Production of biomass

19 cultivars of *Brassica juncea, Sinapis alba* and *Rhaphanus sativus* were grown on the field between June and August 2011 (Fig. 1). Each cultivar was sampled at mid-flowering. Shoot biomass was calculated by weighting all plants of one square meter. Three samples were taken from a plot, each consists of 5 representative plants, which were cut off at the stem basis. Samples were freeze-dried and ground (> 0,5 mm).

Fig. 1: *R. sativus* (left) and *B. juncea* (right)

Bioassay and GSL analysis

Freeze dried, ground shoot tissues of the cultivars were add to a sterile quartz sand filled in gastight glass flasks and artificially infested with 200 microsclerotia/g sand considering the field situation (Fig. 2.1-2). Flasks with GSL-free tissue of *Phacelia* served as controls. Water was added to compensate the water loss of the biomass due to freeze drying and to adjust an optimum moisture level of 60 % of maximum water capacity. Sand, shoot tissue and water were thoroughly mixed to guarantee a homogenous mixture (Fig. 2.3). Flasks were sealed immediately and incubated at 20°C for 48 h. The soil of each flask was air dried and analyzed for viable microsclerotia using the wet sieving detection method described by Neubauer and Heitmann (2011). Additionally GSL concentrations of the biomasses were determined by HPLC and LC-MS according to Ager-Birk (2001) and Opitz et al. (2011).

Fig. 2: Assessment of biomass with bioassay

Results and Discussion

The effects of the shoot tissues in the bioassay reflect the biofumigation potential of the tested brassicaceous amendments under optimal conditions (Fig. 3). They were compared with the maximum amount of ITC release calculated on basis of the measured GSL concentrations (Table 1). Considering the LD_{90} values of the ITCs, determined in a similar test system (unpublished data), the ITC related biofumigation effect of Brassicaceae genotypes can be estimated.

Brassica juncea

Biomasses of *B. juncea* contained Sinigrin in concentrations between 15.5 and 26.5 µmol/g DM (Table 1). In the bioassay the amended *B. juncea* tissues could potentially produce concentrations of 2-propenyl ITC ranging from 50.6-78.1 nmol g^{-1} soil. The amendments reduced the number of viable microsclerotia significantly with efficiencies between 69.3 and 81.3 % (Fig. 3). Assuming that 75 % of Sinigrin is converted into 2-propenly ITC and considering the LD_{90} value of 88.7 nmol/g sand the suppression of microsclerotia can be interpreted widely as an ITC-related biofumigation effect.

In the bioassay freeze-dried tissue with a maximum disruption on cell level was used, whereas in practice the plant tissue is incomplete pulverized so that the release efficiency of ITCs is lower. Also in a natural soil the fate and toxicity of 2-propenyl ITC is reduced, due to ITC sorption on organic matter and microbial degradation. Therefore the biofumigation potential of *B. juncea* could be too low in the field to achieve a significant effect on *Verticillium*.

Fig. 3: Effect of tested brassicaceous amendments on microsclerotia of *V. dahliae* (left) and total ITC liberating of applied tissue on basis of GSL concentration (right).

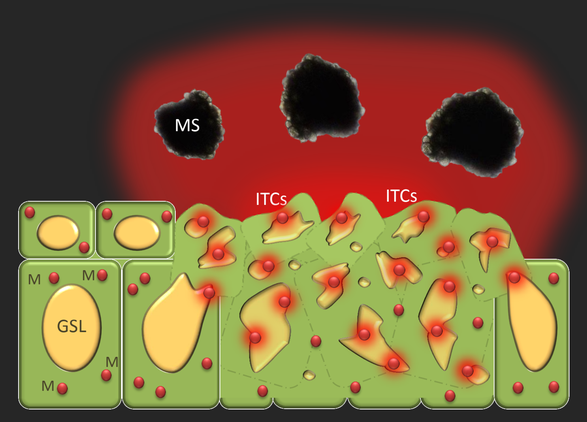

Fig. 4: Prinziple of biofumigation: GSLs are hydrolysed by the myrosinase enzyme (M), which is physically separated from the GSLs in the intact plant tissue. Upon tissue disruption the GSLs and the myrosinase come into contact and the enzyme hydrolyse the GSLs to form ITCs, which have a toxic effect on microsclerotia (MS)

