THE UNIVERSITY *of* EDINBURGH

Cabbage and turnip root flies on resistant and susceptible Brassicas: host selection and chemical interactions.

by

Richard James Hopkins BSc

PhD

University of Edinburgh

1994

DEDICATION

I dedicate this thesis to my wife, Amanda, without whose love and support it would never have come to fruition.

ACKNOWLEDGEMENTS

I acknowledge the overall financial support of The Ministry of Agriculture Fisheries & Food (Studentship AE 8373) and The British Council, The John Fife Memorial Trust and the James Rennie Bequest for supplying travel grants for trips to Switzerland and Holland. I would like to thank Nick Birch of The Scottish Crop Research Institute and Rod McKinlay of SAC for their supervision and admirable patience, which requires no further testing. In addition I must thank those who have gone out of their way to give help, advice and support. Wynne Griffith of SCRI, for constant advice and guidance on chemical analysis and fractionation. The selection of hplc equipment and protocols, performance of injections and interpretation of output associated with hplc analysis are all the work of Wynne Griffith. Ian Morrison of SCRI for explanations, guidance and use of facilities within the Fibres Department at SCRI. Crop Genetics at SCRI for the provision of many of the seeds and advice on sowing. Erich Städler and Robert Baur of The Swiss Federal Research Station, Wädenswil for helpful advice and the production of the electrophysiological data. Frank Wright of SASS for spending so much of his personal time guiding the analysis of the behavioural data. In addition Tony Hunter, Christine Hackett and Jo Hall (now moved on) of SASS for advice on experimental design and in the case of Christine and Jo for writing all the Genstat programs which were used to analyse much of the data. The technical support of the staff at SAC and SCRI who gave what help they could when ever they could. The moral support of Amanda, our families and our friends (particularly Paul Partner and Alisdair Nisbet who had to suffer the shame and indignity of sharing office space). Finally the staff of the former British Leyland for building one Austin Maestro which was reliable and durable, I know because I drove that car.

ABSTRACT

During post-alighting behaviour gravid female turnip root fly, *D.floralis*, select a plant for oviposition predominantly during the initial landing phase; the cabbage root fly, *D.radicum*, also utilise the leaf resting phase. The post-alighting behaviour exhibited by *D.radicum* and *D.floralis* infers that oviposition site selection is primarily based upon positive stimuli present on the leaf surface. Ranking of four genotypes of plants for antixenotic resistance to oviposition by *D.radicum* and *D.floralis* was found to be the same for both fly species, tested in the laboratory (swede cv Doon Major, most susceptible; kale cv Fribor, most resistant) and varied *x*80 (*D.floralis*) and *x*5 (*D.radicum*). Field experiments showed that oviposition (which was dominated by *D.radicum*) varied *x*2 between plant genotypes (swede cv Doon Major, most susceptible; swede cv GRL aga, most resistant). Testing of Brassica leaf surface extracts, applied to surrogate plants, indicated that leaf surface chemicals strongly influence the site of oviposition of *D.floralis*. Methanol soluble polar compounds are the most stimulatory element for *D.floralis* and a fraction which contained aliphatic glucosinolates stimulated oviposition strongly although glucosinolates were not the primary oviposition stimulant. Collaborative experiments indicate that "CIF" (cabbage identification factor) is probably present in this fraction. The concentrations of Brassica root sugars are generally reduced by the damage of both *D.radicum* and *D.floralis* and appear to influence larval development. The percentages of plant fibre and lignin in the roots of Brassicas rise following the damage of *D.floralis*. The concentrations of individual glucosinolates in Brassica roots are radically altered by the damage of *D.floralis* and *D.radicum*. *D.floralis* damage resulted in a rise in the concentration of aromatic glucosinolates and a fall in the concentration of aliphatic glucosinolates. *D.radicum* damage generally resulted in an elevated concentration of both aliphatic and aromatic glucosinolates. There was no clear evidence that glucosinolate profiles were associated with different levels of antibiotic resistance to *D.radicum* and *D.floralis*. GRL aga (SCRI breeding line) was consistently resistant to the oviposition and larval feeding of *D.radicum* and *D.floralis* both in the laboratory and in the field. It was shown that the use of end-of-season chemical analysis to assess the influence of plant chemistry on insect development or host plant resistance in field experiments and the use of damage indexes based on the percentage of a plant root damaged by *D.radicum* may be flawed.

KEY WORDS

Delia radicum(L.), *Delia floralis*(Fall.), Diptera:Anthomyiidae, Brassicaceae, host-plant resistance, insect behaviour,

The species worked on in this thesis

Insects

Diptera:Anthomyiidae

cabbage root fly	*Delia radicum*(L.)
turnip root fly	*Delia floralis*(Fall.)

Plants

Brassicaceae

swede	*Brassica napus*(L.)
kale	*Brassica oleracea*(L.)
rape	*Brassica napus*(L.)

CONTENTS

GENERAL INTRODUCTION

> *All trees, it may be said, have worms, but some less, as fig and apple, some more, as pear. Speaking generally, those least liable to be worm-eaten are those which have a bitter acrid juice.*
>
> Theophrastus, Enquiry into Plants, Book IV, c. 300 BC (Taken from an English translation by Hort 1916, p391)

Theophrastus recorded the earliest known comments on the natural variations in the susceptibility of plants to insect attack and established that not all feeding substrata are equally acceptable to phytophagous insects. The association between the bitter juice of the least damaged fruit and the insect damage links the chemical composition of the feeding substrata with the insects, the essence of resistance. The term resistance has been the subject of a number of definitions, frequently depending upon the circumstances under which the term is defined and may denote different viewpoints of the same events (Horber, 1980). The definition of Painter (1951) placed the stress on the heritable characteristics of a plant.

> "*Resistance* of plants to insect attack may be defined as the relative amount of heritable qualities possessed by the plant which influence the ultimate degree of damage done by the insect. In practical agriculture it represents the ability of a certain variety to produce a larger crop of good quality than do ordinary varieties at the same level of insect population." (Painter, 1951)

Beck (1965) defined plant resistance as "the collective heritable characteristics by which a plant species, race, clone or individual may reduce the probability of successful utilisation of that plant as a host by an insect species, race, biotype, or individual" (Beck 1965). Although essentially similar the definition utilised by Beck (1965) varies most from that of Painter (1951) in that it does not include tolerance. Painter (1941, 1951) believed tolerance to be one of three interrelated characteristics that combined to constitute an overall level of resistance. Painter (1941) explored the nature of resistance in insect-plant interactions and divided resistance to attack by insects into preference, antibiosis and tolerance for which brief notations were included.

"insect **preference** for oviposition, food or shelter"
"**Tolerance**, repair, recovery or ability to withstand infestation"
"**Antibiosis**, adverse effect of plant on biology of the insect"

Painter (1941) proposed the term antibiosis and provided a clear definition.

Antibiosis, "for those adverse effects on the insect life history which result when a resistant host variety or species is used for food. The effects in the insect take the form of reduced fecundity, decreased size, abnormal length of life, and increased mortality".

Painter (1951) expanded and defined preference or non-preference and tolerance, whilst antibiosis retained the above definition.

"**Tolerance**, is a basis of resistance in which the plant shows an ability to grow and reproduce itself or to repair injury to a marked degree in spite of supporting a population approximately equal to that damaging a susceptible host." (Painter, 1951)

"**Preference** or **non-preference** is used to denote the group of plant characters and insect responses that lead to or away from the use of a particular plant or variety, for oviposition, for food or for shelter, or for a combination of the three. Anthropomorphic connotations should not be read into this terminology." (Painter, 1951)

The term non-preference was latterly considered to be unsuitable, due to it being a description of the insect behaviour, and was superseded by the term antixenosis (Kogan & Ortman, 1978). Kogan & Ortman (1978) proposed the term antixenosis to be parallel to antibiosis and in the place of non-preference, but did not provide a formal definition. For the purposes of this thesis the term antixenosis is defined below based on the literature cited above.

Antixenosis, plant properties resulting in negative reactions (non-preference) or total avoidance by insects (Painter, 1941; 1951; Beck, 1965; Kogan & Ortman, 1978; Horber, 1980).

Antixenosis is influenced by plant characteristics, including the effect of volatile leaf and root chemicals in attracting insects to plants, the surface chemicals of the plant and the structure and physical nature of the leaf surfaces. Antibiosis is influenced by factors such as the root hardness, dry matter content, plant fibre component and the concentrations of primary and secondary plant metabolites. Tolerance may be expressed by root factors that discourage deeper larval penetration, by the rapid healing of wounds or by compensatory plant growth. In practical agricultural terms resistance usually reflects the ability of a plant genotype to produce a yield of greater size or quality than would other genotypes exposed to the same

insect population. Resistance is the sum of the interaction of many factors and a high level of resistance may be due to a single large factor or the sum of several factors. Resistance from the viewpoint of the insect can be taken as a reflection of the adequacy of the host for the purposes of the insect. In the case of *Delia radicum* and *Delia floralis* Crucifers are a source of food for larval development. The feeding of the larvae of *D.radicum* and *D.floralis* can result in extensive damage to the roots of Brassica crops (Plates I.1. to I.3.).

The perception of the host plant by the gravid females dictates the site of *D.radicum* and *D.floralis* larval development, which may be strongly influenced by antixenosis. The newly hatched larva must locate the host plant and antixenosis may also play a role in this process. Having located the plant, the development of the larva is influenced by the quality of the host, an antibiotic factor, or the plant may respond in order to compensate for any damage done, a tolerance factor. The manner in which the parts combine to make up the whole is critical to the nature of any mechanism of resistance. *D.radicum* and *D.floralis* are both oligophagous species, the larvae of which feed on a wide range of plants from the Cruciferae (Finch & Ackley, 1977; Ruuth, 1988). The aim of any viable mechanisms of resistance against *D.radicum* and *D.floralis* must be to reduce the occurrence and extent of damage caused by larvae of the two fly species to agricultural Crucifers. Factors which deter oviposition or make the host suboptimal for the larvae may also make the host unsuitable for the consumer and hence are not viable as a resistance mechanism for agricultural crops.

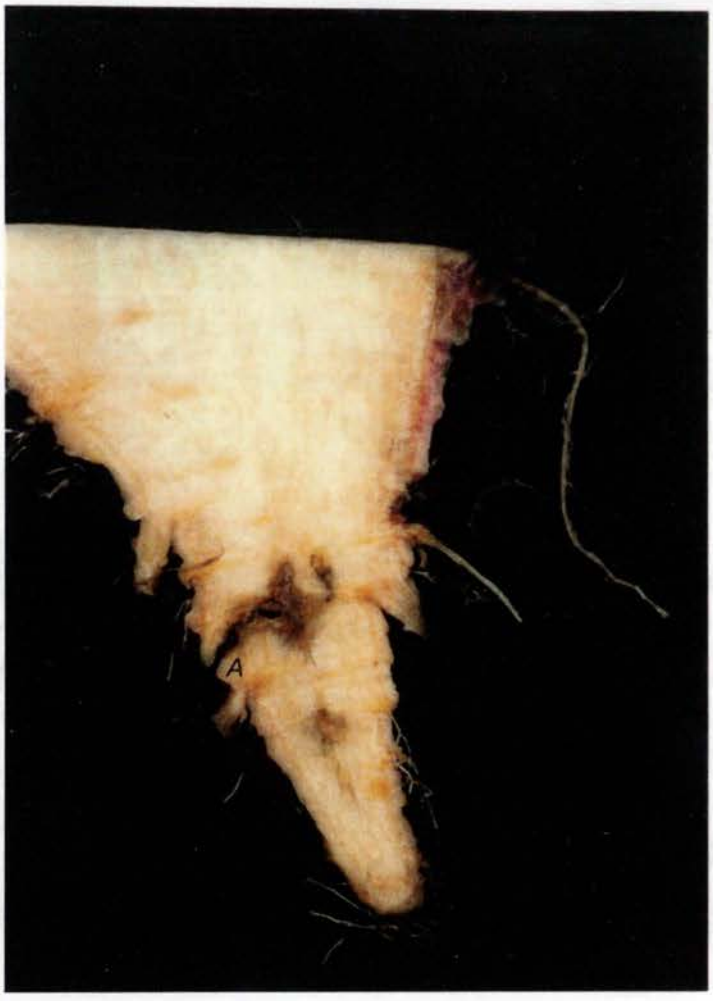
Recognition of the pest status of *D.radicum* and *D.floralis* is a much more recent historical event than Theophrastus. Although it will undoubtedly have been appreciated that Crucifers were damaged and killed by insects since agriculture evolved no record could be found before the middle of the last century. Milburn (1843) outlined the range of insects which were injurious to turnips. This account included note of "ash-grey flies resembling houseflies, but somewhat smaller", which were believed to be "*Anthomyia brassicae* of Bouche". Milburn (1843) noted the destructive power of the root flies and also attributed to them transmission of secondary infestations. The description given in the text is consistent with physical form and damage caused by *D.radicum* and *D.floralis*. The location of the farm on which the observations were made, Yorkshire, would indicate that *D.radicum* was probably the insect concerned. MacDougall (1902) described the existence of a fly which is much more likely to be *D.floralis*. The larvae concerned formed galleries in the roots of swedes and the damage could be traced by a brown trail of decayed material along the route of the maggots' progress.

Within the host range of *D.radicum* and *D.floralis*, the different plants may be distinguished from non-hosts by characters such as structure, leaf shape and secondary plant compounds.

Plate I.1. The surface mining damage of cabbage root fly (*Delia radicum*) larvae on the bulb of a swede plant.

Plate I.2. A cross section of the surface mining damage of the cabbage root fly (*Delia radicum*) on the bulb of a swede plant. Note the extensive purple colouration of the tissue surrounding the damage.

Plate I.3. A cross section of the damage of the turnip root fly (*Delia floralis*) within the bulb of a swede plant. The small entry hole (A) leads to a relatively large internal mine.



Secondary plant compounds have disputed functions: one school of thought holds them to be functional defensive compounds which have evolved in response to the assault of pests and pathogens; the other holds the stance that they are secondary metabolites with other ecological or physiological functions which have a fortuitous effect (Rausher, 1992). It is becoming accepted that neither view is universally correct. The chemical ecology of insect-plant relationships are not all part of one strict law (Futuyma & Keese, 1992). Glucosinolates have been widely linked to the host range and behaviour of insect pests (Louda & Mole, 1991). Städler (1992) reviewed the behavioural responses of insects to secondary plant compounds and cited more than twenty insects which responded to glucosinolates or their volatile hydrolysis products. The effect of glucosinolates or their volatile hydrolysis products on oviposition has been extensively demonstrated in the Lepidoptera (e.g. Reed *et al*, 1989; Traynier & Truscott, 1991) and Diptera (e.g. Städler, 1978; Roessingh *et al*, 1992b). More recent work has shown that other compounds, previously undetected, are more active stimulators of insect behaviour than the glucosinolates with which Crucifer feeding insects are usually linked (Roessingh *et al*, 1992a).

The aim of this project is to identify and quantify sources and mechanisms of natural resistance to *D.floralis* and *D.radicum* and to evaluate how they might be utilised to increase host plant resistance.

Structure of the thesis.

Structurally the thesis is divided into three sections (Figure I.1.):

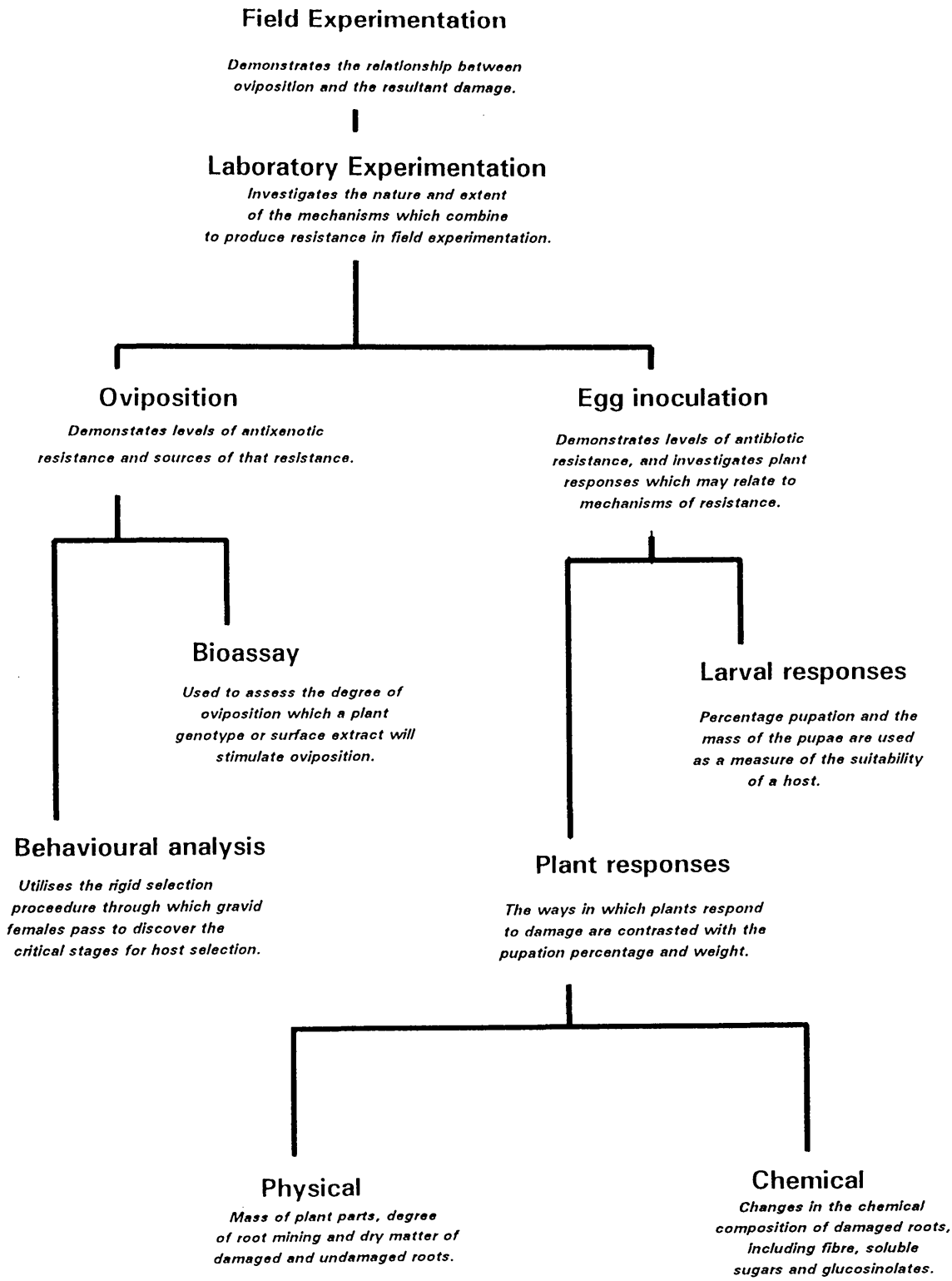
Section A Laboratory studies of antixenotic resistance to *D.floralis* and *D.radicum*.

Section B Laboratory studies of antibiotic resistance to and tolerance of *D.floralis* and *D.radicum*.

Section C The effectiveness of antixenotic and antibiotic resistance to *D.radicum* and *D.floralis* in the field.

Each section contains a brief introduction, literature review, and final discussion of the results achieved in individual chapters. Chapters address specific experimental aims, reviewing relevant literature and discussing the results.

Figure I.1. The structure of the thesis.



SECTION A

LABORATORY INVESTIGATION OF ANTIXENOTIC RESISTANCE TO OVIPOSITION BY *Delia floralis* AND *Delia radicum*

SECTION INTRODUCTION

In a series of field trials between 1955 and 1958, Swailes (1959) found that the numbers of root fly pupae found in a row of swedes could vary nine-fold between plant genotypes. Monitoring of oviposition attributed a part of the resistance to cabbage root fly as being due to antixenosis. The site of larval development for *Delia radicum* and *Delia floralis* is dictated by the gravid females. Gravid root fly females are highly mobile and are capable of powered flight over moderate distances. Finch & Skinner (1975b) demonstrated that some *D.radicum* females migrated up to 1000m per day between mating and oviposition. The search that an insect undertakes for an oviposition site or a food item can be divided in a number of different ways. The most widely accepted is to divide the search into habitat-finding, host finding, host recognition, host acceptance and host suitability. However, the functional changes between many of these stages are difficult to distinguish and there are advantages to simply classify into pre-alighting and post-alighting behaviour (Jones, 1992).

The pre-alighting behaviour of *D.radicum* has been extensively studied. Hawkes & Coaker (1976) utilised ^{32}P-marked flies to demonstrate that only gravid females moved upwind into a host crop that was in the vicinity. They also carried out tests in a wind tunnel which confirmed that orientation to host plant odour was taking place, but that the response was frequently more of a flight upwards than upwind. Finch & Skinner (1982) released marked *D.radicum* at the centre of a circular grass area of 100m radius, surrounded by a 10m wide area of swedes or grass. Using traps containing water or baited with allyl-isothiocyanate it was demonstrated that gravid *D.radicum* females fly upwind without prior stimulation by odours from either a host crop or allyl-isothiocyanate trap. Ellis *et al* (1980) linked *D.radicum* host preference and plant odour. Radish plants exposed to *D.radicum* showed a changing cycle of attractiveness which reached its peak when plants were close to marketable age. Plants that were selected for the high *D.radicum* preference population were at their peak of attraction earlier than those selected for low preference. The preference was correlated with two glucosinolate hydrolysis products, 4-methylthio-3-butenyl-isothiocyanate and 1-cyano-4-methyl-thio-3-butene, that were detected in the ether extracts of macerated radishes made from roots plus hypocotyls. Nottingham & Coaker (1985) showed that *D.radicum* females use anemotaxis both on the ground and in flight to locate odour sources and increase upwind flight in response to

increasing odour. The flight of *D.radicum* females in diffuse odour clouds largely comprised straight fast flights within 30° of upwind and contrasted with discrete plumes in which flights were slower and changed direction more frequently (Nottingham & Coaker, 1987).

During pre-alighting behaviour, *D.radicum* detects plants within host patches by visual stimuli, using leaf area and colour (Prokopy *et al*, 1983a). The ability to distinguish between the different coloured leaf mimics remained unaffected by the nature of the background against which they were placed. The contribution of visual stimuli in eliciting female landing on individual plants within a patch decreased as the distance between plants within a patch was increased. Glasshouse experiments suggested that the final stages of landing site selection were entirely visual, and operated from as far away as 40-60 cm. *D.floralis* males and non-gravid females fly and land preferentially at a height of circa 25cm (Havukkala, 1987). Gravid females fly at a height of 15cm and arrive at the host by a series of 2m upwind flights landing and reorientating between flights.

On finding a potential host, the gravid females of both root fly species carry out a host selection procedure. During host selection, gravid females progress from the leaf to the base of the plant where the eggs are laid. It can be assumed that during host selection the progress of the female is dependent upon receiving the correct stimuli. Positive chemical and/or physical cues will result in continuation of the host selection procedure. If the correct positive cues are not present or if negative cues of sufficient strength are encountered then the potential host plant will be rejected. Städler (1978) demonstrated receptors for sugars and salt on tarsal sensory hairs of female *D.radicum* and characterised a receptor cell with a threshold sensitivity to sinigrin, a glucosinolate, of below 0.0001M. Leaf surface extracts of kale and Chinese cabbage stimulated differing levels of oviposition from *D.floralis* (Alborn *et al*, 1985). It was concluded that antixenotic resistance could control *D.floralis* damage and that the chemical characteristics of a plant were of crucial importance in the selection of an oviposition site. Tarsal contact, acting in synergism with the presence of plant volatiles, was considered the crucial factor in host selection. Plant volatiles alone could not stimulate *D.floralis* to oviposit and experiments on leaf steam washings and methanol extracts were inconclusive (Alborn *et al*, 1985).

Havukkala & Virtanen (1985) elicited a six point behavioural sequence of host plant selection and oviposition for *D.floralis*. The sequence was : (1) Landing; (2) extension of proboscis and examination of the leaf surface; (3) walking over the leaf; (4) running down the stem; (5) walking on the ground at the base of the stem; (6) oviposition. Observations of the behaviour (Havukkala & Virtanen, 1985) found that the most likely site for rejection was the stem run

on a susceptible plant and the leaf walk on a surrogate plant. Flies spent 50% less time walking on the leaves of the model than on those of real plants, but 15 times longer running down and up the stem. The time to proboscis extension was shortened by tarsal contact with sinigrin or allyl-isothiocyanate. Approximately half the flies that progressed onto the stem proceeded to oviposit (29% of the flies that landed on the turnip).

The deterrent effect of the carboxylic acid group on ovipositing *D.radicum* was demonstrated by Cole *et al* (1989) based upon work on the deterrent effect of garden pebble moth frass (Jones & Finch, 1987; Jones *et al*, 1988). Carboxylic acids and compounds which shared various structural features with them were tested to find their effect on otherwise acceptable cauliflower plants. Oviposition deterrence was linked to molecules that contained one or more carboxylic groups and additional carboxylic groups in the molecule did not increase the effect. Studies of *D.radicum* by Städler & Schoni (1990) on surrogate plants (made of plastic coated in paraffin wax) led to the evolution of an ethogram that described the behavioural events of an oviposition sequence. Volatile leaf components produced no detectable effect on gravid *D.radicum*, whilst fractions based on hexane, butanol and water all stimulated oviposition behaviour. Compounds being used in conjunction with surrogate plants retained their potency for 10 days, and it was concluded that the compounds involved were stable and not very volatile. Females discriminated between control and extract treated surrogate leaves just after landing, in the early phases of the oviposition behaviour sequence. The behavioural sequence of gravid *D.radicum* was shown to be the same on natural host plants and surrogate plants. The authors concluded that the stimulation of oviposition was a multi-component function.

Surface extracts of cauliflower leaves contain approximately 60 μM per gram leaf equivalent (gle) of glucosinolates (Roessingh *et al*, 1992b). Testing of leaf surface extracts showed a significant correlation between behavioural discrimination by *D.radicum* and electrophysiological results from tarsal contact receptors. However, a large variation was found in the responses of *D.radicum* to different glucosinolates. The structure of the glucosinolates was unrelated to the stimulation of oviposition, but the length of the glucosinolate side-chain did correlate with its biological activity. It was concluded that compounds other than glucosinolates were also involved in stimulating oviposition by *D.radicum*.

In addition to the chemical aspects of host selection, Roessingh & Städler (1990) noted that physical factors can influence host selection. Gravid *D.radicum* laid most of their eggs around the base of bright green or yellow models, preferring them to red or blue models. The structure of the surrogate plant which received most eggs was with a stem, vertical leaf folds

and a thin covering of paraffin wax, which appeared to be preferred to the smoother surfaces of other coatings tested. Vertical folds of the surrogate leaf surface increased the probability of a behavioural transition from leaf run to stem run and were believed to equate to leaf veins which aid the orientation of flies on real plants. Increasing the surface area increased the number of eggs laid, but not the number of eggs per unit area.

The published data on the host selection sequences of *D.radicum* and *D.floralis* show them to be very similar, and they can be summarised as follows :

PHASE 1: Olfactory stimuli take effect at a relatively close range, which for *D.radicum* varies between 5-15m (Prokopy *et al*, 1983a) and 24m (Hawkes *et al*, 1978). Gravid females approach the prospective host plant, flying up the vapour plume in short bursts of 0.5m for *D.radicum* (Hawkes *et al*, 1978), and 2m for *D.floralis* (Havukkala, 1987), reorientating into the wind between flights (Havukkala, 1987; Hawkes *et al*, 1978; Prokopy *et al*, 1983a). Both males and females fly 15-25cm above the ground, with gravid females flying on average 10cm lower than males or non-gravid females (Havukkala, 1987). Hawkes *et al* (1978) showed that on leaving the vapour plume whilst overshooting the source or moving across the airflow, the majority of *D.radicum* turn downwind, circle and re-enter the plume. Host volatiles including allyl-isothiocyanate have long been known to attract gravid females of both species (Traynier, 1967a). Anemotaxis is used by *D.radicum* females both in flight and on the ground to locate odour sources, upwind flight increasing in response to plant odour (Nottingham & Coaker, 1985). In diffuse odour clouds, *D.radicum* females performed fast straight flights within 30° of upwind whilst in discrete plumes flights were slower and changed direction more frequently (Nottingham & Coaker, 1987).

PHASE 2: In both species, the curved approach associated with anemotaxis in Phase 1 straightens in the last 40-60cm, in what is believed to be a visual response (Havukkala , 1987; Prokopy *et al*, 1983a), leading to landing. Prokopy *et al* (1983a) showed that although females of *D.radicum* could respond at 40-60 cm, a more normal distance for visual responses to take effect was 25cm. In addition to this, work with artificial leaves showed that *D.radicum* demonstrated a degree of discrimination between surrogate leaves, painted in different shades of green to mimic different Brassicas (Prokopy *et al*, 1983b). This work infers that colour (reflected wavelengths of light) plays a part in *D.radicum* oviposition site selection.

PHASE 3 : *D.radicum* and *D.floralis* extend the proboscis onto the leaf surface (a behaviour which may also take place on the stem or at the base of the plant). Although the reason for the

contact with the plant surface remains unknown at present, it is likely that this is a part of the process of contact chemoreception. *D.radicum* has a set of contact chemoreceptors on the proboscis which are sensitive to salt, sugar, water and a number of glucosinolates (Städler, 1978). It is believed that these are specialised for food discrimination. Similar chemoreceptors are present on the proboscis of *D.floralis* (Simmonds, personal communication).

PHASE 4 : Gravid females walk on leaf surfaces or margins, interspersed with pauses or the extension of the proboscis. If the female accepts the potential host up to this point, the fly proceeds down the leaf by following either the leaf margin or one of the leaf veins to the plant stem. The presence of chemoreceptive hairs on the tarsi of female *D.radicum* has been recognised for a long time (Städler, 1978), and it is likely that chemicals sensed in this manner are a part of the same mechanism that includes those detected during the third phase.

PHASE 5 : After reaching the stem, the gravid female walks down, sometimes halting or changing direction, but if the host is acceptable continuing towards the stem base.

PHASE 6 : On reaching the base of the stem, the female fly carries out a circumventive walk around the base of the plant, facing head downwards.

PHASE 7 : On reaching the ground by the base of the stem, the female fly may dig in the soil.

PHASE 8 : Probing may take place between soil particles with the ovipositor.

PHASE 9 : If the female reaches this stage with all necessary stimuli, and no strong deterrents have been encountered, oviposition takes place (Prokopy *et al*, 1983a; Havukkala, 1987; Städler & Schoni, 1989).

PHASE 10 : At any point in the above, the fly may leave the plant.

It can be assumed that during these stages the female carries out a selection procedure, based upon positive and negative, chemical and/or physical cues. The demonstration of tarsal receptors in *D.radicum* (Städler, 1978) has been followed by a large body of published evidence on the influence of host plant chemistry on its oviposition (Jones & Finch, 1987; Jones *et al*, 1988; Cole *et al* 1989; Roessingh & Städler, 1990; Städler & Schoni, 1990; Roessingh *et al*, 1992a; 1992b). The type and concentrations of chemicals encountered at a specific stage may lead to the gravid female continuing to the next stage or rejecting the prospective host. This section investigates differences in the post-alighting oviposition site

selection behaviour of, and the importance of different chemical fractions to, ovipositing *D.radicum* and *D.floralis*.

Chapter A1

The effect of resistant and susceptible Brassica genotypes on the post-alighting behaviour of gravid *Delia floralis* females selecting a host plant for oviposition

INTRODUCTION

The aerial surface of a plant is frequently the interface between insects and their potential host. The diversity of the leaf surface of plants is wide and the genotypic variation in structure is influenced by a number of factors. The surface of the plant may be glaucous, smooth or textured, be hairless or bear hairs which have a wide range of structural types (Jeffree, 1986). The environment in which the plant grows, the temperature, humidity and biotic factors, all interact to modify the surface structure and chemistry of the plant (Baker, 1974; 1982). The variation in the surface of the plant, which results from the interaction of genotype and environment, dictates the stimuli which the insect receives when it makes contact with the surface of the plant.

Contact chemoreception of the plant surface is widely accepted as an important element in the interaction between plants and phytophagous insects (Feeny, 1992; Frazier, 1992; Städler, 1992). Whilst in contact with the surface of a potential host plant, insects explore the environment utilising a range of receptors. During this exploration, the movements on and across the surface of the plant constitute a series of behavioural events. The post-alighting behaviour of *D.floralis* during host selection, as outlined with that of *D.radicum* in the Section Introduction, will include host recognition, host acceptance and host suitability (Jones, 1992). The correct cues for these stages must be received if an insect is to progress through the entire behavioural sequence and oviposit. The progress of the gravid female to an ensuing event is dependent upon the correct stimuli being encountered. The probability of *D.floralis* ovipositing on different plant genotypes varies considerably (Rygg & Sömme, 1971; Ruuth, 1988; Chapter A3). Consequently, the behavioural sequence carried out by gravid females on plants susceptible to oviposition must vary from that carried out on those resistant to oviposition (i.e. possessing antixenotic resistance).

D.floralis discriminates between plants when laying eggs (Rygg & Sömme, 1971; Ruuth, 1988; Chapter A3) and as a consequence it must be assumed that the gravid female progresses towards oviposition in response to one or more cues. Therefore, the discernible responses of gravid females at specific stages in host selection will reflect the presence, absence and potentially the extent and nature of chemical and physical stimulants or deterrents which the female has encountered.

Havukkala & Virtanen (1985) studied the behavioural sequence of *D.floralis* and the effect of host stimuli on host selection. It was concluded that the leaf walk was the most important stage in host selection on surrogate plants and that the stem run was the most important stage in host selection on real plants. However, these conclusions were based upon the percentage of insects on each plant part which rejected within any one stage and upon only partially presented results. The high percentage of flies which rejected on the stem run represented a smaller number of flies than those which had already rejected the plant by that stage. No conclusions were drawn from the fact that insects rejecting after the leaf walk spent longer on the leaf of the plant that those which proceeded onto the stem. The study carried out on *D.floralis* by Havukkala & Virtanen (1985) indicated that variation did occur in the behaviour of *D.floralis* on different potential hosts. However, no publications could be found which contrasted the detailed post-alighting oviposition host selection behaviour of *D.floralis* on known resistant and susceptible plants.

Behavioural analysis of *D.floralis* has two main objectives. Firstly, the point at which resistant genotypes are rejected can be identified, providing indications as to where to look for chemical or physical resistance factors. If the majority of flies rejecting a resistant plant genotype do so at a particular stage in the behavioural sequence, then interest may be focused on the steps which immediately precede rejection. Secondly, the time period spent on a particular phase (duration) may have important inferences for the nature of stimuli for which the insect is searching. For example, when considering a behavioural event, the ensuing behaviour may be classified most simply as either rejection or continued exploration of the potential host plant. The behavioural event which follows immediately after the event being analysed is termed the ensuing behaviour. If the duration of the behaviour being analysed is greater for flies that reject the plant as the ensuing behaviour, than for flies that continue to explore the plant, it suggests the search is for positive cues. Consequently, the rejection may be due to compound(s) being present in insufficient quantities or absent altogether. Failure of gravid females to locate specific cue(s) after a given time threshold may lead to the rejection of that plant. If searching for cues of a specific threshold, longer is likely be spent for an unsuccessful than for a successful search. Consequently, if the flies which reject a plant after a specific stage do so following a shorter period of time than flies which proceed to the next stage, it can be assumed that they have encountered a chemical or physical deterrent factor. If there is no variation in the time spent on a decisive phase in the host plant selection it may be that a quantitative assessment of the leaf surface is taking place rather than assessment on a threshold or on a presence or absence basis.

The experiments in this chapter investigate variations in the behavioural sequence of *D.floralis* on four selected plant genotypes, which have been shown to possess differing levels of antixenotic resistance or susceptibility in previous field and laboratory experiments (Ruuth, 1988; Birch, 1989b; Chapter A3). The principal aim of the behavioural research was to discern at which point *D.floralis* rejected non-selected plants and if rejection patterns varied between the four plant genotypes. This should indicate the location on the plant of the cues which determine host plant selection. A second aim was to determine if the duration of stationary behavioural events was related to the nature of the ensuing event. From this, conclusions can be drawn as to the nature of the cues for host selection. A third element to the study was to determine if, during the host selection procedure, oviposition became a predetermined event. If performing a behaviour increases the probability of a gravid female ovipositing it is likely to attach importance to that element of the host selection behaviour.

MATERIALS AND METHODS

Biological materials

Work was carried out on two genotypes of kale, cvs Fribor and Dwarf Green Curled, and two swedes, a Scottish Crop Research Institute (SCRI) breeding line, GRL aga (Appendix I) and cv Doon Major. The level of antixenotic resistance to *Delia floralis* of three of the four genotypes used in this study has previously been tested in field experiments, although never simultaneously. The kale, Fribor, has demonstrated resistance to *D.floralis* under field conditions (Alborn *et al*, 1985; Ruuth, 1988). Doon Major and GRL aga were included in a larger assessment of swede genotypes in field cage experiments (Birch, 1989). Doon Major was the most susceptible to *D.floralis* attack whilst GRL aga exhibited the strongest resistance to *D.floralis* in the experiment. The results of the laboratory assessment of host preference between these four genotypes is discussed in detail in Chapter A3. In brief, the results confirmed those found by previous workers; the susceptibility ranging from Doon Major, the most susceptible plant genotype, through GRL aga and Dwarf Green Curled to the most resistant plant genotype, kale Fribor. The plant material used was grown in a glasshouse, singly, in 10cm diameter pots which contained a 3:1 mixture of Levington® Universal compost and sand, at 15-20°C, and 16 h photoperiod.

Methodology

Plants were used in the bioassay at the five true leaf stage. Approximately 25 female and 10 male *D.floralis*, aged 7-18 days from a laboratory culture, were placed in the Perspex observation cage (30cm x 30cm x 50cm) at between 0900 and 0930 on the morning that the

observations were due to take place, with food sources identical to those in the culture maintenance cage. The ambient temperature range was 18-24°C and the cage was maintained at a light level of 44 lux using fluorescent tubes. A single plant was then introduced to the flies at 1300 and observations started after 15 minutes. Single gravid females were observed from landing on the plant until oviposition or rejection of the plant. Observations ceased at 1500 or when 25 observations on the plant genotype concerned had been reached.

The use of "Swiss" egg traps (Freuler & Fischer, 1982) was investigated to facilitate egg counting for both bioassays and behavioural studies. The eggs traps are a roll composed of alternating layers of strips of black felt and narrow profiles of adhesive synthetic foam. However, it was found in exploratory experiments that the petioles of the young swede plants which were being used for these experiments tended to be damaged whilst the trap was being placed on the plant. During initial behavioural observations, the Swiss egg trap appeared to interfere with the oviposition behaviour of *D.floralis*. The Swiss egg trap prevented free movement of females at the plant-soil interface and acted as a barrier to soil probing with the ovipositor in the sand immediately adjacent to the stem. To avoid the problems associated with the Swiss egg traps, it was decided to use sieved sand as an oviposition medium for all the laboratory experiments investigating antixenotic resistance to oviposition.

An initial period of time was dedicated to observing the oviposition behaviour of *D.floralis* utilising the six behavioural events which were part of the defined oviposition sequence (Havukkala & Virtanen, 1985).

1) landing
2) extension of the proboscis and examination of the leaf surface
3) walking over the leaf
4) running down the stem
5) walking on the ground at the base of the stem
6) oviposition

However, the above listed behavioural events soon proved to be inadequate for fully describing the detailed behaviour of female *D.floralis*. Consequently, the list of behavioural events was expanded to allow for the wider variety of behavioural events noted in preliminary experiments. The acquisition of the "Observer 2" software package (Noldus, Wageningen, The Netherlands) allowed the recording of a large number of behavioural events accurately. "Observer 2" is IBM-compatible and utilises dedicated keys to record the behavioural events which are taking place. This reduces the amount of time required to record the data when

contrasted with the use of a pencil and paper. As a result, more of the researcher's concentration can be devoted to the observation of the flies.

Initially, events were recorded using a single dedicated key for each behavioural event. However, as the behavioural sequence(s) became more familiar the number of behavioural events increased. As outlined in the Section Introduction, *D.floralis* carries out a sequential selection procedure prior to oviposition on or rejection of a potential host plant. The eight basic phases through which a gravid *D.floralis* female may pass after landing can be expanded to include up to 30 mutually exclusive behavioural events (Table A.1.1.). For the purposes of recording efficiently, it became important to reduce the number of dedicated keys. A number of different configurations were investigated before the final scheme was selected, which divided recorded events into three classes; location, macro-behaviour and micro-behaviour.

location : referred to the point on the plant to which the female had progressed.

macro-behaviour : were exclusive events, largely concerned with modes of movement and oviposition.

micro-behaviour : were non-exclusive events, including grooming when stationary on the plant and proboscis extension.

This scheme reduced the number of dedicated keys to sixteen event keys and an additional rejection key (Table A.1.2.). Behaviour on resistant and susceptible plant genotypes was recorded from landing to oviposition or rejection. The "Observer 2" software package was initially used to carry out preliminary analyses of behavioural data by collation of data and summing of event means. It was also used to produce spreadsheet export files which could be used to facilitate a more detailed statistical analysis using other computer programs. In addition, the behavioural sequence files were exported to Microsoft Excel (version 4.0, Microsoft Corporation) which was used to rearrange the sequence files into a form which could be further analysed. The sequence files were processed utilising a series of Fortran files which were written specifically for the task. The details of the Fortran programming are given later in the Materials and Methods with an example of the most simple program used shown in detail. In addition, a set of three further examples of the Fortran programs written are presented in Appendix II.

Table A.1.1. Mutually exclusive events which may occur whilst a gravid female turnip root fly or cabbage root fly is making a host selection.

1 ; land on leaf.
2 ; walk down leaf surface.
3 ; stand on leaf surface.
4 ; extend proboscis on leaf.
5 ; groom front legs on leaf.
6 ; groom hind legs on leaf.
7 ; groom proboscis on leaf.
8 ; walk up leaf surface.
9 ; rejection, leaf.
10 ; walk down stem.
11 ; stand on stem.
12 ; extend proboscis on stem.
13 ; groom front legs on stem.
14 ; groom hind legs on stem.
15 ; groom proboscis on stem.
16 ; walk up stem.
17 ; circumventive walk of stem.
18 ; rejection, stem.
19 ; walk away from plant on sand.
20 ; stand on sand.
21 ; extend proboscis on sand.
22 ; groom front legs on sand
23 ; groom hind legs on sand
24 ; groom proboscis on sand.
25 ; dig in sand.
26 ; probe with ovipositor in sand.
27 ; oviposit.
28 ; walk towards plant on sand.
29 ; rejection , sand.
30 ; minor flight across plant.

Table A.1.2. Classification of individual keys which are dedicated to give most efficient analysis recording for behavioural events which may take place during host selection by the turnip and cabbage root flies.

CLASS	LOCATION	MACRO-BEHAVIOUR.	MICRO-BEHAVIOUR.
EVENT	Leaf	Landing Phase	Grooming Front Legs.
	Stem	Progressive Walk	Grooming Rear Legs.
	Plant Base	Rest	Grooming Proboscis.
		Regressive Walk	Proboscis Extension.
		Walk, circumventive	
		Pre-oviposition behaviour	
		Oviposition	
		Ovipositor Drag	
		Minor flight	

Definitions of behavioural events on the dedicated keys

For the purpose of event recording, individual keys were dedicated to specific behavioural events defined below.

LOCATION

Leaf: The flag element of the leaf down to the point where it ceases to be continuous with the petiole.

Stem: That part of the petiole which is not connected directly with the leaf and the stalk of the plant, both above and below the hypocotyl down to the interface with the soil surface.

Base: The sieved sand covering the soil surface placed around the base of the plant.

MACRO-BEHAVIOUR

Landing phase: The period of time from the fly first landing on until movement across the leaf surface or flight off leaf.

Progressive walk: Any walking across the plant which constitutes progress towards the site of oviposition. Any walking on the soil surface away from the base of the plant.

Rest: Any period of time which, after a movement across the plant, is spent stationary on the plant or at the base of the plant, with the exception of time spent in pre-oviposition behaviour.

Regressive walk: Any walking on the plant away from the site of oviposition or walking on the soil surface towards the base of the plant.

Circumventive walk: Characterised by the gravid female walking sideways around the stem or the base of the plant with the head towards the soil interface.

Pre-oviposition: Includes the behavioural events which constitute exploration of a potential oviposition site. These are digging in the soil at the base of the plant and probing the soil particles with the ovipositor.

Minor flight: A short flight which the fly takes over the plant surface returning to the surface of the plant within approximately 2 seconds.

Rejection: A flight which permanently leaves the plant.

Oviposition: Any prolonged period of time which the insect spends with its ovipositor deep in the soil and with the abdomen flexing slightly.

MICRO-BEHAVIOUR

Grooming front legs: Rubbing the front legs against each other.

Grooming rear legs: Rubbing the rear legs against each other.

Grooming proboscis: Rubbing the front legs against the proboscis.

Proboscis extension: Extending the proboscis onto the surface of the plant either whilst resting or moving.

Analysis of data

Fortran Processing of Sequence Files

All programs were coded in VAX Fortran-77, version 5.6 (™Digital Corporation), running under the VMS operating system on a local area VAX cluster.

The analysis program, RJH1.FOR, (Figure A.1.1.) exhibits a common Fortran-77 structure, and is sufficiently short not to require coding into procedural sub-routines. After declaration and formatting of all global variable parameters, standard input and output (I/O) channels, channels 3 and 7 respectively, are assigned. The use of standard values provides for a degree of portability (system-independence). A diagrammatic representation of the flow of information through the program is given in Figure A.1.2.

Input column headings are assigned at the time the fixed-format input file (Figure A.1.3.) is read into the program. At this stage, the serial designation of each fly is echoed to the screen to provide a rudimentary error-checking mechanism.

Figure A.1.1. Fortran program, "RJH1.FOR", utilised to extract the duration of the landing phase from input files.

```
      PROGRAM RJH1.FOR
```

* naming of the program for organisational purposes

```
      INTEGER             fly, curfly, reject, flysp,  plsp
      DOUBLE PRECISION    start, landp
      CHARACTER*4         locn, macrob, microb, oldmacrob
      CHARACTER*1         switch
      CHARACTER*3         check
      CHARACTER*100       fname
```

* declaration of the nature of the different inputs and outputs utilised

* whilst the program is running

```
111       FNAME = ' '
          WRITE(*,'(a$)') ' Input file ?   >> '
          READ(*,'(a)') fname
          OPEN(unit=3,file=fname,status='old',err=1)

222       FNAME = ' '
          PRINT*
          WRITE(*,'(a$)') ' Output file ?  >> '
          READ(*,'(a)') fname
          OPEN(unit=7,file=fname,status='new',err=2,
     &         CARRIAGECONTROL='list')
```

* Input and output series, declares a file as input and names an output file

```
1         READ(3,100)check
100       FORMAT(a3)
          IF(check.eq.'CRF')flysp=1
          IF(check.eq.'TRF')flysp=2
          IF(check.eq.'DM') plsp=1
          IF(check.eq.'GRL') plsp=2
          IF(check.eq.'DGC') plsp=3
          IF(check.eq.'FRI') plsp=4
```

* attributes values to the different fly species and crop genotypes

```
          IF (check.ne.'No ') goto 1

2         READ(3,200,end=999) fly, start, locn, macrob, microb, switch
200       FORMAT(i2,2x,f5.1,4x,a4,4x,a4,4x,a4,2x,a1)
```

* reads in the values of the different columns in the specific format of the input files

continues on next page

```
      IF (fly.ne.0) then
         WRITE(*,666)fly
666   FORMAT(' fly is ',i2)
         curfly = (flysp * 1000) + (plsp * 100) + fly
         reject = 0
         oldmacrob = 'temp'
      ENDIF
```

* writes the fly number to which the program has progressed onto the screen this allows the operator
*to know that the program is running sets reject to zero, attributes a nominal value to "oldmacrob"

```
      if ((macrob.ne.'LAND').and.(oldmacrob.eq.'LAND')) then
         landp = start
      endif
```

* defines and recognition point for the end of the landing phase and records
* the time at which it takes place

```
      IF ((locn.eq.'{eo}').and.(oldmacrob.eq.'LAND')) then
         reject = 1
      ENDIF
```

* if end of fly recording (denoted by "{eo}"), follows directly from
* landing phase reject is changed to 1

```
      IF (locn.eq.'{eo}') then
         WRITE(7,300) flysp, plsp, curfly, landp, reject
      ENDIF
300   FORMAT(1x,i2,2x,i2,2x,i4,3x,f5.1,3x,i1)
```

* if it is the end of the behaviour for the individual fly write out the fly
* species, plant genotype, fly number, duration of landing phase and whether
* rejected or not immediately following the landing phase.

```
      oldmacrob = macrob

* set oldmacrob for next loop

      GOTO 2
```

* loop round again

```
999   STOP
      END
```

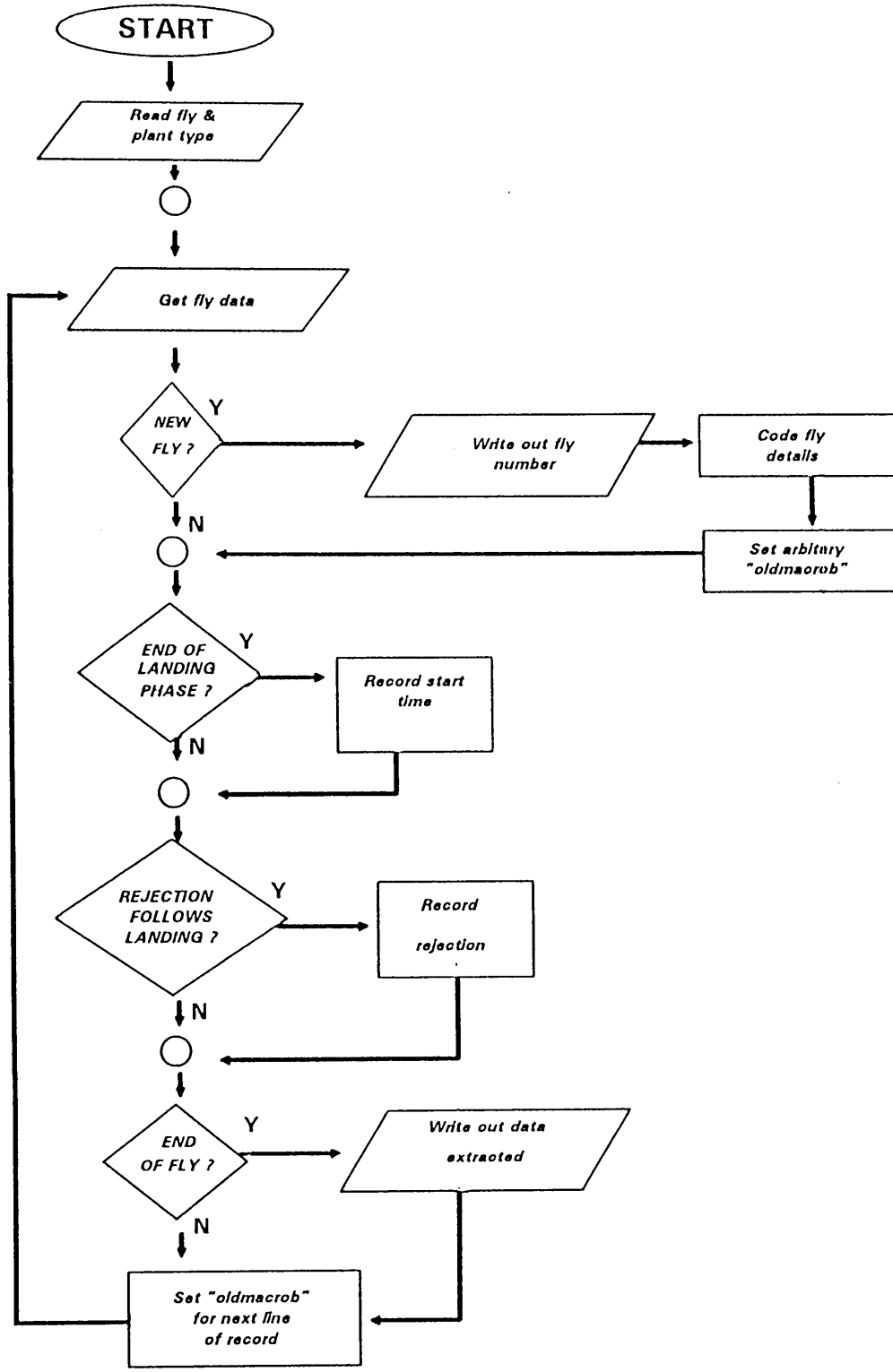


Figure A.1.2. Operation sequence of the fortran programme used to record the length of time spent in landing phase and whether rejected immediately after landing phase.

Figure A.1.3. Example input file for Fortran programs.

```
TRF
GRL
format is (12,2x,f5.1,4x,a4,4x,a4,4x,a4,2x,a1)
12345678901234567890123456789012345678901234567890123456789012345678901234567890
No
 1     0.0    LEAF    LAND                      9.9
       9.9    LEAF    LAND    GR     +          6.8
      16.7    LEAF    LAND    GR     -          3.3
      20.0    LEAF    LAND    EXT    +          1.9
      21.9    LEAF    UP                        5.1
      27.0    LEAF    UP      EXT    -          1.6
      28.6    LEAF    REST                      3.0
      31.6    LEAF    HOP                       1.0
      32.6    LEAF    REST                      6.2
      38.8    {eo}

 2     0.0    LEAF    LAND                      3.5
       3.5    LEAF    LAND    EXT    +          9.7
      13.2    LEAF    LAND    EXT    -          6.2
      19.4    {eo}

 3     0.0    LEAF    LAND                      3.7
       3.7    LEAF    DOWN                      5.7
       9.4    LEAF    DOWN    EXT    +          4.1
      13.5    STEM    DOWN                      3.0
      16.5    STEM    DOWN    EXT    -          5.3
      21.8    STEM    REST                      8.5
      30.3    STEM    DOWN                     11.2
      41.5    STEM    REST                      2.1
      43.6    STEM    REST    GF     +          8.7
      52.3    STEM    REST    GF     -          0.1
      52.4    STEM    DOWN                      0.6
      53.0    BASE    DOWN                      4.6
      57.6    BASE    REST                      3.6
      61.2    {eo}

 4     0.0    LEAF    LAND                     18.4
      18.4    LEAF    HOP                       2.5
      20.9    LEAF    REST                      2.6
      23.5    LEAF    REST    EXT    +          1.7
      25.2    LEAF    REST    EXT    -          3.9
      29.1    LEAF    REST    EXT    +          2.8
      31.9    LEAF    DOWN                      1.4
      33.3    LEAF    REST                      1.1
      34.4    LEAF    REST    EXT    -          1.1
      35.5    LEAF    REST    GF     +         12.1
      47.6    LEAF    REST    GF     -          1.8
      49.4    LEAF    REST    EXT    +          3.6
      53.0    LEAF    REST    EXT    -          0.9
      53.9    LEAF    REST    GR     +          8.9
      62.8    LEAF    REST    GR     -          5.1
      67.9    {eo}
```

A conditional operator determines when a new landing event has taken place (as denoted in the input file), at which point a Boolean switch models the reaction of the fly: rejection of the potential host plant or subsequent macro-behaviour on the plant surface. On encountering the end of a record in the input file, denoted by the "{eo}" string, the reformatted data are written to the output file (Figure A.1.4.). This process continues until the end-of-file marker is reached, at which point, program execution is terminated.

Transitions

The first way in which Fortran processed sequence files was the production of transition matrixes which determined with what frequency ensuing behaviours followed individual behavioural events. Transition matrixes of all behavioural events were not suitable for chi-squared analysis due to the mathematical interdependence of the values (Schnell *et al*, 1985; Kramer & Schmidhammer, 1992). The lateral lines are mathematically related to the vertical lines in the transition matrix. However, tables could be extracted from the transition matrixes which, for specific behaviours, noted the ensuing behaviour of gravid females on the four genotypes. The values in these tables were not interdependent and consequently could be analysed using a chi-squared test on Minitab (Minitab Vax/VMS version 7.1, 1989).

Ensuing event relationships

The Fortran programs were utilised to extract from the sequence files the duration of individual behavioural events and the nature of the ensuing event. The data set comprised durations for individual behavioural events and notations of the nature of the ensuing behaviour and the genotype of the plant on which the behaviour took place. For simplicity, the ensuing events were classified as either rejection or continuation. In the fifth column of an output file (Figure A.1.4.), "1" denotes rejection and "0" denotes continued leaf exploration. A two-way analysis of variance was performed on unbalanced data using the Generalised Linear Model (GLM) command within Minitab (Minitab Vax/VMS version 7.1, 1989). The GLM command was used to fit the model:

Duration of behaviour = A B A*B

Where: A = Genotype

B = Ensuing behaviour

Figure A.1.4. An example of the Fixed-format output file from Fortran program, RJH1.FOR direct import into MINITAB

```
2    2   2201     21.9    0
2    2   2202     19.4    1
2    2   2203      3.7    0
2    2   2204     18.4    0
2    2   2205      2.1    0
2    2   2206      9.3    0
2    2   2207      7.0    0
2    2   2208     23.4    0
2    2   2209      9.3    0
2    2   2210     36.6    1
2    2   2211     21.3    1
2    2   2212     27.7    0
2    2   2213    180.9    0
2    2   2214      4.8    0
2    2   2215    169.6    1
2    2   2216    150.9    1
2    2   2217      8.7    0
2    2   2218      1.6    0
2    2   2219      7.9    1
2    2   2220     13.2    0
2    2   2221     16.0    1
2    2   2222      2.6    0
2    2   2223     20.3    0
2    2   2224     62.6    1
2    2   2225     45.0    1
2    2   2226      9.9    0
2    2   2227    120.6    0
2    2   2228      3.1    0
```

The results of the analysis of variance and the means from the model are shown in the tables of the results section. An arithmetic mean of the duration of individual events is shown in the text and was calculated over all genotypes and for observations for which the ensuing event included both rejection and continued exploration of the potential host plant. The numbers of individuals which progressed onto the stem varied and, consequently, the data were unbalanced. As a result, the stem resting phase was unsuitable for this type of analysis. One-way analyses of variance (Minitab Vax/VMS version 7.1, 1989) were performed on the durations of behavioural events within the resting phase.

Finally, a table of probabilities was calculated. The probability of a gravid female undertaking a specific behaviour continuing to oviposit was calculated. In addition, the probability of an ovipositing female having performed a specific behaviour was calculated. Key behavioural events in the oviposition sequence should be indicated by a high value for both types of probability.

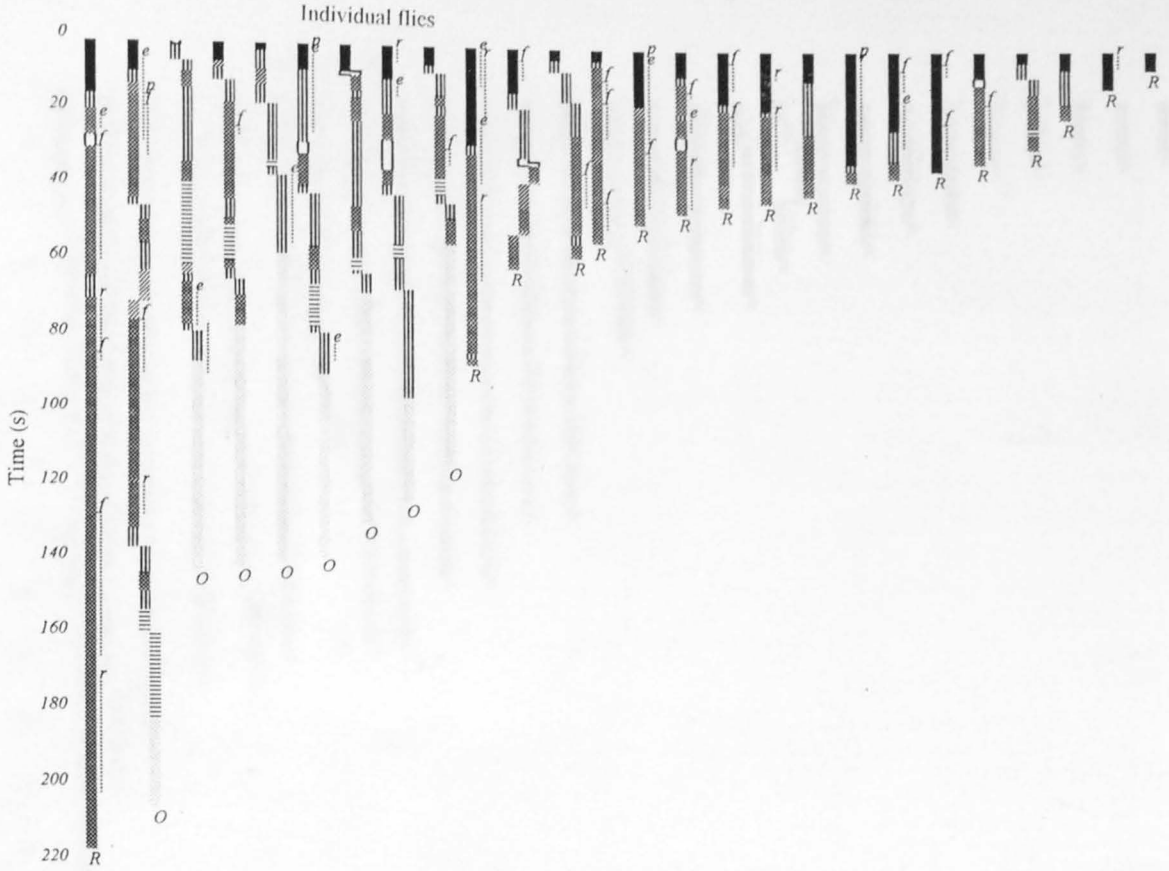
RESULTS

Detailed graphical notations of the behavioural events which individual gravid *D.floralis* females carried out on Doon Major, GRL aga, Dwarf Green Curled and Fribor are to be found in Figures A.1.5. to A.1.8..

Landing Phase

Chi-squared analyses of the event transitions following the landing phase showed that for *D.floralis* the frequency with which different events followed the landing phase varied significantly between plant genotypes (chi-squared = 28.21, df = 9, $P<0.001$). The major components of the chi-squared calculation were the high number of progressive movements in Doon Major and the low number of progressive movements in Fribor and rejections in Doon Major (Table A.1.3.). The classification of events as rejection or continued exploration also produced a significant ($P<0.05$) difference in the distribution of ensuing behaviours following the landing phase.

The mean overall duration (across all plant genotypes and for rejecting and continuing individuals) of the landing phase for *D.floralis* was 24.5s. The mean duration of the landing phase was longest on GRL aga (42.3s) and shortest on Doon Major (13.4s). However, there was no significant difference ($P>0.05$) in the duration of the landing phase on different plant genotypes. The duration of the landing phase of *D.floralis* females for which the ensuing



KEY

	Landing phase
	Progressive movement
	Resting
	Regressive movement
	Circumventive walk
	Short Hopping flight
	Drag
	Preoviposition behaviour
O	*Oviposition*
R	*Rejection*
p	*Groom proboscis*
f	*Groom front limbs*
r	*Groom rear limbs*
e	*Extend proboscis onto plant surface*

Figure A.1.5. Post-alighting oviposition behaviour of *D.floralis* on the swede Doon Major.

All the individuals landed on the leaf surface, transitions from leaf to stem and from stem to base are shown by the column moving to the right. Macro-behaviours performed by the individual are shown by the pattern on the column and micro-behaviours by the letter to the right of the column, the duration of micro-behaviour corresponds to the dotted line which follows the letter.

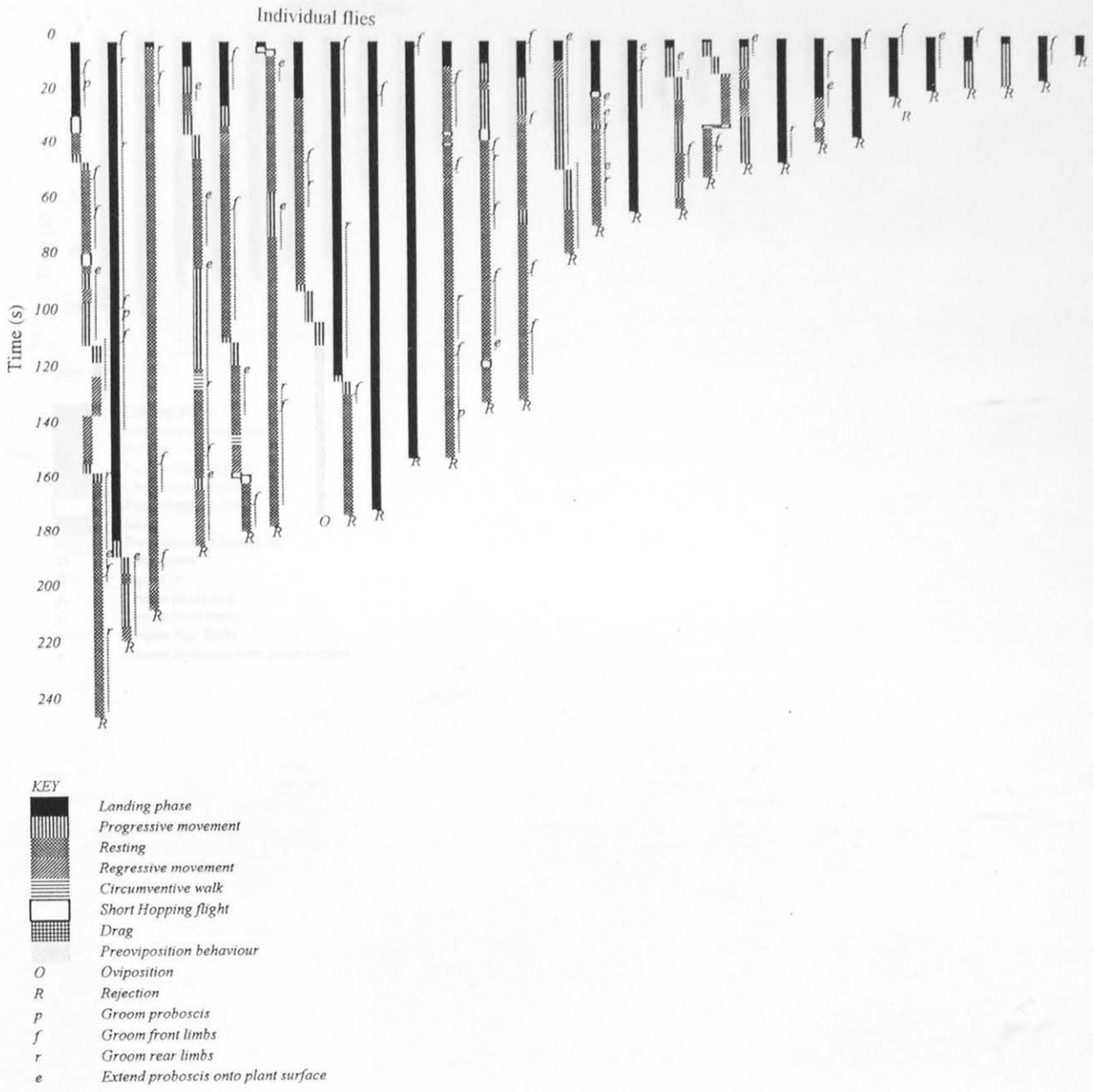


Figure A.1.6. Post-alighting oviposition behaviour of *D.floralis* on the swede GRL aga.

All the individuals landed on the leaf surface, transitions from leaf to stem and from stem to base are shown by the column moving to the right. Macro-behaviours performed by the individual are shown by the pattern on the column and micro-behaviours by the letter to the right of the column, the duration of micro-behaviour corresponds to the dotted line which follows the letter.

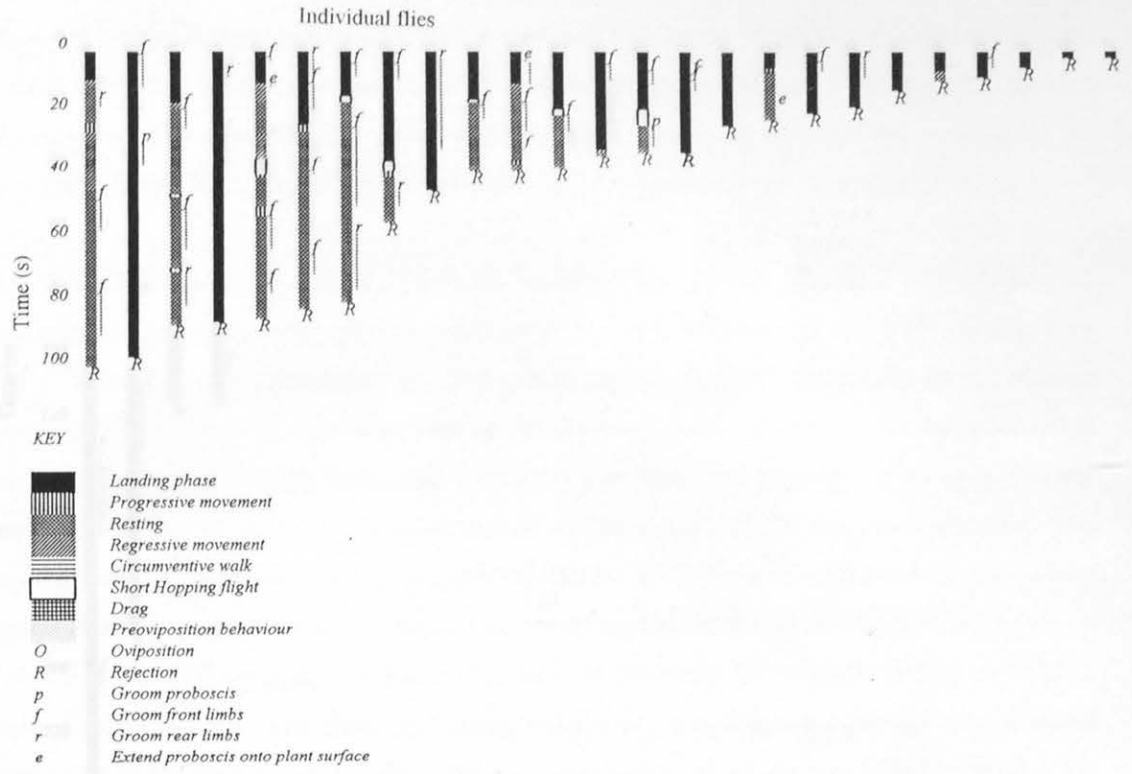


Figure A.1.7. Post-alighting oviposition behaviour of *D.floralis* on the kale Dwarf Green Curled.

All the individuals landed on the leaf surface, transitions from leaf to stem and from stem to base are shown by the column moving to the right. Macro-behaviours performed by the individual are shown by the pattern on the column and micro-behaviours by the letter to the right of the column, the duration of micro-behaviour corresponds to the dotted line which follows the letter.

