About the significance of sulfur nutrition of oilseed rape for honey production

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Abstract

Yellow flowering oilseed rape fields emitting their characteristic scent are a familiar part of the rural landscape and important for honey production. Oilseed rape is an essential European melliferous crop for beekeepers as it is an important foraging plant in early summer. The main pollinators are honey bees, wild bees and bumble bees. Although oilseed rape has the ability of self-pollination, values of up to 95% for the pollination by honey bees were determined. Regularly, macroscopic sulfur deficiency can be found in oilseed rape. The visual symptoms during flowering are a change in color, often together with a reduction of the petal size. These phenological changes are presumably related to a reduced attractiveness for honeybees, something that has been observed repeatedly on production fields. Honey bees are attracted in the order scent >> color > form by honey-bearing plants. Other factors are the nectar and pollen content. The results of the presented research work revealed that severe sulfur deficiency significantly influenced the scent of flowering oilseed rape. Additionally, sulfur deficiency caused a change of the petal color from bright to pale yellow, or even white petals and deformation of petals. Values for the absorbance at 440 nm were about two times higher when plants were sufficiently supplied with sulfur than under conditions of extreme sulfur deficiency. Honey bees, in contrast to humans, are able to see in the UV-range. Spectrometric field measurements revealed also different ultra-violet spectra in relation to the sulfur status. From the deficiency to the sufficiency range petals were modified in size and shape in such way that the mean diameter was reduced from 10.4 to 7.1 mm and the length from 17.0 to 13.7 mm. Neither the nectar, nor the pollen content was influenced by the S nutritional status. The morphological and physiological changes of sulfur-deficient oilseed rape during flowering coincided with a significant reduction of the number of visiting honey bees from on average 5.4 to 3.0 per 0.5 m^2 min^{-1}. The significance of changes in individual parameters of oilseed rape on the behavior of honey bees in relation to the sulfur supply is discussed.

Key words: electronic nose, honeybee, scent, sulfur deficiency

Introduction

Yellow flowering oilseed rape fields emitting their characteristic odor are a familiar part of the rural landscape and important for honey production. Oilseed rape is an essential European melliferous crop for beekeepers as it is an important foraging plant in early summer. In Germany, oilseed rape is grown on an area of about $1.27 \cdot 10^6$ ha (Anon, 2004) and the crop delivers up to 50 kg ha^{-1} honey. The main pollinators in oilseed rape are insects of the family *Apidea* (e.g. honeybees, wild bees and bumble bees) (Corbet, 1992; Williams, 1996) and the significance of honeybees as pollen vectors for seed set and yield has been described in the literature (Steffan-Dewenter, 2003). Oilseed rape provides an important source of nectar and pollen for honeybees, which are attracted by the bright yellow color of the crop in bloom (Pierre et al., 1999). Although oilseed rape has the ability of self-pollination, values of up to 95% for the pollination by honeybees were determined (Olsson, 1960).

Regularly, macroscopic sulfur (S) deficiency can be found in oilseed rape. Neither the nectar, nor the pollen content proved to be influenced by the S nutritional status as experiments carried out under controlled conditions revealed (Brauer, 2007). The visual symptoms of macroscopic S deficiency during flowering are a change in color, often together with a reduction of the petal size (Schnug and Haneklaus, 1994). From the deficiency to the sufficiency range petals were modified in size and shape in such way that the mean diameter was reduced from 10.4 to 7.1 mm and the length from 17.0 to 13.7 mm (Haneklaus et al., 2004). The color changes from bright to pale yellow, or even white petals and petals are regularly deformed if S deficiency occurs early in the vegetation period (Schnug and Haneklaus, 1994). Values for the absorbance of flowers at 440 nm were about two times higher when plants were sufficiently supplied with S than under conditions of extreme S deficiency (Lilienthal and Schnug, 2005). Honeybees, in contrast to humans, are able to see in the UV-range. Spectrometric field measurements revealed also different ultra-violet spectra in relation to the S status (Lilienthal and Schnug, 2005). Sulfur deficiency, however, causes not only these phenological changes, but also influences the scent during flowering, something that has been observed repeatedly on production fields. Consequently, a reduced attractiveness of oilseed rape for honeybees was observed. 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 (Menzel et al. 1993). It was the aim of the presented study to examine the influence of the S nutritional status on the release of volatiles during blooming from oilseed rape flowers and to quantify its impact on the number of foraging honeybees.

