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Secondary metabolites of the leaf surface affected by sulphur fertilisation and perceived by the diamondback moth

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Summary. Oilseed rape, *Brassica napus* L. (cv Express), plants were grown under three different sulphur regimes: sulphur-free (S_0), normal sulphur (S_n, normal field concentration) and a sulphur-rich (S_+, 2 × concentration of S_n). We performed dual choice oviposition assays with the diamondback moth, *Plutella xylostella*, using real plants and, for the first time with this insect, artificial leaves sprayed with methanolic leaf-surface extracts. The results mirrored those of a separate study of preferences for whole plants. Females laid more eggs on surrogate leaves that were treated with S_n extracts than on S_0 plants, while only a slight, not significant, difference was observed between extracts of normal and sulphur-rich plants. This shows that chemical compounds on the leaf surface mediate the oviposition preference and that the female insect can perceive the quality of the host-plants in terms of their fertilisation status.

Since leaf volatiles are known to be oviposition stimulants, we investigated the effects of leaf-surface extracts on insect olfactory responses using electroantennograms (EAGs). In agreement with the behavioural data, we found that extracts of sulphur-treated plants yielded higher EAG amplitudes than the S_0 extracts. Since the leaf content of the volatiles isothiocyanates is influenced by sulphur nutrition, we analysed the extracts for these compounds. Above the detection threshold of our GC-MS system, no isothiocyanates were found. Thus, other compounds present in the surface extracts must be perceived by the antenna.

However, the HPLC analysis revealed 11 different glucosinolates. Progoitrin (2-Hydroxy-3-butenyl) and gluconapoleiferin (2-Hydroxy-4-pentenyl), which belong to the hydroxy-alkene class of glucosinolates, were the most abundant compounds. The total glucosinolate content sharply increased from S_0 to S_n plants, whereas it was slightly lower in S_n versus S_+ plants. Since it is known that glucosinolates can stimulate oviposition, it seems likely that the increased content we observed was influencing the insect preference in this study too.

Key words. Oilseed rape – *Brassica napus* – glucosinolates – isothiocyanates – *Plutella xylostella* – oviposition choice – EAG, HPLC, GC-MS

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Introduction

The diamondback moth, *Plutella xylostella* (L.), is a harmful pest of cruciferous crops throughout the world (Harcourt 1957). In our previous investigation of the effects of sulphur (S) plant nutrition on oviposition of the diamondback moth (Marazzi & Städler, in preparation) we found that moths clearly discriminate between S fertilised and non-fertilised *Brassica napus* oilseed plants. The question arising was which plant characteristics the moth females perceive.

In Brassicaceous plants, S is used in the synthesis of secondary metabolites like glucosinolates (GSLs) (Chew 1988), phytoalexins (Pedras *et al.* 2000) and CIF ("cabbage identification factor"; 1,2-dehydro-3-thia-4,10,10b-triazacyclopenta[.a.]fluorine-1-carboxylic acid) (Hurter *et al.* 1999). Volatile chemicals mainly produced by hydrolysis of non-volatile GSLs (Pivnick *et al.* 1994) are also known to be involved in host-plant location by different insects attacking this plant family (Blight *et al.* 1995). Gupta and Thorsteinson (1960a, b) have demonstrated that constituents of host-plants affected larval feeding and oviposition of *P. xylostella*. Later studies confirmed that host recognition and oviposition by this insect is dependent on GSLs. Reed *et al.* (1989) used plant extraction followed by myrosinase treatment to show that the stimulant activity was greatly reduced after hydrolysis of the GSLs. Individual GSLs were also active but not to the same extent as homogenised plant tissue.

The presence of GSLs in crucifers is strictly dependent on the medium in which the plant is grown (Schnug 1997). In this context, Meyer (2000) showed that soil fertility affects both the degree of defoliation and compensation for herbivory in *Brassica nigra* plants damaged by *Pieris rapae* caterpillars. Oligophagous insects such as diamondback moth larvae accept as food the leaves of only a limited number of plant species. The actual selection of the host-plant is performed by the ovipositing female (reviewed in Marazzi & Städler, in preparation), as the mobility and energy reserves of the first instars are limited and the opportunities to find a suitable host on their own are minimal (Justus & Mitchell 1996).

