Declaration. I hereby declare that this thesis is the result of my own work. The work of others is acknowledged in the text where appropriate.

Patrick Rollo Heywood Hadfield

Cyanogenesis in Bracken (Pteridium aquilinum (L.) Kuhn)

Patrick Rollo Heywood Hadfield

PhD.

University of Edinburgh

1986

Table of Contents

Acknowledgements.

The work described in this thesis was undertaken whilst I was in receipt of an N.E.R.C./C.A.S.E. studentship.

The fieldwork would not have been possible without the permission and help of the following individuals and institutions: The School of Plant Biology, University College of North Wales, Bangor; The National Trust (for permission to work at Penmon Point); The Draper's Field Centre, Betws-y-Coed (Coed Hafod); The Department of Biology, University of Exeter; The Botany School, University of Cambridge; Mr. A.R. Gough and the Elvedon Estate (Lakenheath Warren); The Forestry Commission, Thetford (Brandon Park); The Department of Biology, University of York; The Yorkshire Wildlife Trust (Skipwith Common); Mr. N.W. Clark and the Wildlife Ranger Service, Haddington; East Lothian Regional Council and the Gosford Estate (Gosford Bay); Mr. J.I.McC. Salvesen (Black Hill); Mr. J.E. Hume and the Tyninghame Estate (Binning Wood); Mr. L.H. Wilson and the Shambellie Estate (Shambellie Wood); Mr. A. MacLeod and the West of Scotland Agricultural College, Oban; and the Factor, Blair Atholl Estate (Glen Garry and Clunies Wood).

I received advice from several individuals, including Mr. G.W. Kuroki, Drs. P.A. Crooks, M.A. Hughes, P. Maher, D. Mann, E. Sheffield, I. Till, and Professors E. Dahl, D.A. Jones and J.H. Lawton.

I am indebted to Professor D.M. Henderson, the Regius Keeper of the Royal Botanic Garden, Edinburgh, for his permission to perform much of the work at the Royal Botanic Garden, particularly providing greenhouse space, and for his encouragement in the project. I wish to thank all the staff at the Royal Botanic Garden, Edinburgh, for the interest they have shown in the project. In particular, I must thank Mr. R. Kerby and the horticultural staff, and Mr. M. Matthew and the staff of the Library, for the facilities they made available.

I wish to thank Professor M.M. Yeoman for allowing me to work at the Department of Botany, University of Edinburgh, and the members of the department for their help and companionship. In particular, I would like to thank Messrs. J. and R. McCluskey for their help in the propagation of material and maintaining the greenhouses, and Mrs. H. Quinn for her patient assistance in the Library.

Finally, I extend the greatest thanks to my supervisors, Dr. A.F. Dyer of the Department of Botany, University of Edinburgh, and Dr. C.N. Page, of the Royal Botanic Garden, Edinburgh, for the patience, advice, and boundless enthusiasm they have both provided during the project.

P.R.H.H.

Abbreviations.

Anon.	anonymous
(c)	coast
cm	centimetre(s)
°C	degrees celcius
E	einstein(s)
g	gramme(s)
(h)	heath
I.B.D.	index of bracken dominance
km	kilometre(s)
l	litre(s)
loc.	location
M	molar
m	metre(s)
max.	maximum
mg	milligramme(s)
min.	minimum
ml	millimetre(s)
mM	millimolar
mm	millimetre
µ	micro-
NADPH	nicotinamide adenosine dinucleotide phosphate^{+}
P	probability level
pH	minus log of hydrogen ion concentration
p.s.i.	pounds per square inch
r	correlation coefficient
R.B.G.	The Royal Botanic Garden, Edinburgh.
(scb)	scrub
S.E.	standard error
ssp.	subspecies
temp.	temperature
var.	variety
W	Watt(s)
(w)	wood
[]	concentration
%	per cent

The standard symbols for chemical elements are used throughout.

Abstract.

Cyanogenesis in bracken has been investigated in the field using the picrate test for HCN, and under controlled conditions using the pyridine - pyrazolone test for HCN.

Fieldwork in three habitat types (coastal, open heathland, and woodland) at each of nine locations throughout Britain showed that the amount of HCN released from frond tissues was affected by the habitat type in which the bracken was grown. Other environmental factors were also shown to influence cyanogenesis. It was found that the percentage of cyanogenic fronds and the amount of HCN they released rose rapidly at the start of the season, followed by a slower decline. Some fronds which were acyanogenic early in the season subsequently became cyanogenic.

There was little variation between different years in either the percentage of cyanogenic fronds* or the amount of HCN they release. However, considerable variation was found over small distances within a single habitat. Acyanogenic and cyanogenic fronds were found to be in very close association, and in some cases fronds of the two types were attached to the same rhizome. This showed that the differentiation between the two types was phenotypic and not genotypic. It was found that most fronds which were acyanogenic according to the picrate test released sufficient HCN to produce a positive result with the pyridine - pyrazolone method. Thus the qualitative polymorphism found in the field may be partly due to the sensitivity of the picrate test. Rhizomes transplanted from the wild into garden conditions produced both cyanogenic and acyanogenic fronds, emphasising the lack of genetic control over cyanogenesis.

Under controlled conditions, the effects of shading and nitrogen availability on cyanogenesis were studied separately. As shading was increased, so was the amount of HCN released from tissue samples. Similarly, as the level of nitrogen was increased, greater amounts of HCN were measured. Neither shading nor nitrogen availability had an effect on the amount of HCN released from β-glycoside extracts. There was a large amount of variation within replicates in both experiments. These results were interpreted as demonstrating an effect of the carbon:nitrogen balance on cyanogenesis.

Both gametophytes and juvenile sporophytes were found to be cyanogenic. There was considerable variation within clones in the amount of HCN released from samples.

It was concluded that cyanogenesis in bracken is a highly variable character. There was no evidence of genetic variation, whilst there was considerable evidence of phenotypic variation. Cyanogenesis was not a

* This statement must be reconsidered in the light of the chi-squared analysis of the data in Chapter 6. Please see page 171 and that following page 173.

suitable character with which to study the population biology of bracken.

Chapter 1. Introduction and Literature Review.

1.1. Introduction.

Most characteristics of plants can be variable, differing either continuously or discretely. The study of variation can be used as a tool to investigate the ecology and genetics of plants, these being linked together through selection (Briggs and Walters, 1984). In this way it is possible to study the population ecology and evolution of a species (Briggs and Walters, 1984; Harborne and Turner, 1984; Harper 1977).

This is particularly true of plant species which are polymorphic for secondary plant compounds (Jones, 1972a). Such chemicals do not have a primary function in the metabolism of a species. However, several non-essential functions for secondary chemicals have been proposed. The presence or absence of these chemicals may alter the fitness of the individual plant without being deleterious. It is therefore possible to observe the factors responsible for selecting for a particular trait, within individual plants, populations, varieties (races) and higher taxa.

Genetically polymorphic traits can be used within populations as markers by which particular genotypes can be identified (Briggs and Walters, 1984). This can be particularly useful in studying the population biology of a species, especially if the species forms clonal patches in which separate individuals may represent ramets derived from a single zygote or genets from separate zygotes.

Bracken, Pteridium aquilinum (L.) Kuhn, is one such species. Bracken is a noxious weed in Britain and several other parts of the world. It has an extensive underground rhizome system and forms long-lived populations covering large areas (Watt, 1976). The only aerial parts are the fronds. Although the morphological characteristics of fronds can be used in population studies of bracken (e.g. Oinonen, 1967a,b; but see also Hellum, 1968), bracken exhibits phenotypic variability and plasticity with respect to its morphology (Fletcher and Kirkwood, 1979; Page, 1976). The use of chemical markers to identify fronds belonging to individual plants (genets) is therefore preferable. The population biology of bracken has not been studied systematically, despite the importance of the plant as an invasive weed of marginal agricultural land in several parts of the world (Fletcher and Kirkwood, 1979; Page, 1976; Taylor, 1980). Effective control methods rely on an understanding of the ecology and population biology of a species. This information is missing from our

knowledge and therefore control methods may not be as efficient as possible.

Bracken is cyanogenic, releasing HCN from frond tissues when these are damaged. It has also been shown to be polymorphic for the character (Cooper-Driver et al, 1977; Lawton, 1976). In many other species, polymorphism for cyanogenesis is genetically controlled and thus the character can be used to identify individual genotypes. Cyanogenesis has been used to study the population biology of Trifolium repens L. and Lotus corniculatus L. (sections 1.2.2.6.1 and 1.2.2.6.2). HCN can be easily detected under field conditions, which allows large numbers of samples to be taken and analysed (section 2.3). Cyanogenesis would therefore appear to be a suitable characteristic to use in population studies of bracken.

The purpose of this study was to assess the value of cyanogenesis as a characteristic with which to study the population biology of bracken in a systematic fashion (section 1.3).

1.2. Literature Review.

There has been considerable research into the biology of bracken. Similarly, there has been a lot of work on the genetics, ecology and function of secondary plant compounds in general and cyanogenesis in particular. However, the overlap of these two subjects, namely the role of cyanogenesis in bracken, has rarely been investigated. The background literature to each branch of this research is therefore reviewed separately.

1.2.1. The Biology of Bracken.

There have been several reviews of the literature related to bracken (Braid, 1959; Fletcher and Kirkwood, 1979), and there is a large amount of literature available. In particular, attention is drawn to proceedings of three recent symposia, on "The Biology of Bracken" (Perring and Gardner, 1976), "Bracken in Scotland" (Fletcher and Kirkwood, 1982), and "Bracken: Ecology, land use and control technology" (Smith and Taylor, 1986).

1.2.1.1. The taxonomy of bracken.

Bracken is considered to be one of the five most common plant species (Coquillat, 1951, in Harper, 1977). It is a cosmopolitan species and it can be found on every continent except Antarctica (Tryon, 1941; Page, 1976). The taxonomy of Pteridium aquilinum is therefore complex since it requires the examination of specimens from throughout its worldwide range. The taxonomy of bracken was last revised by Tryon (1941); the taxonomy is critical and it is

possible that a further revision is necessary.

Tryon (1941) recognised twelve varieties of Pteridium which he placed in two subspecies. The two subspecies, P. aquilinum aquilinum and P. aquilinum caudatum, contain eight and four varieties respectively. In general, ssp. aquilinum is limited to the northern hemisphere and ssp. caudatum to the southern hemisphere (Page, 1976). However, ssp. caudatum is absent from Africa, with ssp. aquilinum throughout the continent in its place. The two subspecies overlap in central America and in south east Asia, although it is thought that they are ecologically differentiated (Holttum, 1968; Page, 1976).

The varieties of each subspecies overlap considerably (Page, 1976). There are four varieties present in North America and two are found in Europe. There are authenticated specimens of only one variety, ssp. aquilinum var. aquilinum, in Britain, although it is possible that the other European variety, var. latiusculum was extant at one time. Variety latiusculum has a range that extends from western North America through Europe to Japan (Page, 1976), and it is surprising that the variety is not found in the west of Europe (including Britain). Britain is close to the northerly limit of var. aquilinum (Page, 1976; Tryon, 1941) and it is possible that this significantly affects the ecology of the plant, in particular its reproduction.

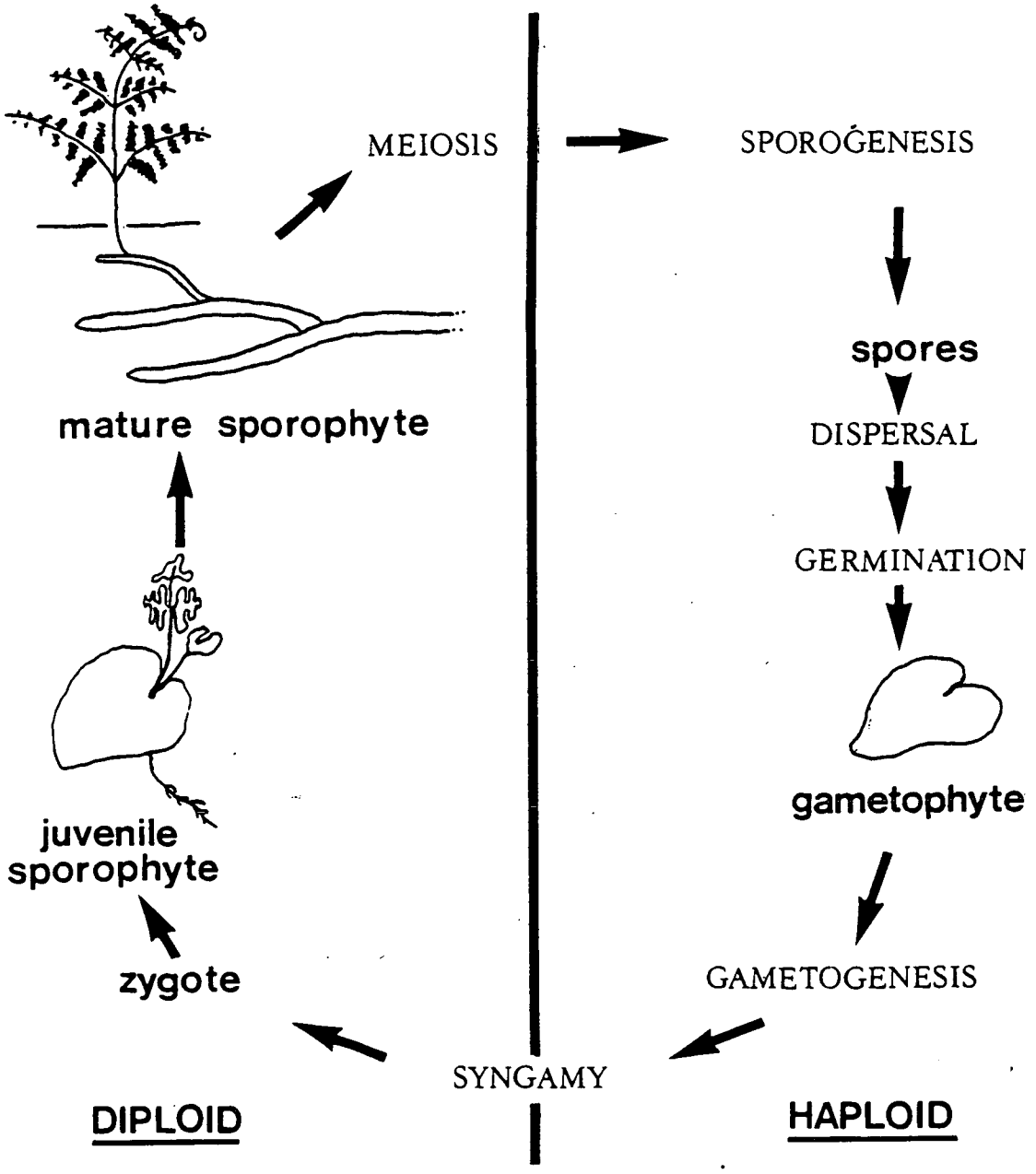
There is no evidence of phytochemical differences between the subspecies or varieties of Pteridium aquilinum (Cooper-Driver, 1976). All the varieties have been shown to have a chromosome number of n=52 or 2n=104 (Page, 1976), although there is also a chromosome count of 2n=208 for var. arachnoideum from the Galapogos Islands (Jarrett et al, 1968; Page, 1976). There is also a count of 2n=52 for a putative species, Pteridium herediae, from Spain (Love and Kjellquist, 1972) although further investigations of similar material from the same area have failed to confirm this (Dr. E. Sheffield, personal communication).

1.2.1.2. The life cycle of bracken.

Pteridium aquilinum has a life cycle typical of homosporous ferns (Sporne, 1975; Fletcher and Kirkwood, 1979) (Figure 1.1). This differs from the life cycle of spermatophytes in several important respects: the gametophyte generation is independent of the sporophyte generation; seeds are not produced; and the main form of dispersal is haploid and forms part of the gametophyte rather than the sporophyte generation (Sporne, 1975; Bell, 1985).

The spores are produced by meiosis (Bell, 1979) within sporangia borne on the mature sporophyte fronds (Conway, 1957). Spore germination is rapid in vitro (Conway, 1949; Schwabe, 1951; Bell, 1958), and is probably as fast under

Figure 1.1. The life cycle of Pteridium aquilinum. The two generations of the life cycle are separated by the bold vertical line. Biological processes (e.g. meiosis) are shown in light upper case characters. Stages in the life cycle (e.g. gametophyte) are shown in bold lower case characters.



natural conditions (Conway, 1949).

The gametophytes can develop quickly, growing into the typical cordate form after 18 - 19 days (Conway, 1949). Gametangia may be apparent after 33 days (Conway, 1949). There is little evidence for self-incompatibility mechanisms within bracken gametophytes (Klekowski, 1972, 1979), although such systems have been reported (Wilkie, 1956). The male gametes (spermatozoids or antherozoids) are produced within antheridia and are motile whilst the female gametes (eggs) remain within the archegonia (Bell and Duckett, 1976). The male gametes are released from the antheridia and are attracted to the archegonia, presumably by chemotaxis, and syngamy results in the formation of a diploid, sporophyte embryo (Bell and Duckett, 1976).

The juvenile sporophyte, or sporeling, develops quickly. The first juvenile frond arises simultaneously with the first roots after approximately one week, before the stem apex is distinct (Gottlieb, 1958). The apex develops an upright rhizome, producing true roots; the initial growth is therefore radial (Gottlieb, 1958; Dasayanake, 1960). The upright rhizome produces eight fronds and then the apex broadens and divides (Gottlieb, 1958; Dasayanake, 1960). The division of the rhizome represents a pseudodichotomy (Dasayanake, 1960) and results in two or more vertical rhizomes (Gottlieb, 1958; Dasayanake, 1960).

The vertical rhizomes grow in opposite directions and grow into the soil (Gottlieb, 1958). They rapidly produce several fronds (Braid and Conway, 1943). The rhizomes branch and can occupy a large area within two seasons growth (Braid and Conway, 1943). After one year from syngamy, the juvenile plant may be indistinguishable from an established plant (Braid and Conway, 1943). The fronds can produce spores within two seasons of growth (Conway, 1949).

1.2.1.3. The morphology of the mature sporophyte.

The mature sporophyte consists of a subterranean rhizome system which produces aerial fronds (Bower, 1928). The rhizome system consists of two types of rhizome. The main rhizome axes are known as long shoots (Watt, 1940). These are dorsiventral and branching by pseudodichotomy (Dasayanake, 1960). Adventitious roots arise radially from the rhizome (Gottlieb, 1958). The long shoot rhizomes are large and do not directly bear fronds (Watt, 1943; Webster and Steeves, 1958; Gottlieb and Steeves, 1961). The long shoots grow rapidly, at between 1 - 3 feet (0.3 - 1m) per year for var. aquilinum (Watt, 1940), and 10 - 17cm per season for var. latiusculum (Webster and Steeves, 1958). Juvenile plants apparently grow faster, in excess of 100cm (Gottlieb, 1958) and 6 feet (approximately 2m) (Braid and Conway, 1943) per season. The

long shoots branch at intervals of 7 - 8 feet (Watt, 1940), approximately 2.3 - 2.6m. The long shoot rhizomes can be found deep in the soil, to depths of 1m in var. aquilinum (Watt, 1940), 40cm (Hellum, 1968) or 60cm (Webster and Steeves, 1958) for var. latiusculum, and between 50cm - 100cm in var. esculentum (O'Brien, 1963).

Fronds are produced by the second rhizome type, commonly called short shoots (Watt, 1940). Short shoots are found closer to the soil surface, at depths of more than 5cm (Watt, 1940; O'Brien, 1963; Hellum, 1968). The short shoots are small and do not grow as quickly as long shoots (Watt, 1940). Short shoots do not branch (Watt, 1940; Webster and Steeves, 1958; O'Brien, 1963). Fronds are produced at intervals of 0.5 - 2cm (O'Brien, 1963; Webster and Steeves, 1958). Short shoots generally produce only a single frond in each season (Watt, 1940; Webster and Steeves, 1958), although the short shoots of juvenile sporophytes can produce several more (Braid and Conway, 1943). Fronds on mature short shoot rhizomes are initiated in the season prior to their emergence (O'Brien, 1963; Webster and Steeves, 1958). Short shoots can remain dormant within the soil for considerable periods (O'Brien, 1963; Watt, 1940, 1976). There are a large number of undeveloped frond buds within an area of soil. Watt (1950) estimated that as many as 80% frond buds were not emergent.

The differentiation between long shoots and short shoots has been the subject of some controversy. A strict dimorphism has been claimed, although intermediate shoots, bearing fronds at 10cm intervals have been recognised (Watt, 1940; Gottlieb, 1958; Gottlieb and Steeves, 1961; Webster and Steeves, 1958). This view has been countered by Dasayanake (1960) and O'Brien (1963) who recognised gradations between shoot types. In particular, both authors maintained that the long shoots can produce fronds, albeit rarely.

The morphology of the frond is very variable, dependent largely on the local environmental conditions (Boodle, 1904; Hellum, 1968; O'Brien, 1963; Watt, 1943). Oinonen (1967a) asserted that the frond morphology was constant enough for several frond characteristics to be used as markers to identify individual genotypes within a stand of fronds. However, the identification of morphological markers suitable for recognising individual clones has not been repeatable (Hellum, 1968) due to the large degree of phenotypic variation between fronds of the same genotype.

Bracken fronds are tripinnately divided (Page, 1982c) and commonly produce 13 to 20 pairs of pinnae, each with 30 to 35 pairs of pinnules (Conway, 1957). At the base of each pinna and, on the large pinnae, each pinnule are

located nectaries (Darwin, 1877; Lawton, 1976; Page, 1982b; Tryon, 1941). The fronds are variable in height above soil level from 0.25m to 3m (O'Brien, 1963; Oinonen, 1967a; Watt, 1943, 1945). The height of the frond above soil level is in part dependent on the depth of the short shoot rhizome in the soil (Watt, 1943) and the age of the the short shoot, as estimated by the number of previous frond buds produced (Watt, 1945).

1.2.1.4. The ecology of the gametophyte generation and the juvenile sporophyte.

Spores are produced and released from mature fronds in late summer in Britain (Conway, 1957; Page, 1976; Watt, 1976). The release of spores is considerably earlier for var. latiusculum in North America, occurring only six weeks after frond emergence in May (Webster and Steeves, 1958). The number of spores released by fronds of var. aquilinum is very high, with an estimated 3 X 10^8 spores produced by a highly fertile frond (Conway, 1957). In Britain spore production is decreased by shading (Boodle, 1904; Conway, 1957; Dring, 1965). In contrast, spore production by var. latiusculum in North America is increased by shade (Webster and Steeves, 1958). It is possible that under tropical conditions there may be continual, or an extended period of, spore production (Gliessman, 1978).

Germination of spores is apparently rare in the field in Britain and does not occur within a stand of bracken fronds (Conway, 1957). There are few reports of sporeling establishment under natural conditions in Britain, and only then under conditions of low competitive stress (Conway, 1949; Farrow, 1915; Melville, 1965). Sporeling plants have been found in a variety of man-made habitats in Britain, particularly in building sites and bombsites (Lousley, 1939, 1944). Gametophytes and juvenile sporophytes are reputedly common in New Zealand (Moore, 1942; Knowles, 1970) and in Costa Rica (Gliessman, 1978). Bracken is often one of the first vascular plants to invade islands and volcanic lava beds (Page, 1976). It would appear that all these habitats are either areas of low competitive stress or which have been subjected to fire (Conway, 1949, 1953; Gliessman, 1978; Knowles, 1970; Page, 1976).

The gametophyte and the juvenile sporophyte of a fern are the establishment phase and as such are subject to intense selection, as is the case for angiosperm seedlings (Harper, 1982). Conway (1949, 1953) and Conway and Stevens (1957) investigated the establishment and survival of gametophytes and sporelings. Spores were subject to predation by soil collembola. It was found that there was a requirement for a lack of competition, and for a lack of soil fungi for the germination and development

of the gametophyte. Gametophytes were susceptible to fungal attack, and this was thought to be a limiting factor. The gametophytes were tolerant of a wide range of substrate pH, although soil pH from 5 - 7.7 was favoured. Gametophytes were sensitive to desiccation. The sporeling plants were susceptible to frosts, and it is possible that the dispersal of spores in late summer in Britain may limit the period between sporophyte establishment and the earliest frosts. Following frost, the juvenile fronds collapsed and were attacked by fungi; similar aged sporelings of Dryopteris filix-mas remained healthy (Conway, 1953).

1.2.1.5. The ecology of the mature sporophyte generation.

The ecology of the mature sporophyte of Pteridium aquilinum differs greatly from the ecology of the gametophyte and the sporeling. However, the location of established sporophytes is dictated by the ecology of the gametophyte. It is possible that the conditions necessary for establishment of bracken are different to those prevalent when bracken has been growing on a site for a long period.

The ecology of bracken has been extensively investigated, particularly in the east of Britain (Watt, 1976). It has a very wide ecological range, and is found in several different habitats (Fletcher and Kirkwood, 1979; Page, 1976; Watt, 1976).

Once established, the plant spreads over an area by rapid growth of its rhizome system (Braid and Conway, 1943; Watt, 1947). The rhizome dies back and rots as it grows forward, thus isolating long shoot branches which were previously connected (Watt, 1940, 1947, 1976). This results in the long shoot rhizomes becoming independent, as co-existing ramets, and may be considered to be a form of vegetative reproduction.

The number of fronds produced per unit area, known as the frond density, is variable, depending on the habitat in which the bracken is growing (Hellum, 1968). Mitchell (1977) gave the density of fronds for heathland sites in Britain as up to 100 fronds m^{-2}. Callaghan et al (1981) found up to 60 fronds m^{-2} in north west England. In contrast, O'Brien (1963) found only 1 frond per 1 foot sq. (approximately 10 fronds m^{-2}) in Australia, and Hellum (1968) found only 7.7 fronds m^{-2} in north east America. Watt (1943, 1947) recognized different regions within a heathland habitat, which he interpreted as different phases of invasion and decline of the bracken, the overall structure being a mosaic of different phases. The frond density at any spot was determined by the phase there (Watt, 1943, 1945). Within an area dominated by bracken, there will be

competition between rhizomes for nutrients and water, and between fronds for light (Watt, 1945).

Watt (1950) emphasised the role of frost in determining the ecology of bracken, particularly in heathland. Both the frond buds and the rhizome buds are susceptible to damage by frost, with as many as 70% of growing frond buds being killed by late frosts. Damaged or dead frond buds can be replaced by dormant buds. The effect of frost on frond buds was shown to be dependent on the depth of the buds within the soil, itself dependent on the age of the short shoots.

Wind was also felt to be an important factor in determining the ecology of bracken, excessive exposure to wind possibly leading to mechanical and physiological damage due to the loss of water (Watt, 1976). Fronds produce large numbers of adventitious roots between the junction with the rhizome and the soil surface. These roots are conspicuous in var. esculentum in Australia and are believed to overcome problems of water stress in Australia by allowing the frond to take advantage of light showers, the water of which may not permeate to the depth of the roots attached to the rhizome (O'Brien, 1963).

Bracken is intolerant of particularly wet conditions, which is thought to be due to the lack of soil oxygen reaching the rhizomes (Poel, 1951, 1961). It has been suggested that the retention of dead frond stipes, rather than their abscission, may provide passage into the soil for oxygen (Anderson, 1961).

Bracken generally grows on acidic and nutrient poor soils (Mitchell, 1977; Watt, 1976). It is not however calcifuge (Braid, 1947, 1959; Conway and Stevens, 1957; De Silva, 1932; Molesworth-Allen, 1968), and its occurrence on nutrient poor soils may reflect previous land use management rather than habitat preference (Watt, 1976). Bracken can produce large amounts of litter (Callaghan et al, 1981; Chen and Lindley, 1981; Frankland, 1976), the decomposition of which can release acidic compounds into the soil (Whitehead, 1964; Whitehead et al, 1982). Mitchell (1977) has shown that the presence of bracken can alter the soil composition, perhaps by its breakdown products.

Light intensity has been shown to have a large effect on bracken. It has been suggested that despite the low light intensity, woodland habitats were favourable to bracken, by minimising temperature variation and water loss, and by increasing nutrient supply (Fletcher and Kirkwood, 1979; Watt, 1976). Boodle (1904) showed that shading affected the frond morphology, a single frond producing both shade and light phenotypes in different pinnae. Shaded fronds are taller, hairless and thinner than exposed fronds (Boodle, 1904; Cook et al, 1979). The relationship between frond height and light intensity was

investigated by Burke (1953), increasing shade reducing frond height. The cuticles of shaded fronds are less well developed, being half the thickness of the cuticles of exposed fronds (Cook et al, 1979). Shaded fronds possess less than half the number of stomata of exposed fronds (Cook et al, 1979).

1.2.1.6. Control of bracken.

Bracken is a noxious weed which is aggressive and invasive (Taylor, 1980). In Britain it occupies large areas of marginal farmland, reducing rough pasture available for grazing (Fletcher and Kirkwood, 1979; MacLeod, 1982; Page, 1982a). Bracken is toxic to livestock which might feed on fronds or rhizomes (Evans, I.A., 1976; Evans, W.C., 1976; Evans et al, 1982) and may represent a danger to man (Taylor, 1980). The spread of bracken probably reflects changes in land management and the decline in the use of bracken within the rural economy (Page, 1982a; Rymer, 1976; Watt, 1976). Because of this spread there has been a large amount of research into control methods (Braid, 1947, 1959; Fletcher and Kirkwood, 1979). When there is available manpower, stands of bracken fronds can be regularly cut which leads to degeneration of the plant within six years (Braid, 1947). This was a suitable method of control when the cut fronds could be put to use as thatch or bedding (Rymer, 1976), but it is uneconomic under current land use. It is possible that the development of biomass as an energy source could result in the mechanical harvesting of bracken (Callaghan et al, 1981a,b).

Bracken can be controlled by deep ploughing, but the land most heavily infested by bracken is often inaccessible and ploughing is difficult (Fletcher and Kirkwood, 1979).

There have been extensive trials with a variety of herbicides (Fletcher and Kirkwood, 1979). The most successful herbicide appears to be 'asulam' (Veerasekaran et al, 1976; Fletcher and Kirkwood, 1979; Kirkwood and Hinshalwood, 1985). The herbicide must be applied at the correct time in the season (Fletcher and Kirkwood, 1979) and application often requires aerial spraying by helicopter. The expense of treating sizeable areas of infested land can be prohibitive (Taylor, 1980). Without suitable after treatment, including reseeding with pasture grasses, bracken can regrow in an area following herbicide treatment (Lee et al, 1982; Martin, 1976).

Research is currently underway to assess the viability of biological control methods. There have been studies on the effect of pathogenic fungi on bracken (Burge and Irvine, 1985) and on the use of foreign insects which feed specifically on bracken (Lawton and MacGarvin, 1985).

1.2.1.7. Interactions between bracken and insects.

It has been stated that ferns in general have comparatively few insect herbivores associated with them (Ehrlich and Raven, 1964; Swain and Cooper-Driver, 1973). This view has been challenged and shown to be spurious in a variety of climates (Balick et al, 1978; Gerson, 1979; Hendrix and Maquis, 1983; Ottoson and Anderson, 1983). The insect fauna associated with bracken has been closely examined in several studies (Kirk, 1977, 1982; Lawton, 1976, 1982, 1984; Lawton and MacGarvin, 1985; Schreiner, 1980). These show that bracken is utilised as a food source by a variety of insects. Lawton and MacGarvin (1985) have shown that the number of insect species feeding on bracken is related to the area covered by bracken in any particular region. The number of each insect on each frond may be low (Lawton, 1976), possibly due to the low nutrient quality of bracken (Lawton and MacGarvin, 1985). This may be partly due to the presence of several potentially harmful phytochemicals (see section 1.2.1.8). The phytochemicals identified from bracken and other ferns can be considered primitive in contrast to the angiosperms (Swain and Cooper-Driver, 1973).

Unlike angiosperms, pteridophytes rarely form stable mutual relationships with insects. There is less potential for mutually beneficial coevolution of insects with the pteridophytes since insects apparently rarely if ever play a role in the dispersal or reproduction of pteridophytes. The only insects that appear to have formed symbiotic relationships with ferns are ants (e.g. Jermy and Walker, 1974; Wagner, 1972; Yapp, 1902). It has been suggested that the presence of nectaries on bracken fronds may attract ants, which might serve to remove or deter insects or other herbivores from the fronds (Darwin, 1877; Lawton, 1976; Page, 1982b; Tryon, 1941). This hypothesis has been investigated in temperate regions in Britain and America and it was found that there was no relationship between the presence of ants on bracken and the level of herbivory (Heads and Lawton, 1984; Lawton and Heads, 1984; Tempel, 1983). It is possible that under different climatic conditions, or with different species of ants, that ants do deter herbivores from bracken (Heads and Lawton, 1984).

1.2.1.8. The chemical ecology of bracken.

Bracken produces a wide variety of secondary plant chemicals, which have various putative functions (Cooper-Driver, 1976; Jones, 1983). Bracken is toxic to animal livestock (Evans, I.A., 1976; Evans, W.C., 1976; Fletcher and Kirkwood, 1979) and it has been suggested that secondary plant compounds may function in general as allelochemics, particularly as herbivore deterrents (Beck and

Reese, 1976; Feeny, 1975; Mattson, 1980; Rhoades, 1983, 1985; Whittaker and Feeny, 1971).

The phytochemicals within bracken vary both genetically and phenotypically (Jones, 1983), although there is no evidence that there are consistent differences between different varieties of bracken (Cooper-Driver, 1976). Variation of particular secondary chemicals in bracken can be both spatial and temporal (Jones, 1983).

Many of the secondary chemicals found in bracken are polymers of phenolic acids. Bracken contains several different, closely related phenolic compounds (Cooper-Driver, 1976). It has been suggested that the phenolic compounds found in bracken may act as herbivore deterrents (Jones, 1983). There is also evidence that phenolic acids are released from bracken (Whitehead, 1964). Bracken has been shown to be allelopathic, releasing phytotoxins which help to maintain the dominance of the plant in both field and laboratory studies (Glass, 1976; Gliessman, 1976; Gliessman and Muller, 1972, 1978; Stewart, 1975). The toxins involved are water soluble, and are released by either living or dead fronds (Gliessman 1976; Gliessman and Muller, 1978). Glass (1976) has shown in laboratory studies that phenolics are produced by bracken at concentrations which are detrimental to other species. However, Whitehead et al (1982) conclude that the levels of phenolic compounds released by bracken are insufficient to affect other species. It is possible that the inability of bracken spores to germinate and of gametophytes to grow within an established bracken stand (Conway, 1957) is due to an allelopathic effect of the mature fronds on the spores or gametophytes. Similar effects are known from other ferns (Davidonis and Ruddat, 1973, 1974).

Bracken fronds contain several flavonols, complex polycyclic compounds derived by polymerisation of phenolic compounds (Cooper-Driver, 1976; Jones, 1983; Swain and Cooper-Driver, 1973). The levels of flavonols vary slightly throughout the season (Cooper-Driver et al, 1977), although the significance of this is unknown. Bracken fronds growing in the open produce higher levels of flavonols than fronds growing in the shade, suggesting that the level of available photosynthate affects the synthesis of these compounds (Cooper-Driver et al, 1977). The flavonols isolated from bracken have been shown to have a variety of effects on insect herbivores, including both feeding stimulation and inhibition of specific insects (Jones, 1983; Jones and Firn, 1979).

The flavonols can be polymerised to form condensed tannins (Cooper-Driver, 1976; Harbourne and Turner, 1984; Jones, 1983). Bracken

contains large quantities of tannins within the fronds (Cooper-Driver, 1976; Cooper-Driver et al, 1977; Tempel, 1981). Like the flavonols, the levels of tannin vary seasonally, increasing during the season (Cooper-Driver et al, 1977; Lawton, 1976). The levels of tannins are higher in open than in shaded fronds (Cooper-Driver et al, 1977). Other environmental effects, such as water stress, are also likely to affect tannin levels in bracken (Tempel, 1981; Jones, 1983). Tannins are believed to function as herbivore deterrents by forming complexes with proteins, both reducing the available protein within a plant and reducing the digestibility of the plant by complexing with the herbivores digestive enzymes (Bate-Smith, 1972; Goldstein and Swain, 1965). Tempel (1981) found no correlation between tannin levels and herbivory in bracken.

Bracken also contains five homologues of insect moulting hormones (phytoecdysteroids) in both fronds and rhizomes (Jones, 1983). These compounds appear to have no specific function within bracken (Jones, 1983), and it has been hypothesised that they act as deterrents to insect herbivores. However, there is no evidence that this is the case (Jones and Firn, 1978). The levels of phytoecdysteroids in fronds rise rapidly in August in Britain (Jones and Firn, 1978). Rhizomes contain larger amounts of phytoecdysteroids than fronds, and it is possible that the function of these chemicals is to protect the rhizomes rather than the fronds (Jones, 1983).

Bracken has been found to contain a total of 29 pterosin sesquiterpene compounds, showing considerable structural variation (Jones, 1983). Jones and Firn (1979) have shown that at least one of these compounds functions to deter several insect herbivores, although at the levels present in bracken it was only deterrent to two insect species. It is possible that these compounds work in concert.

The role of secondary compounds in bracken is uncertain. Despite a large amount of research into their various functions, there is no definitive account of their biological or ecological importance. Indeed, several secondary compounds which might be expected to serve a specific function do not act as expected. For example, the lack of biological activity of phytoecdysones indicates that these compounds are functionless. As with the production of nectar by bracken (section 1.2.1.7), this could be because the secondary compounds are not active under the prevailing conditions of the experiments. Under other environmental conditions, functions may become apparent. It is also possible that secondary compounds interact with each other, so that a specific function for a single compound in isolation could remain obscure. Freeland et al (1985) have shown that the action of tannins and saponins

together may be greater than the combined effect of each class of compounds separately. Given the large number of secondary compounds in bracken, it might be expected that some phytochemicals function only in combination with others.

1.2.2. The Biology of Cyanogenesis.

Cyanogenesis is the production of HCN by biological material (Hegnauer, 1977). Cyanogenesis is common in vascular plants and fungi (Hegnauer, 1977) and in certain arthropod groups (Duffy, 1981). In vascular plants, HCN is released by the enzymatic hydrolysis of cyanogenic glycosides or, rarely, lipids (Siegler, 1981).

1.2.2.1. The structure, biosynthesis, and chemistry of cyanogenic glycosides.

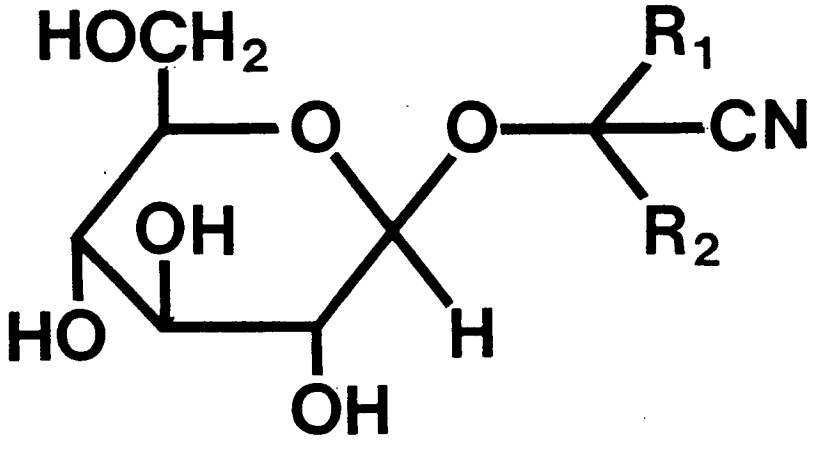
Cyanogenic glycosides consist of a cyanogenic component, commonly an α-hydroxynitrile, linked to a sugar moiety by a β-glycosidic bond (Conn, 1981b; Siegler, 1981). Cyanogenic glycosides can be classified according to the chemical nature of the hyroxynitrile moiety (Eyjolfsson, 1970) or according to their precursor in biosynthesis (Conn, 1981b, 1982; Hegnauer, 1977). Classification according to the precursor allows cyanogenic glycosides to be used in chemotaxonomy (Hegnauer, 1977), and is therefore preferable to a strictly structural treatment.

The structure of a standardised cyanogenic glycoside is shown in Figure 1.2. The nature of R_1 and R_2 will differ according to the particular glycoside. The sugar moiety may also differ. In most of the known cyanogenic glycosides, the sugar group is D-glucose, as shown in Figure 1.2, but in five cyanogenic glycosides this is replaced by a disaccharide (Conn, 1981b).

Cyanogenic glycosides are commonly named after the plant from which they were first isolated. The structure of the aglycone of a cyanogenic glycoside mirrors that of the amino acid from which it was derived (Conn, 1981b; Dewick, 1984; Hegnauer, 1977; Seigler, 1981).

All of the 25 known cyanogenic glycosides are believed to be derived from only six amino acids (Hegnauer, 1977). Some aromatic cyanogenic glycosides (including prunasin, sambunigrin, amygdalin, vicianin, lucumin, holocalin, and zierin) are synthesised from phenylalanine and others (taxiphyllin, dhurrin, and proteacin) from tyrosine. Alkyl cyanogenic glycosides are synthesised from valine (for linamarin), isoleucine (for lotaustralin), and leucine (for acacipetalin, dihydroacacipetalin and cardiospermin). In addition, the non-protein amino acid cyclo-pentyl-glycine is believed to be the precursor to three uncommon

Figure 1.2. The general structure of cyanogenic glycosides. All cyanogenic glycosides are based on this structure. The side groups R_1 and R_2 may be aliphatic or aromatic substituents, or hydrogen. The nature of R_1 and R_2 depends on the amino acid from which a particular glycoside is synthesised. In five glycosides the sugar moiety is a disaccharide.



cyanogenic glycosides, barterin, deidaclin and gynocardin (Conn, 1981b; Dewick, 1984; Hegnauer, 1977; Seigler, 1982).

The synthetic pathways are precisely known for few cyanogenic glycosides and in a limited number of plant species, but the steps are believed to be the same for all the known glycosides for all cyanogenic species (Conn, 1981a; Dewick, 1984). Much of the information about the biosynthesis of cyanogenic glycosides has been obtained by using radio-labelled amino acids and likely intermediates (Conn, 1981a). Enzymatic studies have identified several of the enzymes involved in the synthesis (Conn, 1981a). The enzymes in Sorghum species are contained within microsomes, organised so that the product of one enzyme is channelled to the next enzyme in the biosynthetic sequence (Conn, 1981a). The intermediates are not released between steps. This increases the efficiency of the biosynthetic process and is thought to be the cause of the failure to isolate several intermediates (Conn, 1981b).

Within any single species of plant, only cyanogenic glycosides derived from a single biosynthetic pathway will occur (Conn, 1981a). Apparently without exception, the glycosides linamarin (synthesised from valine) and lotaustralin (synthesised from isoleucine) are found together in the same plant (Conn, 1981b). This is thought to be because the same biosynthetic enzymes can act on both amino acids, which are homologues. However, the relative quantities of the two glycosides may differ. Amygdalin and prunasin, both synthesised from phenylalanine, are also often found in the same species, although each glycoside may be limited to particular organs (e.g. prunasin in the leaves and amygdalin in the seeds of Prunus species). Several cyanogenic glycosides are epimers (e.g. prunasin and sambunigrin; Hegnauer, 1977). Epimers never coexist within a single species, although treatment of the isolated glycosides can cause epimerisation and lead to misinterpretation of the results of extraction of glycosides (Eyjolfsson, 1970).

Cyanogenic glycosides are stable compounds under normal conditions (Conn, 1981b). Under treatment with dilute acids at elevated temperatures (in excess of 60°C) the β-glycosidic bond can be cleaved, producing the sugar moiety and the aglycone hydroxynitrile, the latter being unstable and dissociating into HCN and the aldehyde or ketone (Eyjolfsson, 1970; Nahrstedt, 1981). Upon treatment with concentrated acids, cyanogenic glycosides yield NH_3 and the corresponding hydroxyacid (Uribe and Conn, 1966). Treatment with dilute alkalies can hydrolyse the nitrile group to form the corresponding glycosidic acid (Conn, 1981b).

1.2.2.2. The release of HCN under natural conditions.

HCN is released from cyanogenic glycosides following hydrolysis by β-glycosidases (Hosel, 1981; Figure 1.3). The β-glycosidase cleaves the β-glycosidic bond, producing the free sugar moiety and the α-hydroxynitrile, or cyanohydrin, group. The hydroxynitrile is unstable and can spontaneously breakdown to release HCN and the corresponding aldehyde or ketone (Eyjolfsson, 1970). Certain species contain α-hydroxynitrilases which catalyse the release of HCN from the hydroxynitrile (Conn, 1981b). There is no evidence for an α-hydroxynitrilase in Trifolium repens (Hughes, 1981).

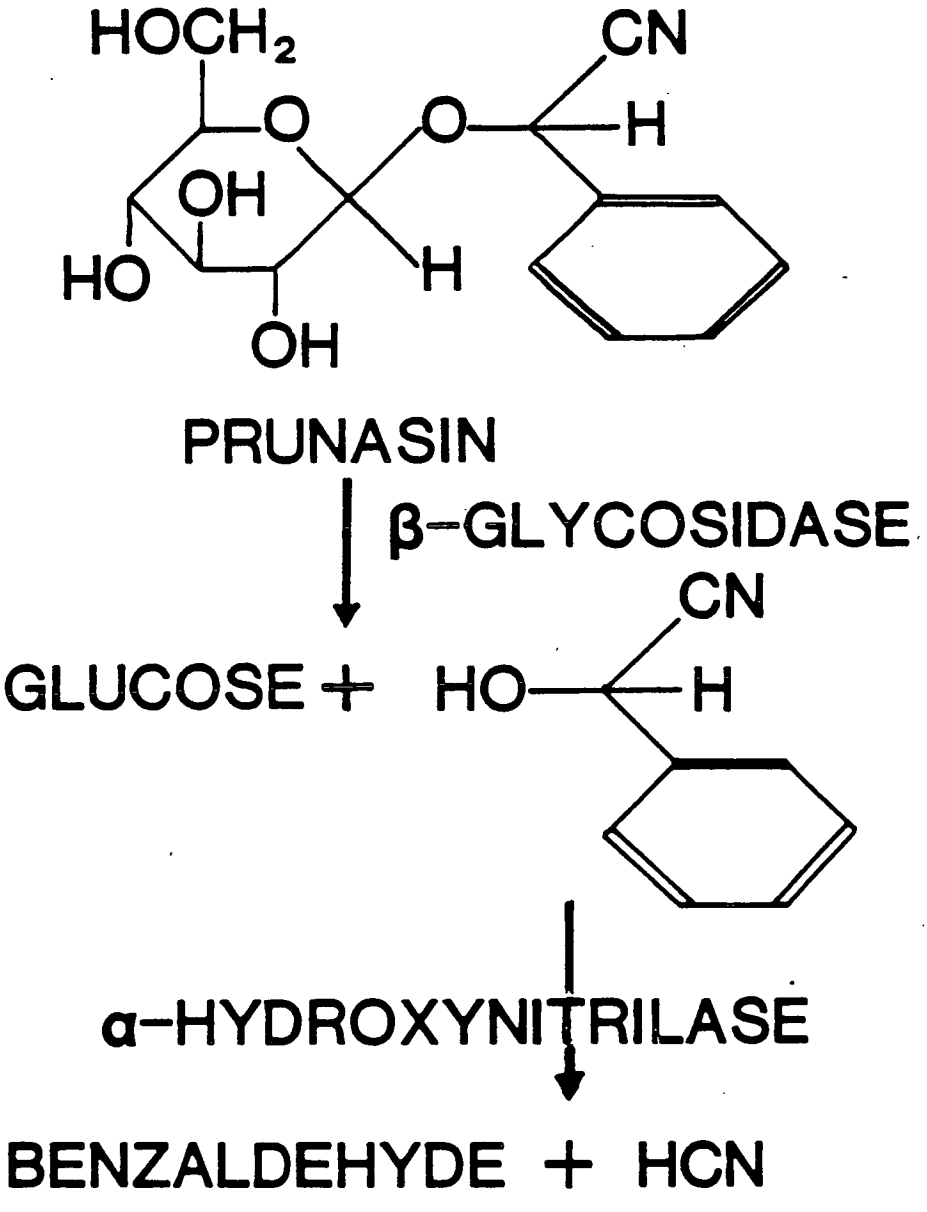
The β-glycosidase found in a particular species is specific to the cyanogenic glycoside present (Hosel, 1981). However, β-glycosidases specific for a particular glycoside maintain their activity for that glycoside regardless of its origin (Hosel, 1981). Thus although linamarase has a low activity to prunasin, it will hydrolyse linamarin from any plant source.

Those cyanogenic glycosides that possess a disaccharide as the sugar moiety possess two β-glycosidases, one of which cleaves the first β-glycosidic bond to release a monosaccharide sugar and the homologous cyanogenic glycoside. This is then hydrolysed by the second β-glycosidase to release the second monosaccharide sugar and the α-hydroxynitrile (Hosel, 1981). Thus amygdalin in Prunus species is first metabolised to its homologue, prunasin, which is hydrolysed in turn to release HCN.

Because of the toxic nature of HCN, the cyanogenic glycoside and its specific glycosidase must be compartmentalised within the cells or tissues of cyanogenic plants (Conn, 1981b). Boersma et al (1983) showed that linamarase in Trifolium repens was localised in the cell wall fragments of homogenised cells, whilst the glycosides present were found in the cytosol. Saunders et al (1977) showed that the dhurrin in Sorghum bicolor L. was contained within the cell vacuole. S. bicolor has C_4 metabolism, and Kojima et al (1974) used the Kranz anatomy common to C_4 plants to separate the tissues of six day old leaves of the plant. They found that dhurrin was localised in the epidermal cells whilst the β-glycosidase and α-hydroxynitrilase were located in the mesophyll cells. Neither of the components was isolated from bundle sheath cells.

Different organs of an individual plant may contain very different levels of cyanogenic glycosides and glycosidases. Thus the young leaves of cassava (Manihot esculenta Crantz) release approximately 600 mg HCN/kg fresh weight, mature leaves release 490 mg HCN/kg fresh weight, the tuber bark releases 760 mg HCN/kg fresh weight and the tuber pulp releases 166 mg HCN/kg fresh

Figure 1.3. The release of HCN from prunasin, the cyanogenic glycoside found in bracken. The β-glycosidic bond of prunasin is first hydrolysed by a specific glycosidase to form glucose and the α-hydroxynitrile, mandelonitrile. The α-hydroxynitrile is further hydrolysed by a hydroxynitrilase to release HCN and benzaldehyde. The α-hydroxynitrile is unstable and may breakdown spontaneously in the absence of a hydroxynitrilase. It is not known whether bracken contains an α-hydroxynitrilase or not.



weight (Gondwe, 1974). Different cultivars of cassava also release different amounts of HCN (Cooke and Coursey, 1981; Gondwe, 1974; Nartey, 1981). Similarly, different strains of Sorghum sudanense L. (Boyd et al, 1938; Nass, 1972) and different cyanogenic varieties of Trifolium repens (Askew 1933; Hughes, 1981; Rogers and Frykolm, 1937) release different amounts of HCN.

1.2.2.3. The distribution of cyanogenic plants.

More than 2000 vascular plant species are known to be cyanogenic, although this is likely to be an underestimate due to the lack of information on most species (Hegnauer, 1977). Indeed, it is believed that all plant species are cyanogenic to a certain degree, in that they may all release trace amounts of HCN if detection methods are sensitive enough (Hegnauer, 1977). Cyanogenic species are known from the pteridophytes, the gymnosperms and the angiosperms (Harper et al, 1976; Hegnauer, 1977). Cyanogenesis is particularly common in the angiosperm families of the Poaceae (=Graminae), the Euphorbiaceae, the Rosaceae, the Ranunculaceae, and the Leguminosae (s.l.) (Hegnauer, 1977; Siegler, 1976, 1981). In most cyanogenic plant species, the specific glycoside involved has not been identified.

Although the presence or absence of a particular cyanogenic glycoside within a species cannot be used for taxonomic purposes, cyanogenesis can be a taxonomically useful character at higher levels of classification (Hegnauer, 1977; Harbourne and Turner, 1984). Within families of vascular plants, it would appear that the amino acid precursors of cyanogenic glycosides and the biosynthetic pathway are more or less constant (Hegnauer, 1977). Thus only phenylalanine-derived cyanogenic glycosides have been isolated from the pteridophytes, and only tyrosine-derived glycosides have been isolated in the gymnosperms (Hegnauer, 1977). Within the angiosperms, tribes of the Rosaceae can be separated on the basis of the precursor of the glycoside found in cyanogenic species, and the same situation is found for tribes of Leguminosae and of Poaceae (Hegnauer, 1977).

Within genera, it is common for both cyanogenic and acyanogenic species to occur. Thus Lotus australis, L. corniculatus, L. japonicus, L. krylorii, and L. tenuis are cyanogenic whilst L. alpinus, L. borbasii, L. caucasius and L. uliginosus are acyanogenic (Blaim and Nowacki, 1979; Grant and Sidhu, 1967; Harbourne and Turner, 1984; Phillips, 1968). Similarly, in the genus Trifolium both cyanogenic and acyanogenic species occur (Blaim and Nowacki, 1979; Gibson et al, 1972). Cyanogenesis can be used as a character in determining the relationships between closely related species (Harbourne and Turner, 1984).

Within a single cyanogenic species, it is common to find both cyanogenic and acyanogenic individuals. Blaim and Nowacki (1979) identified 42 polymorphic Trifolium species out of a total of 48 species tested. Such intraspecific variation means that cyanogenesis cannot be used as a taxonomic marker in the identification of species (Gibbs, 1963; Hegnauer, 1977). However, the study of polymorphism for cyanogenesis can be used in population studies (Jones, 1972a), by identifying individual clones and detecting changing population composition. The study of polymorphic species can reveal selection of one form over another in the field and can therefore help to increase our understanding of evolution (Jones, 1972a; Harper, 1977).

1.2.2.4. The genetics of cyanogenesis.

Species which are polymorphic for cyanogenesis have been studied using cyanogenesis as a genetic marker (Hughes, 1981; Pusey, 1963). Because of their economic importance, the herbage plants Lotus corniculatus and Trifolium repens, both of which are polymorphic for cyanogenesis, have been the subject of genetic investigation for several decades (reviewed by Hughes, 1981).

1.2.2.4.1. The genetics of cyanogenesis in **Trifolium repens**.

The first genetic studies of cyanogenesis in T. repens were by Corkill (1942). He showed that the production of the glycosides linamarin and lotaustralin and of the specific glycosidase is controlled by two genes, Ac and Li respectively. The functional alleles are dominant to the non-functional alleles ac and li, cyanogenic individuals being Ac - Li -. Nass (1972) suggested in addition to the Ac Li genes there may be 'modifying factors', or genes, that influence the amount of HCN released from cyanogenic plants.

Acyanogenic individuals could contain glycoside only (Ac - li li), glycosidase only (ac ac Li -) or neither (ac ac li li) (Hughes, 1981). Maher and Hughes (1973) showed that different Li alleles produced different linamarase activities. These differences were maintained in crosses, indicating that they were genetic rather than being the result of random variation. Plants that are homozygous for the allele li produce no measurable linamarase activity (Maher and Hughes, 1973). Heterozygotes Li li appear to have intermediate activities, suggesting differential production of linamarase (Hughes, 1981; Maher and Hughes, 1973). Similarly, whilst different alleles of Ac produce different amounts of linamarin, the heterozygotes Ac ac produce intermediate levels compared to the homozygotes Ac Ac, and homozygotes ac ac produce no

measurable linamarin (Hughes, 1981).

The observation that the heterozygotes Li li do show linamarase activity shows that the allele li does not inhibit the production of linamarase by the allele Li or the activity of the linamarase it produces (Hughes, 1981). The gene Li could therefore be the structural gene for linamarase or it could be the gene controlling the amount of linamarase synthesised at another site (Hughes, 1981). Hughes (1981) concludes that the gene Li controls the production of linamarase rather than being the structural gene directly responsible for its synthesis, on the basis of certain Li alleles producing low levels of linamarase, below that which would be expected from a normal Li allele.

The steps in the synthesis of linamarin were investigated by supplying radio-labelled precursors, in order to identify the effect of the ac allele on the process (Hughes and Conn, 1976). They identified two different steps in the biosynthetic pathway that were missing in ac ac plants. The differential amount of cyanogenic glycoside produced by different Ac alleles is inherited (Hughes, 1981). This suggests that the gene Ac controls the amount of linamarin produced (Hughes, 1981).

Corkill (1952) investigated the effect of cyanogenesis on the vigour of T. repens plants. He found no correlation between the presence of either Ac or Li alleles and the fitness of individual plants. In contrast, Nass (1972) suggests that breeding for increased vigour in T. repens leads to an increase in cyanogenesis. This might be due to an increased nitrogen uptake or amino acid synthesis. Ennos (1981a) showed that the presence of the Li allele was significantly and positively correlated with leaflet length and width, and that plants containing the Li allele were more competitive than those containing li. He deduced that these two features would combine to make Li plants more successful in the field. The effect of cyanogenesis on the fitness of individuals of T. repens was demonstrated by showing that the competitive ability of plants was affected by cyanogenesis (Ennos, 1981b).

1.2.2.4.2. The genetics of cyanogenesis in **Lotus corniculatus**.

Work on the genetics of the polymorphism of cyanogenesis has been less in Lotus corniculatus than in Trifolium repens. Dawson (1941) identified two phenotypes, cyanogenic and acyanogenic, in Swedish populations of L. corniculatus and he interpreted his data as being consistent with tetrasomic inheritance. This supports the observations that L. corniculatus is a tetraploid species (Harborne and Turner, 1984). Dawson (1941) identified the genes Ac and Li controlling the production of the cyanogenic glycosides, linamarin and

lotaustralin, and the glycosidase repectively. He also suggests that further modifier genes occur. Nass (1972) supports the view of modifier genes controlling cyanogenesis in L. corniculatus.

Professor D.A. Jones and his co-workers have studied the ecological genetics of L. corniculatus (e.g. Jones, 1966, 1981; Ellis et al, 1977a,b; Compton et al, 1983; Ramnani and Jones, 1984, 1985. Section 1.2.2.5). Jones (1966) showed the existence of four phenotypes, one cyanogenic and three acyanogenic. The four phenotypes could result from sixteen genotypes, since L. corniculatus is a tetraploid species. Ellis et al (1977a) found that particular individuals of L. corniculatus were unstable with respect to their phenotype under conditions of varying temperatures. The production of the glycosides, the glycosidase or both could be switched on or off under different temperature conditions. This was further investigated (Ramnani and Jones, 1984, 1985) and it was concluded that the existence of variable phenotypes greatly complicated the analysis of genetic data, since it means that the phenotype cannot be used as an indication of the genotype.

Hughes (1981) suggests that much of the available data on the genetics of cyanogenesis in L. corniculatus is not satisfactory, and she believes that further work is necessary.

1.2.2.4.3. The genetics of cyanogenesis in other species.

Most research on other cyanogenic plant species has concerned Sorghum and Manihot esculenta (cassava), which are both important crops in tropical regions. Both these crops are wholly cyanogenic, although there is large variation between different species of Sorghum and different cultivars of Sorghum and cassava (Cooke and Coursey, 1981; Gondwe, 1974; Nartey, 1981; Nass,1972).

Snyder (1950, in Hughes, 1981) suggested that one gene controlled the level of HCN production in Sorghum sudanense. Nass (1972) believed that a complex multigene system was present in Sorghum, producing variable levels of HCN depending on the genes for cyanogenesis present within the genome. It is possible that there is more than one system or linkage group of genes determining cyanogenesis in Sorghum. Genes for low HCN production are dominant in Sorghum (Nass, 1972). It has been suggested that linkage or pleiotropy may explain the correlation of high HCN production with low vigour in Sorghum species (Nass, 1972).

