Influence of sulfur fertilization on floral scent patterns of crops in full bloom

Elke Bloem*, Silvia Haneklaus*, Rolf Daniels** and Ewald Schnug*

Abstract

Regularly, macroscopic sulfur deficiency can be found in oilseed rape on production fields. Severe sulfur deficiency causes unique flower symptoms with changes in colour from bright to pale yellow and modifications in shape and size of the petals. Less known is the fact that sulfur deficiency alters the scent of flowers. It was shown in previous studies that this has severe implications for pollinating insects such as honeybees. So far no studies exist, which investigated the influence of the sulfur nutritional status on the emission of volatiles by other agricultural plants during flowering. In the present study the influence of the sulfur supply on the floral scent of crops with (white mustard, oil radish) and without sulfur-containing secondary compounds (chamomile, field beans, and peas) was determined. The plant sulfur status was monitored at the start of main growth and the volatile emissions of flowers were characterized three times during main flowering by employing an electronic nose. Sulfur fertilization increased the sulfur content in leaves and flowers of all crops. Significant differences in the emission of volatiles in relation to sulfur fertilization were determined at least at one sampling date for all investigated crops except white mustard. It was possible to distinguish floral scent patterns of beans from peas and chamomile, and that of peas from chamomile and white mustard. Moreover it was possible to discriminate the floral scent of the two glucosinolate-containing crops white mustard and oil radish, too.

Keywords: chamomile, e-nose, Faba beans, floral scent, mustard, oil radish, peas, sulfur

Zusammenfassung

Einfluss der Schwefeldüngung auf das Blütenduftmuster von Kulturpflanzen während der Hauptblüte

Akuter Schwefelmangel an Raps kann regelmäßig auf landwirtschaftlichen Flächen beobachtet werden. Bei makroskopisch sichtbarem Schwefelmangel treten einzigartige Symptome an den Blüten von Raps auf wie zum Beispiel ein Farbwechsel von leuchtend-gelb zu weißlich sowie Blütenblattdeformationen in Größe und Form. Weniger bekannt ist, dass Schwefelmangel den Blütenduft verändert. In vorherigen Untersuchungen wurde gezeigt, dass diese Veränderungen einen nachhaltigen Einfluss auf die Bestäubung durch nützliche Insekten wie die Honigbiene haben. Bislang liegen keine weiteren Studien zur Emission gasförmiger Verbindungen von Kulturpflanzen in Abhängigkeit von der Schwefelversorgung während der Blüte vor. In der vorliegenden Studie wurde der Blütenduft von Kulturpflanzen mit (Weißer Senf, Ölrettich) und ohne schwefelhaltige Sekundärstoffwechselprodukte (Kamille, Faba-Bohnen, Erbsen) untersucht. Der Schwefelversorgungsgrad der Pflanzen wurde zur Hauptwachstumsphase bestimmt und die Muster der Emission gasförmiger Verbindungen durch die Blüten mittels Elektronischer Nase zu drei unterschiedlichen Terminen während der Hauptblüte erfasst. Die Schwefeldüngung erhöhte deutlich den Schwefelgehalt in Blättern und Blüten sämtlicher Kulturen. Die Schwefelzufuhr übte einen signifikanten Einfluss auf die Emission gasförmiger Verbindungen aller Kulturen mit Ausnahme des Weißen Senfs an zumindest einem Beprobungstermin aus. Mit Hilfe der Elektronischen Nase konnte der Blütenduft von Bohne und Erbse beziehungsweise Kamille sowie Erbse und Kamille beziehungsweise Senf klar voneinander unterschieden werden. Auch der Duft der beiden glucosinolathaltigen Kulturen Senf und Ölrettich ließ sich eindeutig von einander unterscheiden.