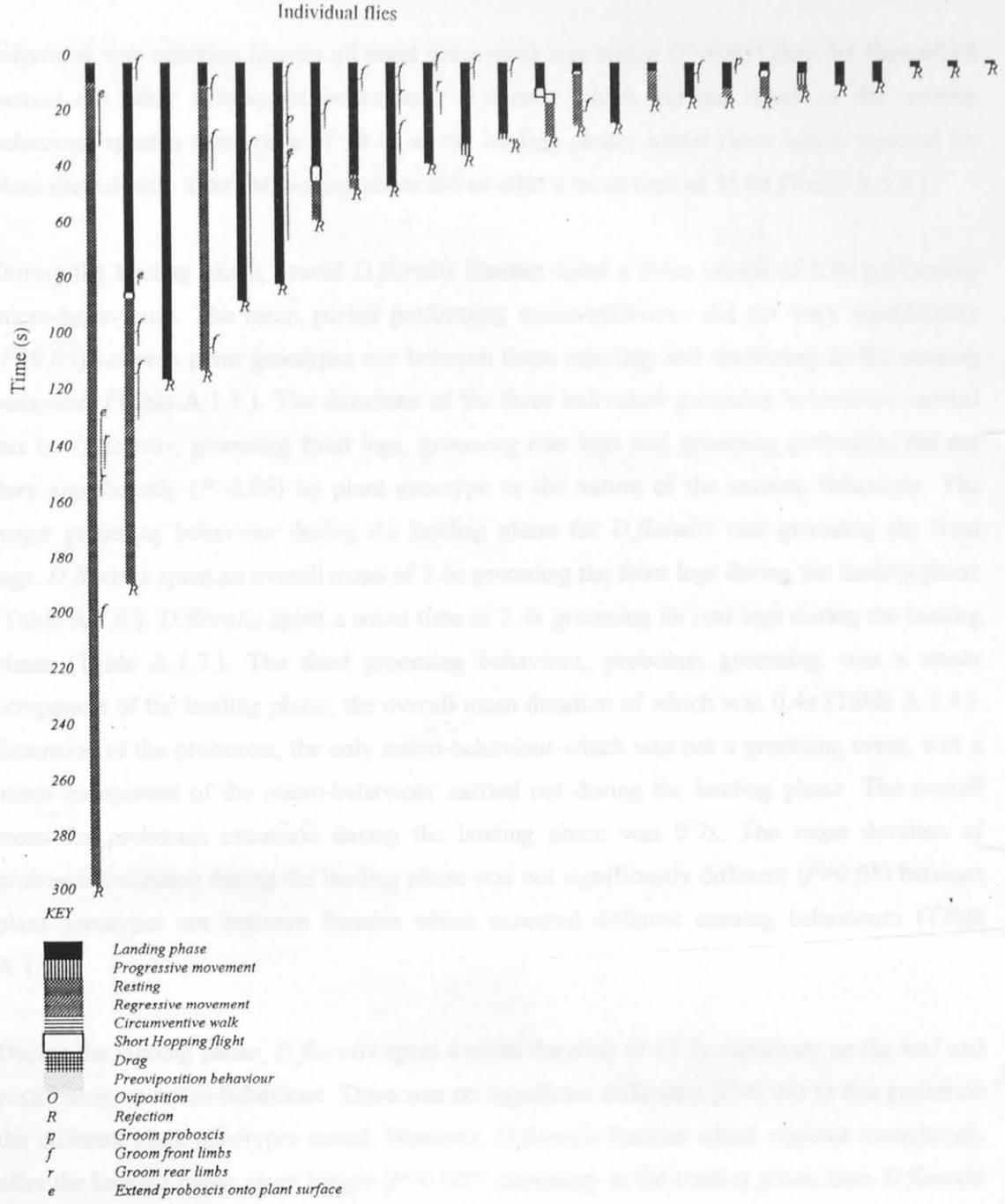


Figure A.1.8. Post-alighting oviposition behaviour of *D.floralis* on the kale Fribor.

All the individuals landed on the leaf surface, transitions from leaf to stem and from stem to base are shown by the column moving to the right. Macro-behaviours performed by the individual are shown by the pattern on the column and micro-behaviours by the letter to the right of the column, the duration of micro-behaviour corresponds to the dotted line which follows the letter.

behaviour was rejection (across all plant genotypes) was higher ($P<0.05$) than for flies which carried out other subsequent behaviours. *D.floralis* which did not reject as the ensuing behaviour spent a mean time of 18.0s in the landing phase, whilst those which rejected the plant immediately after the landing phase did so after a mean time of 35.4s (Table A.1.4.).

During the landing phase, gravid *D.floralis* females spent a mean period of 6.9s performing micro-behaviours. The mean period performing micro-behaviour did not vary significantly ($P>0.05$) between plant genotypes nor between those rejecting and continuing as the ensuing behaviour (Table A.1.5.). The durations of the three individual grooming behaviours carried out by *D.floralis*, grooming front legs, grooming rear legs and grooming proboscis, did not vary significantly ($P>0.05$) by plant genotype or the nature of the ensuing behaviour. The major grooming behaviour during the landing phase for *D.floralis* was grooming the front legs. *D.floralis* spent an overall mean of 3.6s grooming the front legs during the landing phase (Table A.1.6.). *D.floralis* spent a mean time of 2.4s grooming its rear legs during the landing phase (Table A.1.7.). The third grooming behaviour, proboscis grooming, was a minor component of the landing phase, the overall mean duration of which was 0.4s (Table A.1.8.). Extension of the proboscis, the only micro-behaviour which was not a grooming event, was a minor component of the micro-behaviour carried out during the landing phase. The overall mean for proboscis extension during the landing phase was 0.7s. The mean duration of proboscis extension during the landing phase was not significantly different ($P>0.05$) between plant genotypes nor between females which executed different ensuing behaviours (Table A.1.9.).

During the landing phase, *D.floralis* spent a mean duration of 17.7s stationary on the leaf and performing no micro-behaviour. There was no significant difference ($P>0.05$) in this period on the different plant genotypes tested. However, *D.floralis* females which rejected immediately after the landing phase spent longer ($P=0.009$) stationary in the landing phase than *D.floralis* females which performed subsequent behaviours. The mean durations of this behaviour for females rejecting and accepting as the ensuing behaviour for this behaviour event were 27.5s and 12.2s respectively. This result was reflected in the mean duration of this behaviour on the four plant genotypes (Table A.1.10.).

Leaf Resting Phase

When the transitions following *D.floralis* resting on the leaf were subject to chi-squared analysis, there was no significant difference (chi-squared = 8.50, df = 9, $P>0.05$) in the frequencies with which different events followed the leaf resting phase on different plant genotypes (Table A.1.11.).

Table A.1.3. Variation in the ensuing macro-behaviour performed by gravid female *D.floralis* following the landing phase on the four plant genotypes (observed frequencies with expected shown in parentheses).

Genotype	Doon Major	GRL aga	D.G.C.[1]	Fribor	Total[2]
Ensuing behaviour[3]					
Progressive[4]	19(9.75)	13(10.50)	5(9.38)	2(9.38)	39
Hop Flight	2(3.75)	3(4.04)	5(3.61)	5(3.61)	15
Regressive[5]	2(3.75)	3(4.04)	4(3.61)	6(3.61)	15
Rejection[6]	3(8.75)	9(9.42)	11(8.41)	12(8.41)	35
Total[7]	26	28	25	25	104

ChiSq = 28.21 (df=9)

1 D.G.C.=Dwarf Green Curled
2 Total number of occasions on which each ensuing event was performed
3 Behaviours observed to follow the landing phase
4 Movement towards the stem of the plant
5 Movement away from the stem of the plant
6 A flight which does not immediately return to the plant
7 Total number of ensuing events classified which were observed on each genotype

Table A.1.4. Mean duration (s) of landing phase (including micro-behaviour) for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	8890	7846	2615	2.45	0.068
Response	1	7813	5599	5599	5.25	0.024
Geno.Resp	3	1933	1933	644	0.60	0.614
Error	96	102444	102444	1067		
Total	103	121080				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	10.4(6.81, 23)	16.3(18.86, 3)	13.4(10.03, 26)
GRL aga	25.7(7.49, 19)	58.8(10.89, 9)	42.3(6.61, 28)
Dwarf Green Curled	16.6(9.06, 14)	29.1(9.43, 11)	22.9(6.54, 25)
Fribor	19.3(8.73, 13)	37.2(9.85, 12)	28.3(6.58, 25)
Mean[4]	18.0(4.04, 69)	35.4(6.42, 35)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.5. Mean duration (s) of total micro-behaviours during the landing phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	878.9	452.5	150.8	0.54	0.656
Response	1	91.8	81.6	81.6	0.29	0.590
Geno.Resp	3	796.0	796.0	265.3	0.95	0.420
Error	96	26821.0	26821.0	279.4		
Total	103	28587.7				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	4.2(3.49, 23)	6.5(9.65, 3)	5.3(5.13, 26)
GRL aga	13.4(3.84, 19)	7.4(5.57, 9)	10.4(3.38, 28)
Dwarf Green Curled	3.6(4.64, 14)	5.6(4.83, 11)	4.6(3.35, 25)
Fribor	2.0(4.47, 13)	12.1(5.04, 12)	7.1(3.37, 25)
Mean[4]	5.8(2.07, 69)	7.9(3.29, 35)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.6. Mean duration (s) of front grooming during landing phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	345.14	238.91	79.64	1.14	0.337
Response	1	188.31	184.43	184.43	2.64	0.107
Geno.Resp	3	388.37	388.37	129.46	1.85	0.143
Error	96	6703.71	6703.71	69.83		
Total	103	7625.54				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	0.8(1.74, 23)	5.0(4.83, 3)	2.9(2.57, 26)
GRL aga	4.9(1.92, 19)	4.7(2.79, 9)	4.8(1.69, 28)
Dwarf Green Curled	2.5(2.32, 14)	1.9(2.41, 11)	2.2(1.67, 25)
Fribor	1.7(2.23, 13)	11.0(2.52, 12)	6.3(1.68, 25)
Mean[4]	2.5(1.03, 69)	5.6(1.64, 35)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.7. Mean duration (s) rear grooming in landing phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	328.6	159.2	53.1	0.52	0.670
Response	1	13.7	10.8	10.8	0.11	0.746
Geno.Resp	3	267.3	267.3	89.1	0.87	0.458
Error	96	9808.3	9808.3	102.2		
Total	103	10417.8				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	1.9(2.11, 23)	1.5(5.84, 3)	1.7(3.10, 26)
GRL aga	7.2(2.32, 19)	1.0(3.37, 9)	4.1(2.05, 28)
Dwarf Green Curled	0.6(2.80, 14)	3.1(2.92, 11)	1.8(2.02, 25)
Fribor	0.0(2.70, 13)	1.1(3.05, 12)	0.5(2.04, 25)
Mean[4]	2.4(1.25, 69)	1.6(1.99, 35)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.8. Mean duration (s) of proboscis grooming during landing phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	2.240	1.103	0.368	0.14	0.939
Response	1	0.191	0.038	0.038	0.01	0.906
Geno.Resp	3	9.604	9.604	3.201	1.18	0.323
Error	96	261.323	261.323	2.722		
Total	103	273.358				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	0.2(0.34, 23)	0.0(0.95, 3)	0.1(0.50, 26)
GRL aga	0.9(0.38, 19)	0.0(0.55, 9)	0.4(0.33, 28)
Dwarf Green Curled	0.0(0.46, 14)	0.7(0.48, 11)	0.3(0.33, 25)
Fribor	0.2(0.44, 13)	0.8(0.50, 12)	0.5(0.33, 25)
Mean[4]	0.3(0.20, 69)	0.4(0.32, 35)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.9. Mean duration (s) of proboscis extension during landing phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	26.983	12.981	4.327	0.85	0.468
Response	1	0.002	1.045	1.045	0.21	0.651
Geno.Resp	3	18.592	18.592	6.197	1.22	0.306
Error	96	486.883	486.883	5.072		
Total	103	532.461				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	1.6(0.47, 23)	0.0(1.30, 3)	0.8(0.69, 26)
GRL aga	0.4(0.52, 19)	1.7(0.75, 9)	1.1(0.46, 28)
Dwarf Green Curled	0.5(0.62, 14)	0.0(0.65, 11)	0.3(0.45, 25)
Fribor	0.2(0.60, 13)	0.1(0.68, 12)	0.2(0.45, 25)
Mean[4]	0.7(0.28, 69)	0.5(0.44, 35)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.10. Mean duration (s) of landing phase (excluding micro-behaviour) for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	4866.3	4818.3	1606.1	2.66	0.052
Response	1	6211.0	4328.2	4328.2	7.18	0.009
Geno.Resp	3	4235.8	4235.8	1411.9	2.34	0.078
Error	96	57907.2	57907.2	603.2		
Total	103	73220.3				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	6.2(5.12, 23)	9.8(14.18, 3)	8.0(7.54, 26)
GRL aga	12.3(5.63, 19)	51.4(8.19, 9)	31.9(4.97, 28)
Dwarf Green Curled	13.0(6.81, 14)	23.5(7.09, 11)	18.3(4.92, 25)
Fribor	17.2(6.56, 13)	25.1(7.41, 12)	21.2(4.95, 25)
Mean[4]	12.2(3.04, 69)	27.5(4.83, 35)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

The mean overall duration of the leaf resting phase for *D.floralis* was 27.0s and ranged from 39.2s on Fribor to 20.1s on Doon Major (Table A.1.12.). However, there was no significant difference ($P>0.05$) in the duration of the leaf resting phase on different plant genotypes or for those with different ensuing behaviours (either overall or for individual plant genotypes). During the leaf resting phase, micro-behaviours were carried out by gravid *D.floralis* females for a mean period of 10.3s. The mean period performing micro-behaviour did not vary significantly ($P>0.05$) between plant genotypes nor between those rejecting and continuing as the ensuing behaviour (Table A.1.13.). The micro-behaviour performed by *D.floralis* during the leaf resting phase was dominated by grooming behaviours which constituted a mean across all plant genotypes of 9.2s. There was no significant difference ($P>0.05$) in the mean duration of total grooming behaviours between plant genotypes nor between those rejecting and continuing as the ensuing behaviour.

The durations of the three individual grooming behaviours carried out by *D.floralis*, grooming front legs, grooming rear legs and grooming proboscis, were also unaffected by plant genotype or the nature of the ensuing behaviour. The major grooming behaviour for *D.floralis* was grooming the front legs. *D.floralis* spent an overall mean of 6.3s grooming the front legs during the leaf resting phase (Table A.1.14.). *D.floralis* spent a mean time of 2.6s grooming its rear legs during the leaf resting phase (Table A.1.15.). Proboscis grooming was never observed on Fribor, nor for *D.floralis* which continued to explore GRL aga or Dwarf Green Curled. When observed, it remained a minor component of the leaf resting phase; the overall mean duration was 0.3s (Table A.1.16.). On the two kale genotypes, extension of the proboscis was only performed by *D.floralis* females which rejected as the ensuing behaviour. The overall mean period for proboscis extension during the leaf resting phase was 1.2s and was not significantly different ($P>0.05$) between plant genotypes nor between females which executed different ensuing behaviours (Table A.1.17.).

During the leaf resting phase, *D.floralis* spent a mean duration of 16.8s performing no micro-behaviour. There was no significant difference ($P>0.05$) in this period on the four plant genotypes nor for *D.floralis* females which performed different ensuing behaviours (Table A.1.18.).

Stem Resting Phase

On Dwarf Green Curled, *D.floralis* were not observed progressing onto the stem and consequently stem resting was not recorded. Similarly, only one *D.floralis* female was observed resting on the stem of Fribor; the ensuing event was a rejection of the plant. The low numbers of individuals which performed this behaviour resulted in chi-squared analyses for

Table A.1.11. Variation in the ensuing macro-behaviour performed by *D.floralis* following resting upon the leaf on the four plant genotypes (observed frequencies with expected shown in parentheses).

Genotype	Doon Major	GRL aga	D.G.C.[1]	Fribor	Total[2]
Ensuing behaviour[3]					
Progressive[4]	7(5.40)	7(5.60)	0(2.20)	3(3.80)	17
Regressive[5]	4(3.49)	4(3.62)	2(1.42)	1(2.46)	11
Hop Flight	3(2.86)	4(2.96)	0(1.16)	2(2.01)	9
Rejection[6]	13(15.25)	13(15.81)	9(6.21)	13(10.73)	48
Total[7]	27	28	11	19	85

ChiSq = 8.50 (df=9)

1 D.G.C.=Dwarf Green Curled
2 Total number of occasions on which each ensuing event was performed
3 Behaviours observed to follow the landing phase
4 Movement towards the stem of the plant
5 Movement away from the stem of the plant
6 A flight which does not immediately return to the plant
7 Total number of ensuing events classified which were observed on each genotype

Table A.1.12. Mean duration (s) of leaf resting phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	7151	2650	883	0.47	0.705
Response	1	86	624	624	0.33	0.567
Geno.Resp	3	1942	1942	647	0.34	0.794
Error	74	139572	139572	1886		
Total	81	148751				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	18.7(11.21, 14)	21.4(11.61, 13)	20.1(8.07, 27)
GRL aga	33.7(11.21, 15)	23.8(15.36, 13)	28.7(9.51, 28)
Dwarf Green Curled	17.1(17.73, 2)	23.5(12.05, 9)	20.3(10.72, 11)
Fribor	25.2(30.71, 6)	53.2(14.48, 13)	39.2(17.00, 19)
Mean[4]	23.7(9.71, 37)	30.5(6.73, 48)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.13. Mean duration (s) of all micro-behaviours for gravid females *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	102.3	123.1	41.0	0.15	0.927
Response	1	33.2	7.0	7.0	0.03	0.872
Geno.Resp	3	87.9	87.9	29.3	0.11	0.954
Error	74	19781.4	19781.4	267.3		
Total	81	20004.8				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	10.4(4.22, 14)	11.5(4.37, 13)	11.(3.04, 27)
GRL aga	11.0(4.22, 15)	11.6(5.78, 13)	11.3(3.58, 28)
Dwarf Green Curled	5.7(6.68, 2)	10.3(4.54, 9)	8.0(4.04, 11)
Fribor	11.5(11.56, 6)	8.0(5.45, 13)	9.7(6.39, 19)
Mean[4]	9.6(3.66, 37)	10.4(2.53, 48)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.14. Mean duration (s) of front leg grooming for gravid female *D.floralis* during the leaf resting phase on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	55.2	14.6	4.9	0.05	0.987
Response	1	19.2	68.5	68.5	0.64	0.426
Geno.Resp	3	138.3	138.3	46.1	0.43	0.731
Error	74	7893.4	7893.4	106.7		
Total	81	8106.1				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	6.1(2.67, 14)	6.2(2.76, 13)	6.1(1.92, 27)
GRL aga	8.2(2.67, 15)	5.7(3.65, 13)	6.9(2.26, 28)
Dwarf Green Curled	5.3(4.22, 2)	6.9(2.86, 9)	6.1(2.55, 11)
Fribor	11.5(7.30, 6)	3.1(3.44, 13)	7.3(4.04, 19)
Mean[4]	7.7(2.31, 37)	5.5(1.60, 48)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.15. Mean duration (s) of rear leg grooming during the leaf resting phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	58.70	72.99	24.33	0.44	0.726
Response	1	36.85	30.23	30.23	0.54	0.463
Geno.Resp	3	7.78	7.78	2.59	0.05	0.987
Error	74	4106.86	4106.86	55.50		
Total	81	4210.19				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	2.9(1.92, 14)	4.4(1.99, 13)	3.7(1.38, 27)
GRL aga	2.2(1.92, 15)	2.7(2.63, 13)	2.4(1.63, 28)
Dwarf Green Curled	0.4(3.04, 2)	2.7(2.07, 9)	1.6(1.84, 11)
Fribor	0.0(5.27, 6)	1.6(2.48, 13)	0.8(2.91, 19)
Mean[4]	1.4(1.67, 37)	2.9(1.16, 48)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.16. Mean duration (s) of proboscis grooming during the leaf resting phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	3.235	6.053	2.018	0.62	0.606
Response	1	8.047	4.941	4.941	1.51	0.223
Geno.Resp	3	8.375	8.375	2.792	0.85	0.469
Error	74	241.965	241.965	3.270		
Total	81	261.622				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	0.2(0.47, 14)	0.3(0.48, 13)	0.2(0.34, 27)
GRL aga	0.0(0.47, 15)	1.7(0.64, 13)	0.8(0.40, 28)
Dwarf Green Curled	0.0(0.74, 2)	0.6(0.50, 9)	0.3(0.45, 11)
Fribor	0.0(1.28, 6)	0.0(0.60, 13)	0.0(0.71, 19)
Mean[4]	0.0(0.40, 37)	0.6(0.28, 48)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.17. Mean duration (s) for which proboscis was extended during the leaf resting phase for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	82.77	26.93	8.98	0.39	0.759
Response	1	0.04	11.12	11.12	0.49	0.488
Geno.Resp	3	49.02	49.02	16.34	0.71	0.547
Error	74	1694.98	1694.98	22.91		
Total	81	1826.81				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	2.1(1.24, 14)	0.6(1.28, 13)	1.3(0.89, 27)
GRL aga	0.7(1.24, 15)	1.6(1.69, 13)	1.1(1.05, 28)
Dwarf Green Curled	0.0(1.95, 2)	0.0(1.33, 9)	0.0(1.18, 11)
Fribor	0.0(3.38, 6)	4.2(1.60, 13)	2.1(1.87, 19)
Mean[4]	0.7(1.07, 37)	1.6(0.74, 48)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.1.18. Mean duration (s) of the leaf resting phase during which there was no micro-behaviour taking place for gravid female *D.floralis* on different plant genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	7865	2559	853	0.82	0.485
Response	1	12	499	499	0.48	0.490
Geno.Resp	3	2225	2225	742	0.72	0.545
Error	74	76598	76598	1035		
Total	81	86701				

Table of Means

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	8.3(8.31, 14)	9.9(8.60, 13)	9.1(5.98, 27)
GRL aga	22.7(8.31, 15)	12.2(11.38, 13)	17.4(7.04, 28)
Dwarf Green Curled	11.4(13.14, 2)	13.2(8.92, 9)	12.3(7.94, 11)
Fribor	13.8(22.75, 6)	45.3(10.72, 13)	29.5(12.58, 19)
Mean[4]	14.1(7.19, 37)	20.1(4.99, 48)	

Parentheses contain S.D. & N.

1. Those flies which performed another behaviour immediately after performing the behaviour listed
2. Those flies which rejected the plant immediately after performing the behaviour
3. The mean duration of the event listed for all the the flies which performed that behaviour
4. The combined mean duration of the behaviour for flies on all the genotypes tested

which it was highly probable the assumptions were invalid. Consequently, the distribution of ensuing event data were not examined. The mean overall duration of the stem resting phase for *D.floralis* was 15.6s. The duration of the stem resting phase was significantly longer ($P<0.05$) for flies which rejected (32.2s) than for those which continued to explore the plant (12.3s). This result was strongly influenced by the significant ($P<0.05$) difference in the duration of the stem resting phase on GRL aga: flies which rejected had a stem resting phase 44.6s long, whereas flies which continued to explore the plant had a stem resting phase 18.6s long.

Micro-behaviours constituted 6.6s of the stem resting phase of gravid *D.floralis* females. The mean period performing micro-behaviour did not vary significantly ($P>0.05$) between those rejecting and those continuing as the ensuing behaviour. However, whilst micro-behaviours were only observed to occur on the swede genotypes, the mean duration on Doon Major, 0.9s, was lower ($P<0.5$) than that on GRL aga 13.2s.

The micro-behaviour performed by *D.floralis* during the stem resting phase was dominated by extension of the proboscis and grooming of the front legs. Proboscis extension comprised an overall mean of 3.8s and was higher ($P<0.05$) for individuals which rejected than for individuals which accepted as the ensuing behaviour, 11.8s and 2.0s respectively. The major grooming behaviour carried out by *D.floralis* during the stem resting phase was grooming of the front legs. *D.floralis* spent an overall mean of 3.1s grooming the front legs during the stem resting phase. Grooming rear legs took place only on GRL aga and there was no significant ($P>0.05$) difference in the mean duration of the event on rejecting contrasted with continuing individuals, 1.5s and 2.1s respectively. Grooming the proboscis was not observed at all during the stem resting phase. During the stem resting phase, *D.floralis* spent a mean duration of 9.4s performing no micro-behaviour. There was no significant difference ($P>0.05$) in this period on the four plant genotypes. However, female *D.floralis* which rejected spent longer ($P=0.025$) performing this behaviour than those which carried out further behaviour on the plant, 19.2s and 7.1s respectively.

The probability of individuals which carried out a particular behaviour continuing to oviposit and the probability of the individuals which had oviposited carrying out that behaviour showed considerable variation (Table A.1.19.). The probabilities were bulked across the four plant genotypes. The probability of a *D.floralis* female which landed on the plant ovipositing was 0.09. Five behavioural events were always carried out by flies which went on to oviposit: "Landing Phase"; "Progressive Movement" on the leaf; "Progressive Movement" on the stem; "Progressive Movement" on the base; "Preoviposition Behaviour". Only one event showed high probabilities for both probability categories: once a gravid female had performed a

Table A.1.19. Probabilities associated with oviposition and the performance of specific behaviours by gravid female *D.floralis* during oviposition site selection.

Location	Behaviour	Probability of Ovipositing[1]	Proportion of Ovipositors[2]
Leaf	Land	0.09	1.00
Leaf	Land+Groom	0.04	0.22
Leaf	Land+Extend	0.15	0.22
Leaf	Progressive	0.19	1.00
Leaf	Regressive	0.14	0.44
Leaf	Rest	0.10	0.44
Leaf	Rest+Groom	0.06	0.22
Leaf	Extend	0.17	0.33
Stem	Progressive	0.39	1.00
Stem	Regressive	0.29	0.22
Stem	Rest	0.33	0.67
Stem	Groom	0.25	0.22
Stem	Extend	0.13	0.11
Stem	Circumventive walk	0.73	0.89
Base	Progressive	0.69	1.00
Base	Regressive	0.00	0.00
Base	Rest	0.29	0.22
Base	Groom	0.00	0.00
Base	Extend	0.75	0.33
Base	Preoviposition behav.	0.90	1.00
	Hopping flight[3]	0.23	0.33

1 Probability of an individual which carries out the listed behaviour continuing to oviposit on the plant.

2 The probabitlity of an ovipositing female having performed the listed behavioural event.

3 From any point on the plant to any point on the plant

circumventive walk there was a 73% chance of it continuing to oviposit and 89% of the flies which oviposited had performed a circumventive walk on the stem.

DISCUSSION

The visual representations of the post-alighting behaviour of *Delia floralis* (Figures A.1.5. to A.1.8.) show that the behaviour of gravid females varied substantially between plant genotypes. Havukkala & Virtanen (1985) elicited a six point behavioural sequence of host plant selection and oviposition for *D.floralis*. They concluded that the stem run was the most likely site for rejection of a host plant, although leaf walk was the most likely for the surrogate plant which was tested. Havukkala & Virtanen (1985) noted that tarsal contact with sinigrin or allyl-isothiocyanate shortened the time to proboscis extension, which was believed to be important to host plant selection.

Städler (1992) reviewed the behavioural responses of insects to secondary plant compounds and, although many studies have been made of the stimulation of oviposition by such compounds, the detailed host selection behavioural sequence is rarely published. Havukkala & Virtanen (1985) was the most detailed examination of the post-alighting behaviour of *D.floralis* and the conclusions of this study are very different from theirs. They found that 29% of the flies which landed on the turnip ultimately laid eggs. This constituted approximately half the flies which had left the leaf and progressed onto the stem. In this study, only 9% of the flies which landed on a plant progressed to oviposit. This percentage is deceptively low, because no flies oviposited on the resistant kales and only a single female on the partially resistant swede, GRL aga. The swede Doon Major, the only susceptible genotype tested and probably of comparable susceptibility to a turnip seedling, had eight ovipositions from 26 gravid females observed. This constitutes a 31% probability of oviposition taking place. The percentage of flies ovipositing on susceptible plants was therefore very similar in the two studies and the conditions under which the experiment took place can be assumed to have offered similar probabilities of oviposition on a suitable host.

Three results of this behavioural study of host selection by *D.floralis* indicated that the most important stage in host selection was the landing phase. Firstly, chi-squared analyses showed that the differences in ensuing behaviour on the four plant genotypes were very pronounced in the initial landing phase (Table A.1.3.). At no other stage in the selection procedure could statistically significant variations in the distribution of ensuing behaviours between the four plant genotypes be found. If no difference in the pattern of ensuing behaviours between plant genotypes can be found within a selection phase then the differences in the plant genotype are

not influencing the behaviour during that phase. Secondly, on the highly resistant kale genotypes, *D.floralis* was rarely observed to progress beyond the leaf. This behaviour indicates that the antixenotic resistance to *D.floralis* which the kales possess influences *D.floralis* behaviour during the earliest stages of contact with the plant. Thirdly, only the landing phase duration varied significantly when contrasting flies which rejected with those which continued to explore the plant as the ensuing behaviour. Individually, none of the above three results would be conclusive. However, in combination they present clear evidence for the landing phase being the critical stage in host plant selection for *D.floralis* prior to oviposition.

The only exception to the domination of the landing phase in host selection was the stem resting phase of *D.floralis* on GRL aga. For the kale genotypes, the small number of gravid females which progressed to the stem made that phase inconsequential to the resistance demonstrated. The highly susceptible Doon Major had sixteen occurrences of stem resting, only one of which resulted in rejection of the plant as the ensuing behaviour. The four individuals which rejected GRL aga following a stem resting phase did so after stem rests of longer duration than those which continued on the plant. This result may indicate that the mechanism of the resistance to *D.floralis* which GRL aga possesses is not the same as that in the two kale genotypes. Although differentiation was primarily on the leaf, the behavioural pattern on GRL aga was clearly different from Doon Major and the two kale genotypes. In addition, proboscis extension was a major component of stem resting behaviour of GRL aga, when it was a very minor component of the landing and leaf resting phases of all genotypes. This result may relate to an increased use of the chemoreceptors on the proboscis to assess the plant at this stage. Although *D.floralis* are known to possess contact chemoreceptors on the proboscis (Simmonds, personal communication), little is known about their function or sensitivity to compounds.

The significantly longer duration of behavioural phases immediately before the rejection of a plant provides evidence for the hypothesis that the cues for host selection are positive stimuli which are missing or sub-optimal on rejected plants. Several models exist of the host selection behaviour of phytophagous insects (Jaenike, 1978; Rausher, 1985; Courtney *et al*, 1989; Jaenike & Papaj, 1992). However, the models are largely concerned with the changing probability of host acceptance with the period since oviposition last took place. No published evidence could be found which attempted to link variation in the detailed host selection behaviour of an insect with the nature of the stimuli.

The models do present evidence that both egg load and previous experience alter the probability of ovipositing (Jaenike & Papaj, 1992). Papaj & Prokopy (1986) concluded that

female apple maggot flies (*Rhagoletis pomonella*) enhanced their experience following contact with physical and chemical stimuli. Great care was taken during these experiments to use insects in a similar physiological state. However, the *D.floralis* females observed were not naive, in that they had previous contact with host plant stimuli in the form of slices of swede root on which they could oviposit. This action was taken to prevent the increased probability of random oviposition which is associated with insects that have had no opportunity to oviposit (Jaenike, 1978; Jaenike & Papaj, 1992). However, it must be acknowledged that this method may have resulted in a degree of learning which may have influenced the host preference of the flies. The use of flies which had been permitted to oviposit freely prior to experimentation was preferred to the use of naive females which may have been physiologically inclined to oviposit.

The third aim of this behavioural study was to investigate whether oviposition became a predetermined event at any point during the oviposition selection procedure. The table of probabilities clearly shows that this was never strictly the case. However, during the latter stages of host selection, three events achieved high scores in both the columns of the table. Preoviposition behaviour was the event most strongly linked with ovipositing females and flies which carried out a progressive movement at the base of the plant were also likely to oviposit. The earliest point in the oviposition site selection process at which *D.floralis* could be regarded as being strongly linked to oviposition was the circumventive walk on the stem of the plant. Only one female which oviposited failed to carry out this behaviour and females which carried out this behaviour continued to oviposit on over 70% of occasions. However, the small sample of flies which had progressed to this stage on the plant makes it difficult to draw positive conclusions.

An important question stems from these conclusions. Why is the adult flies' host selection for a root feeding site for larvae so strongly biased to the examination of the leaf in the selection process? Exploration of the root at the soil interface by the adult would appear to be a better area in which to assess the environment into which to place its progeny. Traynier (1967b) investigated the media into which the closely related species *D.radicum* preferred to oviposit and demonstrated that sand with a particle size of approximately 1mm was the preferred medium. Although no similar work has been carried out on *D.floralis*, the close relationship between the behaviour, lifecycle and host range of these two species does indicate that they are likely to have similar criteria for oviposition site selection. It is possible that the moist sand and its diameter optimised the conditions and resulted in oviposition being highly probable if all other behavioural criteria had been satisfied. It is possible that assessment of the leaf surface identifies a plant from the host range. Having found a plant within the host range, it

may be most important that the soil is sufficiently moist to prevent desiccation and is of a type to allow easy larval migration.

The energetic advantages of quickly assessing the host quality on the leaf are clear, even if the quality of site assessment is reduced. Use of a leaf assessment would reduce the time period spent on host evaluation and consequently save energy and, potentially, speed the location of a suitable host. In addition, exposure to some predators would be reduced. Carabid beetles are a major egg predator of *D.radicum* and account for approximately 30% of egg mortality (Coaker & Williams, 1963). Sources of adult predation have never been quantified. Minimising the risk of predation to adults may be important to the lifecycle of *D.floralis*.

The confirmation of the leaf as the major site of host selection for *D.floralis* is important for targeting the study of the host selection of this pest. The techniques to extract and fractionate leaf surface chemicals are becoming increasingly refined and knowledge of the nature and location of stimuli will allow a more accurate and efficient search for key compounds to take place. The bioassay of host plant extracts is an important area and will receive more discussion in Chapter A3 and in the Section Discussion.

Chapter A2

The effect of resistant and susceptible Brassica genotypes on the behaviour of gravid *Delia radicum* females selecting a host plant for oviposition

INTRODUCTION

Gravid female *Delia radicum* carry out a rigid series of behavioural events during the selection of an oviposition site. The basic series of events involved is outlined in the section introduction. Zohren (1968) studied a number of aspects of the biology of *D.radicum* including contrasting the oviposition behaviour sequence on real plants and surrogate plants treated with swede juice. It was concluded that contact chemoreception was of greater importance than visual and olfactory stimuli for the selection of host plants. The relationship between the chemical composition of plants and the oviposition of *D.radicum* has been the subject of a number of different studies, and compounds have been characterised which elicit both positive and negative responses by *D.radicum* (Jones *et al*, 1988 Cole *et al*, 1989; Städler & Schoni, 1990; Roessingh *et al*, 1992a; 1992b). Volatile plant compounds and the more stable compounds found on the leaf surface have been associated with oviposition preference and host finding behaviour of *D.radicum*.

Hawkes & Coaker (1976) studied the behavioural responses of *D.radicum* to host plant odours both in the field and in the laboratory. Their conclusions included that female *D.radicum* became responsive to host plant odour when ovarian development was complete. In addition, allyl-isothiocyanate elicited the same responses as host plants. Ellis *et al* (1980) concluded that oviposition by *D.radicum* on high and low preference selections of a radish genotype were subject to cycles of changing attractiveness. In addition, the variations in attractiveness which the radishes showed correlated with concentrations of two volatile glucosinolate hydrolysis products. However, Finch & Skinner (1982) considered that most female *D.radicum* flew upwind regardless of the presence or absence of host plant odours.

However, the detailed post-landing behaviour of the insect on the host plant has received relatively little attention within the area of insect-plant relationships. Variations in the host selection behaviour of *D.radicum* have received little attention. Städler & Schoni (1990) published the most recent and most detailed study on the host selection behaviour of *D.radicum*. The study concerned examined the host selection behaviour of gravid *D.radicum* females on freshly severed cabbage, *Brassica oleracea* var *capitata*, leaves and on surrogate leaves treated with a cabbage extract or a solvent control. Städler & Schoni (1990) found that the behavioural patterns performed on the surrogate plant treated with extract were similar to

those found on the severed leaf. The behavioural patterns performed on the surrogate plant treated with a solvent only were largely restricted to those associated with the upper part of the plant. Although the most thorough study of its kind, Städler & Schoni (1990) did not report the actual behaviours carried out by individual flies but categorised the behavioural patterns. Koštál *et al* (in press) contains detailed observations on the effect of undersowing on the host selection behaviour of *D.radicum*. Gravid female *D.radicum* were found to carry out frequent short flights and host selection was often impaired by collision with undersown plants. Bioassays of leaf and plant extracts are numerous, especially within the Crucifers (Städler, 1992). Several compounds are known to have a profound influence on the host selection of *D.radicum* and this aspect of host plant selection is explored in Chapter A3.

The principal aim of the work reported in this chapter was to identify at what point during host selection for oviposition *D.radicum* rejected plants which were not oviposited on. This behaviour should indicate at which point on the plant the cues which influence oviposition site selection are located. A second aim was to determine if a relationship existed between specific oviposition behaviours and the nature of the ensuing behavioural events. Finally, it was hoped to determine if host acceptance became a pre-determined event at any point in the selection behaviour. If the performance of a behaviour increases the probability of a gravid female continuing to oviposit then the importance of that behavioural event in host selection is highlighted.

MATERIALS AND METHODS

Biological materials

The biological materials utilised for the study of the host selection behaviour of *D.radicum* were identical to those utilised in Chapter A1. Work was carried out on two genotypes of kale, cvs Fribor and Dwarf Green Curled, and two swedes, a Scottish Crop Research Institute (SCRI) breeding line, GRL aga and cv Doon Major. Laboratory assessment of *D.radicum* host preference across these four genotypes is discussed in detail in Chapter A3. The results presented place the plants in the same ranking of preference as was found for *D.floralis*. The most susceptible plant genotype was Doon Major and then, with increasing antixenotic resistance, GRL aga, Dwarf Green Curled and Fribor. The plants were grown in the glasshouse singly in 10cm diameter pots containing a 3:1 mix of Levington® Universal compost and sand, at 15-20°C, and 16 h photoperiod.

Methodology

Plants were used in the bioassay at the five true leaf stage. Flies, aged 7-18 days from a laboratory culture, were placed in the observation cage (30cm x 30cm x 50 cm) at between 0900 and 0930 on the morning that the observations were due to take place with food sources identical to those in the culture maintenance cage. Test plants were then introduced to the flies at 1300 and observations started after 15 minutes. Single gravid females were observed from landing on the plant until oviposition or rejection of the plant. Observations ceased at 1500 or when 25 observations on the plant genotype concerned had been reached. The ambient temperature range was 18-24°C and the light level was maintained at 44 lux using fluorescent tubes. Sieved sand, 1.0-1.4mm diameter, was used as an oviposition medium in these experiments. The use of "Swiss" egg traps (Freuler & Fischer, 1982) was decided against, to avoid the problems associated with them, as discussed in Chapter A1.

The "Observer 2" (Noldus, Wageningen, The Netherlands.) was utilised during both the recording of the behaviour of the flies and during an initial period of time to familiarise the host selection behaviour of *D.radicum*. Initially the behaviour was described using the 30 behaviour events performed by *D.floralis* females (Table A.1.1.). Gravid *D.radicum* females were observed to drag their ovipositors across the plant stem and soil surface prior to oviposition. These behavioural events had only been observed for *D.floralis* as a part of post-oviposition behaviour. The eight phases through which a gravid *D.radicum* female may pass after landing could be expressed as 32 mutually exclusive behavioural events including "ovipositor drag" which took place on the base and the stem of the plant.

The recording scheme used for *D.radicum* was identical to that used for *D.floralis* which divided events in three classes; location, macro-behaviour and micro-behaviour which were defined as before (see Chapter A1, Materials & Methods and Table A.1.2.). The additional macro-behaviour, ovipositor drag , was added to the dedicated keys on the computer. The resulting scheme was:

LOCATION:	*Leaf, Stem, Base.*
MACRO-BEHAVIOUR:	*Landing phase, Progressive walk, Rest, Regressive walk, Circumventive walk, Pre-oviposition, Minor flight, ovipositor drag, Rejection, Oviposition.*
MICRO-BEHAVIOUR:	*Grooming Front legs, Grooming Rear legs, Grooming proboscis, Proboscis Extension.*

Additional definition

Ovipositor drag: Movement by the gravid female with the ovipositor everted and in contact with the substrate.

Insect behaviour was recorded from landing to oviposition or rejection. The "Observer 2" software package was initially used to analyse behavioural data at a superficial level, collation of data and summing of event means, and to produce spreadsheet and sequence export files which could be used to facilitate more detailed analysis. The sequence files were processed utilising the same series of Fortran files written specifically for the task which were described in Chapter A1. All programs were coded in VAX Fortran-77, version 5.6 (Digital Corporation™), running under the VMS operating system on a local area VAX cluster.

The Fortran processed sequence files were used to produce transition matrixes which determined with what frequency ensuing behaviours followed individual behavioural events. Transition matrixes of all behavioural events were not suitable for chi-squared analysis due to the mathematical interdependence of the values (Schnell *et al*, 1985; Kramer & Schmidhammer, 1992) and the low frequency of many behavioural events. To overcome this problem, a set of tables was constructed based on specific behavioural events performed on each genotype. The tables included the ensuing behaviour of gravid females on the four genotypes and the data were not interdependent. These tables were analysed using chi-squared on Minitab (Minitab Vax/VMS version 7.1, 1989).

Ensuing event relationships

Fortran programs were utilised to extract from the sequence files the duration of individual behaviours and the nature of the ensuing event. For simplicity, the events which followed that under analysis were categorised as either rejection or continuation. A two-way analysis of variance was performed on unbalanced data using the Generalised Linear Model (GLM) command within Minitab (Minitab Vax/VMS version 7.1, 1989). The GLM command was used to fit the model:

Duration of behaviour = A B A*B

Where: A = Genotype

B = Ensuing behaviour

The results of the analysis of variance and the means from the model are shown in the tables of the results section. An arithmetic mean of the duration of individual events is shown in the text and was calculated over all genotypes and for observations for which the ensuing event

included both rejection and continued exploration of the potential host plant. The number of individuals which progressed onto the stem of different genotypes varied widely. Consequently, the stem resting phase was unsuitable for the GLM. One-way analysis of variance (Minitab Vax/VMS version 7.1, 1989) was performed on the durations of behavioural events within the resting phase.

Finally, the probability of a gravid female which carried out a specific behaviour continuing to oviposit was calculated, together with the probability of an ovipositing female having carried out a specific behaviour. Key behaviours in the oviposition sequence should be indicated by a high value for both probabilities.

RESULTS

Detailed graphical notations of the behavioural events which individual gravid *D.radicum* females carried out on Doon Major, GRL aga, Dwarf Green Curled and Fribor are to be found in Figures A.2.1. to A.2.4..

Landing Phase

The low frequency of minor flights made by *D.radicum* on the four plant genotypes tested made the chi-squared distribution of events following the landing phase invalid. Subsequent chi-squared analysis excluded this behaviour and the frequency of the remaining event transitions varied significantly between plant genotypes (chi-squared = 13.97, df = 6, $P<0.05$) (Table A.2.1.). The classification of events as rejection or continued exploration also produced a significant ($P<0.05$) difference in the distribution of ensuing behaviours following the landing phase.

The arithmetic mean overall duration (across all plant genotypes and for rejecting and continuing individuals) of the landing phase for *D.radicum* was 27.1 seconds. There was no significant difference ($P>0.05$) in the duration of the landing phase on different plant genotypes. However, the duration of the landing phase of *D.radicum* females (all plant genotypes) for which the ensuing behaviour was rejection was higher than for flies which carried out other subsequent behaviours ($P=0.004$). *D.radicum* which did not reject as the ensuing behaviour spent a mean time of 21.9s in the landing phase, whilst those which rejected the plant immediately after the landing phase did so after a mean time of 37.2s (Table A.2.2.). The interaction effect between plant genotype and ensuing behaviour was not significant ($P>0.05$).

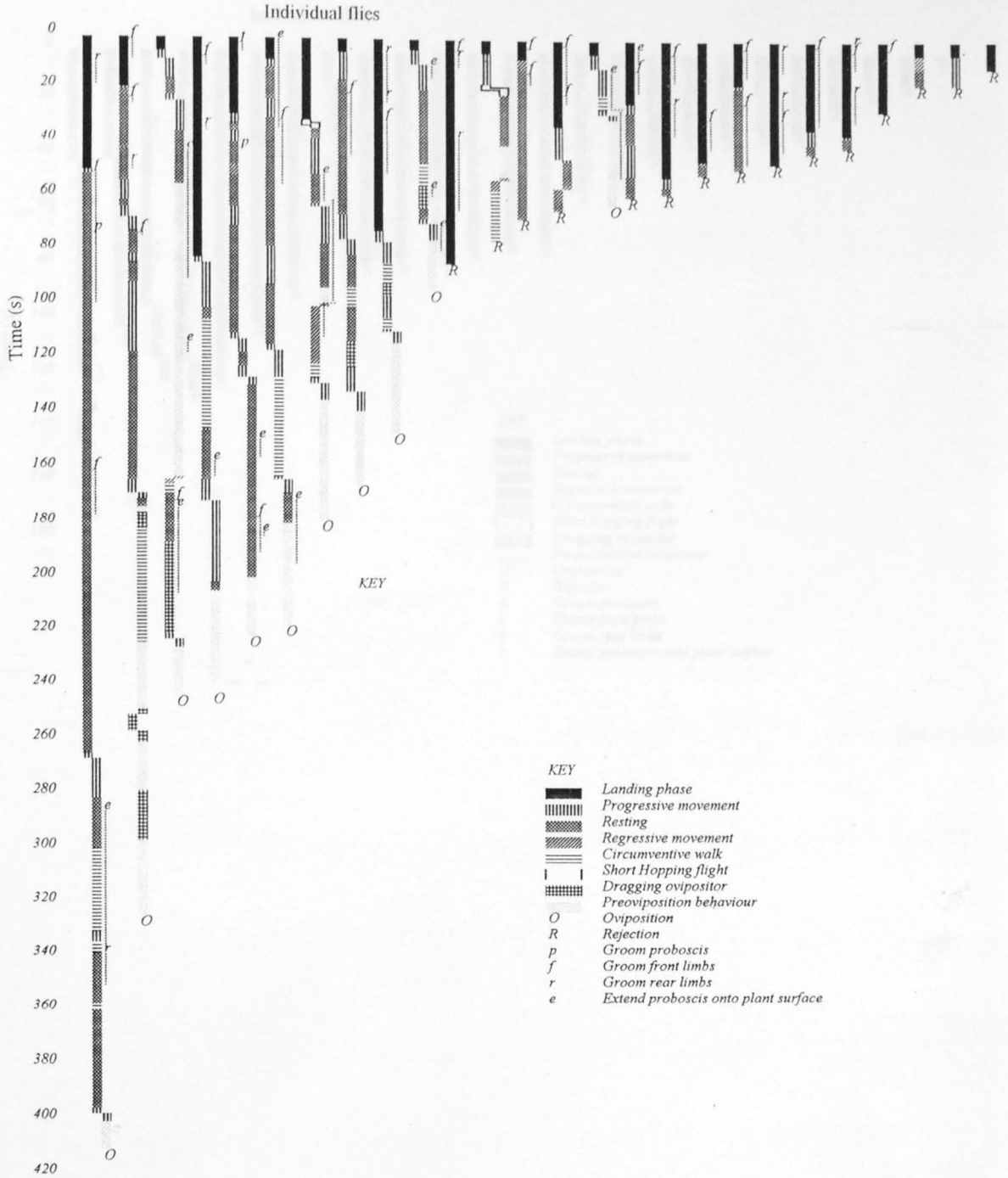


Figure A.2.1. Post-alighting oviposition behaviour of *D.radicum* on the swede Doon Major.

All the individuals landed on the leaf surface, transitions from leaf to stem and from stem to base are shown by the column moving to the right. Macro-behaviours performed by the individual are shown by the pattern on the column and micro-behaviours by the letter to the right of the column, the duration of micro-behaviour corresponds to the dotted line which follows the letter.

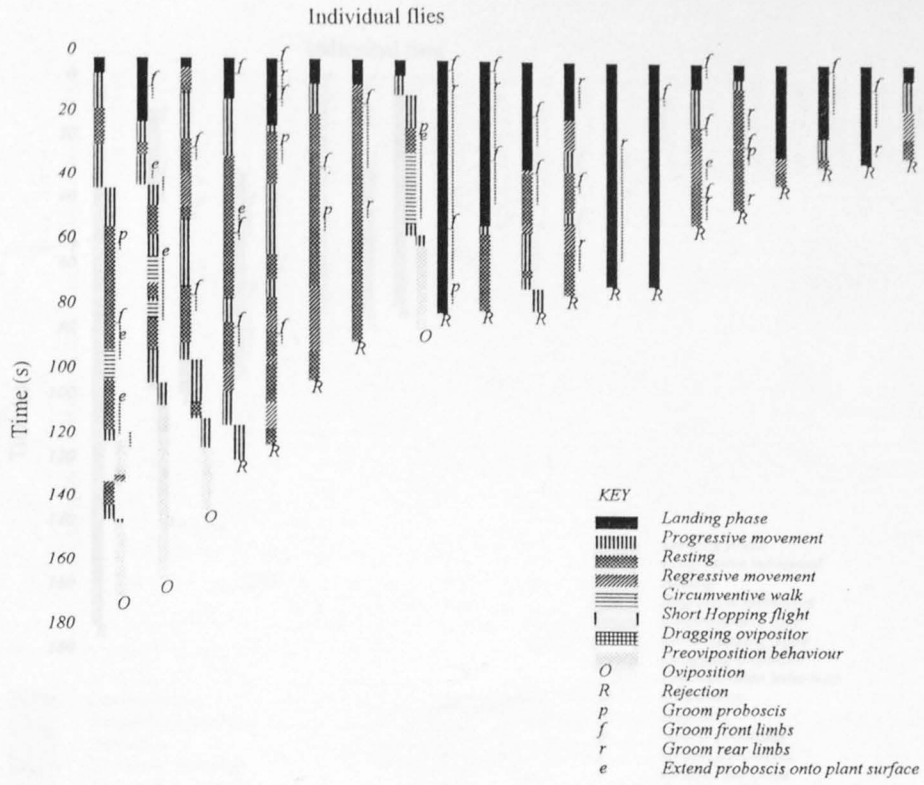


Figure A.2.2. Post-alighting oviposition behaviour of *D.radicum* on the swede GRL aga.

All the individuals landed on the leaf surface, transitions from leaf to stem and from stem to base are shown by the column moving to the right. Macro-behaviours performed by the individual are shown by the pattern on the column and micro-behaviours by the letter to the right of the column, the duration of micro-behaviour corresponds to the dotted line which follows the letter.

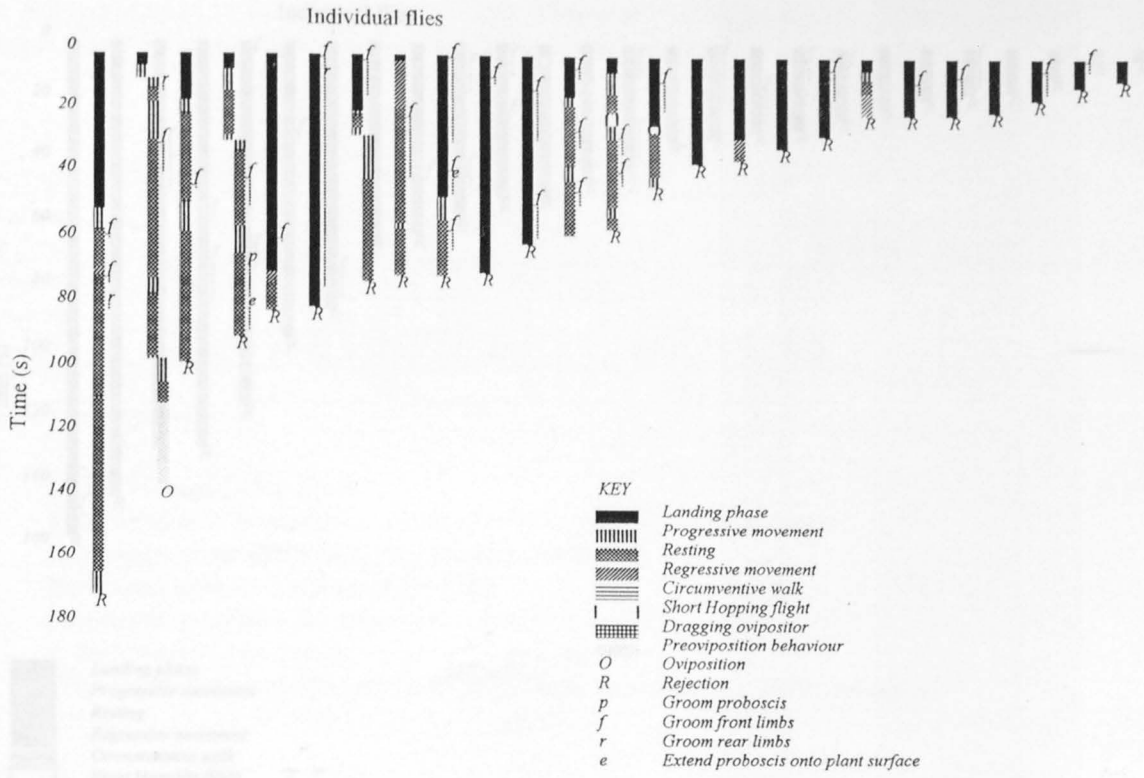


Figure A.2.3. Post-alighting oviposition behaviour of *D.radicum* on the kale Dwarf Green Curled.

All the individuals landed on the leaf surface, transitions from leaf to stem and from stem to base are shown by the column moving to the right. Macro-behaviours performed by the individual are shown by the pattern on the column and micro-behaviours by the letter to the right of the column, the duration of micro-behaviour corresponds to the dotted line which follows the letter.

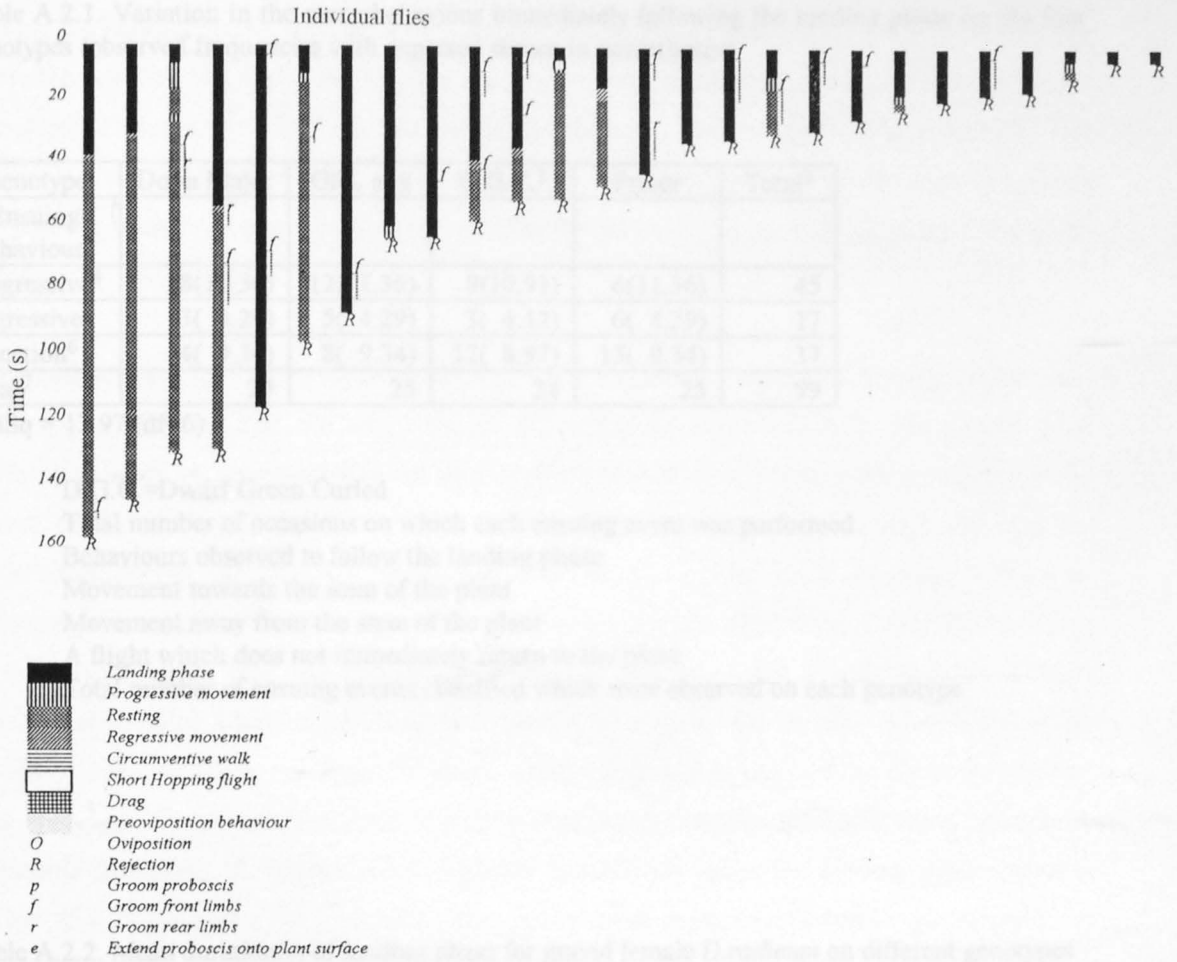


Figure A.2.4. Post-alighting oviposition behaviour of *D.radicum* on the kale Fribor.

All the individuals landed on the leaf surface, transitions from leaf to stem and from stem to base are shown by the column moving to the right. Macro-behaviours performed by the individual are shown by the pattern on the column and micro-behaviours by the letter to the right of the column, the duration of micro-behaviour corresponds to the dotted line which follows the letter.

Table A.2.1. Variation in the macrobehaviour immediately following the landing phase on the four genotypes (observed frequencies with expected shown in parentheses)

Genotype	Doon Major	GRL aga	D.G.C.[1]	Fribor	Total[2]
Ensuing behaviour[3]					
Progressive[4]	18(11.36)	12(11.36)	9(10.91)	6(11.36)	45
Regressive[5]	3(4.29)	5(4.29)	3(4.12)	6(4.29)	17
Rejection[6]	4(9.34)	8(9.34)	12(8.97)	13(9.34)	37
Total[7]	25	25	24	25	99

ChiSq = 13.97 (df=6)

1 D.G.C.=Dwarf Green Curled
2 Total number of occasions on which each ensuing event was performed
3 Behaviours observed to follow the landing phase
4 Movement towards the stem of the plant
5 Movement away from the stem of the plant
6 A flight which does not immediately return to the plant
7 Total number of ensuing events classified which were observed on each genotype

Table A.2.2. Mean duration(s) of landing phase for gravid female *D.radicum* on different genotypes and with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	644.9	534.1	178.0	0.33	0.805
Response	1	4810.7	4731.7	4731.7	8.71	0.004
Geno.resp	3	588.8	588.8	196.3	0.36	0.781
Error	94	51076.5	51076.5	543.4		
Total	101	57121.0				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	26.1(4.97,22)	41.8(11.66,4)	33.9(6.34,26)
GRL aga	15.8(5.65,17)	39.2(8.24,8)	27.5(5.00,25)
Dwarf Green Curled	21.7(6.47,13)	31.7(6.73,12)	26.7(4.67,25)
Fribor	23.9(6.47,13)	36.2(6.47,13)	30.0(4.57,26)
Mean[4]	21.9(2.96,65)	37.2(4.26,37)	

Parentheses = S.D. & N

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

During the landing phase, gravid *D.radicum* females spent a mean period of 7.7s performing microbehaviours. The GLM of the total duration of microbehaviours showed there to be no significant difference ($P>0.05$) in this period on different plant genotypes (Table A.2.3.). The period *D.radicum* females spent performing microbehaviours was higher for flies for which the ensuing behaviour was rejection, 12.4s, than for those which carried out other subsequent behaviours, 5.3s, ($P=0.015$).

The predominant microbehaviour performed by *D.radicum* during the landing phase was grooming of the front legs, for which the overall arithmetic mean was 4.7s. The period spent performing front leg grooming did not vary significantly ($P>0.05$) with plant genotype or the nature of the ensuing behaviour (Table A.2.4.). The period spent grooming rear legs by *D.radicum* during the landing phase was also unaffected by plant genotype ($P>0.05$). However, the nature of the ensuing behaviour was related to a significant ($P=0.006$) variation in the period spent rear leg grooming during the landing phase (Table A.2.5.). Gravid *D.radicum* females which rejected as the ensuing behaviour, did so after spending a mean period of 6.9s grooming rear legs; for those which continued to explore the plant, this period was only 1.1s. *D.radicum* was only observed once performing the third grooming behaviour, proboscis grooming. A female which rejected immediately after the landing phase spent a period of 4.3s grooming the proboscis. This behavioural event was therefore not suitable for statistical analysis. The period of proboscis extension by *D.radicum* did not vary significantly ($P>0.05$) with either plant genotype or the nature of the ensuing behaviour. The overall arithmetic mean for proboscis extension during the landing phase was 0.2s. None of the flies which rejected as the ensuing behaviour were observed to extend their proboscis onto the leaf surface during the landing phase (Table A.2.6.).

During the landing phase, *D.radicum* spent an arithmetic mean duration of 19.4s stationary on the leaf and performing no microbehaviour. There was no significant difference ($P>0.05$) in this period on the different plant genotypes tested nor for those with different ensuing behaviours (Table A.2.7.). However, there was a trend ($P=0.053$) for *D.radicum* females which rejected as the ensuing event to spend a longer period exhibiting this behavioural pattern.

Leaf Resting Phase

The low frequency of minor flights made by *D.radicum* following the leaf resting on the four plant genotypes tested made the chi-squared distribution invalid. Subsequent chi-squared analysis excluded this behaviour and the frequency with which different events followed the leaf resting phase varied significantly between plant genotypes (chi-squared = 15.41, df = 6,

Table A.2.3. Mean duration(s) of total microbehaviours during the landing phase on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	709.3	1038.4	346.1	2.11	0.104
Response	1	1053.5	1001.9	1001.9	6.12	0.015
Geno.resp	3	173.5	173.5	57.8	0.35	0.787
Error	94	15396.8	15396.8	163.8		
Total	101	17333.1				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	9.4(2.73,22)	15.4(6.40, 4)	12.4(3.48,26)
GRL aga	5.6(3.10,17)	17.2(4.53, 8)	11.4(2.74,25)
Dwarf Green Curled	4.8(3.55,13)	11.4(3.70,12)	8.1(2.56,25)
Fribor	1.4(3.55,13)	5.5(3.55,13)	3.5(2.51,26)
Mean[4]	5.3(1.63,65)	12.4(2.34,37)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.4. Mean duration(s) of front grooming during landing phase on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	120.07	83.55	27.85	0.55	0.651
Response	1	70.15	34.77	34.77	0.68	0.410
Geno.resp	3	101.54	101.54	33.85	0.67	0.575
Error	94	4780.86	4780.86	50.86		
Total	101	5072.63				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	6.5(1.52,22)	3.7(3.57,4)	5.1(1.94,26)
GRL aga	3.6(1.73,17)	7.0(2.52,8)	5.3(1.53,25)
Dwarf Green Curled	4.5(1.98,13)	5.8(2.06,12)	5.2(1.43,25)
Fribor	1.4(1.98,13)	4.7(1.98,13)	3.1(1.40,26)
Mean[4]	4.0(0.91,65)	5.3(1.30,37)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.5. Mean duration(s) rear grooming in landing phase on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	242.96	548.43	182.81	2.16	0.098
Response	1	589.08	681.21	681.21	8.03	0.006
Geno.resp	3	215.00	215.00	71.67	0.85	0.473
Error	94	7970.36	7970.36	84.79		
Total	101	9017.40				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	2.4(1.96,22)	11.7(4.60,4)	7.1(2.50,26)
GRL aga	1.9(2.23,17)	9.6(3.26,8)	5.8(1.97,25)
Dwarf Green Curled	0.0(2.55,13)	5.5(2.66,12)	2.8(1.84,25)
Fribor	0.0(2.55,13)	0.8(2.55,13)	0.4(1.81,26)
Mean[4]	1.1(1.17,65)	6.9(1.68,37)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.6. Mean duration(s) of proboscis extension during landing phase (microbehaviour) on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	3.2649	0.9052	0.3017	0.38	0.770
Response	1	0.6705	0.8987	0.8987	1.12	0.292
Geno.resp	3	0.9052	0.9052	0.3017	0.38	0.770
Error	94	75.3536	75.3536	0.8016		
Total	101	80.1941				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	0.5(0.19,22)	0.0 (0.45,4)	0.3(0.24,26)
GRL aga	0.0(0.22,17)	0.0 (0.32,8)	0.0(0.19,25)
Dwarf Green Curled	0.3(0.25,13)	0.0(0.26,12)	0.2(0.18,25)
Fribor	0.0(0.25,13)	0.0(0.25,13)	0.0(0.18,26)
Mean[4]	0.2(0.11,65)	0.0(0.16,37)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

$P<0.025$) (Table A.2.8.). The pattern of ensuing events, when simplified to rejection or continued exploration of the plant, was also significant (chi-squared = 12.41, df = 3, $P<0.01$). The arithmetic mean overall duration of the leaf resting phase for *D.radicum* was 23.2s. However, there was no significant difference ($P>0.05$) in the duration of the leaf resting phase on different plant genotypes or for those with different ensuing behaviours (Table A.2.9).

During the leaf resting phase, microbehaviours were performed by gravid *D.radicum* females for an arithmetic mean period of 4.5s. The mean period performing microbehaviour did not vary significantly ($P>0.05$) between plant genotypes nor between those rejecting and continuing as the ensuing behaviour (Table A.1.10.).

The predominant microbehaviour performed by *D.radicum* during the leaf resting phase was front leg grooming, for which the arithmetic mean was 3.4s. Rear leg grooming, proboscis grooming and extension of the proboscis constituted 0.4s, 0.5s and 0.2s of the microbehaviours respectively. The mean duration(s) of the four microbehaviours performed by *D.radicum* females during the leaf resting phase did not vary significantly ($P>0.05$) between plant genotypes nor for gravid females with different ensuing events (Tables A.2.11. to A.2.14.). The proboscis was not observed to be either groomed or extended by *D.radicum* females on either of the two kale genotypes which were tested.

During the leaf resting phase, *D.radicum* spent an arithmetic mean duration of 18.7s performing no microbehaviour. There was no significant difference ($P>0.05$) in this period on the four plant genotypes nor for *D.radicum* females which performed different ensuing behaviours (Table A.1.15.).

Stem Resting Phase

The distribution of ensuing behaviours for the stem resting phase made the data unsuitable for GLM analysis. On Fribor, *D.radicum* was not observed progressing onto the stem and, consequently, stem resting was not recorded. On 8 occasions *D.radicum* females were observed resting on the stem of GRL aga and on 18 occasions resting on the stem of Doon Major. In both cases, none rejected the plant as the ensuing behaviour. The mean durations of the stem rest for Doon Major and GRL aga were 14.8s and 11.8s respectively. Six observations of stem resting were made for *D.radicum* on Dwarf Green Curled; four flies continued to explore the plant and two rejected as the ensuing behaviour. The mean duration of the stem resting phase on Dwarf Green Curled was 24.3s and there was no significant difference ($P>0.05$) in the duration of the behaviour for observations with different ensuing

Table A.2.7. Mean duration(s) of landing phase (excluding microbehaviour) on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	2119.2	1464.6	488.2	1.36	0.260
Response	1	1361.7	1379.0	1379.0	3.84	0.053
Geno.resp	3	216.4	216.4	72.1	0.20	0.896
Error	94	33789.2	33789.2	359.5		
Total	101	37486.5				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	16.7(4.04,22)	26.4 (9.48,4)	21.5(5.15,26)
GRL aga	10.3(4.60,17)	22.0 (6.70,8)	16.1(4.06,25)
Dwarf Green Curled	16.9(5.26,13)	20.3(5.47,12)	18.6(3.80,25)
Fribor	22.5(5.26,13)	30.6(5.26,13)	26.6(3.72,26)
Mean[4]	16.6(2.41,65)	24.8(3.47,37)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.8. Variation in the macrobehaviour immediately following resting upon the leaf on the four genotypes (observed frequencies with expected shown in parentheses).

Genotype	Doon Major	GRL aga	D.G.C.[1]	Fribor	Total[2]
Ensuing behaviour[3]					
Progressive[4]	10(7.81)	10(10.79)	10(7.44)	2(5.95)	32
Regressive[5]	2(3.42)	9(4.72)	2(3.26)	1(2.60)	14
Rejection[6]	9(9.77)	10(13.49)	8(9.30)	13(7.44)	40
Total[7]	21	29	20	16	86

ChiSq = 15.41 (df=6)

1 D.G.C.=Dwarf Green Curled
2 Total number of occasions on which each ensuing event was performed
3 Behaviours observed to follow the landing phase
4 Movement towards the stem of the plant
5 Movement away from the stem of the plant
6 A flight which does not immediately return to the plant
7 Total number of ensuing events classified which were observed on each genotype

Table A.2.9. Mean duration(s) of leaf resting on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	7044.8	4502.3	1500.8	1.57	0.202
Response	1	940.9	833.5	833.5	0.87	0.353
Geno.resp	3	1836.6	1836.6	612.2	0.64	0.590
Error	77	73401.7	73401.7	953.3		
Total	84	83223.9				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	34.5(8.56,13)	13.0(10.29,9)	23.7(6.69,22)
GRL aga	14.1(7.08,19)	17.5(9.76,10)	15.8(6.03,29)
Dwarf Green Curled	20.9(8.91,12)	14.8(11.67,7)	17.8(7.34,19)
Fribor	46.8(21.83,2)	40.0(8.56,13)	43.4(11.73,15)
Mean[4]	29.0(6.52,46)	21.3(5.07,39)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.10. Mean duration(s) of all microbehaviours on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	238.85	197.15	65.72	0.77	0.514
Response	1	55.27	10.69	10.69	0.13	0.724
Geno.resp	3	254.37	254.37	84.79	1.00	0.400
Error	77	6560.23	6560.23	85.20		
Total	84	7108.71				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	10.1(2.56,13)	3.2 (3.08,9)	6.7(2.00,22)
GRL aga	3.4(2.12,19)	4.3(2.92,10)	3.9(1.80,29)
Dwarf Green Curled	3.8(2.67,12)	1.8 (3.49,7)	2.8(2.20,19)
Fribor	0.0 (6.53,2)	4.4(2.56,13)	2.2(3.51,15)
Mean[4]	4.3(1.95,46)	3.4(1.52,39)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.11. Mean duration(s) of front leg grooming during the leaf resting phase on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	124.13	110.01	36.67	0.72	0.542
Response	1	23.40	2.92	2.92	0.06	0.811
Geno.resp	3	83.91	83.91	27.97	0.55	0.649
Error	77	3909.33	3909.33	50.77		
Total	84	4140.77				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	6.9(1.98,13)	3.2 (2.38,9)	5.1(1.55,22)
GRL aga	2.4(1.64,19)	2.5(2.25,10)	2.4(1.39,29)
Dwarf Green Curled	3.6(2.06,12)	1.8 (2.69,7)	2.7(1.69,19)
Fribor	0.0 (5.04,2)	3.5(1.98,13)	1.8(2.71,15)
Mean[4]	3.2(1.50,46)	2.8(1.17,39)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.12. Mean duration(s) of rear leg grooming during the leaf resting phase on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	8.243	10.459	3.486	1.73	0.169
Response	1	4.167	3.176	3.176	1.57	0.214
Geno.resp	3	9.745	9.745	3.248	1.61	0.194
Error	77	155.579	155.579	2.021		
Total	84	177.734				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	0.2(0.39,13)	0.0 (0.47,9)	0.1(0.31,22)
GRL aga	0.2(0.33,19)	1.6(0.45,10)	0.9(0.28,29)
Dwarf Green Curled	0.2(0.41,12)	0.0 (0.54,7)	0.1(0.34,19)
Fribor	0.0 (1.01,2)	0.9(0.39,13)	0.5(0.54,15)
Mean[4]	0.2(0.30,46)	0.6(0.23,39)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.13. Mean duration(s) of proboscis grooming during the leaf resting phase on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	23.05	14.17	4.72	0.46	0.711
Response	1	11.33	5.91	5.91	0.58	0.450
Geno.resp	3	15.88	15.88	5.29	0.52	0.673
Error	77	790.39	790.39	10.26		
Total	84	840.66				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	2.2(0.89,13)	0.0 (1.07,9)	1.1(0.69,22)
GRL aga	0.6(0.74,19)	0.3(1.01,10)	0.4(0.63,29)
Dwarf Green Curled	0.0(0.92,12)	0.0 (1.21,7)	0.0(0.76,19)
Fribor	0.0 (2.27,2)	0.0(0.89,13)	0.0(1.22,15)
Mean[4]	0.7(0.68,46)	0.1(0.53,39)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.14. Mean duration(s) for which proboscis was extended during the leaf resting phase on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	6.224	4.056	1.352	0.98	0.407
Response	1	2.746	1.441	1.441	1.04	0.310
Geno.resp	3	4.056	4.056	1.352	0.98	0.407
Error	77	106.258	106.258	1.380		
Total	84	119.284				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	1.1(0.33,13)	0.0 (0.39,9)	0.6(0.25,22)
GRL aga	0.2(0.27,19)	0.0(0.37,10)	0.1(0.23,29)
Dwarf Green Curled	0.0(0.34,12)	0.0 (0.44,7)	0.0(0.28,19)
Fribor	0.0 (0.83,2)	0.0(0.33,13)	0.0(0.45,15)
Mean[4]	0.3(0.25,46)	0.0(0.19,39)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

Table A.2.15. Mean duration(s) of the leaf resting phase during which there was no microbehaviour taking place on different genotypes and for gravid females with different ensuing events.

