EFFECT OF CLONAL AND SITE FACTORS ON FEEDING BEHAVIOUR OF LARGE PINE WEEVIL, *HYLOBIUS ABIETIS, (L.)* (COL. CURCULIONIDAE).

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ABSTRACT

Hylobius abietis , the large pine weevil, is the single most destructive insect pest in conifer reforestation areas in Northern Europe.

Four assays involving variables; resin, polyphenols, carbohydrates and nitrogen were carried out on ten clones from full-sib families and one QCI of Sitka Spruce, *Picea sitchensis* to determine clonal and site effect on feeding behaviour of *H. abietis.* The clonal twig material were collected from Brecon in Wales and Dalkeith and Newcastleton in Scotland. Only 1995 growth from the lowest whorl was used. Male weevils were used in the feeding damage.

Weevil weight was found to be significantly positively correlated to feeding damage. A positive relationship exits between the probability of a twig being damaged and twig midpoint diameter. There was significant differences on all the four variables between sites and clones. There was a significant negative correlation between *H. abietis* feeding damage and two response variables; resin and carbohydrates when all sites are pooled. This relationship was attributed to the clonal differences in defence chemicals, nutrients and environmental interacting factors.

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CHAPTER 1.

1.0 GENERAL INTRODUCTION

The large pine weevil, *Hylobius abietis,* is the single most destructive insect pest in conifer reforestation areas in Northern Europe (Lindstrom *et al.*, 1986, Leather *et al.,* 1995, Lindgren *et al.*, 1996). *Hylobius abietis* is a Palearctic forest insect which causes extensive damage to conifer seedlings throughout its range. Although generally referred to as a pine weevil, *H. abietis* attacks and destroys a wide range of coniferous tree species and even attacks young broad-leaved trees (Djeddour, 1996). It feeds on the bark of transplants particularly in restocking areas. This pest is considered to be responsible for the death of approximately one-third of all British conifers planted in restocking sites in recent years (Wilson & Day, 1994).

In Britain, forestry is an important industry, providing material for fuel, building and recreational activities. In 1987 the apparent consumption for raw timber in the UK was 49.4 million cubic metres of which only 5.4% was UK produced (Anon, 1989 in Styles, 1994). According to Evans, (1991) Britain's forest cover amounts to about ten per cent of land surface area; this is more than twice the area recorded at the turn of the century, when it covered just under 5.5%. This increase in afforestation was due to the realisation of the poor state into which British forestry had got over the past centuries.

The British Forestry Commission was established in 1919, one of the aims was to increase the country's forest cover. The increase in afforestation came about due to use of monocultures, especially introduced/exotic species such as Sitka spruce, *Picea sitchensis,* Lodgepole pine, *Pinus contorta,* Douglas fir, *Pseudotsuga menziesii* (Anon, 1989 in Styles 1994).

Before the 1980s, most forest trees were planted on new land, so the risk of damage by *H. abietis* was low. Changes in the British forestry policy were introduced in the mid 1980s. These changes included restriction on new land available for new planting, need to use conifers for restocking was emphasised , and increased need for restocking as post-war plantings of conifers matured were readily harvested (Collins, 1993). In the early 1990's the Forestry Commission (FC) was replanting 8,000 ha per year, but this was expected to double by the year 2000 (Collins, 1993). This high rate of restocking, largely with conifers has increased the importance of *H. abietis* damage. Young saplings are vulnerable to *H. abietis* feeding for the first two growing seasons after planting-out. Feeding by adult weevils girdles and kills millions of seedlings each year, leading to highly significant economic losses (Lindgren *et al.*, 1996). Heritage *et al.,* (1988) have estimated the total cost of damage by *H. abietis* in areas with protection, to be exceeding half a million pounds during the 1985-1986 growing seasons.

In mature plantation crops *H. abietis* has been reported feeding on fallen tops and branches on the ground (Collins, 1993). Munro (1928) observed damage in the upper crown of pines up to 60 feet high. In older plantations, *H. abietis* causes damage by destroying buds and gnawing the bark of leading shoots and branches, however this is minor and economically insignificant compared to damage inflicted on young seedlings in restocking sites (Djeddour, 1996). Due to *H. abietis* activities, survival of conifers in replanted sites is frequently below target stocking densities (Collins, 1993).

1.2 *Hylobius abietis* - Biology.

1.2.1 Taxonomy and Description

Hylobius abietis L. is closely allied to the genus *Pissodes* of the family Curculionidae which forms parts of the super family Rhynchophora of the

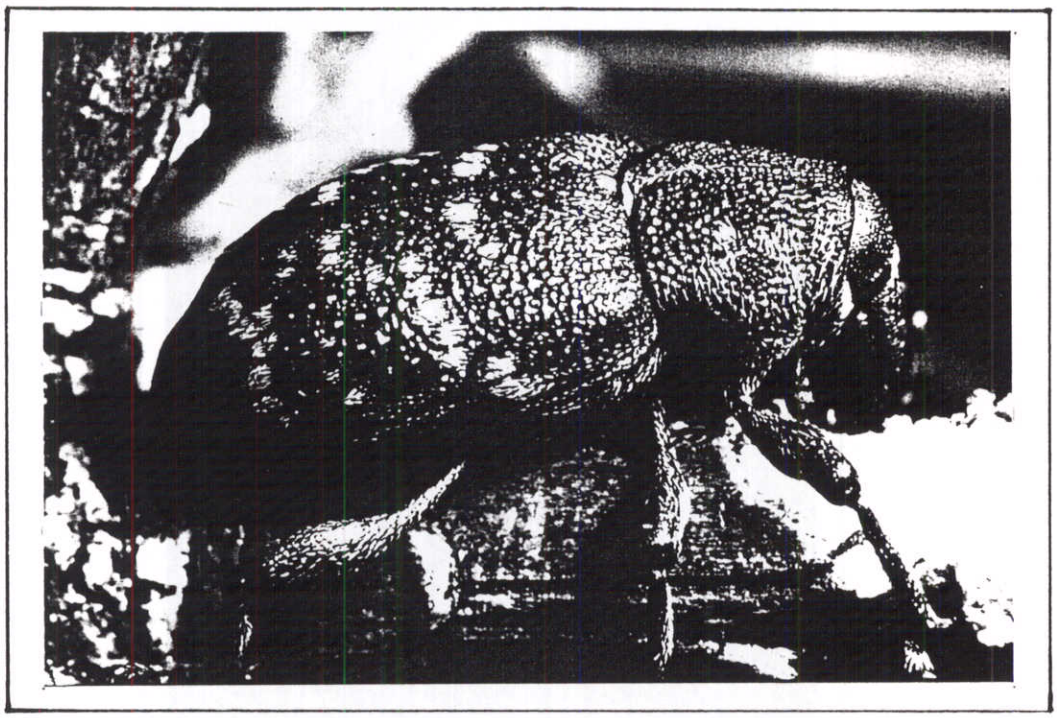


Fig. 1.1 Adult of large pine weevil Hylobius abietis (Magnified x 4)

Fig. 1.2 Larva of large pine weevil Hylobius abietis (Magnified x 3)

Fig. 1.3 Pupa of Hylobius abietis (Magnified x 4)

Fig. 1.5 Extensive brash (waste branches and weeds). Major breeding material of Hylobius abietis

order Coleoptera (Anon. 1964). This is one of the largest British weevils. The adult weevil averages about 15mm but may range from 10-20mm in length. It is a robust and well-armoured beetle with the elytra or wing case completely covering the abdomen. Young adults are purple-brown in colour, later becoming pitchy-black. There are patches of yellow to orange bristles on the elytra, sides of the thorax and on the under side of the body. The thorax is slightly broader than long, with a raised smooth central line. As in other weevils, the head is prolonged to form a rostrum or snout, with powerful chewing mouthparts at the tip. The antennae are elbowed and attached near the end of the rostrum *(Fig 1.1 Adult of large pine weevil. Hylobius abietis).*

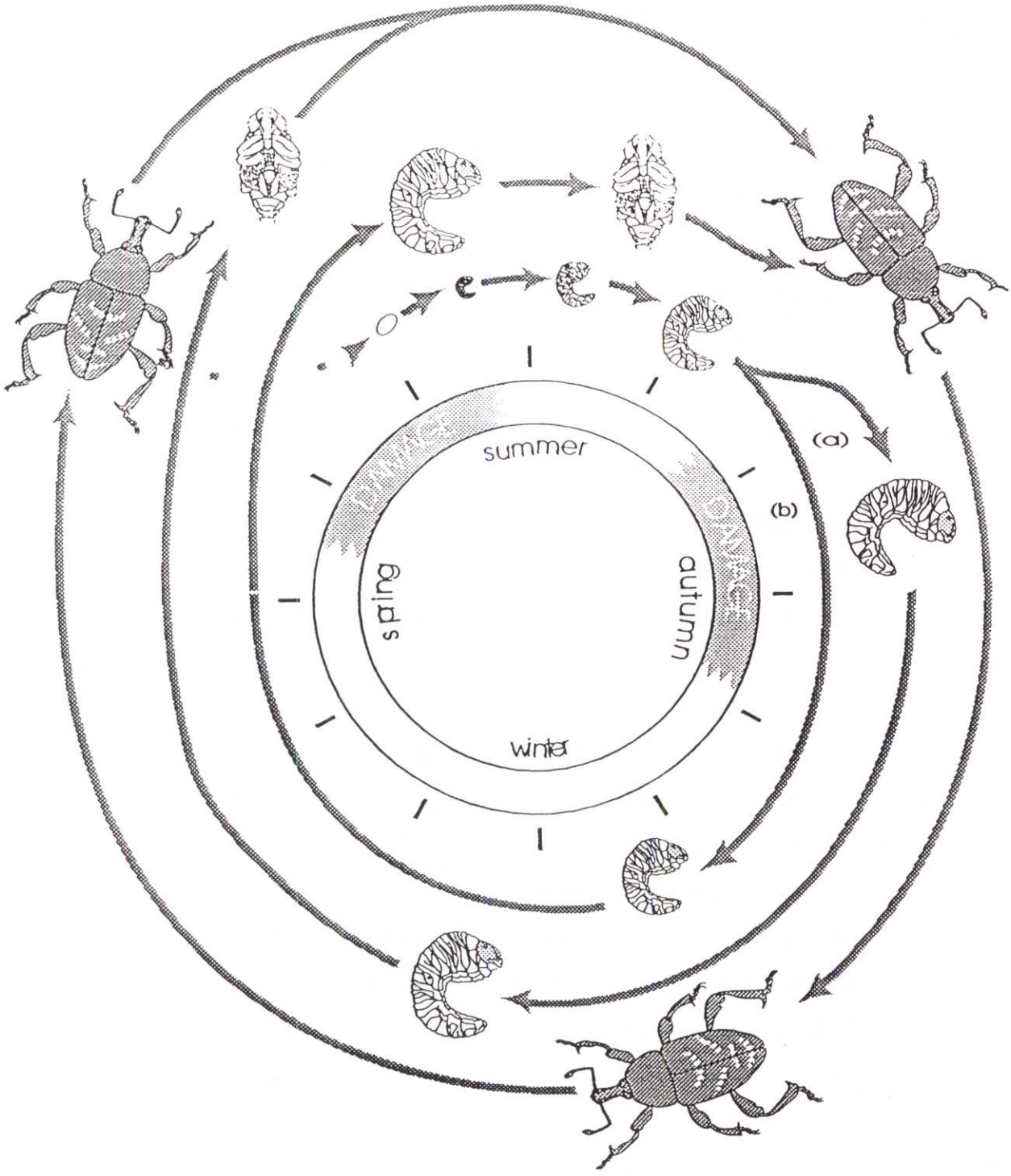
The larva of *H. abietis* has a large wide flattened head. It is soft, cream-coloured and legless with a light brown head. Fully grown larvae measure about 15-20mm in length *(Fig. 1.2. Larva of a large pine weevil and Fig 1.3 Pupa of Hylobius abietis)*.

The most significant feature in its biology that makes it a pest of economic importance is the longevity of the adults. The female adult is able to produce several broods over a 3-4 year period. It has great variability in the length of the life cycle from egg to mature adult (Munro, 1928).

1.2.2 *Hylobius abietis* - General life Cycle

The life cycle of *H. abietis* is well studied by Scott & King (1974), and well reviewed by Collins (1993). Hibernating adult weevils emerge from the humus layer of restock sites in spring and feed on the bark of any woody stemmed plants, including the conifer seedlings. This spring population of weevils causes the first peak of damage observed each year on seedlings. With the help of olfactory orientation, spring and summer females locate stumps of freshly felled conifer trees. Female weevils burrow below the ground, make a hollow into the bark of the stump, or the main root system. The female

Fig. 1.4 The life cycle of *Hylobius abietis* (After Collins, 1993).



(a) Final instar larvae over winter and pupate in the spring.
(b) Larvae continue to feed in the spring and pupate in late summer.

oviposits a single egg inside the hollow. During summer the hatching larvae feed on the phloem under the bark. They make long rambling tunnels increasing in diameter as they develop. There is no characteristic pattern of tunnels formed by larvae. They tunnel randomly under the bark, packing frass behind them and in thick bark excavate ventilation tunnels to the surface.

If larvae have reached the final fifth instar by autumn, they stop feeding and make an overwintering chamber (*Fig. 1.4a)*. In the stumps of some tree species where the bark is thin, eg spruce, the chamber is made partly within wood, while in pine species, where bark is thicker, the chamber is created entirely within bark. The overwintering larvae pupate in spring and adult weevils emerge about 14 days later. If larval development is not complete by autumn, feeding activity resumes after overwintering until the fifth larval instar. When pupation occurs without any further resting period. The adults then emerge and cause extensive feeding damage *(Fig . 1.4b).*

The rate of development from egg to emergent adult depends largely on prevailing climatic conditions, and to some lesser extent upon the time of the year the eggs are laid. In warm and dry conditions, larvae may completely develop by late autumn from eggs laid in the spring, and emerge as adults in the following May or June. Time of emergence is governed by temperature. In unfavourable conditions/climate completion of the life cycle make take two full years. In the northern parts of Britain this may take up to three years (Scott *et al.*, 1974).

Adults emerging at the end of summer are unlikely to lay eggs before hibernating (Scott & King, 1974). However a second phase of adult weevil emergence often results in a second peak of damage sustained on the restocked transplants *(Fig. 1.4).*

H. abietis adults are polyphagous, feeding on a range of tree and understorey species before ovipositing or hibernating over winter. Adult weevils can

survive for three to four years in the field and continue to feed voraciously throughout the period (Collins, 1993).

1.3 *Hylobius abietis* as a pest in restocking sites

Restocking or re-afforestation is the process of replanting with tree seedlings the areas where mature trees have been clearfelled. Stumps remaining after clearfelling allow the rapid build-up of the weevil populations. The tops and side branches cut from the tree and left on site can also provide potentially useful breeding material for *H. abietis* especially when in contact with the ground. Other natural processes like natural fires and windblow can make large quantities of breeding material available.

On clearfelled areas, *H. abietis* adults locate fresh stumps suitable for breeding by olfactory orientation. Adults are strongly attracted to stump chemicals such as monoterpenes and ethanol released when the tree is felled (Tilles *et al.*, 1986a).

Selander *et al.*, (1973) working in Finland and other Nordic countries have suggested a pre- and post- swarming activity of adult *H. abietis.* It is suggested that this is due to changes in olfactory sensitivity and behavioural response to substances present in conifer phloem. These changes were linked to a change in the population behaviour from feeding phase to a swarming phase in search of oviposition sites. In Britain, this distinctive swarming phase has not been observed (Collins, 1993). Restocking sites are thus major areas of population build-up even before planting of the seedlings is done.

On Forestry Commission sites, trees are usually planted from October to April. Planting out is restricted to a particular season because the transplants

must be dormant when moved from the nursery to the restocking site. In some cases the planting period can be extend up to mid-May by keeping the plants under cold storage.

Transplants are at risk of *H. abietis* damage from planting up to the beginning of the third season of establishment. Transplants are only able to survive *H. abietis* feeding damage when they are large enough.

1.4 *Hylobius abietis* management options reviewed.

Hylobius abietis is the main cause of transplant mortality in restock sites. Eidmann (1979) reviewed possible control options for *H. abietis*, which are either targeted at larvae or adult weevils. Methods to reduce the larval population used to include, the use of creosote, lead arsenic, or lime and linseed oil to treat fresh stumps (Nelson *et al.*, 1961). These old generation chemicals which had no significant effect, were environmental pollutants (Munro, 1923).

Burning of brash and other vegetation is not effective. This option causes reduced availability of alternative weevil hosts, thus leading to higher levels of weevil damage on the transplants (Collins, 1993).

Fallow periods of even up to six years have not reduced weevil damage (FC leaflet, 1960 in Collins, 1993). This is likely due to the biology of *H. abietis*, its ability to emerge from pupation after two years and its long survival period of up to four years. The disadvantages of employing a fallow period include loss of revenue incurred and problems of weed growth that cause increased planting/establishment costs. Due to this, the method is unacceptable in UK (RSP 10, 1988).

Information on the relationship between the weevil population size and the damage levels is scanty making it difficult to provide plant protection advice. Forest managers have used the option of delayed planting in restock sites aimed at allowing the weevil population to decline naturally. This has proved to be ineffective, because adults can migrate from nearby clearfell sites.

A number of silvicultural methods have been used to minimise *H. abietis* damage. An increase in stocking density has been used in anticipation of losses from the weevil damage. The final stocking density is achieved by respacing where losses are lower or by beating up (replacing killed trees) if the damage is greater than expected. This technique is costly because the weevil damage is unpredictable and often patchy (RSP 10, 1988). Ploughing or scarification of restock sites before transplanting has been used as a control option. Bakke *et al.*, (1971) suggest that transplants in vegetation often suffer greater levels of feeding damage than those in weed-free sites. However, this method encourages root growth due to the higher temperatures in the soil. The increased plant growth vigour leads to better plant tolerance to weevil damage.

