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Relationship between sulfur deficiency in oilseed rape (Brassica napus L.) and its attractiveness for honeybees

Silvia Haneklaus¹, Anja Brauer¹, Elke Bloem¹ and Ewald Schnug¹

Abstract

Oilseed rape showing macroscopic symptoms of sulfur deficiency influences the behavior of honeybees, something that has been observed repeatedly in production fields. The symptomatology of sulfur deficiency in cruciferous crops is characteristic during the whole vegetation period. The peculiarity of sulfur deficiency symptoms during flowering depends on the moment when sulfur becomes a limiting factor. An early appearance of sulfur deficiency is regularly related to the change of the petal color from bright to pale yellow, or even white petals. At the same time, the petals are modified in size and shape. By comparison, a late occurrence of sulfur deficiency usually results in the change of color mentioned earlier, while other morphological parameters are not affected. It was the aim of this paper firstly to provide a condensed description of sulfur deficiency symptoms in oilseed rape during the vegetation period, secondly to determine the influence of the sulfur supply on morphological characteristic of oilseed rape petals, and thirdly to present for the first time data about the attractiveness of flowering oilseed rape for honey bees in relation to the S supply.

Key words: flower color, honey plants, petal deformation, pollen

Introduction

Macroscopic sulfur (S) deficiency was first observed on production fields in 1981 (Schnug and Haneklaus 1994a), and more than 20 years later it is still the most widespread nutrient disorder in northern Europe (URL: //www.pb.fal.de). The significance of the S supply for crop production, crop quality and plant health has been outlined for example by Schnug and Haneklaus (1998), Schnug (1997) and Haneklaus et al. (2004). Visual symptoms of S deficiency in cruciferous crops are very specific and can be addressed in the field throughout the whole vegetation period. Oilseed rape provides an important source of nectar and pollen for honey bees, which are attracted by the bright yellow color of the crop in bloom (Pierre et al. 1999). During flowering, characteristic changes of macroscopic S deficiency are to be seen in color and shape of the petals. It was observed repeatedly that S deficient oilseed rape is less attractive for honeybees. These findings were, however, subjective, while bias-free experimental studies have not been carried out so far.

Oilseed rape is one of the most important 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 1.27·10⁶ ha (Anon 2004). The main pollinators in oilseed rape are insects of the family Apidae (e.g. honey bees, 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). Although oilseed rape is self-pollinating (Sauré, 2002), the cross-pollination rate, predominately by honeybees, was estimated to be about 20% (Downey et al. 1980). According to Olsson (1960) the cross-pollination rate may vary in relation to genotype and climatic conditions between 5 % and 95 %. By comparison, on fields where composite hybrid oilseed rape varieties are grown or male-sterile lines for breeding of restored hybrid cultivars, these plants have a high dependence on pollination by vectors (Steffan-Dewenter, 2003). Thus, determining the influence of the S supply of oilseed rape on its attractiveness for foraging honeybees is a fundamental contribution from both the agronomic and ecological point of view. It was the aim of this paper to provide a comprehensive description of macroscopic S deficiency symptoms in oilseed rape during the vegetation period with special attention being paid to visual symptoms during flowering, in order to quantify the influence of the S supply on morphological parameters of the flowers and last, but not least to show first results about the attractiveness of flowering oilseed rape for bees in relation to the S supply.

Materials and methods

Two field experiments with winter oilseed rape were conducted at Braunschweig (E 10° 27’, N 52° 18’). In the first experiment S was applied at rates of 50 kg S ha⁻¹ in fall and 100 kg S ha⁻¹ in spring to the cultivar Bristol. N was applied at a rate of 200 kg N ha⁻¹. The plot size was 40 m² and each treatment had

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four replicates. For a detailed description of the experimental design see Salac (2004). Growth stages of oilseed rape were recorded according to the code number of the BBCH scale (Strauss et al. 1994).

Bee traps

A beehive was placed in front of the experimental field on 19 April 2004. At the start of flowering (BBCH 60), in each plot four white and four yellow dishes were positioned at a height corresponding with that of the crop plant. The bees were collected on two following days (20 April 2004 and 21 April 2004). In the second experiment, macroscopic symptoms of S deficiency on leaves and flowers were visible in the cultivar Smart in relation to mineral N fertilization (100 and 200 kg N ha\(^{-1}\)) and application of manure (0 and 4.8 t ha\(^{-1}\)). The plot size was 65 m\(^2\) and each treatment had four replicates. For experimental design see Rogasik et al. (2004).

Plant sampling and analysis

In total, 10 individual flowers from 10 different plants with different degrees of visual S deficiency symptoms (extreme, severe, none) were collected on 4 May 2004 (BBCH 65) and 40 or 80 petals analyzed. The petals were carefully separated by using tweezers and directly fixed on object slides. For the determination of length and diameter of each petal, the object slides were scanned and the images interpreted afterwards automatically by employing the ArcView 3.2 software package (Esri, 1999).

