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# Chemical Changes Induced by Methyl Jasmonate in Oilseed Rape Grown in the Laboratory and in the Field

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The effect of methyl jasmonate (MJ) spraying on the chemistry of *Brassica* plants was investigated. Glucosinolates (GLS) in the leaves, stems, and roots of laboratory-grown oilseed rape (*Brassica rapa* subsp. *oleifera* cv. Tuli and Valo) 3 and 7 days after MJ treatment were analyzed. Volatile organic compounds (VOCs) from whole oilseed rape plants were collected 3 days after MJ treatment. GLS were also analyzed from field-grown oilseed rape (cv. Valo) treated with MJ. The production of indolyl GLS in laboratory-grown oilseed rape, especially the concentration of 4-hydroxy-3-indolylmethyl (4-OH-glucobrassicin) in leaves, stems, and roots, 3-indolylmethyl (glucobrassicin) in stems, and 4-methoxy-3-indolylmethyl (4-methoxyglucobrassicin) in roots, was induced after MJ treatment. The VOC emission profile changed after MJ treatment, and homoterpene (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT) was detected only in MJ-treated plants. The GLS concentration in the field-grown plants was significantly higher in MJ-treated plants than in control plants. These results suggest that spraying with MJ induces the production of secondary compounds, that is, GLS and VOCs, in *Brassica* plants. The induction of VOC emissions in oilseed rape is comparable to that caused by insect feeding damage. Thus, MJ-treated crop plants may become less palatable to insect herbivores and more attractive to natural enemies of herbivores.

**KEYWORDS:** ***Brassica rapa*, oilseed rape; methyl jasmonate; glucosinolates; VOCs**

## INTRODUCTION

Cruciferous plants produce special chemicals, glucosinolates (GLS), for different purposes, for example, to protect themselves from herbivore attack and pathogens. GLS are probably at least one of the reasons why cruciferous plants are so widespread and can survive in a variety of environmental conditions and stresses (*1*). GLS are divided into three groups according to which amino acid they are produced from: aromatic, indolyl, and alkenyl GLS (*2*). The chemical structure of GLS varies, but they always involve a sugar-compound, sulfur, and nitrogen (*3*). The breakdown products of GLS are biologically active compounds that may be toxic or/and volatile (*4*).

Cruciferous plants defend themselves also with chemicals other than GLS. Volatile organic compounds (VOCs) involve isoprene, terpenes, alkanes, alkenes, alcohols, esters, and certain others (*5*−*7*). Green leaf volatiles (GLVs) are emitted rapidly after mechanical or herbivore damage (*8*), while the induced terpenes are emitted more slowly (*6*). The purpose of these volatiles is most likely to reduce herbivory (*5*), but they are also known to attract some herbivores (*9*). Plants also indirectly defend themselves against herbivores, as some predators are attracted to inducible plant volatiles (*10*).

The chemical defense of plants can be divided into basic constitutive defense and induced defense. In the latter, the synthesis pathways of defense compounds are activated as a result of stress or wounding (*10*). The GLS (*1*, *11*) and VOC profiles (*7*, *12*) of *Brassica* plants vary according to plant species and cultivars as well as to the developmental stage of the plant. In addition, many abiotic factors, for example, ambient temperature, water availability, light, and pollution, affect the GLS concentration (*13*, *14*) as well as the VOC emissions (*7*), making them difficult to study. Furthermore, different insect or pathogen attacks probably induce different defense responses in plants (*15*, *16*).

Plant defense can be induced under laboratory conditions with natural hormones (inducers), such as jasmonic acid (JA), a hormone generally found in plants, and especially when plants are wounded (*17*). Methyl jasmonate (MJ) is a volatile methylester of JA and has proven to induce the production of GLS in *Brassica*, especially that of indolyl GLS (*2*, *18*). MJ has also been proven to enhance the activity of the lipoxygenase (LOX) pathway via which GLVs are produced (*19*, *20*). At least on tobacco (*19*) and cotton plants (*21*), common GLVs, (*Z*)-3-hexenyl-acetate and (*Z*)-3-hexen-1-ol, were induced after MJ

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treatment. The activity of the isoprenoid pathway via which terpenoids are produced has been induced by jasmonic acid in gerbera (*22*) and in lima bean (*16*). Furthermore, different inducers have had variable effects on different GLS groups; for example, salicylic acid has been shown to operate on the pathway of aromatic components, inducing especially the level of gluconasturtin (*18*, *23*). The effect of the inducer depends also at least on the plant species (*19*), the concentration of the inducer (*21*, *23*), and the age of a plant (*23*). It has been suggested (*24*) that these specific effects of different inducers could be used as an advantage by combining them in the future.

As MJ is a natural hormone, it could be assumed to have effects similar to those caused by insect wounding. The possibility of exogenously affecting the defense system of plants generates hope of finding an alternative to the use of pesticides. However, it is not known whether chemically induced defense could be used to repel insects (*25*), reduce the amount of pathogens (*26*), and lure predators under field conditions. It is also unclear how investment in plant defense finally affects the primary metabolism of plants. The results have been conflicting, and it is uncertain whether induction may simultaneously reduce the yield and biomass of a plant (*17*, *25*).

