GLUCOSINOLATE ACCUMULATION DURING SEED DEVELOPMENT IN WINTER SOWN OILSEED RAPE (B. napus)

J.K. Fieldsend[1], F.E. Murray[2], P.E. Bilsborrow[2], G.F.J.Milford[1] and E.J. Evans[2]

[1] AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts. UK, AL5 2JQ
[2] University of Newcastle upon Tyne, Newcastle Upon Tyne, NE1 7RU

INTRODUCTION

With the introduction of double-low varieties, seed glucosinolate levels in winter oilseed rape grown in Europe have decreased to below 35 μmol g^{-1} and the newest cultivars are rated to be below 20 μmol g^{-1}. However, the variation in seed glucosinolate concentrations both within and between plants (Ludeke & Evans, 1989; Milford et al., 1989) and between sites and seasons often overrides the genetic low seed glucosinolate character. For instance, variety trials in the United Kingdom, sown with the same certified seed, have consistently shown a two to three-fold range across sites in glucosinolate concentrations in the harvested seed in some cultivars in most years (Kimber, 1988). Similarly, in a survey in 1987 of commercial crops of the cv. Ariana grown from seed certified as containing 13-15 μmol of glucosinolate g^{-1} seed, harvested concentrations ranged from 9 to 35 μmol g^{-1} (Anon, 1988). Comparable variation occurs in France (Merrien & Ribaillier, 1988) and Germany (Schnug, 1988).

It has been shown that seed glucosinolate concentrations are little affected by the husbandry practices used to grow (Evans et al. 1989, 1990) or harvest the crop in the UK (Freer et al., 1989). Particular soil factors and seasonal differences in weather are more likely to be responsible for the observed variation. There is evidence from the UK, France and Germany that applications of nitrogen and sulphur, and perhaps natural variations in the supply of these nutrients from the soil, increase seed glucosinolate concentrations (Evans et al., 1990; Merrien and Riballier, 1988; Schnug, 1988).

The mechanisms by which the site and season variation in seed glucosinolate concentrations and the responses to nitrogen and sulphur occur are not properly understood. Theoretically, concentrations may vary because different amounts of glucosinolate are produced or accumulate in seed, or because glucosinolates are more or less diluted by seed dry mass as a result of faster or more prolonged seed growth. Little is known of the extent to which changes in each of these components of concentration contribute to the observed site and season variation, since up till now measurements on field crops have largely been confined to the final concentrations present in mature seed at harvest. This paper describes some initial measurements on the dynamics of growth and glucosinolate accumulation in the seed of crops at different sites. Such information is essential for a proper physiological understanding of the processes involved.

MATERIALS AND METHODS

The measurements of the patterns of growth and glucosinolate accumulation during normal seed development were made on a series of experimental crops of the cv. Ariana grown at Cockle Park Research Station in the north of the UK and at Rothamsted Experimental Station in the south in 1987/88. The crops were grown according to good commercial practice and fully protected against pest and diseases. Other measurements were made on similar crops of Ariana at Rothamsted in 1989 that were either swathed or were desiccated with diquat (0.60 kg ion ha^{-1} in 520 litres) at different stages during seed development.

In each experiment, the basal 10 pods of the main-stem raceme were collected at intervals from the start of flowering (defined as the time that 50% of the plants had at least one open flower), starting when seeds could easily be dissected from the developing pods. The collected pods were either immediately frozen and lyophilised or gently dried at 45 °C prior to removal of seed, previous measurements having shown that neither method of drying deleteriously affected seed glucosinolates. Seeds were counted and their dry weights and glucosinolate concentrations measured. At both sites, the HPLC protocol of Heaney et al. (1986) was used for glucosinolates, supplemented at Rothamsted, with measurements by the glucose-release method of Heaney et al. (1986) and at Newcastle by X-ray florescence (Schnug & Haneklaus, 1988). Common sets of samples were analysed at each laboratory by each method to ensure that good conformity and uniformity of measurement was maintained throughout the study.

RESULTS AND DISCUSSION

Seed growth

In 1988, flowering started on 2 May at Rothamsted and on 6 May at Newcastle and the patterns of seed growth were very similar at both sites when compared at similar times from the start of flowering, (Fig. 1). Extrapolation of the seed growth curves indicates that the linear phase of rapid seed growth would probably have started within 25-30 days from the start of flowering and was completed within 80 days. The overall durations of the periods of linear seed growth were between 40 and 50 days at mean rates of between 95 and 105 μg DM $seed^{-1}$ day^{-1}. These resulted in final seed weights of between 4.9 and 5.4 mg.