The genetics of cyanogenesis in Manihot esculenta, cassava, is complex because the species is only known in cultivation, although there are about 100

wild species of Manihot. In addition there are possibly several thousand cultivars of cassava, which may have different wild origins and may have been involved in crosses with wild species of Manihot (Cooke and Coursey, 1981). All known species of Manihot are cyanogenic (Cooke and Coursey, 1981). Within M. esculenta, there is apparently continuous variation in the amount of HCN produced by different cultivars (Cooke and Coursey, 1981; Gondwe, 1974), and this polymorphism of the amount of HCN released is inherited (Cooke and Coursey, 1981). There are no known acyanogenic cultivars of M. esculenta, nor are any other species of Manihot polymorphic for the presence of HCN (Cooke and Coursey, 1981).

1.2.2.5. The function of cyanogenesis.

It is generally accepted that cyanogenesis in plants serves to release HCN to act as an allelochemical (Conn, 1979, 1981b; Jones, 1972a). HCN is a highly toxic chemical (Conn, 1979). Alternative functions for cyanogenic glycosides and/or cyanogenesis in plants include roles in intermediary metabolism, or as nitrogen storage compounds (Jones, 1972a). Because cyanogenesis does not occur in all species within a genus, or, in polymorphic species, in all individuals within a population, the function of cyanogenesis cannot be essential to all plants, making a metabolic role unlikely (Jones, 1972a). The leaves of Trifolium repens can contain up to 1% fresh weight of cyanogenic glycosides (Collinge and Hughes, 1982), which suggests that these compounds have a very specific role, as opposed to their being intermediary or waste compounds. It is however possible that a defensive function of cyanogenesis evolved by the over-production of cyanogenic glycosides originally produced for some other purpose, and that the trait was subsequently selected for (Jones, 1979).

Because it is found that there is active turnover of cyanogenic glycosides (Abrol et al, 1966) and that, under high levels of soil nutrients, cyanogenesis is increased (Boyd et al, 1933; see section 1.2.2.6), it has been suggested that cyanogenic glycosides may function as intermediaries in the fixation of organic nitrates into amino acids. However, there is no known pathway for this process that includes cyanogenic glycosides (Jones, 1972a). If radio-labelled precursors are introduced to the biosynthetic pathways of cyanogenic glycosides, no radio-labelled amino acids are subsequently found (Conn, 1980). It is possible that minor involvement of cyanogenic glycosides in amino acid metabolism occurs.

The presence of large amounts of cyanogenic glycosides in seeds (Clegg et al, 1979) suggests that they are not used as a waste nitrogen storage product.

In addition, it had been shown that the glycosides in lima bean (Phaseolus lunatus) are translocated from the cotyledons into the growing seedling (Clegg et al, 1979). This would not be expected if cyanogenic glycosides represented waste products. Several species show a greater concentration of cyanogenic glycosides in young leaves than in mature foliage (Cooper-Driver et al, 1977; Dement and Mooney, 1974; Woodhead and Bernays, 1977). Additionally, it would not be expected that a waste storage product would be present with enzymes to release its toxic products (Boersma et al, 1983). Again, however, it is possible that cyanogenic glycosides play a minor role in waste product metabolism.

There is considerable evidence that HCN released from cyanogenic plants functions as a herbivore deterrent (Conn, 1979; Jones, 1972a). This has been proposed for both Trifolium repens (Angseesing, 1974; Angseesing and Angseesing, 1973; Crawford-Sidebotham, 1972; Horrill and Richards, 1986; Whitman, 1973) and Lotus corniculatus (Crawford-Sidebotham, 1972; Ellis et al, 1976; Jones, 1962, 1966, 1972b; Keymer and Ellis, 1978) from field observations. Miller et al (1975) and Bishop and Korn (1969) found no evidence for preferential eating of cyanogenic or acyanogenic forms of Trifolium repens. Laboratory tests have shown that certain insects graze acyanogenic plant material in preference to cyanogenic (Cooper-Driver and Swain, 1976; Bernays, 1977; Horrill and Richards, 1986). The presence of the cyanogenic glycoside alone had no deterrent effect on the insects in the trials. Woodhead and Bernays (1977) showed that the degree of herbivore feeding on Sorghum bicolor was related to the level of HCN released by the plant. Several authors have noted that cassava has few predators, which Cooke and Coursey (1981) attribute to cyanogenesis in the plant. The genus Acacia contains some species which are cyanogenic; those which are not cyanogenic are apparently protected by symbiotic ant species (Rehr et al, 1973), and the distinction between the two types of Acacia species has been attributed to partitioning of herbivore deterrents. There is seasonal partitioning of allelochemicals in Heteromeles arbutifolia, leaves being cyanogenic early in the season, and then, as the level of cyanogenesis decreases, there is a simultaneous increase in the level of tannin within leaves (Dement and Mooney, 1974).

Several herbivore species have evolved the enzyme system rhodanese, which is capable of detoxifying HCN (Beesley et al, 1985; Davis, 1981; Westley, 1981; Volini and Alexander, 1981). Rhodanese metabolises HCN with sulphane sulphur, producing a thiocyanate salt (Davis, 1981). Rhodanese is widely distributed amongst insects and it has been suggested that its presence in

insect species not normally associated with cyanogenic plants implies that HCN detoxification is not the primary role of the enzyme (Beesley et al, 1985). Certain insect species are thought to have co-adapted to cyanogenic plants to such a degree that they use the HCN to identify their host plant (Jones, 1972a, 1973; Jones et al, 1962; Lane, 1962; Nayar and Frankel, 1963).

There are theoretical arguments that suggest that the palatability of a plant to generalist herbivores increases with the 'apparency' of the plant (Feeny, 1975), and these have been substantiated by field observations (Cates and Orians, 1975). Because of their palatability, it has been predicted that apparent plants are more likely to be protected by allelochemicals. It is noteworthy that several rhizomatous or stoloniferous plant species, which can be considered highly apparent and likely to be available for herbivores, are cyanogenic (e.g. species of Lotus, Trifolium, Pteridium, and several grasses). In addition, young leaves are unapparent whilst mature leaves can be considered apparent (Raupp and Denno, 1983). Apparent plant tissues are more likely to contain quantitative allelochemicals, active in proportion to the quantity ingested (e.g. tannins), whilst non-apparent tissues are likely to contain qualitative allelochemicals, which are generally toxins and produce a behavioural change out of proportion to the amount of allelochemical ingested (e.g. alkaloids, glucosinolates and HCN) (Feeny, 1975). In several plant species, young (non-apparent) leaves are cyanogenic, whilst mature (apparent) leaves contain tannins (Raupp and Denno, 1983).

1.2.2.6. The ecology of cyanogenesis.

The ecology of cyanogenesis has been extensively investigated in the polymorphic species Trifolium repens and Lotus corniculatus, since the polymorphism in these species allows the comparison of cyanogenic and acyanogenic individual plants.

1.2.2.6.1. The ecology of cyanogenesis in **Trifolium repens**.

It has been shown that the amount of HCN released from cyanogenic leaves of plants of T. repens varies throughout the year, particularly with seasonal variation (Askew, 1933; Rogers and Frykolm, 1937). Rogers and Frykolm (1937) found an increase of HCN released with the time of year and the size of each plant. These observations are contrasted with those of Collinge and Hughes (1982), who showed that young seedlings were only cyanogenic after emergence from the soil, the seeds and seedlings below the soil being acyanogenic. On emergence, linamarin and lotaustralin were

synthesised by both light and dark grown seedlings. Dark grown seedlings had 50% greater cyanogenic glycoside content than light grown seedlings, although the dark grown seedlings showed reduced linamarase activity. Synthesis of the glycosides was continuous with leaf growth, maintaining the level of glycosides at 3.5µmoles per 100mg fresh weight. Once the leaflets were fully expanded, the glycoside content was kept constant, application of radio-labelled valine showing that there was no glycoside synthesis, and therefore that there was no turnover of the glycosides (Collinge and Hughes, 1982). This is in contrast to results for other plants (Abrol et al 1966; Hosel, 1981). It has been shown that there can be considerable variation within individual plants of T. repens, leaves of the same age and sampled at the same time releasing very different amounts of HCN, this variation also occurring between leaflets of the same leaf (Dr. Irene Till, personal communication).

Collinge and Hughes (1982) showed that the amount of HCN released from T. repens, in laboratory trials, was altered by the temperature at which the plants were growing. Under extremes of temperature (8°C and 27°C) there was less glycoside production than in the middle range.

H. Daday made an extensive study of cyanogenesis in T. repens in the field. He showed that the gene frequencies of Ac and Li, estimated by the cyanogenic phenotype of the plants he sampled, were related to the latitude (Daday, 1954a) and altitude (Daday, 1954b) at which the plants were growing. The altitudinal sites were not part of a continuous altitudinal transect. He interpreted his results as showing low temperature selection for acyanogenic phenotypes, balanced with selection of cyanogenic phenotypes by herbivores, due to preferential grazing of the acyanogenic phenotypes (Daday, 1965). He further investigated the effect of cyanogenesis on flowering, as a measure of fitness. He found that at low temperatures, in laboratory studies and in the absence of herbivores, that the ac ac li li genotype had the most reproductive vigour and the Ac Ac Li Li phenotype the least reproductive vigour (Daday, 1965). Field observations of flowering and of vegetative growth agreed with his experimental results, acyanogenic plants being more vigorous at low temperatures. He interpreted his results as showing linkage between the genes for cyanogenesis with genes for vigour (Daday, 1965), although Corkill (1942) had shown that the genes for cyanogenesis were independently inherited (i.e. the genes were not linked). Foulds and Grime (1972b) found no differences between the vegetative yield of cyanogenic and acyanogenic phenotypes. Burdon (1980) found no correlation between a wide range of continously variable vegetative and reproductive characters and the frequency of the alleles

Ac and Li.

The effect of altitude on the cyanogenic polymorphism of T. repens was also investigated by De Araujo (1976) and by Brighton and Horne (1977). Unlike Daday's (1954b) investigation, both studies employed continuous altitudinal transects. De Araujo (1976) showed that, for a single altitudinal transect in Wales, the proportion of cyanogenic plants decreased with altitude. Similar results were found by Brighton and Horne (1977) for two altitudinal transects in Scotland. In addition, Brighton and Horne (1977) compared seedling populations with adult populations, and found evidence of selection against the cyanogenic form at high elevations. They concluded that temperature was the factor responsible for the selection.

The effect of temperature on cyanogenic and acyanogenic T. repens was estimated by comparing the rates of photosynthesis and respiration, under laboratory conditions, in order to test the hypothesis that under low temperatures cyanogenic phenotypes may release toxic quantities of HCN (Foulds and Young, 1977). They found no reduction of photosynthesis by detached leaves of acyanogenic phenotypes but a significant reduction of photosynthesis by detached leaves of cyanogenic phenotypes following freezing. However, the cyanogenic phenotype had a higher rate of photosynthesis under normal conditions, the reduced rate being similar to the rate of photosynthesis by acyanogenic phenotypes. Respiration was not affected by low temperatures in either phenotype. No differences in photosynthesis were found when whole plants were frozen. The addition of KCN at normal temperatures inhibited photosynthesis in both phenotypes, whilst not altering respiration. It was suggested that low temperatures could directly affect the cyanogenic phenotype by releasing HCN.

Moisture stress has also been implicated as an environmental factor affecting cyanogenesis. Rogers and Frykolm (1937) suggested that droughting could lead to an increase in the production of HCN by cyanogenic plants of T. repens. Foulds and Grime (1972a,b) found a significant reduction in the frequency of the cyanogenic phenotype under drought conditions, due to a decrease in the frequency of the Ac allele. There was no differential effect of droughting on photosynthesis or respiration in cyanogenic and acyanogenic phenotypes of T. repens (Foulds and Young, 1977). The differential effect of drought stress on the two phenotypes was further investigated by Foulds (1977), who showed that there was no effect on plants related directly to cyanogenesis, but that rather the dominant alleles Ac and Li were linked to, or modifying the action of, genes related to fitness or vigour. It has been

suggested that physical stress will lead to increased production of herbivore deterrents in plants (Rhoades, 1983).

In order to maintain a polymorphism within a population of plants, the selective pressure for one morph must be balanced by selective pressure for the other morph (Jones, 1971). Daday (1965) suggests that this is the case with T. repens. Several studies have indicated that the acyanogenic phenotype of T. repens is grazed in preference to the cyanogenic phenotype (Angseesing, 1974; Angseesing and Angseesing, 1973; Crawford-Sidebotham, 1972; Whitman, 1973). Other studies have found no evidence of selective grazing (Bishop and Korn, 1969; Dritschilo et al, 1979; Miller et al, 1975).

1.2.2.6.2. The ecology of cyanogenesis in **Lotus corniculatus**.

The effect of altitude on cyanogenesis in leaves of L. corniculatus is similar to that found for T. repens, and this was interpreted as an effect of low temperatures reducing the percentage of cyanogenic individuals in a population (Brighton and Horne, 1977). In Canada, Jones (1970) found that cyanogenic and acyanogenic phenotypes showed differential survival of extreme winter conditions. There was no differential effect of temperature on the phenotype of L. corniculatus in England (Jones, 1970).

A differential effect of temperature was found between acyanogenic and cyanogenic phenotypes of L. corniculatus, with respect to photosynthesis (Foulds and Young, 1977). Ellis et al (1977a) found that under controlled conditions, cold temperatures did not act directly against the cyanogenic phenotype. They also showed that some individual plants of L. corniculatus possess unstable phenotypes, which can change under the influence of temperature. Individual plants were found to change phenotypes between all four possible phenotypes at different temperatures (Ellis et al, 1977a). This suggests that there was temperature-sensitive enzyme synthesis, or enzyme activity, resulting in an interaction between genotype and environment determining the phenotype (Ramnani and Jones, 1985).

Foulds and Grime (1972a) found a relationship between the distribution of the acyanogenic phenotype of L. corniculatus and the degree of soil moisture. Abbott (1977, 1981) found that the percentage of cyanogenic individuals was related to exposure and significantly correlated with the percentage of soil moisture at coastal sites in Orkney, Scotland. The distribution cyanogenic plants of L. corniculatus under field conditions was investigated for coastal sites in Anglesey, Wales (Ellis et al, 1977b). The results suggested that the phenotype was determined by exposure, particularly wind and windborne salt,

with a low percentage of cyanogenic individuals under more exposed conditions. Keymer and Ellis (1978) took cuttings from plants of both phenotypes at these sites in Anglesey and grew them under controlled conditions. They found no differences between the fitness of cyanogenic and acyanogenic phenotypes under treatment with salt spray or differential salinity. Ellis et al (1977b) concluded that the selection of phenotypes by exposure was indirect, due to the differential distribution of mollusc species which acted as the selective agents.

In a study which paralleled those by Daday on T. repens (Section 1.2.2.6.1), Ellis et al (1976) and Jones (1977) could find no explanation for the distribution of the cyanogenic form of L. corniculatus throughout Europe. No correlations were found between the distribution of the phenotypes and altitude or latitude, in contrast with the results for T. repens.

Jones (1968) found an interaction between the phenotypes of L. corniculatus and T. repens growing together. In the presence of cyanogenic L. corniculatus, individuals of T. repens were less cyanogenic than individuals growing without L. corniculatus. The nature of the interaction was uncertain, although it could be mediated by herbivores avoiding the patches of a highly cyanogenic Lotus and thus avoiding the acyanogenic Trifolium which would otherwise have been selected against.

The flowers of L. corniculatus are also polymorphic for cyanogenesis (Compton et al, 1983). The distribution of cyanogenic flowers does not strictly follow that of cyanogenic leaves, the two characters being independent. This suggests environmental determination of cyanogenesis since plants which are cyanogenic for either leaves or flowers are genetically cyanogenic, even if they are acyanogenic for the other character. Within a single plant of L. corniculatus, cyanogenic leaves of the same age releasing different quantities of HCN can occur on different shoots (Professor D.A. Jones, personal communication), as has been found for T. repens (Dr. I. Till, personal communication). Compton et al (1983) found no correlation in Norway between cyanogenesis in either leaves or flowers and droughting, temperature or the distribution of invertebrate herbivores. They concluded that selection was due to selective grazing by the small mammalian herbivore, Lemmus lemmus. Ellis et al (1977b,c) found evidence of selection against the acyanogenic leaf phenotype of L. corniculatus by molluscs. Compton and Jones (1985) showed that a range of generalist herbivore insect and mollusc species preferentially grazed acyanogenic leaves and flowers of L. corniculatus.

Jones (1962, 1966) has shown that molluscs selectively graze acyanogenic

leaves of L. corniculatus. However, not all the animals used in the trials showed such preferences. Crawford-Sidebotham (1972) found that molluscs differential grazed acyanogenic plants. Using molluscs identified from field observations, Keymer and Ellis (1978) showed that these species selectively ate acyanogenic plants in laboratory trials.

1.2.2.6.3. The ecology of cyanogenesis in other species.

Lotus australis, a species closely related to L. corniculatus, is also polymorphic for cyanogenesis (Foulds, 1982). However, the phenotype is flexible and dependent on the available moisture, acyanogenic plants becoming cyanogenic under conditions of high moisture (Foulds, 1982).

Sorghum sudanense shows continuous variation in the amount of HCN released from its leaves (Nass, 1972). Boyd et al (1938) investigated the variation of cyanogenesis in Sorghum sudanense. They showed that cyanogenesis in Sorghum was related to the level of available nitrogen in the soil, plants under high nitrogen treatment producing very high levels of HCN. The amount of HCN released from leaves depended upon the age of the tissue and the age of the plant, older tissues and plants releasing low levels of HCN. There were no direct effects of either freezing or droughting, both of which were thought to be important, although it was noted that droughting did not let Sorghum plants develop sufficiently, maintaining a high level of HCN. Sorghum species also showed slight diurnal variation (Boyd et al, 1938). Woodhead and Bernays (1977) also found that the amount of HCN released from leaves of Sorghum bicolor decreased substantially with the age of the plants.

Cassava, Manihot esculenta, also shows continuous variation in the level of HCN produced by its tissues (Cooke and Coursey, 1981). As well as this variation being genetically determined, high soil nutrition is believed to increase the level of HCN produced (Nartey, 1981). High humidity and rainfall may also increase the amount of HCN released. Nartey (1981) found both diurnal and seasonal variation in the amount of HCN released from cassava.

1.2.2.6.4. Conclusions on the ecology of cyanogenesis.

It is possible that cyanogenesis behaves differently in different species of plants, or even in different parts of the world within the same species. The contrasting results for Trifolium repens and Lotus corniculatus are difficult to reconcile, and it must be concluded that different factors may account for the distribution of phenotypes of the two species. There are several contradictory reports concerning the same species (e.g. the effect of low moisture content

on Trifolium repens) which may reflect experimental technique or other factors not measured. Feeding trials are open to experimental bias due to arrangement of the available foods or conditioning of the herbivores. However, the results from several studies indicate that HCN does have a deterrent effect on a variety of herbivores. Abiotic environmental factors have been shown to be important in determining the amount of HCN released from cyanogenic plants.

1.2.2.7. Cyanogenesis in bracken.

In addition to containing the various secondary compounds discussed in section 1.2.1.8, bracken fronds are also cyanogenic. This was first noted by Greshoff (1908; in Cooper-Driver, 1976). Cyanogenesis is common in plants (see section 1.2.2.2). Both subsp. aquilinum and caudatum contain the cyanogenic glycoside prunasin (Bennett, 1968; Kofod and Eyjolfsson, 1966) and an unidentified, but presumably specific (section 1.2.2), β-glycosidase. These components are assumed to be compartmentalised. When frond tissue is damaged, prunasin is brought into contact with the β-glycosidase. The glycoside is hydrolysed to a glucose molecule and an α-hydroxynitrile, mandelonitrile. This further breaks down to produce benzaldehyde and gasous HCN (Figure 1.3). Bracken has also been shown to be polymorphic for cyanogenesis, some fronds within a stand releasing HCN ("cyanogenic") whilst others do not ("acyanogenic") (Cooper-Driver et al, 1977; Lawton, 1976; Schreiner, 1980).

The ecology of cyanogenesis in bracken was first investigated by Moon and Raafat (1951). These workers concentrated on the toxic effects of HCN in bracken and the variation of the amount of HCN released from fronds through the growing season. They were not aware that the character was polymorphic. They sampled comparatively large amounts of bracken over an unspecified area, massing several fronds together, and their method may have entailed a significant time interval between the removal of the fronds and the chemical release of HCN. This could have led to large sampling errors. In addition, error may have been introduced by failing to differentiate between cyanogenic and acyanogenic fronds. They found that the amount of HCN released from fronds was very variable, values from a single site changing from 2mg HCN/100g dry weight to 9mg/100g dry weight within a few days for early season fronds. Late season fronds released larger amounts of HCN, from 5mg/100g to 10mg/100g dry weight.

Cooper-Driver and Swain (1976) and Cooper-Driver et al (1977) isolated four phenotypes of bracken with respect to cyanogenesis from stands of fronds

in south east England. One of these phenotypes was cyanogenic, containing both prunasin and the glycosidase; the other three were acyanogenic, lacking either or both of the necessary components. Lawton (1976) reports similar results for stands of fronds in north east England. These observations can be interpreted as evidence that cyanogenesis is determined by a two gene system, one gene producing prunasin and the second producing the β-glycosidase, although environmental control of phenotype is also possible (Jones, 1983).

Cooper-Driver et al (1977) showed that at one location in south east England, the production of HCN (as arbitrary units) was greater from fronds growing under shaded conditions than those growing in the open. The amount of HCN produced decreased more or less steadily throughout the season. They showed that feeding inhibition of Schistocerca gregaria by bracken was related to the level of HCN released and tannins within the fronds.

Lawton (1976) found no relationship between cyanogenesis in bracken and the insects feeding on it at one site in north east England. He recorded a quantitative decline in the amount of HCN released from massed fronds within a season.

In contrast, Schreiner (1980) showed in north east America that acyanogenic fronds of, presumably, var. latiusculum are subject to greater rates of predation, particularly with respect to sawfly larvae (Strongylogaster spp.). Schreiner (1980) also showed a quantitative seasonal decline in the amount of HCN released from fronds, although her exact sampling method is not clear. She found a higher proportion of cyanogenic fronds under shaded conditions, these fronds releasing greater amounts of HCN, arbitrarily measured. There was no significant difference between the height of cyanogenic fronds and the height of acyanogenic fronds, nor was there any correlation between winter severity, estimated by geographical position, and the percentage of cyanogenic fronds sampled every 100 miles on a 600 mile transect of north east U.S.A.

Tempel (1981) also sampled fronds in north east U.S.A. She found no evidence of cyanogenesis at several sites at two locations, including fronds under shaded conditions. Kirk (1977) reports that both cyanogenic and acyanogenic fronds can be found in Papua New Guinea, presumably fronds of both subsp. aquilinum var. wightianum and subsp. caudatum var. yarrabense. Fronds of var. esculentum in New Caledonia (P.R.H. Hadfield, unpublished results) and in Australia (Professor J. Thompson, personal communication) have also been found to be polymorphic for cyanogenesis.

These studies have shown that cyanogenesis in P. aquilinum is a variable characteristic, and they leave several questions unanswered. Notable amongst

these is the degree to which environmental conditions control cyanogenesis in bracken, and how such control is mediated. The answers to these questions are essential for an understanding of the ecology of cyanogenesis in bracken and the role it plays in determining the interaction between bracken and its herbivores. In addition the extent to which cyanogenesis can be used as a marker in population studies is dependent on the stability and variability the character shows.

1.3. The Objectives of this Investigation.

Cyanogenesis would appear to be a characteristic which is practical to use as a marker in population studies of Pteridium aquilinum. To this end, it is necessary to have a full understanding of the polymorphism of cyanogenesis in bracken, and how cyanogenesis varies within the species. This study therefore set out to establish: a. the extent to which the polymorphism for cyanogenesis in Pteridium aquilinum was distributed throughout Britain, and what factors were responsible for the observed distribution and its maintenance; b. how the polymorphism of cyanogenesis was determined; and c. the extent to which cyanogenesis is phenotypically variable within a population of fronds, and how such variation was controlled. These studies would together indicate the suitability and reliability of cyanogenesis as a population marker in bracken. Without such studies, the feasibility of using biochemical markers in population studies cannot be assessed.

Chapter 2. Materials and Methods.

2.1. Field sampling and site recording.

Fieldwork formed the greater part of the research undertaken. Investigations into the geographic distribution within Britain of the polymorphism of cyanogenesis (chapter 4), the seasonal variation of cyanogenesis (chapter 5), the year to year variation of cyanogenesis (chapter 6) and localised variation (chapter 7) were undertaken. Several of the sites were involved in more than one of these investigations and consequently the selection and the characteristics of the sites are described separately in chapter 3. The present section deals specifically with the sampling procedure used throughout the investigations.

The number of fronds sampled at each site was 100 or 50, usually 50 because of the inaccessibility of several sites and the time needed to complete the sample. When 50 fronds were sampled, a rectangle 18m x 16m was marked out. Permanent pegs were placed at each corner, to allow the site to be found on subsequent occasions. The orientation of the rectangle with regards to magnetic north and any suitable landmarks was noted. An 18m tape, marked at 2m intervals, was placed across the rectangle parallel to the long axis at 4m intervals, to provide 5 transects of 10 points each. This gave 50 sampling points on an 18m x 16m grid. The nearest bracken frond to each point on the grid was tagged so that it could be relocated if necessary. Each frond tagged was identified by its position on the grid, given by the transect (A - E) and position on the transect (1 - 10) at which it was found. When 100 fronds were sampled, the procedure differed only in that an 18m x 18m square was marked out and 10 transects (A - J) of ten points 2m apart were made at 2m intervals, providing 100 points on an 18m x 18m grid.

2.2. Detection and Measurement of Hydrogen Cyanide.

Two different methods were used to detect and measure the amount of HCN released from bracken tissue. The first of these was used as a field test; the second was a laboratory analytic technique.

2.2.1 The Sodium Picrate Test for HCN.

2.2.1.1 Protocol.

Saturated picric acid solution (2,4,6-trinitrophenol; 'Analar' grade, B.D.H. Ltd.) was prepared by soaking crystalline picric acid in distilled water and removing the solution formed. The solubility of picric acid is approximately 13g per litre. The procedure was repeated until a suitable volume (c. 100ml) of saturated solution was obtained. The picric acid solution was then neutralised by the addition of excess (more than 50g per litre of picric acid solution) sodium carbonate (Na_2CO_3, 10 H_2O; 'Analar' grade, B.D.H. Ltd.). The resulting solution was filtered to remove any precipitate. The saturated sodium picrate solution could be stored and used as required.

Whatman #1 filter paper was cut into small strips, 35mm x 7mm in size. These were soaked in saturated sodium picrate solution. The excess solution was allowed to drop from the strips of filter paper. The damp strip of filter paper (referred to throughout as "picrate paper") could then be used to detect HCN released from plant material.

The terminal 4cm of the lowermost pinna on each of the fronds were removed and placed in a 10mm x 50mm glass tube. Three drops (approximately 0.07ml) of toluene (G.P.R. grade, B.D.H. Ltd.) were dropped from a 2ml glass syringe onto the plant material; the material was then lightly crushed with a glass rod. A freshly prepared picrate paper was placed in the mouth of the tube. The paper was held in place by a plastic stopper which sealed the tube and prevented any HCN from leaving the tube. The paper was prevented from touching the sides of the tube, except at the mouth of the tube where the paper was held by the stopper. Only freshly prepared damp picrate papers were used to avoid artefactual effects caused by the papers drying. To ensure that the sample did not plasmolyse and that no HCN was lost from the tissue before the picrate test, the tubes were stoppered within one minute of removing the 4cm sample from the frond. The test procedure was based on that used by Jones (1966).

Each tube was left to stand in a sealed, light-tight box for 24 hours at the ambient temperature. In the presence of HCN gas, the paper changed colour from bright yellow, ultimately to dark brown if sufficient HCN was present. The colour of the paper was assessed against a standard colour chart. If insufficient HCN had been released to cause any change in the colour of the

paper, the sample was said to be acyanogenic ("negative"). If the sample had released sufficient HCN to cause the picrate paper to change colour, the plant material and the frond from which it came was said to be cyanogenic ("positive").

It has been suggested that bacterial contamination of samples being tested for cyanogenesis could result in 'false' positive results, with the bacteria releasing trace amounts of HCN from large quantities of fungal material under test (Saupe et al, 1982). For this reason, Saupe et al (1982) suggest having short incubation periods before scoring for the presence or absence of cyanogenesis. However, the levels of HCN released by bacterial contaminants are expected to be low with the quantities of tissue used in these investigations, and it is felt to be unlikely that these would affect the results presented in this thesis.

2.2.1.2: Calibrating the Sodium Picrate Test for HCN.

It had been noted by several authors that there was a relationship between the colour obtained using the picrate test and the amount of cyanide released from cyanogenic plant material (Melville et al, 1940; Corkill, 1942; Jones, 1966; Blaim and Nowacki, 1979; Boersma et al, 1983). This implied that the test could perhaps be used quantitatively. This would make the picrate test much more useful as a field technique. It was therefore necessary to calibrate the picrate test and to discern the extent to which it could be used quantitatively.

KCN solutions were freshly prepared by dissolving weighed amounts of KCN ('Analar' grade, B.D.H. Ltd.) in 0.1M NaOH solution. The KCN solutions were in the range of 3.85mM to 76.9mM. Different volumes of the KCN solutions were pipetted into 50mm x 12mm glass tubes and a sufficient volume of 0.1M NaOH added to bring the total volume in each tube to 0.2ml.

Fresh picrate papers were prepared as described above. The KCN solutions in the glass tubes were acidified by the addition of 0.3ml concentrated H_2SO_4 (approximately 7M; 'Analar' grade, B.D.H. Ltd.). Each tube was sealed by a stopper holding a picrate paper in place as described above.

The tubes were left in the dark at room temperature for 24 hours. There was no further change in the colour of each test paper with longer time. The resulting colours of the papers were ordered according to their shade, assessed visually. The spectrum of colours obtained was arbitrarily broken up into seven colour classes which were readily discernible. The colour classes were numbered 0 (no change in paper colour: as in the control solutions and those releasing insufficient HCN to cause the picrate papers to change colour)

to 6 (maximum colour change). The midpoint of each colour class was visually matched against colours in the Royal Horticultural Society Colour Chart (Anon, 1966) and a standard colour chart produced.

The calibration of the picrate test was repeated on four occasions distributed over a long period. The results of successive calibrations agreed with each other. The colours produced showed a direct relationship with the logarithm of the amount of HCN released from the KCN solution. When -log[HCN] is plotted against the number of the colour class, a straight line is obtained (Figure 2.1). The correlation coefficient (r) for this line is -0.972; this is highly significant (0.1% level; $P<0.001$). The regression equation for the line is:

$$y = 6.0235 - 0.341\ x$$

This equation was used to predict the value of -log[HCN] for values of x = 1 to x = 6. From these values, estimates for the amount of cyanide corresponding to each colour class were obtained. These values are given in Table 2.1. It should be noted that the range of colour class 0 includes all values of HCN released in the tube less than 1µg, as well as no HCN at all (as in the control tubes); similarly, colour class 6 includes all values in excess of 65µg. The estimates of HCN corresponding to each colour class are similar to those obtained by Jones (1966) and Boersma et al (1983), as shown in Table 2.1.

2.2.1.3. Comparison with Other Methods.

The picrate test has been used variously to study cyanogenesis in plants since the early part of this century (Greshoff, 1906; Mirande, 1912). In some instances it has been used qualitatively whilst in others it has been used quantitatively (see Table 2.2 for references). Those workers who have used the test quantitatively include some who have not calibrated their results or have not published their calibrations. Several workers using the test qualitatively have suggested that it might be used quantitatively but that this was not necessary for their work.

There are several variants of the methods used by different authors. Several authors have used filter papers soaked in sodium picrate solution as described in section 2.2.1.1. Many authors have failed to publish exact details of their methods, particularly the size of glass tubes used and the size of filter paper used, and it is therefore difficult to compare their results directly.

Schroder (1977) only briefly mentioned the picrate paper test, emphasising

Figure 2.1. The calibration curve for the picrate paper test. The picrate test was calibrated by the release of known amounts of HCN from solutions of KCN by acidification, and recording the colour of the picrate paper produced by this amount of HCN. The colours were classified into seven classes, 0 - 6. The curve was prepared by the regression of -log[HCN] on the colour class.

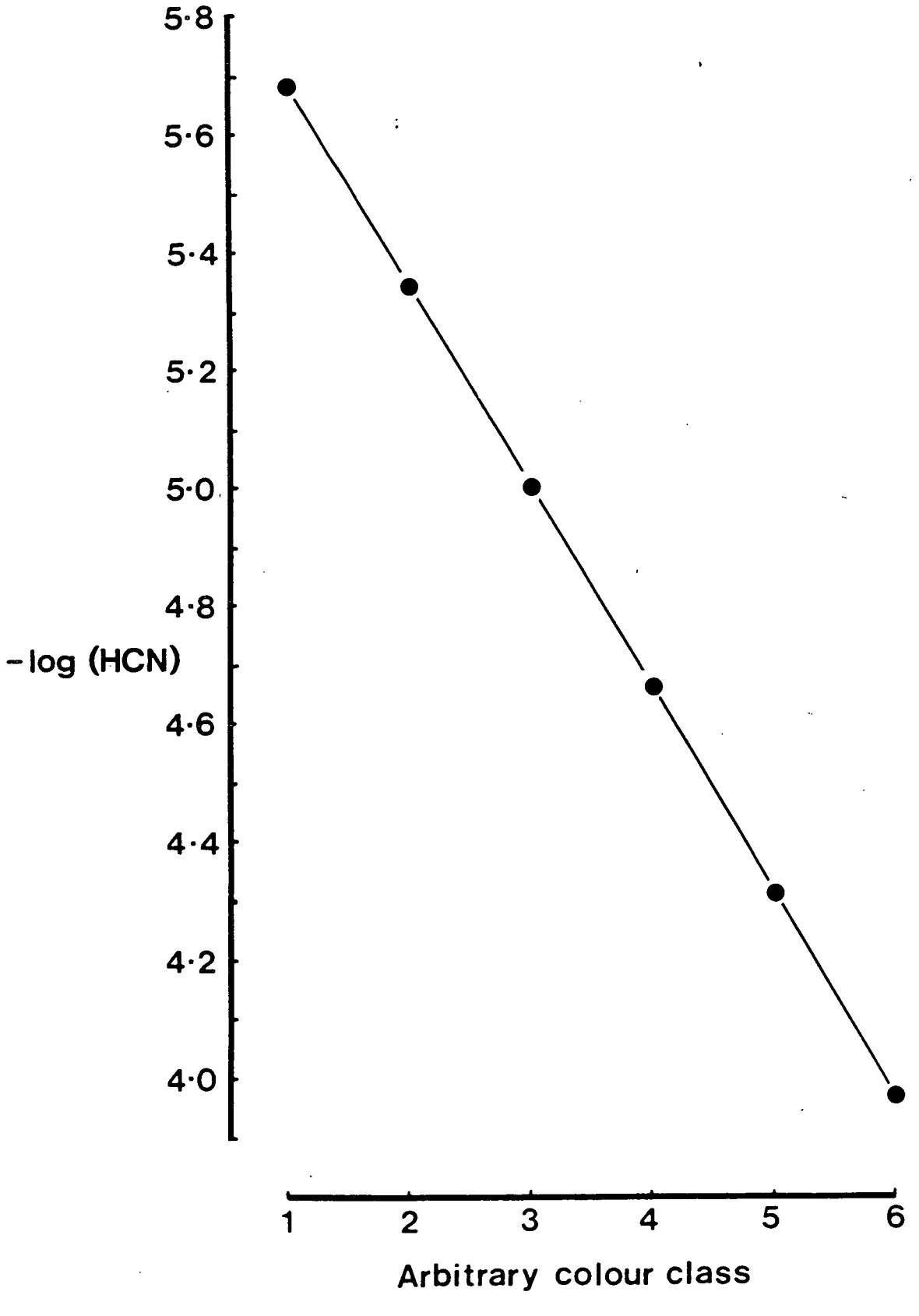


Table 2.1. The results of the calibration of the sodium picrate paper test for HCN and a comparison with the results of Jones (1966) and Boersma et al (1983). The range of colours produced in the calibration was arbitrarily divided into seven classes (0 - 6) and a standard assessed against the RHS Standard Colour Chart (Anon., 1966). The estimate of HCN required to produce the standard colour in each class was calculated from the regression line of -log[HCN] against the colour class. The values of HCN given are µg.

Colour Class	0	1	2	3	4	5	6
Colour Standard	4A	20A	22A	167A	170B	166C	174B
HCN Equivalent	0-1	>1-<3	>3-7.5	>7.5-16	>16-30	>30-75	>75
HCN Estimate	0	2.08	4.56	10.00	21.98	48.19	105.68
Reported by Jones (1966)	-	0.4	1.7	2.7	3.57	13.9	27.7
Reported by Boersma et al (1983)	<1	1	2	5	10	20	50

Table 2.2. Publications referring to the use of the picrate test to detect HCN gas liberated from cyanogenic plant tissues.

a. Publications referring to the use of the picrate test as a qualitative technique, arranged in order of publication date. It should be noted that several authors included in the Table have described the picrate test in several papers; only representative examples are listed in the Table.

Reference	Species Studied	Notes
Greshoff, 1906	several	
Armstrong et al, 1913	*Trifolium repens* and *Lotus corniculatus*	
Dawson, 1941	*Lotus corniculatus*	
Daday, 1954a,b	*Trifolium repens*	use of glycosidase to detect glycoside only
Pusey, 1963	*Trifolium repens*	suggests low scores are glycoside only
Foulds and Grime, 1972a,b	*Trifolium repens*	after Daday, 1954a
Rehr et al, 1973	*Acacia* spp.	after Dawson, 1941
De Araujo, 1976	*Trifolium repens*	scored after two hours
Abbott, 1977	*Lotus corniculatus*	after Jones, 1966
Ellis et al, 1977a,b,c	*Lotus corniculatus*	after Jones, 1966
Kirk, 1977	*Pteridium aquilinum*	
Ennos, 1981	*Trifolium repens*	after De Araujo, 1976
Foulds, 1982	*Lotus australis*	after Daday, 1954a
Kaplan et al, 1983	108 species	scored after 1 - 3 hours

Table 2.2 - continued.

b. Publications referring to the use of the picrate test as a quantitative technique without calibration of the results, arranged in order of publication date.

Reference	Species Studied	Notes
Ware, 1925	Trifolium repens	scored 1 - 3
Rogers and Frykolm, 1937	Trifolium repens	scored against standards, 0 - 5
Jones, 1962	Lotus corniculatus	after Corkill, 1942; scored 0 - 6
Bishop and Korn, 1969	Trifolium repens	after Jones, 1966
Angseesing and Angseesing, 1973	Trifolium repens	scored 0 - 6 but treated qualitatively
Cooper-Driver et al, 1977	Pteridium aquilinum	after Eyjolffson, 1970. Scored 0 - 4 with possibility of colorimetry. Results presented as 'arbitrary' units
Tempel, 1981	Pteridium aquilinum	scored standards 0 - 5 or read colorimetrically. No cyanogenic material found.

Table 2.2. – continued. c. Publications referring to the use of the picrate test as a quantitative technique with calibration, arranged in order of publication date.

Reference	Species Studied	Notes
Boyd et al, 1938	Sorghum sudanense	liquid colorimetry
Melville et al, 1940	Trifolium repens	paper test, scored 0 – 6.
Corkill, 1942	Trifolium repens	after Melville et al, 1940
Jones, 1966	Trifolium repens	paper test, scored 0 – 6.
Gilchrist et al, 1967	herbage species	eluate paper/ liquid colorimetry
Burns et al, 1970	Sorghum spp.	liquid picrate colorimetry
Gondwe, 1974	Manihot esculenta	after Burns et al, 1970
Mitchell, 1974	Brassica oleracea	paper test with reflectance colorimetry
Schroder, 1977	Cynodon spp.	liquid picrate colorimetry (also paper test)
Blaim and Nowacki, 1979	48 Trifolium spp and 12 Lotus spp.	paper test scored zero, low, medium, high, with calibration
Schreiner, 1980	Pteridium aquilinum	after Schroder, 1977
Boersma et al, 1983	Trifolium repens	paper test scored 0 – 6.
Horrill and and Richards, 1986	Trifolium repens	after Mitchell, 1974

its sensitivity, but presented no data on his results or calibration. He also noted that dry picrate papers should be used, in contrast to most authors who have suggested that only freshly prepared damp papers should be used for consistent results and greater sensitivity.

Jones (1966) recorded the picrate paper test to be sensitive to amounts of HCN greater than 0.425µg (given as 25µg HCN/g fresh weight for a typical Lotus leaf of 17mg) in a 50mm x 10mm glass tube. Blaim and Nowacki (1979) state that the sensitivity of their test is approximately 1µg (given as 0.01mg HCN/g fresh weight for 0.1g leaf sample) in a 50mm x 15mm glass tube. Boersma et al (1983) give the sensitivity of the test as 1µg HCN in a standard tube of unspecified size.

Several workers have used sodium picrate solution as a quick method of colorimetry (Boyd et al, 1938; Burns et al, 1970; Gondwe, 1974; Schroder, 1977; Schreiner, 1980), using distillation methods to collect HCN released by plant material in KOH or NaOH traps and then analysing the solution in the trap by adding sodium picrate solution and measuring the absorbance of the mixture. Calibration was against known aliquots of CN^- in KOH or NaOH added to sodium picrate solution. This has the advantage of being quantitative and removing the subjectivity required in visually assessing the colour class in the picrate paper test. However, more sensitive colorimetric methods are available (Zitnak, 1973) and although these tend to be more complex and time consuming, the increased sensitivity and accuracy makes such methods preferable. Gilchrist et al (1967) used a combination of the picrate paper test and picrate colorimetry. Using typical picrate paper methods, they then eluted the HCN-picrate complex from the filter paper and used this in colorimetry. Although removing the subjectivity of the paper test, this method maintains any errors in the paper test due to technique (e.g. variation in the size of filter paper; variation in the amount of sodium picrate absorbed by the filter paper). Errors may be increased by elution of the complex into solvent. In particular, picrate papers can become bleached by sunlight and after long periods of time (personal observations), and therefore elution must be done promptly. This method seems therefore to combine the poor qualities of both methods. It is particularly inappropriate where large numbers of plants are to be sampled or as a field test where laboratory conditions cannot be maintained for elution and samples would otherwise have to be kept for long periods of time before colorimetry was possible. If a small number of plants is to be sampled, it is advisable to use a more sensitive and accurate analytic method of HCN detection and measurement.

An alternative to the picrate paper test is the 'tetrabase' spot test (Fiegl and Anger, 1966). This involves mixing solutions of copper (II) ethylacetoacetate and tetramethyl diamino diphenylmethan in $HCCl_3$. Filter papers are soaked in a 1:1 mixture of these solutions. The papers are then left to dry, and they are used in this state, fresh papers not being required. The tetrabase test is reputedly more sensitive than the picrate paper test, although the limits of detection given by Fiegl and Anger (1966) are 1µg (in an unspecified volume of air), similar for those given by Blaim and Nowacki (1979), Boersma et al (1983) and reported here, for the picrate paper test. In a comparison of the two tests, similar results were usually obtained but some fronds of Pteridium which were acyanogenic by the picrate paper test gave positive results with the tetrabase test using different pinna tips (Dr. A.F. Dyer, personal communication). This might be due to greater sensitivity of the tetrabase test but it might equally reflect differences between pinna tips. In addition, the tetrabase test is supposed to show a greater specificity than the picrate test, which has been reported to be also sensitive to the presence of sulphides and other substances (Fiegl, 1960; Snell and Snell, 1959). However, the tetrabase test can only be used qualitatively, and this disadvantage outweighs the reported advantages over the picrate paper test mentioned above.

2.2.2. The Pyridine – Pyrazolone Test for HCN.

2.2.2.1. Protocol.

The laboratory technique for the detection and estimation of HCN employed a very sensitive and accurate colorimetric technique. It was, however, time consuming, and required the use of noxious chemicals and strict control over the physical conditions, in particular temperature. For these reasons, it was not possible to use this technique for fieldwork.

The method of Mao et al (1965) was followed. The plant material to be tested was weighed and placed in the reaction vessel of a Thunberg tube (Figure 2.2). 2ml or 1ml of 0.1M NaOH (depending on the amount of the material sampled and the amount of HCN expected to be released) was pipetted into the upper vessel of the Thunberg tube and the joint was lubricated with a small amount of silicon grease. Two drops of toluene were placed on the plant material before sealing the tube with the upper vessel. The Thunberg tube was then left for at least 24 hours. After this time, all the HCN released has been absorbed by the NaOH trap; this was confirmed by drawing

Figure 2.2. The Thunberg tube. An amount of 0.1M NaOH is placed in the side arm of the upper vessel. A known amount of plant material is placed in the lower vessel, and toluene is added. The plant material is lightly crushed and the lower vessel is sealed by the upper vessel. Any HCN released by the plant material is absorbed by the NaOH which is then assayed by the pyridine - pyrazolone method.

air from the Thunberg tube through the side arm with a syringe, and expelling the air through another NaOH trap. Any HCN which had not been absorbed by the trap in the Thunberg tube would have been absorbed by the second trap. On no occasion was any HCN found in the second trap. This procedure was repeated for several tubes in each run of analyses initially, but this was later deemed unnecessary. Aliquots of the NaOH solution trap from the Thunberg tube were tested for the presence of CN^- ions.

70mg of 'pyrazolone' (3-methyl-1-phenyl-5-pyrazolone; G.P.R. grade, B.D.H. Ltd.) and 14mg of 'bispyrazolone' (3,3'-dimethyl-1,1'-diphenyl-[4,4'-bi-2-pyrazoline]-5,5'-dione; G.P.R. grade, B.D.H. Ltd.) were mixed with 25ml of pyridine ('Analar' grade, B.D.H. Ltd.); if larger quantities were required, the ratios were maintained. The pyrazolone - pyridine reagent had to be freshly prepared on each occasion, although the pyrazolone - bispyrazolone mixture could be stored in the ratio 5:1 (Dr. P. Maher, Open University; personal communication).

From each NaOH trap to be tested, an aliquot of suitable size was taken. The size of each aliquot was 0.1 - 1ml depending on the expected amount of HCN; generally, 0.1ml was tested initially, with 1ml later being tested if a very low amount of HCN was measured on the first occasion. Each aliquot was placed in a sealable reaction tube (for convenience, screw topped 70mm x 15mm glass tubes were used). The volume was made up to 1ml by the addition of 0.1M NaOH. To this was added 1ml of 0.15M NaH_2PO_4 (G.P.R. grade, B.D.H. Ltd.). The tubes were then placed on ice because the products of subsequent steps were volatile. 0.5ml of freshly prepared 5mM chloramine-t (G.P.R. grade, B.D.H. Ltd.) were added and the tubes shaken to thoroughly mix their contents. The tubes were then left for more than 2 minutes.

After this length of time, 1ml of the pyrazolone - pyridine reagent was added and the mixture turned pink if CN^- was present. The mixture can then be left to stand at room temperature. After thirty minutes the mixture has turned blue. The blue pigment is fully developed after sixty minutes. It is stable at least overnight, but the absorbance of the mixture was measured after ninety minutes as standard practice. The absorbance was read at 620nm using a Corning 252 Bench Colorimeter. Greater accuracy could be obtained using a spectrophotometer set to 620nm, but because of the hazardous nature of pyridine the absorbance had to be read in a fume cupboard, which was only possible using a bench colorimeter.

A standard curve was prepared for each set of analyses. This was obtained by using 0 - 100µl of 0.185mM KCN in 0.1M NaOH ($\equiv$ 4.8µg HCN ml^{-1})

in place of 0.1ml from the NaOH trap. A straight line calibration curve is obtained for amounts of CN^- up to 1µg in the reaction mixture (Figure 2.3). There was little variation between the calibration curves obtained on different occasions. The limit of detection was approximately 0.01µg CN^- in the reaction mixture.

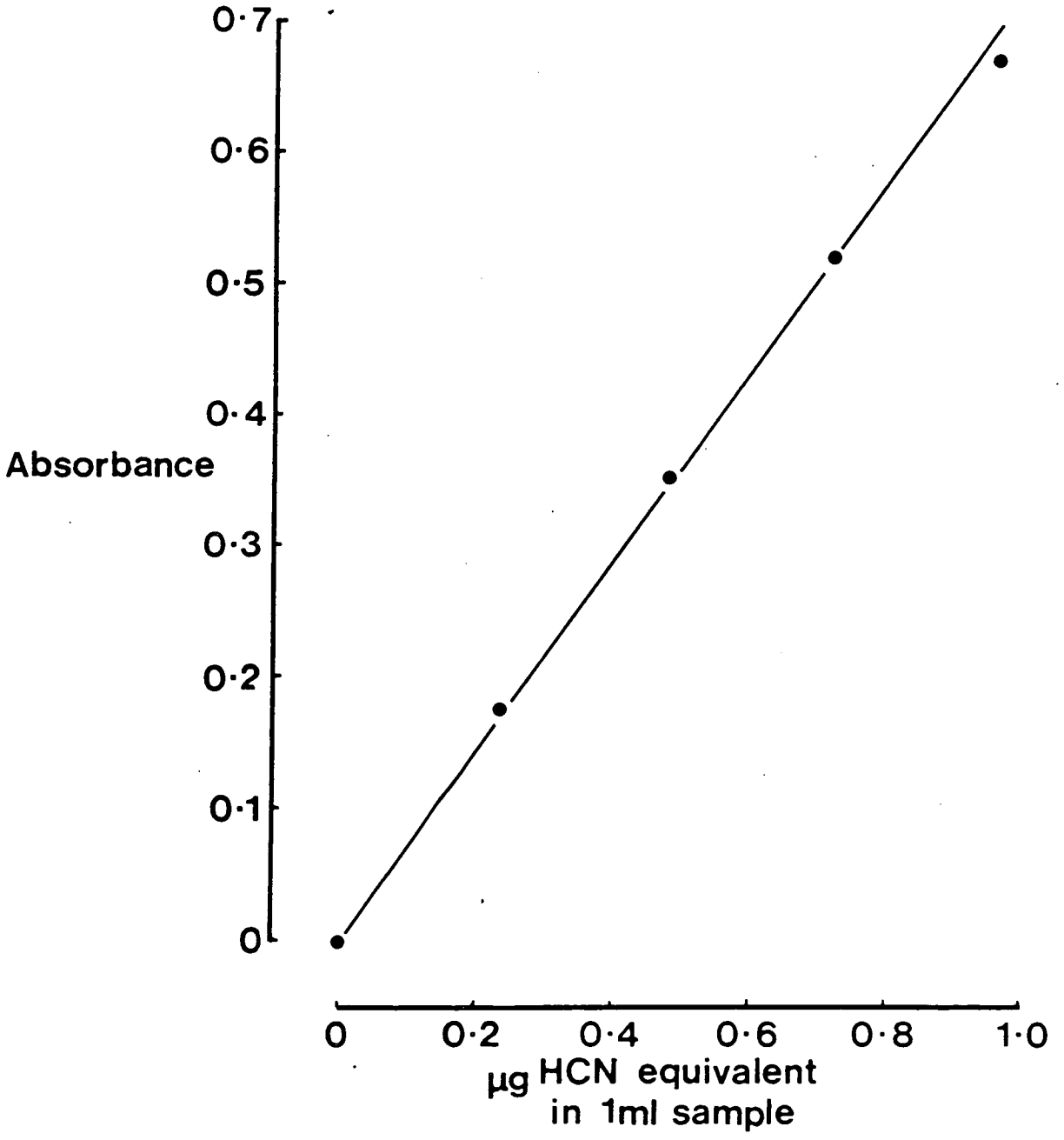
2.2.3. The Preparation of β-glycoside Extracts.

The β-glycoside from bracken, prunasin, was extracted from frond samples in order to provide an estimate of the β-glycoside content independently of the enzyme activity within the tissue. The method of extraction is based on that given by Pusey (1963).

Samples of bracken fronds were taken and weighed. The samples were placed in pyrex boiling tubes, together with two drops of water to prevent scorching, and autoclaved at 20 p.s.i. for 20 minutes. The use of the autoclave destroyed the β-glycosidase enzyme without affecting the glycoside, which is stable at high temperatures (Conn, 1980). Each sample was then ground in a homogeniser containing 1ml of Na_2HPO_4-citric acid buffer at pH5. The homogenate was placed in a centrifuge tube and the homogeniser washed out with a further 0.5ml of buffer, also put into the centrifuge tube. The homogenate was spun in a bench centrifuge at high speed for five minutes to remove any insoluble plant debris. The homogenate from each sample was then decanted into a test tube and placed on ice.

Commercially produced β-glycosidase, extracted from Prunus species and active on prunasin (Sigma Ltd.), was resuspended in 50ml of Na_2HPO_4-citric acid buffer at pH5 and placed on ice. Thunberg tubes were cooled in ice and 1ml of the enzyme suspension was placed in the lower vessel of each tube. Into the upper vessel of each tube was placed 2ml of 0.1ml NaOH. 0.5ml of the β-glycoside extract was then put into the lower vessel, the Thunberg tube was sealed by the upper vessel and the enzyme suspension and glycoside extract were gently shaken so that they were well mixed. The Thunberg tubes were then placed in a water bath at 35°C for 2 hours. The Thunberg tubes were then left overnight to ensure that all the HCN released from the glycoside extracts was absorbed by the NaOH trap. The NaOH in each trap was then analysed by the pyridine - pyrazolone test as described above (section 2.2.2).

Figure 2.3. The calibration curve for the pyridine - pyrazolone method of CN^- detection. The curve is prepared by the regression of the absorbance of the CN^- - pyridine - pyrazolone mixture at 620nm against known concentrations of CN^- in 0.1M NaOH solution. The standard curve was prepared with each run of the pyridine - pyrazolone test.



2.3. Plant Material and Growth Conditions.

2.3.1. Gametophytes.

2.3.1.1. Spore Material.

Gametophytes were initially grown from spores collected from individual fronds from Blackhill, Midlothian (map reference NT1964) (spore stock B10) and from Botley Hill, Titsey, Surrey (map reference TQ4055) (spore stock Ti) by Dr. A.F. Dyer in the summer of 1982. Later collections were made by myself of sporing fronds at my field sites in the Lothians (chapter 3). These fronds had previously been tested for cyanogenesis in the summers of 1983 and 1984. The spores remain viable for several years, although the percentage of spores germinating decreases with time (Dyer, 1979). There was no reason to believe that the material was anything other than Pteridium aquilinum ssp. aquilinum var. aquilinum, which is believed to be the only form of Pteridium that grows in Britain (Tryon, 1941; Page, 1976).

2.3.1.2. Spore Sterilisation.

It is preferable to sterilise fern spores grown on mineral media in order to maintain replicable conditions, since fungal and bacterial contaminants may unduly affect the growth and development of gametophytes in an unpredictable way. However, because pteridophyte spores are single cells with little protection from the sterilising agent, techniques used for sterilising angiosperm tissues cannot be used because they may kill the spores as well as the contaminants. The surfaces of fern spores are sculptured and may frequently have bacteria and fungal spores adhering.

Several techniques have been published for sterilising fern spores to maintain sterility under culture. The most common method reported is the use of dilute concentrations of $CaCl_3O$ (calcium hypochlorite) (Steeves et al, 1955; Dyer, 1979), a technique frequently used to surface sterilise angiosperm seeds or tissues. Steeves et al (1955) suspended spores of P.aquilinum vars. latiusculum and aquilinum in 3.5% solution of $CaCl_3O$ for ten minutes. They did not report what percentage of germination was achieved or whether surface sterilisation affected the subsequent growth of the gametophytes.

Schwabe (1951) used two methods of surface sterilisation of spores of P. aquilinum. The first involved the use of 0.036% $HgCl_2$ (mercuric chloride) in which the spores were suspended. This was sufficient to remove contamination but the growth and development of the gametophytes was

severely retarded. The second method employed repeated washing of the spores in sterile water. He noted that 10 to 12 washes were sufficient to remove all contaminants, effectively surface sterilising the spores without affecting the growth of the gametophytes. For this reason, he adopted the latter method.

In preliminary trials both 1% and 0.5% (by weight) solutions of $CaCl_3O$ were used. The spores were suspended in the solutions for two minutes and then centrifuged using a bench centrifuge at high speed. The sterilant was pipetted off and the spores were resuspended in sterile water, shaken to remove traces of the sterilant and centrifuged again. The spores were washed twice more and then plated onto nutrient media. The 1% $CaCl_3O$ solution reduced the germination of the spores to zero whilst the 0.5% solution reduced the germination to 2% of untreated controls.

In contrast, washing the spores in sterile water ten times reduced contamination to zero with no adverse effects on gametophyte growth and development. Because of its simplicity and efficiency without affecting the growth of the plants, this technique was adopted throughout in the culture of gametophytes from spores.

2.3.1.3. Culture Medium.

Fern gametophytes have been grown on a wide range of experimental nutrient media, with or without the addition of agar (see Dyer (1979) for a review of different media). A modified Mohr's medium was used for all the material grown from spores in these investigations (Dyer, 1979; Table 2.3). The fresh medium had a pH of 6.8. No micronutrients were included. The medium was autoclaved at 126°C and 20 p.s.i. for 20 minutes, and then plated out onto 5cm diameter sterile petri dishes or 3.5cm six-well multiwell dishes (Sterilin Ltd.).

2.3.1.4. Spore Sowing.

Following washing (section 2.3.1.2), the spores were centrifuged and resuspended in 1ml of sterile water. A drop of this was placed on a haemocytometer and the number of spores in the sample was counted. The number in subsequent samples was adjusted by the addition of further sterile water to the suspension until there were 300 spores ml^{-1}. Approximately 30 spores, contained within 0.1ml of this suspension, were plated onto the agar in a petri dish or the dish of a multiwell plate. The drop of suspension was spread over the whole surface of the plate. The plate was then sealed with

Table 2.3. Modified Mohr's Medium. The contents of the medium are based on a general medium (Dyer, 1979). The medium was used as a growth medium for gametophytes.

Mineral	Concentration in medium, g l^{-1}
$MgSO_4$, $7H_2O$	0.51
KNO_3	0.12
$FeCl_3$	0.017
$Ca(NO_3)_2$, $4H_2O$	1.44
K_2HPO_4	0.25
Agar (Oxoid #3)	5.00

'Parafilm' (American Can Corporation). Sterile conditions were maintained throughout the sowing to ensure that the plates were uncontaminated. Any plates showing signs of contamination were discarded.

2.3.1.5. Culture Conditions.

The petri dishes and multiwell plates were placed on black trays floating in a water bath adapted as a culture tank by Grant's of Cambridge (Dyer, 1979) (Figure 2.4). The temperature of the water bath was maintained at 20°C ± 0.5°C by means of a thermostatically controlled water heater working antagonistically with a refrigeration unit. The cultures were supplied with continuous illumination from two 2ft (61cm) 'Ekco 40W Daylight white G.P. Quickstart' fluorescent lights with a colour temperature of 4300K. The height of these lights above the cultures was adjusted to provide 20μE m^{-2} sec^{-1} at the level of the cultures. The intensity of the illumination was regularly measured using a portable light meter and the height of the lights was adjusted if necessary. These lights provided the only source of illumination; the black trays prevented illumination from the sides or reflecting from the bottom of the tank. The tank was covered by glass which maintained a high humidity within the black trays. Under these conditions the gametophytes grew quickly, following a normal pattern of development.