The first objective of this study was [1] to examine the effect of MJ treatment on oilseed rape plants. The main hypothesis was that MJ would increase GLS production in plants. We wanted to discover also [2] if there are differences in GLS production between cultivars and various plant parts. The GLS profile of roots in particular has not been studied to any degree; even it is thought to differ most from that of above-ground parts (the leaves and stem) (*11*). In addition, we wanted to examine [3] the amount of GLS in the parts of the plants that had grown after the MJ treatment (new parts) and compare those to the directly treated parts. Finally, we wanted [4] to establish the tradeoff between different chemical defense pathways by investigating GLS production and the emitted VOCs under the same MJ treatment and [5] to compare the effects of MJ under field and laboratory conditions.

## MATERIALS AND METHODS

**Laboratory Experiment.** *Plant Material and Treatments.* For laboratory experiment, *Brassica rapa* subsp. *oleifera* (cv. Valo and Tuli, Boreal Ltd., Jokioinen, Finland) were sowed (35 plants per cultivar) in 0.8-L plastic pots filled with a mixture of garden soil (Kekkilä), peat (P2), and sand (2:1:1 v/v). The plants were grown in a growth chamber at 19 °C:12 °C (day:night), using a 22 h light:2 h darkness photoperiod (350 $\mu$mol/m$^2$/s PAR during the light period). The plants were watered daily with tap water and fertilized weekly with 0.1% Superex 9 (19:5:20 N:P:K, Kekkilä, Finland). The fertilization started at the time when 2−3 leaves had grown. Half of the 4 week old seedlings were sprayed over the leaf surfaces with 5 mL of methyl jasmonate (MJ) (dissolved in 2% ethanol in aqueous solution) at a concentration of 1 mg/mL. The control plants were correspondingly sprayed with 2% ethanol in aqueous solution during the same day. MJ-treated and control plants were transferred to separate growth chambers with the growth conditions described above. The studies were carried out in the summer of 2001.

*VOC Collection.* The VOCs were collected 3 days after treatment from 20 randomly selected plants (5 plants per cultivar per treatment). Whole plants with pots were individually enclosed inside 1.5-L glass cuvettes, and the growth medium of pots was isolated from the rest of the plant with aluminum foil while the joint between the glass cuvette and lid was tightened with Parafilm. The cuvette had one inlet for purified air and one for sampling. The airflow was calibrated with a mini-Buck calibrator (model M-5, A.P. Buck, Inc., Orlando, FL) and was set to 150 mL/min for filtered air and 100 mL/min for sampling. Collection was performed at room temperature and at approximately 250 $\mu$mol/m$^2$/s (additional lamps were kept above the plants). VOCs were collected for 1 h in purified collection tubes filled with approximately 150 mg of collecting resin (Supelco, Tenax TA mesh 60/80). The glass material used for collecting was purified in a heating chamber (120 °C) overnight. The VOC samples were analyzed using GC-MS (Hewlett-Packard GC type 6890, MSD 5973), and trapped compounds were desorbed with a thermal desorption unit (ATD 400 Automatic Thermal Desorption System, Perkin-Elmer) at 250 °C for 10 min, cryofocused at −30 °C, and injected onto a HP-5 capillary column (50 m × 0.2 mm i.d. × 0.5 $\mu$m film thickness, Hewlett-Packard). The temperature program began at 40 °C for 1 min, followed by increases of 5 °C/min to 210 °C and 20 °C/min to 250 °C during the run. Compounds were identified by comparison of the mass spectra with those in the Wiley library and also with pure standards. Emissions were expressed as ng/plant/h.

*GLS.* After collection of VOCs, the same plants were used for the GLS analyses. Therefore, leaves, stem, and roots were separately harvested into liquid nitrogen and stored at −80 °C until they were freeze-dried for analysis. The second harvest for GLS analyses was done 7 days after the treatments, as was done previously for the other 20 plants (5/cultivar/treatment). In addition, the shoot material that had been grown after the treatments (new parts) was taken for GLS analyses from 20 additional plants (5/cultivar/treatment).

The freeze-dried plant material was homogenized with a mortar and pestle, and an approximately 300 mg sample was weighed into test tubes. The extractions of GLS from plants were based on the method reported in the Official Journal of the European Communities (*27*) and also as described by Reddy et al. (*28*). Certified reference seed material (RMs 367, Community Bureau of Reference) was used to verify the correct application of the method. The GLS were extracted and afterward desulfated with purified sulfatase (*Helix pomatia* type H1) overnight. The desulfo-GLS were eluted from columns with 0.5 mL of water the following morning.

The desulfo-GLS were separated using HPLC (Hewlett-Packard series 1050,1040 M series II detection system) using the capillary column (HP Lichrospher 100 RP-18e, 5 $\mu$m, 250 × 4 mm), and an 0.8 mL/min flow rate at 30 °C by elution gradient from 100% water into 25% acetonitrile in 45 min. GLS were detected at the wavelength of 229 nm. Individual GLS were identified by comparing the retention times of samples with the retention times of reference seeds. The quantities of the GLS identified were calculated by using the peak area of each compound and the peak area and concentration of the internal standard (sinigrin). The result was multiplied with the equivalent response factor of each desulfoglucosinolate given in the Official Journal of the European Communities (*27*).

