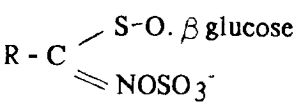
GCIRCBUL7*18

Factors that determine levels of glucosinolates in oilseed rape

G.F.J. MILFORD and Jane K. FIELDSEND
AFRC Institute of Arable Crops Research, Rothamsted Experimental Station, Harpenden, Herts. AL5 2JQ
E.J. EVANS and P.B. BILSBORROW, Department of Agriculture,
University of Newcastle upon Tyne, Newcastle upon Tyne, NE1 7RU

Glucosinolates are compounds which occur in all brassicas including cabbage, brussel sprouts and oilseed rape. All glucosinolates have the general structure:

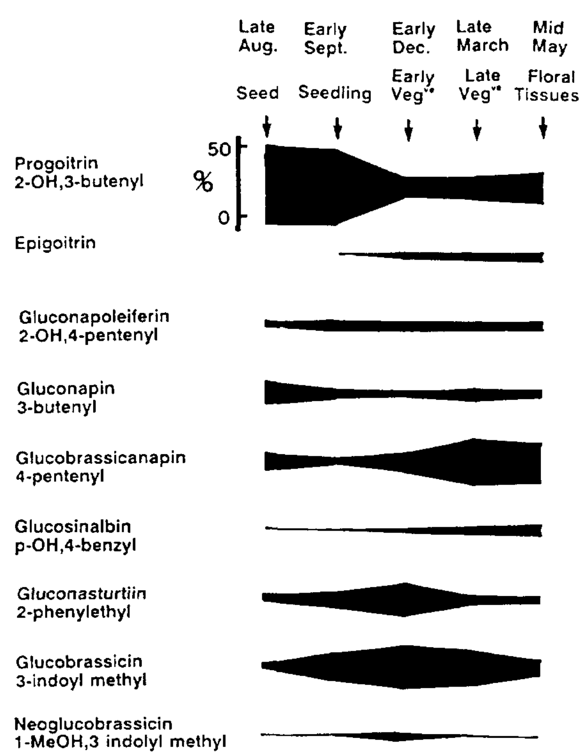


but differences in the side chain, R, create a range of distinct compounds. R might be an alkenyl, aromatic of indolyl group. Different brassicas and even different tissues in the same plant contain different types or concentrations of glucosinolates. The functions of glucosinolates are not known, but they are thought to be important in protecting plants against pests and diseases. In vegetables they contribute to flavour.

Growers of oilseed rape are interested in glucosinolates for entirely different reasons; they wish to ensure that the average concentration in their harvested seed is below an EEC-imposed limit. This is currently 35 micromol g-1 of seed but is likely to decrease to 20 micromol g-1 in 1992. The limit is set because high concentrations of glucosinolates in the rapemeal by-product that is left when oil is extracted can have harmful toxicological effects if the meal is fed to animals. However, low glucosinolates make the rapemeal potentially as valuable as the oil.

The level of seed glucosinolates in oilseed rape is under genetic control and new low-glucosinolate varieties have been developed which are assisting growers to meet the EEC quality criteria. However, there is now increasing evidence that environmental factors can over-ride the genetic factor and lead to levels of glucosinolates that exceed the proposed limits. Evidence for this has come from a joint, MAFF-funded study involving scientists from the Crop Management Department of IACR-Rothamsted and the Department of Agriculture, University of Newcastle-upon-Tyne. This study has shown that considerable variation occurs between sites and seasons in seed glucosinolate concentrations even in the new low-glucosinolate material entering the national variety lists. Figure 1 provides an example of a twofold range in seed glucosinolates that occurred when the same double-low variety, Ariana, was grown at Rothamsted in successive years. Agronomic trials have shown that little of the variation is attributable to the husbandry used to grow the crop, implying that certain combinations of soil and weather are likely to be responsible. Nutrition appears to be important because applications of nitrogen and sulphur fertilisers, and perhaps variations in the ability of soil to supply these nutrients, increase glucosinolate concentrations, but it is not clear how.

Fig. 1: Seasonal differences in glucosinolate concentrations in developing rapeseed



Interest in the mechanisms by which differences in seed glucosinolates develop are centring on the inferface between the pod wall and the seed which may act as a barrier to the production or relocation of glucosinolates or their precursors to the seed. It has not yet been conclusively demonstrated whether the seeds themselves are capable of synthesising glucosinolates and current work on glucosinolate biosynthesis at IACR-Rothamsted aims to do this.