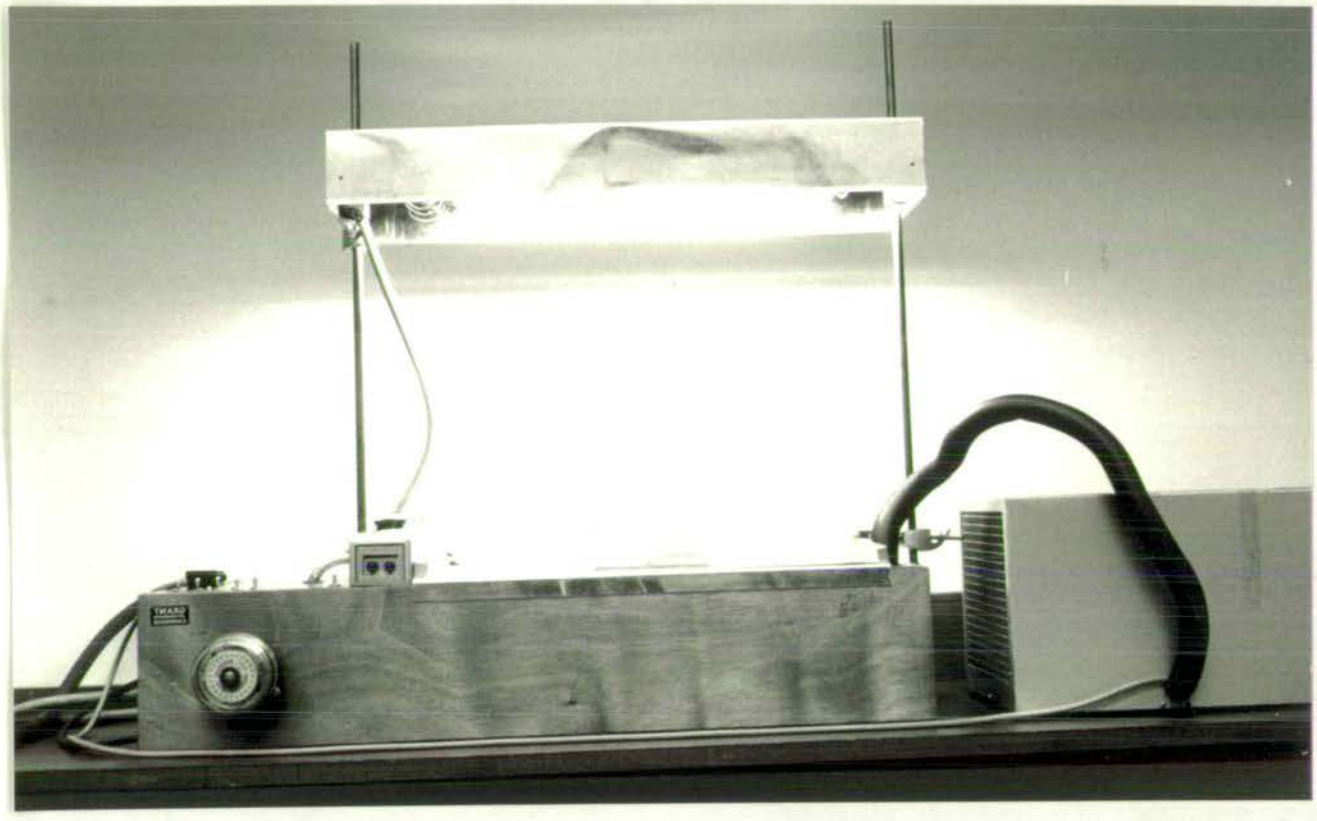
During four periods of equipment failure, gametophyte cultures were placed in a growth cabinet. The intensity of the light in the cabinet and the temperature were kept at the same levels as for the culture tank. However, the humidity in the growth cabinet would have been somewhat lower than in the culture tank as there was no water source in the cabinet. Since the petri dishes were tightly sealed the humidity within each petri dish or multiwell dish would have been maintained, the different conditions having no effect on the gametophytes.

2.3.1.6. Preparation of Gametophyte Clonal Material.

Miller (1968) noted that plasmolysis of gametophytes could lead to the proliferation of gametophytic tissue from the plasmolysed area. Such growth is probably caused by single cells within the gametophyte becoming isolated from neighbouring cells due to damage to their plasmodesmata (Nakazawa, 1963), allowing each cell to develop independently.

This response was used as a method of obtaining clonal gametophyte material. This is necessary for experimentation in order to remove the

Figure 2.4. The water bath adapted as a growth chamber for gametophytes and juvenile sporophytes. The temperature is maintained by the use of a heating unit and a refridgeration unit working in antagonism, controlled by a thermostat. The plants were grown on mineral agar media in petri dishes and multiwell dishes. The dishes were placed on black trays which were floated on the water surface. Constant illumination was provided by lights suspended above the growth tank. (Photograph: A.F. Dyer).



variation encountered in dealing with material from more than one spore source.

0.1M solution of $CaCl_2$ (G.P.R. grade, B.D.H. Ltd.) was sterilised by autoclaving for 20 minutes at 126°C and at 20 p.s.i. Maintaining sterile conditions, a large gametophyte (approximately 0.5cm in diameter) was removed from the culture medium and floated on a small amount of the $CaCl_2$ solution in a petri dish. The petri dish was placed under a dissection microscope so that the progress of plasmolysis could be observed. When a large number of cells had been plasmolysed (typically after 2 minutes), the gametophyte was taken from the solution and placed in a clean petri dish which was then flooded with sterile water and left until all the cells had become fully turgid. The gametophyte was then placed on fresh nutrient medium prepared as described in section 2.3.1.3. and replaced in the culture tank or growth cabinet. Excessive plasmolysis, due to the use of too high a concentration of $CaCl_2$ or too long a treatment, resulted in the gametophyte becoming necrotic and generally led to its death.

Two to three weeks after plasmolysis, outgrowths of tissue from the surface of the gametophyte were visible. These developed as individual gametophytes and at the same rate as gametophytes raised from spore. Six weeks after plasmolysis (Figure 2.5) the cloned gametophytes were large enough to be separated from the parent gametophyte and transferred to individual petri dishes or multiwell dishes, to grow on in isolation. The multiwell dishes were particularly useful since they allowed gametophytes of common origin to be kept together. The daughter gametophytes can be used experimentally or themselves plasmolysed to produce more clonal material.

2.3.2. Sporophytes.

2.3.2.1. Sporophytes Raised from Spores.

After six to eight weeks, gametophytes grown according to the method given in 2.3.1 become fertile. Gametophytes of Pteridium can bear both antheridia and archegonia, although unisexual gametophytes are frequently encountered. On gametophytes which produce both types of gametangia, the antheridia are formed first, the antherozoids usually being released before the archegonia are fertile. This is likely to minimise intra-gametophytic selfing. The archegonia then develop and become fertile. Archegonia can be long-lived, and if the archegonia of a gametophyte remain unfertilised, secondary antheridia may be formed to promote intra-gametophytic selfing.

Figure 2.5. A plasmolysed gametophyte growing on mineral agar medium. The gametophyte was plasmolysed in 0.25M $CaCl_2$ and then plated onto fresh agar medium. After two - three weeks outgrowths are visible, which grow into small gametophytes which can be removed and grown on. In this way clonal stocks of gametophytes were built up.

Usually, however, within a culture of gametophytes several gametophytes would be at different stages of fertility, and inter-gametophytic selfing (Klekowski, 1979) would be most likely to occur. Since within any one culture of gametophytes the spores came from the same frond, this is the equivalent of self fertilisation in the angiosperms. Apogamous sporophyte formation was not observed.

Fertilisation of the archegonium produces the diploid zygote and initiates the sporophyte generation. Within ten days the first sporophyte frond is visible (Figure 2.6) and the sporophyte root system develops. The second frond develops within a further ten days. The later fronds develop rapidly. After approximately four weeks the gametophyte dies, the sporophyte being wholly independent from an early stage.

After the second or third frond had been produced, the young sporophytes with attached gametophytes were removed from the gametophyte culture. If this was not done, the growing fronds quickly became constricted by the lid of the petri dish or multiwell dish, the developing root systems became tangled, and the culture overcrowded. The sporophytes were grown on in agar made up according to the instructions in section 2.3.1.3, within sterilised 50mm x 20mm glass tubes stoppered by sterilised corks to facilitate gaseous exchange. The tubes were then replaced in the culture tank or growth chamber and grown under the same conditions as the gametophyte cultures (section 2.3.1.5). After several fronds had been produced, the growing sporophytes were transferred to a closed propagation unit in the greenhouse and the corks removed. The propagation unit maintained the high humidity required by the sporophytes at this stage. Ventillation in the propagation unit was increased to acclimatise the sporophytes. After two weeks the sporophytes were pricked out into 50mm square pots filled with commercial fine peat and 'Perlite' (Fisons Ltd.) in the ratio 3:1. The pots were replaced in the propagation unit for a further two weeks, and were then placed on the open greenhouse bench.

After nine fronds had been produced the aerial rhizome system divided, producing two horizontal rhizomes (Dasayanake, 1960). At this stage the plants were repotted to allow for rhizome growth. The plants were placed into the appropriate size of pot. Regular repotting was required since the rhizome system of each sporophyte divided repeatedly and grew rapidly, as had been reported by other authors (Conway, 1953; Webster and Steeves, 1955; Dasayanake, 1960). The young sporophytes remained in their juvenile form whilst they were grown in the greenhouse. They did not produce spores under greenhouse conditions, although according to other workers this is not always

Figure 2.6. Juvenile sporophytes growing on mineral nutrient medium. The first sporophyte fronds are formed a week or so after fertilisation of the female gametophyte.

the case (Professor R. Kirkwood, University of Strathclyde, personal communication; Dr. E. Sheffield, University of Manchester, personal communication).

2.3.2.2. Greenhouse Conditions.

2.3.2.2.1. The Botany Department of Edinburgh University.

Sporophytes grown at the Botany Department were grown on in an unshaded greenhouse. From August 1st of each year until May 31st of the following year natural light was supplemented by several 400W mercury vapour lamps (Philips Ltd.) suspended 1.5m above the bench surface to maintain a daylength of 16 hours. Throughout the year the minimum temperature within the greenhouse was 16°C. The maximum temperature within the greenhouse was that of the ambient temperature, if greater than 16°C.

2.3.2.2.2. The Royal Botanic Garden (R.B.G), Edinburgh.

Sporophytes were also grown in a greenhouse at the R.B.G. Only natural light was available. From October 15th of each year until March 15th of the following year the greenhouse was shaded by the application of white-wash over the glass. This was the policy of the R.B.G. During this period sporophytes at the R.B.G. were grown on but not used for experiments. Throughout the year the minimum night temperature was maintained at 13°C, the minimum day temperature was 15.5°C and the maximum temperature was 27°C.

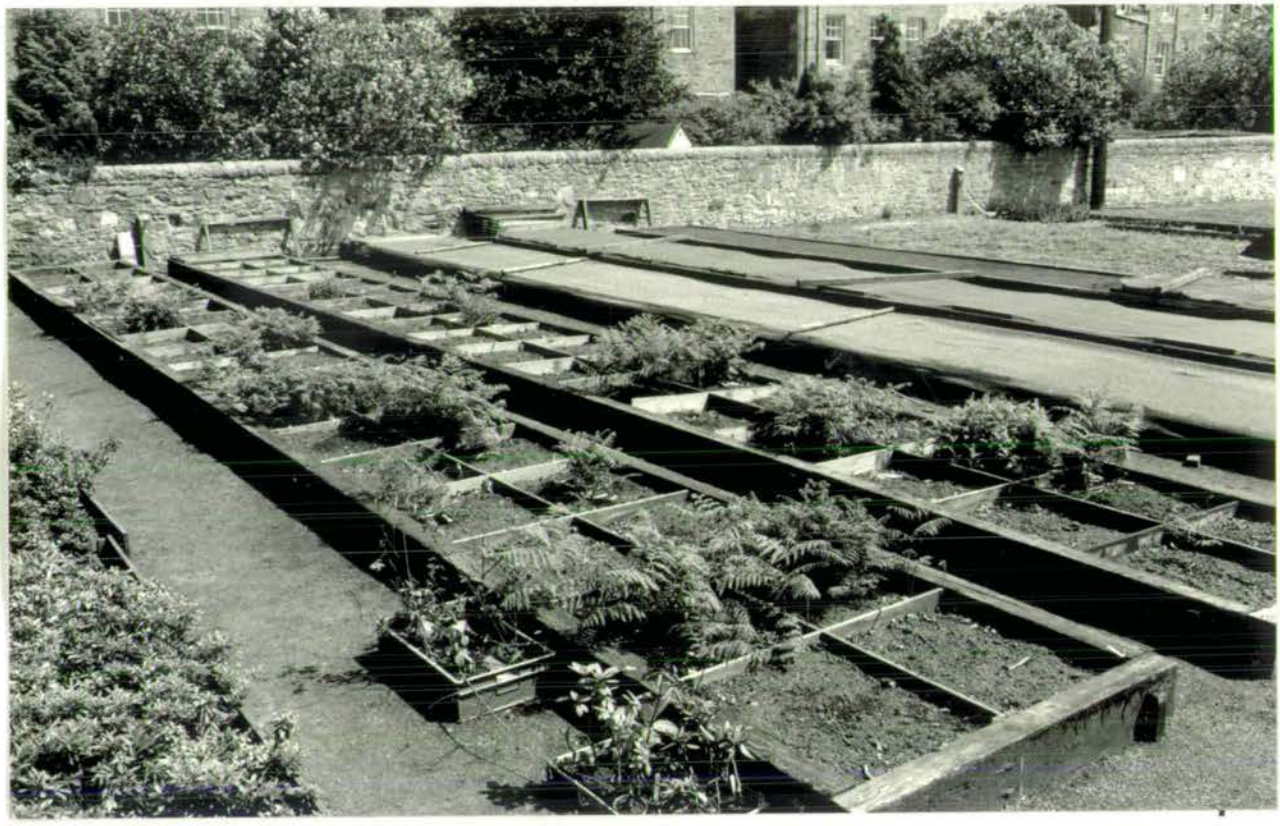
2.3.2.3. Sporophytes Grown From Wild Rhizome Collections.

Collections of living rhizome material were made from five sites at two locations in Scotland (chapter 3). Fronds at these sites had previously been tagged and tested for cyanogenesis using the picrate paper test (section 2.2.1.1) by Dr. A.F. Dyer (in 1982) or by myself (in 1983). The fronds were relocated after they had senesced in the autumn. Segments of rhizomes still attached to the tagged fronds were dug up and detached from the ramifying rhizome system.

The rhizome segments, 10 - 15cm in length, were placed in 20cm diameter pots containing fine peat. They were left in a sheltered position between the greenhouses at the R.B.G. throughout the winter.

The collections made in autumn 1982 were transplanted into 1m x 1m plots in the nursery at the R.B.G. (Figure 2.7). The plots were filled with fine peat to

Figure 2.7. Transplanted bracken growing in the nursery plots at the Royal Botanic Garden, Edinburgh. The nursery was sheltered but offered no shading. Each plot was approximately 1 X 1m.



a depth of 35cm. The plots were separated by creosoted wooden railway sleepers. The plots were fed every three weeks from the end of April until the beginning of September each year with 'Maxicrop Triple Strength' (Maxicrop International Ltd.). The stock solution was diluted 1:1000, each plant receiving 7l of the diluted medium on each occasion (Table 2.4).

The rhizome collections made in autumn 1983 were treated as for the earlier material. However, because of lack of space, they were not planted out into nursery plots. Instead the rhizome segments were placed in 30cm pots containing 3:1 fine peat/ 'Perlite' mix and grown under greenhouse conditions at the R.B.G (Figure 2.8). These plants were fed weekly with plant feed, which was freshly prepared from mineral salts.

Approximately 50% of all the rhizomes collected from the wild produced fronds in the following season, the other 50% remaining dormant or dying.

2.3.2.4. Preparation of Sporophyte Clonal Material.

It was necessary to prepare clonal sporophyte material for experimental work in order to minimise variation within treatments which could be attributed to genetic differences. Sporophytes proved as easy to clone as gametophytes. Only sporophytes raised from spores were cloned but there was no indication that there would be any difficulty in applying the same technique to sporophyte rhizomes collected from the wild; indeed the cloning technique used was basically the same as that used to introduce rhizome segments from the wild into cultivation.

After the juvenile sporophyte was pricked out into the 3:1 peat/'Perlite' mix (section 2.3.2.1), the upright rhizome system divided to produce two divergent horizontally growing rhizomes. These divided repeatedly, as is reported for the plant in the wild (Watt, 1940; 1976). The development of the rhizome system was rapid, and the plants became pot-bound.

Ten centimetre sections of rhizome were excised from the plant to be cloned by cutting with a scalpel or pruning knife. These were repotted in 3:1 peat/'Perlite' mix in a pot of the appropriate size, depending on the use for the clone. The rhizomes grew quickly from dormant buds and produced fronds after four or five weeks, although this time was up to 10 weeks if the material was cloned in the winter. The rhizomes branched and could themselves be cloned once they had developed sufficiently. Cloning was usually performed on pot-bound plants. The cloning procedure is similar to the natural development of clones as described by Watt (1940), where the older part of the branched rhizome system dies and rots after a time, leaving separate individual branched rhizomes.

Table 2.4. The mineral content of the stock medium given to outdoor rhizome transplants. The analysis was provided by M.F. Perry (Maxicrop International Ltd., personal communication). Each nursery plot received 7l of the solution diluted 1:1000, every three weeks from April until September.

Macronutrients	$g\ l^{-1}$
N (as NH_4 and NH_2CONH_2)	33
P_2O_5	16
K_2O	33
Chelated micronutrients	$g\ l^{-1}$
Fe	0.120
Mg	0.120
Mn	0.026
Cu	0.020
Zn	0.020
B	0.030
Mo	0.001.

Figure 2.8. Transplanted bracken growing in pots in the greenhouse at the Royal Botanic Garden, Edinburgh.

Chapter 3. Field Study Sites.

Because of the importance of fieldwork in the various investigations, the details of the several field sites are grouped together in one chapter, describing the selection of the sites, measurements made at each site, and the characteristics of each site.

3.1. Site Selection.

The sites were chosen at each of nine locations, numbered one to nine (Figure 3.1). The particular reasons for selecting a location varied: each was chosen because there had been a large amount of research into the biology of bracken in the region, either published (locations 3 and 4; see Watt (1976) and Lawton (1976), respectively) or unpublished (locations 5 and 6; Dr. A.F. Dyer, personal communication); because the region was well known to have a large area of bracken infested land (locations 1, 7, and 9); and in order to provide the greatest geographical range (locations 2 and 8). Location 5 was used for most of the studies for reasons of practical convenience. Wherever possible, the help of local botanists was sought to aid in the selection of the sites at each location.

At each location, three habitats were studied whenever possible (Figure 3.1). These were a coastal habitat, an open heathland habitat, and a woodland habitat. These three habitat types were chosen as representing typical, but very different, habitats in which bracken is commonly found. The choice of these three habitats would therefore provide a wide ecological range within which salient features of the biology of the polymorphism in cyanogenesis could be identified.

The site selection for each of these habitats at each location was made after consultation with local botanists (locations 1, 2, 3, 4, 5, and 7), because a large amount of work into the biology of bracken had been done at the site (locations 3, 4, and 6) and after consultation of the latest edition of 1:50000 scale 'Landranger' Ordnance Survey Maps. The selection of particular sites also had to reflect the access to the site and permission to work at each site.

Possible sites for each habitat at each location were visited. Sites were only selected if the area of land on which bracken was growing exceeded 25m x 25m. If there were several possible sites within the selected habitat, the most vigorous patch of bracken was selected; this was judged subjectively. Although this process may have decreased the randomness and objectivity of

Figure 3.1. The locations and sites in Britain at which stands of bracken fronds were sampled. The numbers of the locations (1 – 9) correspond to those in Table 3.1. The map reference for each site is given in Table 3.1. ▲ coastal habitat sites; ■ open habitat sites; ● woodland habitat sites.

the site selection, it did mean that each site was the most vigorous in the area and that each site was selected using the same criteria, minimising differences between locations due to plant fitness. Several sites were subject to some disturbance due to man or to animals, either wild (especially deer and rabbits) or domesticated (mainly sheep although sometimes cattle as well). This reflects the nature of the land infested by bracken rather than any factor in the site selection.

Once a suitable site was selected, the procedure outlined in section 2.1 was performed. Table 3.1 gives the map references and names of each of the sites. It should be noted that it was not possible to find suitable sites for a coastal habitat at either location 4 (Yorkshire) or location 9 (Perthshire), despite attempts to do so. Similarly, a woodland site at location 5 (Lothians) could not be found until the field season of 1984, rather than that of 1983 as for all the other sites; it is for this reason that the woodland site at location 5 is numbered out of sequence. This site was not used in the analyses of the effects of habitat type and environmental factors on cyanogenesis.

3.2. Associated Measurements.

At each site, the physical characteristics were recorded in situ and from 1:10000 scale Ordnance Survey Maps. These characteristics were the altitude, the slope and the aspect of each site. They are given in Table 3.2.

Certain biological characteristics of each site were also measured. The mean frond height was obtained by measuring the maximum height (i.e. the distance from the frond tip to the ground along the rachis) of each frond sampled, using a 2m rule; it should be remembered that fronds often curved and that the real height of the frond above the ground might not be at the frond tip and may have been less than the height of the fully extended frond. The frond density (the number of fronds per square metre) of each site was measured in the centre of the site. Neither of these two measures was absolute, since the height of the frond will increase over the season, as will the frond density; emerging fronds were noted at the sites in location 5 throughout the season until September.

The index of bracken dominance (I.B.D.) is the product the mean frond height multiplied by the frond density, divided by 100. The I.B.D. has been used as a measure of vigour (Mitchell, 1977) and is similar to the assessment of frond form and the state of the whole plant used by Watt (1943). It must be noted that although the I.B.D. is an objective measure, it is not a real value; rather, it allows the state of the plant to be envisaged. The frond density and

Table 3.1. The Location and Field Sites. Sites were selected at nine locations around Britain. At each location bracken fronds were sampled at each of three habitats: coastal, open, and woodland. Coastal sites were not available at locations 4 (Yorkshire) or 9 (Perthshire). All locations and sites are number sequentially, except for the woodland site (site 25) at location 5 (Lothians) which was not sampled as part of the field survey for which all the other sites were used.

Location	Site	Habitat	Map Reference	Local Name
1. North Wales	1	Coastal	SH637813	Penmon Point
	2	Open	SH872605	adjacent to Llwyn Saint Farm
	3	Woodland	SH808578	Coed Hafod
2. Devon	4	Coastal	SX828372	Start Point
	5	Open	SX745900	Prestonbury Common
	6	Woodland	SX744897	Hore Wood
3. East Anglia	7	Coastal	TM475623	Sizewell Beach
	8	Open	TL756815	Lakenheath Warren
	9	Woodland	TL765848	Brandon Park
4. Yorkshire	10	Open	SE638377	Skipwith Common
	11	Woodland	SE655376	Skipwith Common
5. Lothians	12	Coastal	NT445779	Gosford Bay
	13	Open	NT191643	Blackhill
	25	Woodland	NT602802	Binning Wood
6. Galloway	14	Coastal	NX892548	Sandyhills Bay
	15	Open	NX882608	Clawbelly Hill
	16	Woodland	NX948678	Shambellie Wood
7. Argyllshire	17	Coastal	NM816297	Slatrach Bay
	18	Open	NM912274	Torr-an-tuirc (close to Creag Bhan)
	19	Woodland	NM892272	above Loch Nell
8. Sutherland	20	Coastal	NC682632	Aird Torrisdale
	21	Open	NC710582	adjacent to Achnabourin
	22	Woodland	NC711579	opposite Rhinovie
9. Perthshire	23	Open	NN779679	Glen Garry
	24	Woodland	NN778677	Clunies Wood, Glen Garry

Table 3.2. The Physical Characteristics of the Field Sites. The altitude, slope and aspect were all taken from the latest edition of 1:10000 (six inch) series of Ordnance Survey Maps. The altitude was measured to the nearest 5m unless there was a "spot-height" on the map to indicate more accurate measurement. The slope was estimated from a 100m transect running perpendicular to the contours and centred on the site, unless the slope was variable over 100m, in which case the slope was estimated between points over which it was constant. The aspect was measured perpendicular to the contours over the site. The key to the locations and sites can be found in Table 3.1.

Location	Site	Habitat	Altitude m	Slope (percent)	Aspect (compass point)
1	1	coastal	15	10	NNE
	2	open	275	25	ESE
	3	woodland	90	12	NNW
2	4	coastal	25	28	NNE
	5	open	215	40	WSW
	6	woodland	150	20	NE
3	7	coastal	5	5	E
	8	open	12	0	0
	9	woodland	10	0	0
4	10	open	10	0	0
	11	woodland	10	0	0
5	12	coastal	4	5	NNW
	13	open	300	12	NNW
	25	woodland	15	0	0
6	14	coastal	12	0	0
	15	open	135	23	NE
	16	woodland	40	10	SE
7	17	coastal	5	5	NNE
	18	open	120	12	SW
	19	woodland	50	30	NW
8	20	coastal	12	25	ESE
	21	open	10	5	ENE
	22	woodland	10	12	ENE
9	23	open	300	12	SSW
	24	woodland	285	15	SSW

the frond height are affected by environmental factors, particularly shading. In woodland sites the fronds are typically sparse, with a low frond density, and tall, being somewhat etiolated. This obviously alters the I.B.D. In addition, the increase in both frond height and frond density during the season will have an effect. However, all the measurements were made at the height of the season from late June until early August, several weeks after the initial flush of fronds in early May, and these measurements are likely to be reasonably constant at any one site during this period. These measurements are given for the 1983 field season for all sites except site 25, for which data are only available for 1984, in Table 3.3. Watt (1943) has shown that the frond height, and in particular the length of the petiole, is related to several features of the growing plant, especially the depth of the frond-bearing rhizome in the soil. The depth in the soil of the short shoot rhizomes was measured at each corner and in the centre of each site; the minimum depth only was recorded.

The mean sample weight was the mean fresh weight of 4cm tips of the lowermost pinnae from a representative sample of 10 or 20 fronds within the site, weighed in situ.

The number of associated species was recorded by identifying the plants found in five quadrats, each 1 metre square in size, placed at the corners and in the centre of each site. In addition, any plants prominent within the site or in the immediate vicinity but not found within any of the five quadrats were identified. It is possible that certain plant species not within the quadrats occurred at the site but were not noticed, particularly those that were not flowering at the time the site was visited. The measurements are given for the 1983 field season, except for site 25 for which the data collected in 1984 are given, in Table 3.4.

In order to assess the effect of the climate on the distribution of the polymorphism of cyanogenesis in bracken, it was necessary to estimate climatic variables for each site: the large number of study sites and their inaccessibilty from Edinburgh meant that individual recordings of climatic variables could not be undertaken. The climatic data for each site (Table 3.6) were estimated by the use of equations recently published with a set of climatic maps (White and Smith, 1982). The equations were devised using multiple regression analyses based upon the physical characteristics of the sample sites, a total of 61 weather stations recorded over ten years, and allow the prediction of values for several climatic characteristics. Only those climatic features with a high predictive accuracy or felt to be relevant to the ecology of bracken were estimated. These climatic variables were the mean air

Table 3.3. The characteristics of bracken fronds at each field site. The mean frond height is taken from the maximum height of each frond sampled at the site in the summer of 1983, except for the woodland site at location 5 (site 25) which was sampled in the summer of 1984. The maximum frond height was taken to be the distance from the soil surface to the tip of the frond. The frond density was the number of fronds inside a 1m X 1m quadrat placed in the centre of the site. The index of bracken dominance (I.B.D.) is mean frond height multiplied by the frond density divided by 100. It can be used as a measure of vigour of frond growth (Mitchell, 1977) to compare sites. The key to the locations and sites can be found in Table 3.1.

Location	Site	Habitat	Mean Frond Height cm, ±S.E.	Frond Density fronds m^{-2}	I.B.D.
1	1	coastal	95.5 ±2.22	24	22.92
	2	open	107.9 ±2.39	25	26.97
	3	woodland	98.0 ±3.19	18	17.64
2	4	coastal	102.9 ±1.90	35	36.01
	5	open	115.6 ±2.99	32	36.99
	6	woodland	113.5 ±2.81	18	20.39
3	7	coastal	100.4 ±2.91	25	25.10
	8	open	93.5 ±2.11	25	23.37
	9	woodland	131.8 ±4.82	18	19.77
4	10	open	102.4 ±1.59	34	34.82
	11	woodland	166.9 ±2.54	30	50.07
5	12	coastal	142.9 ±2.06	42	60.02
	13	open	69.4 ±1.86	56	38.86
	25	woodland	127.7 ±3.65	14	17.88
6	14	coastal	136.5 ±2.79	36	49.14
	15	open	119.9 ±4.18	37	44.36
	16	woodland	155.0 ±3.38	21	32.55
7	17	coastal	130.8 ±3.41	37	48.40
	18	open	108.5 ±3.19	27	29.29
	19	woodland	89.7 ±3.00	20	17.94
8	20	coastal	67.3 ±1.69	27	18.17
	21	open	93.7 ±2.39	24	22.50
	22	woodland	112.3 ±2.38	33	37.06
9	23	open	157.9 ±1.95	32	50.53
	24	woodland	98.7 ±2.03	19	18.75

Table 3.4. The sample weight, rhizome depth and number of associated species at each field site. The mean sample weight is the mean of 10 or 20 representative 4cm lowermost pinna tips taken at random from within the site and weighed in situ. The rhizome depth is the minimum depth in the soil of short shoot rhizomes, taken from the depth of short shoot rhizomes at the centre of the site and at each corner. The associated vascular plant species were identified within 1m X 1m quadrats placed at the centre and each corner of the site; any species which were present at the site and identified were also included. The measurements were made in the summer of 1983, except for the woodland habitat at location 5 (site 25) for which measurements made in the summer of 1984 are given. The key to the locations and sites can be found in Table 3.1.

Location	Site	Habitat	Mean sample weight mg, ±S.E.	Minimum rhizome depth, cm	Number of associated vascular plant Species
1	1	coastal	164.5 ±7.97	25	7
	2	open	205.0 ±11.42	13	14
	3	woodland	114.2 ±12.10	10	13
2	4	coastal	189.2 ±20.98	8	14
	5	open	154.5 ±22.15	17	12
	6	woodland	87.2 ±3.64	16	14
3	7	coastal	130.8 ±20.57	21	7
	8	open	143.4 ±20.70	21	5
	9	woodland	75.8 ±13.69	18	11
4	10	open	137.6 ±21.44	15	0
	11	woodland	58.2 ±3.79	8	5
5	12	coastal	170.6 ±20.44	22	20
	13	open	87.8 ±4.63	11	10
	25	woodland	72.9 ±4.30	19	4
6	14	coastal	279.3 ±33.05	32	13
	15	open	173.3 ±19.30	5	12
	16	woodland	96.5 ±5.60	12	13
7	17	coastal	160.8 ±7.53	4	17
	18	open	137.4 ±9.15	5	9
	19	woodland	88.8 ±5.09	10	18
8	20	coastal	232.7 ±8.14	10	18
	21	open	151.3 ±7.01	10	13
	22	woodland	98.2 ±3.74	12	11
9	23	open	125.9 ±3.93	8	8
	24	woodland	93.7 ±6.72	7	9

Table 3.5. Analyses of soil macronutrients at each field site. The soil pH was measured within two hours after soil samples were taken from the centre and each corner of each site. 50g of soil was mixed with 50ml of distilled water and left to settle for 15 minutes, after which time the pH was measured using a portable pH meter. The remaining soil was kept for laboratory analysis of available phosphorus (P), potassium (K), magnesium (Mg), and total nitrogen (N). The levels of P,K, and Mg were obtained by extracting these minerals by shaking the soil for 30 minutes in ammonium acetate/acetic acid buffer and measuring the concentration of the ions in solution. The total N was measured by Kjeldahl digestion and determination. The analysis of macronutrients was machine-run and carried out at the Chemical Analytical Laboratory, East of Scotland College of Agriculture, Edinburgh by Dr. P.A. Crooks. The key to the locations and sites can be found in Table 3.1.

Location	Site	Habitat	Mean Soil pH ±S.E.	Levels of Available Nutrients			
				P	K	Mg	N
				mg kg^{-1}			%
1	1	coastal	5.52 ±0.17	0.8	201	191	0.224
	2	open	4.62 ±0.03	4.1	92	37	0.521
	3	woodland	4.13 ±0.10	1.3	67	53	0.662
2	4	coastal	4.98 ±0.17	0.8	275	313	0.582
	5	open	3.78 ±0.16	0.8	193	72	1.054
	6	woodland	3.64 ±0.20	3.3	184	172	0.711
3	7	coastal	3.84 ±0.16	4.2	21	49	0.118
	8	open	4.88 ±0.27	1.1	38	12	0.092
	9	woodland	4.07 ±0.05	4.3	29	17	0.098
4	10	open	3.42 ±0.05	5.6	222	36	0.308
	11	woodland	3.88 ±0.41	4.1	113	94	1.042
5	12	coastal	4.70 ±0.18	1.5	34	125	0.146
	13	open	3.44 ±0.12	2.3	70	66	0.878
	25	woodland	3.55 ±0.04	2.6	49	62	0.573
6	14	coastal	5.74 ±0.38	9.9	28	101	0.133
	15	open	3.32 ±0.08	2.6	131	111	1.055
	16	woodland	3.78 ±0.009	1.0	65	70	0.677
7	17	coastal	4.86 ±0.04	3.0	205	341	0.998
	18	open	4.07 ±0.18	1.6	101	94	1.026
	19	woodland	4.65 ±0.14	0.8	57	106	0.735
8	20	coastal	4.98 ±0.48	0.8	106	272	0.497
	21	open	4.54 ±0.10	1.1	62	119	0.327
	22	woodland	4.29 ±0.12	5.9	204	384	0.673
9	23	open	3.96 ±0.17	1.3	74	45	0.368
	24	woodland	3.65 ±0.115	1.4	64	69	0.532

Table 3.6. The estimated daily mean air temperature at 0900 hours, 1.3m above ground level, for each field site. The values were estimated using published equations obtained by multiple regression analysis of values recorded in the field (White and Smith, 1982). The key to the locations and sites can be found in Table 3.1.

Location	Site	Habitat	Temperature, °C				
			Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Annual Mean
1	1	coastal	4.83	11.37	14.80	7.95	9.75
	2	open	2.66	10.26	16.32	5.47	8.75
	3	woodland	4.19	11.22	14.39	6.72	9.13
2	4	coastal	5.70	12.36	17.27	9.25	11.15
	5	open	4.91	10.75	10.94	7.75	8.59
	6	woodland	5.12	11.40	13.77	7.85	9.56
3	7	coastal	4.37	12.22	16.22	7.70	10.13
	8	open	3.93	12.55	15.91	6.70	9.77
	9	woodland	3.92	12.55	15.90	6.69	9.77
4	10	open	3.94	12.02	15.13	6.41	9.38
	11	woodland	3.94	12.02	15.13	6.41	9.38
5	12	coastal	3.95	10.75	14.14	6.82	8.92
	13	open	2.14	8.89	11.12	4.94	6.77
	25	woodland	4.06	10.96	14.92	6.54	9.12
6	14	coastal	4.37	11.00	14.42	7.36	9.29
	15	open	3.43	10.61	16.56	6.12	9.18
	16	woodland	3.60	10.98	13.19	6.09	8.47
7	17	coastal	4.15	10.41	13.57	6.88	8.75
	18	open	3.09	9.85	12.18	5.45	7.64
	19	woodland	4.39	10.17	10.64	5.45	6.61
8	20	coastal	3.13	10.13	15.12	6.09	8.63
	21	open	3.65	10.37	14.79	6.21	8.75
	22	woodland	3.65	10.37	14.79	6.21	8.75
9	23	open	1.45	8.76	10.63	3.90	6.19
	24	woodland	1.60	8.91	10.78	4.02	6.33

temperature at 9am, 1.3m above ground level, and the mean rainfall on the sites. It must be noted that these data are estimates for each quarter (January to March; April to June; July to September; and October to December) and as such only reflect the mean of each characteristic, rather than the extremes. It is possible in the case of those data referring to temperature that extremes are of greater importance to the ecology of bracken at any one site than estimates of the mean values. For this reason, values for the extremes of temperature were estimated from values recorded at weather stations in the locality of each study site, taken from Meteorological Office records for the thirty year period from 1930 to 1960 (Anon., 1972). These figures are means of the means of monthly minimum temperatures for the month of January; the means of the monthly minimum temperature early in the growing season (April - June) and the means of monthly maximum temperatures for the month of July. Alternative measurements are available for the mean daily and the absolute values for minimum and maximum temperatures but these were felt to be of less biological importance. Bracken has been shown to be affected by frost (Watt, 1950) and the best predictor of frost appears to be the mean monthly minimum temperatures, since the mean daily minima will smooth the extremes; similarly, the absolute minimum temperature for a site over the thirty year period of meteorological measurements is likely to be a rare event of little predictive value.The mean monthly maximum and minimum temperatures have then been adjusted for each study site by calculating the difference due to the lapse rate of temperature with increasing altitude, taken as -0.5°C per 100m increase in altitude for minimum temperatures and -0.7°C per 100m increase in altitude for maximum temperatures (Mr. Brown, Enquiries Officer, Meteorological Office, Edinburgh; personal communication). It must be noted that these figures for temperature do not take account of the topography of the site, which the quarterly estimates do (White and Smith, 1982). This may be important in establishing the effect of 'exposure', which is a combination of several features of the environment, or of predicting late frosts which may occur later at some sites due to 'frost hollows'; for instance, site 10 has occasionally suffered frosts into July (Professor J.H. Lawton, University of York; personal communication), causing frost damage to bracken fronds, although this is not evident from the data predicted for the site. The minimum and maximum temperatures for each study site are given in Table 3.7.

3.3: Site descriptions.

Certain observations made of the study sites are inadequately described in

Table 3.8. Estimates of quarterly rainfall for each field site. The values were estimated using published equations obtained by multiple regression analyses of values taken in the field (White and Smith, 1982). The key to the locations and sites can be found in Table 3.1.

Loc.	Site	Habitat	Mean Rainfall, cm				
			Jan-Mar	Apr-Jun	Jul-Sep	Oct-Dec	Total
1	1	coastal	19.01	21.34	26.45	27.37	94.43
	2	open	27.16	25.47	32.59	36.24	121.80
	3	woodland	23.70	22.91	27.63	31.92	106.47
2	4	coastal	18.10	18.80	19.20	21.94	78.25
	5	open	26.06	24.43	26.40	33.84	111.04
	6	woodland	24.55	21.90	24.91	31.99	103.65
3	7	coastal	12.98	14.09	15.49	18.19	60.93
	8	open	12.31	14.03	16.49	17.56	60.55
4	9	woodland	12.31	14.03	16.48	17.56	60.54
	10	open	11.58	14.20	17.87	18.31	62.13
	11	woodland	11.58	14.20	17.87	18.31	62.13
5	12	coastal	14.94	15.88	21.55	17.55	70.11
	13	open	27.85	25.85	31.59	31.71	116.66
	25	woodland	16.34	15.95	20.85	18.78	72.12
6	14	coastal	19.59	21.66	25.53	25.53	92.48
	15	open	23.60	22.76	29.32	30.16	106.14
	16	woodland	21.69	20.75	25.80	27.46	95.98
7	17	coastal	30.95	26.99	36.62	41.30	136.23
	18	open	33.20	29.02	38.71	43.45	144.79
	19	woodland	32.02	27.71	36.82	41.95	138.88
8	20	coastal	21.41	18.30	25.84	28.82	94.64
	21	open	21.70	19.22	25.58	29.13	95.91
	22	woodland	21.70	19.22	25.58	29.13	95.91
9	23	open	31.15	28.37	34.48	37.75	132.11
	24	woodland	30.99	28.08	33.90	37.50	130.83

a tabular form, particularly the species composition of the associated flora and morphological variations of the fronds of bracken. The vascular plant species found within the sites were identified using 'Excursion Flora of the British Isles' (Clapham, Tutin and Warburg, 1981), 'Grasses' (Hubbard, 1982), 'The Wild Flower Key' (Rose, 1981) and 'The Ferns of Britain and Ireland' (Page, 1982). The taxonomy used follows these references throughout. Using these works the species found were classified according to their usual habitat type (coastal or dunes, c; heathland and grassland, h; woodland, w; arable land, ar; wasteland, wst; and scrub, scb.). It is likely that at some sites not all the species present may have been identified, particularly those that were not in flower.

Location 1, site 1: Penmon Point, Anglesey (Figures 3.2 and 3.3). The coastal site in North Wales was on a moderately exposed point on a rocky coastline. The area was open to the public but there were no signs of recent disturbance. Certain fronds were scorched and this may have been the result of wind or salt burn. The associated flora consisted of: Crataegus monogyna (scb); Hyacynthoides non-scripta(w); Lonicera periclymenum (scb); Potentilla erecta (h); Rubus fruticosus agg. (scb); Sambucus nigra (w/scb); and Silene dioica (w/c).

Location 1, site 2: adjacent to Llywn Saint Farm (Figures 3.4 and 3.5). The open site in North Wales was below a field used for sheep grazing, which was separated from the site by a fence. It was adjacent to a dense wood, which contained no substantial populations of Pteridium aquilinum. The site was on a very steep slope and could not be grazed. There was no evidence of recent disturbance. The associated flora consisted of: Anemone nemorosa (w); Brachypodium sylvaticum (w); Chamaenerion angustifolium (wst/w); Galium aparine (scb); Holcus mollis (w); Hyacinthoides non-scripta (w); Lolium perenne (h); Lysimachia nemorum (w); Oxalis acetosella (w); Potentilla anglica (w); Rubus fruticosus agg. (scb); Silene dioica (w/c); Stellaria holostea (w); Ulmus glabra (w); and Viola riviniana (w). It should be noticed that several of these plants are characteristically woodland species, and that the only heath and grassland species was Lolium perenne, although Potentilla anglica is found on shaded heaths as well as woodland. It is possible that this site represents a formerly wooded site, although the preponderance of shade tolerant or woodland species may result from the shading of the habitat by the bracken fronds. Within the site were several fronds which had a dwarf morphology, although only one of these fronds was sampled (this being by chance rather than by design). The dwarf frond, A5, was 35cm in height compared to the mean height of 98.0 $\pm$3.19cm. It is possible that such dwarf

Figure 3.2. Penmon Point, Anglesey. The coastal habitat site at location 1. The photograph was taken from the south west.

Figure 3.3. The locality of Penmon Point, Anglesey. The position of the coastal habitat site at location 1 is shown by ▲. The bold numbers show grid reference lines. (Scale: 1/10000).

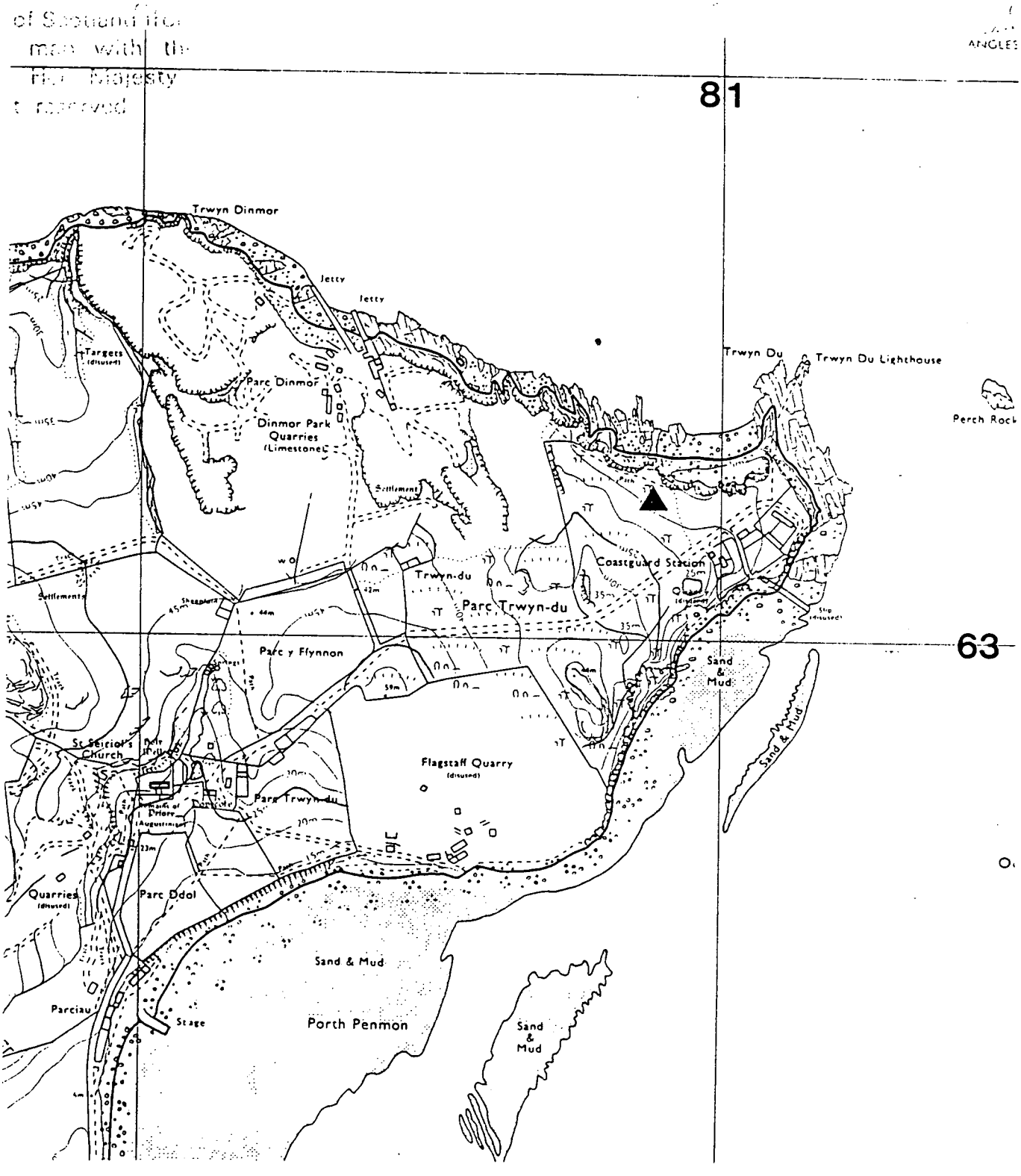
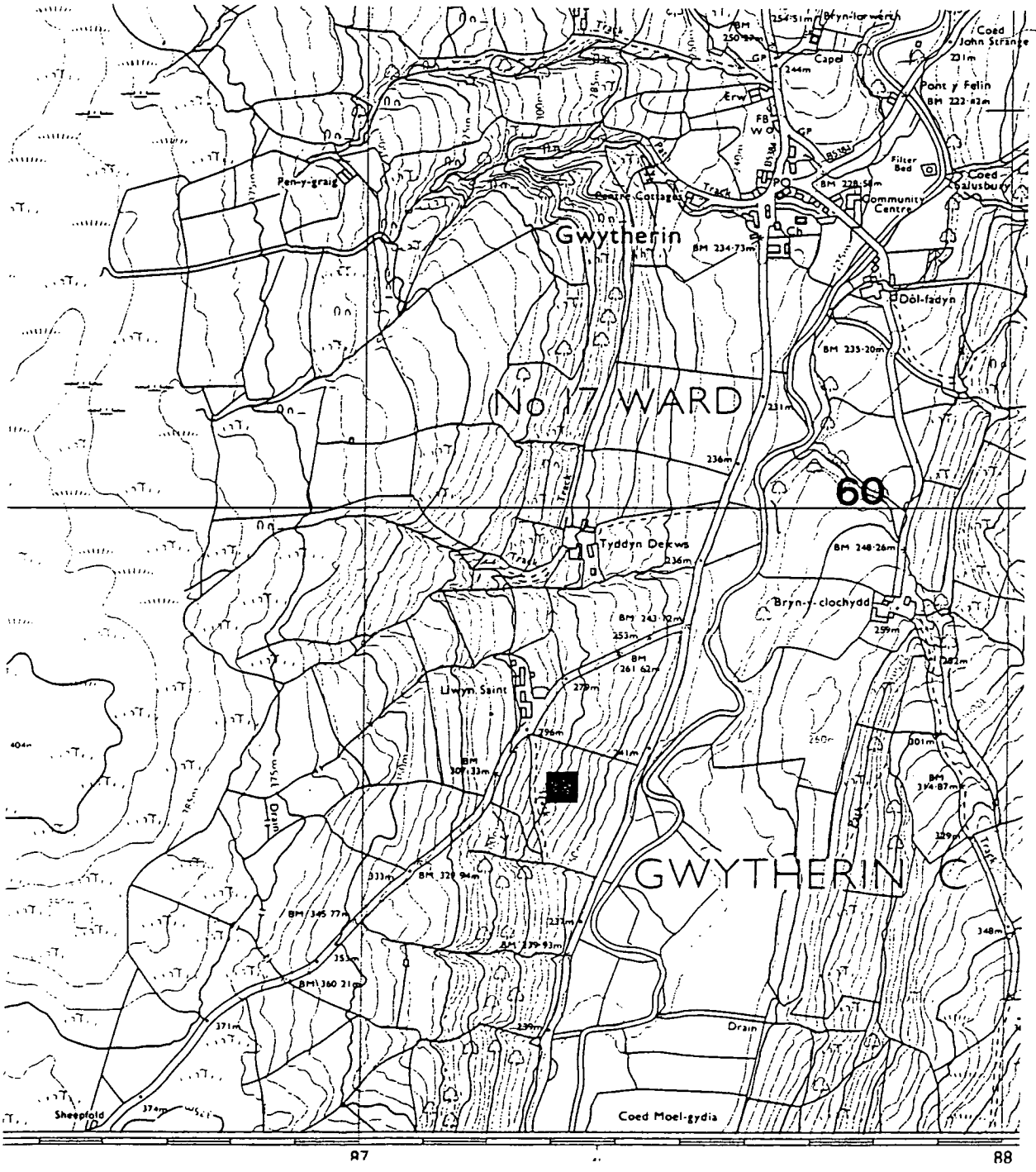


Figure 3.4. Llywn Saint Farm, North Wales. The open habitat site at location 1. The photograph was taken from the south.

Figure 3.5. The locality of Llywn Saint Farm, North Wales. The position of the open habitat at location 1 is shown by ■. The bold number shows the northing grid reference line. (Scale: 1/10000).



fronds represent a different clone within a population (see, for instance, Oinonen, 1967a,b) although such a great morphological difference may simply reflect the variation present within the plant or the physical conditions of the rhizome: Watt (1943) showed that the height of the frond was proportional to the depth of the rhizome in the soil, and dwarf fronds such as A5 may simply emerge from very shallow rhizomes, for instance those that have been forced towards the surface by an obstruction in the soil. Dwarf fronds were found at several other of the field sites; where they occurred it will be noted under that particular site.

Location 1, site 3: Coed Hafod Wood (Figures 3.6 and 3.7). The North Wales woodland site was in a large wood of Quercus petraea. It was on protected land although the wood was used for teaching and there was possibly some disturbance due to this. The associated flora consisted of: Anthoxanthum odoratum (h); Betula pubescens ?(seedling) (w); Deschampsia caespitosa (w); Dryopteris affinis subsp. borreri (w) Festuca gigantea (w); Holcus mollis (w); Hyacinthoides non-scripta (w); Ilex aquifolium (seedling) (w); Lonicera periclymenum (scb); Oxalis acetosella (w); Rubus fruticosus (scb); Polystichum setiferum (w); Stellaria holostea (w). There were large patches of the mosses Polytrichum juniperum and Rhytidiadelphus squarrosus.

Location 2, site 4: Start Point (Figures 3.8 and 3.9). The coastal site in south west England was on cliffs on an exposed point, although bracken was not found in the most exposed situations on the point: the site itself was to the north of the tip. There was scorching on several of the fronds and small trees nearby, suggesting wind or salt burn. The associated flora was: Acer pseudoplatanus (w); Arum italicum subsp. neglectum (fruiting) (c); Digitalis purpurea (w/h); Galium saxatile (h); Holcus lanatus (h); Hyacinthoides non-scripta (fruiting) (w); Lotus hispidus (c); Medicago arabica (c); Potentilla erecta (h); Ranunculus bulbosus (h); Rumex acetosa (h); Veronica sp. (vegetative); and Viola sp. (vegetative).

Location 2, site 5: Prestonbury Hill (Figures 3.10 and 3.11). The open location in south west England was adjacent to a sheep pasture, and several fronds on the site appeared to have been damaged by sheep: pinnae were broken and in some cases removed, although it is possible that ramblers or wind may have caused similar damage. There were several holes which were taken to be rabbit burrows although there was no evidence of droppings; the burrows may have been deserted. The fronds were very tough and leathery, probably due to the exposed nature of the site. Some fronds within the site

Figure 3.6. Coed Hafod, North Wales. The woodland habitat site at location 1. The photograph was taken from the south.

Figure 3.7. The locality of Coed Hafod, North Wales. The position of the woodland habitat site at location 1 is shown by ● . The bold number shows the easting grid reference line. (Scale: 1/10000).

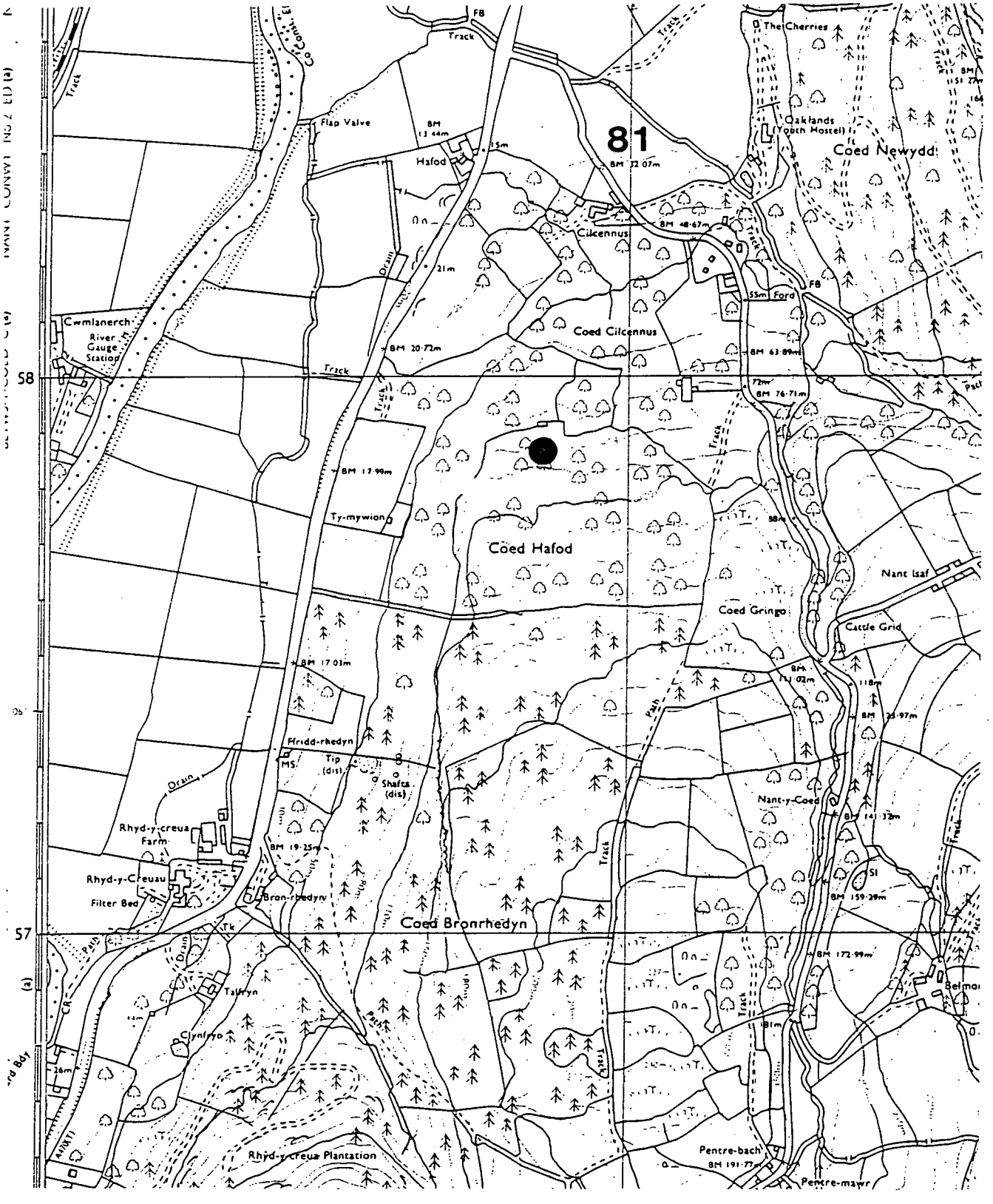


Figure 3.8. Start Point, Devon. The coastal habitat site at location 2. The photograph was taken from the south west.

Figure 3.9. The locality of Start Point, Devon. The position of the coastal habitat site at location 2 is shown by ▲. The bold numbers show grid reference lines. (Scale: 1/10560).

Figure 3.10. Prestonbury Hill, Devon. The open habitat site at location 2. The photograph was taken from the east.

differed markedly in morphology, the lamina of these being crispate with narrower, parallel-edged pinnules; none of these fronds were amongst those sampled. The associated flora consisted of: Acer pseudoplatanus (w); Digitalis purpurea (w); Erica tetralix (h); Fragaria vesca (h); Galium saxatile (h); Hedera helix (w); Potentilla erecta (h); Quercus petraea saplings (w); Rubus fruticosus agg. (scb); Rumex acetosella (h); Sorbus aucuparia (w); and Viola canina (h).

Location 2, site 6: Charles Wood (Figures 3.12 and 3.13). The woodland site in south west England was in a protected woodland which was subject to frequent amenity use. There was some evidence of damage by large herbivores or visitors; the upper pinnae had been removed from some fronds. Only one of these fronds was sampled, its lower pinnae being intact. It was reported that the wood may have contained a population of deer. The wood was made up of trees of Fagus sylvatica and Quercus petraea with some Betula pubescens. As well as these species, the associated flora consisted of: Corylus avellana (w); Digitalis purpurea (w); Dryopteris affinis subsp. robusta (w); Galium aparine (w/scb); Hedera helix (w); Hyacinthoides non-scripta (w); Ilex aquifolium (w); Lonicera periclymenum (scb); Oxalis acetosella (w); Rubus fruticosus agg. (scb); and Vaccinium myrtillus (w/h).

Location 3, site 7: Sizewell Beach (Figures 3.14 and 3.15). The coastal site in East Anglia was on a beach commonly used as an amenity. There was however no obvious disturbance to the bracken by members of the public. There were rabbit droppings, but no warrens were visible. Some fronds showed evidence of scorching; although indicative of salt-burn, other populations of fronds inland in East Anglia also showed signs of scorching. It is possible that this was due to droughting or perhaps late frost damaging already expanded fronds. All the fronds at this site were very tough and leathery. The associated flora consisted of: Ammophila arenaria (c); Carex arenaria (c); Elymus farctum (c); Festuca rubra (h/c); Galium verum (c/h); Rubus fruticosus agg. (scb); and Rumex acetosella (h).

Location 3, site 8: Lakenheath Warren (Figures 3.16 and 3.17). The open site in East Anglia also showed scorching, although only to isolated fronds. The bracken was very impenetrable although not particularly dense in terms of fronds m^{-2}; there was very little growing beneath the fronds. The only associated species were: Agrostis capillaris (h); Carex binervis (h); Festuca tenuifolia (h); Nardus stricta (h); and Urtica dioica (scb).

Location 3, site 9: Brandon Park (Figures 3.18 and 3.19). The woodland site in East Anglia was in a deciduous woodland bordering a conifer plantation. The

Figure 3.12. Charles Wood, Devon. The woodland habitat at location 3. The photograph was taken from the south.