For the determination of the amount of pollen produced the anthers of 10 flowers were placed in 1.5 ml Eppendorf tubes. The pollen was dissolved from the anthers by using dimethyl ether. Then the anther peduncles were removed, the ether vapourized and finally the amount of pollen weighed.

The differences in color of the oilseed rape petals were determined colorimetrically by employing the method of Miyamjima et al. (2000). From each level of S supply (extreme S deficiency, severe S deficiency, sufficient S supply) 100 petals from at least 25 different plants were collected and shock frozen in liquid nitrogen and freeze-dried before analysis.

Statistical analysis

The software package CoHort (Anon, 1990) was used for ANOVA (Tukey-Kramer test).

Results and discussion

Sulfur deficiency symptoms of oilseed rape during the vegetation period

Severe S deficiency symptoms were often described in the literature as being less specific and more difficult to identify than other nutrient deficiency symptoms (Bergmann, 1993). Brassica species such as oilseed rape, however, reveal characteristic macroscopic symptoms of S deficiency that can be found throughout the vegetation period. As a supplement to the description of S deficiency symptoms, illustrative digital colour images (WWW No.) can be retrieved from the World Wide Web (URL://www.fal.pb.de). Physiological changes in plant metabolism as a result of S deficiency are described for instance by Schnug (1988) and Schnug and Haneklaus (1994a).

Macroscopic S deficiency symptoms of oilseed rape before winter (BBCH 1-19)

Even before winter, during the early growth of oilseed rape, leaves may start to develop symptoms of S deficiency (WWW 2). Though the plants are still small, symptoms can cover the entire plant (WWW 3). Sulfur fertilization before or at sowing will ensure a sufficient S supply, particularly on light, sandy soils and promote the natural resistance of plants against fungal diseases (Haneklaus et al., 2004).

Macroscopic S deficiency symptoms of oilseed rape from the start of the main vegetation period until appearance of inflorescences above upper leaves (BBCH 30 - 59)

Plants suffering from severe S deficiency, show a characteristic marbling of the leaves. The chlorosis starts from the leaf's edge spreading over intercostal areas but the zones along the veins always remain green (Schnug, 1988) (WWW 4). Deficiency symptoms in younger, fully developed leaves of oilseed rape at the start of stem elongation begin to appear when the total S concentration drops below 3.5 mg g\(^{-1}\) S in double low varieties (Schnug and Haneklaus, 1994a, b).

Chlorosis very rarely turns into necrosis (Schnug, 1988, Ulrich et al., 1993) as it does with nitrogen and magnesium deficiency, which is an important criterion for differential diagnosis. Even under conditions of extreme S deficiency where an oilseed rape plant shows severe disorders it will not wilt (WWW 6).

A characteristic secondary symptom of severe S deficiency is the reddish purple color due to the enrichment of anthocyanins in the chlorotic parts of Brassica leaves (WWW 8). Under field conditions, the formation of anthocyanins starts 4 - 7 days after chlorosis. In particular those leaves not fully expanded produce spoon-like deformations when struck by S deficiency (WWW 9). The reason for this is a reduced cell growth rate in the chlorotic areas along the edge of the leaves, while normal cell growth continues in the green areas along the veins,
so that S deficient leaves appear to be more succulent. The grade of the deformation is stronger the less expanded the leaf is when the plant is struck by S deficiency (WWW 10). Marbling, deformations and anthocyanin accumulation can be detected up to the most recently developed small leaves inserted in forks of branches (WWW 11).

Macroscopic S deficiency symptoms of oilseed rape plants during flowering (BBCH 60 - 69)

During flowering S deficiency causes one of the most impressive symptoms of nutrient deficiency: the 'white blooming' of oilseed rape (WWW 12). The white color presumably develops from an overload of carbohydrates in the cells of the petals caused by disorders in the protein metabolism, which finally ends up in the formation of leuco-anthocyanins (Schnug and Haneklaus, 1995). As with anthocyanins in leaves, the symptoms develop strongest during periods of high photosynthetic activity. Besides the remarkable modification in color, size and shape of oilseed rape the petals change, too. This apparently influences the attractiveness of oilseed rape for honeybees as according to initial personal observations this is seen as well as changes in the petal color, a weaker scent and a reduced number of bees. A verification of this appraisal would be of utmost significance for beekeepers and farmers alike in order to warrant a high yielding oilseed rape crop and honey harvest. In two field experiments the influence of the S supply on morphological parameters of oilseed rape flowers and the behavior of bees was investigated and the first results are presented below.