Glucosinolates normally increase in amount and concentration during seed grothw and not during seed maturation (Fig.2). However, seed growth and glucosinolate accumulation can be uncoupled. If a plant desiccant, such as diquat, is applied very early in the pod development stage when seed are still growing they greatly increase seed glucosinolate concentrations. The effect is smaller the later in seed growth the desiccant is applied, and there is no effect when seed are mature (Fig. 2) The increase in concentration results from the dual effects of an accelerated accumulation of glucosinolates in the seed -there may be a fivefold increase in rate within a day of the application of the desiccant - and an inhibition of seed dry matter growth. A consequence is that seed of treated plants may remain very small yet contain similar amounts of glucosinolate as seed of untreated plants. This suggests that glucosinolates, or glucosinolate precursors, approximating in quantity to the content of mature seed are present in the seeds, pod wall or adjacent tissues at the early stages of pod development and their relocation or synthesis is accelerated by pod dehydration, maturation or senescence.

Because of the role that glucosinolates may have in protecting brassicas, there is concern that the move to low glucosinolate varieties will lead to an increased incidence of pests and diseases in oilseed rape crops. However, the research has shown that, except perhaps for the very early seedling stage, there is no immediately obvious relationship between concentrations of glucosinolates in the seed and those in the plants that are grown from them. Both the types and concentrations of glucosinolates present in the different tissues of the oilseed rape plant change with time (see Fig.3). Other measurements have shown that, despite large differences in seed concentrations, total glucosinolate concentrations in the vegetative and floral tissues of the first generation of commercial double-low varieties are similar to those in the older single-low types. However, preliminary data indicate that some second generation double-low varieties that have very low seed glucosinolate concentrations also have low concentrations in vegetative tissues. Whether such varieties are more susceptible to pests and diseases has not yet been tested.

Glucosinolates are primarily located within cell vacuoles. Measurements of glucosinolate concentrations expressed on a tissue water basis, which closely reflect vacuolar concentrations, have shown that the concentrations of some, but not all, compounds are within the range known to be toxic to certain pathogens *in vitro* (Table 1). The tissue water concentrations, which range from 30 to 600 µM for individual compounds, can change from toxic to non-toxic concentrations at different stages of growth. Other researchers, also at IACR, are attempting to relate these changes with disease incidence.

Table 1: Tissue water concentrations (µM) of glucosinolates in leaves of the oilsed rape cv. Ariana and the effective concentrations (ED 50) at which particular pathogens are known to be controlled by specific glucosinolates.

	Early vegetative stage	Late vegetative stage	ED_{50}
Glucobrassicanapin	282	478	
Progoitrin	128	162	
Gluconapin	34	125	
Gluconapoleiferin	80	35	186⁺
Epiprogoitrin	51	91	
Gluconasturtiin	350	68	100*
Glucobrassicin	650	222	142⁺
Neoglucobrassicin	26	0	189⁺

⁺ *Leptosphaeria maculans*
* *Alternaria spp*

This research has revealed the complex nature of the interactions between the environment and genetic factors determining seed glucosinolate levels in oilseed rape. The strong influence of site and season on seed glucosinolate concentrations has implications for future methods of regulating the quality of rapeseed. The results suggest that reliance on seed certification of low glucosinolate varieties without testing harvested seed may not lead to desired improvements in the quality of rapemeal. Certification rests on the assumption that genetic factors will always outweigh environmental influences but the research clearly shows that this is not always so. It also indicates that tests of the glucosinolate content of vegetative tissues might be advisable during breeding programmes if the natural immunity to pests and diseases that is thought to be conferred by glucosinolates is not to be lost as breeders strive for even lower levels of glucosinolates in seed.

Fig. 2: Changes in glucosinolates concentrations during seed development and the effects of different times of crop desiccation. Crops are usually mature at 1100-1200°C days from 50% flowering when most of the seed is black.

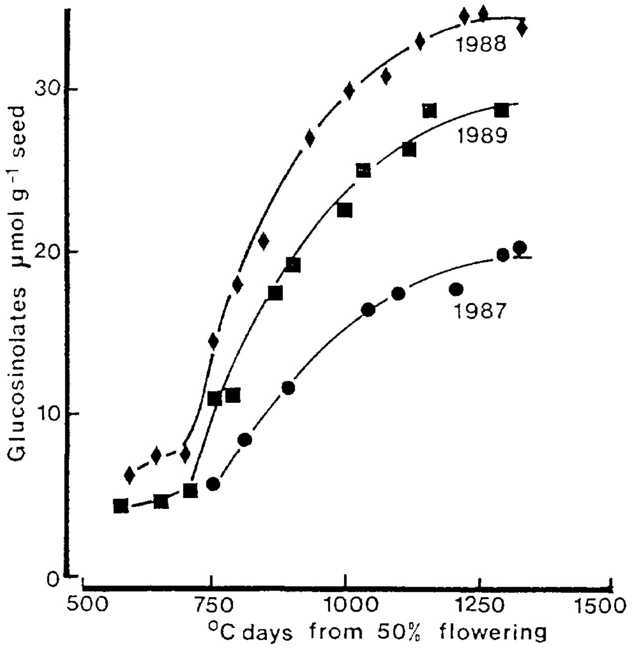


Fig. 3: Changes in the types and concentrations of glucosinolates in vegetative and floral tissues during the development of oilseed rape.