In the present work, S was used as a variable environmental factor, since this mineral is clearly involved in the production of secondary plant metabolites that have key roles as attractants, feeding (Renwick 2002) and oviposition stimulants (Justus & Mitchell 1996; Hughes *et al.* 1997; Spencer *et al.* 1999) for *Plutella xylostella*. Gupta and

Thorsteinson (1960a) first studied the effect of S mineral nutrition in two *Brassica* species (*B. alba* and *B. nigra*). They found that *P. xylostella* laid more eggs on the S fertilised plants than the S deficient controls. Although these differences were not significant, they seemed to be suggestive. This prompted us to study the indirect influence of S fertilisation on the behavioural and physiological responses of female diamondback moths to *Brassica napus* (L.) plant extracts hoping to find the plant characteristics related to the observed preference.

Materials and methods

Insects

All the *Plutella xylostella* (L) (Lepidoptera: Plutellidae) required for the bioassays came from our laboratory culture established with approximately 200 individuals during spring 2000 and reared as described by Marazzi & Städler (in preparation). Briefly, an equal number of male and female mature moths (approximately 100 per cage) were held in mesh cages (50 × 45 × 45 cm) in a climate-controlled room (21 ± 1°C, 70 % RH and 16 h photophase), where they were allowed to mate and oviposite only on the leaves of potted (∅ 13 cm) *B. napus* cv CC-Cross F1 at the pre-bolt stage. The moths had access to a source of water and to 10 % sugar-water.

Plants

The oilseed rape (*B. napus* cv. Express) was grown as described by Marazzi & Städler (in preparation). Briefly, a modified "Hoagland" nutrient solution was used to provide the three different S levels of fertilisation: S_n = 1 mM of $MgSO_4$ (normal S concentration in a Swiss field); S_+ = 2 mM of $MgSO_4$ (high sulphur level) and S_0 (S-free level), which was obtained by replacing $MgSO_4$ with $MgCl_2$ (1 mM). All the experiments were conducted using plants with 3–4 true leaves (30–35 days after planting), comparable in size.

Oviposition choice assay

Bioassay with real plants: We attempted to relate our oviposition data on real plants with the glucosinolate content of the three samples of *B. napus* plants (S_0, S_n and S_+). For this purpose we used the data concerning the oviposition choice assay with real plants from Marazzi and Städler (in preparation), where we tested 4 *B. napus* plants at a time (2 plants per cage for each treatment, i. e. S_n vs S_0 or S_n vs S_+) for a period of 24 hours, during which about one hundred 1–2 day-old diamondback moths were released into each cage and were allowed to mate and oviposit on plants (n = 20).

Bioassay with artificial leaves: We used the same extraction procedure as described by Baur *et al.* (1996) to obtain wax-free methanolic leaf-surface extracts of S_0, S_n and S_+ plants at the 3–4 true leaf stage. Amounts and concentrations of samples were expressed in gle (gram leaf equivalent) or gle/ml, respectively. One gle represents the amount of leaf surface extract obtained by dipping 1 g of fresh leaf material.

The oviposition substrates used in the experiments consisted of paraffin-coated green paper model leaves developed originally for the cabbage root fly by (Roessingh & Städler 1990) with a surface projection of 7 × 7 cm, 1.5 cm wide vertical folds and a flat stem of 5 × 1.5 cm. Leaves were individually inserted into hydroscopic foam (Smithers-Oasis, Germany D-67269) cylinders (8 cm in diameter, 5 cm high) and placed in plastic containers (9 cm in diameter, 5 cm high). The top of each container was covered with a black plastic layer (9 cm in diameter). Surrogate leaves were sprayed using an airbrush (Aerograph Sprite, Devilbiss) in a fume hood with 1 gle of the extract to test.