Schlüsselwörter: Ackerbohne, Blütenduft, Elektronische Nase, Erbse, Kamille, Ölrettich, Schwefel, Weißer Senf

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Introduction

The scent of flowers is produced by a multitude of different chemical compounds. More than 700 metabolites have been identified in the volatiles of 60 different plant families (Knudsen et al., 1993). In volatiles of oilseed rape 34 different compounds were determined (Tollsten and Bergström, 1988; Robertson et al., 1993; McEwan and Smith, 1998). It is hardly feasible to quantify changes in the concentration of all known metabolites in relation to sulfur (S) supply and crop species so that an electric nose system seems a promising approach to determine complex modifications due to variations in the S supply and to deliver results on basic differences between crop species.

Macroscopic S deficiency causes characteristic symptoms in all vegetative plant parts of *Brassica* crops (Haneklaus et al., 2006). During flowering of oilseed rape a change in color, often together with modifications in size and shape of the petals can be observed (Schnug and Haneklaus, 1994). Besides these phenological changes S deficiency causes also changes in the release of volatiles by oilseed rape flowers (Haneklaus et al., 2007; 2009).

In glucosinolate-containing crops such as *Brassica rapa* nitriles and isothiocyanates, which are degradation products from glucosinolates were found in the floral scent in large quantities (Omura et al., 1999). Previous studies have shown that the S supply increases significantly the glucosinolate content in vegetative plant parts, seeds and petals of oilseed rape and other *Brassica* (Schnug, 1993; He, 1999). Thus it may be assumed that the S nutritional status affects the composition of the scent of glucosinolate-containing crops such as *Brassica*.

Honeybees are attracted by scent, color and form of the honey-bearing plants, but it is the scent, which is the fastest and strongest signal for pollen and nectar (Menzel et al., 1993). Morphological changes and modifications in the pattern of volatiles in relation to S fertilization have been described for oilseed rape, but it is most likely the striking resemblance of a pollinated and fading rapeseed flower with an unpollinated, S-deficient one, which makes them less attractive to honey bees (Schnug and Haneklaus, 1994 and 2005; Haneklaus et al., 2007).

S fertilization influenced the infestation of winter oilseed rape with pests; obviously a higher S supply favored specialist insects (Haneklaus et al., 2008 and 2009). So far, no data exist about the influence of the S supply on the release of volatiles by agricultural plants during flowering though they might be an indicator for the infestation of crops by pests.

Electric nose (e-nose) systems can discriminate a wide range of different scents and are suitable for different research purposes. Traditionally e-nose systems have been used to distinguish differences in aroma and quality of a product as for instance vegetables and fruits (Broda, 2000), spicery (Madsen and Grypa, 2000), wine (Martin et al., 2008), beer, tobacco, coffee and cosmetic products (Bartlett et al., 1997). In food and cosmetic industries a consistent quality, odor and taste is highly important and e-nose systems are a fast, cheap and reproducible possibility to transform complex mixtures of headspace volatiles into electrical responses that characterize the headspace (Sinesio et al., 2000) for the control of the product quality. Other applications of e-nose systems imply the detection of toxic or malodorous odors from landfill sites and industries, the detection of mine fields or bombs in military areas, and in medicine for the diagnosis of diseases (Hatt and Dee, 2009). In the agricultural sector, Laothawornkitkul et al. (2008) demonstrated the potential use of e-nose technology as a real-time pest and disease monitoring system in agriculture.

It was the aim of the present study to determine the influence of S fertilization on the floral scent of different crops and to investigate if patterns in the release of volatiles by flowers of different plant species can be distinguished.

Materials and Methods

In 2008, a field trial was conducted at the experimental station of the Julius Kühn-Institute (JKI) in Braunschweig (E 10°27´, N 52°18´). The climate is temperate and characterized by frequent changes in temperature, humidity, and winds. The soil type is a Cambisol with a loamy sand soil texture (6.5 % clay; 47 % sand) with a low water retention capacity and a high leaching rate. The soil pH value was 5.5.

