# Weed Suppression by Release of Isothiocyanates from Turnip-Rape Mulch

Jan Petersen,* Regina Belz, Frank Walker, and Karl Hurle

## ABSTRACT

**The allelopathic potential of isothiocyanates (ITC) released by turnip–rape mulch [*Brassica rapa* (Rapifera Group)–*Brassica napus* L.] was evaluated. Six different ITCs were identified from chopped turnip–rape by HPLC-DAD/HPLC-MS. All plant parts contained 2-phenylethyl-ITC. In the shoot *n*-butyl and 3-butenyl-ITC dominated. Younger leaves, flowers, and buds also contained small amounts of benzyl and allyl-ITC. Furthermore, marginal amounts of 4-pentenyl-ITC were detected. In the soil, where turnip–rape mulch was incorporated, only low amounts of ITCs were detected. It was shown that the $DT_{50}$ of ITCs in soil are very short. Germination tests with weed seeds in aqueous ITC solutions showed, that aryl-ITCs were the most suppressive compounds. Within the alkyl-ITCs, the activity decreased with increasing molecular mass. The susceptibility of different weed species to ITCs mainly depended on seed size. Smaller seeds tended to be more sensitive. Further studies demonstrated a high biological activity of ITCs in the vapor phase. *n*-Butyl-ITC was more suppressive in the vapor phase than in aqueous solution, while 2-phenylethyl-ITC showed the opposite effect. Results demonstrated that weed suppression observed in the field was probably due to the high amounts of ITCs identified in turnip–rape mulch. Isothiocyanates were strong suppressants of germination on tested species—spiny sowthistle [*Sonchus asper* (L.) Hill], scentless mayweed (*Matricaria inodora* L.), smooth pigweed (*Amaranthus hybridus* L.), barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.], blackgrass (*Alopecurus myosuroides* Huds.), and wheat (*Triticum aestivum* L.)—and probably interact with weed seeds in the soil solution and as vapor in soil pores.**

Species of the *Brassica* family are often used as cover or green manure crops (de Almeida, 1985; Brown and Morra, 1995; Boydston and Hang, 1995; Al-Khatib et al., 1997; Krishnan et al., 1998). Superficial incorporation (upper soil surface, 0–10 cm) of green mulch of these species caused a temporary weed suppression, which is probably due to secondary plant metabolites (Al-Khatib et al., 1997). *Brassica* spp. contain high amounts of glucosinolates (Fenwick et al., 1983). Although the biological activity of these secondary plant metabolites is very low (Fenwick et al., 1983), they play a key role for weed suppression as they can be converted to the corresponding isothiocyanates (ITC) by the plant enzyme myrosinase. Besides ITCs, also other breakdown products of glucosinolates (nitriles, thiocyanates, and oxazolidinethiones) can occur, depending on various factors [e.g., pH, Fe(II) concentration, side chain-substitution, and plant species] (Bones and Rossiter, 1996). The main breakdown products at pH 7 are ITCs (Bones and Rossiter, 1996), which are phytotoxic (Fenwick et al., 1983). Living plants do not actively release high amounts of ITCs (Börner, 1961), because glucosinolates are located in the vacuole and myrosinase is bound to the cell wall (Björkman, 1976). As long as this separation exists, there is only a low ITC content in the cells (Tang, 1971). Larger amounts of ITCs can only be released by the breakdown of the cells, e.g., during decomposition of dead plant material (Bell and Muller, 1973), or even faster by incorporating green plant material into the soil. If *Brassica* spp. plant tissues are incorporated into the soil, it is possible to control weeds in the following crop by ITCs released from the mulch (Brown and Morra, 1995; Boydston and Hang, 1995; Al-Katib et al., 1997). This might be a chance to reduce the use of herbicides and could be an additional tool to control herbicide-resistant weeds.

The objectives of this study were to evaluate the allelopathic potential of ITCs released by turnip–rape mulch. Therefore, the content of ITCs in different parts of turnip–rape, and in the soil after incorporation was determined, and the phytotoxicity of different ITCs on several weed species—spiny sowthistle [*Sonchus asper* (L.) Hill], scentless mayweed (*Matricaria inodora* L.), smooth pigweed (*Amaranthus hybridus* L.), barnyardgrass [*Echinochloa crusgalli* (L.) Beauv.], blackgrass (*Alopecurus myosuroides* Huds.), and wheat (*Triticum aestivum* L.)—was investigated.

## MATERIALS AND METHODS

### Plant and Soil Material

Winter turnip–rape [*Brassica rapa* (L.) var. *rapa* ssp. *oleifera* (DC.) Metzg. cv. Perko] was planted (10 kg $ha^{-1}$) 20 Aug. 1997 at Hohenheim University, Stuttgart, Germany. The soil type was a loam (pH 6.7) with an organic matter content of 1.8%. The average plant density in April 1998 was 66 plants $m^{-2}$. On 24 Apr. 1998 the shoots of the plants were cut off (2–4 cm above soil level) and 1 wk later, the turnip–rape mulch was incorporated 10 cm deep into the soil with a rotary tiller. Plant and soil material for chemical analysis and germination tests were taken from this field (fresh plant samples 23 Apr. 1998 and soil samples 7, 15, and 28 d after mulch incorporation) and stored at −20°C until analysis.