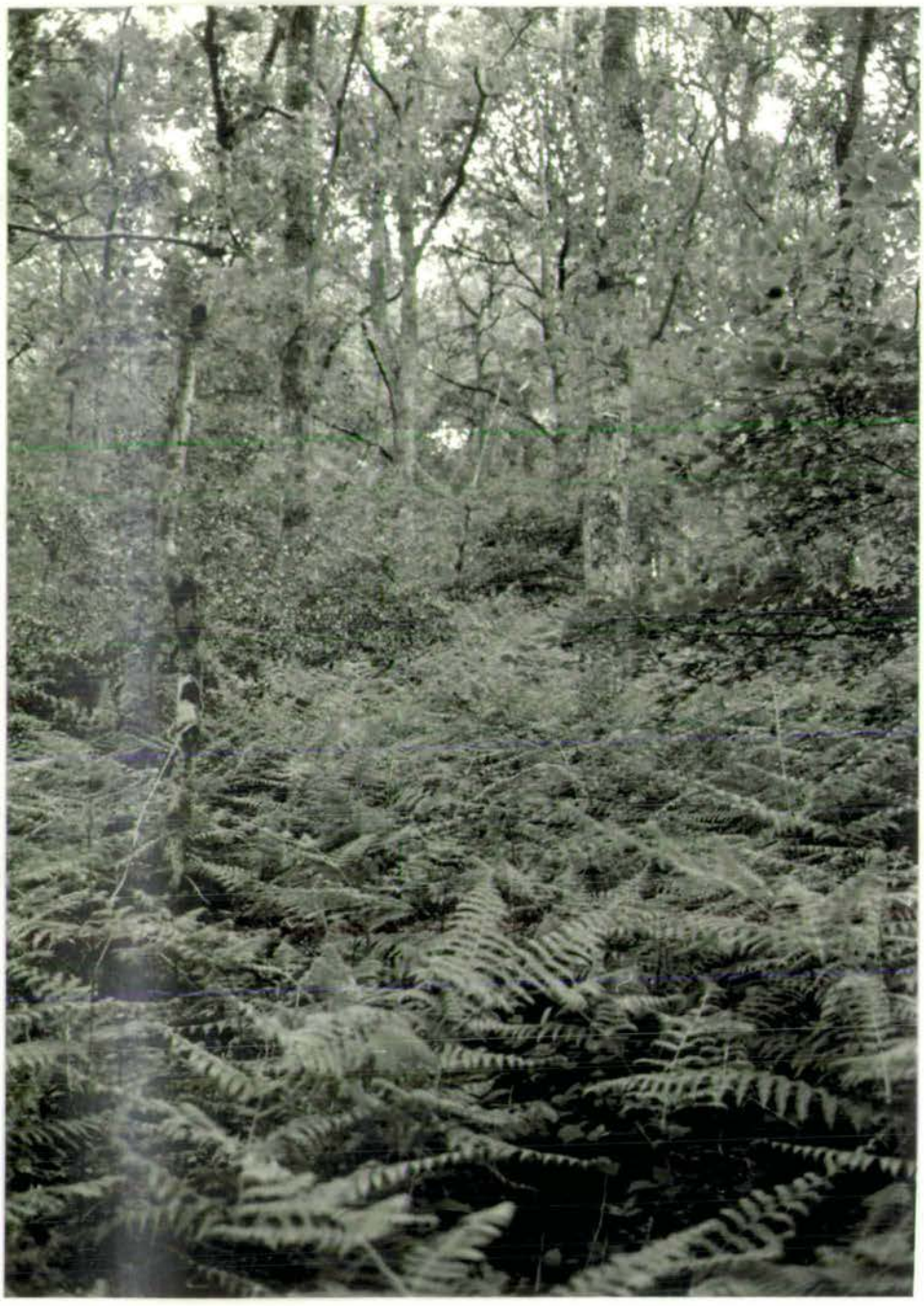


Figure 3.13. The locality of Charles Wood, Devon. The position of the woodland habitat site at location 2 is shown by ●. (Scale: 1/10560).

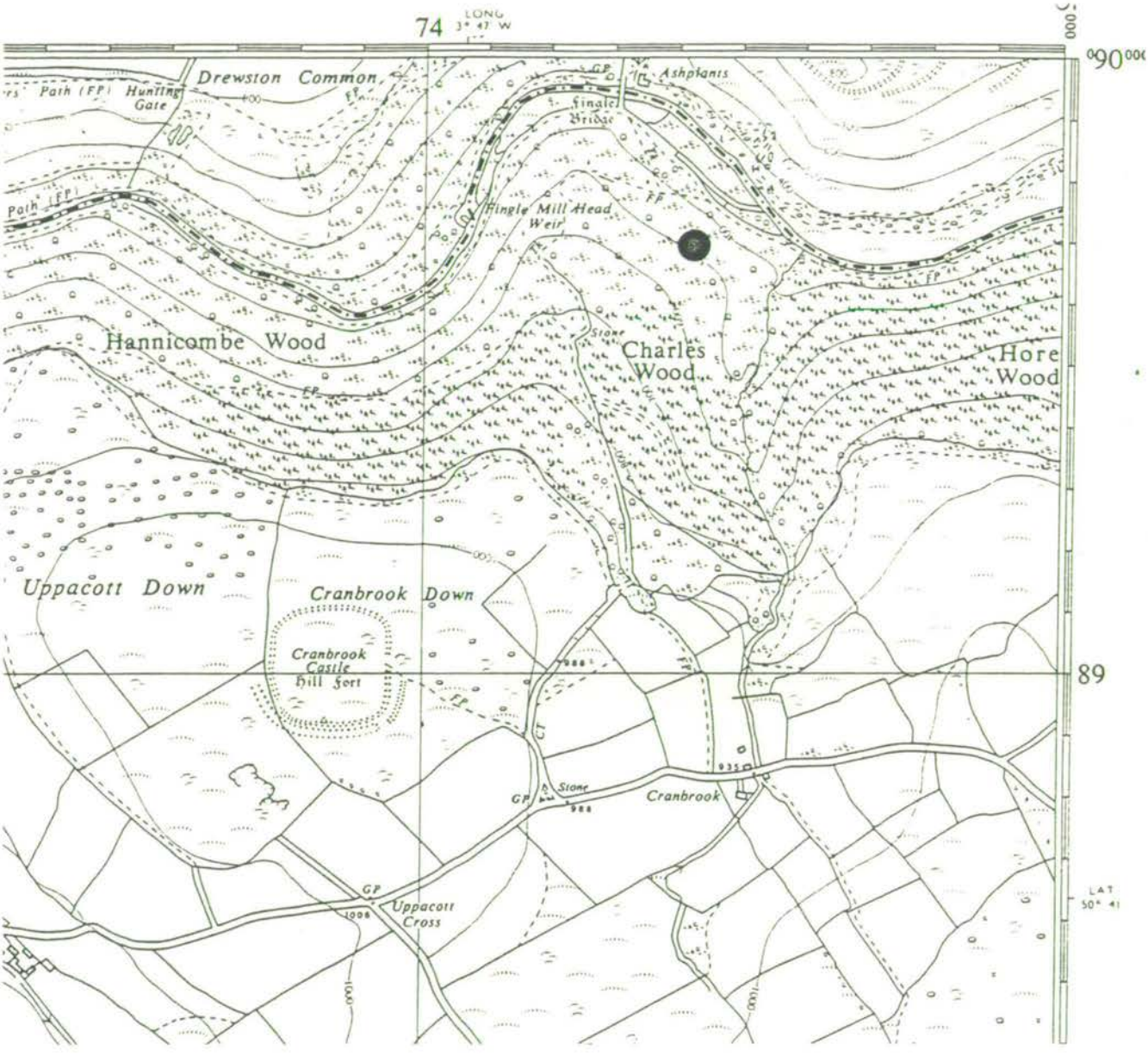


Figure 3.14. Sizewell Beach, Suffolk. The coastal site at location 3. The photograph was taken from the west.

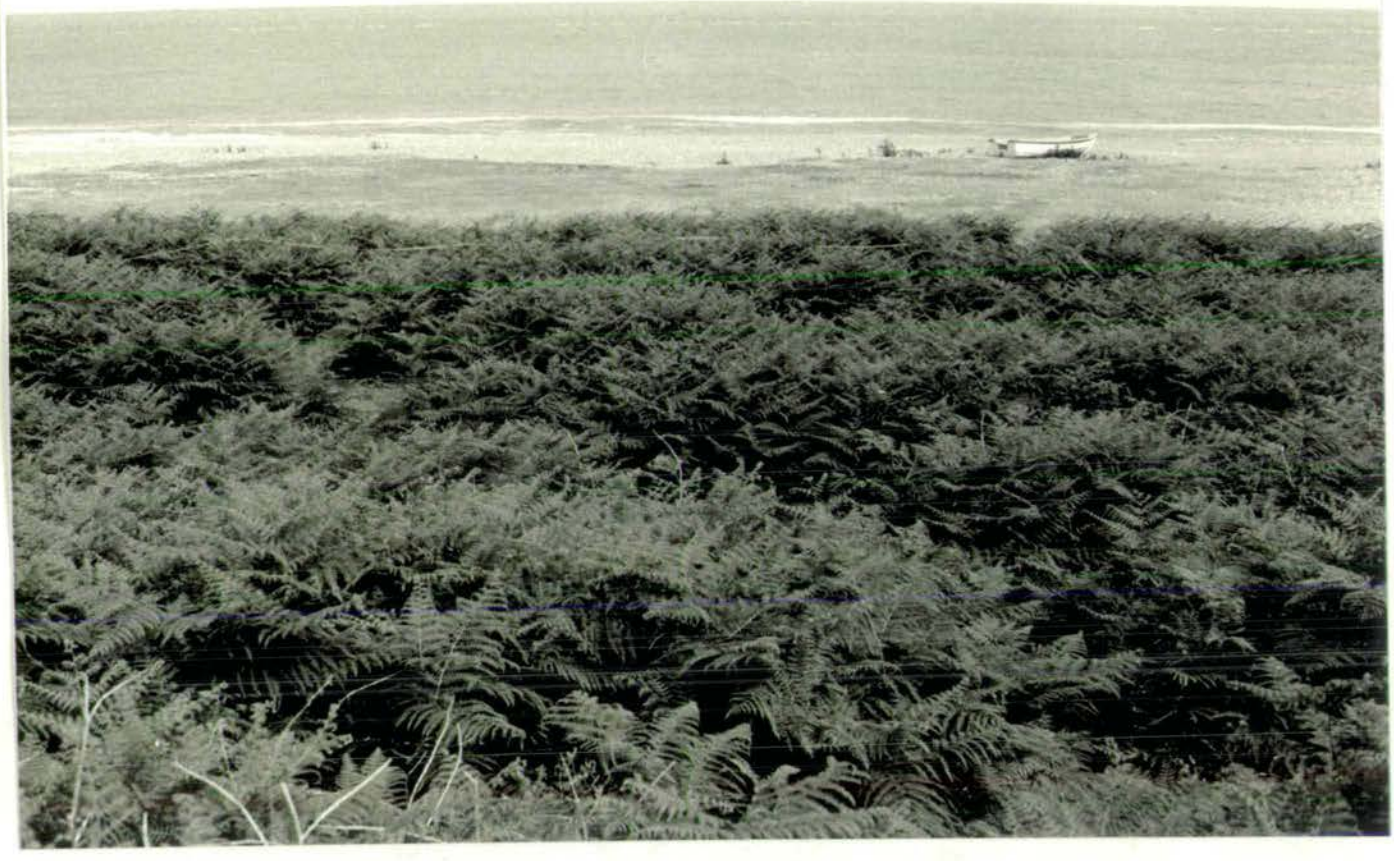


Figure 3.15. The locality of Sizewell Beach, Suffolk. The position of the coastal habitat site at location 3 is shown by ▲. The bold numbers show grid reference lines. (Scale: 1/10000).

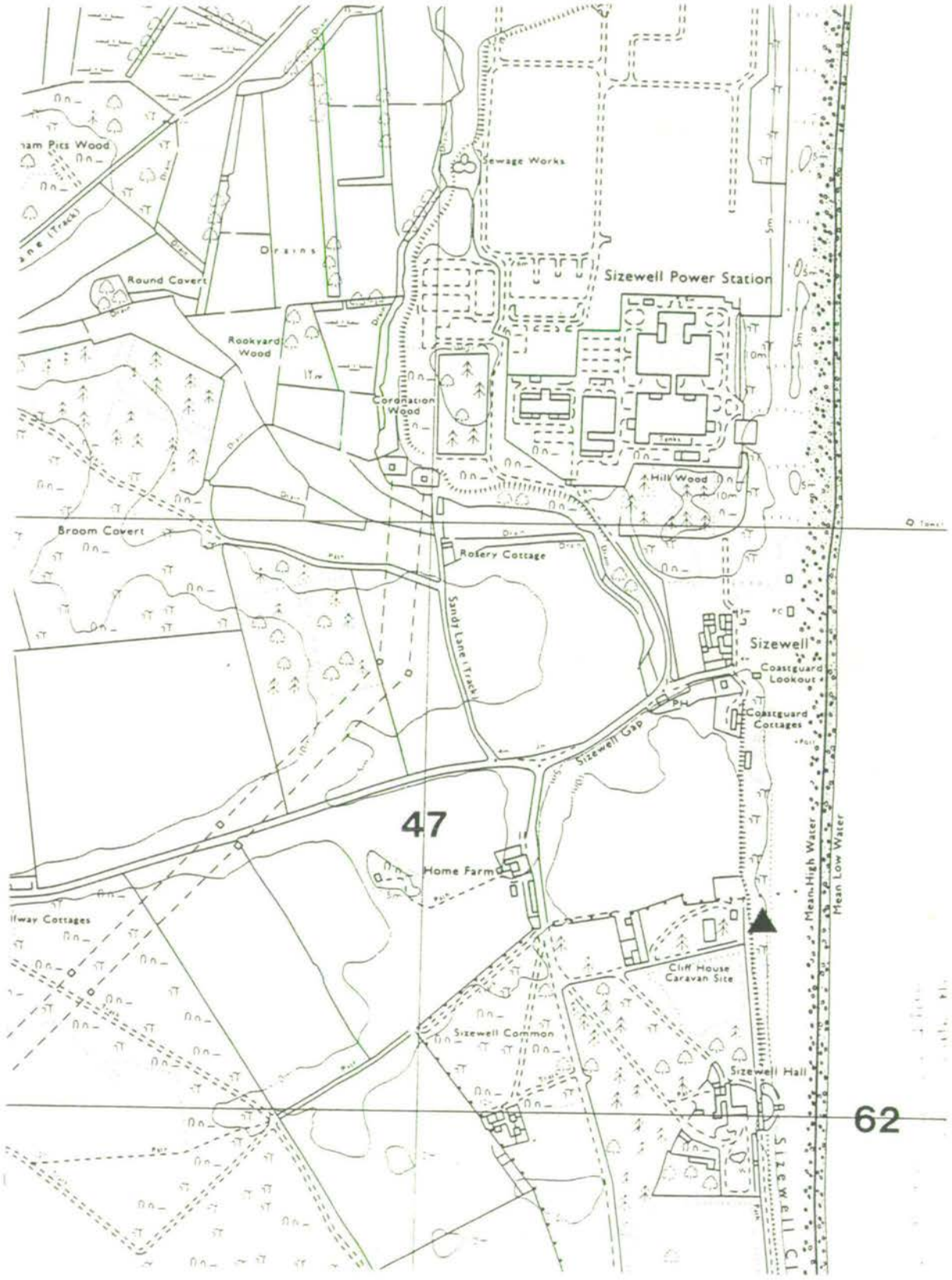


Figure 3.16. Lakenheath Warren, Suffolk. The open habitat site at location 3. The photograph was taken from the east.

Figure 3.17. The locality of Lakenheath Warren, Suffolk. The position of the open habitat site at location 3 is shown by ■. (Scale: 1/10000).

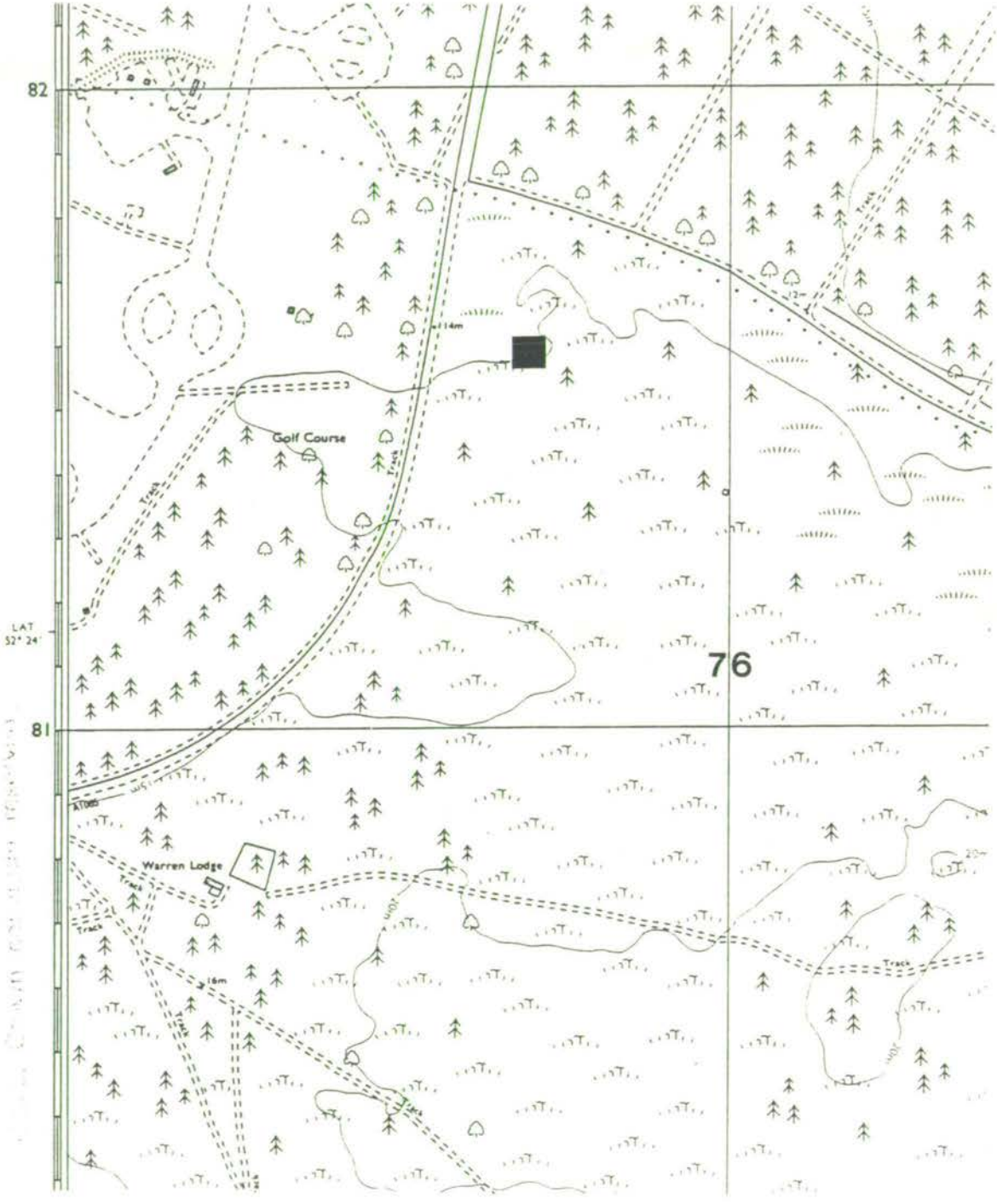


Figure 3.18. Brandon Park, Suffolk. The woodland habitat site at location 3. The photograph was taken from the south east.

trees were Betula pendula with scattered Quercus robur. There were several 'dwarf' fronds on the site, including five fronds which were sampled. In addition there were two other distinct morphologies: several fronds were still unfurling at the tips of the frond, although the lower pinnae were fully extended, whilst the majority of fronds were fully extended. Those fronds still unfurling were very tall, and grew straight, the rachis being stiff, whilst those fronds which were fully developed were shorter but arched over. Whether these differences are due to genotypic differences between plants or phenotypic variation could not be established. There were a large number of fronds which had been damaged by the removal of the lamina, leaving a stump of the rachis. Because the rachis stumps apparently bore tooth marks, these appeared to have been damaged by large herbivores, possibly deer, presumably as the fronds emerged and started to expand. There was no debris that was obviously from that season's growth, rather than the previous season's litter, suggesting that the fronds had been eaten. The wood and plantation are part of a park which was reported to shelter a herd of deer. There was no evidence of rabbits. As well as the dominant tree species, the associated species at the site consisted of: Agrostis canina (h); Brachypodium sylvaticum (w); Fagus sylvatica (seedling) (w); Holcus lanatus (h); Lonicera periclymenum (scb); Pinus sp. (seedling) (w); Quercus sp. (seedling) (w); Rubus fruticosus (scb); and Stachys sylvatica (w).

Location 4, site 10: Skipwith Common (Figures 3.20 and 3.21). The open site in north east England was on common land which is now protected. Because this site had been selected earlier in the year and had easy access, the sample consisted of 100 fronds. This enabled the samples of 50 and 100 fronds to be compared; there was no difference in the results, which was the same outcome as the comparison of sample size at location 5. The current season growth was very dense and there was a large amount of the previous seasons' litter. There was noticeable activity from bees which appeared to be visiting the nectaries on the fronds. No other species were found on the site, presumably due to the impenetrability of the bracken.

Location 4, site 11: Skipwith Common (Figures 3.21 and 3.22). The woodland site in north east England was adjacent to the open site (above). The wood consisted of Betula pendula trees. Several fronds were scandent upon trees and other fronds. The associated flora was sparse, consisting only of: Betula pendula (seedling) (w); Brachypodium sylvatica (w); Deschampsia caespitosa (w); and Urtica dioica (scb).

Location 5, site 12: Gosford Bay (Figures 3.23 and 3.24). The coastal site in

Figure 3.20. Skipwith Common, Yorkshire. The open habitat site at location 4. The photograph was taken from the south.

Figure 3.21. The locality of Skipwith Common, Yorkshire. The position of the open habitat site at location 4 is shown by ■, and the woodland habitat by ●. (Scale: 1/10000).

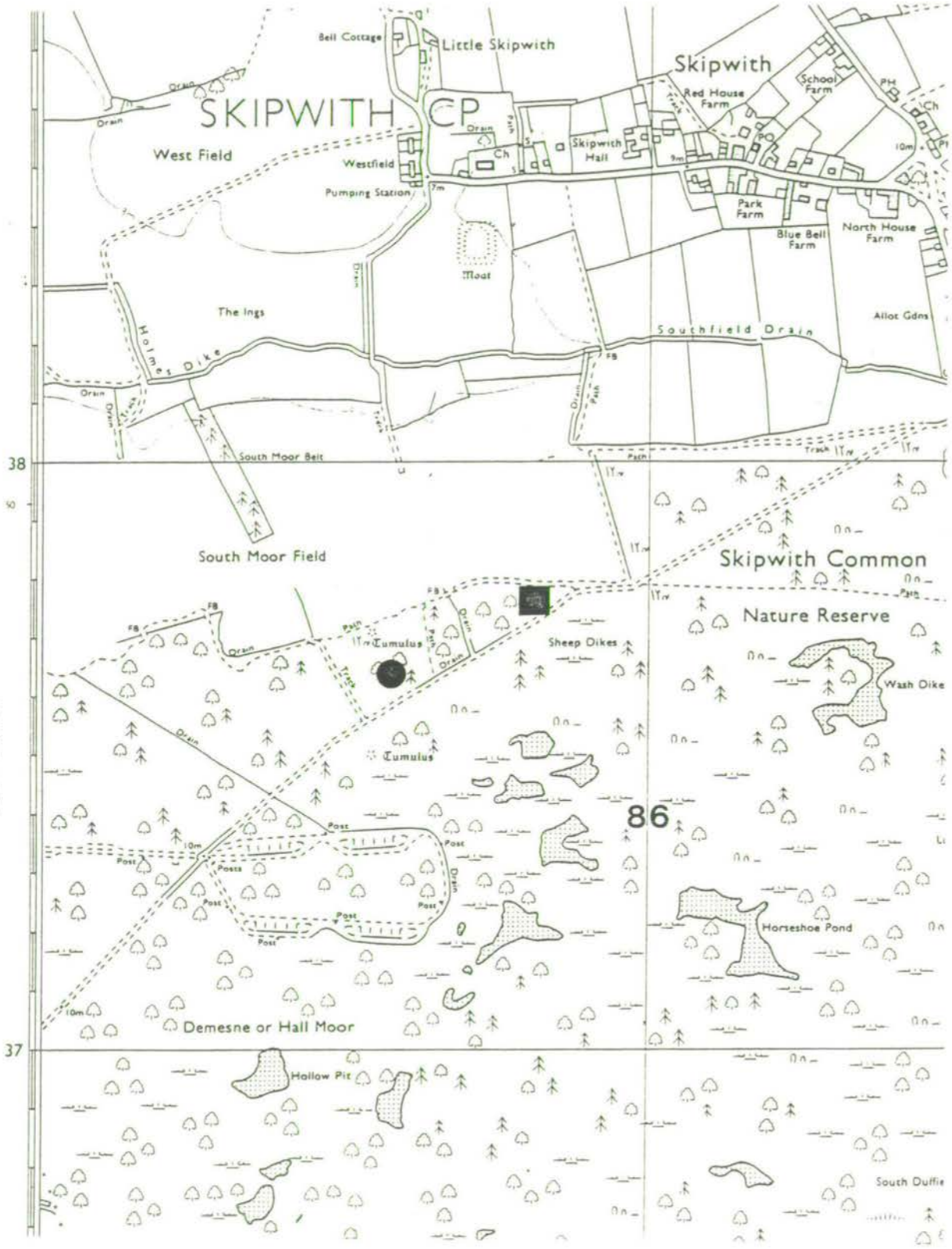
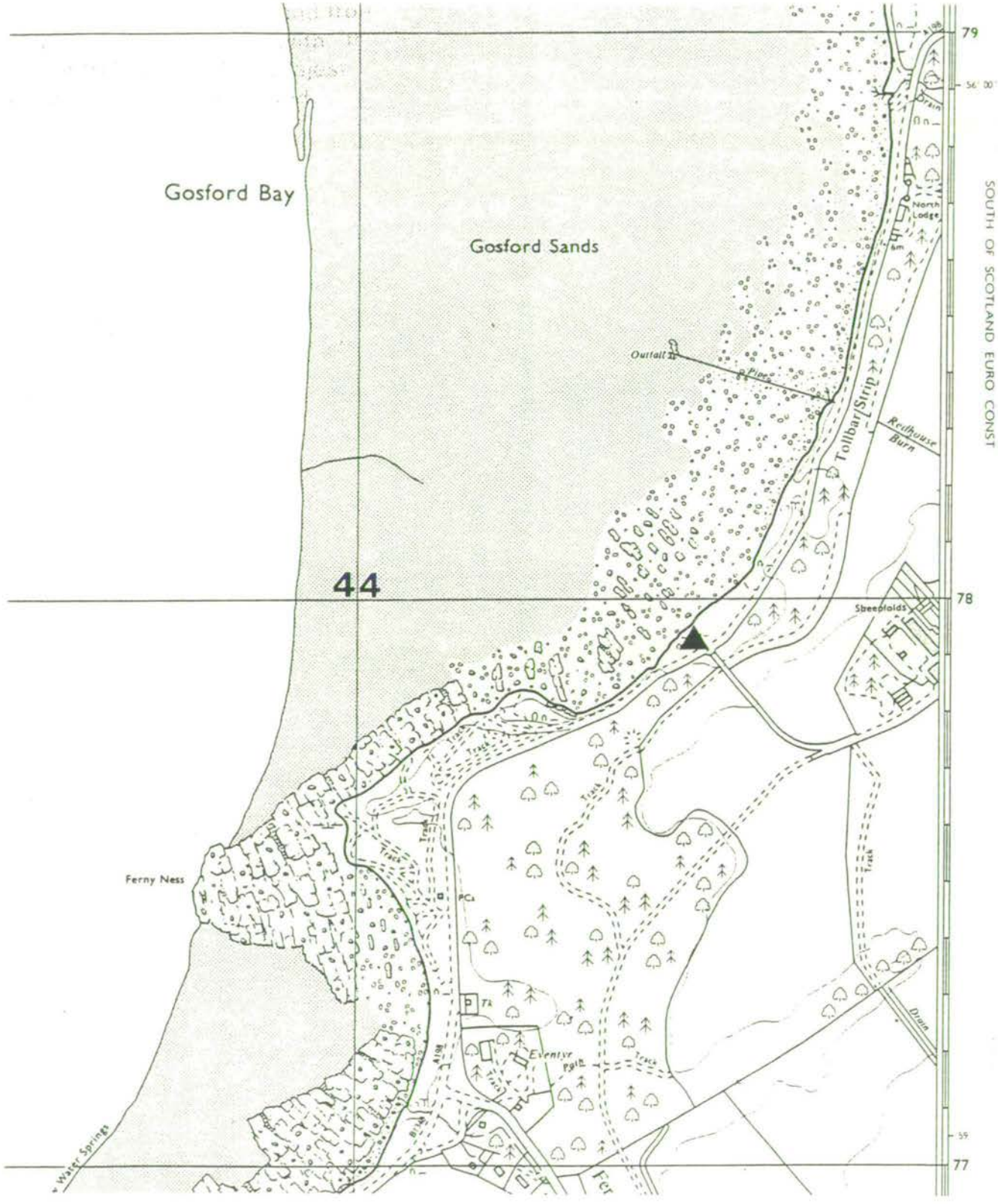


Figure 3.22. Skipwith Common, Yorkshire. The woodland habitat site at location 4. The photograph was taken from the south.

Figure 3.23. Gosford Bay, East Lothian. The coastal site at location 5. The photograph was taken from the south west.

Figure 3.24. The locality of Gosford Bay, East Lothian. The position of the coastal habitat at location 5 is shown by ▲. The bold number shows the grid easting reference line. (Scale: 1/10000).



south east Scotland was close to a point called Ferny Ness, which implies that the area, if not the site, has contained populations of bracken for a long period. The site is on the Forth Estuary; however the estuary is fully tidal at this point and the north shore is 21km from the site at its closest (the distance due north is 25km). This can therefore be treated as open sea rather than a sheltered estuary. The area is subject to severe storms with a northerly prevailing wind: later in the season the fronds tend to be damaged by storms blowing over the fronds; there was some evidence of wind or salt burn, particularly on fronds to the windward (coastal) side of the site, outside the site. This stretch of coast is popular with visitors and there were some traces of previous fire damage on the site; the frequency of fires is not known. There were several ants' nests on the site and some evidence of rabbit activity; there were some holes which could be abandonned warrens, as well as rabbit droppings on turf close to the site. This site was involved in several studies throughout 1983, 1984, and 1985, and the associated flora for the site is therefore well recorded:
Acer pseudoplatanus (saplings) (w); Alliaria petiolata (w); Armeria maritima (c) Arenaria serpyllifolia (scb); Arrhenatherum elatius (scb/c); Cardamine flexuosa (scb); Crataegus monogyna (w/scb); Elymus repens (scb); Festuca rubra var. arenaria (c); Galium aparine (scb/c); Glechoma hederacea (scb/w); Heracleum sphondylium (scb); Holcus lanatus (scb/w); Montia perfoliata (scb); Myosotis arvensis (w/c); Primula veris (scb); Rosa canina agg. (R. caesia?) (scb); Rubus fruticosus agg. (scb); Rumex crispus (c); Senecio jacobea (scb/c); Silene dioica (c/w); Taraxacum officinale agg. (h/scb); Urtica dioica (scb); and Veronica chamaedrys (w/h).

Location 5, site 13: Blackhill (Figures 3.25 and 3.26). The open site in south east Scotland was on land used for sheep grazing. Sheep had free access to the site and there were several sheep paths running through the site. These had the effect of clumping fronds in several cases, so that the frond density was very variable from point to point. Early in the season there was some evidence of sheep eating the emerging fronds, in a similar manner to that discussed for Brandon Park (location 3, site 9; above). There was no obvious damage to fronds by sheep later in the season and it is possible that the sheep selected palatable fronds before the development of toxic compounds in the fronds (see Cooper-Driver and Swain, 1976; Cooper-Driver et al, 1977). There was some evidence of frost damage to emerging fronds as well; the croziers of affected fronds turn brown and die. Several fronds were noticed which had only a single lowermost pinna; these could not be tested since it was necessary to sample at the site more than once and thus these fronds could

Figure 3.25. Black Hill, Midlothian. The open habitat site at location 5. The photograph was taken from the north east.

Figure 3.26. The locality of Black Hill, Midlothian. The position of the open habitat site at location 5 is shown by ■. (Scale: 1/10560).

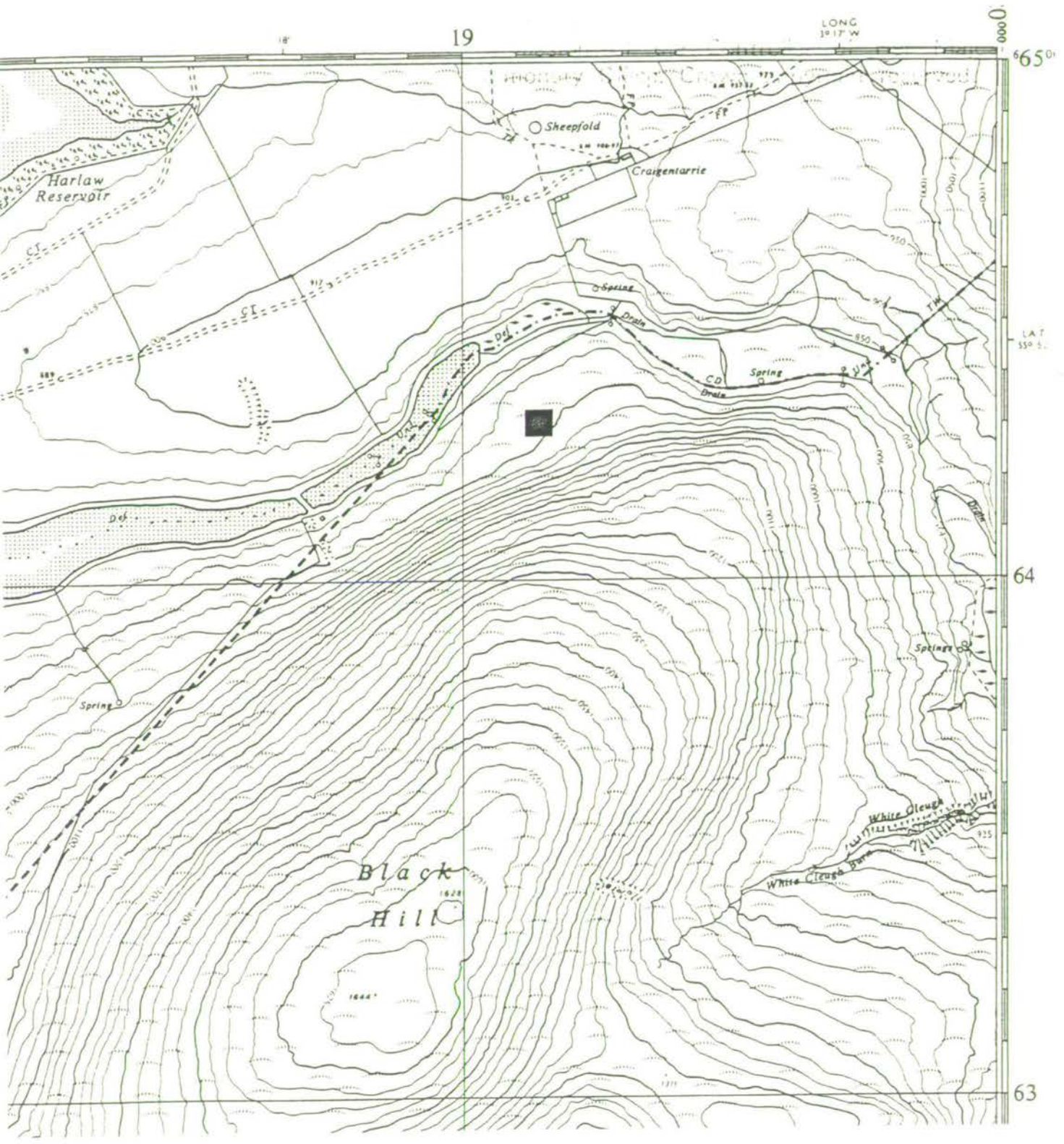


Figure 3.27. Binning Wood, East Lothian. The woodland habitat at location 5. The photograph was taken from the north.

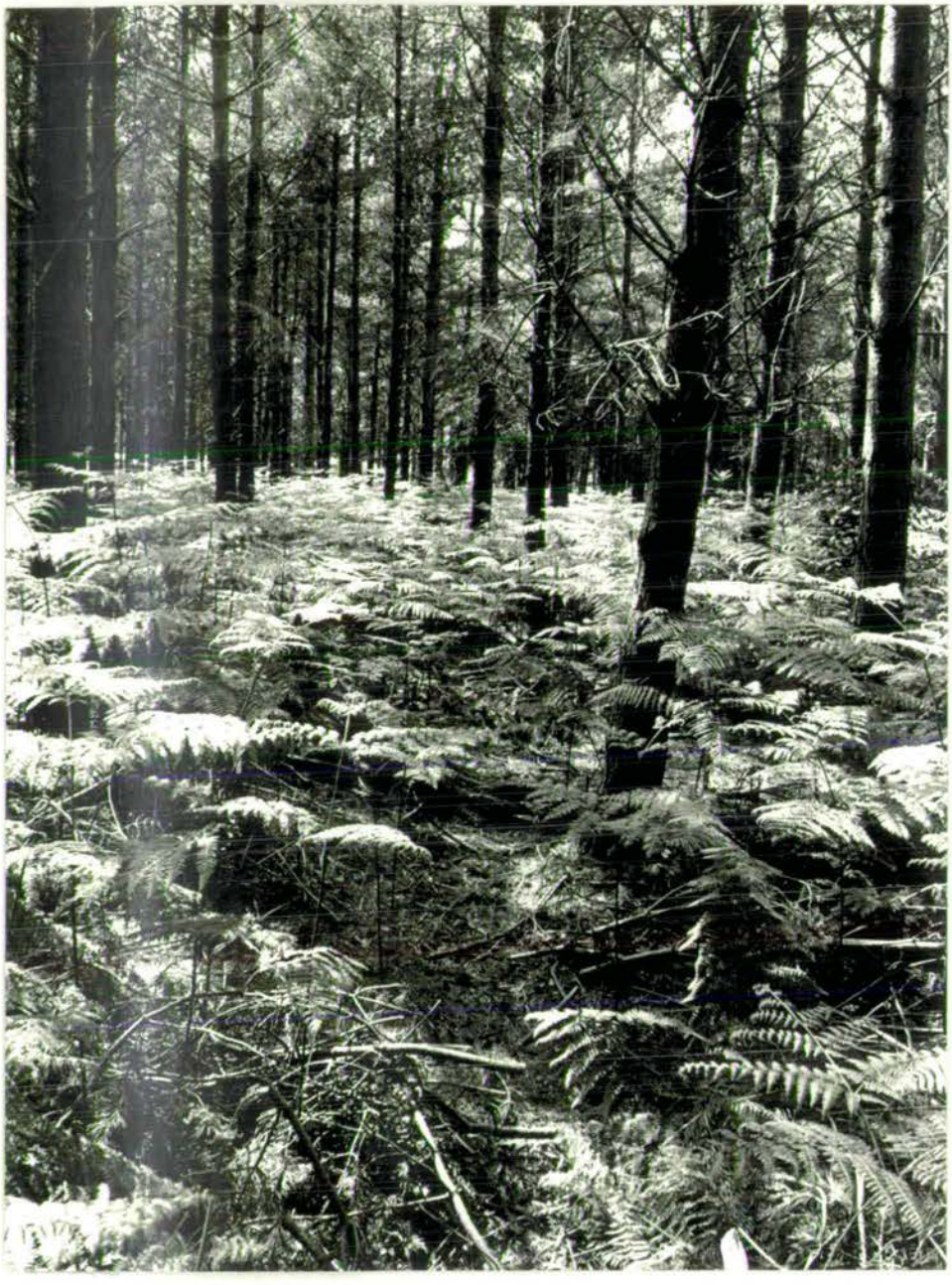


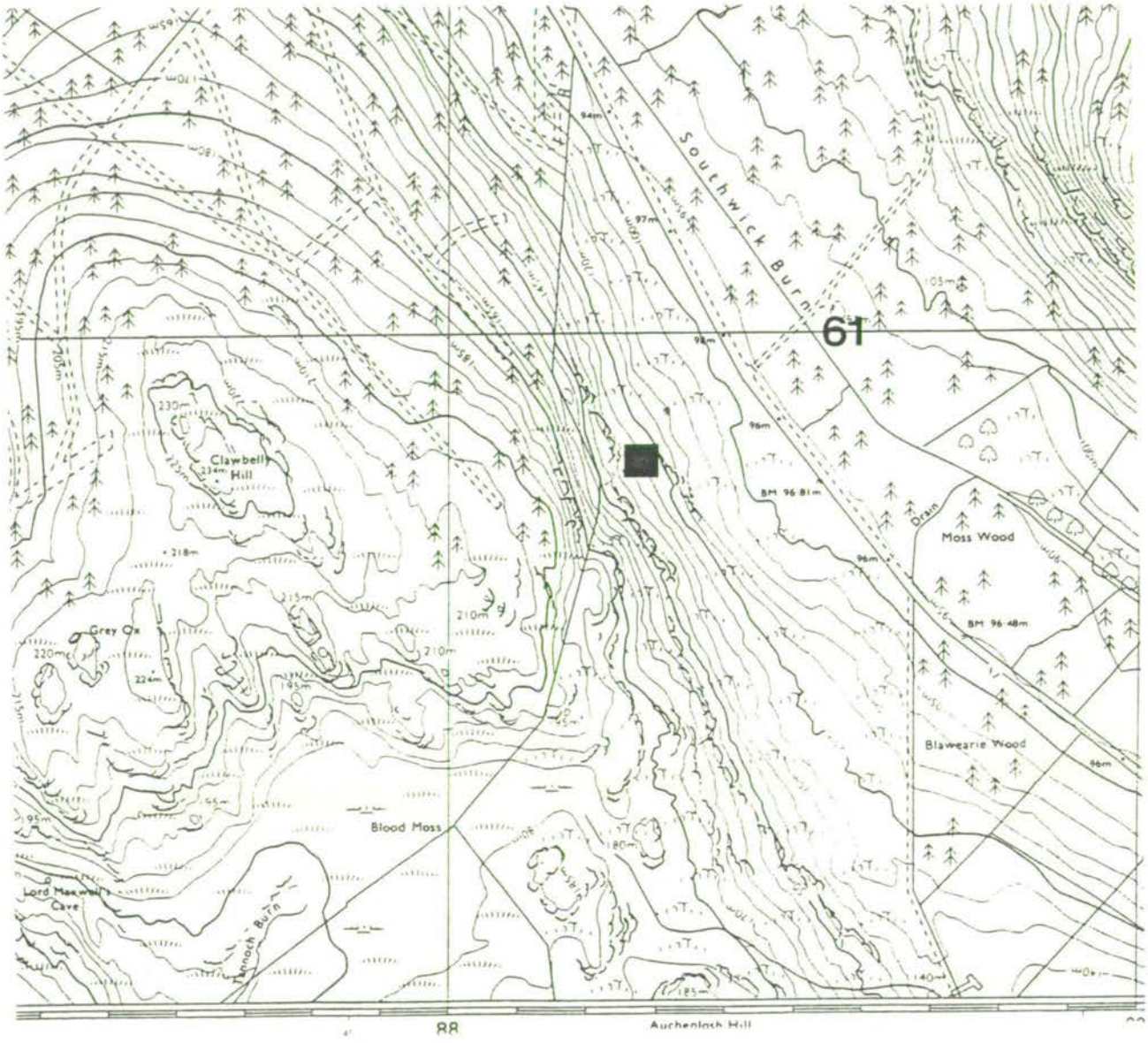
Figure 3.28. The locality of Binning Wood, East Lothian. The position of woodland habitat site at location 5 is shown by ●. (Scale: 1/10560).

Figure 3.29. Sandyhills Bay, Dumfriesshire. The coastal habitat site at location 6. The photograph was taken from the west.

Figure 3.30. The locality of Sandyhills Bay, Dumfriessire. The position of the coastal habitat site at location 6 is shown by ▲. (Scale: 1/10000).

Figure 3.31. Clawbelly Hill, Dumfriessire. The open habitats site at location 6. The photograph was taken from the south.

Figure 3.32. The locality of Clawbelly Hill, Dumfriessire. The position of the open habitat site at location 6 is shown by ■. The bold number shows the northing grid reference line. (Scale: 1/10000).



south west Scotland was visited annually between 1983 and 1985. Within 10m and 50m distance were two sites which were studied several times before 1983 and again in 1984 (Dr. A.F. Dyer, unpublished results). These sites are discussed in chapter 6. The hillside was used for grazing livestock but they rarely entered the bracken. The associated flora consisted of: Blechnum spicant (h/w); Calluna vulgaris (h); Dactylorhiza maculata (h); Deschampsia flexuosa (h); Erica cinerea (h); Festuca ovina (h); F. rubra var. rubra (h); Galium saxatile (h); Holcus lanatus (h/w); Hyacinthoides non-scripta (fruiting) (w); Narthecium ossifragrum (h); Oxalis acetosella (w); Potentilla erecta (h); Rubus fruticosus (scb); and Vaccinium myrtillus (h).

Location 6, site 16: Shambellie Wood (Figures 3.33 and 3.34). Like the previous site, this site was visited annually between 1983 and 1985, and was very close to two sites studied several times prior to 1983 and again in 1984 (Dr. A.F. Dyer, unpublished results, discussed in chapter 6). The wood consisted of large trees of Betula pubescens, Fraxinus excelsior, and Quercus petraea. There was a plantation of Pinus next to the wood. The associated flora consisted of: Dactylis glomerata (h); Deschampsia caespitosa (w); Dryopteris dilatata (w); Fagus sylvatica (sapling) (w); Ilex aquifolium (w); Lonicera periclymenum (scb); Oxalis acetosella (w); Polystichum setiferum (w); Potentilla erecta (h); Quercus sp. (seedling) (w); Rubus fruticosus agg. (scb); Sorbus aucuparia (w); and Teucrium scorodonia (w).

Location 7, site 17: Slatrach Bay (Figures 3.35 and 3.36). The coastal site in west Scotland was on the island of Kerrera in the Firth of Lorn, facing the island of Mull which was 7km away. The associated flora consisted of: Achillea millefolium (h); Dactylis glomerata (h); Festuca ovina (h); Filipendula ulmaria (w); Holcus lanatus (w); Hyacinthoides non-scripta (w); Juncus sp. (h); Leontodon hispidus (c/h); Lotus corniculatus (h); Potentilla vulgaris (h); Primula veris (w); Prunella vulgaris (w/h); Ranunculus repens (h); Rumex crispus (c); Trifolium repens (h); Urtica dioica (scb); and Vicia cracca (scb).

Location 7, site 18: Torran Tuich (Figures 3.37 and 3.38). The open site in west Scotland was on land used for open grazing by sheep. There appeared to have been little disturbance by sheep however. The associated flora consisted of: Achillea millefolium (h); Calluna vulgaris (h); Carex flacca (h); Erica cinerea (h); Festuca ovina (h); Galium saxatile (h); Luzula campestris (h); Nardus stricta (h); Potentilla erecta (h); Vaccinium myrtillus (h); and Viola riviniana (w/h).

Location 7, site 19: Loch Nell (Figures 3.39 and 3.40). This site was also open to sheep but there appeared to have been little disturbance. The wood

Figure 3.33. Shambellie Wood, Dumfriesshire. The woodland habitat site at location 6. The photograph was taken from the east.

Figure 3.34. The locality of Shambellie Wood, Dumfriesshire. The position of the woodland habitat site is shown by ●. (Scale: 1/10000).

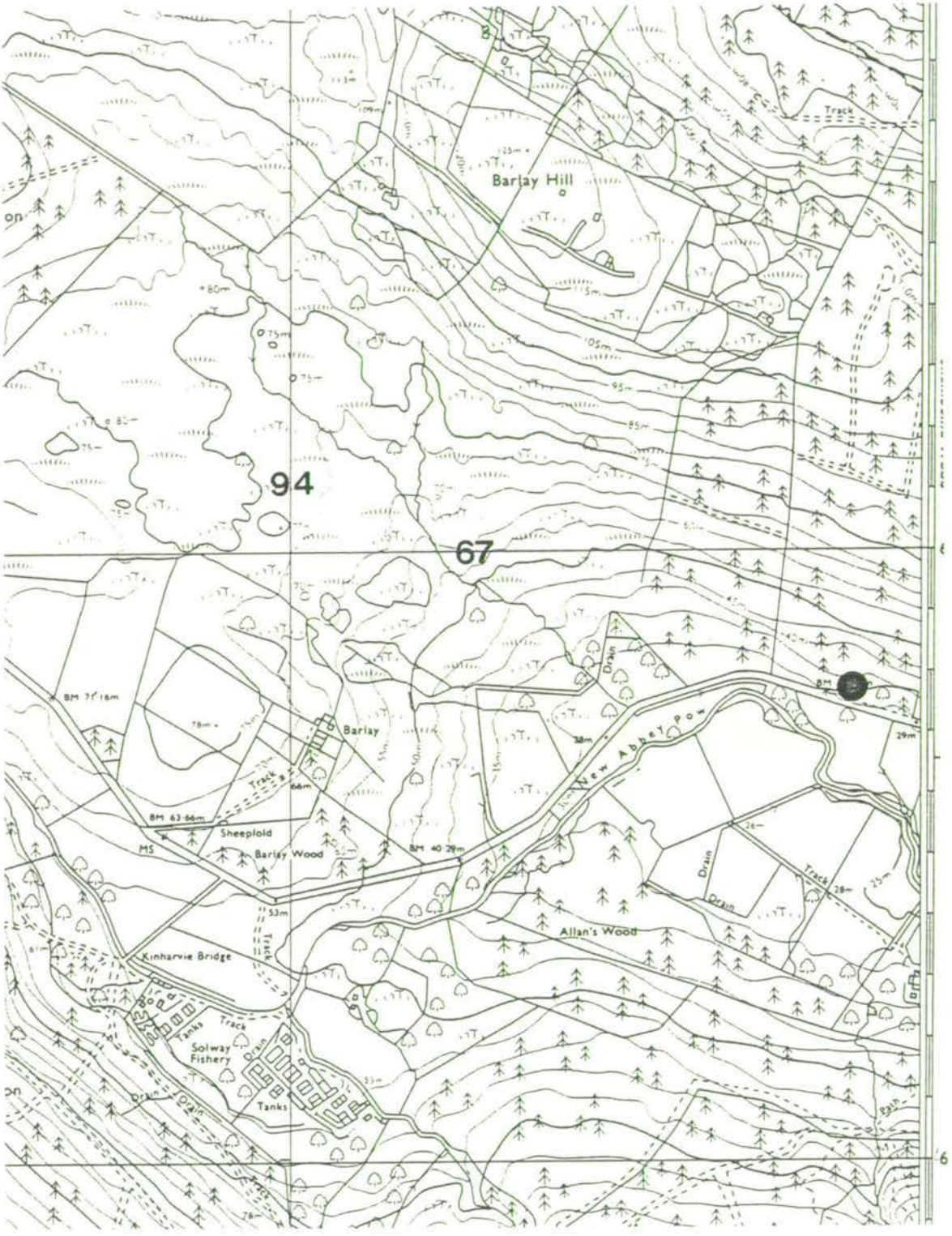


Figure 3.35. Slatrach Bay, Argyllshire. The coastal habitat site at location 7. The photograph was taken from the south east.

Figure 3.36. The locality of Slatrach Bay, Argyllshire. The position of the coastal habitat site at location 7 is shown by ▲. (Scale: 1/10000).

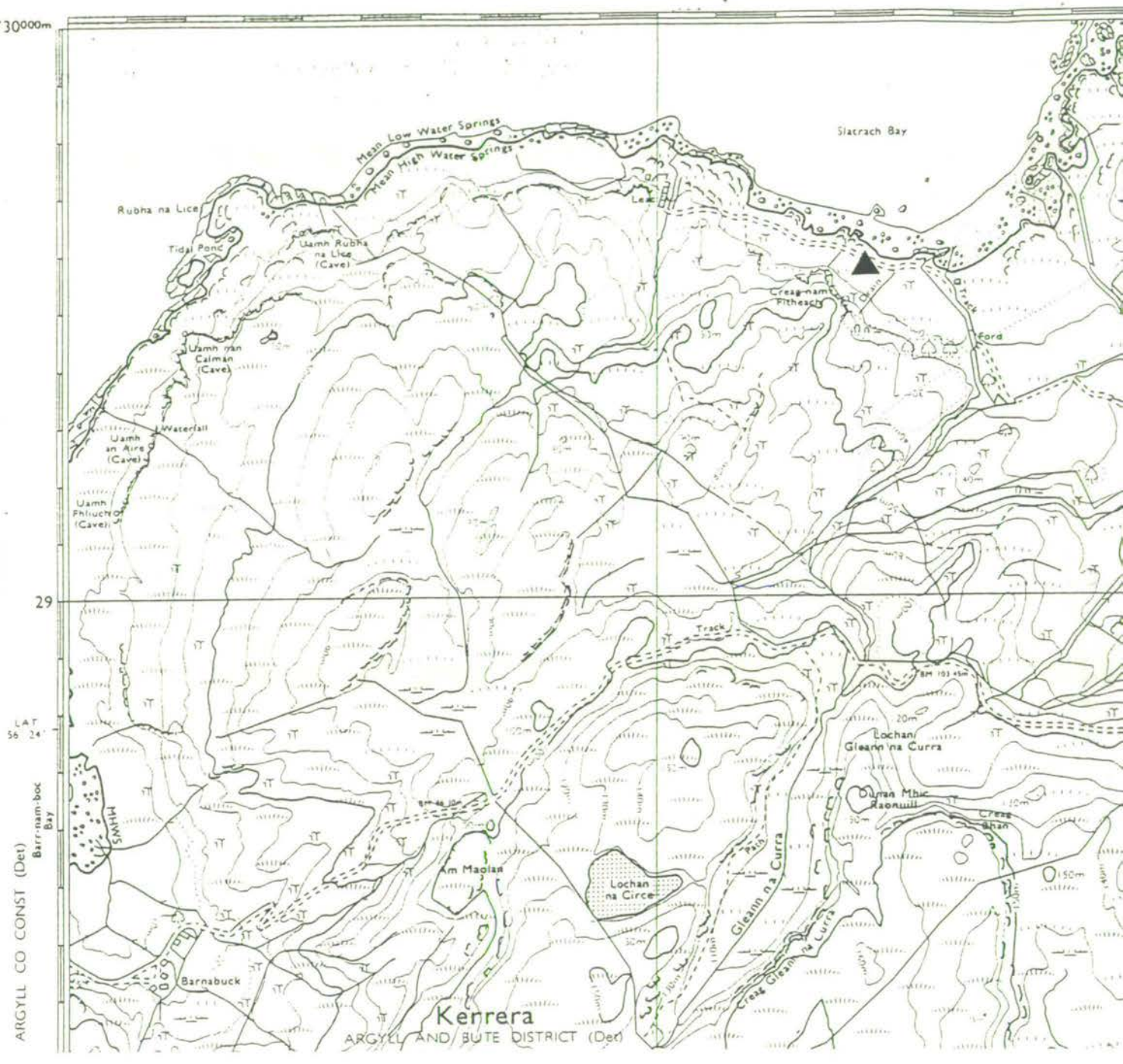


Figure 3.37. Torr-an-tuirc, Argyllshire. The open habitat site at location 7. The photograph was taken from the south west.

Figure 3.38. The locality of Torr-an-tuirc, Argyllshire. The position of the open habitat site at location 7 is shown by ■. The bold number shows the easting grid reference line. (Scale: 1/10000).

