CHANGES IN LEVELS OF GLUCOSINOLATES IN LEAVES OF OILSEED RAPE (BRASSICA NAPUS) DURING GROWTH

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INTRODUCTION

Glucosinolates are secondary metabolites present in all species of the genus Brassica. A wealth of information is available on the amount and kind of glucosinolates present in the seed of oilseed rape. In contrast, little information is available on the amount and kind of glucosinolates in the vegetative parts of oilseed rape and their metabolic function remains unresolved. It has been suggested that products released by enzymatic breakdown may function as antifungal agents towards Leptosphaeria maculans (Mithen et al. 1986; Peterka and Schlösser 1989).

Considerable differences in relative amounts of individual glucosinolates were observed between different parts of the same plant (Buchner 1988; Macfarlane Smith and Griffiths 1988; Sang et al. 1984; Uppström 1983). The amounts of individual glucosinolates within plant parts also changed over time with plant development.

Previous studies have been conducted in the greenhouse or growth cabinet. This may not influence the amount of glucosinolates in seedlings, but may considerably influence the amount of glucosinolates in later growth stages. This paper reports preliminary results of a field experiment undertaken to determine the amount and concentration of glucosinolates in leaves of five cultivars of spring-type oilseed rape. At a later stage, these findings will be examined in relation to resistance to L. maculans.

MATERIALS AND METHODS

The experiment was conducted on fallow-sown, spring-type oilseed rape (Brassica napus) at Winnipeg, Manitoba in 1990. Two cultivars with high levels of glucosinolates in the seed: Brutor and Midas, and three cultivars with low levels of glucosinolates in the seed: Legend, Maluka and Westar were arranged in a randomized complete block design with three replications.

From each plot, a sample of at least five plants was taken once or twice a week. Plants were dissected into stem and leaves, weighed and stored at -20 °C. Frozen plant material was ground and suspended in methanol (Macfarlane Smith and Griffiths 1988). After filtration, methanol was removed by rotary evaporation in vacuo. Glucosinolates were desulphated and analyzed by gas chromatography (Daun and McGregor 1981).

RESULTS AND DISCUSSION

Fresh weight (FW) and dry weight (DW) increased for all leaf positions from leaf emergence until leaf senescence. FW at senescence was approximately 0.1, 0.8, 1.5, 3.3, 4.5 and 5.5 g for cotyledon, first leaf, second leaf, third leaf, fourth leaf and fifth leaf, respectively. The ratio DW/FW decreased from 0.085 to 0.055 throughout the observation period.

Large quantities of 2-hydroxy 3-butenyl glucosinolate (HOBUT) and indol 3-ylmethyl glucosinolate (INDOL) were obtained. Smaller quantities of 2-hydroxy 4-pentenyl, 3-butenyl, 4-pentenyl, methoxy indol 3-ylmethyl, and phenylethyl glucosinolate were obtained.

For cultivars with high levels of glucosinolates in the seed, the amount of HOBUT decreased throughout the observation period in the cotyledons and first leaf (Fig. 1). In the second and later leaves, the amount of HOBUT initially increased and subsequently decreased.

For cultivars with low levels of glucosinolates in the seed, the amount of HOBUT was very low in cotyledons, first leaf and second leaf throughout the observation period (Fig. 1). In the third leaf and later leaves, the amount of HOBUT increased slightly before it decreased.

No consistent differences were observed for amount of INDOL between the cultivars with high levels of glucosinolates in the seed and those with low levels in the seed. For all cultivars, amount of INDOL initially increased and subsequently decreased in cotyledons, second leaf, third leaf, fourth leaf and fifth leaf (Fig. 2). Only the first leaf showed a continuous decrease in amount of INDOL.

The amount of HOBUT and INDOL obtained from the cotyledons of Midas closely coincides with the amount reported by McGregor (1988). The amounts of HOBUT obtained in this study coincide with those reported by Uppström (1983) for cultivars with high levels of glucosinolates in the seed as well as for those with low levels in the seed. The amounts of INDOL reported by Uppström (1983) were very variable. Values obtained in this study show less variation and fall within the reported range.