### Germination Tests

Germination tests were performed in glass petri dishes (9-cm diam.) containing two layers of filter paper (9-cm diam., Macherey-Nagel), moistened with 8 mL of distilled water and 50 seeds of the test species were placed on the filter paper. For different concentrations the hydrophobic ITCs were initially dissolved in methanol and then known amounts of the metha-

J. Petersen, current address: Inst of Sugar Beet Research, Holtenser Landstr. 77, 37079 Goettingen, Germany; and R. Belz, F. Walker, and K. Hurle, Inst. of Phytomedicine, Weed Science Dep., Hohenheim Univ., 70593 Stuttgart, Germany. Received 29 Nov. 1999. *Corresponding author (petersen@ifz-goettingen.de).

Published in Agron. J. 93:37–43 (2001).

**Abbreviations:** DT, disappearance time; ED, effective dosage; HPLC, high-performance liquid chromatography; ITC, isothiocyanate; MS, mass spectrometry; PAD, photodiode array detector; TSM, thousand seed mass.

nol–ITC solution were added to the destilled water. The methanol concentration in each petri dish (except the water control) was 0.4% (vol.). The methanol control (0.4%, vol.) had no effect on germination and growth of the test plants (results not shown—similar to water control). The ITCs were applied in concentrations between 0.1 and 200 mg $L^{-1}$. The tests were performed with methyl, allyl, *n*-propyl, *n*-butyl, benzyl, and 2-phenylethyl-ITC (Lancaster Synthesis), respectively. The petri dishes were sealed with parafilm (American National Can) and placed in a growth chamber at 24/16°C and 50/0 μmol $m^{-2}$ $s^{-1}$ light intensity (12 h/12 h). Each treatment was repeated six times. After 7 d, the germination percentage was evaluated by counting number of germinated seeds (radical length >2 mm) and by setting germination percentage of the control as 100%. A test period of 7 d was necessary, because the ITCs caused a delay in germination.

The effect of volatile ITCs was tested in 1-L glass jars. Germination percentages were evaluated for smooth pigweed. At the bottom of the jars two layers of filter paper (11-cm diam.) were moistened with 10 mL of distilled water and 50 seeds were put in. In the center of each filter paper a glass stopper with a 2.7-cm diam. glass fiber paper (Schleicher & Schuell) was placed (Fig. 1). The test compounds were applied on the glass fiber paper, which was taped to the glass stopper. 2-Phenylethyl and *n*-butyl-ITC were dissolved in methanol and tested in different amounts (6.25–2000 μg) per glass jar. Finally, the jar was closed with a glasscover and sealed with parafilm. Due to the high vapor pressure of the compounds (>1 hPa, 25°C) the ITCs volatilized rapidly. The same amount of methanol was applied to each treatment. Test parameters and climatic conditions were the same as mentioned above. Each treatment was repeated five times.

To describe the sensitivity of different species to ITCs, another germination test in petri dishes was performed. Different dosages (1–100 mg $L^{-1}$) of methyl-ITC were tested on six species (smooth pigweed, blackgrass, barnyardgrass, scentless mayweed, spiny sowthistle, and 'Toronto' winter wheat), and after 7 d the germination percentages were determined.

An additional germination test was performed using the field soil samples. The samples were taken 1 wk after incorporation of the turnip–rape mulch. The soil (0–10 cm depth) was passed through a 4-mm sieve and placed in 18 by 13 by 5 cm plastic pots. In each pot, 200 seeds of a weed species were added and watered continuously by glass fiber wicks (Vitrulan). The test was conducted in an open greenhouse in summer (temperature during daytime 30°C). Three different weed species were tested: smooth pigweed, scentless mayweed, and spiny sowthistle. Each treatment was repeated six times. After 7 d emergence percentage was determined.

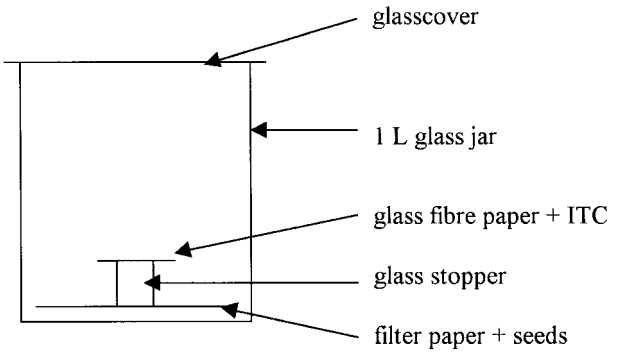


**Fig. 1. Design for testing the effect of volatile ITCs on germination of smooth pigweed.**

**Table 1. Linear regression of external standards for isothiocyanate quantification.**

| Isothiocyanate | Linear regression | $r^2$ (0.95) |
|---|---|---|
| Allyl | $y = 0.4849\ x$ | 0.71 |
| Benzyl | $y = 0.2102\ x$ | 0.54 |
| *n*-Butyl | $y = 1.1430\ x$ | 0.90 |
| 2-Phenylethyl | $y = 0.2917\ x$ | 0.80 |

## Data Analysis

Germination percentages were subjected to nonparametric h-test (Kruskal and Wallis, 1953), because of inhomogeneous variances. Means with different letters indicated significant differences (Kruskal and Wallis/Tukey-Kramer; $\alpha = 0.05$). To compare the activity of different isothiocyanates, a four parameter logistic regression model was fitted to the data by nonlinear regression analysis (Streibig, 1988). To cope with inhomogeneous variances, a weighting procedure by using inverse SD was applied (Michel et al., 1999). The assumption of parallel dose–response curves was tested by an *F*-test (Seefeldt et al., 1995).

