STUDIES OF INSECTS ASSOCIATED WITH CRUCIFERS: HOST PLANT SELECTION AS A FUNCTION OF ALLELOCHEMICS PRESENT IN THE PLANTS. PROBLEMS RELATED TO RAPE CONTAINING HIGH AND LOW CONCENTRATIONS OF GLUCOSINOLATES.

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Summary.

Investigations of the insect fauna associated with oilseed rape have been performed. Particular attention has been assigned to the possibilities of changes in the insect pest problems caused by changes from growing rape with a high glucosinolate content to growing the new double low rape varieties. The studies have comprised field and semi-field experiments with the blossom beetle (Meligethes aeneus F.), the cabbage seed weevil (Ceutorhynchus assimilis Payk.) and the Brassica pod midge (Dasyneura brassicae Winn).

Relative damage caused by these insects on several new double low rape varieties was compared to corresponding damage on rape containing high concentrations of glucosinolates by counting the insect attacks throughout the growth season. Determination of allelochemics including glucosinolates and carboxylic acids present in the plants has been performed using different parts of the plants harvested at different times during the growth season.

Introduction.

A number of insects feed solely on plants belonging to the family Cruciferae. These plants are known especially for their content of glucosinolates and thioglucoside glucohydrolase EC 3.2.3.1 (myrosinase). When the plants are crushed glucosinolates and myrosinases are mixed, and a number of different autolysis products are formed[1].

Many insects recognise their host plants at least partially by their content of glucosinolates. Glucosinolates stimulate feeding and (or) oviposition, while some of the volatile hydrolysis products (e.g. isothiocyanates) attract the insects from some distance. On the other hand glucosinolates are toxic to many insects which do not normally feed on plants containing glucosinolates[2]. Thus, glucosinolates can be considered as defensive plant compounds, but a few insects have been adapted metabolically and behaviourally to these compounds, and now they utilise them as host specific cues.

It might therefore be expected that the new cultivars of spring rape with low contents of glucosinolates would be less acceptable to insects which are adapted to glucosinolates, but on the other hand more susceptible to attack from non-adapted species.

Investigations on these problems have been performed during 1982. Focus has been placed on the most important pests of oilseed rape in Denmark, the blossom beetle (Meligethes aeneus F.), the cabbage seed weevil (Ceutorhynchus assimilis Payk.), and the Brassica pod midge (Dasyneura brassicae Winn.). Because of their phenology the seed weevil and the pod midge are particularly important on winter rape crops, but their responses to spring rape cultivars are investigated in the present study as winter rape cultivars with low contents of glucosinolates are not yet available.

These three insect species are crucifer-specialists, as they reproduce only on plants belonging to this family. Blossom beetles feed on nectar and pollen from a number of plant species, but they oviposit only on crucifers[3]. The behavioural responses of these three insect species to allelochemics from crucifers have not been very well investigated compared to those of other crucifer-specialists. They are attracted to some hydrolysis products of glucosinolates[2], but the role played by glucosinolates in feeding and oviposition behaviour has not been studied. Åhman[4] did not detect any difference in infestation levels of the pod midge in oilseed rape cultivars with high and low contents of glucosinolates. Nothing is known about the effect of other allelochemics on host plant selection of these insect species.

In the same way little is known about the content of glucosinolates or other allelochemics in plant parts of oilseed rape attacked by insects. In the present study the relative acceptability of a number of spring rape cultivars was evaluated. Chemical analyses were performed at relevant growth stages of plants grown under conditions exactly identical to those used in the experiments with insects.

Materials and Methods.

Experiments with blossom beetles and seed weevils were performed in cages under semi-field conditions. Plants were grown in 15 l buckets containing a standard soil mixture. Plants and buckets were covered with a nylon netting. Details on the design of these cage experiments, growing conditions of plants etc. will be given elsewhere[5].

In each bucket a high glucosinolate reference cultivar, Gulliver (G) was grown together with a low glucosinolate test cultivar. These were the Brassica napus cultivars Erglu (E), Karat (K), Line (L), and Mary (M), and the B. campestris cultivar Candle (C).