All the oviposition bioassays were conducted in the same cages as those described for the rearing (about 100 adult moths per cage). Four plants (or four surrogate leaves) were arranged in a circle on the floor of the cage (2 for each treatment: S_n (control) vs S_0 or S_n (control) vs S_+). After an oviposition period of 24 hours, the eggs laid on each plant or artificial leaf were counted and expressed as a percentage of the total number of eggs laid on all plants within one replicate. Thus, the resulting preference values for the treatments compared (S_n vs S_0 and S_n vs S_+ respectively) totalled 100 %. A Mann-Whitney U-test was performed to determine the significant differences in preference between treatments. For each pair-wise assay, 9 (S_0 vs S_n) and 13 (S_n vs S_+) repetitions were performed and after each replicate, the plant or artificial leaf positions were changed clockwise.

Chemical analysis of glucosinolates

The surface extracts of *B. napus* grown under the S_0, S_n or S_+ regimes and a total leaf extract (homogenate) of each plant group were assayed for their GSL content. For each treatment, 15 plants were removed from the pots and the sand was gently washed from the roots using the same nutrient solution to prevent contamination. Plants were then separated into roots and aerial parts, frozen in liquid nitrogen, crushed into small pieces and stored at –20°C until needed. Prior to analysis, samples of both plant parts were ground to a fine powder at –18°C with a coffee mill, 10 g weighed out, placed in 50 ml glass flasks and stored at –20°C until needed.

We used the same extraction procedure described by Griffiths *et al.* (2001) to obtain desulfoglucosinolates. The glucosinolates, twenty µl aliquots (representing approximately 2 leaves), were analysed by HPLC. The analytical column used was equipped with a Lichrospher (100 RP 18, 5 µm, 4 × 250 mm). The binary mobile phase system was composed of distilled water (A) and water : acetonitrile, 80 : 20 (B). The analysis was run with the following gradient program: 0 to 45 min linear gradient 0 to 100 % B and then held for 5 min on 100 % B. The flow rate was 1 ml/min and the detection of desulfoglucosinolates was monitored with an UV/VIS detector at 230 nm.

Quantifications were based on 2 GSL standard solutions (Doon Major and Dwarf), prepared and quantified at the SCRI in Dundee, Scotland. The Jasco HPLC system was equipped with Chromeleon software, which was used for data acquisition and analyses. Reported quantifications are the means of five separate extractions and analyses. The amounts of GSLs from individual peaks were summed and these values were analysed by ANOVA to determine differences between treatments. The GSLs nomenclature is based on the identification list of Griffiths *and al.* (2001).

Chemical analysis of isothiocyanates

Isothiocyanates in the plant extracts were analysed by combined gas chromatography and mass spectrometry using a Finnigan Voyager GC-MS System. The GC was equipped with a fused silica capillary column: DB-5 (30 m, 0.25 mm, 0.25 µm coating). Each plant eluates were prepared by shaking 2 ml crude extract with 2 ml hexane. Two µl of the hexane fraction of each sample were injected splitless using an auto sampler (PAL, CTC-Analytics). Identifications and quantifications were made by external calibration, comparing obtained mass spectra and retention times with those of 4 pure isothiocyanatae (phenyl-, butenyl-, benzyl- and allyl-isothiocyanates). The detection threshold was 1.5 µg/ml crude extract.

Electrophysiology

Female diamondback moths, 1–2 days old, were cooled in a refrigerator (5.5°C) for 1–4 hours to reduce their activity. The wings and the legs of the cooled insects were amputated. The body was mounted ventral side up in the groove of a Plexiglas® holder and positioned so that the antennae were attached to a sticky wax layer, using strips of transparent tape (Scotch® 3M 810, 19 mm × 33 m). The head and scape of the antenna were fixed to the support with histology paraffin (mp 45°C) melted locally with a temperature controlled soldering iron (50°C). The recordings preparation was