## Analysis of Isothiocyanates

A method was developed to extract and analyze ITCs in soil and plant tissues. To avoid losses during the sample extraction process, the ITCs were converted to their corresponding amines by alkaline hydrolysis. Therefore, plant material was homogenized in 0.1 *M* NaOH using an Ultra Turrax (Janke & Kunkel). After this step each sample was divided in two subsamples. The subsamples were extracted separately and analyzed, and mean was calculated. Because of complex extraction method replications, and statistical analysis could not be conducted. The samples (soil 50 g; plant tissue 0.25–5 g) were added into a 2-L retort and 1 L of distilled water, 5 mL of 10 *M* NaOH, and 100 μL of silicone antifoaming emulsion (Roth) were added. The whole mixture was heated to convert the ITCs to the corresponding amines, which were depleted by steam extraction for 30 min. Steam was conducted through

**Table 2. Isothiocyanate content in different organs of turnip rape.**

| Organ | Dry wt. | Isothiocyanate | Amount ITC | Relative ITC | Sum ITC |
|---|---|---|---|---|---|
| | mg $m^{-2}$ | | mg $m^{-2}$ | % | mg $m^{-2}$ |
| Flowers + buds | 36.2 | Allyl | 2.2 | 17.2 | 55.7 |
| | 6.0 | Butenyl‡ | 6.9 | 7.6 | 12.0% |
| | | Benzyl | 1.7 | 4.4 | |
| | | Butyl | 37.2 | 28.5 | |
| | | Phenylethyl | 7.8 | 4.1 | |
| Younger leaves† | 71.1 | Allyl | 1.4 | 11.4 | 28.6 |
| | 11.7 | Butenyl | 9.9 | 10.9 | 6.2% |
| | | Benzyl | 9.0 | 23.5 | |
| | | Butyl | 4.1 | 7.1 | |
| | | Phenylethyl | 4.1 | 2.2 | |
| Older leaves | 142.8 | Butenyl | 39.0 | 43.1 | 73.7 |
| | 23.6 | Butyl | 9.8 | 9.8 | 15.9% |
| | | Phenylethyl | 24.9 | 13.1 | |
| Stem | 289.5 | Butenyl | 31.3 | 34.6 | 156.2 |
| | 47.8 | Benzyl | 27.8 | 78.4 | 33.8% |
| | | Butyl | 78.4 | 60.2 | |
| | | Phenylethyl | 18.7 | 9.8 | |
| Tap-root (0–15 cm) depth | 65.8 | Allyl | 9.0 | 71.4 | 148.5 |
| | 10.9 | Butenyl | 3.3 | 3.6 | 2.1% |
| | | Butyl | 0.8 | 0.6 | |
| | | Phenylethyl | 135.4 | 70.9 | |

† The 8 youngest leaves.
‡ Butenyl isothiocyanate content was assessed with *n*-butyl-ITC regression.

**Table 3. Isothiocyanate content in soil (μg kg$^{-1}$ dry soil) when turnip–rape was grown, with and without mulch incorporation.**

| ITC | Incorporation† | Inital‡ | Days after mulch incorporation 7 | 15 | 28 |
|---|---|---|---|---|---|
| Allyl | With | 97 | 0 | 0 | 0 |
| | Without | | 0 | 0 | 0 |
| Benzyl | With | 296 | 0 | 0 | 0 |
| | Without | | 0 | 0 | 0 |
| Butenyl | With | 695 | 42 | 31 | 16 |
| | Without | | 13 | 53 | 18 |
| *n*-Butyl | With | 1000 | 0 | 16 | 45 |
| | Without | | 28 | 47 | 33 |
| 2-Phenylethyl | With | 1468 | 92 | 101 | 28 |
| | Without | | 158 | 40 | 128 |

† With, turnip–rape mulch incorporated into the soil. Without, turnip–rape mulch on the soil surface.
‡ ITC, amount in fresh turnip–rape mulch (Calculated per kg soil − Initial conc.).

a cooling pipe and condensed into an Erlenmeyer flask containing 2 mL of acetonitrile and 5 mL of water. For detection of the amines by HPLC-DAD (diode array detector) the compounds were derivatized with dabsylchloride (dimethylaminoazobenzene-4-sulfonylchloride, Fluka). Therefore, 300 μL of dabsylchloride solution (0.5% in acetonitrile) and 1 mL of 1% $Na_2CO_3$ solution were added to the sample, and were kept in a water bath at 30°C for 30 min. Afterward, a solid phase extraction (solid phase ENV$^+$, IST) was performed to extract the amines from the reaction medium. External standards of ITCs were used to quantify the amines. They were treated in the same way as the samples. The results of the high performance liquid chromatography (HPLC)-DAD were confirmed by HPLC-MS.

### High Performance Liquid Chromatography Conditions

High performance liquid chromatography was performed using a Pharmacia LKB gradient pump coupled to a photodiode array detector (Kontron Instruments DAD 440). The column was a RP-18 CP3, 250 by 4 mm (5 μm, Grom), with a gradient of 25% acetonitrile and 75% of 10 mmol phosphate-buffer (pH 2.4) for 24 min, followed by 100% acetonitrile for an additional 14 min. Injection volume was 30 μL. The dabsylchloride derivates were detected at 275 nm.