Materials and Methods

Field experiments with winter oilseed rape (cv. *Lion*) were carried out in 2005 and on two neighboring fields (I ('Suedfeld') and II ('Nordfeld')) in 2006 in Braunschweig, Lower Saxony (E 10° 37`, N 52° 00`). Sulfur was fertilized at rates of 50 kg ha^{-1} before sowing and 50 and 100 kg ha^{-1} at start of the vegetation period in 2005 and 2006, respectively. Each block had a size of 135 in 2005, and 1800 and 2000 m^2 in 2006; blocks were separated by at least 200 m.

In 2005, flowers and leaf material were collected from a production field in Gross Schenkenberg, Schleswig Holstein (E 10° 31′, N 53° 47′) at full blooming of the oilseed rape crop.

In younger, fully developed leaves the total S content was determined at start of stem elongation and at full blooming, respectively. Volatiles in the headspace of oilseed rape flowers during full blooming were analyzed by means of an electronic nose (e-nose) with a sensor system consisting of 32x conductive polymer sensors (CPS; AromaScan® A32/50S). The number of visiting honeybees was determined during full blooming by visual reckoning using a counting frame. The statistical analysis was performed by the General Linear Model (GLM) procedure employing the LSD test (α=0.05) and principal component analysis (PCA). A complex description of the experimental design, analytical methods and statistical procedures is given by Brauer (2007).

Results and Discussion

Oilseed rape is the only major crop in German agriculture that delivers pollen and nectar to honey bees in significant amounts. Phenological changes of oilseed rape caused by S deficiency may have significant influence on its attractiveness to honeybees. But it is putatively a changed pattern of volatiles in the headspace of oilseed rape flowers which has the strongest influence on honeybees. A total of 34 different compounds were found in volatiles of oilseed rape (Tollsten and Bergström, 1988; Robertson et al., 1993; McEwan and Smith, 1998). The main volatiles from oilseed rape flowers were 3-hydroxy-2-butanone > 2,3-butanedione > dimethyl disulfide >> formaldehyde > 3-methyl-2-butanone > dimethyl trisulfide (Robertson et al., 1993). Omura et al. (1999) determined nitriles and isothiocyanates in large quantities in the floral volatiles of *Brassica rapa*. Honeybees use volatiles for discrimination whereby a conditioning threshold was determined for individual components (Pham-Delégue et al., 1993). Previous studies have shown that the S supply increases the glucosinolate content in vegetative plant tissues, seeds and petals of oilseed rape so that a connection with the release of isothiocyanates may be assumed (Schnug, 1988 and 1993).

Expectedly, S fertilization increased the total S content significantly. In 2005, the total S content increased from 6.8 to 14.4 and in 2006 from 4.1/3.5 to 8.4/9.7 mg g^{-1} S (d.w.) in field experiment I and II. While in 2005, the S supply was optimum/excessive, in 2006 the S status corresponded to that of moderate/severe S deficiency in the control plots according to Haneklaus et al. (2006) and Haneklaus et al. (2007). Plants in the control plots showed characteristic symptoms of severe S deficiency on leaves and petals. On the production fields the visual analysis of the S status corresponded with the chemical analysis of 2.0 and 7.9 mg g^{-1} S (d.w.), too.

Principal component analysis (PCA) of the sensor data was carried out in relation to the S nutritional status, deficient or sufficient so that it was possible to calculate whether differences in the emission of volatiles were related to the S supply of the plants. The determination of the volatiles in the headspace of oilseed rape flowers revealed that severe S deficiency significantly influenced the scent of flowering oilseed rape as the results from PCA of field experiment I and the production field show (Figure 1). Here, a quality factor of > 2 was determined, which indicates significant differences in the scent of S deficient and sufficiently supplied oilseed rape (Brauer, 2007). Differences between these two groups proved to be, however, not significant in field experiment II. As results from greenhouse experiments showed climatic factors such as air temperature had a strong effect on the results of the e-nose measurements (Brauer, 2007) and most likely masked the S effect in field experiment II. This again might have influenced the measurements by e-nose as this equipment proved to be sensitive against moisture and temperature of the samples (Haugen and Kvaal, 1998).