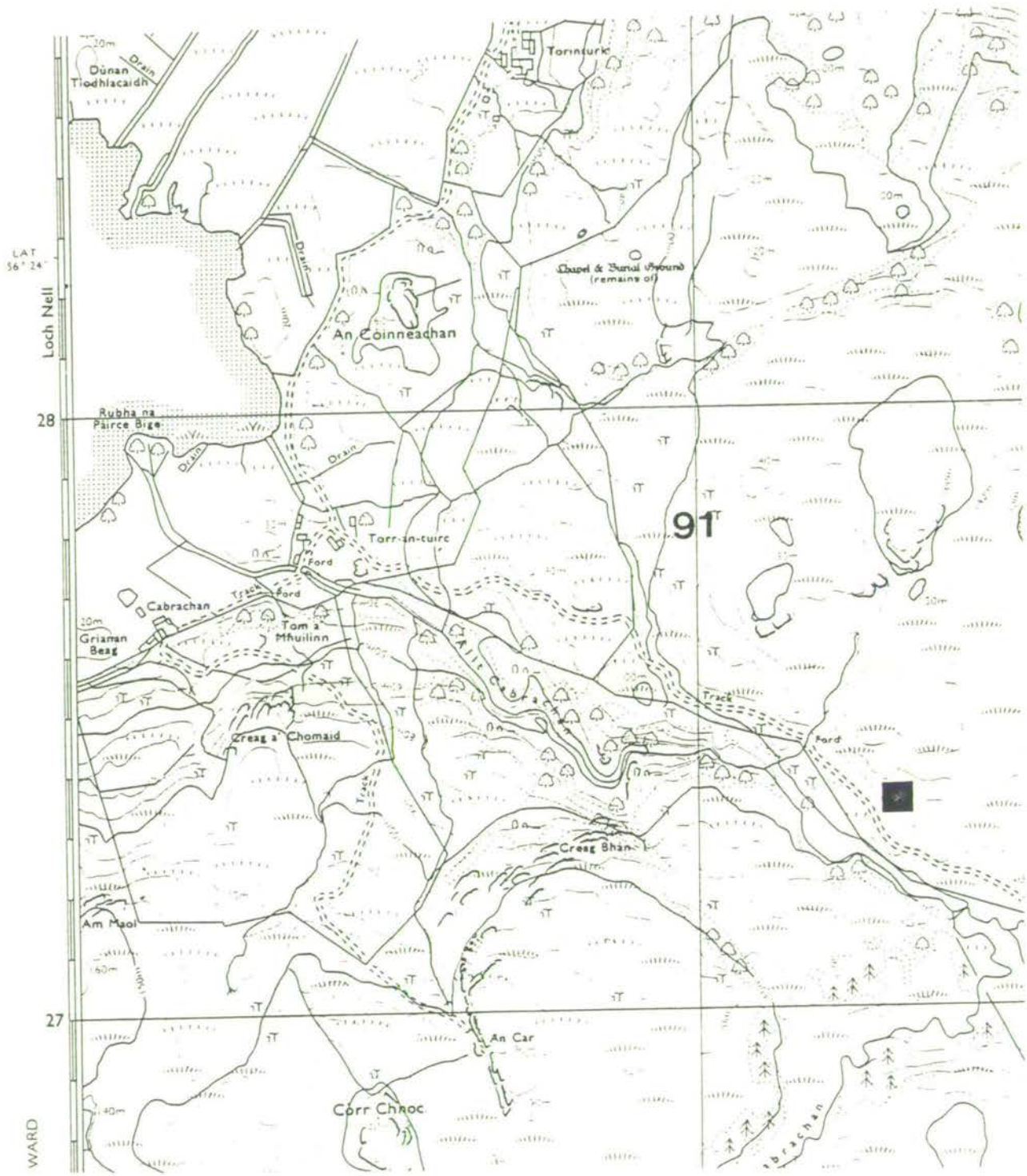
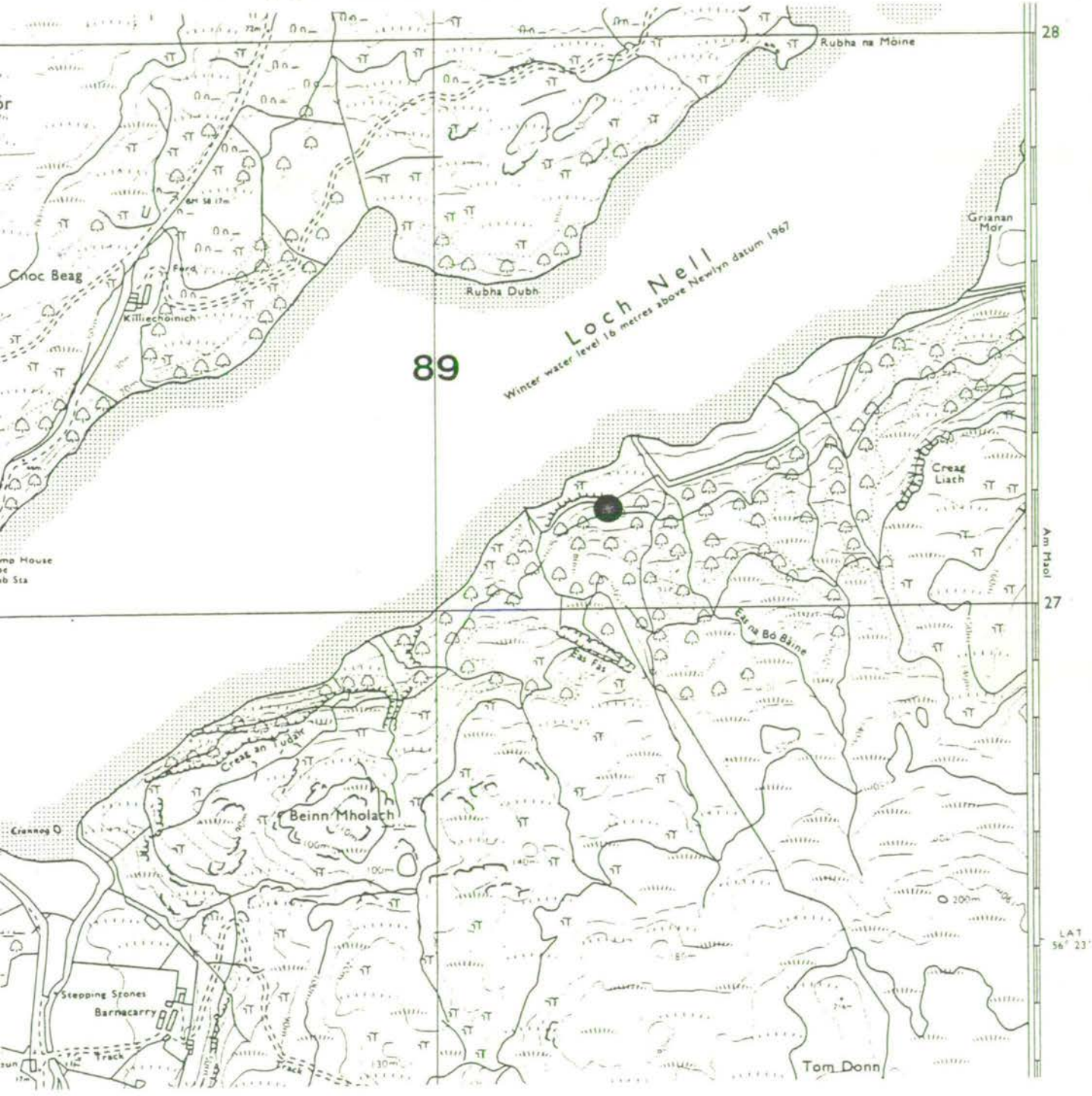


Figure 3.39. Loch Nell, Argyllshire. The woodland habitat site at location 7. The photograph was taken from the east.

Figure 3.40. The locality of Loch Nell, Argyllshire. The position of the woodland habitat site at location 7 is shown by ●. The bold number shows the easting grid reference line. (Scale: 1/10000).



consisted of Betula pubescens trees, some of which were apparently dead. Some of the fronds produced during the previous season appeared to be still standing. The associated flora consisted of: Betula pubescens (seedling) (w); Blechnum spicant (w/h); Brachypodium sylvaticum (w); Calluna vulgaris (h); Cerastium holosteoides (h/w); Deschampsia caespitosa (w); Digitalis purpurea (w/h); Euphrasia officinalis (h); Festuca rubra var. rubra (h); Galium saxatile(h); Holcus mollis (w); Oxalis acetosella (w); Poa trivialis (w); Potentilla erecta (h); Primula vulgaris (w); Prunella vulgaris (w/h); Sorbus aria agg. (w/scb); Veronica officinalis (w/h); and Viola ?riviniana (vegetative) (w).

Location 8, site 20: Aird Torrisdale (Figures 3.41 and 3.42). The north Scottish coastal site was on low cliffs facing the Atlantic Ocean. The site was exposed and showed some signs of wind or salt-burn. The site backed onto heathland. The associated species were: Aegopodium podagraria (scb); Aira preacox (h); Armeria maritima (c); Arenaria serpyfolia (c); Betula sp. (seedling) (w); Calluna vulgaris (h); Erica cinerea (h); Euphrasia officinalis agg. (h); Festuca rubra var. arenaria (c); Galium aparine (w/c); Hypericum humifusum (h/w); Nardus stricta (h); Poa subcaerulea (h/c); Plantago maritima (c); Primula sp. (?scotica) (vegetative) (h); Prunella vulgaris (w/h); Ranunculus bulbosus (h/c); Stachys sylvatica (w); Teucrium scorodonia (w); Thymus drucei (c/h); Trifolium repens (h); and Viola riviniana (w/h).

Location 8, site 21: Achnabourin (Figures 3.43 and 3.44). The open site in north Scotland was on the flood plain of the River Naver. The site was open to livestock, which sometimes entered the stand of bracken. The associated species were: Achillea millefolium (h); Agrostis stolonifera (h); Cerastium holosteoides (h/w); Cirsium palustre (h/w); Deschampsia flexuosa (h); Festuca rubra var. rubra (h); Galium saxatile (h); Glechoma hederacea (scb/w); Luzula campestris (h); Potentilla erecta (h); Prunella vulgaris (w/h); Ranunculus repens (h); Rumex acetosella (h); Trifolium repens (h); Veronica officinalis (w/h); and Viola riviniana (w/h).

Location 8, site 22: opposite Rhinovie (Figures 3.44 and 3.45). The woodland site in the north of Scotland was very close to the open site, on slightly higher ground overlooking the River Naver. The wood consisted of Betula pubescens. The associated species were: Blechnum spicant (w/h); Deschampsia flexuosa (w); Euphrasia officinalis agg. (h); Galium saxatile (h); Oxalis acetosella (w); Poa trivialis (w); Potentilla erecta (h); Primula veris (w); Ranunculus repens (h); Sorbus aucuparia (seedling) (w); Veronica officinalis (w/h); and Viola riviniana (w/h).

Location 9, site 23: Glen Garry (Figure 3.46 and 3.47). The open site in

Figure 3.41. Aird Torrisdale, Sutherland. The coastal habitat site at location 8. The photograph was taken from the north east.

Figure 3.42. The locality of Aird Torrisdale, Sutherland. The position of the coastal habitat site at location 8 is shown by ▲. The bold numbers show grid reference lines. (Scale: 1/10560).

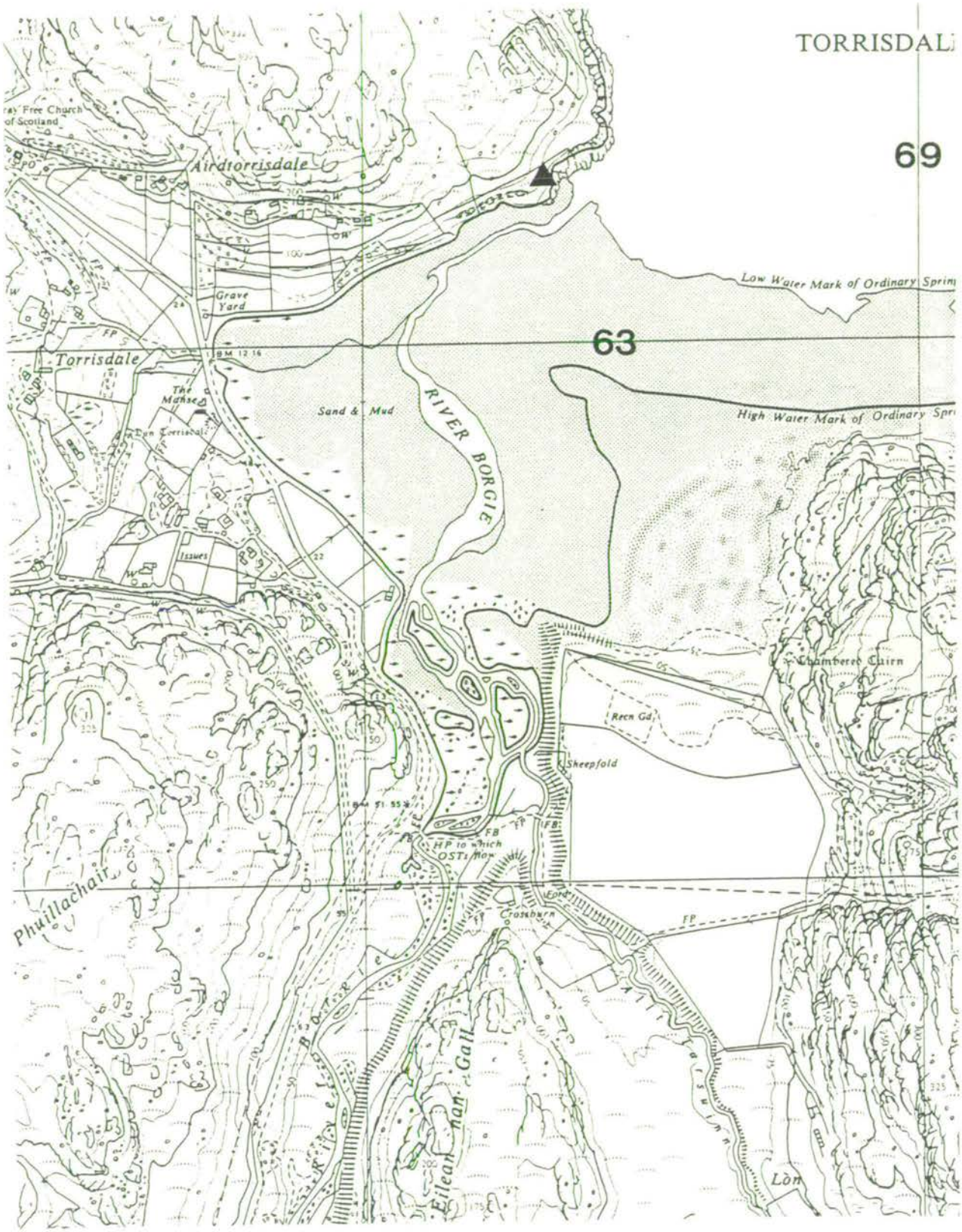


Figure 3.43. Achnabourin, Sutherland. The open habitat site at location 8. The photograph was taken from the south west.

Figure 3.44. The locality of Strath Naver, Sutherland. The position of the open habitat site at location 8 is shown by ■, and the woodland habitat site by ●. The bold number shows the easting grid reference line. (Scale: 1/10560).

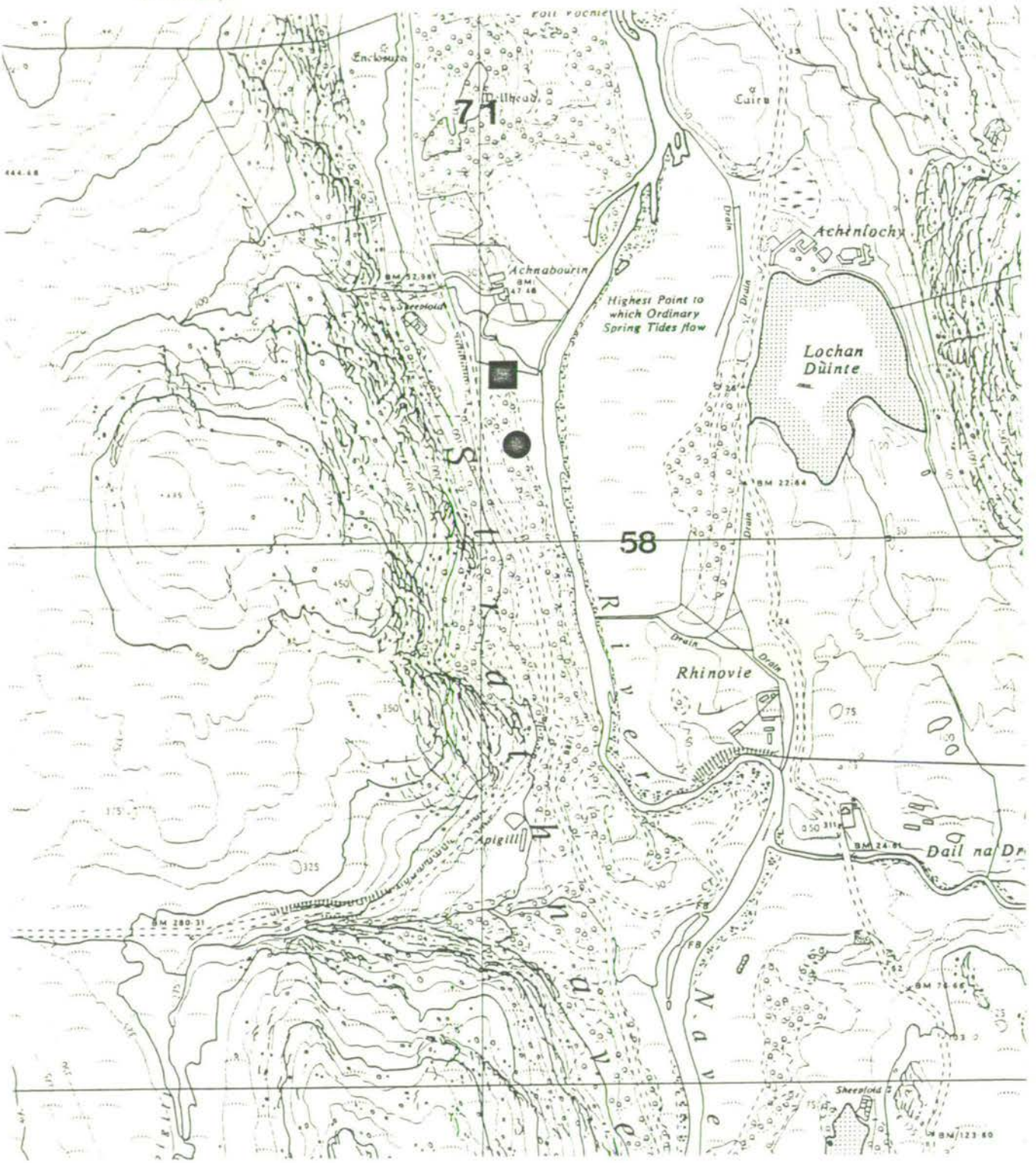


Figure 3.45. Strath Naver, Sutherland. The woodland habitat site at location 8. The photograph was taken from the north.

central Scotland was on land open for live stock but there appeared to have been no disturbance nor any other evidence of livestock in the area. The growth of bracken was thick and only one of the five 1 sq. m quadrats contained any other species. These were: Campanula rotundifolia (h); Euphrasia officinalis agg. (h); Galium saxatile (h); Plantago lanceolata (h); Ranunculus repens (h); Rumex acetosella (h); Trifolium repens (h); and Urtica dioica (scb).

Location 9, site 24: Clunies Wood, Glen Garry (Figures 3.47 and 3.48). The woodland site in central Scotland was directly below the open site, in well developed Betula pendula wood. The associated species were: Brachypodium sylvaticum (w); Campanula rotundifolia (h); Deschampsia flexuosa (w); Festuca rubra var. rubra (h); Galium saxatile (h); Hyacinthoides non-scripta (fruiting) (w); Polygala vulgaris (h); Potentilla erecta (h); Sorbus aucuparia (w); Vaccinium myrtillus (h); and Viola riviniana (w/h).

Figure 3.46. Glen Garry, Perthshire. The open habitat site at location 9. The photograph was taken from the west.

Figure 3.47. The locality of Glen Garry, Perthshire. The open habitat at location 9 is shown by ■, and the woodland habitat by ●. The bold numbers show grid reference lines. (Scale: 1/10000).

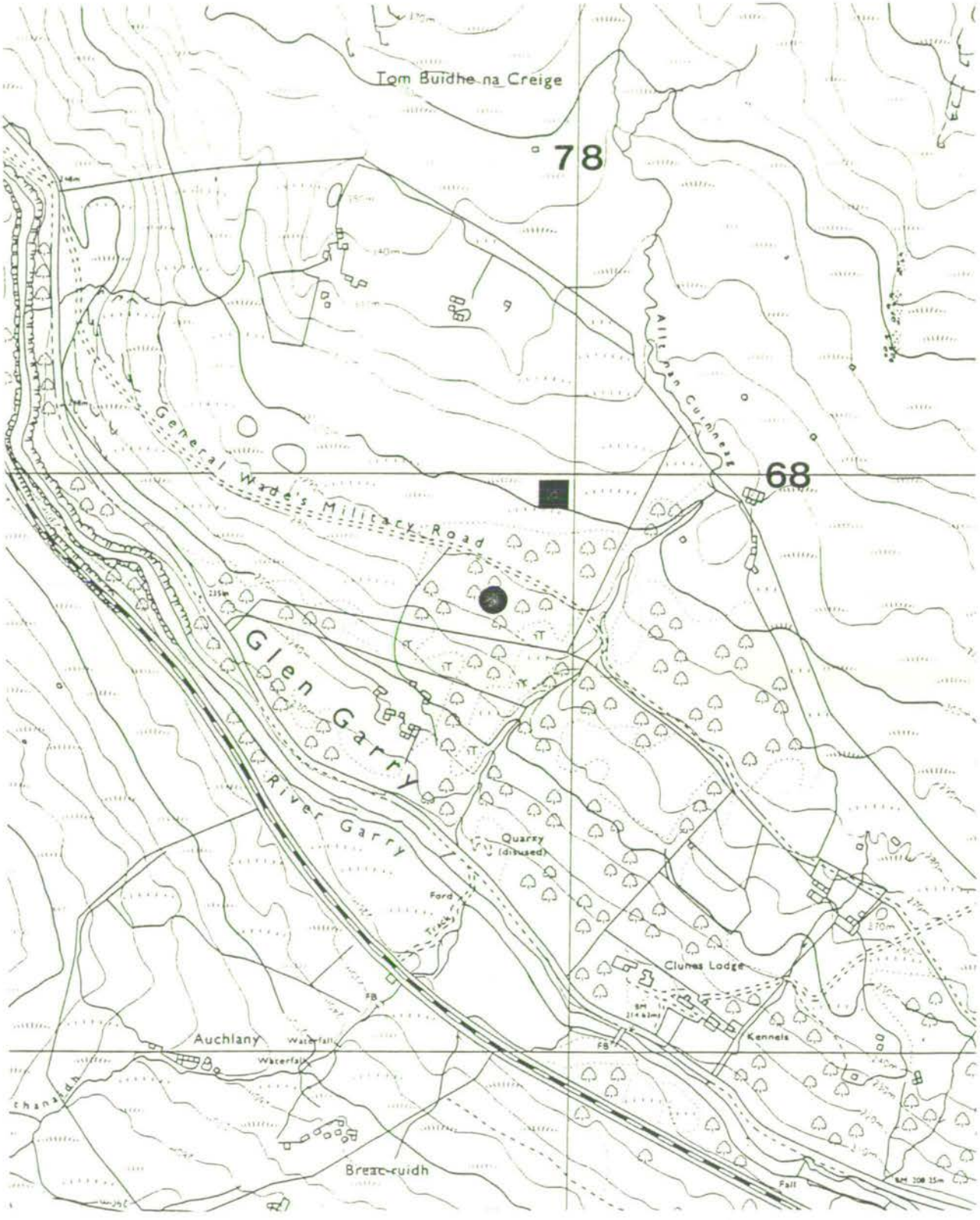


Figure 3.48. Clunies Wood, Perthshire. The woodland habitat site at location 9. The photograph was taken from the east.

Chapter 4. A Survey of Cyanogenesis in Pteridium aquilinum throughout Britain.

In order to ascertain the extent of the polymorphism of cyanogenesis in Pteridium aquilinum in Britain, a nationwide survey of cyanogenesis was undertaken in the summer of 1983. In addition, the object of the survey was to demonstrate the effect of the habitat and other environmental variables upon cyanogenesis. In particular climatic variables were investigated, since these had been implicated as affecting cyanogenesis in other species (e.g. Daday, 1954a,b; Jones, 1970, 1977).

4.1. Methods.

Sites 1 to 24 (chapter 3; figure 3.1) were visited and the fronds of bracken at each site were tested using the sodium picrate test (chapter 2.2.1) and the sampling procedure described (chapter 2.1). The number of cyanogenic fronds sampled at each site was used to produce an estimate of the percentage of cyanogenic fronds at the site, and the mean amount of HCN released by cyanogenic fronds at each site was estimated. The associated data were recorded at each site as described earlier (chapter 3).

Studies of sites 12 and 13 at location 5 formed part of a longer term project and had been sampled previously in the season; some of the initial sample of 100 fronds at each of these sites had died in the interim.

All of the picrate tests were examined after 24 and 48 hours; several sites showed a greater percentage of positive results after 48 hours than after 24 hours (chapter 2.2.1). These results are open to different interpretations. Two possible hypotheses are either that those frond samples scoring positive only after 48 hours released their HCN at a slower rate or that they lacked the β-glycosidase and that the release of HCN was due to degradation by general hydrolases as the tissues broke down. The only frond samples that showed a change between 24 and 48 hours were those that had scored 0 after 24 hours and 1 after 48 hours. It seems likely that these fronds contained both the glycosidase and prunasin, but at low levels so that insufficient HCN had been released to produce a positive score after 24 hours, whereas enough HCN had accumulated after 48 hours to attain a picrate score of 1. The activities of β-glycosidases involved in cyanogenesis are highly specific for particular cyanogenic glycosides (Hosel, 1981), whilst hydrolysis by non-specific degrading enzymes is likely to occur over a longer time period. De Araujo (1976) scored his picrate test after only three hours to overcome positive

scores from glycoside-only plants of Trifolium repens, and Pusey (1963) suggested that plants of T. repens scoring after 48 hours was indicative of glycoside-only phenotypes releasing HCN following autolysis. However, there is no evidence given to support his suggestion and other workers dispute it (Dr. M.A. Hughes, University of Newcastle; personal communication). Fronds that scored positively only after 48 hours are here taken to contain both the glycoside and the glycosidase. It is only scores of 0 that changed after 48 hours. Because of the logarithmic spacing between the colour classes, it requires far more HCN to turn a picrate score from 1 to 2, or 2 to 3, than from 0 to 1. The fronds which took 48 hours to achieve a positive result only produced picrate scores of 1. Although the percentage of cyanogenic fronds at these sites was higher after 48 hours, the estimate of the mean amount of HCN released by fronds at these sites has decreased, but this was not significant (see below).

4.2. Results.

Stands of bracken fronds were found to be polymorphic for cyanogenesis throughout Britain. At any one site, from 0% to 100% of the fronds sampled released HCN in sufficient quantities to produce a positive result with the picrate paper test after 24 hours, with a mean (± standard error) of 36.56 ± 7.75%; after 48 hours, the mean percentage of cyanogenic fronds was 41.15 ± 7.74%. Cyanogenic fronds were estimated to release from 15.8 to 402.5 µg HCN g^{-1} fresh weight, with a mean of 85.0 ± 22.0 µg HCN g^{-1} fresh weight when scored after 24 hours (Table 4.1). After 48 hours, between 15.0 and 402.5 µg HCN g^{-1} fresh weight of HCN were released, with a mean estimate of HCN released of 81.63 ±22.19 µg HCN g^{-1}. There was no significant difference between the results taken after 24 hours and those taken after 48 hours, when analysed using a blocked analysis of variance treating the two results for each site as replicates (habitat/replicate stratum F=0.096, d.f.=3,14. Not significant). The differences between 24 and 48 hours have therefore been ignored.

4.3. Analysis of results.

4.3.1. The Effect of Habitat Type.

The results were subjected to a blocked analysis of variance. By using this analysis any effect of the habitat type upon cyanogenesis should be evident. The locations were treated as blocks and the habitat types as treatments. In this way the differences in the date of sampling at each location can be

Table 4.1. The percentage of cyanogenic fronds in each sample and the estimated mean amount of HCN released from cyanogenic pinna tips from sites throughout Britain in 1983. The results were recorded by the picrate test, scored after 24 hours (with the results from the picrate test after 48 hours in brackets, if different). The date given is the date on which the fronds were sampled. The transformed percentage of cyanogenic fronds is the value of the percentage after the angular (arcsine) transformation, necessary for statistical analysis. The key to the sites is given in Table 3.1. (Loc., locations; %, the percentage of cyanogenic fronds in each sample; t[%], the transformed percentage of cyanogenic fronds in each sample; HCN, the estimated amount of HCN, $\mu g\ g^{-1}$ fresh weight).

Loc.	Site	Habitat	Date	%	t[%]	HCN
1	1	coastal	20.6	2	8.13	133.6
	2	open	21.6	100	90.0	277.3
	3	woodland	22.6	10 (72)	18.43 (58.05)	59.8 (29.8)
2	4	coastal	27.6	58	49.60	44.1
	5	open	26.6	18 (20)	25.10 (26.56)	24.5 (23.8)
	6	woodland	25.6	96	78.46	116.5
3	7	coastal	1.7	4 (14)	11.54 (21.97)	50.2 (31.8)
	8	open	29.6	98	81.87	143.0
	9	woodland	30.6	100	90.0	402.5
4	10	open	3.7	18 (21)	25.10 (27.27)	33.7 (31.5)
	11	woodland	4.7	100	90.0	335.4
5	12	coastal	16.7	37.5 (51)	37.76 (45.57)	53.0 (40.5)
	13	open	14.7	18 (25.5)	25.10 (30.33)	32.0 (27.9)
6	14	coastal	23.7	0	0	0
	15	open	21.7	0	0	0
	16	woodland	22.7	2	8.13	103.6
7	17	coastal	27.7	0	0	0
	18	open	25.7	60	50.77	39.4
	19	woodland	26.7	30	33.21	70.2
8	20	coastal	30.7	6 (8)	14.18 (16.43)	16.0 (15.0)
	21	open	1.8	16	23.58	15.8
	22	woodland	31.7	0	0	0
9	23	open	3.8	66 (76)	54.33 (60.67)	32.4 (31.1)
	24	woodland	4.8	38	38.06	47.0
Means, ±SE			24 hours		36.56 ±7.75	84.58 ±21.96
			48 hours		41.15 ±7.74	81.66 ±22.19

Table 4.1 - continued.

Mean transformed percentage of cyanogenic fronds, t[%] ±S.E.

Coastal	17.4 ±7.2
Open	41.7 ±9.9
Woodland	44.6 ±13.0
Grand mean	35.4 ±10.6

Mean amount of HCN released from samples of cyanogenic fronds (HCN), $\mu g\ g^{-1}$ ±SE

Coastal	42.41 ±17.45
Open	66.46 ±29.63
Woodland	141.87 ±51.49
Grand mean	84.5 ±21.96

Mean amount of HCN released from cyanogenic fronds at all sites excluding site 2, location 1 (HCN#) $\mu g\ g^{-1}$ ±SE.

Coastal	42.41 ±17.45
Open	40.15 ±15.35
Woodland	141.87 ±51.49
Grand mean	76.22 ±21.20

overcome. The significance of the different locations and sampling date can be separated from the effect of habitat. The percentages of cyanogenic fronds at each site were transformed using the angular transformation,

$$\theta = \sin^{-1} (n/100)^{-2}$$

prior to the analysis, to render data suitable for analysis (Sokal and Rohlf, 1969).

Neither locations nor habitat had any effect upon the results of the picrate test scored after 24 hours as the percentage of cyanogenic fronds or the estimated mean amount of HCN released by cyanogenic pinna tips (Table 4.2). However, it was noticed that the mean amount of HCN estimated for cyanogenic fronds at site 2, the open site at location 1, was almost twice as much as the second highest mean for an open habitat: 277.3 µg HCN g^{-1} fresh weight for site 2 compared with 143.0 µg HCN g^{-1} fresh weight for site 8. In chapter 3, it was seen that the associated flora at site 2 was composed almost wholly of woodland species. Although many of these are common in the open sites, site 2 lacks any open or heathland species, and in this respect it is anomalous. This site was adjacent to a wood and part of a farm. It is possible that this site represented a woodland habitat that had been cleared comparatively recently; attempts to verify whether or not this was the case were not successful. It should be noted that the results from this site are anomalous only with regards to the estimate of the mean amount of HCN released by pinna tips: the high percentage of cyanogenic fronds is in keeping with the large degree of variation found throughout the survey for this character. However, it was felt appropriate to repeat the analyses of variance of the estimates of the mean amount of HCN released after excluding the results for site 2 (Table 4.2). The estimated mean amount of cyanide released from cyanogenic pinna tips after 24 hours showed a statistically significant effect, at the 5% level, due the habitat type (Table 4.2). In no case is the effect of location and time of sampling significant.

The means given in Table 4.1 show that there is very little difference between the coastal and open habitats: the mean amount of HCN released from cyanogenic pinna tips from coastal sites is 42.41 ± 17.45 µg HCN g^{-1} fresh weight and that from open sites is 40.15 ± 15.35 µg HCN g^{-1} fresh weight. This indicates that the statistically significant result in the analysis of variance is solely due to the high levels of HCN released from cyanogenic pinna tips in woodland habitats, with a mean amount of HCN released of 141.87 ± 51.49 µg

Table 4.2. The results of analysis of variance of the transformed percentage of cyanogenic fronds (t[%]), the estimated mean amount of HCN released from cyanogenic fronds at all sites (HCN), and the estimated mean amount of HCN released from cyanogenic fronds excluding site 2, location 1 (HCN#). Full analysis of variance tables are given in Appendix 1, Tables A1.1 - A1.3.

Character	Variation between	Variance ratio	Degrees of freedom	Significance
t[%]	locations	1.27	8,13	N.S.
t[%]	habitats	1.99	2,13	N.S.
HCN	locations	1.68	8,13	N.S.
HCN	habitats	2.52	2,13	N.S.
HCN#	locations	1.85	8,12	N.S.
HCN#	habitats	4.10	2,12	*

N.S. not significant; * significant at 5% level ($P<0.05$).

HCN g^{-1} fresh weight. The effect of habitat on the estimated mean amount of HCN released from cyanogenic pinna tips is therefore an effect of woodland rather than of habitat generally.

4.3.2. Other Environmental Effects.

The environmental variables measured at each site or estimated later were analysed to ascertain whether or not there were any environmental effects other than that due woodland. Regression and correlation analyses were used on the results from each habitat type separately and also on the results of all sites together. Both the transformed percentage of cyanogenic fronds at each site and the estimated mean amount of HCN released from cyanogenic pinna tips were analysed in this way (Tables 4.3 - 4.5).

4.3.2.1. The percentage of cyanogenic fronds.

Of immediate interest are the correlations found between the percentage of cyanogenic fronds sampled and the estimated mean amount of HCN released from cyanogenic pinna tips (Tables 4.3 and 4.5). These two features were correlated for both open ($r=0.845$, $P<0.01$) and woodland ($r=0.829$, $P<0.05$) habitat types, and for all the sites taken together ($r=0.765$; $P<0.001$). The transformed percentage of cyanogenic fronds at all sites was also significantly correlated with the monthly maximum July temperature ($r=0.445$, $P<0.05$. Table 4.3). No other factors measured or estimated for each site have a significant correlation with the percentage of cyanogenic fronds at all sites (Table 4.3).

There are no factors which were significantly correlated with the transformed percentage of cyanogenic fronds sampled at coastal sites (Table 4.3). Those factors with the largest correlation coefficients are the amount of phosphorus present in the soil ($r=-0.530$), the estimated mean summer temperature temperature ($r=0.581$), the actual July maximum temperature ($r=-0.554$) and the amount of rainfall ($r=-0.543$ for the amount of annual rainfall).

The percentage of cyanogenic fronds sampled at open sites is significantly correlated with soil pH ($r=0.851$; $P<0.01$) and soil magnesium levels ($r=-0.690$; $P<0.05$) (Table 4.3). Most of the climatic variables estimated for the open sites have very low correlation coefficients with the percentage of cyanogenic fronds sampled at open sites.

In addition to the amount of HCN released, the only correlation with the percentage of cyanogenic fronds sampled at woodland sites was the maximum July temperature ($r=0.846$; $P<0.01$) (Table 4.3). The other climatic variables

Table 4.3. The correlation coefficients, r, of the transformed percentages of cyanogenic fronds scored after 24 hours at sites of different habitat types and at all sites correlated with the environmental variable measured at each site or estimated subsequently. The different measurements made are discussed fully in chapter 3.

Environmental factor	Coastal sites (df=5)	Open sites (df=7)	Woodland sites (df=6)	All sites (df=22)
Mean amount of HCN	0.213	0.845**	0.829*	0.765***
Soil pH	-0.216	0.851**	-0.279	-0.113
I.B.D.	0.159	-0.432	0.080	-0.150
Soil P	-0.530	-0.002	0.292	-0.074
Soil K	0.275	-0.467	-0.095	-0.151
Soil Mg	0.164	-0.690*	-0.397	-0.387
Soil N	-0.056	-0.496	-0.114	-0.095
Winter temp.	0.418	-0.390	0.261	-0.109
Spring temp.	0.420	0.026	0.602	0.244
Summer temp.	0.581	0.021	0.345	0.095
Autumn temp.	0.474	-0.287	0.352	-0.095
Jan.min.temp.	-0.163	-0.110	-0.094	-0.232
May min.temp.	-0.059	-0.170	-0.138	-0.267
Jun.min.temp.	-0.127	-0.136	-0.021	-0.201
July max.temp.	0.544	0.082	0.846**	0.445*
Winter rain	-0.456	0.013	-0.529	-0.173
Spring rain	-0.504	0.026	-0.497	-0.169
Summer rain	-0.557	0.035	-0.575	-0.216
Autumn rain	-0.575	0.065	-0.519	-0.166
Annual rain	-0.543	0.038	-0.535	-0.183

* significant at 5% level ($P<0.05$); ** significant at 1% level ($P<0.01$); *** significant at 0.1% level ($P<0.001$).

Table 4.4. The correlation coefficients, r, of the estimated mean amount of HCN released after 24 hours from cyanogenic pinna tips sampled at sites of different habitat types correlated with the environmental variables measured at the site or subsequently estimated. The different measurements made are discussed fully in chapter 3.

Environmental factor	Coastal sites (df=5)	Open sites (df=7)	Woodland sites (df=6)	All sites (df=22)
Soil pH	0.060	0.707*	-0.170	-0.025
I.B.D.	-0.373	-0.467	0.272	-0.189
Soil P	-0.485	0.293	0.340	0.101
Soil K	0.233	-0.286	-0.343	-0.208
Soil Mg	-0.201	-0.584	-0.487	0.361
Soil N	-0.410	-0.352	-0.260	-0.149
Winter temp.	0.374	-0.223	0.182	0.004
Spring temp.	0.382	0.143	0.793*	0.433*
Summer temp.	0.192	0.417	0.580	0.286
Autumn temp.	0.385	-0.050	0.307	0.022
Jan.min.temp.	0.568	0.175	-0.047	-0.016
May min.temp.	0.621	0.142	-0.161	-0.075
Jun.min.temp.	0.530	0.185	0.003	0.019
July max.temp.	0.066	-0.010	0.941***	0.474*
Winter rain	-0.429	-0.072	-0.820*	-0.382
Spring rain	-0.247	-0.044	-0.776*	-0.346
Summer rain	-0.282	-0.022	-0.791*	-0.348
Autumn rain	-0.324	-0.011	-0.817*	-0.329
Annual rain	-0.332	-0.035	-0.810*	-0.355

* significant at 5% level ($P<0.05$); *** significant at 0.1% level ($P<0.001$).

Table 4.5. The correlation coefficients, r, of the estimated mean amount of HCN released after 24 hours from cyanogenic pinna tips sampled at both open habitat sites and all habitat sites, excluding site 2, correlated with the environmental variables measured at each site or later estimated. The different measurements made are discussed fully in chapter 3.

Environmental factor	Open sites (df=6)	All sites (df=21)
Percentage of cyanogenic fronds	0.865**	0.726***
Soil pH	0.781*	-0.071
I.B.D.	-0.495	-0.162
Soil P	-0.215	0.053
Soil K	-0.426	-0.204
Soil Mg	-0.724*	-0.323
Soil N	-0.570	-0.151
Winter temp.	0.099	0.113
Spring temp.	0.574	0.528**
Summer temp.	0.207	0.219
Autumn temp.	0.138	0.083
Jan.min.temp.	-0.251	-0.063
May min.temp.	-0.456	-0.101
Jun.min.temp.	-0.282	-0.009
July max.temp.	0.682	0.625**
Winter rain	-0.481	-0.477*
Spring rain	-0.478	-0.450*
Summer rain	-0.501	-0.454*
Autumn rain	-0.488	-0.437*
Annual rain	-0.493	-0.460*

* significant at 5% level ($P<0.05$); ** significant at 1% level ($P<0.01$); *** significant at 0.1% level ($P<0.001$).

estimated from the sites typically have high but not significant, values of r. Of particular interest is the correlation coefficient associated with the mean spring temperature (r=0.602). None of the soil characteristics recorded from woodland sites correlate with the percentage of cyanogenic fronds sampled at these sites, and these variables typically have low values of r with the percentage of cyanogenic fronds sampled from woodland sites.

4.3.2.2. The amount of HCN released.

The estimate of the mean amount of HCN released by the cyanogenic pinna tips sampled at coastal sites had generally low values of the correlation coefficient, r, when correlation analysis with the associated measurements was carried out (Table 4.4). There were no significant correlations. However, the greatest values of r were obtained for the estimated minimum temperatures for January (r=0.568), May (r=0.621), and June (r=0.530).

The estimated mean amount of HCN released from cyanogenic fronds at open habitat sites gave similar results to the percentage of cyanogenic fronds when correlated with the variables (Table 4.4). Soil pH was significantly correlated with the amount of HCN released (r=0.707; $P<0.05$). The estimated climatic variables again gave low values of r, particularly the seasonal and annual amount of rainfall. The correlation coefficients for the estimated mean amounts of HCN released from open habitat sites excluding site 2, the anomalous site discussed earlier, are of more interest (Table 4.5). The soil pH was still significantly correlated (r=0.781; $P<0.05$). The amount of soil magnesium also gave a significant correlation (r=−0.724; $P<0.05$), as it did with the percentage of cyanogenic fronds sampled at open sites. The correlation coefficients of several other variables also changed when site 2 was omitted, although they do not become significant. In particular, the value of the correlation coefficients associated the maximum July temperature (from r=−0.010, including site 2, to r=0.682, excluding site 2) and the amount of rainfall (for annual rainfall, from r=−0.035, including site 2, to r=−0.493, excluding site 2) were altered.

The estimated mean amount of HCN released from cyanogenic pinna tips sampled at woodland habitat sites correlated significantly with several of the associated factors (Table 4.4). The correlation coefficient between the amount of HCN released and the average spring temperature was significant (r=0.793; $P<0.05$). The equivalent correlation coefficient for the amount of HCN released with the percentage of cyanogenic fronds sampled at woodland sites was also high. The maximum July temperature correlated significantly with the amount

of HCN released at woodland sites ($r=0.941$; $P<0.001$). The January, May and June minimum temperatures had very low correlation coefficients with the estimated mean amount of HCN released from cyanogenic fronds sampled at woodland sites. There were significant correlations between the estimated mean amount of HCN released and each of the seasonal rainfall values ($r=-0.810$; $P<0.05$, for annual rainfall).

Taking all the sites together, the estimated mean amount of HCN released from cyanogenic pinna sampled at each site correlated significantly with two of the environmental variables (Table 4.4). These were the average spring temperature ($r=0.433$; $P<0.05$) and the maximum July temperature ($r=0.474$; $P<0.05$), both of which correlated significantly with the amount of HCN released from cyanogenic fronds sampled at the woodland habitat sites. The effect of removing site 2 from the analysis increases the significance of the correlation coefficients of both the average spring temperature ($r=0.528$; $P<0.01$) and the maximum July temperature ($r=0.625$; $P<0.01$) (Table 4.5). In addition, the correlation coefficients for seasonal amounts of rainfall are all significant ($r=-0.460$; $P<0.05$, for annual rainfall).

4.4. Discussion.

4.4.1. The effect of habitat type upon cyanogenesis.

The effect of habitat type upon the estimated mean amount of HCN released from cyanogenic pinna tips can be seen to be due to an effect of woodland: the means and standard errors of coastal and open habitat types are very similar and do not account for much of the variance found (Table 4.2). Bracken growing in woodland could be affected by several factors. Firstly, there could be a direct effect of shade on the carbon/nitrogen balance: under low levels illumination, carbon might be limiting whereas nitrogen is likely to be limiting under high levels of illumination. Secondly, the large amount of litter and the increased organic matter in the soil could lead to increased mineral nutrition of bracken, resulting in excess nitrogen. Thirdly, the trees could stimulate microbial activity in the soil, either directly (as a result of root exudation, for instance) or indirectly (as a result of the large amount of litter and the detritus), again resulting in increased mineral nutrition of the bracken growing as an understorey, leading to excess nitrogen. It is likely that all these mechanisms are active to some degree. Each is likely to increase the amount of nitrogen available to bracken relative to the amount of carbon fixed.

Plants grown in open sunlight achieve maximum rates of photosynthesis at

light intensities of approximately 150 - 200 W m^{-2}, or about 40 - 50% of full sunlight (Fitter and Hay, 1983). At illuminations lower than this, photosynthesis is not optimised: light becomes the limiting factor. For plants grown in the shade, the photosynthetic apparatus is adapted to lower intensities of illumination and saturation of photosynthesis occurs at levels as low as 15 - 20% of full sunlight (Etherington, 1982). Differences are found between genotypically adapted shade plants and phenotypically adapted shade plants, although both affect the physiology of the plants (Fitter and Hay, 1983). Boodle (1904) showed that a single bracken frond grown under experimental conditions could show both sun and shade leaf characteristics if distal and proximal pinnae were kept under different light regimes. This suggests that shade tolerance in bracken is a phenotypic response rather than a fixed genotypic response, although ferns generally have low photosynthetic capacities, with light saturation occurring at low levels of illumination (Raven,1985). In either case, the efficiency of carbon fixation by bracken growing as an understorey plant will be reduced.

The amount of light reaching the understorey of a wood is reduced to between 37% and 0.5% of the external value, with values for deciduous woods in the range of 22% to 7% (Anderson, 1964; Etherington, 1982; Harper, 1977). The amount of light reaching the bracken understorey in a coniferous plantation in Thetford Chase (contiguous with Brandon Park, site 9) was 13% of the value outside the tree canopy (Roberts et al, 1984). At this level of illumination, photosynthesis will be sub-optimal even for a shade leaf. Carbon assimilation will therefore be limiting, rather than mineral nutrition as is likely to be the case in conditions of full illumination (Harper, 1977; Fitter and Hay, 1983). Most plants respond to low levels of illumination by reducing root growth (Mooney, 1972) and there is some evidence that the activity of nitrate reductase is reduced in shaded leaves (Beevers and Hageman, 1980), therefore reducing mineral uptake and nitrate utilisation. Nitrogen will be available in excess under these conditions. This has been shown for Impatiens parviflora DC. in experimental trials: with shading, a 10-fold dilution of nutrient medium had no effect upon growth, although at high levels of illumination there were marked effects of such a dilution (Hughes, 1966). Thus at low levels of illumination, low mineral concentrations are the optimum, and mineral concentrations which might be limiting under full illumination are not so under shading.

The second and third hypotheses - that the large amount of litter present in a woodland could increase the uptake of mineral nutrients, and that the

presence of tree roots could actively stimulate mineralisation - are difficult to separate. The increased proportion of organic matter in woodland soil would stimulate rhizosphere activity compared to a heathland soil (Etherington, 1982). Acid heathland soils are typically 'mor' in form, whilst woodlands have better developed brown-forest soil, or 'mull' (Etherington, 1982). Mor soils have a deep persistent litter layer and a slow rate of decomposition due to limited rhizosphere activity. Mull soils are characterised by rapid breakdown and mineralisation compared to mor soils. This leads to efficient nutrient cycling despite the large nutrient pool that remains held in the standing crop of the trees (Etherington, 1982). The development of an extensive root system by a woodland community can also encourage rhizosphere activity by increasing aeration and the amount of organic matter in the soil.

The increased availability of nitrogen to shaded plants is also indicated by the increased nutritional quality of such plants to herbivores (Blair et al, 1983). Nutritional quality is largely determined by the amount of protein available to herbivores relative to the amounts of carbohydrate, plants being poor suppliers of nitrogen to herbivores (McNeill and Southwood, 1978; Mattson, 1980). Thus both the absolute and the relative quantities of nitrogen available to a phytophagous insect are important in determining herbivory (McNeill and Southwood, 1978).

Cooper-Driver et al (1977) showed that at a single site in southern England, shaded bracken produced greater amounts of HCN than unshaded bracken, and that the converse was true for tannins, a quantitative allelochemical. The results reported in this chapter support the differentiation between systems of defence in bracken. The production of cyanogenic compounds by shaded bracken could result from a phenotypic response to the low light intensity, due to physiological and biochemical mechanisms discussed above. Under high light intensities, in the open, nitrogen utilisation and plant defence in bracken would be maximised by the use of carbon-based allelochemicals, including tannins.

Louda and Rodman (1983) showed an effect of shade on the production of glucosinolates in Cardamine cordifolia: removal of natural shade decreased the amount of glucosinolates produced, with a significant effect on the extent of herbivory on exposed plants. A similar result was found when naturally occurring shade and sun plants were compared. The authors concluded that individual plants differed in their pattern of secondary compound production in response to environmental factors.

Because of the ecology of Trifolium repens and Lotus corniculatus,

comparisons cannot be made between plants growing naturally under open and shaded conditions: neither species is found in woodland, being limited to open grassy places (Clapham et al, 1981).

Both Lotus corniculatus and Trifolium repens show different distributions of cyanogenic and acyanogenic phenotypes in different habitat types. In particular, populations of Lotus corniculatus contain a greater frequency of cyanogenic plants under heathland conditions than coastal (Ellis et al, 1977b; Keymer and Ellis, 1978). These differences have been attributed to exposure, especially the effect of salt spray (Keymer and Ellis, 1978) and water deficit (Foulds and Grime, 1972a,b; Foulds and Young, 1977; Foulds, 1977). Exposure has also been implicated as a selective agent for cyanogenesis along a stretch of variable coastline (Abbott, 1977, 1981). These various workers have suggested that exposure, saline spray and/or extreme water deficit (both of which could produce physiological drought), could either be effective directly on the plants or by mediation through animal herbivores. Kaplan et al (1983) also showed that exposure, measured as proximity to the sea, significantly reduced the frequency of cyanogenesis in a total of 108 species, and that this was related to insect pressure. In the absence of herbivores, particularly molluscs (Crawford-Sidebotham, 1972), cyanogenesis at exposed sites is of little advantage and is thus selected against (Ellis et al, 1977b,c).

The results given here show no differentiation between open and coastal habitat types, with respect to either the percentage of cyanogenic fronds or the estimate of the amount of HCN such fronds release. This could be because Pteridium aquilinum is not subject to the same selection pressures as Lotus and Trifolium, particularly with regard to herbivore pressure. The stature of bracken fronds compared to the small leaves of Lotus and Trifolium make this very likely.

4.4.2. The effect of climate upon cyanogenesis.

Work on Trifolium has also implicated winter temperature as a selective agent against cyanogenesis, either directly upon cyanogenic phenotypes (Daday, 1954a,b), through linkage with other temperature sensitive characteristics (Daday, 1965) or due to temperature altering the distribution of herbivores (Bishop and Korn, 1969; Brighton and Horne, 1977). Ennos (1981) has shown that cyanogenic genotypes in Trifolium may be linked with genes for morphological characteristics, and it is possible that these are selected for by herbivores or temperature (Cahn and Harper, 1976). Ellis et al (1977a) show no effect of temperature on cyanogenesis in Lotus corniculatus, although

Foulds and Young (1977) found that frosting selected against cyanogenic phenotypes.

Neither the mean winter temperature nor the minimum winter temperature correlated with cyanogenesis in bracken in any of the three habitat types (Tables 4.5 - 4.7). This is possibly because, unlike either Lotus or Trifolium, the perennial parts of Pteridium are subterranean, and thus less susceptible to frost damage. Watt (1950) has shown however that frost can be an important factor in determining the ecology of bracken, and that frond buds were susceptible to frost damage prior to emergence. If cyanogenic frond buds are differentially frost sensitive, it is possible that the cyanogenic fronds were those that emerged later in the season or from deeper in the soil, or both.

There was a significant correlation between the amount of HCN released by cyanogenic pinna tips and the mean spring temperature at woodland habitat types and all sites taken together, and it was believed that this could be due to an amelioration of low temperatures affecting emerging fronds. To test this hypothesis, the minimum temperatures of May and June were investigated, since any late frosts could damage the emerging fronds. No significant result was obtained. The correlation with spring temperature is therefore difficult to explain.

In contrast to the lack of correlation with low temperatures, bracken showed a correlation between the maximum July temperature and both the percentage of cyanogenesis and the amount of HCN released by cyanogenic fronds (Tables 4.3 - 4.5); there was no such correlation when the mean summer temperature was investigated. It should be noted that although the correlation with maximum July temperature was present when all the sites were taken together, a correlation was found only for the woodland habitat when the three habitat types are analysed separately. The same is true of the correlation between the amount of HCN released and the mean spring temperature. It is therefore likely that the correlation for all the sites was wholly due to the correlation between cyanogenesis and maximum July temperature at woodland sites. This is curious in itself: the correlation is likely to be due to an effect of woodland rather than an effect of temperature directly on bracken, since such an effect should produce a correlation for each habitat type. In addition, there was a very significant correlation between the maximum July temperature at woodland habitat sites and the annual rainfall at these sites ($r=-0.865$, $P<0.01$). A similar correlation between July maximum temperature and annual rainfall was found at open habitat sites ($r=-0.871$, $P<0.01$) but not at coastal habitat sites ($r=-0.571$, not significant). Taking all

the sites together, there was a highly significant correlation between the maximum July temperature and the amount of annual rainfall ($r=-0.785$, $P<0.001$). It is possible that the correlation between maximum July temperature and cyanogenesis at woodland habitat sites is due to a relationship between rainfall and cyanogenesis (Table 4.4). The correlation between July maximum temperature and the amount of HCN released by cyanogenic pinna tips at woodland habitat sites is far more significant ($r=0.941$, $P<0.001$) than that between rainfall and the amount of HCN released ($r=-0.810$, $P<0.05$). There is a correlation between July maximum temperature and the percentage of cyanogenic fronds sampled at woodland habitat sites ($P<0.01$, significant at 1%), whilst there is no correlation between the annual rainfall and the percentage of cyanogenic fronds at the same sites. Therefore it is likely that although the correlations between July maximum temperature and cyanogenesis at woodland habitat sites does not necessarily indicate a direct effect of temperature upon cyanogenesis, it is not due to a relationship between temperature and rainfall.

Because the correlations between July maximum temperature and cyanogenesis are not found with the other habitat types, it is unlikely that the correlation is due the maximum temperature having a profound effect on cyanogenesis. Possible effects of temperature on cyanogenesis could be mediated by effects on either i) the cycling or uptake of mineral nutrients, possibly by affecting the rhizosphere; ii) the synthesis, or turnover, of prunasin from phenylalanine (possibly due to an effect of temperature on other metabolic pathways, e.g. the conversion of phenylalanine into aromatic compounds by PAL); iii) the activity of β-glycosidase or hydroxynitrilase; or iv) some other factor, or factors, connected with the woodland habitat. The synthesis of prunasin has not been sufficiently well characterised for a statement to be made about the likelihood of any of these mechanisms.

The correlation between the amount of seasonal and annual rainfall and the amount of HCN released by cyanogenic pinna tips has been mentioned above, and it is possible that this correlation is due to a relationship between rainfall and maximum July temperatures. The correlation between cyanogenesis and rainfall is negative in all cases, showing that as rainfall decreases the amount of HCN released increases. In no study of cyanogenesis in other species has increasing water deficit been shown to increase cyanogenesis, although Boyd et al (1938) expected droughting to increase cyanogenesis in Sorghum. Again, the correlation between rainfall and cyanogenesis in bracken is limited to woodland habitat types (Table 4.4), although it is also significant when all sites

are taken together if site 2 is excluded from the analysis (Table 4.5).

There are two environmental characteristics that show correlations with cyanogenesis at open habitat sites only. These two characteristics are soil pH and soil Mg. Both show significant correlations with the percentage of cyanogenic fronds sampled at each site (Table 4.3) and with the amount of HCN released from cyanogenic pinna tips at open habitat sites excluding site 2 (Table 4.5). The correlation with pH is positive in both cases whilst that with soil Mg is negative. There was no correlation between the soil pH and the soil Mg, which suggests that they are unconnected. Both soil pH and Mg can affect the mineral nutrition of plants, both by altering the accessibility of calcium (Ca) (Fitter and Hay, 1983). In addition, low pH inhibits nitrification and can cause phosphate deficiency through reaction with aluminium hydroxides, and reduce the availability of other nutrients (Fitter and Hay, 1983). Thus at acid pH, the nutrition of bracken might be adversely affected. Indeed, low pH may be considered characteristic of nutrient poor soils (Etherington, 1982). Bracken growing in open habitats dominates the vegetation and can lead to a change in the soil, reducing the mineral status of the soil and lowering pH (Mitchell, 1977). The reduction of pH by bracken might be due to the large amounts of phenolic acids produced by the plant and released from the litter (Whitehead, 1964), which may also have an allelopathic effect (Gliessman and Muller, 1978; Whitehead et al, 1982; Stewart, 1975). The soil pH at coastal habitat sites will be ameliorated by the nature of the soil (often containing large amounts of shell fragments) and the action of the sea introducing calcium and other minerals. Similarly, the presence of other vegetation, and the large amount of litter and organic matter in the soil may have a buffering effect, although woodland habitat sites often had similar soil pH values to open habitat sites (see Table 3.5). Low soil pH can lead to slow rates of litter decomposition by limiting the activity of the rhizosphere (Etherington, 1982). Soil Mg can compete with Ca for uptake by plant roots, and this may lead to a build up of toxic Mg in plant tissues (Fitter and Hay, 1983). The correlations between both the percentage of cyanogenic fronds sampled at open habitat sites and the amount of HCN released by cyanogenic pinna tips at all open habitat sites except site 2 with soil Mg can be taken as an indication that the mineral nutrition of bracken in the field may have an effect on cyanogenesis, although a causal relationship is not necessary.

The correlation between the percentage of cyanogenic fronds sampled at both open and woodland habitat sites and the estimated amount of HCN released by pinna tips of these fronds would be expected if the same factors

determined both characteristics, but although the soil pH at open habitat sites and the July maximum temperature correlate with both characteristics, there is no evidence that this is so.

4.5. Conclusions.

The results of this survey show that the environment can have considerable influence on cyanogenesis in Pteridium aquilinum. The wide ecological tolerance of bracken may explain why this influence is particularly apparent in this species. A major effect of environment on cyanogenesis in bracken would appear to be mediated by nutrient relationships. This is not surprising given the effect which the diversion of nitrogen into the production of prunasin and β-glycosidase could represent in a nutrient-poor environment.

5. Seasonal variation of cyanogenesis in Pteridium aquilinum.

Cooper-Driver et al (1977) reported a seasonal decline of cyanogenesis in bracken for one season at one site in the south of England. However, no quantitative measure of cyanogenesis was used. Dement and Mooney (1974) and Kaplan et al (1983) report the feature for other plant species. It was decided to investigate the seasonal effect on cyanogenesis in three different habitats to see what qualitative and quantitative changes occurred in populations of fronds through the season.

5.1. Method.

A coastal, an open and a woodland habitat were selected at location 5 (chapter 3; Figures 3.1, 3.22 - 3.27). The coastal and open sites were studied for two successive seasons (1983 and 1984), whilst the woodland site was only available for the second of these seasons. The sampling procedure was similar for both seasons and followed that described in chapter 2. In the first season (the summer of 1983) a permanent plot at each site then available was established, and an 18m X 18m grid was marked out. One hundred fronds were selected on this grid and tagged with plastic labels to enable them to be found again. These fronds were sampled on four occasions from June until September. The first sample of 100 fronds at each site was not taken until the lowermost pinnae were sufficiently well developed to allow the removal of the terminal 4cm without significantly damaging the frond. After both the lowermost pinnae had been sampled (i.e. after the frond had been sampled twice), the lowermost intact pinna was sampled.

In 1984 it was decided to sample fronds from early in the season, before the croziers had expanded, to obtain information on cyanogenesis at this time. Sampling bracken fronds this early in the season would require the removal of comparatively large quantities of frond tissue in its unfurled state, which could alter the frond physiology and the behaviour of cyanogenesis. Because of this, in the second season (the summer of 1984) the procedure was altered. The fronds were not initially tagged. Instead, the sampling point on the grid was marked with a permanent peg to allow it to be found again and the closest frond to the peg which had not previously been sampled was sampled on each occasion. Before the third set of samples were taken in mid June 1984, when the fronds were sufficiently developed so that repeated sampling on a single frond could be carried out without that frond sustaining too much damage, the

closest unsampled frond to each grid point was tagged and that frond was subsequently sampled on each occasion as in 1983. This allowed the amount of HCN to be followed within a single frond as it changed during the season. The samples taken from fronds early in the season were of either the terminal 4cm of the lowermost pinna, or the whole unfurled pinna, whichever was least. Following tagging, the terminal 4cm of each of the two lowermost pinna were sampled for cyanogenesis.

The sodium picrate test for cyanogenesis was used to provide data on both the percentage of cyanogenic fronds at each site and an estimate of the mean amount of HCN released by cyanogenic pinna tips. Each site was sampled on four occasions in 1983 and on seven occasions in 1984.

The height of each frond sampled was recorded. An additional 20 pinna tips were removed from fronds within the site and weighed in situ. These pinna tips were then dried at 80°C until there was no further weight change, and the dry weight was measured.

5.2. Results.

Both the percentages of cyanogenic fronds sampled at each site and the estimated mean amount of HCN released by cyanogenic fronds at each site decreased with time during both seasons (Table 5.1; Figures 5.1 and 5.2). However, there was an initial increase in the amount of cyanide released by cyanogenic fronds at all sites in 1984, with a peak in late June for the open site and in early July for the coastal and woodland sites. This peak occurs at the open and woodland habitat sites after the fronds had been permanently tagged, and it is therefore not an artefact of the sampling method. A similar peak may have been missed by the later sampling regime in 1983. In addition, the amount of HCN is an estimate of the mean amount of HCN released by cyanogenic fronds, and as such it is not affected by the number of acyanogenic fronds in the sample.

The fresh weight, dry weight, and dry weight as a percentage of fresh weight of frond samples, and the height of fronds sampled change throughout the season in both 1983 and 1984 (Tables 5.2 and 5.3). These measurements give an impression of the development of the fronds during the season.