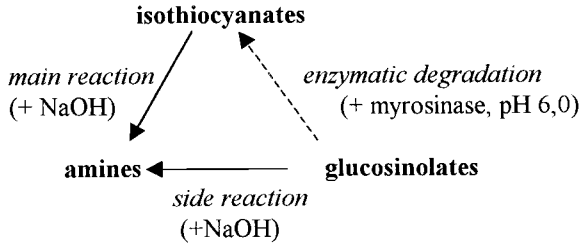


**Fig. 3. Disappearance of *n*-butyl and 2-phenylethyl isothiocyanate from soil.**

## RESULTS AND DISCUSSION

### Isothiocyanates in Turnip–Rape Mulch

Five different ITCs (allyl, *n*-butyl, 3-butenyl, benzyl, and 2-phenylethyl-ITC) were identified in turnip–rape mulch. These results were confirmed by HPLC-MS analysis. Furthermore, small amounts of 4-pentenyl-ITC could be identified. This compound was not detectable with HPLC-DAD. The results confirmed findings from Fenwick et al. (1983), Daxenbichler et al. (1991), and El-Sayed and El-Sakhawy (1995). However, *n*-butyl-ITC was not detected by these authors. In contrast with the authors mentioned above, the hydroxyalkyl and indolyl-ITCs were not detected. This could be due to different *B. rapa* ssp. and different growth stages when samples were taken.

The ITCs were quantified using external standards in three different concentrations. Each standard concentration was analyzed four times. In Table 1, the linear regressions are shown. Although the applied method only gave an estimate of the magnitude of ITC concen-

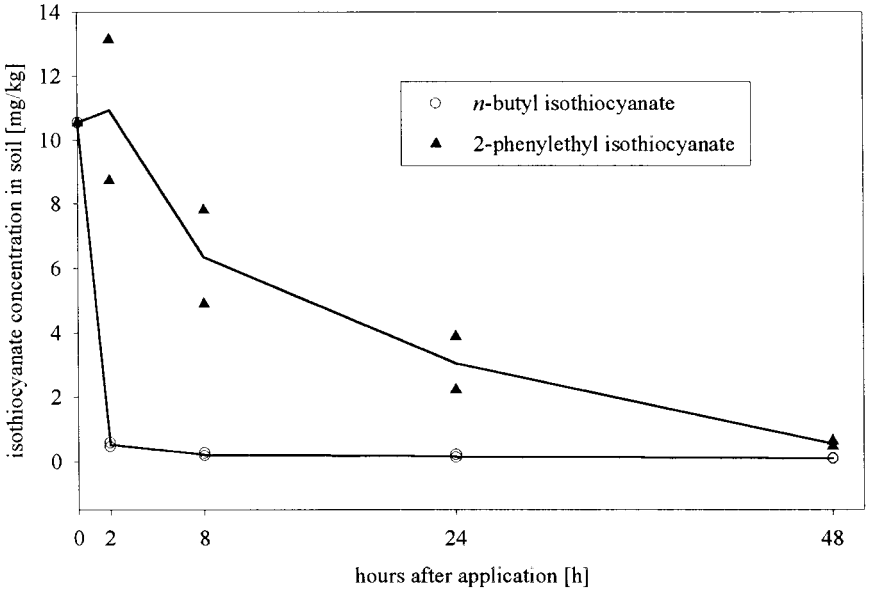


**Fig. 2. Disappearance of *n*-butyl and 2-phenylethyl isothiocyanate from soil.**

**Table 4. Conversion of sinigrine to allyl-isothiocyanate by alkaline hydrolysis.**

| Sinigrine | Potential allyl-ITC† | Real allyl-ITC‡ | Conversion |
|---|---|---|---|
| | μg | | % |
| 2 000 | 499 | 45 | 9.0 |
| 4 000 | 998 | 112 | 11.2 |
| 4 000 + myrosinase | 998 | 598 | 59.9 |
| 25 000 | 6 237 | 914 | 14.7 |

† Theoretical max amount of allyl-ITC after sinigrine breakdown.
‡ Determined amount of ITC after sinigrine breakdown.

**Table 5. Effect of turnip–rape mulch on emergence percentages (rel.) of different weeds.**

| Weed species | Control† | Without‡ | With |
|---|---|---|---|
| Smooth pigweed | 100 (117.6)§a | 97.5a | 124.7a |
| Scentless mayweed | 100 (150.6)a | 106.7a | 73.9ab |
| Spiny sowthistle | 100 (118.0)a | 95.8ab | 47.8ab |

† Soil from the same field, but where no turnip–rape was grown.
‡ With, turnip–rape mulch incorporated into the soil. Without, turnip–rape mulch on the soil surface.
§ Average number of seeds that emerged in control soil = 100%.

tration, the method is sufficient for determination of differences in ITC content.

Table 2 shows the average of plant dry weight and average ITC content for the different parts of the plant. The plant samples were taken immediately before the turnip–rape was cut off (flowering stage of the plants). The total ITC content was 463 mg $m^{-2}$. The main component was 2-phenylethyl-ITC (190.9 mg $m^{-2}$), followed by *n*-butyl (130.2 mg $m^{-2}$), and butenyl-ITC (90.4 mg $m^{-2}$). While 2-phenylethyl-ITC was the main compound in the tap root, the shoot mainly contained *n*-butyl and butenyl-ITC. Younger leaves, flowers, and buds also contained small amounts of benzyl (38.5 mg $m^{-2}$) and allyl-ITC (12.6 mg $m^{-2}$).

## Isothiocyanates in Soil Samples

Results indicate that there is a high potential for the release of ITCs after incorporating turnip–rape mulch into the soil (Table 2). Consequently, the next step was to quantify the ITC content in the turnip–rape soil. To describe the dynamics of the ITC releasing process, soil samples were taken 7, 15, and 28 d after mulch incorporation. Soil samples contained only low concentrations of ITCs (Table 3). Butenyl, *n*-butyl, and mainly 2-phenylethyl-ITC could be identified. But, there was no obvious relationship between ITC concentration and sampling time, and there was no effect of tillage on ITC concentration. The maximum concentration (200 μg $kg^{-1}$) was found at the first sampling date; however, this concentration was about 2300 times less than in fresh turnip–rape tissues.