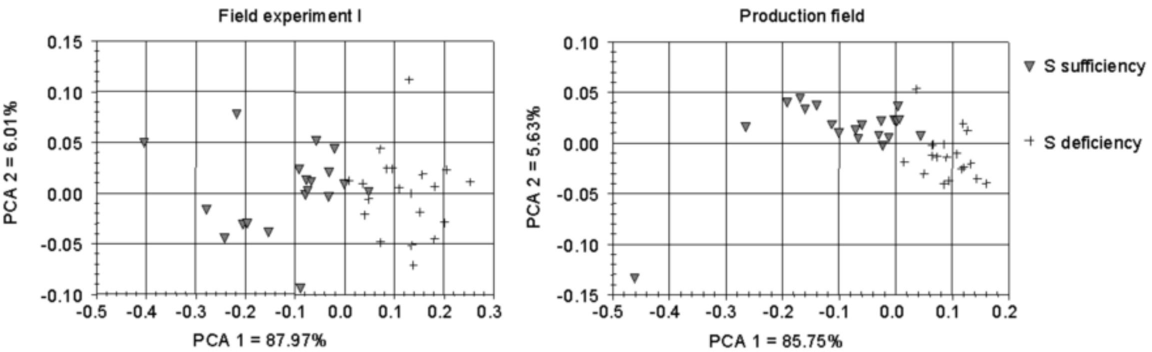

Figure 1. Two-dimensional PCA plot for headspace volatiles from flowers with and without S deficiency symptoms in relation to S fertilization/status under field conditions in 2006 (left) and on a production field in 2005 (right).

A weak, however, significant linear correlation was found between total S content in younger, fully developed leaves and resistance (dR/R) of the sensors ($Y = 1.952 \cdot X + 5.724$; $r^2= 26\%$), which indicates that the intensity of the scent is related to the S nutritional status of the plant.

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 e-nose are that collection of samples is easy, analysis rapid, costs per sample are low and patterns of metabolites yielding different scents can be measured. The major disadvantage is that no single component can be identified quantitatively. At the moment it is speculative whether the S nutritional status is linked to the release of any of the S-containing volatiles. Investigations about the influence of the S supply on H_2S emissions of oilseed rape and other crops revealed that such relationship depended on continuous S amendments and thus indicates regulatory mechanisms prior or after cysteine formation (Bloem et al., 2007). In comparison, the results of the e-nose suggest that the release of volatiles is related to the internal S pool of the plant and their synthesis regulated by the availability of S-containing precursors such as glucosinolates.

S fertilization coincided with a significant increase in the number of visiting honeybees from on average 5.4 to 2.9 per 0.5 m^2 min^{-1} during flowering in 2005 (Table 1). This effect was not consistent with the results from 2006, where on both fields a similar number of honeybees was counted independent on the S supply though the petals showed morphological changes in form and color (Table 1). These findings suggest that S fertilization intensifies the scent over a wide range of S supply. Striking is that in 2006 a distinctly lower number of visiting honeybees was determined than in the previous year. Obviously other plants provided a better source for pollen and nectar in 2006, which might have blurred the significance of the S nutritional status for bee behavior.

Table 1. Influence of the S nutritional status of oilseed rape on the number of visiting honeybees.

Experiment	S nutritional status	Comparative counts(n)	Number of bees			
			min	max	mean	cv (%)
Field study 2005	S sufficiency	20	2	4	2.9	20
	S excess		5	6	5.4	10
Field study I 2006	Severe S deficiency	32	0	2	0.4	150
	S sufficiency		0	2	0.5	143
Field study II 2006	Severe S deficiency	32	0	3	0.7	113
	S sufficiency		0	2	0.8	107

With view to honey production from oilseed rape a corresponding reduction of its contribution by up to 45% on severely S-deficient fields can be expected.

A quantification of the influence of form and color of petals/flower, and scent was not feasible under given field conditions. This could be preferably tested by using greenhouse plants with a S nutrition that yields reduced/normal petal sizes in white/yellow color as foraging crops and their acceptance as foraging plants being tested in an open fly hall. Though scent has a much stronger impact than form or color, it might be possible that S deficiency inverts this common fact. A small white petal might mimic that of a sufficiently supplied crop which has been pollinated and is recognized as such by honeybees. This would also explain why the message that pollen and nectar is available is obviously not transferred from bees foraging the S-deficient oilseed rape crop to other bees of the hive.