Analysis of Variance for Duration

Source	DF	Seq SS	Adj SS	Adj MS	F	*P*
Genotype	3	6711.6	4779.0	1593.0	2.58	0.060
Response	1	540.1	655.4	655.4	1.06	0.306
Geno.resp	3	927.4	927.4	309.1	0.50	0.683
Error	77	47530.4	47530.4	617.3		
Total	84	55709.5				

Table of Means for Duration

Genotype	Accept[1]	Reject[2]	Overall[3]
Doon Major	24.3(6.89,13)	9.7 (8.28,9)	17.0(5.39,22)
GRL aga	10.7(5.70,19)	13.2(7.86,10)	12.0(4.85,29)
Dwarf Green Curled	17.1(7.17,12)	13.0 (9.39,7)	15.0(5.91,19)
Fribor	46.8(17.57,2)	35.5(6.89,13)	41.1(9.44,15)
Mean[4]	24.7(5.24,46)	17.9(4.08,39)	

Parentheses contain S.D. & N.

1 Those individuals which remain on the plant as the ensuing behaviour
2 Those individuals which leave the plant as the ensuing behaviour
3 All the individuals which accept or reject the plant as the ensuing behaviour
4 The combined mean duration of the behaviour for flies on all the genotypes tested

events. There was also no significant difference ($P>0.05$) in the duration of the behaviour for observations on the three plant genotypes.

Microbehaviours constituted 7.3s of the stem resting phase of gravid *D.radicum* females. The mean period performing microbehaviour did not vary significantly ($P>0.05$) between those rejecting and continuing as the ensuing behaviour on Dwarf Green Curled, for which the means were 9.0s and 5.1s respectively. On Doon Major and GRL aga, microbehaviours comprised 5.1s and 12.6s respectively. The mean period performing microbehaviour did not vary significantly ($P>0.05$) between the three plant genotypes tested.

The microbehaviour performed by *D.radicum* during the stem resting phase was dominated by extension of the proboscis and grooming of the front legs. Proboscis extension comprised an overall mean of 3.6s and did not vary significantly between genotypes ($P>0.05$). The mean period spent extending the proboscis on Doon Major was 5.1s and on GRL aga was 2.1s. None of the observations which resulted in continued exploration of the Dwarf Green Curled plant included proboscis extension, but one of the two flies which rejected spent 7.1s with the

proboscis extended. The predominant grooming behaviour carried out by *D.radicum* during the stem resting phase was grooming of the front legs for which the overall mean was 0.8s. *D.radicum* was rarely observed performing the other grooming behaviours during the stem resting phase. Grooming of the rear legs was observed for one individual on both Doon Major and Dwarf Green Curled, for which the durations were 12.5s and 1.9s respectively. Both of the individuals concerned continued to explore the surface of the plant. Grooming of the proboscis was also infrequently observed being performed by *D.radicum*. One of the individuals which rejected Dwarf Green Curled included 10.6s of proboscis grooming and two of the observations on GRL aga included proboscis grooming of 1.4s and 3.5s.

The probability of *D.radicum* individuals which carried out a particular behaviour continuing to oviposit and the probability of ovipositing individuals having performed a particular behaviour showed considerable variation (Table A.2.16.). The probabilities were bulked across the four plant genotypes. The probability of a *D.radicum* female which landed on the plant ovipositing was 0.16. Three behavioural events were always carried out by flies which went on to oviposit: "Landing Phase"; "Progressive Movement" on the stem; "Preoviposition Behaviour". The probability of a *D.radicum* female ovipositing became very high as soon as progressive movement took place on the stem of the plant. Behaviours associated with the stem and base were nearly all associated with probabilities of ovipositing which were greater than 0.80 . The exceptions were regressive movements on the stem and base. The probabilities of flies which oviposited having performed specific behaviours were slightly less well defined. A high proportion of flies which oviposited had performed stem resting and/or circumventive walks on the stem of the plant.

DISCUSSION

Visual representations of the host selection behaviour of *Delia radicum* showed apparent variation on the four plant genotypes tested. The results obtained by analysing the behaviour on the plant show defined antixenotic resistance to *D.radicum* being displayed in the kale genotypes Fribor and Dwarf Green Curled, and in the swede GRL aga, when compared to the swede genotype Doon Major. The kale genotypes were consistently rejected during the exploration of the leaves of the plants, whilst *D.radicum* was observed to progress down swede plants and, in the case of Doon Major, to oviposit on more than 40% of occasions. This behaviour contrasted with the behaviour on the swede GRL aga, on which approximately 15% of the *D.radicum* females which landed went on to oviposit.

Table A.2.16. Probabilities for specific behavioural relationships

Location	Behaviour	Probability of Ovipositing[1]	Proportion of Ovipositors[2]
Leaf	Land	0.15	1.00
Leaf	Land+Groom	0.11	0.38
Leaf	Land+Extend	0.33	0.06
Leaf	Progressive	0.29	0.94
Leaf	Regressive	0.13	0.25
Leaf	Rest	0.15	0.50
Leaf	Rest+Groom	0.21	0.38
Leaf	Extend	0.40	0.13
Stem	Progressive	0.80	1.00
Stem	Regressive	0.60	0.19
Stem	Rest	0.86	0.75
Stem	Groom	0.83	0.31
Stem	Extend	0.90	0.56
Stem	Circumventive walk	0.92	0.75
Stem	Ovipositor drag	1.00	0.25
Base	Progressive	1.00	0.87
Base	Regressive	0.75	0.19
Base	Rest	0.86	0.38
Base	Groom	1.00	0.06
Base	Extend	1.00	0.43
Base	Ovipositor drag	1.00	0.13
Base	Preoviposition behav.	0.94	1.00
	Hopping flight[3]	0.67	0.13

1 Probability of an individual which carries out the listed behaviour continuing to oviposit on the plant.
2 The probability of an ovipositing female having performed the listed behavioural event.
3 From any point on the plant to any point on the plant

The only previous published detailed study of the post-alighting oviposition behaviour of *D.radicum* (Städler & Schoni, 1990) described only the behaviour that took place on the severed leaf of a plant. As a consequence, the authors drew no conclusions as to the point during the post-alighting oviposition behaviour when the potential host was being selected or rejected. However, leaf surface extracts applied to a surrogate plant stimulated similar behaviour to that found on the leaf and they concluded that leaf surface chemicals were important in the host selection process. The results obtained in this study demonstrate that, for *D.radicum,* post-alighting oviposition selection is largely determined by behavioural events during the leaf exploration phases.

The evidence for *D.radicum* oviposition host selection taking place on the leaf comes from four sources. The first piece of evidence which supports the selection having taken place on the leaf was the variation in the ensuing events which relate to the landing and leaf resting phases. The chi-squared analyses of the ensuing behaviours associated with the landing and leaf resting phases showed that in both cases the distribution varied significantly. In both cases the number of rejections on the highly resistant kale Fribor, was much higher than random rejection would predict from the chi-squared distribution. If *D.radicum* was not differentiating between the different potential host plants during these two phases, the distribution of ensuing events would show no significant differences.

The second piece of evidence which supports host selection taking place on the leaf was the significantly longer duration of the landing phase for flies which rejected the plant immediately after the landing phase. This behaviour was at its most extreme on the swede GRL aga, for which the landing phase of *D.radicum* which continued was 15.8s long and those which rejected was 39.2s long. However, this behaviour was not seen for *D.radicum* females which rejected immediately after the leaf resting phase. There was no significant difference in the duration of the leaf resting phase for *D.radicum* with different ensuing behaviours.

The third piece of evidence supporting the importance of the leaf was the high probabilities of oviposition associated with behavioural events on the stem of the plant. Having landed on a plant, the probability of a *D.radicum* female ovipositing was 0.15, approximately one in seven. Once a *D.radicum* female progressed onto the stem of the plant, the probability of ovipositing increased considerably. The progression from leaf exploration, for which the probabilities of oviposition taking place were all below 0.40, to stem exploration, for which the probabilities of oviposition taking place were mostly greater than 0.80, was particularly marked.

The fourth piece of evidence in support of the leaf as the site of host selection was the very low number of rejections which were observed to take place on the stem of the genotypes being tested. The stationary behavioural events were invariably those which preceded rejection. Individuals were recorded leaving the plant from movement behaviours, but these rejections were associated with individuals which "fell" from the plant. Powered flight from the plant was normally preceded by the flies momentarily lowering their abdomen to the leaf surface, which necessitated at least a short halt in their movement across the plant. Only two of the 32 individuals which were observed resting on the stem of the plant rejected as the

ensuing behaviour. It can be concluded that the differentiation taking place at this stage in the host selection behaviour was minimal.

The results produced in this study clearly support the previously published findings (Zohren, 1968; Städler & Schoni, 1990) which concluded that contact with the leaf surface and stem of host plants was essential to host plant recognition prior to oviposition by *D.radicum*. Previous studies have utilised leaves severed from the plant as an oviposition medium. As a consequence, the part played by the plant below the petiole has remained unexplored. In addition to the confirmation of the importance of the leaf surface on intact host plants, examination of leaves still attached to the plant has allowed a more thorough assessment of the host selection behavioural processes. Consequently, the importance of the leaf has been reinforced by the knowledge that the behaviour lower down the plant, although varied, consistently resulted in continuation to oviposit.

The durations of behavioural events associated with different ensuing events were not as distinct as had been the case with *D.floralis* (Chapter A1). Chi-squared analyses showed that oviposition site selection was taking place both during the landing phase and during the leaf resting behavioural events. The duration of the landing phase was significantly longer for rejecting individuals, when contrasted with those that continued to explore the plant. However, there were no significant differences associated with the duration of the leaf resting phase. This result makes it difficult to conclude what is taking place during the leaf resting phase. The differentiation during the landing phase can be attributed to *D.radicum* seeking positive oviposition stimuli, the longer period being associated with failure to find adequate stimulation. The durations of the leaf resting phase make it impossible to draw conclusions as to the nature of the stimuli involved. The absence of proboscis extension for individuals rejecting following the landing phase, and all proboscis-associated behaviour during the leaf resting phase on the 2 kales, indicate that the proboscis may have a role in oviposition site selection. The pattern of absence described above indicates that the proboscis may have a secondary function after tarsal sensilla provide primary site identification.

Although detailed studies of the behaviour of phytophagous insects on the host plant are uncommon, bioassays of the surface extracts of the host plants of cabbage root fly have also produced evidence that the leaf is important in the host selection process. Zohren (1968) concluded that swede juice applied to a surrogate leaf was more stimulatory than the real leaf of the plant. This effect may have been due to a number of factors including elevated concentrations of important compounds in the extract, absence of negative leaf structure features or simply the application of high extract concentrations. Städler & Schoni (1990) not

only studied the behaviour of *D.radicum* on severed leaves but also bioassayed surface extracts of cabbage plants and demonstrated that chemicals extracted from the leaves stimulated oviposition. Roessingh & Städler (1990) found that variation in the form of the leaves of surrogate plants altered the host preference of *D.radicum*. Their work demonstrated that, amongst many other factors, the possession of a defined stem was important to oviposition by *D.radicum*. Consequently, although this study confirms the importance to host selection of the leaf surface, the presence of stem features is also important for progression to oviposit.

Surface extracts of host plants of Crucifer-feeding insects are frequently used to bioassay for compounds which have a stimulatory effect on insects (Traynier & Truscott, 1991; Roessingh *et al*, 1992a; 1992b; reviewed Städler, 1992), whilst the detailed host selection behaviour is a neglected area. The reasons why the leaf is so important in host selection has received some discussion in Chapter A1. The significance of the oviposition behaviour of phytophagous insects on their host plants is discussed in more depth in the Section Discussion.

Chapter A3

The chemical basis of antixenotic resistance to oviposition by *Delia radicum* and *Delia floralis*

INTRODUCTION

Antixenotic resistance (Kogan & Ortman, 1978) to *Delia radicum* and *Delia floralis* oviposition is a product of the structure and chemical composition of the plant with which the gravid female makes contact. The influence of plant colour and form on *D.radicum* host selection is well documented. Prokopy *et al* (1983a; 1983b) demonstrated that, whilst the area of surrogate leaves was important to host plant selection, the leaf colour also influenced host choice. Surrogate plants mimicking real plants which had a whitish wax bloom or red coloured foliage were less detectable by or less attractive to *D.radicum.* Tuttle et al (1988) found that yellow sticky traps were most effective when placed at ground level, a height which corresponded to that of the host plant. The structure of surrogate leaves has also been shown to influence oviposition site selection. Possession of a waxy surface, a stem and vertical folds, to aid orientation by gravid female *D.radicum*, has been found to increase the oviposition on surrogate plants (Roessingh & Städler, 1990).

Host chemistry is widely accepted as a major influence on the host selection of Crucifer-feeding insects, including *D.radicum* and *D.floralis* (reviewed Städler, 1992). The role of volatile leaf and root chemicals in attracting *Delia* spp. to plants is well documented. Wallbank & Wheatley (1979) utilised the responses of *D.radicum* to the volatile plant compound allyl-isothiocyanate, to improve the performance of water traps seven-fold. Ellis *et al* (1980) found that oviposition on radish by *D.radicum* was positively correlated with two glucosinolate volatile hydrolysis products. Further work with the cabbage root fly on insect orientation and responses to visual and tactile stimuli confirmed the role of glucosinolates and their breakdown products as allelochemics (Nottingham and Coaker, 1985; Tuttle *et al*, 1988).

The concentrations of primary and secondary plant products on the leaf surface is highly influential in the oviposition host selection of phytophagous insects (Berenbaum & Seigler, 1992). The chemical ecology of the interactions between insects and Brassicas is particularly well documented. Glucosinolates and their breakdown products are synonymous with the Crucifers (Kjaer, 1976; Louda & Mole, 1991) and have been linked with the oviposition specificity or feeding stimulation of more than twenty Crucifer-feeding insects (reviewed

Städler, 1992) and deterrence of many generalists. Bodnaryk (1991) found that high concentrations of sinalbin in young cotyledons and leaves deterred the feeding of the flea beetle *Phyllotreta cruciferae,* and larvae of the bertha armyworm, *Mamestra configura.* However, in older leaves lower concentrations of sinalbin did not deter *P.cruciferae,* a Crucifer specialist, whilst *M. configura*, a generalist, was deterred from feeding. The cabbage butterfly, *Pieris rapae*, can be stimulated to oviposit by concentrations of 3-indolemethyl glucosinolate (glucobrassicin) as low as 10^{-6}M when associated with water. Higher concentrations elicited faster oviposition and stronger visual responses, but its enzyme hydrolysis products failed to influence oviposition (Traynier & Truscott, 1991). Sachdev-Gupta *et al* (1992) concluded that *P.rapae* responded preferentially to oviposition sites which contained aromatic glucosinolates. van Loon *et al* (1992) carried out an investigation of the influence of leaf surface chemicals on the oviposition of *Pieris brassicae* (Lepidoptera:Pieridae). Fractionation of a methanol extract of the leaf of cabbage, *Brassica oleracea,* demonstrated that the oviposition of *P.brassicae* could be largely attributed to the presence of 3-indolyl-methyl glucosinolate.

Insects possess the ability to "taste" utilising gustatory receptor cells which are located in cuticular structures called sensilla associated with the mouthparts, buccal cavity, tarsi, antennae and ovipositors (Frazier, 1992). Städler (1978) demonstrated a receptor cell in tarsal sensory hairs of female *D.radicum*, sensitive to sinigrin, a glucosinolate, with a threshold sensitivity below 0.0001M, in addition to the presence of receptors for sugars and salt. The presence or concentration of chemicals strongly influence gravid *D.radicum* females during exploration of the leaf surface. The deterrent effect of sinapic (3,5-dimethoxy-4-hydroxycinnamic) acid in the frass of garden pebble moth reduced *D.radicum* oviposition by 60% (Jones & Finch, 1987; Jones *et al*, 1988). Oviposition by *D.radicum* was reduced by over 50% by a range of carboxylic acids (Cole *et al*, 1989). Roessingh *et al* (1992b) investigated the role of seven glucosinolates in stimulating the oviposition of *D.radicum*. This species exhibited a clear dose response only to 3-indolyl-methyl glucosinolate, to which the flies chemoreceptors were particularly responsive. In addition, following fractionation, the most stimulatory part of the extract contained no glucosinolates and the authors concluded that other compounds were of greater importance. The glucosinolates are a group of compounds particularly characteristic of the family Cruciferae (Kjaer, 1976) and nearly half of the British Cruciferae have been confirmed as supporting the larval feeding and development of *D.radicum* (Finch & Ackley, 1977).

Städler (1992) concluded that not all secondary plant compounds were linked to host feeding specificity and their role in the oviposition of *D.radicum* and *D.floralis* is unclear. For both

cabbage and turnip root fly, the extent to which particular glucosinolates stimulate oviposition varies with the structure of individual molecules and may be related to both the length of side chains and the presence of individual structures (Roessingh *et al*, 1992b). Whilst isolated individual compounds have positive and negative influences upon oviposition, it can be assumed that in combination on the plant they alter the motivation of gravid females during a selection procedure. The host selection sequence is interrupted on plant genotypes exhibiting antixenotic resistance (Chapters A1 and A2) and the leaf surface is critical in the selection of an oviposition site.

Oviposition on different plant genotypes can be assessed using two basic methods, "choice" and "no choice", both of which have advantages and disadvantages. No choice tests more accurately reflect the situation in modern agriculture where a particular genotype dominates the range of plants available for oviposition. However, no choice tests are subject to wide variations in daily oviposition which have been demonstrated for *D.radicum* (Zohren, 1968) and to variations in physiological need to oviposit. Choice tests provide a more direct contrast between genotypes at the cost of less accurately reflecting the situation in the field. Laboratory "choice test" methods for the study of antixenotic resistance to *D.radicum* were much improved with the advent of a turntable to decrease the effect of the positioning of plants within a cage (Ellis & Hardman, 1975). The application of such methods to the testing of leaf extracts by bioassay should much improve the reliability of results.

The aim of the work in this chapter was to investigate the contribution of leaf surface compounds to the host selection for oviposition of *D.radicum* and *D.floralis*. This objective was investigated by extracting chemicals from the leaf surface, fractionating these extracts and using surrogate plants to test their activities as oviposition stimuli. Secondarily, the concentrations of individual glucosinolates in the leaf surface extracts were quantified.

MATERIALS AND METHODS.

Biological materials

Work has been carried out on two genotypes of kale, cvs Fribor and Dwarf Green Curled, and two genotypes of swede, a Scottish Crop Research Institute (SCRI) breeding line, GRL aga and cv Doon Major. The genotypes were selected for the reasons given in Chapters A1 and A2. Plants were glasshouse grown in 10cm pots containing a 3:1 mixture of Levington® Universal compost and sand. The growth conditions were a 16:8h light:dark regimen and a temperature range of 16-21°C. *D.floralis* came from a culture established in 1985 at SCRI

and *D.radicum* came from a culture established from pupae collected in the field in 1991 in Scotland and supplemented with pupae which were kindly supplied by the Entomology Department of Horticulture Research International, Wellesbourne.

Bioassay

During bioassays a single replicate, one of each plant genotype or treatment was placed on each layer of the turntable. When assessing oviposition preference on whole plants, an undamaged plant grown under the same conditions as for Chapters A1 and A2, at the 5-8 true leaf development stage, was selected and its compost covered with a paper gasket. The gasket was then covered with sand, which had been sieved to produce a size range of 1.0 to 1.4 mm. Flies, 30 to 40 females and 10 males, aged 7-18 days were introduced to the turntable (Plate A.3.1. & A.3.2.) at 0900. The turntable chamber contained the food sources used in culture, placed off the turntable to avoid the position of the food influencing host selection. Flies were allowed 4 hours to acclimatise to the change of cage and recover from the transfer process before each test began. Plants were introduced at 1300 and exposed to the gravid females for 24h. The eggs were then recovered by floating out and sieving (0.2mm mesh), and counted. The number of eggs laid on each plant was then expressed as a percentage of the total number of eggs laid on all plants during the 24h of the test. When less than 200 eggs were laid during 24h, the results of that replicate of the choice test were disregarded. Results were analysed utilising Statview 4.0 (Abacus Concepts Inc., Berkley, California) to perform a Kruskal-Wallis test on the percentage eggs laid and Mann-Whitney U test on paired groups.

Leaf surface chemistry

Leaf surface chemicals were extracted from plants (5-8 true leaf stage) using methods based on Roessingh *et al* (1992b). The extraction method used involved two dips into solvents with different polarities. Three or four undamaged leaves were dipped into dichloromethane (a non-polar solvent) for five seconds, removed and allowed to drip back into the solvent for a further ten seconds. The same leaves were next dipped into methanol (a relatively polar solvent) for five seconds, removed and allowed to drip back into the methanol for ten seconds before being discarded. In this way, 100g of leaves were surface extracted per 300-400ml of each solvent. All the organic solvents were then removed under vacuum using a rotary evaporator with a water bath at 35-40°C. The dichloromethane extract was reduced to a waxy white residue which was then redissolved in dichloromethane. The methanol extract was reduced to a viscous liquid which contained a light green suspension. The suspension was removed from the methanol extract using glass filters (Whatman GF/D, glass microfibre filters, 4.7cm diameter) to leave a viscous orange liquid. Filters were cut to the smallest size possible to minimise the loss of extract. Both extracts were stored in 4ml screw-top glass vials with a

Plate A.3.1. The turntable used to remove the influence of plant position during the bioassays of plants and surrogate plants treated with leaf surface extracts.

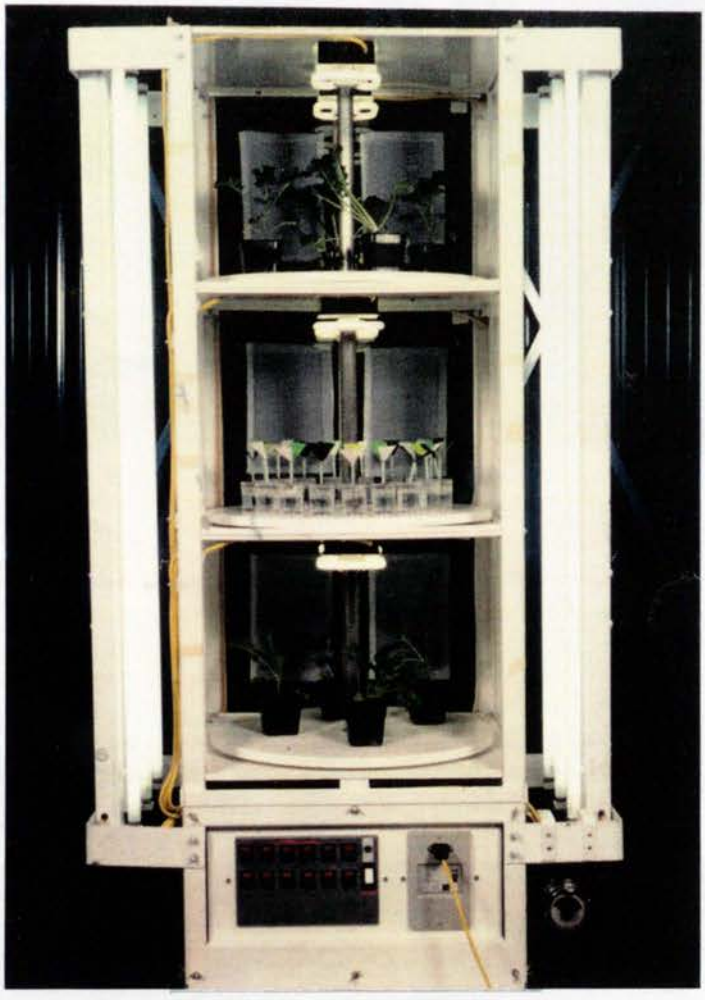
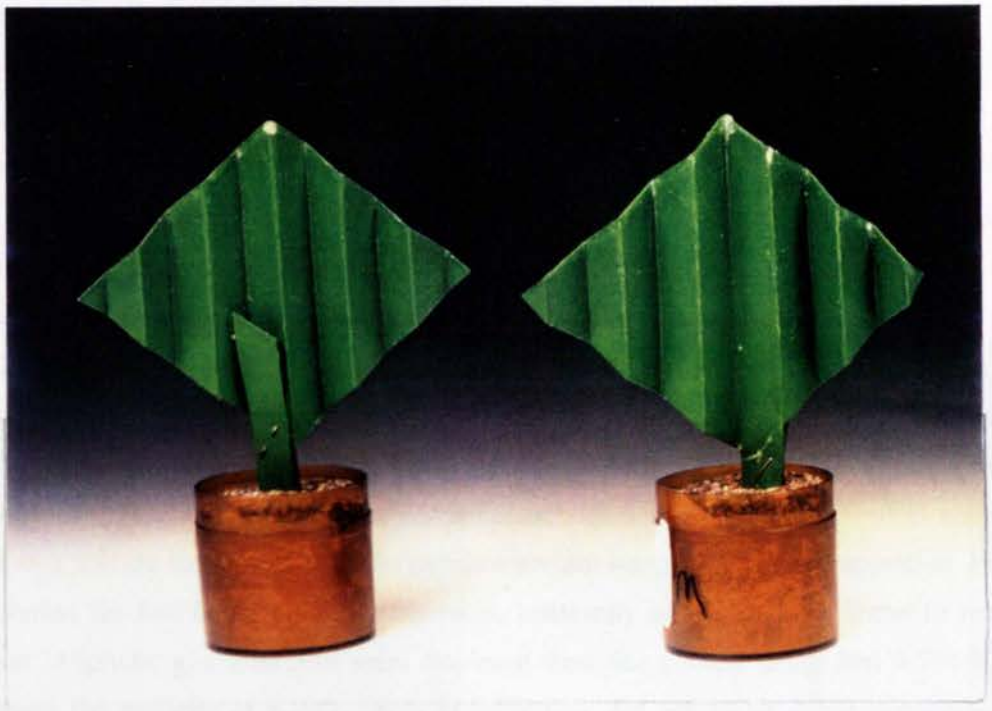


Plate A.3.2. Four plants placed within one of the three shelves within the turntable.

Plate A.3.3. Surrogate plants manufactured by Stifung Behindertenbetriebe im Kanton Schwyz. The surrogate plants were made of green cardboard coated in paraffin wax and embedded in the pot using paraffin wax that was then covered with a layer of sieved sand.



Teflon-lined seal at -20°C. Extracts were diluted with the minimum quantity of the extraction solvent necessary to solubilise at room temperature. The methanol extract was fractionated according to the scheme described later.

The testing of leaf surface extracts applied to surrogate plants was similar to the testing of real plants described above. Initial investigations were made with different colours of card coated in paraffin wax and attached to a drinking straw for use in bioassays. Ultimately, the development of these plants was rejected in favour of a manufactured surrogate leaf. Surrogate leaves were manufactured in Switzerland, by Stiftung Behindertenbetriebe im Kanton Schwyz, to a standard pattern with collaborators at Wädenswil and The Royal Botanical Gardens, Kew. The surrogate plants are manufactured from green card and covered with paraffin wax, and are based on a larger model developed by Schoni and Städler (1990). The use of the manufactured plant was adopted to give consistency with other workers in the field and to make more efficient use of the time available for research. The surrogate leaves were embedded in paraffin wax in a 4cm diameter pot and the wax covered with a 1cm layer of 1.0-1.4 mm diameter sand (Plate A.3.3.). When surrogate plants were being used for the bioassay they were sprayed in a fume hood with 1.25 gram leaf equivalents (gle) of the chemical extract. The extracts were applied between 0900 and 0930 on the day of the bioassay using an air-brush. Sprayed surrogate plants were kept in a fume hood until they were introduced to the flies permitting volatiles from the solvents used to disperse. The methanol and dichloromethane extracts of the four plant genotypes were tested with solvent controls in a 10 way "choice" test. Combinations of dichloromethane extracts and a stimulatory fraction were used to test for oviposition deterrence.

Fractionation of extracts

A-25 DEAE sephadex minicolumns were prepared and stabilised according to the methods for the extraction of glucosinolates in Chapter B3. 10 gle of the methanol extract to be fractionated was loaded onto the column in 2ml of distilled water. The column was then rinsed with 2ml of distilled water. Total intact glucosinolates were removed from the column using 2x 1ml of 0.5M pyridine acetate, initial experiments having discovered that this chemical would remove all the glucosinolates intact from the column. The pyridine acetate was then removed under vacuum using a rotary evaporator with a water bath at 35-40°C and water added as a solvent for the extract. The pyridine acetate was removed by evaporation, keeping the solution for two hours on a rotevaporator, constantly adding distilled water to retain in solution. Aliphatic glucosinolates were displaced from the column using 5ml 0.5M K_2SO_4. Increasing the molarity of K_2SO_4, even to saturation, did not result in the displacement of aromatic glucosinolates, which could only be displaced using pyridine acetate. Aromatic

Plate A.3.4. Gravid female *Delia floralis* performing a leaf margin run on a surrogate plant.

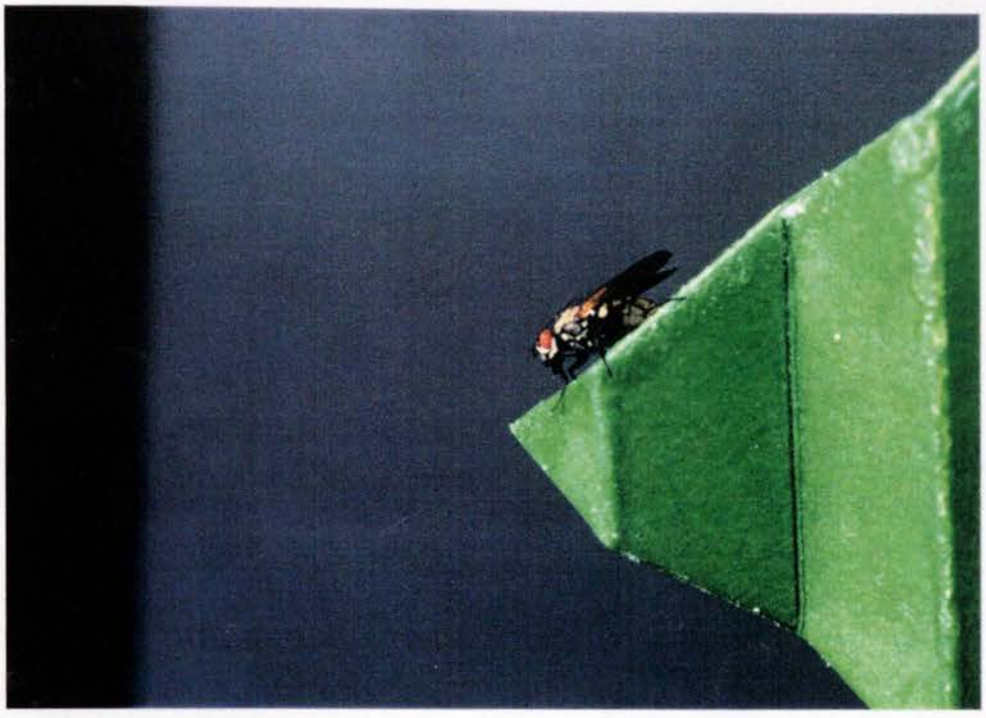


Plate A.3.5. Gravid female *Delia floralis* ovipositing in the sieved sand at the base of a surrogate plant.

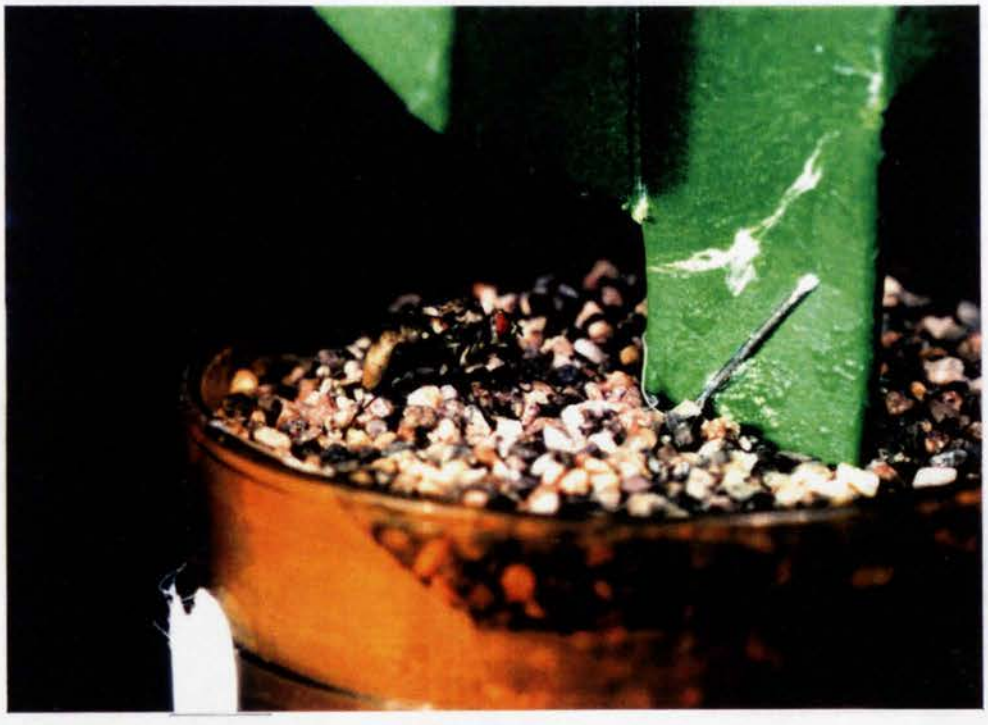
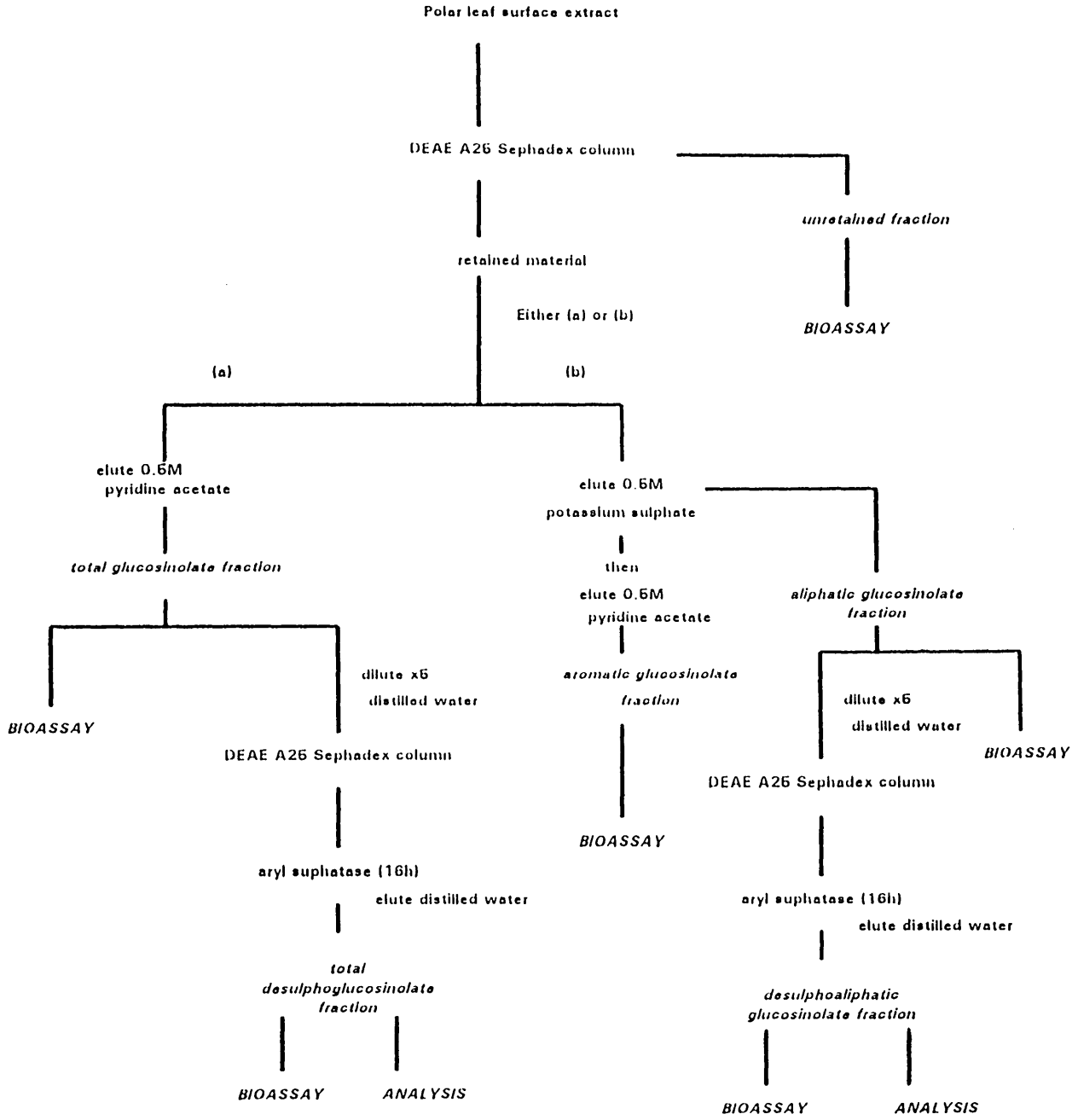


Figure A.3.1. Production of fractions from the polar leaf surface extracts of the four Brassica genotypes.



glucosinolates were extracted separately by first removing the aliphatic glucosinolates, then using the method for total glucosinolates on the same column. In this way it was possible to divide the original methanol extract of each genotype into four fractions with complementary constituents:

Total fraction, those molecules which could be removed from the column by 0.5M pyridine acetate (which included the glucosinolates and unidentified compounds).

Aliphatic+ fraction, that which was removed from the column by 0.5M K_2SO_4 (which included the aliphatic glucosinolates and unidentified compounds).

Aromatic+ fraction, the remaining molecules after 0.5M K_2SO_4 application which could be removed from the column by 0.5M pyridine acetate (which included the aromatic glucosinolates and unidentified compounds).

Unretained fraction, the extract and rinse water which passed straight through the column when added to it.

Glucosinolates were desulphated for bioassays by the addition of 0.2ml of sulphatase enzyme, which was then left on the column overnight. The following morning desulphated glucosinolates were washed from the column using distilled water. The desulphation of glucosinolates was also used to remove them from the fraction in order to test the activity of the non-glucosinolate element of each fraction.

Glucosinolate analysis

The glucosinolate content of the four methanol extracts was determined by analysis of desulphoglucosinolates, using the method of Heaney and Fenwick (1980). Individual glucosinolates were separated by High Performance Liquid Chromatography (hplc). The methods involved are detailed in Chapter B3.

Sensory studies

As part of a British Council funded collaboration, involving exchange visits to Switzerland, fractionated extracts which had been previously prepared for bioassay were taken to Dr Erich Städler and Dr Robert Baur at the Swiss Federal Research Station, Wädenswil for chemosensory analysis on tarsal receptors of *D.radicum*.

Electrophysiology methods

An electrophysiological study of extracts and derived fractions was carried out by Dr Robert Baur and Dr Erich Städler at the Swiss Federal Research Station, Wädenswil. The study utilised established methods (Städler, 1978; Roessingh *et al*, 1992b) to investigate the responses of contact receptors on the tarsi of *D.radicum* females to polar (methanol soluble) leaf surface extracts produced as a part of this study. After decapitation, female insects were placed in a channel in a Plexiglas block with the front legs taped to the surface of the block, exposing sensilla on the ventral surface of the tarsi. Analyses were carried out in a Faraday cage to minimise electrical interference and a humidified air stream (0.5m/sec at room temperature, 19-25°C) was directed over the insect to prevent desiccation. An indifferent electrode (tip diameter 1 μm), filled with 100mM NaCl was inserted in the tibia. This preparation allowed stable recordings for many hours using standard tip recording techniques (Städler, 1978; 1984). A non-blocking amplifier with a 10^{13} Ω input impedance and a maximal input bias current of 150 fA was used. Recordings were made from C and D sensilla present on the ventro-lateral surface of each tarsomere (Städler, 1978). To distinguish the different sensilla, an index was used that specifies the tarsal segment, ie D_4 is a D sensillum on the 4th tarsal segment, counted from proximal to distal (Roessingh *et al*, 1992b). All extracts and fractions were dissolved in 10mM KCl which evoked less than 10 spikes per second on its own (Figure A.3.8.) and were contacted on the sensillum using stimulus pipettes which were drawn to a tip diameter of appoximately 0.1mm. Stimulus pipettes were freshly filled immediately before each series of recordings, and an interval of at least two minutes was maintained between stimulations of the same sensillum. The first 50 ms of a recording were distorted by contact artefact (Roessingh, 1992b). Consequently, the total spike counts in the period 50-1050ms were used in the analysis. All counting was carried out from the digitised recordings using a data acquisition and analysis programme developed in Wädenswil and running on a Macintosh II computer.

Two of the fractions produced at SCRI were tested on a number of sensilla, to investigate the nature of the chemical stimuli contained in them. The aliphatic+ and the desulphoaliphatic+ fractions were tested at two concentrations, 0.1 gle/ml and 1.0 gle/ml. The fractions were tested on three sensilla which are known to possess different sensitivities to glucosinolates and "Cabbage Identification Factor" (CIF). CIF is a polar molecule of unknown structure which stimulates *D.radicum* oviposition more strongly than glucosinolates (Roessingh *et al*, 1992a). The sensilla to which the fractions were applied are listed below, together with their sensitivity to glucosinolates and CIF.

D2 sensilla: not sensitive to glucosinolates or CIF

D3 & D4 sensilla: sensitive to glucosinolates, not sensitive to CIF

C5 sensilla: sensitive to glucosinolates and CIF

RESULTS

Turnip Root Fly

Whole plant bioassay

The percentage of eggs which were laid by *D.floralis* on each plant genotype differed ($P<0.001$, N=10) by a factor of approximately 80. The ranking order for oviposition was Doon Major, GRL aga, Dwarf Green Curled and Fribor which received 77.0, 18.9, 3.3 and 0.9% respectively (Figure A.3.2.).

Bioassay of surface extracts and their fractions

The methanol extracts of the four plant genotypes tested had higher proportions ($P<0.001$, N=13) of eggs laid upon them than the dichloromethane extracts. Solvent controls of methanol and dichloromethane received 0.5 and 0.2% respectively of the eggs laid. The dichloromethane extracts of Doon Major, GRL aga, Dwarf Green Curled and Fribor received 1.8, 0.8, 0.1 and 0.2% respectively of the total eggs laid. The percentage eggs laid on the methanol extracts of the surface of the four plant genotypes of Brassica differed significantly ($P<0.05$) between extracts. The methanol extracts of Doon Major, GRL aga, Dwarf Green Curled and Fribor received 40.6, 32.5, 19.0 and 4.4% respectively of the total eggs laid (Figure A.3.3.).

The deterrent effect of the dichloromethane extracts on oviposition was investigated by applying 1.25 gle of each extract to a dummy already sprayed with the highly stimulatory Doon Major methanol fraction. The results indicated that Doon Major and Fribor were not significantly different ($P>0.05$, N=9) from the control which had been sprayed with dichloromethane (Table A.3.1.). GRL aga and Dwarf Green Curled received greater ($P<0.05$, N=9) proportions of the eggs than the control. It was concluded that the dichloromethane extract was not deterrent when applied to a stimulatory extract. Dichloromethane extracts had consequently been shown to stimulate little oviposition compared to methanol extracts and to have no deterrent effect on top of a stimulatory fraction.. Thus, it was decided to concentrate on further fractionation of the methanol extracts of the leaves of the four Brassica genotypes.

Figure A.3.2. Percentage of total eggs laid by *D.floralis* on different Brassica genotypes during a choice experiment.

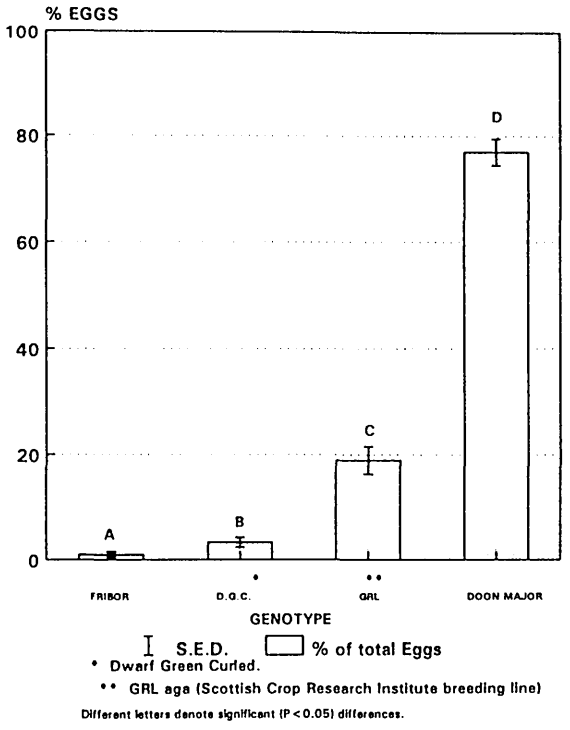


Figure A.3.3. Percentage of total eggs laid by *D.floralis* on surrogate plants sprayed with two leaf surface extracts of Brassica genotypes during a choice experiment.

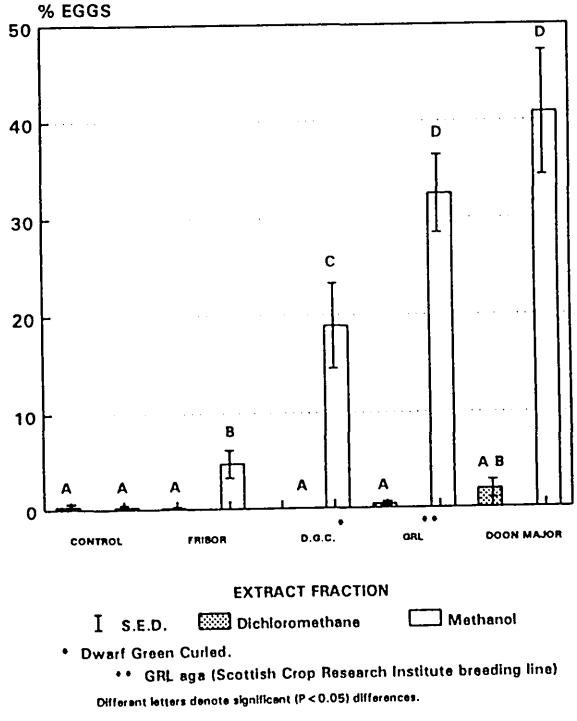


Table A.3.1. The influence of dichloromethane extracts of the four test plant genotypes on oviposition on the methanol extract of Doon Major.

Treatment	Mean % eggs	SE
Control	13.95	3.93
Fribor	14.05	2.53
Dwarf Green Curled	32.41	5.88
GRL aga	22.84	4.14
Doon Major	16.75	2.37

N = 9

Investigation of the activity of desulphoglucosinolates showed them to stimulate little or no activity when compared to that of the raw methanol fraction. Only the desulphoglucosinolates from GRL aga received any eggs at all (Mean = 0.8%, N=4). Oviposition on GRL aga desulphoglucosinolates was less than on the control plant which had been sprayed with pure methanol. The oviposition on the raw methanol extracts reflected that in earlier experiments.

Testing of the different glucosinolate fractions demonstrated that the most active fraction was the aliphatic+ fraction (Figure A.3.4.). The activity of the aliphatic+ fraction was approximately 60% of the activity of the raw methanol fraction. Significantly lower ($P<0.001$, N=6) proportions of eggs were laid on the total glucosinolate, aromatic+ and unretained fractions.

Application of the aliphatic+ fraction of the methanol extract of the four plant genotypes to surrogate plants showed that there were differences ($P<0.05$, N=8) in the proportions of eggs laid on different extract fractions (Figure A.3.5.). Doon Major received less eggs (30.0%) than GRL aga (48.8%), whilst the ranking of the kale genotypes was consistent with the whole plant and methanolic extract bioassays.

To investigate whether the activity in the aliphatic+ fraction was due to the glucosinolates or other compound(s), it was contrasted with the desulphoaliphatic+ fraction. A higher proportion of eggs was laid on the latter (Figure A.3.6.) but there was no significant difference ($P>0.05$, N=10) between the two extracts. Two controls, a control from a blank column treated with sulphatase and a solvent control both received significantly ($P<0.05$, N=10) lower proportions of eggs than the two fractions under test.

Figure A.3.4. Percentage of total eggs laid by *D.floralis* on surrogate plants sprayed with different fractions of the methanol (polar) leaf surface extract of GRL aga during a choice experiment.

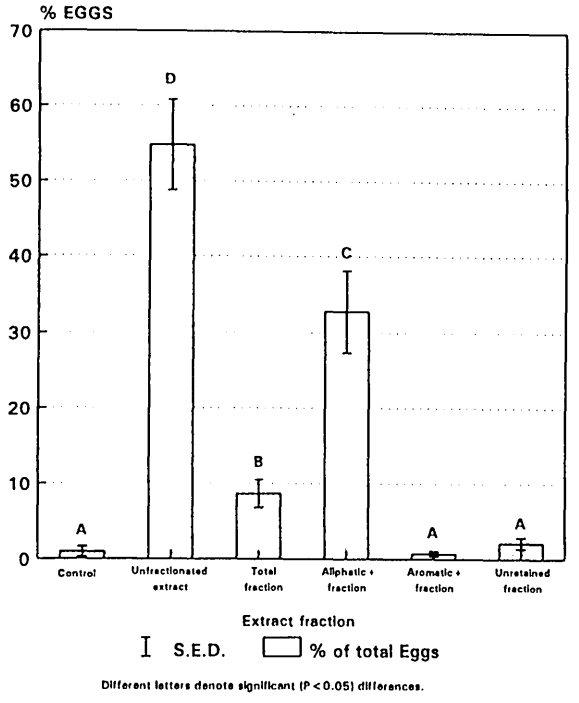


Figure A.3.5. Percentage of total eggs laid by *D.floralis* on surragate plants sprayed with the different aliphatic+ fractions of the methanol (polar) leaf surface extract of Brassica genotypes during a choice experiment.

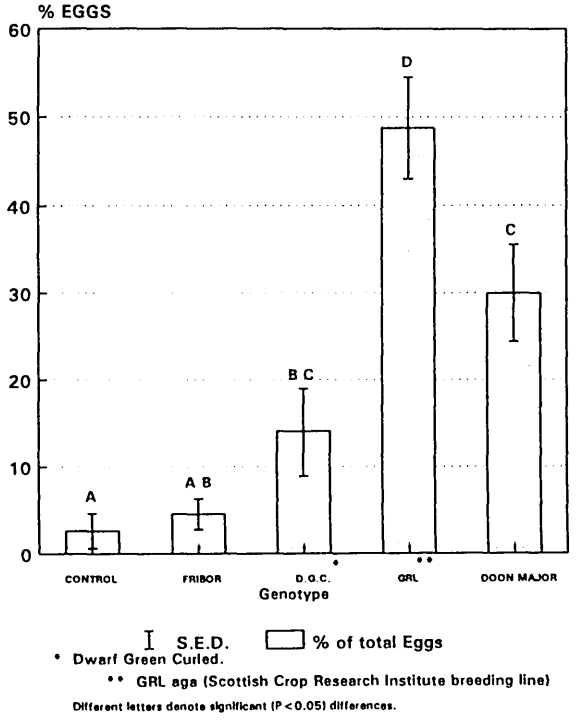


Figure A.3.6. Percentage of total eggs laid by *D.floralis* on surrogate plants sprayed with different treatments of the aliphatic+ fraction of GRL aga during a choice experiment.

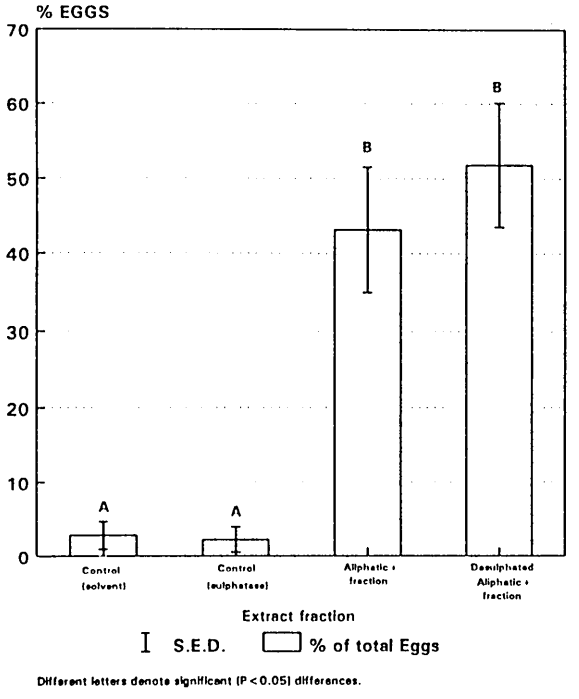
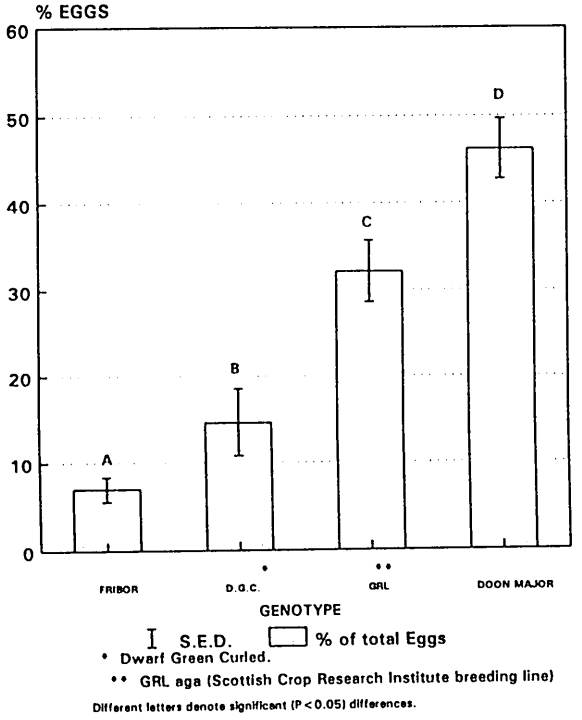


Figure A.3.7. Percentage of total eggs laid by *D.radicum* on different Brassica genotypes during a choice experiment.



Cabbage root fly

Whole plant bioassay

Oviposition tests in the laboratory using cabbage root fly showed an oviposition profile similar to that for turnip root fly, though the differences were not quite so extreme (Figure A.3.7.). The majority of eggs were still laid on cv Doon Major, which received an average of 46.2% of the eggs, and progressively lower proportions of eggs on GRL aga, Dwarf Green Curled and Fribor.

Leaf surface Glucosinolates

Analysis of the glucosinolate content of the methanol leaf surface extracts demonstrated that detectable concentrations of glucosinolates were present. Total glucosinolate content of the methanol extracts ranged from 66.4 nM/gle (Dwarf Green Curled) to 173.6 nM/gle (Doon Major) (Table A.3.2.). Thirteen individual glucosinolates were detected, six of which were found in all the extracts tested.

Table A3.2. Concentrations of glucosinolates (nM/gle)found in the methanoic fraction of leaf surface extracts of four Brassica genotypes.

Crop type	Swede	Swede	Kale	Kale
Glucosinolate / Genotype	Doon Major	GRL aga	D.G.C.[1]	Fribor
3 methylsulphinyl propyl*	n/d	n/d	15.2	32.4
2-hydroxy-3-butenyl	22.5	5.4	tr	tr
Prop-2-enyl	n/d	n/d	2.1	7.3
4-methyl sulphinyl propyl*	1.2	1.4	3.4	5.2
unknown	4.1	2.2	n/d	n/d
2 hydroxy pent-4-enyl / 4-methyl sulphinyl but-3-enyl	27.4	5.1	n/d	n/d
But-3-enyl	tr	1.1	n/d	n/d
3-methyl thiopropyl*	62.7	42.7	tr	tr
Pent-4-enyl	7.0	n/d	n/d	n/d
3-indolemethyl	30.5	29.5	40.2	19.5
4-methoxy-3-indolemethyl	16.7	12.3	3.5	3.1
1-methoxy-3-indolemethyl	1.5	7.5	0.8	tr
TOTAL	173.6	107.2	65.2	67.5

1 Dwarf Green Curled
* indentification based on retention time
n/d not detected
tr trace (less than 0.5 nM/gle)

Figure A.3.8. The electrophysiological responses of the C5 sensillum of *D.radicum* tarsal receptors to a CIF containing fraction of the extracts of Doon Major, GRL aga, Dwarf Green Curled and Fribor (produced by Dr R. Baur, Swiss Federal Research Station, Wädenswil).

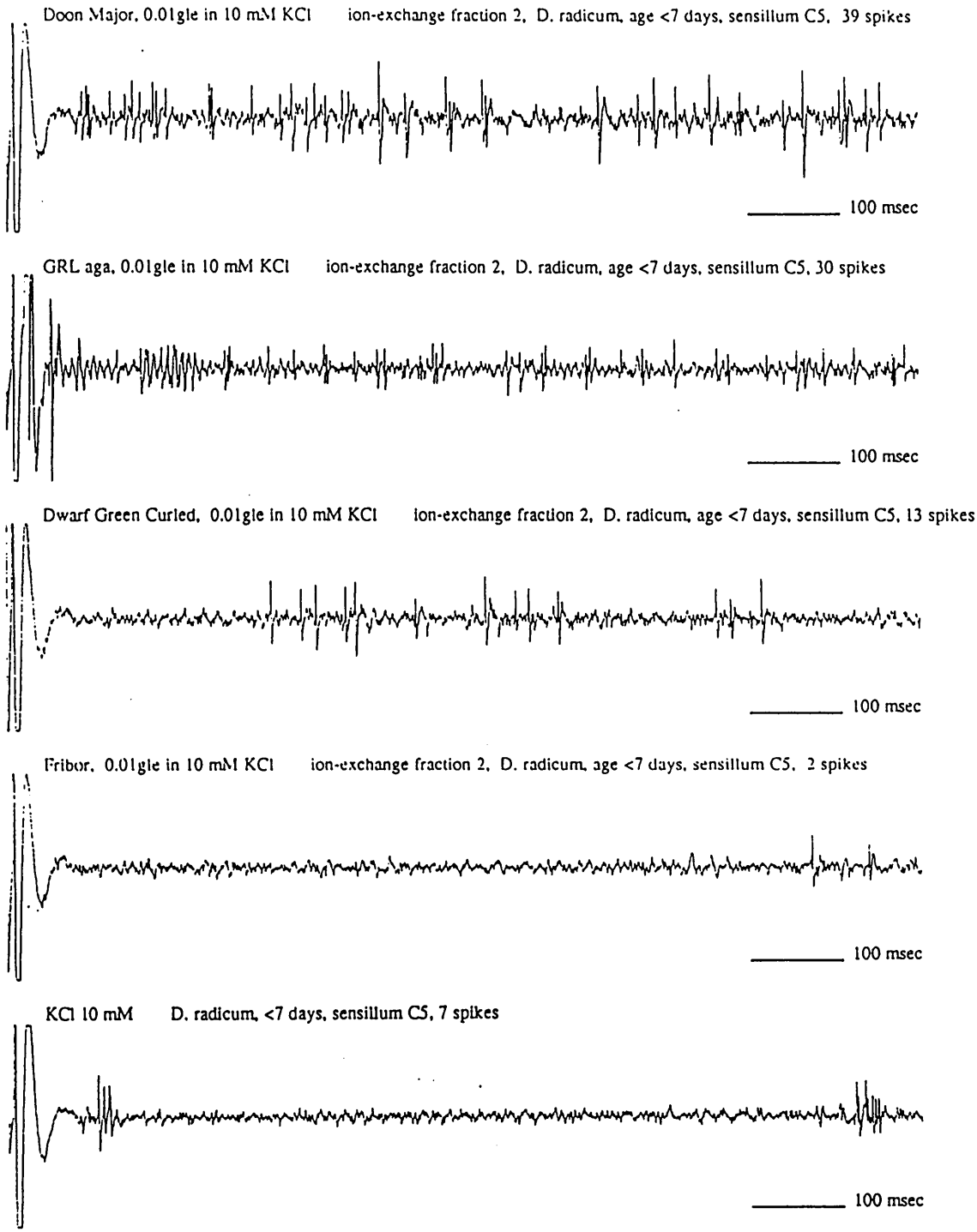
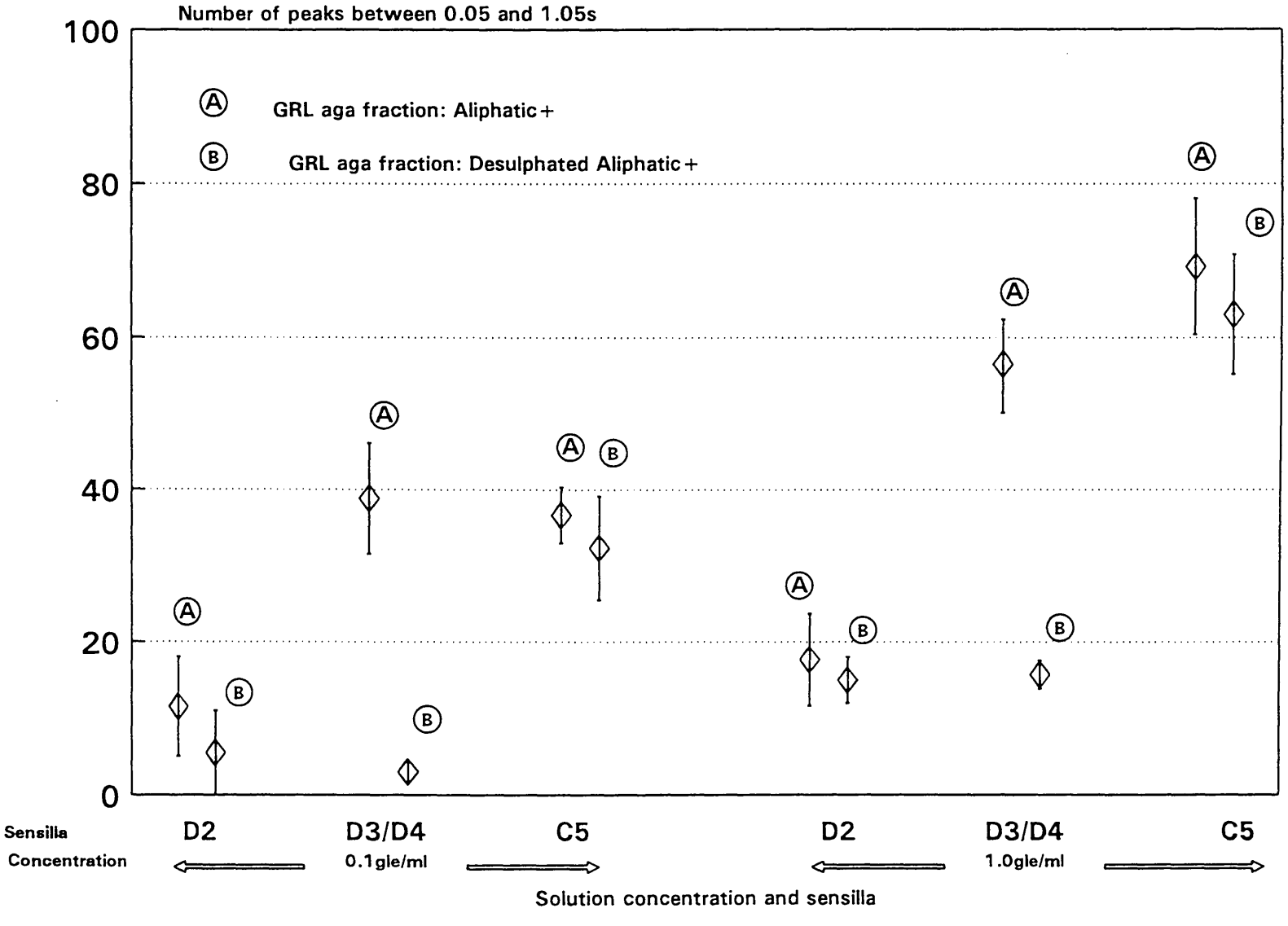


Figure A.3.9. Response of different tarsal sensillae of the cabbage root fly to fractions of GRL aga polar leaf surface extract.



Electrophysiology of extracts with the cabbage root fly

Explorative investigations of the raw methanol extracts demonstrated that the ranking of the stimulatory responses was similar to the ranking of the bioassay carried out in SCRI (Figure A.3.8.). The study investigated the stimulation of the listed sensilla to the Aliphatic+ and Desulphated Aliphatic+ fractions. Figure A.3.9. shows the mean number of spikes per second produced by the two extracts on the three sensilla. The pattern which was produced with a concentration of 0.1 gle/ml was also reflected at 1.0 gle/ml. The response of the D2 sensilla was very low and unaffected by the nature of the fraction applied. The D3/D4 sensilla was highly stimulated by the Aliphatic+ fraction and stimulated very little by the Desulphated Aliphatic+ fraction. The C5 sensilla was highly stimulated by both aliphatic+ and desulphoaliphatic+ fractions, with a small but non-significant reduction in the desulphoaliphatic+ fraction at both concentrations tested.

DISCUSSION

The results obtained using four genotypes of Brassica show a clearly defined scale of antixenotic resistance. *Delia floralis* and *Delia radicum* laid fewer eggs on the kale genotypes than the swede genotypes. The consistently high proportion of eggs laid on Doon Major in the choice test may be interpreted as an indication of a distinct host preference being displayed by *D.floralis* and *D.radicum*. The clarity of the apparent preference shown led to the choice of the four plant genotypes as a group for a series of behavioural analyses, which are discussed in detail in the two previous chapters (Chapters A1 and A2).

Following the determination of the importance of the leaf stage in the rejection of the kale genotypes tested, it was elected to investigate the properties of the leaf surface extracts of different polarities. Gravid *D.floralis* females were presented with a choice of methanol and dichloromethane extracts of the four plant genotypes. Methanol fractions stimulated far higher oviposition levels than dichloromethane fractions. The ranking in root fly oviposition stimulated by real plants for *D.radicum* and *D.floralis* and by the methanol extracts in *D.floralis* was identical. In addition, the methanol extracts of the leaf surface of the four genotypes also produced the same ranking for *D.radicum* (Baur *et al*, in prep.). The minimal oviposition on both methanol and dichloromethane controls indicated that neither solvent was stimulatory. The stimulation of *D.floralis* to oviposit by methanol extracts reflects previously published work (Havukkala & Virtanen, 1985) and the results found with leaf extracts and *D.radicum* (Städler and Schöni, 1990; Roessingh *et al*, 1992a; 1992b; Koštál, 1993).

The dichloromethane fraction of the four genotypes was influencing the oviposition of *D.floralis* when added to the stimulatory Doon Major fraction. However, extracts from different genotypes only caused minor modifications in the distribution of eggs laid in a choice test. The influence of the dichloromethane fraction on *D.floralis* oviposition was not strong, nor did it reflect the oviposition on real plants. The application of dichloromethane extracts to surrogate plants which had been treated with a Doon Major methanol extract did show some effect. Dwarf Green Curled and GRL aga dichloromethane extracts appeared to make the methanol extract of Doon Major more stimulatory to ovipositing *D.floralis*. Consequently, it was decided to concentrate investigations on the methanol extracts and the stimulatory compounds within them. The dichloromethane fractions although not stimulatory, may contain many compounds which influence the host selection of *D.radicum* and *D.floralis*. The constraints of time placed upon this project precluded further exploration of a number of aspects of host plant resistance, including the nature of the compounds contained in the dichloromethane extracts. The wax structures of the leaf surface have been observed to vary considerably between Brassica genotypes in preliminary studies (Hopkins, unpublished observations) but was similarly beyond the scope of this study.

Chemical analyses of the methanol fraction demonstrated that the concentrations of glucosinolates in the leaf surface extracts were of the same order of magnitude as those which stimulate *D.radicum* and *D.floralis* (Städler, 1978; Roessingh *et al*, 1992b; Simmonds *et al*, in prep). It has been demonstrated that different glucosinolates and concentrations of glucosinolates vary widely in their stimulation of *D.radicum* and *D.floralis* (Roessingh *et al*, 1992b; Simmonds *et al*, in prep). However, the concentrations of individual glucosinolates found in the different methanol fractions did not fully account for the oviposition preference amongst them. The results shown in Figure A.3.5. were initially believed to infer that the activity present in the methanol fraction was due to aliphatic glucosinolates. The very low oviposition on fractions produced by using pyridine acetate was suspected to be linked to failure to remove all of this compound from samples. However, increasing the effort to remove pyridine acetate did not increase the stimulation of the fractions and the aliphatic+ fraction had approximately 60% of the activity of the raw methanol fraction. Consequently, effort could justifiably be concentrated on the aliphatic+ fraction. Having confirmed that desulphoglucosinlates had no significant stimulatory activity, it was decided to investigate further the activity of this fraction by desulphating the glucosinolates. The results shown in A.3.5. indicate that the aliphatic glucosinolates are not the primary oviposition stimulant in the methanol extract, as desulphation did not significantly reduce the oviposition stimulated by this fraction. Therefore, an oviposition stimulant is present in the methanol fraction which is

not a glucosinolate. Although this compound (or compounds) currently remains unidentified, and it would require a great deal of work to isolate and identify the compound concerned, there are certain characteristics which have been defined by the extraction procedure used to reach this stage. The compound is polar, stable, is not volatile and is not degradable with the enzyme sulphatase. Further fractionation and electrophysiological study of the fractions by Dr Robert Baur at the Swiss Federal Research Station, Wädenswil have yielded further information. The fraction concerned stimulates the same *D.radicum* sensilla as CIF (Cabbage Identification Factor), an as yet unidentified non-glucosinolate molecule which strongly stimulates *D.radicum* to oviposit (Roessingh *et al*, 1992a; 1992b). Figure A.3.9. clearly demonstrates a number of characteristics of the extract. The high stimulation of C5 sensilla by both fractions indicates that CIF is probably the compound concerned. If the stimulation of both C5 and D3/D4 sensilla were significantly higher for Aliphatic+ than Desulphoaliphatic+ it would indicate that glucosinolates were the main stimulants in the extract. Further collaborative work between The Scottish Crop Research Institute and The Swiss Federal Research Station, Wädenswil is in progess to discover if CIF is responsible for the main oviposition stimulation in the extracts.

It was unfortunate that there was insufficient time either to pursue this line of investigation further, or to test the extracts in bioassays and on tarsal receptors of both *D.radicum* and *D.floralis*. However, it was known when this line of work was undertaken that the final goals of the project, the isolation and identification of active oviposition deterrents and stimulants, were likely to require a longer term research programme involving collaboration of several individuals with complementary skills (chemists, entomologists and plant physiologists, for example). The results achieved to date have however provided basic information which, combined with the continuing efforts at SCRI and elsewhere, will prove useful in achieving long term objectives.

The use of the most active fraction of a series of extracts does not necessarily lead to the most stimulatory molecule, nor to the molecule which has the most influence on the host selection procedure. This conclusion arises simply because the fraction which is the most active may be so as a result of the combination of molecules which it contains. In addition, the activity of a fraction may not simply be the sum of the individual compounds within it but is subject to synergistic and antagonistic interactions.

The presence of unidentified stimulatory molecules in highly stimulatory fractions of leaf extracts does question the overall role of glucosinolates as the principal mediators of insect behaviour on Brassicas. Much of the work which associates glucosinolates with insect host

selection consists of correlations between glucosinolate profiles and insect damage or activity and it is accepted that such data are suggestive but not definitive (Louda & Mole, 1991). Whilst *D.radicum* behaviour has been known to be influenced by glucosinolates for some time, glucosinolates role compared to molecules such as CIF is not fully understood yet. More work is clearly needed to identify the structure and distribution of CIF on the leaf surface of a wider range of Brassicas and to search for other molecules which may, as yet undetected, also be influencing the behavioural patterns of Brassica-feeding insects.

In addition to *D.radicum* and *D.floralis,* a large number of insects have had their behaviour linked to glucosinolates. Städler (1992) cited approximately twenty papers which linked glucosinolates with oviposition or feeding of insects. The papers concerned fall into two broad categories: indirect evidence from correlative data between insect damage and host plant chemical composition; and more direct evidence from testing of compounds on surrogate plants or using electrophysiological techniques. The data from the first type of study are known to be suggestive rather than definitive and are sometimes presented as such. However, the very high activity of compounds of low molecular weight which have remained unrecognised until the most recent work (Roessingh *et al*, 1992a) does cast some doubt on bioassays which utilise compounds of only moderate purity.

