# Glucosinolate hydrolysis compounds for weed control

Natalia Bellostas, Per Nielsen Kudsk, Jens C. Sørensen and Hilmer Sørensen

***Abstract* – Glucosinolates are allelochemicals present in all Brassica plants. Upon hydrolysis by endogenous enzymes they produce a series of biologically active compounds, such as isothiocyanates and their derivatives among others. These compounds have marked fungicidal, nematocidal and herbicidal effects and therefore their use as biodegradable natural products for crop protection has attracted much attention in the last years. A number of these compounds, either individually or in combination, were tested against *Sinapis alba* and *Lollium perenne* in Petri dishes bioassays. $C_{50}$ values as low as 0.7 and 0.2 mM were obtained. This may open the possibility for using glucosinolate hydrolysis products as herbicides.[1]**

## Introduction

Glucosinolates are amino acid derived allelochemicals present in all plants of the order Capparales and some few other species (Sørensen, 1990). Myrosinase isoenzymes (EC 3.2.1.147) co-exist with glucosinolates and catalyze the hydrolysis of these compounds releasing an aglucone that further rearranges to a variety of products depending on the parent glucosinolate and the environmental conditions (Sørensen, 1990). Glucosinolate-containing plants have traditionally attracted much attention due to the physiological effects of the various glucosinolate degradation compounds. Oxazolidine-2-thiones have appreciable antinutritional effects on monogastric animals. Isothiocyanates are fungicidal (Chan and Close, 1987), nematocidal (Buskov et al., 2002) and herbicidal (Brown and Morra, 1997). Other glucosinolate and isothiocyanate derived products (e. g. dithioureas) are also known for the biological activity.

The concept "natural plant protection agents (NAPPA)" refers to the compounds originally produced by agricultural plants that have the ability to inhibit the growth of microbes or weeds and that can therefore be used as natural pesticides or herbicides. The term NAPPA is used in this paper to describe glucosinolates and hydrolysis products from Brassica plants.

## Materials and methods

*Preparation of glucosinolate hydrolysis compounds and compounds tested:*

Stock solutions of different glucosinolates (1 M) were prepared and allowed to react with myrosinase in order to produce the active degradation compounds. Isothiocyanates were prepared from the parent glucosinolate by degradation of the glucosinolate in 100 mM phosphate buffer pH 6.5. Oxazolidine-2-thiones were formed in myrosinase catalysed degradation of 2-hydroxy substituted glucosinolates. Thioureas were produced by allowing the corresponding isothiocyanates to react with ammonia in an ethanol solution. Concentration and purity of the above mentioned compounds was determined spectrophotometrically and by capillary electrophoresis (Sørensen et al., 2000).

*Formulation of NAPPA solutions:*

Fifteen different formulas were developed, including the compounds individually and in combination. Hydrolysed rapeseed oil was used as an emulsion agent for the solubilisation of the compounds.

*Test system:*

Twenty seeds of either *Sinapis alba* L. or *Lolium perenne* L. were germinated in Petri dishes, on filter paper wetted with either water or the NAPPA solutions at six different concentrations (0.04-100 mM). The Petri dishes were sealed to prevent evaporation and the germination percentage was counted after 1, 4, 6 and 8 days. Two separate trials were performed. The tests were performed at the Danish Institute of Agricultural Sciences at Flakkebjerg or at Grønt Center in Holeby following their standard methods. Inhibition effect in percent was calculated according to: (growth of control-growth of seeds treated with NAPPAs)*100 / (growth of control).

## Results

The concentrations of *NAPPA's* needed to achieve a 50% inhibition of germination after 4 days were calculated on the basis of doses response curves (Figures 1 and 2).

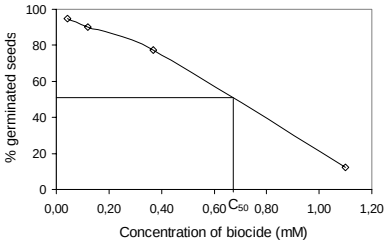


**Figure 1.** Seeds of *Sinapis alba* germinated on NAPPA (Bio-9.1) wetted paper in covered Petri dishes. The number of germinated seeds was counted at different days (day 4 is shown) and a germination percentage was calculated. $C_{50}$ values are presented in Table 1.

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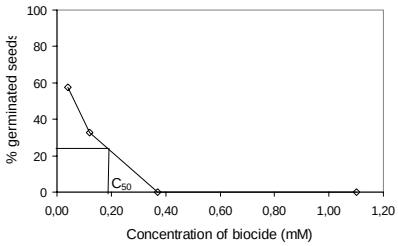


**Figure 2.** Seeds of *Lolium perenne* L. germinated on NAPPA (Bio-9.1) wetted paper in covered Petri dishes. The number of germinated seeds was counted at different days (day 4 is shown) and a germination percentage was calculated. $C_{50}$ values are presented in Table 1.