5.3. Discussion.

The percentages of cyanogenic fronds sampled at the coastal and open habitat sites in 1984 are greater than those sampled in 1983. Both sites in 1983 show a steady decline in the percentage of cyanogenic fronds sampled; in

Table 5.1. The percentage of cyanogenic fronds sampled and the estimated mean amount of HCN released by cyanogenic fronds (µg HCN g^{-1} fresh weight) for each sampling during 1983 and 1984. Up to the third sample taken in 1984, different fronds on each site were tested for cyanogenesis; following the third sample, the fronds were tagged and the same fronds were tested on each subsequent occasion. The number of surviving fronds from the original sample size of 100 at each site is given in brackets after the percentage of cyanogenic fronds.

a. The percentage of cyanogenic fronds sampled at each site during 1983.

Sample	1	2	3	4
Date	3-6.6	13-16.7	18-19.8	1-2.9
Habitat				
coastal	68(100)	35(96)	9(83)	3(76)
open	18(100)	16(94)	12(62)	9(55)

b. The estimated mean amount of HCN released by cyanogenic fronds, µg g^{-1} fresh weight, at each site sampled during 1983.

Sample	1	2	3	4
Date	3-6.6	13-16.7	18-19.8	1-2.9
Habitat				
coastal	67	59	32	21
open	107	30	27	45

c. The percentage of cyanogenic fronds sampled at each site in 1984.

Sample	1	2	3	4	5	6	7
Date	7-16.5	25-29.5	12-15.6	4-6.7	29.7-6.8	21-23.8	12-17.9
Habitat							
coastal	95(100)	95(100)	79(100)	89(99)	58(90)	24(87)	5(81)
open	21(100)	13(100)	43(100)	57(95)	21(75)	11(63)	7(46)
woodland	54(100)	60(100)	51(100)	56(100)	36(100)	27(99)	9(90)

d. The estimated mean amount of HCN released by cyanogenic fronds at each site, µg g^{-1} fresh weight, in 1984.

Sample	1	2	3	4	5	6	7
Date	7-16.5	25-29.5	12-15.6	4-6.7	29.7-6.8	21-23.8	12-17.9
Habitat							
coastal	38	44	131	69	47	34	14
open	79	45	85	115	80	46	13
woodland	35	68	133	411	348	255	115

Figure 5.1. The percentage of cyanogenic fronds fronds sampled at each of the sites at location 5 throughout the growing seasons in 1983 and 1984. △, coastal habitat site, 1983; ▲, coastal habitat site, 1984; □, open habitat site, 1983; ■, open habitat site, 1984; ●, woodland habitat site, 1984.

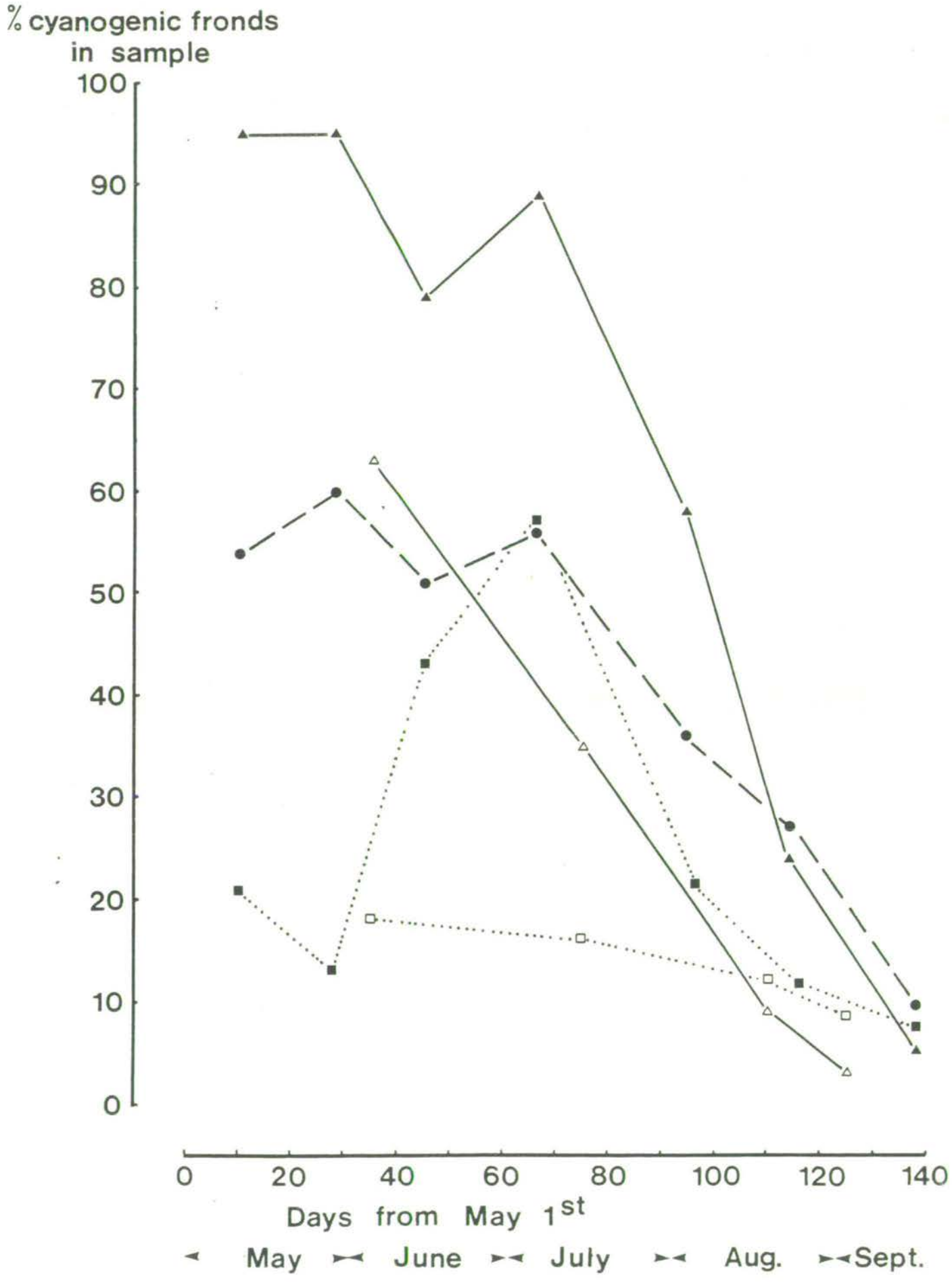

% cyanogenic fronds in sample
100
90
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Days from May 1st
May
June
July
Aug.
Sept.

Figure 5.2. The estimated mean amount of HCN released from cyanogenic pinna tips sampled at each of the sites at location 5 throughout the growing seasons in 1983 and 1984. △ , coastal habitat site, 1983; ▲ , coastal habitat site, 1984; □ , open habitat site, 1983; ■ , open habitat site, 1984; ● , woodland habitat site, 1984.

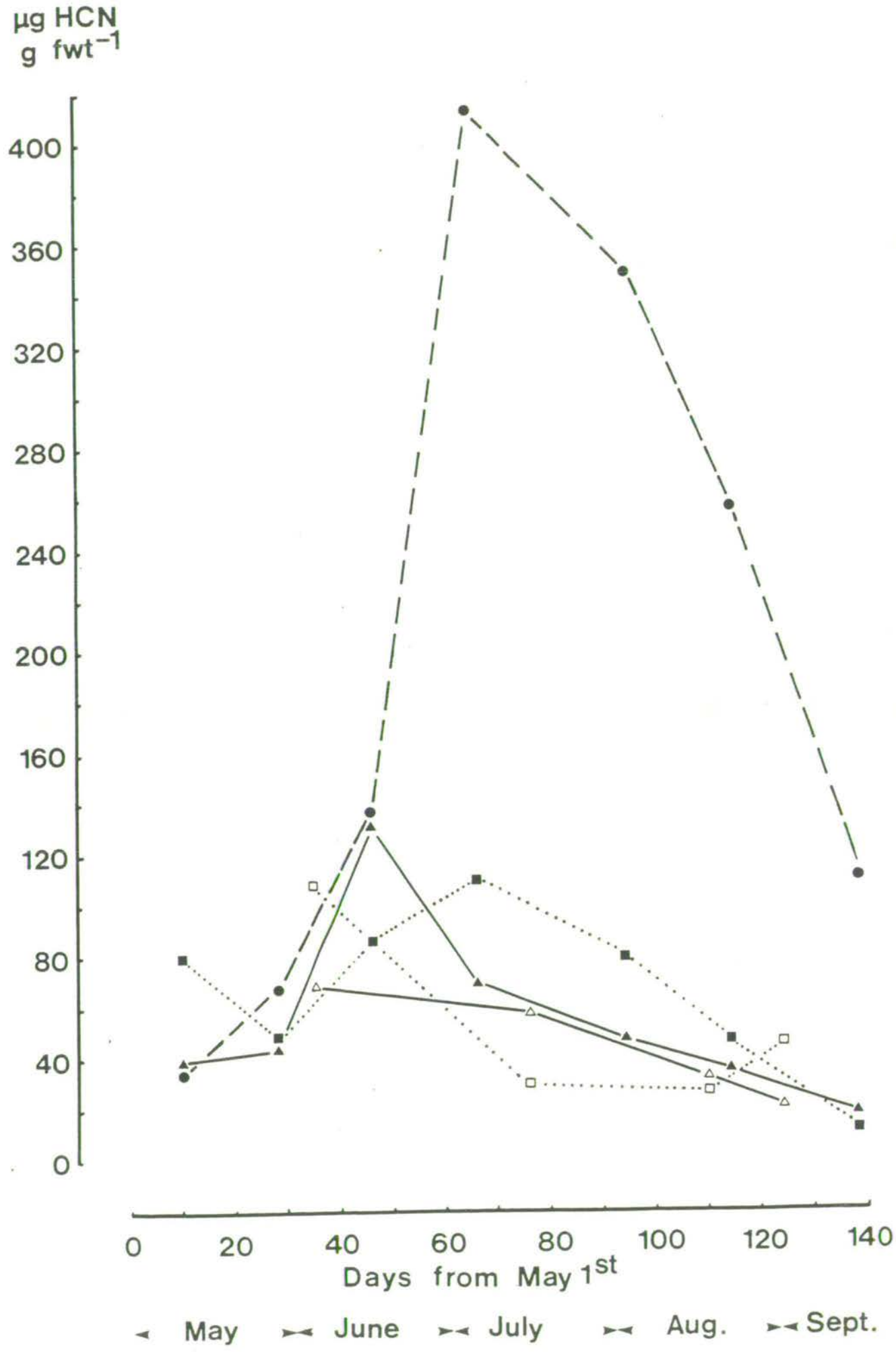

µg HCN
g fwt^{-1}
400
360
320
280
240
200
160
120
80
40
0
0
20
40
60
80
100
120
140
Days from May 1st
May
June
July
Aug.
Sept.

Table 5.2. The fresh weights (1983 and 1984) and dry weights (1984 only) of 20 representative pinna tips of a size equivalent to those tested for cyanogenesis from field sites at location 5.

a. The fresh weight of pinna tips sampled at each site during 1983, mg ± S.E.

Sample	1	2	3	4
Date	3-6.6	13-16.7	18-19.8	1-2.9
Habitat				
coastal	87.8±3.0	170.6±20.4	214.9±18.4	274.2±19.0
open	87.6±4.6	166.8±12.5	181.7±13.4	193.5±14.0

b. The fresh weight of pinna tips sampled at each site during 1984, mg ± S.E.

Sample	1	2	3	4	5	6	7
Date	7-16.5	25-29.5	12-15.6	4-6.7	29.7-6.8	21-23.8	12-17.9
Habitat							
coastal	173.5±40.0	309.9±13.7	192.3±10.8	235.4±19.0	271.7±16.2	278.4±9.5	298.1±13.3
open	160.5±23.6	327.1±11.9	192.1±8.7	170.2±11.8	226.0±9.5	239.0±10.5	238.7±9.1
woodland	148.4±17.6	260.4±20.8	182.9±14.9	72.9±4.3	78.7±2.8	93.8±4.0	119.7±3.5

c. The dry weight of pinna tips sampled at each site in 1984, mg ±S.E.

Sample	1	2	3	4	5	6	7
Date	7-16.5	25-29.5	12-15.6	4-6.7	29.7-6.8	21-23.8	12-17.9
Habitat							
coastal	31.3±6.1	58.6±2.6	37.4±2.4	59.1±5.9	74.0±5.0	79.9±3.4	87.4±3.9
open	25.9±3.9	54.7±2.1	36.3±1.7	49.8±4.1	72.5±3.2	83.3±3.9	77.0±2.7
woodland	24.0±3.5	42.5±4.7	28.6±2.5	16.6±1.2	19.0±0.9	25.5±1.3	27.7±0.8

d. The dry weight of pinna tips sampled in 1984 as a percentage of the fresh weight, % ± S.E.

Sample	1	2	3	4	5	6	7
Date	7-16.5	25-29.5	12-15.6	4-6.7	29.7-6.8	21-23.8	12-17.9
Habitat							
coastal	17.6±0.4	19.0±0.3	19.4±0.7	24.7±0.9	28.4±0.7	29.7±1.1	29.4±0.3
open	16.2±0.3	16.7±0.2	19.0±0.6	29.1±1.0	32.1±0.5	34.8±0.4	32.3±0.5
woodland	17.0±0.4	16.3±0.8	15.6±0.3	23.0±1.3	24.0±0.6	27.1±0.6	23.4±0.6

Table 5.3. The mean height of fronds sampled throughout the season in 1983 (coastal and open habitat sites only) and 1984 (all three habitat sites), cm ±S.E.

a. Fronds sampled throughout the season in 1983.

Sample	1	2	3	4
Date	3–6.6	13–16.7	18–19.8	1–2.9
Habitat				
coastal	52.8±1.4	142.9±2.1	152.3±2.4	153.5±2.4
open	27.7±0.8	69.4±1.9	75.5±2.2	75.2±2.4

b. Fronds sampled during the season in 1984.

Sample	1	2	3	4	5	6	7
Date	7–16.5	25–29.5	12–15.6	4–6.7	29.7–6.8	21–23.8	12–17.9
Habitat							
coastal	11.0±0.7	36.3±1.5	66.4±1.9	94.1±2.0	98.7±2.0	102.6±2.1	99.9±1.9
open	8.7±0.5	26.2±1.1	35.9±1.5	56.4±1.6	58.6±1.8	66.5±2.2	70.1±2.5
woodland	13.1±1.3	45.1±2.9	71.1±2.7	127.7±3.6	138.6±4.6	138.6±4.2	147.3±3.5

(Table 5.1); this finding is in keeping with the results presented for sites throughout Britain (chapter 4).

Both sites sampled in 1983 show a decline in the estimated mean amount of HCN released from cyanogenic fronds during the season, although the open habitat site shows a late season increase (Figure 5.2). This late season increase is in fact due to a single frond, which consistently scored 3 on the picrate test whilst other fronds at the site declined in the amount of HCN they released; several fronds with picrate scores of only 1 died between the third and fourth samplings at this site in 1983, resulting in an increase in the mean amount of HCN released.

All three habitat sites sampled in 1984 show an increase in the estimated mean amount of HCN released from cyanogenic fronds early in the season. The open habitat site shows an initial decline but following this there is an increase. The coastal habitat site shows a maximum estimated mean amount of HCN released at the third sampling in 1984 (mid June), whilst both the coastal and woodland habitat sites show their maxima at the fourth sampling (early July).

The large increase in the mean amount of HCN released from cyanogenic fronds sampled at the woodland habitat site between the third and fourth samplings in 1984 must be due to a substantial increase in the amount of HCN that each cyanogenic frond is releasing: weakly cyanogenic fronds in the earlier sample were highly cyanogenic in the later sample. To a lesser extent, the same happens at the coastal and open sites. This shows that the amount of HCN released from cyanogenic fronds is not fixed, but instead is variable. The increase could be due to increased nitrogen uptake from the soil as water is absorbed for flushing the fronds, increased de novo synthesis of prunasin to protect the expanding fronds, increased mobilisation of prunasin from the rhizomes into the fronds, or a combination of these. It is possible that the synthesis of prunasin requires active photosynthesis,and therefore as the fronds expand and start to photosynthesise there is an increase in the amount of prunasin in the frond. It has been shown that the in vitro synthesis of taxiphyllin from tyrosine requires NADPH as a source of metabolic energy (Hosel and Nahrstedt, 1980). NADPH is formed by the light reaction of photosynthesis.

The lowermost pinnae were not fully expanded until late June, between the third and fourth samplings in 1984, as the percentage dry weight of the pinna samples indicates with an increase. Before this, the fronds are unlikely to be photosynthetically self-sufficient, importing photosynthate for growth rather

than exporting excess assimilated carbon. It is possible that there is insufficient NADPH to use in the synthesis of prunasin before the pinna or frond is photosynthetically fully active.

There is evidence that cyanogenic glycosides (Conn, 1980a) and other nitrogen-based secondary plant compounds (Krischik and Denno, 1983) are easily transported around plants, although whether this is the case for bracken or not is not known. It has been suggested that the production of nitrogen-based allelochemicals may be a form of nitrogen storage as well as defensive chemicals, in that such compounds can be metabolised later (when more effective defensive chemicals are available) to release the nitrogen for growth (Krischik and Denno, 1983). Jones (1972a) suggested that cyanogenesis as a defensive mechanism could have evolved from a system of nitrogen storage. Rhizomes of Pteridium aquilinum from greenhouse grown plants which were tested for cyanogenesis and for the presence of prunasin gave no indication of HCN release (chapter 11). It is possible however that bracken in the wild translocates prunasin or its precursors from the fronds to the rhizomes at the end of each season, and that the glycoside is stored in the rhizomes during the winter and transferred to the developing fronds at the start of the following season.

In 1984 all three habitat sites show a decline in the amount of HCN released by cyanogenic fronds after the third (mid June, coastal habitat site only) or fourth (early July, open and woodland habitat sites) sampling. The rate of decline at the coastal site is similar to that recorded in 1983 (Figure 5.2). Following the dramatic increase in the amount of HCN released by cyanogenic fronds sampled at the woodland site, there is an equivalent decrease.

It has been shown that some plants possess inducible plant defences (Haukioja and Niemala, 1976; Haukioja, 1982; Rhoades, 1983, 1985). It is possible that repeated sampling of the same fronds, as was carried out in the throughout summer of 1983 and from mid June in 1984, could induce an increase in cyanogenesis or the production of other allelochemicals in Pteridium aquilinum, which could alter either the number of cyanogenic fronds or the amount of HCN such fronds release. There is no evidence from the results presented here that the sampling of fronds had any effect on the percentage of cyanogenic fronds sampled at later dates or the amount of HCN released by the pinna tips of such fronds. It is possible that other secondary chemicals produced by bracken were affected by the repeated sampling at the three sites.

Several field studies have shown that the production of secondary plant

compounds changes during the season. Dement and Mooney (1974) showed that Heteromeles arbutifolia produced both cyanogenic glycosides and tannins, prunasin being predominant in young leaves whilst tannins were more important in mature leaves. Mooney et al (1980) showed that seasonal variation of resin production in Diplacus aurantiacus was correlated with the life cycle of a prominent insect herbivore. Kraft and Denno (1982) showed a similar relationship between the production of resin by Baccharis halimifolia and herbivory by a beetle. Feeny (1968, 1970) showed that the amount of tannin present in leaves of Quercus robur increases throughout the season, and related this to the palatability of leaves to insect herbivores.

The results presented here are similar to those reported by Cooper-Driver et al (1977), and show a quantitative as well as qualitative variation throughout the season in all three habitat types. As well as a seasonal decline in cyanogenesis (measured in arbitrary units), Cooper-Driver et al (1977: Figure 3) also showed an early season increase in early June, although this was not commented on. They also reported greater cyanogenesis in shaded fronds compared to unshaded fronds, as found here. The method employed by Cooper-Driver et al (1977) required the sampling of approximately 1.5g from the second pinna from the apex of the frond; this pinna is small and the same frond could not be sampled on different occasions. Thus these authors were looking at larger amounts of tissue than reported here, and did not record cyanogenesis in the same fronds during the season. In addition, Cooper-Driver et al (1977) measured cyanogenesis in only 25 fronds for each sample. Thus their results are likely to be subject to greater sampling error than the results presented here.

Moon and Raafat (1951) investigated cyanogenesis in bracken on the Pentland Hills, the range of mountains which includes the open habitat site (location 5, site 13) for which results are presented here; unfortunately, Moon and Raafat do not give precise locations for their field sites. They employed a large scale method of harvesting, homogenising many fronds. They did not discriminate between cyanogenic and acyanogenic fronds, and it must be assumed that they were not aware of any polymorphism. They found a large amount of day to day variation, possibly due to harvesting from areas containing different proportions of cyanogenic and acyanogenic fronds. In addition, they found a decrease in the amount of HCN released by a mass of fronds throughout the season, in keeping with the results presented here. The levels of HCN they found were similar to those reported for the open habitat site in this study.

Schreiner (1980) sampled 15 fronds of bracken from six different stands in the north eastern United States, and tested for cyanogenesis. The exact sampling method used is not given. She too found a decrease in the amount of HCN produced by cyanogenic fronds during the season, although in contrast with the results presented here they found that the emergent croziers were the most cyanogenic.

Cooper-Driver et al (1977) also report an increase in tannins, carbon-based quantitative herbivore deterrent compounds, as cyanogenesis declines. This is in keeping with the differentiation between qualitative and quantitative plant defences discussed above, and their paper has been quoted in support of these theories (Krischik and Denno, 1983). Tempel (1981) found very variable levels of tannins in bracken during the season. Tannins act as herbivore deterrents by complexing the herbivores' digestive enzymes (Bate-Smith, 1973). Tannins have been demonstrated to inhibit non-specific β-glycosidases in vitro under particular conditions (Goldstein and Swain, 1965). It is possible that tannins inhibit the glycosidase enzyme responsible for the release of HCN (Goldstein and Spencer, 1985). Cooper-Driver et al (1977) report a substantial increase late in the season in the amount of tannin found in unshaded fronds, but not in shaded fronds. The effect of increased tannins on cyanogenesis cannot be estimated.

5.3. Conclusions.

The changes in cyanogenesis found in bracken throughout the season are in keeping with those found in other studies, both on Pteridium aquilinum and other species. However, cyanogenesis was found to be variable, some acyanogenic fronds (according to the picrate test) becoming cyanogenic later in the season, some time after emergence. The estimated mean amount of HCN released from cyanogenic fronds changed throughout the season, there being a steady decrease in 1983 whilst in 1984 there was an early season increase followed by a steady decrease. The differences noted between the two seasons could be due to differences in the timing of the start of the growing season and the start of sampling. The decline in cyanogenesis during the season can be explained in terms of the partitioning of available resources for plant secondary compounds to give the most efficient predation deterrent.

Chapter 6. Annual variation of cyanogenesis in Pteridium aquilinum.

The results presented in chapter 5 show variation in the percentage of cyanogenic fronds sampled at the coastal and open habitat sites between the two seasons, in 1983 and 1984. This raises questions as to the constancy of cyanogenesis from year to year at any one site, and to what extent the results from a single season can be applied more widely. This chapter reports an investigation into the variation over a three year period of cyanogenesis in bracken growing in three different habitats.

6.1. Method.

Each of the three sites at location six (chapter 3) were visited annually from 1983 to 1985, and 50 fronds sampled for cyanogenesis using the sodium picrate test (chapter 2). The sites were visited at approximately the same time of year each season, the third week in July. This does not take account of differences in the timing of each season, but it was felt that to attempt to allow for this would rely on a subjective assessment and that it would be more systematic to sample each year according to the calendar.

Each habitat site had been selected in 1983 and marked with permanent pegs to allow the 18 X 18m grid to be accurately established each season. All four corner pegs were found each year at the open and woodland habitat sites, the grid coinciding precisely with that originally established in 1983; unfortunately only one corner peg could be found at the coastal habitat site in 1984, so that there might be a slight discrepancy between the alignment of the grid in 1983 and 1984, when the corners of the grid were again marked. In 1985, only two pegs could be found at the coastal habitat site, so there might again have been a discrepancy in setting up the grid. The difference in the relocation of the grid between the seasons at the coastal habitat site is unlikely to have exceeded 0.5m.

The results from the picrate test were used to compare both the percentage of cyanogenic fronds sampled at each habitat site each year and the estimated mean amount of HCN released from cyanogenic fronds at each habitat site each year.

6.2. Results.

The percentages of cyanogenic fronds sampled at the open and woodland habitat sites increased each successive year (Table 6.1). No cyanogenic fronds

Table 6.1. The percentage of cyanogenic fronds sampled at three sites at location six on successive years. The angular transformation of each percentage is in parentheses.

Habitat	Year 1983	1984	1985	Mean angular transformation ±S.E.
coastal	0	0	0	0
open	0	4 (11.54)	16 (23.58)	11.7 ±6.3
woodland	2 (3.13)	10 (18.43)	28 (31.95)	19.5 ±6.9
Grand mean				10.4 ±4.0

Table 6.2. The estimated mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, released from cyanogenic pinna tips sampled annually from 1983 to 1985 at three sites at location 6.

Habitat	Year 1983	1984	1985	Mean, ±S.E.
coastal	0	0	0	0
open	0	25.6	21.7	15.8 ±8.0
woodland	103.6	135.9	153.6	131.0 ±14.6
Grand mean				48.9 ±21.2

were sampled at the coastal habitat site in any of the three years. The rank order of the percentages of cyanogenic fronds sampled at each site was the same from year to year, the woodland site always having a greater number of cyanogenic fronds than the other two sites.

The estimated mean amounts of HCN released from cyanogenic pinna tips also varied from year to year (Table 6.2). The cyanogenic fronds at the open habitat site released less HCN in 1985 than in 1984; since no cyanogenic fronds were sampled at the open habitat site in 1983, there was no estimate of the mean amount of HCN released for this site in that year. Cyanogenic fronds at the woodland habitat site released more HCN in each successive year.

The mean fresh weights of representative pinna tips sampled at each site in each year also varied from year to year (Table 6.3), as did the mean heights of fronds sampled for cyanogenesis (Table 6.4). These features of the fronds can be used as an indication of the development of the fronds in each year.

The results were analysed using a blocked analysis of variance. The percentages of cyanogenic fronds at each site in each year were transformed by the angular transformation to render the data suitable for analysis (Sokal and Rohlf, 1969; chapter 4). The percentage of cyanogenic fronds sampled at each site in each year did not differ significantly between years or between habitats (Table 6.5).* The estimated mean amount of HCN released from cyanogenic fronds at each site did not differ significantly between years, but did differ significantly between the three habitat types ($P<0.001$; Table 6.5). The mean fresh weights of representative pinna tips from each site do not differ significantly between years or between habitat types (Table 6.5). The height of fronds sampled for cyanogenesis differ significantly both between years ($P<0.05$) and between habitat types ($P<0.01$; Table 6.5).

6.3. Discussion.

The analysis of variance shows that the differences of both the percentage of cyanogenic fronds sampled and the estimate of the mean amount of HCN released by such fronds at each site between different years are not significant (Table 6.5),* and therefore that over the three years covered by the study the sites were consistent. The lack of effect of habitat on the percentage of cyanogenic fronds sampled is similar to that found throughout Britain (chapter 4), whilst the effect of the habitat type on the estimated mean amount of HCN released from cyanogenic fronds is consistent with the results found throughout Britain (chapter 4).

The fresh weight of representative pinna tips is not affected either by the

* This statement must be reconsidered in the light of a chi-squared analysis of the data in Table 6.1. Please see page 171 and that following page 173.

Table 6.3. Mean fresh weights of 10 (1983) or 20 (1984 and 1985) pinna samples taken at the three habitat sites at location 6 from 1983 to 1985, mg ± S.E.

	Year			
Habitat	1983	1984	1985	Mean, ±S.E.
coastal	279.3 ±33.0	154.2 ±7.2	158.5 ±7.3	197.3 ±41.0
open	173.3 ±19.3	178.4 ±10.6	156.0 ±7.9	169.2 ±6.8
woodland	96.5 ±5.6	84.6 ±5.4	94.4 ±3.3	91.8 ±3.7
Grand mean				152.8 ±19.8

Table 6.4. The mean heights of fronds sampled for cyanogenesis at the three habitat sites at location 6 from 1983 to 1985, cm ± S.E.

	Year			
Habitat	1983	1984	1985	Mean, ±S.E.
coastal	136.5 ±2.8	109.9 ±3.8	124.7 ±2.6	123.7 ±7.7
open	119.9 ±4.2	104.1 ±4.5	96.0 ±4.7	106.7 ±7.0
woodland	155.0 ±3.4	134.1 ±4.6	133.3 ±4.8	118.0 ±5.8
Grand mean				123.7 ±6.1

Table 6.5. The results of analysis of variance of the transformed percentage of cyanogenic fronds (t[%]), the estimated amount of HCN released from cyanogenic fronds (HCN), the mean fresh weight of samples (weight), and the mean height of sampled fronds (height), made over three years at sites at location six. Complete tables of the results of analysis of variance are given in Appendix 2, Tables A2.2 - A2.4.

Character	Variation between	variance ratio	degrees of freedom	significance
t[%]	years	3.988	2,4	N.S.
t[%]	habitats	6.123	2,4	N.S.
HCN	years	2.752	2,4	N.S.
HCN	habitats	87.583	2,4	***
weight	years	1.305	2,4	N.S.
weight	habitats	5.669	2,4	N.S.
height	years	11.780	2,4	*
height	habitats	27.27	2,4	**

N.S., not significant; *, significant at 5% level ($P<0.05$); **, significant at 1% level ($P<0.01$); ***, significant at 0.1% level ($P<0.001$).

Table 6.5a. The results of the reanalysis of the number of cyanogenic fronds sampled at the open and woodland habitat sites over three successive years (Table 6.1) using chi-squared analysis (Sokal and Rohlf, 1969).

Habitat	Degrees of freedom	X^2	Significance
Open	2	11.16	**
Woodland	2	15.33	***

, signifcant at 1%; *, significant at 0.1% level.

year to year variation or the habitat type. This suggests that there was little or no difference in the developmental state of the fronds between the three years and that comparisons between different years are valid. In contrast, the mean height of fronds sampled shows both an effect due to the year and due to the habitat type. The effect due to different years suggests that there were in fact developmental or environmental differences and that perhaps comparisons between years are not valid. These points are difficult to reconcile. However, because there were no effects of different years on either aspect of cyanogenesis, it can be assumed that the difference between years found with respect to the height of fronds has no effect on cyanogenesis.*

The consistent difference in the estimated mean amount of HCN released from samples taken from different habitats shows that either the environmental factors determining cyanogenesis are constant from year to year or that cyanogenesis is fixed in a population of bracken fronds, as would be the situation if cyanogenesis was genetically determined in Pteridium aquilinum. It is not possible to separate these two alternatives on the evidence available.

The observed differences in the various measurements between years at each site, whether significant or not, may be due to damage to the sites caused by repeated sampling at each site. However none of the differences found occurred throughout all the sites and thus it would appear that sampling at each site over an extended period has not changed the development or ecology of the plant. In particular, cyanogenesis does not appear to have been affected, and therefore has not been induced, at least not over the period of sampling. It is however still possible that other secondary plant chemicals have been affected by the repeated visits to these sites.

Other sites at location six were sampled for cyanogenesis in Pteridium aquilinum between 1978 and 1984, under the supervision of Dr. A.F. Dyer. The open and woodland sites sampled in this study were very close to, but not the same as, the open and woodland habitat sites for which results have been given. In this survey only the percentage of cyanogenic fronds in each sample of 100 fronds was recorded, using the picrate paper test. The results, presented in part elsewhere (Dyer and Hadfield, 1985), show little variation over the seven year period (Table 6.6). These results have been subject to analysis of variance following angular transformation. The analysis shows no effect between years (Table 6.7) but a very significant effect between habitats. These results and their analysis support the results reported here.*

There have been few studies of secondary plant metabolites which have compared levels of metabolites over several seasons. Jones (1977) reported on

* This statement must be reconsidered in the light of a chi-squared analysis of the data in Table 6.1. Please see page 171 and that following page 173.

Table 6.6. The percentage of cyanogenic fronds sampled at each of four sites at location six from 1978 to 1984. The sample size was 100 fronds. The data was collected under the supervision of Dr. A.F. Dyer, taken in part from Dyer and Hadfield (1985). The angular transformation of each percentage, necessary for statistical analysis, is given in parentheses.

	Year					
Habitat	1978	1979	1980	1981	1982	1984
coastal (exposed)	4(11.5)	3(10.0)	7(15.3)	2(8.1)	4(11.5)	-
coastal (sheltered)	59(50.2)	73(58.7)	79(62.7)	53(46.7)	54(47.3)	-
open	34(35.7)	80(63.4)	67(54.9)	62(51.9	68(55.5)	48(43.8)
woodland	35(36.3)	38(38.1)	51(45.6)	27(31.3)	33(35.1)	37(37.5)

Mean angular transformation of the percentage of cyanogenic fronds sampled, ±S.E.

Coastal (exposed)	11.3 ±1.2
Coastal (sheltered)	53.1 ±3.2
Open	50.9 ±4.0
Woodland	37.3 ±1.9
Grand mean	38.7 ±3.8

Table 6.7. The results of analysis of variance of the transformed percentage of cyanogenic fronds (t[%]) measured at four sites at location 6 over a period of six years. Complete analysis of variance tables are given in Appendix 2, Table A2.5.

Character	Variation between	Variance ratio	Degrees of freedom	Significance
t[%]	years	2.840	5,13	N.S.
t[%]	habitats	71.840	3,13	***

N.S., not significant; ***, significant at 0.1% level ($P<0.001$).

The analysis of the annual variation in the percentage of cyanogenic fronds sampled using chi-squared analysis shows that there significant differences between different years at both the open and the woodland habitat sites (Table 6.5a, page 171). It cannot be taken that the percentage of cyanogenic fronds sampled at any one site will be consistent from year to year. This observation shows that the variation between different years fits in with the results from other fieldwork which shows a large degree of variability with respect to the percentage of cyanogenic fronds sampled (chapters 4 and 7).

The amount of HCN released from cyanogenic fronds remains consistent between years (Tables 6.2 and 6.5).

Chapter 7. Local variation of cyanogenesis in Pteridium aquilinum.

The results from the survey of cyanogenesis in Pteridium aquilinum throughout Britain (chapter 4) showed that, in certain cases, differences between habitat types occurred over comparatively small distances. The results from the study of variation of cyanogenesis between different years (chapter 6) showed that these differences were constant from one year to another, at least over a relatively short period.* It is therefore of interest to know how cyanogenesis in bracken varies over small distances within habitat types.

7.1. Method.

The open and woodland habitat sites at location 6 (chapter 3) were close to sites sampled by Dr. A.F. Dyer from 1978 to 1982 (chapter 6; Dyer and Hadfield, 1985). In 1984, three sites within each of the three habitat types were sampled: the three sites originally studied by myself (chapters 3 and 4; within each habitat type, denoted site 1), the open and woodland sites studied by Dr. Dyer (chapter 6; within each habitat type, site 2) and one additional site in each habitat (site 3; Figures 7.1, 7.2, 7.3, and 7.4). The coastal habitat sites sampled by Dr. Dyer were not available for study, and two additional sites close to the original coastal site studied by myself at location 6 were selected (sites 2 and 3; Figures 7.5 and 7.6).

The sites sampled in both the open and woodland habitat types were very close to each other. The three sites sampled at the open habitat were within 150m of each other, and two were within 20m of each other (Figure 7.1). The three sites studied at the woodland habitat were within 500m of each other (Figure 7.3). There were no notable environmental differences between the sites in either habitat type. At both the open and woodland habitat types, bracken was continuous between each of the sites. It is possible therefore that single clones of bracken were present at each of the three sites at both habitat types.

The populations sampled at the coastal habitat types were discrete and more distant from each other, being spread over at least 1000m (Figure 7.5). It is likely that these represent different populations. The coastal sites were at different points in a bay and thus subject to different degrees of exposure. In addition, one was sheltered by a row of trees (site 3). Although this makes the results from the coastal habitat type somewhat difficult to analyse, they are

* This statement must be reconsidered in the light of the chi-squared analysis of the data in Chapter 6. Please see page 171 and that following page 173.

Figure 7.1. The three open sites at location 6 from which fronds were sampled in 1984. (Scale: 1/10000).

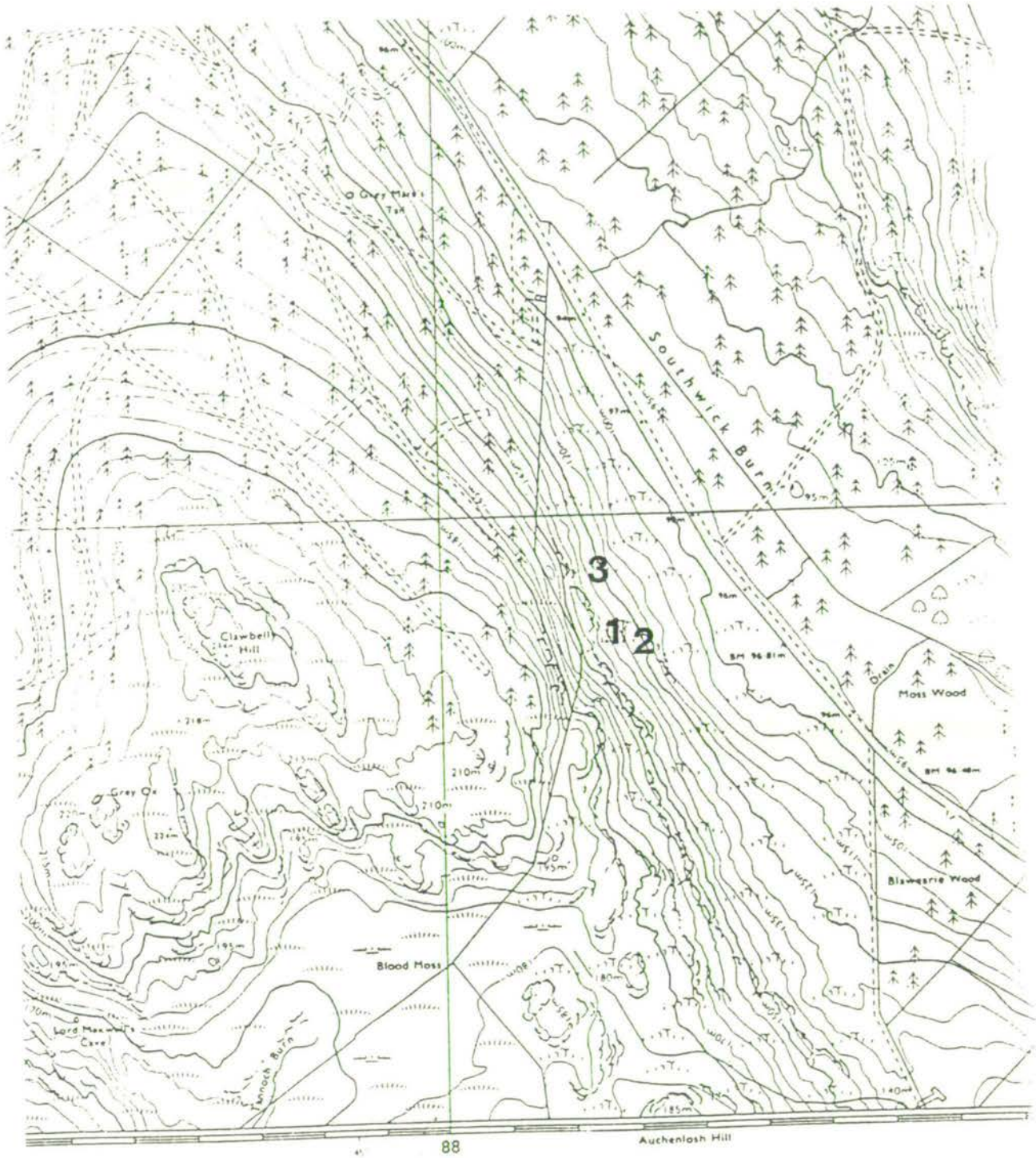
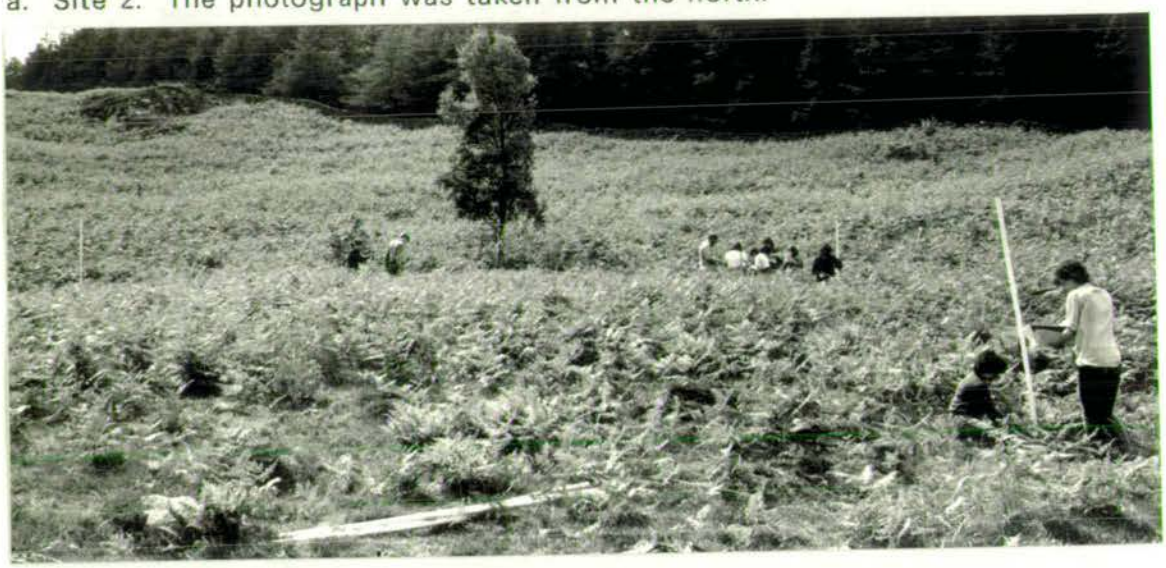


Figure 7.2. The additional sites in the open habitat from which fronds were sampled in 1984. Site 1 is illustrated in Figure 3.31.

a. Site 2. The photograph was taken from the north.



b. Site 3. The photograph was taken from the south east.

Figure 7.3. The three woodland sites at location 6 from which fronds were sampled in 1984. (Scale: 1/10000).

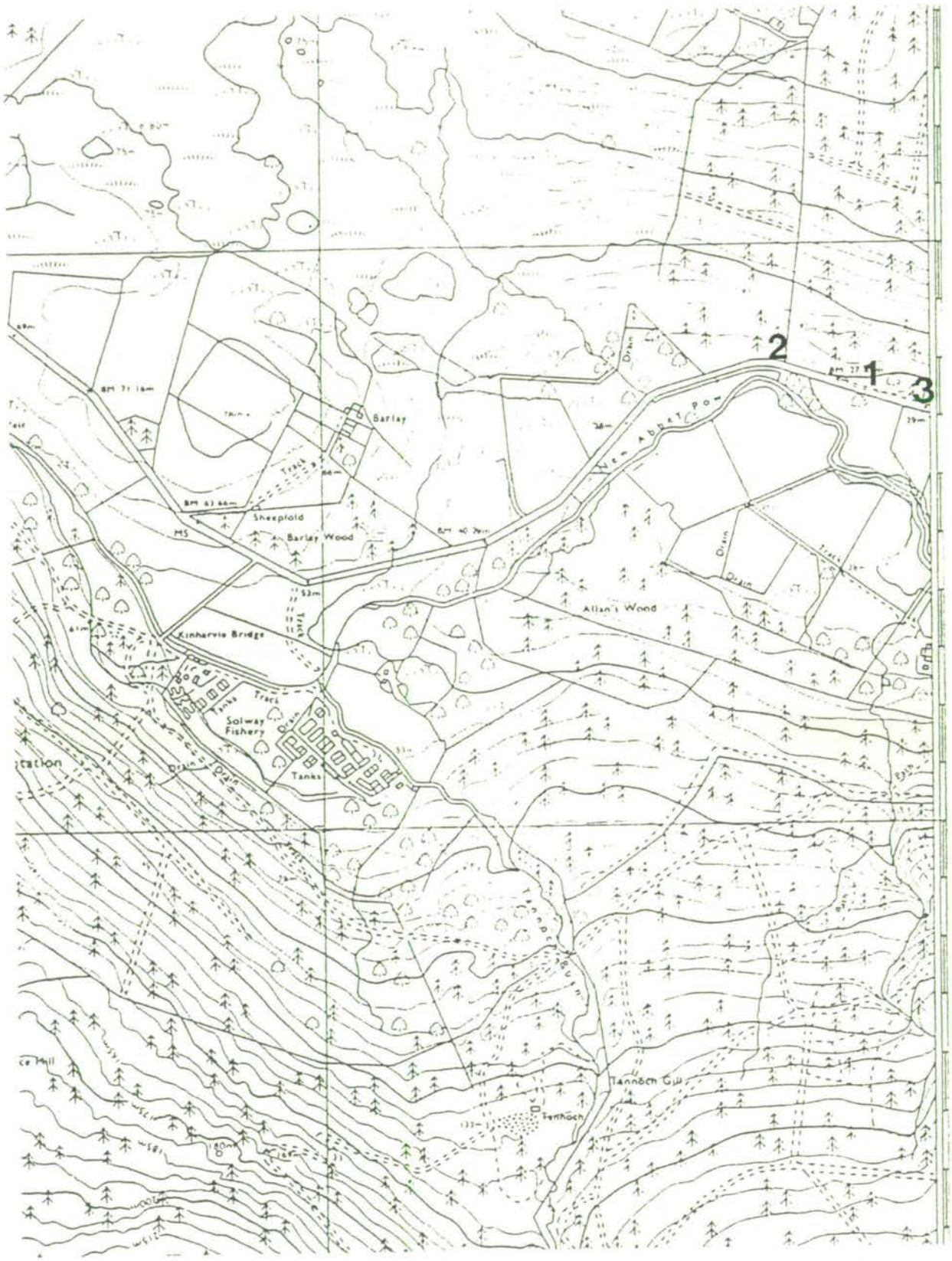


Figure 7.4. The additional sites in the woodland habitat from which fronds were sampled in 1984. Site 1 is illustrated in Figure 3.33.

a. Site 2. The photograph was taken from the south west.

Figure 7.4 – continued

b.Site 3. The photograph was taken from the south.

Figure 7.5. The three coastal sites at location 6 from which fronds were sampled in 1984. (Scale: 1/10000).

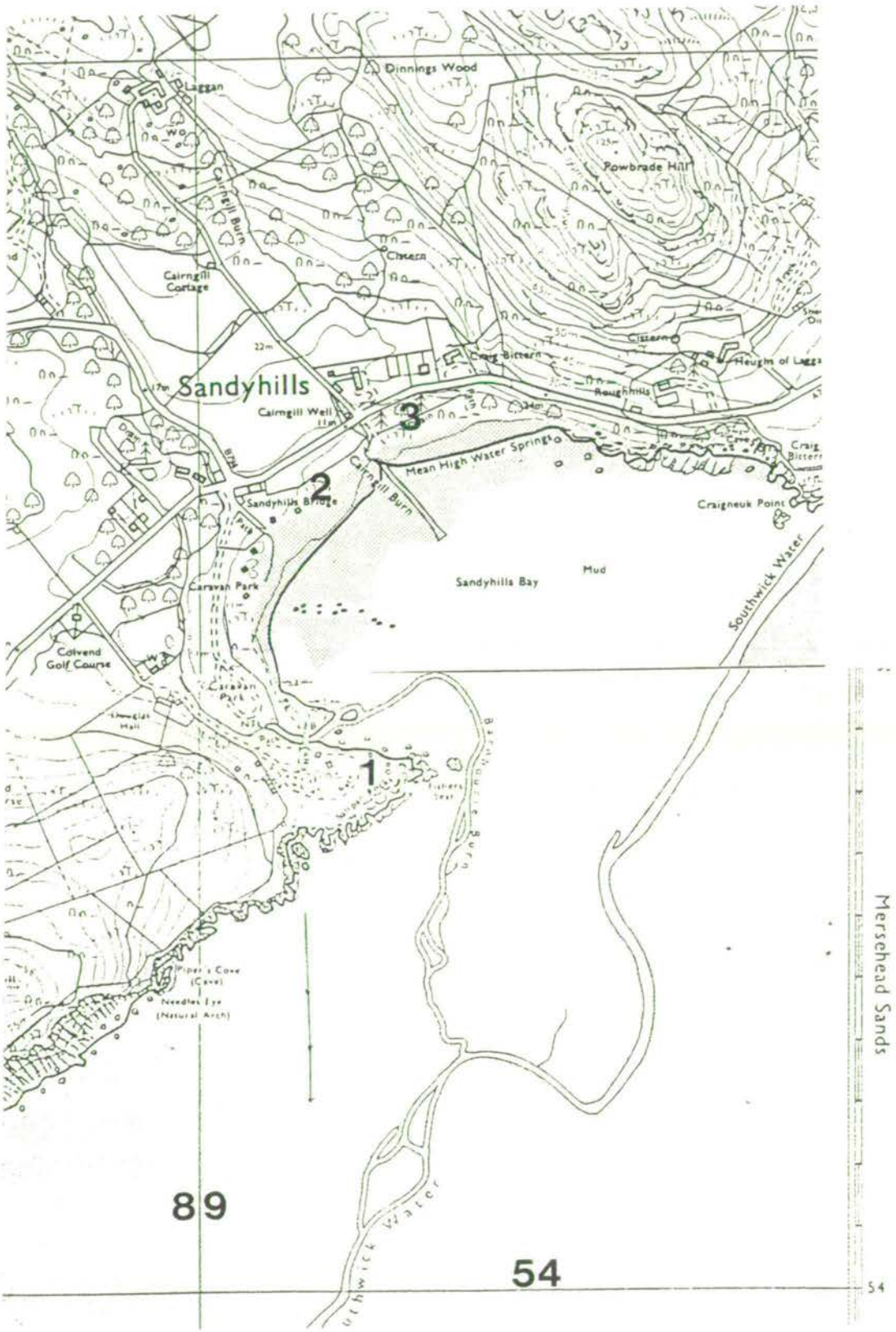


Figure 7.6. The additional sites in the coastal habitat from which fronds were sampled in 1984. Site 1 is illustrated in Figure 3.29.

a. Site 2. The photograph was taken from the south.

b. Site 3. The photograph was taken from the north.

included for comparison.

The sites originally studied by Dr. Dyer and the additional sites were sampled by students of,the University of Edinburgh in the last week of June 1984, under the supervision of Dr. Dyer and myself; the sites sampled originally in 1983 were sampled by myself in the third week of July, 1984.

At each site an 18 X 18m grid was set up as described in chapter 2. Samples of 100 (sites sampled in June) or 50 (site sampled in July) fronds selected on the grid were tested for cyanogenesis using the sodium picrate test (chapter 2). The biological variables of frond height, frond density and sample fresh weight were measured. Soil samples were taken for measurement of soil pH and laboratory analysis of mineral elements.

7.2. Results.

There was considerable variation found in the percentage of cyanogenic fronds sampled at each of the three sites within each habitat type (Table 7.1). In contrast, the estimated mean amount of HCN released from cyanogenic pinna tips shows little variation (Table 7.2). The results were analysed using an unblocked analysis of variance, with the different sites within each habitat type as treatment replicates. This analysis shows that after the percentage of cyanogenic fronds sampled was transformed by the angular transformation (Sokal and Rohlf, 1969), the variance within habitat types (i.e. between sites 1,2, and 3 of each habitat) is almost as great as the variance between habitat types, and therefore the difference between the habitat types is not significant (Table 7.4). In contrast, analysis of variance of the estimated mean amount of HCN released from cyanogenic pinna tips gives a highly significant result between habitat types (Table 7.4).

The mean fresh weight of pinna tips differed between sites within habitat types as well as between habitat types (Table 7.3). Unfortunately some of these values were based on gross rather than individual measurements of 20 pinna tips, and therefore standard errors can not be given. The most variation occurred between sites in the coastal habitat type. The woodland habitat sites were very similar with respect to frond height, whilst the coastal and open habitat sites both varied considerably (Table 7.5).

The soil characteristics also differ greatly between sites within each habitat type. The soil pH varies considerably within the coastal habitat type, and this may reflect the degree of exposure (Table 7.6). The values for soil pH in the other two habitat types do not differ to the same extent, but the differences might be important given the proximity and apparent uniformity of conditions

Table 7.1. The percentage of cyanogenic fronds sampled at sites within the same habitat type in June and July 1984. For each habitat type, site 1 is that sampled in 1983 (chapter 3); site 2 is that sampled by Dr. A.F. Dyer, 1978 - 1982 (Dyer and Hadfield, 1985), except for the coastal habitat where site 2 was the exposed beach site; and site 3 is an additional site sampled in 1984 only, which in the case of the coastal habitat represented a sheltered site. In each habitat type, 50 fronds were sampled at site 1 and 100 fronds each at sites 2 and 3. The angular transformation of each percentage, necessary for statistical analysis of the data, is given in parentheses.

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	0	23 (28.7)	17 (24.3)	13.3 ±6.9
open	4 (11.5)	48 (43.8)	2 (8.13)	18.0 ±15.0
woodland	10 (18.4)	37 (37.5)	76 (60.7)	41.0 ±19.2
Grand mean				24.1 ±1.6

Table 7.2. The estimated mean amount of HCN, μg g^{-1} fresh weight, released from cyanogenic pinna tips sampled at each of three sites in three habitat types in 1984. The key to the site numbers is given in Table 7.1.

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	0	26.0	40.7	22.2 ±11.9
open	25.6	42.7	21.0	29.8 ±6.6
woodland	135.9	127.1	111.6	124.9 ±7.1
Grand mean				59.0 ±8.5

Table 7.3. The mean fresh weight, mg, of representative pinna tips from each of three sites in three habitat sites sampled for cyanogenesis in 1984. Some of these values were taken from gross rather than individual measurements of 20 pinna tips and therefore no estimates of standard error are available. The key to the site numbers is given in Table 7.1.

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	154.2	208.0	115.4	159.2 ±26.8
open	178.4	158.5	158.0	165.0 ±6.7
woodland	96.5	108.4	118.1	107.7 ±6.3
Grand mean				143.9 ±12.2

Table 7.4. The results of analysis of variance of the transformed percentage of cyanogenic fronds (t[%]) and the estimated mean amount of HCN released from cyanogenic fronds (HCN), sampled at each of three sites in three different habitat types at location 6. Complete analysis of variance tables are given in Appendix 3, Table A3.1.

Character	Variation between	Variance ratio	Degrees of freedom	Significance
t[%]	habitats	1.082	2,6	N.S.
HCN	habitats	41.669	2,6	***

N.S., not significant; *** significant at 0.1% ($P<0.001$).

Table 7.5. The mean height, ±S.E., of fronds sampled for cyanogenesis at each of three different sites in three habitat types at location 6 in 1984. The key to the site numbers is given in Table 7.1.

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	109.9 ±3.8	128.8 ±2.1	135.2 ±2.4	124.6 ±2.7
open	104.1 ±4.5	79.6 ±1.7	49.2 ±1.4	77.6 ±9.2
woodland	134.1 ±4.6	138.6 ±2.9	134.9 ±3.2	135.9 ±1.4
Grand mean				112.7 ±2.10

Table 7.6. The mean soil pH of each of three sites in three different habitat types, ±S.E. Soil samples were taken from each corner and from the centre of each of the sites, the value given being the mean of the five pH values. The key to the site numbers is given in Table 7.1.

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	5.74 ±0.38	4.36 ±0.37	3.78 ±0.20	4.63 ±0.58
open	3.32 ±0.08	4.08 ±0.10	3.46 ±0.06	3.62±0.08
woodland	3.78 ±0.09	3.60 ±0.07	4.10 ±0.12	3.83 ±0.10
Grand mean				4.02 ±0.24

at these sites (Table 7.6). The mineral content of the soil shows very wide variation between sites within each habitat type for all four minerals measured (Table 7.7). This particularly true of the open habitat type. On the whole, the levels of mineral nutrients found can be considered below average (Dr. P.A. Crooks, Edinburgh School of Agriculture, personal communication).

7.3. Discussion.

That the percentage of cyanogenic fronds can vary over distances as small as 150m within the open and the woodland habitat types in this study is most interesting. It is important to note that bracken was continuous over the whole area for the open habitat site, although it became very sparse at the edge of site 3, which represented the end of a very large bracken stand. The bracken between the sites in the woodland habitat type was sparser, as commonly found in woodland (e.g. Table 3.3), but was still continuous between sites. The observed differences within habitat types of the percentage of cyanogenic fronds sampled therefore reflect either population differences within seemingly single stands (i.e. genetic differences) or environmental differences within seemingly uniform habitats. It is significant that no such differences were found regarding the estimated mean amounts of HCN released from cyanogenic fronds within habitat types. This suggests that the factors determining the amount of HCN are independent of those determining the percentage of cyanogenic fronds (although it should be remembered that there was a strong correlation between the percentage of cyanogenic fronds sampled and the estimated amount of HCN released by these fronds as shown in chapter 4, Table 4.7).

Unlike the open and woodland habitat types, there was not continuous bracken between the sites sampled at the coastal habitat sites, and the three different sites might well have represented different populations or have been subject to different environmental conditions. However, these three sites showed a similar degree of variation with respect to the percentage of cyanogenic fronds to that found under both the other habitat types, and the estimated mean amount of HCN released varied similarly to that for the sites sampled under the open habitat type. This suggests that despite the discrete nature of the stands of bracken sampled in the coastal habitat type, the differences between the three sites are no greater than those found between the three closer sites at each of the other two habitat types.

As was found for sites throughout Britain, there was no difference between the estimated mean amount of HCN released by cyanogenic fronds sampled in

Table 7.7. The mineral content of soils from each of three sites in three habitat types sampled in 1984. Although soil samples were taken from each corner and the centre of each site, these combined together for the soil analysis. The key to the site numbers is given in Table 7.1.

a. The phosphorus content of the soil, mg kg^{-1}.

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	2.2	0.69	3.1	2.00 ±0.70
open	3.2	0.48	0.38	1.35 ±0.92
woodland	1.4	2.8	1.4	1.87 ±0.47
Grand mean				1.74 ±0.37

b. The potassium content of the soil, mg kg^{-1}.

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	57	25	36	39.3 ±9.4
open	245	68	109	140.7 ±53.5
woodland	57	48	56	53.7 ±2.8
Grand mean				77.9 ±22.3

c. The magnesium content of the soil, mg kgh−1

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	165	52	82	99.7 ±33.8
open	115	35	34	61.3 ±26.8
woodland	63	33	35	43.7 ±9.7
Grand mean				68.2 ±15.2

d. The nitrogen content of the soil, %.

	Site			
Habitat	1	2	3	mean, ±S.E.
coastal	0.19	0.12	0.21	0.17 ±0.05
open	0.77	0.48	0.54	0.60 ±0.09
woodland	0.41	0.50	0.43	0.45 ±0.03
Grand mean				0.41 ±0.07

the coastal or the open habitat types, but there were large differences between the estimated mean amount of HCN released from fronds sampled at the woodland habitat sites and the other two habitat types. This is therefore further evidence of an effect of habitat, notably woodland, upon cyanogenesis.

The characteristics of fronds sampled show similar differences between the three sites within each habitat type. As might be expected, the bracken in the woodland habitat appears most homogeneous with respect to the height of fronds.