At various growth stages of the plant[6] insects were introduced to the cages. Four experimental series were carried out:

1) blossom beetles were introduced at growth stage 3.1 (Inflorescence visible at centre of rosette). Numbers of beetles on each of the two cultivars were counted 2-3 times each day during one week by visual inspection through the netting.
2) blossom beetles were introduced at growth stage 4.1 (First flower open). Insect counts were made as in series 1.
3) blossom beetles were introduced at growth stage 3.1. They stayed in the cages until normal harvest time when the number of podless stalks was counted.
4) seed weevils were introduced at growth stage 4.3 (Lower pods starting to fill). Insect counts were made as in series 1. After two weeks the insects were removed. At normal harvest time the number of pods with larval exit holes were counted.

Different densities of beetles were used: 5/plant in series 1 and 4, 10/plant in series 2,and 2/plant in series 3.

All experiments were run in five replicates. Plants were used only once.

Plants used for chemical analyses were freeze dried and stored at -20 °C. Extractions were performed in a way excluding autolysis. The plant constituents were separated into well defined groups by column chromatography (unpubl. results). Total glucosinolate content was determined by the amount of glucose released from purified glucosinolate fractions after treatment with myrosinase, while individual glucosinolates were determined by HPLC[7]. Analysis of carboxylic acids and other quantitatively dominating compounds was performed by use of traditional methods including HVE, PC, GC, and HPLC.

Results and Discussion.

The glucosinolate concentrations of green parts of oilseed rape are very low (Fig. 1). In leaves they are below 0.5 µmole/g dry weight, while concentrations in 12 other crucifers ranged between 15 and 200 µmole/g dry weight[8]. There are great variations in glucosinolate concentrations depending on the growth stage of the plant[9] (Fig. 1). Generally the concentrations are higher in inflorescences than in leaves.

The concentrations of glucosinolates in seeds are about 10 times higher in Gulliver than in the other cultivars tested[10]. In green parts the differences are less pronounced, but the highest concentrations are invariably found in Gulliver (Figs. 1 and 2). Indolyl glucosinolates are the quantitatively dominating glucosinolates in green parts (unpubl. results).

No positive correlation was found between glucosinolate levels and the acceptability of the cultivars to blossom beetles (Fig. 2). At growth stage 3.1 more beetles were observed on Candle than on Gulliver, even though Candle had the lowest glucosinolate level of all the investigated cultivars. The small differences between beetle numbers counted on the B. napus cultivars were not correlated with glucosinolate concentrations. On stage 4.1 no significant differences were found in numbers of beetles counted on different cultivars even though the differences in glucosinolate levels were still apparent (Fig. 2 B).

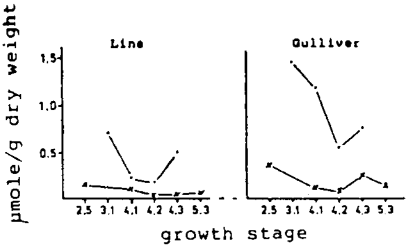

Fig. 1. Glucosinolate content in two cultivars of oilseed rape at different growth stages

x—x: leaves •—• : inflorescences

Neither is there any correlation between glucosinolate levels and the number of podless stalks. A distinction was made between terminal podless stalks which are situated above the uppermost fertile pod and non-terminal podless stalks which are situated below the uppermost fertile pod. In Fig. 2 C the relative numbers of non-terminal podless stalks are shown as a function of glucosinolate concentrations found