**Field Experiment.** *Plant Material.* For the field experiment in the Kuopio University Garden, surface soil (up to a depth of 20 cm) from an area 7 × 10 m in size was removed, and soil rich in organic matter was added 1 week before planting. This was done to obtain an appropriate growth medium for the species studied and to equalize the growth conditions in the field. Two days before planting, the soil was fertilized with 4 kg of phosphorus−potassium fertilizer, 2 kg of y-fertilizer, and 1 kg of potassium sulfate. The oilseed rape plants (*Brassica rapa*, subsp. *oleifera*, cv. Valo) were precultivated in pots before planting in the growth medium as described above. In the middle of June, the plants were transplanted into a total of 4 blocks, each of them 1.75 × 1.5 m in size inside the 7 × 10 m experimental area so that next to an oilseed rape block there were always two similar sized white cabbage blocks (*Brassica oleracea* var. *capitata*) and two similar sized broccoli blocks (*Brassica oleracea* var. *italica*). Blocks with various plant species were arranged in alternating order. Eighty individual seedlings of each plant species were thus needed. The field was irrigated when needed, and the rape plants had to be backed up with sticks to support them while growing. The first MJ treatment (concentration 1 mg MJ/L of 2% ethanol in aqueous solution, 4 mg/plant) was sprayed 1 month after planting. A second similar treatment was given 2 weeks later.

*GLS.* For GLS analyses, the leaves (2−4 leaves) were harvested 2 days after the second treatment systematically from every corner of every oilseed rape block only. GLS were analyzed as described before.

**Table 1.** Concentration (±SD, $\mu$mol/g d.w.) of Individual GLS in Control (Treated with Water) and MJ-Treated Oilseed Rape Cv. Tuli and Valo, 3 Days after the Treatment[a]

| | treatment | concentration ($\mu$mol/g d.w.) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRG | GRP | GNP | GNA | 4GL | GBN | GER | GBR | GNS | 4MG | NGB |
| | | | | | | Cv. Valo | | | | | | |
| leaves | W | 0.00 | 0.26 ± 0.17 | 0.03 ± 0.06 | 0.00 | 0.04 ± 0.05 | 0.70 ± 0.91 | 0.00 | 0.16 ± 0.21 | 0.15 ± 0.23 | 0.00 | 0.00 |
| | MJ | 0.00 | 0.37 ± 0.09 | 0.20 ± 0.27 | 0.00 | 3.08 ± 2.97* | 0.00 | 0.00 | 1.85 ± 1.07†† | 0.31 ± 0.69 | 0.01 ± 0.03 | 0.01 ± 0.03 |
| roots | W | 0.00 | 0.44 ± 0.18 | 0.05 ± 0.11 | 0.00 | 0.07 ± 0.04 | 0.00 | 0.00 | 0.29 ± 0.06 | 7.22 ± 2.03 | 1.74 ± 0.33 | 0.00 |
| | MJ | 0.00 | 0.37 ± 0.12 | 0.00 | 0.00 | 0.74 ± 0.56* | 0.29 ± 0.39 | 0.00 | 0.32 ± 0.22 | 9.00 ± 5.16 | 4.70 ± 1.76* | 0.00 |
| stems | W | 0.00 | 0.09 ± 0.15 | 0.05 ± 0.12 | 0.02 ± 0.05 | 0.04 ± 0.05 | 0.61 ± 0.63 | 0.00 | 0.09 ± 0.07 | 0.31 ± 0.43 | 0.01 ± 0.02 | 0.01 ± 0.03 |
| | MJ | 0.00 | 0.20 ± 0.33 | 0.03 ± 0.07 | 0.00 | 2.25 ± 2.31* | 0.08 ± 0.18 | 0.00 | 1.18 ± 0.7* | 0.51 ± 0.68 | 0.16 ± 0.22 | 0.04 ± 0.09 |
| | | | | | | Cv. Tuli | | | | | | |
| leaves | W | 0.03 ± 0.07 | 0.19 ± 0.12 | 0.07 ± 0.16 | 0.09 ± 0.16 | 2.41 ± 4.96 | 1.17 ± 1.34 | 0.00 | 2.15 ± 4.46 | 0.49 ± 0.57 | 0.00 | 0.00 |
| | MJ | 0.00 | 0.14 ± 0.19 | 0.05 ± 0.12 | 0.52 ± 0.72 | 5.40 ± 4.94 | 0.20 ± 0.44 | 0.00 | 3.85 ± 4.46 | 0.08 ± 0.17 | 0.06 ± 0.09 | 0.02 ± 0.05 |
| roots | W | 0.00 | 0.31 ± 0.22 | 0.00 | 0.00 | 0.18 ± 0.35 | 0.09 ± 0.13 | 0.04 ± 0.09 | 0.51 ± 0.59 | 7.05 ± 4.32 | 3.39 ± 5.28 | 0.00 |
| | MJ | 0.00 | 0.56 ± 0.28 | 0.00 | 0.00 | 1.25 ± 1.47 | 0.20 ± 0.44 | 0.00 | 0.63 ± 0.63 | 10.88 ± 5.98 | 6.04 ± 7.42 | 0.00 |
| stems | W | 0.23 ± 0.53 | 0.11 ± 0.18 | 0.09 ± 0.20 | 0.14 ± 0.32 | 0.57 ± 1.07 | 1.09 ± 2.29 | 0.00 | 0.14 ± 0.27 | 0.29 ± 0.42 | 0.22 ± 0.49 | 0.03 ± 0.07 |
| | MJ | 0.00 | 0.08 ± 0.19 | 0.32 ± 0.44 | 0.00 | 1.38 ± 0.85 | 0.00 | 0.00 | 1.07 ± 0.65†† | 0.57 ± 0.78 | 0.45 ± 0.74 | 0.15 ± 0.22 |

[a] Statistical differences between the treatments among each plant part according to the Mann–Whitney *U*-test, *$P \leq 0.05$, or according to the independent samples *t*-test (log-transformed data), † $P \leq 0.05$ or †† $P \leq 0.01$. Treatments: MJ, methyl jasmonate; W, water. Glucosinolates: PRG, progoitrin; GRP, glucorafanin; GNP, gluconapoleiferin; GNA, gluconapin; 4GL, 4-OH-glucobrassicin; GBN, glucobrassicanapin; GER, glucoerucin; GBR, glucobrassicin; GNS, gluconasturtin; 4MG, 4-methoxyglucobrassicin; NGB, neoglucobrassicin.