Billet traps have been used in attempts to reduce *H. abietis* populations (Eidmann, 1979). Traps are positioned around the edges of restock sites to attract incoming weevils. They are checked regularly and all weevils are collected and destroyed. This method does not reduce the weevil population enough to reduce damage significantly. The method has the disadvantage that it is labour intensive, costly and unreliable as a single control option (Munro, 1923).

The most effective control methods are those targeted on protecting the plants from contact with weevils. There are two physical barriers to prevent weevil access to the plant. The Teno collar is a polyethylene collar for use

with containerised or bare-rooted stock. This technique is only effective in weed and brash-free sites, as there should be no bridge to cross the barrier. This method is expensive as it increases planting time and cost (Lindstrom *et al.*, 1986). This method is used by the Forestry Commission(FC) in restock sites (Collins, 1993). Vinetta Stocking is the second type of physical barrier. It consists of a knitted stocking surrounding the plant. This prevents weevils from reaching the plant. The application cost of this method is twice that of standard chemical treatment (Eidmann *et al.*, 1984). This method is not used by the FC.

The standard method for plant protection is the application of insecticides. New generation insecticides like synthetic pyrethroids are used by FC (Eidmann, 1979). Chemical application can be divided into pre- and post-planting treatments. Pre-planting chemical treatments are done on bare-rooted stock. The disadvantages of this techniques is that the insecticide deposits must be dried to ensure weather fitness. Plants are at risk of root desiccation during drying and also physical damage due to the extra handling involved. At present a second preplanting treatment is done when the plants are on the conveyor belt, they are sprayed with the insecticide using an electrodyn system in an enclosed chamber. This method is still at an experimental stage. Its main advantages includes less exposure of the chemicals to the forestry nursery workers and reduced handling of plants (RSP 10, 1988).

Post-planting treatment involves the spraying of the plants with insecticides. This usually increases the level of protection remaining from dipping treatment after a period of weathering. As a single treatment, it only provides protection for part of a season. The disadvantage is that the chemical deposit must dry on the plant before it becomes rain-fast; thus good weather is required for plants treated outside (Collins, 1993).

The disadvantages with the use of insecticides to control *H. abietis* is the fact that some chemicals have unacceptable phytotoxic effects, particularly when treated plants are cold stored in bags (Tabbush, 1985). Some insecticides have inherent problems in their use eg, lindane is relatively slow acting and, pyrethroids although having low mammalian toxicity can act as local irritants.

1.5 Sitka spruce and chemical defence traits

Sitka Spruce, *Picea sitchensis* (Bong) Carr. is Britain's most extensively planted timber species. The bulk of Sitka spruce genetic material presently grown in Britain, originates from Queen Charlotte Islands (QCI) in Canada (Lee, 1991). Through years of breeding this conifer species has undergone considerable selection to increase gain in growth and form (Rook 1992).

Secondary plant metabolites have been implicated as defensive agents for plants in plant-plant (allelopathic), plant-pathogen, and plant-herbivore interactions (Rhoades, 1975). There are recent several reviews of evidence for antiherbivore function of secondary substances (Rhoades *et al.*, 1976, Feeny, 1976). Two main lines of evidence have been used to show antiherbivore activity of these substances. The first involves deterrence, and the second involves antibiosis. In the first, the preference of herbivores for a series of plant tissues is assessed in relation to the concentration of secondary metabolites in these tissues. If the herbivore is deterred, a negative correlation should result. Both within-plant and between plant comparisons are possible. In the second method, some fitness measure such as growth rate, fecundity, or , ideally, number of viable progeny is measured for herbivores fed the various tissues, individual plants, or morphs, and the correlation between secondary metabolite content and herbivore fitness is examined (Rhoades, 1975). These antiherbivore plant secondary metabolites are genetically controlled in plants and are heritable from one generation to

the next. The selection of the individuals that possess the antiherbivory traits, is very important in breeding for resistant varieties particularly against bark weevils.

It is important to select and restock with Sitka spruce genotypes (varieties) that are high yielding, have good stemform and are resistant to insect pests like *H. abietis*. The desirability of selecting Sitka spruce clones that are genetically resistant to *H. abietis* cannot be overemphasised. Characteristics of plants that can affect their selection as hosts by insects include morphology, chemical constituents, and nutritional status. Sitka spruce could be assessed for the factors that may be involved in antixenosis, such as the presence or absence of volatile attractants, feeding and oviposition stimulants and deterrants. Other factors involved are in antibiosis, such as the ability of the host to resist attack through the production of resin (Tomlin *et al.*, 1996a). In conifers, resin often plays a very important role in defence against insect pests and pathogens (Tomlin *et al.*, 1996b).

Primary (constitutive) resin is considered to be the first line of defence of conifers, acting as a wound cleansing agent, in some cases it deters further attack and prevents fungal growth (Berryman, 1972). Conifer resin is composed of diterpene resin acids, monoterpenes, sesquiterpenes, and terpene alcohols, the predominant fraction being the diterpene resin acids. The composition and physical properties of resin acids are under strong genetic control (Hanover, 1975). There is evidence that the composition or the amount of resin acids may be involved in resistance of Sitka spruce against weevils like white pine weevil, *Pissodes strobi* (Peck) (Tomlin *et al.*, 1996b).

The amount of defensive secondary chemicals present in plant tissue contributes to the effectiveness of the defence and so the causes of variation in concentration of these chemicals are of considerable ecological interest.

Genetic, environmental and plant physiological factors may influence the expression of plant defensive traits (Wainhouse *et al.*, 1996). Plant growth is known to be increased by application of fertiliser where nutrients are limiting (Savill *et al.*, 1986). This carbon-demanding response to fertilization can reduce resources allocated to carbon based defences as predicted by resource-availability models of defence (Herms *et al.*, 1992). According to Mugasha *et al.*, (1993), in general there is increase in nitrogen content in plant tissues after fertiliser application. However, levels of defence may fall (Herms *et al.*, 1992), and may allow the plant to become more vulnerable to herbivore attack. In plant breeding programmes there is always a trade-off between selection for traits of high growth rate and those of quantitative defence (Bryant *et al.*, 1985).

1.6 Aims of the project

The primary aim of the project is to measure the genetic and environmental variation in the amount of feeding by adult *H. abietis* on Sitka spruce clonal plants. Other questions include;

1. Are there differences between and within sites in defensive secondary chemicals (resins and polyphenols) in the selected clones.

2. Are there differences between and within sites in damage by *H. abietis* on the selected clones.

3. Is there a relationship between the amount of *H. abietis* feeding damage to clones and the concentration of chemicals and nutrients between and within sites.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Field Collection of clonal material

Clonal material from full-sib families of Sitka spruce, *Picea sitchensis (Bong) Carr.* was planted in 1994 by the TIB (Tree Improvement Branch of the Forestry Commission) at four sites; Brecon in Wales, Dalkeith, Kintyre and Newcastleton in Scotland. At each site there were 15 blocks of single-tree plots of 118 clones plus two unimproved Queen Charlotte Islands provenances (QCI). The experiment was part of a TIB clonal growth performance test and permission was granted for removal of required branch tissue from some of the clonal trees on condition there was minimal damage on the plants. Using recent growth performance data from Dalkeith, the fastest growing clone in each of the ten families was selected for this project, together with one QCI. The clone numbers were retained for reference and the selected QCI referred to as 'clone' 1. In order to investigate the interaction between site, clones and feeding damage of *H. abietis*, three sites were selected. They were located at Brecon in Wales and Newcastleton and Dalkeith in Scotland.

In order to obtain enough experimental twig material for the 5 experimental bioassays, the blocks were re-grouped together to form 'super-blocks', here referred to as replicates (*See Figures 2.1-2.3)*. Sections of the branches used were only of the 1995 growth and restricted to the first whorl from the soil-level. The layout of these super-blocks (replicates) was determined by the slope of the land or other factors mentioned below. Thus the layout varied from one site to the other.

Fig 2.1 Brecon site - Experiment Brecon 46.

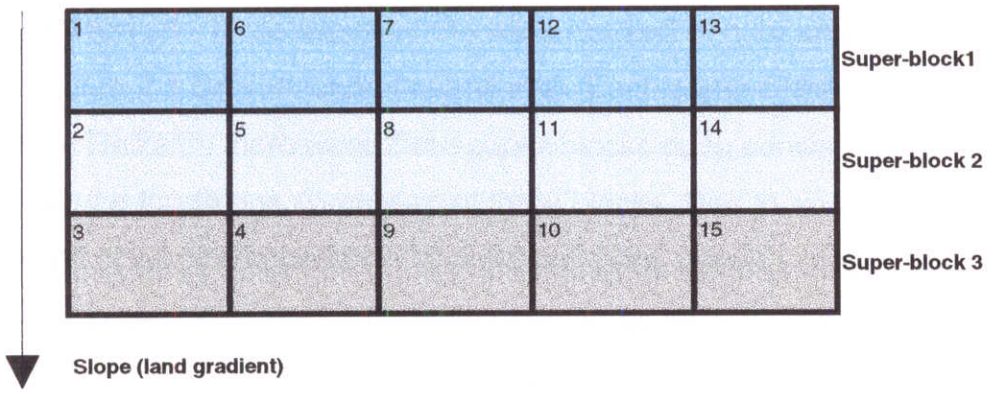


Fig 2.2 Dalkeith site - Experiment P94 SS Clonal

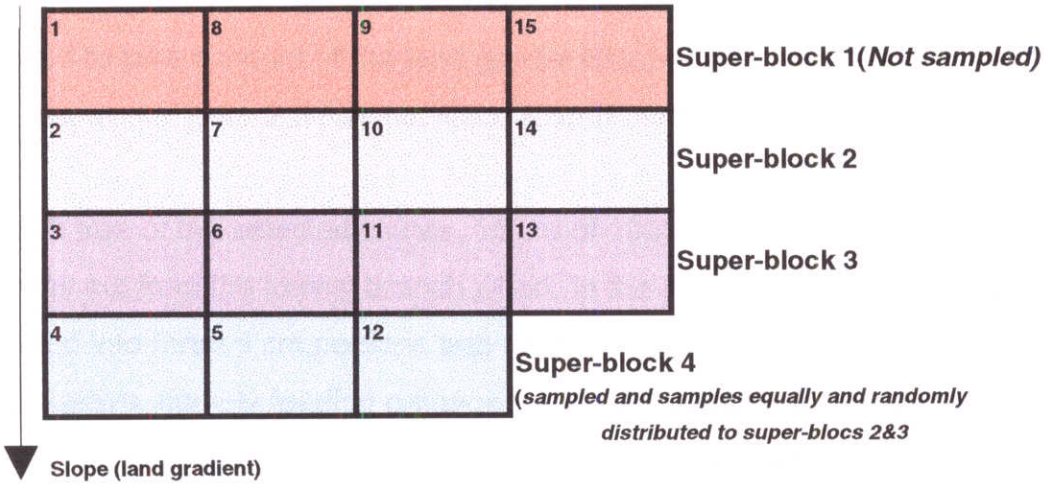
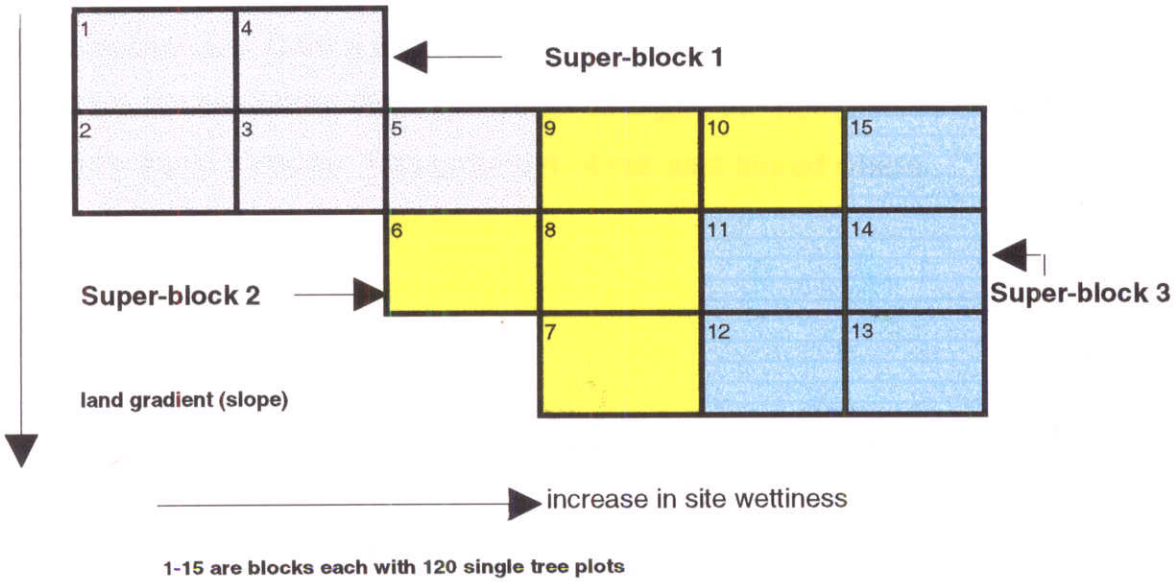


Fig. 2.3 Newcastleton Experimental site



1-15 are blocks each with 120 single tree plots

At the Brecon site, three super-blocks were marked at right angles to the land slope (*Figure 2.1 Brecon experimental site*). Each super-block consisted of 5 blocks. At Dalkeith, there were three super-blocks each consisting of 4 blocks, and a fourth one composed of three blocks. Due to poor tree growth, as a result of weed competition (Wainhouse. *pers. comm.*), super-block one was not sampled, (*Figure 2.2 Dalkeith experiment layout*). The material for the fourth super-block was divided equally and randomly added to the other two super-blocks to provide enough material to carry out the different bioassays. At the Newcastleton experimental site, three super-blocks were established, each consisting of five blocks. The super-blocks were established to take account of the land gradient(slope) and also changes in the site wetness (*Figure 2.3 Newcastleton experimental site)*

From each tree of the selected clones, 15 cm of 1995 branch internode growth was cut from the lowest branch whorl. In the laboratory, each sample was divided into three 5 cm portions and randomly allocated to each of the three bioassays, namely feeding damage, resin and polyphenols (secondary plant chemicals), and carbohydrates and nitrogen (nutrient) extraction. Samples for each super-block were pooled. Samples for nutrient and secondary plant metabolite extraction were stored at -70°C and those for damage bioassay at 2°C for a short period. For resin and polyphenol analysis, needles were removed after dipping in liquid nitrogen, the bark peeled off and ground under liquid nitrogen. This ground material was stored where necessary at -70°C.

2.2 Experiment 1.

2.2.1 Feeding Damage Bioassay

The feeding damage bioassays were carried out in clear plastic boxes measuring 11 x 4 x 1.5 cm. Assays were arranged as a choice test with

several (1-5) twigs of a clone from a given super-block for each weevil. Each twig sample measured 4 cm. The needles were carefully removed with a sharp blade without damaging the bark. The needles were cut at the scion (a zone of dead cells) so that resin oozing was prevented. The twig diameter was measured at the midpoint to determine the twig size. Male weevils used in the bioassays were from a culture reared and maintained by Entomology branch (Forestry Commission at Alice Holt Lodge). Larvae and pupae were collected and reared through to adults on pine logs. The females were used in another experiment. Prior to the start of the bioassay, the weevils were starved for at least 24 hours and weighed. A moistened tissue paper was put at the bottom of the box to avoid weevil desiccation. Small holes were made in the top of the box to allow air exchange and reduce high water vapour formation. The bioassay was carried out in a controlled environment photoperiod of 16L:8D and temperatures of 20^{0}C. After the bioassay the weevils were starved for 48 hours and weighed again.

After a period of four days the feeding damage was assessed, using a modified method described by Leather *et al.*, (1994). The damaged twigs were wrapped in cellotape and the shape of the feeding scar or the area of damage showing bark consumption was traced using a fine, water resistant black marker pen. The damage was differentiated into shallow and deep damage. Deep damage was defined as visible exposure of the white cambium compared to shallow damage where the cambium was not visible. The marked shape was transferred to tracing paper and shaded black. Using a computer DT-scanner programme set at resolution of 75, the black surface area was calculated in square millimetres. This was recorded for every replicate.

Since the bioassay used plant material for 1995 growth, the thickness of the bark was taken to be standard and thus it was necessary to differentiate the volume of the bark consumed by the weevil in the two types of feeding

damage. The deep type of feeding was taken as the normal feeding as thus the shallow damage was divided by two. The sum of the two gave the total damage on the twig.

2.3 Experiment 2

2.3.1 Extraction of Polyphenols.

From the ground bark material, 0.2 g was weighed into a centrifuge tube and 20 ml of 80% methanol (MeOH) was then added and homogenised for one minute at maximum speed. The homogeniser rod was rinsed with 70% alcohol when changing from one replicate to the next so as to avoid any contamination. The homogenate was held at 40^{0}C in a water bath for one hour. The tubes were balanced as tube pairs (tubes with equal weighs paired together) and centrifuged at 5000 revolutions per minute for 6 minutes. 8-10 ml of the supernatant was removed and put into labelled specimen tubes. At this point, the material was stored (if necessary) at -18^{0}C for several weeks to avoid degradation.

The Folin-Ciocalteu method outlined by Sauvestry *et al.* (1991) was used to estimate the total polyphenols present in the clones. Due to the sensitivity of the amounts of reagents used the accuracy of the transfer pipettes was checked using distilled water. The principle behind this calibration is that 1 ml of distilled water weighs 1 g. Using a transfer pipette measure 1 ml of distilled water and find out whether that water weighs exactly 1 g. If not, the transfer pipette is adjusted till this is obtained.

