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SELECTIVE INDUCTION OF GLUCOSINOLATES IN OILSEED RAPE LEAVES BY METHYL JASMONATE

KEVIN J. DOUGHTY, GUY A. KIDDLE, BARRY J. PYE, ROGER M. WALLSGROVE and JOHN A. PICKETT

Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Hertfordshire AL5 2JQ, U.K.

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Key Word Index—*Brassica napus*; Cruciferae; oilseed rape; induction; glucosinolates; methyl jasmonate.

Abstract—Oilseed rape (*Brassica napus*) plants sprayed with a solution of methyl jasmonate or exposed to methyl jasmonate vapour accumulated indolyl glucosinolates in their leaves in amounts that depended on the concentration applied. At the highest concentration of methyl jasmonate, the total glucosinolate concentration increased up to 20-fold. The predominant components of the response were 3-indolylmethyl- and 1-methoxy-3-indolylmethyl-glucosinolates, which together comprised 90% of the total glucosinolates in treated leaves. This selective induction of indolyl glucosinolates contrasts with the response to fungal infection and to treatment with other abiotic elicitors. The implications of these findings are discussed in terms of the role of both methyl jasmonate and the glucosinolates in plant defence.

INTRODUCTION

Jasmonic acid and its volatile methyl ester, methyl jasmonate (MJ), are ubiquitous plant compounds with inhibitory and promotory effects, often similar to those of abscisic acid, on many plant physiological processes [1]. Among these effects is the triggering of various biosynthetic pathways associated with reactions to wounding, herbivory and infection [2–4]. This paper reports the accumulation of particular glucosinolates in oilseed rape (*Brassica napus*) leaves in response to treatment with MJ. Glucosinolates are a group of thioglucosides found in all cruciferous plants. When tissues are damaged, they are hydrolysed by a thioglucosidase enzyme (myrosinase, EC 3.2.3.1) to release various products, some of which contribute to the plant's defence against microorganisms and pests [5]. Glucosinolates are constitutive to oilseed rape tissues, but they also accumulate in response to fungal infection [6], actual and simulated insect damage [7, 8] and other forms of stress [9].

RESULTS

Spraying oilseed rape plants with MJ solutions or exposing them to MJ vapour caused them to accumulate glucosinolates in both developing (sixth) and mature (fourth) leaves. In sprayed (Fig. 1) and vapour-treated plants (Fig. 2), almost all of the increase was in the indolyl compounds, 3-indolylmethyl- (**7**), and 1-methoxy-3-indolylmethyl (**8**) glucosinolate: plants sprayed with MJ accumulated slightly higher concentrations of these glucosinolates than those exposed to MJ vapour. This selective induction meant that indolylglucosinolates comprised most of the total in treated leaves. For example, in leaves exposed to the highest concentration of MJ vapour, the proportion of indolylglucosinolates changed from 20% (fourth leaves) and 35% (sixth leaves) of the total in control leaves to *ca* 90% of the total in both leaves from treated plants.

Analysis of variance of log-transformed data from two replicate experiments for each type of treatment showed that plants sprayed with MJ at 150 mg ml^{-1} had significantly greater concentrations of 3-indolylmethylglucosinolate (**7**) in fourth and sixth leaves, and of 1-methoxy-3-indolylmethylglucosinolate (**8**) in sixth leaves ($P < 0.05$) [but not of the latter in fourth leaves: $P = 0.052$], than plants which had received MJ solutions at lower concentrations, Triton (surfactant) alone, or sterile distilled water. Similarly, plants exposed to pure MJ vapour had significantly greater concentrations of **7** and **8** in fourth ($P < 0.05$) and sixth ($P < 0.01$) leaves than plants treated with lower concentrations of vapour, or ethanol (solvent) alone. Sprayed plants also accumulated 2-hydroxy-3-butenylglucosinolate (**3**) in fourth leaves and 4-pentenylglucosinolate (**2**) in sixth leaves, but an accumulation of these compounds also occurred in plants sprayed with Triton alone. The change in the concentration of 2-phenylethylglucosinolate (**5**) in sixth leaves from plants sprayed with 150 mg ml^{-1} MJ was not significant.