The difference in the ITC content between plant material and soil samples results primarily from the dilution of ITC after incorporation in the soil, and volatilization and hydrolyzation of the compounds. To estimate degradation and disappearance of ITCs in the soil, defined amounts of *n*-butyl and 2-phenylethyl-ITC were added to uncontaminated soil (where no *Brassicaceae* had grown), and analyzed after 2, 8, 24, and 48 h (Fig. 2).

Because *n*-butyl-ITC is sensitive to hydrolysis and is very volatile, only 5% of the initially applied amount could be recovered after 2 h. In comparison to *n*-butyl, 2-phenylethyl-ITC is less sensitive to hydrolysis and less volatile. Therefore, the disappearance time $DT_{50}$ for 2-phenylethyl-ITC in soil was about 16 h and for *n*-butyl-ITC <1 h. Disappearance of the ITCs was promoted by water-saturated soil and high temperatures (30°C). Thus, only small amounts of the compounds could be quantified in the soil samples. Under field conditions, the disappearance might be slower than in our laboratory test system. However, results of Brown and Morra (1995) showed, that ITCs are very unstable in soil. Within 24 h >90% of the ITC amount disappeared, mainly due to volatilization. For disappearance of ITCs in soil temperature and soil moisture content are the most important factors (Borek et al., 1995). Not very clear is the importance of microorganisms for breakdown of ITCs in the soil (Borek et al., 1995). However, results of Parthipan and Mahadevan (1994) showed that soil mircoorganisms are sensitive to methyl-ITC, indicating that other factors than microbiological activity of the soil are of greater importance.

With our analytical method we were able to detect the biological active ITCs (main reaction, see Fig. 3). However, it would be important to determine the amount of glucosinolates from which the ITCs develop. Therefore, the conversion rate of a glucosinolate (sinigrine, Lancaster Synthesis) to the corresponding ITC (allyl-ITC) was investigated using alkaline hydrolysis (Table 4). The rate of the side reaction of sinigrine to allyl-ITC by alkaline hydrolysis was about 11.6% compared with the theoretically max amount. When the enzyme myrosinase [myrosinase from white mustard (*Sinapis alba* L.), Sigma-Aldrich] was added (reaction time 30 min at pH 6.0, phosphate-buffer) before the alkaline hydrolysis, the conversion rate increased to

**Table 6. Effect of methyl-isothiocyanate concentration on the germination percentage (rel.) of different plant species.**

| Species | TSM | 0 mg $L^{-1}$ | 1 mg $L^{-1}$ | 5 mg $L^{-1}$ | 10 mg $L^{-1}$ | 100 mg $L^{-1}$ |
|---|---|---|---|---|---|---|
| | g | | | | | |
| Spiny sowthistle | 0.2 | 100 (41.2)†a | 99.0ab | 20.9ab | 0b | 0b |
| Scentless mayweed | 0.3 | 100 (45.4)a | 77.5a | 26.0ab | 0b | 0b |
| Smooth pigweed | 0.5 | 100 (47.4)a | 96.2ab | 47.8ab | 0b | 0b |
| Barnyardgrass | 0.8 | 100 (45.2)a | 98.2a | 51.8ab | 0b | 0b |
| Blackgrass | 1.7 | 100 (29.2)a | 78.1a | 83.6ab | 19.9b | 0b |
| Wheat | 45.3 | 100 (47.0)a | 96.2a | 94.9ab | 67.2ab | 12.3b |

† Average number of seeds that germinated in untreated control = 100%.