50 ml of gallic acid standard was made by dissolving 0.2 g of gallic into 100 ml of distilled water. This was used as the standard solution when measuring the absorbancy. Gallic acid gives similar absorbance to tannic acid. This was serially diluted into labelled specimen tubes to give concentrations of 7.5, 15,

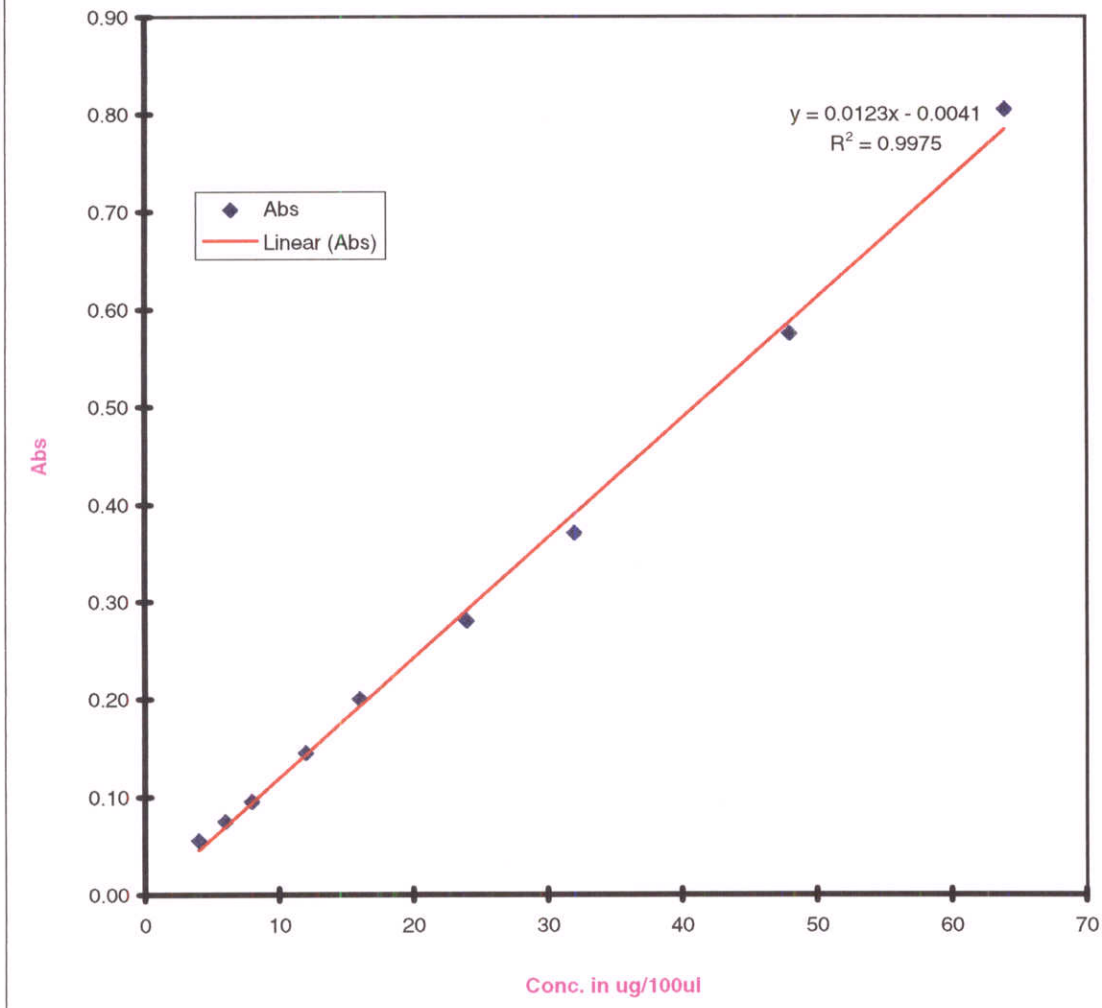
30 µg per 100 µl. These were used as the standard samples for each sample run, to calibrate the colorimeter.

100 µl of the sample were pipetted into 25 ml graduated tubes with poly-stoppers. 10 ml of distilled water were added, followed by 0.5 ml of Folins reagents, and the samples were then stoppered and shaken. Care was taken to add an accurate volume of only 0.5 ml of the Folins reagent as it is known that absorbance increases in direct proportion to volume of Folin reagent. After exactly three minutes, 1.2 ml of 20 g/100 ml of sodium carbonate solution was added. The stopper was replaced and the sample shaken again.

After exactly one hour, each sample was poured into a cuvette, and the absorbancy was read at 766 nm. Timing was very important here as absorbance increases with time. 760 nm filter is the optimum for the colour produced but due to the filter not being available, a 766 nm filter which is close enough to the optimum was used (Sauvestry *et al.* 1991). Distilled water, the blank, was used to zero the colorimeter which was warmed up for one hour before the readings could be taken.

Polyphenol absorbancy readings were made more meaningful by calibrating with gallic acid as the standard. This standardisation also imparts more immediate information to the reader when he reads that a plant sample contains the equivalent of 40 mg/g gallic acid. To convert absorbance reading to quantities of phenolics, a correlation of absorbance against known concentrations of serially diluted gallic acid (µg/100µl) was established. As the assay obeys the Beer-lambert law, then this plot gives a straight line. This is paradoxically termed as a standard curve. The equation of this relationship was established and used in the conversion, where y equals the absorbancy and x is the concentration in µg/100 µl gallic acid *(Fig 2.4 Standard curve of absorbance and gallic acid concentration).* By calculating the bark dry weight/fresh weight ratio from the samples for carbohydrate extraction,*(see*

Fig. 2.4 Standard curve of absorbance and gallic acid concentration



Polyphenol absorbance conversion to standard - gallic acid concentration equivalents.

y = absorbance
x = gallic acid concentration

From the equation above;

Abs. = 0.0123conc. - 0.0041

Therefore;

conc. = (Abs. + 0.0041) / 0.0123

section 2.1 above the relationship of the samples) the 0.2 g bark fresh weight used in the polyphenol extraction can be converted to dry weight. The amount of polyphenols in the clones sampled was expressed as milligrams gallic acid equivalent per gram of bark dry weight (mg GAE /g dry wt.)

2.4 Experiment 3

2.4.1 Resin Extraction

The ground material of the bark was removed from -70°c, and weighed out to between 0.7 and 1.0 g into a TEFLON^{R} centrifuge tube. The sample was then homogenised in 10 ml of 98% pentane for one minute at maximum speed. The solvent and the extract was then filtered off through qualitative filter paper (Whatman No. 1) into a pre-weighed sample tube, the sample and the filter paper was then rinsed and the rinsing also collected. The solvent was then evaporated off using nitrogen gas until constant weight.

Using the bark dry weight/fresh weight ratio the amount of resin extracted was expressed as milligrams resin per gram dry weight.

2.5 Experiment 4

2.5.1 Carbohydrates and Nitrogen Assay

The Forestry Commission, Research Agency at Alice Holt Lodge Research Station, who assisted in this assay have developed a simple method for the quantification of total non-structural carbohydrates in coniferous tissues. This method was used and is based on a water extraction procedure and the quantification procedure utilises *p*-hydroxybenzoic acid hydrazide, (*p*-HBAH) (Ward *et al.,* 1993).

The carbohydrate sample twigs were removed from the -70°C and needles were removed by dipping into liquid nitrogen, the fresh bark was peeled off

and weighed to obtain the bark wet/fresh weight. The material was placed in an oven at 75°C for three days to obtain a constant weight. The dried samples were then ground to pass a 0.5 mm screen and stored in sealed tubes in the dark at room temperature.

Before analysis, the stored ground tissues were remixed to ensure uniformity in the samples. For each sample, 100 mg of ground dried tissue was transferred to each of two 100 ml conical flasks. At this time an additional small sample was weighed and then oven dried at 100°C to determine moisture content at the time of analysis. To one flask, 15 ml of deionized water and some anti-bumping granules were added, the mixture was then boiled for two minutes to gelatinise the starch. After cooling, 10 ml of acetate buffer and 10 ml of fresh amyloglucosidase solution were added. To the second flask, 35 ml of deionized water were added, then the necks of both flask were covered with aluminium foil to exclude contaminants. Thus after extractions, the solvents in the flasks contained soluble sugars derived from starch plus the original soluble sugar component (flask 1), or simply the water soluble carbohydrate fraction (flask 2). For each batch of samples, blanks lacking sample materials and sample comprising standard plant materials of known carbohydrates content are included for quality control assessment. Apple and Poplar standard samples were used. All samples were then rotated at 120 r. p. m. at a temperature of 55°C for two days. After incubation the samples were filtered through Whatman No. 1 filter paper, the residues on the filter being washed with deionized water, the final filter volumes were adjusted to 50 ml in volumetric flasks.

The extracted soluble sugars were quantified in a continuous flow auto-analyser system. Because the *p*-HBAH regent does not detect non-reducing sugars, on line acid hydrolysis is incorporated in the chemistry manifold to reduce sucrose to its component reducing sugars. The yellow coloured

complex formed by *p*-HBAH in hot alkaline conditions in the presence of reducing sugars was quantified colorimetrically at 410 nm.

Finally, the estimates of carbohydrate content were corrected for actual dry weight as determined from the small samples redried before analysis and expressed as percentage of the dry weight.

Equal parts of the ground material were analysed for nitrogen. Material was digested in sulphuric acid/hydrogen peroxide mixture to produce a clear solution. Nitrogen was determined colorimetrically as ammonia by the reaction with salicylate and dichloroisocyanurate using nitroprusside as catalyst. The total amount of nitrogen was expressed as a percentage of the dry weight.

2.6 Statistical Design and Methods

Both Genstat 5.3 and SAS statistical packages, provided at Statistics and Computing Branch at Alice Holt Lodge, FC Research Station, were used to fit logistic regression analysis and linear mixed models including analysis of covariance.

Logistic regression analysis was used to investigate the relationship between the probability of twig feeding damage and the twig diameter.

Analysis of covariance was used for analysing data from a designed experiment where in addition to the response variable, one or more continuous variables were measured on each experimental unit. In this experiment the effect of individual weevil weight was used as a covariate in examining at the relationship between twig damage and site and clonal effects.

Some of the weevils did not survive the experiment which led to an unbalanced data set for the designed linear mixed model. To overcome this problem and to be able to produce estimates of the different variance components a residual maximum likelihood algorithms (REML) was applied to the analysis. This technique, also known as restricted maximum likelihood , requires that a model is defined into its fixed and random effects (treatments). Fixed effects describe those treatments that are imposed on the experiment where the effect of those specific choices of treatment are of interest. Random effects are generally used to describe those effects where the values present in the experiment represent a random selection of values from a larger homogeneous population. In this project sites and clones were considered to be fixed effects whilst replicates within sites were considered random. The interaction between sites and clone can be considered fixed whilst all other terms crossed with replicate were considered to be random effects. The REML algorithm then provides efficient estimates of the treatment effects for the unbalanced data set by combining variance information from all levels of the design.

CHAPTER 3

3.0 RESULTS

3.1 Feeding Damage Bioassay

3.1.1 Twig Size and Feeding Damage

The twig midpoint diameter used in this bioassay ranged from 2.8 mm to a maximum of 9.4 mm (mean = 5.3). This was used as a measure of twig size used in the feeding damage bioassay.

A logistic regression shows a relationship exists between the probability of a twig being damaged in a box containing at least three twigs and a maximum of five. The probability of a twig being damaged by a weevil increased as the twig size increased (Fig. 3.1). Table 3.1 shows twig diameter classified into five classes with percentage number damaged and undamaged. This shows that the number of damaged twigs increased as the twig diameter class increased. From the fitted model, it was found that the probability, p, of a twig being damaged when its midpoint diameter, t is known can be obtained by the equation below;

$$p(\text{damage}) = e^{(-1.744 + 0.653t)} / (1 + e^{(-1.744 + 0.653t)})$$ (Appendix 1)

There was no significant effect of twig size on the amount of feeding damage. The different twig sizes were evenly distributed amongst the clones (Appendix 1).

Fig. 3.1 Twig diameter class (mm) and Damage/Undamaged

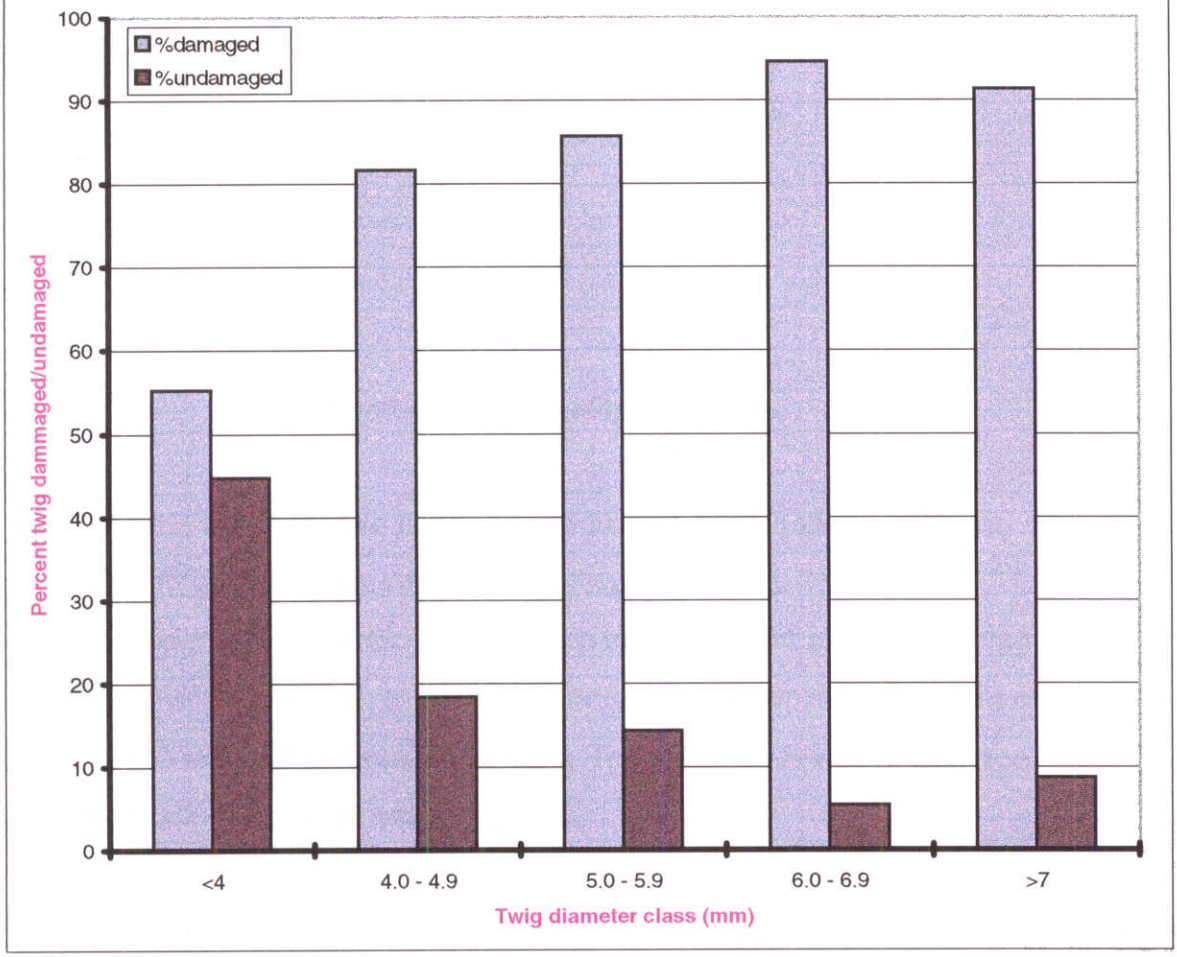


Table 3.1 Relationship between twig size and damage

Twig size class(mm)	% damaged	% undamaged
<4.0	55.3	44.7
4.0-4.9	81.7	18.3
5.0-5.9	85.7	14.3
6.0-6.9	94.6	5.4
>7.0	91.3	8.7

3.1.2 Weevil weight and Feeding Damage

The weight of the male beetles used ranged from 0.0714 g to a maximum weight of 0.1636 g. The mean weevil weight was 0.1119 g. There was a highly significant positive correlation between weevil weight and the feeding damage ($F_{1,32}$=8.19, $p<0.01$). In ANCOVA analysis a mean weevil weight of 0.11g is used to adjust the feeding damage. The adjusted levels of damage for site is graphically represented in Fig. 3.2.

3.1.3 Site and Clone effect on Feeding Damage

There was a highly significant difference on adjusted feeding damage between the three sites ($F_{2,5}$=29.09, $p<0.01$). There was a significant difference in mean feeding damage between Brecon and the other two sites, but no significant difference between Dalkeith and Newcastleton. Brecon was found to have very high significant clonal differences ($F_{9,32}$=5.56, $p<0.001$) as well as Dalkeith ($F_{9,32}$=3.75, $p<0.01$). Newcastleton showed no significant difference between clones (appendix 2).