Treated plants showed some chlorosis and slight decreases in total fresh weight. These effects were greatest at the highest concentrations applied in each experiment. For example, total plant fresh weights were 15.4 and 17.3% less than corresponding controls for plants sprayed with 150 mg ml^{-1} in solution, and those exposed to the highest concentration of MJ vapour, respectively.

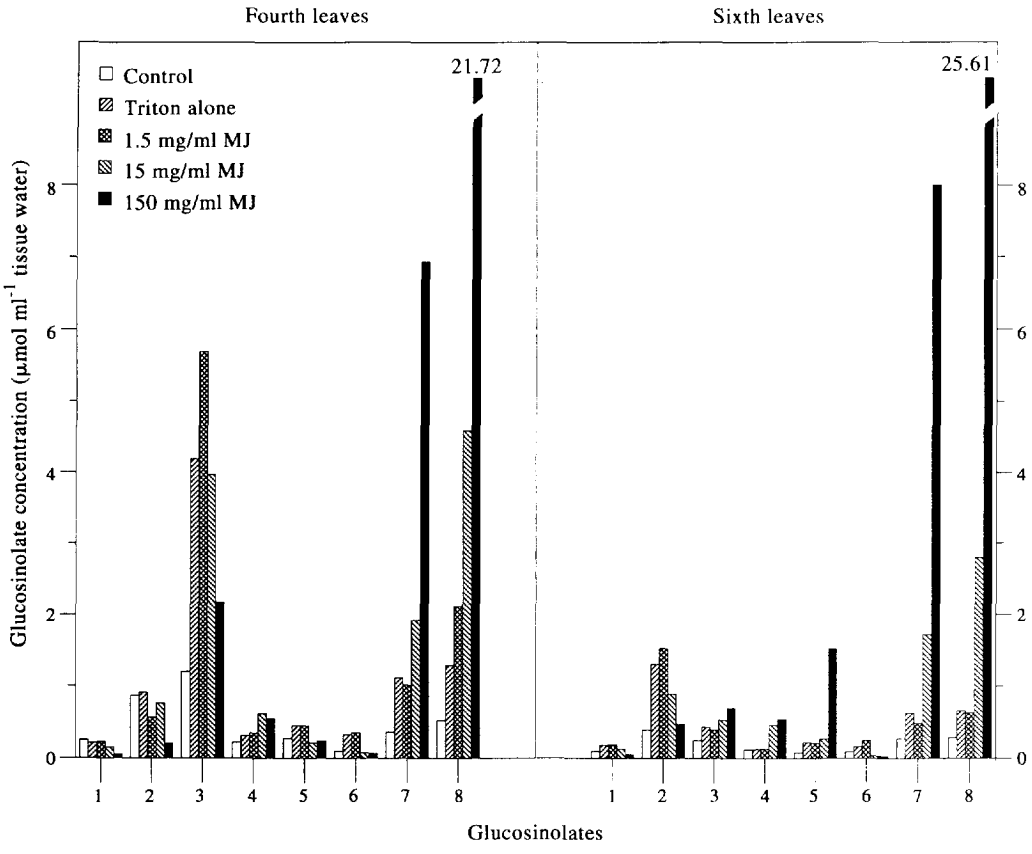

Fig. 1. Concentrations of glucosinolates in fourth and sixth leaves from oilseed rape plants, seven days after spraying with MJ solutions at three different concentrations. Controls consisted of sterile distilled water or Triton X-100 alone. Glucosinolates: **1**, 3-butenyl; **2**, 4-pentenyl; **3**, 2-hydroxy-3-butenyl; **4**, 2-hydroxy-4-pentenyl; **5**, 2-phenylethyl; **6**, *p*-hydroxybenzyl; **7**, 3-indolylmethyl; **8**, 1-methoxy-3-indolylmethyl.