SECTION DISCUSSION

The survival of the larvae of oligophagous insects, such as *Delia radicum* and *Delia floralis*, is largely dependent on the ability of the female to identify plants on which their progeny may successfully develop. Singer (1986) considered the selection of an oviposition site a critical stage in the life cycle of many phytophagous insects. The larvae of *D.radicum* can migrate several centimetres to the root of a host plant (Zohren, 1968) and orientate to a wide range of volatile host plant compounds (Koštál, 1992). However, this trivial migration of *D.radicum* larvae is a minor element of the host selection process. The ability to migrate over such a small distance will only be of advantage if the egg is placed adjacent to or in the immediate vicinity of the host plant. The broader aspects of the location of the host plant have already been reviewed in some depth in the Section Introduction. The pre-alighting behaviour of *D.radicum* has been regularly associated with host plant odour (e.g. Hawkes *et al*, 1978; Nottingham & Coaker, 1985) and colour (e.g. Prokopy *et al*, 1983a; 1983b).

The work within this Section dealt largely with the post-alighting oviposition behaviour of *D.radicum* and *D.floralis*. The studies of the detailed host selection behaviour of *D.radicum* and *D.floralis* were concerned exclusively with this element of insect-plant interactions. The bioassays which were performed will also have included an element of pre-alighting behaviour. It can be regarded as a short-coming of the research that no counts were made of the landing rate of *D.radicum* and *D.floralis* on the four plant genotypes studies. Even at the short ranges from which the landing site was selected, no more than 50cm, the foliar form and colour may have influenced the landing rate of the flies. This omission is of lesser importance for the bioassays which were performed on the extracts and fractions applied to standardised surrogate plants than those on the leaves of the four real plants. The surrogate plants used were of consistent size and colour and the extracts produced had been evaporated down at reduced pressure and elevated temperature, minimising any volatile content.

The low numbers of eggs laid on the genotypes GRL aga, Dwarf Green Curled and Fribor clearly indicate that they display antixenotic resistance (Kogan & Ortman, 1978) to *D.radicum* and *D.floralis* when contrasted to the susceptible Doon Major. Alternatively, this result can be described as *D.radicum* and *D.floralis* displaying host preference (Painter, 1941; 1951) for Doon Major in a "choice" situation. While the former view describes the nature of the plant, the latter view comments on the oviposition which took place. Preference does not

actually attribute a liking for Doon Major to the two fly species, but simply comments on a non-random distribution of the eggs when offered a number of oviposition sites (Singer, 1986).

The behavioural studies which were carried out on both species of fly indicated that the selection of plants for oviposition was primarily taking place on the leaf. For *D.floralis*, differentiation of behavioural patterns was most pronounced during the landing phase and for *D.radicum* during a combination of the landing and leaf resting phases. The site of oviposition for both species is usually in the soil at the base of plants. The larvae of *D.radicum* and *D.floralis* both feed predominantly on the roots of Crucifers (Coaker and Finch, 1971). This study shows the adults select the site of larval development primarily as a result of leaf characteristics of the plant. This behaviour quite clearly shows that direct assessment of the site of larval development is not the primary cause of the rejection of non-selected plants despite the proximity of oviposition to the site of larval feeding. The gravid female clearly gains from the ability to assess the potential host plant on initial contact with the leaf surface. Avoiding an unnecessary period moving to and assessing the base of the plant conserves both time and energy and may reduce exposure to predators and entomopholous fungi and nematodes in the soil. The efficiency of host plant assessment may be impaired by utilising a limited exploration of the plant. However, the relationship between oviposition preference and larval performance has not been properly explored for *D.radicum* and *D.floralis* and the relationship between these two characteristics is known to be very variable (Thompson, 1988). The use of the differentiation between "rejection" and "non-rejection" behaviours as the main criteria on which to categorise ensuing behaviours is not the most obvious way in which to divide the behaviours. The categorisation of a range of behaviours under the broad classification of not rejecting is simplistic. But having demonstrated, using chi-squared analyses on the pattern of ensuing behaviours, that differentiation is occurring it was decided that this division would be most suitable. It avoids debate over the exact nature of a behavioural response by using a broad distinct categorisation.

The selection of a host plant by both *D.radicum* and *D.floralis* is the product of a complex of sensory inputs. The behavioural events observed taking place during the host selection procedures of *D.radicum* and *D.floralis* are the product of the insect responding to a number of different stimuli. The colour of leaves (e.g. Prokopy *et al*, 1983a; 1983b), the structure of the plant (e.g. Roessingh, 1990; Koštál, 1993) and the chemical composition of the plant, particularly the phylloplane (leaf surface) (reviewed, Städler; 1992), have all been demonstrated to influence *D.radicum* host selection. The behavioural evidence (Chapters A1 and A2) also indicates that the cues which drive host selection are positive. In the case of

D.floralis, many flies which reject a host plant for oviposition do so having not even moved across the leaf surface. Secondary plant compounds are widely accepted as mediators in host plant selection and their influence in cruciferous insects has been widely explored (Städler, 1992). Secondary plant compounds are distributed throughout the plant although concentrations may vary in different plant parts and with the growth stage of the plant. The concentrations of glucosinolates and S-Methylcysteine Sulphoxide (SMCO) in the bulb and leaves of maturing swede plants varied widely from July onwards (Griffiths *et al*, 1991). The oviposition preferences of *D.radicum* have been linked to the volatile hydrolysis products of radishes and the variation which took place in these chemicals at different plant growth stages (Ellis *et al*, 1980). In this section, plants of similar size and identical growth stage were used to minimise the effect of changes in the host chemistry which may take place. It must also be noted that under field conditions changes in host plant chemistry due to the interaction between genotype and environment will be important to the consistency of the resistance mechanisms.

The complexity of assessing the interactions which take place between two organisms is compounded by the variation within species. Although a certain degree of consistency is present in the genetic material of both plant and insect, the variability is considerable. In addition, the prevailing environment will have a strong influence on both the plant and the insect. As a consequence, the variation in the behavioural events during host plant selection may be quite wide. The genetic variation in the potential host plants is reduced by the use of breeding lines and commercial varieties but remains wide. The use of laboratory cultured flies also reduces their genetic variability, in addition to the usual advantages of the use of flies of known age, fitness and health. However, selection of insects for survival under laboratory conditions can alter the genetic composition of the population which may result in changes in the biology of the insect (Wolfson, 1988). The genetic consistency of insects from a laboratory culture is one which may have a price in terms of the influence on the results of the experiments. The influences on selection in a laboratory culture are different from those in the field and the genetic composition of laboratory cultured flies will therefore be different from those in the field. *D.radicum* was used which had originated from Scotland, Wellesbourne and Switzerland in various aspects of this study. The consistency of the results achieved indicates that the genetic origin of cultured flies may not be a critical factor. However, differences due to the selection pressure of laboratory cultures may remain a problem.

The results presented in the three chapters in this section show that demonstrable levels of antixenotic resistance to *D.floralis* and *D.radicum* can be found in the Brassicas tested. This, within itself, is unremarkable as antixenotic resistance to both species of flies has been known

to exist for many years and incidence of such has been listed in the introductions to the section and to the individual chapters. However previous laboratory studies have concentrated on the range of resistance to an individual root fly species. In this case, it has been possible to demonstrate that within the small range of plant genotypes tested the resistance to *D.floralis* and *D.radicum* was coincident. The ranking of the four plant genotypes was identical and the relative proportions of eggs laid on the four genotypes by *D.floralis* and *D.radicum* were similar.

The work carried out on the responses of *D.floralis* to leaf surface extracts (Chapter A3) can be combined with the preliminary electrophysiological studies of Swiss collaborators on *D.radicum* (Chapter A3; Baur *et al*, in prep.). A proportion of the antixenotic resistance present can be attributed to the influence of the chemical composition of the phylloplane of Brassicas influencing oviposition by female root flies.

Glucosinolates and their volatile hydrolysis products have figured largely in the assessments of the chemical ecology of *D.radicum* and *D.floralis*. The influence of the existence of techniques to easily measure and quantify glucosinolates may well have strongly influenced their prominence in the literature. More recent work has demonstrated that a new (but to date unidentified) compound, far more stimulatory than glucosinolates, was present in the polar leaf surface extracts of Crucifers (Roessingh *et al*, 1992a). The electrophysiological studies carried out by Robert Baur at Wädenswil on the fractions supplied in this study indicate that the activity of the fractions tested on *D.floralis* may be partially attributed to this compound. The unfractionated methanol leaf surface extracts tested on *D.floralis* as part of this thesis resulted in a near identical oviposition profile with *D.radicum* at Wädenswil (Baur *et al*, in prep). These two results indicate that glucosinolates may not be the dominant oviposition stimulant in the contact chemoreception of Brassica-feeding insects which was formerly believed to be the case. Further work will be needed to confirm this relationship, but the indications are that, despite the dominance of glucosinolates in the literature, they may not be the primary stimulant of oviposition for *D.radicum* and *D.floralis*.

The identification of oviposition contact repellents to *D.radicum* has been less fruitful. However, the repellent nature of garden pebble moth, *Evergestis forficalis*, frass (Jones & Finch, 1987) led to the identification of sinapic acid as an oviposition deterrent (Jones *et al*, 1988). The effect was later found to be universal among the carboxylic acids tested (Cole *et al*, 1989). In addition, the effect of experience on oviposition cannot be ignored. Traynier & Truscott (1991) tested solutions of glucobrassicin (3-indolylmethyl glucosinolate) and sinigrin (allyl glucosinolate) as oviposition stimulants for *Pieris rapae*. Oviposition substrates had

different shades of green and those treated with glucobrassicin were preferred to those treated with sinigrin. When offered the choice of shades two hours later 17 out of 18 insects landed on a substrate, and 15 of those landed on the colour associated with glucobrassicin. For these reasons, care was always taken in the experiments presented in this Section to minimise the experience of flies used. Koštál (1993) tested a range of plant models confirming the importance of chemistry and demonstrating that *D.radicum* would lay eggs even when denied access to both host plant models and to host plant chemicals. Therefore, it remains important to balance the minimisation of experience with allowing gravid females to oviposit freely.

Were the relative proportions of eggs laid on the kale genotypes tested in the laboratory to occur under field conditions, it would reduce root damage to an extent which would be equivalent, or even superior to existing chemical control. Reduced pesticide doses in combination with the use of existing moderately resistant genotypes of swede can provide satisfactory control for the cabbage root fly (McKinlay & Birch, 1992; Tadskal, 1992). The levels of antixenotic resistance demonstrated in this Section could considerably raise the economic threshold for application of pesticide. However, increased levels of resistance to *D.floralis*, which have to date only been found within the kales, have been known some time (Alborn *et al*, 1985; Ruuth, 1988). The antixenotic resistance demonstrated by GRL aga remains of relatively moderate status, when contrasted with that of the kales. If this difference in resistance levels can be narrowed, then the antixenotic resistance of swedes to the oviposition of *D.radicum* and *D.floralis* will have achieved levels where chemical control in field crops may be unnecessary in many instances.

SECTION B

ANTIBIOTIC RESISTANCE TO *Delia floralis* AND *Delia radicum* AND CHEMICAL INTERACTIONS WITH BRASSICA ROOTS

SECTION INTRODUCTION

Delia floralis and *Delia radicum* extensively mine the root surface and, in the case of the former, the inner cortex of the roots of Brassica crops (Plates I.1. to I.3.). Such damage can restrict the growth or kill the plant and, in the case of root crops, results in the need for extensive root trimming of harvested plants. It may also allow the entry of secondary pathogens causing soft rots and further reducing the value of the crop (Doane & Chapman, 1964a; 1964b). Knowledge of the chemical and genetic nature of mechanisms of resistance against a crop pest is desirable to direct a plant breeding programme.

Swailes (1960) initiated a glasshouse study of antibiotic resistance to *D.radicum* following field results which indicated that swede genotypes varied in their susceptibility to cabbage root fly (Swailes 1959). Swailes (1960) demonstrated that on plugs of root with the cortex removed percentage pupation on swede varied from 60% to 81%. The percentage pupation was higher than achieved in field experiments, a fact which was attributed to larvae establishing better due to the removal of the root cortex. Survival rates of *D.radicum* were linked to the rate of establishment of root feeding by first instar larvae (Swailes, 1960) confirming the importance of skin characteristics (Swailes, 1968). Finch & Coaker (1969) found that inoculation of increasing numbers of eggs of *D.radicum* led to a reduction in mean pupal weight. The weight of the pupa determined the weight of the adult fly which emerged from it, and also correlated with the fecundity of the fly. A variation in weight from 20 mg to 24 mg led to a rise in fecundity of approximately 30%. The ability to suppress the reproduction of successive generations of root flies by this margin would be a valuable contribution to their control. When Skinner & Finch (1983) evaluated swede for the rearing of cabbage root fly, a number of criteria were assessed. Among the criteria they included pupae production, for which there was a twofold variation. Although the aims of the study were not to assess resistance within swede, it did indicate the natural variation in food quality that existed.

Resistance in big leafed turnip to *D.floralis* was demonstrated by Varis (1958). However, the resistance was not attributed to any mode of action, but was merely assessed on the basis of the damage sustained by different genotypes by the end of the growing season. Rygg &

Sömme (1972) concluded that differences in the field resistance of swedes and turnips could be largely attributed to differences in numbers of eggs being laid by *D.floralis*. The variation in egg numbers laid on resistant and susceptible genotypes was close to tenfold. However, Rygg & Sömme (1972) also noted differences in the percentage of larvae surviving to pupate on different hosts. They concluded that differences in the chemical composition of the roots led to a variation in the extent to which larvae were stimulated to penetrate the root. They also concluded that variations in the root form, its length and size, could affect the success of larvae migrating to and penetrating near the root tip.

Levels of partial resistance to *D.floralis* have been attributed to root dry matter (Shaw, 1982; 1984; 1985; 1993; Birch, 1988). Field experiments confirmed moderate resistance to *D.floralis* attack in the cultivars Angus and Melfort. Assessments of root characteristics at the end of the growing season showed that resistant genotypes had consistently higher dry matter content than those susceptible to damage. Laboratory experiments have demonstrated that larval feeding on some resistant swede genotypes can be restricted to relatively shallow mining (Birch, 1988). The feeding mines of *D.floralis* on resistant genotypes had a mean maximum depth which was a quarter of that on susceptible genotypes. This type of restricted larval feeding was found to be correlated with root dry matter content, but was apparently not related to tissue hardness. Attempts to correlate root glucosinolates to *D.floralis* resistance (Birch *et al*, 1990; Birch *et al*, 1992) have proved inconclusive. The glucosinolate composition of the roots of Brassicas was substantially altered by the damage of *D.floralis*, but there was no apparent link between resistance and glucosinolates or groups of glucosinolates.

Genotypic variation in the larval development of *Delia* spp. may be related to an increase in phagostimulation. Bernays & Simpson (1982) reviewed the control of food intake in insects concluding that most insects would not start to feed unless an appropriate stimulus from the substrate indicated to them that it was of a suitable nature. Sugars are the most widespread phagostimulants (Bernays & Simpson, 1982) and have been shown to have a phagostimulatory effect on the larvae of other Anthomyiid flies (Mochizuki *et al*, 1985; Honda & Ishikawa, 1987). *D.radicum* and *D.floralis* feed on Brassica roots, the composition of which is little understood, particularly the sugar and fibre contents. Genotypic variations are known to exist in the concentrations of different sugars in the roots of mature swede plants (Bradshaw & Griffiths, 1990). The phagostimulatory nature of sugars (which is discussed in the Introduction to Chapter B1) and the genotypic variations in their concentrations are likely to influence the feeding rate of insects on different Brassica genotypes. The influence of pest damage is also important to crop quality, especially for forage and horticultural crops.

Before the effect of the chemical composition of roots on the development of both *D.radicum* and *D.floralis* can be fully appreciated, it is necessary to understand the changes that the roots undergo when being fed upon by the larvae of these two species. Such knowledge will allow the root composition to be related more closely to the development of insects feeding upon them. Work with *D.floralis* feeding on the roots of Brassicas has found that the glucosinolates in Brassica roots are radically altered following damage (Birch *et al*, 1990). However, the glucosinolate composition changes which occur with increasing densities of *D.floralis* have not been addressed. Interactions between glucosinolates and *D.radicum* larvae have been restricted to work on the volatile hydrolysis products of glucosinolates (Koštál, 1992). The relationship between root feeding insects and the primary constituents of plant roots seems to have been largely uninvestigated. This Section seeks to investigate the relationship between the composition of Brassica roots and the feeding and development of the larvae of *D.radicum* and *D.floralis*.

Chapter B1

The relationship between the feeding of *Delia floralis* larvae and the sugar content of swede roots

INTRODUCTION

Bernays & Simpson (1982) reviewed the control of food intake in insects. They noted that sugars have been tested on a variety of insects which include locust (*Locusta migratoria, Schistocerca gregaria*), beetles (*Hypera postica, Leptinotarsa decemlineata*) and caterpillars (*Pieris brassicae, Spodoptera* spp.). Pentoses, hexoses, disaccharides and trisaccharides have all been tested on the range of insects listed above. Pentoses all possess no or very low activity at best. Hexoses, disaccharides and trisaccharides have a range of activity which varies with different sugars and insects. Of the hexoses the most active was D-fructose, which has proved active against all the insects listed except *Pieris brassicae*. D-glucose, which is also a hexose, was generally less active than fructose but was stimulatory for all the insects tested. Amongst the disaccharides D-sucrose was clearly the most stimulatory sugar. D-sucrose was highly stimulatory against all the insects which were listed, and was the most stimulatory sugar of all those which had been tested (Bernays & Simpson, 1982) .

Mochizuki *et al* (1985) tested methanol extracts of onion on onion fly larvae, *Hylemya antiqua* (Meigen), an Anthomyiid species related to the turnip root fly. Basic, neutral and acidic fractions of a raw onion extract were tested and phagostimulatory activity was found to exist only in the neutral fraction. The neutral fraction contained glucose, sucrose and fructose in concentrations of 0.09M, 0.07M and 0.06M. When pure solutions of these sugars were tested at 0.05M, 0.10M and 0.50M, glucose was not stimulatory. Fructose and sucrose were stimulatory only at 0.10M. Honda & Ishikawa (1987) demonstrated that the anterior organs of onion fly larvae, *H.antiqua*, are stimulated by 1 molar solutions of eight sugars including glucose, sucrose and fructose. As the anterior organs are believed to have a gustatory function (Honda & Ishikawa, 1987), the presence of sugars may be associated with feeding stimulation. *D. radicum* larvae possess at least three groups of chemoreceptors which may have a gustatory function (Ryan & Behan, 1973). No chemoreception tests were carried out on the larvae concerned, but three types of structure were found to be present. The three structures found, styloconic, dome-shaped and campaniform, were consistent with chemoreceptors, odour receptors and mechanoreceptors respectively.

Sugar concentrations are known to vary in the roots of mature swede plants. Bradshaw & Griffiths (1990) analysed the concentration of glucose, fructose and sucrose in nineteen genotypes of swede. The concentration of fructose varied from 189.5 g kg^{-1} DM (grammes per kilogramme of dried-matter) to 252.9 g kg^{-1} DM. The concentration of glucose was higher than the fructose concentration in all the genotypes tested, and varied from 289.2 g kg^{-1} DM to 350.4 g kg^{-1} DM. The concentrations of sucrose were far lower than those of fructose and glucose, ranging from 17.1 g kg^{-1} DM to 62.5 g kg^{-1} DM.

The phagostimulatory nature of sugars and the genotypic variations in their concentrations are likely to influence the feeding rate of insects on different Brassica genotypes. The stimulatory nature of sugars and their variation in roots makes them attractive as a means of modifying *D.floralis* behaviour and development. The influence of pest damage is also important to crop quality, especially for forage and horticultural crops. The concentration of sugars in the roots of swede plants prior to bulb development is unknown. In this study, the interaction between swede root sugar content and *D.floralis* larval feeding and development is examined.

MATERIALS AND METHODS

Eight swede genotypes, including five commercial cultivars, Angela, Angus, Doon Major, Marian, and Melfort, and three Scottish Crop Research Institute breeding lines, GRL aga , M4M2 a-c and Nmm3 a-d, were selected to represent a range of characteristics. Angus and, to a lesser extent, Melfort have exhibited partial resistance to turnip and cabbage root flies in a number of field experiments (Birch, 1988; Ruuth, 1988; Wilson *et al*, 1990). Doon Major is used as a standard susceptible genotype in experiments against both turnip root fly and cabbage root fly (Birch, 1988; Birch, 1989B; Ruuth, 1988; Wilson *et al*, 1990). Marian and derived lines (M4M2 a-c and Nmm3 a-d) had shown tolerance to cabbage root fly damage at the seedling stage (Wilson *et al*, 1990), and GRL aga had been shown to be resistant to turnip root fly (Birch, 1989). Previous work on mature swede plants had shown them to exhibit a range in root sugar content (Bradshaw & Griffiths, 1990).

Plants were grown in 10cm diameter pots containing a 3:1 mixture of Levington® Universal compost and sand, in a glasshouse with a 16:8 h light:dark regimen and a temperature range of 16-21° C. *Delia floralis* eggs less than 24 hours old from a laboratory culture were inoculated onto the plants at the 8-10 true leaf stage, at a rate of 5, 10 or 20 eggs per plant. Following inoculation, plants were arranged in a series of randomised blocks with eight replicates per treatment. Control plants of the same developmental stage were grown under

identical conditions. Dead leaves were removed daily to avoid migration of larvae between plants. Six weeks after inoculation, pupae were washed from each pot using a 1.4mm sieve. Pupae floated out of the compost were air dried and then weighed individually. Plants were harvested and divided into root and aerial parts at the hypocotyl. Roots and aerial parts were weighed separately and plants from the first four and last four replicates were bulked prior to freeze drying and milling for chemical analysis.

Sugar extraction and analysis.

Freeze dried swede samples were extracted using both water and 80% v/v aqueous ethanol. In a preliminary experiment, comparison of the results for individual sugar contents revealed no significant differences between the values obtained using the two procedures. The use of the 80% ethanol extraction appeared to prolong the operational life of the chromatographic column compared with the water extracts. Consequently, 80% aqueous ethanol was used for the extraction of sugars in all subsequent swede sample analyses.

The constituent sugars, fructose, glucose and sucrose were separated and quantified by high performance liquid chromatography (hplc) using a method based on that described for tropical root crops by Tamate & Bradbury (1985). All separations were performed on a Gilson isocratic hplc system (Anachem Ltd. Luton, Beds U.K.) fitted with an Apex-NH_2 (5µ, 15cm x 4.6mm id.) cartridge column (Jones Chromatography, Hengoed, Mid-Glam.,U.K.). The eluant, 75% v/v acetonitrile, was monitored using a refractive index detector (Gilson model 131. Anachem Ltd., Luton, Beds., U.K.) and the resultant peaks quantified on a Schimadzu C-R3 integrator (Anachem Ltd., Luton, Beds., U.K.).

Statistical analysis

Data were analysed on Genstat (Genstat, Sun/Unix version 5.0, release 2.2, 1990) using analysis of variance on untransformed data. Correlations were calculated on Minitab (Minitab Vax/VMS version 7.1, 1989).

RESULTS

Plant responses to root damage

The effects of genotype and inoculum level on root weights are given in Table B.1.1. An analysis of variance showed root weight to vary significantly ($P<0.001$) with both genotype and inoculum level but there was no significant interaction ($P=0.089$). Increasing the number of eggs inoculated onto the root of the swede led to significant ($P<0.001$) reductions in the

root weight of all swede genotypes. The overall mean root weight of control plants was 22.18g, calculated from the mean root weight of all genotypes. This was significantly greater than the overall mean root weights for all levels of inoculation ($P<0.001$). The overall mean root weights of the 5, 10 and 20 egg inoculation levels were 14.54g, 12.01g and 6.06g respectively, the roots from the 20 egg inoculation level being significantly ($P<0.001$) smaller than those from both the 5 and the 10 egg inoculation levels. There were also significant ($P<0.001$) differences in the root weights between genotypes, the smallest being Angus, 8.46g, and the largest being Angela, 19.03g (Table B.1.1).

Development of turnip root fly larvae on inoculated roots

Neither the level of inoculation nor the swede genotype had a significant ($P>0.05$) effect on the percentage pupation of *Delia floralis* larvae. Percentage pupation ranged from a minimum of 51% on cvs Angela and Melfort to a maximum of 59% on cv Doon Major.

The analysis of mean pupal weights showed significant effects of genotype ($P<0.001$) and inoculum ($P=0.018$). The interaction was not significant ($P=0.62$). Increasing the number of eggs inoculated onto the roots led to a decrease in pupal weight. There were significant differences ($P<0.001$) between the 5, 10 and 20 egg levels of inoculation, the mean weight of pupae for each treatment being 24.51 mg, 22.75 mg and 20.81 mg respectively. There was also a significant ($P<0.02$) difference between swede genotypes, mean pupal weights varying between Angela, 21.17 mg, and Doon Major, 24.28 mg (Table B.1.2).

Sugar concentrations

Total sugar

Total sugar concentration was determined by summing the individual values for glucose, fructose and sucrose. The concentration of total sugar in control roots differed significantly ($P<0.001$) between the genotypes studied, the lowest value being GRL aga, 6.78 grammes per 100g of freeze dried matter (g/100gFDM), and the highest sugar concentration, 18.77 g/100gFDM, in SCRI breeding line Nmm2 a-d (Table B.1.3). In all the genotypes, the concentration of sugars was significantly higher in the control roots. In the inoculated roots, the sugar concentration fell with increasing numbers of eggs. The mean sugar content of the 5, 10 and 20 egg-inoculated roots fell by 29%, 39% and 49% respectively, of the mean value of the control. Averaged over all treatments the total sugar concentration in the roots differed significantly ($P<0.001$) between genotypes. The lowest concentration was in SCRI breeding line GRL aga, with a mean of 5.37 g/100gFDM, and the highest sugar concentration was recorded in cv Angela, with a mean across all treatments of 13.91 g/100gFDM (Table B.1.3). No statistically significant interactions were found between genotype and inoculation level

Table B.1.1. Mean root weight (g) of swede genotypes inoculated with varying numbers of turnip root fly eggs.

	Eggs per plant				Genotype
Genotype	Control	5	10	20	Mean
Angela	21.50	22.15	20.23	12.25	19.03
Nmm3 a-d	31.28	13.88	12.71	8.80	16.67
Marian	27.58	15.03	14.33	8.06	16.25
Doon Major	26.83	17.28	14.34	4.40	15.71
Melfort	21.54	16.86	10.10	2.83	12.83
M4M2 a-c	17.78	7.80	13.09	4.27	10.73
GRL aga	15.40	12.40	6.51	5.35	9.92
Angus	15.59	10.96	4.77	2.50	8.46
Inoculum Mean	22.18	14.54	12.01	6.06	13.70

INOCULATIONS, SED=1.35, DF=3. residual DF = 216
GENOTYPE, SED=1.91, DF=7.
INOC.GENOTYPE, SED=3.83, DF=21.

Table B.1.2. Mean pupal weight (mg) on the root of swede genotypes inoculated with varying numbers of turnip root fly eggs per plant.

	Eggs per plant			Genotype
Genotype	5	10	20	Mean
Doon Major	27.95	24.53	20.37	24.28
Nmm3 a-d	25.54	23.08	22.43	23.68
Melfort	24.07	23.66	21.30	23.01
M4M2 a-c	25.20	22.27	20.66	22.71
Angus	24.43	22.52	20.51	22.49
Marian	23.29	22.34	21.54	22.39
GRL aga	23.27	22.53	19.48	21.76
Angela	22.33	21.02	20.17	21.17
Inoculum Mean	24.51	22.75	20.81	22.69

INOCULATIONS, SED=0.545, DF=2. residual DF = 160
GENOTYPE, SED=0.890, DF=7.

TABLE B.1.3. Mean total sugar concentration (g/100gFDM) of the root of swede genotypes inoculated with varying numbers of turnip root fly eggs per plant.

	Eggs per plant				Genotype
Genotype	Control	5	10	20	Mean
Nmm3 a-d	18.77	12.26	10.08	5.99	11.77
Angela	18.41	15.79	11.54	9.89	13.91
Doon Major	16.40	13.84	11.51	10.98	13.18
M4M2 a-c	14.45	7.44	9.49	7.27	9.66
Marian	12.07	7.24	7.30	7.85	8.61
Melfort	11.79	8.21	6.38	5.90	8.07
Angus	8.47	5.96	4.13	3.09	5.41
GRL aga	6.78	5.55	5.31	3.86	5.37
Inoculum Mean	13.39	9.53	8.22	6.85	9.50

INOCULATIONS, SED=1.84, DF=3. residual DF = 27
GENOTYPE, SED=0.92, DF=7.
INOC.GENOTYPE, SED=0.65.

TABLE B.1.4 Mean fructose concentration (g/100gFDM) of the root of swede genotypes inoculated with varying numbers of turnip root fly eggs per plant.

	Eggs per plant				Genotype
Genotype	Control	5	10	20	Mean
Nmm3 a-d	5.76	3.56	2.45	1.14	3.23
Angela	5.92	4.77	3.26	2.72	4.17
Doon Major	4.67	3.55	2.86	2.26	3.33
M4M2 a-c	4.51	1.77	2.51	1.60	2.60
Marian	3.47	1.76	2.06	2.27	2.39
Melfort	3.49	2.24	1.68	1.36	2.19
Angus	2.37	1.55	0.88	0.55	1.34
GRL aga	1.70	1.26	1.09	0.75	1.20
Inoculum Mean	3.98	2.55	2.10	1.58	2.55

INOCULATIONS, SED=0.63, DF=3. residual DF = 27
GENOTYPE, SED=0.32, DF=7.
INOC.GENOTYPE, SED=0.23.

(P>0.05) for either total or individual sugars.

Individual sugars

The fructose concentration in control roots differed significantly (P<0.001) between the genotypes studied, ranging from 1.70 g/100gFDM in SCRI breeding line GRL aga to 5.92 g/100gFDM in cv Angela (Table B.1.4). In all the genotypes, inoculation with *D.floralis* eggs resulted in a statistically significant (P<0.001) fall in the concentration of fructose, concentration decreasing with increasing egg numbers. The mean fructose content of the 5, 10 and 20 egg inoculated roots was 64%, 53% and 40% of the control respectively. The concentration of fructose in all roots averaged over treatments differed significantly (P<0.001) between genotypes. The lowest overall mean concentration of fructose, 1.20 g/100gFDM, was found in SCRI breeding line GRL aga. The highest overall mean concentration of fructose, 4.17 g/100gFDM was found in cv Angela (Table B.1.4).

The concentration of glucose in control roots also differed significantly (P<0.001) between the genotypes studied, ranging from 3.24 g/100gFDM in SCRI breeding line GRL aga to 9.95 g/100gFDM in SCRI breeding line Nmm2 a-d (Table B.1.5). The concentration of glucose was significantly (P<0.001) higher in the control roots and fell with increasing numbers of eggs. The mean glucose content of the 5, 10 and 20 egg inoculated roots was 64%, 54% and 45% of the control respectively. The concentration of glucose in all roots averaged over treatments differed significantly (P<0.001) between genotypes. The lowest concentration of glucose, 2.44 g/100gFDM, was found in GRL aga, and the highest concentration, 6.91 g/100gFDM, was found in cv Angela.(Table B.1.5)

The glucose content of the roots was highly correlated with the fructose content of the root, r=0.99, and the ratio of glucose : fructose was approximately 5:3. As would be expected, fructose (r=0.98) and glucose (r=0.98) were both closely correlated to total sugar content.

The concentration of sucrose in control roots differed significantly (P<0.001) between the genotypes studied, the lowest concentration being in cv Marian, 1.57 g/100gFDM and the highest in cv Doon Major, 3.20 g/100gFDM (Table B.1.6). Inoculation had no significant effect (P>0.05) on the sucrose content of roots. The concentration of sucrose in roots, averaged across all treatments, was significantly different (P<0.001) between genotypes. The lowest level was found in cv Angus (1.36 g/100gFDM) and the highest level was found in cv Doon Major (3.68 g/100gFDM) (Table B.1.6).

TABLE B.1.5. Mean glucose concentration (g/100gFDM) of the root of swede genotypes inoculated with varying numbers of turnip root fly eggs per plant.

	Eggs per plant				Genotype
Genotype	Control	5	10	20	Mean
Nmm3 a-d	9.95	6.09	4.74	2.95	5.93
Angela	9.83	7.80	5.31	4.72	6.91
Doon Major	8.54	6.21	5.62	4.31	6.17
M4M2 a-c	7.73	3.55	4.58	3.08	4.74
Marian	7.04	4.06	3.62	3.99	4.67
Melfort	6.58	3.87	3.03	3.54	4.26
Angus	4.32	3.01	1.78	1.38	2.62
GRL aga	3.24	2.32	2.35	1.88	2.44
Inoculum Mean	7.15	4.61	3.88	3.23	4.72

INOCULATIONS, SED=1.00, DF=3. residual DF = 27
GENOTYPE, SED=0.50, DF=7.
INOC.GENOTYPE, SED=0.35,.

TABLE B.1.6. Mean sucrose concentration (g/100gFDM) of the root of swede genotypes inoculated with varying numbers of turnip root fly eggs per plant.

	Eggs per plant				Genotype
Genotype	Control	5	10	20	Mean
Nmm3 a-d	3.07	2.61	2.89	1.90	2.62
Angela	2.67	3.23	2.97	2.46	2.83
Doon Major	3.20	4.08	3.03	4.41	3.68
M4M2 a-c	2.22	2.12	2.40	2.60	2.33
Marian	1.57	1.42	1.63	1.60	1.55
Melfort	1.73	2.10	1.68	1.01	1.63
Angus	1.79	1.41	1.37	0.87	1.36
GRL aga	1.85	1.98	1.87	1.24	1.73
Inoculum Mean	2.26	2.37	2.23	2.01	2.22

INOCULATIONS, SED=0.69, DF=3. residual DF = 27
GENOTYPE, SED=0.34, DF=7.
INOC.GENOTYPE, SED=0.24.

Relationship between sugar concentrations and turnip root fly pupal weights

Initial correlations between *D.floralis* pupal weights and either total or individual sugar contents were not statistically significant ($P>0.05$). However, plotting the data showed the values for cv Angela to be widely separated from the data points for other genotypes on all the graphs. When the correlation coefficients were recalculated, omitting the data for cv Angela, statistically significant correlations between the remaining seven swede genotypes' sugar contents and pupal weights were found. Total sugar content was significantly (r=0.90 [DF=5], $P<0.01$) correlated with pupal weights (Figure B.1.1.). All the individual sugar contents were significantly ($P<0.02$) correlated with pupal weights (fructose r=0.87 [DF=5] (Figure B.1.2.); glucose r=0.87 [DF=5] (Figure B.1.3.); sucrose r=0.84 [DF=5] (Figure B.1.4.)).

DISCUSSION

Total and individual sugar contents of the control roots of undamaged swedes varied widely between swede genotypes, and did so in a manner that was more extreme and different from results found previously in mature swedes (Bradshaw & Griffiths, 1990). The five named cultivars used in this study were all tested as mature roots in the study carried out by Bradshaw & Griffiths (1990). The concentrations of fructose and glucose found in the young roots analysed in this study were all approximately 20% and 25% respectively, of those previously found in mature swede roots. As the roots of swedes are storage organs and the plants used in this study were young, this result is not unusual. The relationship between the sucrose content of young swede roots, and published data on mature roots, was less clear. The concentration in young Doon Major was approximately 150% of the concentration found in mature roots. The young roots of the other genotypes tested were between 78% and 29% of the concentration found in mature roots. The close link between glucose and fructose found in this experiment resulted in them having a strong influence on the total sugar content of the root, together accounting for approximately 75% of the total root sugars.

Within the range tested, increasing the number of *D.floralis* eggs inoculated onto a plant had no effect on larval survival, as shown by percentage pupation. The competition for food resources in the root did not become so limiting as to lead to larval mortality. There was no significant difference ($P>0.05$) between the percentage of eggs which survived to pupation at different pest densities. The percentage pupations at 5, 10 and 20 eggs per plant were 54.1%, 56.6% and 55.2% respectively. However, the mean pupal weight produced at higher egg densities was reduced for all swede genotypes, indicating that the food resource was either restricted quantitatively or reduced qualitatively by increasing numbers of larvae per root.

Figure B.1.1. The correlation between total root sugar concentration (g/100gFDM) of seven swede genotypes and the mean weight of *D.floralis* pupae (mg) for each genotype.

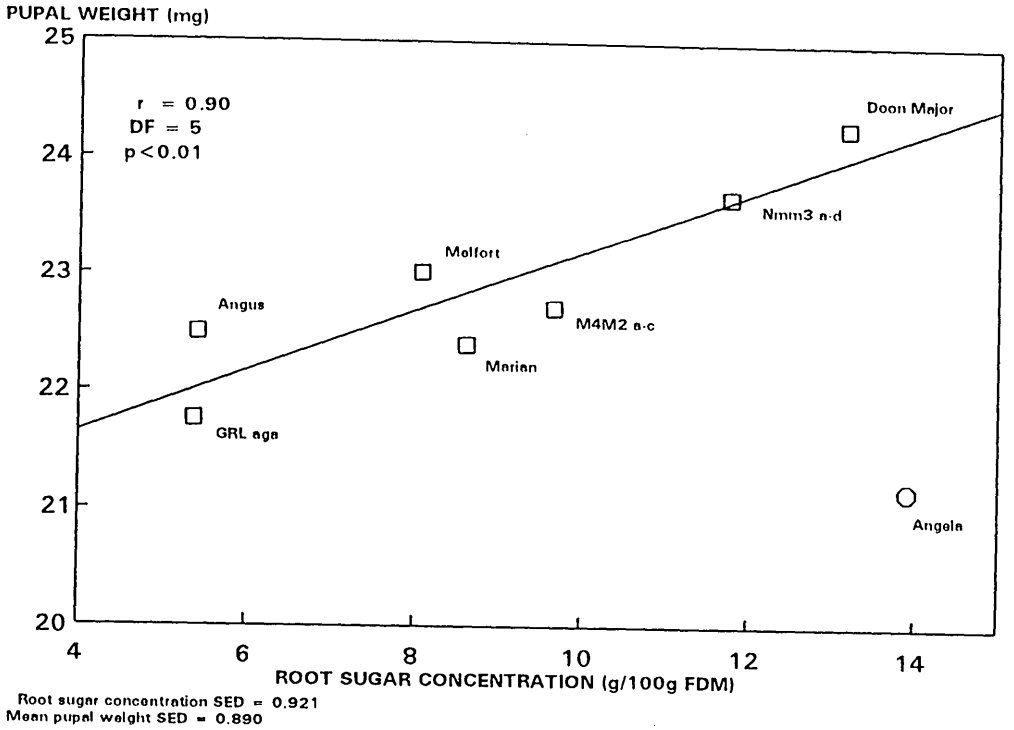


Figure B.1.2. The correlation between root fructose concentration (g/100gFDM) of seven swede genotypes and the mean weight of *D.floralis* pupae (mg) for each genotype.

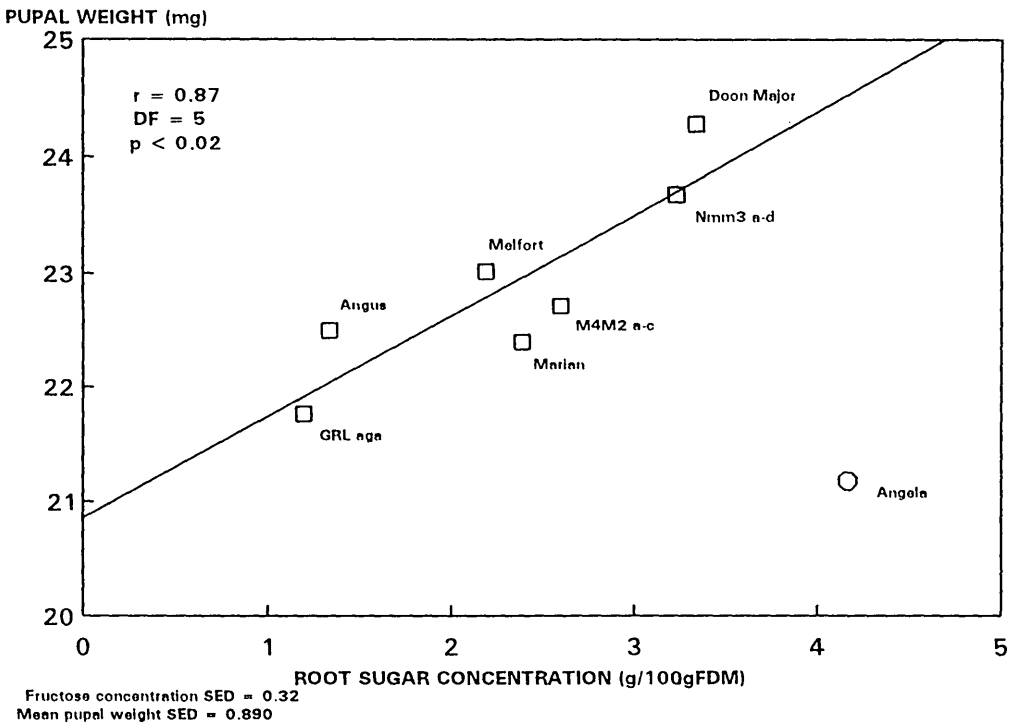


Figure B.1.3. The correlation between root glucose concentration (g/100gFDM) of seven swede genotypes and the mean weight of *D.floralis* pupae (mg) for each genotype.

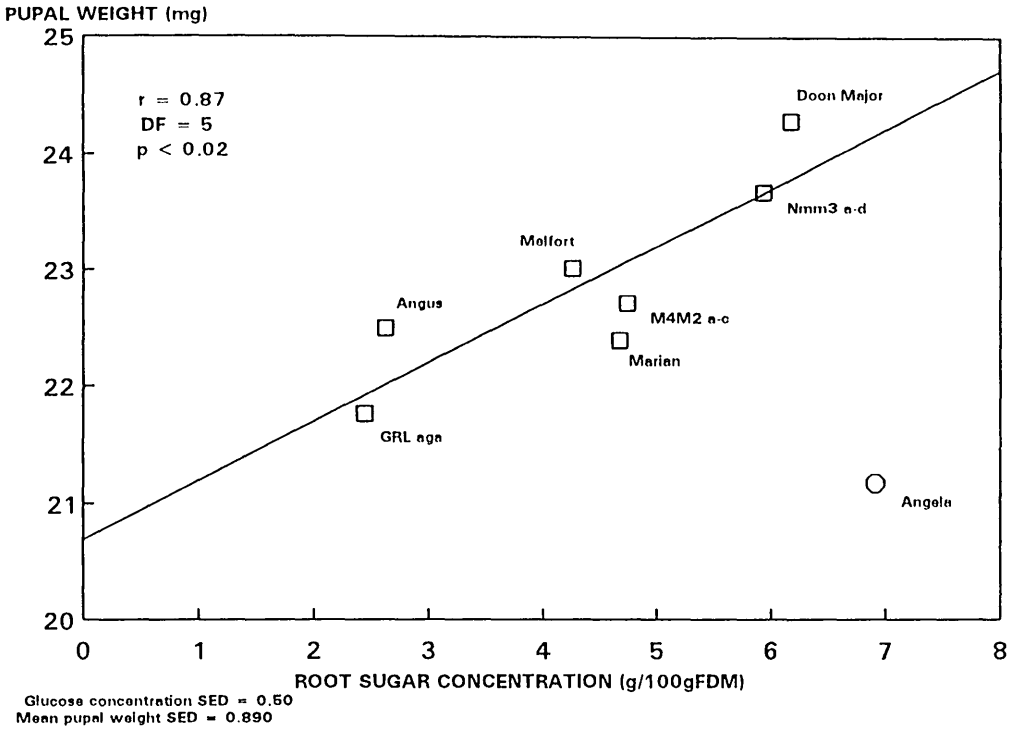
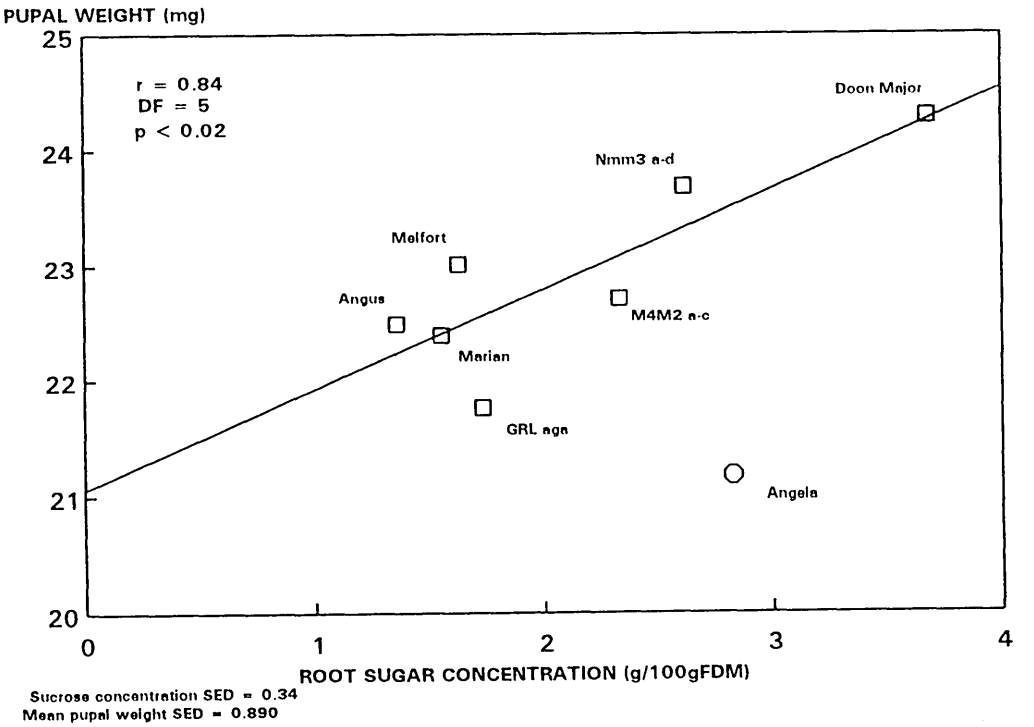


Figure B.1.4. The correlation between root sucrose concentration (g/100gFDM) of seven swede genotypes and the mean weight of *D.floralis* pupae (mg) for each genotype.



Finch & Coaker (1968) also found that inoculation of increasing numbers of eggs of the closely related species, *Delia radicum*, led to a reduction in mean pupal weight. The weight of the pupa determined the weight of the adult fly which emerged from it, and also correlated with the fecundity of the adult fly. They also found that variation in weight from 20 mg to 24 mg led to a rise in fecundity of approximately 30%. The variation in pupal weight with genotype can be attributed to variation in the food quality of different host plants. There was no evidence to indicate any variation in the development time of *D. floralis* on different genotypes during this experiment. Insects can increase development time on sub-optimal hosts and the Brassica pod midge, *Dasineura brassicae* is an example of a pest which reduces its development time on sub-optimal oilseed rape plants (Åhman, 1985). At the time that plants were sampled, all the larvae had pupated and few adults had emerged from the pupae. There was no discernible pattern to the distribution of emerged adults at the time of sampling, though the experimental design was not suited to measuring variations in the development time of *D.floralis* on different genotypes.

Plant genotypic variation in the larval development of turnip root fly may be related to an increase in phagostimulation. Bernays & Simpson (1982) reviewed the phagostimulatory nature of sugars on insects and the phagostimulatory effect of sugars on larvae of the onion fly, *Hylemya antiqua*, was demonstrated by Mochizuki *et al* (1985). The correlation between root sugars of swede and pupal weights indicated that the sugar content of the root influenced the development of *D. floralis* larvae in all genotypes except cv Angela. In the inoculation experiments Angela performed differently to the other swede genotypes tested. For example, inoculation of turnip root fly eggs onto the roots generally reduced the quantity of root tissue produced by all genotypes, except for cv Angela, which was not significantly affected by the 5 or 10 egg inoculation level (Table B.1.1). The percentage pupation which took place for these two inoculation levels on Angela indicated that comparatively greater root weight could not be attributed to reduced numbers of larvae developing on the host plant. Angela may be tolerant of damage and/or have a compensatory growth response to damage. This suggestion would not, however, explain Angela as an outlier in the correlation between the concentration of sugars and size of pupae produced. On Angela, the combination of smaller pupae, unaffected root production when inoculated with up to 10 eggs, and failure to fit the sugar/pupae correlation would indicate the presence of a deterrent or antibiotic factor in the roots. Alternatively, Angela may lack other phagostimulants needed fully to stimulate *D.floralis* larval feeding, or this cultivar may contain an antifeedant, making it less palatable. The swede genotype Angela is worthy of further study.

The results suggest that, in seven of the eight genotypes examined, sugar content exerts an influence on the host quality of swede plants for *D. floralis* larvae. The close relationship between different individual sugars and the relationship between glucose and fructose and total sugar content makes differentiation of the effects of each sugar difficult. The correlations produced indicate that total sugar concentration is more important than the concentration of the individual sugars. However, the relationship between the total sugar concentration and the individual concentrations of glucose and fructose makes a certain conclusion to the question of which is most important impossible on the strength of the data presented. To have a certain answer to the part which is played by different sugars in turnip root fly development, it would be necessary to use an artificial diet. No known artificial diet is currently available for Brassica root flies; the need for one is discussed in more detail in the section discussion. However, sugars have been shown to have a phagostimulatory effect on the larvae of other Anthomyiid flies (Mochizuki *et al*, 1985; Honda & Ishikawa, 1987), although *D.floralis* has not been studied. This type of partial resistance mechanism on its own is unlikely to be sufficient to reduce significantly rootfly damage. However, minor manipulations of root sugar content may serve to complement other mechanisms of resistance or tolerance. Used in combination with a swede having antixenotic resistance to oviposition and tolerance to root damage, the combined effect could substantially reduce insecticide requirements to control this pest.

The reduced sugar content after larval damage may be the product of two effects. Firstly, larvae may be selectively feeding on sections of the root which contain relatively high proportions of sugar, thus reducing the average sugar content of the remaining root. Secondly, it is probable that sugar production is adversely affected by the reallocation of resources due to the damage being sustained by the plant. The combination of the reduced sugar concentration in the remaining root tissue and reduced root weight causes a large loss in the amount of sugar in the root as a whole. The size of the roots of plants inoculated with 20 turnip root fly eggs was between 56.9% and 13.1% of the size of control plants. The total concentration of sugars in the roots of plants inoculated with 20 turnip root fly eggs was between 31.9% and 67.0% of the concentration of control plants. The cumulative effect of the reductions in root weight and in concentration was a reduction in total sugar yield per root of between 80.4% and 91.8%. The large effect of larval damage on root sugar content has a number of implications. Changes in the sugar content of swedes may reduce their nutritive value to livestock and humans. It has however been previously shown (Gemmell *et al*, 1990) that swede genotypes with low total sugar contents early in the growing season may compensate for this before harvest. Gemmell *et al* (1990) demonstrated that the concentrations of sugar in different genotypes of swede did not all follow the same accumulation curve from

June to November. In some genotypes, the concentration of sugar in the roots changed little over this time period. But in those genotypes which started with very low concentrations of sugars in June, most increased the concentration of sugar in the roots at a much greater rate than those with relatively high sugar concentrations in June. The long term effect of reductions in swede root sugar concentration due to insect damage have not been studied, and may be important to the nutritional status of the crop.

The implication for the use of field experiments to assess the effect of root chemical factors on pests of swede may also be considerable. Previous work on *D. floralis* attributed the extent of the root damage to the chemical composition at harvest (Shaw, 1982; 1984; 1985; 1993). Turnip root fly damage varies with levels of oviposition (Ruuth, 1988) and results, as demonstrated here, in changes to the chemical composition of the plant root if damage occurs around the eight true leaf stage. It may, therefore, be misleading to predict susceptibility to damage based solely on the chemical composition of swedes at harvest.

Chapter B2

Development of *Delia floralis* on swedes, rapes and kales, its relationship to root dry matter, sugar, plant fibre and lignin content

INTRODUCTION

The chemical composition of root material ingested by root fly larvae varies considerably. The major component of growing plant material is water, and cell walls and cellular contents will be suspended in it when roughly masticated by feeding turnip root fly larvae. The variation in the ingested plant root components will dictate the dietary intake of the insect.

Plant cell walls, or fibre, are composed of non-starch polysaccharides, cellulose, hemicelluloses and pectins, as well as the aromatic polymer lignin (Selvendran & Robertson 1990). Cellulose, a major constituent of plant cell walls, is indigestible by the majority of insects (Fonty & Gouet; 1989, Prins & Kreulen, 1991). It is a polymer of glucose (Selvendran & Robertson 1990) and perhaps it is surprising that the majority of insects are not able to utilise this apparently abundant source of energy. Insects which do utilise cellulose, such as wood wasps, silverfish, termites and wood boring beetles, do so as part of a specialist diet, taking advantage of an otherwise vacant ecological niche (Fonty & Gouet, 1989, Prins & Kreulen, 1991). These insects ferment cellulose and other plant polysaccharides utilising a range of cellulose-degrading, symbiotic, microorganisms which are usually harboured in the hindgut (Prins & Kreulen, 1991). The digestion of cellulose is completed by the activity of insect-synthesised enzymes such as cellobiases which are present in salivary glands and midgut tissue to degrade the products further to glucose. The abilities of *Delia* spp. to digest cell wall components remains unstudied, but there is no evidence for insects occupying a similar ecological niche to the turnip root fly utilising this resource (Prins & Kreulen, 1991). However, there have been indications that root flies interact with microbes present in the plant. Doanne & Chapman (1964b) demonstrated that larvae of the cabbage root fly could not survive on plugs of swede which had been heat-sterilised. However, it could not be concluded whether this effect was due to removal of microbial activity or due to chemical changes in the plant tissue brought about by the application of heat.

The inability of most phytophagous insects to digest cellulose leaves a major carbohydrate source of energy not utilised. However, amino acids are usually the limiting factor in insect development because they are needed for growth. Insects usually ingest an excess of sugars

and starches in order to assimilate the quantities of amino acids which they require (Young, 1991). Larvae of the onion maggot, *Delia antiqua* (Diptera:Anthomyiidae), have been reared on an artificial diet (Eyman & Friend, 1985). The use of the artificial diet for the onion maggot demonstrated the relative importance of carbohydrates and amino acids in the development of a species closely related to the turnip root fly. The onion maggot usually develops on decaying onion tissue and can develop on an onion agar which has been supplemented with 10 essential amino acids. The omission of all the amino acids from the mixture prevented development from taking place whilst the omission of single amino acids resulted in less marked effects. Omission of choline, thiamine, pyridoxine or pantothenic acid had no significant effect on the development of the onion fly. Omitting biotin or nicotinic acid resulted in sub-optimal development to the adult stage. The omission of riboflavin or folic acid resulted in the development of adults which did not lay viable eggs (Eyman & Friend, 1985). Herein may lie the reasons for the inability of many phytophagous insects to utilise cellulose. Readily available carbohydrate already constitutes a major part of the diet of many phytophagous insects, and consequently there is little evolutionary pressure to develop the ability to assimilate more.

In addition to their nutritive importance, amino acids and carbohydrates are important as phagostimulants to many insects which will not initiate or continue feeding unless they receive appropriate stimulation from the substrate (Bernays & Simpson, 1982). The phagostimulatory activity of a particular compound generally increases with concentration to a threshold, beyond which the effect may be reduced or reversed. Sugars, particularly sucrose, are the best known and most widespread insect phagostimulants (Bernays & Simpson, 1982). Consequently, the extent to which insects are stimulated to feed may vary with changes in the sugar concentration in feeding substrates. The relationship between *D.floralis* larval feeding and Brassica root sugar concentrations has been explored in some detail in Chapter B1.

The composition of plant fibre is not a constant factor. Lignification of plant cell walls takes place to increase the strength of plant tissue, allowing them to resist environmental stress. In addition, lignification has long been identified as a polygenic resistance mechanism, which protects plants against a large number of potential pathogens and pests (Wood, 1982). This aspect of insect-plant interactions between root flies and their host plants appears to have remained unexplored, although larval stages of the pests of sugarcane had their development impaired by encountering an increased proportion of fibre (Agarwal, 1969). Crude fibre content of the inner whorl tissue of corn, *Zea mays*, was linked to resistance to the southwestern cornborer, *Diaetraea grandiosella* (Hedin *et al*, 1984).

Although unable to digest cellulose and free the stored energy, the fibre content in the insect diet will influence the nutrition of phytophagous insects. Increasing the fibre content of the feeding substrate will reduce the efficiency of feeding, increasing the rate of ingestion needed to sustain the same nutritive value. To date, it is uncertain whether fibre has any nutritional value for the turnip root fly, but it is improbable. Consequently, to raise the fibre content of the food material may adversely effect the development of turnip root fly larvae. To date, no data have been published on the relationship between *D.floralis* and plant fibre or free sugars. The aim of this chapter is to investigate the relationship between *D.floralis* larval feeding and plant fibre content and dry matter composition of Brassica roots. In addition, the relationship between *D.floralis* larval feeding and the sugar content of the roots of Brassicas will be further investigated.

MATERIALS AND METHODS

Experimental material

Six Brassica genotypes were selected to include two each of kale, swede, and rape. Seed sown on 23 February 1990 was pricked out at the dicotyledon stage and plants were grown singly in 15cm diameter pots containing a 3:1 mix of Levington® Universal compost and sand, in a glasshouse at 15-20°C, 16 h photoperiod. After 32 days growth (at the 3-4 true leaf stage), 12 plants of each genotype were inoculated with 10 eggs of *D.floralis*, which were less than 24 hours old and come from a laboratory culture. The inoculated plants were arranged in a randomised block design (12 blocks) with 12 control (uninoculated) plants of each genotype and grown for a further 8 weeks. At harvest, each plant was divided into leaf, root and stem material and each part weighed. Plants from the first six and second six replicates were bulked, freeze-dried and milled for chemical analysis.

Percentage freeze-dried matter (FDM) was calculated by dividing the dry weight of the bulked sample after freeze drying by the sum of the fresh weight of those plant parts which went into the sample.

Extraction of plant fibre

Plant fibre was extracted from freeze-dried material using a method based on that of Goering & van Soest (1970). A neutral detergent solution was produced (30g sodium lauryl sulphate + 18.61g disodium ethylene-diamine-tetra-acetate + 6.81g sodium borate decahydrate + 4.56g anhydrous disodium hydrogen phosphate + 10ml 2-ethoxyethanol in 1000ml water). A 1g sub-

sample of the freeze-dried material was placed in a 200ml round-bottomed flask to which was added 100ml of the neutral detergent solution. The round-bottomed flask was then linked to a condenser and refluxed for 1 hour. The suspension was then filtered through a weighed glass fibre filter paper, rinsed with 100ml hot distilled water (approx 60°C), followed by 100ml methanol and 100ml acetone, leaving behind a cell wall preparation. The filter paper was then air dried overnight and weighed, the weight of the filter paper subtracted from the total weight and the sum multiplied by 100 to give the percentage plant fibre.

Lignin analysis

Lignin content of the total fibre was measured by the acetyl bromide method (Morrison, 1972a; 1972b; Iiyama & Wallis, 1988). A 10-20mg sub-sample of the cell wall preparation made during the neutral detergent extraction was weighed into a 3.5ml brown glass vial. To the sample was added 2ml of a solution of 25% acetyl bromide in acetic acid with 100µl of 70% perchloric acid. The vial was capped with a Teflon lined seal and heated at 70°C for 30 minutes, shaking every 10 minutes. After 30 minutes the sample was removed from the heater block and allowed to cool. The contents of the vial were transferred to a 50ml volumetric flask containing 5ml of acetic acid and 0.45ml of 2M sodium hydroxide, rinsing twice with acetic acid. The volume was then made up to approximately 40ml with acetic acid, to which was added 0.8ml of 0.5M hydroxylammonium chloride before filling the flask to the 50ml mark with acetic acid. The absorbance of the solution was read at 280nm in silica cells concurrently with a reagent blank, each sample was replicated three times. The percentage lignin was then calculated according to the formula below:

$$\text{Percentage lignin} = \frac{(\text{OD} \times 3.36) - 1.11}{\text{Conc. of sample (g l}^{-1})}$$

OD = optical density of sample

Sugar extraction and analysis.

Freeze-dried Brassica samples were extracted using 80% v/v aqueous ethanol. The constituent sugars, fructose, glucose and sucrose, were separated and quantified by high performance liquid chromatography (hplc) using the method described in Chapter B1.

Statistical analysis

Data were analysed on Genstat (Genstat, Sun/Unix version 5.0, release 2.2, 1990) using analysis of variance on untransformed data. Correlations were calculated on Minitab (Minitab Vax/VMS version 7.1, 1989).

Plant responses to larval feeding by turnip root fly

Growth of Brassica leaves after attack by *D.floralis* larvae, as estimated by the difference after 8 weeks between leaf weights of control and inoculated plants, differed significantly ($P<0.001$) between Brassicas, although there was no clear separation between the three Brassica crop types (Table B.2.1.). Greatest leaf weight losses (23-27%) were recorded on swede, cv Doon Major, and rape, cv Ariana. Stem weight losses also differed significantly ($P<0.001$) between Brassicas and showed similar trends, swede, cv. Angus, suffering higher (17-20%) losses than the other Brassicas (Table B.2.2.). Larval feeding damage on the roots differed significantly between Brassicas ($P<0.001$). The swede, cv. Doon Major, gave highest control root weight and percentage loss (46%), other Brassicas being reduced by 25-37% (Table B.2.3.). Within pairs of each Brassica crop type, only the two swede genotypes responded differently to larval feeding ($P<0.001$).

Pupal development on different genotypes

Differences in larval survival, as measured by percentage pupation, were found between the genotypes (Table B.2.4.). Although percentage pupation was not significantly different for all six Brassicas ($P=0.06$), survival on the two kales was significantly lower than on the two swedes ($P<0.05$). The lowest percentage pupation was on kale KHSCN, 38.3% and the highest on Ariana, 59.2%. The percentage pupation on cv Hobson, 40.8%, was significantly ($P<0.05$) lower than on cv Ariana. Significantly ($P<0.001$) greater total and individual pupal weights were recorded after feeding on the two swede genotypes and on rape, cv Ariana, than on the other three genotypes. The mean individual pupal weights of insects on Ariana, Doon Major and Angus were 19.8, 19.9 and 20.0 mg respectively. In contrast, the larvae which developed on KHSCN, Hobson and KLSCN produced much smaller pupae, 12.5, 13.8 and 14.4 mg respectively (Table B.2.4.)

Total dry matter content

The overall mean of the freeze-dried matter (FDM) content of all treatments on all genotypes was 19.5 g/100g. There was no significant difference ($P>0.05$) for controls between or within crop types (Table B.2.5.). Although dry matter content was highest in the kales, FDM 20.3 g/100g, and lowest in the rapes, FDM 18.4 g/100g, the differences were not significant ($P=0.079$). Similarly, the mean FDM of control roots, 20.1 g/100g, was not significantly different ($P=0.099$) from the mean FDM of inoculated roots, 18.9 g/100g. No significant differences ($P>0.05$) for any of the factors related to the dry matter content were found.

Table B.2.1. Mean weight of leaves (g) of control plants of each genotype and those inoculated with *Delia floralis* at the rate of ten eggs per plant.

		Leaf weight		
Crop type	Genotype	Control	Inoculated	% Leaf loss
Kale	KLSCN	10.23	9.23	9.5
Kale	KHSCN	11.65	10.16	12.8
Rape	Hobson	14.14	12.99	8.1
Rape	Ariana	10.37	7.60	26.7
Swede	Angus	9.62	8.41	12.5
Swede	Doon Major	6.70	5.18	22.7
SED		0.72		
residual D.F.		116		

Table B.2.2. Mean weight of stems (g) of control plants of each genotype and those inoculated with *Delia floralis* at the rate of ten eggs per plant.

		Stem weight		
Crop type	Genotype	Control	Inoculated	% Stem loss
Kale	KLSCN	13.37	11.43	14.5
Kale	KHSCN	13.63	12.68	6.9
Rape	Hobson	15.36	14.51	5.5
Rape	Ariana	6.50	5.38	17.2
Swede	Angus	9.44	7.51	20.4
Swede	Doon Major	5.46	4.51	17.3
SED		0.68		
residual D.F.		116		

Table B.2.3. Mean weight of roots (g) of control plants of each genotype and those inoculated with *Delia floralis* at the rate of ten eggs per plant.

		Root weight		
Crop type	Genotype	Control	Inoculated	% Root loss
Kale	KLSCN	15.46	9.68	37.4
Kale	KHSCN	14.69	9.22	37.2
Rape	Hobson	16.40	10.76	34.4
Rape	Ariana	15.34	10.93	28.8
Swede	Angus	16.63	12.32	25.9
Swede	Doon Major	26.85	14.51	46.0
SED		1.17		
residual D.F.		116		

Table B.2.4. Percentage pupation and mean weights of individual and total pupae per root (mg) of *Delia floralis* pupae after larval feeding with ten eggs per plant

		Pupal weight / root		Pupation
Crop type	Genotype	Total	Mean	Percentage
Kale	KLSCN	60.2	14.4	39.2
Kale	KHSCN	48.8	12.5	38.3
Rape	Hobson	57.3	13.8	40.8
Rape	Ariana	117.8	19.8	59.2
Swede	Angus	103.4	20.0	50.8
Swede	Doon Major	117.7	19.9	58.3
SED		17.0	1.2	9.2
residual D.F.		55	55	55

Plant fibre content

The overall mean of the plant fibre as a component of FDM content of all treatments was 56.1 grammes / 100 grammes of FDM, which converts to 10.9 g/100g fresh weight (Table B.2.6.). Significant ($P<0.001$) differences occurred between the plant fibre content of crop types as a component of both FDM and fresh weight. The mean plant fibre component of the FDM of the kales, rapes and swedes, averaged for control and inoculated roots, was 65.5, 63.6 and 39.3 g/100gFDM respectively. This corresponded to the kales, rapes and swedes having fibre contents of 13.3, 11.7 and 7.7 g/100g fresh weight respectively. There were significant differences between the control plants of crop types and genotypes. The controls of the two swedes had a mean fibre content of 34.0 g/100gFDM, whilst the kales and rapes had a mean of 61.5 g/100gFDM and 61.0 g/100gFDM respectively. Within crop types, the controls of the two genotypes of the kales and rapes did not differ significantly in fibre content ($P>0.05$). However, within the swedes, the controls of Angus (29.0 g/100gFDM) were lower ($P=0.004$) than Doon Major (39.0 g/100gFDM).

Turnip root fly larval feeding had a significant ($P<0.001$) effect on the fibre component of the FDM of the Brassicas. Control Brassicas had a mean fibre component of 52.2 g/100gFDM and inoculated Brassicas had a mean 60.1 g/100gFDM, a 15% rise in the fibre after attack. These figures correspond to the controls having a fibre component of fresh weight of 10.4 g/100g and inoculated Brassicas having a mean fibre component of fresh weight of 11.3 g/100g, which although not significantly different ($P=0.077$), corresponds to a numerical rise in the fibre component of fresh weight of 8.72% after attack.

Significant ($P=0.004$) differences between the genotypes of Brassicas could only be demonstrated in the fibre content of FDM. There was no significant difference ($P>0.05$) within crop types in the corresponding content of fresh weight. The only genotypes within the Brassicas which showed significant differences were the swedes, in which the fibre content of FDM of Doon Major and Angus were 43.3 and 35.3 g/100gFDM respectively. There was no significant difference ($P>0.05$) between the genotypes of kales or rapes.

Lignin content

The overall mean lignin content of all plants was 9.5 g/100g fibre, which converts to 5.8 g/100gFDM or 1.1 g/100g fresh weight. There were significant differences in lignin content between the controls of Brassica crop types (Table B.2.7.) and genotypes (Table B.2.8.). Significant ($P<0.001$) differences occurred between the lignin content of Brassicas as a

Table B.2.5. Mean freeze-dried matter content (g/100g fresh weight) of the roots of control plants of each genotype and those which have been damaged by turnip root fly.

Crop type	Genotype	control	inoculated	Mean
Kale	KLSCN	21.15	19.86	20.51
Kale	KHSCN	20.66	19.45	20.06
Rape	Hobson	18.55	18.08	18.31
Rape	Ariana	19.77	17.03	18.40
Swede	Angus	22.76	19.65	21.20
Swede	Doon Major	17.84	19.52	18.68
SED		1.62		1.15
residual D.F.		11		11

Table B.2.6. Mean Fibre content (g/100g) of control plants and those inoculated with turnip root fly presented as a proportion of the Freeze-dried Matter (FDM) and Fresh Weight (FW) for each genotype.

		Plant Fibre / FDM (g/100g)			Plant Fibre / FW (g/100g)		
Crop type	Genotype	0	10	Mean[1]	0	10	Mean[1]
Kale	KLSCN	61.5	69.5	65.5	13.0	13.9	13.4
Kale	KHSCN	61.5	69.5	65.5	12.7	13.5	13.1
Swede	Angus	29.0	41.5	35.3	6.6	8.2	7.4
Swede	Doon Major	39.0	47.5	43.3	7.0	9.3	8.1
Rape	Hobson	60.5	67.5	63.8	11.1	12.2	11.6
Rape	Ariana	62.0	65.0	63.5	12.3	11.1	11.7
SED		2.3		1.7	1.1		0.8
residual D.F.		11		11	11		11

1 Mean of control and inoculated root

component of fibre, FDM and fresh weight (Table B.2.7.). The lignin content of the fibre of the kales, rapes and swedes was 13.0, 10.7 and 4.9 g/100g respectively. The lignin content of the FDM of the kales, rapes and swedes was 8.6, 6.9 and 2.0 g/100gFDM respectively. These measurements corresponded to the kales, rapes and swedes having a lignin content of fresh weight of 1.7, 1.2 and 0.4 g/100g respectively.

Larval feeding had a significant ($P<0.001$) effect on lignin as a component of the fibre, FDM and fresh weight of the Brassicas (Table B.2.7.). Control Brassicas had a mean lignin content of 7.8 g/100g fibre and inoculated Brassicas had a mean lignin content of 11.2 g/100g fibre, corresponding to a 43% increase. The lignin content of dry matter of control and inoculated plants was 4.5 g/100gFDM and 7.1 g/100gFDM respectively, a 59% increase after attack. These measurements corresponded to the controls having a lignin content of fresh weight of 0.9 g/100g and inoculated Brassicas having a lignin content of fresh weight of 1.3 g/100g, a 48% increase after attack.

Significant differences between the genotypes of Brassicas could be demonstrated for lignin as a component of fibre, FDM and fresh weight (Table B.2.8.). Lignin content of fibre was significantly different for genotype ($P=0.036$). There was no significant difference ($P>0.05$) between the lignin concentrations of the rapes and the swedes. The low thiocyanate ion kale contained significantly ($P=0.036$) greater concentrations of lignin than the high thiocyanate ion kale, the former being 15.1 g/100g fibre and the latter being 10.9 g/100g fibre. Similarly, lignin content of dry matter was significantly different for the kales ($P=0.024$). The low thiocyanate ion kale (KLSCN) contained significantly greater concentrations of lignin than the high thiocyanate ion kale (KHSCN), 9.9 and 7.2 g/100gFDM respectively. As a component of fresh weight, the lignin was significantly ($P=0.006$) different in the two kales: KLSCN contained higher concentrations of lignin than KHSCN, the former being 2.0 g/100g fresh weight and the latter being 1.4 g/100g fresh weight (Table B.2.8.). There was no significant interaction ($P>0.05$) between crop types or genotypes in their lignification response to larval attack.

Total sugar content

The overall mean total sugar content of Brassicas was 6.32 g/100gFDM. There were significantly different ($P<0.001$) concentrations of total sugar in the different Brassicas (Table B.2.9.). The concentration in swedes, rapes and kales being 10.45, 5.09 and 3.44 g/100gFDM respectively. *D.floralis* larval feeding had a significant ($P=0.002$) effect on the total sugar content of the Brassicas, control roots containing a mean of 6.93 g/100gFDM and

Table B.2.7. Variation in the lignin content of different crop types with larval feeding, as a percentage of Fibre, Dry matter and Fresh Weight.