**Chemicals.** VOC collection: (*Z*)-3-hexenyl acetate (Aldrich) and 1-chloro-octane (Fluka). GLS extraction: Sulfatase, *Helix pomatia* type H1 (Sigma), DEAE Sepharose Cl-6B resin (Fluka), imidatsolformate (Sigma), sodium acetate buffer (Riedel-deHaën), sinigrin (Fluka), DEAE-Sephadex A25 resin (Fluka), and certified reference seed material (the Community Bureau of Reference, BCR program of the Commission of the European Communities). HPLC: acetonitrile (Rathburn) and methanol (Rathburn).

**Statistical Analyses.** In the laboratory experiment, either the Kruskal−Wallis test or ANOVA (log-transformed data) was used to detect the main effect of MJ treatment on GLS concentrations. Depending on the normality of the data, either the Mann−Whitney *U*-test with Bonferroni correction or independent samples *t*-test (log-transformed data) was used to test differences in GLS concentration between treatments among the plant parts and the cultivars. A multifactorial ANOVA was conducted to detect the main effects of both variety and plant part on glucosinolate concentrations. To analyze the GLS concentration of the field grown plants, the Mann−Whitney *U*-test was used as above. The results of VOCs collected were represented as ng/plant/h. The concentrations were calculated by using the internal standard (1-chloro-octane), and the results were again analyzed with the Mann−Whitney *U*-test. All significances of differences were determined at a level of $P < 0.05$.

## RESULTS

**Laboratory Experiment.** *Individual GLS.* A total of 12 individual GLS were identified in the oilseed rape samples: four indolyl, one aromatic, and eight alkenyl GLS. The quantity and quality of individual GLS varied depending on the cultivar, the part of the plant, the day harvested, and the treatment.

MJ treatment significantly increased ($P < 0.05$) the concentration of 4-OH-glucobrassicin in all parts of cv. Valo in the first harvest (**Table 1**) and in the leaves and new parts in the second harvest (**Table 2**). Also, in the leaves ($P = 0.001$, log-transformed data) and in the roots ($P < 0.001$, log-transformed data) of cv. Tuli the concentration of 4-OH-glucobrassicin showed an increase in the second harvest (**Table 2**).

The concentration of glucobrassicin was significantly higher ($P < 0.05$) in the MJ-treated stems and leaves of cv. Valo and stems of cv. Tuli than in the controls in the first harvest (**Table 1**). In the second harvest, the glucobrassicin concentration was higher ($P < 0.05$) in the MJ-treated stems and new parts of cv. Valo and leaves of cv. Tuli than in the controls (**Table 2**). In the controls, the concentration of glucobrassicin was almost nonexistent.

In the roots of cv. Valo (first harvest) and cv. Tuli (second harvest, log-transformed data), the concentration of 4-methoxyglucobrassicin increased significantly ($P < 0.05$) after MJ treatment (**Table 1**). The concentration of gluconasturtin increased in all parts of the MJ-treated plants in the first harvest, but the concentration had fallen in the second harvest (leaves and stems of both the cultivars $P < 0.05$, log-transformed data) (**Tables 1** and **2**).

In addition to observed differences between the treatments, the GLS concentrations showed dependence on the plant parts. The concentration of glucoraphanin was dependent on the plant part in both cultivars in the first ($P < 0.05$) and second ($P = 0.001$) harvest. Similarly, the concentration of gluconasturtin was dependent on the plant part in both of the varieties ($P < 0.001$) in both harvests (**Tables 1** and **2**). In addition, in cv. Tuli the concentration of 4-methoxybrassicin in the first harvest (**Table 1**) and the concentration of glucobrassicanapin and brassicanapin in the second harvest (**Table 2**) were affected by the plant part ($P < 0.05$).

*The Total GLS Concentration.* In general, the total GLS concentration had increased due to MJ treatment in the first harvest (**Figures 1a** and **b**). In the stems and leaves of cv. Valo, the concentration had risen significantly ($P < 0.05$, log-transformed data) (**Figure 1b**). In addition to the MJ treatment, the total GLS concentration of cv. Tuli ($P < 0.05$) and cv. Valo ($P < 0.001$) was affected by the plant part (**Figures 1a** and **b**). The highest total GLS concentration was in the roots, producing approximately half of the total GLS in both varieties, and the lowest in the stems. In the first harvest, the total GLS concentration in control plants of cv. Tuli was approximately two-thirds of that in MJ-treated plants (**Figure 1a**). Similarly, cv. Valo produced approximately 60% more glucosinolates in MJ-treated roots, 4-fold more in MJ-treated leaves, and 2.5-fold more in MJ-treated stems as compared to the controls (**Figure 1b**).