Within each leaf, the amount of glucosinolates changed very rapidly over time. The peak amount of HOBUT and INDOL increased from the first leaf to the fifth leaf, indicating a synthesis of glucosinolates during this period. These results agree with those reported by Buchner (1988) and McGregor (1988). The later decrease in glucosinolates may be due to translocation or enzymatic breakdown of the glucosinolates.

Antifungal activity towards L. maculans is not determined by the amount of glucosinolates in the vegetative tissues, but by the concentration on a fresh weight basis. In addition, the activity depends on the kind of glucosinolate, e.g. HOBUT does not inhibit mycelial growth whereas INDOL has an ED50 of 139 nmol/ml (Mithen et al. 1986). In the cotyledons and all leaves, the concentration of INDOL was highest during the early stages of leaf growth, and rapidly decreased during leaf expansion. The highest concentration of INDOL was 10.4 µmol/g FW in the second leaf of Legend. After nine days, this concentration decreased to 417 nmol/g FW. The observed concentrations of INDOL may inhibit growth of L. maculans when the cell membranes lose their integrity during infection and the glucosinolates are attacked by myrosinase. In addition, INDOL may be involved in the biosynthetic pathway for the production of phytoalexins (Takasugi et al. 1988).

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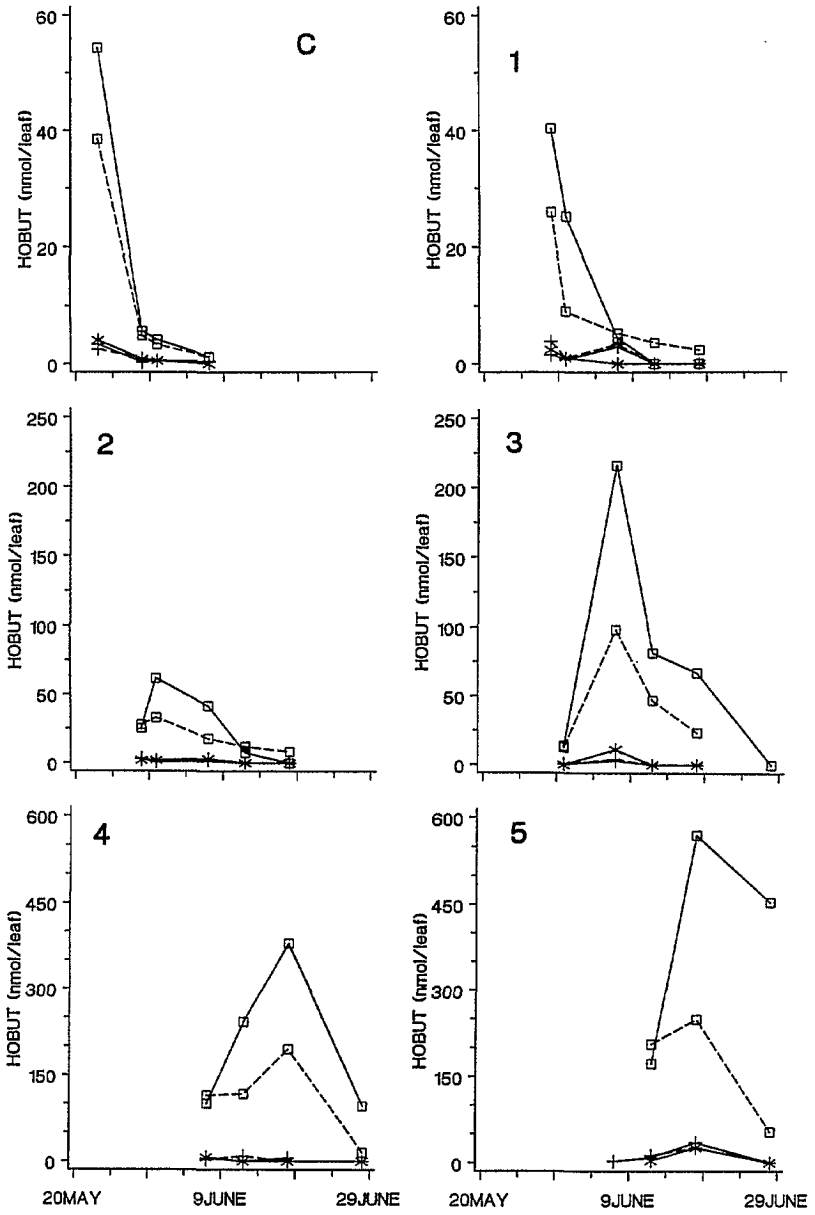

Fig. 1. Amount of 2-hydroxy 3-butenyl glucosinolate (HOBUT) in the cotyledons (C) and five true leaves (1-5) for five cultivars of Brassica napus (□ – – □: Brutor; □——□: Midas; + – – +: Legend; +——+: Maluka; *——*: Westar) on seven observation days.