The presented examples show the response obtained by using the most effective compound (Bio-9.1) in the herbicidal tests. All compounds have been evaluated in the same way to give the data presented in Table 1. The tested glucosinolate degradation products had different effect on inhibition of the germination of seeds from *Sinapis alba* and *Lolium perenne* L. (Table 1).

**Table 1.** Germination inhibition exerted by NAPPAs on two different seed types. The table shows the concentrations ($C_{50}$) at which 50 % of the seeds germinated relative to a control (monitored at day 4).

| *NAPPA* type (tested concentration range) | *Sinapis alba* | *Lolium perenne* |
|---|---|---|
| Bio-1.0 (0.04 – 10 mM) | 8 mM | 2.5 mM |
| Bio-1.1 (0.04 – 10 mM) | n.d. | 10 mM |
| Bio-2.0 (0.04 – 10 mM) | n.d. | 0.8 mM |
| Bio-2.1 (0.04 – 0.4 mM) | n.d. | n.d. |
| Bio-3.0 (0.04 – 10 mM) | n.d. | n.d. |
| Bio-3.1 (0.04 – 10 mM) | n.d. | 8 mM |
| Bio-4.0 (0.04 – 10 mM) | 8 mM | 0.8 mM |
| Bio-4.1 (0.04 – 10 mM) | n.d. | 6 mM |
| Bio-5 (0.04 – 3.3 mM) | n.d. | n.d. |
| Bio-6.0 (0.04 – 1.1 mM) | n.d. | n.d. |
| Bio-7 (0.04 – 10 mM) | > 10 mM | 8 mM |
| Bio-8 (0.04 – 1.1 mM) | n.d. | n.d. |
| Bio-9.0 (0.04 – 10 mM) | > 10 mM | 1 mM |
| Bio-9.1 (0.04 – 1.1 mM) | 0.7 mM | 0.2 mM |
| Bio-10 (0.04 – 10 mM) | > 10 mM | > 10 mM |

For *Lolium perenne* L. (Figure 2) the $C_{50}$ value was calculated at 23 % germination, since there was only 47 % germinated seeds in the control sample. Determinations of $C_{50}$ values are very dependent on the choice of method. Evaluation of the effects should be conducted at a fixed moment in time (Figure 3). Hence, the $C_{50}$ value evaluated at day 4 would be seven times lower than the $C_{50}$ value evaluated at day 8 (Figure 3). Direct comparison with literature values must therefore be conducted with caution.

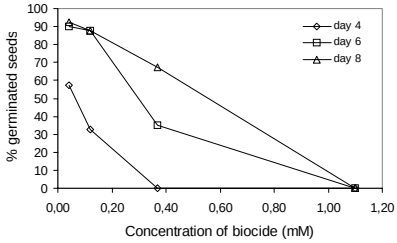


**Figure 3.** Percentage of germination of *Lolium perenne* L. seeds at different concentrations of Bio-9.1 measured at day 4, 6 and 8 after imbibition.

*Lolium perenne* L. seeds are without exception more affected in terms of reduced germination by the tested NAPPAs than *Sinapis alba* L. seeds (Table 1). The compounds Bio-1.0 and Bio-4.0 were equally effective towards germination reduction of *Sinapis alba* L. seeds ($C_{50}$ = 8 mM), whereas Bio-4.0 ($C_{50}$ = 0.8 mM) was more effective on *Lolium perenne* L. than Bio-1.0 was ($C_{50}$ = 2,5 mM). Bio-2.0 and Bio-9.0 did not show stronger inhibition effects on germination of either seeds than Bio-1.0 and Bio-4.0. The compound mixture Bio-9.1 showed the best activity towards germination of the two tested plants. Surprisingly, Bio-2.1 did not show similar effects, which could have been expected owing to the close structural resemblance to Bio-9.1. On the other hand, Bio-4.1 and Bio-1.1 gave some inhibition on germination especially on *Lolium perenne* L. Bio-7 gave also effects on both *Lolium perenne* L. and *Sinapis alba* L. seeds, and with quite similar strength, which was not seen for any other tested compound. Bio-5 was tested in lower concentrations and did therefore not give any $C_{50}$ values (Table 1).

## CONCLUSIONS

The biodegradability of the compounds is an important factor to consider if NAPPAs are to be used in agriculture. The NAPPAs used in the present experiments seem to be quite easily biodegraded since their effect was based on growth inhibition rather than toxicity of the seeds, as delays in germination were observed.

## REFERENCES

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