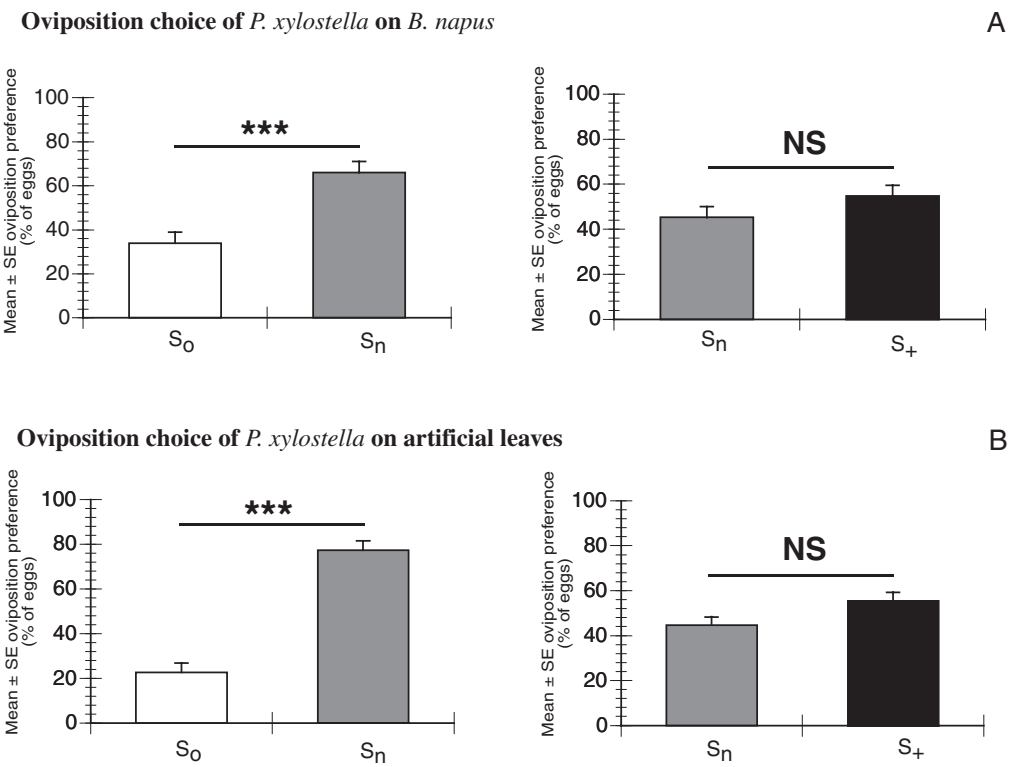

Fig. 1 Effect of sulphur fertilisation on *P. xylostella* oviposition choice. (**A**) Oviposition choice assay with *B. napus* plants. Number of replicates: n = 9 for S_0/S_n and n = 13 for S_n/S_+. These data are the same as in Marazzi & Städler (2003) and shown here for comparison. (**B**) Oviposition choice assay with leaves treated with *B. napus* plant surface extracts (1 gle). Number of replicates: n = 9 for S_0/S_n and n = 13 for S_n/S_+. S_0 = sulphur-free; S_n = normal field-concentration of sulphur; S_+ = sulphur-rich