In the second harvest, 7 days after the treatment, the GLS concentration in the leaves of cv. Tuli was significantly higher due to MJ treatment ($P = 0.001$) (**Figure 1c**). The total GLS concentration was also dependent on the plant part in both cultivars ($P \leq 0.001$) (**Figures 1c** and **d**). In general, the GLS concentration of the roots had risen further, and that of the stems had fallen as compared to the other parts of the plants. In the new parts grown after the treatment, the total GLS concentration

**Table 2.** Concentration (±SD, μmol/g d.w.) of Individual GLS in MJ-Treated and Control Oilseed Rape, Cv. Tuli and Valo, 7 Days after the Treatments (Second Harvest)[a]

| | treat-ment | concentration (μmol/g d.w.) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | PRG | GRP | GNP | GNA | 4GL | GBN | GER | GBR | GNS | 4MG | NGB |
| | | | | | | Cv. Valo | | | | | | |
| leaves | W | 0.13 ± 0.12 | 0.17 ± 0.05 | 0.09 ± 0.09 | 0.03 ± 0.07 | 0.10 ± 0.09 | 0.22 ± 0.25 | 0.00 | 0.16 ± 0.20 | 0.27 ± 0.14 | 0.00 | 0.02 ± 0.03 |
| | MJ | 0.28 ± 0.39 | 0.21 ± 0.11 | 0.64 ± 0.85 | 0.00 | 3.36 ± 4.86* | 0.00 | 0.26 ± 0.59 | 1.45 ± 1.53 | 0.05 ± 0.11† | 0.06 ± 0.12 | 0.00 |
| roots | W | 0.13 ± 0.18 | 0.34 ± 0.32 | 1.01 ± 1.68 | 0.00 | 3.11 ± 5.19 | 0.05 ± 0.09 | 0.00 | 0.86 ± 0.79 | 11.04 ± 12.62 | 1.14 ± 1.08* | 0.00 |
| | MJ | 0.27 ± 0.18 | 0.51 ± 0.13 | 0.22 ± 0.19 | 0.00 | 0.70 ± 0.30 | 0.6 ± 1.21 | 0.00 | 0.50 ± 0.11 | 10.34 ± 8.24 | 6.24 ± 1.66* | 0.00 |
| stems | W | 0.21 ± 0.21 | 0.16 ± 0.05 | 0.05 ± 0.07 | 0.01 ± 0.03 | 0.06 ± 0.03 | 0.44 ± 0.25 | 0.16 ± 0.36 | 0.02 ± 0.03 | 0.63 ± 0.35 | 0.02 ± 0.03 | 0.00 |
| | MJ | 0.17 ± 0.24 | 0.19 ± 0.16 | 0.03 ± 0.07 | 0.00 | 0.35 ± 0.26 | 0.12 ± 0.26 | 0.15 ± 0.34 | 0.24 ± 0.14* | 0.03 ± 0.04* | 0.20 ± 0.18 | 0.00 |
| new parts | W | 0.19 ± 0.21 | 0.18 ± 0.14 | 0.08 ± 0.09 | 0.00 | 0.04 ± 0.04 | 0.63 ± 0.48 | 0.00 | 0.02 ± 0.03 | 0.34 ± 0.24 | 0.00 | 0.00 |
| | MJ | 0.25 ± 0.54 | 0.08 ± 0.12 | 0.35 ± 0.46 | 0.18 ± 0.25 | 1.84 ± 1.84* | 1.73 ± 1.53 | 0.00 | 1.84 ± 2.52* | 0.33 ± 0.37 | 0.23 ± 0.48 | 0.03 ± 0.07 |
| | | | | | | Cv. Tuli | | | | | | |
| leaves | W | 0.13 ± 0.20 | 0.19 ± 0.03 | 0.36 ± 0.56 | 0.06 ± 0.12 | 0.11 ± 0.04 | 0.62 ± 0.61 | 0.08 ± 0.15 | 0.23 ± 0.33 | 0.58 ± 0.33 | 0.01 ± 0.01 | 0.02 ± 0.03 |
| | MJ | 0.28 ± 0.27 | 0.22 ± 0.12 | 1.48 ± 0.84† | 0.00 | 5.37 ± 3.60†† | 0.17 ± 0.23 | 0.00 | 1.66 ± 0.98† | 0.03 ± 0.08†† | 0.07 ± 0.01 | 0.07 ± 0.1 |
| roots | W | 0.15 ± 0.12 | 0.45 ± 0.03 | 0.18 ± 0.15 | 0.00 | 0.20 ± 0.14 | 0.29 ± 0.41 | 0.00 | 0.22 ± 0.09 | 11.03 ± 3.01 | 1.51 ± 0.15 | 0.00 |
| | MJ | 0.62 ± 0.55 | 0.53 ± 0.09 | 0.75 ± 0.25†† | 0.00 | 1.56 ± 0.47†† | 0.50 ± 0.88 | 0.11 ± 0.22 | 0.53 ± 0.29 | 16.21 ± 9.80 | 5.59 ± 2.77† | 0.00 |
| stems | W | 0.19 ± 0.29 | 0.26 ± 0.24 | 0.10 ± 0.17 | 0.01 ± 0.01 | 0.03 ± 0.03 | 0.73 ± 0.55 | 0.00 | 0.02 ± 0.02 | 0.49 ± 0.34 | 0.00 | 0.00 |
| | MJ | 0.25 ± 0.24 | 0.17 ± 0.04 | 0.20 ± 0.04 | 0.00 | 0.67 ± 0.47 | 0.25 ± 0.32 | 0.00 | 0.21 ± 0.16 | 0.07 ± 0.15† | 0.07 ± 0.08 | 0.00 |
| new parts | W | 0.44 ± 0.60 | 0.32 ± 0.19 | 0.26 ± 0.19 | 0.39 ± 0.36 | 0.21 ± 0.13 | 1.60 ± 0.92 | 0.07 ± 0.15 | 0.07 ± 0.12 | 0.58 ± 0.73 | 0.00 | 0.00 |
| | MJ | 0.63 ± 1.30 | 0.21 ± 0.19 | 0.98 ± 1.59 | 0.17 ± 0.38 | 4.98 ± 4.94 | 1.45 ± 1.68 | 0.00 | 4.55 ± 7.28 | 0.65 ± 0.86 | 0.09 ± 0.21 | 0.06 ± 0.13 |