Macroscopic S deficiency symptoms of oilseed rape during ripening (BBCH 71 - 99)

The strongest yield component affected by S deficiency in oilseed rape is the number of seeds per pod, which decreases significantly (WWW 16) (Schnug, 1988). As described earlier for leaves, the branches and pods of S deficient plants are often red or purple colored due to the accumulation of anthocyanins. Extremely low numbers of seeds per pod, in some cases seedless 'rubber pods' are characteristic symptoms of extreme S deficiency.

Influence of the S supply on morphological parameters of oilseed rape flowers and the attractiveness for honey bees

Honeybees are attracted by scent, colour and form of the honey-bearing plants, but it is the scent which has the fastest and strongest impact (Menzel et al. 1993). Honey bees might assess the amount and concentration of nectar in each flower by employing different senses: directly by visual access to the nectar (Throp et al. 1975, Willmer et al. 1994), or by olfactory sensation (Heinrich 1979; Galen and Kevan, 1983), indirectly by an indicator of the reward for foraging such as colour (Gori, 1983; Weis, 1991), flower size (Galen and Nepport, 1987; Eckhart 1991), or the particular floral structures (Bell et al., 1984; Gonzalez et al., 1995).

Influence of the S supply on volatiles from oilseed rape flowers

Volatile released during flowering of plants facilitate flower recognition by the honeybee and thus increase their foraging efficiency. The chemical analysis of volatiles from various plant species revealed a multiplex composition of floral odors with

Table 1:
Influence of the S nutritional status on the shape of petals in field grown oilseed rape plants at main flowering (BBCH 65).

<table>
<thead>
<tr>
<th>S Status</th>
<th>(n)</th>
<th>Mean diameter (D) (mm)</th>
<th>Mean length (L) (mm)</th>
<th>Mean D:L ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme S deficiency</td>
<td>40</td>
<td>5.2</td>
<td>12.5</td>
<td>0.41</td>
</tr>
<tr>
<td>Severe S deficiency</td>
<td>80</td>
<td>6.0</td>
<td>13.5</td>
<td>0.45</td>
</tr>
<tr>
<td>Sufficient S supply</td>
<td>80</td>
<td>10.0</td>
<td>16.4</td>
<td>0.61</td>
</tr>
<tr>
<td>LSD&lt;sub&gt;5%&lt;/sub&gt;</td>
<td></td>
<td>0.29</td>
<td>0.40</td>
<td>0.015</td>
</tr>
</tbody>
</table>

Table 2:
Influence of the S nutritional status on the absorbance at 440 nm of rapeseed petals at main flowering (BBCH 65).

<table>
<thead>
<tr>
<th>S status</th>
<th>Sample (mg)</th>
<th>Absorbance at 440 nm</th>
<th>Absorbance g&lt;sup&gt;-1&lt;/sup&gt; dry matter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme S deficiency</td>
<td>21.8</td>
<td>0.654</td>
<td>30.0</td>
</tr>
<tr>
<td>Severe S deficiency</td>
<td>28.5</td>
<td>0.952</td>
<td>35.6</td>
</tr>
<tr>
<td>Sufficient S supply</td>
<td>21.2</td>
<td>1.575</td>
<td>74.3</td>
</tr>
</tbody>
</table>
more than 700 different compounds that were found in 60 families of plants (Knudsen et al. 1993). The mechanisms by which honeybees process this complex chemical information and adapt their behavior accordingly are as yet unknown (Wadhams, 1994).

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-Deleuté et al., 1993). Previous studies have shown that the S supply increases the glucosinolate in vegetative plant tissue, seeds and petals of oilseed rape (Schnug, 1988, 1993). Additionally, 2-phenylethyl isothiocyanate yielded limited conditioned responses in honey bees, but was an active component after being learned in a complex mixture of volatiles (Laloi et al., 2000). Thus a relationship between the S-containing compound, intensity of the scent and finally the attractiveness to honey bees seems possible.

Influence of the S supply on the size and shape of petals of oilseed rape

Severe S deficiency also causes deformations of leaves and petals (Schnug and Haneklaus, 1994a). If S deficiency strikes the plant early in the vegetation period, the size of the petals is reduced most severely and instead of a bright yellow color, the characteristic white flowering can be observed (see above). In comparison, if S deficiency occurs later in the vegetation period the reduction in size and changes in color are distinctly less. In cases where S deficiency sets in shortly before flowering, the petal size remains unaffected, while changes in color can still be seen.

Egg shaped petals are characteristic of extreme and severe S deficiency, which are a result of the reduction in diameter and length of the petals. The progression of deformations in relation to the S supply was assessed by establishing the relationship between the diameter of the petals and the quotient of diameter and length (Figure 1). Similar results were found by Schnug and Haneklaus (1994a). A classification of plants into three groups of S supply (extreme S deficiency, severe S deficiency and sufficient S supply) revealed that the petal diameter may be reduced by 50 % and petal length by 24 % as a result of enduring S deficiency (Table 1).