Glucosinolate accumulation during seed growth

In contrast to the seed growth, there were distinct differences between sites in the patterns of glucosinolate accumulation (Fig. 2). After a lag, seed glucosinolate concentrations increased almost linearly with seed age. At Newcastle, the phase of linear increase in concentration started 30 days after the start of flowering and lasted for a further 30 days so that glucosinolate concentrations remained more or less constant for the final 30 days of seed growth. At Rothamsted, concentrations started to increase only after 45 days from the start of flowering and continued to increase for a further 45 days, almost up to seed maturity (Fig. 3). During the linear phase of change, glucosinolates increased, on average by about

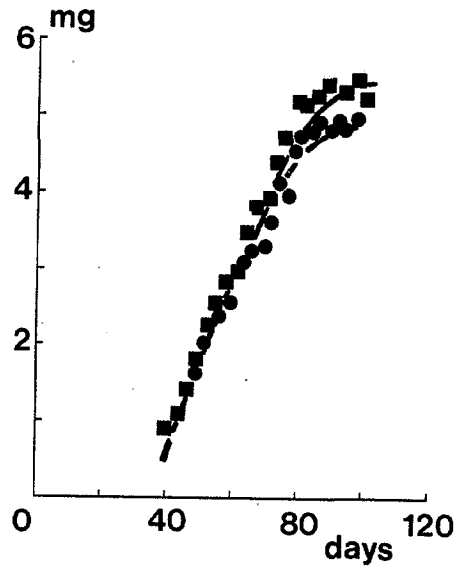

Fig. 1. Changes in seed dry mass with days after the start of flowering at Rothamsted (●) and Newcastle (■).

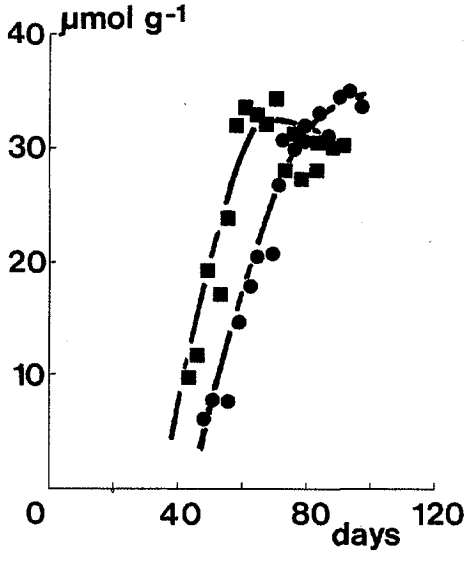

Fig. 2. Changes in glucosinolate concentration (μmol g^{-1} DM) with days from the start of flowering at Rothamsted (●) and Newcastle (■).

1.3 μmol g^{-1} day^{-1} at Newcastle and by about 0.9 μmol g^{-1} day^{-1} at Rothamsted. Nevertheless, the very different patterns of glucosinolate accumulation resulted in similar final concentrations in the mature seed (33-35 μmol g^{-1}) because the faster rate of increase in concentration at Newcastle was offset by the shorter period during seed growth. The changes in glucosinolate concentration during seed growth observed at Newcastle are similar to those reported for German crops by Buchner (1988) and those of Rothamsted are similar to patterns observed in France by Merrien and Ribaillier (1988).

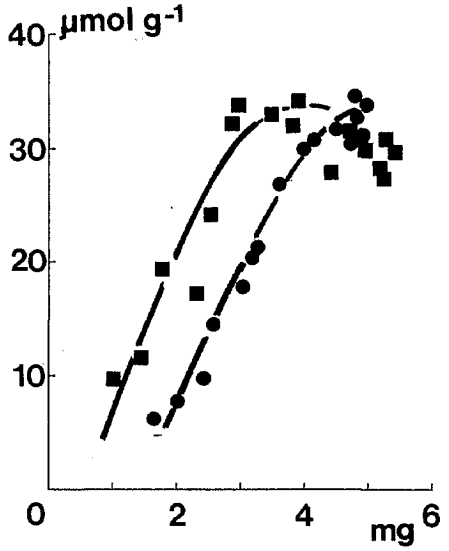

Fig. 3. Changes in glucosinolate concentration (μmol g^{-1} DM) as a function of dry mass during seed growth at Rothamsted (●) and Newcastle (■).

Effects of harvesting practices

Glucosinolates only accumulated in seed during active seed growth and not during seed maturation at the two sites. However, the occurrence of different patterns of glucosinolate accumulation in seed with similar patterns of seed dry matter growth suggests that the processes of growth and glucosinolate accumulation are not strongly interdependent and are probably affected by different environmental or physiological factors.

Further evidence that glucosinolate accumulation can be uncoupled from seed growth was obtained from an experiment at Rothamsted in which crops were swathed or were desiccated with diquat at different stages of seed growth (Fig. 4). Seed growth was immediately stopped by both treatments when they were applied at early or very early stages of seed development, i.e. at 70 and 60 days from the start of flowering respectively. Both treatments also induced large and rapid increases in the concentrations of glucosinolates.