Five different crops: white mustard (*Sinapis alba*), oil radish (*Raphanus sativus var. oleiformis*), chamomile (*Matricaria chamomilla*), field beans (*Vicia faba*) and peas (*Pisum sativum*) were grown in plots each with a size of 288 m^2. Plots were split in half and were fertilized with 100 kg S ha^{-1} as ammonium sulphate nitrate (+S) which was applied in two equal doses of 50 kg ha^{-1} at sowing and at the start of the main growth. The other half of the plot received no S (-S). Nitrogen was fertilized at the following rates: white mustard and oil radish: 100 kg ha^{-1} N; chamomile, field beans and peas: 30 kg ha^{-1} N. Potassium was applied at an equal rate of 115 kg ha^{-1} K.

Samples were taken at geo-referenced sampling locations in an area of about 1 to 2 m^2 around the sampling point (Figure 1). Younger, fully differentiated leaves were sampled in each plot at the start of the main vegetative growth in fourfold repetition for mineral analysis (Figure 1). During full blooming flowers were taken in each plot on three different days to investigate the S status and the scent of flowers. Unpollinated flowers were cut at the insertion of the pedicel. Time between sampling dates varied in relation to the crop-specific progression of blossom and was at minimum 3 days (peas and chamomile) and at maximum 17 days (oil radish). For the mineral analysis leaves and flowers pooled from the three sampling dates were dried until constancy of weight in a ventilated oven at 60 °C. The plant material was fine-ground to a particle size of < 0.1mm using a *Retsch* ultracentrifugal mill.

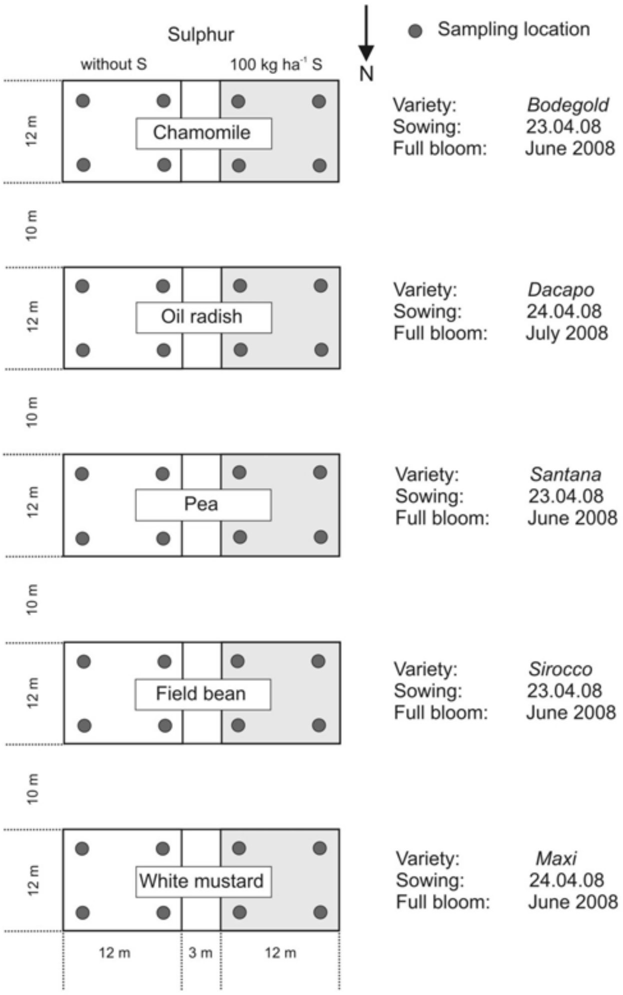


Figure 1:

Field trial for the determination of changes in the release of volatiles by different agricultural plants during flowering

The total S content in leaves and flowers was determined by a high temperature combustion method, which operates at 1150 °C for complete S recovery with subsequent gas analysis for S employing the *Elementar Vario Max CNS* equipment.