Fraction	Fibre (g/100g)		FDM (g/100g)		Fresh Weight (g/100g)	
Crop type	control	inoculated	control	inoculated	control	inoculated
Kale	11.75	14.26	7.22	9.89	1.50	1.95
Rape	8.29	13.16	5.10	8.72	0.97	1.53
Swede	3.45	6.26	1.13	2.80	0.23	0.55
SED	1.25		0.76		0.13	
residual D.F.	11		11		11	

Table B.2.8. Variation in the lignin content of different genotypes with larval feeding, as a proportion of Fibre, Dry matter and Fresh Weight.

		Fibre (g/100g)		FDM (g/100g)		Fresh Weight (g/100g)	
Crop type	Genotype	con	inoc	con	inoc	con	inoc
Kale	KLSCN	14.39	15.87	8.88	11.01	1.86	2.19
Kale	KHSCN	9.11	12.65	5.57	8.77	1.15	1.71
Rape	Hobson	7.14	13.40	4.34	9.06	0.78	1.62
Rape	Ariana	9.44	12.93	5.86	8.39	1.16	1.43
Swede	Angus	4.26	5.73	1.23	2.37	0.28	0.46
Swede	Doon Major	2.65	6.79	1.03	3.23	0.18	0.63
SED		1.77		1.07		0.18	
residual D.F.		11		11		11	

inoculated roots containing a mean of 5.72 g/100gFDM, representing a mean decrease of 18%. Within crop types, significant differences ($P<0.001$) could also be found in the concentrations of total sugar in the roots. There was no significant difference ($P>0.05$) between the genotypes of rape or kale. In the swedes, Doon Major had a significantly ($P<0.001$) higher concentration of total sugar than Angus, the concentrations being 12.23 and 8.66 g/100gFDM respectively.

There was no significant difference in the effect of larval feeding on different crop types ($P>0.05$). However, the different genotypes responded differently to larval feeding ($P=0.04$). Although the total sugar concentration fell in all genotypes with larval feeding, this result was only significant ($P<0.05$) in the genotypes Doon Major and Ariana (Table B.2.9.).

Individual sugars

The overall mean fructose content of all Brassicas was 0.96 g/100gFDM. There were significantly different ($P<0.001$) concentrations of fructose in the different Brassica crop types (Table B.2.10.). The concentrations of fructose in swedes, rapes and kales were 2.30, 0.20 and 0.23 g/100gFDM respectively. Larval feeding had a significant ($P=0.046$) effect on the concentration of fructose in the Brassicas, control roots containing a mean of 1.04 g/100gFDM and inoculated roots containing a mean of 0.78 g/100gFDM, a fall of 25%. Significant differences ($P=0.014$) could also be found in the concentrations of fructose in the roots of genotypes. There was no significant ($P>0.05$) difference between the fructose content of genotypes of rape or kale. In the swedes, Doon Major had a higher concentration of fructose than Angus, the concentrations being 2.70 and 1.90 g/100gFDM respectively.

The effect of larval feeding on root fructose concentration differed significantly ($P=0.010$) between crop types. There was no significant ($P>0.05$) difference between the concentrations of fructose in control and inoculated roots of both kales and rapes. However, the different genotypes of swede responded differently to larval feeding. Although the fructose concentration fell in both swede genotypes with larval feeding, this result was only significant ($P=0.027$) in Doon Major, in which fructose concentration decreased from 3.50 to 1.90 g/100gFDM, representing a reduction to 54% of the control (Table B.2.10.).

The overall mean glucose content of Brassicas was 2.01 g/100gFDM. There were significantly different ($P<0.001$) concentrations of glucose in the different Brassicas (Table B.2.11.). The concentration of glucose in swedes, rapes and kales was 4.34, 1.06 and 0.63 g/100gFDM respectively. Larval feeding had no significant ($P>0.05$) effect on the glucose

Table B.2.9. Mean total sugar concentration (g/100gFDM) of the roots of control plants and those inoculated with the turnip root fly for each genotype.

Crop type	Genotype	control	inoculated	Mean
Kale	KLSCN	3.83	3.25	3.59
Kale	KHSCN	3.48	3.09	3.29
Rape	Hobson	5.40	5.34	5.37
Rape	Ariana	5.71	3.90	4.81
Swede	Angus	8.93	8.40	8.66
Swede	Doon Major	14.10	10.36	12.23
SED		0.76		0.53
residual D.F.		11		11

Table B.2.10. Mean fructose concentration (g/100gFDM) of the roots of control plants and those inoculated with the turnip root fly for each genotype.

Crop type	Genotype	control	inoculated	Mean
Kale	KLSCN	0.20	0.34	0.27
Kale	KHSCN	0.18	0.19	0.18
Rape	Hobson	0.26	0.27	0.26
Rape	Ariana	0.12	0.16	0.13
Swede	Angus	1.97	1.83	1.90
Swede	Doon Major	3.50	1.90	2.70
SED		0.28		0.20
residual D.F.		11		11

concentration in the Brassicas. However, significant differences (P=0.004) could be found in the concentrations of glucose in the roots of genotypes. There was no significant (P>0.05) difference between the genotypes of rape or kale. In the swedes, Doon Major had a higher concentration of glucose than Angus, the concentrations being 5.01 and 3.67 g/100gFDM respectively.

The effect of larval feeding on root glucose concentration differed significantly (P<0.001) between crop types. The concentration of glucose in inoculated roots of both kales and swedes fell by between 10% and 40% when compared to control roots. The glucose concentration of the inoculated roots of rapes was 80% higher than the controls (Table B.2.11.). However, within crop types the different genotypes of swedes and rapes responded differently to larval feeding, (P=0.018). Although the glucose concentration fell in both swede genotypes with larval feeding, it only underwent a significant (P<0.05) fall in the genotype Doon Major, the concentration falling from 6.26 to 3.75 g/100gFDM. In the rape genotypes, the glucose concentration rose with larval feeding, but it only underwent a significant (P<0.05) rise in cv Hobson (Table B.2.11.).

The overall mean sucrose content of Brassicas was 3.41 g/100gFDM. There were significantly different (P<0.001) concentrations in the different Brassicas (Table B.2.12.). The concentration of sucrose in swedes and rapes was higher than in kales, the concentrations being 3.81, 3.83 and 2.58 g/100gFDM respectively. Larval feeding had a significant (P<0.001) effect on the concentration of sucrose in the Brassicas, control roots having a mean of 3.72 g/100gFDM and inoculated roots having a mean of 3.10 g/100gFDM. Significant differences (P<0.001) could also be found in the concentrations of sucrose in the roots of genotypes. There was no significant (P>0.05) difference between the genotypes of rape or kale. For the swedes, Doon Major had a higher concentration of sucrose than Angus, the concentrations being 4.53 and 3.10 g/100gFDM respectively.

The effect of larval feeding on root sucrose concentration differed significantly (P<0.001) between crop types. There was no significant (P>0.05) difference between the concentration of sucrose in control and inoculated roots of swede. However, in both kales and rapes, the sucrose content fell significantly (P<0.05) (Table B.2.12.). Within crop types, there was no significant difference (P>0.05) in the way in which different genotypes responded to larval damage.

Table B.2.11. Mean glucose concentration (g/100gFDM) of the roots of control plants and those inoculated with the turnip root fly for each genotype.

Crop type	Genotype	control	inoculated	Mean
Kale	KLSCN	0.71	0.60	0.65
Kale	KHSCN	0.67	0.54	0.61
Swede	Angus	3.87	3.47	3.67
Swede	Doon Major	6.26	3.75	5.01
Rape	Hobson	0.66	1.52	1.09
Rape	Ariana	0.87	1.21	1.04
SED		0.39		0.28
residual D.F.		11		11

Table B.2.12. Mean sucrose concentration (g/100gFDM) of the roots of control plants and those inoculated with the turnip root fly for each genotype.

Crop type	Genotype	control	inoc	Mean
Kale	KLSCN	3.03	2.31	2.67
Kale	KHSCN	2.64	2.36	2.50
Swede	Angus	3.10	3.11	3.10
Swede	Doon Major	4.34	4.72	4.53
Rape	Hobson	4.50	3.56	4.03
Rape	Ariana	4.72	2.55	3.64
SED		0.32		0.23
residual D.F.		11		11

Relationship between root composition and turnip root fly pupation

Calculation of correlations between the *D.floralis* percentage pupation and the composition of the roots (total and individual sugars, fibre and lignin) of the Brassica genotypes tested revealed no significant correlations ($P>0.05$). The mean pupal weight of *D.floralis* was significantly inversely correlated with the fibre content as an element of fresh weight of the root ($P<0.05$, r=-0.783 [DF=5]). Significant correlations could be achieved for most of the characteristics of the roots tested by the removal of Ariana from the data set. Although unpresented, for the reasons given below, correlations performed in this manner would produce significant correlations between both percentage pupation and mean pupal weight ($P<0.05$ and $P<0.001$ respectively), and all the root factors except FDM and sucrose. However, due to the aggregated nature of the points when plotted, most points were at the extremes. It was deemed inappropriate to disregard one point from a data set of six. In addition, the correlation found for all six points between the mean pupal weight of *D.floralis* and fibre content as an element of fresh weight of the root should be viewed cautiously. Ten root components were correlated with two pupal characteristics (20 correlations), consequently it might reasonably be expected for one to be significant at the 5% probability level.

DISCUSSION

The six Brassicas showed a range of responses to root damage after *Delia floralis* larval feeding. In the susceptible genotypes, plants suffered up to 46% tissue losses due to larval feeding. The variation in the percentage pupation and the pupal weights of *D.floralis* indicates that the quality of different genotypes as hosts for *D.floralis* varied considerably. The rape Hobson, and the kales KLSCN and KHSCN, were classified as poor hosts when contrasted with the rape Ariana, and the swedes Angus and Doon Major. The pupal weights on these poor host plants were reduced by approximately 30% when contrasted with those from good host plants. Percentage pupation on the poor hosts was also reduced to approximately 40%, which contrasted with the 50-60% pupation on the good hosts. Although Ariana was a suitable host for larval development, it was also relatively tolerant to the damage of *D.floralis*. This effect has been attributed to the regrowth of roots (Birch, 1988). Hence the root weight was relatively unaffected whilst the leaf and stem weights were substantially reduced. In the more resistant genotypes (both kales and rape cv Hobson), plant tissue losses were generally smaller, percentage pupation reduced and mean pupal weight lower than for those which had fed on susceptible roots. Angus was relatively resistant to *D.floralis* attack when compared to Doon Major, as found in previous studies (Birch, 1988).

The fibre content of the Brassicas was, however, affected by the damage caused by the turnip root fly. Insect damage has been shown to influence the fibre content of plants. Fibre content of alfalfa, as measure by the neutral detergent method, was increased by the damage of the potato leafhopper, *Empoasca fabae* (Hutchins *et al*, 1989). The mean pupal mass and percentage pupation of *D.floralis* were not related to the fibre content of the Brassica genotypes tested. The difference in the susceptibility of the two rape genotypes could not be explained by differences in fibre content of the roots. The resistance demonstrated in cv Hobson may be explained by the pronounced lignification of the roots which took place with larval feeding. The control plants of Hobson had intermediate lignin contents (0.78 g/100g of fresh weight) when compared to the other genotypes. However, the lignin content of the fresh weight of turnip root fly-damaged Hobson was 1.62g/100g, more than double the root lignin content of the control roots. Staining of inoculated roots has demonstrated that lignification takes place in the area immediately around the turnip root fly feeding site (Hopkins, unpublished observations). This localised effect will therefore be far greater than the two-fold increase which takes place when averaged over the whole root.

The plant responses to turnip root fly damage, as measured by total and individual sugar concentrations of the roots, varied considerably with different crop types and genotypes. The total sugar concentration of roots always decreased after turnip root fly attack, although not always significantly. There are two possible reasons for the observed decrease in root sugar concentration. Firstly, production of sugar may be limited by the damage to the root caused by larval feeding. In this case, as the root size increases the rate of sugar production is not as high as in the control roots and, as a consequence, the concentration of sugar in the inoculated root is reduced at harvest. Secondly, it is unlikely that the distribution of sugars is uniform throughout the roots of the Brassicas studied. It is therefore possible that the parts of the root which are higher in sugar will be preferentially fed upon due to the phagostimulatory nature of sugars. If feeding takes place on the areas of the root with higher sugar concentrations, it will result in a decrease in the mean sugar concentration of the whole root. Further work is needed to study the distribution of different compounds in the roots of Brassicas and their effects on the larval feeding and development of the larvae of the turnip root fly.

The relationship between the fibre, lignin and sugar contents of the root of the Brassicas tested remains unclear. Whilst Ariana was clearly influencing the correlation calculation sufficiently to justify its removal, the loss of one point from six would be questionable and the result is consequently inconclusive. The FDM and the sucrose content of the Brassica roots tested were clearly not influencing the development of *D.floralis* larvae (as measured by percentage

pupation and mean pupal weight). FDM content of the roots of the genotypes tested was unaffected by turnip root fly larvae feeding on the roots. The resistance to turnip root fly in three of the genotypes tested clearly was not related to the FDM content of the roots. This result contradicts results gained by testing the dry matter content of mature swede at the end of the season (Shaw, 1982; 1984; 1985; 1993), where the dry matter content appeared to be related to resistance. Birch (1988) demonstrated that restricted larval feeding could be correlated with some components of root dry matter but was not directly related to tissue hardness. In this experiment, resistance was clearly not directly related to root dry matter. The wide variation in the mean pupal weight, from 12.5mg to 20.0mg, had no demonstrable link with the dry matter content of the roots on which the larvae developed. This result reinforces the arguments previously put forward in this thesis (Chapter B1) which question the use of end of season measurements to determine the source of a mechanism of resistance from earlier in the growing season. The ability of the root of the Brassicas tested to function in the uptake of water and nutrients is likely to have long term effects and this area has not yet been researched.

The rise in the plant fibre of the roots of the Brassicas tested does provide an indication as to the reason for the fall in the fructose and glucose content which has been demonstrated here and in Chapter B1. Cellulose is the main component of plant fibre and is an unbranched polymer of glucose residues. Correlations performed previously (Chapter B1) have shown that the percentages of glucose and fructose in the roots of swede were intimately linked. Consequently, if large amounts of glucose residues were being utilised to construct cellulose chains to repair insect damage then a reduction in the concentrations of glucose and fructose in the roots of Brassicas would be expected. The use of radio-labelled compounds to trace the destination of glucose and fructose in the roots of damaged Brassicas suggests itself as a method for testing this hypothesis.

Although unwilling to remove Ariana from correlation calculations in this instance, it is apparent that both here and in previous publications (Birch *et al*, 1990; 1992) Ariana is more susceptible to *D.floralis* attack than other rape genotypes. Birch *et al* (1990) suggest that compensatory root growth, which provides both food for larvae and large changes in root metabolism, is the reason for this susceptibility. However, the mean root weight of control and inoculated roots of Ariana provides no evidence of a large compensatory root growth acting to aid tolerance to attack.

Chapter B3

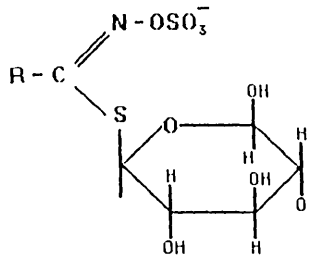
Modification of swede (*Brassica napus* ssp. *rapifera*) root glucosinolate content by different densities of turnip root fly (*Delia floralis*) larvae

INTRODUCTION

Glucosinolates are a family of allelochemics which occur in all Brassica crops, including swede (Kjaer, 1976). Glucosinolates conform to the general structure shown in Figure B.3.1, but differences in the side chain create a range of at least 100 known distinct compounds (Louda & Mole, 1991) of which 15 are acknowledged to exist in swede (Fenwick *et al*, 1983; Birch *et al*, 1992). The importance of the concentration of individual and total glucosinolates to agricultural produce is indicated by the European Communities limit of 35 μmol g^{-1} imposed on oilseed rape. Their acknowledged goitrogenicity, mammalian toxicity and flavour taint make them important as a factor in the diet of both humans and livestock (Fenwick *et al*, 1983). Boag *et al* (1990) observed that wild roe deer avoided feeding on oilseed rape at times when it contained high concentrations of glucosinolates. The effect which glucosinolates have on mammals is partly a result of their breakdown products. Intrarumenally infused allyl cyanide, the nitrile breakdown product of prop-2 enyl glucosinolate, led to a reduction in the voluntary food intake of sheep (Duncan & Milne, 1992). This effect was coincident with a reduction in liver function and changes in cellular respiration. In monogastric farm animals, the major effects of glucosinolates are impaired iodine utilisation, thyroid and liver function linked to a reduction in palatability and growth (Huisman & Tolman, 1992). Darroch *et al* (1991), incorporated indole glucosinolate extracts in the feed of mice. Increasing the glucosinolate content of the diet linearly decreased the feed intake of the mice. At higher levels this resulted in palatability problems, and ultimately intake was so reduced that mortality increased. However, thyroid function, hepatic/renal weights and morphology were not affected.

The toxicological importance of glucosinolates is combined with the status of glucosinolates and their breakdown products as allelochemics. Wallbank & Wheatley (1979) noted the responses of *D.radicum* to allyl isothiocyanate, which, when added to water traps on fallow ground, improved their performance by approximately seven-fold when contrasted with unbaited traps. Ellis *et al* (1980) noted that within radish cabbage root fly egg laying was positively correlated with two volatile glucosinolate hydrolysis products. Further work with the cabbage root fly on insect orientation and responses to visual and tactile stimuli confirmed

Figure B.3.1. The structures of different glucosinolates.



General structure

R=position of sidechain

Aliphatic sidechains

Prop-2-enyl

$CH_2 = CH - CH_2 -$

But-3-enyl

$CH_2 = CH - CH_2 - CH_2 -$

Pent-4-enyl

$CH_2 = CH - CH_2 - CH_2 - CH_2 -$

2-hydroxy but-3-enyl

$CH_2 = CH - \underset{OH}{CH} - CH_2 -$

2-hydroxy pent-4-enyl

$CH_2 = CH - CH_2 - \underset{OH}{CH} - CH_2 -$

3-methylthiopropyl

$CH_3 - S - CH_2 - CH_2 - CH_2 -$

4-methylthiobutyl

$CH_3 - S - CH_2 - CH_2 - CH_2 - CH_2 -$

3-methylsulphinylpropyl

$CH_3 - SO - CH_2 - CH_2 - CH_2 -$

4-methylsulphinylbutyl

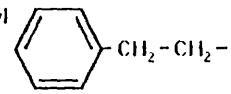
$CH_3 - SO - CH_2 - CH_2 - CH_2 - CH_2 -$

4-methylsulphinylbut-3-enyl

$CH_3 - SO - CH = CH - CH_2 - CH_2 -$

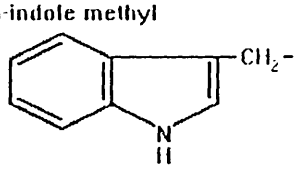
Aromatic sidechains

Phenylethyl

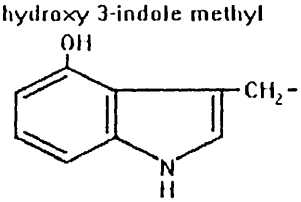


Indole-Aromatic sidechains

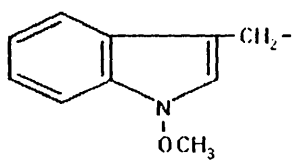
3-indole methyl



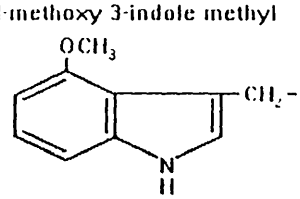
4-hydroxy 3-indole methyl



1-methoxy 3-indole methyl



4-methoxy 3-indole methyl



the role of glucosinolates and their breakdown products as allelochemics (Nottingham & Coaker, 1985; Tuttle *et al*, 1988).

Although adults of *D.radicum* are highly mobile, larvae can only move limited distances and eggs are often laid close to rather than on the plant. Consequently, orientation and host plant location are important to newly hatched larvae. Volatile glucosinolate hydrolysis products are also kaironomics of newly hatched larvae of *D.radicum* which exhibited positive taxis to allyl and ethyl isothiocyanate (Finch & Skinner, 1974; Koštál, 1992). However, at very high concentrations, the same compounds were repellent. The position of glucosinolates as behaviour-mediating compounds remains far from clear and the nature of the role they play in *D.radicum* and *D.floralis* host selection has been discussed in Section A.

The role of glucosinolates and their breakdown products as mediators of behaviour is not restricted to cabbage root fly, but is widespread throughout Crucifer-feeding insects (reviewed Städler, 1992). Larvae of the chrysomelid beetle, *Psyllioides chrysocephala,* would only penetrate and feed on plants which contained glucosinolates (Bartlet & Williams, 1991). However, adults would not accept non-food plants to which sinigrin had been added. Unidentified parts of the solvent fractions of rejected plants could lead to rejection of otherwise acceptable hosts (Bartlet & Williams, 1991). Bodnaryk (1991) found that sinalbin was a component of both antixenotic and antibiotic resistance to insects. High concentrations of sinalbin in young cotyledons and leaves deterred the feeding of the flea beetle, *Phyllotreta cruciferae,* and larvae of the bertha armyworm, *Mamestra configura.* The lower concentrations of sinalbin found in older leaves did not appear to confer any protection from *P.cruciferae* upon the plant. Although no protection was gained against this oligophagous insect, *M.configura*, a polyphagous insect, was deterred from feeding. Traynier & Truscott (1991) found that glucobrassicin, 3-indolemethyl glucosinolate, stimulated oviposition by the cabbage butterfly, *Pieris rapae*. The glucosinolate was only effective when associated with water and its enzyme hydrolysis products failed to influence oviposition. Investigation of the oviposition stimulants in leaf surface extracts demonstrated that glucobrassicin was also important to the large cabbage butterfly, *P.brassicae* (van Loon *et al*, 1992). Glucobrassicin was identified as the stimulant in the most attractive fraction of a methanol extract. Glucobrassicin, at the same concentration as in the most attractive fraction, was equally stimulatory. Oilseed rape glucosinolate concentrations and proportions are extensively changed by slug damage (Glen *et al*, 1990). They found that the number and leaf area of seedlings with damage symptoms of the slug were strongly and inversely related to the total glucosinolate concentrations in seed and one week old seedlings.

It has clearly been demonstrated by previous authors that it is possible to find good correlations between glucosinolates and the feeding damage of Brassica feeders. It has also been demonstrated that the association between glucosinolates and *D.floralis* feeding is far from clear. Birch *et al* (1992) investigated the glucosinolate concentrations of roots of a range of Brassicas which had been damaged by *D.floralis*. Neither total nor individual glucosinolates were consistently associated with either resistance or susceptibility to attack by *D.floralis*. However, feeding damage caused by *D.floralis* did result in widespread changes to the glucosinolate composition of the roots. Feeding damage to plants induces changes in individual and total glucosinolate concentrations. Birch *et al* (1990) found that, within oilseed rape plants which had been damaged by *D.floralis*, the concentrations of individual glucosinolates and their relative proportions were dramatically altered. The increase in individual indole glucosinolates was up to 88% whilst aliphatic glucosinolates were largely reduced or unaffected. Further work (Birch *et al* 1992) demonstrated that this effect was also to be found in kales and swedes damaged by *D.floralis*.

Work on swede glucosinolate content has predominantly concentrated on varietal variation which can be considerable in mature roots (Mullin *et al* 1980; Carlson *et al*, 1981; Adams *et al*, 1989). Little is known about *D.floralis* damage and the changes in glucosinolate content which occur in different swede genotypes. The effect of pest density on changes in glucosinolate concentrations has also remained unknown. This chapter addresses both these questions.

MATERIALS AND METHODS

Biological Material

The biological material utilised in this chapter was the same as was used in Chapter B1 and consisted of eight genotypes of swede selected to represent a range of characteristics, susceptibility, tolerance and partial resistance to turnip root fly. Plants were grown in 10cm pots in a glasshouse with a temperature range of 16-21° C. *D.floralis* eggs were inoculated onto the plants at a rate of 5, 10 or 20 eggs per plant and arranged with control plants in a series of randomised blocks with eight replicates per treatment. Plants were harvested and divided into root and aerial parts at the hypocotyl. Roots and aerial parts were weighed separately and frozen prior to freeze drying and milling. These methods are given in more detail in the Materials and Methods of Chapter B1.

Chemical analysis

Glucosinolates were extracted from the freeze-dried and milled roots by boiling a 1g sub-sample in 20ml of 70% v/v aqueous methanol. The extraction was carried out in a 100ml round-bottomed flask brought to and maintained at 80°C for 15 minutes in a water bath. The contents of the flask were filtered using a Buchner funnel rinsing the flask with 70% v/v aqueous methanol and retaining the filtrate. The residue and filter paper were then returned to the round-bottomed flask with a further 20ml of 70% v/v aqueous methanol, and returned to the water bath at 80°C for 5 minutes. The contents of the flask were filtered and the filtrates combined. The combined filtrates which contained the glucosinolates in solution were then rotorevaporated down to approximately 6ml and transfered to a 10ml volumetric flask, rinsing and making up to volume with distilled water. The extracted glucosinolates were then transferred to a 20ml polythene container and frozen for storage at -20°C.

Batches of extracts were desulphated by a method based on that of Heaney & Fenwick (1980a). A-25 DEAE Sephadex columns were prepared using pasteur pipettes which had been partially blocked with glass wool to restrict the flow. For each column, 0.1 g of A-25 DEAE Sephadex was suspended in 2ml of 0.5M pyridine acetate (40ml pyridine+30ml acetic acid+930ml of distilled water) and transfered onto the glass wool. The columns were then washed with one rinse of 2ml of 0.5M pyridine acetate and four rinses of water. A 4ml sample was loaded onto the column and rinsed with 2ml of 0.02M pyridine acetate. The column was left overnight with 0.02ml of aryl sulphatase to convert glucosinolates to desulphoglucosinolates. The desulphoglucosinolates were eluted the following morning with one rinse of 1ml of distilled water and two rinses of 0.5ml of distilled water.

Individual glucosinolates were separated and quantified by high performance liquid chromatography (hplc). All the separations were performed on a Gilson hplc system (Anachem Ltd, Luton, Beds, UK) utilising a C-18 reverse phase column (Jones Chromatograph, Hengoed, Mid-Glamorgan, UK) by Dr D.W. Griffiths. Peaks were detected utilising UV detection, 230nm and quantified using Schimadzu C-R3 integrator (Anachem Ltd, Luton, Beds, UK). Identification of desulphoglucosinolate peaks was by coelution with standards supplied by the Food Research Institute, Norwich unless otherwise stated in Table B.3.1.

Statistical Analysis

Data were analysed on Genstat (Genstat, Sun/Unix version 5.0, release 2.2, 1990) using analysis of variance on untransformed data.

The genotypic variation in root weight, pupal weight and responses to turnip root fly damage are discussed in Chapter B1.

Total glucosinolate content of swede roots

Hplc analysis of the roots of eight swede genotypes revealed detectable concentrations of 14 glucosinolates. Nine of the glucosinolates detected had aliphatic side chains, the other five had aromatic side chains (Table B.3.1.). The control roots of swedes contained a mean glucosinolate concentration of 30.6 mmol kg^{-1}. There was no significant ($P>0.05$) difference between the glucosinolate concentrations found in the control roots of the eight swede genotypes tested. The glucosinolate concentrations of roots damaged by *D.floralis* larvae were not significantly different ($P>0.05$) from the roots of control plants (Table B.3.2.). The mean total glucosinolate concentrations of plants inoculated with 5, 10 and 20 eggs of the turnip root fly were 32.3, 33.7 and 31.5 mmol kg^{-1} respectively. There was, however, considerable variation in the concentrations of individual glucosinolates in the swede roots tested and also between control and insect-damaged roots.

Aliphatic glucosinolate content of swede roots

There was no significant difference ($P>0.05$) in the aliphatic glucosinolate content of the different genotypes of swede averaged over all the treatments (Table B.3.3.). The control swedes contained a mean aliphatic glucosinolate concentration of 18.8 mmol kg^{-1}, which represented 62% of the total glucosinolate content (Table B.3.4.). The concentration of aliphatic glucosinolates was lower ($P<0.001$) in roots damaged by *D.floralis*. The mean aliphatic glucosinolate concentrations of plants inoculated with 5, 10 and 20 eggs of the turnip root fly were 12.0, 11.0 and 9.4 mmol kg^{-1} respectively. There was no significant ($P>0.05$) difference between the aliphatic glucosinolate concentrations of the swedes inoculated at the three levels. Aliphatic glucosinolates comprised 35, 32 and 30% of the total glucosinolate content of swedes inoculated with 5, 10 and 20 *D.floralis* eggs respectively. The percentage of total glucosinolates which had aliphatic sidechains was significantly ($P<0.001$) lower for plants which had suffered *D.floralis* damage.

Aromatic glucosinolate content of swede roots

There was a significant variation ($P=0.005$) in the aromatic glucosinolate content of the roots of the swedes tested (Table B.3.5.). The aromatic glucosinolate content of control swedes varied from 7.1 mmol kg^{-1} (Nmm3 a-d) to 27.0 mmol kg^{-1} (Melfort). The control swedes contained a mean aromatic glucosinolate concentration of 12.0 mmol kg^{-1}. The percentage of

Table B.3.1. The sequence of glucosinolate peaks eluted, their nature and their method of identification from the roots of swede.

PEAK	NATURE	NAME
Peak 1	Aliphatic	2-hydroxy-3-butenyl
Peak 2	Aliphatic	Prop-2-enyl
Peak 3	Aliphatic	4-methyl sulphinylbutyl*
Peak 4	Aliphatic	4-methyl sulphinyl but-3-enyl
Peak 5	Aliphatic	2-hydroxy Pent-4-enyl
Peak 6	Aliphatic	But-3-enyl
Peak 7	Indole-Aromatic	4-hydroxy 3-indole methyl
Peak 8	Aliphatic	3-methylthiopropyl*
Peak 9	Aliphatic	Pent-4-enyl
Peak 10	Aliphatic	4-methyl thiobutyl
Peak 11	Indole-Aromatic	3-indole methyl
Peak 12	Aromatic	Phenylethyl
Peak 13	Indole-Aromatic	4-methoxy 3-indole methyl
Peak 14	Indole-Aromatic	1-methoxy 3-indole methyl

*Tentative identification only, based on retention times published under similar chromatographic conditions.

Table B.3.2. Variation in the total glucosinolate concentration (mmol kg^{-1}) of the roots of swedes between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	26.2	23.7	35.4	38.5	31.0
Nmm3 a-d	21.3	27.1	32.0	28.6	27.3
Melfort	53.6	34.5	35.1	34.3	39.4
Angus	23.8	42.8	34.8	21.5	30.7
GRL aga	27.9	26.7	23.6	31.4	27.4
Marian	29.7	34.6	35.8	30.6	32.7
Doon Major	31.9	35.4	46.8	26.2	35.1
M4M2 a-c	30.4	33.3	26.4	41.2	32.8
Mean[2]	30.6	32.3	33.7	31.5	32.0

INOCULATIONS, SED = 2.8, DF = 3 residual D.F. = 27
GENOTYPE, SED = 4.0, DF = 7
INOC.GENOTYPE, SED = 7.9, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.3. Variation in the concentration of aliphatic glucosinolates (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of the turnip root fly.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	16.5	7.4	10.1	11.3	11.3
Nmm3 a-d	14.2	10.3	10.0	7.1	10.4
Melfort	26.6	9.5	7.8	6.2	12.5
Angus	15.8	19.1	12.8	8.3	14.0
GRL aga	16.9	9.9	6.6	8.3	10.4
Marian	21.2	15.4	13.0	8.5	14.7
Doon Major	20.0	14.9	17.2	9.5	15.4
M4M2 a-c	17.4	8.8	10.5	16.1	13.2
Mean[2]	18.6	12.0	11.0	9.4	12.7

INOCULATIONS, SED = 1.6, DF = 3 residual D.F. = 27
GENOTYPE, SED = 2.7, DF = 7
INOC.GENOTYPE, SED = 4.5, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.4. Variation in the proportion of aliphatic glucosinolates (percentage of total glucosinolates) in swede roots with genotype and *D.floralis* larval numbers.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	62.5	25.1	28.2	28.9	36.2
Nmm3 a-d	65.7	37.9	31.2	27.3	40.5
Melfort	50.4	26.0	22.8	17.7	29.2
Angus	62.1	43.9	36.2	36.8	44.7
GRL aga	62.0	35.0	27.9	25.3	37.6
Marian	71.1	45.0	35.6	25.0	44.2
Doon Major	62.9	41.4	36.9	37.4	44.7
M4M2 a-c	57.2	26.0	38.3	38.4	40.0
Mean[2]	61.7	35.1	32.1	29.6	39.6

INOCULATIONS, SED = 2.6, DF = 3 residual D.F. = 27
GENOTYPE, SED = 3.7, DF = 7
INOC.GENOTYPE, SED = 7.5, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.5. Variation in the concentration of aromatic glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	9.8	16.3	25.3	27.3	19.7
Nmm3 a-d	7.1	16.8	22.0	21.5	16.8
Melfort	27.0	25.0	27.4	28.1	26.9
Angus	7.9	23.7	22.0	13.2	16.7
GRL aga	11.0	16.9	17.0	23.1	17.0
Marian	8.5	18.7	22.8	22.1	18.0
Doon Major	11.9	20.5	29.5	16.8	19.7
M4M2 a-c	13.0	24.5	15.9	25.1	19.6
Mean[2]	12.0	20.3	22.7	22.1	19.3

INOCULATIONS, SED = 1.7, DF = 3 residual D.F. = 27
GENOTYPE, SED = 2.4, DF = 7
INOC.GENOTYPE, SED = 4.9, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.6. Variation in the concentration of indole glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	6.2	11.3	19.2	21.8	14.6
Nmm3 a-d	3.0	11.7	16.6	17.5	12.2
Melfort	15.8	19.2	22.0	23.4	20.1
Angus	5.5	19.9	19.4	11.1	14.0
GRL aga	6.1	12.9	14.1	18.9	13.0
Marian	3.6	13.6	17.2	18.2	13.1
Doon Major	5.9	16.3	23.3	13.3	14.7
M4M2 a-c	5.2	17.7	12.3	18.0	13.3
Mean[2]	6.4	15.3	18.0	17.8	14.4

INOCULATIONS, SED = 1.4, DF = 3 residual D.F. = 27
GENOTYPE, SED = 1.9, DF = 7
INOC.GENOTYPE, SED = 3.8, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

total glucosinolates which had aromatic sidechains was inversely related to the percentage which had aliphatic sidechains. The concentration of aromatic glucosinolates was higher ($P<0.001$) in *D.floralis*-damaged roots. The mean aromatic glucosinolate concentrations of plants inoculated with 5, 10 and 20 eggs of the turnip root fly were 20.3, 22.7 and 22.1 mmol kg^{-1} respectively, and did not differ significantly ($P>0.05$) across the three inoculation levels. Following *D.floralis* damage, the percentage of total glucosinolates which had aromatic sidechains was significantly ($P<0.001$) higher than the control plants.

Indole glucosinolate content of the roots of swedes

Indole glucosinolate content of the roots tested varied significantly ($P=0.010$) between genotypes (Table B.3.6.). The indole glucosinolate content of control swedes varied from 3.0 mmol kg^{-1} (Nmm3 a-d) to 15.8 mmol kg^{-1} (Melfort). The control swedes contained a mean aromatic glucosinolate concentration of 6.4 mmol kg^{-1}, representing 21% of the total glucosinolate content (Table B.3.7.). The concentration of indole glucosinolates was higher ($P<0.001$) in *D.floralis*-damaged roots. The mean indole glucosinolate concentrations of plants inoculated with 5, 10 and 20 eggs of the turnip root fly were 15.3, 18.0 and 17.8 mmol kg^{-1} respectively. There was no significant ($P>0.05$) difference between the indole glucosinolate concentrations of the swedes inoculated at the three levels. As a percentage of total glucosinolates, the indole-based group was significantly ($P<0.001$) higher for plants which had suffered *D.floralis* damage than for controls (Table B.3.7.).

Individual Aliphatic Glucosinolates

The predominant aliphatic glucosinolate was 2-hydroxy-3-butenyl glucosinolate which constituted an overall mean of 25% of the total glucosinolate content of the roots of the swedes tested. The 2-hydroxy-3-butenyl glucosinolate content of the swede roots tested did not vary significantly ($P>0.05$) between genotypes (Table B.3.8.). The 2-hydroxy-3-butenyl glucosinolate content of control swedes varied from 9.5 mmol kg^{-1} (Angela) to 13.9 mmol kg^{-1} (Marian). The control swedes contained a mean 2-hydroxy-3-butenyl glucosinolate concentration of 11.4 mmol kg^{-1}. The concentration of 2-hydroxy-3-butenyl glucosinolate was lower ($P<0.001$) in *D.floralis*-damaged roots. Plants inoculated with 5, 10 and 20 *D.floralis* eggs contained 7.4, 6.8 and 5.6 mmol kg^{-1} of 2-hydroxy-3-butenyl glucosinolate respectively. There was no significant ($P>0.05$) difference between the 2-hydroxy-3-butenyl glucosinolate concentrations of the swedes inoculated at the three levels.

Table B.3.7. Variation in the percentage of total glucosinolates which had indole sidechains, with genotype and number of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	23.4	54.4	54.2	57.1	47.3
Nmm3 a-d	14.1	44.5	51.9	60.1	42.6
Melfort	30.1	57.8	62.3	69.4	54.9
Angus	28.3	47.7	56.6	53.7	46.6
GRL aga	20.8	52.1	59.8	62.1	48.7
Marian	11.8	40.2	48.8	62.4	40.8
Doon Major	18.6	47.5	49.9	47.2	40.8
M4M2 a-c	17.2	54.2	48.3	44.2	41.0
Mean[2]	20.5	49.8	54.0	57.0	45.3

INOCULATIONS, SED = 2.5, DF = 3 residual D.F. = 27
GENOTYPE, SED = 3.6, DF = 7
INOC.GENOTYPE, SED = 7.1, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.8. Variation in the concentration of 2-hydroxy-3-butenyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	9.5	5.2	5.5	5.4	6.4
Nmm3 a-d	10.2	6.9	6.7	4.3	7.0
Melfort	13.3	5.1	3.5	2.8	6.2
Angus	9.5	13.6	9.4	6.3	9.7
GRL aga	10.3	5.4	4.0	4.2	6.0
Marian	13.9	10.2	8.5	5.9	9.6
Doon Major	13.2	8.2	9.9	4.9	9.1
M4M2 a-c	11.1	4.8	6.8	11.1	8.4
Mean[2]	11.4	7.4	6.8	5.6	7.8

INOCULATIONS, SED = 1.2, DF = 3 residual D.F. = 27
GENOTYPE, SED = 1.7, DF = 7
INOC.GENOTYPE, SED = 3.5, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.9. Variation in the concentration of prop-2-enyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	0.14	0.06	0.08	0.09	0.09
Nmm3 a-d	0.12	0.07	0.07	0.06	0.08
Melfort	0.20	0.05	0.07	0.03	0.09
Angus	0.14	0.24	0.16	0.13	0.16
GRL aga	0.16	0.07	0.06	0.08	0.09
Marian	0.16	0.13	0.10	0.07	0.11
Doon Major	0.18	0.07	0.15	0.05	0.11
M4M2 a-c	0.09	0.03	0.07	0.13	0.08
Mean[2]	0.15	0.09	0.09	0.08	0.10

INOCULATIONS, SED = 0.02, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.03, DF = 7
INOC.GENOTYPE, SED = 0.06, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.10. Variation in the concentration of 4-methyl sulphinylbutyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	1.11	0.15	0.35	0.36	0.49
Nmm3 a-d	0.98	0.21	0.19	0.36	0.43
Melfort	2.32	0.43	0.57	0.31	0.91
Angus	2.44	0.73	0.73	0.42	1.08
GRL aga	1.27	0.66	0.33	0.59	0.71
Marian	1.34	1.30	0.50	0.31	0.86
Doon Major	1.01	0.89	0.89	0.25	0.76
M4M2 a-c	0.48	0.18	0.39	0.40	0.36
Mean[2]	1.37	0.57	0.49	0.37	0.70

INOCULATIONS, SED = 0.18, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.25, DF = 7
INOC.GENOTYPE, SED = 0.51, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

The prop-2-enyl glucosinolate content of control swedes varied from 0.09 mmol kg^{-1} (M4M2 a-c) to 0.20 mmol kg^{-1} (Melfort), but was not significantly ($P>0.05$) different between genotypes (Table B.3.9.). The control swedes contained a mean prop-2-enyl glucosinolate concentration of 0.15 mmol kg^{-1} which was higher ($P=0.007$) than in *D.floralis*-damaged roots. There was no significant ($P>0.05$) difference between the prop-2-enyl glucosinolate concentrations of the swedes inoculated at the three levels. Plants inoculated with 5 and 10 *D.floralis* eggs contained a mean of 0.09 mmol kg^{-1} of prop-2-enyl glucosinolate and those inoculated with 20 eggs contained 0.08 mmol kg^{-1}.

The 4-methyl sulphinylbutyl glucosinolate content of the roots of the swedes tested did not vary significantly ($P>0.05$) between genotype (Table B.3.10.) controls or overall. The 4-methyl sulphinylbutyl glucosinolate content of control swedes varied from 0.48 mmol kg^{-1} (M4M2 a-c) to 2.44 mmol kg^{-1} (Angus). The control swedes contained a mean 4-methyl sulphinylbutyl glucosinolate concentration of 1.37 mmol kg^{-1} which was higher ($P<0.001$) than in *D.floralis*-damaged roots. The mean 4-methyl sulphinylbutyl glucosinolate concentrations of plants inoculated with 5, 10 and 20 *D.floralis* eggs were 0.57, 0.49 and 0.37 mmol kg^{-1} respectively. There was no significant ($P>0.05$) difference between the 4-methyl sulphinylbutyl glucosinolate concentrations of the swedes inoculated at the three levels.

There were significant ($P<0.001$) differences in the 4-methylsulphinyl but-3-enyl glucosinolate content of the roots of the genotypes tested (Table B.3.11.). The 4-methylsulphinyl but-3-enyl glucosinolate content of control swedes varied from 0.05 mmol kg^{-1} (Doon Major) to 1.44 mmol kg^{-1} (M4M2 a-c). The 4-methylsulphinyl but-3-enyl glucosinolate genotypic mean concentration across all treatments varied from 0.05 mmol kg^{-1} (Angus) to 1.26 mmol kg^{-1} (Doon Major). The control swedes contained a mean 4-methylsulphinyl but-3-enyl concentration of 0.61 mmol kg^{-1} which was not significantly different ($P>0.05$) from *D.floralis*-damaged roots. There was no significant ($P>0.05$) difference between the 4-methylsulphinyl but-3-enyl glucosinolate concentration of the swedes inoculated at the three levels. The response of the different genotypes to the damage of the turnip root fly larvae differed significantly ($P=0.019$). The concentration of 4-methylsulphinyl but-3-enyl glucosinolate in the majority of genotypes was unaffected by the damage of the turnip root fly larvae. However, in Doon Major, which had the lowest concentration of 4-methylsulphinyl but-3-enyl glucosinolate in the control roots, the concentration rose with inoculation and was the highest for all swedes across all inoculation levels. In cv Marian, the 4-methylsulphinyl but-3-enyl glucosinolate concentration fell in all inoculated roots and was significantly ($P<0.05$) lower in the 20 egg inoculation than in the control roots.

Table B.3.11. Variation in the concentration of 4-methyl sulphinyl but-3-enyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	0.51	0.42	0.55	0.62	0.52
Nmm3 a-d	0.78	0.79	0.85	0.63	0.76
Melfort	0.35	0.17	0.18	0.26	0.24
Angus	0.11	0.04	0.06	0.00	0.05
GRL aga	0.36	0.27	0.24	0.31	0.29
Marian	1.29	0.67	0.76	0.49	0.80
Doon Major	0.05	1.46	1.95	1.60	1.26
M4M2 a-c	1.44	0.94	0.58	1.01	0.99
Mean[2]	0.61	0.59	0.64	0.61	0.61

INOCULATIONS, SED = 0.12, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.17, DF = 7
INOC.GENOTYPE, SED = 0.35, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.12. Variation in the concentration of 2-hydroxy pent-4-enyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	4.30	0.95	2.35	3.00	2.65
Nmm3 a-d	0.42	0.89	0.94	1.29	0.88
Melfort	4.94	1.47	1.97	1.68	2.51
Angus	0.87	0.07	0.27	0.06	0.32
GRL aga	2.24	1.88	0.99	1.93	1.76
Marian	2.03	1.67	1.34	0.75	1.45
Doon Major	2.12	2.82	2.14	1.88	2.24
M4M2 a-c	2.03	0.93	1.52	1.08	1.39
Mean[2]	2.37	1.33	1.44	1.46	1.65

INOCULATIONS, SED = 0.33, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.47, DF = 7
INOC.GENOTYPE, SED = 0.94, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

The 2-hydroxy pent-4-enyl glucosinolate content of the roots of the swedes tested varied significantly ($P<0.001$) between genotypes (Table B.3.12.). The 2-hydroxy pent-4-enyl glucosinolate content of control swedes varied from 0.4 mmol kg^{-1} (Nmm3 a-d) to 4.9 mmol kg^{-1} (Melfort), a mean concentration of 2.4 mmol kg^{-1}. The 2-hydroxy pent-4-enyl glucosinolate genotypic mean concentration across all treatments varied from 0.3 mmol kg^{-1} (Angus) to 2.6 mmol kg^{-1} (Angela). The concentration of 2-hydroxy pent-4-enyl glucosinolate was lower ($P=0.013$) in *D.floralis*-damaged roots. The mean 2-hydroxy pent-4-enyl glucosinolate concentrations of plants inoculated with 5, 10 and 20 eggs of the turnip root fly were 1.3, 1.4 and 1.5 mmol kg^{-1} respectively. There was no significant ($P>0.05$) difference between the 2-hydroxy pent-4-enyl glucosinolate concentration of the swedes inoculated at the three levels.

The but-3-enyl glucosinolate content of control swedes varied from no detectable content (M4M2 a-c) to 1.17 mmol kg^{-1} (Melfort) (Table B.3.13.). This variation was significant ($P<0.001$), as was the but-3-enyl glucosinolate genotypic mean concentration across all treatments which varied from no detectable content (M4M2 a-c) to 0.41 mmol kg^{-1} (Melfort). The but-3-enyl glucosinolate concentration of control roots was 0.35 mmol kg^{-1} which was higher ($P=0.002$) than in *D.floralis*-damaged roots. The mean but-3-enyl glucosinolate concentrations of plants inoculated with 5, 10 and 20 *D.floralis* eggs were 0.12, 0.05 and 0.08 mmol kg^{-1} respectively, which were not significantly different ($P>0.05$). Neither the control plants nor any of the inoculated swedes M4M2 a-c contained any detectable quantities of but-3-enyl glucosinolate.

The 3-methylthiopropyl, pent-4-enyl and 4-methyl thiobutyl glucosinolate contents of the swede roots tested did not vary significantly ($P>0.05$) between genotypes (Tables B.3.14. to B.3.16.). The 3-methylthiopropyl glucosinolate content of control swedes varied from 0.15 mmol kg^{-1} (Angela) to 0.50 mmol kg^{-1} (Melfort) with a mean across all genotypes of 0.28 mmol kg^{-1}. The pent-4-enyl glucosinolate content of the controls of Angus was not detectable whilst Melfort contained 0.34 mmol kg^{-1}. The 4-methyl thiobutyl glucosinolate content of control swedes varied from 0.4 mmol kg^{-1} (Angela) to 3.5 mmol kg^{-1} (Melfort). The control swedes contained a mean 4-methyl thiobutyl glucosinolate concentration of 1.9 mmol kg^{-1}, which was not significantly different ($P>0.05$) from *D.floralis*-damaged roots. The concentration of 3-methylthiopropyl and pent-4-enyl glucosinolate were also not significantly ($P>0.05$) affected by *D.floralis* damage.

Table B.3.13. Variation in the concentration of but-3-enyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	0.19	0.07	0.03	0.38	0.17
Nmm3 a-d	0.06	0.03	n / d	n / d	0.02
Melfort	1.17	0.32	0.12	0.03	0.41
Angus	0.70	0.18	0.21	0.11	0.30
GRL aga	0.59	0.31	0.08	0.13	0.27
Marian	0.05	0.03	n / d	n / d	0.02
Doon Major	0.03	n / d	n / d	n / d	0.01
M4M2 a-c	n / d	n / d	n / d	n / d	n / d
Mean[2]	0.35	0.12	0.05	0.08	0.15

INOCULATIONS, SED = 0.076, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.107, DF = 7
INOC.GENOTYPE, SED = 0.215, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

n / d no detectable quantity

Table B.3.14. Variation in the concentration of 3-methylthiopropyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae..

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	0.15	0.12	0.10	0.21	0.14
Nmm3 a-d	0.26	0.24	0.12	0.13	0.19
Melfort	0.50	0.16	0.10	0.23	0.25
Angus	0.10	0.15	0.12	0.15	0.13
GRL aga	0.39	0.16	0.11	0.21	0.22
Marian	0.23	0.17	0.15	0.06	0.15
Doon Major	0.36	0.18	0.25	0.25	0.26
M4M2 a-c	0.30	0.99	0.10	0.23	0.40
Mean[2]	0.28	0.27	0.13	0.18	0.22

INOCULATIONS, SED = 0.09, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.12, DF = 7
INOC.GENOTYPE, SED = 0.24, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.15. Variation in the concentration of pent-4-enyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	0.20	0.09	0.08	0.59	0.24
Nmm3 a-d	0.09	0.08	0.05	n / d	0.05
Melfort	0.34	0.10	0.06	n / d	0.12
Angus	n / d	n / d	n / d	0.03	0.01
GRL aga	0.32	0.15	n / d	0.11	0.14
Marian	0.07	0.12	0.06	n / d	0.06
Doon Major	0.19	0.05	0.20	0.10	0.13
M4M2 a-c	0.12	0.05	n / d	0.04	0.05
Mean[2]	0.16	0.08	0.05	0.11	0.10

INOCULATIONS, SED = 0.05, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.07, DF = 7
INOC.GENOTYPE, SED = 0.14, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

n / d no detectable quantity

Table B.3.16. Variation in the concentration of 4-methyl thiobutyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	0.43	0.29	1.02	0.66	0.60
Nmm3 a-d	1.32	1.15	1.13	0.34	0.99
Melfort	3.50	1.69	1.22	0.87	1.82
Angus	1.95	4.01	1.85	1.13	2.24
GRL aga	1.28	1.00	0.73	0.72	0.93
Marian	2.19	1.71	1.60	1.00	1.62
Doon Major	2.85	1.28	1.72	0.52	1.59
M4M2 a-c	1.86	0.93	1.08	2.20	1.52
Mean[2]	1.92	1.51	1.29	0.93	1.41

INOCULATIONS, SED = 0.38, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.53, DF = 7
INOC.GENOTYPE, SED = 1.06, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.17. Variation in the concentration of phenylethyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	3.7	5.1	6.2	5.4	5.1
Nmm3 a-d	4.2	5.1	5.4	4.0	4.7
Melfort	11.2	5.9	5.4	4.7	6.8
Angus	2.4	3.9	2.5	2.1	2.7
GRL aga	4.9	3.9	2.9	4.2	4.0
Marian	4.9	5.0	5.6	3.9	4.9
Doon Major	6.0	4.2	6.2	3.5	5.0
M4M2 a-c	7.8	6.8	3.6	7.1	6.3
Mean[2]	5.6	5.0	4.7	4.4	4.9

INOCULATIONS, SED = 0.6, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.8, DF = 7
INOC.GENOTYPE, SED = 1.7, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.18. Variation in the concentration of 4-hydroxy 3-indole methyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	0.85	0.55	0.80	0.67	0.71
Nmm3 a-d	0.49	0.38	0.35	0.22	0.36
Melfort	1.65	0.73	0.49	0.27	0.78
Angus	0.41	0.52	0.27	0.20	0.35
GRL aga	0.50	0.08	0.18	0.27	0.26
Marian	0.40	0.18	0.27	0.19	0.26
Doon Major	0.23	0.46	0.54	0.45	0.42
M4M2 a-c	0.95	0.51	0.41	0.50	0.59
Mean[2]	0.68	0.42	0.41	0.34	0.47

INOCULATIONS, SED = 0.09, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.13, DF = 7
INOC.GENOTYPE, SED = 0.26, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Individual Aromatic Glucosinolates

The phenylethyl glucosinolate content of the roots of the swedes tested varied significantly ($P<0.001$) between genotypes (Table B.3.17.) from 2.4 mmol kg^{-1} (Angus) to 11.2 mmol kg^{-1} (Melfort). The phenylethyl glucosinolate genotypic mean concentration across all treatments varied from 2.7 mmol kg^{-1} (Angus) to 6.8 mmol kg^{-1} (Melfort). The control swedes contained a mean phenylethyl glucosinolate concentration of 5.6 mmol kg^{-1} which was not significantly ($P>0.05$) different from *D.floralis*-damaged roots.

Individual Indole-aromatic Glucosinolates

The 4-hydroxy 3-indole methyl glucosinolate content of the roots of the swedes tested varied significantly ($P<0.001$) between genotypes (Table B.3.18.). The 4-hydroxy 3-indole methyl glucosinolate content of control swedes varied from 0.23 mmol kg^{-1} (Doon Major) to 1.65 mmol kg^{-1} (Melfort). The 4-hydroxy 3-indole methyl glucosinolate genotypic mean concentration across all treatments varied from 0.26 mmol kg^{-1} (Doon Major) to 0.78 mmol kg^{-1} (Melfort). The control swedes contained a mean 4-hydroxy 3-indole methyl glucosinolate concentration of 0.68 mmol kg^{-1} which was higher ($P=0.005$) than *D.floralis*-damaged roots. The mean 4-hydroxy 3-indole methyl glucosinolate concentrations of plants inoculated with 5, 10 and 20 eggs of the turnip root fly were 0.42, 0.41 and 0.34 mmol kg^{-1} respectively. There was no significant ($P>0.05$) difference between the 4-hydroxy 3-indole methyl glucosinolate concentrations of the swedes inoculated at the three levels.

There was a significant ($P<0.001$) variation in the 3-indole methyl glucosinolate content of the genotypes tested (Table B.3.19.). The 3-indole methyl glucosinolate content of control swedes varied from 0.25 mmol kg^{-1} (Nmm3 a-d) to 2.78 mmol kg^{-1} (Melfort). Similarly, the 3-indole methyl glucosinolate genotypic mean concentration across all treatments varied from 0.99 mmol kg^{-1} (Nmm3 a-d) to 3.13 mmol kg^{-1} (Melfort). The control swedes contained a mean 3-indole methyl glucosinolate concentration of 1.11 mmol kg^{-1}. The concentration of 3-indole methyl glucosinolate was higher ($P<0.001$) in *D.floralis*-damaged roots than in control roots. The mean 3-indole methyl glucosinolate concentrations of plants inoculated with 5, 10 and 20 eggs of the turnip root fly were 1.77, 2.30 and 2.82 mmol kg^{-1} respectively. The plant concentration of 3-indole methyl glucosinolate became higher ($P<0.001$) with successive inoculation levels.

Significant ($P<0.001$) variations were found in the 4-methoxy 3-indole methyl glucosinolate content of the roots of the eight genotypes tested (Table B.3.20.). The 4-methoxy 3-indole

Table B.3.19. Variation in the concentration of 3-indole methyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	1.36	1.97	2.97	4.28	2.64
Nmm3 a-d	0.25	0.81	1.36	1.55	0.99
Melfort	2.78	2.65	2.84	4.24	3.13
Angus	0.82	2.00	2.62	2.92	2.09
GRL aga	1.48	2.13	2.11	3.22	2.23
Marian	0.52	1.16	1.74	2.19	1.40
Doon Major	1.28	2.19	3.78	2.27	2.38
M4M2 a-c	0.38	1.31	0.98	1.90	1.14
Mean[2]	1.11	1.78	2.30	2.82	2.00

INOCULATIONS, SED = 0.22, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.31, DF = 7
INOC.GENOTYPE, SED = 0.62, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.20. Variation in the concentration of 4-methoxy 3-indole methyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	2.05	3.43	5.55	6.88	4.48
Nmm3 a-d	0.99	2.18	2.87	4.05	2.52
Melfort	4.32	4.60	5.15	4.47	4.64
Angus	1.27	5.24	4.76	1.47	3.19
GRL aga	1.53	3.14	3.31	4.52	3.12
Marian	1.15	2.48	3.85	2.83	2.58
Doon Major	2.36	3.93	6.10	2.86	3.81
M4M2 a-c	1.14	3.67	2.01	3.58	2.60
Mean[2]	1.85	3.59	4.20	3.83	3.37

INOCULATIONS, SED = 0.37, DF = 3 residual D.F. = 27
GENOTYPE, SED = 0.52, DF = 7
INOC.GENOTYPE, SED = 1.03, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

Table B.3.21. Variation in the concentration of 1-methoxy 3-indole methyl glucosinolate (mmol kg^{-1}) between different genotypes of swede when damaged by different numbers of *D.floralis* larvae.

	Eggs per plant				
Genotype	control	5	10	20	mean[1]
Angela	1.9	5.1	9.9	10.0	6.8
Nmm3 a-d	1.3	8.3	12.0	11.6	8.3
Melfort	7.0	11.2	13.5	14.4	11.5
Angus	3.0	12.1	11.8	6.5	8.4
GRL aga	2.6	7.6	8.5	10.9	7.4
Marian	1.5	9.8	11.4	13.0	8.9
Doon Major	2.0	9.7	12.9	7.7	8.1
M4M2 a-c	2.7	12.2	8.9	12.0	9.0
Mean[2]	2.8	9.5	11.1	10.8	8.5

INOCULATIONS, SED = 0.88, DF = 3 residual D.F. = 27
GENOTYPE, SED = 1.24, DF = 7
INOC.GENOTYPE, SED = 2.47, DF = 21

1. For control and all inoculation levels of one genotype
2. For all the genotypes tested at one inoculation level or control

methyl glucosinolate content of control swedes varied from 0.99 mmol kg^{-1} (Nmm3 a-d) to 4.32 mmol kg^{-1} (Melfort). The 4-methoxy 3-indole methyl glucosinolate genotypic mean concentration across all treatments was lowest in Nmm3 a-d (2.52 mmol kg^{-1}) and highest in Melfort (4.64 mmol kg^{-1}). The control swedes contained a mean 4-methoxy 3-indole methyl glucosinolate concentration of 1.85 mmol kg^{-1}, which was lower ($P<0.001$) than *D.floralis*-damaged roots. The response of the different genotypes to the damage of the turnip root fly larvae differed significantly ($P=0.011$). The concentration of 4-methoxy 3-indole methyl glucosinolate was raised by the damage of the turnip root fly larvae for all genotypes tested. However, the rise in the concentration of 4-methoxy 3-indole methyl glucosinolate was never significant ($P>0.05$) for any one genotype across all inoculation levels. In cv Melfort the 4-methoxy 3-indole methyl glucosinolate concentrations of inoculated plants was never significantly ($P>0.05$) higher than the concentrations in control plants.

The predominant aromatic glucosinolate was 1-methoxy-3-indole methyl glucosinolate which comprised an overall mean of 27% of the total glucosinolate content of the swede roots tested. The 1-methoxy-3-indole methyl glucosinolate content of the roots of the swedes tested varied significantly ($P=0.030$) between genotypes (Table B.3.21.). The 1-methoxy-3-indole methyl

glucosinolate content of control swedes varied from 1.5 mmol kg^{-1} (Nmm3 a-d) to 7.0 mmol kg^{-1} (Melfort). The 1-methoxy-3-indole methyl glucosinolate genotypic mean concentration across all treatments varied from 6.8 mmol kg^{-1} (Angela) to 11.5 mmol kg^{-1} (Melfort). The control swedes contained a mean 1-methoxy-3-indole methyl glucosinolate concentration of 2.8 mmol kg^{-1}. The concentration of 1-methoxy-3-indole methyl glucosinolate was higher ($P<0.001$) in *D.floralis*-damaged roots than in control roots. The mean 1-methoxy-3-indole methyl glucosinolate concentrations of plants inoculated with 5, 10 and 20 eggs of the turnip root fly were 9.5, 11.1 and 10.8 mmol kg^{-1} respectively. The concentration of 1-methoxy-3-indole methyl glucosinolate was not significantly ($P>0.05$) different between the swedes inoculated at the three levels.

DISCUSSION

The concentrations of individual glucosinolates and their structural groupings varied considerably between genotypes. Of the fourteen glucosinolates detected, only three, 3-methylthiopropyl, pent-4-enyl and 4-methylthiobutyl, did not vary with either genotype or inoculation level. The three glucosinolates listed are all aliphatic and relatively minor components of the total glucosinolate content. 4-methylthiobutyl glucosinolate had the highest concentration of the three listed above, a mean across all treatments of 1.41 mmol kg^{-1}, less than 4% of total glucosinolate concentration. 3-methylthiopropyl and pent-4-enyl both comprised a mean of less than 1% of the total glucosinolate content of the roots of the swede genotypes tested. Major changes took place in the concentration of the remaining eleven glucosinolates detected in the roots of swedes during this experiment. The net effect of the changes which took place in the concentrations of individual glucosinolates was that no statistically significant variation occurred in the total glucosinolate content. The variation in individual glucosinolate concentrations coincided so as to oppose and cancel each other out.

Birch *et al*, (1992) demonstrated the presence of fourteen glucosinolates in the undamaged roots of two swede genotypes, Angus and Doon Major, although three of the fourteen glucosinolates detected were only detectable in one of the genotypes. This study, which tested a much wider range of genotypes, confirmed the presence of the same fourteen glucosinolates and found them all to be present in both Angus and Doon Major. The control roots of Angus, like those of Birch *et al,* (1992), contained no detectable quantity of pent-4-enyl glucosinolate, but it was detected in low concentrations in the roots of swede which had been inoculated with twenty eggs of the turnip root fly. The only glucosinolate which was not detected in all the genotypes of swede was but-3-enyl glucosinolate. The SCRI breeding line, M4M2 a-c,

contained no detectable quantities of but-3-enyl glucosinolate in either the controls or the *D.floralis*-damaged roots.

The changes in individual glucosinolate concentrations which took place following insect damage were generally not dose dependent. In the majority of cases, the concentration of an individual glucosinolate would rise or fall following the damage caused by the application of 5 eggs per plant. The response of the plant to larval feeding damage, following the inoculation of 10 or 20 turnip root fly eggs was different from the control, but not from the application of 5 eggs per plant. The exception to this was 3-indole methyl glucosinolate in which the response to inoculation did increase with successive numbers of eggs being inoculated onto the plant.

The response of the majority of glucosinolates to insect damage was the same for the range of genotypes tested. Two glucosinolates were the exception to this, 4-methylsulphinyl but-3-enyl glucosinolate and 4-methoxy 3-indole methyl glucosinolate. The response of 4-methylsulphinyl but-3-enyl glucosinolate varied considerably between genotypes. In the majority of genotypes, the concentration of these two compounds were not affected by *D.floralis* damage. The responses to *D.floralis* damage exhibited by genotypes included both increases and decreases in concentration with damage. The plant response in the majority of genotypes was an elevated concentration of 4-methoxy 3-indole methyl glucosinolate at one or two treatment levels, but not consistently across all treatment levels. The responses of 4-methoxy 3-indole methyl glucosinolate to turnip root fly damage in Melfort were not different from the control plants.

The switch in the glucosinolate composition of damaged swede roots was the result of two factors: rising concentration of indole glucosinolates and falling concentration of aliphatic glucosinolates. The concentration of phenylethyl glucosinolate, the only aromatic glucosinolate which did not have an indole group, was not altered by *D.floralis* damage. The pattern of indole and aliphatic glucosinolates was remarkably similar. In both cases, the pattern of response was consistent within the classes of glucosinolate, but was dominated by the concentration of a single glucosinolate, 1-methoxy 3-indole methyl glucosinolate. This compound was the dominant indole glucosinolate and 2-hydroxy-3-butenyl glucosinolate was the dominant aliphatic glucosinolate. Across all treatment and genotype combinations, the proportion of 2-hydroxy-3-butenyl glucosinolate was between 44.5% and 76.0% (mean 59.2%) of total aliphatic glucosinolate. The proportion of 1-methoxy 3-indole methyl glucosinolate in the total indole glucosinolates was between 31.0% and 72.9% (mean 57.4%). Consequently, these two glucosinolates dominated the response of the swede plants to

D.floralis damage, but this response was also reflected in the changes which took place in the concentrations of structurally similar glucosinolates.

The role of glucosinolates in plant metabolism remains unclear. Clossais-Besnard & Larher (1991) concluded from studies on oilseed rape (*Brassica napus* L.) that glucosinolates act as a storage molecule for nitrogen, carbon and sulphur. Glucosinolates are accumulated at the growing points of plants when young seedlings perform photosynthesis and increase their biomass rapidly. The accumulation of glucosinolates in the shoots of growing plants was especially marked for 2-hydroxy-3-butenyl glucosinolate (Clossais-Besnard & Larher, 1991). The rapid fall in the root concentration of 2-hydroxy-3-butenyl glucosinolate in this study may be associated with either a change in root function from growth to repair following damage or with the movement of the compound to leaves to allow compensatory growth to take place.

Glucosinolates, like many secondary plant compounds, appear to stimulate monophagous and oligophagous insects whilst deterring polyphagous insects. It is probable that changes in the glucosinolate composition of plants can alter the host status of a plant (Birch *et al*, 1992). Indole glucosinolates act as oviposition or feeding stimulants to a wide range of Brassica feeding insects. Oviposition is particularly stimulated by indole glucosinolates in turnip root flies (Simmonds *et al*, in press), cabbage root fly (Roessingh *et al*, 1992a), diamondback moth (Reed, 1989), and cabbage butterfly (Traynier & Truscott, 1991). Cabbage stem flea beetle, *Psyllioides chrysocephala*, feeding is influenced by the presence of glucosinolates in the feeding substrate (Bartlet & Williams, 1991). Conversely, the feeding of the field slug, *Deroceras reticulatum,* is inversely proportional to the concentration of total glucosinolates in oilseed rape seedlings (Glen *et al*, 1990). The inverse relationship between glucosinolate concentration and *D.reticulatum* feeding may be a product of the generalist nature of the field slug. The other species listed are specialist Brassica feeding insects and, as a generalist, the field slug may be biologically ill-suited to breaking down glucosinolates.

Clearly, the interactions which take place between phytophagous insects and host plant secondary metabolites are only partially understood. Whilst knowledge of the metabolism, function and perception of secondary plant products remains largely incomplete, the part they play in insect-plant relationships will also be little understood. Berenbaum & Seigler (1992) estimated that more than 20,000 individual secondary plant compounds have been isolated and characterised from the Angiosperms alone. Such diversity is constrained within individual species by a combination of abiotic factors and biotic interactions. The role of glucosinolates as mediators of herbivore host specificity is widely accepted although frequently constrained by other factors (Louda & Mole, 1992). The uncertainty which surrounds the role of

glucosinolates is partly explained by the complex nature of the systems with which they interact. Dicke & Sabelis (1988) point out that the costs and benefits of a specific trait are not constant. A consequence of this is that the function of a chemical within a specific interaction is difficult to assess. Secondary metabolites as defensive chemicals may not even have a cost if their production is part of a process which yields other benefits (Simms, 1992). What is becoming clear is that whatever the evolutionary reasons and the primary functions associated with glucosinolates, the concentration found in host plants influences phytophagous insects.

In this case, the narrow range of resistance which is present in the swede genotypes tested does not allow for a full exploration of the part which is played by glucosinolates. The glucosinolates which varied between genotypes did so in a way which clearly precluded a direct relationship between pupal weight and glucosinolate concentrations. The combination of the non-dose response to *D.floralis* and the similarity of the responses found here to that of a wide range of pests, diseases and artificial damage indicates that the changes which take place are of a general nature and not pest specific. From the evidence presented, it is not possible to be certain whether the changes in glucosinolates are defensive or merely a by-product of repairing damage to the plant tissue.

Chapter B4

Interactions between larvae of the cabbage root fly (*Delia radicum*) and dry matter and sugar content of Brassica roots

INTRODUCTION

An extensive study of plants, both cultivated and wild, which would support development of cabbage root fly was undertaken by Finch & Ackley (1977). The cultivated host range of *Delia radicum* are members of the Cruciferae, but only about half the wild plants tested would support the development of *D.radicum* (Finch & Ackley, 1977). The susceptibility of the cultivated Brassicas to *D.radicum* is less well defined. The genotypes of Brassica which are grown change constantly with time which results in variations in relative susceptibilities of different crop types. Swailes (1959) investigated the relationship between *D.radicum* and nine swede genotypes assessing oviposition, pupal size and number and damage on different genotypes. The number of eggs laid on the different genotypes varied by a factor of approximately five. However, the number of eggs laid upon a genotype was not reflected in the root damage which genotypes suffered or the numbers of pupae associated with each genotype. The genotype which was least damaged and produced the fewest puparia was cv Wilhelmsburger, which was quite heavily oviposited upon. The pupae from Wilhelmsburger were the smallest, but not very different from other genotypes, and consequently the author concluded that establishment of larval feeding was the critical factor. Swailes (1968) restricted *D.radicum* larvae to a small portion of the root and contrasted feeding on the intact skin with that on inner tissues. It was concluded that skin characteristics were critical to the survival rates of *D.radicum* larvae. Finch & Coaker (1968) found the cortex of the root was not a barrier to larval penetration. Although no genotype was specified for this work, it does reinforce the general variability within results found within crop types.

Initial rearing techniques for *D.radicum* utilised slices of swede (Read, 1960) which were constantly replaced to avoid soft rot. If adequate food resources were not always available, then pupation took place prematurely. The rearing of cabbage root fly could also be improved by selecting swede roots which did not begin to rot when stored in saturated sphagnum moss or vermiculite for two weeks prior to use (Read & Welch, 1962). Read (1965) believed that removal of the surface of the root to expose the inner tissues would increase the survival of larvae. Read (1965) considered that swedes were the best plants on which to rear *D.radicum*.

Work on the host range and variations in development of *D.radicum* on different host plants is fairly extensive. However, the perception of macro-molecules by *D.radicum* is far less well investigated. Ryan & Behan (1973) discovered chemoreceptors on *D.radicum* larvae which are believed to have a gustatory function and the importance of sugars as phagostimulants to anthomyiid flies has already been reviewed earlier in Section B. Honda & Ishikawa (1987) demonstrated the stimulatory effect of sugars on the larvae of the onion fly, *Hylemya antiqua*. Genotypic variations between the concentrations of different sugars in the roots of young Brassicas have already been demonstrated in Chapters B1 and B2. Earlier chapters have also shown a link between Brassica root sugar concentrations and *D.floralis* larval development. However, there are marked differences in the feeding strategies which have been adopted by *D.floralis* and *D.radicum*. Whilst *D.floralis* usually mines deeply within the root, *D.radicum* feeds in shallower mines which rarely penetrate the cortex. To date, no evidence has been published on the distribution of sugars within the roots of young Brassicas and their influence on the intake of feeding larvae. Consequently, even if the dietary requirements of *D.radicum* and *D.floralis* are identical, the effect of different genotypes will not necessarily be the same owing to variation in feeding sites within the roots.

The aim of this element of the study was to assess the interaction between Brassica roots and *D.radicum* larval feeding and development. As a part of this study, the genotypic variation in root size, freeze-dried matter content (FDM) and sugar content was examined along with the changes which take place in these factors following *D.radicum* damage. Links between these factors and larval fitness and survival on different Brassica genotypes were explored, and contrasts are drawn with the results from similar experiments using *D.floralis* (Chapters B1 and B2).

MATERIALS AND METHODS

Biological material

Nine Brassica genotypes were selected to represent a range of characteristics which included the level of resistance to the turnip root fly in earlier experiments. The nine Brassica genotypes comprised five genotypes of swede (Angela, Angus, Doon Major, GRL aga and Marian), two genotypes of rape (the oilseed rape Ariana, and the forage rape Hobson) and two genotypes of kale (KHSCN-high thiocyanate ion and KLSCN-low thiocyanate ion). Angus has exhibited partial resistance to turnip and cabbage root flies in a number of field experiments (Birch, 1988; Ruuth, 1988; Wilson *et al*, 1990). Doon Major is often used as a standard susceptible genotype in experiments against both turnip root fly and cabbage root fly (Birch, 1988; Birch,

1989b; Ruuth, 1988; Wilson *et al*, 1990). Marian, at the seedling stage, has shown tolerance to cabbage root fly damage (Wilson *et al*, 1990) and GRL aga has been shown to be resistant to turnip root fly (Birch, 1989b). Previous work on mature swede plants had also shown them to exhibit a range of root sugar contents (Bradshaw & Griffiths, 1990). Angela is of particular interest, owing to both its apparent antibiotic resistance to turnip root fly (Chapter B1) and the difference in response compared with the other swede genotypes.