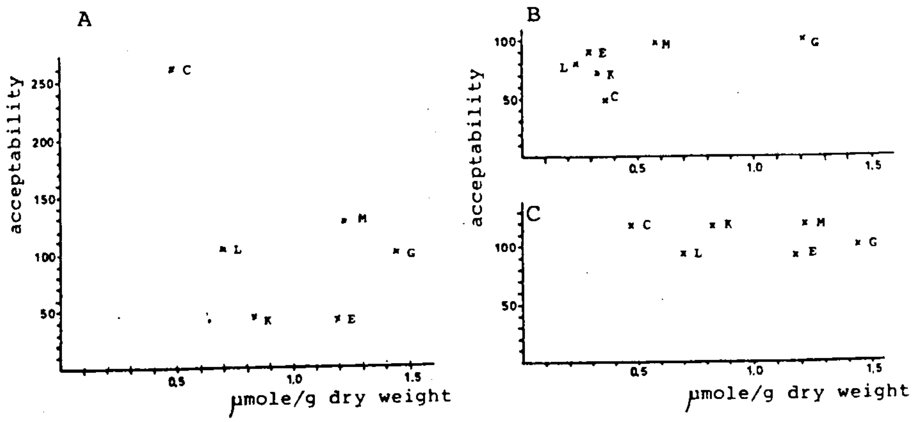

Fig. 2. Relations between the relative acceptability of cultivars of oilseed rape (Gulliver = 100) to blossom beetles and the glucosinolate content of inflorescences.

A: Growth stage 3.1; acceptability estimate based on counts of adult beetles (series 1)

B: Growth stage 4.1; acceptability estimate based on counts of adult beetles (series 2)

C: Growth stage 3.1; acceptability estimate based on number of non-terminal podless stalks.

in buds at the time when beetles were introduced. The number of non-terminal podless stalks is better correlated with beetle counts than the total number of podless stalks, and it is probably a better estimate of beetle damage as a number of terminal buds never develop into pods even in the absence of attack by blossom beetles[11].

Even in the absence of blossom beetle attack we found some cultivar differences in the number of podless stalks (unpubl. results). Counts of adult beetles may also be misleading if beetles are counted when they are only resting on the plants without actually feeding on them. Thus, there is a need for more adequate methods for detecting differences in susceptibility of plants to attack by blossom beetles.

In Fig. 3 two estimates of relative acceptability of cultivars to the seed weevil are compared. No significant differences are found between Gulliver and any of the test cultivars with either of these estimates. However, the two estimates are fairly well correlated.

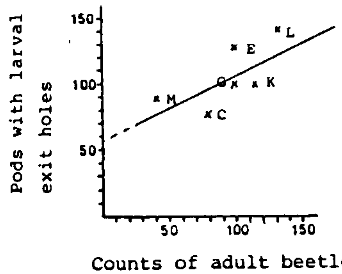

Fig. 3. Relation between two estimates of the relative acceptability of oilseed rape cultivars (Gulliver = 100) to seed weevils

We have yet no final data on the concentrations of glucosinolates in pod walls used as oviposition sites for seed weevils, but when whole inflorescences are examined the usual cultivar differences are apparent (Fig. 1). Line is at least as acceptable as Gulliver (Fig. 3) and thus there are no indications that the seed weevils prefer cultivars with high glucosinolate concentrations.

In the present study it was possible to relate very closely biological and chemical data. However, the glucosinolate levels found under these semi-field conditions may be different from those found in plants grown in the field.

When the same cultivars (except Karat) were grown in the field, no differences were found in infestation levels of seed weevils and pod midges, but the infestation levels were low. The relative acceptability of cultivars at growth stages 3.1 and 4.1 were very similar in field and semi-field experiments.

Winfield[12] did not detect any differences between mustard and oilseed rape species in their susceptibility to attack by blossom beetles. Differences in glucosinolate levels in buds of species of oilseed rape and mustard are higher than those found between oilseed rape cultivars in the present study (unpubl. results). Our failure to detect any differences between cultivars related to glucosinolate levels may therefore not be very surprising. However, if blossom beetles recognise their host plants partially by means of glucosinolates, as many other crucifer-specialists do[2], then it is reasonable to presume that there is a lower detection limit. It seems as if the concentrations found in cultivars included in the present study are still above this limit.

In the semi-field experiments a negative correlation was found between the acceptability of oilseed rape cultivars to blossom beetles and the presence of certain carboxylic acids. At present we do not know whether this is merely coincidence or whether these compounds do affect beetle behaviour or performance.

Further studies are being conducted to evaluate the role played by glucosinolates and other allelochemics in host plant selection of blossom beetles and seed weevils. These studies proceed mainly along two lines: 1) chemical studies e.g. determinations of concentrations of individual glucosinolates and other allelochemics and 2) behavioural studies, where insect responses to pure compounds and plant extracts are investigated.

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