| Phyton (Austria) Special issue: "D. Grill" | **Vol. 45** | Fasc. 3 | (79)-(95) | 1.9.2005 |
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# Sulphur Deficiency Symptoms in Oilseed Rape (*Brassica napus* L.) - The Aesthetics of Starvation

By

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A scientific bunch of flowers dedicated to our friend Dieter Grill

Key words: Macroscopic symptoms, mineral nutrition, sulphur.


## Summary

SCHNUG E. & HANEKLAUS S. 2005. Sulphur deficiency symptoms in oilseed rape (*Brassica napus* L.) - the aesthetics of starvation. - Phyton (Horn, Austria) 45 (3): (79)-(95).

Deficiency symptoms are the visual response of plants to starvation from essential mineral elements. General characteristics of mineral nutrient deficiencies are the loss of chlorophyll (chlorosis), often followed by the dying off of the plant tissue (necrosis). Only a few nutrient deficiencies produce symptoms in plants, which are unique so that the visual diagnosis can be used for, instance as a tool for fertilizer management. Among all types of mineral nutrient disorders an insufficient sulphur supply of oilseed rape is presumably the only one, which produces characteristic and unmistakable deficiency symptoms at all development stages of the crop. Despite the tragic occurrence of sulphur starvation itself, the symptoms are remarkably outstanding and their detailed description is the objective of this contribution.


## Introduction

With decreasing atmospheric sulphur (S) inputs (DAEMMGEN & al. 1997) and changes in fertiliser practices towards low or no S containing products for nitrogen (N) and phosphorous (P) (CECCOTTI & al. 1997), S became a major limiting factor for plant growth in industrialised as well as remote rural areas in northern Europe. Today there are only a few locations left where the mean S input from atmospheric and fertiliser sources satisfies the demand of the crop plants. However, not on all sites with a negative S balance plants will show S deficiency symptoms, and the crop response to S fertilisation is not universal. The reason is that S is a

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geogenically abundant element (CLARK 1979) in contrast to, for instance N, which origin is predominantly anthropogenic in agro-ecosystems. Vast amounts of S are bound in minerals (e.g. gypsum and pyrite) and delivered by groundwater to the surface. For example some springs, like the Rhume spring in the German Harz mountain area in northern Germany (51.592°N; 10.304°E) discharges annually 7092 t of S bound to gypsum (HERRMANN 1969), which is twice the amount of S sold in the whole country as potassium-sulphate fertilisers and theoretically enough to fertilise 10% of all oilseed rape that is cropped in Germany with S. Plant roots may also have access to sufficient S from S being dissolved in soil water and shallow groundwater bodies on sites without access to S from minerals (ERIKSEN & al. 1997, BLOEM & al. 1997, HANEKLAUS & al. 2001, 2003). Soil water and shallow groundwater show distinctly higher sulphate concentrations than precipitation water because of natural S sources in the soil or the charging by atmospheric sources (ERIKSEN & al. 1997).

Deficiency symptoms are the visual response of plants to starvation with essential mineral elements. General characteristics of a deficiency with an essential mineral element is the loss of chlorophyll (chlorosis), often followed by the dying off of the plant tissue (necrosis). Only a restricted number of nutrient deficiencies is associated with symptoms, which are specific and unique so that they are useful for visual diagnosis in the field.

First of all, the visual diagnosis of plants identifies symptoms of severe nutrient deficiencies caused by physiological disorders and altered or damaged plant tissues. A lack of nutrients without producing deficiency symptoms ('hidden deficiency'), may be significant for the productivity of a crop, but has no pathological impact on the organism.

Literature describes symptoms of S deficiency as being less specific and more difficult to identify than other nutrient deficiency symptoms (BERGMANN 1992, 1993, CHAPMAN 1966, ROBSON & SNOWBALL 1986, SAALBACH 1970). Visual diagnosis is an expert's job and requires much more efforts for reliable interpretation than a simple comparison of pictures and descriptions given in literature with symptoms observed. In a perfect diagnostic system visual diagnosis always must come together either with soil, or plant analysis (BENNETT 1993).

S starvation in oilseed rape is most likely the only example where characteristic deficiency symptoms are to be seen at all development stages. Despite the tragic of starvation itself those symptoms are of outstanding beauty and worth a detailed description, not only from scientific point of view.