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References

Anon (2004) Statistisches Bundesamt Deutschland http://www.destatis.de/basis/d/forst/forsttab7.php

Bloem E, Haneklaus S, Salac I, Wickenhaeuser P, Schnug E (2007) Facts and fiction about sulphur metabolism in relation to plant-pathogen interactions. J Plant Biol (submitted)

Brauer A (2007) Einfluss der Schwefelversorgung auf morphologogische und physiologische Parameter von Rapsblüten (*Brassica napus* L.) und deren Wirkung auf das Verhalten von Honigbienen. PhD thesis, Braunschweig University (in preparation)

Corbet S A (1992) Wild bees for pollination in the agricultural landscape. In: Bruneau E (ed) Bees for pollination. Proc. EC workshop, Brussels, 2-3 March 1992, pp 175-188

Haneklaus S, Brauer A, Bloem E, Schnug E (2004) Relationship between sulfur deficiency in oilseed rape (*Brassica napus L.*) and its attractiveness for honey bees. FAL – gric. Research 283 (special issue): 37-43

Haneklaus S, Bloem E, Schnug E, De Kok L, Stulen I (2006) Sulphur. In: Barker AV and Oilbeam DJ (eds) Handbook of Plant Nutrition, CRC Press, Boca Raton, pp 183-238

Haneklaus S, Bloem E, Schnug E (2007) Sulfur interactions in crop ecosystems. In: MJ Hawkesford and LJ De Kok (eds) Sulphur in plants - an ecological perspective. Kluwer Academic Publ., Leiden (in press)

Haugen JE, Kvaal K (1998) Electric nose and artificial neural networks. Meat Sci. 49: 273-286

Lilienthal H, Schnug E (2005) Eignung spektralre Signaturen zur Lokalisierung von Schwefelmangel in Raps mit Hilfe der Fernerkundung. FAL – Agric. Research 286 (special issue): 47-54

McEwan M, Smith WHM (1998) Identification of volatile organic compounds emitted in the field by oilseed rape (*Brassica napus ssp. oleifera*) over the growing season. Clinical Exp. Allergy 28:332-338

Menzel R, Greggers U, Hammer M (1993) Functional organization of appetitive learning and memory in a generalist pollinator, the honey bee. In: Papaj DR, Lewis AC (eds) Insect Learning. Chapman Hall, New York, pp. 79-125

Olsson G (1960) Species crosses within the genus Brassica. II. Artificial Brassica napus L. Hereditas 46: 351-386

Omura H, Honda K, Hayashi N (1999) Chemical and chromatic bases for preferential visiting by the cabbage butterfly, *Pieris rapae*, to rape flowers. J Chem Ecol 25:1895-1906

Pierre J, Mesquida J, Marilleau R, Pham-Delegue MH, Renard M (1999) Nectar secretion in winter oilseed rape, Brassica napus-quantitative and qualitative variability among 71 genotypes In: Plant Breeding, H. 118:471-476

Robertson G W, Griffiths DW, Smith MW, Butcher RD (1993) The application of thermal-desorption-gas-chromatography mass spectrometry to the analysis of flower volatiles form 5 varieties of oilseed rape. Phytochem Anal 4:152-157

Schnug E (1988) Quantitative und qualitative Aspekte der Diagnose und Therapie der Schwefelversorgung von Raps (*Brassica napus* L.) unter besonderer Berücksichtigung glucosinolatarmer Sorten. Habilitationsschrift (Dsc thesis) Agrarwiss. Fakultät der Christian-Albrechts-Universität zu Kiel

Schnug E (1993) Physiological functions and environmental relevance of sulphur-containing secondary metabolites. In: De Kok LJ et al. (eds) Sulfur Nutrition and Sulfur Assimilation in Higher Plants. SPB Academic Publishing bv, The Hague, pp 179-190

Schnug E, Haneklaus S (1994) Sulphur deficiency in *Brassica napus*: biochemistry, symptomatology, morphogenesis. Landbauforschung Völkenrode 144:1-31

Steffan-Dewenter I (2003) Seed set of male-sterile and male-fertile oilseed rape (Brassica napus) in relation to pollinator density. Apidologie 34:227-235

Tollsten L, Bergström G (1988) Headspace volatiles of whole plants and macerated plant parts of Brassica and Sinapis. Phytochemistry, 27 (2): 4013-4018

Williams IH (1996) Aspects of bee diversity and crop pollination in the European Union. In: Matheson A, Buchmann SL, O'Toole C, Westrich P, Williams IH (eds) The Conservation of Bees. Academic Press, London, pp 63-80