Plants were grown in 10cm diameter pots containing a 3:1 mixture of Levington® Universal compost and sand in a glasshouse with a 16:8 h light:dark regime and a temperature range of 16-21°C. At the 8-10 true leaf stage, five *D.radicum* eggs less than 24 h old from a laboratory culture, were inoculated onto the plants. (The original design for the experiment had more than one inoculation level. However, the laboratory culture of *D.radicum* failed to provide sufficient eggs and the design was altered to one of a single inoculation level, five eggs being the largest number of eggs per plant which could be adequately replicated). Inoculated plants were arranged with control plants in a series of randomised blocks with twelve replicates per treatment. Dead leaves were removed daily to avoid migration of larvae between plants. Five weeks after egg inoculation, pupae were washed from each pot and collected using a 1.4mm meshed sieve prior to being air dried and then weighed individually. Plants were harvested and divided into root and aerial parts at the hypocotyl which were weighed separately and frozen prior to freeze drying and milling in two bulked samples. Freeze-dried samples were weighed and used to calculate the FDM of the bulked samples.

Sugar extraction and analysis.

Freeze-dried and milled Brassica root samples were extracted using 80% v/v aqueous ethanol. The constituent sugars, fructose, glucose and sucrose, were separated and quantified by high performance liquid chromatography (hplc) using methods, chemicals and equipment identical to those described in Chapter B1.

Statistical analysis

Data were subjected to analysis of variance on Genstat (Genstat, Sun/Unix version 5.0, release 2.2, 1990) and the data for percentage pupation of larvae were found to be unsuitable for this treatment because the distribution of the data were not normal. Consequently, these data were subjected to a binomial analysis on Genstat, a form of generalised linear model which allows for the distribution of the data. The data for individual and total sugar concentrations required angular transformation and the root weight data required log transformation to stabilise the variances. The range of untransformed values is shown in the text and the angular transformed values are presented in the tables with untransformed values

shown in parentheses.

Correlations were performed between the mean values (of control and inoculated) for the dry matter content, total sugar content and individual sugar content, and the probability of pupating and the mean pupal mass. Correlations were performed on Minitab (Minitab Vax/VMS version 7.1, 1989) using the command "correlation" to produce a Pearson product moment correlation coefficient and *P* values were then looked up in statistical tables.

RESULTS

Development of cabbage root fly larvae on inoculated roots

The analysis of mean pupal weights showed variation ($P<0.001$) on the different crop types. There were significant ($P<0.05$) differences between the kales, rapes and swedes with pupal masses of 6.68, 10.52 and 12.34mg respectively. There were no significant differences ($P>0.05$) between the pupal weights associated with the individual Brassica genotypes within a crop type (Table B.4.1.).

The estimate of regression coefficients showed that the lowest probability of an egg developing to pupate was 0.45 on the kale, KHSCN. Application of the deviance ratio showed that this was not significantly ($P>0.05$) different from the kale KLSCN, the rape Hobson, nor the swede GRL aga. The probabilities of an egg surviving to pupate were significantly ($P<0.05$) higher on all other genotypes (Table B.4.1.).

Plant responses to root damage

The overall mean root weight for all genotypes (control and inoculated plants) was 5.58g. The mean root weight of the controls for all genotypes was 6.33g and of inoculated roots was 4.82g. The log transformed root weight of controls was higher ($P<0.001$) than inoculated plants, the means being 1.69 and 1.43 respectively. The overall root weight of the swedes across treatment and genotype was higher ($P<0.001$) than the kales and rapes. Root weights of the control plants of individual genotypes ranged from 3.23g, kale KLSCN, and rape Ariana, to 13.52g, swede Angela (Table B.4.2.).

The response of different genotypes to inoculation varied ($P=0.009$), but in all genotypes, root weight was reduced after inoculation, although not always significantly ($P>0.05$). There was a reduction ($P<0.001$) in the root weight of the kale KHSCN, and in the swedes Angela, Angus, GRL aga and Marian after *D.radicum* damage (Table B.4.2.).

Table B.4.1. Probability of surviving to pupate (and error on that probability), and mean pupal weight (mg) of cabbage root fly which had developed on different Brassica genotypes.

Crop Type	Genotype	Probability	S.E.	Pupal Weight
Kale	KHSCN	0.45	0.06	6.89
Kale	KLSCN	0.47	0.06	6.46
Rape	Ariana	0.78	0.05	11.56
Rape	Hobson	0.62	0.06	9.48
Swede	Angela	0.67	0.06	12.55
Swede	Angus	0.77	0.05	11.67
Swede	Doon Major	0.73	0.06	11.78
Swede	GRL aga	0.57	0.06	12.96
Swede	Marian	0.68	0.06	12.74

PUPAL WEIGHT, SED=1.01, DF=6

Table B.4.2. Angular transformation of mean root weight (g) of undamaged Brassica genotypes and those damaged by cabbage root fly (untransformed figures are shown in brackets).

Crop Type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	1.18 (3.32)	0.98 (2.70)	1.08 (3.01)
Kale	KLSCN	1.14 (3.23)	1.01 (2.81)	1.07 (3.02)
Rape	Ariana	1.17 (3.23)	1.01 (2.77)	1.09 (3.00)
Rape	Hobson	1.29 (3.71)	1.16 (3.21)	1.23 (3.46)
Swede	Angela	2.59(13.52)	2.37(11.13)	2.48(12.33)
Swede	Angus	1.92 (6.92)	1.30 (3.80)	1.61 (5.36)
Swede	Doon Major	2.04 (7.90)	1.96 (7.24)	2.00 (7.57)
Swede	GRL aga	1.72 (5.70)	1.52 (4.74)	1.62 (5.22)
Swede	Marian	2.21 (9.42)	1.58 (5.00)	1.89 (7.22)
SED		0.09		0.07
residual D.F.		17		17

Dry-matter content of Brassica roots

The overall mean FDM content of the Brassicas tested was 23.5%. Overall, inoculation had no significant (P>0.05) effect on FDM. However, for the kales inoculation caused an increase (P=0.017) in FDM, with control kales having a mean FDM of 25.7%, and inoculated kales a mean FDM of 28.1%.

The FDM content of the three crop types varied significantly (P<0.001); kales, rapes and swedes contained 26.9, 24.4 and 21.8% FDM respectively. Between genotypes, the dry matter content varied (P<0.001) from 27.1% in kale KLSCN, to 19.9% in swede Angela (Table B.4.3.). The effect of inoculation on individual genotypes was not significantly different (P>0.05) from the effect on crop type.

Sugar concentrations

Total sugar

Total sugar concentration was determined by summing the individual values for glucose, fructose and sucrose. The overall mean total sugar concentration of the roots was 7.78 g per 100g of freeze dried matter (g/100gFDM). Inoculation reduced (P<0.001) the total concentration of sugars in the roots of Brassicas from 9.05 to 6.50 g/100gFDM. The total sugar concentration in control roots differed (P<0.001) between the genotypes studied, ranging from 3.13 g/100gFDM in kale KHSCN, to 15.80 g/100gFDM in swede Angela (Table B.4.4.). In all the genotypes, the total concentration of sugar was higher (P<0.001) in the control roots than in the inoculated roots, but in GRL aga this difference was not significant (P>0.05). The combined mean total sugar concentration of damaged and control roots differed significantly (P<0.001) between genotypes. The lowest value found was for kale KHSCN (2.61 g/100gFDM), whilst the highest sugar concentration, 14.50 g/100gFDM, was found in swede Angela (Table B.4.4.).

Individual sugars

The fructose content of kales and rapes was very low, usually only a trace less than 0.1 g/100gFDM which was too small to be measured. Consequently, there was no significant difference (P>0.05) in the concentration of fructose in the roots of kales or rapes. The concentration of fructose in swede control roots differed (P<0.001) from 1.27 g/100gFDM in GRL aga to 3.42 g/100gFDM in Angela (Table B.4.5.). Over all swede genotypes, the concentration of fructose was lower (P<0.001) in *D.radicum*-damaged roots than in control roots, although this difference was only significant (P<0.05) in Angus, GRL aga and Marian. The fructose concentration in *D.radicum*-damaged roots of Doon Major was higher than that

Table B.4.3. Mean root freeze dried matter content (g/100g) of undamaged Brassica genotypes and those damaged by cabbage root fly.

Crop Type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	24.9	28.4	26.7
Kale	KLSCN	26.4	27.8	27.1
Rape	Ariana	25.1	21.9	23.5
Rape	Hobson	24.9	25.8	25.3
Swede	Angela	19.9	20.0	19.9
Swede	Angus	22.4	22.0	22.2
Swede	Doon Major	20.7	19.3	20.0
Swede	GRL aga	27.3	26.5	26.9
Swede	Marian	19.1	20.8	20.0
SED		1.2		0.8
residual D.F.		17		17

Table B.4.4. Mean total sugar concentration (g/100gFDM) angular transformations (untransformed in parentheses) in the roots of undamaged Brassica genotypes and damaged by the cabbage root fly.

Crop	Genotype	Control	Inoculated	Mean
Kale	KHSCN	10.18 (3.13)	8.31 (2.09)	9.25 (2.61)
Kale	KLSCN	11.41 (3.93)	9.74 (2.86)	10.57 (3.40)
Rape	Ariana	14.87 (6.59)	11.21 (3.78)	13.04 (5.19)
Rape	Hobson	15.69 (7.33)	12.15 (4.43)	13.92 (5.88)
Swede	Angela	23.42(15.80)	21.28(13.20)	22.35(14.50)
Swede	Angus	18.28 (9.84)	14.83 (6.55)	16.55 (8.20)
Swede	Doon Major	22.49(14.63)	20.84(12.66)	21.67(13.65)
Swede	GRL aga	15.61 (7.24)	14.85 (6.57)	15.23 (6.91)
Swede	Marian	21.10(12.96)	14.63 (6.38)	17.87 (9.67)
SED		0.57		0.40
residual D.F.		17		17

Table B.4.5. Mean fructose concentration (g/100gFDM) angular transformations (untransformed in parentheses) of the root of undamaged Brassica genotypes and damaged by the cabbage root fly.

Crop	Genotype	Control	Inoculated	Mean
Kale	KHSCN	(tr)	(tr)	(tr)
Kale	KLSCN	(tr)	(tr)	(tr)
Rape	Ariana	(tr)	(tr)	(tr)
Rape	Hobson	(0.14)	(tr)	(0.12)
Swede	Angela	10.66 (3.42)	10.15 (3.12)	10.40 (3.27)
Swede	Angus	8.53 (2.20)	6.24 (1.18)	7.38 (1.69)
Swede	Doon Major	9.16 (2.54)	9.65 (2.81)	9.40 (2.68)
Swede	GRL aga	6.47 (1.27)	5.41 (0.89)	5.94 (1.08)
Swede	Marian	9.87 (2.94)	6.13 (1.14)	8.00 (2.04)
SED		0.46		0.33
residual D.F.		9		9

tr=concentration<0.1 g/100gFDM
Kales and rapes were not included in the statistical analysis because they contained only trace quantities of fructose.

Table B.4.6. Mean glucose concentration (g/100gFDM) angular transformations (untransformed in parentheses) of the roots of undamaged Brassica genotypes and damaged by the cabbage root fly.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	1.81 (0.10)	1.81 (0.10)	1.81 (0.10)
Kale	KLSCN	3.12 (0.31)	2.76 (0.26)	2.94 (0.29)
Rape	Ariana	1.81 (0.10)	3.90 (0.47)	2.86 (0.29)
Rape	Hobson	4.56 (0.64)	2.98 (0.27)	3.77 (0.46)
Swede	Angela	16.02 (7.62)	14.67 (6.43)	15.34 (7.03)
Swede	Angus	13.14 (5.17)	9.51 (2.73)	11.32 (3.95)
Swede	Doon Major	14.23 (6.04)	13.69 (5.60)	13.96 (5.82)
Swede	GRL aga	9.39 (2.66)	8.40 (2.14)	8.90 (2.40)
Swede	Marian	16.08 (7.68)	9.39 (2.66)	12.74 (5.17)
SED		0.60		0.42
residual D.F.		17		17

of the control plants, although the difference was not significant ($P>0.05$).

The mean overall concentration of glucose in control roots was 3.37 g/100gFDM. The concentration of glucose in control roots differed ($P<0.001$) between genotypes, ranging from a trace in kale KHSCN, and rape Ariana, to 7.68 g/100gFDM in Marian (Table B.4.6.). Overall, the effect of cabbage root fly damage was to reduce ($P<0.001$) the concentration of glucose. The glucose concentration was lower ($P<0.001$) in inoculated roots of Angus, Marian, Angela and Hobson and higher ($P<0.001$) in inoculated roots of Ariana. The root glucose concentrations of remaining genotypes were not significantly ($P>0.05$) altered by *D.radicum* damage. The mean glucose concentration of the Brassicas tested correlated (r=0.743 [DF=8], P<0.02) with the mean fructose content of the Brassicas tested.

The mean concentration of sucrose in control roots was 4.26 g/100gFDM and differed ($P<0.001$) between genotypes. The lowest sucrose concentration in the control roots was found in Marian (2.34 g/100gFDM) whilst the highest concentration (6.55 g/100gFDM) was found in Hobson (Table B.4.7.). *D.radicum*-damaged roots of the kales and rapes had significantly ($P<0.001$) reduced sucrose concentrations. Within the swedes, the sucrose content of Angela and Doon Major were reduced ($P<0.001$) by the *D.radicum* damage. The combined mean sucrose concentration (damaged and control roots) differed significantly ($P<0.001$) between genotypes. The lowest value was found in kale KHSCN (2.41 g/100gFDM), and the highest sucrose concentration, 5.31 g/100gFDM, in Hobson (Table B.4.7.).

Table B.4.7. Mean sucrose concentration (g/100gFDM) angular transformations (untransformed in parentheses) of the roots of Brassica genotypes damaged by the cabbage root fly and control plants.

Crop	Genotype	Control	Inoculated	Mean
Kale	KHSCN	9.85 (2.93)	7.90 (1.89)	8.88 (2.41)
Kale	KLSCN	10.80 (3.52)	9.10 (2.50)	9.95 (3.01)
Rape	Ariana	14.64 (6.39)	10.32 (3.21)	12.48 (4.80)
Rape	Hobson	14.82 (6.55)	11.62 (4.06)	13.22 (5.31)
Swede	Angela	12.60 (4.76)	11.01 (3.65)	11.80 (4.21)
Swede	Angus	9.04 (2.47)	9.35 (2.64)	9.20 (2.56)
Swede	Doon Major	14.24 (6.05)	11.89 (4.25)	13.06 (5.15)
Swede	GRL aga	10.48 (3.31)	10.84 (3.54)	10.66 (3.43)
Swede	Marian	8.79 (2.34)	9.24 (2.58)	9.02 (2.46)
SED		0.41		0.29
residual D.F.		17		17

Relationship between FDM and sugar content and cabbage root fly pupae

The correlations between the probability of *D.radicum* pupating and the components of the Brassica roots tested showed that the probability of pupating was inversely linked to the FDM of the Brassica roots (r=-0.704 [DF=8], $P<0.05$). The correlations between the probability of pupating and the sugar content of the roots were not significant ($P>0.05$) (total sugar, r=-0.622 [DF=8]; fructose, r=-0.474 [DF=8]; glucose, r=-0.495 [DF=8]; sucrose, r=-0.393 [DF=8]).

The correlations between the mean pupal weight of *D.radicum* and the components of the Brassica roots tested showed that the pupal weight was inversely linked to the FDM of the Brassica roots (r=-0.660 [DF=8], $P<0.05$). The correlation between the mean pupal weight and the total sugar content of the roots was statistically significant (r=-0.768 [DF=8], $P<0.01$). The concentrations of fructose and glucose were both significantly ($P<0.05$) correlated with the pupal weight (fructose, r=-0.679 [DF=8]; glucose r=-0.698 [DF=8]). There was no significant ($P>0.05$) correlation between the sucrose concentration in the roots tested and the mean pupal weight of *D.radicum* (r=-0.216 [DF=8]).

DISCUSSION

The Brassicas which were tested in this chapter demonstrated a range of antibiotic resistance to the cabbage root fly, *D.radicum*. Antibiotic resistance to the development of *D.radicum* was most clearly demonstrated in the two genotypes of kale, KHSCN and KLSCN, in which the percentage pupation was below 50% and the mean pupal weight below 7mg. The rape genotype Hobson also produced a low percentage pupation and relatively small pupae, whilst Ariana demonstrated a marked susceptibility to *D.radicum*. The pupae produced on the five swede genotypes were all heavier than the pupae from KHSCN, KLSCN and Hobson. However, the probability of pupation on GRL aga was far lower than on the other swede genotypes and was not different from the probability of pupating in the kales. Low pupation percentages linked to pupae of a normal size have been attributed to difficulty in establishing feeding (Swailes, 1960). It may be the case that such a mechanism is present in GRL aga causing early mortality in some larvae.

The size of food resource available to the developing *D.radicum* larvae, as measured by the weight of root tissue, varied widely. There was no difference between the weights of the control roots of kales and rapes, all of which weighed between 3g and 4g (fresh weight). However, the control roots of the swedes varied from approximately two to four times the

weight of the kales and rapes. This substantial variation in the size of the food resource is almost certain to affect the degree of larval feeding competition that takes place on the different genotypes. Larval density and food quality have been linked to *D.radicum* pupal size and premature pupation (Read, 1960; Finch & Coaker, 1968). Premature *D.radicum* pupation occurred if the swede rotting limited the feeding resource, and consequently pupae were smaller. The optimum ratio for larval development on mature swede was 1 larva for every 1.6 grammes of root tissue available (Finch & Coaker, 1968). Smaller pupae were produced if excessive numbers of eggs were placed around the root and the size of the fly which emerged from these pupae was directly proportional to pupal size. The fecundity of the fly was also found to be related to the weight of the pupa. Finch & Coaker (1968) used mature swede bulbs in their study, onto which large numbers of eggs were inoculated. Consequently, it is not ideal to compare their root weight to egg numbers ratio with the values from this study. A root weight:egg ratio of 1.6:1 would indicate that five larvae would require 8 grammes (fresh weight) of root on which to develop. The very small weight of the root of GRL aga makes a limitation on food availability a possible explanation for the low pupation. However, the large pupal weight and small reduction in root size on inoculated GRL aga (Table B.4.1.) would indicate that competition for food resources is not the explanation. As indicated in the Materials and Methods of this chapter, it was originally intended to explore the effect of different numbers of *D.radicum* eggs on Brassica roots. However, the persistent problems which were encountered in culturing *D.radicum* made this impossible. The effect on pupation of changing *D.radicum* density is an important unanswered question for Brassicas of this age.

Coupled with the variation in the weight of the root tissue were the differences in its composition to be found in different genotypes. The FDM content of the roots varied widely between and within crop types, which contrasted with the results of the inoculations which were carried out with *D.floralis*. The control plants of the rapes and kales had much larger FDM content than had been found previously (Chapter B2). The plants were grown in different glasshouses (but on the same site) in which the temperatures and subsidiary lighting were set to identical specifications. However, the conditions within the glasshouse will not have been identical owing to variation in weather conditions and position of the glasshouse. No firm conclusions can be drawn as to what caused the controls of the rapes and kales to differ from those in the *D.floralis* experiment in Chapter B2. It is likely that the environmental variations listed above contributed to the variation. The FDM content of the kales rose in roots which had been damaged by *D.radicum*. This rise in the FDM content of the kales opposed the trend which was demonstrated with *D.floralis*. When inoculated with *D.floralis*, all the genotypes except Doon Major reduced their FDM content, although the effect was not statistically significant (Table B.2.5.). These changes may be a result of the different feeding

strategies which are used by the two species. The kales displayed the highest degree of antibiotic resistance to *D.radicum:* the probability of pupation was lowest on the two kales and the pupae were the smallest (Table B.4.1.).

The concentrations of individual sugars, and consequently the total sugar concentration of the roots of the Brassicas tested, varied both between and within crop types. The concentrations of fructose in the rape and kale genotypes were detectable, but below the threshold at which they could be accurately measured. Similarly, the rape and kale controls contained between 0.1 and 0.64 g/100gFDM of glucose. In swedes, the control roots contained between 1.27 and 3.42 g/100gFDM of fructose and between 2.66 and 7.68 g/100gFDM of glucose. Rape is essentially an annual crop and does not require to build up sugar stores with which to overwinter and fuel a second year's development. Consequently, it is to be expected that no large carbohydrate reserves are found in the roots. The roots of swede are storage organs which are accumulating energy reserves for flower and seed production in the second season of growth. Kale is also a biennial crop, but whilst the roots are the storage organs of swedes, kales build up reserves in the stem and largely retain their leaves over winter. Root concentrations of glucose and fructose are closely linked, as demonstrated in Chapter B1. The concentration of sucrose appears to be independent of both of these molecules in this experiment and in earlier chapters.

The total sugar concentration in the roots of all the Brassicas tested reduced in cabbage root fly-inoculated roots. This result arose from reduced concentrations of all the sugars measured in most of the genotypes tested. With the exception of one genotype, the concentration of individual sugars in inoculated roots was less than, or not statistically significantly different from, control roots. The glucose concentration of Ariana was higher in damaged than in control roots. However, the reduction in total root sugar concentration which took place in GRL aga was not statistically significant. The total sugar content of the roots of GRL aga were the lowest of any of the swede genotypes which were tested. The control plants of GRL aga had a total sugar concentration which was not different from the controls of the two rape genotypes. This result reflects the results found for GRL aga during Chapter B1 and confirms that GRL aga has very low sugar content during its early growth stages.

The glucose content of the Brassica roots tested varied in the control plants by a factor of nine. The swede genotypes tested were particularly high in glucose with the exception of GRL aga, which contained a comparatively low concentration. Glucose and fructose were once again highly correlated. The extremely low values for the fructose content of rapes and kales reflect those found during the experimental work on the turnip root fly in Chapter B2. The

results of the analyses for individual sugars were generally lower in this experiment than those in earlier chapters e.g. the concentrations of fructose were reduced to trace levels in the kales and the rapes.

The rise in the concentration of some sugars may be due to differences in the distribution of various sugars within the roots of Brassicas. The more surface-oriented mining by *D.radicum* larvae could conceivably be removing the part of the root which contains the lowest proportion and concentration of some sugars. The net effect would be to raise the mean sugar concentration of the root. This hypothesis merits further investigation in the light of the results achieved here and in earlier chapters.

The low probability of *D.radicum* developing to pupate on roots of GRL aga, which was not significantly different from the more resistant rapes and kales, is of great interest. GRL aga was never contrasted directly with the kales and rapes during the series of inoculation experiments which were carried out with *D.floralis*. *D.floralis* pupae which developed on GRL aga had a low weight, but the percentage pupation on different swede genotypes was not different. When used in field experimentation against the cabbage root fly GRL aga had previously only demonstrated intermediate resistance characteristics. Wilson *et al* (1990) found the variation in the weight of cabbage root fly pupae was small and there was no difference between the swede genotypes tested.

The swede Angela, which was included in this experiment partly as a result of its apparent antibiotic resistance to *D.floralis* (Chapter B1), proved to be quite susceptible to cabbage root fly. Flies developing on cvs Angela, Angus and Marian all produced pupae of similar size to flies developing on Doon Major and the probability of pupation taking place on these four genotypes was relatively high (67% to 77%). The susceptibility of Ariana to *D.radicum* and the resistance shown by Hobson reflect the results achieved when inoculating *D.floralis*. Hobson and Ariana share fundamentally different characteristics as feeding substrates for *D.radicum* and *D.floralis*.

Low percentage pupation coincident with normal pupal weight has previously been linked with difficulty in establishing feeding (Swailes, 1960). The basis of this suggestion is that those larvae which do establish feeding can consume sufficient root tissue to meet dietary requirements. Consequently, the larvae which fail to establish feeding result in low survival rates, but the reduction in mean pupal weight which characterises poor dietary quality is not displayed. In field experiments which tested swede genotypes, Shaw *et al* (1993) found a correlation between *D.floralis* damage and root dry matter content. In this experiment, the

probability of *D.radicum* pupating and the weight of the pupae were inversely correlated to the FDM of the Brassica roots tested. In addition, the weight of the pupae on the different Brassica genotypes was positively correlated with the glucose, fructose and total sugar concentrations of the different Brassica genotypes. Higher FDM of Brassica roots reduces the probability of *D.radicum* surviving to pupate. Furthermore, the mean weight of individuals which pupate is adversely affected by the FDM of the roots on which they were feeding. Increasing the total sugar content of the root, particularly glucose and fructose, clearly exerts a positive influence on the mean weight of the individuals which pupate. The reductions in the weight of the pupae found are clearly adequate to produce a moderate control of *D.radicum*. The mechanism is probably unsuitable for use on crops such as swede where the eating quality of the root would be adversely affected by such changes in its composition. However, for crops where the root is not harvested, this mechanism of control merits further investigation to confirm its efficacy and mode of action.

McKinlay & Birch (1992) demonstrated that *D.radicum* damage to a resistant swede genotype treated with a half dose of insecticide was no greater than the damage to a susceptible genotype treated with a full dose of insecticide. Taksdal (1992) found that combining reduced pesticide applications with resistant genotypes could give adequate field control against both cabbage and turnip root flies. The genotype GRL aga, has already shown high levels of antixenotic resistance to both pests when contrasted with other swede genotypes (Birch, 1989; Section A; Section B; Section C). The improvement in resistance offered by GRL aga may lead to further reductions in the amount of pesticide required to give adequate field control. In addition to the results from glasshouse experiments shown here, GRL aga has shown some field resistance to cabbage root fly damage (Chapters C1 and C2). The potential of this genotype for management of root flies is discussed in more depth in the General Discussion of the thesis.

Chapter B5

Modification of Brassica root glucosinolate content by *Delia radicum* larvae

INTRODUCTION

Glucosinolates are a group of secondary plant compounds synonymous with the Cruciferae (Kjaer, 1976) and found in all Brassica crops, including swede, kale and rape. Work on swede glucosinolate content has demonstrated considerable varietal variation in mature roots (Carlson *et al*, 1981; Adams *et al*, 1989). Birch *et al* (1990; 1992) have found total glucosinolate content in the roots of Brassicas ranging from between 3.40 to 35.29 mmol kg^{-1} and including 15 different glucosinolates. Glucosinolates and their breakdown products have been linked with the host specificity or feeding stimulation of more than twenty Crucifer-feeding insects (Städler, 1992). Many of the individual papers published have already been cited at some length in earlier parts of the thesis. Glucosinolates and related compounds have been extensively associated with stimulating the behaviour of Lepidoptera, Coleoptera and Diptera (Städler, 1992). Ellis *et al* (1980) noted that, within radish, *D.radicum* egg laying was positively correlated with two glucosinolate volatile hydrolysis products. *D.radicum* larvae exhibited positive and negative taxis to different concentrations of allyl and ethyl isothiocyanate, volatile glucosinolate hydrolysis products (Koštál, 1992). However, at very high concentrations the same compounds were repellent to larvae.

It has clearly been demonstrated by previous authors that it is possible to find good correlations between total and individual glucosinolates and the damage of Brassica-feeding insects. The number and concentration of glucosinolates in different plants have also been shown to vary widely between genotypes and also with the damage caused by insects.

It has also been clearly demonstrated that the glucosinolate profile of Brassicas is altered by *D.floralis* feeding. Birch *et al* (1990; 1992) investigated the glucosinolate concentrations of roots of a range of Brassicas which had been damaged by *D.floralis*. Feeding damage caused by *D.floralis* resulted in widespread changes to the glucosinolate composition of the roots. Feeding damage to plants induces changes in individual and total glucosinolate concentrations. Birch *et al* (1990) found that, within oilseed rape plants which had been damaged by *D.floralis*, the concentrations of individual glucosinolates and their relative proportions were dramatically altered. The increase in individual indole glucosinolates was up to 88% whilst aliphatic glucosinolates were largely reduced. Further work demonstrated that this effect was

also to be found in kales and swedes damaged by *D.floralis* (Birch *et al*, 1992). However, neither total nor individual glucosinolates were consistently associated with either resistance or susceptibility to *D.floralis*.

The responses of *D.radicum* larvae to glucosinolates and their hydrolysis products have received little investigation (Koštál, 1992) and no published material could be found on effects of *D.radicum* damage on the glucosinolate content of Brassica roots. The latter question is addressed in this chapter of the thesis.

MATERIALS AND METHODS

Biological Material

Nine Brassica genotypes were selected to represent a range of characteristics which included the level of resistance to the turnip root fly in earlier experiments. The nine Brassica genotypes comprised five genotypes of swede (Angela, Angus, Doon Major, GRL aga and Marian), two genotypes of rape (the oilseed rape Ariana, and the forage rape Hobson) and two genotypes of kale (KHSCN-high thiocyanate ion and KLSCN-low thiocyanate ion). The biological material used was the same as that used in Chapter B4. The inoculation protocol is described in detail in the Materials and Methods of that chapter.

Chemical analysis

Glucosinolates were extracted from the freeze dried and milled roots by boiling a 1 g sub-sample in 70% v/v aqueous methanol and converted to desulphoglucosinolates using the method of Heaney and Fenwick (1980a). Individual glucosinolates were separated by high performance liquid chromatography (hplc). The methods are described in detail in Chapter B3.

Statistical analysis

Data were square root transformed to stabilise the variance on the mean and subjected to analysis of variance using Genstat (Genstat, Sun/Unix version 5.0, release 2.2, 1990).

RESULTS

Biological data

The genotypic variation in root weight, pupal weight and responses to *D.radicum* damage are discussed in Chapter B4.

Total glucosinolate content of Brassica roots

Hplc analysis of the roots of eight swede genotypes revealed detectable concentrations of 11 glucosinolates (Table B.5.1.). Seven of the glucosinolates detected had aliphatic side chains and the remainder had aromatic side chains (Table B.5.1.). In addition, a peak (number 12) was eluted at 6 minutes for which no confirmation of its identity was available. Peak 12 was included in the total glucosinolate content.

The control roots of the Brassicas tested contained a mean total glucosinolate concentration of 11.89 mmol kg^{-1}. The total glucosinolate concentration varied significantly ($P<0.001$) both between and within crop types. The highest total glucosinolate concentration in the control roots was found in the rape Hobson, which contained 28.84 mmol kg^{-1}. The lowest concentration of glucosinolates in control roots was in the swede Angela (4.58 mmol kg^{-1}). The glucosinolate concentrations of roots damaged by *D.radicum* larvae were not significantly different ($P>0.05$) from the roots of control plants in the rapes and the kales (Table B.5.2.). However, the glucosinolate concentrations of swede plants inoculated with *D.radicum* were all greater ($P<0.001$) than the control plants. The total glucosinolate content of control swedes ranged from 19.14 mmol kg^{-1} in GRL aga to 42.14 mmol kg^{-1} in Angus. The composition of the total glucosinolate content of the roots tested varied considerably between different genotypes and between control and insect-damaged roots.

Aliphatic glucosinolate content of Brassica roots

There were no significant differences ($P>0.05$) in the aliphatic glucosinolate concentration of the different crop types and genotypes tested (Table B.5.3.). The control plants contained a mean aliphatic glucosinolate concentration of 7.93 mmol kg^{-1}, 62.3% of the total glucosinolate content (Table B.5.4.). The concentration of aliphatic glucosinolates was highest in Hobson (23.52 mmol kg^{-1}) and lowest in Angela (2.76 mmol kg^{-1}). The concentration of aliphatic glucosinolates was consistently lower in the kales and rapes with *D.radicum*-damaged roots, but not significantly ($P>0.05$) lower. The roots of swedes which had been damaged by *D.radicum* contained significantly ($P<0.001$) elevated concentrations of aliphatic glucosinolates compared with controls (Table B.5.3.). As a percentage of the total glucosinolate content, aliphatic glucosinolates consistently fell ($P<0.001$) across all crop types. The percentage of total glucosinolates which were aliphatic in control plants was between 49.8% and 81.8%. In *D.radicum*-damaged plants, the percentage of glucosinolates which were aliphatic was between 27.6% and 63.9%.

Table B.5.1. The sequence of glucosinolate peaks eluted, their nature and their method of identification from the roots of Brassicas.

Peak	Nature	Name
Peak 1	Aliphatic	2-hydroxy-3-butenyl
Peak 2	Aliphatic	Prop-2-enyl
Peak 3	Aliphatic	4-methyl sulphinylbutyl*
Peak 4	Aliphatic	2-hydroxy pent-4-enyl
Peak 5	Aliphatic	But-3-enyl
Peak 6	Indole-Aromatic	4-hydroxy 3-indole methyl
Peak 7	Aliphatic	Pent-4-enyl
Peak 8	Aliphatic	4-methyl thiobutyl
Peak 9	Indole-Aromatic	3-indole methyl
Peak 10	Aromatic	Phenylethyl
Peak 11	Indole-Aromatic	1-methoxy 3-indole methyl
Peak 12	Unidentified peak	eluted at approximately 6 minutes

*Tentative identification only, based on published retention times under similar chromatographic conditions.

Table B.5.2. Variation in the total glucosinolate concentration (mmol kg^{-1}) square root transformed (untransformed data in brackets) of the roots of different Brassica genotypes damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	3.38 (11.45)	3.25 (10.60)	3.32 (11.03)
Kale	KLSCN	3.54 (12.54)	3.59 (12.93)	3.57 (12.73)
Rape	Ariana	3.24 (10.48)	4.51 (20.37)	3.87 (15.42)
Rape	Hobson	5.35 (28.84)	5.19 (26.94)	5.27 (27.84)
Swede	Angela	2.07 (4.58)	5.00 (25.04)	3.54 (14.81)
Swede	Angus	3.60 (13.47)	6.47 (42.14)	5.04 (27.80)
Swede	Doon Major	2.93 (8.59)	5.42 (29.59)	4.17 (19.09)
Swede	GRL aga	2.37 (5.75)	4.37 (19.14)	3.37 (12.44)
Swede	Marian	3.38 (11.41)	5.72 (32.72)	4.55 (22.06)
Total		3.32 (11.89)	4.84 (24.38)	4.08 (18.13)

INOCULATIONS, SED = 0.148, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.314, DF =6
INOC.GENOTYPE, SED = 0.444, DF =6

Table B.5.3. Variation in the concentration of aliphatic glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by the *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	2.39 (5.71)	2.03 (4.11)	2.21 (4.91)
Kale	KLSCN	2.84 (8.10)	2.43 (5.89)	2.63 (6.99)
Rape	Ariana	2.46 (6.07)	2.60 (6.88)	2.53 (6.47)
Rape	Hobson	4.83 (23.52)	4.14 (17.18)	4.49 (20.35)
Swede	Angela	1.60 (2.76)	3.25 (10.56)	2.42 (6.66)
Swede	Angus	2.84 (8.68)	4.19 (17.76)	3.52 (13.22)
Swede	Doon Major	2.41 (5.81)	3.56 (12.76)	2.99 (9.29)
Swede	GRL aga	1.68 (2.87)	2.30 (5.32)	1.99 (4.09)
Swede	Marian	2.80 (7.86)	3.33 (11.10)	3.07 (9.48)
Total		2.65 (7.93)	3.09 (10.17)	2.87 (9.05)

INOCULATIONS, SED = 0.132, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.279, DF =6
INOC.GENOTYPE, SED = 0.395, DF =6

Table B.5.4. Variation in the percentage of total glucosinolates in swede roots which had aliphatic sidechains by genotype and *D.radicum* damage.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	50.1	39.1	44.6
Kale	KLSCN	65.0	45.6	55.3
Rape	Ariana	58.0	33.2	45.6
Rape	Hobson	81.6	63.9	72.7
Swede	Angela	58.9	42.4	50.7
Swede	Angus	60.7	41.8	51.2
Swede	Doon Major	67.8	43.4	55.6
Swede	GRL aga	49.8	27.6	38.7
Swede	Marian	68.9	33.9	51.4
Total		62.3	41.2	51.8

INOCULATIONS, SED = 1.83, DF =1 residual D.F. = 17
GENOTYPE, SED = 3.89, DF =6
INOC.GENOTYPE, SED = 5.50, DF =6

Aromatic glucosinolate content of Brassica roots

The aromatic glucosinolate content of the control roots tested varied ($P<0.001$) from 1.83 mmol kg^{-1} in Angela to 5.22 mmol kg^{-1} in Hobson (Table B.5.5.). The control plants contained a mean aromatic glucosinolate concentration of 3.58 mmol kg^{-1}. For all the crop types tested, the concentration of aromatic glucosinolates was higher ($P<0.001$) in *D.radicum*-damaged roots. The aromatic glucosinolate content of *D.radicum*-damaged roots ranged from 4.78 mmol kg^{-1} in KHSCN to 24.38 mmol kg^{-1} in Angus. The mean aromatic glucosinolate content of plants inoculated with *D.radicum* was 13.98 mmol kg^{-1}. The percentage of total glucosinolates which had aromatic sidechains was inversely related to the percentage which had aliphatic sidechains.

Indole glucosinolate content of Brassica roots

The indole glucosinolate content of the roots of the Brassicas tested varied ($P<0.001$) both within and between crop types (Table B.5.6.). The indole glucosinolate content of control plants ranged from 0.69 mmol kg^{-1} (Angela) to 2.43 mmol kg^{-1} (Angus). The control plants contained a mean indole glucosinolate concentration of 1.17 mmol kg^{-1}, 12.3% of the total glucosinolate content (Table B.5.7.). The percentage of total glucosinolate of control plants which indole glucosinolates comprised ranged from 2.5% in Hobson to 20.7% in Angus. In all cases, the concentration of indole glucosinolates was higher ($P<0.001$) in *D.radicum*-damaged roots both in real terms and as a percentage of total glucosinolate content. The mean indole glucosinolate concentration of plants inoculated with *D.radicum* was 9.54 mmol kg^{-1}, 37.9% of the total glucosinolates. The percentage of total glucosinolates which had indole sidechains was significantly ($P<0.001$) higher for plants which had suffered *D.radicum* damage (Table B.5.7.).

Individual Aliphatic Glucosinolates

The concentration of 2-hydroxy-3-butenyl glucosinolate in the roots tested varied significantly ($P<0.001$) between crop types and genotypes (Table B.5.8.). The 2-hydroxy-3-butenyl glucosinolate content of control Brassicas ranged from 0.68 mmol kg^{-1} (KHSCN) to 14.36 mmol kg^{-1} (Hobson). The control Brassicas contained a mean 2-hydroxy-3-butenyl glucosinolate concentration of 3.64 mmol kg^{-1}. The mean 2-hydroxy-3-butenyl glucosinolate concentration of the control swedes (2.44 mmol kg^{-1}) was higher ($P<0.001$) than the kales (0.74 mmol kg^{-1}) and lower ($P<0.001$) than the rapes (7.43 mmol kg^{-1}). The 2-hydroxy-3-butenyl glucosinolate content of kale and rape roots was unaltered ($P>0.05$) by *D.radicum* damage. However, in swede roots *D.radicum* damage resulted in a rise ($P<0.001$) which left the roots containing between two and four times the concentration of 2-hydroxy-3-butenyl

Table B.5.5. Variation in the concentration of aromatic glucosinolates (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas when damaged by *D.radicum* larvaeand undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	2.00 (4.01)	2.18 (4.78)	2.09 (4.39)
Kale	KLSCN	1.63 (2.86)	2.65 (7.04)	2.14 (4.95)
Rape	Ariana	2.10 (4.41)	3.67 (13.48)	2.88 (8.94)
Rape	Hobson	2.28 (5.22)	3.11 (9.68)	2.69 (7.40)
Swede	Angela	1.32 (1.83)	3.80 (14.48)	2.56 (8.15)
Swede	Angus	2.18 (4.80)	4.93 (24.38)	3.56 (14.59)
Swede	Doon Major	1.66 (2.78)	4.05 (16.57)	2.86 (9.67)
Swede	GRL aga	1.68 (2.87)	3.71 (13.82)	2.70 (8.35)
Swede	Marian	1.86 (3.47)	4.65 (21.62)	3.26 (12.55)
Total		1.86 (3.58)	3.64 (13.98)	2.75 (8.78)

INOCULATIONS, SED = 0.101, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.213, DF =6
INOC.GENOTYPE, SED = 0.302, DF =6

Table B.5.6. Variation in the concentration of indole glucosinolates (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	1.06 (1.13)	1.63 (2.67)	1.34 (1.90)
Kale	KLSCN	1.11 (1.24)	2.00 (4.01)	1.56 (2.63)
Rape	Ariana	0.80 (0.64)	2.91 (8.52)	1.85 (4.58)
Rape	Hobson	0.84 (0.72)	2.34 (5.46)	1.59 (3.09)
Swede	Angela	0.80 (0.69)	3.01 (9.14)	1.91 (4.91)
Swede	Angus	1.56 (2.43)	4.15 (17.25)	2.85 (9.84)
Swede	Doon Major	1.04 (1.10)	3.43 (11.91)	2.24 (6.50)
Swede	GRL aga	1.10 (1.24)	3.21 (10.32)	2.16 (5.78)
Swede	Marian	1.16 (1.35)	4.07 (16.60)	2.62 (8.97)
Total		1.05 (1.17)	2.97 (9.54)	2.01 (5.36)

INOCULATIONS, SED = 0.078, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.165, DF =6
INOC.GENOTYPE, SED = 0.234, DF =6

Table B.5.7. Variation in the percentage of total glucosinolates which had indole sidechains, by genotype and *D.radicum* damage.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	9.9	25.0	17.4
Kale	KLSCN	10.0	31.1	20.5
Rape	Ariana	6.1	42.8	24.5
Rape	Hobson	2.5	20.3	11.4
Swede	Angela	15.3	36.3	25.8
Swede	Angus	20.7	41.1	30.9
Swede	Doon Major	12.6	40.0	26.3
Swede	GRL aga	21.3	54.2	37.8
Swede	Marian	11.8	50.6	31.2
Total		12.3	37.9	25.1

INOCULATIONS, SED = 1.37, DF =1 residual D.F. = 17
GENOTYPE, SED = 2.91, DF =6
INOC.GENOTYPE, SED = 4.11, DF =6

Table B.5.8. Variation in the concentration of 2-hydroxy-3-butenyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas and when damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	0.82 (0.68)	0.70 (0.50)	0.76 (0.59)
Kale	KLSCN	0.91 (0.84)	1.03 (1.06)	0.97 (0.95)
Rape	Ariana	1.67 (2.80)	1.93 (3.76)	1.80 (3.28)
Rape	Hobson	3.78 (14.36)	3.15 (9.90)	3.46 (12.13)
Swede	Angela	0.95 (0.98)	2.07 (4.30)	1.51 (2.64)
Swede	Angus	2.33 (5.88)	3.46 (12.08	2.89 (8.98)
Swede	Doon Major	1.41 (2.00)	2.43 (5.90)	1.92 (3.95)
Swede	GRL aga	1.14 (1.34)	1.55 (2.44)	1.35 (1.89)
Swede	Marian	1.98 (3.92)	2.58 (6.70)	2.28 (5.31)
Total		1.67 (3.64)	2.10 (5.18)	1.88 (4.41)

INOCULATIONS, SED = 0.010, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.212, DF =6
INOC.GENOTYPE, SED = 0.299, DF =6

glucosinolate. The mean 2-hydroxy-3-butenyl glucosinolate concentration of plants inoculated with *D.radicum* was 5.18 mmol kg^{-1}.

The prop-2-enyl glucosinolate content of control Brassicas varied from no detectable quantity in the swedes Angela and GRL aga, to 2.37 mmol kg^{-1} in KLSCN (Table B.5.9.). There were significant ($P<0.001$) differences in the prop-2-enyl glucosinolate content of the Brassicas tested both between and within crop types and in the response of genotypes to *D.radicum* damage. The control kales of KHSCN and KLSCN contained 1.94 and 2.37 mmol kg^{-1} of prop-2-enyl glucosinolate respectively. These concentrations were significantly higher ($P<0.001$) than those in the swedes and rapes and did not show any significant ($P>0.05$) response to *D.radicum* damage. The lower concentrations of prop-2-enyl glucosinolate in rapes and swedes responded differently ($P<0.001$). The rape genotypes were unaffected by *D.radicum* damage as was the swede GRL aga, in which prop-2-enyl glucosinolate was undetected in both control and inoculated plants. The concentration of prop-2-enyl glucosinolate was lower ($P<0.001$) in *D.radicum*-damaged roots of Doon Major and Marian and higher ($P<0.001$) in *D.radicum*-damaged roots of Angela and Angus.

The 4-methyl sulphinylbutyl glucosinolate content of the roots of the Brassicas tested varied significantly ($P<0.001$) between crop types and genotypes (Table B.5.10.). 4-methyl sulphinylbutyl glucosinolate was not detected in the controls of Doon Major, but was present in the roots of all other control Brassicas. The highest concentration of 4-methyl sulphinylbutyl glucosinolate in a control was 2.64 mmol kg^{-1} in KLSCN. *D.radicum* damage resulted in a rise ($P<0.001$) in the 4-methyl sulphinylbutyl glucosinolate content of the roots of all genotypes of swede. The mean 4-methyl sulphinylbutyl glucosinolate content of rapes was not significantly ($P>0.05$) changed by *D.radicum*. The swede genotypes tested all increased the concentration of 4-methyl sulphinylbutyl glucosinolate in inoculated roots.

The 2-hydroxy pent-4-enyl glucosinolate content of the roots tested varied significantly ($P<0.001$) between and within crop types (Table B.5.11.). The 2-hydroxy pent-4-enyl glucosinolate content of control roots was lowest in the kale KHSCN, 0.03 mmol kg^{-1}, and highest in the rape Hobson, 5.92 mmol kg^{-1}. The concentration of 2-hydroxy pent-4-enyl glucosinolate was higher ($P=0.015$) in some *D.radicum*-damaged roots whilst others were not significantly ($P>0.05$) affected. The genotypes in which the 2-hydroxy pent-4-enyl glucosinolate concentrations of inoculated plants were significantly higher were KLSCN, Angela, Doon Major and GRL aga.

Table B.5.9. Variation in the concentration of prop-2-enyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvaeand undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	1.39 (1.94)	1.32 (1.75)	1.36(1.84)
Kale	KLSCN	1.54 (2.37)	1.62 (2.62)	1.58(2.50)
Rape	Ariana	0.24 (0.06)	0.24 (0.06)	0.24(0.06)
Rape	Hobson	0.54 (0.29)	0.40 (0.16)	0.47(0.23)
Swede	Angela	n/d (n/d)	0.20 (0.04)	0.10(0.02)
Swede	Angus	0.27 (0.08)	0.45 (0.21)	0.36(0.14)
Swede	Doon Major	1.15 (1.32)	0.20 (0.04)	0.67(0.68)
Swede	GRL aga	n/d (n/d)	n/d (n/d)	n/d(n/d)
Swede	Marian	1.51 (2.29)	0.10 (0.02)	0.81(1.15)
Total		0.74(0.93)	0.50(0.54)	0.62(0.74)

INOCULATIONS, SED = 0.024, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.051, DF =6
INOC.GENOTYPE, SED = 0.073, DF =6
n/d means no detectable quantity of prop-2-enyl glucosinolate

Table B.5.10. Variation in the concentration of 4-methyl sulphinylbutyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	1.61 (2.61)	1.22 (1.49)	1.42 (2.05)
Kale	KLSCN	1.62 (2.64)	1.32 (1.76)	1.47 (2.20)
Rape	Ariana	0.62 (0.38)	0.66 (0.44)	0.64 (0.41)
Rape	Hobson	1.15 (1.32)	1.08 (1.16)	1.11 (1.24)
Swede	Angela	0.50 (0.26)	0.99 (0.98)	0.74 (0.62)
Swede	Angus	1.17 (1.37)	1.46 (2.12)	1.31 (1.75)
Swede	Doon Major	n/d (n/d)	1.16 (1.35)	0.58 (0.68)
Swede	GRL aga	0.59 (0.35)	0.89 (0.80)	0.74 (0.58)
Swede	Marian	0.19 (0.70)	0.61 (0.37)	0.40 (0.22)
Total		0.83 (1.00)	1.04 (1.16)	0.93 (1.08)

INOCULATIONS, SED = 0.043, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.091, DF =6
INOC.GENOTYPE, SED = 0.129, DF =6

Table B.5.11. Variation in the concentration of 2-hydroxy pent-4-enyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas and when damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	0.11 (0.03)	0.42 (0.19)	0.26 (0.11)
Kale	KLSCN	0.22 (0.05)	0.67 (0.45)	0.45 (0.25)
Rape	Ariana	1.17 (1.38)	1.34 (1.82)	1.26 (1.60)
Rape	Hobson	2.42 (5.92)	2.33 (5.41)	2.38 (5.67)
Swede	Angela	1.15 (1.40)	2.23 (4.97)	1.69 (3.18)
Swede	Angus	0.39 (0.16)	0.62 (0.40)	0.51 (0.28)
Swede	Doon Major	1.56 (2.45)	2.18 (4.83)	1.87 (3.64)
Swede	GRL aga	0.88 (0.77)	1.43 (2.05)	1.15 (1.41)
Swede	Marian	1.25 (1.58)	1.04 (1.19)	1.15 (1.38)
Total		1.02 (1.53)	1.36 (2.36)	1.19 (1.94)

INOCULATIONS, SED = 0.069, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.147, DF =6
INOC.GENOTYPE, SED = 0.208, DF =6

Table B.5.12. Variation in the concentration of but-3-enyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	0.66 (0.43)	0.42 (0.18)	0.54 (0.31)
Kale	KLSCN	0.73 (0.55)	n/d (n/d)	0.37 (0.27)
Rape	Ariana	0.64 (0.41)	n/d (n/d)	0.32 (0.20)
Rape	Hobson	0.62 (0.39)	0.15 (0.05)	0.39 (0.22)
Swede	Angela	0.15 (0.05)	0.22 (0.05)	0.19 (0.05)
Swede	Angus	0.55 (0.32)	0.34 (0.23)	0.44 (0.27)
Swede	Doon Major	n/d (n/d)	0.11 (0.03)	0.06 (0.01)
Swede	GRL aga	0.26 (0.07)	0.11 (0.03)	0.19 (0.05)
Swede	Marian	n/d (n/d)	n/d (n/d)	n/d (n/d)
Total		0.40 (0.24)	0.15 (0.06)	0.28 (0.15)

INOCULATIONS, SED = 0.050, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.107, DF =6
INOC.GENOTYPE, SED = 0.151, DF =6
n/d means no detectable quantity of but-3-enyl glucosinolate

But-3-enyl glucosinolate comprised no more than 0.55 mmol kg^{-1}, the concentration in the control roots of KLSCN. But-3-enyl glucosinolate varied between crop types (P<0.001) and within crop types (P=0.012) (Table B.5.12.). In the control roots of the swedes Marian and Doon Major, but-3-enyl glucosinolate was not detectable. The control plants of the rapes and kales contain higher (P<0.001) but-3-enyl glucosinolate concentrations than those plants which had *D.radicum*-damaged roots. The but-3-enyl glucosinolate content of swedes inoculated with *D.radicum* was not significantly (P>0.05) different from the control plants.

The pent-4-enyl glucosinolate content of the roots of the Brassicas tested varied significantly (P<0.001) between and within crop types and in the response of the different genotypes to damage (Table B.5.13.). The pent-4-enyl glucosinolate content of control plants was highest in Ariana (1.05 mmol kg^{-1}) and not detectable in the swedes Angus and Marian. The concentration of pent-4-enyl glucosinolate in Angus and Marian rose significantly (P<0.001) with damage. The concentration of pent-4-enyl glucosinolate in all other genotypes was not significantly (P>0.05) affected by *D.radicum* damage, although in GRL aga and the two kale genotypes it was undetected in inoculated roots.

4-methyl thiobutyl glucosinolate was only detected in six of the nine genotypes and in either the control or the inoculated plants of those genotypes, never both (Table B.5.14.). The control plants of KLSCN, Hobson and Angus contained 1.63, 0.34 and 0.87 mmol kg^{-1} of 4-methyl thiobutyl glucosinolate respectively. In these three genotypes, the reduction of the 4-methyl thiobutyl glucosinolate concentration to below detectable levels which took place following *D.radicum* damage was a significant (P=0.004) reduction. In the remaining six genotypes, there was no significant difference (P>0.05) between the control and *D.radicum*-damaged roots. However, following *D.radicum* damage, Ariana, Angela and Marian contained detectable quantities of 4-methyl thiobutyl glucosinolate.

Individual Aromatic Glucosinolates

The phenylethyl glucosinolate content of the roots of the Brassicas tested varied (P<0.001) between crop types and in the response of different crop types to *D.radicum* damage (Table B.5.15.). The phenylethyl glucosinolate content of control plants varied from 1.14 mmol kg^{-1}(Angus) to 3.77 mmol kg^{-1} (Ariana). There was no significant difference (P>0.05) in the concentration of phenylethyl glucosinolate between the control plants of the swedes and the kales, but the control rapes were higher (P=0.008) than swede and kale controls. The phenylethyl glucosinolate concentration of *D.radicum*-damaged roots was only significantly (P<0.05) different from the control plants in the case of the swedes. *D.radicum*-damaged roots

Table B.5.13. Variation in the concentration of pent-4-enyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	0.11 (0.03)	n/d (n/d)	0.06 (0.01)
Kale	KLSCN	0.11 (0.03)	n/d (n/d)	0.06 (0.01)
Rape	Ariana	1.02 (1.05)	0.62 (0.76)	0.82 (0.91)
Rape	Hobson	0.95 (0.91)	0.70 (0.50)	0.83 (0.70)
Swede	Angela	0.19 (0.07)	0.35 (0.12)	0.27 (0.10)
Swede	Angus	n/d (n/d)	1.62 (2.74)	0.81 (1.37)
Swede	Doon Major	0.22 (0.05)	0.73 (0.62)	0.48 (0.34)
Swede	GRL aga	0.41 (0.34)	n/d (n/d)	0.21 (0.17)
Swede	Marian	n/d (n/d)	1.65 (2.74)	0.82 (0.37)
Total		0.34 (0.27)	0.63 (0.83)	0.48 (0.55)

INOCULATIONS, SED = 0.100, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.211, DF =6
INOC.GENOTYPE, SED = 0.299, DF =6
n/d means no detectable quantity of pent-4-enyl glucosinolate

Table B.5.14. Variation in the concentration of 4-methyl thiobutyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvaeand undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	n/d (n/d)	n/d (n/d)	n/d (n/d)
Kale	KLSCN	1.25 (1.63)	n/d (n/d)	0.62 (0.82)
Rape	Ariana	n/d (n/d)	0.20 (0.04)	0.10 (0.02)
Rape	Hobson	0.57 (0.34)	n/d (n/d)	0.29 (0.17)
Swede	Angela	n/d (n/d)	0.23 (0.11)	0.12 (0.05)
Swede	Angus	0.75 (0.87)	n/d (n/d)	0.38 (0.44)
Swede	Doon Major	n/d (n/d)	n/d (n/d)	n/d (n/d)
Swede	GRL aga	n/d (n/d)	n/d (n/d)	n/d (n/d)
Swede	Marian	n/d (n/d)	0.21 (0.09)	0.10 (0.04)
Total		0.29 (0.32)	0.07 (0.03)	0.18 (0.17)

INOCULATIONS, SED = 0.080, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.170, DF =6
INOC.GENOTYPE, SED = 0.240, DF =6
n/d means no detectable quantity of 4 methyl thiobutyl glucosinolate concerned

Table B.5.15. Variation in the concentration of phenylethyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	1.70 (2.88)	1.45 (2.11)	1.57 (2.49)
Kale	KLSCN	0.99 (1.61)	1.74 (3.03)	1.37 (2.32)
Rape	Ariana	1.94 (3.77)	2.20 (4.96)	2.07 (4.36)
Rape	Hobson	2.12 (4.50)	2.05 (4.22)	2.08 (4.36)
Swede	Angela	1.04 (1.14)	2.31 (5.34)	1.68 (3.24)
Swede	Angus	1.53 (2.37)	2.67 (7.13)	2.10 (4.75)
Swede	Doon Major	1.30 (1.69)	2.15 (4.66)	1.72 (3.17)
Swede	GRL aga	1.27 (1.63)	1.87 (3.51)	1.57 (2.57)
Swede	Marian	1.46 (2.12)	2.24 (5.03)	1.85 (3.57)
Total		1.48 (2.41)	2.08 (4.44)	1.78 (3.43)

INOCULATIONS, SED = 0.105, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.222, DF =6
INOC.GENOTYPE, SED = 0.315, DF =6

Table B.5.16. Variation in the concentration of 4-hydroxy 3-indole methyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	n/d (n/d)	n/d (n/d)	n/d (n/d)
Kale	KLSCN	0.32 (0.12)	n/d (n/d)	0.16 (0.06)
Rape	Ariana	n/d (n/d)	0.07 (0.01)	0.04 (0.01)
Rape	Hobson	n/d (n/d)	n/d (n/d)	n/d (n/d)
Swede	Angela	0.12 (0.03)	0.12 (0.03)	0.12 (0.03)
Swede	Angus	n/d (n/d)	0.12 (0.03)	0.06 (0.02)
Swede	Doon Major	n/d (n/d)	n/d (n/d)	n/d (n/d)
Swede	GRL aga	0.26 (0.07)	n/d (n/d)	0.13 (0.04)
Swede	Marian	n/d (n/d)	n/d (n/d)	n/d (n/d)
Total		0.08 (0.02)	0.04 (0.01)	0.06 (0.02)

INOCULATIONS, SED = 0.029, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.061, DF =6
INOC.GENOTYPE, SED = 0.087, DF =6
n/d means no detectable quantity of 4-hydroxy 3-indole methyl glucosinolate

of swedes contained between 3.51 mmol kg^{-1} (GRL aga) and 7.13 mmol kg^{-1} (Angus).

Individual Indole-aromatic Glucosinolates

4-hydroxy 3-indole methyl glucosinolate was only found in low concentrations in the roots of five of the genotypes tested. There was no significant difference ($P>0.05$) in the concentration of this glucosinolate within or between genotypes and it was not significantly ($P>0.05$) affected by damage from *D.radicum*. No detectable level of 4-hydroxy 3-indole methyl glucosinolate was found in either control or inoculated roots of KHSCN, Hobson, Doon Major or Marian. The only genotype in which 4-hydroxy 3-indole methyl glucosinolate was found in both control and inoculated roots was Angela (Table B.5.16.).

The concentration of 3-indole methyl glucosinolate in the roots of the Brassicas tested varied significantly between ($P<0.001$) and within ($P=0.020$) crop types (Table B.5.17.). The 3-indole methyl glucosinolate content of control Brassicas varied from 0.05 mmol kg^{-1} (Hobson) to 0.62 mmol kg^{-1} (Angus). In all the genotypes tested, the effect of *D.radicum* damage was to raise the 3-indole methyl glucosinolate concentration. However, this effect was only significant ($P<0.001$) in the rape and the swede genotypes.

1-methoxy 3-indole methyl glucosinolate was the dominant aromatic glucosinolate in the roots of the Brassicas tested. The concentration of 1-methoxy 3-indole methyl glucosinolate in the control roots ranged from 0.48 mmol kg^{-1} (Angela) to 1.81 mmol kg^{-1} (Angus). The concentration of 1-methoxy 3-indole methyl glucosinolate varied significantly ($P<0.001$) both between and within crop types (Table B.5.18.). The concentration of 1-methoxy 3-indole methyl glucosinolate was higher ($P<0.001$) in *D.radicum*-damaged plants than in control plants. The proportionate rise in the concentration of 1-methoxy 3-indole methyl glucosinolate in inoculated roots was greater in Ariana, in which the roots damaged by *D.radicum* contained more than fifteen times the concentration of this glucosinolate found in the control roots.

Peak 12 (unidentified compound)

Peak 12 was eluted at approximately six minutes and, although it conformed to the general characteristics of a glucosinolate, the elution time did not correspond precisely with those published for glucosinolates. Peak 12 was detected only at low concentrations in the control plants of the kales, one swede genotype and one rape genotype. Hobson contained 0.10 mmol kg^{-1} and Marian 0.08 mmol kg^{-1} of Peak 12. The control kales contained higher ($P<0.001$) concentrations. KHSCN and KLSCN contained 1.74 and 1.58 mmol kg^{-1} respectively.

Table B.5.17. Variation in the concentration of 3-indole methyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	0.35 (0.12)	0.44 (0.19)	0.39 (0.16)
Kale	KLSCN	0.26 (0.07)	0.46 (0.21)	0.36 (0.14)
Rape	Ariana	0.39 (0.15)	0.85 (0.73)	0.62 (0.44)
Rape	Hobson	0.22 (0.05)	0.56 (0.32)	0.39 (0.19)
Swede	Angela	0.41 (0.18)	1.28 (1.64)	0.84 (1.91)
Swede	Angus	0.75 (0.62)	1.11 (1.24)	0.93 (1.93)
Swede	Doon Major	0.42 (0.19)	1.10 (1.25)	0.76 (1.72)
Swede	GRL aga	0.38 (0.15)	0.92 (0.84)	0.65 (0.50)
Swede	Marian	0.37 (0.14)	0.86 (0.76)	0.62 (0.45)
Total		0.40 (0.19)	0.84 (0.80)	0.62 (0.49)

INOCULATIONS, SED = 0.045, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.096, DF =6
INOC.GENOTYPE, SED = 0.135, DF =6

Table B.5.18. Variation in the concentration of 1-methoxy 3-indole methyl glucosinolate (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvaeand undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	1.00(1.01)	1.57 (2.48)	1.28 (1.75)
Kale	KLSCN	1.02(1.05)	1.95 (3.80)	1.48 (2.43)
Rape	Ariana	0.69(0.49)	2.78 (7.78)	1.74 (4.14)
Rape	Hobson	0.81(0.67)	2.27 (5.14)	1.54 (2.90)
Swede	Angela	0.68(0.48)	2.72 (7.47)	1.70 (3.97)
Swede	Angus	1.33(1.81)	3.99(15.98)	2.66 (8.90)
Swede	Doon Major	0.95(0.91)	3.25(10.66)	2.10 (5.78)
Swede	GRL aga	1.00(1.02)	3.08 (9.48)	2.04 (5.25)
Swede	Marian	1.10(1.21)	3.98(15.84)	2.54 (8.52)
Total		0.95(0.96)	2.84 (8.74)	1.90 (4.85)

INOCULATIONS, SED = 0.075, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.158, DF =6
INOC.GENOTYPE, SED = 0.223, DF =6

Table B.5.19. Variation in the concentration of peak 12 (mmol kg^{-1}) square root transformed (untransformed data in brackets) between different genotypes of Brassicas damaged by *D.radicum* larvae and undamaged.

Crop type	Genotype	Control	Inoculated	Mean
Kale	KHSCN	1.31 (1.74)	1.31 (1.72)	1.31 (1.73)
Kale	KLSCN	1.26 (1.58)	n/d (n/d)	0.63 (0.79)
Rape	Ariana	n/d (n/d)	0.10 (0.02)	0.05 (0.01)
Rape	Hobson	0.32 (0.10)	0.27 (0.08)	0.29 (0.09)
Swede	Angela	n/d (n/d)	n/d (n/d)	n/d (n/d)
Swede	Angus	n/d (n/d)	n/d (n/d)	n/d (n/d)
Swede	Doon Major	n/d (n/d)	0.51 (0.26)	0.25 (0.13)
Swede	GRL aga	n/d (n/d)	n/d (n/d)	n/d (n/d)
Swede	Marian	0.28 (0.08)	n/d (n/d)	0.14 (0.04)
Total		0.35 (0.39)	0.24 (0.23)	0.30 (0.31)

INOCULATIONS, SED = 0.027, DF =1 residual D.F. = 17
GENOTYPE, SED = 0.057, DF =6
INOC.GENOTYPE, SED = 0.081, DF =6
n/d means no detectable quantity of the glucosinolate concerned

DISCUSSION

The responses of the total glucosinolate content of different crop types to *D.radicum* damage were not consistent, rising in the swede genotypes and not changing in the kales and the rapes. The total glucosinolate content of *D.radicum*-damaged roots was reduced in Hobson and KHSCN but this was not statistically significant.

The changes in total glucosinolate composition of damaged Brassica roots were the result of the differing interactions of aliphatic and aromatic glucosinolates. In the kale and the rape genotypes, the aliphatic glucosinolate contents were not changed by *D.radicum* damage. However, in the swede genotypes, considerable increases took place in the aliphatic glucosinolate content when these plants were damaged by *D.radicum*. Aromatic glucosinolates, with or without an indole group, were considerably increased in all three crop types. The concentrations of individual glucosinolates varied considerably between different crop types and genotypes to produce the changes above.

The glucosinolate content of the control roots was hugely variable. Previous studies of plants at a similar growth stage (Birch *et al*, 1990; 1992) had demonstrated glucosinolate

concentration of a similar magnitude, but far less variability between control plants of the different crop types and genotypes. The concentration of glucosinolates in the swedes examined in Chapter B3 of this thesis was higher and more consistent than in this case.

Although eleven glucosinolates were detected, only five were present in both control and inoculation plants of all the genotypes tested. Of the four aromatic glucosinolates detected, three, phenylethyl, 3-indole methyl and 1-methoxy 3-indole methyl, were present in control and inoculation plants of all genotypes. The remaining aromatic glucosinolate, 4-hydroxy 3-indole methyl, was present only in very low concentrations in approximately half the genotypes of all crop types.

The seven aliphatic glucosinolates were less well distributed: 2-hydroxy-3-butenyl and 2-hydroxy pent-4-enyl glucosinolates were present in both control and inoculated plants of all genotypes. In addition, both rapes and both kales contained two glucosinolates in control and inoculated plants: prop-2-enyl and 4-methyl sulphinylbutyl.

The range of glucosinolates found was slightly less extensive than might have been expected from previously published work. Birch *et al* (1992) found 4-methoxy-3-indole methyl glucosinolate in a range of Brassicas which included both the rapes and the kales and two of the swedes tested in this experiment. In addition, 3-methylsulphinylpropyl glucosinolate was present in moderate concentrations in the roots of kales and 4-methylthiopropyl glucosinolate was present in the roots of all genotypes except one. 4-methylsulphinyl but-3-enyl glucosinolate, which has been shown to be present in swede and rape (Chapter B3; Birch *et al*, 1992), was not detected during this experiment. The very low concentrations of many of the glucosinolates combined with the failure to detect others which have previously been detected within the genotypes tested does raise some questions regarding the plants' glucosinolate metabolism. However, several of the glucosinolates detected were present in high concentrations. Therefore, it can be concluded that no deficiencies were preventing the general formation of glucosinolates. It was unfortunate that on this occasion the plants could not be inoculated with a much wider range of numbers of *D.radicum* eggs. A wider range of inoculation levels would have given clearer indications of the mechanisms which were interacting.

The questions of the variability in the results of this experiment and their difference from similar experiments are not helped by a lack of clarity as to the role of glucosinolates in plant metabolism. Clossais-Besnard & Larher (1991) concluded that glucosinolates are storage molecules for nitrogen, carbon and sulphur. The accumulation of glucosinolates at the

growing points of young seedlings during photosynthesis and biomass increase was especially marked for 2-hydroxy-3-butenyl glucosinolate (Clossais-Besnard & Larher, 1991). The rise in the root concentration of 2-hydroxy-3-butenyl glucosinolate in this study may be associated with the nature of *D.radicum* damage. The surface-oriented mining of *D.radicum* will lead to it damaging the active growing points of the plant in which Clossais-Besnard & Larher (1991) found that 2-hydroxy-3-butenyl glucosinolate accumulated. Such direct damage to the accumulation point may be leading to the formation of scar and callous tissue containing elevated levels of 2-hydroxy-3-butenyl glucosinolate.

D.radicum is a specialist Brassica feeder, and such insects are frequently stimulated to feed by the presence of glucosinolates (Städler, 1992). The interactions between phytophagous insects and host plant secondary metabolites are not fully understood. Knowledge of the metabolism of glucosinolates is fairly complete (Chew, 1988). However, the perception of glucosinolates by the larvae of *D.radicum* has not been studied although the larvae are known to orientate to the volatile hydrolysis products of glucosinolates (Finch & Skinner, 1974; Koštál, 1992). The function of many secondary plant metabolites remains open to debate and is often little understood. What is becoming clear is that the concentrations of secondary plant metabolites influence feeding larvae of many phytophagous insects.

The range of susceptibility to *D.radicum* in the genotypes tested was wide, but the role played by glucosinolates remains unclear. The variation in glucosinolates did not coincide directly with susceptibility. It is not ideal to attempt to discover the relationships between glucosinolates and feeding comparing the glucosinolates in plants with damage. The changes which happen are very complicated, and consequently to associate a constantly changing factor with the biological result is difficult. Electrophysiological techniques could provide essential data as to what can be detected by the larvae and a link to the use of artificial diets could then confirm the concentrations at which the effect of glucosinolates are stimulatory. The responses which took place to *D.radicum* damage are not typical of the responses which have been induced by other insects and artificial damage.

SECTION B

SECTION DISCUSSION

D.floralis usually mines deeply within the root whilst *D.radicum* feeds in shallower mines. A consequence of the different feeding strategies which have been adopted by *D.floralis* and *D.radicum* is that, even if the dietary requirements of *D.radicum* and *D.floralis* are identical, the effect of different genotypes may not be. The Brassicas tested in this section have exhibited a range of tolerance and antibiotic resistance to both *D.radicum* and *D.floralis*. The forage rape, Hobson, and the kale genotypes were poor host plants, whilst the oilseed rape, Ariana, and the swedes were good hosts for the development of *D.radicum* and *D.floralis*. It has been established for a considerable time that *D.radicum* and *D.floralis* larvae develop well on swede (Read, 1960; Varis, 1958). Amongst the swede genotypes tested in this section for resistance, GRL aga has demonstrated some resistance to both species of fly. GRL aga was shown to possess both antixenotic and antibiotic resistance to *D.floralis* in field cage experiments (Birch, 1989), but was susceptible to oviposition by first generation *D.radicum* in field experiments (Wilson *et al*, 1990). A gravid female insect will lower the threshold for oviposition as the period since last oviposition increases (Jaenike & Papaj, 1992), an aspect which may weaken the effect of antixenotic resistance used in monoculture. Consequently, the properties of the larval feeding substrate will always remain important and the potential reduction in fecundity associated with reduced pupal weight (Finch & Coaker, 1968) may also contribute to controlling *D.radicum.*

The size of the food resource available to *D.radicum* and *D.floralis* larvae did vary widely. However, the relative sizes of roots with differing susceptibilities indicated that competition for food resources was not the cause of differing pupal survival and size. The FDM of the roots was also unrelated to resistance to *D.floralis*, contradicting published findings (Shaw, 1982; 1984; 1985; 1993; Birch, 1988). However, a relationship was demonstrated between *D.radicum* pupation and the FDM of the Brassica roots tested. Clearly, this relationship merits further investigation which may be best performed using an artificial diet. FDM of Brassica roots was unaffected by *D.floralis* damage and *D.radicum* only raised FDM in the kales. The variation in pupal weight with genotype must be attributed to variation in food quality or food intake by developing larvae. The chemical composition of root material dictates the dietary intake of the insect by both its influence on the extent to which a larva feeds and the limitations placed on the development of the larva by the composition of the food eaten. The former characteristic is a combination of the effect of phagostimulants and deterrents influencing the attempts to feed by the larva and the physical characteristics of the

root, hardness and other factors influencing intake. Consequently, the interactions which take place between a feeding larva and the root of the plant are complicated. Individual compounds may play a dual role in the relationship, acting as both a phagostimulant and an aspect of dietary limitations. Rygg & Sömme (1972) noted differences in the percentage of *D.floralis* larvae surviving to pupate on different hosts. They concluded that differences in the chemical composition of the roots led to a variation in the extent to which larvae were stimulated to penetrate the root. Resistance to *D.radicum* in swede has also been attributed to the rate of establishment of root feeding by first instar larvae (Swailes, 1960). In both the above cited cases, the authors concluded that the early stages of larval development were critical and it is likely the stimulation of a larva to feed and the provision of easily available energy for the larva make sugars influential in this stage. The reduction in the concentration of sugars (found in this Section) is, undoubtedly, partly the result of a diversion of glucose to cellulose production. However, it is also possible that larvae may be selectively feeding on the parts of the root which contain high levels of certain compounds. The different feeding habits of the two species may well explain the differences in the responses of some Brassicas to the damage resulting from feeding larvae. The marked rise in fibre content may be the cause of the decrease which took place in the concentrations of fructose and glucose in damaged roots. *D.radicum* and *D.floralis* both generally reduced sugar content after larval damage which may be the product of selective feeding or of adversely affected production by the reallocation of resources owing to the damage being sustained by the plant. Sugars are a major source of non-structural carbohydrate in plants and the concentrations of non-structural carbohydrate are reduced in radish exposed to multiple stresses (Pell *et al*, 1990). The specific response to root fly damage is impossible to separate from general responses to stress.