Volatiles in the headspace of the flowers were analyzed by means of an e-nose with a sensor system consisting of 32 conductive polymer sensors (CPS; AromaScan® A32/50S, UK). The measurement was conducted with 7 fresh flowers of peas, beans and chamomile and 10 flowers of mustard or oil radish. All flowers were directly sealed gas-tightly in glass vials (22 ml capacity) on the field. The measurement was conducted at 40 °C after an equilibration time of 15 minutes. Headspace volatiles were sampled for a period of 1 minute. After each measurement the system was rinsed with synthetic air to clean the sensors. Data analysis was based on the values of maximum change in resistance ($\Delta R)/R_0$ of the sensors, which were interpreted by standard mapping techniques, using software supplied by the manufacturer (AromaScan, 1995). E-nose data set clusters are compared in a two-dimensional principal component analysis (PCA) plot, which provides a pictorial reflection of similar patterns or differences from the data files after data reduction (AromaScan, 1995). PCA is a technique used to reduce multidimensional data sets to a lower dimension for analysis to identify the meaning of investigated factors for the characterized values. The quality factor (QF), which is calculated by the software, is an index to segregate significantly different groups from each other (AromaScan, 1995).

For statistical analysis differences between means of treatments were tested by employing a t-test (Simons, 1995).

Results and Discussion

Previous studies revealed that the S nutritional status of a crop is not only related to the content of primary and secondary S-containing compounds in vegetative and generative plant tissue (Haneklaus et al., 2006) and the emission of S-containing gases such as H_2S (Bloem et al. 2007), but also to the emission of volatiles, which are important for the scent of oilseed rape flowers (Brauer, 2007).

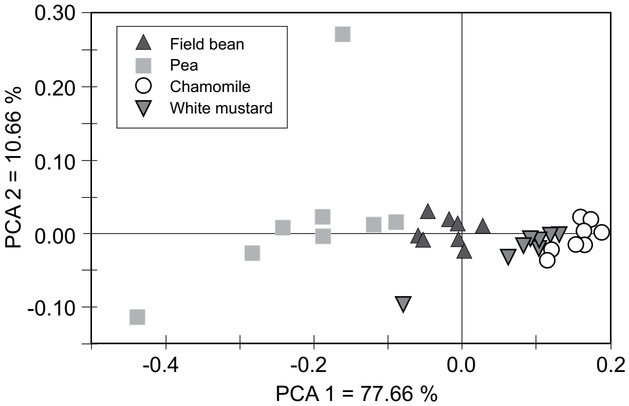
S fertilization increased distinctly the S content of leaves and flowers (Table 1). These differences proved to be statistically significant in leaf tissue of oil radish and chamomile and in the flowers of all crops except pea.

Table 1:

Influence of S fertilization on the total S content in leaves and flowers of agricultural crops.

Crop	Leaves [mg S g^{-1} d.w.]			Flowers [mg S g^{-1} d.w.]		
	- S	+ S	*Sig*[1]	- S	+ S	*Sig*[1]
White mustard (*Sinapis alba*)	6.8	8.6	ns	13.6	15.4	**
Oil radish (*Raphanus sativus var. oleiformis*)	7.4	9.3	*	16.8	18.8	*
Chamomile (*Matricaria chamomilla*)	4.3	6.1	**	3.8	4.1	*
Field beans (*Vicia faba*)	4.6	4.6	ns	2.6	2.9	**
Pea (*Pisum sativum*)	3.7	4.1	ns	4.6	4.6	ns

Sig[1]: Level of significance



2 dimensional PCA map

Class	Class	QF
Bean	Pea	2.66
Bean	Chamomile	5.25
Bean	Mustard	1.98
Pea	Chamomile	5.30
Pea	Mustard	2.78
Chamomile	Mustard	1.66

Figure 2:

Two-dimensional PCA plot for headspace volatiles from flowers of peas, beans, chamomile and mustard sampled on 27th June 2008 (data include measurements for –S and +S treatments)

The total S content in leaves of all crops proved to be sufficiently high for producing maximum yields (Haneklaus et al., 2006). Based on the studies of Brauer (2007) a distinct effect of a change in the S nutritional status on the emission of volatiles by flowers was expected when going from sufficiency to excess, too.