mounted under a stereomicroscope and continuously humidified with a water-saturated air stream (1 m/s, 22 ± 3°C). We used basically the same method as described by Guerin and Visser (1980). Briefly, the airflow was split into continuous and stimulatory airstreams at a 9 : 1 ratio, which converged prior to the electrophysiological preparation. The stimulatory air stream passed through a Pasteur pipette (20 µm Ø) containing the test compound (100 µl) spread on a folded filter paper (1.6 × 5 cm, paper from Schleicher & Schuell) and upon activation of a valve, was injected into the continuous air stream. The indifferent electrode, filled with a saline solution (Kaissling 1995), was inserted in the base of the antenna and the recording electrode, containing saline plus 0.01 % (v/v) of polyvinylpyrolidone solution, was brought in contact with the antennal tip. The EAG signal was recorded using a lab-built amplifier with high input impedance (10^{13} Ω) and low bias current (< 10 pA). The signals were filtered (electronic high-pass with cornering frequency of 0.001 Hz), amplified (100 ×) and digitised using SuperScope II 3.0 software (GW Instruments, Somerville, Massachusetts) on a Macintosh computer. The EAG amplitudes were determined using PowerChrom v2.2.4 (AD Instruments, Springs, Colorado) software. The S_0, S_n and S_+ plant extracts were tested at a concentration of 0.04, 0.1 and 0.4 gle. Trans-2 hexanal (1 and 10 µg) was used as control for antennal activity at the beginning and end of a test sequence. The set of stimuli was exposed sequentially to the insect in the following order: from S_0, S_n to S_+, always starting with the lowest plant extract concentration. Responses to extracts were expressed as the mean of 5 pulses of 0.5 s with an interval of 5 s. EAG measurements were replicated on 5 female antennae.

The EAG responses to plant extracts between the 3 groups of differently fertilised plants were analysed by ANOVA with the amplitude (mV) of the EAGs as the dependent factor, type of treatment (S_0, S_n and S_+) and concentration (0.04, 0.1 and 0.4 gle) as independent factors (n = 5). We performed multiple comparisons using a Bonferroni post-hoc test within each group of plant extracts to detect differences between concentrations.

Results

Oviposition choice on artificial leaves treated with surface extracts

The *B. napus* plants differing in S fertilisation tested by Marazzi and Städler (in preparation) (Fig. 1A) were extracted and yielded the material for these experiments. As shown in Fig. 1A, the oviposition choice of *P. xylostella* on real plants was affected by S concentration (Mann-Whitney U-test, S_0 vs S_n: n = 9, p = 0.0013 and S_n vs S_+: n = 13, p = 0.0956). The wax-free methanolic leaf-surface extracts of plants fertilised with S proved to be active in the oviposition bioassays of *P. xylostella* (Fig. 1B). The number of eggs laid was significantly higher (Mann-Whitney U-test, n = 9, p = 0.0003) on artificial leaves sprayed with S_n plant extracts than on artificial leaves sprayed with S_0 plant extract, representing an increase of nearly 250 % on S_n plant extracts. Similarly, a slight but not significant difference was found between the number of eggs laid on artificial leaves sprayed with S_n and S_+ plant extracts (Mann-Whitney U-test, n = 13, p = 0.0833). Compared to S_n, S_+-sprayed artificial leaves showed a 24 % increase in oviposition.

Chemical analysis of B. napus plant extracts

Eight GSLs were consistently detected in all tissue from the three populations of *B. napus* tested (Fig. 2A). These included the alkene GSL, 3-butenyl (gluconapin), two hydroxy-alkenes GSLs, 2-hydroxy-3-butenyl (progoitrin)