Cellulose, a polymer of glucose, is a major constituent of plant cell walls and indigestible by the majority of insects (Fonty & Gouet, 1989; Prins & Kreulen, 1991). Lignification of cell walls has also been identified as a polygenic resistance mechanism which protects plants against a large number of potential pathogens and pests (Wood, 1982). Larval stages of some insect pests have had their development impaired by increased levels of fibre (Agarwal, 1969; Hedin *et al*, 1984) and others have caused an increase in the fibre content of alfalfa (Hutchins *et al*, 1989). The mean pupal mass and percentage pupation of *D.floralis* were not related to the fibre content of the Brassica genotypes tested. The differing susceptibilities of the two rape genotypes could not be explained by differences in fibre content of their control roots nor by their responses to root damage. However, the fibre content was elevated in *D.floralis*-damaged roots and the resistance demonstrated in Hobson was coincident with a pronounced lignification following *D.floralis* damage. Staining of inoculated roots has demonstrated that lignification takes place in the area immediately around *D.floralis* feeding sites (Hopkins,

unpublished observations). The selection of genotypes which were capable of lignifying a root wound site is potentially of great use. However, the value of such a mechanism might be restricted by the adverse effects of lignification on plant metabolism and on the crop quality of plants for which the root is the edible part. For plants where the aerial parts are to be harvested, a penalty in yield resulting from changes in the metabolism following lignification may be the only adverse effect. If this proves to be the case, the yield penalty may well be offset by reductions in production associated with reduced pesticide application costs.

Insects will not initiate feeding unless they receive appropriate stimulation from the substrate (Bernays & Simpson, 1982) and failure to initiate feeding has been noted as a mechanism of resistance against both *D.floralis* (Rygg & Sömme, 1972) and *D.radicum* (Swailes, 1968). Sugars are widespread insect phagostimulants (Bernays & Simpson, 1982) and have a phagostimulatory effect on the larvae of some Anthomyiid flies (Mochizuki *et al*, 1985; Honda & Ishikawa, 1987). *D.radicum* larvae possess at least three groups of chemoreceptors which may have a gustatory function (Ryan & Behan, 1973). Consequently, the extent to which insects are stimulated to feed may vary with changes in the sugar concentration in feeding substrates. Within swede plants, genotypic variation in sugar concentrations was linked to the larval development of *D.floralis,* possibly through an increase in phagostimulation. The close inter-relationship between individual and total sugars makes differentiation of the effects of each sugar on root fly development difficult. To have a certain answer to the part which is played by different sugars in *D.radicum* and *D.floralis* development, it would be necessary to use an artificial diet.

There has been considerable work on the relationship between adult *D.radicum* and glucosinolates (Wallbank & Wheatley, 1979; Ellis *et al*, 1980; Nottingham & Coaker, 1985; Tuttle *et al*, 1988; Roessingh *et al,* 1992a; 1992b). In addition, *D.radicum* larvae exhibited positive and negative taxis to different concentrations of allyl and ethyl isothiocyanate (Finch & Skinner, 1974; Koštál, 1992). The role of glucosinolates and their breakdown products is not restricted to effects on cabbage root fly, but is widespread throughout Crucifer-feeding and polyphagous insects (Bartlet & Williams, 1991; Bodnaryk, 1991; Traynier & Truscott 1991; Städler, 1992). It is possible to find good correlations between glucosinolates and the feeding damage of Brassica-feeding insects. However, the association between glucosinolates and *D.floralis* and *D.radicum* feeding is far from clear. Both in this study and previously (Birch *et al*, 1990; 1992), the glucosinolate concentrations of roots of a range of Brassicas were not consistently associated with either resistance or susceptibility to attack by *D.radicum* and *D.floralis*.

However, feeding damage caused by *D.radicum* and *D.floralis* did result in widespread changes to the glucosinolate composition of the roots. The concentrations of individual glucosinolates and their relative proportions were dramatically altered by the damage of both species. The damage of both *D.floralis* and *D.radicum* resulted in large rises in the concentrations of indole glucosinolates. In *D.floralis*-damaged swede roots, a fall in the concentration of aliphatic glucosinolates was found which was not repeated in the roots damaged by *D.radicum*. Glucosinolates, like many secondary plant compounds, appear to stimulate Crucifer-feeding insects whilst deterring others. It is probable that changes in the glucosinolate composition of plants can alter the host status of a plant (Birch *et al*, 1992). Indole glucosinolates act as oviposition or feeding stimulants to a wide range of Brassica-feeding insects (Chew, 1988; Städler, 1992). In addition, they have structure similarities to the indole phytoalexins. Phytoalexins are low molecular weight compounds which possess anti-microbial activity and are synthesised *de novo* following attack by pathogens. Recent research has shown that they are widespread within the Brassicae and that their abiotic elicitation can be linked to disease resistance (Rouxel *et al*, 1991).

Study of the Angiosperms has isolated more than 20,000 individual secondary plant metabolites, the diversity of which within individual species is constrained by abiotic factors and biotic interactions (Berenbaum & Seigler, 1992). The metabolism, function and perception of plant secondary metabolites by herbivores are only partially understood. However, the role of glucosinolates as mediators of host specificity is widely accepted although frequently constrained by other factors (Louda & Mole, 1992). The complicated interactions of glucosinolates partly explains the uncertainty of their function. Dicke & Sabelis (1992) point out that the costs and benefits of a specific trait are not constant and, consequently, the function of a chemical within a specific interaction is difficult to assess. What is becoming clear is that whatever evolutionary reasons for the primary function of glucosinolates within plants, their concentrations in host plants influence phytophagous insects and are influenced by them. From the evidence presented, it is not possible to be certain if the changes in glucosinolates are defensive or merely a by-product of repairing damage to the plant tissue.

The agricultural consequences of the changes in the chemical composition of Brassica roots on crop quality are quite diverse. Changes in the sugar content of Brassicas may reduce their nutritive value to livestock and humans. The acknowledged goitrogenicity, mammalian toxicity and flavour taint of glucosinolates mean that changes in their concentration may result in palatability and physiological problems (Fenwick *et al*, 1983; Darroch *et al*, 1991; Boag *et al*, 1990; Duncan & Milne, 1992; Huisman & Tolman, 1992). However, the concentrations of

sugars and glucosinolates are both highly variable throughout the growing season. The long term effect of changes in sugar and glucosinolate concentration due to insect damage have not been studied, and may be important to the nutritional status of the crop. The implication for the use of field trials to assess the effect of plant chemicals on pests may be considerable. Previous work on *D.floralis* has attributed the extent of the root damage to chemical composition at harvest (Shaw, 1982; 1984; 1985; 1993). However, oviposition varies widely with plant genotype and insect damage changes the chemical composition of the plant. If the changes brought about are enduring, it may be misleading to predict susceptibility to damage based solely on the chemical composition of plants at harvest.

The role of other plant compounds in the nutrition of *D.radicum* and *D.floralis* merits further investigation. Amino acids are frequently the limiting factor in insect development (Young, 1991). Larvae of the onion maggot, *D.antiqua* (Diptera:Anthomyiidae), can develop on an onion agar which has been supplemented with 10 essential amino acids. The omission of all the amino acids from the mixture prevented development from taking place whilst the omission of single amino acids resulted in less marked effects (Eyman & Friend, 1985). The effects demonstrated with *D.radicum* and *D.floralis* are clearly only a part of a much wider picture and cannot be fully appreciated without knowledge of the proteins and amino acids which are present in the roots of Brassicas and which of them are essential to the development of root fly larvae.

The relationship between root fly damage and the chemical composition of the root is more clear cut than is the relationship between the chemical composition of the root and the development of the root flies. There is clearly a degree of interdependent regulation between the chemical composition of the roots of swede and the feeding of *D.radicum* and *D.floralis*. The feeding undoubtedly varies with the swede chemical composition which in turn influences the feeding. Further work is needed to study the distribution of different compounds in the roots of Brassicas and their effects on larval feeding and development. Further investigations would clearly benefit from an artificial diet for *D.radicum* and *D.floralis* to allow separation of the two effects.

The partial antibiotic resistance to *D.radicum* and *D.floralis* demonstrated may serve to complement other mechanisms of resistance or tolerance. The combination of a number of mechanisms may reduce insecticide requirements for Brassica crops and reduce selection pressure for insecticide resistance. McKinlay & Birch (1992) and Taksdal (1992) found that combining reduced pesticide applications with resistant genotypes could give adequate field control against *D.radicum* and *D.floralis* . GRL aga has shown antixenotic and antibiotic

resistance to both pests when contrasted with other swede genotypes. The improvement in resistance which GRL aga can contribute to this relationship may lead to further reductions in the amount of pesticide required to give field control.

SECTION C.

FIELD BASED STUDIES OF MECHANISMS OF HOST PLANT RESISTANCE TO TURNIP AND CABBAGE ROOT FLIES

SECTION INTRODUCTION.

Field based studies provide a valuable method for assessing the relative influence of different mechanisms of resistance operating simultaneously. Glasshouse and laboratory studies can be used effectively to assess the variation of a small number of factors under controlled environmental conditions. However, glasshouse and laboratory studies do not adapt well to the investigation of the complex interactions between mechanisms of resistance and other factors. In the field, the action of a mechanism of resistance can be investigated whilst subject to biotic and abiotic interactions. Accurate field observation is the only means of assessment likely to reveal the more subtle interactions that are likely to take place when a resistant genotype is used in the field. A mechanism of resistance is only of practical value if it can be successfully transferred to the field and retain its mode of action under a range of environmental conditions. Ultimately, resistant genotypes must be tested with field experiments.

Field-based experiments have been carried out for a number of years against both *Delia radicum* and *Delia floralis*. Rygg & Sömme (1972) concluded that differences in the field resistance to *D.floralis* of swedes and turnips were due to differences in oviposition, but they also noted differences in the percentage of larvae successfully developing to pupate on different hosts. They concluded that differences in the chemical composition of the roots led to a variation in the extent to which larvae were stimulated to penetrate the root. They also concluded that variations in the root form, length and size could affect the success of larvae migrating to the root tip before penetrating the root near the growing tip. Resistance to *D.radicum* in swede has also been studied (Swailes, 1959). Survival rates of *D.radicum* were attributed to the rate of establishment of root feeding by first instar larvae (Swailes, 1960). Varis (1958) demonstrated resistance to *D.floralis* in the big leafed turnip, but did not categorise the nature of the resistance. Levels of partial resistance to *D.floralis* in swede have been confirmed and attributed to root dry matter (Shaw, 1982; 1984; 1985; 1993; Birch, 1988). Larval feeding on some resistant swede genotypes is restricted to relatively shallow mining (Birch, 1988). This type of restricted larval feeding correlates with root dry matter content, but is unrelated to tissue hardness.

Previous work has been carried out in the field on the interactions which take place between

D.radicum and *D.floralis* and plant genotype, prevailing weather conditions, plant age and other insect species. The effect of different plant genotypes as hosts of both *D.radicum* and *D.floralis* is well documented and is discussed elsewhere within this thesis. However, the density of plants is also influential on the lifecycle of *D.radicum* and variation in plant diversity will influence the lifecycle of *D.radicum* and *D.floralis* through its effect on insect diversity. The development of *D.radicum* in the field is influenced by temperature, which dictates the occurrence of diapause and the process of diapause development (Collier & Finch, 1983), and by its effect on insect flying, will dictate oviposition. Ellis *et al* (1980) found that the attractiveness of radish varied considerably with the age of the plant under test. The variations in the actual and physiological age of a plant in the field may be considerable in different growing areas and between different seasons. Finch & Jones (1988) concluded that *D.radicum* host selection was considerably influenced by the competition of other Crucifer-feeding insects. They demonstrated that the effect of frass from different insect species could be deterrent, neutral or stimulatory to the host selection process of *D.radicum*. Consequently, in a field situation, the effect of host selection on other species could influence host selection by *D.radicum* or *D.floralis*. Host selection by either root fly species could be more strongly influenced by the presence or absence of other pest species than by the direct effect of variation in genotype.

The primary aim of the field experimentation was to discover which of the mechanisms of resistance to *D.radicum* and *D.floralis*, antixenosis, antibiosis or tolerance, are active in the field. Secondly, field experiments aimed to discover which of the mechanisms studied individually in the laboratory was dominant in the field and to what extent resistance to *D.radicum* and *D.floralis* was expressed under field conditions.

GENERAL METHODS

Oviposition monitoring

The number of eggs laid by flies was monitored using the marked plant method (Rygg & Sömme, 1972). In the 1990 and 1991 field experiments, plots were composed of four rows of plants. The outer two rows in each plot were used as guard rows within the plot and were not monitored. Within the central two rows, individual plants were marked, using a white plastic plant label placed in the soil 15-25cm away from the plant. At set time intervals, the soil 4cm around marked plants was removed to a depth of 1cm using a spoon, bulked for the row and returned to the laboratory where it was stored in a refrigerator (temp 4°C) until an egg count could be carried out. Soil taken during sampling was replaced with egg-free soil collected

from the field earlier in the year and cleaned of eggs in laboratory. Eggs were separated from the soil by flotation using water, sieved (mesh size 0.2mm) and counted. The egg numbers per sample are presented as *D.radicum* and *D.floralis*. The type of damage and pupae identification may indicate that the oviposition is dominated by one species, but it would be wrong to assume so as eggs are very similar and some pupae cannot be identified to species.

Assessment of Root fly damage

The 1990 and 1991 experiments were both harvested in November, when plants in the central two rows which had not been used for oviposition monitoring were hand-lifted and washed. Roots were scored visually for external damage, and quartered longitudinally to mark the presence or absence of internal damage. The score for external damage on roots was on a scale of zero to 4, similar to I.O.B.C. standard methods (Thompson, pers. comm. [to A.N.E. Birch]) and Jörgensen (Jörgensen, 1957) index scores were calculated. The 50% to 100% damage score category used in the above indexes was divided to improve the accuracy of the scoring. For purposes of scoring, a root was placed into one of the five categories below:

0: undamaged
1: less than 25% of root area damaged
2: 25-50% of root area damaged
3: more than 50% of root area damaged
4: more than 75% of root area damaged

A mean root score for each genotype could then be calculated by summing the scores for each root and dividing by the number of roots. Root damage scores were also used to calculate a Jörgensen index score for each genotype (Jörgensen, 1957). A Jörgensen index score is based upon the percentage of all roots of each genotype which fall into certain categories. For the purposes of scoring a Jörgensen type index, the numbers of roots in categories 3 and 4 were combined. Hence, each root was placed into one of four categories :

A: undamaged
B: less than 25% of root area damaged
C: 25-50% of root area damaged
D: more than 50% of root area damaged

The percentage of roots falling into each of the above categories was then calculated and a Jörgensen index score produced for each genotype according to the formula :

$$\text{Index} = 100 - \frac{(3A + 2B + 1C + 0D)}{3}$$

The Jörgensen index is a score based upon the percentage of plants falling into each category across a whole experiment. Because the score is produced from the results across the whole experiment, and not on individual plots, it is not suitable for statistical analysis because it is unreplicated. The use of different scoring systems for the measurement of *D.radicum* damage has been shown to have no effect on the ranking of genotypes under test (Soni, pers. comm. [to A.N.E. Birch])

For the purposes of this study, it is assumed that cabbage root fly damage is characterised by shallow surface mines which do not penetrate beyond the cortex. It is also assumed that deep penetrating feeding mines which formed tunnels entering the tissue beneath the cortex characterise the damage caused by the turnip root fly. Birch (1988) demonstrated that *D.floralis* may produce shallow mines on some plant genotypes and the identification of the species causing damage was supported by identifying the species of pupae collected at the time of harvesting the field experiment.

Chapter C1

1990 Field Experiment

Resistance to *Delia radicum* and *Delia floralis* in swede (*Brassica napus* ssp. *rapifera*)

INTRODUCTION

In agricultural terms, the importance to swede of damage by *D.radicum* and *D.floralis* can be particularly high. Many of the Crucifers utilised in horticulture are grown for the aerial part of the plant: cabbage and cauliflower are good examples. Other Crucifers, swede, turnip and radish amongst them, are grown for the root. Whilst damage to the root of those which are grown for the aerial part may reduce yield, damage to a root which is for human consumption may make the crop unmarketable. Consequently, mechanisms of resistance to root feeding pests are of particular importance to root crops, and swede has long been recognised as a good host for *D.radicum* (Read, 1960). Varis (1958) demonstrated that different genotypes of the big-leafed turnip (*Brassica rapa* ssp *rapifera*) varied in their naturally occurring susceptibility to both oviposition and damage by *D.radicum* and *D.floralis*. The swede genotype cv Wilhelmsberger showed consistent resistance to damage, and produced the fewest pupae in all tests. However, levels of oviposition on Wilhelmsberger were at no time particularly low. More recently, Birch (1988) tested swede genotypes against *D.radicum* and *D.floralis* under both field and glasshouse conditions, demonstrating that oviposition on the susceptible genotype, Doon Major, was between four and eight times greater than on the resistant genotype, Angus. Birch (1988) concluded that antixenosis was the major component in resistance to the root flies, *D.floralis* and *D.radicum*, in the field. Swedes have also been tested in field cage experiments in which the density of the turnip root fly was artificially raised at the site of the experiment (Birch, 1989b). The site of the field experiment was enclosed within a frame covered in "Tygan" netting and *D.floralis* released within the field cage. Using this method, cv Doon Major was again shown to be highly susceptible to turnip root fly damage and the swede breeding line GRL aga was found to possess antixenotic resistance to *D.floralis* (Birch, 1989b). The published results of field experiments to assess the susceptibility of different plant genotypes to *D.radicum* and *D.floralis* have been extensively reviewed in previous chapters. Resistance to both species has been previously attributed to both antixenosis and antibiosis or to a combination of the two mechanisms.

The aim of the 1990 field experiment was to differentiate between the susceptibility to oviposition on and the resultant damage to ten genotypes of swede (*Brassica napus* ssp. *rapifera*) by *D.radicum* and *D.floralis*.

MATERIALS AND METHODS

Plant Material

Ten swede genotypes, including seven commercial cultivars, Angela, Angus, Doon Major, Marian, Määtta, Melfort, Sator Otofte and Vige, and three Scottish Crop Research Institute breeding lines, GRL aga, M4M2 a-c and Nmm3 a-d, were chosen to represent a range of resistance to *Delia* spp.. Angus, and to a lesser extent Melfort, have exhibited partial resistance to turnip and cabbage root flies in a number of field experiments (Birch, 1988; Ruuth, 1988; Shaw, 1982; 1984; 1985; 1993; Wilson *et al*, 1990) and also in field cage experiments (Birch, 1989b). Vige and Määtta have also exhibited resistance to the turnip root fly in field experiments in Scandinavia (Ruuth, 1988), the former genotype also showing resistance to the turnip root fly in field cage experiments (Birch, 1989b). Doon Major and Sator Otofte are used as standard susceptible genotypes in experiments against both the turnip root fly and the cabbage root fly (Birch, 1988; 1989b; Ruuth, 1988; Wilson *et al*, 1990). Marian and derived lines (M4M2 a-c and Nmm3 a-d) had shown tolerance to cabbage root fly damage at the seedling stage (Wilson *et al*, 1990), and GRL aga had been shown to be resistant to turnip root fly in field cage experiments (Birch, 1989b), but susceptible to first generation cabbage root fly (Wilson *et al*, 1990). In the field cage experiments, GRL aga had the lowest oviposition, pupal numbers per plant and root damage index, and also the highest percentage of roots falling into marketable categories.

Experimental site

The experiment was carried out at Goshen Farm, Musselburgh, East Lothian, courtesy of Mr D. Chalmers. The site is approximately 25m above sea level with a very slight northern aspect. The soil was a brown sandy loam (soils in the area are a mixture of brown calcareous and brown forest which are derived from carboniferous sediments). The results of a soil analysis from the site are shown in Table C.1.1.. The soil nutrient status of the site was adequate for the growing of swedes, consequently, no additional fertiliser was added. The sandy nature of the soil was suited to root fly oviposition monitoring. No herbicide was applied as it was deemed that this might have an unknown influence on the chemistry of the swede genotypes.

Experimental design

Five replicates of each genotype were sown in a row and column design, produced by T.Hunter of the Scottish Agricultural Statistics Service. Each plot consisted of four rows 5 metres long and 70 cm apart. Seed was hand sown on the 9th and 10th of May 1990. The plots were hand thinned (average of five plants per metre) on the 12th and 13th of June 1990.

Analysis	Status
pH	7.0
Extractable Ions	
Phosphorus	High
Potassium	Moderate
Magnesium	High
Sulphur	Low
Manganese	Moderate
Copper	High
Zinc	High
Boron	High

Table C.1.1. Soil nutrient status of the 1990 field experiment site.

Oviposition sampling

On the 9th of July, the plants in each plot which were to be used for monitoring oviposition on the trial site were marked with a plastic plant label. The soil from around the base of the marked plants was removed and replaced four days before monitoring commenced. This was to ensure that the sample taken on the first occasion was consistent, in terms of disturbance and oviposition period, with those taken on subsequent dates. Monitoring took place every five days throughout the season, starting on the 13th of July and finishing when egg numbers diminished on 17th August.

Damage scoring

The field experiment was harvested on 19th November 1990. Twenty roots were taken at random from the centre two rows of each plot. Roots which had been used for oviposition monitoring were not used to assess root damage. On the same day that roots were harvested, five soil cores were taken from each plot. Soil cores (15cm deep, 15cm diameter) were taken from directly beneath a plant in the centre two rows of a plot which had not been used for oviposition monitoring and pupae were extracted as in Section C, General. Harvested roots were then washed and scored visually for external and internal damage as outlined in Section C, General. The roots which sustained less than 50% surface area damage were regarded as those which, with trimming, could be marketable. This figure is derived by summing the numbers of roots which were assessed as being in scoring categories 0, 1 and 2 of the initial

scoring scheme.

Statistical Analysis

An analysis of variance was performed on the data using Genstat (Genstat, Sun/Unix version 5.0, release 2.2, 1990). The variance of the residuals increased with the mean, and the data were square root transformed and reanalysed using analysis of variance on Genstat.

RESULTS

Oviposition

The numbers of *D.radicum* and *D.floralis* eggs which were laid on the marked plants of each genotype were monitored at five-day intervals on eight occasions between 13th July 1990 and 17th August 1990. On seven occasions, there was no significant difference ($p>0.05$) in the numbers of eggs which were laid on different genotypes. The exception was the sample taken on 28th July 1990 when the numbers of eggs laid on different genotypes over the preceeding five days were significantly different ($p=0.043$). On this monitoring date, the highest levels were recorded on cv Sator Otofte which received 5.38 eggs per sample and the lowest was cv M4M2 a-c which received 1.82 eggs per sample (Table C.1.2.).

Total *D.radicum* and *D.floralis* oviposition over the monitored season was not significantly different between genotypes ($p>0.05$), although the probability level achieved ($p=0.064$) suggested that there may have been a trend in the results (Table C.1.2.). The trend which was developing is best illustrated in a graph of the cumulative totals for oviposition on different genotypes (Figure C.1.1.). Oviposition remained consistently below the overall mean on Nmm3 a-d, GRL aga, M4M2 a-c, Määtta, Angela and Marian. Sator Otofte and Doon Major were clearly the most susceptible to oviposition.

Root Damage

The percentages of plants which were undamaged by the cabbage root fly or had cabbage root fly damage which could be trimmed were significantly different between genotypes ($p=0.018$). Identification of pupae confirmed that *D.radicum* was the dominant species at the field experiment site. The roots falling into the categories of undamaged and damage categories 1 and 2 were combined and classified as marketable. The genotype with the highest percentage of marketable bulbs was cv Vige at 100% (Table C.1.3.). The genotype which had the lowest percentage of marketable bulbs was SCRI breeding line M4M2 a-c, at 88% (Table C.1.3.).

TABLE C.1.2. *D.radicum* oviposition (square root transformed) mean levels of cabbage root fly on different swede genotypes. Number of eggs on the date 28th July 1990, and total number of eggs for all monitoring dates.

Swede Genotype	28/7/1990	Total
Sator Otofte	2.32 (5.38)	5.94 (35.28)
Doon Major	2.23 (4.97)	5.50 (30.25)
Angus	2.15 (4.62)	5.08 (25.81)
Vige	1.48 (2.19)	4.93 (24.30)
Angela	1.67 (2.79)	4.42 (19.54)
Nmm 3a-d	1.89 (3.57)	4.26 (18.15)
GRL aga	1.80 (3.24)	4.20 (17.64)
M4M2 a-c	1.35 (1.82)	4.20 (17.64)
Marian	1.39 (1.93)	4.13 (17.06)
Määtta	1.51 (2.28)	3.71 (13.76)
SED	0.34	0.69
DF	9	9

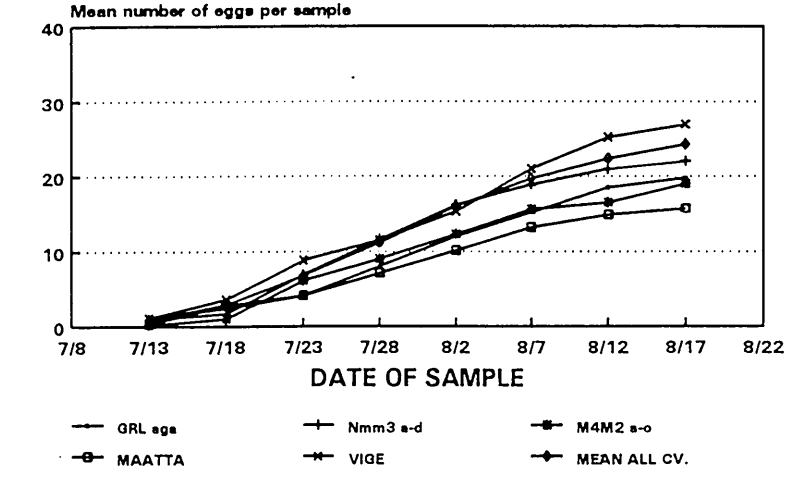
Eggs per five plant sample. Detransformed numbers are shown in brackets.

TABLE C.1.3. Percentage of each swede genotype which fell into marketable *D.radicum* damage categories and mean number of plants per sample with internal damage.

Swede Genotype	% marketable roots[1]	Number damaged internally[2]
Nmm 3a-d	90	1.20
M4M2 a-c	88	3.00
Marian	93	3.20
Doon Major	95	3.80
Angela	97	2.00
Sator Otofte	95	3.60
GRL aga	98	1.00
Angus	98	0.60
Vige	100	0.20
Määtta	99	0.92
SED	3.5	0.78
DF	9	9

1 Damage categories 0, 1 & 2
2 Per 20 plant sample

Figure C.1.1. Cumulative *D.radicum* and *D.floralis* combined oviposition on different swede genotypes during the 1990 field experiment.



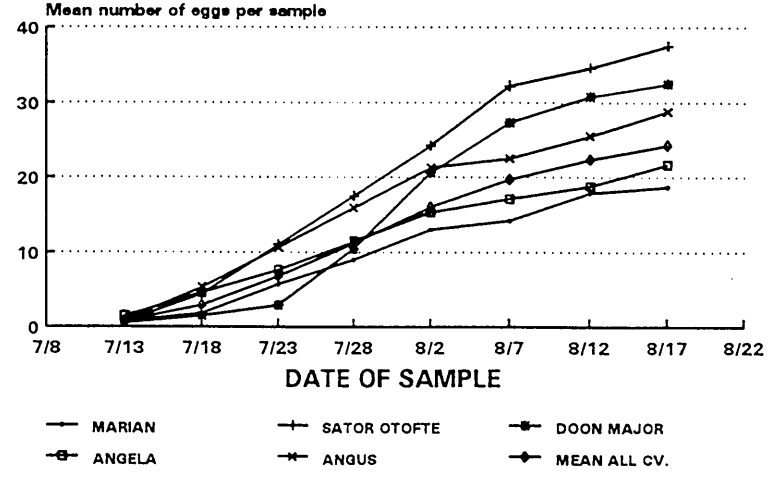


TABLE C.1.4. Mean cabbage root fly damage score of each swede genotype (scale 0-4) and the genotype Jörgensen Index score.

Swede Genotype	Mean root damage Score	Jörgensen Index
Nmm3 a-d	1.23	40.0
M4M2 a-c	1.10	35.0
Marian	0.79	26.0
Doon Major	0.72	24.0
Angela	0.65	22.0
Sator Otofte	0.66	22.0
GRL aga	0.43	14.0
Angus	0.28	0.9
Vige	0.22	0.7
Määtta	0.10	0.4
SED	0.16	n/a
DF	9	n/a

n/a = not applicable

The mean root damage score was significantly different ($p<0.001$) between genotypes. The mean scores ranged from a minimum of 0.10, cv Määtta, to a maximum of 1.23, SCRI breeding line Nmm2 a-d (Table C.1.4.). Conversion of the scores for cabbage root fly damage on the surface of the swede to Jörgensen indexes for the genotypes tested in the trial produced a 100-fold variation in scores. The Jörgensen indexes ranged from 4, cv Määtta, to 40, SCRI breeding line Nmm2 a-d (Table C.1.4.).

Internal root damage scores which were attributed to the damage of *D.floralis* were significantly different for the genotypes tested in the experiment ($p<0.001$). The mean number of plants per 20 plant sample which showed signs of internal damage varied from 0.2, cv Vige, to 3.8, cv Doon Major (Table C.1.3.). None of the pupae which were identifiable could be confirmed as being *D.floralis*.

DISCUSSION

The first generation of cabbage root fly was not monitored. Germination of the swedes in the experiment was slow and the initial growth was also impeded by dry weather conditions causing drought. The swedes were not large enough to thin out for five weeks after sowing,

and were not large enough to monitor for a further four and a half weeks. The removal of the soil from around the base of the plant requires the plant to be quite robust. The problem of monitoring oviposition on other fragile brassicas can usually be overcome by the use of egg traps (Freuler & Fischer, 1982). However, two problems exist with the use of the egg traps. Firstly, the use of the traps requires the young plant to be quite robust; use of the traps in the laboratory frequently resulted in swede plants being broken. The trap is designed to go round a plant stem and the petioles of young swede plants are often too fragile to survive undamaged. Secondly, the root of the swede grows rapidly and the the egg traps are soon too small to encircle the root of the plants. Earlier attempts which were made to sample sections of the guard rows showed the plants to be too fragile to monitor, petioles breaking on contact. The slow germination and slow development of the swede plants in the experiment resulted in the first generation of cabbage root fly having passed before monitoring could commence. The combination of missing the first generation of cabbage root fly, and the generally low oviposition rate resulted in low cumulative egg totals.

The absence of a significant difference in the total oviposition on individual genotypes, despite the trend, made it improper to attempt to correlate oviposition with damage scores, or with pupal numbers. The numbers of eggs found were low when contrasted with published results (Birch, 1988, Wilson *et al*, 1990). The low egg numbers at the experimental site can be attributed to a combination of seasonal variation and influence of the site of the experiment. A large area of the fields immediately surrounding the site of the experiment was planted out with cabbages during the course of the experiment. These fields may have served to dissipate the local population of root flies as a large number of alternative host sites were available close to the field experiment.

The root damage scores for the cabbage root fly on swede genotypes grown showed a tenfold range in Jörgensen index, and a twelvefold range in the mean damage score (Table C.1.4.). However, the score for cv Määtta must be interpreted with a degree of caution. Plots of Määtta had very low germination which resulted in low plant density. All the plots of Määtta had low plant numbers and two plots could not be used at all because the plant density was less than 10 plants per row. Plant density has been shown to have an effect on the density of cabbage root flies on plots (Finch *et al*, 1976) and the plant density could barely provide enough for monitoring. Internally damaged plants, which may be regarded as those which have been attacked by the turnip root fly, made up a relatively low proportion of the roots which suffered attack. None of the pupae sampled could be positively identified as *D.floralis*, but the damage is highly distinctive. There may have been *D.floralis* pupae amongst those which were not identifiable.

The scores for the Jörgensen index are low compared to the results of previous swede trials (Ruuth, 1988; Birch, 1989). Nine of the ten genotypes tested had more than 90% of the roots falling into categories 0, 1 or 2, which may be regarded as marketable roots. The low root damage scores are probably linked to the very low oviposition on the experimental site during the season concerned. The delayed germination and slow early growth may have reduced the attractiveness of the swede as an oviposition site.

Wilson *et al* (1990) measured the survival of swede seedlings against early first generation attack of cabbage root fly. No measurement of this type was possible in this case owing to the lateness of emergence of seedlings. Observations of the young plants on the experimental site prior to the thinning of the experiment showed that plants were not showing symptoms of root fly damage. Thinned plants examined in the laboratory also showed minimal signs of *D.radicum* attack. Marian, Nmm3 a-d and M4M2 a-c have all achieved very high rates of survival following first generation cabbage root fly attack (Wilson *et al*, 1990). The resistance, in the form of tolerance to first generation attack, demonstrated by Wilson *et al* (1990) was not reflected in the results of the 1990 field experiment. There was no sign of first generation attack on thinned plants and second generation attack did not appear to relate to the information published on resistance to first generation attack. Marian, Nmm3 a-d and M4M2 a-c were the three genotypes which produced the highest proportion of unmarketable roots in the 1990 field experiment. The genotype GRL aga, which was the most susceptible genotype in the experiment performed by Wilson *et al* (1990), was amongst the least damaged by second generation attack in this experiment (Table C.1.3). The differences between the results presented by Wilson *et al* (1990) and those gained in this experiment may be due to either exposure to a different generation of the cabbage root fly or may be a consequence of the differences in growth stage and growth conditions of the two experiments. The changes in swede genotype susceptibility to cabbage root fly attack with age have not been investigated, but, as both the structure and the chemical composition of plants change with age, it is likely that susceptibility to plant pests also varies. Ellis et al (1980) demonstrated that the susceptibility of radish to oviposition by *D.radicum* varied with the age of the plant. The late development of the plants in the 1990 field experiment may have resulted in a reduction in their susceptibility to oviposition.

Chapter C2

1991 Field Experiment
Resistance to *Delia radicum* and *Delia floralis* in Brassicas

INTRODUCTION

During the 1991 field season, two field experiments were carried out at the field station of the Edinburgh School of Agriculture-Organic Farming Centre (Jamesfield Farm, Nr Newburgh, Fife). The first field experiment consisted of five swede genotypes which were selected based upon the results achieved in the 1990 field season combined with previously published work (Birch, 1988; Ruuth, 1988; Wilson *et al*, 1990). The second field experiment consisted of two genotypes of swede and two genotypes of kale and is detailed in the Materials and Methods of this chapter.

For the original experiment, seed was drilled into a prepared seedbed on 2nd of May 1991 in a randomised plot design with ten replicates of each genotype. For the three weeks prior to sowing, the experimental site received little or no rain and the soil was consequently very dry. The dry soil conditions were compounded by the weather during the remainder of May, rainfall in the Perth area was extremely low. Rainfall records from Meteorological Station 1481, Strathallan School, Perth (approximately four miles from the site of the experiment) recorded only 15mm of rain during May. As a consequence of the prevailing weather conditions, the germination of the experiment was very poor. Four rows of swede plants, five metres long, contain approximately 100 plants, and requires more to allow plants to be thinned to an even distribution. Less than 25% of the plots in the field experiment contained enough plants. On this basis, it was decided no realistic monitoring or replication of results could take place and that to continue with the experiment was highly unlikely to yield data with sufficient replication for meaningful analysis. Consequently, the experimental site was ploughed in.

Following the failure of the initial experiment, it was decided that a field experiment was still possible to assess resistance to the second generation of *D.radicum*. In order to increase the probability of achieving a stand of plants which could be monitored, it was decided to germinate seed in modules and plant out through a polythene mulch to reduce weed competition. It was decided that under such a growing regimen the four Brassica genotypes which had been the subject of laboratory experiments in Section A could be tested in a field experiment. Laboratory experiments discussed in Section A demonstrated that antixenotic resistance to *D.floralis* and *D.radicum* is present in the swede breeding line GRL aga, and

also the kales cv Dwarf Green Curled and cv Fribor, when contrasted with the susceptible swede, cv Doon Major.

MATERIALS AND METHODS

Plant Material

Four Brassica genotypes were selected to complement the laboratory experiments. The Brassicas were two swede genotypes, the commercial cultivar Doon Major and the Scottish Crop Research Institute breeding line GRL aga. In addition, two kale genotypes were used, cv Dwarf Green Curled and cv Fribor. Doon Major has been used as a standard susceptible genotype in experiments against both *D.floralis* and *D.radicum* (Birch, 1988; 1989b; Ruuth, 1988; Wilson *et al*, 1990). GRL aga had been shown to be resistant to *D.floralis* in field cage experiments (Birch, 1989b). In the field cage experiments, GRL aga had the lowest oviposition and pupal numbers per plant, and also had the lowest root damage index, with the highest percentage of roots falling into marketable categories. Fribor has shown resistance to *D.floralis* in field experiments (Alborn *et al*, 1985; Ruuth, 1988). No publication could be found which tested the resistance of Dwarf Green Curled to *D.radicum* or *D.floralis*. Seed was sown in 15mm modules in a 3:1 Levington® Universal compost : sand mix. When plants reached the two true leaf stage, they were planted out at the experimental site.

Experimental site

The experiment was carried out at the field centre of the Edinburgh School of Agriculture-Organic Farming Centre (Jamesfield Farm, Nr Newburgh, Fife). The site was 15m above sea level with a slight northern aspect. The soil was a sandy loam (Carpow/Carey soil series). The results of a soil analysis from the site are shown in Table C.2.1.. The analysis showed no deficiencies which would adversely affect Brassica growth, consequently no additional fertiliser was added to the site, nor was any herbicide applied.

Experimental design

Eight replicates of each genotype were laid out in a randomised block design. Each plot consisted of four rows 50cm apart, 4 metres long containing 20 plants, each 20cm apart. Plants were hand planted at the two true leaf stage through holes cut in a polythene mulch (50 gauge, 4m wide, LBS polythene, Cotton Tree, Nr Kolle, Lancashire) on the 16th and 17th of July 1991. On 22nd of July, the experiment was inspected and plants which had not survived planting out were replaced. On this date, it was decided that the plants were not sufficiently strong to have the soil cleared as a prerequisite to monitoring.

Analysis	Status
pH	6.7
Extractable Ions	
Phosphorus	High
Potassium	High
Magnesium	High
Sulphur	Moderate
Manganese	Very Low
Copper	Moderate
Boron	Low

TABLE C.2.1. Nutrient status of the soil on the site of the 1991 field experiment site.

Oviposition sampling

On the 29th of July, the 4 plants in each plot which were to be used for monitoring oviposition were marked with a plastic plant label and the soil taken from around those plants and replaced with soil which contained no eggs. Monitoring took place every week, starting on the 5th of August 1991 and finishing when egg numbers diminished on 9th September.

Damage scoring

After oviposition monitoring had ceased, the experiment was left until it was harvested on 5th November 1991. Sixteen roots were taken at random from the centre two rows of each plot, leaving roots which had been used for oviposition monitoring. On the day that roots were harvested, five soil cores were taken from each plot. Soil cores (15cm deep, 15cm diameter) were taken directly beneath a plant which had not been used for oviposition monitoring in the centre two rows of a plot and pupae were extracted as in the Section C, General Methods. Harvested roots were then washed and scored visually for external and internal damage as outlined in Section C, General Methods. In addition to the assessments previously used, the area of cabbage root fly damage was also scored according to the square root of the area of the damage, rounded up to the nearest integer. Although not an accepted manner of scoring damage, it was included as an estimate of the amount damage caused by *D.radicum*. The presence or absence of cabbage root fly damage on the bulb and the side roots of the genotypes was also recorded.

Statistical Analysis

An analysis of variance was performed on the data using Genstat (Genstat, Sun/Unix version 5.0, release 2.2, 1990). The variance on the oviposition levels for individual weeks was stabilised by performing a square root transformation on the data. The data from the total oviposition levels did not require transformation. The pupal counts from the four soil cores were aggregated owing to there being a large number of zeros in the data. The data from the aggregated pupal cores were square root transformed to stabilise the variance and then an analysis of variance performed. A binomial model was fitted to each of the four assessments of damage (index, area, presence of damage on side roots and presence of damage on root bulb) using Genstat. This type of test was needed because the distribution of the data was not normal and a binomial analysis accommodated the distribution of the data.

RESULTS

Oviposition

The data from the weekly samplings of root fly egg numbers were square root transformed in order to give a normal distribution (Figure C.2.1. and Figure C.2.2.). For weeks 1, 2 and 4, there was no significant difference ($P>0.05$) between the number of eggs oviposited on different genotypes. Significant ($P<0.001$) differences were observed during week 3, cv Dwarf Green Curled receiving more eggs than all other genotypes, and cv Doon Major receiving more eggs than cv Fribor and breeding line GRL aga. In weeks 5 and 6, cv Doon Major received significantly more eggs than the other three genotypes, the probabilities were $P=0.016$ and $P=0.006$ respectively. Total oviposition, which did not require transformation, showed that significantly different numbers of eggs were laid on each genotype ($P=0.008$). The ranking for total oviposition was cv Doon Major, cv Dwarf Green Curled, cv Fribor and breeding line GRL aga (Table C.2.2.).

Root damage

The mean area of root damaged was significantly ($P<0.001$) different for the genotypes tested. The mean area score split the four genotypes into two groups, the high scoring group containing kale Fribor and swede Doon Major, the low scoring group containing kale Dwarf Green Curled and swede genotype breeding line GRL aga (Table C.2.3.). The mean score according to percentage area produced the same ranking order as above and also divided into two significantly ($P=0.002$) different groups. In this case, there was no significant difference ($P>0.05$) between cv Fribor, cv Doon Major and cv Dwarf Green Curled, which were all

Figure C.2.1. Combined *D.radicum* and *D.floralis* oviposition on swede genotypes during the 1991 field experiment (mean number of eggs per 4 plant sample).

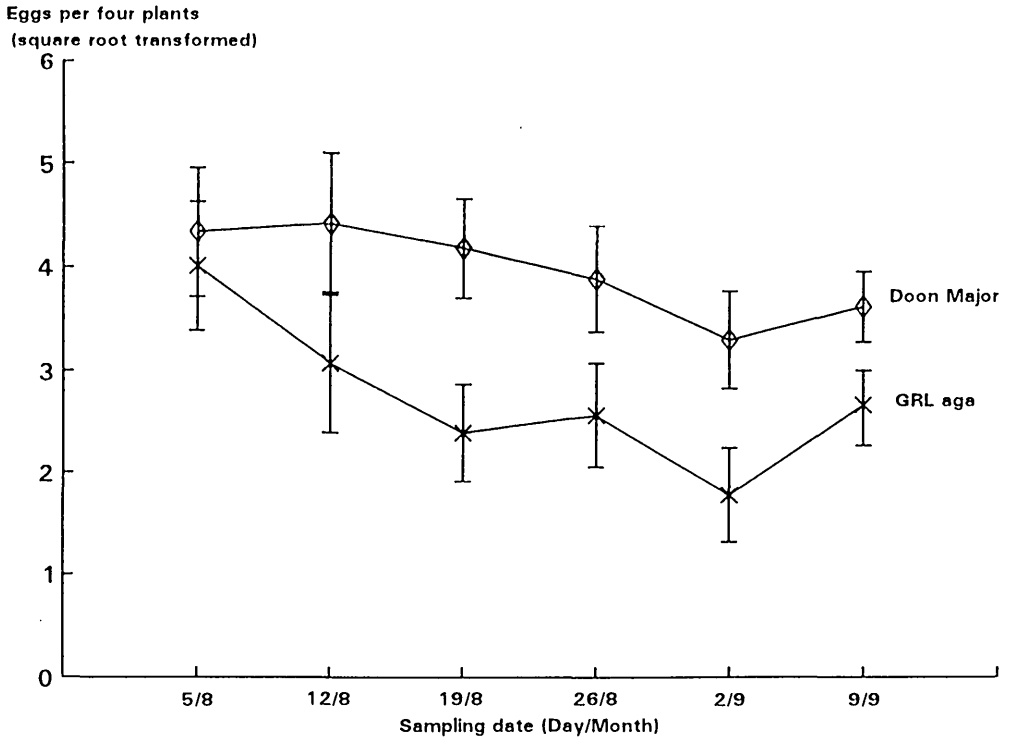


Figure C.2.2. Combined *D.radicum* and *D.floralis* oviposition on kale genotypes during the 1991 field experiment (mean number of eggs per 4 plant sample).

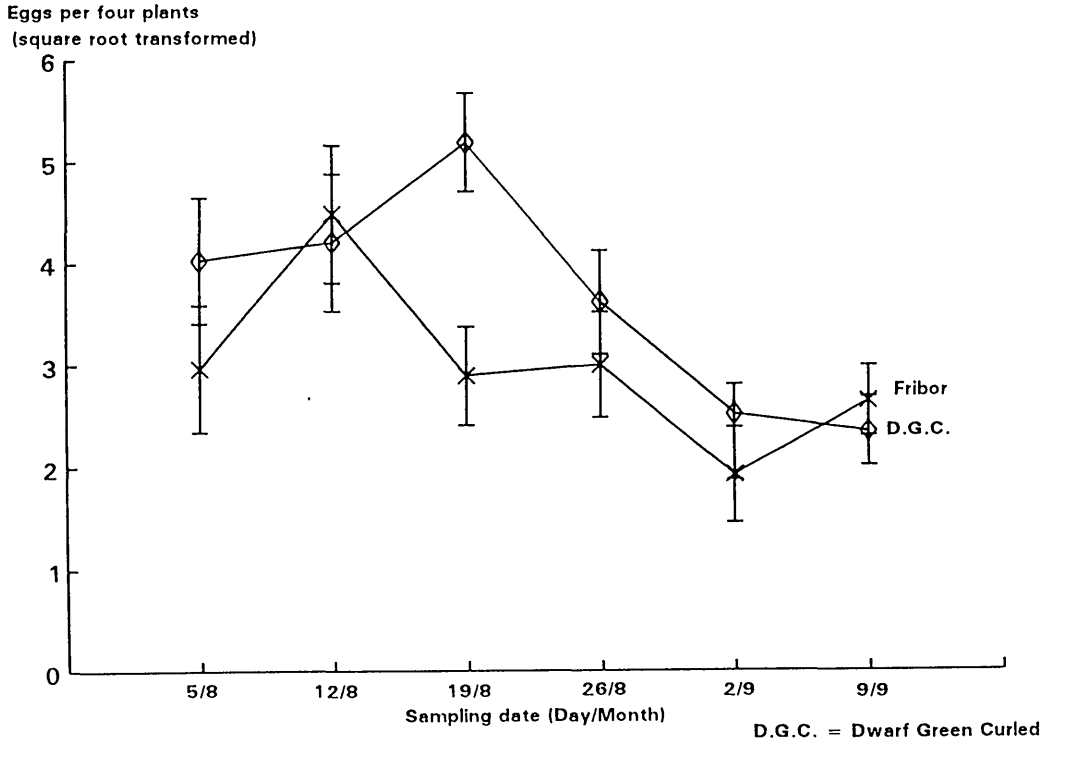


TABLE C.2.2 Mean of total *D.radicum* and *D.floralis* oviposition on each genotype (untransformed) and mean numbers of pupae at the end of the season (square root transformed, detransformed figures in parentheses) for the 1991 field experiment.

Crop type	KALE	KALE	SWEDE	SWEDE	
Genotype	Fribor	D.G.C.*	GRL aga	Doon Major	SED
Total egg numbers[1]	67.9	94.0	58.9	106.3	13.7
Mean number of pupae / plot[2] (Detransformed)	0.85 (0.72)	1.99 (3.96)	0.73 (0.53)	2.90 (8.41)	0.42 (n/a)

* D.G.C.=Dwarf Green Curled

1 The mean of the total number of eggs laid over the 6 weeks of monitoring

2 per four plants sampled

n/a = not applicable

TABLE C.2.3 Mean *D.radicum* damage scores for each genotype, Jörgensen index and mean area of damage

Crop type	KALE	KALE	SWEDE	SWEDE	
Genotype	Fribor	D.G.C.*	GRL aga	Doon Major	SED
Mean damage score[1]	2.4	2.0	1.6	2.2	n/a
Jörgensen index	48.44	41.36	27.08	43.49	n/a
Area of Damage	2.12	1.46	1.20	1.98	0.19

* D.G.C.=Dwarf Green Curled

1 Mean arithmetic score only, analysis is given in the table below

n/a = not applicable

TABLE C.2.4. Predictions from regression models of mean *D.radicum* damage index scores and proportions of roots damaged on the central bulb or on the side roots during the 1991 field experiment (predictions from models are shown with approximate SE).

Crop type	KALE	KALE	SWEDE	SWEDE
Genotype	Fribor	D.G.C.*	GRL aga	Doon Major
Mean Damage Score	-0.514	-0.793	-1.362	-0.685
SE	0.127	0.133	0.153	0.131
Log Score#	0.38(2.4)	0.31(2.0)	0.21(1.6)	0.34(2.2)
Central Bulb	-0.383	-0.094	-0.158	0.760
SE	0.185	0.182	0.182	0.194
Probability	0.41	0.48	0.46	0.68
Side Roots	2.043	0.588	-0.727	-0.159
SE	0.264	0.180	0.184	0.174
Probability	0.88	0.64	0.33	0.46

* D.G.C. = Dwarf Green Curled

logarithmic value of damage score with detransformed arithmetic score in brackets

significantly ($P<0.05$) higher in their damage scores than swede genotype, GRL aga.

The distribution of damage on the roots also yielded significant ($P<0.05$) results between genotypes (Table C.2.4.). Doon Major was more frequently damaged on the central bulb ($P=0.002$) than all other genotypes, which were not significantly different from each other ($P>0.05$). The presence or absence of damage on the side roots was also significantly ($P<0.001$) different. All genotypes could be separated from the other three and the ranking order was Fribor, Dwarf Green Curled, Doon Major and GRL aga. There was no significant difference ($P>0.05$) between genotypes for the presence or absence of internal damage.

Pupal numbers

All the pupae which were identifiable from the experimental site were cabbage root fly pupae. Pupal numbers were bulked per plot and square root transformed in order to approximate a normal distribution (Table C.2.2.). The results were significant ($P<0.001$), cv Doon Major having more pupae than all other genotypes, and cv Dwarf Green Curled having more pupae than cv Fribor and breeding line GRL aga. There was no significant difference ($P>0.05$) in pupal numbers arising from cv Fribor and Breeding line GRL aga. The mean number of pupae for each genotype was significantly ($P=0.025$) correlated with total oviposition ($r=0.955$ [DF=3]).

DISCUSSION

The results from the root fly oviposition monitoring carried out during this field experiment serve to complement the results achieved in laboratory experiments discussed in Section A. Although no significant difference ($P>0.05$) occurred between levels of oviposition during three of the six weeks monitored, there was some consistency across the six weeks. The swede, cv Doon Major, received more eggs than the swede breeding line GRL aga on all sampling dates. The kale, cv Dwarf Green Curled, received more eggs than kale cv Fribor on four of the six weeks. The numbers of eggs laid on the swede genotypes confirm previously published findings (Birch, 1988, Birch, 1989a). GRL aga has partial antixenotic resistance to the oviposition of *D.radicum* and *D.floralis*. The oviposition was probably predominantly *D.radicum* as the majority of damage was of that type and all the pupae which could be identified were *D.radicum*. The total oviposition on the genotypes tested has similarities to the results achieved in the oviposition work and behavioural analysis discussed in Section A. Within crop type, the ranking of genotypes is consistent with that demonstrated in the laboratory. GRL aga consistently had less eggs laid on it than Doon Major, whilst Fribor had

less eggs laid on it than Dwarf Green Curled. However, the strong antixenotic resistance which kales demonstrated in the laboratory when contrasted with swedes was not repeated in this field experiment. The likely reasons for the differences between the laboratory and field results are discussed in detail in Section C Discussion.

Oviposition, pupae numbers and damage to roots were, overall, low during this experiment. The mean root damage score of the susceptible swede (Doon Major) was lower than in recent studies of cabbage root fly from Scotland (McKinlay & Birch, 1992). The validity of the root scoring methods is also questionable when scoring two such different root types. The addition of an indication of the area of the damage and the scoring of central bulbs and side roots for presence or absence of damage are both considered improvements to the existing scoring system. Whilst classifying roots into one of four broad damage categories is probably adequate for breeding purposes, where many criteria are under consideration, it is felt that when *D.radicum* damage is the primary purpose of a study such schemes are too inaccurate.

The correlation between oviposition levels and pupal numbers at the end of the season would appear to indicate that antixenotic resistance was the dominant factor in determining end-of-season pest numbers during the course of this experiment. The dominance of antixenotic resistance over environmental factors and antibiotic factors agrees with the conclusions drawn by Rygg & Sömme (1972) for the closely related turnip root fly. Rygg & Sömme (1972) concluded that the differences in the susceptibility of swedes and turnips to attack by the turnip root fly depended largely on differences in oviposition, although establishment of newly hatched larvae was also an important factor.

The nature of the damage caused to the roots of different genotypes is of great interest. No previous author has published results on the distribution of root fly damage on the roots of Brassicas. The results clearly indicate that the damage which is done to roots not only varies in its area and in the percentage of the root surface which it covers, but also does so in its distribution. The roots of kale plants had a high probability of suffering *D.radicum* damage to the side roots. In swedes, damage was much more likely to have taken place on the larger central bulb. Closer recording of the distribution of damage over the surface of the root may lead to knowledge of the feeding preferences and capabilities of different instars of the Brassica root flies. The depth of damage caused to the root by *D.radicum* is not the same on all genotypes and crop types. No measurements of the depth of damage were made, but the damage to the kales appeared shallow when compared to that on the swede genotypes. Such damage is difficult to assess as part of a large scale field trial. Birch (1988) took measurements of *D.floralis* damage during an inoculation experiment and two field

experiments and found reduced mine depth on certain swede genotypes. Accurate assessment of the distribution and depth of the feeding mines of *D.radicum* could provide valuable information on the feeding behaviour of this species. The feeding behaviour may later be related to the distribution of nutrients in different root tissues available to feeding larvae.

SECTION C

SECTION DISCUSSION

The results which were achieved in the laboratory (Sections A & B) demonstrated that some genotypes of Brassicas possess partial antixenotic and antibiotic resistance to *Delia radicum* and *Delia floralis*. The discrepancy between the very consistent results achieved by laboratory methods and the inconsistent ones achieved at both field experiments requires discussion. Field-based work is exposed to a wide variety of variables which can be excluded in the laboratory or glasshouse. Not least amongst these variables is pest density itself. Work carried out in the laboratory is done at artificially high pest densities; in the field, the best that can be achieved is to attempt to locate a site at which a relatively high field density of flies is probable in most growing seasons. Alternatives have been sought which are intermediate between field and laboratory methods. Birch (1989b) utilised a field cage to raise the level of the turnip root fly population in the field. However, this method does not allow the influence of long range host finding to be taken into account. Hence, the field cage method, whilst giving a consistency of attack, neither allows long range selection influences to take place, nor gives gravid females the option to reject the available site altogether. Oviposition by insects is influenced by the availablity of oviposition sites. The use of field cages may result in oviposition taking place on plants which in a field experiment would not receive eggs.

Long range host selection by *D.radicum* is one of the influences which comes into play in the field which earlier work in this thesis could not take into account. Gravid females migrate moderately long distances, in the region of 2000-3000 m in total, and do not necessarily stop at the first block of host plants which they encounter (Finch & Skinner, 1975). Flight by gravid females is upwind and not necessarily stimulated by the presence of host volatiles (Finch & Skinner, 1982). Wallbank & Wheatley (1979) demonstrated that allyl isothiocyanate and hexyl acetate, both Crucifer volatiles, could influence the behaviour of *D.radicum* in an olfactometer. Finch & Skinner (1982) concluded that the encounter of host plant volatiles was the first stage in host plant selection, taking place whilst the insect was still in flight.

In addition to long range host selection taking place in the field, variations in host selection are likely to take place at the contact stage. The importance of the chemical composition of a potential host plant of *D.radicum* and *D.floralis* has already been discussed in earlier chapters and has previously been demonstrated (Städler & Schoni, 1990). The physical component of host plant selection by *D.radicum* was investigated by Roessingh & Städler (1990) who demonstrated that the size, shape, colour and surface coating of a model could influence the

host selection procedure. The presence or absence of a stem and the presence and orientation of three-dimensional folds in the leaf model also influenced the host selection procedure of *D.radicum*. These are all factors which are subject to continuous change with the growth of a plant. As a plant grows the leaf area increases, and the colouration and proportion of the leaves varies during development towards maturity. Ellis *et al* (1980) demonstrated that plant age was an important influence on non-preference, antixenotic resistance, for the cabbage root fly. As the attraction of different plant genotypes will vary with growth stage and age, it is inevitable that their relative attraction will vary over time. Consequently, the succession of gravid females, all of which will naturally vary in their perception of the environment, will be presented with a series of potential hosts which will be constantly varying with the development of the plants and the prevailing environmental conditions. To have practical applications in field agriculture, a mechanism of resistance must be strong enough to override these environmental factors.

Following host plant selection in a field situation, the egg and subsequently the larva are exposed to a wider series of influences than the inoculated egg in the laboratory. Laboratory inoculated eggs are placed carefully at the base of the stem and carry out their development in a controlled environment. Firstly, the eggs of root flies in a field situation are, not necessarily laid directly against the side of the stem which means that the newly hatched larvae will first have to migrate from the point of oviposition to the side of the stem, prior to attempting to commence feeding. Koštál (1992) noted the orientation of newly hatched cabbage root fly larvae to volatiles. Both allyl and ethyl isothiocyanate elicited taxis in newly hatched cabbage root fly larvae which may allow them to migrate to a host plant in their immediate vicinity. Secondly, during these early stages and throughout the larval phase of the lifecycle, root flies are exposed to a large number of parasites and predators in the field which would not threaten them in the glasshouse. Hymenopterous parasitoids frequently attack root fly: Coaker & Finch (1971) list five species of Braconidae, three species of Cynipidae and four species of Ichneumonidae which will attack cabbage root fly. The cynipid, *Idiomorpha rapae*, has on occasions been found parasitising 60% of *D.radicum* pupae. The eggs, larvae and pupae of root flies are also common prey to polyphagous predators living in the soil. The influence of the host plant on these interactions is as yet indeterminate: no published work is available detailing the use made of Brassica volatiles by predators and parasites seeking their prey. However, it is very likely that interactions are taking place and that different Brassica genotypes will differentially attract predators and parasites. The regular watering of plants in the glasshouse also protects eggs and larvae from desiccation.

It is difficult to draw positive conclusions from the results of two field experiments which

approached the problem with separate strategies. The 1990 field experiment used only swede genotypes, which are of near identical growth form when contrasted with the kale plants grown in the 1991 field experiment. The short notice in which the 1991 field experiment was designed limited the use of genotypes and made it impossible to do an experiment which linked with the 1990 experiment. The second years' field experiments drew strongly on the results of the first year and the findings in the laboratory to produce a smaller, but much more detailed analysis of the interactions taking place in the field. The performance of GRL aga in the field was consistently good during both the 1990 and the 1991 field experiments. Although no differences in oviposition occurred between different genotypes during the 1990 field experiment, GRL aga received far fewer eggs than the average, and was consistently low in all the measurements of damage. In the 1991 field experiment, GRL aga performed far better than the susceptible Doon Major and with more consistency than the kale genotypes which had exhibited a greater degree of antixenotic resistance in the laboratory.

The changes made to the damage scoring scheme between 1990 experiment and the 1991 experiment were definite improvements, but did not bring the assessment of the different types of roots to a satisfactory level. The advantages of a more accurate scoring system have already been raised in Chapter C2 Discussion. The structure of the roots of swede and kale are totally different. Assessment of the percentage of a swede root which suffered damage was not difficult whilst the complex root structure of the kales made it difficult to be sure of the proportion of root which was damaged. In addition to the site of damage, a way must be found of taking into account the depth to which the root is mined. The volume of kale root consumed was certainly not as high as the scores for the area or percentage of root damaged would lead one to believe. The evidence for a reduced depth of *D.radicum* damage is not based upon measurements but upon observation. Possible improvements to the assessment of root fly damage are included in the General Discussion.

The balance of power: Antixenosis versus Antibiosis

The results presented in this thesis indicate that, within the host range tested, oviposition by *D.radicum* and *D.floralis* in the laboratory varied between plant genotypes by multiples of approximately *x*5 and *x*80 respectively. Oviposition at the field experiment sites was less extreme: rootfly eggs varied by up to *x*2 over the season. The inoculation experiments indicated that, within the range tested, the weight of pupae on different genotypes varied by *x*2 for both species whilst the probability of eggs developing through to pupae varied by approximately *x*1.5. On balance, it can be concluded that within the range of Brassicas tested the potential for mechanisms of resistance was most strongly demonstrated by antixenotic resistance to oviposition, particularly by *D.floralis*. The range of Brassicas tested for antixenosis and antibiosis was not the same, although some genotypes were tested for both. There was some evidence from laboratory studies that antixenotic resistance and antibiotic resistance within the swede genotypes may be related. GRL aga, when contrasted with Doon Major, was shown to have both antixenotic resistance and antibiotic resistance to *D.radicum* and *D.floralis*. As Fribor and Dwarf Green Curled were not inoculated with either species of fly, it would be unwise to draw too much in the way of conclusions from this observation. This study was not intended to assess the levels of different mechanisms within genotypes, but to explore the nature of mechanisms of resistance found in genotypes. The genotypes were selected to test particular individual mechanisms of resistance and not to examine the relationship between antixenosis and antibiosis for *D.radicum* and *D.floralis*. The relationship between different mechanisms of resistance remains a biologically important one, but the subject for a different type of study.

The relationship between antixenotic resistance and antibiotic resistance to a pest species (also termed as preference and performance) is not necessarily a close one (Thompson, 1988). For *D.radicum* and *D.floralis*, it was apparent to early researchers that antixenosis and antibiosis were often not linked. Swailes (1959) investigated *D.radicum* in field experiments and found that the swede genotype least damaged, Wilhelmsburger, was a genotype which was oviposited on heavily. The weight of pupae found on Wilhelmsburger was no less than on other genotypes. Consequently, he concluded that the dominant form of resistance was one of establishment of larvae, and that neither oviposition nor larval development was affected.

Rygg & Sömme (1972) studied resistance to oviposition and larval development of *D.floralis* on swedes and turnips. They found that the $x2$ variation in oviposition on some genotypes related to an overall reduction of between approximately 30% and 90% in larvae numbers.

Jaenike & Papaj (1992) combined theories of behavioural plasticity during host selection by phytophagous insects and concluded that antixenosis alone will not be sufficient to control a pest species. Firstly, the physiological state of an insect changes as the period since its last oviposition event increases (Jaenike & Papaj, 1992). The result of this change is that the insect becomes increasingly more likely to oviposit on a sub-optimal host of constant acceptability. Consequently, the stimulus threshold for oviposition falls, as the period since oviposition rises. This general theory is confirmed by the findings of Koštál (1993) that *D.radicum* would oviposit without host plant chemicals when deprived of access to host plant chemicals or models. Secondly, for phytophagous insects the probability of ovipositing on a host plant increases with the number of ovipositions which have previously taken place on that plant (Jaenike & Papaj, 1992). This effect was termed "learning" but has never been tested in either *D.radicum* or *D.floralis*. Traynier & Truscott (1991) utilised two different shades of green as oviposition sites for *Pieris rapae* treated with glucobrassicin or sinigrin. The shade treated with glucobrassicin was preferred for oviposition. However, when offered the choice of the two shades of green two hours later, neither treated with either stimulant, the shade which had been treated with glucobrassicin in earlier "conditioning" experiments was preferred for landing by 17 out of 18 butterflies tested. Fifteen of the 17 butterflies which landed went on to oviposit, despite the lack of chemical stimuli.

When a sub-optimal host is the dominant plant in an insect's environment, physiological changes within the insect will result in oviposition on that plant. Thereafter, the high frequency of encounter and constant reinforcing of previous host experience may result in a breakdown of the resistance if a more susceptible oviposition site is not available.

The problems associated with the breakdown of antixenotic resistance may be avoided by a combination of antixenotic and antibiotic resistance within a single genotype. If those individuals (insects) which develop on the sub-optimal host have their fitness penalised for doing so then the breakdown of resistance may be avoided. The progeny of individuals prepared to oviposit on a sub-optimal host will be less fit and, consequently, contribute less readily to the genetic pool of the species. Antixenotic resistance may be best utilised in

combination with a trap-crop which will increase the encounters with optimal hosts and diminish the development of the trend towards the sub-optimal host. Miller & Cowles (1990) suggest the use of a similar mechanism, termed "stimulo-deterrent diversion" to reduce the development of insecticide resistance in the onion fly. It is apparent that the work which has been carried out to date on *D.radicum* and *D.floralis* is only adequate to allow the application of general theories of host selection. It is essential to the utilisation of developing resistance mechanisms that the consequences of their utilisation are fully understood. Careless and excessive use of chemical methods of insect control have frequently led to their premature breakdown because of insect resistance. Host plant resistance is unlikely ever to present a universal panacea for insect pest problems and the best use of it will only be made if care is taken in its application (Kogan, 1986). Use of host plant resistance as part of a structured Integrated Pest Management system, where it is only one of several factors reducing pest fitness, may be the best strategy in the long term.

The work produced in the body of this thesis indicates that the swede genotype, GRL aga, not only has antixenotic resistance to oviposition by both *D.radicum* and *D.floralis*, but also has antibiotic resistance to the larvae of both these species of flies. However, the moderate level of antixenotic and antibiotic resistance found to *D.radicum* and *D.floralis* may still be inadequate to provide sufficient and sustainable protection for a field crop. The combination of reduced pesticide input and resistant swede genotypes has already been shown to provide control equal to that of a susceptible genotype with full pesticide input (McKinlay & Birch, 1992; Taksdal, 1992).

Finch (1993) reviewed the integrated management of *D.radicum* and concluded that the contribution to be made by plant resistance was "relatively minor". The results in this thesis indicate that this stance may be underestimating the usefulness of host plant resistance in an Integrated Pest Management system. Laboratory experiments demonstrated great potential for reducing the number of eggs laid on Brassica crops. If an insect can be hindered in its attempts to oviposit on a plant, then the resultant reduction in damage by the larvae of the pest will provide protection to the crop. In addition to the direct advantage to the crop, antixenotic resistance provides benefits by increasing the searching effort of the gravid females. The energy used in this search must be replenished and the result may adversely influence the overall fecundity of the female. The increases in movement by the female will increase the probability of predation and other causes of adult mortality. Koštál (in press) has shown that

undersowing Brassica crops can lead to increased rejection of a plant owing to the obstruction of *D.radicum* movement by the foliage of the undersow. *D.radicum* females which encountered the foliage of undersow during short flights on the potential host plant normally flew away from the potential plant after escaping the undersow. The effect of combining resistant genotypes, which have a higher rejection frequency, with undersowing is potentially an area of great interest.

The results of the laboratory experiments were far more clearly defined than the results of the field experiments. Unfortunately the field experimentation with Doon Major, GRL aga, Dwarf Green Curled and Fribor was carried out only once. The restricted nature of a PhD study limited the potential to return to the field to provide further data on this area. The results achieved in the field showed that, although antixenotic resistance could be demonstrated within the range of plants tested, it was not present to the same extent. No field data were produced for antixenotic resistance to *D.floralis*. Antixenotic resistance to *D.radicum* in the lab was demonstrated as *x*5 oviposition on susceptible plants, in the field the largest margin was approximately *x*2.

Two major factors are known to interact in the field which are not usually a major element of laboratory studies. Firstly, the environmental interactions with the plant genotype are far more pronounced in the field environment than is the case in the laboratory. The constraints on the environment in the glasshouse are fairly strong. The fluctuations of daylight and temperature are reduced by the addition of artificial light and heat, water is plentiful and the compost in which the plants are grown can be consistently mixed from known sources and in known proportions. Baker (1974) demonstrated that the number and structure of the wax crystals on the leaf surface are radically altered by changes in environmental conditions. Environmental factors are combined with the interactions which take place in the field between *D.radicum* and *D.floralis* and other animals. The repellent effect of chemical components of the frass of the garden pebble moth are extensive (Jones & Finch, 1987; Jones *et al*, 1988; Cole *et al*, 1989). Also, the presence of aphids has been shown to reduce the host selection of *D.radicum* (Finch & Jones, 1988). The combination of the factors explored in Section C Discussion and above give good indications of why the very strong antixenotic resistance found in the laboratory was found to be reduced in the field. In addition, the low levels of oviposition and damage which were recorded suggest that the population of flies was fairly low for both field experiments. Low populations of insect pests during field experiments may be linked to a

reduction in discrimination taking place between host plants of different genotypes.

Host Plant Resistance & Chemical Ecology

Host plant chemistry is just one aspect of the resistance to insects which plants may possess. The work which has been carried out on the effect of the structure of surrogate plants on host plant selection (Roessingh & Städler, 1990; Koštál, 1993) indicates that plant structure can have an important part to play in host selection. The leaf surface extracts which were tested in Chapter A3 indicated that although host selection was strongly influenced by plant chemistry, it was not wholly explained by it. Roessingh & Städler (1990) found that the inclusion of leaf folds was critical to successful host plant selection by gravid *D.radicum* females. The kale plants which were tested in Section A were of a radically different structure to the more susceptible swedes. The more elongate stems of the kale genotypes were unlikely to be of influence because the kales were usually rejected before the stem was explored. However, the curly leaves of the kales provided a physical barrier to the free movement of gravid females across the leaf surface. Although not quantified in this study, it was also clear that the leaves of the kales obstructed free movement of the flies onto the petioles. The influence of the leaf structure remains largely unexplored and there is potential to breed plants with such characteristics. This area of study recommends itself for further research as strongly as does the chemical ecology of the leaf surface.

The status of chemicals such as glucosinolates in the ecology of cruciferous plants is an area already under a degree of debate. The function of many secondary plant compounds is uncertain and a number of theories exist to explain their evolutionary status in insect-plant relationships. The theories concerned define the function of secondary plant compounds as either specific defence molecules or minor metabolic compounds which have an additional and fortuitous defence capability (Rausher, 1992). Many secondary compounds have several well defined functions which include the storage of nutrients, acting as precursors and defensive compounds. The evolutionary perspective may be of secondary importance to the deployment of secondary plant compounds in the defence of agricultural crops.

However, many assertions as to the status of secondary plant compounds are becoming increasingly uncertain. The discovery of compounds such as CIF (Roessingh *et al*, 1992) indicates that compounds which strongly influence host selection behaviour can remain undiscovered for considerable periods. The indications that CIF is found in other Brassica

crops beside the cabbage on which it was first discovered imply that it may play an important role in *D.radicum* and *D.floralis* host selection. The high activity of the desulphoglucosinolate fraction when contrasted with the aliphatic+ fraction in Chapter A3 may ultimately develop to be of wider importance to Crucifer-insect interactions. Many of the published papers linking glucosinolates to insect host selection are based upon correlation of glucosinolate profiles with the host range or feeding of insects. The inferences from such data are often promoted to facts when quoted later. This error was nearly repeated in Chapter A3 of this thesis when a fraction containing aliphatic glucosinolates was found to reflect the bioassay of whole plants. However, the fraction retained its activity when the glucosinolates were structurally damaged (desulphated) to remove their activity. The electrophysiological data produced by Dr R. Baur (Wädenswil, Switzerland) do support the hypothesis that CIF is the primary stimulatory compound in the desulphoglucosinolate fraction. The compound stimulating oviposition in this case may or may not be CIF, but the question of purity of compounds used in bioassays will also need to be addressed. There may be many low molecular weight compounds influencing the results of chemical ecology experiments, which are difficult to isolate by fractionation procedures and equally difficult to identify.