In comparison to GC-MS analysis, which is used to analyze individual components quantitatively, the e-nose determines changes in the spectrum of several compounds and thus works qualitatively. The advantages of the determination by e-nose are that a high number of samples can be easily and fast analyzed and the costs per sample are low. A major disadvantage is that it is not possible to quantify the concentration of individual components.

In a first attempt the scent of different flowers was compared (Figure 2) to test the suitability of the e-nose measurement for the characterization of flower scent. Paillan-Legue (1987) showed that climatic factors such as air temperature have a strong effect on the results of the e-nose measurement. Therefore all flowers were sampled and measured on the same day (Figure 2) so that any change of climatic factors had no impact on the results obtained. PCA of the sensor data was performed and the calculation of so-called quality factors (QF) revealed statistically significant differences (Figure 2).

Groups are significantly different from each other when QF's higher than 2 were determined. QF's were determined between crops and on different days in relation to the S supply, each on basis of four replicate measurements per plot. The results of the e-nose measurements revealed significant differences in the patterns of volatiles between crops (Figure 2) and in relation to the S supply (Figure 3).

The data in Figure 2 reveal further that beans and peas, beans and chamomile, peas and chamomile and peas and mustard can be discriminated by their scent irrespective of the S supply as results of both treatments were included in

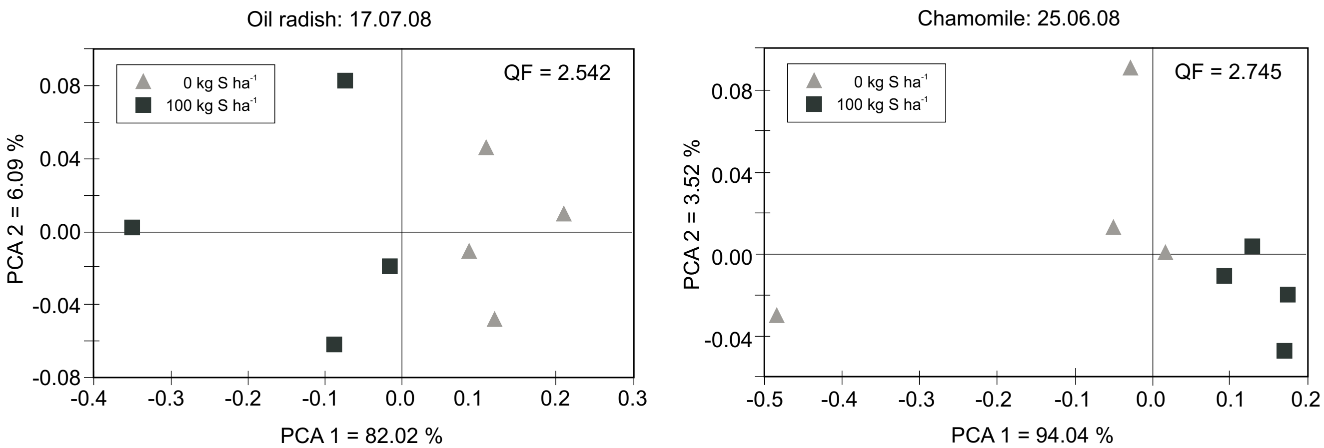


Figure 3:

Two-dimensional PCA plot for headspace volatiles from oil radish and chamomile flowers in relation to S application.

the data set. In comparison it was not possible to discriminate mustard and beans and mustard and chamomile by their release of volatiles on that particular day. A differentiation between -S and +S plots delivered the same result. This finding suggests that plants synthezising secondary S-containing metabolites such as mustard do not necessarily show a different pattern in scent, which can be detected by e-nose. Interestingly to note is the fact that it was possible to discriminate the scent of the two legume crops. Similar results were determined for different pairs of crops at the other sampling dates and the two glucosinolates-containing crops white mustard and oil radish were discriminated by their scent, too.