## Macroscopic symptoms of S deficiency on single plants

When grown side by side under conditions of S starvation, different crops start to develop S deficiency symptoms in the order oilseed rape, followed by potato, sugar beet, beans, peas, cereals, and finally maize. The total S concentration in plant tissue corresponding with the first appearance of deficiency symptoms is highest in oilseed rape (3.5 mg $g^{-1}$ S) and lowest in gramineous crops (1.2 mg $g^{-1}$ S). Potato and sugar beet show symptoms at higher concentrations (2.1 - 1.7 mg $g^{-1}$) than beans and peas (1 - 1.2 mg $g^{-1}$). This order reflects basically the general S requirement of different plants. The S demand of oilseed rape is elevated, because of its high content of proteins and S containing glucosinolates, and only low in plants which produce mainly carbohydrates as for instance sugar beet.

*Brassica species* such as oilseed rape develop the most distinctive expression of symptoms of any crop deficient in S. The symptoms are very specific and thus are a reliable guide towards S deficiency. There is no difference in the symptomatology of S deficiency between high and low glucosinolate containing varieties (SCHNUG 1988). In the following chapter S deficiency symptoms are described, which are characteristic of *Brassica* species. They may be taken as general for dicotyledonous plants, except when specific variations are mentioned in the text.

S deficiency symptoms of leaves

Even at very early growth stages leaves of oilseed rape start to develop symptoms of S starvation. Fig. 1 shows a crop of winter oilseed rape at rosette forming in autumn. As S is fairly immobile within the plant, symptoms always show up first in the youngest leaves. However, when the plants are still small, symptoms may cover the entire plant. Deficiency symptoms in young foliar tissue of oilseed rape begin to appear when the total S concentrations falls below 2.8 mg $g^{-1}$ and 3.5 mg $g^{-1}$ S in high glucosinolate and low glucosinolate containing cultivars, respectively (SCHNUG & HANEKLAUS 1994a).

Leaves starving from S begin to develop chlorosis (BURKE & al. 1986, DIETZ 1989a & b, ERGLE & EATON 1951, HAQ & CARLSON 1993, STUIVER & al. 1997). Fig. 2 shows that the chlorosis starts from the leaf's edge spreading over intercostate areas, whilst the zones along the veins remain always green (LOBB & REYNOLDS 1956, SCHNUG 1988). The reason for the green areas around the veins is most likely the reduced intercellular space in that part of the leaf tissue, resulting in shorter transport distances and a more effective transport of sulphate. However, high anthocyanine contents in leaves, naturally occurring in some varieties of oilseed rape (e.g. *Bronowsky*) may mask this symptom, but they are still easy to recognise in translucent light.

In comparison, S deficient potato leaves show the same characteristic colour pattern and veining as oilseed rape, but sugar beet, peas and beans simply begin to develop a chlorosis evenly spread over the leaf without any veining (HALL & SCHWARTZ 1993, ULRICH & al. 1993).

Chlorosis caused by S deficiency never turns into necrosis (SCHNUG 1988, ULRICH & al. 1993) as do for instance N and magnesium (Mg) deficiencies, which is an important criterion for differential diagnosis. The intensity of S deficiency symptoms on leaves is related to the N supply of the plants. In general, a high N supply promotes the expression of S deficiency symptoms, and vice versa (WALKER & BOOTH 1994).

However, even under extreme S deficiency with severe disorders of oilseed rape it will not wither. Fig. 3 shows an oilseed rape plant totally deformed due to S starvation, however, without any tendency towards final decay.

Fig. 1. Severe S deficiency symptoms in a *Brassica napus* crop before winter.

Fig. 2. Intercostate chlorosis causing characteristic marbling of *Brassica napus*.

Fig. 3. Extreme S deficiency of *Brassica napus* in a pot experiment.

Fig. 4. Anthocyanine enrichments in *Brassica napus*.

Fig. 6. Intercostate chlorosis, spoonlike leaf deformations and anthocyanine enrichments in *Brassica napus* leaves.