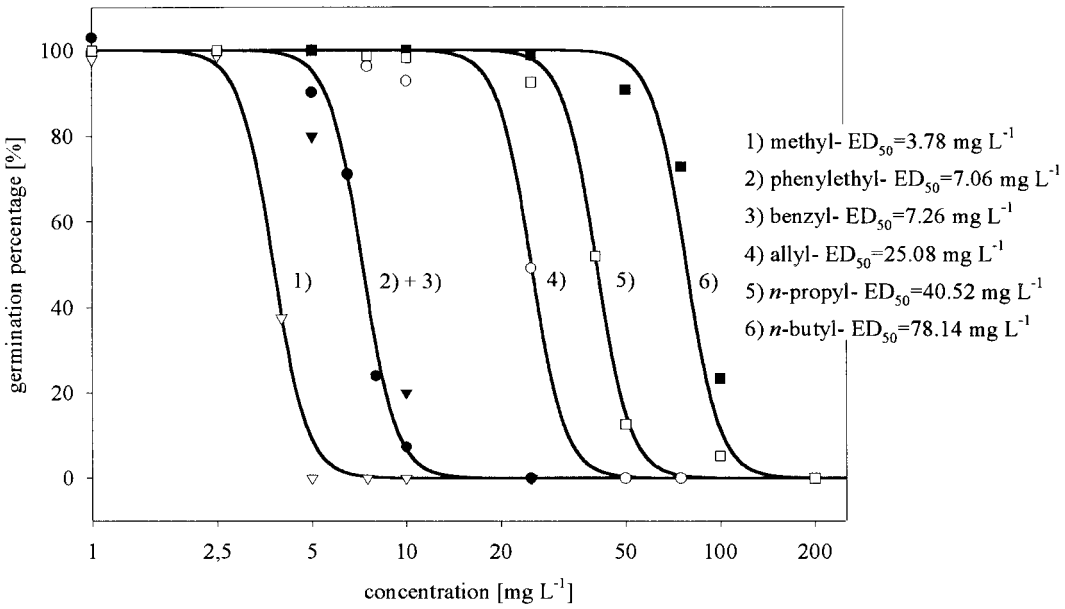


**Fig. 4. Effect of different isothiocyanates on the germination percentage of smooth pigweed.**

60%. It is assumed that the total amount of ITCs released from turnip–rape mulch was 5 to 10 times higher than the concentrations given in Table 2. Homogenization of the fresh plant samples were done under alkaline conditions. Under these conditions plant myrosinase could not work. Consequently, most of the interesting compounds were glucosinolates and not ITCs, when starting the extraction.

## Effect of Isothiocyanates on Seed Germination/Emergence

The bioassay with the field soil samples, where turnip–rape was grown, showed that turnip–rape mulch residues on the soil surface did not influence seed emergence. When the mulch was incorporated into the soil, the emergence percentage of smooth pigweed increased, while that of scentless mayweed and of spiny sowthistle decreased (Table 5).

The susceptibility to methyl-ITC to different species depended on the seed size [≅thousand seed mass (TSM), Table 6]. Smaller seeds tended to be more sensitive. Consequently, spiny sowthistle was the most sensitive and wheat the most resistant species (Table 6).

The effect of different ITCs on the germination percentage of smooth pigweed showed parallel dose–response relationships (Fig. 4). This indicates, that all ITCs have the same mode of action. The primary targets for the biological action of ITCs are the enzymes of glycolysis and respiration (Drobnica et al., 1977). The slope arround $ED_{50}$ of the dose–response relationship is very steep, which indicates the effects of ITCs increases rapidly, if a certain concentration is exceeded. Methyl-

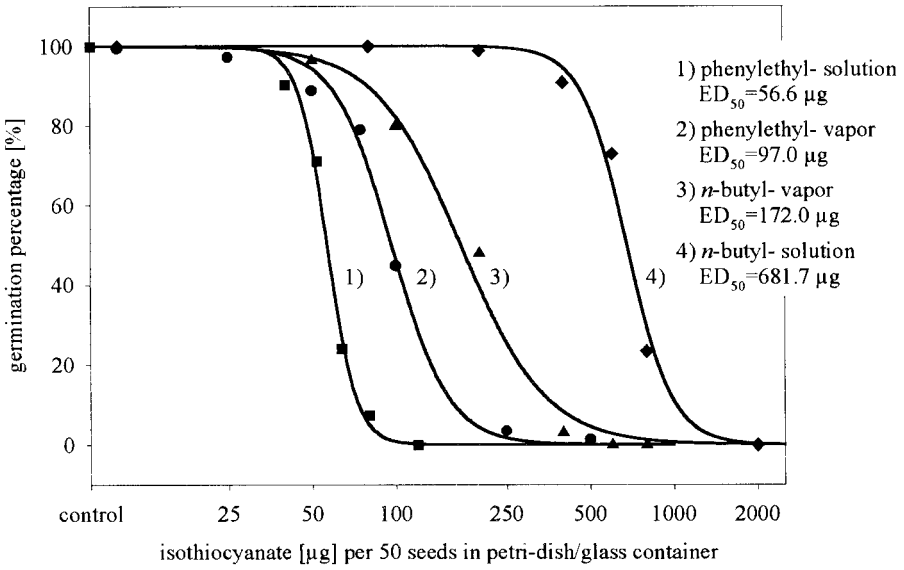


**Fig. 5. Comparison between the effect of *n*-butyl and 2-phenylethyl isothiocyanate as vapor and in aqueous solution on germination percentage of smooth pigweed.**

ITC is the most reactive compound and *n*-butyl-ITC is more than 20-fold less active. Dose–response relationships of the aryl-ITCs (benzyl and phenylethyl-ITC) showed no significant difference. However, within the alkyl-ITC, the activity decreased due to increased molecular mass. Because each ITC has only one reactive NCS-group, the reactivity decreased because of increasing alkyl chain length. The aryl-ITCs do not behave in the same manner, because they are more stable in aqueous solutions and can remain biologically active over a longer period of time.

In contrast to Bialy et al. (1990), we found much lower active concentrations of ITCs. This could be due to the fact that we used methanol (0.4%) to improve the solubility of ITCs, while Bialy et al. (1990) used ether as solvent. In both solvents ITCs are completely soluble, but methanol is the better negotiator between water and ITCs.

Low ITC concentrations delayed germination, but the ungerminated seeds were still viable (results of conducted tetrazolium tests not shown). Consequently, low ITC concentrations can induce secondary seed dormancy. Higher concentrations of ITCs may penetrate into the seeds in larger amounts and react with enzymes. These seeds loose their viability, because the reactions with the enzymes are irreversible (Drobnica et al., 1977).

As mentioned before, some ITCs are very volatile. Volatilization might be a way for ITCs to reach weed seeds in the soil. Therefore, a germination test was performed in 1-L glass jars, where the ITCs could only reach the smooth pigweed seeds as vapor. For seven different concentrations of *n*-butyl and 2-phenylethyl-ITC dose–response relationships were established and compared with the results of the petri dish tests (Fig. 5). The dose–response relationships of the different test systems were not parallel.