Table 1: Field grown species and cultivars of Brassicaceae 2011: Production of glucosinolates, which are converted into toxicological relevant isothiocyanates, and potential isothiocyanat concentrations in soil.

	Brassica juncea					*Raphanus sativus*							*Sinapis alba*					
Cultivar	Terrafit	Energy	101	102	103	Defender	Contra	Adagio	Consul	Siletta N	Colonel	Picobello	Commodore	Luna	Cover	Absolut	Maxi	Accent	Samba
Fresh matter [kg/m²]	2.9	2.8	3.6	3.2	1.9	7.3	5.6	8.3	4.4	6.1	5.6	4.4	6.3	2.0	2.6	2.8	2.3	2.7	1.7
Dry matter [kg/m²]	0.5	0.5	0.5	0.5	0.4	0.9	0.7	1.0	0.5	0.77	0.8	0.5	0.7	0.3	0.5	0.5	0.4	0.5	0.3
Glucosinolate concentration [µmol/g DM ± SD]	SINIGRIN					GLUCORAPHSATIN								GLUCOTROPAEOLIN					
	20.1 ± 2.9	26.5 ± 2.2	21.9 ± 1.6	15.5 ± 1.9	24.4 ± 4.7	17.1 ± 4.1	14.0 ± 4.1	19.6 ± 0.3	15.3 ± 4.1	4,0 ± 3.0	7.7 ± 1.1	9.4 ± 0.5	18.2 ± 2.4	2.4 ± 0.6	0.9 ± 0.2	2.0 ± 0.2	1.8 ± 0.2	2.6 ± 0.7	2.4 ± 0.7
Glucosinolate production [mmol/m²]	SINIGRIN					GLUCORAPHSATIN								GLUCOTROPAEOLIN					
	10.6	12.1	11.9	7.5	9.2	14.9	9.8	19.2	8.1	3.1	6.0	4.7	13.3	0.8	0.4	1.0	0.8	1.2	0.8
Total ITC-liberating* [nmol/g soil]	2-PROPENYL ITC					4-METHYLTHIOBUTENYL ITC								BENZYL ITC					
	78.2	89.4	87.8	55.8	67.8	111	72,8	142	59.8	22.8	44.6	34.8	98.2	5.9	3.2	7.4	5.7	8.9	5.6

* Total ITCs added to the soil assuming a release rate of 100 %, incorporation depth 10 cm, and a soil bulk density of 1,3 g cm^{-3}.

Raphanus sativus and *Sinapis alba*

The amendments with cultivars of *R. sativus* und *S. alba* had compared to *B. juncea* a much lower effect on the microsclerotia. Mortalities of microsclerotia were determined between 9.8 % for the poorest and 37.5 % for the best variety (Fig. 3). In case of *Raphanus* the potential ITC concentrations or the conversion of GSLs into ITCs was too low regarding to the LD_{90} value. The tissues of *S. alba* genotypes could produce total amounts of benzyl ITC near or above the LD_{90} value of 8.4 nmol g/sand, nevertheless their effect was very poor. Probably the release efficiency was also too low or benzyl ITC was directly sorbed by organic matter of the applied biomass itself. The results show that *S. alba* and *R. sativus* are no alternatives to *B. juncea*.

References: NEUBAUER C., HEITMANN B. , 2011: Quantitative detection of *Verticillium dahliae* in soil as a basis for selection of planting sites in horticulture. Journal für Kulturpflanzen, 63 (1). S. 1-8, 2011

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