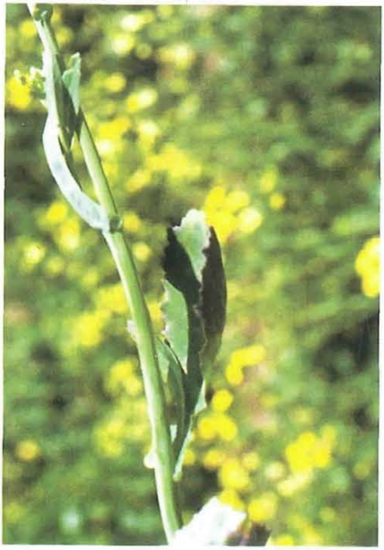

Fig. 5. Spoonlike leaf deformations and anthocyanine enrichments in *Brassica napus* leaves.

With the duration of severe S deficiency the chlorotic parts of *Brassica* leaves gain a reddish purple colour due to the enrichment of anthocyanines (Fig. 4). Under field conditions, the formation of anthocyanines starts 4 - 7 days after chlorosis. A high photosynthetic activity promotes this process. The phenomenon is initialised by the enrichment of carbohydrates in the cells following the inhibition of the protein metabolism. To avoid physiological disorders caused by too high carbohydrate concentrations, plants detoxify these in form of anthocyanates derived from the reaction with cell-borne flavonols (DELOCH & BUSSLER 1964, EATON 1935, 1941, 1951, HARBORNE 1967, 1968, NIGHTINGALE & al. 1932). Many other nutrient deficiencies are also accompanied by the formation of anthocyanins, which on its own therefore is a less specific indicator for S deficiency.

In particular, not fully expanded leaves produce spoonlike deformations when struck by S deficiency (Fig. 5). The reason is a reduced cell growth rate in the chlorotic areas along the edges of the leaves, while normal cell growth continues in the green areas along the veins. The grade of the deformation is stronger the less expanded the leaf is when S deficiency starts. Marbling, deformation and anthocyanine accumulation can be detected up to the most recently developed small leaves inserted in the forks of branches (Fig. 6).

Leaves of S deficient plants appear to have a higher succulence (BERGMANN 1993, BUGAKOVA & al. 1969). DELOCH & BUSSLER 1964 suspected an increase in the chloride (Cl) uptake caused by a lack of sulphate as the physiological background for this phenomenon. But with an increase of Cl concentrations by 0.4 mg $g^{-1}$ on the account of a decrease of S concentrations by 1 mg $g^{-1}$ in leaves, this effect seems to be too small to justify this hypothesis (SCHNUG 1988). It is more likely that the appearance of increased succulence is caused by the mechanical effects of distortion (see above) together with a cell wall thickening from the accumulation of starch and hemicellulose (SINCLAIR 1993).

S deficiency symptoms of flowers

S deficiency causes one of the most impressive symptoms of nutrient deficiency in plants: the 'white blooming' of oilseed rape flowers. Since the beginning of the 1990s this symptom has become so widespread in northern Europe that it has been accidentally picked up by artists or photographers, who were attracted by the beauty of an oilseed rape field in full bloom (LINCOLN 1995).

Fig. 7 shows an oilseed rape field in Germany painted by an artist in the early 1980s when the S supply in many places was still high and severe S deficiency with symptoms was still rare. The painting in Fig. 8 was completed 10 years later in England when atmospheric S depositions were drastically reduced and oilseed rape with S deficiency symptoms could be observed regularly. On an extremely S deficient site an entire oilseed rape crop may show white flowers, which becomes a very prominent landmark in the landscape (Fig. 9). But more often a mixture of white and yellow flowering plants in an oilseed rape crop can be found which remains often hidden, because it takes the human eye at least 10 - 15 minutes to identify white objects in front of a yellow background (Fig. 10).

The white colour develops from an overload of petal cells with carbohydrates caused by disorders in the protein metabolism, which finally ends up in the formation of leuco-anthocyanines (SCHNUG & HANEKLAUS 1995). As with anthocyanines in leaves, the symptom develops strongest during periods of high photosynthetic activity. Additionally, Figs. 11 and 12 show that colour, size and shape of oilseed rape petals are modified by S deficiency, too (SCHNUG & HANEKLAUS 1994a, HANEKLAUS & al. 2005).