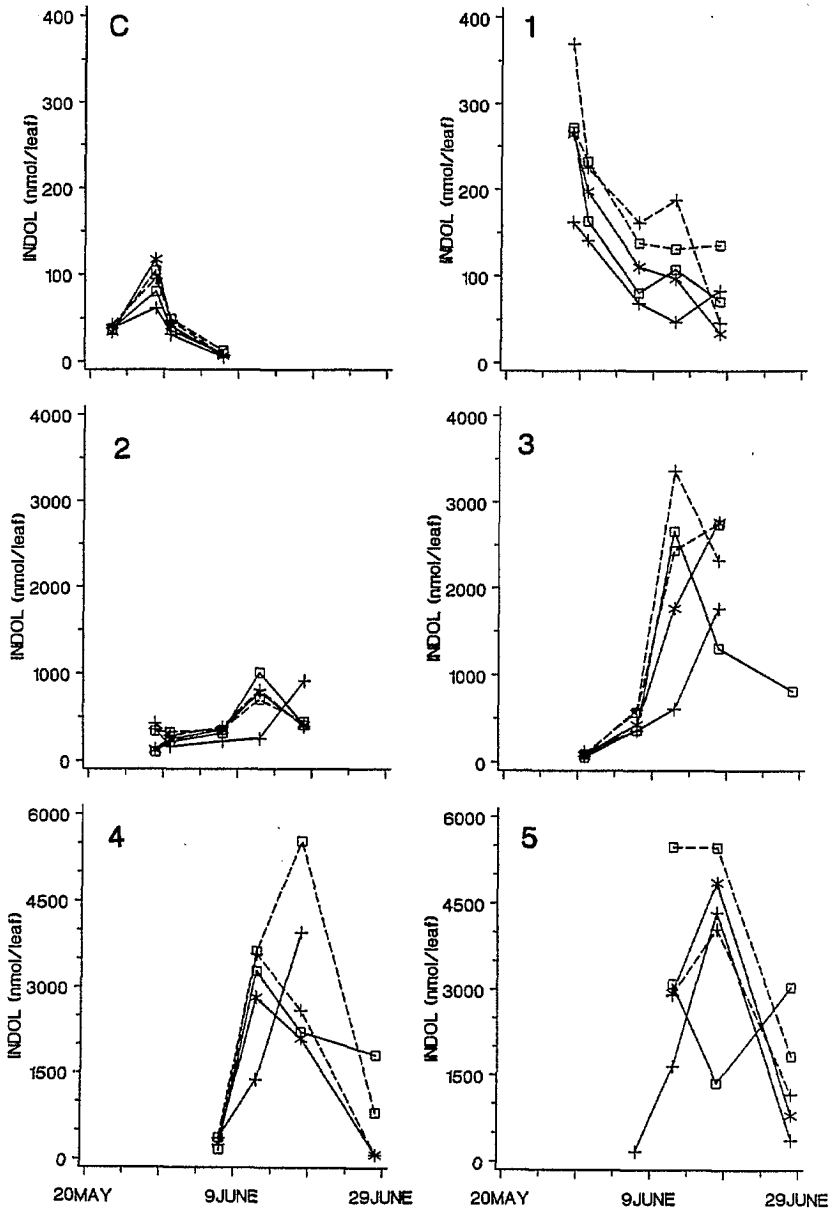

Fig. 2. Amount of indol 3-ylmethyl glucosinolate (INDOL) in the cotyledons (C) and five true leaves (1-5) for five cultivars of Brassica napus (□ – – □: Brutor; □——□: Midas; + – – +: Legend; +——+: Maluka; *——*: Westar) on seven observation days.