Appendix 3 tabulates the feeding damage per replicate for each site. The mean feeding damage per site is also shown. Appendix 4 is a graphically representation of trends of feeding damage in each of the three different

Estimated damage levels for sites/clones with average beetle size of 0.11

Figure 3.2

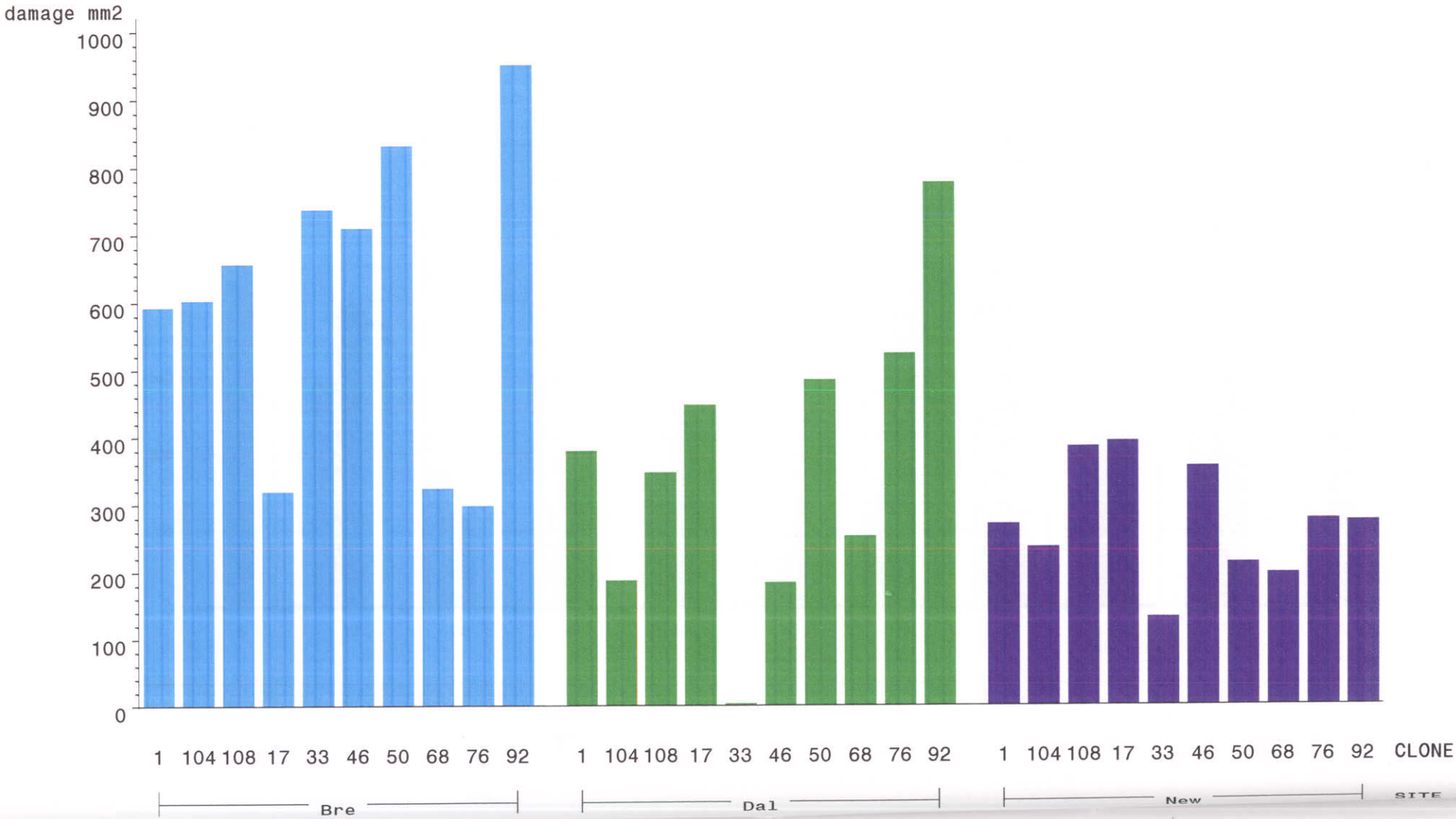
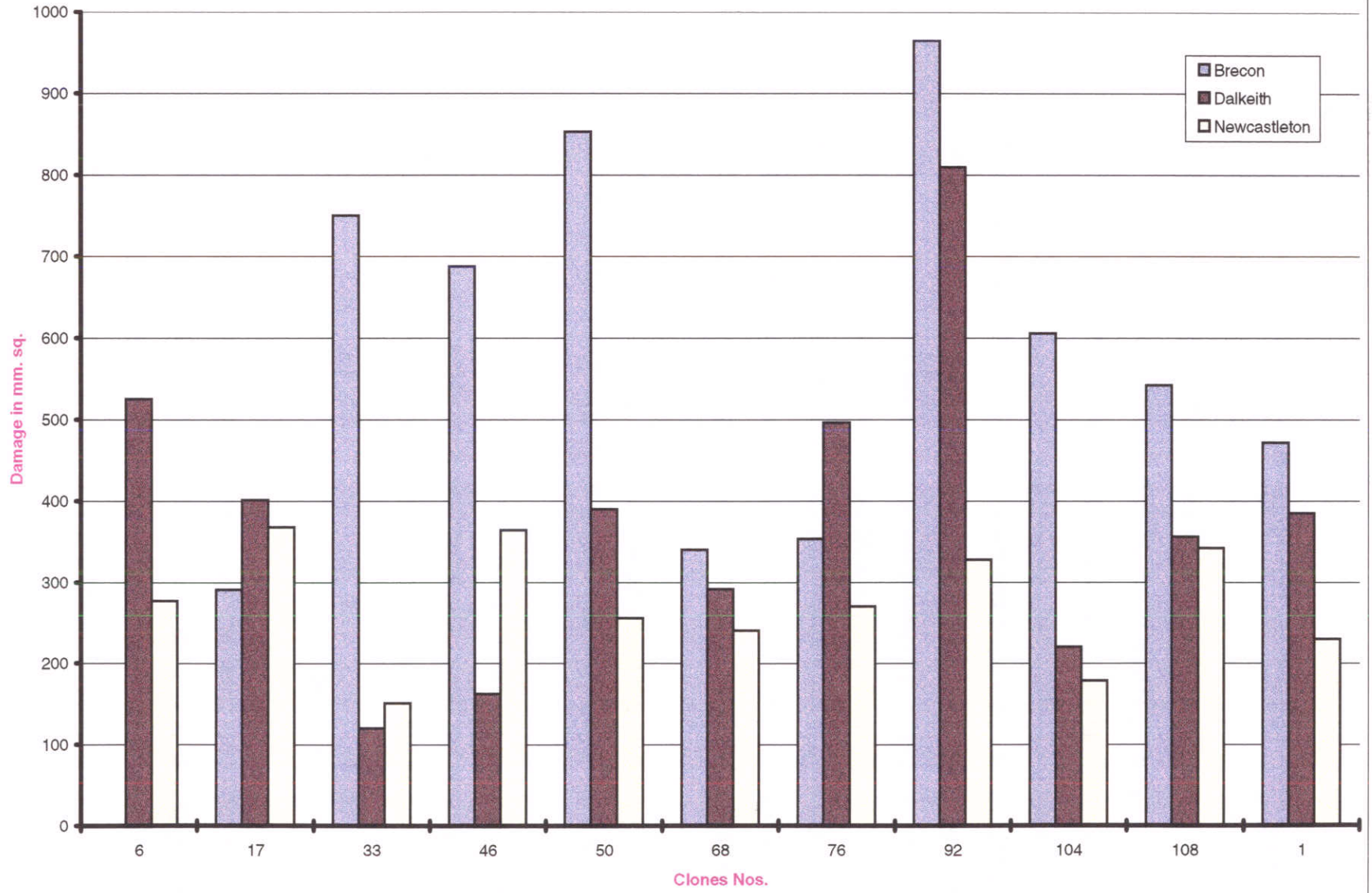


Fig. 3.3 FEEDING DAMAGE ON CLONES FROM THE THREE SITES



Missing Bar show that the weevil died before end of the Bioassay

sites. There is considerable variation in the feeding damage in the three sites (Fig. 3.3). When feeding damage is adjusted to weevil mean weight of 0.11g, a general trend becomes more apparent. There was low feeding damage on Newcastleton clones compared to Brecon clones. Dalkeith had intermediate damage levels (Fig. 3.2). Note that in all cases where the *H. abietis* weevil died before end of the bioassay, these were excluded from the analysis.

3.2 Resin assay Analysis

Appendix 5 shows the summary data on the resin assay expressed as milligrams of resin per gram dry weight (mg resin /g dry wt.). The maximum, minimum and mean per site is shown.

Appendix 6 is a graphical representation on the trend/variation of resin per site. Figure 3.4 shows the mean trend of resin in the three sites.

For analysis, the data was transformed to logarithms (log link) to meet the assumption of equal sampling errors from one treatment to another. There was significant differences between the three sites ($F_{2,5}$=5.79, $p<0.05$) in the amount of resin. Applying Tukey's multiple range test, average resin levels were significantly different between Brecon and Newcastleton (t=-3.31, $p<0.05$, df=5). Dalkeith contained intermediate resin levels and could be grouped with either of the other two sites. There was a very highly significant difference in resin concentration between the clones ($F_{10,47}$=10.12, $p<0.001$). There was also significant of site/clone interaction ($F_{20,47}$=1.82, $p<0.05$) (appendix 7). Clone 17 contains the highest amount of resin for both Newcastleton and Dalkeith, but the highest in Brecon site was observed to be clone 104. Clone 76 contains the smallest amount of resin for Brecon and Newcastleton sites, however at Dalkeith clone 1 contains the lowest (Figure 3.4).

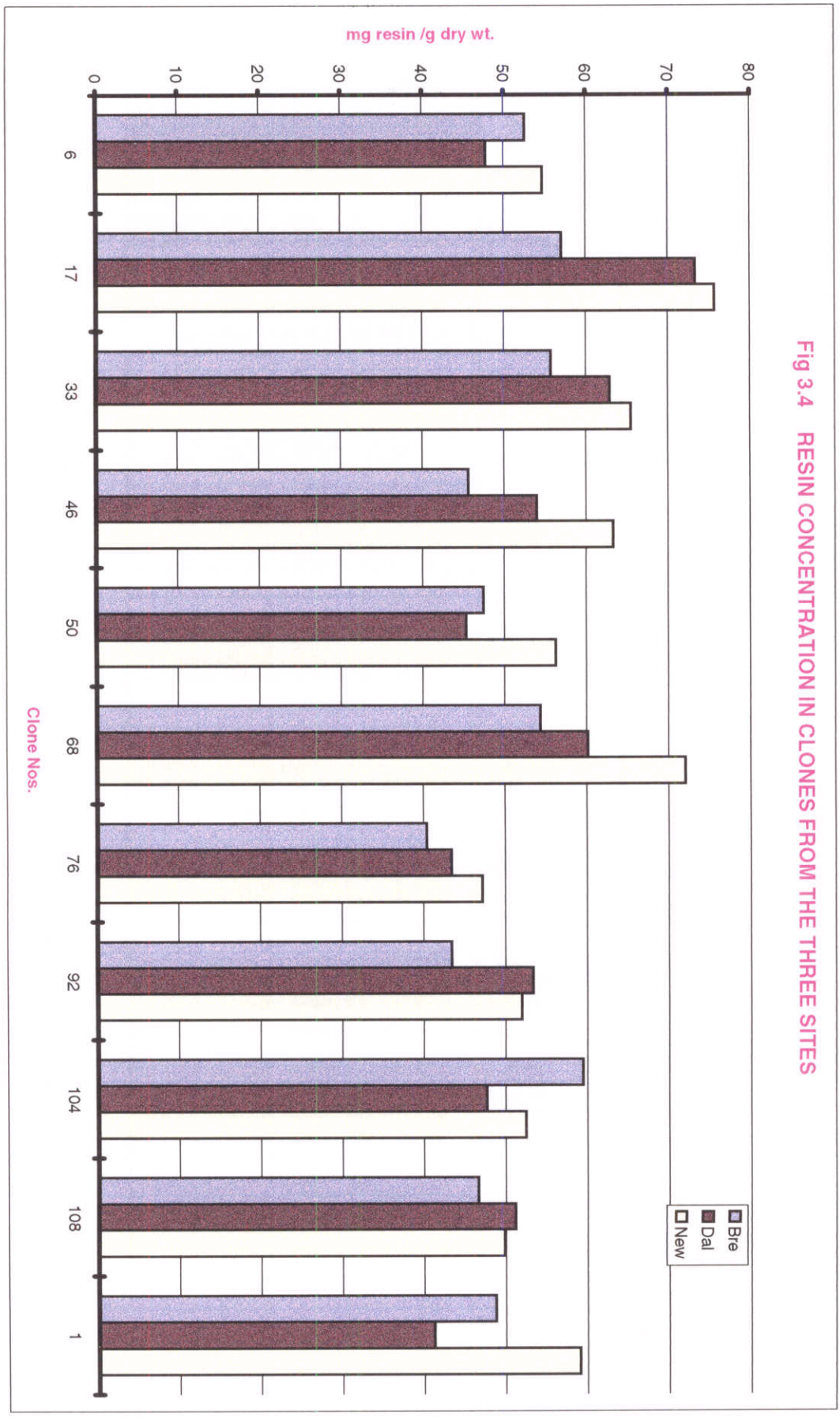


Fig 3.4 RESIN CONCENTRATION IN CLONES FROM THE THREE SITES

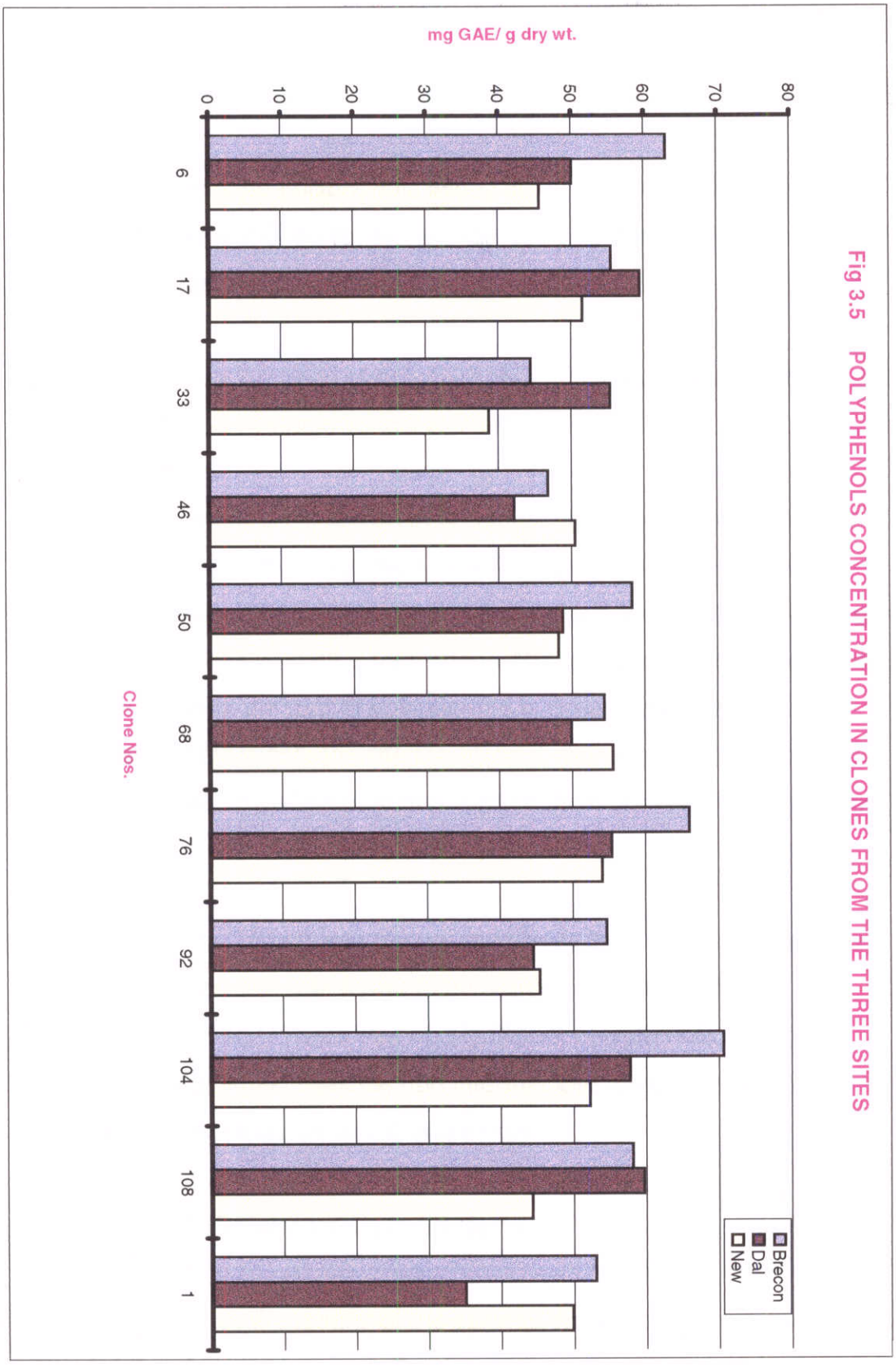


Fig 3.5 POLYPHENOLS CONCENTRATION IN CLONES FROM THE THREE SITES

3.3 Polyphenols assay Analysis

Appendix 8 shows the polyphenols assay summary data for each site. The range on the amount of polyphenols expressed as milligram GAE per gram dry weight for each site is shown.

Appendix 9 shows the trend of polyphenols within each site while figure 3.6 shows how the polyphenols varied in the three sites.

ANOVA for polyphenols is shown in appendix 10. There was a significant difference between the three sites ($F_{2,5}$=7.29, $p<0.05$) in the amount of polyphenols. Brecon and Newcastleton were significantly different on mean polyphenols (t=3.69, $p<0.05$, df=5). Clone 104 and clone 108 were observed to have the highest amount of polyphenols in Brecon and Dalkeith sites respectively. At Newcastleton site the highest was observed on clone 68. Clone 33 had the lowest amount of polyphenols at both Newcastleton and Brecon.

At Dalkeith, clone 1 (QCI) was observed to have the lowest amount. The amount of polyphenols was found to be highly significant different ($F_{10,46}$=3.57, $p<0.01$). There was no significant polyphenols site/clone interaction effect. Again Dalkeith had intermediate levels of polyphenols compared to Newcastleton and Brecon sites.

3.4 Carbohydrate Data Analysis

Appendix 11 shows the results of the carbohydrates assay from the three sites. Total carbohydrates, including free sugars and starch are expressed as a percentage dry weight. The minimum, maximum and mean per site are shown.

Figure 3.6 a diagrammatic representation of the trend of percentage total carbohydrates per dry weight in the three sites.

There was a very high significant difference in total carbohydrate concentration between sites ($F_{2,5}$=94.24, p<0.001). Newcastleton was very highly significantly different from both Brecon and Dalkeith(t=-11.38, p<0.001, df=5). Clone 6 and clone 17 contained the highest amount of carbohydrates in Newcastleton and Brecon respectively. At Dalkeith, clone 1 was observed to have the highest. Clone 68, 92 and 50 had the lowest carbohydrates in Brecon, Dalkeith and Newcastleton respectively. However there was no significant difference between Brecon and Dalkeith. There was a highly significant difference in carbohydrate concentration between clones ($F_{10,46}$=3.07, p<0.01). Dalkeith was observed to have intermediate levels of carbohydrates compared to other two sites. There was no significant site/clone interaction effect (appendix 12).

3.5 Nitrogen assay results

Appendix 13 shows the summary data for the total nitrogen expressed as a percentage of the dry weight. The minimum, maximum and mean in each site is shown.