In Figure 3 the influence of S fertilization on the release of volatiles is shown for oil radish and chamomile. These crops were selected exemplary because of their significant response to S fertilization (Table 1). The results clearly reveal that S fertilization and the S nutritional status of the crop significantly influenced the release of volatiles by flowers so that it was possible to distinguish between the +S and –S treatment of both crops.

A weak, however, significant linear correlation was found between the total S content in flowers (X) of oil radish and the resistance (dR/R) of all 32 sensors (Y = 0.405X – 0.13; r^2 = 66.3 % for the medium resistance of all 32 sensors). This indicates that the intensity of the scent was related to the S status of the plant.

Abbey et al. (2005) studied the impact of S fertilization on the emission of volatiles by different genotypes of spring onions employing an e-nose. S fertilization proved to have a significant effect, while it was not possible to distinguish between all genotypes.

Global radiation and temperature are the most important climatic factors influencing the scent of crops (Brauer, 2007; Paillan-Legue, 1987). Accordingly the QF's varied strongly for one crop species between sampling dates (Table 2). Under unfavorable climatic conditions, it seems generally difficult to unfold treatment effects. Even under controlled greenhouse conditions variations of climatic factors had a strong effect on the release of volatiles and masked the S effect (Brauer, 2007). Thus it can be concluded that single e-nose measurements might not reveal the true impact of S fertilization and crop type on the release of volatiles.

White mustard proved to be the only crop for which at none of the sampling dates significant differences between -S and +S treatment were found (Table 2). The fact that the S supply in the control plots was in the sufficiency range (Haneklaus et al., 2006) might have blurred a treatment effect, which may exist when going from deficiency to sufficiency range. In general, the S supply of all crops in the control plots was sufficient for maximum productivity so that the question remains open how severe, macroscopic S deficiency will affect the release of volatiles.

From agro-environmental point of view the significance of changes in the floral scent, which were determined between crops and in dependence on the S supply in this study, for the pollination by insects, and the infestation with pests remains open. In the presented field experiment a higher abundance of butterflies of the genus *Pieris* was

Table 2:

Influence of S fertilization on patterns of volatiles, expressed by quality factors (QF), emitted by flowers of different crops at different sampling dates

Plant	Date	Temperature [°C]		Global radiation [J cm^{-2}]	QF
		Mean	Maximum		
Glucosinolate-containing crops					
White mustard (*Sinapis alba*)	26.06.08	18.5	23.2	2623	1.260
	27.06.08	16.3	20.2	1291	1.614
	01.07.08	20.0	27.1	2869	1.634
Oil radish (*Raphanus sativus Var. oleiformis*)	01.07.08	20.0	27.1	2869	**2.149**
	07.07.08	16.7	22.2	2030	1.480
	17.07.08	14.6	19.1	1430	**2.542**
Glucosinolate-free crops					
Chamomile (*Matricaria chamomilla*)	25.06.08	19.1	24.2	1579	**2.745**
	26.06.08	18.5	23.2	2623	**2.081**
	27.06.08	16.3	20.2	1291	0.832
Field beans (*Vicia faba*)	18.06.08	17.8	23.7	2534	**2.122**
	25.06.08	19.1	24.2	1579	0.753
	27.06.08	16.3	20.2	1291	1.970
Pea (*Pisum sativum*)	25.06.08	19.1	24.2	1579	1.170
	26.06.08	18.5	23.2	2623	**2.848**
	27.06.08	16.3	20.2	1291	1.391

counted in plots of white mustard and oil radish, which received S fertilization. This observation is in agreement with the studies of Haneklaus et al. (2008, 2009), which showed that S fertilization, resulted in a higher population of specialist insects in oilseed rape. Last, but not least the significance of the S supply for pollination by modifying intensity and pattern of volatiles emitted by plants might be worth to examine if crop productivity depends on cross-pollination, and here on a scale from severe S deficiency to excessive S supply.

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