The trigger for the change in colour is most likely the increasing sugar concentration in the tissue due to disorders in the protein metabolism. By pigment formation, plants prevent an excessive accumulation of free sugars. Thus, white flowering in S deficient crops will most likely occur during periods of high photosynthetic activity. Two mechanisms could be involved in this phenomenon: one major pigment causing the yellow colour of rapeseed flowers is the flavonol quercetagetin, and its isorhamnetin 3-glycoside (BROUILLARD 1988, HARBORNE 1967). The glycosylation of flavonols, however, has a hypsochromic effect, which might shift the absorption spectra to the UV range, which is invisible for human eyes. The second hypothesis is that the increasing sugar concentrations promote the formation of anthocyanines, which would occur in the form of colourless leuco-anthocyanines. Like any other S deficient tissue of oilseed rape, white petals show lower cysteine, γ-glutamyl cysteine, glutathione and ascorbate concentrations, but an increased peroxidase activity (SCHNUG & al. 1995).

In relation to the duration of S deficiency, not only the colour, but also the size and shape of oilseed rape flowers are affected. Breakdowns of the S supply for a short time are the reason for white, but normally shaped petals. This phenomenon is characteristic on sites where S deficiency due to decreasing environmental S inputs is just beginning to develop. In regions with an established low S input, such as in all northern European growing areas, S deficient white rapeseed flowers are significantly smaller with more oval shaped petals (SCHNUG & HANEKLAUS 1994a, b). 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 & al. 2001).

Although oilseed rape is self-pollinating (SAURE 2002), the cross-pollination rate, predominately by honeybees, was estimated to be about 20% (DOWNEY & 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 depend on pollination by vectors (STEFFAN-DEWENTER 2003). First observations in field-grown composite hybrids show increased problems with pollination of hybrids in low sulphur environments. This problem can be attributed to the processes discussed next. Oilseed rape provides an important source of nectar and pollen for honeybees, which are attracted by the bright yellow colour of the crop in bloom (PIERRE & al. 1999). Oilseed rape is one of the most important European melliferous crops for beekeepers as it is an important foraging plant in early summer. The

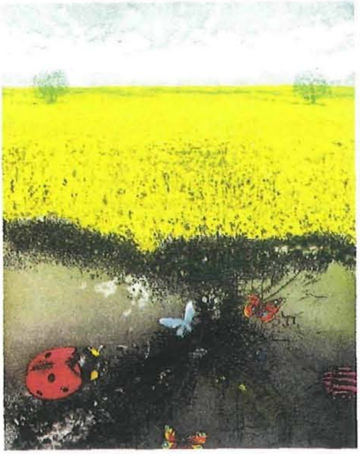

Fig. 7. Oilseed rape by SCHIEL 1987.

Fig. 8. Oilseed rape by LINCOLN 1995.

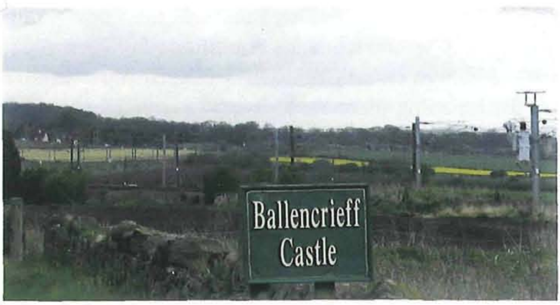


Fig. 9. Blooming, neighbouring fields of *Brassica napus* with severe S deficiency symptoms ('white flowering') and a sufficiently supplied crop.

Fig. 10. White flowering *Brassica napus* crop among yellow flowering plants.

Fig. 11. Yellow petals of a *Brassica napus* plant sufficiently supplied with S.

Fig. 12. White petals of an S deficient *Brassica napus* plant.

Fig. 13. Reduced number of pods and anthocyanine enrichments in a *Brassica napus* crop.

main pollinators in oilseed rape are insects of the family Apidea (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).

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 & 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 & al. 1975, WILLMER & al. 1994), or by olfactory sensation (HEINRICH 1979, GALEN & KEVAN 1983); indirectly by an indicator of the reward for foraging such as colour (GORI 1983, WEIS 1991), flower size (GALEN & NEWPORT 1987, ECKHART 1991), or the particular floral structures (BELL & al. 1984, GONZALEZ & al. 1995).