Figure 3.7 is a histogram showing the trend/variation of percentage total nitrogen per dry weight in the three sites. There was a higher nitrogen concentration in the Newcastleton clones compared to Dalkeith. Clones at the Brecon site had intermediate levels. Within each site plants had a fairly uniform nitrogen contents showing only small levels of natural variation.

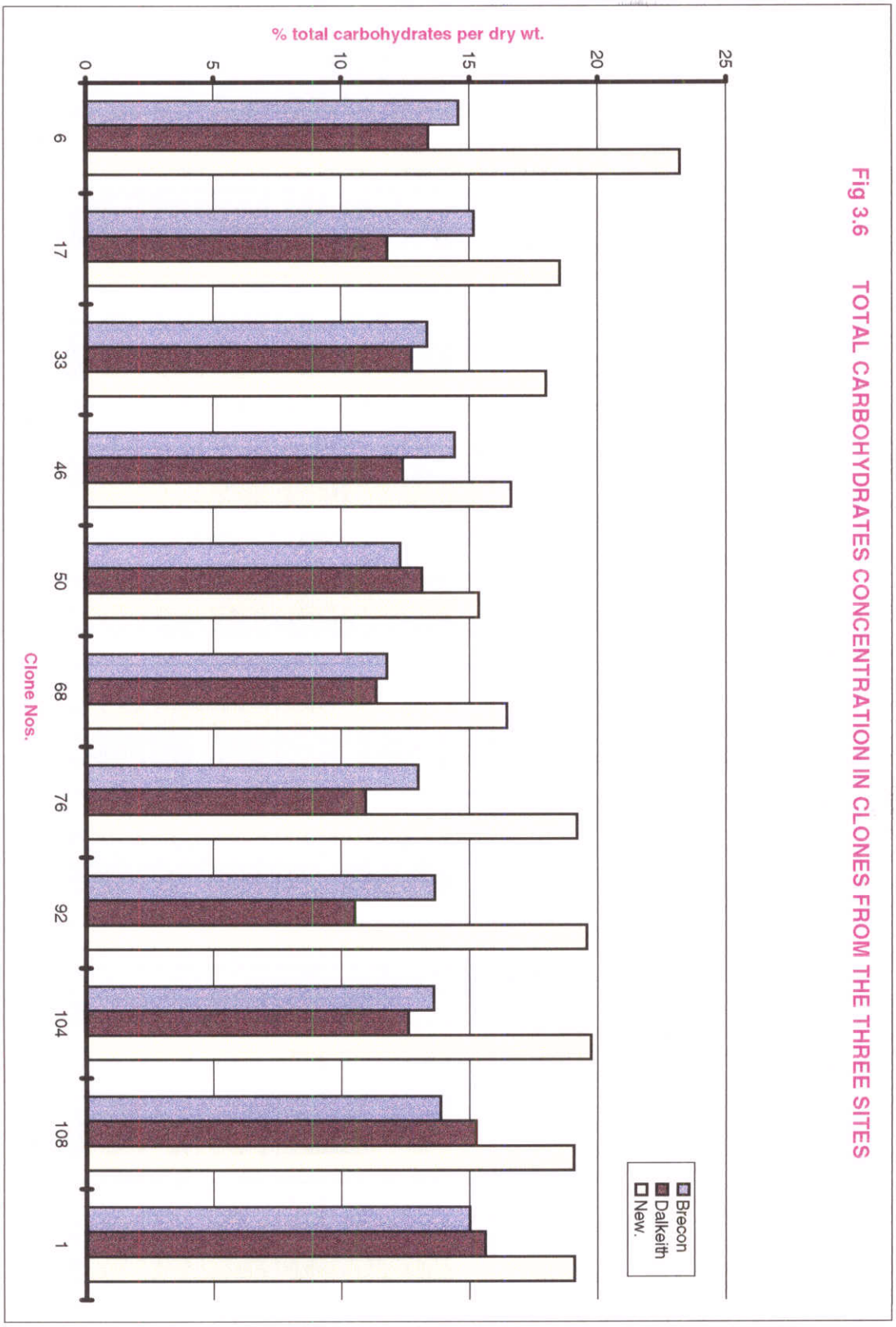


Fig 3.6 TOTAL CARBOHYDRATES CONCENTRATION IN CLONES FROM THE THREE SITES

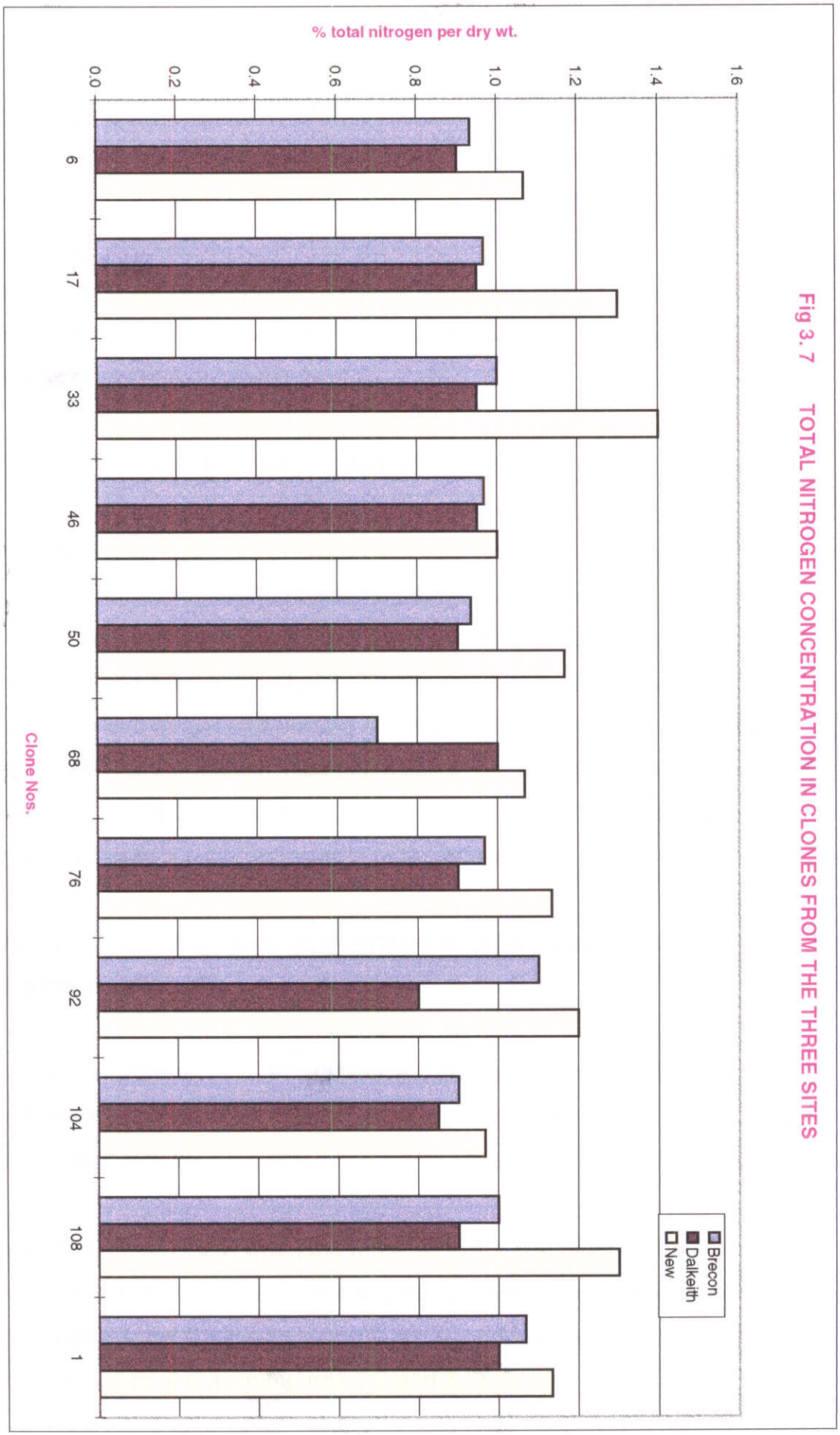


Fig 3. 7 TOTAL NITROGEN CONCENTRATION IN CLONES FROM THE THREE SITES

There were highly significant differences between the three sites in nitrogen concentration ($F_{2,5}$=33.17, p<0.01). There was significant difference in nitrogen concentration between clones ($F_{10,46}$=2.26, p<0.05).

Newcastleton is highly significant different from Brecon (t=-6.78, p<0.01, df=5) and Dalkeith (t=-6.99, p<0.01, df=5) sites but Brecon is not significantly different from Dalkeith (appendix 14).

3.6 Feeding Damage Correlation Analysis

One of the major objective of this project was to determine whether a significant correlation existed between the response variables; resin, polyphenols, carbohydrates and nitrogen and the *H. abietis* feeding damage. In subsection 3.1.2 it was noted that there was a significant relationship between weevil weight and feeding damage and so partial correlations were used to look for the relationships that exist. Appendix 15 shows the details of partial correlation analysis of the feeding damage and the other four variables. Below is a summary table of the analysis.

Table 3.2 Feeding damage Pearson Partial Correlation Coefficients (All sites combined).

Variable	**Resin**	**Polyphenols**	**Carbohydrates**	**Nitrogen**
Coefficient r	-0.3151	0.0812	-0.3438	-0.2332
Probability	p<0.05	n.s.	p<0.01	n.s.

The analysis shows that when the three sites are combined, a highly significant negative correlation exists between feeding damage and carbohydrates and there was a significant negative correlation between feeding damage and resin (figures 3.8 and 3.9).

Table 3.3 Feeding damage Pearson Partial correlation Coefficients per site (appendix 16)

Variables	Resin	Polyphenol	Carbohydrates	Nitrogen
Brecon	-0.3670	-0.2141	0.1036	0.0570
Dalkeith	-0.0711	-0.1391	-0.3838	-0.3498
Newcastleton	0.0642	-0.1632	0.0744	0.2584

There was no significant correlation in any of the four variables when analysis was done for each site.

Figure 3.8 Relationship between Feeding Damage and Resin (All sites combine

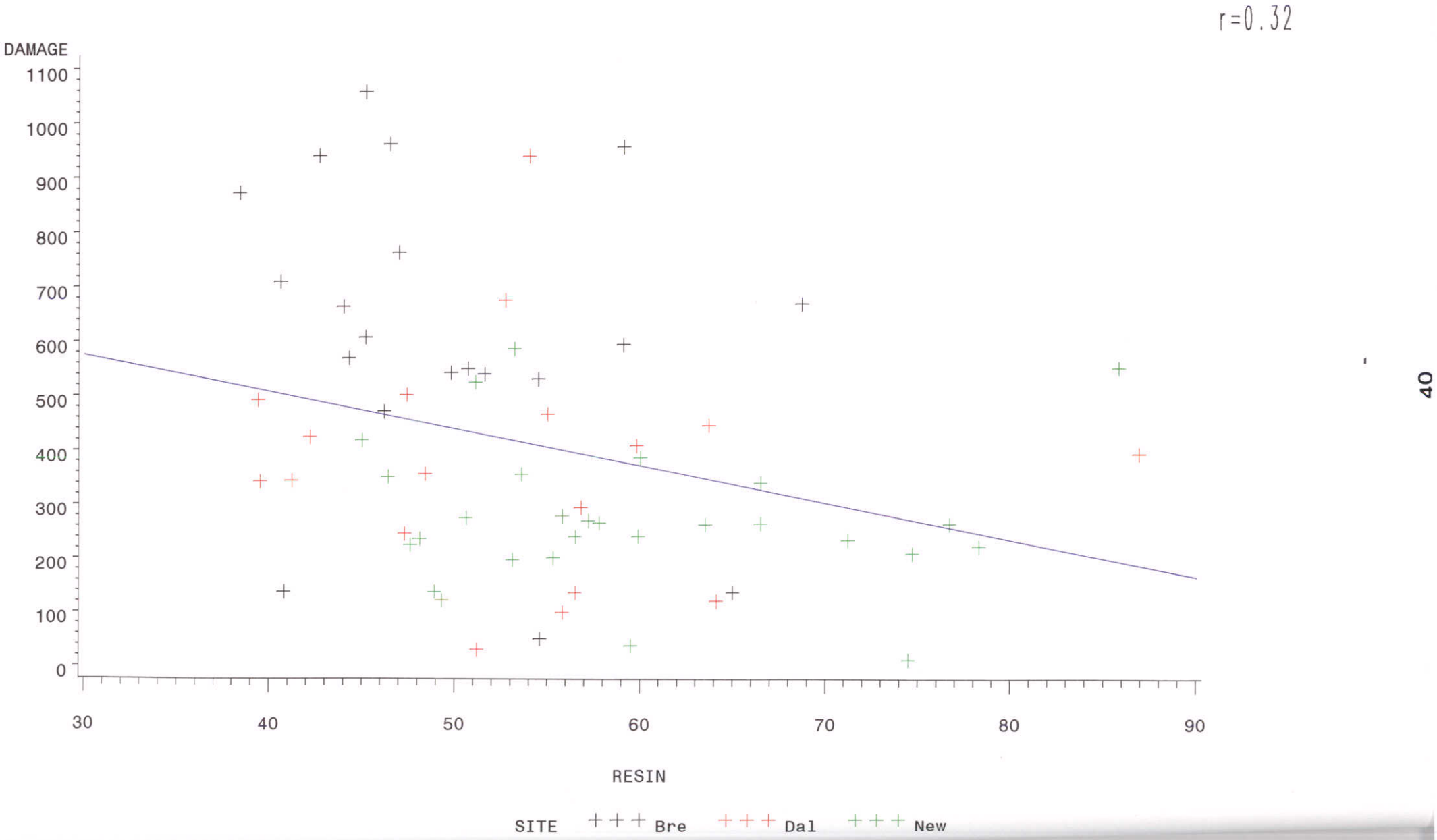
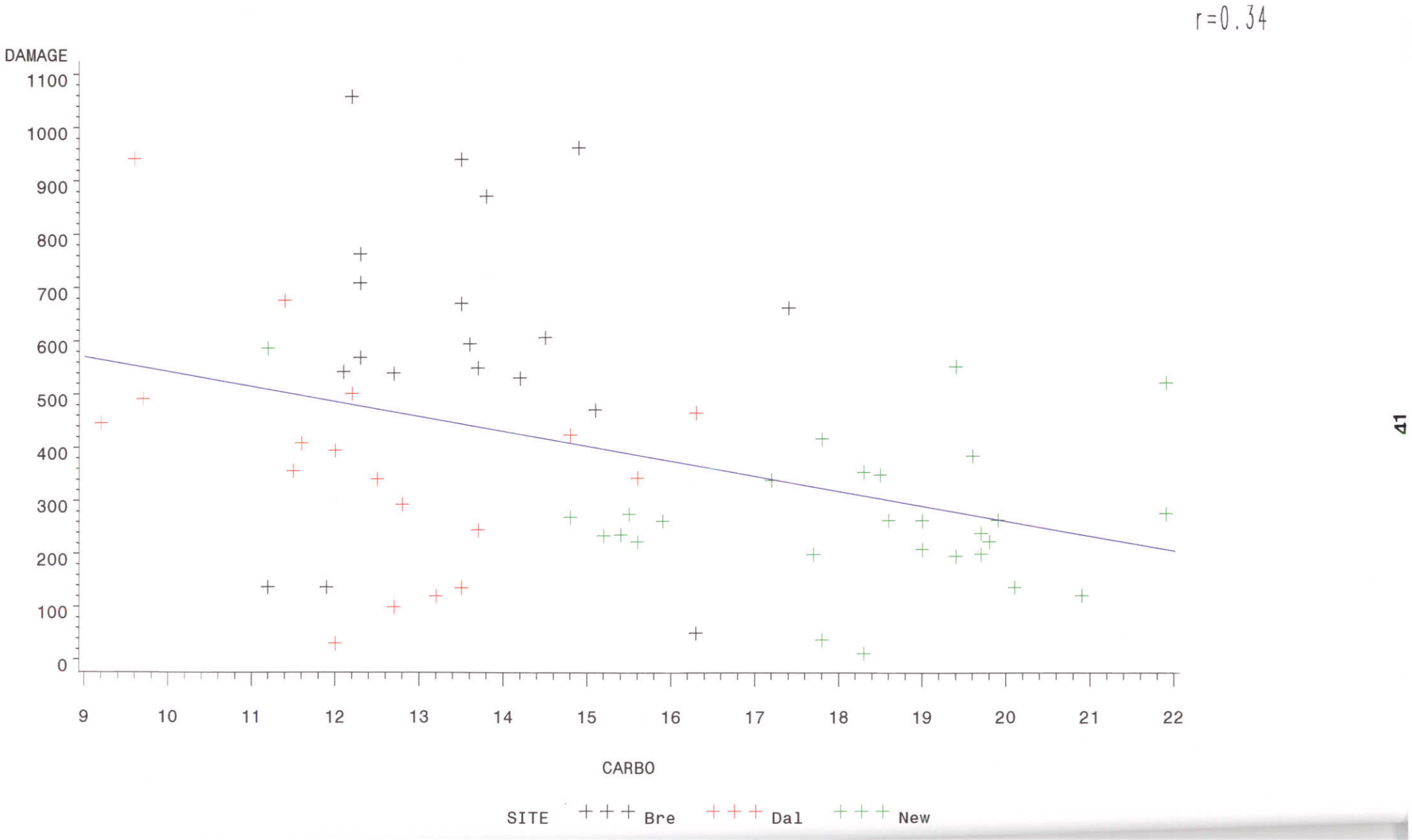


Figure 3.9 Relationship between Feeding Damage and Carbohydrates (All sites combine



CHAPTER 4

4.0 DISCUSSION

4.1 Influence of Twig size and weevil weight on amount of feeding

Although there was no significant relationship between the amount of feeding damage and twig diameter, the probability of a twig being damaged increases with twig diameter. Within the size range used in the choice test feeding damage bioassays, large twigs with diameter greater 6 mm are predicted to have a more than 90% chance of being attacked (Fig 3.1). This relationship could influence the probability of attack on transplants in the forest with damage more likely and possibly mortality higher on transplants with optimal diameter for *Hylobius* feeding. New transplants have been identified to be prone to Hylobius attack leading to mortality during their first and second growing season (Collins, 1993). This may be because they are in the diameter class (6-9 mm) that has the highest probability of being attacked. Past *H. abietis* feeding damage bioassays have adopted the use of twigs with a standard diameter of about 1 cm (Leather *et al.,* 1994, Manlove, *et al.,* 1997). These past studies cannot provide information for comparison with the present finding. However there is need for further investigation with a well designed choice test incorporating twigs of a wide range of sizes.