The soil characteristics show that the three sites within each habitat actually differ considerably, despite appearing superficially uniform. However, there is no pattern discernible between either aspect of cyanogenesis measured and any of the soil minerals. Similarly, there is no apparent connection between any of the frond characteristics and the soil mineral content. The heterogeneity of the three habitat types over such small distances, especially in the case of the open habitat type, is somewhat surprising. Given that the soil analyses were performed on the five amalgamated soil samples, it is unlikely that the apparent heterogeneity is due to sampling error. It is possible that large differences occurred within each site, and that the five samples taken showed the differences found due to random variation.

The differences found in cyanogenesis cannot be attributed to any of the factors measured at each site. Whether this is because the variation is random or varies in accordance to some other factor which shows changes over comparatively short distances cannot be established.

The results found in this field survey are interesting in the light of investigations involving other species. There has been a large amount of work regarding the distribution of cyanogenic and acyanogenic phenotypes of Trifolium repens (Daday, 1954a,b, 1958; de Araujo, 1976) and Lotus corniculatus (Jones, 1972b, 1977; Ellis et al, 1976, 1977b; Abbott, 1977, 1981) over both large and small distances. If these species differ on a small scale to the same degree as Pteridium aquilinum, it is possible that studies such as these contain much sampling error. This is particularly likely to be the case in those surveys carried out on a national, continental or worldwide scale (e.g. Daday, 1954a, 1958; Jones, 1972b, 1977), since localised (but undetected) differences could be as great as the large scale differences for which trends are described.

Abbott (1977, 1981) investigated several populations of Lotus corniculatus along the coast Orkney, separated by distances similar to the different sites studied in the coastal habitat type in the current survey. He showed that the

large differences in the distribution of the cyanogenic phenotype were related to the soil moisture content, highly cyanogenic populations correlating with high water availability, as was shown experimentally by Foulds and Grime (1972a,b). It is possible that the variation of percentage of cyanogenic bracken fronds found between sites within the open and woodland habitat types, and between the discontinuous stands of bracken sampled in the coastal habitat, reflect differences in the soil moisture over very small distances. There was no correlation between the percentage of cyanogenic bracken fronds sampled at sites throughout Britain and rainfall (chapter 4). However, the available soil moisture depends largely on the soil type and its characteristics as well as the amount of rainfall (Fitter and Hay, 1983), and it is possible that the localised differences in the percentage of cyanogenic fronds sampled is related to soil moisture.

7.4. Conclusions.

The results given here show that the percentage of cyanogenic fronds sampled can vary considerably over comparatively small distances. In contrast, the estimated mean amount of HCN released from cyanogenic fronds was relatively constant. If cyanogenesis is genetically determined, it is necessary to explain why and how the genotype can differ substantially over small distances. If cyanogenesis is environmentally determined, those factors responsible for the control of cyanogenesis must vary over small distances. Whichever is the case, the mechanism by which cyanogenesis in fronds is determined must therefore be variable over such small distances. It is possible this reflects a feature of the soil, since most other environmental factors would be more or less constant over distances such as those involved in this survey.

Chapter 8. Small Scale Patterns of Cyanogenesis in **Pteridium aquilinum**.

The previous chapter showed that when populations of fronds were sampled within apparently uniform environments, variation was found in the percentage of cyanogenic bracken fronds sampled at different sites over small distances (less than 1km) within each of three habitat types. In that and all the previous surveys, each frond sampled at these sites was 2m from the nearest neighbouring frond sampled. Given that the density of fronds at any one site varied from 14 to 56 fronds m^{-1} (chapter 3), a sample of 100 fronds represents only 0.55 - 2.2% of the total number of fronds at the site, and a sample of 50 only 0.27 - 1.1%. It has been found for sites throughout Britain (chapter 4) that over the 18 X 18m sampling grid the distribution of sampled cyanogenic bracken fronds was apparently random, and that the pattern of fronds was typically a mosaic of the two types (Figure 8.1), except at the few sites where only cyanogenic or acyanogenic fronds were sampled. However, non-random distribution of fronds on a small scale would not have been detected by this sampling technique; cyanogenic fronds occurring in discrete patches would have given similar results to individual cyanogenic fronds randomly distributed over the whole area of a site.

In order to find out whether the recorded patterns accurately reflected the true distribution of cyanogenic fronds, it was decided to test every frond in a square 1 X 1m in area at each of the three habitat types at location 5. In addition to giving information about the small scale distribution of cyanogenic fronds, investigating an area of 1 X 1m would allow the rhizomes to be excavated and the connections between fronds to be traced, and the frond number in each of the small squares would be low enough to permit the testing of all those fronds which were acyanogenic according to the picrate test to be analysed for the release of HCN using the pyridine - pyrazolone test (chapter 2), which would not be feasible with the large sample size.

8.1. Method.

At each of the three habitat sites at location 5 (chapter 3), a square 1 X 1m was marked out at the centre of the site and all the fronds within the square were tagged. Each square was orientated to include as many fronds as possible. Because of the low frond density at the woodland habitat site, an additional 1 X 1m square at the edge of the site was sampled. The terminal 4cm of the lowermost pinna of each frond was sampled in mid-June, 1985,

Figure 8.1. The distribution of cyanogenic and acyanogenic fronds over the 18 X 18m grid at the three habitats sampled at location 5 in 1984. The results of the third sample (in mid June) are given. The position of each frond on the sampling grid is recorded by a letter (A - J) and a number (1 - 10). Cyanogenic fronds: +; acyanogenic fronds: -.

a. Coastal habitat site. Percentage of cyanogenic fronds sampled: 79%.

	1	2	3	4	5	6	7	8	9	10
A	+	+	-	+	-	+	+	+	+	+
B	+	+	+	+	+	+	+	+	+	+
C	-	+	+	+	+	+	+	+	+	+
D	+	+	+	+	+	+	+	+	+	+
E	-	+	-	-	+	+	+	-	+	+
F	-	-	-	+	+	+	+	+	+	+
G	+	+	-	+	+	+	+	-	+	+
H	+	-	-	+	+	+	+	+	-	+
I	+	-	-	+	-	-	+	+	+	+
J	+	+	+	+	-	-	+	+	+	+

b. Open habitat site. Percentage of cyanogenic fronds sampled: 43%.

	1	2	3	4	5	6	7	8	9	10
A	-	+	+	+	+	+	-	-	-	+
B	+	+	+	-	-	+	-	-	+	-
C	+	+	+	+	-	+	+	-	-	-
D	+	+	-	+	-	+	-	-	-	-
E	+	+	+	+	+	-	-	-	-	-
F	+	+	-	-	+	-	-	+	+	+
G	+	+	-	-	-	-	-	-	-	-
H	-	+	-	-	+	-	-	+	-	+
I	-	-	-	+	-	-	-	+	-	+
J	-	-	-	-	-	-	-	+	-	+

c. Woodland habitat site. Percentage of cyanogenic fronds sampled: 51%

	1	2	3	4	5	6	7	8	9	10
A	+	+	-	-	-	+	-	+	-	-
B	+	+	-	-	-	-	+	+	+	+
C	+	+	+	+	-	+	-	+	+	-
D	+	-	-	-	-	-	+	+	-	-
E	-	-	-	+	+	+	+	+	+	+
F	-	-	+	+	+	-	+	+	-	-
G	+	-	-	+	-	-	-	-	+	+
H	-	-	+	+	-	+	+	-	+	+
I	-	+	-	-	-	-	+	+	+	+
J	-	-	-	+	-	-	-	+	+	+

using the picrate test for HCN (chapter 2). The height of each frond sampled, and the mean fresh weight of the samples, were measured (chapter 3). The squares were visited again in early August and larger frond samples of approximately 1.5g were removed from the fronds which were acyanogenic according to the picrate test. These were packed in ice and transported for analysis of HCN released under laboratory conditions, using the pyridine - pyrazolone test of Mao et al (1965; chapter 2). The soil within the square was removed and the rhizomes exposed; this was not possible at the open site since the soil proved impossible to dig without severing the rhizomes, thereby providing no useful results. It is also likely, from what was seen at this site when as much soil as possible was removed, that it would have proved very difficult to identify individual rhizomes due to the very large number of rhizomes present in the soil. Soil samples were taken from the depth of the short shoot rhizomes at all sites, and the soil pH was measured in the laboratory. The remaining soil was analysed for potassium, phosphorous, magnesium and nitrogen.

8.2. Results.

Each of the four 1 X 1m squares sampled contained both cyanogenic and acyanogenic fronds, as defined by the sodium picrate test (Table 8.1). The estimated mean amounts of HCN released from cyanogenic fronds at each site differ somewhat from the equivalent values recorded in 1984 (Table 8.2), but they are of a similar order of magnitude. The cyanogenic fronds were apparently randomly distributed within each 1 x 1m square, the resulting mosaic being similar to that found for distantly spaced fronds sampled over the larger 18 X 18m area (Figures 8.1 and 8.2). Cyanogenic fronds were in close association with acyanogenic fronds.

The excavation of the rhizomes of the 1 X 1m square in the coastal habitat showed that each frond was attached to a short shoot rhizome (sensu Watt, 1940) which in turn was attached to a long shoot rhizome (Figure 8.3). Each short shoot rhizome produced only a single frond, and there were several dormant or dead rhizomes. No two short shoot rhizomes were attached to the same long shoot rhizome within the area excavated, and therefore no two fronds were linked together, within the area of the square. There were also some living long shoot rhizomes that traversed the excavated area without producing a short shoot rhizome, passing straight through the square. It is likely that there were rhizome connections between some of the long shoot rhizomes outside the excavated area. However, it is not possible from the

Table 8.1. The percentage of cyanogenic fronds sampled in a 1 X 1m square at each of the three habitat sites at location 5. The total number of fronds sampled is given in parentheses, and for comparison the results from the third sample taken in 1984 are also provided (see Table 5.1). Both sets of samples were taken in mid June.

Habitat	1985	1984
coastal	88 (42)	79
open	45 (62)	43
woodland 1	20 (10)	51
woodland 2	38 (13)	51
woodland 1+2	30 (23)	51

Table 8.2. The estimated mean amounts of HCN, $\mu g\ g^{-1}$ fresh weight, released from cyanogenic fronds sampled in each 1 X 1m square in three habitat types. The value estimated for the third sampling in 1984 is given for comparison.

Habitat	1985	1984
coastal	36	131
open	45	85
woodland 1	101	133
woodland 2	101	133
woodland 1+2	101	133

Figure 8.2. The distribution of cyanogenic and acyanogenic fronds over four squares each 1 X 1m, one in the coastal habitat site, one in the open habitat site and two in the woodland habitat site at location 5. The relative positions of fronds are shown to scale. The squares in the woodland habitat site were excavated and the rhizomes exposed; the rhizomes are shown in the two diagrams. Open numbers, cyanogenic fronds according to the picrate test; ◯, acyanogenic fronds according to the picrate test; □, acyanogenic fronds according to the pyridine - pyrazolone test; →, growing rhizome apex; – –, rhizome dying back.

a. coastal habitat site.

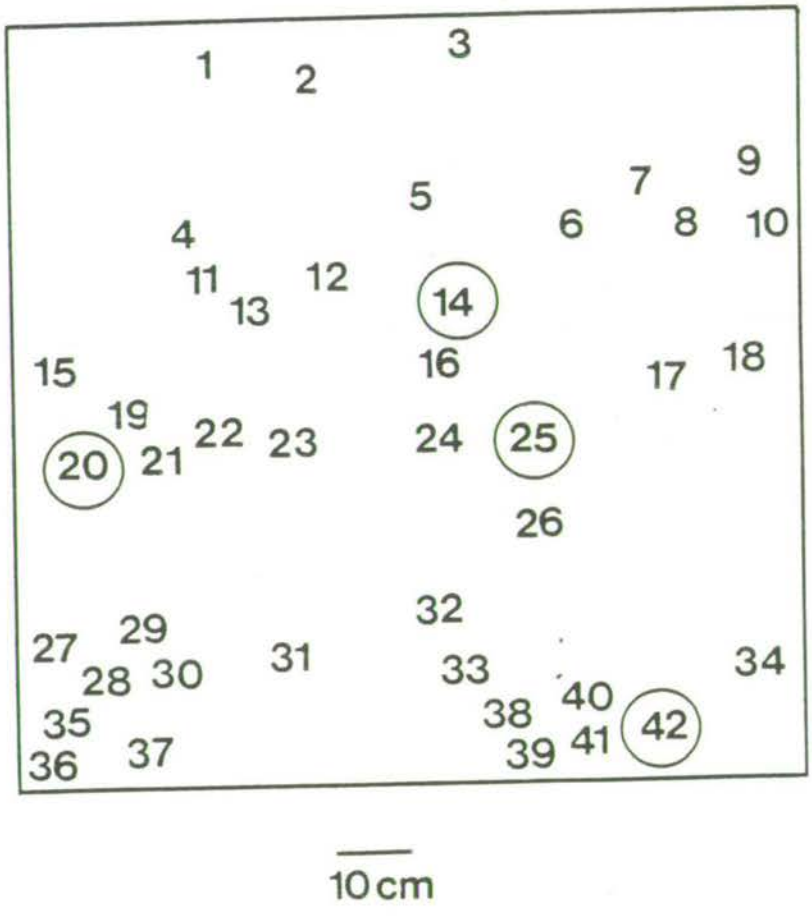
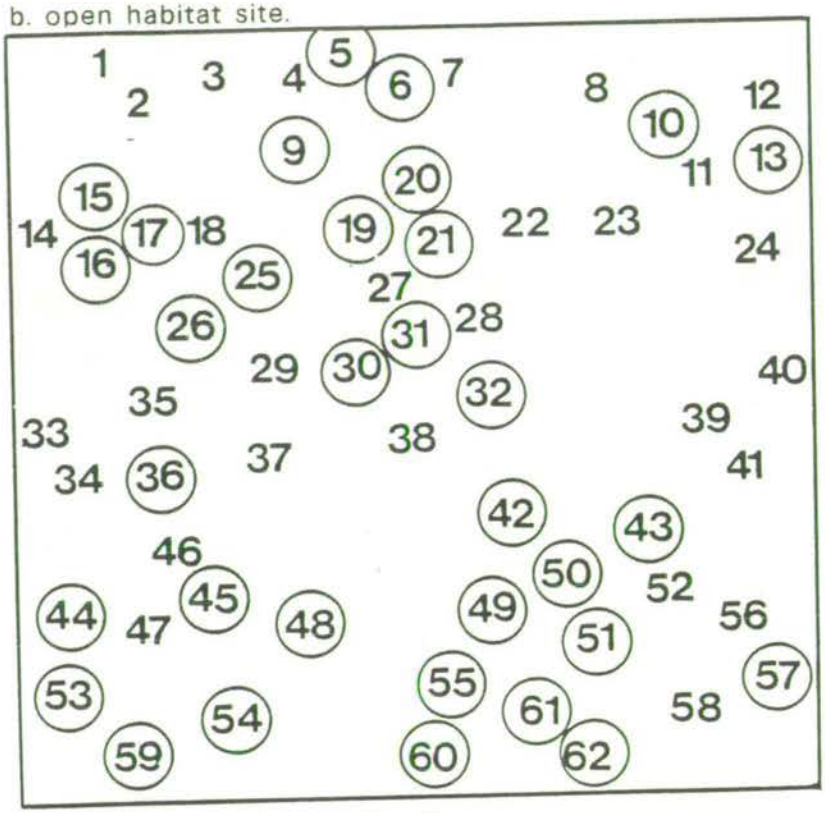
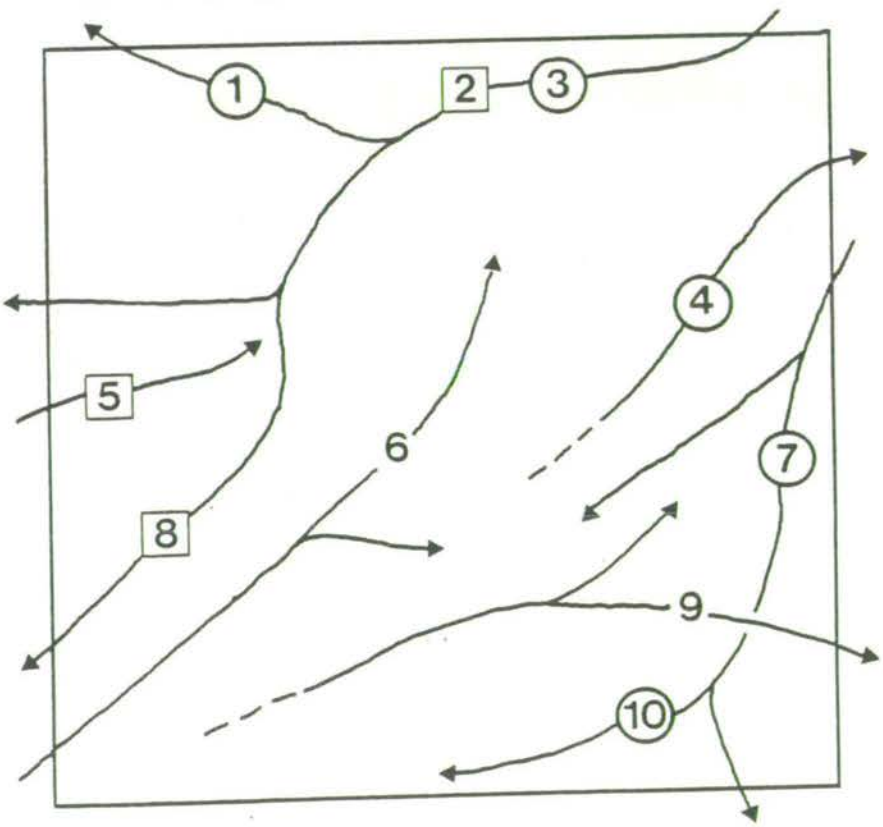


Figure 8.2 – continued.

b. open habitat site.



c. woodland habitat, square 1.



10cm

Figure 8.2 – continued.

d. woodland habitat, square 2.

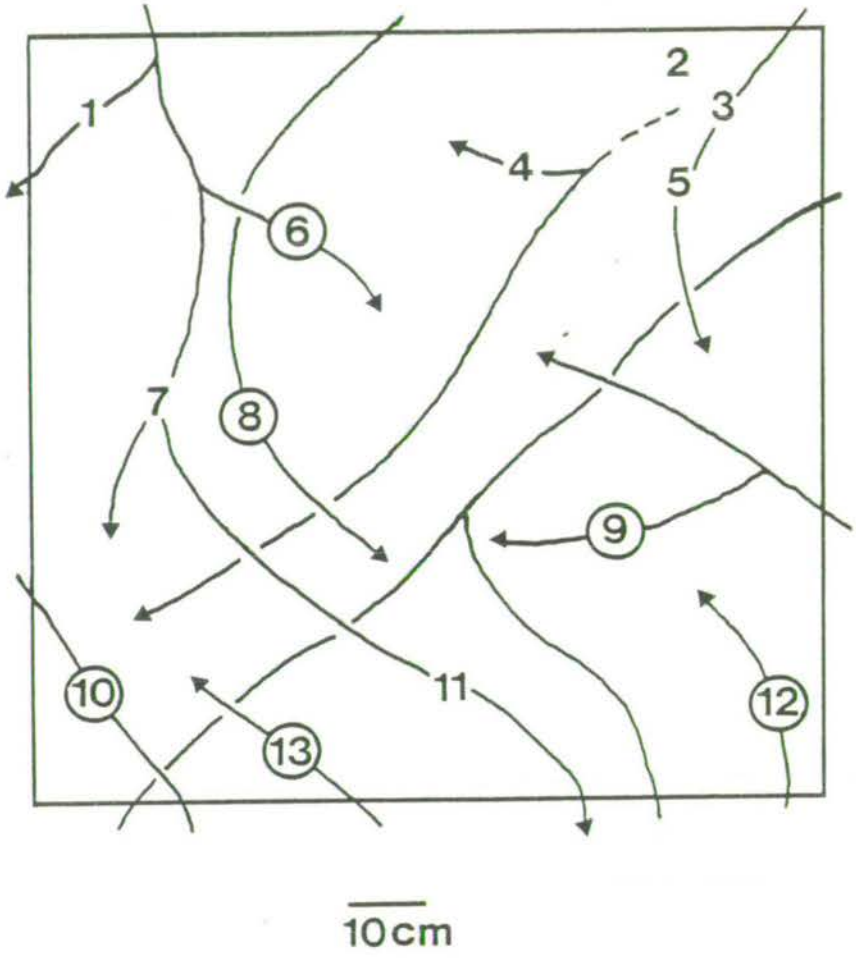


Figure 8.3. The excavation of rhizomes within an area 1 X 1m at the coastal habitat site at location 5. This shows the large number of long shoot rhizomes, none of which branch, within the area excavated.

observations made to discern which fronds were attached to the same rhizome system.

In contrast, the two 1 X 1m squares excavated at the woodland habitat site showed several rhizomes connected within each square (Figures 8.2c and d, 8.4 and 8.5). The ten fronds in the first square were borne on six rhizome systems unconnected within the area excavated (Figure 8.2c), one of which produced four fronds and another producing two fronds; all these fronds were acyanogenic according to the picrate test. Each frond was produced by a different short shoot rhizome.

The second square contained 13 fronds attached to eight separate rhizomes, one of which produced four fronds, and another, two (Figure 8.2d). One of the fronds (no. 2 in Figure 8.2d) was accidently severed and its rhizome could not therefore be satisfactorily identified. Of the four fronds connected to the same rhizome system, three were cyanogenic according to the picrate test whilst the fourth was acyanogenic (Figures 8.2d and 8.6).

At each site, several of the fronds which were acyanogenic according to the picrate test were dead when the further frond samples were taken for laboratory analysis. One acyanogenic frond had died at the coastal habitat site, 15 at the open habitat site, one at the first woodland habitat site and one at the second. All those fronds from the coastal and open habitats which were acyanogenic according to the picrate test gave positive results with the pyridine - pyrazolone method, although the amounts of HCN were far less than those estimated to be released from those fronds which the picrate paper test showed to be cyanogenic (Table 8.5). The first square at the woodland habitat contained three fronds which released no HCN detectable by the pyridine - pyrazolone test, whilst all the other fronds tested at the site released measurable amounts of HCN.

The soil pH, measured from the depth of the short shoot rhizomes, gave similar values for the coastal and open habitat sites to those measured in 1983 (Table 8.6). The levels of mineral nutrients gave somewhat different values to those measured in 1983 (Table 8.7), although the levels of available nitrogen were similar for the two years. The mineral nutrients at the two squares in the woodland habitat varied considerably between the two squares (Table 8.7), an indication that the apparently uniform habitats may differ substantially over short distances.

8.3. Discussion.

The results from this study are of exceptional interest. The mosaic pattern

Table 8.3. The mean fresh dry weights, ± S.E., of 20 representative pinna tips sampled at the three habitat sites at location 5 in 1985. The values from the third sample taken in 1984 are given for comparison.

a. Fresh weights, mg.

Habitat	1985	1984
coastal	241.6±10.6	192.3±10.8
open	194.3±13.2	192.1±8.7
woodland	131.6±10.8	182.9±14.9

b. Dry weight, mg; the dry weight as a percentage of fresh weight is given in parentheses after each value.

Habitat	1985	1984
coastal	34.3±2.9 (14.2)	37.4±2.4 (19.4)
open	32.1±2.2 (16.5)	36.7±1.7 (19.4)
woodland	19.9±1.8 (15.2)	28.6±2.5 (15.6)

Table 8.4. The mean height, cm ±S.E., of fronds sampled for cyanogenesis in each 1 X 1m square at the three habitat sites at location 5 in 1985. The values for the same sites recorded in 1984 are given for comparison.

Habitat	1985	1984
coastal	74.8±2.0	66.4±1.9
open	58.8±2.2	35.9±1.5
woodland 1	109.0±10.0	71.1±2.7
woodland 2	96.1±9.4	71.1±2.7
woodland total	101.7±6.8	71.1±2.7

Table 8.5. The results of the pyridine - pyrozolone test for HCN released from fronds which were negative according to the picrate test.

Habitat	Number of fronds tested	Mean sample weight, g	Mean HCN released, $\mu g\ g^{-1}$ fresh weight.
coastal	4	2.30±0.1	0.69±0.12
open	19	1.65±0.1	0.14±0.05
woodland 1	7	1.11±0.1	0.03±0.02
woodland 2	5	1.08±0.0	0.22±0.17
woodland total	12	1.09±0.0	0.11±0.07

Table 8.6. The pH of soil removed from the depth of the short shoot rhizomes at the four 1 X 1m squares at three habitat sites at location 5 in 1985. Values are given for the soil pH from the depth of the short shoot rhizomes at the coastal and open habitat sites, sampled in 1983, for comparison.

Habitat	1985	1983
coastal	4.2	4.7
open	3.4	3.4
woodland 1	3.6	-
woodland 2	3.5	-

Table 8.7. The levels of available mineral nutrients in soil removed from the depth of the short shoot rhizomes at the four 1 X 1m squares at the three habitat sites at location 5. Values are given from the depth of the short shoot rhizomes at the coastal and open habitat sites, sampled in 1983, for comparison.

a. Phosphorous (P), mg kg^{-1}

Habitat	1985	1983
coastal	24	15
open	23	23
woodland 1	36	-
woodland 2	13	-

b. Potassium (K), mg kg^{-1}

Habitat	1985	1983
coastal	19	34
open	168	70
woodland 1	57	-
woodland 2	37	-

c. Magnesium (Mg), mg kg^{-1}

Habitat	1985	1983
coastal	112	125
open	168	66
woodland 1	73	-
woodland 2	38	-

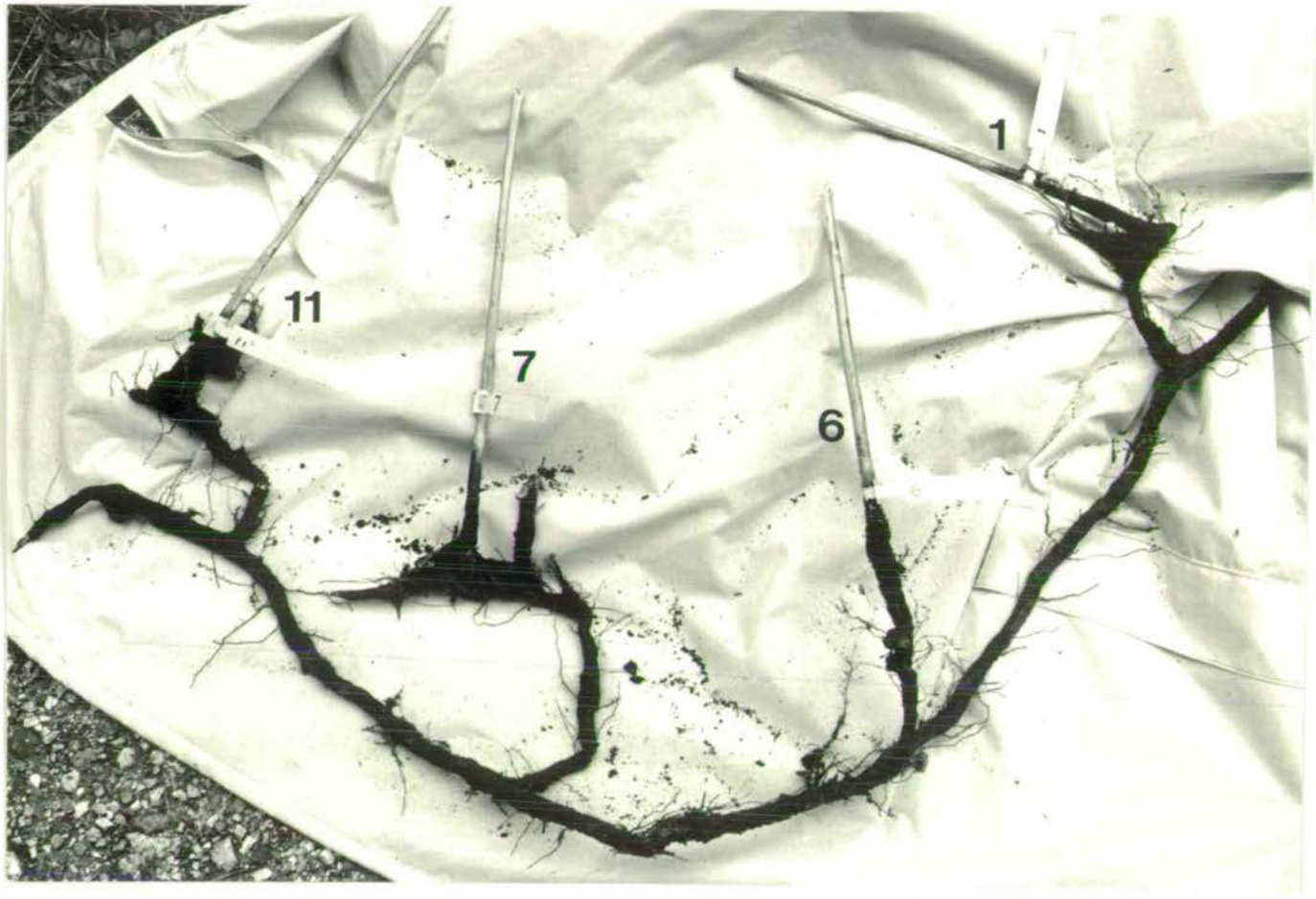
d. Nitrogen (N), %

Habitat	1985	1983
coastal	0.194	0.146
open	1.004	1.055
woodland 1	0.341	-
woodland 2	0.232	-

Figure 8.4. The excavation of rhizomes within an area 1 X 1m at the woodland habitat site, square 1, at location 5. This shows the low number of long shoot rhizomes, several of which branch, within the area excavated.

Figure 8.5. The excavation of rhizomes within an area 1 X 1m at the woodland habitat site, square 2, at location 5. This shows the low number of long shoot rhizomes, several of which branch, within the area excavated. In addition, the long shoot rhizome producing both cyanogenic and acyanogenic fronds, according to the picrate test, is arrowed.

Figure 8.6. A detail of the the long shoot rhizome from the second square excated at the woodland habitat which produced both cyanogenic and acyanogenic fronds, according to the picrate test. The fronds are numbered according to the map of the area excavated, Figure 8.2d.



formed by fronds sampled at 2m intervals over the large 18 X 18m area is similar to that found when every frond is sampled within only 1 X 1m. The factors determining the differences between cyanogenic fronds (according to the picrate test) and acyanogenic fronds must either be variable over the small distances involved, on a scale of centimetres, or else cyanogenesis is not determined by small scale environmental factors to any great extent but rather is determined by factors affecting rhizomes. Such factors could be effective some distance from the frond, since rhizome connections may be continuous over large distances: Watt (1943) measured rhizomes in the soil in excess of 1300 feet (approximately 427m).

It is significant that there did not appear to be any extensive clumping of cyanogenic or acyanogenic fronds at the open habitat site. This site had a high frond density with 62 fronds being sampled in the square, and 45% of sampled fronds were cyanogenic. This site therefore provides a more informative sample than the other two sites which had either a very high percentage of cyanogenic fronds or a comparatively low frond density. The distribution of cyanogenic fronds at this site shows that cyanogenesis is more or less randomly distributed between fronds within a small area and apparently also over a considerably larger area.

The values of soil pH from the coastal and the open habitat sites do not differ much between 1983 and 1985. Similarly, the two squares at the woodland habitat site have approximately the same soil pH. In contrast, the levels of nutrients present in the soil samples analysed differ substantially from those found at the coastal and open habitat sites in 1983 (Table 8.7). This is particularly true of the levels of potassium and magnesium. However, the percentage of nitrogen found in the soil is similar in the two sets of analyses. The levels of nutrients analysed from the two squares in the woodland habitat also differ substantially for all nutrients analysed. It is not possible to say whether this results from differential uptake between the two squares, or heterogeneity in the distribution of soil nutrients within an area.

The excavation of rhizomes at the coastal and the woodland habitat sites was very informative. It confirmed reports by Webster and Steeves (1958) and Watt (1976) that short shoot rhizomes usually bear only one frond each season. The high density of fronds at the open habitat site would imply that there was a very large number of rhizomes present, which, together with the shallow and rocky soil, may explain why the square was so difficult to excavate without damaging the rhizome connections. This was unfortunate since it would have been useful to trace the connections between the several short shoot rhizomes

present at this site.

The lack of rhizome branching within the 1 X 1m square at the coastal habitat site is of interest. The possibility cannot be discounted that the rhizomes were connected outside the square, but it is noteworthy that each long shoot rhizome produced only one frond bearing short shoot within the square. In contrast to those excavated at the coastal habitat site, the long shoot rhizomes excavated at the two squares at the woodland habitat site showed repeated branching. It is possible that the branching found under woodland conditions reflects a low level of intra-specific competition for 'space', allowing free branching when this might be inhibited under the conditions of higher frond density at the coastal site. Watt (1940) suggests that mature rhizomes systems may well be in competition with each other within established stands, whilst rhizomes invading ground uncolonised by bracken would not be. Alternatively, it is possible that the high density of rhizomes at the coastal site represents the result of repeated branching over a great length of time, and that the visible branching at the woodland habitat site occurs in order to maximise frond production.

The most important observation from the squares excavated at the woodland habitat site was the rhizome connections between cyanogenic and acyanogenic fronds (Figure 8.6). This single observation raises several points. It is clear that if both cyanogenic and acyanogenic fronds can be linked to the same rhizome system, the determination of cyanogenesis in Pteridium aquilinum cannot primarily be due to a genetic polymorphism, since all the fronds attached to a single long shoot rhizome must be genetically identical, assuming that there has been no somatic mutation to alter the genetic makeup of the frond bearing short shoot. The determination of cyanogenesis must therefore be presumed to be environmental. That cyanogenic and acyanogenic fronds can occur together attached to the same rhizome system in close proximity negates the possibility that environmental factors affecting the long shoot rhizomes some distance from the frond play a part in determining cyanogenesis. This therefore suggests that cyanogenesis is determined either by small scale local environmental factors, which differ between fronds over comparatively small distances, or by physiological or developmental differences between fronds or between short shoot rhizomes. It is likely that environmental differences on such a scale would be related to the characteristics of the soil, since other factors are likely to be stable over distances of less than 1m. It is not possible to say from the data collected here whether or not developmental differences between fronds have been

important in determining cyanogenesis. The depth of the short shoot in the soil, the age of a short shoot, the date of frond emergence and the rate of frond expansion are all factors which could affect the partitioning of nutrients within a frond or a rhizome system and which could alter the production of secondary metabolites by a frond. The number of frond bases on a short shoot would perhaps give an indication of the age of the shoot, but it has been suggested by Watt (1940) that short shoot rhizomes can remain dormant in the soil, which would make estimation of the age of the short shoot unreliable. There is also likely to be severe competition between differentiated frond buds for resources, and this is likely to be very important in changing the development of the buds: Watt (1950) has shown that less than 20% of differentiated frond buds produce emergent fronds. It is not possible to estimate the effect of cyanogenesis upon the development of the frond buds; there remains the possibility that one function of cyanogenesis is to protect developing buds from predation by soil organisms, prior to the emergence of the frond. There is no evidence to support or refute this idea.

The results of the laboratory tests for HCN release from acyanogenic fronds, according to the picrate test, show that 91% of the fronds tested released measurable amounts of HCN, although in insufficient quantities to produce a positive score using the picrate test. The three fronds which did not release a measurable amount of HCN were all in the first square of the woodland habitat site. Two of these fronds were attached to the same rhizome system, to which were also attached fronds releasing measurable quantities of HCN; the third was not connected to any other frond within the square. It seems likely, therefore, that these two "acyanogenic" fronds were releasing insufficient HCN to produce a positive result even with the pyridine – pyrazolone test. That they were connected to the same rhizome system as fronds releasing detectable HCN shows that there is nothing intrinsically different about the rhizomes to which these fronds were connected.

This observation requires a re-evaluation of the nature of the polymorphism which has been seen to be widespread. It had been assumed that the polymorphism had been absolute, and that fronds were either cyanogenic or acyanogenic. It now seems likely that in fact the polymorphism is an artefact of the picrate test for HCN, since fronds have been shown to release HCN at levels below those which would produce a positive score using this test. It must be noted however that the amount of HCN released by these "acyanogenic" fronds is substantially less than that required to produce a positive picrate result, given the size of pinna samples taken, and that the

polymorphism observed in the field probably represents a disjunction as to the amount of HCN released by a frond: the "cyanogenic" fronds in the woodland habitat site released approximately 900 times the amount of HCN that was released by "acyanogenic" fronds, whilst those "cyanogenic" fronds in the coastal and open habitat sites released 52 times and 320 times as much respectively as the "acyanogenic". The polymorphism thus relates to the amount of HCN released rather than whether or not HCN is released. This may however affect the understanding of the polymorphism: it becomes necessary to rely on what may be an arbitrary measure, that of the picrate test. It is possible that HCN released at very low levels from fronds may represent a base level of HCN production, perhaps necessary for amino acid metabolism rather than being active as an allelochemical. Hegnauer (1977) has suggested that all plants are cyanogenic to a limited degree and that those recognised as cyanogenic represent plants which produce HCN in excess of the usual metabolic requirements. Jones (1972a) suggested that the evolution of cyanogenesis as an allelochemical resulted from selection for just such an overproduction of HCN. That it is possible to have both high and low levels of cyanogenesis in two fronds assumed to be genetically identical still produces difficulties in understanding the control of cyanogenesis in bracken.

The biological importance of cyanogenesis is difficult to assess if the polymorphism found in the field relates to the amount rather than to the absolute production of HCN. There are several cyanogenic plant species which show a continuous variation in the amount of HCN released, most notably Manihot esculenta (cassava), which although having undergone repeated selection for low HCN production remains cyanogenic (Cooke and Coursey, 1981). The importance to plants of maintaining variable levels of allelochemicals may be due to minimising adaptation by herbivores (Whitham et al, 1984), and it may therefore be advantageous to bracken to produce fronds which release very different amount of HCN.

8.4. Conclusions.

The results presented in this chapter are of great importance in relation to the results presented in previous chapters. They show that cyanogenesis in bracken differs over very small distances, and that "cyanogenic" and "acyanogenic" fronds occur in small areas as a mosaic, much as was found for the larger areas studied. That "cyanogenic" and "acyanogenic" fronds can be produced by the same rhizome system limits the extent to which polymorphism of cyanogenesis can be interpreted as being genetically controlled, and requires

any environmental control to be very localised, possibly to the region immediately surrounding the frond. Lastly, it seems likely that all fronds are cyanogenic to a certain degree, and that the polymorphism found reflects variation in the amount of HCN being released rather than whether or not a frond is absolutely cyanogenic. The species however appears to be phenotypically polymorphic with regard to the amount of HCN released by fronds, since such large differences have been found between fronds.

Chapter 9. Transplantation Experiments.

The previous chapter provided evidence which shows that both cyanogenic and acyanogenic fronds, according to the picrate test, can be produced by the same rhizome system and that the polymorphism for cyanogenesis observed in field surveys of Pteridium aquilinum is not genetically determined. However, it has also been shown that the frequency of cyanogenic fronds within a population was nevertheless constant from year to year, indicating that the phenotype is fixed (chapter 6).* It was therefore decided to investigate the extent to which the cyanogenic phenotype was fixed within particular rhizomes, transplanted from their natural habitats into cultivation, so that conditions could be controlled. The amount of variation of cyanogenesis found would reflect inherent differences (i.e. genetic or fixed in the phenotype) of the rhizomes, rather than environmentally induced phenotypic differences.

9.1. Method.

Rhizome segments were transferred from their wild environment into experimental garden plots or into the greenhouse. The conditions under both regimes were controlled to an extent not obtainable in the field, although absolute control was not be possible for the outdoor plots.

Two series of rhizome collections were made (chapter 2). The first set of rhizomes was taken from four sites in three habitat types at location 5 which had previously been tested for cyanogenesis by the picrate method by Dr. A.F. Dyer (see chapter 6). The fronds tested by Dr. Dyer had been tagged to allow them to be found following the die-back of the fronds at the end of the season, when the rhizome would have stopped growing and entered dormancy. The rhizomes were collected in early December of 1982. Only rhizomes which were connected to labelled fronds were collected. These were necessarily short shoot rhizomes. Rhizomes attached to fronds of either phenotype were dug up and 10 - 15cm sections excised. Forty four collections were made, consisting of 17 rhizomes segments attached to cyanogenic fronds, 24 attached to acyanogenic fronds, and three attached to fronds which were 'borderline positives' (barely producing a colour change with the picrate test). None of the borderline positives survived transplantation to produce fronds. The rhizomes were kept outdoors in pots, protected by peat, throughout the winter and were then planted out into garden plots the following spring (chapter 2).

Frond emergence from the transplanted rhizomes was comparatively late in

* This statement must be reconsidered in the light of the chi-squared analysis of the data in Chapter 6. Please see page 171 and that following page 173.

the season, occurring from mid June, but continued throughout the season and well into the autumn. Thirteen of the rhizome segments produced a total of 49 fronds in the first season (1983); the following year, fourteen of the segments produced 152 fronds. It is assumed that those segments which did not produce fronds were dead.

It was felt that the transplantation of the rhizomes could affect the development of the fronds, and the results have only been analysed for the second growing season in cultivation in order to minimise differences between rhizome segments prior to transplantation (e.g. the nutrient status of the soil or the effect of the rhizosphere in the original habitat) and to allow the rhizome segments to acclimatise to cultivation. All the fronds produced were tested for cyanogenesis by the picrate test for HCN (chapter 2). The terminal 4cm of the lowermost pinna of each frond was removed and weighed individually prior to the picrate test. This allowed direct comparisons to be made between the sample weights of cyanogenic and acyanogenic fronds, and a more accurate estimate of the mean amount of HCN released from cyanogenic pinna tips. This estimate was based on the mean amount of HCN released per gramme fresh weight of cyanogenic pinna tips only.

The second set of rhizome collections was made from sites at location 8 where low levels of cyanogenesis were detected in the summer of 1983. The intention was to attempt to identify wholly acyanogenic plants, and hence only rhizomes attached to phenotypically acyanogenic fronds (as determined earlier in the season using the picrate test) were excised and grown on. Rhizomes were collected from the coastal and open habitats only at location 8, because it was felt that these habitats were more likely to maintain acyanogenic plants than the woodland habitat type, despite the lack of cyanogenic fronds sampled at the woodland habitat (chapter 4). Fifteen rhizomes segments attached to acyanogenic fronds were collected from each habitat type, in late September 1983. These were placed in pots and grown on under greenhouse conditions (chapter 2). Only nine rhizome segments survived to produce fronds. The fronds were tested for the release of HCN by the pyridine - pyrazolone method (chapter 2).

9.2. Results.

All rhizome segments from both series of transplants produced cyanogenic fronds, regardless of the phenotype of the frond tested prior to the removal of the frond from its wild habitat (Tables 9.1 and 9.4). Several rhizome segments produced both cyanogenic and acyanogenic fronds.

The estimated mean amount of HCN released by cyanogenic fronds from each rhizome segment in the first series varied greatly, from 12.9 to 309.7 µg HCN g^{-1} (Table 9.1). There was a similar degree of variation in the more accurate results from the second series, which had the amount of HCN released measured by the pyridine - pyrazolone method (Table 9.4).

The sample fresh weights and frond heights were similar for all the rhizome transplants in the first series (Tables 9.1 and 9.2).

9.3. Analysis of results.

The results were analysed using an unblocked analysis of variance.There was no difference between the estimated mean amount of HCN released from cyanogenic fronds produced by rhizomes which had been attached to cyanogenic fronds when they were first sampled in the field and the estimated mean amount of HCN released by cyanogenic fronds produced by rhizomes which had been attached to acyanogenic fronds in the field. In other words, the type of frond produced previously by the rhizome segments has no effect on the amount of HCN released by cultivated fronds (Table 9.3).

The estimated mean amount of HCN released by fronds produced by the first series of rhizome transplants show no effect of their original habitat type (Table 9.3).

To test whether there were any effects due to the phenotypes of the fronds attached to the rhizomes prior to transplantation and development of the fronds, both mean pinna tip sample fresh weight (Table 9.1) and mean frond height (Table 9.2) were subject to analysis of variance. The results of this analysis show that neither the mean fresh weight of fronds (Table 9.3) nor the height of fronds (Table 9.3) was affected by the phenotype of the fronds attached to the rhizome segments prior to their transplantation.

The effect of the original habitat from which the rhizome segments were removed upon the fronds was investigated in a similar fashion. Neither the mean fresh weight of pinna samples (Table 9.3) nor the mean height of fronds (Table 9.3) was affected by the habitat type from which the rhizome segments were removed. The data is given for the two coastal sites amalgamated, but the low variance ratio indicates that treating the two sites separately would have little effect on the results.

To ascertain whether there was any difference between cyanogenic and acyanogenic fronds apart from the ability to release HCN, the fresh weights and heights of each frond were subject to analysis of variance with their phenotype

Table 9.1. The results of the first series of rhizome transplants, 1984. The total number of fronds produced by rhizome segments collected from four sites at location 6 in December 1982, in July of 1984. Each rhizome collection is identified by the grid number of the frond to which it was attached prior to transplantation and the phenotype of that frond when tested by the picrate test in June 1982 (+: cyanogenic; −: acyanogenic). The phenotype of each frond produced was tested by the picrate test and the mean amount of HCN released from cyanogenic pinna samples was estimated. The mean fresh weight of cyanogenic pinna tips was measured in situ, immediately before the picrate test was carried out.

Habitat	Plant	Number of fronds	Number of cyanogenic fronds	Mean sample weight mg ±S.E.	Mean weight of cyanogenic pinna tips mg ±S.E.	Estimated mean amount of HCN released µg HCN g^{-1}
coastal 1	A9 (−)	22	11	145.8± 8.6	154.4±15.4	16.4
	E4 (−)	9	4	176.2±10.0	161.3±12.3	12.9
coastal 2	I3 (−)	17	14	151.4±11.7	147.9±13.1	92.5
	I2 (−)	13	5	150.6± 8.4	148.8±15.2	14.0
	H1 (−)	6	6	135.5±15.0	135.5±15.0	258.9
open	B4 (+)	9	4	129.4±18.3	146.2±28.0	86.5
	D2 (+)	5	3	186.4±12.8	179.7±20.0	101.7
	D6 (+)	10	9	142.3± 9.7	142.9±10.8	135.7
	G7 (−)	15	15	156.7±13.2	156.7±13.2	309.7
woodland	G9 (+)	9	9	154.2±15.1	154.2±15.1	95.3
	I9 (+)	7	7	164.4±20.0	164.4±20.0	281.1
	A4 (−)	3	3	167.0±25.7	167.0±25.7	160.0
	J3 (−)	12	7	135.2±16.6	122.0±19.5	85.1
	E4 (−)	15	15	106.4± 6.6	106.4± 6.6	165.6
Totals		152	112			
Means				146.4± 3.6	144.7± 4.3	129.7±25.8

Table 9.1 - continued.

Mean amounts of HCN released, classified according to phenotype of fronds attached to the rhizome segments prior to their transplantation, µg HCN g^{-1} ±S.E.

acyanogenic transplants	123.9 ± 36.2
cyanogenic transplants	140.1 ± 36.2

Mean amounts of HCN released, classified according to the original habitat of the transplanted rhizomes, µg HCN g^{-1} ±S.E.

coastal 1	14.6 ± 1.7
coastal 2	121.8 ±72.2
open	158.4 ±51.5
woodland	157.4 ±35.0

Mean fresh weight of pinna tip samples, classified according to the phenotype of fronds attached to the rhizome segments prior to their transplantation, mg, ± S.E.

acyanogenic	147.2 ±6.8
cyanogenic	155.3 ±9.7

Mean fresh weight of pinna tip samples, classified according to the habitat from which the rhizomes were transplanted, mg ± S.E.

coastal	151.9 ±6.7
open	153.7 ±12.2
woodland	145.4 ±11.2

Mean fresh weight of pinna tip samples, classified according to the results of the picrate tests of the fronds from which the samples were removed, mg ± S.E.

"acyanogenic"	150.6 ±7.0
"cyanogenic"	146.1 ±4.3

Table 9.2. The physical characteristics of fronds produced by the first series of rhizome transplants in July 1984. The mean heights of fronds produced by rhizomes transplanted from four sites at location 6 into garden plots in Edinburgh. The key to the rhizomes is given in Table 9.1.

Habitat	Rhizome	Mean height of fronds cm, ± S.E.	Mean height of cyanogenic fronds cm, ± S.E.	Mean height of acyanogenic fronds cm, ± S.E.
coastal 1	A9 (−)	41.1±2.7	39.2±5.1	43.5±2.2
	E4 (−)	42.2±3.2	35.0±3.5	48.0±3.4
coastal 2	I3 (−)	38.7±4.1	39.6±4.4	36.7±9.3
	I2 (−)	49.2±3.7	46.0±6.4	51.2±4.8
	H1 (−)	43.3±5.6	43.3±5.6	-
open	B4 (+)	36.2±3.7	30.2±2.3	40.6±4.7
	D2 (+)	41.0±4.0	38.3±6.0	45.0±5.0
	D6 (+)	42.5±4.4	44.4±4.4	25.0
	G7 (−)	36.7±2.8	36.7±2.8	-
woodland	G9 (+)	41.1±4.8	41.1±4.8	-
	I9 (+)	35.7±6.0	35.7±6.0	-
	A4 (−)	20.0±7.6	20.0±7.6	-
	J3 (−)	42.5±4.8	50.0±6.4	32.0±4.4
	E4 (−)	44.7±3.0	44.7±3.0	-
Grand means		39.6±1.8	38.9±2.0	40.3±3.1

Mean height of fronds produced by transplanted rhizome segments, according to the phenotype of fronds attached to the segments prior to their transplantation, cm ± S.E.

acyanogenic	39.8 ±2.7
cyanogenic	38.9 ±1.6

Mean height of fronds produced by transplanted rhizome segments, according to the habitat from which the segments were removed, cm ± S.E.

coastal	42.9 ±1.7
open	38.6 ±1.9
woodland	36.8 ±4.4
Grand mean	147.2 ±5.2

Mean height of fronds produced by transplanted rhizome segments, classified according to the result of the picrate tests of samples removed from the fronds, cm ± S.E.

acyanogenic	39.7 ±1.2
cyanogenic	39.7 ±2.1

Table 9.3. The results of analysis of variance of the estimated amounts of HCN released from cyanogenic fronds (HCN), the mean fresh weight of samples taken from fronds (weight) and the mean height of fronds (height) produced by rhizome segments removed from four sites under three habitat types at location 6 and transplanted into nursery plots. Complete analysis of variance tables are given in Appendix 4, Tables A4.1 - A4.8.

Character	Variation between	Variance ratio	Degrees of freedom	Significance
HCN	rhizome phenotype	0.084	1,12	N.S.
HCN	original habitat	1.087	2,11	N.S.
weight	rhizome phenotype	0.492	1,12	N.S.
weight	original habitat	0.186	2,11	N.S.
weight	frond phenotype	0.301	1,150	N.S.
height	rhizome phenotype	0.055	1,12	N.S.
height	original habitat	1.068	2,11	N.S.
height	frond phenotype	0.001	1,150	N.S.

N.S. not significant.

Table 9.4. The mean amount of HCN released by fronds produced by rhizome segments removed from location 8 and grown on in pots under greenhouse conditions at Edinburgh. The rhizomes are identified by habitat type from which they were removed and the grid position of the the frond to which they were attached prior to transplantation. The phenotypes of the fronds to which the rhizomes were attached were all acyanogenic, when sampled by the picrate test in the field. The mean amount of HCN released from fronds was estimated from the results of the pyridine - pyrazolone test on individual fronds.

Habitat	Rhizome	Number of fronds produced	Number of cyanogenic fronds		Mean amount of HCN released µg HCN g^{-1}, ±S.E.
coastal	A4	4	4		269.5 ±67.9
	A8	5	5		99.8 ±28.2
	C7	6	6		38.0 ±26.9
	B1	1	1		1.5
	B2	5	5		114.0 ±64.4
	B4	3	2		1.4 ±0.5
open	A1	5	3		63.9 ±59.5
	C4	5	5		49.8 ±21.6
	E7	2	2		40.8 ±38.9
Total		36	33	Mean	75.4 ±27.4

Mean amount of HCN released from cyanogenic fronds, classified according to the original habitat from which the rhizome segments were removed, µg HCN g^{-1} fresh weight ± S.E.

coastal	103.5 ±26.1
open	53.2 ±19.5

as the treatment. Neither the fresh weight of fronds (Table 9.3) nor the height of fronds (Table 9.3) showed any effect due the phenotype of the frond.

All the rhizome segments in the second series of collections produced cyanogenic fronds (Table 9.4). Each of these rhizomes had produced an acyanogenic frond, according to the picrate test, in the field prior to collection. The amount of HCN released by cyanogenic fronds varied considerably both between fronds on the same rhizome (as shown by the very large standard errors given in Table 9.4) and between rhizomes (Table 9.4). There were three fronds which released no detectable HCN whilst other fronds on the same rhizome were cyanogenic.

The results from the second rhizome collection were analysed using analysis of variance to ascertain whether the original habitat type from which the rhizome segments were removed had any effect on the amount of HCN released by cyanogenic fronds. The effect of habitat was not significant (Table 9.5).

The results were similarly analysed to see whether different rhizomes removed from the same habitat type produced fronds which released similar amounts of HCN. The coastal and open habitats were analysed separately (Table 9.5). There was an effect of rhizome upon the amount of HCN released by fronds from those rhizome collected from the coastal habitat (Table 9.5) but no effect for those rhizomes collected from the open habitat (Table 9.5).

9.4. Discussion.

The transplantation of rhizome segments from different habitats into similar conditions has shown that the effects of habitat type upon cyanogenesis, as shown in chapters 4, 5, 6 and 7, are not fixed in the rhizome (Tables 9.1 and 9.4). In the first series of collections, rhizomes which had produced either cyanogenic or acyanogenic fronds in the field, according to the picrate test, were transplanted. There was no evidence of an effect of the phenotype of fronds produced in the field on the estimated mean amount of HCN released from cyanogenic fronds produced following transplantation (Table 9.3). Analysis of the physical characteristics of the fronds produced by the first series of rhizome transplants showed no differences between either the phenotype of fronds produced by the rhizomes segments prior to their transplantation or the original habitat of the rhizomes segments on either the fresh weight of the pinna tips sampled or on the height of fronds. It would therefore appear that the differences found between habitat type in previous chapters are due to environmental features rather than selection for genotype by the habitat.

Table 9.5. The results of analysis of variance on the amounts of HCN released from cyanogenic fronds produced from rhizomes transplanted from the coastal and open habitat sites at location 8 into greenhouse pots. Complete analysis of variance tables are given in Appendix 4, Tables A4.9 - A4.10.

Character	Variation between:	Variance ratio	Degrees of freedom	Significance
HCN	original habitat	1.502	1,31	N.S.
HCN	'coastal' rhizomes	4.306	4,18	*
HCN	'open' rhizomes	0.040	2,9	N.S.

N.S. not significant; * significant at 5% level ($P<0.05$).

In addition, in both series of transplants, some rhizomes produced both cyanogenic and acyanogenic fronds, which shows that fronds of both phenotypes can be produced by the same rhizome system. In the second series of transplants, several rhizomes produced fronds that would have appeared acyanogenic if they had been tested with the picrate test rather than the pyridine-pyrazolone method, and only acyanogenic fronds would have been scored for rhizomes B1 and B4 from the coastal habitat (Table 9.4).

These results therefore show that there is no intrinsic difference between rhizomes producing cyanogenic fronds and those producing acyanogenic fronds. That rhizomes in the seconds series of transplantations produced both types of frond suggests that the rhizomes were not subject to somatic mutation, since the rhizome growth was limited by the size of the pot and the rhizomes had divided few times compared to the rhizomes planted in nursery plots which allowed free growth up to 1m in any direction. It had been suggested that somatic mutation was one explanation for the single acyanogenic frond attached to a rhizome producing cyanogenic fronds excavated at the woodland habitat site at location 5 (chapter 8).

In contrast to the pattern of cyanogenic and acyanogenic (according to the picrate test) fronds found in the field (chapter 8), the rhizome transplants growing in nursery plots and pots were growing in homogeneous soil media, and they were fed to maintain the level of nutrients available to fronds. With the second series of transplants in particular, differences between the conditions under which fronds developed were minimal. It is therefore very difficult to explain the differences between fronds that were found with respect to the amount of HCN released. It is not possible to differentiate between fronds from either series of rhizome collections, but the lack of evidence of physical differences between cyanogenic and acyanogenic fronds in the first series of transplants suggests that there are no developmental differences between fronds of the two phenotypes (Tables 9.3). It is therefore not evident what is determining cyanogenesis, or perhaps more correctly, what is determining a high rate of HCN production in some fronds and a low rate in others.

The transplanted rhizomes were growing under conditions of high nutrient application. Schreiner (1980) applied nitrogen fertiliser to plots of cyanogenic bracken, and found that cyanogenesis increased in shaded habitats only. Professor J.H. Lawton has also fertilised field plots and increased the amount of HCN released by bracken (personal communication). It is possible that the production of cyanogenic fronds by rhizomes that previously produced

acyanogenic fronds in the transplantation series was simply a response to the availability of soil nutrients, although it is therefore difficult to explain the differentiation between cyanogenic and acyanogenic fronds (according to the picrate test) in the first series, and between high and low production of HCN in the second series, within the same rhizome system.

Ellis et al (1977a) found that certain plants of Lotus corniculatus were unstable in their phenotype expression, and that some replicates within a clone had different phenotypes. This was difficult to explain, given the genetics of the species. It had been thought that the instability might be temperature dependent, but experiments showed that this was not strictly the case. The authors concluded that the instability in L. corniculatus was due to a genotype/environment interaction, further influenced by a genetic system independent of the two gene system responsible for the determination of the presence of the cyanogenic glycoside and β-glycosidase. Daday (1965) and Jones (1972a) suggested that cyanogenesis in Trifolium repens might be influenced by pleiotropic modifying genes, particularly with respect of the apparent selection by low temperatures against cyanogenesis. A similar situation is believed to occur in Lotus corniculatus (Nass, 1972). The existence of multigene systems has been used to explain the observed continuous variation found in both polymorphic and wholly cyanogenic plant species (Nass, 1972; Hughes, 1981). Although such complex genetic systems may explain the unstable phenotypes found by Ellis et al (1977a) in L. corniculatus, they cannot explain variation between clonal replicates grown under the same environmental conditions, nor the differentiation between fronds produced by the same rhizome in the transplantation series described here.

It has been suggested that dividing and transplanting rhizomes as has been done here may result in the rejuvenation of the rhizome system (Dr. C.N. Page, personal communication). This could affect cyanogenesis. The apparent growth of the rhizomes in the nursery plots was such that after two seasons' growth the rhizomes had begun to escape from their 2m X 2m plots, suggesting a rate of growth in excess of 0.5m $year^{-1}$. This is considerably less than the rate of growth for juvenile plants reported by Braid and Conway (1943) under similar nursery conditions. In addition, the frond morphology of the nursery transplants appeared indistinguishable from adult fronds growing in the wild. Although these measures are subjective, they would indicate that the rhizomes had not undergone rejuvenation. It is not possible to determine whether the development of the rhizome, rather than the frond, was of a juvenile or adult form, although the rhizomes had in certain cases at least

differentiated into short shoot and long or intermediate shoot rhizomes (sensu Watt, 1940). Given the large number of fronds produced by the nursery transplants (Table 9.1), it would appear likely that some short shoot rhizomes produced more than one frond in the season. The rhizomes were not excavated to check whether in fact this was the case since it had been hoped to continue the investigation for a third season. However, at the start of the third season some of the rhizomes had grown out of the plots and hence the fronds they produced could not be differentiated. As a consequence, work on the transplants ceased.