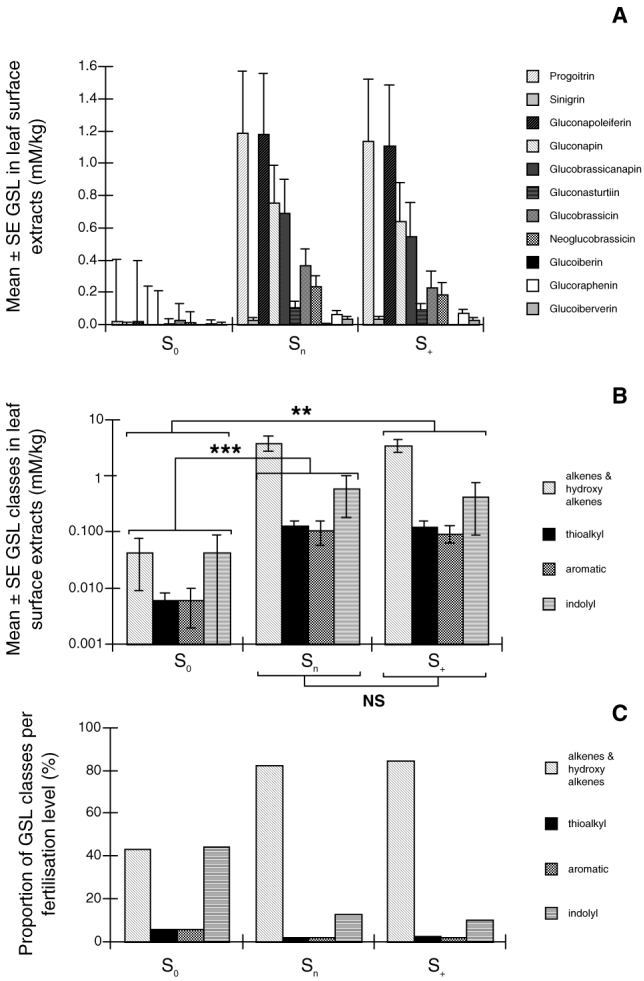

Fig. 2 Analytical data of the glucosinolates content of *B. napus* plants. (**A**) Profiles of individual glucosinolates for S_0, S_n and S_+ *B. napus* plants. Number of replicates: 5. Abbreviations: as in Fig. 1. (**B**) Profiles of glucosinolate in a logarithmic scale. Number of replicates: 5. (**C**) Proportion of the glucosinolate classes. Interaction (ANOVA) between sulphur fertilisation vs group of GSL: $p < 0.001$

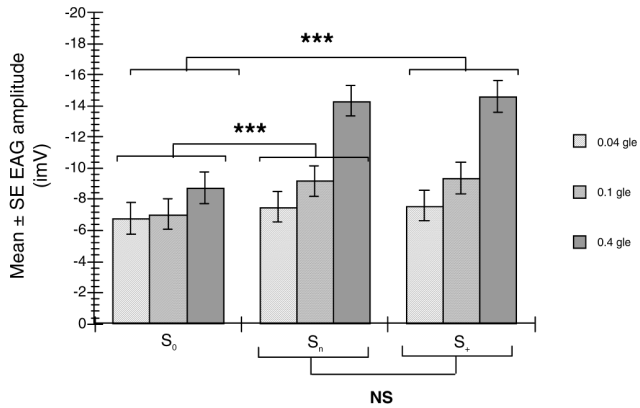

Fig. 3 Electroantennogram responses to *B. napus* surface extracts of plants differing in S nutrition. The plant extracts were tested in three different concentrations: 0.04, 0.1 and 0.4 gle (gram leaf equivalent). Values are given as means ± of 5 stimulations per antenna (insect) and per concentration. Number of replicates: 5 insects. The dose-dependent relationship between concentration and female antennal response was tested by a Bonferroni-test, n = 5, 0.04–0.1 gle: $p = 0.0029$, 0.04–0.4 gle, $p < 0.0001$, 0.1–0.4 gle: $p < 0.0001$. Abbreviations: as in Fig. 1

and 2-hydroxy-4-pentenyl (gluconapoleiferin), two thioalkyl GSLs, 4-methylsulphinyl-3-butenyl (glucoraphenin) and 3-methylthiopropyl (glucoiberverin); two indolyl GSLs, 3-indolylmethyl (glucobrassicin) and 1-methoxy- 3-indolylmethyl (neoglucobrassicin) and the aromatic GSL 2 phenylethyl (gluconasturtiin). Moreover, we found three additional GSLs in S_n and S_+ plant extracts: two alkene GSLs, 2-propenyl (sinigrin) and 4-pentenyl (glucobrassicanapin), and a thioalkyl GLS, 3-methylsulphinylpropyl (glucoiberin) (Fig. 2A). The concentrations given in mM/kg of leaf extract of the individual GSLs detected in S_n and S_+ plant extracts were overall higher than those found in S_0 plant extracts (Fig. 2B). The proportions of individual GSLs classes varied considerably also between the plant extracts (Fig. 2C). The proportion of progoitrin observed in S_n and S_+ plant extracts ($S_n = 1.188$, $S_+ = 1.141$ mM/Kg leaf surface) was around 60 times larger than that found in S_0 plant extracts (0.019 mM/Kg leaf surface). Similarly, the proportion of gluconapoleiferin was about 50 times larger in S_n and S_+ than in S_0 plant extracts. The greatest proportional difference was found with gluconapin, whose concentration was between 650 and 750 times higher in S_n and S_+ versus S_0 plant extracts. Quantitatively, the total GSLs detected showed differences that were between 10 and 30 times higher in S_n and S_+ versus S_0 plant extracts (Fig. 2A). The potential presence of volatile breakdown products of GSLs, the isothiocyanates, was assed by GC-MS analysis. Isothiocyanates were not present in any of the tested plant extracts (detection threshold: 1.5 µg/ml crude extract).