Volatiles released during flowering of plants facilitate flower recognition by the honey bee and thus increase their foraging efficiency. The chemical analysis of volatiles from various plant species revealed a multiplex composition of floral odors with more than 700 different compounds that were found in 60 families of plants (KNUDSEN & al. 1993). The mechanisms by which honeybees process this complex chemical information and adapt their behavior accordingly are as yet unknown (WADHAMS & al. 1994). A total of 34 different compounds were found in volatiles of oilseed rape (TOLLSTEN & BERGSTRÖM 1988, ROBERTSON & al. 1993, MCEWAN & 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 & al. 1993). OMURA & 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 & 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-phenyl-ethyl isothiocyanate yielded limited conditioned responses in honey bees, but was an active component after being learned in a complex mixture of volatiles (LALOI & al. 2000). Thus a relationship between the S-containing compound, intensity of the scent and finally the attractiveness to honey bees seems possible.

Crops visited by bees show earlier petal fall, probably because they set flowers earlier, resulting in a more uniform pod ripening and ease of harvest. This may, therefore, result in higher yields (WILLIAMS & COOK 1982). Nectar, however, is the bee's source of carbohydrate and their hovering is the one of the most energy expensive forms of flight. The reflective pattern of flowers provides visitors with clues as to the age of the flowers and presence of food rewards (KEVAN & BAKER 1983). During senescence of rapeseed flowers, which begins immediately after pollination, the yellow petal colour vanishes and the petals shrink quickly before falling to the ground. A pollinated and fading rapeseed flower is therefore similar to an unpollinated S deficient one and thus less attractive to honey bees. BARTH 1982 reported that bees prefer yellow flowers to white ones and consequently in S deficient fields, much lower bee activity has been observed than in S sufficient crops, which are bright yellow.

Smaller, whiter flowers may be less attractive to bees only after previous experience and not because of a specific signalling. Even if sufficiently with S supplied rapeseed flowers would be 'instinctively' more attractive to honey bees, the animals are known to adapt their behaviour rapidly, in this case in favour of white(r) and smaller flowers if the reward will be satisfying. DE JONG 1998 (personal communication) emphasised that bees are extremely fast in associating relevant cues with a reward. S-deficiency in rapeseed, therefore, will probably only have the negative bee-related effects when the bees can not distinguish pollinated from non-pollinated flowers as reliable as they can in rapeseed that is sufficiently supplied with S.

Who could have imagined at the beginning of the 1980s that the reduction of $SO_2$ emissions from burning fossil fuels (SENDNER 1985) would have an impact on honey production twenty years later?

Flowers of severely S deficient oilseed rape develop no pods or pods with significantly lower numbers of seeds than normal plants (Fig. 13). As before, the leaves as well as the branches and pods of S deficient plants are often red or purple coloured caused by the accumulation of anthocyanins.

Though not many investigations report on the influence of the S supply on root development, there is sufficient evidence that S deficiency reduces root mass and increases the number of secondary roots (HOLOBRADA 1969, RIVERO 1996 (personal communication), SMITH & al. 1993).

S deficiency and structure

Visual diagnosis of plants concentrates usually on changes in the appearance of individual plant organs and as mentioned before only is applicable to identify a severe nutrient deficiency. Severe S deficiency is characterised by blocking or breakdown of certain metabolic pathways, which finds its final expression in physiological disorders and visible symptoms. But long before true metabolic calamities occur, a shortage of S supply will limit the plants ability to fully exploit yield.

Yield, however, is a combination of individual parameters (GEISLER 1983) and abnormal changes in the yield structure of a crop may indicate any shortage of growth factors earlier than first visible deficiency symptoms take place. Changes in yield structure can be identified by visual assessment, too. Although changes in yield structure might not be specific at all, they provide a valuable piece of information for differential diagnosis of S deficiency, particularly when symptomatic interferences with pests and diseases may occur.

The strongest yield component affected by S deficiency in oilseed rape is the number of seeds per pod, which decreases highly significantly with S deficiency (SCHNUG 1988). The number of branches per plant, flower insertion, fertility and seed weight remain nearly unaffected, even under conditions of severe S deficiency (SCHNUG 1988, SCHNUG & HANEKLAUS 1994a). Abnormally low numbers of seeds per pod, down to seedless 'rubber pods' are a characteristic hint towards S deficiency, particularly in late growth stages, when no photosynthetically active leaves for visual assessment or chemical analysis are available. Under severe S starvation plants may also fail to commence pod growth after fertilisation. The little remains of the fertilised stigma fall off after some time faking an image of sterility of flowers or a lowered degree of fertilisation.

