



Mineralization of the allelochemical sorgoleone in soil

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ARTICLE INFO

Article history:

Received 4 February 2009

Received in revised form 17 April 2009

Accepted 17 April 2009

Available online 2 June 2009

Keywords:

Lipid benzoquinone

Sorgoleone

Sorghum

Allelopathy

Soil fate

ABSTRACT

The allelochemical sorgoleone is produced in and released from the root hairs of sorghum (*Sorghum bicolor*). Studies have confirmed that it is the release of sorgoleone that causes the phytotoxic properties of sorghum, and sorgoleone has a potential to become a new natural herbicide, or the weed suppressive activity of sorghum can be utilized in integrated weed management. Since sorgoleone is released into soil, knowledge of the fate of sorgoleone in soil is essential if it is to be utilized as an herbicide. Fate studies will characterize the persistence and mobility of the compound. Three types of radioactively labelled sorgoleone were produced and used to study mineralization (complete degradation to CO₂) of this lipid benzoquinone in four soils, two from the United States of America (Mississippi) and two from Denmark. The studies showed that sorgoleone was mineralized in all soils tested. The methoxy group of sorgoleone was readily mineralized, whereas mineralization of the remaining molecule was slower. Mineralization kinetics indicated that microorganisms in American soils were able to use sorgoleone as a source of energy.

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1. Introduction

Sorgoleone (2-hydroxy-5-methoxy-3-[(Z,Z)-8',11',14'-penta-decatriene]-p-benzoquinone) is an allelochemical produced in the root hairs of sorghum species (Czarnota et al., 2001; Dayan et al., 2007) along with a variety of structural analogues (Kagan et al., 2003) responsible for the weed inhibiting properties of sorghum often observed in the field (Rimando et al., 2003). It is exuded dynamically from the root hairs of sorghum as oily droplets that are released directly in the soil. Therefore, its action is similar to a pre-plant incorporated herbicide. The fact that sorgoleone is released from the roots of sorghum continually during the growing season may prolong the presence of sorgoleone in soil (Weidenhamer, 2005; Dayan, 2006; Dayan et al., 2009). Laboratory studies have confirmed that sorgoleone inhibits photosystem II and *p*-hydroxyphenylpyruvate dioxygenase (Meazza et al., 2002; Rimando et al., 2003), and it also affects water uptake (Rimando et al., 2003; Hejl and Koster, 2004). Thus, sorgoleone has the potential to be used as a natural herbicide (Macias et al., 2007) either by application of the purified compound or by utilization of the weed suppressive activity of sor-

ghum in integrated weed management. Additionally, this allelochemical can stimulate the germination of the parasitic weed witch-weed (*Striga hermotica*), which can seriously reduce sorghum growth and yield (Wigchert and Zwanenburg, 1999; Erickson et al., 2001) and for this reason as well it is of interest to investigate the fate of sorgoleone in soil.

Sorghum has been known for a long time to have a negative effect on subsequent crops (Breazeale, 1924), which suggests that sorgoleone may be highly persistent in soil. A study showed that sorgoleone can easily be recovered within 1 h of soil application, and the recovery decreases rapidly over time (Weston et al., 1997), but another study (Weston and Czarnota, 2001) showed that sorgoleone was detectable seven weeks after applying sorgoleone to the soil. However, the soil behavior of sorgoleone, such as mobility and persistence, is not well understood, though such knowledge is important to predict its herbicidal activity, environmental fate, and to identify potential limitations in its use. As for all herbicides it is preferable if sorgoleone persists at a critical concentration to suppress weeds after which a rapid disappearance is desired to minimize non-target effects.

This study investigated the mineralization (complete degradation to inorganic constituents) of sorgoleone in soil. Both surface soils and sub-surface soils from four locations in Denmark and the United States of America were used to investigate the effect of soil type. Sorgoleone labelled with ¹⁴C in two distinct positions

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(methoxy and benzoquinone) in addition to uniformly labelled sorgoleone was used to investigate the susceptibility to degradation of different moieties of the molecule. Finally, the effect of two sorgoleone concentrations on mineralization was also investigated.