Electroantennogram response to B. napus plant extracts

The EAG responses of female moths of *P. xylostella* to leaf surface extracts of S_0, S_n and S_+ tested at three increasing concentrations are shown in Fig. 3. Mean EAG responses elicited by the test compounds ranged from about 0.1 (at 0.04 gle, mainly S_0 plant extracts) to 3 mV (at 0.4 gle, mainly S_n and S_+ plant extracts). All concentrations of S_0 extracts tested induced significantly lower responses than those of S_n or S_+ extracts (Bonferroni, n = 5, $p < 0.001$,). Conversely, no significant difference was found between the EAG recordings for S_n- and S_+-plant extracts (Bonferroni, n = 5, $p = 0.5490$, Fig. 3A). EAG amplitudes were clearly concentration dependent and peaked at the highest dose tested (0.4 gle) for all three plant extracts tested.

Correlations between behaviour and chemical analysis

The lack of S nutrition dramatically affected the GSL content of the plants, corresponding well to the results of the behavioural assay described in Fig. 1. The significant differences in the female antennal perceptions revealed by EAG recordings were exactly mirrored by the GSL content of plant extracts.

Discussion

Oviposition preference influenced by leaf surface extracts

The acceptability of *B. napus* for *P. xylostella* was markedly affected by the S nutrient levels of the plant. The lack of S in the plant nutrient solution reduced oviposition on both real plants and artificial leaves. Surprisingly, the differences detected with sprayed surrogate leaves were more pronounced. Our study seems to be the first showing the direct role of fertilisation on plant secondary metabolites in influencing oviposition behaviour. Our results are in contrast with previous publications comparing *Brassica* species differing in GSL levels (Pivnick *et al.* 1994; Bodnaryk 1997). These authors concluded that species like *P. xylostella* are insensitive to sinigrin and suggested that its pest status on low-GSL lines would be likely to remain unchanged. Possible reasons for this disparity might be the followings: 1) "low GSL varieties" are not comparable to the effect of plant S-deficiency, 2) these plants are known to have a lower content of GSLs only in the seeds, but not necessarily in other plant parts (Fahey *et al.* 2001) and 3) in our experiments, extreme variations in the levels of S-supply were used, making the differences between the three entries more evident. It is therefore conceivable that in the behavioural choices made by the insect, only the threshold between undetectable/detectable presence of GSLs was qualitatively important and not the quantitative difference. However, other plant metabolites are certainly also influenced by the S shortage and may therefore have affected the oviposition behaviour positively or negatively. Further, some of these metabolites are not volatile and thus cannot be detected by the olfactory sensilla. In addition, the final response of an insect in accepting or rejecting a particular plant is mediated by a balance of positively and negatively interpreted sensory signals evoked by plant chemicals. In this respect, it should be noted that negative (inhibitory) effects of the plant extracts were not more specifically tested.