The final number of seeds per pod is determined immediately after fertilisation and maintained irrespectively of the nutritional status of the plant. Even if sufficient S would be available for the plant after pollination, the pods would not be able to benefit from it. Thus the distribution of failed pod insertion and pods with abnormally low seed numbers along the branch are also a record of the time when S starvation hit the plant in the past.

Up to approximately one week after blooming, even oilseed rape plants with severe S starving are able to restart and continue flowering, pod insertion and seed filling and may regain full yield if sufficient S is supplied and taken up by the plant (SCHNUG 1988). The ability of oilseed rape to compensate fully for earlier S deficiency makes correct visual diagnosis in that crop vital as it allows in principle the correction of S deficiency at any time during the main vegetative period. Practical limits under field conditions, however, are technical constraints for fertiliser application in larger crops and the problem that there might be not enough vegetative time left to complete the delayed vegetation cycle of the plant and allow seeds to mature.

Field-scale S deficiency symptoms

Symptoms of nutrient deficiency are usually described for individual plants or plant parts. However, there are some characteristic features in the appearance of fields, which can provide an early evidence of S deficiency.

S deficiency develops first on the light textured sections of a field. From a bird's eye view these areas appear in an early oilseed rape crop as irregularly shaped plots with lighter green colour ('wash outs'). The irregular shape distinguishes the phenomenon from the regular shape of areas caused by N deficiency, which is usually caused by inaccurate fertiliser applications. Due to frequent soil compaction and limited root growth, S deficiency develops first along the headlands and tramlines or otherwise compacted areas of a field. In agricultural systems S uptake and S off-take are significantly higher than for instance in forest ecosystems and the significance of their regional expansion for the S balance of landscapes is discussed by HANEKLAUS & al. 2003.

The appearance of S deficient oilseed rape fields is more obvious at the beginning of blooming: white flowers of oilseed rape are often distinctively smaller and therefore much more of the green undercover of the crop is shining through the canopy of the crop. Characteristic in blooming oilseed rape with severe S deficiency is also a reduced activity of honey bees (see above). Another characteristic indicator for an S deficient site is the so-called 'second flowering' of the oilseed rape crop. Even if an S deficient crop has finished flowering it may come back to full bloom if sufficient S is supplied. Then the typical climatic situation is a wet and rainy spring season until the end of blooming, followed suddenly by warm and dry weather. During the wet period precipitation water, which has 10 to 100 times lower S concentrations than the entire soil solution, dilutes or leaches the sulphate in the rooting area of the plants finally causing S starvation. With the warmer weather, evaporation increases and S rich subsoil water becomes plant available, thus causing the second flowering of the crop. During maturity S deficiency in oilseed rape crops is revealed by a sparse, upright standing crop.

Microscopic S deficiency symptoms

Some authors suggested that long before being visible to the human eye, deficiency symptoms might be detectable at a microscopic level (BUSSLER 1978, FINCK 1979). In extensive studies PISSAREK 1977, however, could find no early changes in the cell structure of S deficient plants other than a reduction of the chlorophyll content in the chloroplasts, particularly in the chloroplasts of cells from young leaves (BURKE & al. 1986, HAQ & CARLSON 1993).

## Conclusions

Deficiency symptoms are the visual response of plants to the level of mineral nutrition. General characteristics are the loss of chlorophyll (chlorosis), followed by a dying off of the plant tissue (necrosis). Only a few nutrient deficiencies come along with symptoms, which are characteristic in a way that they are useful for visual diagnosis. Visual diagnosis on single plants requires specific deficiency symptoms, which in case of S can be easily identified in dicotyledoneous, but not in gramineous plants. In cereal crops an irregular appearance of the whole field indicates S deficiency. Due to unique symptoms on leaves, flowers and yield structure, the visual identification of severe S deficiency in *Brassica* species is particularly easy and unmistaken. The application of microscopy for earlier identification of S deficiency symptoms provides no significant advantage.

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