2. Materials and methods

2.1. Soils

Soils from Denmark were collected in 2003, and the American soils were collected in 2006. The soils were stored frozen with the water content they had at the time of sampling. The Danish soils were agricultural soils sampled at Jyndevad and Sjællands Odde (Sj. Odde) and were selected to represent the two most common soil types encountered in Denmark and the north-western part of Europe. Samples were taken from the A (0–25 cm) and B (25–65 cm) horizons. The Jyndevad site is situated in the south western part of Denmark on a glacial outwash plain, and the soil is coarse sandy. The Sj. Odde site is situated in the north eastern part of Denmark and is formed on calcareous glacial till. According to USDA's Soil Taxonomy (Soil, 1999) the Jyndevad soil is a Humic Psammentic Dystrudept and the Sj. Odde soil is a Typic Argiudoll.

The American soils (Sharkey and Dundee) were sampled in the state of Mississippi, in the flat alluvial plain of the Mississippi River. Historically, the predominant crops in this region have been cotton and soybeans, but sorghum and corn are increasingly grown. In the locations where these soils were collected, there is no history of sorghum being grown. Recent history of the Sharkey soil location is a corn to cotton rotation for the past five years. The Sharkey soil is formed on clayey alluvium and is classified as a Chromic Epiaquert. Recent history of the Dundee soil location is five years of soybean followed by five years of cotton. The Dundee soil is formed on loamy alluvium and is classified as a Typic Endoaqualf.

The soil characteristics differed in texture as well as in organic matter content (Table 1). Jyndevad had a very low content of clay, but a higher content of organic carbon than Sj. Odde, which, compared to other Danish soils, has a high content of clay. The two American soils had a high content of clay and silt and their organic matter content was comparable to the Danish soils. Sj. Odde had a slightly higher pH than the Jyndevad soil, especially in the B horizon. The pH in the American soils was lower than in the Danish soils.

2.2. Mineralization experiment

It is not possible to purchase radioactively labelled sorgoleone, so this was produced *in vitro* as described by Dayan et al. (2003). Twenty sorghum seeds were germinated in plastic Petri dishes in the presence of 3 mL of sterile water containing either ^{14}C -methyl-D-methionine (50 mCi/mmol), ^{14}C -acetate (100 mCi mmol $^{-1}$), or

^{14}C -sucrose (620 mCi/mmol) (American Radiolabeled Chemicals, Inc., St. Louis, MO). The dishes were sealed and incubated in the dark at 25 °C in a CU-32L plant growth chamber (Percival Scientific Inc. Perry, IA 50220, USA) for 10 d. All labelling procedures were done under low-intensity green light to prevent the formation of anthocyanins by sorghum roots. Sorgoleone crude extract was obtained by dipping excised roots in CHCl_3 for 3 min. Sorgoleone was purified by thin layer chromatography (TLC) on 20 × 20 cm silica F_{254} glass-backed preparative plates (Analtech, Newark, DE) developed in chloroform–ethylacetate (7:3, v/v). The band containing sorgoleone ($R_f = 0.35$) was scraped off the plate and eluted with CHCl_3 . The material was dried under nitrogen and stored at –20 °C. Unlabelled sorgoleone was produced in the same way.

Seedlings grown in the presence of ^{14}C -methionine produced sorgoleone with its 5-methoxy carbon being labelled. Those grown in the presence of ^{14}C -acetate produced sorgoleone with the quinone ring labelled, but not the hydrophobic tail. Seedlings grown in the presence of ^{14}C -glucose produced uniformly labelled sorgoleone (Fig. 1) (Dayan et al., 2003).

As sorgoleone is highly hydrophobic with a log K_{ow} of 6.1 (Trezzi et al., 2006) it is not possible to apply it to the soil as an aqueous solution. Instead the method proposed by Brinch and co-workers (2002) was used to ensure sufficient contact with the soil and to cause as little disturbance to the microorganisms as possible.

The mineralization experiments were conducted by weighing 5 g field moist soil (Table 1) into glass flasks into which 500 μL acetone with sorgoleone at either 1 mg mL $^{-1}$ or 10 mg mL $^{-1}$ concentrations were added. The solutions were a mixture of trace amounts of ^{14}C -labelled sorgoleone and unlabelled sorgoleone yielding a final specific radioactivity of ~50,000 dpm per glass flask. The flasks were left open overnight in a fume hood to allow the evaporation of acetone. The following day, 1 mL water and 15 g of moist soil was added and mixed with the initial 5 g. A test tube with 1 mL 1 M NaOH was placed inside the flasks to serve as a CO_2 trap and the flasks were closed with airtight lids. NaOH was removed at intervals and replaced with fresh NaOH. The NaOH solutions were transferred to a diffuse scintillation vial with 10 mL Optiphase 'Highsafe' 3 scintillation cocktail from Perkin Elmer and the activity was measured on a Wallac 1409 DSA liquid scintillation counter. The amount of radioactivity applied initially to the soil was measured by applying 500 μL of the solutions applied to the soil directly to a scintillation vial.