Non-volatiles in leaf surface extracts

In the present study, we found that the higher the S application, the higher the total GSL content. Mainly the abundance of the alkenes and the thioalkyl increased whereas the aromatic and indolyl classes were comparatively unaffected. It is interesting that S affected not only the quantities of GSLs but also the qualitative proportions (Fig. 2C). The different patterns in the four groups of GSLs in response to S can be explained by their amino acid precursors (Schnug & Hanecklaus 1994). Indolyl and aromatic GSLs are derived from tryptophan and tyrosine respectively, and their biosynthesis requires two S-containing compounds: cysteine and adenosyl-5'-phosphosulphate (APS) (Mithen 2001). In contrast the synthesis of alkenes and thioalkyl GSLs depends on three S sources: methionine, cysteine and APS. Given this difference in S requirement at the biosynthetic level, thioalkyl and alkene (synthesised via modification of the side chain of thioalkyl) GSLs might be more sensitive to the plant S status. The effect of S application on the total GSL content is in agreement with the observations of Schnug & Hanecklaus (1994) showing that the S nutritional status of the plants exerts a considerable effect on the GSL content. Similarly Mailer (1989) and Scherer (2001) reported that S application up to 100 μg mL^{-1} raised the GSL concentration in the rapeseed cv. Wesbrook (*B. napus* L.). Interestingly, Mailer (1989) found that a nutrient solution provided with an excess of S (200 μg mL^{-1}), lowered the GSL concentration. This could explain why, in our results, S_+ values were not always higher than S_n.

After landing on a host plant, contact stimuli can influence oviposition. In the case of *P. xylostella*, individual GSLs, including sinigrin and glucobrassicin, certainly play an important role in host recognition and oviposition stimulation (Reed *et al.* 1989; Renwick 2002), as well as in larval feeding, as reported by Van Loon *et al.* (2002). This function is synergised by surface waxes, as demonstrated by Spencer *et al.* (1999), who reported only limited oviposition stimulant activity of sinigrin or cabbage homogenates in the absence of wax.

Volatiles in leaf surface extracts

In agreement with the behavioural and analytical data, antennal olfactory responses (EAGs) to the extracts were also significantly stronger for S_n and S_+ plants than for S_0 plants. Extracts of host-plant volatiles are most likely involved in host location as shown by Palaniswamy *et al.* (1986) and also enhance egg deposition rate in *P. xylostella*. Reed *et al.* (1989) identified intact glucosinolates in host-plant extracts stimulating oviposition and found that degradation with myrosinase or sulphatase largely eliminated the activity, thus excluding isothiocyanates, as active principles. Pivnick *et al.* (1994) studied attraction of the moths and found that allyl-isothiocyanates was an attractive component in homogenised plant volatiles but it was virtually absent from intact plant volatiles. The authors performed a gas chromatographic fractionation of these volatiles and found a terpene-containing fraction to be most attractive, whereas no isothiocyanates were present. Justus *et al.* (unpubl.) recorded GC-EAGs of headspace extracts from two host and non-host plants of *P. xylostella* and identified several EAG active components, but again no evidence was found that isothiocyanates matched the most stimulating compounds. All these results seem to be in agreement with our findings that isothiocyanates do not, or only in minute quantities, occur in the behaviourally active extracts. On the other hand, Renwick *et al.* (in preparation) have found convincing evidence that some isothiocyanates stimulate oviposition. These same compounds also produced matching EAG activities, so that apparently isothiocyanates do have some role to play in host-plant selection of *P. xylostella*. Renwick and Radke (1990) provided clear evidence that several chemically different compounds are involved in the stimulation of the diamondback moth oviposition. Therefore, it can be assumed that the extracts tested in our study contain also a mixture of compounds that might, individually or in combination, stimulate oviposition. The highly active plant extracts obtained in this study would be a good basis to investigate the relative importance of volatile and non-volatile compounds, because as we showed, they match the response to intact plants very well indeed.

The most important result of practical interest of this work is the fact that no significant differences between S_n and S_+ plants were noted. Consequently, as sulphur fertilisation approaches the optimal level for rape, the crop plant will have no further influence on attack or population increase rates of its pest. Within the range tested in this investigation, S fertilisers that allow optimal harvests do not lead to increased infestation by *P. xylostella*.

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