Corresponding to results of Al-Khatib et al. (1997), effects of ITCs in the vapor phase on seed germination were found. It is obvious that there are larger differences in the activity of the same ITC, depending on the application method (vapor or aqueous solution). *n*-Butyl-ITC is more effective in the vapor phase than in aqueous solution. However, with 2-phenylethyl-ITC it was the opposite. This can be explained by the higher volatilization rate of *n*-butyl compared with 2-phenylethyl-ITC and the higher stability to hydrolysis and vice versa. These specific properties are decisive for the activity of different ITCs in the soil environment. Oleszek (1987) showed that volatiles from chopped *Brassica* spp. leaves affected germination of different test species. Volatilization of ITCs from incorporated *Brassica* mulch might therefore contribute to the reduction of weed germination.

Our experiments clearly demonstrate that high amounts of ITCs are released from turnip–rape mulch, and there is a high probability that these compounds are responsible for suppression of weed germination after incorporation of green turnip–rape mulch into soil. This effect can be used for the following crop, especially when planting is done without tillage. Then it is likely that less or no additional herbicides are necessary. However, results of Al-Khatib et al. (1997) showed that some weed species still germinate after incorporation of green *Brassica* mulch, and as a consequence, additional weed control methods are required.

In regions where winter crops are grown, cover crops can be integrated into crop rotation easily. Particularly winterhardy cover crops, e.g., turnip–rape, can be grown before late-planted crops [e.g., maize (*Zea mays* L.)] in the spring. In this case, not only weed suppression but also reduced soil erosion and leaching of nutrients, and suppression of some soil-borne diseases can be achieved (Mithen, 1992; Angus et al., 1994). Results of field trials showed that maize growth and yield was not influenced by turnip–rape grown before as cover crops. However, weed density in the maize was reduced compared with a control without cover crop (Petersen, 1999, p. 43–50).

It seems that the potential of cover crops to release ITCs has been overlooked as a tool in integrated crop production.

## ACKNOWLEDGMENTS

The authors thank Dr. W. Armbruster, Hohenheim University, Institute of Food Chemistry, for technical assistance and the Aventis Crop Science company, Frankfurt/a.M., for financial support.

## REFERENCES

Al-Khatib, K., C. Libbey, and R. Boydston. 1997. Weed suppression with *Brassica* green manure crops in green pea. Weed Sci. 45: 439–445.

Angus, J.F., P.A. Gardner, J.A. Kirkegaard, and J.M. Desmarchelier. 1994. Biofumigation: Isothiocyanates released from *Brassica* roots inhibit growth of the take-all fungus. Plant Soil 162:107–112.

Bell, D.T., and C.H. Muller. 1973. Dominance of California annual grasslands by *Brassica nigra*. Am. Midl. Nat. 90:277–299.

Bialy, Z., W. Oleszek, J. Lewis, and G.R. Fenwick. 1990. Allelopathic potential of glucosinolates (mustard oil glycosides) and their degradation products against wheat. Plant Soil 129:277–281.

Björkman, R. 1976. Properties and function of plant myrosinase. *In* J.G. Vaughan et al. (ed.) The biology and chemistry of the Cruciferea. Academic Press, London.

Bones, A.M., and J.T. Rossiter. 1996. The myrosinase–glucosinolate system, its organisation and biochemistry. Physiol. Plant. 97: 194–208.

Borek, V., M.J. Morra, P.D. Brown, and J.P. McCafferey. 1995. Transformation of the glucosinolate-derived allelochemicals allyl isothiocyanate and allylnitril in soil. J. Agric. Food Chem. 43:1935–1940.

Börner, H. 1961. Untersuchungen zur Frage der Wurzelausscheidungen mit der Hilfe radioaktiver Isotopen am Beispiel von Ackersenf (*Sinapis arvensis* L.). Z. Pflanzenkr. Pflanzenschutz 5:626–633.

Boydston, R., and A. Hang. 1995. Rapeseed (*Brassica napus*) green manure crop suppresses weeds in potato (*Solanum tuberosum*). Weed Technol. 9:669–675.

Brown, P.D., and M.J. Morra. 1995. Glucosinolate-containing plant tissues as bioherbicides. J. Agric. Food Chem. 43:3070–3074.

Daxenbichler, M.E., G.F. Spencer, D.G. Carlson, G.B. Rose, A.M. Brinker, and R.G. Powell. 1991. Glucosinolate composition of seeds. Phytochemistry 30:2623–2638.

de Almeida, F.S. 1985. Effect of some winter crop mulches on the soil weed infestation. p. 651–659. *In* Proc. Br. Crop Prot. Conf. Weeds, London.

Drobinca, L., P. Kristian, and J. Augustin. 1977. The chemistry of the –NCS group. *In* S. Patai (ed.) The chemistry of cyanates and their thio derivates. Part 2. John Wiley & Sons, New York.