In order to better evaluate the role that microorganisms have on sorgoleone mineralization, a companion experiment was conducted utilizing the same treatments and soils as described previously, but the soils used were sterilized by autoclaving three times.

To estimate mineralization of the carbons in the aliphatic chain in the molecule, it was assumed that all carbons are labelled equally when using sucrose as the substrate, which has been shown by Dayan and co-workers (2003). Furthermore, the contribution of the methoxy group and the quinone ring was assessed in the same experiments with these two types of labelling. As

Table 1
Properties of the soils used. Silt is 2–20 μm .

Location	Horizon	pH (0.01 M CaCl_2)	C (%)	Clay (%)	Silt (%)	Sand (%)	Moisture (%)
Dundee	A	6.0	1.38	19	29	52	13.1
USA	B	6.4	0.44	22	26	52	10.7
Jyndevad	A	6.9	2.43	5	3	92	4.0
Denmark	B	6.1	1.00	5	1	94	2.4
Sharkey	A	5.8	2.21	43	28	29	19.2
USA	B	5.8	1.12	45	20	35	14.5
Sj. Odde	A	7.2	1.25	19	18	63	5.5
Denmark	B	7.6	0.30	31	26	43	9.2

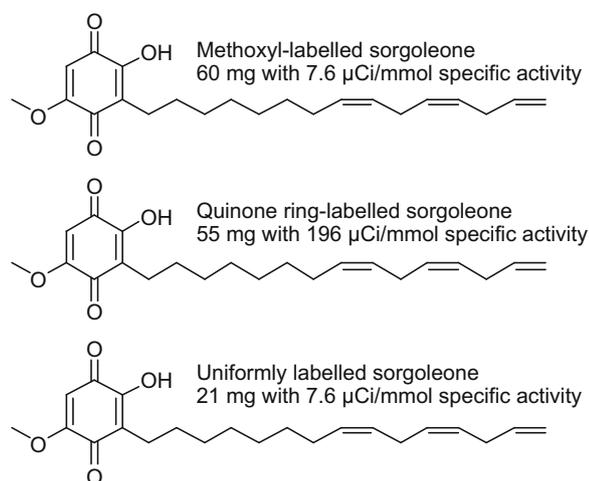


Fig. 1. Structure of sorgoleones with different ^{14}C labelling patterns and their respective specific activities.

the carbon in the methoxy group constitute 1/22 of the carbons in the entire molecule and the carbons in the ring constitute 6/22, then the mineralization of the aliphatic chain (with 15/22 of the carbons in the molecule) can be estimated as:

$$\frac{22 \cdot \%Min_{Total} - 1 \cdot \%Min_{Methoxy} - 6 \cdot \%Min_{Ring}}{15}$$

where $\%Min_{Total}$ is the percentage mineralization of the uniformly labelled sorgoleone, $\%Min_{Methoxy}$ is the percentage mineralization of the methoxy labelled sorgoleone and $\%Min_{Ring}$ is the percentage mineralization of the quinone ring labelled sorgoleone.

3. Results

The amount of mineralized sorgoleone residues was calculated as the percentage of applied sorgoleone, and the results of the experiments are shown in Fig. 2. The figure shows mineralization of the three forms of differently labelled sorgoleone at the two different concentrations investigated. The two upper panels show the quinone ring labelled form. Notice that the kinetics for the Danish soils and the Dundee B were the same irrespective of the initial concentration, whereas kinetics for the Sharkey and Dundee A soils differed depending on the amount of sorgoleone added. This was also observed for the uniformly labelled form (two lower panels in Fig. 2), while for the methoxy group labelled form (the two middle panels in Fig. 2) the initial mineralization was rapid, but for the 25 ppm scenarios levelled out after approximately 14 d. For the 250 ppm scenarios, mineralization levelled out after 20–30 d, and a higher percentage was mineralized than for 25 ppm.

Fig. 3 shows the mineralization of the different parts of the molecule 76 d and 77 d after application of 250 ppm and 25 ppm sorgoleone, respectively. The methoxy group was mineralized to a higher extent than the other parts of the molecule, and it is worth noticing the differences between the two initial concentrations. When 250 ppm was added, a much higher percentage of the methoxy group was mineralized compared to 25 ppm in all soils. For the American soils, a much higher percentage of all components of the molecule were mineralized at 250 ppm compared to 25 ppm.