Understanding the relationship between twig size and feeding behaviour could help in developing pest management strategies and in improving the knowledge of *H. abietis* population dynamics and its feeding behaviour. Knowledge of the stem diameter of transplants most likely to attacked would help targeting control methods to that diameter class in the forest. This would reduce the cost and amount of chemical used in the field spraying against

Hylobius. Transplanting in the forest could be carried out with seedlings whose stem diameter is above that which is most prone to attack.

Weevil weight was found to be significantly positively correlated to amount of feeding damage. Although similar results were reported by Djeddour, (1996), the differences were related to the sex of the weevil, the larger females causing more damage than the smaller males. This relationship was explained as due to the female's physiological requirement for egg production hence the need for more energy (Djeddour, 1996). Although the present results cannot be taken to be conclusive, the explanation given based on sexual dimorphism proposed by Djeddour is likely not satisfactory. Results are probably best explained by the simple relationship between body size and amount eaten.

4.2 Site/clonal effect on defensive and nutrient chemicals

Site factors had a large effect on the concentration of resin and polyphenols in the bark of Spruce. Polyphenol and resin content of plant tissues are influenced by both light intensity and soil nutrient concentration and these environmental factors may have varied between the different sites. Other secondary metabolites like lignin in Sitka spruce bark have also been shown to be influenced by environmental factors (Wainhouse, *et al.* 1996).

The significant differences between clones in the concentration of defensive chemicals may have been due to the differences in their growth rate at the different sites. Wainhouse *et al.* (1996) reported that slow growing provenances of Spruce had a high concentration of lignin, a secondary metabolite with a defensive role. This may confer relatively good resistance to attack by insects, but the metabolism of lignin production may result in slow growth. Further analysis on the correlation of the defensive chemicals and growth performance of the clones will be undertaken later to help interpretation of results by the Forest Commission, Entomology Branch. This

site induced effect is of considerable practical interest as it could influence the susceptibility of trees to pests (eg *H. abietis)* and diseases when planted on different sites.

As well as clonal and site-induced variation in the concentration of secondary chemicals, the concentration of nutrients (carbohydrates and nitrogen) also varied between sites and clones. At Newcastleton carbohydrates and nitrogen concentrations were significantly higher than both Dalkeith and Brecon. One of the main causes of this may have been the effect of frost on the trees at Newcastleton. At this site, a spring frost killed the new shoots on many of the trees, removing an important sink for stored carbohydrates which therefore remain at high levels within the tree. Another explanation for the lower concentration of carbohydrates at Brecon is likely due to growth differences between Brecon and Newcastleton. There is a possibility that the spring growing period, starts earlier in the south as compared to the north and thus, by the time of sampling, Brecon clones could have utilised the bark-stored carbohydrates during the initial bud burst which was likely to have been earlier than that at Newcastleton and Dalkeith.

4.3 Feeding damage Relationships

It is apparent from the results that there is a negative relationship between the amount of resin in bark and *Hylobius* feeding damage. At Newcastleton, trees had relatively high amounts of resin in bark and low levels of adjusted feeding damage whereas at Brecon there was low amount of resin and high levels of feeding damage (Fig 3.2 and 3.4). It was likely that the high amount of resin concentration on the Newcastleton clones combined with other interacting factors could have contributed to the low feeding damage observed (Fig. 3.2 and 3.4). This conclusion is supported by the results of Tomlin *et al.,* 1996a and Alfaro, *et al.,* 1980 on feeding deterrence of white pine weevil that was associated with differences in amounts of resin on

different specific genotypes of Sitka spruce. The quantity and quality of resin acids on Sitka spruce have been shown to provide resistance against bark weevils (Tomlin *et al.*, 1996b). Sitka spruce provenances with higher density of outer resin ducts have been associated with resistance to white pine weevil, *Pissodes strobi* due to high resin flow (Tomlin *et al.*,1994).

Within sites however, there was a non-significant negative correlation between feeding damage and resin. Only when all the three sites were pooled together was the amount of feeding damage significantly negatively correlated to resin (Table 3.2). This might be explained from the origin of the clones. The clones in each site originate from ten families (with the exception of the QCI - 'clone 1') and only the best growth performance clone was chosen per family. Work by Wainhouse *et al.* (Unpublished data) show a positive correlation between resin and growth performance of Sitka spruce provenances. This being the case, the fact that the fastest growing clones were selected for the present study may mean that there was only a small range in the amount of defensive chemicals produced by these clones. When the sites are combined the resin range increases and thus resulting in a significant relationship. At all the three sites, polyphenols were not significantly correlated to feeding damage (Table 3.3).

Nutrient chemicals show a non-significant relationship at site level. However when the sites are pooled together, a significant negative correlation ($p<0.01$) between carbohydrates and feeding damage was observed (Table 3.2 and fig. 3.9). Wainhouse, *et al* (unpublished data) have reported a positive association between carbohydrates and secondary chemicals concentrations, this was due to carbon/nutrient balance. In this case, the sites which had relatively high carbohydrates also had relatively low feeding damage. It is likely that the available carbon was being used in the biosynthesis of defensive chemicals, eg resin, possibly deterring feeding by the weevils. However the correlation between carbohydrates and feeding damage is only

observed when sites are combined explaining the difference in the concentration of the carbohydrates at the different sites. Due to the effect of frost it would be worthwhile to recommend a further investigation.

4.4 Conclusion

- There were significant differences between sites in the amounts of defensive chemicals and nutrients in the selected clones. This was attributed to interacting factors between the clones and the environment.

- There were significant differences in feeding damage of *H. abietis* between sites and clones. This was attributed to the differences in defensive chemicals acting as deterrents and the differences in the amounts of nutrients.

- The results were not conclusive on the significant relationship between feeding damage by *H. abietis* and amounts of defensive chemicals and nutrients at site level. However a negative correlation exits between feeding damage and two chemicals namely resin and total carbohydrates when the sites are pooled together.

The results from this project emphasise the complexity of site and clonal effects on plant quantitative defences and nutrients. It is likely that the nutrients have a complex relationship with the quantity and quality of the plant defences. The interaction of different factors involved are likely to determine the feeding behaviour of *H. abietis* on Sitka spruce clones.

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Appendix 1

1. ANOVA
Site. Brecon
variate: Twig size

Source of variation	d f.	s.s	m.s.	v.r.	F pr.
clone	10	24.84	2.48	1.34	0.22
Residual	117	217.58	1.86		
Total	127	237.79			

Site. Dalkeith
variate: Twig size

Source of variation	d f.	s.s	m.s.	v.r.	F pr.
clone	10	14.18	1.42	1.18	0.32
Residual	65	77.87	1.20		
Total	75	87.19			

Site. Newcastleton
variate: Twig size

Source of variation	d f.	s.s	m.s.	v.r.	F pr.
clone	10	16.26	1.63	1.11	0.36
Residual	84	122.88	1.46		
Total	94	131.77			

Regression Analysis

Response variate	:	Damage
Binomial totals	:	1
Distribution	:	Binomial
Link function	:	Logit
Fitted terms	:	Constant + twig size

	df	deviance	mean deviance	deviance ratio
Regression	1	17.0	16.9655	19.97
Residue	212	189.2	0.8925	
Total	213	206.2	0.9680	

Estimates of regression coefficients

	estimate	s.e	t
Constant	-1.744	0.837	-2.08

Appendix 2.

Estimated damage levels for site/clones with average weevil size of 0.11g

The Mixed Procedure

Class Level information

Class	Levels	Values
SITE	3	Bre Dal New
CLONE	10	1 104 108 17 33 46 50 68 76 92
REP	3	1 2 3

Tests for Fixed effects.

Source	NDF	DDF	Type I F	Pr. > F
Weevil wt.	1	32	5.01	0.0324
Site	2	5	34.64	0.0012
Clone	9	32	3.98	0.0017
Site*Clone	18	32	3.12	0.0024

Source	NDF	DDF	Type IIIF	Pr. > F
Weevil wt.	1	32	8.19	0.0074
Site	2	5	29.09	0.0018
Clone	9	32	4.68	0.0005
Site*Clone	18	32	3.12	0.0024

clone (site) interactions

Site	Weevil wt.	NDF	DDF	F	Pr>F
Brecon	0.11	9	32	5.56	0.0001
Dalkeith	0.11	9	32	3.75	0.0026
Newcastleton	0.11	9	32	1.00	0.4565

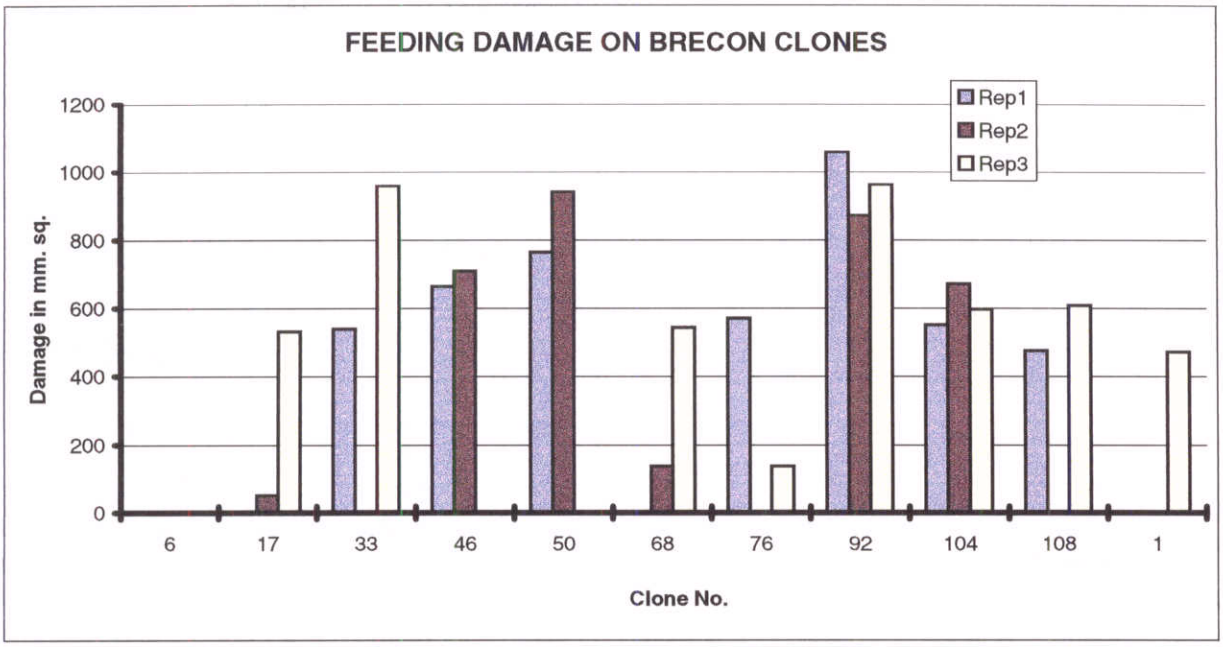
SUMMARY: DAMAGE BIOASSAY DATA (sq.mm)

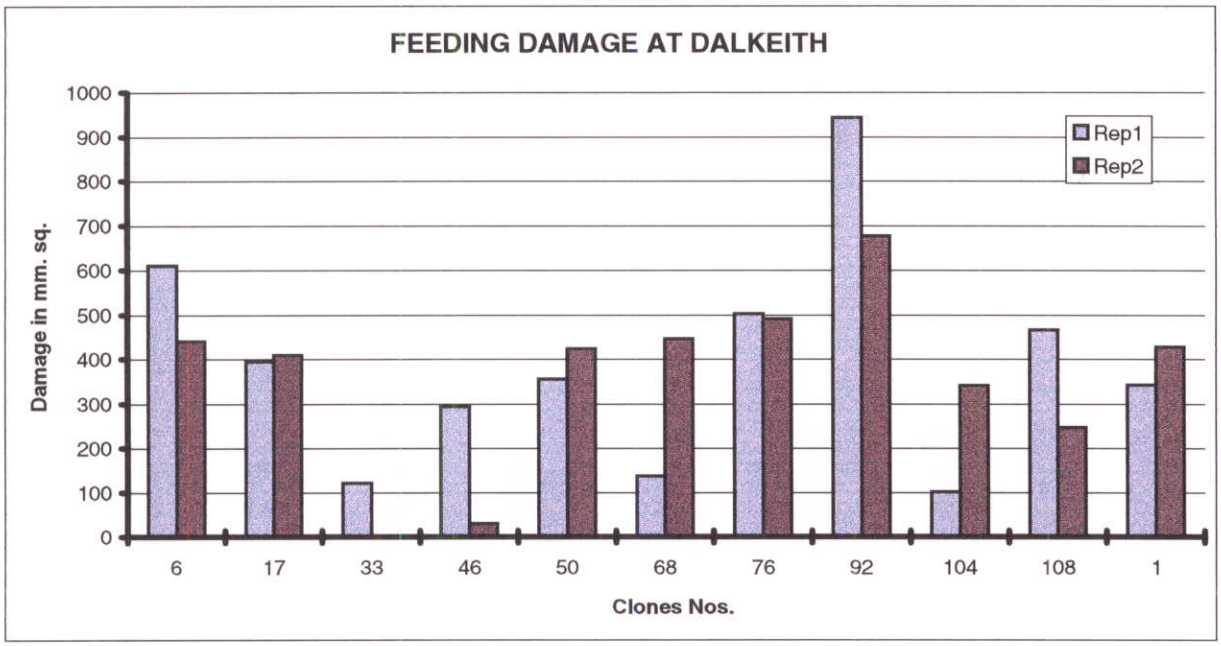
	BRECON				DALKEITH			NEWCASLTETON			
Clone	Rep1	Rep2	Rep3	Brecon	Rep1	Rep2	Dalkeith	Rep1	Rep2	Rep3	Newcastleton
6	*	*	*	*	610.52	439.74	525.13	185.69	481.49	164.82	277.33
17	*	51.04	531.39	291.22	394.56	408.31	401.44	209.66	554.60	339.72	367.99
33	540.33	*	959.32	749.83	120.54	*	120.54	12.62	200.49	239.71	150.94
46	664.88	709.90	*	687.39	293.74	30.73	162.24	240.11	264.08	587.01	363.73
50	764.16	941.53	*	852.85	355.95	423.52	389.74	235.92	269.07	262.13	255.71
68	*	137.39	543.02	340.21	136.60	446.16	291.38	222.74	234.56	264.37	240.56
76	570.15	*	137.01	353.58	501.80	491.23	496.52	122.61	417.88	*	270.25
92	1058.92	872.67	963.56	965.05	941.88	676.35	809.12	277.73	354.86	350.39	327.66
104	550.25	671.54	595.40	605.73	100.01	340.98	220.50	200.79	137.87	197.05	178.57
108	476.16	*	607.94	542.05	466.12	245.39	355.76	274.93	525.42	224.69	341.68
1	*	*	471.51	471.51	342.94	426.67	384.81	265.01	385.66	38.37	229.68

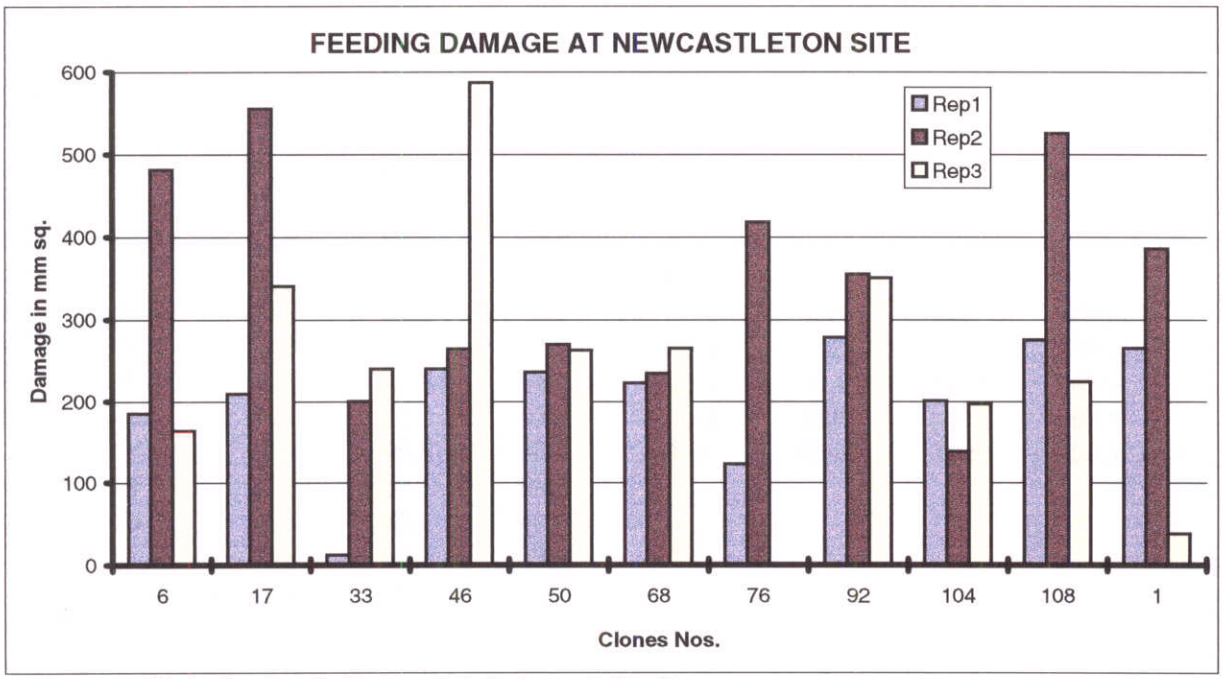
Damage in the site rep.	Brecon	Dalkeith	ecastleton
Min	51.04	30.73	12.62
Max.	1058.92	941.88	587.01
Mean	610.38	390.18	273.19

Damage range per site			
Min	291.22	120.54	150.94
Max.	965.05	809.12	367.99
Mean	585.94	377.92	273.10

* - Weevil dead before end of bioassay or no sample avaiable







Missing Bar show that the weevil died before end of the Bioassay

RESIN BIOASSAY DATA SUMMARY

Resin expressed as mg resin per g dry wt.