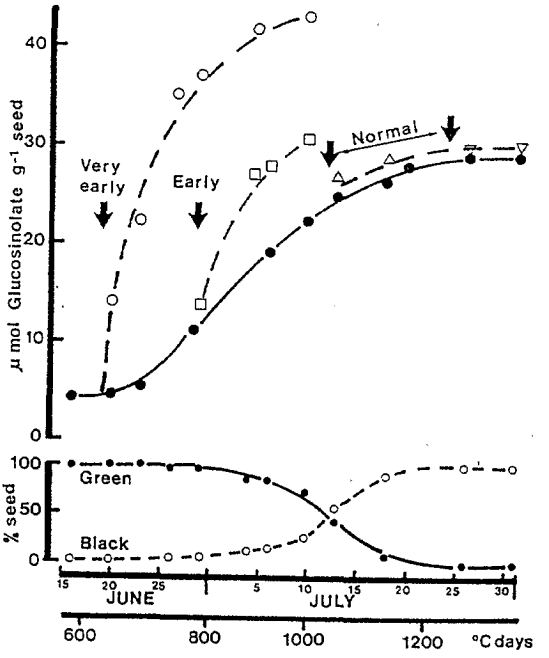

Fig. 4. Effects on seed glucosinolate concentrations of desiccation with diquat at different stages of seed development. Seed maturation indicated by the proportions of green and black seed.

During the 5 days following treatment, the desiccant increased seed glucosinolate concentrations by about 40 μmol g^{-1} when applied very early in seed growth in mid June and, and by 15 μmol g^{-1} when applied in early July. During the 5 days following the mid June treatment, the absolute amounts of glucosinolate increased by over 100 nmol per seed compared with 40 nmol per seed in the control crop, and by 60 nmol per seed compared with 35 nmol per seed following the early July treatment. Neither swathing nor desiccation had deleterious effects on seed growth or seed glucosinolate concentrations if applied when seeds were mature.

CONCLUSIONS

These detailed and coordinated studies of the dynamics of seed growth and glucosinolate accumulation have shown that the patterns of glucosinolate accumulation can differ in crops with similar patterns of seed growth at different sites and yet result in similar final concentrations of glucosinolate in mature seed. Corresponding data from crops grown in different seasons, but not reported here, show similar independence of seed growth and glucosinolate accumulation that result in differences in glucosinolate concentrations in harvested seed. It is likely that seed growth and glucosinolate accumulation are each influenced by different edaphic and environmental factors.

REFERENCES

BUCHNER, R. 1988. Analyse und Biologie der Glucosinolate in Raps (Brassica napus L.). Ph D Thesis, University of Gottingen.

EVANS, E.J., BILSBORROW, P.E., LUDECKE, F.H., MILFORD, G.F.J., FIELDSEND, J.K., RAWLINSON, C.J. and SPINK, J. 1989. Variation in seed glucosinolate levels as influenced by agronomic inputs. Aspects of Applied Biology 23: 151-158.

EVANS, E.J., BILSBORROW, MILFORD, G.F.J., FIELDSEND, J.K., PORTER, A.J.R. and RAWLINSON, C.J. 1990. Agronomic factors influencing the glucosinolate content of double-low winter-sown oilseed rape in the United Kingdom. Groupe Consultatif International de Recherche sue le Colza, Bulletin 6: 80-84.

FREER, J.B.S., OGILVY, S.E., EVANS, E.J., BILSBORROW, P.E., RAWLINSON, C.J., MILFORD, G.F.J., and FIELDSEND, J.K. 1989. The effects of time and method of harvest on seed yield and glucosinolate concentrations of winter oilseed rape. Aspects of Applied Biology 23: 145-150.

HEANEY, R.K., SPINKS, E.A., HANLEY, A.B. and FENWICK, G.R. 1986. Analysis of glucosinolates in rapeseed. Technical Bulletin, AFRC Food Research Institute, Norwich. 22 p.

KIMBER, D.S. 1988. Glucosinolate levels obtained in 1987 in the UK. Proceedings of the Double-low Rapeseed Forum, London 1988: 38-40.

LUDECKE, F.H. and EVANS, E.J. 1989. Seed glucosinolate content in winter oilseed rape (B. napus) as influenced by sampling technique. Aspects of Applied Biology 23: 159-164.

MERRIEN, A. and RIBAILLIER, D. 1988. Les glucosinolates chez le colza d'hiver. CETIOM Bulletin 99: 10-14.

MILFORD, G.F.J.,FIELDSEND, J.K., SPINK, J. and CHILD, R.D. 1989. Between and within plant variation in seed glucosinolate concentrations in the winter oilseed rape cv. Ariana. Aspects of Applied Biology 23: 173-176.

SCHNUG, E. 1988. Fluctuations in the glucosinolate content in seeds of 0 and 00 oilseed rape. Proceedings of the Double-low Rapeseed Forum, London 1988: 38-42.

SCHNUG, E. and HANEKLAUS, S. 1988. Theoretical principles of the indirect determination of the total glucosinolate content in rapeseed and meal by quantifying the sulphur concentrations via X-ray fluorescence (X-RF method). J. Sci. Fd. Agric. 45: 243-254.