Results with sterilized soil showed negligible mineralization, less than 1% at the end of the experiment.

4. Modelling

The mineralization data was modelled using the three-half order models proposed by Brunner and Focht (1984). Three different

forms of the model were fitted to the data. The first model assumes linear growth:

$$P = S \cdot [1 - \exp(-k_1 \cdot t - 1/2 \cdot k_2 \cdot t^2)] + k_0 \cdot t \quad (1)$$

where t is time measured in days (d), P is the product in this case ^{14}C labelled CO_2 and has the unit % mineralized of applied, k_0 is the zero order rate constant and has the unit d^{-1} , k_1 is the first order rate constant and has the unit d^{-1} and k_2 is the increase of the first order rate constant with time and has the unit d^{-2} . In Brunner and Focht (1984), S is given as S_0 which they interpreted as the substrate concentration at time zero (Brunner and Focht, 1984). However, S has the same unit as P (% mineralized of applied) so this interpretation of S makes little sense. Instead, in cases where k_0 has a low value, S will determine the maximum value of P as P is equal to $S + k_0 \cdot t$ when t increase and $-k_1 \cdot t - 1/2 \cdot k_2 \cdot t^2 < 0$.

The second model assumes no growth, and in this case $k_2 = 0$ and the model then have the form:

$$P = S_0 \cdot [1 - \exp(-k_1 \cdot t)] + k_0 \cdot t \quad (2)$$

The last model tested assumes exponential growth and has the form:

$$P = S_0 \cdot \left[1 - \exp\left(-k_1 \cdot t - \frac{E_0}{\mu} (\exp(\mu \cdot t) - 1)\right) \right] + k_0 \cdot t \quad (3)$$

where E_0 is the initial cell concentration and μ is the growth rate constant. All other constant are as for model 1.

Data were fitted to the three models using Sigma Plot (Systat Software, Inc. Chicago, IL 60606). None of the data could be described by the model with exponential growth (model 3). The mineralization of quinone ring labelled and uniformly labelled sorgoleone in Sharkey A and B and Dundee A and B ring labelled at 250 ppm could be fitted by model 1 assuming linear growth and the rest of the data was well described by model 2 assuming zero growth. The best fit was chosen based on an F -tests ($P < 0.05$) (Robinson, 1985), which was further confirmed by an evaluation of the R^2 -value and a visual inspection of the fit. The parameters for the different models and R^2 are presented in Tables 2 and 3.

5. Discussion

The results presented demonstrate that sorgoleone can be mineralized in soils with very different physical and chemical characteristics and of different origin. When comparing with sterilized soil where negligible amounts were mineralized, it is apparent that sorgoleone mineralization was due to microbial activity. Access to ^{14}C -sorgoleone with three labelling patterns applied at two concentrations was useful in demonstrating variable kinetics for different components of this allelochemical. The methoxy group was easily mineralized as shown by the fast kinetics and a high S value in the models, e.g. in the Sj. Odde B soil at 25 ppm applied 26% was mineralized after only two days. The mineralization was rapid at both 25 ppm and 250 ppm, but the kinetics was different at 25 ppm and 250 ppm. If 25 ppm was added, the mineralization was rapid for the first 10 d and then slowed with very little additional mineralization thereafter. Mineralization of the 250 ppm sample did not decrease until after 15–25 d. In the modelling, this resulted in higher k_1 values for the 25 ppm experiments compared to the 250 ppm (Tables 2 and 3). The only exception was with the Jyndeved A soil, where k_1 was approximately the same in both the experiments. For all soils, a higher percentage of the applied sorgoleone was mineralized when 250 ppm was added compared to 25 ppm, and hence S was higher in the 250 ppm experiments (compare Tables 2 and 3). Interestingly, mineralization of sorgoleone was among the lowest in Sharkey soil when 25 ppm was added,