NECASTLETON SITE

Clone No	Rep1	Rep2	Rep3	New
6	67.1	52.2	44.9	54.7
17	74.7	85.8	66.5	75.6
33	74.5	*	56.5	65.5
46	59.9	76.7	53.2	63.3
50	48.1	57.2	63.3	56.2
68	78.3	71.2	66.5	72.0
76	49.3	45.0	*	47.2
92	55.8	53.6	46.4	51.9
104	55.3	48.9	53.1	52.4
108	50.6	51.1	47.6	49.8
1	57.8	60.0	59.5	59.1

	Min	Max	Mean
Rep 1	48.1	78.3	61.0
Rep 2	45.0	85.8	60.2
Rep 3	44.9	66.5	55.8
New	47.2	75.6	58.9

DALKEITH SITE

Clone No	Rep1	Rep2	Dal
6	49.4	46.2	47.8
17	86.9	59.8	73.3
33	64.1	61.7	62.9
46	56.8	51.2	54.0
50	48.4	42.2	45.3
68	56.5	63.7	60.1
76	47.4	39.4	43.4
92	54.0	52.7	53.4
104	55.8	39.5	47.7
108	55.0	47.3	51.1
1	41.2	*	41.2

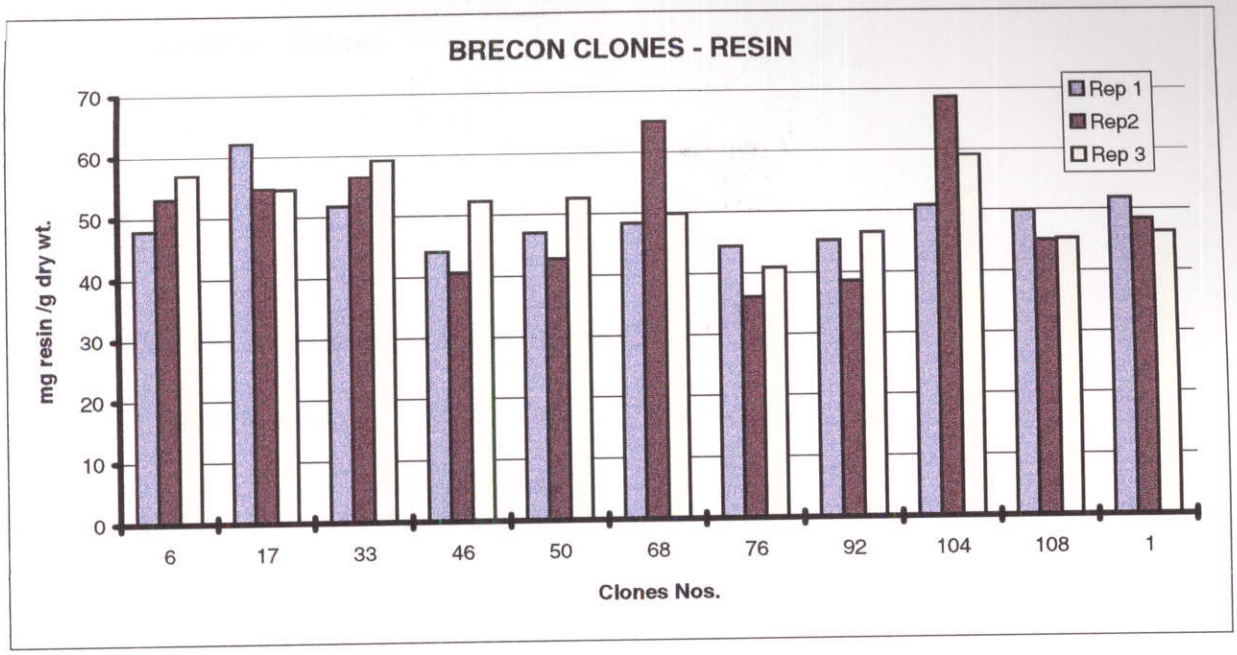
	Min	Max	Mean
Rep 1	41.2	86.9	55.9
Rep 2	39.4	63.7	50.4
Dal	41.2	73.3	52.7

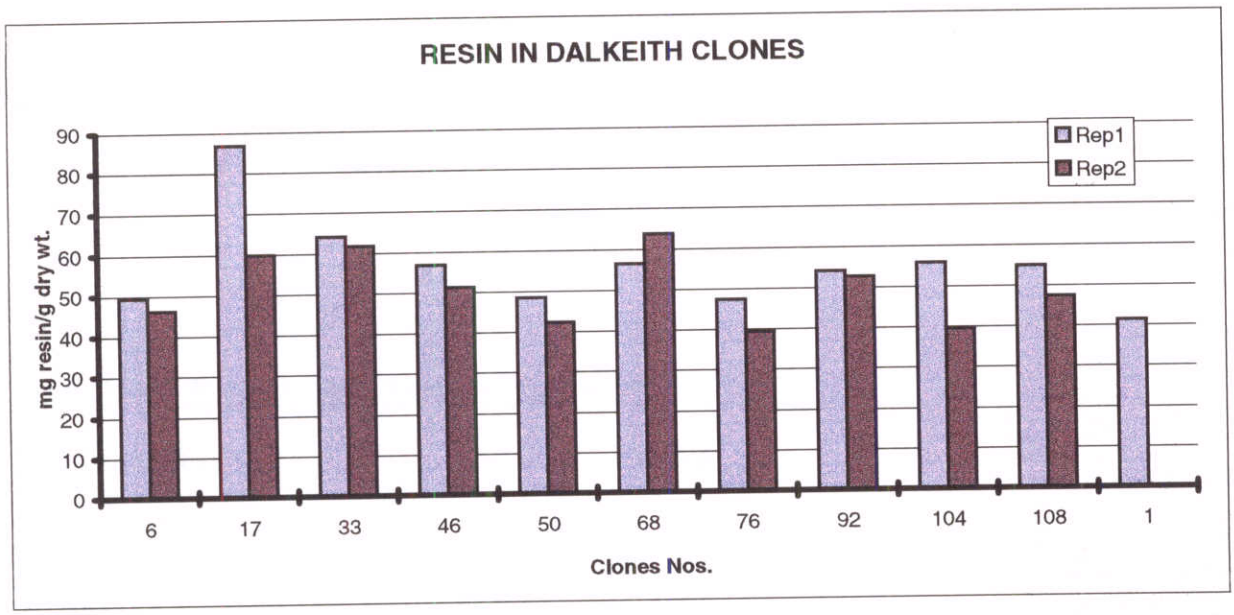
BRECON SITE

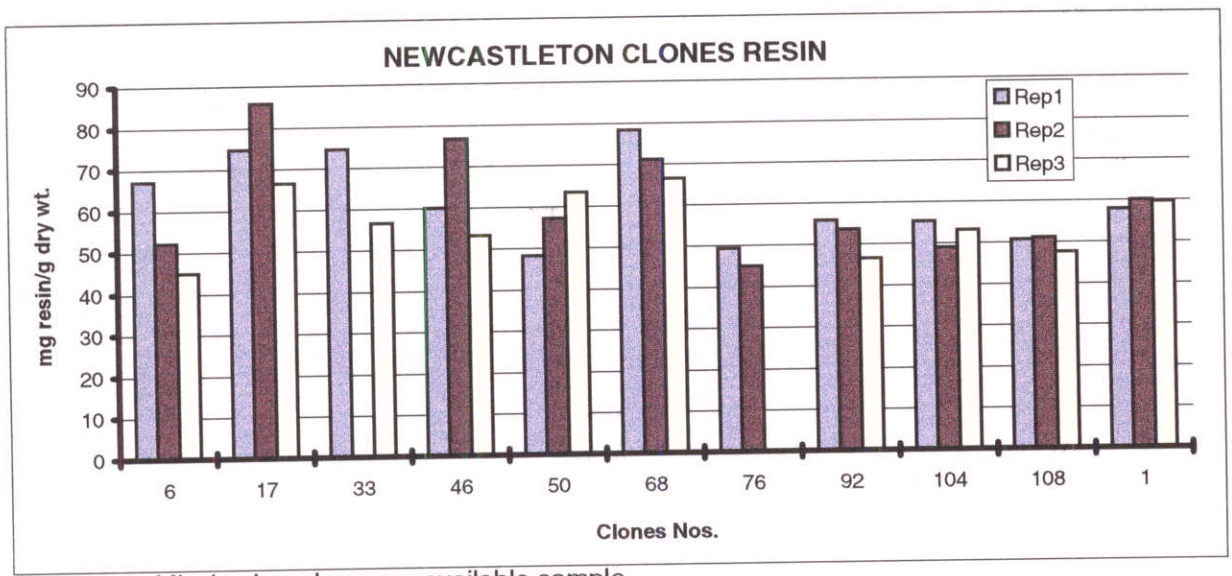
Clone No	Rep 1	Rep2	Rep 3	Bre
6	47.9	53.0	56.9	52.6
17	62.0	54.6	54.5	57.0
33	51.6	56.4	59.1	55.7
46	44.0	40.6	52.2	45.6
50	47.0	42.7	52.5	47.4
68	48.3	65.0	49.8	54.4
76	44.3	36.1	40.8	40.4
92	45.2	38.4	46.5	43.4
104	50.7	68.7	59.1	59.5
108	49.8	45.0	45.2	46.6
1	51.7	48.3	46.2	48.8

	Min	Max	Mean
Rep 1	44.0	62.0	49.3
Rep 2	36.1	68.7	49.9
Rep 3	40.8	59.1	51.1
Bre	40.4	59.5	50.1

* - no sample available







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Appendix 7

The MIXED Procedure

Class Level Information

Class	Levels	Values
SITE	3	Bre Dal New
CLONE	11	1 104 108 17 33 46 50 6 68 76 92
REP	3	1 2 3

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-138.9558668	
1	2	-141.9484925	0.00000155
2	1	-141.9486035	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
REP(SITE)	0.00212036
Residual	0.01213310

Model Fitting Information for LRESIN

Description	Value
Observations	85.0000
Res Log Likelihood	23.1895
Akaike's Information Criterion	21.1895
Schwarz's Bayesian Criterion	19.2383
-2 Res Log Likelihood	-46.3790

Tests of Fixed Effects

Source	NDF	DDF	Type I F	Pr > F
SITE	2	5	5.91	0.0482
CLONE	10	47	9.94	0.0001
SITE*CLONE	20	47	1.82	0.0466

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
SITE	2	5	5.79	0.0499
CLONE	10	47	10.12	0.0001
SITE*CLONE	20	47	1.82	0.0466

Least Squares Means

Effect	SITE	LSMEAN	Std Error	DF	t	Pr > |t|
SITE	Bre	3.90395283	0.03277888	5	119.10	0.0001
SITE	Dal	3.94358183	0.04080451	5	96.65	0.0001
SITE	New	4.05875103	0.03330030	5	121.88	0.0001

Differences of Least Squares Means

Effect	SITE	_SITE	Difference	Std Error	DF	t
SITE	Bre	Dal	-0.03962900	0.05233988	5	-0.76
SITE	Bre	New	-0.15479820	0.04672649	5	-3.31
SITE	Dal	New	-0.11516920	0.05266800	5	-2.19

Differences of Least Squares Means

Pr > |t|	Adjustment	Adj P
0.4831	Tukey-Kramer	0.7429
0.0212	Tukey-Kramer	0.0470
0.0804	Tukey-Kramer	0.1667

POLYPHENOL BIOASSAY DATA SUMMARY

Polyphenols expressed as mg GAE per g dry wt.

NEWCASTLETON

Clone No.	Rep 1	Rep 2	Rep 3	New
6	50.1	41.9	44.9	45.6
17	50.2	59.6	44.9	51.6
33	44.3	*	33.0	38.7
46	49.4	55.6	46.4	50.5
50	46.9	47.7	49.9	48.2
68	64.4	55.1	47.3	55.6
76	60.8	47.3	*	54.0
92	49.1	45.9	41.1	45.4
104	59.2	54.3	43.2	52.2
108	50.2	43.8	38.8	44.3
1	47.2	47.1	55.1	49.8

	Min	Max	Mean
Rep 1	44.3	64.4	52.0
Rep2	41.9	59.6	49.8
Rep 3	33.0	55.1	44.4
New	38.7	55.6	48.7

BRECON

Clone No.	Rep 1	Rep 2	Rep 3	Brecon
6	58.3	67.7	63.0	63.0
17	53.8	51.2	61.4	55.5
33	33.9	51.8	47.5	44.4
46	48.0	47.2	45.1	46.8
50	68.3	51.4	55.0	58.2
68	42.1	67.0	54.1	54.4
76	71.4	62.0	64.5	66.0
92	50.6	56.9	56.3	54.6
104	65.7	75.4	70.7	70.6
108	71.7	51.2	51.6	58.2
1	64.0	52.2	42.8	53.0

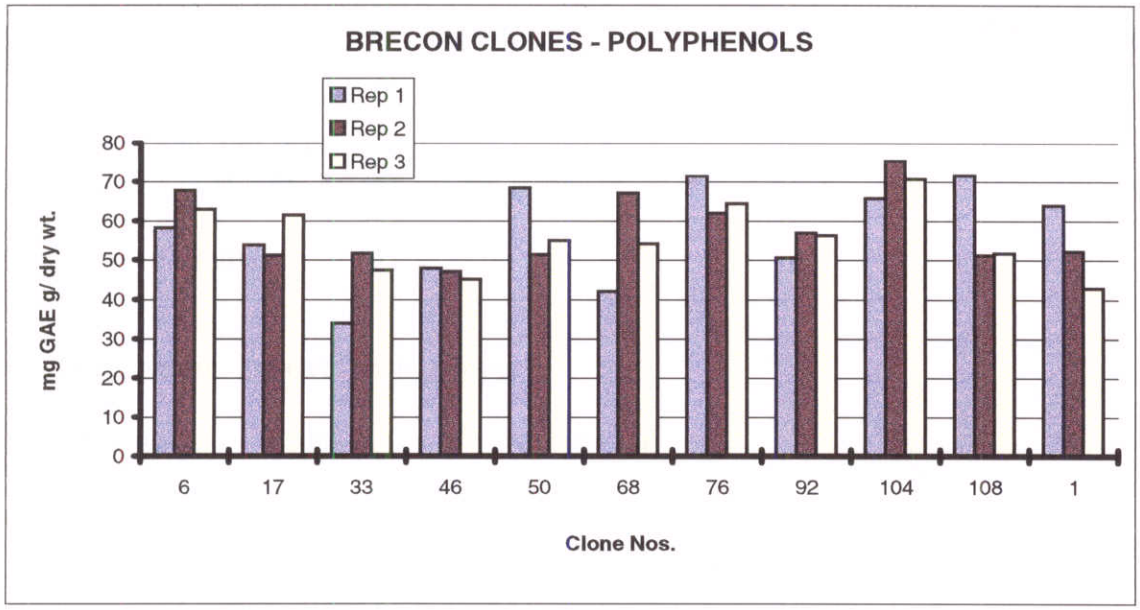
	Min	Max	Mean
Rep 1	33.9	71.7	57.1
Rep2	47.2	75.4	57.6
Rep 3	42.8	70.7	55.6
Brec	44.4	70.6	56.8

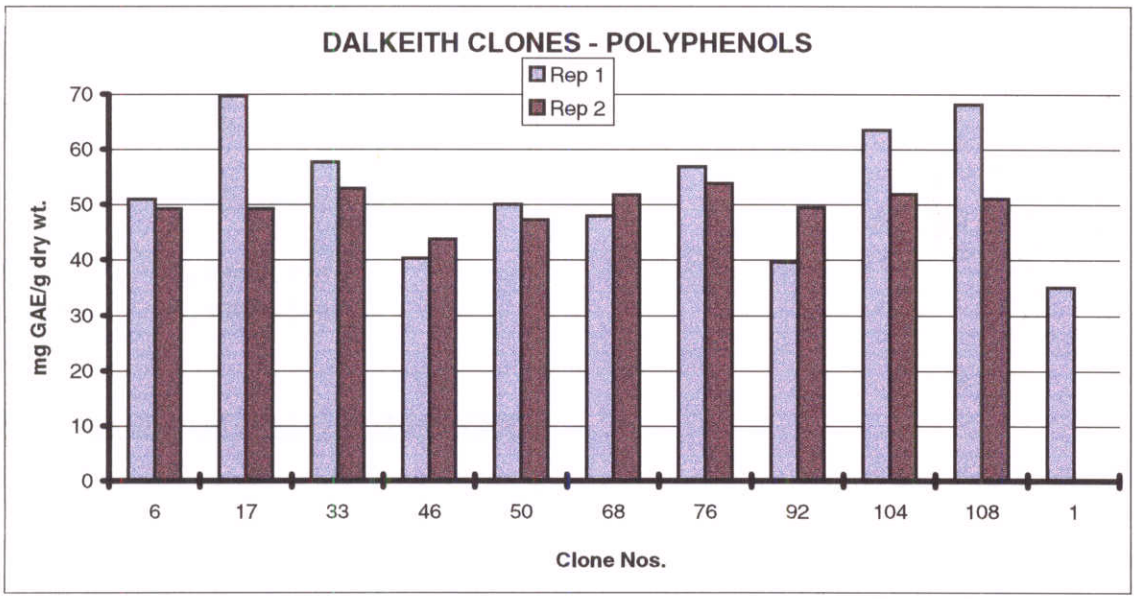
DALKEITH

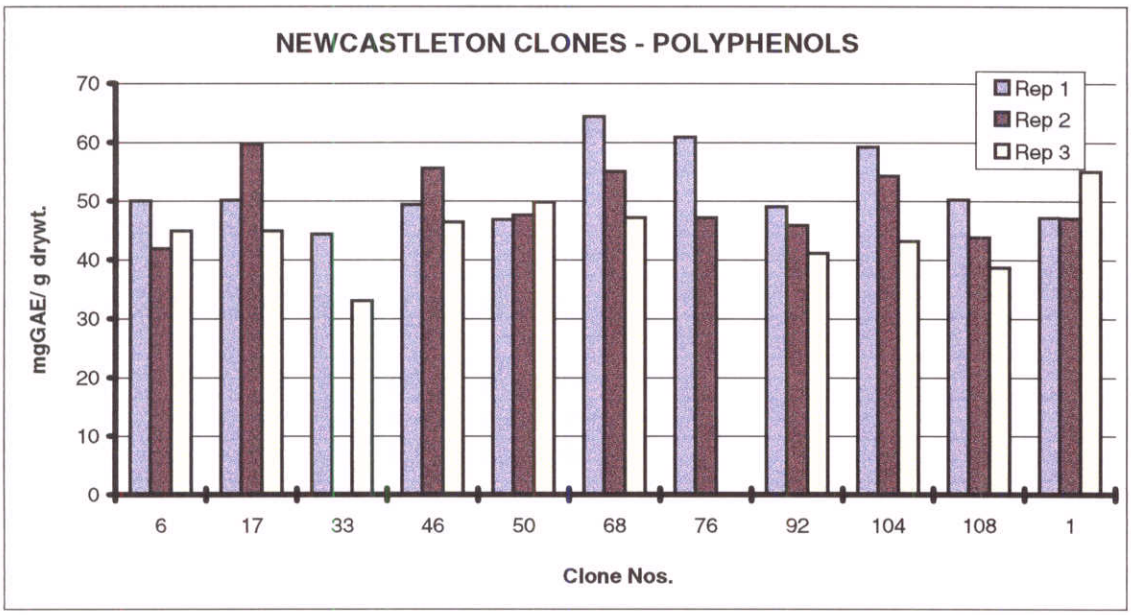
Clone No.	Rep 1	Rep 2	Dal
6	51.0	49.2	50.1
17	69.6	49.3	59.5
33	57.7	52.9	55.3
46	40.3	43.8	42.1
50	50.1	47.3	48.7
68	48.0	51.7	49.8
76	56.9	53.8	55.4
92	39.5	49.5	44.5
104	63.5	51.9	57.7
108	68.1	51.1	59.6
1	35.0	*	35.0