The size of flowers was an important criterion for bumble bees as with decreasing diameter, from 25 to 8 mm, the time for searching was drastically prolonged from 10.4 to 124.3 seconds (Spaethe et al., 2001).

Influence of the S supply on the petal color of oilseed rape

On S deficient sites, yellow and white petals exist side by side, thus excluding genetic influences and indicating nutritional effects. Changes in the colour of the oilseed rape petals are possibly related to increasing sugar concentrations in the plant tissue due to disorders in the protein metabolism. By pigment formation, plants prevent excessive accumulation of free sugars. One major pigment causing the yellow colour of rapeseed flowers is the flavonol quercetagetin and its isorhamnetin 3-glycoside (Harborne, 1967). Glycosylation of flavonols has a hypsochromic effect, which might lead to a shift of the absorption spectra to the UV range, which is invisible to the human eye. Another hypothesis to explain the change in color is that the synthesis of colorless anthocyanins is increased (Schnug and Haneklaus, 1995). The influence of the S nutritional status on the absorbance at 440 nm is shown in Table 2.

The differences in the absorbance were strongest between petals showing extreme S deficiency and those plants with a sufficient supply, but also verifiable for extreme and severe S deficiency (Table 2). The results are in agreement with those found by Schnug and Haneklaus (1995).

Influence of the S supply on the pollen content of oilseed rape

Oilseed rape offers ample pollen, which is of high relevance for the development of the honeybee population after winter (von der Ohe and von der
Ohe, 2002). Besides this, the pollen supply contributes to a satisfying and healthy development of the bee hive (von der Ohe and von der Ohe, 2002). Von der Ohe and von der Ohe (2002) showed that genotypical differences in the pollen content were not significant, while abiotic factors such as climatic conditions had a distinct impact. The determination of the pollen content revealed that S deficiency did not affect the supply (Table 3).

Table 3.
Influence of the S nutritional status on the pollen content of oilseed rape at main flowering (BBCH 65).

<table>
<thead>
<tr>
<th>S status</th>
<th>Pollen content (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extreme S deficiency</td>
<td>0.020</td>
</tr>
<tr>
<td>Severe S deficiency</td>
<td>0.022</td>
</tr>
<tr>
<td>Sufficient S supply</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Ongoing studies investigating the influence of the S supply on the nectar content and quality of oilseed rape in relation to the S supply under greenhouse conditions revealed that both parameters were not influenced by the treatment. Thus it may be concluded that S deficient oilseed rape offers a nutrient, which is comparable to that of a sufficiently supplied plant in both the amount and quality of pollen and nectar, respectively. Differences in the attractiveness of S deficient oilseed rape therefore must be related exclusively to scent and morphological features.

Influence of the S supply on the attractiveness of flowering oilseed rape for honey bees

For studying the attractiveness of oilseed rape for foraging honey bees in relation to the S supply under field conditions the experimental design of the field experiments was not appropriate because of the missing spatial distance of at least 200 m (von der Ohe, 2004) between S deficient and plants with a sufficient S supply. This is essential for assessing behavioral differences related to this nutritional factor. The collection of honeybees in white and yellow dishes in plots with different S application rates must therefore only be treated as strictly indicative for the behaviour under natural conditions with white and yellow flowers (Figure 2).

Hill et al. (2001) found out that the foraging behavior of honeybees was related among other things to the colour of the flowers and that a white and yellow colour, together with blue yielded discriminative behavior in relation to reward volume and quality. It is also interesting in this context that some insects such as syrphid flies preferred yellow flowering wild radish plants to white flowering cross-wild F-1 hybrids, while bumble bees showed no such preference (Lee and Snow, 1998).

The dishes were only installed for two days in order to limit the losses of honeybees. Yellow dishes are attractants for honeybees which use yellow flowering plants for foraging (Saure, 2002). The results reveal that a significantly lower number of honeybees was attracted and finally collected in the white dishes than in the yellow ones. This result was consistent on both days. During the second day a significantly lower number of bees was gathered, which suggests a rapid messaging within the bee-hive.

Figure 2:
Number of collected bees in relation to sampling date and dish colour (Y=Yellow; W=White) at main flowering (BBCH 65).

Conclusions

S deficiency results in significant morphological changes such as shape and color. Additionally, the scent might also be related to the S nutritional status of the plant. In contrast, pollen and nectar content and quantity are obviously not influenced by the S nutrition, so both factors can be excluded from being the causal reason for different attractiveness of S deficient and sufficiently supplied plants for honey bees. Bees proved to be more attracted to yellow than white dishes so that next to scent and shape this parameter seems to be relevant for foraging honeybees. Further research with free flying bees will be carried out in field experimentation in order to answer these open questions.

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