[a] Statistical differences between the treatments among each plant part according to the Mann–Whitney *U*-test, $*P \leq 0.05$, or according to the independent samples *t*-test (log-transformed data), $^{\dagger}P \leq 0.05$ or $^{\dagger\dagger}P \leq 0.01$. Treatments: MJ, methyl jasmonate; W, water. Glucosinolates: PRG, progoitrin; GRP, glucorafanin; GNP, gluconapoleiferin; GNA, gluconapin; 4GL, 4-OH-glucobrassicin; GBN, glucobrassicanapin; GER, glucoerucin; GBR, glucobrassicin; GNS, gluconasturtin; 4MG, 4-methoxyglucobrassicin; NGB, neoglucobrassicin.

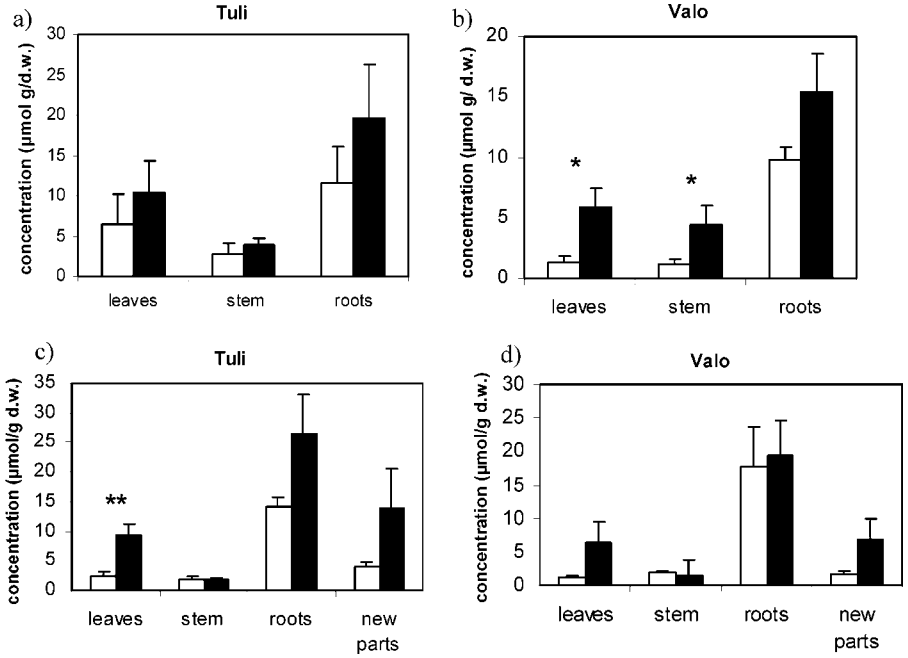


**Figure 1.** Total GLS concentration (+SE, μmol/g d.w.) in control (open bars) and MJ-treated (solid bars) leaves, stems, and roots of oilseed rape 3 days after the treatments in cultivars (**a**) Tuli and (**b**) Valo and 7 days after the treatments in cultivars (**c**) Tuli and (**d**) Valo. An asterisk denotes a statistically significant difference ($P \leq 0.05$), and a double-asterisk denotes a statistically very significant ($P \leq 0.01$) difference between the treatments according to the independent samples *t*-test (data log-transformed).

was approximately the same as in the leaves. The differences between the treatments became more significant than differences in the samples collected in the first harvest (**Figures 1c** and **d**).

*VOC Collection.* The identified VOCs in oilseed rape samples were 3-methyl-2-butanone, methyl-*d*-31-dideuterio-2-propenyl ether, 1,3,5-cycloheptatriene, 6-methyl-5-hepten-2-one, (*Z*)-3-hexenyl acetate, (*E*)-4,8-dimethyl-1,3,7-nonatriene (DMNT), and (*E*,*E*)-α-farnesene. The major compound in emissions from MJ-treated plants was DMNT, constituting 46% of emissions in cv. Tuli and 36% in cv. Valo. In the control plants, DMNT was not found at all, leading to a significant difference between treatments both in Valo ($P < 0.01$) and in Tuli ($P < 0.05$) (**Figure 2a**). The other dominating VOCs were (*Z*)-3-hexenyl-acetate (**Figure 2b**) and (*E*,*E*)-α-farnesene (**Figure 2c**), the concentrations being significantly ($P < 0.05$) higher after MJ treatment in cv. Tuli only and in both cultivars, respectively.

**The Field Experiment.** *Individual GLS.* In the field-grown oilseed rape, 11 individual GLS in the leaves were found. The most commonly found GLS were mainly indolyl GLS: 4-OH-glucobrassicin, glucobrassicin, 4-methoxyglucobrassicin, and one aromatic GLS, gluconasturtin (**Table 3**).