The changes in the glucosinolate profile of roots damaged by *D.radicum* and *D.floralis* may be of importance to host location by adult females and larvae of both species. The attractive nature of isothiocyanates for many insects has already been discussed at some length in other parts of the thesis. Koštál (1991) demonstrated that, whilst low concentrations of isothiocyanates were attractive to the larvae of *D.radicum*, higher concentrations were repellent. Consequently, the effect of changes in the concentrations of glucosinolates is likely to have an important effect on the concentration of volatile hydrolysis products released, especially when the root tissue is damaged.

Methodology

The work performed in this thesis has utilised as wide a range of methods as was practicable to study host plant resistance. The methods used in this thesis were chosen to draw together a number of aspects of the resistance to *D.radicum* and *D.floralis* which are to be found in the Brassicas. These results show that antixenosis and antibiosis are both capable of significantly reducing the effect of both *D.radicum* and *D.floralis* in the laboratory. Testing of the genotypes in the field produced results that were less certain, but were based on only one experiment when *D.radicum* and *D.floralis* populations relatively low.

Laboratory methods used to investigate the nature of host plant resistance to *D.radicum* and *D.floralis* fell into three broad categories: established techniques, novel techniques and established techniques utilised for novel purposes. Established techniques, such as the testing of antixenotic resistance in the turntable, have been combined with novel techniques, such as the sequential analysis of host plant selection behaviour. In Section A, the results of the two approaches complemented and confirmed findings. In addition, the application of techniques outside their normal area, such as the analysis of the fibre content of plant roots, has yielded a number of very interesting results. Fibre analysis is more normally applied within the animal feed industry, but the measurement of fibre in the roots of the Brassicas tested proved interesting. As a result, the data produced in different parts of this thesis sometimes concur and sometimes contradict. The use of behavioural studies to observe the host plant rejection point of both species particularly supports the data produced by extracting the leaf surface chemicals and leads to a very strong case for the application of similar techniques elsewhere. Other relatively new techniques within entomology, such as Electrical Penetration Graph technique, might benefit from sequential analysis of behavioural data rather than those based on percentage of total time for each event. The methods for sequence analysis were particularly difficult to evolve, but once learnt can be very easily applied to similar situations.

Measurement of damage to roots

With the exception of the Chapters in Section C, measurement of the area of damage caused to a root has largely been avoided throughout this thesis. The use of techniques measuring the proportion of a root which has been visibly damaged by insect larvae was considered inappropriate on the young plants which were used in the inoculation experiments. The damage caused often varied considerably in depth, especially in the experiments when different crop types were used, concurring with Birch (1988). The measurement of damage to swede roots, using established techniques and based on a small number of categories defined by the percentage of the total root area affected, was also shown to be inadequate. The additional assessment of the location of the damage improved the biological data considerably. However, because the system is based on the percentage of total area, it is as much an assessment of root size as it is an assessment of damaged area. The area of swede root exposed to *D.radicum* damage may be regarded equal to half the surface area of a sphere of the diameter of the root in question. Alternatively, the form of the root may be regarded as a cone which is mathematically a more complicated object, so far as calculating surface area is

concerned. The principle is unaffected by which model of root shape you choose to accept. The surface area of both shapes increases rapidly with changes in the diameter of the root. Consequently, a genotype with a mean diameter 10% greater than average will consistently produce artificially low scores. To correct this, it would be better to produce a score based on the area of the damage which, in combination with monitoring of egg numbers, would more accurately reflect the quantity of larval feeding per egg laid. In addition, an index based upon the actual area of damage to the root of the Brassicas, would not be so strongly influenced by the structure of the root. When measuring root damage, it is important to concentrate on the damage which has taken place; traditional scoring methods have been too strongly influenced by the size of the root of the plant.

Conclusions

- During post-alighting oviposition site selection behaviour, gravid female *D.floralis* accept or reject a potential host plant primarily on the basis of an assessment of the surface of the leaf during the landing phase.

- During post-alighting oviposition site selection behaviour, gravid female *D.radicum* accept or reject a potential host plant primarily on the basis of an assessment of the surface of the leaf, during landing and leaf resting phases.

- The pattern of behaviour exhibited by gravid female *D.radicum* and *D.floralis* infers that oviposition site selection is based upon positive stimuli.

- Ranking of antixenotic resistance to oviposition by *D.radicum* and *D.floralis* was found to be the same for the four genotypes of plants tested in the laboratory (Doon Major, most susceptible, Fribor, most resistant):
  D.floralis, laboratory oviposition varied *x*80 between plant genotypes.
  D.radicum, laboratory oviposition varied *x*5 between plant genotypes.
  D.radicum, field oviposition varied *x*2 between plant genotypes (Doon Major, most susceptible, GRL aga, most resistant).

- Use of surrogate plants treated with leaf surface extracts of a range of Brassicas indicates that leaf surface chemicals strongly influence the site of oviposition of *D.floralis*. Polar compounds are the most stimulatory element for *D.floralis* and a fraction which contained aliphatic glucosinolates stimulated oviposition strongly, although the glucosinolates were not the primary oviposition stimulant. Collaborative experiments indicate that CIF (Cabbage Identification Factor, a substance for which there is no published structure) is probably present in this fraction as the primary oviposition stimulant.

- Field experiments showed that antixenotic resistance to oviposition influenced the numbers of *D.radicum* pupae on plant genotypes at the end of the growing season.

- The concentrations of Brassica root sugars are generally reduced by the damage of both *D.radicum* and *D.floralis*.

- The sugar content of the roots of the Brassica genotypes tested appears to influence the development of the larvae of both *D.radicum* and *D.floralis*.

- The percentage of plant fibre in the roots of Brassicas rises following the damage of *D.floralis* as does the lignin content as a percentage of root fibre and plant weight (fresh and dry).

- The concentrations of individual glucosinolates in Brassica roots are radically altered by the damage of both *D.radicum* and *D.floralis*. *D.floralis* damage results in a rise in the concentration of aromatic glucosinolates and a fall in the concentration of aliphatic glucosinolates. *D.radicum* damage generally resulted in an elevated concentration of both aliphatic and aromatic glucosinolates. There was no clear evidence that glucosinolate profiles were associated with different levels of antibiotic resistance to *D.radicum* and *D.floralis*.

- The swede genotype, GRL aga was consistently resistant to the oviposition and larval feeding of *D.radicum* and *D.floralis* both in the laboratory and, to an extent, in the field.

- The use of "end of season" chemical analyses to assess the influence of plant chemistry on insect development or host plant resistance in field experiments may be fundamentally flawed owing to the wide-ranging changes in the chemical composition of plants found to occur as a result of insect damage.

- The use of damage indices based on the percentage of a plant root damaged by *D.radicum* may be flawed owing to the influence of root size as dictated by plant genotype. This problem is particularly important when contrasting damage between different Brassica crop types (e.g. kales and swedes).

- The influence of known chemical deterrents on the post-alighting oviposition site behaviour of *D.radicum* and *D.floralis*. Particularly the influence of compounds on the relationship between duration of events and ensuing behaviour.

- The confirmation, or otherwise, of CIF and related compounds as the stimulatory compound(s) for *D.floralis* and *D.radicum* in Brassica leaf surface extracts. This work is in progress at The Scottish Crop Research Institute and Swiss Federal Research Institute, Wädenswil.

- The long term effect of insect damage on the chemical composition and growth of Brassica genotypes and the ecological significance of damage-induced responses. (e.g. the impact of first generation cabbage root fly damage on the host plant selection of second generation cabbage root fly).

- The development of an artificial diet to test the influence of root chemistry on *D.radicum* and *D.floralis* larval feeding.

- The quantification and analysis of the micro-distribution of leaf surface chemicals which are important as oviposition stimulants and deterrents. Investigation of how such compounds are detected in the wax matrix by female flies.

- The distribution of nutrients, feeding stimulants and antifeedants in the roots of Brassicas in relation to larval feeding and damage.

- The significance of changes in fibre content and composition to the feeding of *D.radicum* and *D.floralis* larvae.

- The nature of the antibiotic and antixenotic resistance to *D.radicum* and *D.floralis* which appears to be present in GRLaga, and the antibiotic resistance to *D.floralis* of Angela.

Appendix I

The origin of the SCRI breeding line material utilised in this thesis.

GRL aga

GRL = cv. Melfort x BRdf

GRL aga = F_4 family, bulked up.

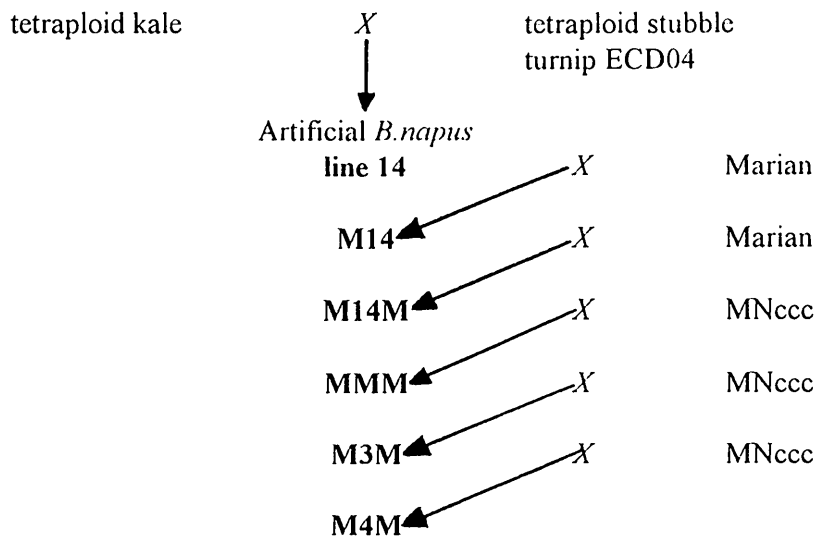
Where BRdf is a breeding line from cv. Ruta Otofte by two generations of selfing.

Nmm3 a-d

Complete details are not available for this breeding line.

The line is derived from cv. Marian with additional clubroot resistance backcrossed in from breeding line RN5 (derived from a Dutch stubble turnip).

M4M2 a-c



MN ccc = breeding line derived from Marian with 3 generations of selfing.

Three plants were then selected from Family 2 of M4M (**M4M2**) and selfed to give M4M2a, M4M2b and M4M2c.

M4M2 a-c is the bulk of these three selfed families.

Appendix II

Examples of Fortran programming utilised in Chapters A1 and A2.

Example 1, program LANDGF.FOR, extracts the amount of time spent grooming the front legs during the landing phase.

```
      program landgf


      integer            fly, curfly, reject, flysp, plsp, x
      double precision   start, gfs, gfe, gfp
      character*4        locn, oldlocn, macrob, microb, oldmacrob
      character*1        switch
      character*3        check
      character*100      fname

111     fname = ' '
        write(*,'(a$)') ' Input file ?   >> '
        read(*,'(a)') fname
        open(unit=3,file=fname,status='old',err=1)

222     fname = ' '
        print*
        write(*,'(a$)') ' Output file ?  >> '
        read(*,'(a)') fname
        open(unit=7,file=fname,status='new',err=2,
     &       carriagecontrol='list')


1     read(3,100)check
100   format(a3)
      if(check.eq.'CRF')flysp=1
      if(check.eq.'TRF')flysp=2
      if(check.eq.'DM') plsp=1
      if(check.eq.'GRL') plsp=2
      if(check.eq.'DGC') plsp=3
      if(check.eq.'FRI') plsp=4


      if (check.ne.'No ') goto 1

2     read(3,200,end=999) fly, start, locn, macrob, microb, switch
200   format(i2,2x,f5.1,4x,a4,4x,a4,4x,a4,2x,a1)
```

continues on next page

```
      if (fly.ne.0) then
         write(*,666)fly
666   format(' fly is ',i2)
         curfly = (flysp * 1000) + (plsp * 100) + fly
         reject = 0
         prop = 0
         x = 0
         oldmacrob = 'junk'
         oldlocn = 'crud'
      endif

4     if ((x.eq.0).and.(macrob.eq.'LAND').and.(microb.eq.'GF')
     *.and.(switch.eq.'+')) then
         gfs = start
       else
         gfs = 0
      endif

      if ((x.eq.1).and.(macrob.eq.'LAND').and.(microb.eq.'GF')
     *.and.(switch.eq.'-')) then
         gfe = start
       else
         gfe = 0
      endif

      if ((macrob.eq.'LAND').and.(microb.eq.'GF')
     *.and.(switch.eq.'+')) then
         x = x + 1
      endif

      if ((macrob.eq.'LAND').and.(microb.eq.'GF')
     *.and.(switch.eq.'-')) then
         x = x - 1
      endif

      if ((x.ne.0).and.(macrob.ne.'LAND')
     *.and.(oldmacrob.eq.'LAND')) then
         gfe = start
      endif

        gfp = gfp + gfe - gfs

      if ((locn.eq.'{eo}').and.(oldmacrob.eq.'LAND')) then
         reject = 1
      endif

      if (locn.eq.'{eo}')
     * then
          write(7,300)flysp, plsp, curfly, gfp, reject
300   format(1x,i2,2x,i2,2x,i4,3x,f10.1,3x,i1)

       gfp = 0
       x = 0
      endif

       oldmacrob = macrob
       oldlocn = locn

      goto 2

999   stop
      end
```

Example 2, program LEREGR.FOR, extracts the amount of time spent grooming during the leaf resting phase.

```
      program leregr (allgrooming)

      integer            fly, curfly, reject, flysp, plsp, x
      double precision   start, grs, gre grp
      character*4        locn, oldlocn, macrob, microb, oldmacrob
      character*1        switch
      character*3        check
      character*100      fname

111     fname = ' '
        write(*,'(a$)') ' Input file ?   >> '
        read(*,'(a)') fname
        open(unit=3,file=fname,status='old',err=1)

222     fname = ' '
        print*
        write(*,'(a$)') ' Output file ?  >> '
        read(*,'(a)') fname
        open(unit=7,file=fname,status='new',err=2,
     &       carriagecontrol='list')

1     read(3,100)check
100   format(a3)
      if(check.eq.'CRF')flysp=1
      if(check.eq.'TRF')flysp=2
      if(check.eq.'DM') plsp=1
      if(check.eq.'GRL') plsp=2
      if(check.eq.'DGC') plsp=3
      if(check.eq.'FRI') plsp=4


      if (check.ne.'No ') goto 1

2     read(3,200,end=999) fly, start, locn, macrob, microb, switch
200   format(i2,2x,f5.1,4x,a4,4x,a4,4x,a4,2x,a1)

      if (fly.ne.0) then
         write(*,666)fly
666   format(' fly is ',i2)
         curfly = (flysp * 1000) + (plsp * 100) + fly
         reject = 0
         micp = 0
         x = 0
         oldmacrob = 'junk'
         oldlocn = 'crud'
      endif
```

continues on next page

```
      if ((locn.eq.'LEAF').and.(macrob.eq.'REST')
     *.and.((microb.eq.'GF').or.(microb.eq.'GR')
     *.or.(microb.eq.'GP')).and.(switch.eq.'+')) then
         grs = start
         x = x + 1
       else
         grs = 0
      endif

      if ((locn.eq.'LEAF').and.(macrob.eq.'REST')
     *.and.((microb.eq.'GF').or.(microb.eq.'GR')
     *.or.(microb.eq.'GP')).and.(switch.eq.'-')) then
         gre = start
         x = x - 1
       else
         gre = 0
      endif

      if ((oldlocn.eq.'LEAF').and.(oldmacrob.eq.'REST')
     *.and.(x.ne.0).and.((locn.eq.'{eo}')
     *.or.(macrob.ne.'REST'))) then
         gre = start
      endif

        grp = grp + gre - grs

      if ((locn.eq.'{eo}').and.(oldmacrob.eq.'REST')) then
         reject = 1
      endif

      if ((oldlocn.eq.'LEAF').and.(macrob.ne.'REST')
     *.and.(oldmacrob.eq.'REST')) then
          write(7,300)flysp, plsp, curfly, grp, reject
300   format(1x,i2,2x,i2,2x,i4,3x,f10.1,3x,i1)

       grs = 0
       gre = 0
       grp = 0
       x = 0
      endif

       oldmacrob = macrob
       oldlocn = locn

      goto 2

999   stop
      end
```

Example 3, program STREEXT.FOR, extracts the amount of time spent with the proboscis extended during the landing phase.

```
      program streext

      integer            fly, curfly, reject, flysp, plsp, x
      double precision   start, mics, mice, micp
      character*4        locn, oldlocn, macrob, microb, oldmacrob
      character*1        switch
      character*3        check
      character*100      fname

111     fname = ' '
        write(*,'(a$)') ' Input file ?   >> '
        read(*,'(a)') fname
        open(unit=3,file=fname,status='old',err=1)

222     fname = ' '
        print*
        write(*,'(a$)') ' Output file ?  >> '
        read(*,'(a)') fname
        open(unit=7,file=fname,status='new',err=2,
     &       carriagecontrol='list')


1     read(3,100)check
100   format(a3)
      if(check.eq.'CRF')flysp=1
      if(check.eq.'TRF')flysp=2
      if(check.eq.'DM') plsp=1
      if(check.eq.'GRL') plsp=2
      if(check.eq.'DGC') plsp=3
      if(check.eq.'FRI') plsp=4


      if (check.ne.'No ') goto 1

2     read(3,200,end=999) fly, start, locn, macrob, microb, switch
200   format(i2,2x,f5.1,4x,a4,4x,a4,4x,a4,2x,a1)

      if (fly.ne.0) then
         write(*,666)fly
666   format(' fly is ',i2)
         curfly = (flysp * 1000) + (plsp * 100) + fly
         reject = 0
         micp = 0
         x = 0
         oldmacrob = 'junk'
         oldlocn = 'crud'
      endif
```

continues on next page

```
4     if ((x.eq.0).and.(locn.eq.'STEM').and.(macrob.eq.'REST')
     *.and.(microb.eq.'EXT').and.(switch.eq.'+')) then
        mics = start
      else
        mics = 0
      endif

      if ((x.ne.0).and.(locn.eq.'STEM').and.(macrob.eq.'REST')
     *.and.(microb.eq.'EXT').and.(switch.eq.'-')) then
        mice = start
      else
        mice = 0
      endif

      if ((x.ne.0).and.(locn.eq.'STEM').and.(macrob.eq.'REST')
     *.and.(oldmacrob.ne.'REST')) then
        mics = start
      endif

      if ((x.ne.0).and.((locn.eq.'{eo}').or.(locn.eq.'STEM')
     *.and.(macrob.ne.'REST').and.(oldmacrob.eq.'REST'))) then
        mice = start
      endif

      if ((microb.eq.'EXT').and.(switch.eq.'+')) then
        x = x + 1
      endif

      if ((microb.eq.'EXT').and.(switch.eq.'-')) then
        x = x - 1
      endif

        micp = micp + mice - mics

      if ((locn.eq.'{eo}').and.(oldmacrob.eq.'REST')) then
        reject = 1
      endif

      if ((oldlocn.eq.'STEM').and.(macrob.ne.'REST')
     *.and.(oldmacrob.eq.'REST')) then
          write(7,300)flysp, plsp, curfly, micp, reject
300   format(1x,i2,2x,i2,2x,i4,3x,f10.1,3x,i1)

       micp = 0

      endif

       mics = 0
       mice = 0

       oldmacrob = macrob
       oldlocn = locn

      goto 2

999   stop
      end
```

REFERENCES & BIBLIOGRAPHY

ADAMS, H., VAUGHAN; J.G., FENWICK; G.R. (1989). The use of glucosinolates for cultivar identification in swede, *Brassica napus* L. *var. napobrassica*. (L.)Peterm. Journal of the Science of Food and Agriculture, **46**, 319-324.

AGARWAL, R.A. (1969). Morphological characteristics of sugarcane and insect resistance. Entomologica Experimentalis et Applicata, **12**, 767-776.

ÅHMAN, I. (1985a). Oviposition behaviour of *Dasineura brassicae* on a high- versus a low-quality brassica host. Entomologica Experimentalis et Applicata, **39**, 247-253.

ÅHMAN, I. (1985b). Larval Feeding period and growth of *Dasineura brassicae* (Diptera) on Brassica host plants. Oikos, **44**, 191-194.

ALBORN, H., KARLSSON, H., LUNDGREN, L., RUUTH, P., STENHAGEN, G. (1985). Resistance in crop species of the genus *Brassica* to oviposition by the turnip root fly, *Hylemya floralis*. Oikos, **44(1)**, 61-69.

ANDERSON, M.D., PENG, C., WEISS, M.J. (1992). Crambe, *Crambe abyssinica* Hochst, as a Flea Beetle Resistant Crop (Coleoptera: Chrysomelidae). Journal of Economic Entomology, **85(2)**, 594-600.

BAKER, E.A. (1974). The influence of the environment on leaf wax development in *Brassica oleracea* var. *gemmifera*. New Phytologist, **73**, 955-966.

BAKER, E.A. (1982). Chemistry and morphology of plant epicuticular waxes. In D.F. Cutler, K.L. Alvin and C.E. Price, Eds. *The Plant Cuticle*. Academic Press, New York, 1982.

BAKER, E.A., HUNT, G.M. (1981). Developmental changes in leaf epicuticular waxes in relation to foliar penetration. New Phytologist, **88**, 731-747.

BARTLET, E., WILLIAMS, I.H. (1991). Factors restricting the feeding of the cabbage stem flea beetle (*Psylliodes chrysocephala*). Entomologica Experimentalis et Applicata, **60**, 233-238.

BECK, S.D. (1965). Resistance of plants to insects. Annual Review of Entomology, **10**, 207-232.

BELL, W.J. (1990). Searching behavior pattern in insects. Annual Review of Entomology, **35**, 447-467.

BERENBAUM, M.; SEIGLER, D.S. (1992). Biochemicals: Engineering Problems for Natural Selection. In: Insect Chemical Ecology An Evolutionary Approach. Eds. ROITBERG, B.D.; ISMAN, M.B.. Chapman & Hall. 1992.

BERENBAUM, M.R.; ZANGERL, A.R. (1992). Genetics of Secondary Metabolism and Herbivore Resistance in Plants. In: Herbivores: Their interactions with secondary plant metabolites (Second Edition) Volume II: Evolutionary and Ecological Processes. Academic Press, 1992.

BERNAYS, E.A., SIMPSON, S.J. (1982). Control of food intake. Advances in Insect Physiology, **16**, 59-118.

BIRCH, A.N.E. (1988). Field and glasshouse studies on components of resistance to root fly attack in swedes. Annals of Applied Biology, **113**, 89-100.

BIRCH, A.N.E. (1989a). Interactions between root flies and brassica roots - clues to alternative control strategies. Roots and the soil environment. Aspects of Applied Biology, **22**, 289-295.

BIRCH, A.N.E. (1989b). A field cage method for assessing resistance to turnip root fly in brassicas. Annals of Applied Biology, **115**, 321-325.

BIRCH, A.N.E., GRIFFITHS, D.W., MacFARLANE-SMITH, W.H. (1990). Changes in forage and oilseed rape (*Brassica napus* L.) root glucosinolates in response to attack by turnip root fly (*Delia floralis* Fall.). Journal of the Science of Food and Agriculture, **51**, 309-320.

BIRCH, A.N.E., GRIFFITHS, D.W., HOPKINS, R.J., MacFARLANE-SMITH, W.H., McKINLAY, R.G. (1992). Glucosinolate in Responses of Swede, Kale, Forage and Oilseed Rape to Root Damage by Turnip Root Fly (*Delia floralis*) Larvae. Journal of the Science of Food and Agriculture, **58**, 1-9.

BIRCH, A.N.E., STÄDLER, E., HOPKINS, R.J., SIMMONDS, M.S.J., BAUR, R., GRIFFITHS, D.W., RAMP, T., HURTER, J., McKINLAY, R.G. (1993). In: Proceedings of the IOBC/WPRS Working Group. Breeding for Resistance to Insects and Mites. Editors: P.R. Ellis & J.Freuler.

BIRCH, N. (1985). Preliminary observations on swedes with resistance and susceptibility to turnip root fly (*Delia floralis*) in Scotland. In:Proceeding of the CEC / IOBC Experts Group Meeting .Rennes Nov. 1985. Progress on pest management in field vegetables. Editors: R. Cavalloro & C. Pelerents.

BIRD, L.S. (1982). The M.A.R. (Multi-Adversity Resistance) System for genetic improvement of cotton. Plant Diseases, **66(2)**, 172-176.

BOAG, B., MACFARLANE-SMITH, W.H., GRIFFITH, D.W., SMITH, W.H.M. (1990). Observations on the grazing of double low oilseed rape and other crops by roe deer. Applied Animal Behavioural Science, **28(3)**, 213-220.

BODNARYK, R.P. (1991). Developmental profile of sinalbin (*p*-hydroxybenzyl glucosinolate) in mustard seedlings, *Sinapis alba* L.,and its relationship to insect resistance. Journal of Chemical Ecology, **17(8)**, 1543-1556.

BRACKEN, G.K. (1988). Seasonal occurence and infestation potential of cabbage maggot, (*Delia radicum*) (L.) (DIPTERA : ANTHOMYIIDAE), attacking rutabaga in Manitoba as determined by captures of females using water traps. Canadian Entomologist, **120**, 609-614.

BRADSHAW, J.E., BIRCH, A.N.E., GEMMELL, D.J., WILLIAMSON, C.J. (1989). Progress at S.C.R.I. in breeding swedes (*Brassica napus* L. ssp. *rapifera*) with improved disease and pest resistance. Aspects of Applied Biology, **23**, 15-21.

BRADSHAW, J.E., GRIFFITHS, D.W. (1990). Sugar content of swedes for stockfeeding. Journal of the Science of Food and Agriculture, **50**, 167-172.

BUENDGEN, M.R., COORS, A.W., GROMBACHER, RUSSEL, W.A. (1990). European corn borer resistance and cell wall composition of three maize populations. Crop Science, **30**, 505-510.

CAPRIO, M.A.; TABASHNIK, B.E. (1992). Evolution of Resistance to Plant Defensive Chemicals in Insects. In: Insect Chemical Ecology An Evolutionary Approach. Eds. ROITBERG, B.D.; ISMAN, M.B.. Chapman & Hall. 1992.

CHEW, F.S. (1988). Biological Effects of Glucosinolates. In: Biologically Active Natural Products, American Chemical Society.

CARLSON, D.G., DAXENBICHLER, M.E., VANETTEN, C.H., TOOKEY, H.L., WILLIAMS, P.H. (1981). Glucosinolates in crucifer vegetables: turnips and rutabagas. Journal of Agriculture and Food Chemistry, **29**, 1235-1239.

CLOSSAIS-BESNARD, N., LARHER, F. (1991). Physiological Role of Glucosinolates in *Brassica napus*. Concentration and Distribution Pattern of Glucosinolates among Plant Organs during a Complete Life Cycle. Journal of the Science of Food and Agriculture, **56**, 25-38.

COAKER, T.H., FINCH, S. (1971). The cabbage root fly, *Erioischia brassicae* (Bouché). Report of The National Vegetable Research Station for 1970 (1971), 23-42.

COAKER, T.H., WILLIAMS, D.A. (1963). The importance of some Carabidae and Staphylinidae as predators of the cabbage root fly, *Erioischia brassicae* (Bouché). Entomologica Experimentalis et Applicata, **6**, 156-164.

COCHRAN, W.G. (1938). Some difficulties in the statistical analysis of replicated experiments. Empire Journal of Experimental Agriculture, **6(22)**, 157-175.

COLE, R.A. (1980). The use of porous polymers for the collection of plant volatiles. Journal of the Science of Food and Agriculture, **31**, 1242-1249.

COLE, R.A., JONES, T.H., FINCH, S. (1989). Deterent effect of carboxylic acids on cabbage root fly oviposition. Annals of Applied Biology, **115**, 39-44.

COLE, R.A., PHELPS, K. (1979). Use of canonical variate analysis in the differentiation of swede cultivars by gas-liquid chromatography of volatile hydrolysis products. Journal of the Science of Food and Agriculture, **30**, 669-676.

COLLIER, R.H., FINCH, S. (1983). Completion of diapause in field populations of the cabbage root fly (*Delia radicum*). Entomologica Experimentalis et Applicata, **34**, 186-192.

COURTNEY, S.P., CHEN, G.K., GARDNER, A. (1989). A general model for individual host selection. Oikos, **55**, 55-65.

DAPSIS, L.J., FERRO, D.N. (1983). Effectiveness of baited cone traps for monitoring adult cabbage maggots: with notes on female ovarian development. Entomologica Experimentalis et Applicata, **33**, 35-42.

DARROCH, C.S., BELL, J.M. (1991). Potential goitrogenic and toxic effects of indole glucosinolate extract injected in to the developing chick embryo. Canadian Journal of Animal Science, **71**, 481-487.

DAXENBICHER, M.E., VANETTEN, C.H., WILLIAMS, P.H. (1979). Cruciferous vegetables, analysis of 14 varieties of chinese cabbage. Journal of Agriculture and Food Chemistry, **27(1)**, 34-37.

DENNA , D.W. (1970). Transpiration and the waxy bloom in *Brassica oleracea* L.. Australian Journal of Biological Science, **23**, 27-31.

DETHIER, V.G., BARTON-BROWN, L., SMITH, C.N. (1960). The designation of chemicals in terms of the responses they elicit from insects. Journal of Economic Entomology, **53**, 134-136.

DICKE, M., SABELIS, M.W. (1988). Infochemical terminology: based on cost benefit analysis rather than origin of compounds? Functional Ecology, **2**, 131-139.

DOANE, J.F., CHAPMAN, R.K. (1962). Oviposition preference of the cabbage maggot, *Hylemya brassicae* (Bouché). Journal of Economic Entomology, **55(1)**, 137-138.

DOANE, J.F., CHAPMAN, R.K. (1964a). The relation of the cabbage maggot, *Hylemya brassicae* (Bouché), to decay in some cruciferous crops. Entomologica Experimentalis et Applicata, **7**, 1-8.

DOANE, J.F., CHAPMAN, R.K. (1964b). Development of the cabbage maggot, *Hylemya brassicae* (Bouché), on aseptic and decaying rutabaga tissue. Entomologica Experimentalis et Applicata, **7**, 115-119.

DOUGHTY, K.J., PORTER, A.J.R., MORTON, A.M., KIDDLE, G., BOCK, C.H., WALLSGROVE, R. (1991). Variation in the glucosinolate content of oilseed rape (*Brassica napus* L.) leaves. II. Response to infection by *Alternaria brassicae* (Berk.) Sacc. Annals of Applied Biology, **118**, 469-477.

DUNCAN, A.J., MILNE, J.A. (1992). Effect of Long-term Intra-ruminal Infusion of the Glucosinolate Metabolite Allyl Cyanide on the Voluntary Food Intake and Metabolism of Lambs. Journal of the Science of Food and Agriculture, **58**, 9-14.

DUNN, J.P., POTTER, D.A., KIMMERER, T.W. (1990). Carbohydrate reserves, radial growth, and mechanisms of resistance of oak trees to phloem-boring insects. Oecologia, **83**, 458-468.

ELLIS, P.R., COLE, R.A., CRISP, P., HARDMAN, J.A. (1980). The relationship between cabbage root fly egg laying and volatile hydrolysis products of radish. Annals of Applied Biology, **95**, 283-289.

ELLIS, P.R., DOWKER, B.D., FREEMAN, G.H., HARDMAN, J.A. (1985). Problems in field selection for resistance to carrot fly (*Psila rosae*) in carrot cv. LONG CHANTENAY. Annals of Applied Biology, **106**, 349-356.

ELLIS, P.R., HARDMAN, J.A. (1975). Laboratory methods for studying non-preference resistance to cabbage root fly in cruciferous crops. Annals of Applied Biology, **79**, 253-264.

ELLIS, P.R., HARDMAN, J.A., CRISP, P., JOHNSON, A.G. (1976). Laboratory studies of non-preference resistance to cabbage root fly in radish. Annals of Applied Biology, **84**, 81-89.

EYMAN, M., FRIEND, W.G. (1985). Development of Onion Maggots (Diptera: Anthomyiidae) on Bacteria-free Onion Agar Supplemented with Vitamins and Amino Acids. Annals of the Entomological Society of America, **78(2)**, 182-185.

van EMDEN, H.F. (1990). The effect of *Brevicoryne brassicae* on leaf area, dry matter distribution and amino acids of the Brussels sprout plant. Annals of Applied Biology, **116**, 199-204.

FEENY, P. (1992). The Evolution of Chemical Ecology: Contributions from the study of Herbivorous Insects. In: Herbivores: Their interactions with secondary plant metabolites (Second Edition) Volume II: Evolutionary and Ecological Processes. Academic Press, 1992.

FENWICK, G.R., HEANEY, R.K., MULLIN, W.J. (1983). Glucosinolates and their breakdown product in food and food plants. CRC, Critical Reviews in Food Science and Nutrition, **18(2)**, 123-210.

FINCH, S. (1971). The fecundity of the cabbage root fly *Erioschia brassicae* under field conditions. Entomologica Experimentalis et Applicata, **14**, 147-160.

FINCH, S. (1974). Feeding and associated behaviour of the adult cabbage root fly *Erioischia brassicae* (Bch.) (Dipt., Anthomyiidae) under laboratory conditions. Bulletin of Entomological Research, **63**, 661-671.

FINCH, S. (1978). Volatile plant chemicals and their effect on host plant finding by the cabbage root fly (*Delia brassicae*). Entomologica Experimentalis et Applicata, **24**, 150-159.

FINCH, S. (1986). Assessing Host-Plant Finding by Insects. In: Insect-Plant Interactions. Eds: Miller, J.R. & Miller, T.A.. Springer-Verlag, New York.

FINCH, S. (1993). Integrated pest management of the cabbage root fly and carrot fly. Crop Protection, **12(6)**, 423-430.

FINCH, S., ACKLEY, C.M. (1977). Cultivated and wild host plants supporting populations of the cabbage root fly. Annals of Applied Biology, **85**, 13-22.

FINCH, S., COAKER, T.H. (1969). A method for the continuous rearing of the cabbage root fly *Erioschia brassicae* (Bouché) and some observations on its biology. Bullitin of Entomological Research, **58**, 619-627.

FINCH, S., COLLIER, R.H. (1989). Effects of the angle of inclination of traps on the numbers of large Diptera caught on sticky boards in certain vegetable crops. Entomologica Experimentalis et Applicata, **52**, 23-27.

FINCH, S, COLLIER, R.H., SKINNER, G. (1986). Local population differences in emergence of cabbage root fly from S.W. Lancashire: implications for pest forecasting and population divergence. Ecological Entomology, **11(2)**, 139-145.

FINCH, S., JONES, T.H. (1987). Interspecific competition during host plant selection by insect pests of cruciferous crops. In: Insects-Plants. Dr W.Junk (publisher) Eds. Labeyrie, V., Fabres, G., Lachaise, D.

FINCH, S., JONES, T.H. (1988). Naturally-occuring deterents to cabbage root fly egg-laying. Proceeding, Brighton Crop Protection Conferance, Pests and Diseases-1988, 263-268.

FINCH, S., JONES, T.H. (1989). An analysis of the deterent effect of aphids on cabbage root fly (*Delia radicum*) egg-laying. Ecological Entomology, **14**, 387-391.

FINCH, S, SKINNER, G. (1973). Distribution of cabbage root flies in brassica crops. Annals of Applied Biology, **75**, 1-14.

FINCH, S., SKINNER, G. (1974). Some factors affecting the efficiency of water-traps for capturing cabbage root flies. Annals of Applied Biology, **77**, 213-226.

FINCH, S., SKINNER, G. (1975a). An improved method of marking cabbage root flies. Annals of Applied Biology, 79, 243-246.

FINCH, S., SKINNER, G. (1975b). Dispersal of the cabbage root fly. Annals of Applied Biology, 81, 1-19.

FINCH, S., SKINNER, G. (1982). Upwind flight by the cabbage root fly, *Delia radicum*. Physiological Entomology, 7, 387-399.

FINCH, S., SKINNER, G., FREEMAN, G.H. (1975). The distribution and analysis of cabbage root fly egg populations. Annals of Applied Biology, 79, 1-18.

FINCH, S., SKINNER, G., FREEMAN, G.H. (1976). The effect of plant density on populations of cabbage root fly on four cruciferous crops. Annals of Applied Biology, 83, 191-197.

FINCH, S., SKINNER, G., FREEMAN, G.H. (1978). Distribution and analysis of cabbage root fly pupal population. Annals of Applied Biology, 88, 351-356.

FONTY, G., GOUET, P. (1989). Fibre-degrading Microorganisms in the monogastric digestive tract. Animal Feed Science and Technology, 23, 91-107.

FOSTER, S.P., HARRIS, M.O. (1992). Foliar chemicals of wheat and related grasses influencing oviposition by Hessian fly, *Mayetiola destructor* (Say) (Diptera: Cecidomyiidae). Journal of Chemical Ecology, 18(11), 1965-1980.

FRAZIER, J.L. (1992). How Animals Perceive Secondary Plant Compounds. In: Herbivores: Their interactions with secondary plant metabolites (Second Edition) Volume II: Evolutionary and Ecological Processes. Academic Press, 1992.

FREULER, J., FISCHER, S. (1982). Description d'un piege a oeufs pour la mouche du chou, *Delia brassicae* WIEDMANN. Bulletin de la Societe Entomologique Suisse, 55, 77-85.

FULLER, B.W., REAGAN, T.E. (1989). The relationship of sweet sorghum plant fibre and survival of the sugarcane borer, *Diatraea saccharalis* (F.) (Lepidoptera: Pyralidae). Journal of Agricultural Entomology, 6(2), 113-118.

FUTUYMA, D.J. (1983). Selective Factors in the Evolution of Host Choice by Phytophagous Insects. In: Herbivorous Insects: Host-Seeking Behaviour and mechanisms. Ed. S. Ahmad, Academic Press, New York.

FUTUYMA, D.J., KEESE, M.C. (1992). Evolution and Coevolution of Plants and Phytophagous Arthropods. In: Herbivores: Their interactions with secondary plant metabolites (Second Edition) Volume II: Evolutionary and Ecological Processes. Academic Press, 1992.

GEMMEL, D.J., GRIFFITHS, D.W., BRADSHAW, J.E. (1990). Effect of Cultivar and harvest date on Dry-matter Content, Hardness and Sugar Content of Swedes for Stockfeeding. Journal of the Science of Food and Agriculture, 53, 333-342.

GLEN, D.M., JONES, H., FIELDSEND, J.K. (1990). Damage to oilseed rape seedlings by the field slug *Deroceras reticulatum* in relation to glucosinolate concentration. Annals of Applied Biology, 117, 197-207.

GOERING, H.K., VAN SOEST, P.J. (1970). Forage Fibre Analysis (Apparatus, Reagents, Proceedures, and Some Applications). Agriculture Handbook 379, U.S. Department of Agriculture.

GOULD, F. (1991). Arthropod behaviour and the efficacy of plant protectants. Annual Review of Entomology, **36**, 305-330.

GOWERS, S., MUNRO, I.K., GEMMELL, D.J. (1984). Turnip root-fly resistance in swedes. Cruciferae Newsletter, **9**, 22-23.

GRIFFITHS, D.W., BRADSHAW, J.E., TAYLOR, J., GEMMELL, D.J. (1991). Effect of Cultivar and Harvest Date on the Glucosinolate and S-Methylcysteine Sulphoxide Content of Swedes (*Brassica napus* ssp *rapifera*). Journal of the Science of Food and Agriculture, **56**, 539-549.

HAGERMAN, A.E., BUTLER, L.G. (1991). Tannins and Lignins. In: Herbivores: Their interactions with secondary plant metabolites (Second Edition) Volume II: Evolutionary and Ecological Processes. Academic Press, 1992.

HARDMAN, J.A., ELLIS, P.R. (1978). Host plant factors influencing the susceptibility of cruciferous crops to cabbage root fly attack. Entomologica Experimentalis et Applicata, **24**, 193-197.

HARRIS, C.R., SVEC, H.J. (1966). Mass rearing of the cabbage maggot under controlled environmental conditions with observations on the biology of cyclodiene susceptible and resistant strains. Journal of Economic Entomology, **59(3)**, 569-573.

HAVUKKALA,I. (1987). Odour source finding behavior of the turnip root fly , *Delia floralis* (FALL.) (DIPTERA : ANTHOMYIIDAE) in the field. Journal of Applied Entomology, **104**, 105-110.

HAVUKKALA, I. (1988). Non-chemical control methods against cabbage root flies *Delia radicum* and *Delia floralis* (ANTHOMYIIDAE). Annales Agriculturae Fenniae, **27**, 271-279.

HAVUKKALA, I., VIRTANEN, M. (1985). Behavioral sequence of host selection and oviposition in the turnip root fly, *Delia floralis* (FALL) (ANTHOMYIIDAE). Zeitschrift fur Angewante Entomologie, **100(1)**, 39-47.

HAWKES, C. (1972). The diurnal periodicity and cycle of behavior of the adult cabbage root fly (*Erioischia brassicae*) Annals of Applied Biology, **70**, 109-118.

HAWKES, C. (1975). Physiological condition of adult cabbage root fly (*Erioischia brassicae* (BOUCHE)) attracted to host plants. Journal of Applied Ecology, **12**, 497-506.

HAWKES, C., COAKER, T.H. (1976). Behavioral responses to host-plant odours in adult cabbage root fly, (*Erioischia brassicae* [Bouché]). Symposium Biology Hungary, **16**, 85-89.

HAWKES, C., PATTON, S., COAKER, T.H. (1978). Mechanisms of host plant finding in adult cabbage root fly, *Delia brassicae*. Entomologica Experimentalis et Applicata, **24**, 219-227.

HEANEY, R.K., FENWICK, G.R. (1980a). Glucosinolates in brassica vegetables. Analysis of 22 varieties of brussels sprout. (*Brassica oleracea* var. *gemmifera*). Journal of the Science of Food and Agriculture, **31**, 785-793.

HEANEY, R.K., FENWICK, G.R. (1980b). The glucosinolate content of brassica vegetables. A chemotaxonomic approach to cultivar identification. Journal of the Science of Food and Agriculture, **31**, 794-801.

HEDIN, P.A., DAVIS, F.M., WILLIAMS, W.P., SALIN, M.L. (1984). Possible factors of leaf-feeding resistance in corn to the southwestern corn borer. Journal of Agriculture and Food Chemistry, **32**, 262-267.

HONDA, I., ISHIKAWA, Y. (1987). Electrophysiological studies on the dorsal and Anterior Organs of the Onion Fly Larvae, *Hylemya antiqua* MEIGEN (Diptera: Anthomyiidae). Applied Entomology and Zoology, **22**, 410-416.

HORBER, E. (1972). Plant Resistance to Insects. Agricultural Science Review, **10(2)**, 1-10.

HORBER, E. (1980). Types and classification of resistance. In: Breeding Plants Resistant to Insects. Maxwell, F.G., Jennings, P.R., John Wiley & Sons.

HUANG, X., RENWICK, J.A.A. (1993). Differential selection of host plants by two *Pieris* species, the role of oviposition stimulants and deterents. Entomologica Experimentalis et Applicata, **68**, 59-69.

HUANG, X., RENWICK, J.A.A., SACHDEV-GUPTA, K. (1993). A chemical basis for differential acceptance of *Erysimum cheiranthoides* by two *Pieris* species. Journal of Chemical Ecology, **19(2)**, 195-210.

HUGHES, R.D., SALTER, D.D. (1959). The natural mortality of *Erioischia brassicae* (Bouché) (DIPTERA : ANTHOMYIIDAE) during the egg stage of the first generation. Journal of Animal Ecology, **28**, 343-357.

HUISMAN, J., TOLMAN, G.H. (1992). Antinutritional factors in the plant proteins of diets for non-ruminants. In: Recent Advances in Animal Nutrition, Garnsworthy, P.C., Haresign, w., Cole, D.J.A. (Eds), Butterworth-Heinemann Ltd.

HUNT, G.M., BAKER, E.A. (1982). Developmental and environmental variations in plant epicuticular waxes: some effects on the penetration of naphthylacetic acid. In: The Plant Cuticle. Eds. CUTLER, D.F., ALVIN, K.L, PRICE, C.E..Academic Press. London. 1982.

HUTCHINS, S.H., BUXTON, D.R., PEDIGO, L.P. (1989). Forage quality of alflfa as affected by potato leafhopper feeding. Crop Science, **29**, 1541-1545.

IIYAMA, K., WALLIS, A.F.A. (1988). An improved acetyl bromide proceedure for determining lignin in woods and wood pulps. Wood Science and Technology, **22**, 271-280.

JAENIKE, J.; PAPAJ, D.R. (1992). Behavioural Plasticity and Patterns of Host Use by Insects. In: Insect Chemical Ecology An Evolutionary Approach. Eds. ROITBERG, B.D.; ISMAN, M.B.. Chapman & Hall. 1992.

JAENIKE, J. (1978). On Optimal Oviposition Behaviour in Phytophagous Insects. Theoretical Population Biology, **14**, 350-356.

JEFFREE, C.E. (1986). The cuticle, epicuticular waxes and trichomes of plants, with reference to their structure, functions and evolution. In: Insects and the plant surface. Eds.JUNIPER, B., SOUTHWOOD, R.. Edward Arnold LTD.1986.

JONES, R.E. (1992). Search behaviour: strategies and outcomes. In: Proceedings of the 8th International Symposium on Insect-Plant Relationships. Eds. MENKEN, S.B.J.; VISSER, J.H.; HARREWIJN, P. Kluwer Academic Publishers. 1992.

JONES, T.H., COLE, R.A. and FINCH S. (1988). A cabbage root fly oviposition deterent in the frass of garden pebble moth caterpillars. Entomologica Experimentalis et Applicata, **49(3)**, 277-282.

JONES, T.H., FINCH, S. (1987). The effect of a chemical deterent, released from the frass of caterpillars of the garden pebble moth, on cabbage root fly oviposition. Entomologica experimentalis et applicata, **45**, 283-288.

JONES, F.G.W., JONES, M.G. (1984). Pests of Field Crops. (3rd Edition). Edward Arnold. 1984.

JÖRGENSEN, J. (1957). Den store kalflue (Cortophila floralis Fall.). Nyere undersøgelser vedrørende dens biologi, parasitering og bekaempelse. Tiderisk for Planteavl, **60**, 657-712.

JU, H., CHONG, C., BIBLE, B., MULLIN, W.J. (1980). Seasonal variation in glucosinolate composition of rutabaga and turnip. Canadian Journal of Plant Science, **60**, 1295-1302.

JUDD, G.J.R., BORDEN, J.H. (1992). Aggregated oviposition in *Delia antiqua* (Meigen): A case for mediation by semiochemicals. Journal of Chemical Ecology, 18(4), 621-633.

KING, G.J. (1990). Molecular genetics and breeding of vegetable brassicas. Euphytica, **50**, 97-112.

KJAER, A. (1976). Glucosinolates in the cruciferae. In: The Biology and Chemistry of the Cruciferae. Eds. VAUGHAN, J.G., MacLEOD, A.J., JONES, B.M.G. Academic Press. 1976.

KOGAN, M. (1977). The role of Chemical Factors in Insect/Plant Relationships. In: Proceedings of The XV International Congress of Entomology.

KOGAN, M., ORTMAN, E.F. (1978). Antixenosis-A New Term Proposed to Define Painter's "Nonpreference" Modality of Resistance. Bulletin of the Entomological Society of America, **24(2)**, 175-176.

KORITSAS, V.M. (1990). Biochemical and physiological responses of oilseed rape (*Brassica napus* L.) to infestation by the cabbage stem flea beetle (*Psylliodes chrysocephala* L.). PhD thesis. University of London.

KORITSAS, V.M., LEWIS, J.A., FENWICK, G.R. (1989). Accumulation of indole glucosinolates in *Psylliodes chrysocephala* L.-infested or damaged tissues of oilseed rape. (*Brassica napus* L.). Experimentia, **45**, 493-495.

KORITSAS, V.M., LEWIS, J.A., FENWICK, G.R. (1991). Glucosinolate responses of oilseed rape, mustard and kale to mechanical wounding and infestation by cabbage stem flea beetle (*Psylliodes chrysocephala*) Annals of Applied Biology, **118**, 209-221.

KOŠTÁL, V. (1991). Orientation Behavior of Newly Hatched Larvae of the Cabbage Maggot, *Delia radicum* (L.) (Diptera:Anthomyiidae), to Volatile Plant Metabolites. Journal of Insect Behaviour, **5(1)**, 61-70.

KOŠTÁL, V. (1992). Physical and chemical factors influencing landing and oviposition by the cabbage root fly on host-plant models. Entomologica Experimentalis et Applicata, **66**, 109-118.

KRAMER, M., SCMIDHAMMER, J. (1992). The chi-squared statistic in ethology: use and misuse. Animal Behaviour, **44**, 833-841.

KURPPA, S. (1989). Pests of cultivated plants in Finland during 1988. Annales Agriculturae Fenniae, **28**, 97-102.

LAMB, D.J. (1984). Monitoring and forecasting the activity of root fly pests of brassicas in the West of Scotland. Proceedings, Crop Protection in Northern Britain, 1984.

LAMMERINK, J., MacGIBBON, D.B., WALLACE, A.R. (1984). Effect of the cabbage aphid (*Brevicoryne brassicae*) on total glucosinolate in the seed of oilseed rape (*Brassica napus*). New Zealand Journal of Agricultural Research, **27**, 89-92.

LANDOLT, P.J. (1993). Effects of host plant leaf damage on cabbage looper moth attraction and oviposition. Entomologica Experimentalis et Applicata, **67**, 79-85.

LICHTENSTEIN, E.P., TRONG, F.M., MORGAN, D.G. (1962). Identification of 2-phenylethylisothiocyanate as an insecticide occuring naturally in the edible part of turnips. Journal of Agriculture and food chemistry, **10**, 30-33.

van LOON, J.J.A., BLAAKMEER, A., GRIEPINK, F.C., van BEEK, T.A., SCHOONHOVEN, L.M., de GROOT, A. (1992). Leaf surface compound from *Brassica oleracea* (Cruciferae) induces oviposition from *Pieris brassicae* (Lepidoptera: Pieridae). Chemoecology, **3**, 39-44.

LOUDA, S., MOLE, S. (1991). Glucosinolates: Chemistry and Ecology. In: Herbivores: Their interactions with secondary plant metabolites (Second Edition) Volume I: The Chemical Participants. Academic Press, 1991.

MacFARLANE-SMITH, W.H., GRIFFITHS, D.W. (1988). A time-course study of glucosinolates in the ontogeny of forage rape (*Brassica napus* L.). Journal of the Science of Food and Agriculture, **43**, 121-134.

MacDOUGALL, R.S. (1902). Disease in turnips and swedes. Transactions of the Highland and Agricultural Society of Scotland, **5, (XV)**, 244-263.

McCLOSKY, C., ISMAN, M.B. (1993). Influence of foliar glucosinolates in oilseed rape and mustard on feeding and growth of the bertha armyworm, *Mamestra configura* Walker. Journal of Chemical Ecology, **19(2)**, 249-266.

McDANELL, R., McLEAN, A.E.M., HANLEY, A.B., HEANEY, R.K., FENWICK, G.R. (1988). Chemical and biological properties of indole glucosinolates (glucobrassicins): a review. Food and Chemical Toxicology, **26(1)**, 59-70.

McDONALD, R.S., SEARS, M.K. (1991). Effects of root damage by cabbage maggot, *Delia radicum* (L.) (DIPTERA:ANTHOMYIIDAE), on canola, *Brassica campestris* L., under laboratory conditions. The Canadian Entomolgist, **123**, 861-867.

McKINLAY, R.G., BIRCH, A.N.E. (1992). Integrated control of root flies in swede. In: Proceedings of the IOBC Working Group, Integrated Control in Field Vegetable crops, Eds Finch, S., Freuler, J..

MILBURN, M.M. (1843). An account of the insects injurious to turnips. Transactions of the Highland Agricultural Society, **8**, 47-54.

MILFORD, G., EVANS, E. (1990). Factors that Determine Levels of Glucosinolates in Oilseed Rape. AFRC News, October 1990, 8-10.

MILLER, J.R., COWLES, R.S. (1990). Stimulo-deterent diversion: a concept and its possible application to onion maggot control. Journal of Chemical Ecology, **16**, 3197-3212.

MILLER, R.W., EARLE, F.R., WOLFF, I.A. (1965). Search for new industrial oils. XIII. Oils from 102 species of cruciferae. Journal of the American oil chemistry Society, **42**, 817-821.

MOCHIZUKI,A., ISHIKAWA, Y., MATSUMOTO, Y. (1985). Sugars as Phagostimulants of the Onion Fly Larvae, *Hylemya antiqua* MEIGEN (Diptera: Anthomyiidae). Applied Entomology and Zoology, **20**, 465-469.

MORISON, G.D. (1939). The turnip root fly (*Phorbia floralis* Fallen) in Northern Scotland. Proceeding 7th International Congress of Entomology, Berlin, 1938, 2576-2584.

MORRISON, I.M. (1972a). A Semi-micro Method for the Determination of Lignin and its Use in Predicting the Digestibility of Forage Crops. Journal of the Science of Food and Agriculture, **23**, 455-463.

MORRISON, I.M. (1972b). Improvements in the Acetyl Bromide Technique to Determine Lignin and Digestibility and its Application to Legumes. Journal of the Science of Food and Agriculture, **23**, 1463-1469.

MOSSOBA, M.M., SHAW, G.J., ANDRZEJEWSKI, D., SPHON, J.A., PAGE, S.W. (1989). Application of gas chromatography/matrix isolation/fourier transform infrared spectrometry to the identification of glucosinolates from *brassica* vegetables. Journal of Agriculture and Food Chemistry, **37**, 367-372.

MUKERJI, M.K. (1969). Oviposition preference and survival of *Hylemya brassicae* on some cruciferous crops. Canadian Entomologist, **101**, 153-158.

MULLIN, W.J. (1980). Hydrolysis products from glucosinolates in rutabaga (*Brassica napobrassica* Mill). Journal of Food Technology, **15**, 163-168.

MULLIN, W.J., PROUDFOOT, K.G., COLLIN, M.J. (1980). Glucosinolate content and clubroot of rutabaga and turnip. Canadian Journal of Plant Science, **60**, 605-612.

MULLIN, W.J., SAHASRABUDHE, M.R. (1977). Glucosinolate content of cruciferous vegetable crops. Canadian Journal of Plant of Plant Science, **57**, 1227-1230.

NAIR, K.S.S., McEWEN, F.L. (1976).Host selection by the adult cabbage maggot, *Hylemya brassicae* (DIPTERA : ANTHOMYIIDAE): effect of glucosinolates and common nutrients on oviposition. Canadian Entomologist, **108**, 1021-1030.

NEWMAN, R.M., HANSCOM, Z., KERFOOT, W.C. (1992). The watercress glucosinolate-myrosinase system: a feeding deterrent to caddisflies, snails and amphipods. Oecologica, **92**, 1-7.

NOLDUS, L.P.J.J., VAN DE LOO, E.L.H.M., TIMMERS, P.H.A. (1989). Computers in behavioral research. Nature, **341**, No.6244, 767-768.

NOTTINGHAM, S.F. (1988). Host-plant finding for oviposition by adult cabbage root fly, (*Delia radicum*). Journal of Insect Physiology, **34(3)**, 227-234.

NOTTINGHAM, S.F., COAKER, T.H. (1985). The olfactory response of cabbage root fly, *Delia radicum*, to the host plant volatile allylisothiocyanate. Entomologica Experimentalis et Applicata, **39**, 307-316.

NOTTINGHAM, S.F., COAKER, T.H. (1987). Changes in flight track angles of cabbage root fly, *Delia radicum*, in diffuse clouds and discrete plumes of the host plant volatile allylisothiocyanate. Entomologica Experimentalis et Applicata, **43**, 275-278.

OUDEN, H.den, THEUNISSEN, J. (1988). Preference and non-preference in monitoring cabbage root fly , *Delia radicum/D.brassicae*, oviposition by traps. Acta Horticulturae, **219**, 11-14.

PAINTER, R.H. (1941). The Economic Value and Biological Significance of Insect Resistance Plants. Journal of Economic Entomology, **34**, 358-367.

PAINTER, R.H. (1951). Insect Resistance in Crop Plants. The MacMillan Company, 1951.

PAPAJ, D.R., PROKOPY, R.J. (1986). Phytochemical basis of learning in *Rhagoletis pomonella* and other herbivorous insects. Journal of Chemical Ecology, **12(5)**, 1125-1143.

PELL, E.J., WINNER, W.E., VINTEN-JOHANSEN, C., MOONEY, H.A. (1990). Response of radish to multiple stresses. New Phytologist, **115**, 439-446.

POND, D.D., DIONE, L.A., WHITE, R.G., MOORE, C.A. (1962). Note on egg-laying response of two species of root maggot on turnips bred for resistance to the cabbage root maggot, *Hylemya brassicae* (Bouché). Canadian Journal of Plant Science, **42**, 530-531.

de PONTI, O.M.B. (1982). Plant resistance to insects: a challenge to plant breeders and entomologists. In: Proceedings of the 5th International Symposium on Insect-Plant Relationships. P. Kluwer Academic Publishers. 1982.

PRINS, R.A., KREULEN, D.A. (1991). Comparative aspects of plant cell wall digestion in insects. Animal Feed Science and Technology, **32**, 101-118.

PROKOPY, R.J., COLLIER, R.H., FINCH, S. (1983a). Visual detection of host plants by cabbage root flies. Entomologica Experimentalis et Applicata, **34**, 85-89.

PROKOPY, R.J., COLLIER, R.H., FINCH, S. (1983b). Leaf color used by cabbage root flies to distinguise among host plants. Science, **221**, 190-192.

RAUSHER, M.D. (1985). Variability for host preference in insect populations: mechanistic and evolutionary models. Journal of Insect Physiology, **31(11)**, 873-889.

RAUSHER, M.D. (1992). Natural Selection and the Evolution of Plant-Insect Interactions. In: Insect Chemical Ecology An Evolutionary Approach. Eds. ROITBERG, B.D.; ISMAN, M.B.. Chapman & Hall. 1992.

READ, D.C. (1960). Mass rearing of the Cabbage Maggot, *Hylemya brassicae* (Bouché) (Diptera: Anthomyiidae) in the Greenhouse. The Canadian Entomologist, **92**, 574-576.

READ, D.C. (1965). Rearing Root Maggots, Chiefly *Hylemya brassicae* (Bouché) (Diptera: Anthomyiidae) for bioassay. The Canadian Entomologist, 97, 136-141.

READ, D.C., WELCH, H.E. (1962). Establishing and Maintaining a Culture of *Hylemya brassicae* (Bouché) (Diptera: Anthomyiidae) in the Greenhouse or Laboratory. The Canadian Entomologist, 94, 458-460.

REED, D.W., PIVNICK, K.A., UNDERHILL, E.W. (1989). Identification of chemical oviposition stimulants for the diamondback moth, *Plutella xylostella*, present in three species of Brassicaceae. Entomologica Experimentalis et Applicata, 53, 277-286.

ROBERTSON, J.A., MURISON, S.D., CHESSON, A. (1992). Particle Size Distribution and Solubility of Dietary Fibre in Swede- (*Brassica napus*) based and Wheat-Bran-based Diets during Gastrointestinal Transit in the Pig. Journal of the Science of Food and Agriculture, 58, 197-205.

ROESSINGH, P.; STÄDLER, E. (1990) Foliar form, colour and surface characteristics influence oviposition behaviour of the cabbage root fly *Delia radicum*. Entomologica Experimentalis et Applicata, 57, 93-100.

ROESSINGH, P.; STÄDLER, E.; HURTER, J.; RAMP, T. (1992a). Oviposition stimulant for the cabbage root fly: important new cabbage leaf surface compound and specific tarsal receptors. In: Proceedings of the 8th International Symposium on Insect-Plant Relationships. Eds. MENKEN, S.B.J.; VISSER, J.H.; HARREWIJN, P.. Kluwer Academic Publishers. 1992.

ROESSINGH, P.; STÄDLER, E.; FENWICK, G.R., LEWIS, J.A., NIELSON, J.Kvist, HURTER, J.; RAMP, T. (1992b). Oviposition and tarsal chemoreceptors of the cabbage root fly are stimulated by glucosinolates and host plant extracts. Entomologica Experimentalis et Applicata, 65, 267-282.

ROGERSON, J.P., DIXON, G.M. (1976). The distribution of cabbage root fly eggs in large fields of swedes. Plant Pathology, 25, 73-80.

ROUXEL, T., KOLLMAN, A., BOULIDARD, L., MITHEN, R. (1991). Abiotic elicitation of indole phytoalexins and resistance to *Leptosphaeria maculans* within Brassiceae. Planta, 184, 271-278.

RUUTH, P. (1988). Resistance of cruciferous crops to turnip root fly. Journal of Agricultural Science in Finland, 60, 269-279.

RYAN, M.F., BEHAN, M. (1973). Cephalic sensory receptors of the cabbage-root fly, *Erioischia brassicae* (B.) (Diptera: Anthomyiidae). International Journal of Insect Morphology and Embryology, 2(2), 83-86.

RYGG, T. SÖMME, L. (1972). Oviposition and Larval development of *Hylemya floralis* (Fallén) (Diptera: Anthomyiidae) on Varieties of Swedes and Turnips. Norsk Entomologisk Tidsskift, 19, 81-90.

SANG, J.P., MINCHINTON, I.R., JOHNSTONE, P.K., TRUSCOTT, R.J.W. (1984). Glucosinolate profiles in the seed, root, and leaf tissue of cabbage, mustard, rapeseed, radish, and swede. Canadian Journal of Plant Science, 64, 77-93.

SCHONI, R., STÄDLER, E. (1987). Host and non-host plant chemicals influencing the oviposition behaviour several herbivorous insects. In: Labeyrie, V., Fabres, G., Lachaise, D. (Eds), Proceedings of The 6th International Symposium on Insect-Plant Relationships. Dr W. Junk Publishers, Dordrecht, 31-36.

SCHNELL, G.D., WATT, D.J., DOUGLAS, M.E. (1985). Statistical comparison of proximity matrices: applications in animal behaviour. Animal Behaviour, **33**, 239-253.

SEARS, M.K., DUFAULT, C.P. (1986). Fight activity and oviposition of the cabbage maggot, *Delia radicum*, (DIPTERA : ANTHOMYIIDAE), in relation to damage to rutabagas. Journal of Economic Entomology, **79(1)**, 54-58.

SELVENDRAN, R.R., ROBERTSON, J.A. (1990). The chemistry of dietary fibre - an holistic view of the cell wall matrix. In: Dietary Fibre: Chemical and Biological Aspects (Eds. Southgate, D.A.T., Waldron, K.W., Johnson, I.T., Fenwick, G.R.). Special Publication No. 83, Royal Society of Chemistry, London.

SHATTUCK, V.I., KAKUDA, SHELP, B.J. (1991). Effect of low temperature on the sugar and glucosinolate content of rutabaga. Scientia Horticulturae, **48**, 9-19.

SHAW, G.J., ANDRZECJEWSKI, D., ROACH, J.A.G., SPHON, J.A. (1989). Separation and identification of glucosinolates from Brassica vegetables using high-performance capillary gas chromatography (GC)-positive-ion chemical ionisation mass spectrometry (PICIMS) and GC-PICIMS/MS. Journal of Agriculture and Food Chemistry, **37**, 372-378.

SHAW, M.W. (1982). Control of turnip root fly, *Delia floralis* (FALLEN). Research Investigations and field trials 1980-1981, School of Agriculture, University of Aberdeen, 196-197.

SHAW, M.W. (1984). Control of turnip root fly *Delia floralis*. Research Investigations and field trials 1980-1981, School of Agriculture, University of Aberdeen, 204-206.

SHAW, M.W. (1985). Control of turnip root fly *Delia floralis*. Research Investigations and field trials 1980-1981, School of Agriculture, University of Aberdeen, 229-231.

SHAW, M.W., ALLAN, R.M., HUNTER, E.A., MUNRO, I.K. (1993). A relationship between dry matter and damage by larvae of the turnip root fly *Delia floralis* in cultivars of the swedish turnip *Brassica napus*. Entomologica Experimentalis et Applicata, **67**, 129-133.

SINGER, M.C. (1986). The Definition and Measurement of Oviposition Preference in Plant-Feeding Insects. In: Insect-Plant Interactions. Miller, J.R., Miller, T.A. (Eds), Springer Verlag.

SKINNER, G., FINCH, S. (1983). Evaluation of swede cultivars for rearing the cabbage root fly in the laboratory. Annals of Applied Biology, Tests of Agrochemicals & Cultivars, **102**, 140-141.

SKINNER, G., FINCH, S. (1986). Reduction of cabbage root fly (*Delia radicum*) damage by protective discs. Annals of Applied Biology, **108**, 1-10.

SLANSKY, F. (Jr). (1992). Allelochemical-Nutrient Interactions in Herbivore Nutritional Ecology. In: Herbivores: Their interactions with secondary plant metabolites (Second Edition) Volume II: Evolutionary and Ecological Processes. Academic Press, 1992.

STÄDLER, E. (1978). Chemoreception of host plant chemicals by females of *Delia (Hylemya) brassicae*. Entomologica Experimentalis et Applicata, **24**, 711-720.

STÄDLER, E. (1980). Chemoreception in arthropods: sensory physiology and ecological chemistry. In: Animals and environmental fitness. Eds. GILLES, R. Pergamon Press, Oxford and New York, 1980.

STÄDLER, E. (1984). Contact chemoreception. In: Chemical-ecology of Insects. Eds. BELL, W.J., CARDE, R.T. Chapman and Hall LTD, 1984.

STÄDLER, E. (1992). Behavioural Responses of Insects to Plant Secondary Compounds. In: Herbivores: Their interactions with secondary plant metabolites (Second Edition) Volume II: Evolutionary and Ecological Processes. Academic Press, 1992.

STÄDLER, E., ROESSINGH, P. (1990). Perception of surface chemicals by feeding and ovipositing insects. In: Szentesi, A., Jermy, T. (Eds), Proceedings of The 7th International Symposium on Insect-Plant Relationships. Symp. Biol. Hung., **39**, 71-86.

STÄDLER, E., SCHONI, R. (1990). Oviposition behavior of cabbage root fly, *Delia radicum* (L.), influenced by host plant extracts. Journal of Insect Behaviour, **3(2)**, 195-209.

STONER, K.A. (1990). Glossy Leaf Wax and Plant Resistance to Insects in *Brassica oleracea* Under Natural Infestation. Environmental Entomology, **19(3)**, 730-739.

SUETT, D.L. (1987). Influence of treatment of soil with carbofuran on the subsequent performance of insecticides against cabbage root fly (*Delia radicum*) and carrot fly (*Psila rosae*). Crop Protection, **6**, 371-378.

SWAILES, G.E. (1959). Resistance in Rutabagas to the cabbage maggot, *Hylemya brassicae* (Bouché) (Diptera: Anthomyiidae). The Canadian Entomologist, **91**, 700-703.

SWAILES, G.E. (1960). Laboratory evaluation of resistance in resistance in rutabaga varieties to the cabbage maggot, *Hylemya brassicae* (BOUCHE), (DIPTERA:ANTHOMYIIDAE). Canadian Entologist, **92**, 958-960.

SWAILES, G.E. (1961). Laboratory studies on mating and oviposition of *Hylemya brassicae* (BOUCHE) (DIPTERA:ANTHOMYIIDAE). Canadian Entomologist, **93**, 940-943.

SWAILES, G.E. (1968). Feeding through root surfaces of rutabaga by *Hylemya brassicae* (Diptera: Anthomyiidae). The Canadian Entomologist, **100**, 1061-1064.

TAKSDAL, G. (1992). The complementary effects of plant resistance and reduced pesticide dosage in field experiments to control the turnip root fly, *Delia floralis*, in swedes. Annals of Applied Biology, **120**, 117-125.

TAMATE, J.,BRADBURY, J.H. (1985). Determination of sugars in tropical root crops using 13C n.m.r. spectroscopy: Comparison with the HPLC method. Journal of the Science of Food and Agriculture **36**, 1291-1302.

THOLEN, J.T., SHIFENG, S., TRUSCOTT, R.J.W. (1989). The thymol method for glucosinolate determination. Journal of the Science of Food and Agriculture, **49**, 157-165.

THOMPSON, J.N. (1988). Evolutionary ecology of the relationship between oviposition preference and performance of offspring in phytophagous insects. Entomologica Experimentalis et Applicata, **47**, 3-14.

TRAYNIER, R.M.M. (1967a). Effect of host plant odour on the behaviour of the adult cabbage root fly , *Erioischia brassicae.* Entomologica Experimentalis et Applicata, **10** , 321-328.

TRAYNIER, R.M.M. (1967b). Stimulation of oviposition by the cabbage root fly *Erioischia brassicae*. Entomologica Experimentalis et Applicata, **10** , 401-412.

TRAYNIER, R.M.M., TRUSCOTT, R.J.W. (1991). Potent natural egg-laying stimulant for cabbage butterfly *Pieris rapae*. Journal of Chemical Ecology, **17(7)**, 1371-1380.

TRUSCOTT, R.J.W., JOHNSTONE, P.K., MINCHINTON, I.R., SANG, J.P. (1983). Indole Glucosinolate in swede (*Brassica napobrassica* L. MILL.). Journal of Agriculture and Food Chemistry, **31** , 863-867.

TUTTLE, A.F., FERRO, D.N., IDOINE, K. (1988). Role of visual and olfactory stimulil in host finding of adult cabbage root flies, *Delia radicum*. Entomology Experimentalis et Applicata, **47**, 37-44.

UDA, Y., OZAWA, Y., TAKAYAMA, M., SUZUKI, K., MAEDA, Y. (1988). Free and soluble bound phenolic acids in some cruciferous vegetables. Nippon shokuhin kogyo gakkaishi, **35(5)**, 360-366.

VARIS, A-L. (1958). On the susceptibility of the different varieties of big-leafed turnip to damage caused by cabbage maggots (*Hylemyia* ssp.) Journal of the Scientific Agricultural Society of Finland, **30**, 271-275.

VARIS, A.L. (1967). Studies on the biology of the cabbage root fly (*Hylemya brassicae* BOUCHE.) and the turnip root fly (*Hylemya floralis* FALL.). Annales Agriculturae Fenniae, **6**, 1-13.

WALLBANK, B.E., WHEATLEY, G.A. (1979). Some responses of cabbage root fly (*Delia brassicae*) to allyl isothiocyanate and other volatile constituents of crucifers. Annals of Applied Biology, **91**, 1-12.

WARD, E.R., UKNES, S.J., WILLIAMS, S.C., DINCHER, S.S., WIEDERHOLD, D.L., ALEXANDER, D.C., AHL-GOY, P., MÉTRAUX, J.-P., RYALS, J.A. (1991). Coordinate Gene Activity in Response to Agents That Induce Systemic Acquired Resistance. The Plant Cell, **3**, 1085-1094.

WESTON, P.A., KELLER, J.E., MILLER, J.R. (1992). Oviposition Stimulus Deprivation and Its Effect on Lifetime Fecundity of *Delia antiqua* (Meigen) (Diptera: Anthomyiidae). Environmental Entomology, **21(3)**, 560-565.

WILLIAMSON, B., DUNCAN, G.H. (1989). Use of cry-otechniques with scanning electron microscopy to study infection of mature red raspberry fruits by *Botrytis cinerea*. New Phytologist, **111**, 81-88.

WILSON, R.N., BIRCH, A.N.E., BRADSHAW, J.E. (1990). Differences in susceptibility of twelve swede genotypes to cabbage root fly attack. Tests of Agrochemicals and cultivars, **11**, Annals of applied Biology, **116**(Supplement), 116-117.

WOLFSON, J.L. (1988). Bioassay techniques, an ecological perspective. Journal of Chemical Ecology, **14(10)**, 1951-1963.

WOOD, R.K.S. (1982). Active Defense Mechanisms in Plants. Plenum Press.

WOODHEAD, S., PADGHAM, D.E. (1988). The effect of plant surface characteristics on resistance of rice to the brown planthopper, *Nilaparvata lugens*. Entomologica Experimentalis et applicata, **47**, 15-22.

YOUNG, S. (1991). How plants fight back. New Scientist, 1st June 1991, 41-45.

ZOHREN, E. (1968). Laboruntersuchungen zu Massenanzucht, Lebensweise, Eiablage und Eiablageverhalten der Kohlfliege, *Chortophila brassicae* Bouché *(Diptera, Anthomyiidae)*. Zeiscrift fur Angewandte Entomologie, **62**, 139-188.

1425g (unbound)