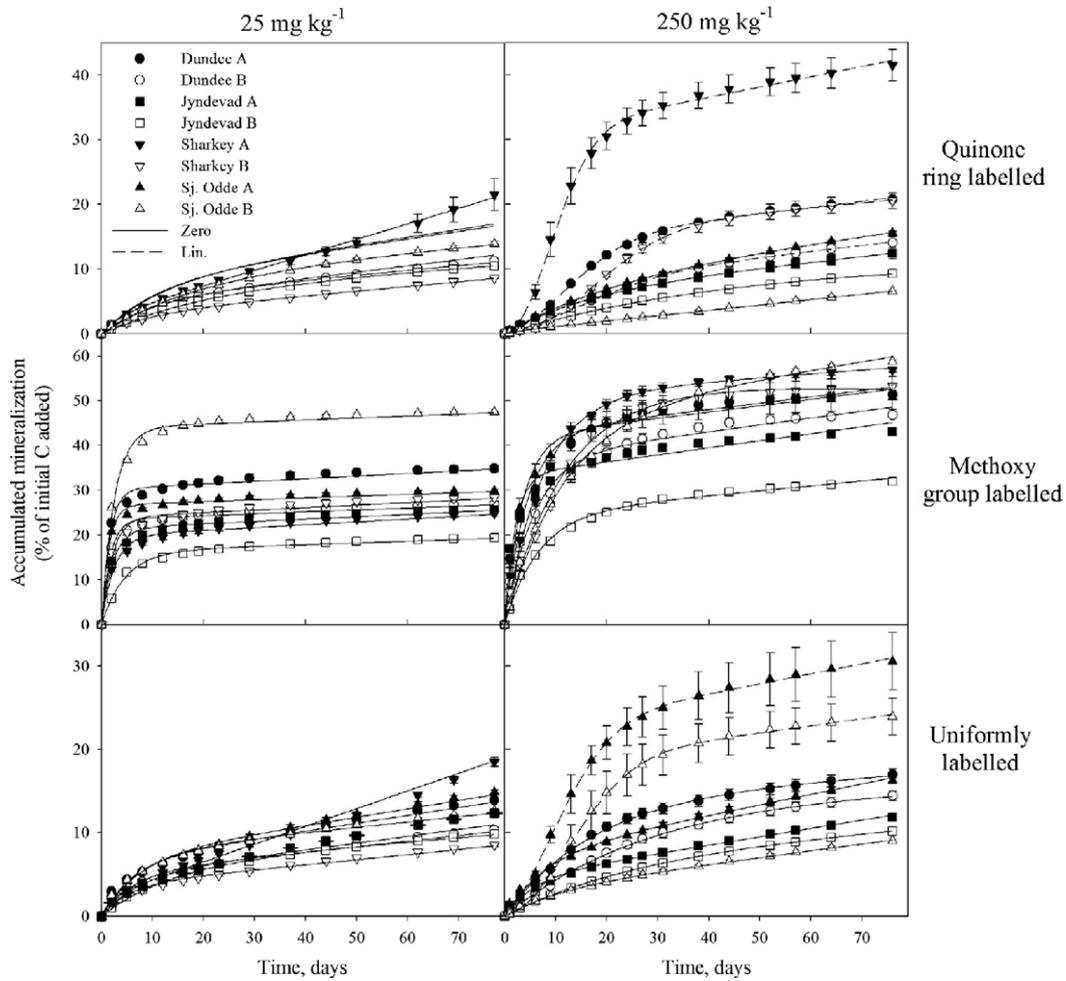


Fig. 2. Mineralization of the quinone ring labelled, the methoxy labelled and the uniformly labelled sorgholeone. Data was modelled using the three-half order model with linear or no growth. The error bars show the standard deviation. Notice the different scales on the y-axis.

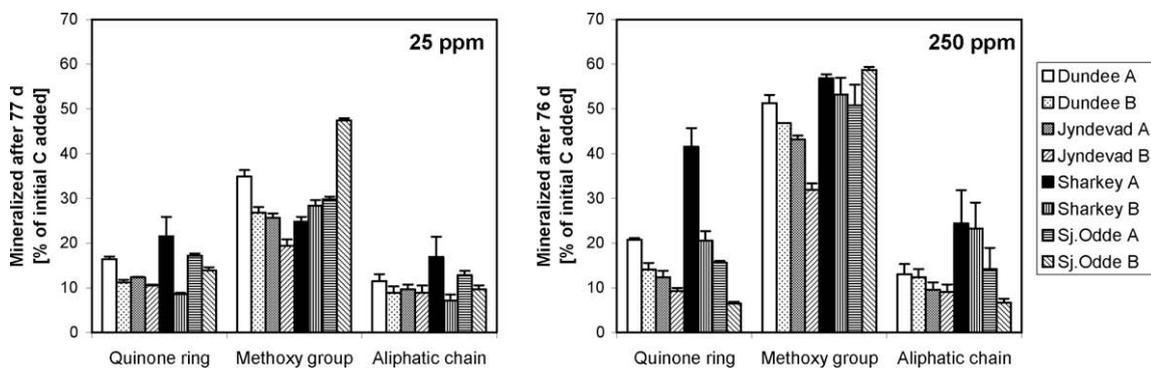


Fig. 3. Mineralization of the different parts of the molecule after 76–77 d at the two concentrations applied.