The concentration of gluconapoleiferin was 10-fold higher and the concentration of glucobrassicanapin almost 20-fold higher in MJ-treated plants than in the controls ($P < 0.01$ in

**Table 3.** Concentration (±SD, μmol/g d.w.) of Individual GLS Identified in the Leaves of Field-Grown Oilseed Rape Cv. Valo, 2 Days after the Treatment[a]

| treat-ment | concentration (μmol/g d.w.) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | GLI | PRG | EPR | GRP | GNP | GNA | 4GL | GBN | GBR | GNS | 4MG |
| W | 0.0 0 | 0.21 ± 0.14 | 0.00 | 0.23 ± 0.06 | 0.10 ± 0.12 | 0.04 ± 0.12 | 1.95 ± 3.97 | 0.07 ± 0.20 | 0.61 ± 0.54 | 0.63 ± 0.73 | 0.64 ± 0.68 |
| MJ | 0.0 0 | 0.51 ± 0.36 | 0.00 | 0.21 ± 0.11 | 1.04 ± 0.48** | 0.03 ± 0.09 | 3.86 ± 4.22 | 1.93 ± 1.63** | 3.96 ± 4.19* | 0.80 ± 0.93 | 0.96 ± 1.67 |

[a] Statistically significant difference between the treatments among each compound according to the Mann–Whitney *U*-test, *$P \leq 0.05$, **$P \leq 0.01$. Treatments: MJ, methyl jasmonate; W, water. Glucosinolates: GBI, glucoiberin; PRG, progoitrin; EPR, epiprogoitrin; GRP, glucorafanin; GNP, gluconapoleiferin; GNA, gluconapin; 4GL, 4-OH-glucobrassicin; GBN, glucobrassicanapin; GBR, glucobrassicin; GNS, gluconasturtin; 4MG, 4-methoxyglucobrassicin.

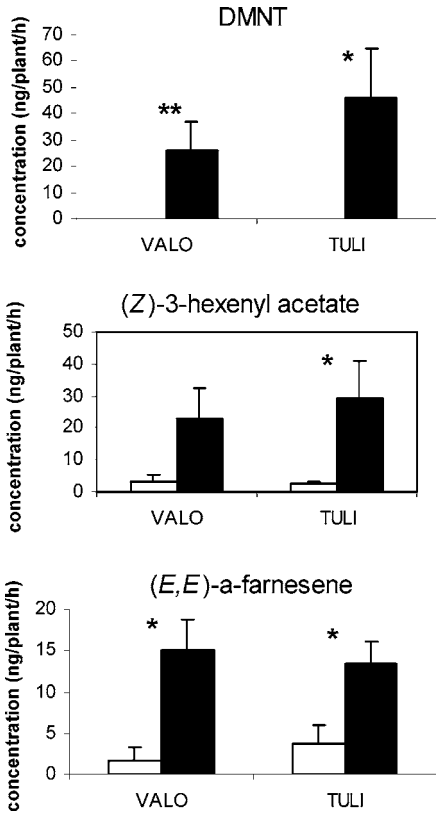


**Figure 2.** Influence of MJ on the concentration of (**a**) (*E*)-4,8-dimethyl-1,3,7-nonatriene, (**b**) (*Z*)-3-hexenyl-acetate, and (**c**) (*E*,*E*)-α-farnesene (+SE, ng/plant/h) in control (open bars) and MJ-treated (solid bars) oilseed rape cultivars Tuli and Valo. An asterisk denotes a statistically significant difference ($P \leq 0.05$), and a double-asterisk denotes a statistically very significant ($P \leq 0.01$) difference between the treatments according to the Mann–Whitney test.

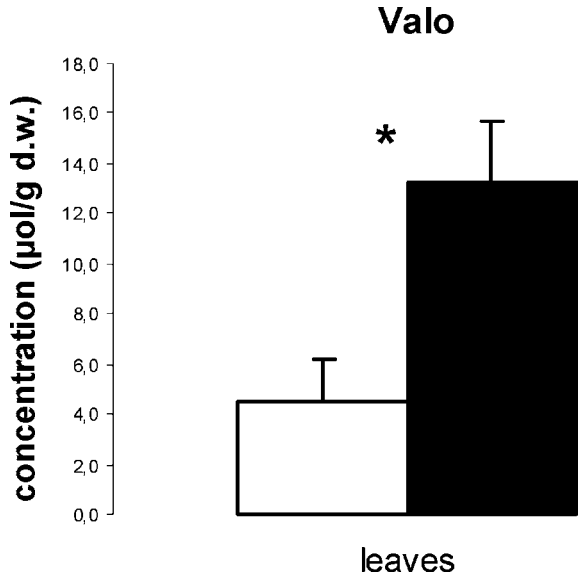


**Figure 3.** Total GLS concentration (+SE, μmol/g d.w.) in control (open bars) and MJ-treated (solid bars) oilseed rape in the field 2 days after the second treatment. An asterisk denotes a statistically significant difference between the treatments ($P \leq 0.05$, Mann–Whitney).

both). There was also a significant increase ($P < 0.05$) in the concentration of glucobrassicin in MJ-treated plants. As well, the concentrations of progoitrin, 4-OH-glucobrassicin, and 4-methoxyglucobrassicin were higher in the leaves of MJ-treated plants as compared to the controls, but the differences were insignificant.

*The Total GLS Concentration.* The total GLS concentration was significantly higher ($P < 0.05$) in the leaves of MJ-treated oilseed rape as compared to the controls (**Figure 3**).