	Min	Max	Mean
Rep 1	35.0	69.6	52.7
Rep2	43.8	53.8	50.1
Dal	35.0	59.6	50.7

* - no sample material available







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Appendix 10

The MIXED Procedure

Class Level Information

Class	Levels	Values
SITE	3	Bre Dal New
CLONE	11	1 104 108 17 33 46 50 6 68 76 92
REP	3	1 2 3

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	286.57043666	
1	2	286.04435281	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
REP(SITE)	2.71339495
Residual	48.13504642

Model Fitting Information for PHENOL

Description	Value
Observations	85.0000
Res Log Likelihood	-190.807
Akaike's Information Criterion	-192.807
Schwarz's Bayesian Criterion	-194.758
-2 Res Log Likelihood	381.6140

Tests of Fixed Effects

Source	NDF	DDF	Type I F	Pr > F
SITE	2	5	6.79	0.0375
CLONE	10	47	3.48	0.0017
SITE*CLONE	20	47	1.76	0.0570

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
SITE	2	5	7.29	0.0330
CLONE	10	47	3.57	0.0014
SITE*CLONE	20	47	1.76	0.0570

Least Squares Means

Effect	SITE	LSMEAN	Std Error	DF	t	Pr > |t|
SITE	Bre	56.78181818	1.53723868	5	36.94	0.0001
SITE	Dal	50.63195526	1.93668006	5	26.14	0.0001
SITE	New	48.65114558	1.58052333	5	30.78	0.0001

Differences of Least Squares Means

Effect	SITE	_SITE	Difference	Std Error	DF	t
SITE	Bre	Dal	6.14986292	2.47261651	5	2.49
SITE	Bre	New	8.13067260	2.20480311	5	3.69
SITE	Dal	New	1.98080968	2.49975672	5	0.79

Differences of Least Squares Means

Pr > |t|	Adjustment	Adj P
0.0554	Tukey-Kramer	0.1176
0.0142	Tukey-Kramer	0.0318
0.4640	Tukey-Kramer	0.7234

CARBOHYDRATES ASSAY SUMMARY DATA

Total carbohydrates expressed as % dry wt.

Site	Clone	Rep 1	Rep 2	Rep 3	Mean
Brecon	6	13.7	14.7	15.3	14.6
	17	15.0	16.3	14.2	15.2
	33	12.7	14.0	*	13.4
	46	17.4	12.3	13.6	14.4
	50	12.3	13.5	11.1	12.3
	68	12.0	11.2	12.1	11.8
	76	12.3	14.8	11.9	13.0
	92	12.2	13.8	14.9	13.6
	104	13.7	13.5	13.6	13.6
	108	12.5	14.6	14.5	13.9
	1	14.6	15.3	15.1	15.0
Dalkeith	6	13.4	*		13.4
	17	12.0	11.6		11.8
	33	13.2	12.3		12.8
	46	12.8	12.0		12.4
	50	11.5	14.8		13.2
	68	13.5	9.2		11.4
	76	12.2	9.7		11.0
	92	9.6	11.4		10.5
	104	12.7	12.5		12.6
	108	16.8	13.7		15.3
	1	15.6	*		15.6
Necastleton	6	22.5	23.0	24.1	23.2
	17	19.0	19.4	17.2	18.5
	33	18.3	17.7	*	18.0
	46	19.7	19.0	11.2	16.6
	50	15.4	14.8	15.9	15.4
	68	15.6	15.2	18.6	16.5
	76	20.9	17.8	18.9	19.2
	92	21.9	18.3	18.5	19.6
	104	19.7	20.1	19.4	19.7
	108	15.5	21.9	19.8	19.1
	1	19.9	19.6	17.8	19.1

* - no sample

Clone Nos.	Brecon	Dalkeith	New.
6	14.6	13.4	23.2
17	15.2	11.8	18.5
33	13.4	12.8	18.0
46	14.4	12.4	16.6
50	12.3	13.2	15.4
68	11.8	11.4	16.5
76	13.0	11.0	19.2
92	13.6	10.5	19.6
104	13.6	12.6	19.7
108	13.9	15.3	19.1
1	15.0	15.6	19.1

site	Min	Mean	Max.
Brecon	11.8	13.7	15.2
Dalkeith	10.5	12.7	10.5
New.	15.4	18.6	23.2

Appendix 12

The MIXED Procedure

Class Level Information

Class	Levels	Values
SITE	3	Bre Dal New
CLONE	11	1 104 108 17 33 46 50 6 68 76 92
REP	3	1 2 3

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	135.85755197	
1	1	135.85755197	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
REP(SITE)	0.00000000
Residual	2.95513072

Model Fitting Information for CARBO

Description	Value
Observations	84.0000
Res Log Likelihood	-114.795
Akaike's Information Criterion	-116.795
Schwarz's Bayesian Criterion	-118.726
-2 Res Log Likelihood	229.5893

Tests of Fixed Effects

Source	NDF	DDF	Type I F	Pr > F
SITE	2	5	100.76	0.0001
CLONE	10	46	3.78	0.0009
SITE*CLONE	20	46	1.57	0.1030

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
SITE	2	5	94.24	0.0001
CLONE	10	46	3.07	0.0046
SITE*CLONE	20	46	1.57	0.1030

Least Squares Means

Effect	SITE	LSMEAN	Std Error	DF	t	Pr > |t|
SITE	Bre	13.69848485	0.30597360	5	44.77	0.0001
SITE	Dal	12.68181818	0.39843028	5	31.83	0.0001
SITE	New	18.62424242	0.30597360	5	60.87	0.0001

Differences of Least Squares Means

Effect	SITE	_SITE	Difference	Std Error	DF	t
SITE	Bre	Dal	1.01666667	0.50236096	5	2.02
SITE	Bre	New	-4.92575758	0.43271201	5	-11.38
SITE	Dal	New	-5.94242424	0.50236096	5	-11.83

Differences of Least Squares Means

Pr > |t|	Adjustment	Adj P
0.0989	Tukey-Kramer	0.2016
0.0001	Tukey-Kramer	0.0002
0.0001	Tukey-Kramer	0.0002

SUMMARY NITROGEN ASSAY DATA
(expressed as percentage total Nitrogen per Dry weight).

Site	Clone	Rep 1	Rep 2	Rep 3	Mean
Brecon	6	0.9	0.9	1.0	0.9
	17	1.0	1.0	0.9	1.0
	33	0.8	1.2	*	1.0
	46	1.0	0.8	1.1	1.0
	50	0.9	1.0	0.9	0.9
	68	0.4	0.9	0.8	0.7
	76	0.9	0.8	1.2	1.0
	92	1.1	1.1	1.1	1.1
	104	1.0	0.9	0.8	0.9
	108	0.9	1.1	1.0	1.0
	1	1.1	1.0	1.1	1.1
Dalkeith	6	0.9	*		0.9
	17	1.0	0.9		1.0
	33	0.9	1.0		1.0
	46	1.1	0.8		1.0
	50	0.9	0.9		0.9
	68	1.1	0.9		1.0
	76	0.9	0.9		0.9
	92	0.8	0.8		0.8
	104	0.9	0.8		0.9
	108	0.9	0.9		0.9
	1	1.0	*		1.0
Necastleton	6	1.0	1.0	1.2	1.1
	17	1.2	1.4	1.3	1.3
	33	1.3	1.5	*	1.4
	46	1.1	1.0	0.9	1.0
	50	1.2	1.1	1.2	1.2
	68	1.1	0.9	1.2	1.1
	76	1.2	1.1	1.1	1.1
	92	1.1	1.3	1.2	1.2
	104	1.1	0.9	0.9	1.0
	108	1.3	1.3	1.3	1.3
	1	1.2	1.1	1.1	1.1

* no sample

Summary

Clone	Brecon	Dalkeith	New
6	0.9	0.9	1.1
17	1.0	1.0	1.3
33	1.0	1.0	1.4
46	1.0	1.0	1.0
50	0.9	0.9	1.2
68	0.7	1.0	1.1
76	1.0	0.9	1.1
92	1.1	0.8	1.2
104	0.9	0.9	1.0
108	1.0	0.9	1.3
1	1.1	1.0	1.1

site	Min	Mean	Max.
Brecon	0.7	1.0	1.1
Dalkeith	0.8	0.9	0.8
New.	1.0	1.2	1.4

Appendix 14

The MIXED Procedure

Class Level Information

Class	Levels	Values
SITE	3	Bre Dal New
CLONE	11	1 104 108 17 33 46 50 6 68 76 92
REP	3	1 2 3

REML Estimation Iteration History

Iteration	Evaluations	Objective	Criterion
0	1	-138.1166647	
1	1	-138.1166647	0.00000000

Convergence criteria met.

Covariance Parameter Estimates (REML)

Cov Parm	Estimate
REP(SITE)	0.00000000
Residual	0.01372549

Model Fitting Information for NITROGEN

Description	Value
Observations	84.0000
Res Log Likelihood	22.1925
Akaike's Information Criterion	20.1925
Schwarz's Bayesian Criterion	18.2606
-2 Res Log Likelihood	-44.3849

Tests of Fixed Effects

Source	NDF	DDF	Type I F	Pr > F
SITE	2	5	32.47	0.0014
CLONE	10	46	2.87	0.0072
SITE*CLONE	20	46	1.71	0.0669

Tests of Fixed Effects

Source	NDF	DDF	Type III F	Pr > F
SITE	2	5	33.17	0.0013
CLONE	10	46	2.26	0.0301
SITE*CLONE	20	46	1.71	0.0669

Least Squares Means

Effect	SITE	LSMEAN	Std Error	DF	t	Pr > |t|
SITE	Bre	0.95757576	0.02085257	5	45.92	0.0001
SITE	Dal	0.91818182	0.02715363	5	33.81	0.0001
SITE	New	1.15757576	0.02085257	5	55.51	0.0001

Differences of Least Squares Means

Effect	SITE	_SITE	Difference	Std Error	DF	t
SITE	Bre	Dal	0.03939394	0.03423667	5	1.15
SITE	Bre	New	-0.20000000	0.02948998	5	-6.78
SITE	Dal	New	-0.23939394	0.03423667	5	-6.99

Differences of Least Squares Means

Pr > |t|	Adjustment	Adj P
0.3019	Tukey-Kramer	0.5279
0.0011	Tukey-Kramer	0.0025
0.0009	Tukey-Kramer	0.0022

Appendix 15

```
1 'PARTIAL' Variables:  BWT
4 'WITH'    Variables:  RESIN    PHENOL    CARBO    NITROGEN
1 'VAR'     Variables:  DAMAGE
```

Simple Statistics

Variable	N	Mean	Std Dev	Sum
BWT	64	0.109345	0.020988	6.998100
RESIN	64	54.795313	11.171796	3506.900000
PHENOL	64	52.507813	9.181353	3360.500000
CARBO	64	15.190625	3.297412	972.200000
NITROGEN	64	1.026563	0.160596	65.700000
DAMAGE	64	399.519531	245.615815	25569

Simple Statistics

Variable	Minimum	Maximum	Partial Variance	Partial Std Dev
BWT	0.071400	0.163600	.	.
RESIN	38.400000	86.900000	126.002485	11.225083
PHENOL	33.900000	75.400000	85.599036	9.251975
CARBO	9.200000	21.900000	10.484398	3.237962
NITROGEN	0.800000	1.400000	0.026094	0.161536
DAMAGE	12.620000	1058.920000	59401	243.722500

Pearson Partial Correlation Coefficients
/ Prob > |R| under Ho: Partial Rho=0 / N = 64

	DAMAGE
RESIN	-0.31510 0.0119
PHENOL	0.08121 0.5269
CARBO	-0.34381 0.0058
NITROGEN	-0.23323 0.0658

---------- SITE=Bre ----------

Correlation Analysis

1 'PARTIAL' Variables: BWT
4 'WITH' Variables: RESIN PHENOL CARBO NITROGEN
1 'VAR' Variables: DAMAGE

Simple Statistics

Variable	N	Mean	Std Dev	Sum
BWT	19	0.109453	0.016692	2.079600
RESIN	19	49.205263	8.168739	934.900000
PHENOL	19	57.284211	10.973162	1088.400000
CARBO	19	13.552632	1.591773	257.500000
NITROGEN	19	0.963158	0.121154	18.300000
DAMAGE	19	599.083684	273.182115	11383

Simple Statistics

Variable	Minimum	Maximum	Partial Variance	Partial Std Dev
BWT	0.071400	0.134300	.	.
RESIN	38.400000	68.700000	70.527131	8.398043
PHENOL	33.900000	75.400000	108.977510	10.439229
CARBO	11.200000	17.400000	2.230976	1.493645
NITROGEN	0.800000	1.200000	0.015514	0.124556
DAMAGE	51.040000	1058.920000	75970	275.627041

Pearson Partial Correlation Coefficients
/ Prob > |R| under Ho: Partial Rho=0 / N = 19

	DAMAGE
RESIN	-0.36703 0.1341
PHENOL	-0.21408 0.3937
CARBO	0.10360 0.6825
NITROGEN	0.05705 0.8221

---------- SITE=Dal ----------

Correlation Analysis

1 'PARTIAL' Variables: BWT
4 'WITH' Variables: RESIN PHENOL CARBO NITROGEN
1 'VAR' Variables: DAMAGE

Simple Statistics

Variable	N	Mean	Std Dev	Sum
BWT	18	0.110778	0.023189	1.994000
RESIN	18	53.438889	11.325772	961.900000
PHENOL	18	51.516667	9.295935	927.300000
CARBO	18	12.461111	1.926942	224.300000
NITROGEN	18	0.911111	0.090025	16.400000
DAMAGE	18	373.156111	217.009078	6716.810000

Simple Statistics

Variable	Minimum	Maximum	Partial Variance	Partial Std Dev
BWT	0.078500	0.152100	.	.
RESIN	39.400000	86.900000	134.923387	11.615653
PHENOL	35.000000	69.700000	89.586636	9.465022
CARBO	9.200000	16.300000	3.915053	1.978649
NITROGEN	0.800000	1.100000	0.008289	0.091044
DAMAGE	30.730000	941.880000	50036	223.687021

Pearson Partial Correlation Coefficients
/ Prob > |R| under Ho: Partial Rho=0 / N = 18

	DAMAGE
RESIN	-0.07110 0.7863
PHENOL	-0.13909 0.5945
CARBO	-0.38385 0.1282
NITROGEN	-0.34980 0.1687

---------- SITE=New ----------

Correlation Analysis

1 'PARTIAL' Variables: BWT
4 'WITH' Variables: RESIN PHENOL CARBO NITROGEN
1 'VAR' Variables: DAMAGE

Simple Statistics

Variable	N	Mean	Std Dev	Sum
BWT	27	0.108315	0.022811	2.924500
RESIN	27	59.633333	11.132765	1610.100000
PHENOL	27	49.807407	6.282633	1344.800000
CARBO	27	18.162963	2.422991	490.400000
NITROGEN	27	1.148148	0.139698	31.000000
DAMAGE	27	276.661111	135.869257	7469.850000

Simple Statistics

Variable	Minimum	Maximum	Partial Variance	Partial Std Dev
BWT	0.073900	0.163600	.	.
RESIN	45.000000	85.800000	123.508927	11.113457
PHENOL	38.800000	64.400000	40.505402	6.364385
CARBO	11.200000	21.900000	4.662382	2.159255
NITROGEN	0.900000	1.400000	0.019552	0.139828
DAMAGE	12.620000	587.010000	15003	122.487142

Pearson Partial Correlation Coefficients
/ Prob > |R| under Ho: Partial Rho=0 / N = 27

	DAMAGE
RESIN	0.06425 0.7552
PHENOL	-0.16320 0.4257
CARBO	0.07441 0.7179
NITROGEN	0.25844 0.2024