but it was one of the highest when 250 ppm was added. Sharkey had a potentially higher retention capacity than the other soils due to a higher clay and organic C content. This may have contributed to lower bioavailability at the 25 ppm sorgholeone concentration. In the Sharkey and Sj. Odde soil mineralization at 25 ppm was higher in the B than in A horizon perhaps because the content of organic matter was lower in the B-horizons thus rendering it more bioavailable. Sorgholeone has a $\log K_{ow}$ of 6.2 (Trezzini et al., 2006) and is therefore expected to sorb strongly to the organic mat-

ter in soil, so a lower organic matter content will most likely result in less being sorbed and hence in a higher bioavailability.

Mineralization of the quinone ring was different from that of the methoxy group. Mineralization was slow for all soils with the 25 ppm treatment, and after 77 d none of the soils reached more than 21% mineralized. The same type of kinetics also was observed for the Danish soils at 250 ppm, and at the end of the 76-d experiment, only 6–16% of the applied sorgholeone was mineralized (see Figs. 2 and 3). When 250 ppm was applied, the American soils had

Table 2

Fitting of the three-half order model with no growth (model 1) to the data with 25 ppm sorgoleone added. Model parameters with the standard error in parentheses and R^2 for the fit of the model to the data.

Soil	Labelled form	S	k_0	k_1	R^2
Dundee A	Methoxy	30.21 (0.40)	0.058 (0.0061)	0.64 (0.048)	0.97
	Ring	7.80 (0.25)	0.11 (0.0032)	0.091 (0.0059)	1.00
	Uniformly	6.40 (0.19)	0.095 (0.0027)	0.17 (0.015)	0.99
Dundee B	Methoxy	23.45 (0.30)	0.043 (0.0049)	0.60 (0.046)	0.97
	Ring	5.37 (0.31)	0.074 (0.0040)	0.098 (0.012)	0.98
	Uniformly	5.03 (0.24)	0.066 (0.0034)	0.16 (0.023)	0.97
Jyndevad A	Methoxy	21.38 (0.30)	0.054 (0.0050)	0.46 (0.035)	0.97
	Ring	4.66 (0.14)	0.097 (0.0018)	0.11 (0.0072)	1.00
	Uniformly	4.67 (0.16)	0.081 (0.0022)	0.14 (0.013)	0.99
Jyndevad B	Methoxy	16.25 (0.43)	0.039 (0.0064)	0.22 (0.020)	0.95
	Ring	6.47 (0.19)	0.054 (0.0021)	0.050 (0.002)	1.00
	Uniformly	5.97 (0.43)	0.049 (0.0052)	0.073 (0.0090)	0.98
Sharkey A	Methoxy	19.73 (0.32)	0.064 (0.0052)	0.40 (0.032)	0.97
	Ring	2.61 (0.86)	0.24 (0.013)	0.25 (0.31)	0.93
	Uniformly	2.26 (0.20)	0.21 (0.0032)	0.52 (0.25)	0.99
Sharkey B	Methoxy	23.88 (0.35)	0.056 (0.0057)	0.48 (0.038)	0.97
	Ring	2.98 (0.12)	0.072 (0.0015)	0.099 (0.0083)	1.00
	Uniformly	3.69 (0.14)	0.061 (0.0020)	0.19 (0.023)	0.99
Sj. Odde A	Methoxy	26.66 (0.21)	0.040 (0.0035)	0.70 (0.036)	0.99
	Ring	7.89 (0.30)	0.12 (0.0037)	0.085 (0.0060)	1.00
	Uniformly	6.86 (0.26)	0.10 (0.0036)	0.14 (0.014)	0.99
Sj. Odde B	Methoxy	43.88 (0.27)	0.045 (0.0044)	0.40 (0.012)	0.99
	Ring	8.43 (0.62)	0.071 (0.0068)	0.053 (0.0053)	0.99
	Uniformly	7.45 (0.15)	0.064 (0.0020)	0.13 (0.0068)	0.99

Table 3

Fitting of the three-half order model with no growth (model 1) or with linear growth (model 2) to the data with 250 ppm sorgoleone added. Model parameters with the standard error in parentheses and R^2 for the fit of the model to the data.