## DISCUSSION

**GLS Groups.** MJ induced mainly the concentration of indolyl GLS, but not aromatic or alkenyl GLS, being in agreement with Kiddle et al. (*2*). The constitutive GLS profile in the leaves, stem, and new parts of both oilseed rape cultivars involved more indolyl GLS than other GLS, but after MJ treatment the distribution became more distinctive. Except for one alkenyl GLS, gluconapoleiferin, only the induction of indolyl GLS to the MJ treatment was significant in the laboratory grown plants. As in the earlier studies (*2*, *18*), our study showed the induction of one alkenyl GLS after MJ treatment. On the other hand, the concentration of another alkenyl compound, glucobrassicanapin, decreased as a result of MJ treatment; furthermore, the concentration was lower in the second harvest than in the first.

Our results prove that MJ has specific effects on the GLS and is targeted to increase the concentration of indolyl GLS. On this basis, it seems unlikely that MJ mimics the production of GLS induced by natural stresses if a stress additionally induces other than indolyl GLS. However, it is not likely that different natural stresses would induce similar reactions in plants' chemical defense on every occasion. In fact, the special effect of MJ could also be strength when applying it in practical applications.

**GLS between Cultivars, Parts of the Plant, and Developing Stage.** In many surveys (*11*, *28*, *29*), it has been shown that there is variability in the GLS concentration between plant cultivars. Our study gives support to this where the studied oilseed rape cultivars are concerned. The GLS concentration of cv. Tuli is naturally higher than in cv. Valo, but Valo reacts to MJ more strongly than Tuli. Thus, it looks likely that MJ cannot raise the GLS concentration in plants above a certain threshold level. It may be that higher production of the defense compounds is no longer economical because it deprives growth of too many resources as compared to the original harm from the stresses.

Our results also support the idea that the GLS concentration differs between developmental stages (*14*) and various parts of the plant (*1*, *11*). The GLS concentration was clearly dependent on the part of the plant and was most distinct in the roots as compared to the other parts. In the stems, the GLS concentration was lowest, and in the roots it was highest during both harvests. In addition to indolyl GLS, one aromatic compound, gluconasturtin, was one of the major GLS occurring constitutively in the control roots, but the reaction of this compound to the MJ treatment was a decrease in concentration.

The GLS concentration in all parts was smaller in the second harvest than in the first harvest. In the second harvest, only in the new parts was the GLS concentration as high as in the leaves and stems harvested first. These results show that the GLS concentration is higher in young plant tissue than in old tissue (*29*). This also indicates that the effect of MJ lasts longer than only a few days and covers the whole plant, not only the parts sprayed.

**VOCs.** The collection of VOCs verifies the former results (*30*) that there are no radical differences between cultivars in the VOC profiles. The cultivars also reacted to MJ quite similarly by increasing the amount of one homoterpene, DMNT, one GLV, (*Z*)-3-hexenyl acetate, and one sesquiterpene, (*E*,*E*)-α-farnesene. Both cultivars started to produce DMNT only after MJ treatment. According to these and earlier results from other plant species (*16*, *20*−*22*), it seems that the response to herbivore damage could be mimicked using MJ at least to some extent. Furthermore, an advantage of MJ might be that only natural enemies are attracted by volatiles from MJ-treated plants, while more herbivores could be attracted by volatiles emitted from herbivore-damaged plants (*21*).

**GLS in the Field.** The effect of environmental stress can be indicated in the control plants grown in the field as they contained compounds (i.e., 4-OH-glucobrassicin, progoitrin, 4-methoxyglucobrassicin) that occurred only in minor quantities in the leaves of laboratory-grown control plants. In any case, the effect of UV-B radiation was missing from the laboratory, and it was previously reported that plants' chemical defense might have a role in UV protection in the field (*31*, *32*). The effect of UV-B on GLS, too, should be studied more. However, because the concentration of 4-OH-methoxybrassicin was approximately the same in controls growing in the field as in MJ-treated examples grown in the laboratory, it is likely that the rape plants react to environmental stress mainly with these, also MJ-induced, compounds. This also gives support to the fact that MJ can imitate those natural stresses that plants have to face anyway in the field to some extent.

The total GLS concentration in the laboratory-grown plants was only 30% of that in the field-grown. Also, it seemed that MJ had a lower influence on the field-grown oilseed rape plants than on the laboratory-grown ones. The latter might be explained by different treatment, as the dose of MJ treatment in the field was only 80% of the dose given in laboratory. On the other hand, we do not know the differences between the effects of one given treatment (in the laboratory) or two (in the field) or the optimum time left between the two treatments. However, it is clear that differences between the laboratory and field-grown oilseed rape plants might very well be explained by different growing conditions (*1*, *13*), pollution in the air (*33*), UV-B-radiation (*31*, *32*), and/or the inability of a plant to raise the GLS concentration higher than to a certain level.

There are different opinions (*17*, *25*, *34*−*36*) about the effects of induced defense on the biomass of a plant and on the harvest. Our other corresponding field study showed clearly that MJ treatment reduces the growth of rape and the size of the harvest at least in the MJ concentrations given here (data not shown). Consequently, it seems unlikely that MJ could be used as an inductor of defense compounds on the cultivated crop, at least in the current concentrations which induce such strong allocation of resources to the defense. More likely, the strength of MJ could be in inducing the GLS content in other plants that could be used as herbivore attractants or repellents.

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