El-Sayed, A.M., and F.S. El-Sakhawy. 1995. Identification of principal

volatile flavours of *Brassica rapa* seeds. Bull. Fac. Pharm. Cairo Univ. 33:91–93.
Fenwick, G.R., R.K. Heaney, and W.J. Mullin. 1983. Glucosinolates and their breakdown products in food and food plants. Crit. Rev. Food Sci. Nutr. 18:123–301.
Krishnan, G., D.L. Holshauser, and S.J. Nissen. 1998. Weed control in soybean (*Glycine max*) with green manure crops. Weed Technol. 12:97–102.
Kruskal, W.H., and W.A. Wallis. 1953. Use of ranks in one-criterion variance analysis. J. Am. Stat. Assoc. 48:907–911.
Michel, A., J. Petersen, M.N. Dogan, and V. Ernst. 1999. Anleitung zur Anlage und Auswertung von Versuchen zur Erstellung quantitativer Dosis-Wirkungsbeziehungen am Beispiel der Wirksamkeit von Herbiziden. Gesunde Pflanz. 1:10–19.
Mithen, R. 1992. Leaf glucosinolate profiles and their relationship to pest and disease resistance in oilseed rape. Euphytica 63:71–83.
Oleszek, W. 1987. Allelopathic effects of volatiles from some cruciferae species on lettuce, barnyard grass and wheat growth. Plant Soil 102:271–273.
Parthipan, B., and A. Mahadevan. 1994. Effects of methyl isothiocyanate on soil microflora and the biochemical activity of soils. Environ. Pollut. 87:283–287.
Petersen, J. 1999. Untersuchungen zur Unkrautbekaempfung mit Bodendeckern in herbizidresistentem Mais. Cuvillier Verlag, Goettingen.
Seefeldt, S.S., J.E. Jensen, and P.E. Fuerst. 1995. Log-logistic analysis of herbicide dose response relationship. Weed Technol. 9:218–227.
Streibig, J.C. 1988. Herbicide bioassay. Weed Res. 28:479–484.
Tang, C.S. 1971. Benzyl isothiocyanate of papaya fruit. Phytochemistry 10:117–121.

# Laboratory Bioassay for Phytotoxicity: An Example from Wheat Straw

Bader Al Hamdi, Inderjit,* Maria Olofsdotter, and Jens C. Streibig

## ABSTRACT

**Allelopathy involves complex plant × plant chemical interactions. Although a large number of laboratory bioassays have proposed to demonstrate allelopathy, most of them have little or no relevance in terms of explaining behavior in the field. In this paper, we discuss the phytotoxicity of wheat (*Triticum aestivum* L.) straw leachate to the seedling growth of perennial ryegrass (*Lolium perenne* L.). The objective of this study was to discuss the significance of (i) soil, (ii) leachate concentrations in bioassays of plant debris and soil, (iii) the role of N fertilizer in overcoming plant growth inhibition, (iv) organic molecules in plant inhibition, and (v) actual assay species. The results show the phytotoxic nature of wheat straw leachate (WSL) and the possible involvement of organic molecules in the growth inhibition of perennial ryegrass. However, laboratory studies can not demonstrate allelopathy as the sole factor responsible for the observed growth inhibition.**

Concerns are often raised about the relevance of laboratory bioassays for allelopathy (Connell, 1990; Inderjit and Olofsdotter, 1998; Inderjit and Dakshini, 1995, 1999). Willis (1985, p. 72) listed a six-point protocol necessary to demonstrate allelopathy in natural systems: "(i) a pattern of inhibition of one species or plant by another must be shown, (ii) the putative aggressive plant must produce a toxin, (iii) there must be a mode of toxin release from the plant into the environment, (iv) there must be toxin transport and/or accumulation in the environment, (v) the afflicted plant must have some means of toxin uptake, and (vi) the observed pattern of inhibition cannot be explained solely by physical factors or other biotic factors, especially competition and herbivory." Blum et al. (1999) recently concluded that no study has ever demonstrated all of these criteria. Nature is too dynamic to be solely explained by a mechanism of plant interference. The observed growth pattern is better explained by a synergistic action of several mechanisms of interference (Inderjit and Del Moral, 1997). It is almost impossible to demonstrate allelopathy by following the above six criteria. We, therefore, will restrict our discussion to phytotoxicity. We argue that laboratory bioassays can generate some meaningful data, provided that attention is paid to following points: (i) soil, (ii) several concentrations of phytotoxic material, (iii) elimination of possible inhibition by N deficiency due to added organic material, (iv) involvement of organic molecules in plant inhibition, and (v) assay species. A study with a wheat straw–perennial ryegrass system is designed to address the above criteria. Activated charcoal was added to the system, as suggested, to isolate the interference by organic molecules (Mahall and Callaway, 1992; Inderjit and Foy, 1999).

Many ecologists often argue that the addition of plant debris, leachate, or both into the soil results in enhanced microbial activity, which causes N depletion. Any growth suppression, they argue, is due to N depletion, rather than organic molecules (Harper, 1977). To address this concern and invoke the probable involvement of organic molecules in growth suppression, a series of experiments was conducted. These experiments investigated the effect of soil amended with WSL on the seedling growth of perennial ryegrass and whether the interference due to wheat straw is modified after the addition of activated charcoal and different amounts of N fertilizers. The objective of this paper is to demonstrate that laboratory bioassays for phytotoxicity can generate some meaningful data, provided that experiments are conducted under realistic conditions.

## Why Wheat Straw?

Wheat straw has been reported to possess allelopathic activities (Guenzi and McCalla, 1962; Guenzi et al., 1967). Guenzi and McCalla (1966) found phytotoxicity of phenolic acids, particularly *p*-coumaric acid, from

B. Al Hamdi, M. Olofsdotter, and J.C. Streibig, Dep. of Agric. Sci. (Weed Sci.), The Royal Veterinary and Agric. Univ., Thorvaldsensvej 40, DK-1871 Frederiksberg C, Copenhagen, Denmark; Inderjit, Dep. of Botany, Panjab Univ., Chandigarh 160014, India. Received 20 Nov. 1999. *Corresponding author (allelopathy@satyam.net.in).

Published in Agron. J. 93:43–48 (2001).

**Abbreviations:** FS, full strength; WSL, wheat straw leachate.