DISCUSSION

In this study on rosette-stage plants of oilseed rape cv. Bienvenu, indolyl glucosinolates were selectively induced by methyl jasmonate, and 1-methoxy-3-indolylmethylglucosinolate accumulated more than did 3-indolylmethylglucosinolate. Our findings agree with those published recently by Bodnaryk [10], except that in his study, only 3-indolylmethylglucosinolate accumulated in seedlings of oilseed rape cv. Westar. This difference suggests that, like the glucosinolate accumulation that occurs in response to infection [6], the response to MJ can differ between cultivars.

The selective accumulation of indolylglucosinolates in response to MJ closely resembles the response of oilseed rape leaves either to artificial wounding [7, 8] or to infestation of petioles by the cabbage stem flea beetle (*Psylliodes chrysocephala*) [7]; Bodnaryk [10] has suggested that MJ may be involved in the signalling that takes place during the response to these stresses. There is much evidence for a general role for MJ in the signalling that follows wounding or infection [11–15] and MJ is known to be physiologically active in various tissues of *Brassica* species and other crucifers [15–18]. However, the failure of MJ to cause an accumulation of aliphatic or aromatic glucosinolates means that it does not mimic the response of oilseed rape to infection by the fungal pathogen, *Alternaria brassicae* [6], suggesting that additional or other signals must be involved. In another study [19], treating potato disks with MJ also failed to mimic the response to infection, as induced by an elicitor from the fungus, *Phytophthora infestans*. The reduction in growth and the loss of chlorophyll that occurred following treatment of oilseed rape in our study, and of related species in other studies [16, 18], suggest that MJ may not so much signal stress, as cause it directly, at least at high concentrations.

In similar experiments with oilseed rape, we have found that glucosinolates are capable of being induced by a second abiotic elicitor, salicylic acid; this too is selective, inducing only 2-phenylethylglucosinolate [20]. Selective induction of particular types of glucosinolates by these elicitors demonstrates that different controls operate on the biosynthetic pathways of methionine-, phenylalanine- and tryptophan-derived glucosinolates (i.e., aliphatic, aromatic and indolylglucosinolates, respectively).