Soil	Labelled form	S	k_0	k_1	k_2	R^2
Dundee A	Methoxy	42.46 (1.04)	0.14 (0.023)	0.23 (0.017)		0.98
	Ring	11.38 (1.25)	0.074 (0.020)	0.060 (0.0080)	0.0021 (0.0018)	0.98
	Uniformly	14.01 (1.88)	0.039 (0.027)	0.058 (0.0092)		0.98
Dundee B	Methoxy	36.93 (1.23)	0.15 (0.026)	0.18 (0.016)		0.97
	Ring	7.11 (1.35)	0.093 (0.022)	0.011 (0.0096)	0.0034 (0.0015)	0.97
	Uniformly	14.19 (4.39)	0.015 (0.048)	0.035 (0.0094)		0.98
Jyndevad A	Methoxy	33.20 (0.65)	0.16 (0.016)	0.49 (0.042)		0.97
	Ring	6.08 (1.56)	0.084 (0.023)	0.062 (0.020)		0.96
	Uniformly	4.50 (0.19)	0.10 (0.0040)	0.17 (0.019)		0.99
Jyndevad B	Methoxy	24.40 (0.52)	0.11 (0.010)	0.15 (0.0077)		0.99
	Ring	11.43 (11.64)	0.00060 (0.10)	0.021 (0.015)		0.97
	Uniformly	6.80 (1.51)	0.049 (0.018)	0.040 (0.0083)		0.99
Sharkey A	Methoxy	50.86 (1.30)	0.085 (0.025)	0.14 (0.0076)		0.99
	Ring	30.18 (1.84)	0.16 (0.037)	-0.0044 (0.016)	0.014 (0.0038)	0.96
	Uniformly	21.83 (2.93)	0.12 (0.054)	0.021 (0.021)	0.0071 (0.0043)	0.91
Sharkey B	Methoxy	53.77 (3.12)	-0.015 (0.052)	0.086 (0.0077)		0.98
	Ring	14.89 (1.80)	0.076 (0.030)	-0.0072 (0.0070)	0.0043 (0.0010)	0.98
	Uniformly	17.71 (2.98)	0.085 (0.053)	0.0025 (0.018)	0.0063 (0.0031)	0.91
Sj. Odde A	Methoxy	41.85 (1.54)	0.14 (0.035)	0.29 (0.037)		0.93
	Ring	7.93 (0.95)	0.10 (0.012)	0.046 (0.0055)		1.00
	Uniformly	7.04 (0.32)	0.13 (0.0062)	0.13 (0.013)		0.99
Sj. Odde B	Methoxy	45.72 (1.39)	0.19 (0.02)	0.09 (0.01)		1.00
	Ring	0.27 (0.12)	0.83 (0.0027)	0.26 (0.37)		0.98
	Uniformly	2.98 (0.27)	0.081 (0.0046)	0.092 (0.014)		0.99

a very different sigmoid shaped kinetic with an initial short lag phase, followed by rapid mineralization for approximately 30 d, after which it levelled off for a slower phase. Hence these data sets were fitted better with the three-half order model suggesting linear growth (model 1) than with the model for zero growth (model 2).

After 76 d up to 41.5% was mineralized, indicating that these soils harboured microorganisms capable of utilizing sorgoleone

as an energy source. Other studies have demonstrated that in soils where an allelochemical was present, the soil microbial community adapted to using that compound as an energy source, resulting in its enhanced degradation (Kong et al., 2008). The soils used in this study had no history of a sorghum crop where sorgoleone may have been secreted. However, the wild sorghum johnsongrass (*Sorghum halapense*) is a commonly encountered weed in the southern USA. Although it is possible that johnsongrass may have

occurred in the areas where the Sharkey and Dundee soils were collected, weed control with tillage and herbicides prevented infestations. It is therefore unlikely that sorgoleone-adapted microorganisms were a significant contributing factor to enhanced degradation in Dundee and Sharkey in this study. Since johnson-grass is not found in Denmark, the potential does not exist for sorgoleone deriving from that source.

Another aspect that must be considered is that the soils were stored frozen until they were used in the experiment. Freezing would depress the microbial community in the soils, perhaps resulting in a lag time for recovery of microbial populations once the soils were restored to ambient temperatures. Variations in the lag time among the soils may have resulted in the observed differences during the experiment. Of course, the ideal situation would have been to use fresh soils, but this was not practically possible. However, studies have shown that freezing soil during storage is an acceptable way of storing soil for use in microbial experiments. Mortensen and Jacobsen (2004) found that frozen storage of a Danish soil did not affect the mineralization of the pesticides MCPA and metribuzin, even in the subsoil, which is not usually exposed to freeze/thaw events under *in situ* field conditions. Other experiments have shown that while frozen storage does induce changes in the microbial activity and communities, storage in a refrigerator at 4 °C induced changes similar to those of freezing. Studies also have shown that air drying is the least satisfactory method for storing soils used for microbiological evaluations (Zelles et al., 1991; Shishido and Chanway, 1998).