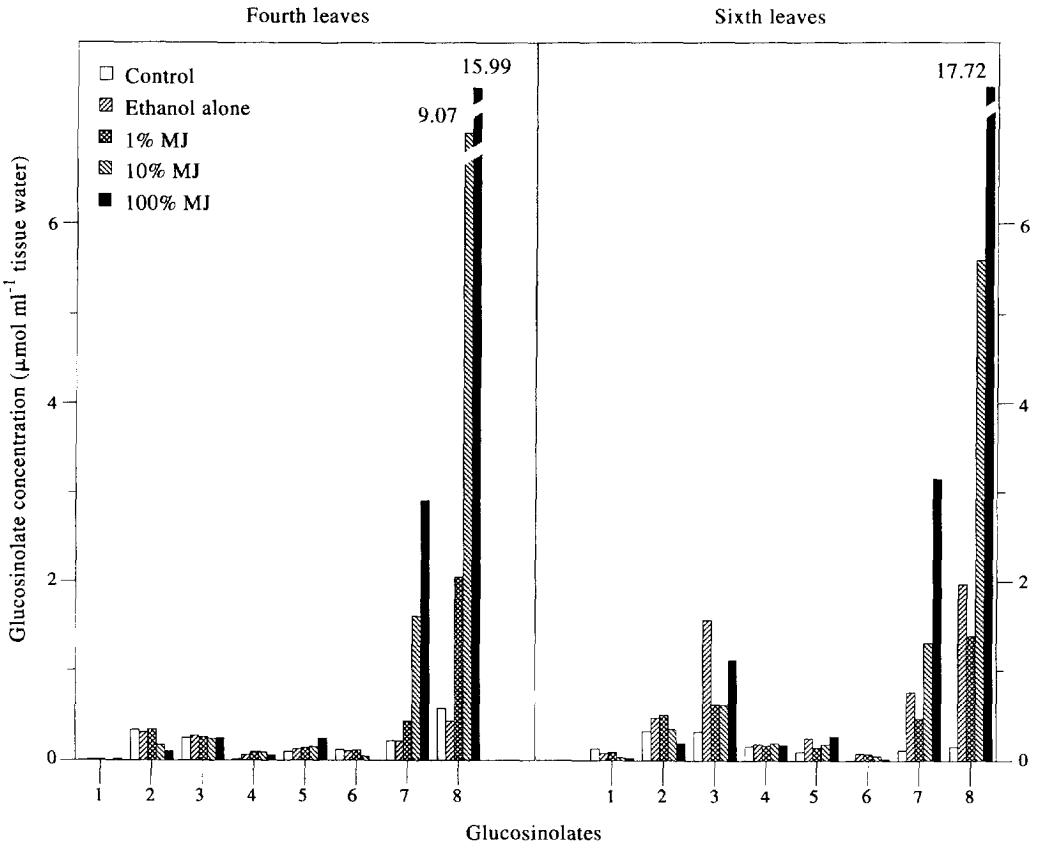

Fig. 2. Concentrations of glucosinolates in fourth and sixth leaves from oilseed rape plants, seven days after initial exposure to three concentrations of volatile MJ. Controls consisted of sterile distilled water or ethanol alone. Glucosinolates: **1**, 3-butenyl; **2**, 4-pentenyl; **3**, 2-hydroxy-3-butenyl; **4**, 2-hydroxy-4-pentenyl; **5**, 2-phenylethyl; **6**, *p*-hydroxybenzyl; **7**, 3-indolylmethyl; **8**, 1-methoxy-3-indolylmethyl.

The induction of glucosinolates by various stresses is obviously a complex response, or series of responses, but MJ (and salicylic acid) may prove useful tools in the study of the biosynthesis of glucosinolates and perhaps also in investigations of their role in resistance to pests and diseases.

EXPERIMENTAL

Plants. Seed of the winter oilseed rape (*B. napus* L.) cv. Bienvenu (low erucic acid, high glucosinolate) was sown in peat based compost in 2 l pots. Seedlings were thinned down to 2 per pot. Plants were maintained in a glasshouse compartment until treatment, and for the period of incubation following treatment, under the following conditions: 18–21°; 16/8 hr light/darkness, with supplementary lighting (400 W sodium lamps) providing light levels up to 280 μmol $m^{-2} s^{-1}$.

Treatment of plants. Plants were treated at growth stage 1.6–1.8 (*ca* 30 days old, 6–8 true leaves expanded [21]), either by spraying them with MJ solns or by exposing them to MJ vapour. In each of 2 replicate expts, plants were sprayed with MJ at 1.5, 15 or 150 mg ml^{-1} in aq. solns containing 0.2% Triton X-100, using a track-mounted electrostatic rotary atomizer [22]. The speed and swath width of the sprayer was such that plants received 2–5 mg MJ each at the highest concn applied. Controls consisted of plants sprayed either with 0.2% Triton X-100 alone or with sterile distilled H_2O. Eight pots (16 plants) were sprayed at each concn or control treatment. Plants were exposed to MJ vapour by placing them in sealed, low-density polythene tanks (capacity 84 l) lined with Al foil to prevent absorption of MJ into the walls of the tanks. Plants were watered before being placed in the tanks. Three pots (6 plants) were placed in each tank. MJ was released into the airspace within the tanks surrounding the test plants by allowing it to evaporate from filter paper disks (7 cm). Immediately before the tanks were sealed, 2 disks in each tank were inoculated with 25 μl each of 1 of the following: pure MJ (giving a vapour phase concn of *ca* 0.6 μg^{-1}), 10 or 1% (w/v) MJ solns in EtOH or EtOH as control. In each of 2 replicate expts, each treatment was tested twice in separate tanks. During 72 hr incubation of plants with MJ vapour, tanks were covered with a glass plate and sealed using a double layer of Lasso tape. Tanks were located in a glasshouse compartment under conditions similar to those under which plants were raised.