One other factor that may have altered the microbial activity in the soils is that the natural structure of the soils was disturbed when the soil was transferred to the laboratory microcosm, thereby altering the microorganisms accessibility to oxygen and soil organic matter. Again, varying response among the soils to these changes in conditions may have enhanced or masked differences.

Mineralization of the uniformly labelled sorgoleone resembles the mineralization of the quinone ring labelled sorgoleone, except that a lower maximal level of mineralization was observed. When 25 ppm was applied, only a little more than 18% was mineralized after 77 d in the Sharkey A soil, which was the soil with the highest mineralization potential. As for the mineralization of 250 ppm of the quinone ring labelled compound, the Sharkey A soil with 250 ppm also had a sigmoid kinetic involving a lag-phase followed by rapid mineralization, and, at last, a slower phase, which were best characterized by the model suggesting linear growth. Mineralization of the uniformly labelled treatment reached only 30.5% in Sharkey A after 76 d, whereas 41.5% was mineralized when the quinone ring labelled form was used.

Given the mineralization kinetics of three differently labelled forms of sorgoleone, we were able to calculate mineralization of the aliphatic chain. This calculation showed that after 77 d, 6.7–24.4% of the carbons in the chain were mineralized when 250 ppm was applied (Fig. 3). After 76 d, 7.2–16.9% was mineralized when 25 ppm was applied. All of these mineralization data demonstrate that all parts of the sorgoleone molecule are vulnerable to degradation in soil.

Regarding the activity of sorgoleone as an allelochemical in soil, it is unknown how much of the molecule has to be degraded before the activity is gone. It may be sufficient that activity is lost if only the methoxy group is degraded. If that is the case, the rapid mineralization of the methoxy group demonstrated in the present study will have serious implications for the use of sorgoleone as an herbicide.

The results presented herein clearly illustrate that mineralization of sorgoleone varies depending on which part is labelled, the sorgoleone concentration added to soils, and the type of soils investigated. Had only the methoxy labelled form been used at a concentration of 250 ppm, the conclusion would have been that

sorgoleone is easily and completely mineralized in soil, whereas if the uniformly labelled form was used at a concentration of 25 ppm, the conclusion would have been that mineralization is slow and incomplete, and not related to presence of specific microorganisms. Instead, using the three different labelling patterns, the two different concentrations, and eight different types of soils provides a more complete picture of the degradation and mineralization of sorgoleone in soil.

Comparing mineralization of sorgoleone to mineralization of other natural or synthetic compounds, e.g. pyrene, 2,4-D, glyphosate, atrazine, metribuzin, saponins from alfalfa (*Medicago sativa*) or glucosinolates and isothiocyanates from Brassicas (Locke and Harper, 1991; Okumura et al., 1999; Gimsing et al., 2004; Farenhorst et al., 2006; Haderlein et al., 2006; Zablutowicz et al., 2006; Baelum et al., 2008; Poulsen et al., 2008), it is seen that natural compounds are not mineralized faster or to a greater extent than synthetic compounds. Therefore, it is obvious that naturally occurring compounds will not have a more environmentally benign behavior simply because they are natural. The present study clearly shows that many variables such as soil type, concentration of test compound, and labelling pattern, will influence mineralization, and this will be the case for both natural and synthetic compounds alike. Also the structure of the compound will be most important and not whether the compound is synthesized by a plant or in a factory.

Based on our study it can be concluded that the allelochemical sorgoleone can be mineralized in all of the soils tested. However, the different chemical groups of the molecule are not mineralized equally. A high percentage of the methoxy group is rapidly mineralized whereas the other parts of the molecule mineralize more slowly. The study also showed that apparently there are microorganisms in the American soils that are capable of using sorgoleone as a carbon source, which is the basis for a higher degree of mineralization and thereby a reduced risk of persistence. In the Danish soils, on the other hand, where no growth related mineralization was observed, there may be problems with persistence.

Acknowledgement

The Carlsberg Foundation and the Danish Research Council for Technology and Production are thanked for their financial support.

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