Glucosinolate analysis. Fourth (mature) and sixth (developing) leaves were sampled for glucosinolate analysis in all cases 7 days after the time of spraying or after the start of incubation with volatile MJ, respectively. In each expt, two samples were taken for each combination of leaf age and treatment. For plants sprayed with MJ, the 2 samples were taken from separate groups of 4 pots (8 plants) each. For plants exposed to volatile MJ, 2 samples of 6 plants each were taken from separate tanks, and a further set of control samples was taken from plants that had not been incubated in tanks. Leaf samples were weighed, immersed in liquid N_2, freeze-dried and finally milled before storage at $-50°$. Glucosinolates were extracted and measured by HPLC [23]. Glucosinolate levels are expressed as concns in soln in tissue H_2O, which probably reflect best the *in planta* concns confronting herbivores or invading microorganisms. Presented data are mean values from 2 expts.

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REFERENCES

1. Sembdner, G. and Parthier, B. (1993) *Annu. Rev. Plant. Physiol. Plant Mol. Biol.* **44**, 569.
2. Farmer, E. E. and Ryan, C. A. (1990) *Proc. Nat. Acad. Sci. U.S.A.* **87**, 7713.
3. Andresen, I., Becker, W., Schlüter, K., Burges, J., Parthier, B. and Apel, K. (1992) *Plant Mol. Biol.* **19**, 193.
4. Kauss, H., Krause, K. and Jeblick, W. (1992) *Biochem. Biophys. Res. Commun.* **189**, 304.
5. Chew, F. S. (1988) in *Biologically Active Natural Products: Potential Use in Agriculture* (Cutler, H. G., ed.), p. 155. American Chemical Society, Washington D.C.
6. Doughty, K. J., Porter, A. J. R., Morton, A. M., Kiddle, G., Bock, C. H. and Wallsgrove, R. M. (1991) *Ann. Appl. Biol.* **118**, 469.
7. Koritsas, V. M., Lewis, J. A. and Fenwick, G. R. (1991) *Ann. Appl. Biol.* **118**, 209.
8. Bodnaryk, R. P. (1992) *Phytochemistry* **31**, 2671.
9. Mithen, R. F. (1992) *Euphytica* **63**, 71.
10. Bodnaryk, R. P. (1994) *Phytochemistry* **35**, 301.
11. Creelman, R. A., Tierney, M. L. and Mullet, J. E. (1992) *Proc. Nat. Acad. Sci. U.S.A.* **89**, 4938.
12. Farmer, E. E. and Ryan, C. A. (1992) *Plant Cell* **4**, 129.
13. Gundlach, H., Müller, M. J., Kutchan, T. M. and Zenk, M. H. (1992) *Proc. Nat. Acad. Sci. U.S.A.* **89**, 2389.
14. Hildmann, T., Ebneth, M., Peña-Cortés, H., Sánchez-Serrano, J. J., Willmitzer, L. and Prat, S. (1992) *Plant Cell* **4**, 1157.
15. Melan, M. A., Dong, X., Endara, M. E., Davis, K. R., Ausubel, F. M. and Peterman, T. K. (1993) *Plant Physiol.* **101**, 441.
16. Herrmann, G., Lehmann, J., Peterson, A., Sembdner, G., Weidhase, R. A. and Parthier, B. (1989) *J. Plant Physiol.* **134**, 703.
17. Wilen R. W., van Rooijen, J. H., Pearce, D. W., Pharis, R. P., Holbrook, L. A. and Moloney, M. M. (1991) *Plant Physiol.* **95**, 399.
18. Staswick, P. E., Su, W. and Howell, S. H. (1992) *Proc. Nat. Acad. Sci. U.S.A.* **89**, 6837.
19. Choi, D., Bostock, R. M., Avdiushko, S. and Hildebrand, D. F. (1994) *Proc. Nat. Acad. Sci. U.S.A.* **91**, 2329.
20. Kiddle, G. A., Doughty, K. J. and Wallsgrove, R. M. (1994) *J. Exp. Bot.* **45**, 1343.
21. Sylvester-Bradley, R. (1985) *Aspects Appl. Biol.* **10**, 395.
22. Arnold, A. J. and Pye, B. J. (1981) *Proc. Br. Crop Prot. Conf. Pests Diseases* 661.
23. Porter, A. J. R., Morton, A. M., Kiddle, G., Doughty, K. J. and Wallsgrove, R. M. (1991) *Ann. Appl. Biol.* **118**, 461.