9.5. Conclusions.

The results of these transplantation experiments have shown that the differences found between populations of bracken fronds growing under different habitat conditions in the wild are not genetically fixed, and that there are no differences between fronds produced by rhizomes in cultivation despite differences being found between fronds attached to the same rhizomes in the field. It could therefore be concluded that such differences found in the field are due to environmental differences between the populations of fronds. However these experiments also showed that cyanogenic and acyanogenic fronds could be produced by the same rhizome system in cultivation, despite adequate nutrients being supplied to the rhizomes and environmental differences within a rhizome system being small if present. It is possible that the development of the frond is important in determining cyanogenesis, but there is no evidence for this from the fresh weight of pinna tips or the height of fronds. The development of the frond below the soil, from its initiation, may well be important. It is not possible to say how the status of the rhizome at frond initiation could affect the frond.

Chapter 10. The effect of shading on cyanogenesis in **Pteridium aquilinum**.

The survey of cyanogenesis throughout Britain showed that bracken growing in woodland habitats released greater quantities of HCN than bracken growing in open and coastal habitats (chapter 4), and similar results have also been shown in other surveys (chapters 5, 6, and 7). Although this may not be a direct effect of shading by trees upon the physiology of bracken, it was decided to investigate experimentally the effect of differential shading on clonal plants of bracken to ascertain the importance of shading in determining cyanogenesis.

10.1. Method.

A shade frame was built in a greenhouse at the Royal Botanic Garden, Edinburgh, using white coarse weave shade cloth (Figure 10.1). One, two, or three layers used to produce three different levels of shading. Each layer of shade cloth reduced the light intensity by approximately 50%, giving values of 50%, 25%, and 12.5% of the ambient light intensity. At 1200 hours, GMT, on a bright but cloudy day in mid May, the ambient light flux was 150 $\mu E\ m^{-2}\ s^{-1}$, and the flux in the shade frame 70, 37, and 18 $\mu E\ m^{-2}\ s^{-}1$ respectively. Such levels are similar to those found in light open woodland of the kind typically containing bracken (Hughes, 1964; Fitter and Hay, 1983). It was not possible to have an unshaded control since the ventilation within the frame was restricted by the shade cloth and it was not feasible to replicate this without shading. Producing an unshaded control by covering the whole frame in transparent polythene, and thereby reducing the ventilation throughout the frame, would have raised the temperature and humidity to levels at which fungal contamination would have been a risk. No attempts were made to produce an altered light quality as would naturally occur in woodland.

Sporophyte material, consisting of a single clone grown up from spore, Ti:5, was cloned by dividing the rhizome system developed into segments 10cm long (chapter 2). Each rhizome segment was of long shoot rhizome material and included a single active rhizome apex; it is possible that dormant apices or buds were not detected. These were planted into trays containing a mixture of fine peat and perlite in a ratio of 3:1. Six replicate rhizomes were used in each level of shading. Not all the replicates produced fronds. This does not appear to have been due to shading, since all the replicates in the 25% treatment survived whilst only half in both the 50% and 12.5% treatments survived. The rhizomes were divided and planted in early March, prior to the onset of the

Figure 10.1. The shade frame. This was assembled from dexion in the greenhouse at the Royal Botanic Garden, Edinburgh, and provided three levels of shading. The shade cloth used was close weave standard thickness.

season. It was felt that before this insufficient light would have been available for the development of the plants under shaded conditions.

Each replicate in each shading treatment was given 200ml of liquid nutrient medium (Table 10.1) fortnightly, to maintain a moderate nutrient status for each replicate. The level of nitrogen supplied to each replicate was kept low, since preliminary trials indicated that shading had little effect on cyanogenesis if nitrogen was freely available, as might be expected. Within each shade treatment, the replicates were moved at random when given nutrient medium to reduce the possibility of differences within the frame affecting the replicates.

Two sets of samples were taken, in mid May and in late July. The fronds were numbered approximately in the order of which they emerged. Frond samples were removed and individually weighed prior to the measurement of the amount of HCN produced by the pyridine - pyrazolone method of Mao et al (1965; chapter 2). The fronds in all treatments appeared etiolated.

When the frond samples were taken in mid May, additional samples of frond tissue were removed for a direct estimation of the β-glycoside content, as given by the amount of HCN released by the action of exogenous glycosidase on glycoside extracts (chapter 2). Attempts to measure the activity of β-glycosidase in frond samples failed.

10.2. Results.

All replicate rhizomes produced at least some fronds that released measurable quantities of HCN (Table 10.2). Some fronds in the 50% treatment did not release measurable HCN. The fronds from each replicate rhizome and from replicate rhizomes within each treatment were very variable (Table 10.2). The fronds released amounts of HCN similar to the estimated mean amount of HCN released from cyanogenic fronds at woodland sites (chapter 4).

10.2.1. The effect of shading.

The results of the amount of HCN, as $\mu g\ g^{-1}$ fresh weight, produced by each cyanogenic frond were analysed using analysis of variance. The two sets of samples were analysed separately (Table 10.3). The first sets showed that the shading treatment had a significant effect on the amount of HCN released by cyanogenic fronds (5% level, $P<0.05$; Table 10.3a; Figure 10.2). By analysing the treatments separately, it is seen that there is no significant difference between the 50% and 25% treatments (Table 10.3a) nor between the 25% and 12.5% treatments (Table 10.3a), but that there was a significant difference between the 50% and 12.5% treatments (1% level, $P<0.01$; Table 10.3a).

Table 10.1. The nutrient medium supplied to clonal replicates under conditions of differential shading. The medium is based on that used for growing gametophytes on agar (Dyer, 1982), with 75% of the nitrogen (as nitrate) replaced by chloride. Each replicate rhizome was given 200ml at weekly intervals.

Mineral nutrient	$g\ l^{-1}$
$MgSO_4, 7H_2O$	0.546
KNO_3	0.032
KCl	0.084
$FeCl_3, 6H_2O$	0.018
$CaCO_3, 4H_2O$	0.386
$CaCl_2$	0.543
K_2HPO_4	0.268

Table 10.2. The mean amount of HCN released from pinna tips of fronds from each replicate rhizome. The replicate rhizomes were under three different shading treatments, representing 50%, 25% and 12.5% of the ambient light intensity. The HCN was released by the action of toluene upon whole tissue samples, which were weighed prior to the liberation of HCN. The HCN released was measured by the pyridine - pyrazolone test. Two sets of frond samples were taken.

a. First sample, taken May 19th 1985.

Treatment	Replicate	Number of fronds	Number of cyanogenic fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
50%	1	4	3	2.57 ±1.30
	2	2	2	6.73 ±4.97
	3	3	3	179.15 ±42.39
25%	1	5	5	204.92 ±30.44
	2	3	3	88.53 ±33.10
	3	4	4	105.75 ±45.48
	4	3	3	200.66 ±18.09
	5	2	2	108.44 ±60.08
	6	4	4	106.01 ±91.70
12.5%	1	2	2	228.46 ±44.38
	2	4	4	174.21 ±43.71
	3	3	3	179.78 ±21.34
Means				
50%				69.83 ±34.90
25%				140.05 ±19.65
12.5%				188.11 ±21.68
Grand mean				136.46 ±15.27

Table 10.2 - continued.

b. Second sample, taken July 29th 1985.

Treatment	Replicate	Number of fronds	Number of cyanogenic fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, $\pm$ S.E.
50%	1	6	2	1.95 $\pm$0.16
	2	4	3	2.76 $\pm$1.13
	3	6	6	88.20 $\pm$43.83
25%	1	7	7	66.58 $\pm$18.42
	2	7	7	97.68 $\pm$34.10
	3	7	7	84.14 $\pm$27.56
	4	6	6	115.77 $\pm$21.27
	5	5	5	150.47 $\pm$37.89
	6	4	4	81.20 $\pm$18.21
12.5%	1	6	6	117.37 $\pm$34.42
	2	4	4	111.81 $\pm$43.71
	3	3	3	122.21 $\pm$7.78

Means	μg HCN g^{-1} $\pm$S.E.
50%	49.21 $\pm$26.58
25%	97.51 $\pm$11.49
12.5%	116.78 $\pm$16.95
Grand mean	92.83 $\pm$15.92

Table 10.3. The results of analysis of variance of the amount of HCN released from samples taken from fronds grown under three shading treatments. The HCN was released by the action of toluene upon whole tissues. Complete analysis of variance tables are given in Appendix 5, Tables A5.1 – A5.5

a. First sample.

Variation between		Variance ratio	Degrees of freedom	Significance
all treatments		4.213	2,33	*
50% and 25% treatments		3.465	1,25	N.S.
50% and 12.5% treatments		8.711	1,15	**
25% and 12.5% treatments		2.211	1,26	N.S.
Variation between	**within**	**Variance ratio**	**Degrees of freedom**	**Significance**
replicates	50% treatment	13.234	2,5	**
replicates	25% treatment	1.943	5,13	N.S.
replicates	12.5% treatment	0.430	2,6	N.S.

b. Second sample.

Variation between		Variance ratio	Degrees of freedom	Significance
all treatments		3.625	2,57	N.S.
50% and 25% treatments		3.625	1,45	N.S.
50% and 12.5% treatments		4.883	1,22	*
25% and 12.5% treatments		0.789	1,47	N.S.
Variation between	**within**	**Variance ratio**	**Degrees of freedom**	**Significance**
replicates	50% treatment	1.392	2,8	N.S.
replicates	25% treatment	1.064	5,30	N.S.
replicates	12.5% treatment	0.021	2,10	N.S.

c. First and second samples.

Variation between	within	Variance ratio	Degrees of freedom	Significance
samples	50% treatment	0.229	1,17	N.S.
samples	25% treatment	3.998	1,53	N.S.
samples	12.5% treatment	6.882	1,20	*

N.S. not significant; * significant at 5% level ($P<0.05$); ** significant at 1% level ($P<0.01$).

The second set of samples showed no difference between the treatments when they were analysed together (Table 10.3b), nor between either the 50% and 25% treatments or the 25% and 12.5% treatments (Table 10.3b). There was however again a significant difference between the 50% and 12.5% treatments (5% level; $P<0.05$; Table 10.3b; Figure 10.2).

The amounts of HCN released by β-glycosidase from β-glycoside extracts taken from frond tissue in mid May (Table 10.4; Figure 10.2) are similar to those released by the action of toluene upon whole tissues (Table 10.2), although replicates 1 and 2 in the 50% shading treatment released far higher amounts of HCN following extraction of the β-glycoside and its hydrolysis by β-glycosidase than they did from the whole tissues. All the fronds produced measurable amounts of HCN from the action of exogenous β-glycosidase on β-glycoside extracts. The results of the amount of HCN released from the β-glycoside extracts were subjected to analysis of variance to see whether there was a discernible effect of shading. There was no significant difference between shading treatments (Table 10.5).

10.2.2. Variation between replicate rhizomes within each treatment.

The amounts of HCN released by cyanogenic fronds produced by each replicate rhizome within each treatment were also subject to analysis of variance to examine whether there were significant differences between rhizomes within treatments. The replicate rhizomes were treated separately for each of the two samples. There was a very significant difference between replicate rhizomes in the amount of HCN released by cyanogenic fronds in the 50% treatment sampled in mid May (1% level; $P<0.01$. Table 10.3a), but neither of the other two treatments showed significant differences between replicates (Table 10.3a). There were no significant differences between replicate rhizomes in the amount of HCN released from cyanogenic fronds in any of the treatments sampled in late July (Table 10.3b).

Within each treatment, the amounts of HCN released by the action of β-glycosidase upon β-glycoside extracts from fronds produced by each replicate rhizome were compared by analysis of variance (Table 10.5). The rhizomes in the 50% shading treatment showed highly significant differences between replicates (1% level; $P<0.01$. Table 10.5) whilst there were no such differences between the replicate rhizomes in either the 25% or 12.5% shading treatments (Table 10.5).

Table 10.4. The amount of HCN released from glycoside extracts. The mean amount of HCN released from by the action of exogenous β-glycosidase on glycoside extracts from frond tissue under three shade treatments. The fronds were sampled in mid May. The HCN was released from the glycoside extracts by the addition of commercially available glycosidase and measured using the pyridine - pyrazolone method. All frond extracts released measurable quantities of HCN.

Treatment	Replicate	Number of fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
50%	1	4	88.37 ±21.25
	2	2	67.13 ±2.35
	3	3	242.06 ±10.76
25%	1	5	197.94 ±19.62
	2	3	134.88 ±19.51
	3	4	164.11 ±24.17
	4	3	166.49 ±7.60
	5	2	173.29 ±34.4
	6	4	213.29 ±47.88
12.5%	1	2	203.99 ±13.40
	2	4	185.91 ±65.30
	3	3	193.79 ±21.52
Means			
50%			134.88 ±28.49
25%			180.59 ±13.25
12.5%			192.55 ±27.58
Grand mean			172.38 ±20.32

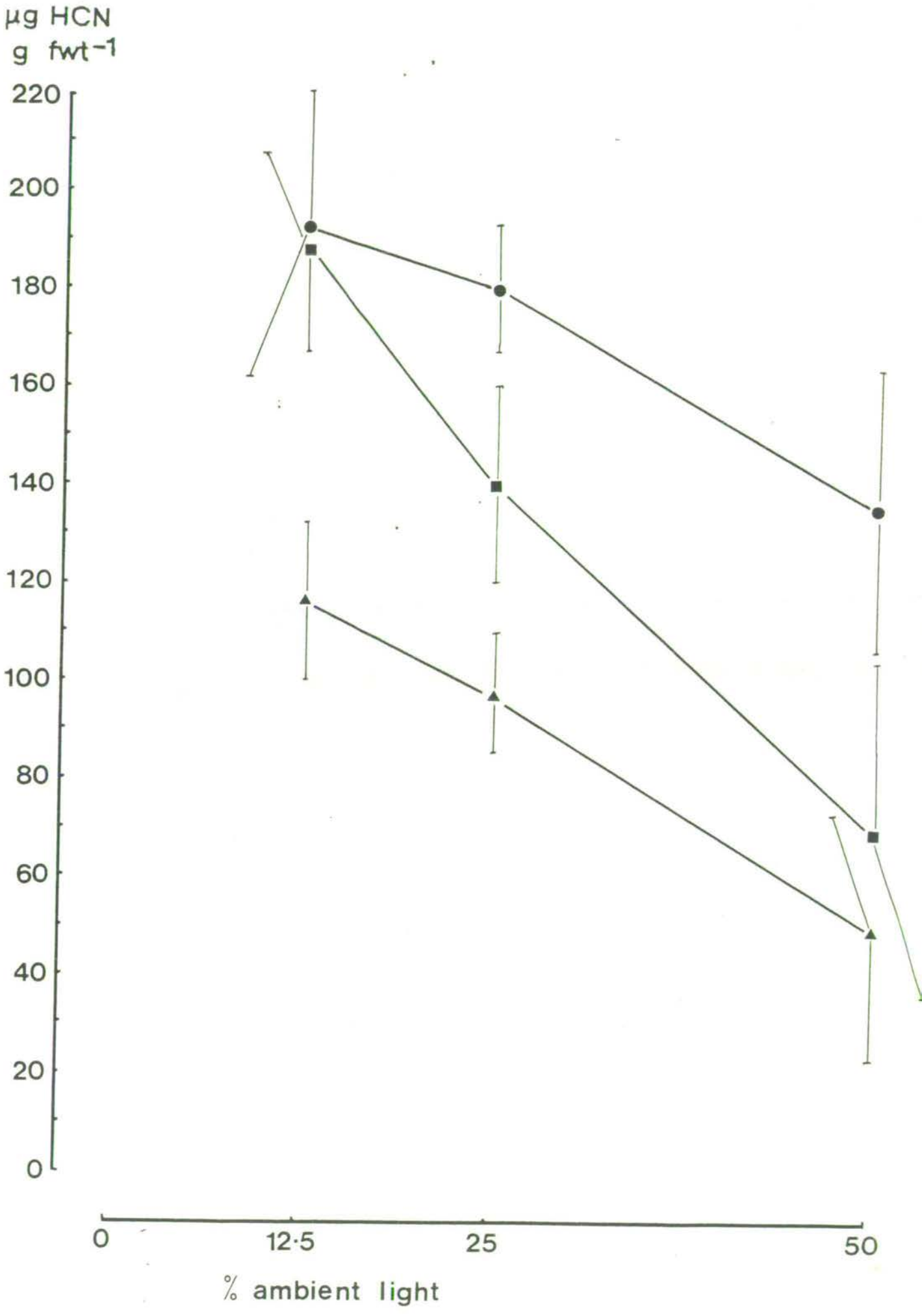
Table 10.5. The results of analysis of variance of the amount of HCN released from glycoside extracts taken from fronds grown under three shading treatments. The HCN was released by the action of exogenous glycosidase on the extracts. Complete analysis of variance tables are given in Appendix 5, Tables A5.6 - A5.7.

Variation between		Variance ratio	Degrees of freedom	Significance
treatments		1.727	2,34	N.S.

Variation between	within	Variance ratio	Degrees of freedom	Significance
replicates	50% treatment	25.599	2,6	***
replicates	25% treatment	1.799	5,13	N.S.
replicates	12.5% treatment	0.024	2,6	N.S.

N.S., not significant; ***, significant at 0.1% level ($P<0.001$).

Figure 10.2. The amount of HCN released from frond samples grown under three shading treatments. Two series of samples were taken (■, first series; ▲, second series). In addition, β-glycoside extracts were prepared at the same time as the first series of samples. These were treated with β-glycosidase to release HCN (●).


µg HCN
g fwt⁻¹
220
200
180
160
140
120
100
80
60
40
20
0
0
12·5
25
50
% ambient light

10.2.3. Variation between sets of samples.

It is possible to compare the two sets of samples taken in mid May and in late July directly by analysis of variance of the amount of HCN released by cyanogenic fronds produced by replicate rhizomes in each treatment on each occasion. There were no significant differences between the mid May and late July samples for either the 50% treatment or the 25% treatment (Table 10.3c) in the amount of HCN released by cyanogenic fronds. The 12.5% treatment showed a significant difference between the two samples in the amount of HCN released (5% level, $P<0.05$. Table 10.3c).

10.2.4. Comparison of the methods of HCN release from tissues.

The results of the amount of HCN released by the two different methods (the action of toluene on whole tissues and the action of β-glycosidase upon β-glycoside extracts) were compared by analysis of variance of the results for each shading treatment separately. It was necessary to analyse each treatment separately due to the different numbers of replicate rhizomes and fronds in each treatment, which would have complicated a blocked analysis of variance. None of the shading treatments showed a significant difference between the two methods (Table 10.6).

10.3. Discussion.

10.3.1. The effect of shading.

The results presented here indicate that increased shading can have a direct influence on the amount of HCN released by cyanogenic bracken fronds, shown by the whole tissue analysis, as has been demonstrated in the field. The effect is not however in direct relation to the level of shading, and it is possible that this is due to the availability of nitrogen; had nitrogen been supplied to replicates at a lower rate, there may have been a greater relationship between the amount of HCN released and the level of shading. It is of note that despite the overall significance of the differences between the whole tissue samples in May (Table 10.3a), there was no significant difference between the 50% and 25% shading treatments, nor between the 25% and 12.5% shading treatments. The statistical difference found was due largely to the large difference between the 50% and 12.5% shading treatments (Table 10.3a). This is probably due more to the very large standard errors associated with the results, since there would appear to be some difference between the 50% and 25% shading treatments.

Table 10.6. The results of analysis of variance of the amount of HCN released from fronds by two methods. The fronds were grown under three shading treatments. The two methods used to release the HCN were the action of toluene on whole tissue frond samples, and the action of exogenous glycosidase on glycoside extracts of frond samples. Complete analysis of variance tables are given in Appendix 5, Table A5.8.

Variation between	within	Variance ratio	Degrees of freedom	Significance
methods	50% treatment	2.914	1,16	N.S.
methods	25% treatment	2.925	1,36	N.S.
methods	12.5% treatment	0.016	1,16	N.S.

N.S., not significant.

The second series of samples, taken more than two months after the first, show no overall statistical differences between the three shading treatments (Table 10.3b), although there remains a significant difference between the 50% and 12.5% shading treatments (Table 10.3b). This lack of an effect due to shading in the second series of samples is confusing, but may be in part explained by the decrease in the means of each treatment, compared to the first series (Table 10.2) whilst the variability, as shown by the large standard errors associated with the means, remains at a similar level.

The analysis of the results from the glycoside extraction show only slight effects of the shading treatments on HCN production (Table 10.4) which are not statistically significant (Table 10.5). This is in contrast to the results from the whole tissues treated with toluene.

The effect of shading on cyanogenesis and possible mechanisms of control has been discussed in chapter 4. The results presented here show that shading can have an effect on the level of HCN released from frond samples independent of any effect due to woodland on bracken. This suggests that the increase in the amount of HCN released is determined to a certain extent by physical rather than biological effects.

10.3.2. Differences between sampling date.

Between the first set of samples in May and the second in July, there was a general decrease in the amount of HCN released (Table 10.2). When the two series of samples were compared statistically, no significant difference was found within the 50% and 25% shading treatments (Table 10.3c), although a significant difference was found between the two samples taken of the 12.5% shading treatment. Again, this is largely due to the large variability, and hence large standard errors, associated with each of the shading treatments for both of the series of samples. The differences found between the two samples were large, but equally large was the variation within each sample.

It is possible that the decrease in the amount of HCN released between the first and second series of samples is due to growth of the rhizomes, producing more fronds attached to each rhizome. This may reduce the allocation to each frond of nutrients within each rhizome system, since the volume of nutrient feed was kept constant throughout the course of the experiment. However, although two of the rhizome replicates in the 12.5% shading treatment did not produce any more fronds between the two series of samples, they did still show a substantial decrease from May to July in the amount of HCN that their fronds released.

It should be remembered that the experiment was set-up in early March, with the fronds being produced in late March and early April, at the beginning of the season. Between the emergence of the fronds and the first series of samples the fronds were subject to illumination associated with spring; between the first and second series of samples, the intensity of illumination will have changed from that of spring to mid summer, and although the relative intensities under each shading treatment will have remained constant, the absolute light intensities for each of the treatments will have increased. The carbon: nitrogen balance will thus be changed, resulting in a relative decrease in nitrogen to each plant as more carbon is fixed under the increased light intensities. This may explain the decrease in the amount of HCN noted between the first and second series of samples.

10.3.3. Differences between replicate rhizomes within treatments.

The results from the 50% shading treatment were very variable under constant conditions for both series of samples (Tables 10.3a and 10.3b), despite the rotation of the replicates within the shade frame to reduce any variation in the conditions within the each treatment in the shading frame. This is difficult to explain, since it suggests an intrinsic difference between rhizome segments taken from clones of the same plant, which should be identical, growing under uniform conditions. The difference between replicates within the 50% shading treatment was significant for the first series of samples (Table 10.3a), but no such difference was found for the second sample (Table 10.3b) nor for either of the other two treatments in either series of samples (Tables 10.3). However, the variation between fronds of the same replicate rhizomes of the 50% shading treatment in the second series of samples was large, and it seems possible from the means (Table 10.2b) that this variation is obscuring a difference between the replicates.

10.3.4. Differences between method of HCN release.

Although differences between the results obtained by the two different methods of HCN release in the first series of samples are not significantly different, they do show large differences (c.f. Tables 10.2a and 10.4). Notable amongst these are the amounts of HCN released from the glycoside extracts from the replicate rhizomes in the 50% shading treatment. Replicate rhizomes 1 and 2 released very low levels of HCN from the whole tissues (Table 10.2a), whilst much larger quantities of HCN were released from the glycoside extracts derived from the same fronds (Table 10.4). The fronds which released no

measurable HCN from the whole tissue released substantial amounts of HCN from the glycoside extract. Similar differences are not found for the other two shading treatments. It therefore seems likely that this result shows a real difference between the two methods which is only relevant to the 50% treatment and that this is due to the incomplete release of HCN from the whole tissue sample from particular rhizomes, since an artefactual difference due to technique would be likely to show for all replicates and treatments. In addition, the extraction may be expected to result in the loss of HCN or glycoside, rather than its increase, and an increase in the variation found within replicates of all treatments, instead of the decrease that was found (compare the standard errors associated with the release of HCN from whole tissues (Table 10.2) with those associated with the release of HCN from glycoside extracts (Table 10.4)). The differences between the two methods in the amount of HCN released from replicates of the 50% shading treatment are therefore likely to be due to the functioning of the mechanism of cyanogenesis, notably the activity of the glycosidase, rather than the method of liberation of HCN from the tissue.

It was hoped to show this by studying the activity of β-glycosidase extracted from fronds of all three treatments, but unfortunately the technique failed to give any results at all, probably because the samples of frond tissue removed for this were too small. It was not possible to perfect the technique in the time available. However, it would seem likely that in replicate rhizomes 1 and 2 of the 50% shading treatment the release of HCN was restricted due to the enzyme activity rather than due to a lack of substrate. This could be explained as an effect of high illumination on plants with restricted nitrogen availability, resulting in decreased synthesis of non-essential enzymes. It must of course be noted that the third replicate under this treatment produced large amounts of HCN regardless of the method of release employed, and it is therefore not possible to conclude that there is an effect of light intensity upon the production of glycosidase. It is interesting that replicate rhizome 3 of the 50% treatment released significantly more HCN from the glycoside extract than the other two replicates, as was also found for the release of HCN from the whole tissue. There is no reason why this should be so, although it supports the idea that the third replicate differed intrinsically from the other two.

10.3.5. Variation between fronds within replicate rhizomes.

In all replicate rhizomes, HCN production in both series of samples and according to both methods of HCN release, was very variable between fronds, as shown by the standard errors associated with their means (Tables 10.2 and

10.4). This large degree of variation is hard to explain, although similar variation was obtained in the results from field surveys (chapters 7 and 8) and the results from the transplantation experiments (chapter 9). Furthermore, all three of these chapters reported acyanogenic fronds attached to rhizomes also producing cyanogenic fronds, as is reported here for replicates 1 and 2 of the 50% treatment. It should be noted that the pyridine - pyrazolone test was used in several of these investigations, and that acyanogenic fronds are not therefore an artefact of the picrate paper test. It is possible that the variation found in this experiment within each replicate rhizome system was due to developmental differences between fronds. The rate of frond production may change as the rhizome system develops. In addition, the use of small rhizome segments as experimental material and the culture method used may result in the material reverting to juvenile characteristics. The fronds did not spore under the greenhouse conditions, and appeared to maintain the morphology of juvenile fronds. There was no direct relationship between the order of emergence of each frond and the amount of HCN released from the frond samples, although in general the earliest fronds to emerge produced slightly more HCN. However, it is difficult to tell whether this trend is due to developmental aspects of the rhizome system, due to the decreasing availability of nitrogen in proportion to the number of fronds, or perhaps simply due to chance. The order of frond-bud production may differ from the order of frond emergence, which was only approximated.

The results presented here support the conclusions drawn in chapter 4 regarding the influence of shading on cyanogenesis in bracken. Of great importance is that shading can have a direct effect upon cyanogenesis; the influence of trees on the ecology and physiology of bracken is also likely to be of importance and may affect cyanogenesis.

10.3. Conclusions.

The intensity of light has been shown to have a direct effect on cyanogenesis in clonal replicates of bracken, although the effect was not in proportion to the light intensity. Not all of the three shading treatments produced significantly different amounts of HCN within frond tissues. Variation within treatments and within replicate rhizomes was very great, which substantiates observations made with respect to field surveys and transplant experiments in preceding chapters.

Chapter 11. The effect of nitrogen availability on cyanogenesis in *Pteridium aquilinum*.

The effect of shading on cyanogenesis in *Pteridium aquilinum* has been discussed in chapters 4 and 10, and the hypothesis was proposed that the effect of shading was due to the alteration of the carbon/nitrogen balance. Schreiner (1980) has shown that applying nitrogen fertiliser to field plots under open conditions can increase the amount of HCN released from cyanogenic fronds; similar results have been reported by Professor J.H. Lawton (personal communication). However, there has been no systematic study of the effect of differential nitrogen fertilisation on cyanogenesis in bracken under controlled conditions. In order to investigate the effect of differential nitrogen nutrition on clonal replicates of bracken, two series of trials were set up. It would have been preferable to examine the combined effect of differential nutrition and differential shading within the same experiment, but the space required for the large number of replicates of bracken rhizomes for the completion of such an experiment was not available.

11.1. Methods.

The first series of trials was established in January 1984, and the second in mid March of 1985. Both series used replicate long shoot rhizome segments from the bracken clone Ti:5. This was the same clone that was used in shading trials (chapter 10). The replicates were set up as described in chapter 2. The replicates were placed on a bench in a greenhouse at the Department of Botany, University of Edinburgh. The greenhouse conditions are described in chapter 2.

In the first series of trials, three different levels of nitrogen were given, with four replicates in each treatment. The nutrient media used were based on a general fern nutrient medium (Dyer, 1979). Nitrogen was supplied in the relative amounts 10, 1, and 0 (Table 11.1a). Each replicate in each treatment received 200ml of nutrient medium at weekly intervals. The plants received additional illumination to supplement the ambient light supply to allow growth to continue over winter (chapter 2).

The second series of trials used four different nutrient media, containing nitrogen in the relative amounts 10, 5, 2.5, and 1, with three replicates in each treatment (Table 11.1b).

The fronds produced by each replicate rhizome in each treatment in each

Table 11.1. The nutrient media used in the two series of trials investigating the effect of differential nitrogen nutrition. The media are based upon a general fern nutrient medium (Dyer, 1979). Three levels of nitrogen were supplied in the first trial and four levels of nitrogen were supplied in the second trial. The amounts of nutrients are given as g l^{-1} of media. Each rhizome replicate of each treatment was supplied with 200ml of medium at weekly intervals.

a. First trial.

Nutrient	Relative amount of nitrogen supplied		
	10	1	0
$MgSO_4$, $7H_2O$	0.51	0.51	0.51
KNO_3	0.12	0.012	0
KCl	0	0.080	0.088
$FeCl_3$, $6H_2O$	0.017	0.017	0.017
$Ca(NO_3)_2$	1.44	0.144	0
$CaCl_2$	0	1.20	1.33
KH_2PO_4	0.25	0.25	0.25

b. Second trial.

Nutrient	Relative amount of nitrogen supplied			
	10	5	2.5	1
$MgSO_4$, $7H_2O$	0.51	0.51	0.51	0.51
KNO_3	0.12	0.06	0.03	0.012
KCl	0	0.044	0.066	0.080
$FeCl_3$, $6H_2O$	0.017	0.017	0.017	0.017
$Ca(NO_3)_2$	1.44	0.72	0.36	0.144
$CaCl_2$	0	0.67	1.00	1.20
KH_2PO_4	0.25	0.25	0.25	0.25

series of trials were tagged and numbered in the order that they emerged from the soil. This allowed the amount of HCN produced by each frond to be followed over the period of the experiment. This was particularly of interest for the first series of trials which lasted for four months, with three months between the first and fourth (final) fronds samples being taken.

Uniform frond samples, by weight approximately 100mg, were used throughout the trials. Four sets of samples were removed from fronds produced by the rhizomes in the first series of trials, from late February to mid April. Two sets of samples were removed from the fronds produced by rhizomes in the second series of trials, in May and July. Each frond sample taken was weighed. The HCN was released from the frond samples by the addition of toluene to the tissue within a Thunberg tube (chapter 2), and the amount of HCN liberated was analysed by the pyridine - pyrazolone method (chapter 2). A third set of frond samples was taken from the fronds produced by rhizomes in the second series of trials and β-glycoside extracts were prepared from the frond tissues (chapter 2). HCN was released from each extract by the action of exogenous β-glycosidase and the amount of HCN released was estimated by the pyridine - pyrazolone method (chapter 2).

In addition to the fronds samples, rhizome samples were also taken in the second series. The terminal 1.5cm of a single long shoot rhizome branch was excised from each replicate rhizome system. The 1.5cm section was treated similarly to the frond tissue to obtain the β-glycoside extracts (chapter 2). These extracts were treated with exogenous glycosidase and any HCN released was collected in a 0.1M NaOH trap.

The physical characteristics of the replicate rhizome systems were recorded after the second set of samples was taken from fronds in the second series of trials. The length of each living frond was measured. The fronds were removed, weighed, dried in an oven at 80°C until there was no further weight loss, and weighed again to obtain a value for the dry weight of the fronds. The rhizomes were measured, the number of long shoot rhizome branches counted, and the number of short shoot rhizomes counted. The total number of dormant frond buds was also counted. The fresh weight of the rhizomes was measured, and the rhizomes were dried in an oven at 80°C until there was no further weight loss, to obtain the dry weight value.

11.2. Results.

11.2.1. The first series.

All rhizomes in the first series of trials produced cyanogenic fronds, although not all the fronds produced were cyanogenic (Table 11.2; Figure 11.1). In general, the mean amount of HCN produced by fronds of each replicate in each treatment decreased as the experiment progressed (Table 11.2; Figure 11.2). The results within each replicate were rather variable, as is shown by the large standard errors associated with the means. Some fronds which were acyanogenic at the first sample became cyanogenic at later samples, whilst others that were cyanogenic at the first sample became acyanogenic during the course of the experiment.

Each set of samples taken in the first series of trials was analysed for treatment effects by analysis of variance (Table 11.3). All the analyses showed significant treatment effects at the 0.1% level ($P<0.001$). Fronds sampled from the high nitrogen (10) treatment released significantly more HCN than both the low nitrogen (1) and nitrogen-free treatments ($P<0.01$ or $P<0.001$) when each treatment was compared separately. However, there were no significant differences found between the low nitrogen (1) and nitrogen-free (0) treatments in any of the four sets of samples. Thus the significant treatment effect was wholly due to the very large amounts of HCN released by cyanogenic fronds produced by rhizomes under the high nitrogen treatment.

The four sets of samples taken were compared by analysis of variance within treatments to see how the treatments had changed over the course of the experiment (Table 11.3e). All three treatments showed significant differences between the different sets of samples in the amounts of HCN released from fronds, at the 0.1% level ($P<0.001$) for the high nitrogen (10) treatment and at the 5% level ($P<0.05$) for both the low nitrogen (1) treatment and the nitrogen-free (0) treatment.

11.2.2. The second series.

All the rhizomes in the second series of trials produced cyanogenic fronds, although some also produced acyanogenic fronds (Table 11.4). The mean amounts of HCN released by frond samples of each replicate in each treatment were associated with large standard errors (Table 11.4; Figure 11.3), indicating the amount of variability found within each replicate. The mean amount of HCN released by fronds in each replicate decreased between the taking of the first and the second samples (Table 11.4).

Table 11.2. The means of the amount of HCN released by all fronds of each replicate of each treatment in the first series of trials. Those replicates which produce only one cyanogenic frond have no standard error associated with the mean, since the standard error is equal to the mean in these cases (marked n.a., not applicable). Some fronds died in between the sample dates.

a. First sample.

Treatment	Replicate	Number of fronds	Number of cyanogenic fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
10	1	5	4	205.23 ±57.09
	2	4	3	329.48 ±118.28
	3	9	9	301.89 ±45.74
	4	4	4	353.05 ±8.90
1	1	2	1	157.71 (n.a.)
	2	5	2	72.41 ±45.62
	3	1	1	100.54 (n.a.)
	4	2	2	138.57 ±12.23
0	1	2	2	107.98 ±84.09
	2	5	3	43.11 ±38.59
	3	4	4	179.84 ±74.62
	4	1	1	110.31 (n.a.)

Means.

Nitrogen treatment.	
10	298.46 ±31.90
1	99.37 ±38.08
0	102.12 ±31.46

Table 11.2 - continued.

b. Second sample.

Treatment	Replicate	Number of fronds	Number of cyanogenic fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
10	1	5	5	317.31 ±69.45
	2	5	5	284.36 ±76.26
	3	8	8	329.89 ±39.23
	4	7	7	419.01 ±34.33
1	1	2	2	32.24 ±9.54
	2	5	2	20.50 ±16.79
	3	1	1	11.16 (n.a.)
	4	3	2	92.38 ±46.73
0	1	2	2	94.40 ±68.50
	2	6	4	57.70 ±26.90
	3	3	2	10.27 ±8.91
	4	1	1	90.92 (n.a.)
Means.				
10				343.21 ±26.16
1				41.39 ±16.51
0				54.73 ±17.84

Table 11.2 - continued.

c. Third sample.

Treatment	Replicate	Number of fronds	Number of cyanogenic fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
10	1	8	7	162.00 ±42.37
	2	5	5	222.29 ±46.54
	3	10	8	197.49 ±41.83
	4	12	11	217.05 ±27.19
1	1	2	1	30.26 (n.a.)
	2	4	1	19.42 (n.a.)
	3	1	0	0
	4	3	2	31.95 ±21.76
0	1	3	2	105.31 ±94.06
	2	6	1	2.15 (n.a.)
	3	4	2	69.15 ±63.03
	4	1	1	31.64 (n.a.)
Means.				
10				198.08 ±18.35
1				23.40 ±10.60
0				43.35 ±26.47

Table 11.2 - continued.

d. Fourth sample.

Treatment	Replicate	Number of fronds	Number of cyanogenic fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
10	1	8	7	171.57 ±45.65
	2	5	5	202.75 ±59.93
	3	11	9	161.43 ±47.55
	4	12	12	255.23 ±31.73
1	1	2	1	2.61 (n.a.)
	2	5	1	3.48 (n.a.)
	3	1	0	0
	4	4	3	17.42 ±9.67
0	1	3	1	34.52 (n.a.)
	2	7	0	0
	3	5	1	1.61 (n.a.)
	4	1	0	0
Means.				
10				200.69 ±22.35
1				7.69 ± 3.85
0				6.99 ± 6.46

Table 11.3. The result of analysis of variance of the amount of HCN released from frond samples in the first series of trials. The fronds were produced by replicate rhizomes under three different nutrient treatments. The HCN was released by the action of toluene on samples of whole tissues from fronds. Complete analysis of variance tables are given in Appendix 6, Tables A6.1 – A6.5.

a. First sample.

Variation between	Variance ratio	Degrees of freedom	Significance
All treatments	12.210	2,40	***
10 and 1	13.146	1,28	**
10 and 0	17.024	1,32	***
1 and 0	0.003	1,20	N.S.

b. Second sample.

Variation between	Variance ratio	Degrees of freedom	Significance
All treatments	48.600	2,45	***
10 and 1	53.693	1,34	***
10 and 0	52.172	1,35	***
1 and 0	0.298	1,21	N.S.

c. Third sample.

Variation between	Variance ratio	Degrees of freedom	Significance
All treatments	19.802	2,56	***
10 and 1	28.833	1,43	***
10 and 0	21.299	1,47	***
1 and 0	0.371	1,22	N.S.

Table 11.3 - continued.

d. Fourth sample.

Variation between	Variance ratio	Degrees of freedom	Significance
All treatments	28.002	2,61	***
10 and 1	24.414	1,46	***
10 and 0	32.485	1,50	***
1 and 0	0.007	1,26	N.S.

e. Between samples.

Variation betweenwithin	Variance ratio	Degrees of freedom	Significance
samples treatment 10	8.366	3,108	***
samples treatment 1	4.063	3,38	*
samples treatment 0	3.280	3,50	*

N.S. not significant; * significant at 5% level ($P<0.05$); ** significant at 1% ($P<0.01$); *** significant at 0.1% ($P<0.001$).

Table 11.4. The means of the amount of HCN released by all fronds of each replicate of each treatment in the second series of trials. Those replicates which produce only one cyanogenic frond have no standard error associated with the mean, since the standard error is equal to the mean in these cases (marked n.a., not applicable). Some fronds died in between the sample dates.

a. First sample.

Treatment	Replicate	Number of fronds	Number of cyanogenic fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
10	1	4	4	301.73 ±91.95
	2	5	3	7.24 ±4.38
	3	4	4	108.18 ±40.30
5	1	4	3	91.14 ±43.69
	2	3	3	33.95 ±32.26
	3	3	3	63.52 ±37.10
2.5	1	1	1	41.30 (n.a.)
	2	2	1	2.67 (n.a.)
	3	3	2	4.58 ±2.35
1	1	1	1	146.92 (n.a.)
	2	1	1	17.36 (n.a.)
	3	3	1	34.10 (n.a.)

Means.

10	128.91 ±45.04
5	65.70 ±21.87
2.5	10.06 ±6.38
1	53.31 ±30.11

Table 11.4 - continued.

b. Second sample.

Treatment	Replicate	Number of fronds	Number of cyanogenic fronds	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
10	1	9	9	143.67 ±11.52
	2	5	5	47.32 ±5.22
	3	5	5	106.69 ±33.69
5	1	4	4	63.14 ±17.66
	2	2	2	21.31 ±5.53
	3	3	3	57.25 ±24.11
2.5	1	2	2	27.18 ±6.07
	2	2	2	17.36 ±1.58
	3	4	4	10.57 ±2.92
1	1	3	2	39.53 ±29.32
	2	3	2	7.79 ±5.52
	3	2	2	3.23 ±0.48
Means.				
10				108.58 ±13.54
5				51.88 ±11.64
2.5				16.42 ±3.14
1				18.55 ±11.55

Figure 11.1. The amount of HCN released by samples of fronds grown under three nitrogen treatments in the first series of nitrogen trials. Four sets of samples were taken (●, first set; ■, second set; ▲, third set; ○, fourth set).

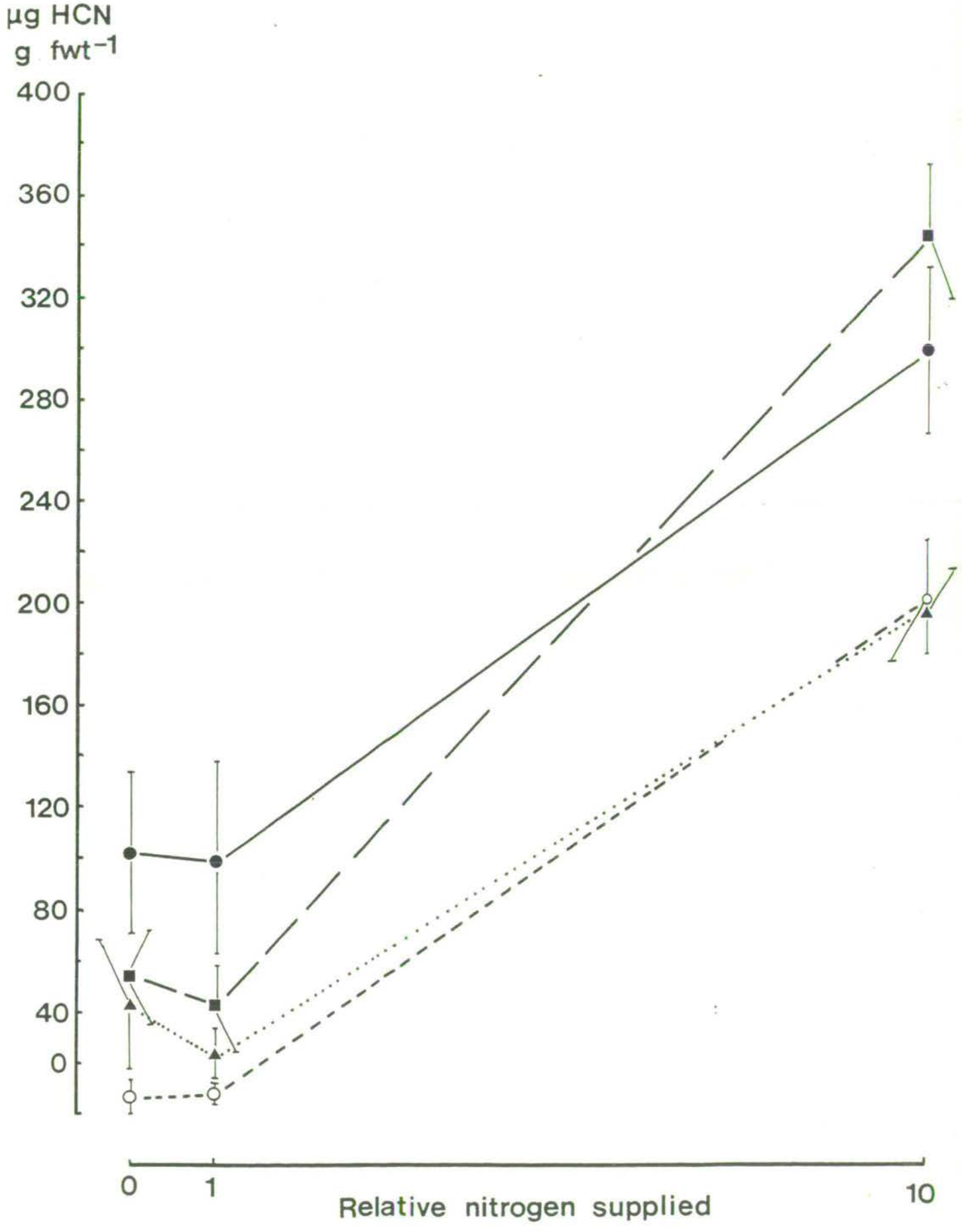


Figure 11.2. The amount of HCN released by samples of fronds grown under three nitrogen treatments in the first series of nitrogen trial, throughout the period of the experiment (▲ , nitrogen-free (0) treatment; ■ , low nitrogen (1) treatment; ● , high nitrogen (10) treatment).

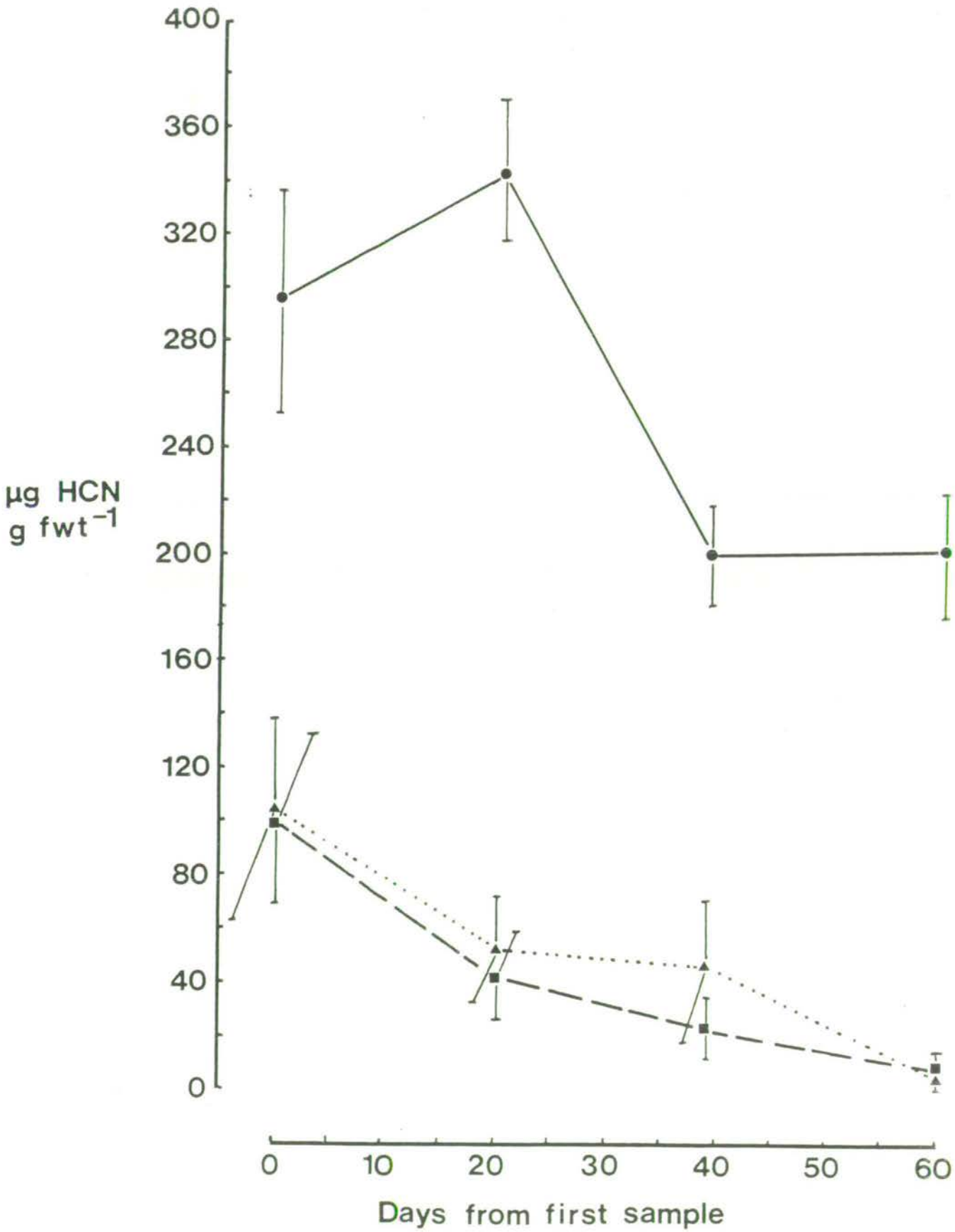
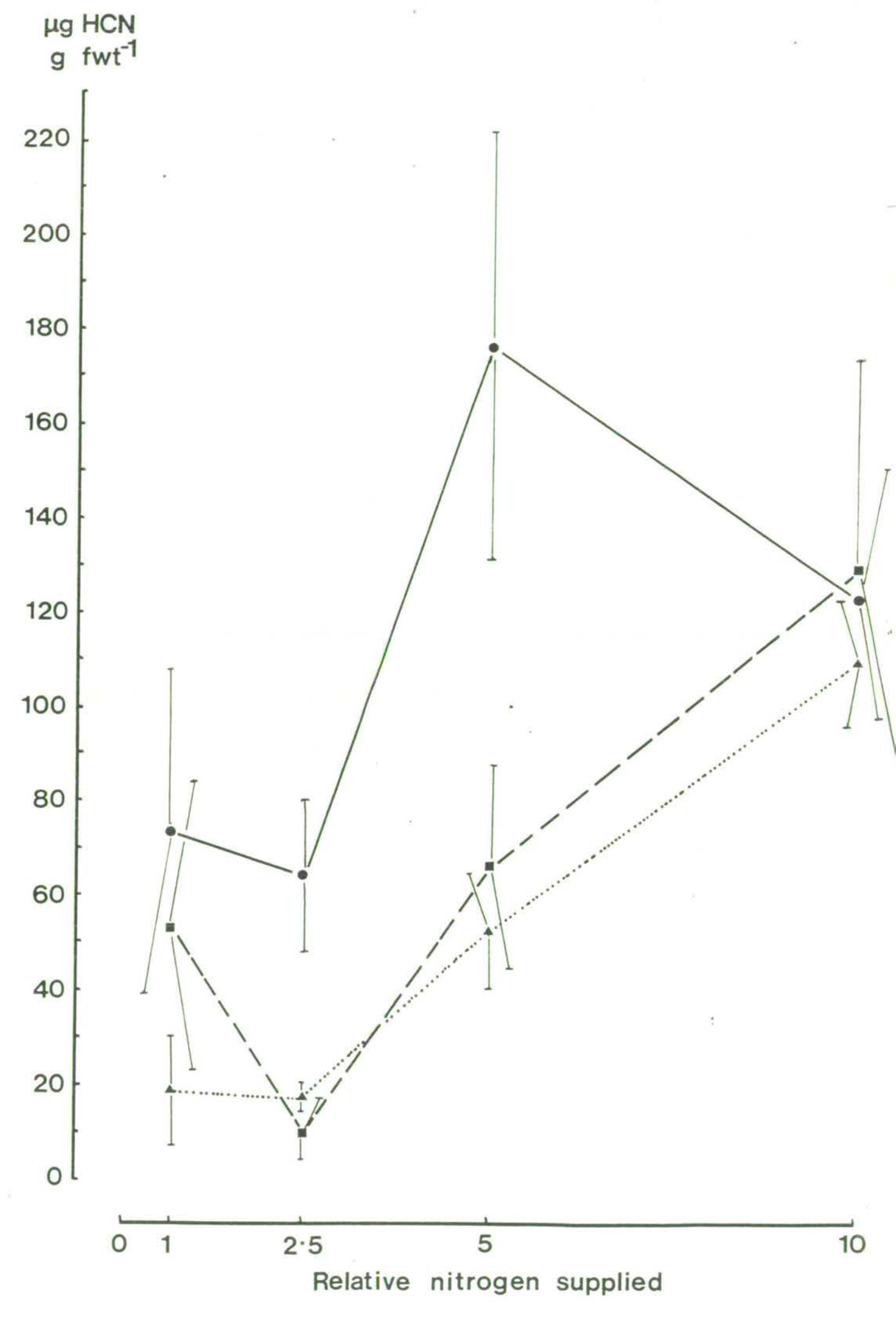


Figure 11.3. The amount of HCN released by samples of fronds grown under four nitrogen treatments in the second series of nitrogen trials. Two sets of samples were taken (■, first set; ▲, second set). In addition, β-glycoside extracts were prepared at the same time as the first set of samples was taken. These were treated with β-glycosidase to release HCN (●).


µg HCN
g fwt^{-1}
220
200
180
160
140
120
100
80
60
40
20
0
0
1
2·5
5
10
Relative nitrogen supplied

The results of the two sets of samples were analysed by analysis of variance. There were no significant treatment effects for the first set of samples, either taking all the treatments together or analysing pairs of treatments (Table 11.5a). In contrast, the second set of samples show highly significant treatment effects (Table 11.5b), both between all treatments taken together (0.1% level; $P<0.001$) and between all pairs of treatments except between the medium nitrogen (5) and lowest nitrogen (1) treatments and between the low nitrogen (2.5) and the lowest nitrogen (1) treatments (Table 11.5b). The two sets of samples were compared by an analysis of variance of each treatment separately, to assess whether there had been a significant decrease in the amount of HCN released from the frond tissues; none of the four treatments showed any significant change (Table 11.5c).

11.2.3. The glycoside extracts.

The amounts of HCN released from the β-glycoside extracts, sampled at the same time as the first set of samples in the second series of trials, were similar to those released from the whole tissues under the action of toluene (Table 11.6; Figure 11.3). The analysis of variance of these results showed no significant effect due to treatments (Table 11.7), a result similar to that of the analysis of variance of the amount of HCN released from the whole tissue frond samples taken at the same time (Table 11.5a).

The amounts of HCN released from the first set of whole tissue frond samples and the β-glycoside extracts were compared by analysis of variance, each treatment analysed separately; of the four treatments, only the low nitrogen (2.5) treatment showed any significant effect (1% level; $P<0.01$) due to the method of release of HCN from fronds (Table 11.8).

There was no evidence that HCN was released from the glycoside extracts taken from the rhizome sections. No HCN was found in any of the NaOH traps used in this part of the experiment.

11.2.4. The physical characteristics of replicate rhizomes and their fronds.

The physical characteristics of the fronds varied both within and between treatments (Table 11.9). The number of fronds produced by each replicate (Table 11.9) was significantly different between treatments (5% level; $P<0.05$. Table 11.11). The highest mean values for frond fresh and dry weight were produced by the high nitrogen (10) treatments (Table 11.9a), but the analysis of variance of the frond fresh weight (Table 11.11) and the frond dry weight (Table 11.11) showed no significant differences due to treatments. The dry weights as

Table 11.5. The results of the analysis of variance of the amounts of HCN released from fronds in the second series of trials. The fronds were produced by replicate rhizomes under four different nutrient treatments. The HCN was released from samples of whole tissue by the action of toluene. Complete analysis of variance tables are given in Appendix 6, Tables A6.6 - A6.8.

a. First sample.

Variation between	Variance ratio	Degrees of freedom	Significance
All treatments	1.740	3,30	N.S.
10 and 5	1.319	1,21	N.S.
10 and 2.5	3.102	1,17	N.S.
10 and 1	0.987	1,16	N.S.
5 and 2.5	3.672	1,14	N.S.
5 and 1	0.109	1,13	N.S.
2.5 and 1	2.372	1,9	N.S.

b. Second sample.

Variation between	Variance ratio	Degrees of freedom	Significance
All treatments	12.219	3,40	***
10 and 5	7.049	1,26	*
10 and 2.5	18.911	1,25	***
10 and 1	16.264	1,25	***
5 and 2.5	7.752	1,15	*
5 and 1	4.095	1,15	N.S.
2.5 and 1	0.032	1,14	N.S.

c. Between samples.

Variation between	within	Variance ratio	Degrees of freedom	Significance
samples	treatment 10	0.252	1,30	N.S.
samples	treatment 5	0.291	1,17	N.S.
samples	treatment 2.5	0.937	1,12	N.S.
samples	treatment 1	1.597	1,11	N.S.

N.S. not significant; * significant at 5% level ($P<0.05$); ** significant at 1% level ($P<0.01$); *** significant at 0.1% level ($P<0.001$).

Table 11.6. The means of the amount of HCN released under the activity of exogenous β-glycosidase from β-glycoside extracts taken from all fronds of each replicate of each of four treatments in the second series of trials. All fronds produced β-glycoside extracts which released measurable quantities of HCN. Those replicates which produced only one frond have no standard error associated with the mean, which is the single value of HCN released in these cases (marked n.a., not applicable).

Treatment	Replicate	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ± S.E.
10	1	151.90 ±62.85
	2	73.72 ±33.05
	3	136.56 ±35.18
5	1	198.65 ±78.98
	2	90.50 ±40.20
	3	146.52 ±31.77
2.5	1	124.79 (n.a.)
	2	65.35 ±16.30
	3	43.66 ±17.21
1	1	102.33 (n.a.)
	2	44.72 (n.a.)
	3	73.28 ±60.73
Means.		
10		123.72 ±26.55
5		176.31 ±45.14
2.5		64.41 ±15.54
1		73.38 ±34.49

Table 11.7. The results of analysis of variance of the amount of HCN released by the action of glycosidase of glycoside extracts. The glycoside extracts were prepared from samples removed from fronds produced by replicate rhizomes grown under four different nutrient-treatments. The frond samples were removed soon after after the first set of samples were taken (c.f. Table 11.4). Complete analysis of variance tables are given in Appendix 6, Table A6.9.

Variation between	Variance ratio	Degrees of freedom	Significance
All treatments	2.443	3,30	N.S.

N.S. not significant.

Table 11.8. The analysis of variance of the amount of HCN released from frond samples by two different methods. HCN was released from whole tissue samples, removed from fronds, by the action of toluene (Table 11.3) and from glycoside extracts, prepared from the same fronds, by the action of exogenous glycosidase (Table 11.5). The fronds were produced by replicate rhizomes grown under four nutrient treatments. Complete analysis of variance tables are given in Appendix 6, Table A6.10.

Variation between	within	Variance ratio	Degrees of freedom	Significance
methods	treatment 10	0.009	1,23	N.S.
methods	treatment 5	4.216	1,18	N.S.
methods	treatment 2.5	10.460	1,10	**
methods	treatment 1	0.192	1,8	N.S.

N.S. not significant; ** significant at 1% level ($P<0.01$).

Table 11.9. The mean physical characteristics of the fronds produced by replicate rhizomes under four different nitrogen treatments. The fresh weight of fronds was measured after excision from the rhizome; the fronds were then placed in an oven at 80°C until there was no further weight loss, and the dry weight was measured. The number of distinct pinnae was recorded, and this value does not include the terminal pinnae of a frond which merge and cannot be conveniently counted. The length of the lamina was measured from the base of first pinnae to the tip of the frond. The length of the stipe was measured from the junction of the frond with the rhizome. The frond was removed at this point and the total length of the frond measured from the junction of the frond stipe with the rhizome and tip of the frond. The measurements were made after the second set of samples was taken, and some fronds had died in the interim.

a. Frond weight.

Treatment	Replicate	Number of fronds	Mean fresh weight g, ±S.E.	Mean dry weight g, ±S.E.	Mean % dry/fresh weight ±S.E.
10	1	9	2.66 ±0.57	0.53 ±0.14	21.16 ±0.88
	2	5	5.42 ±0.82	1.01 ±0.20	21.10 ±2.88
	3	6	3.78 ±1.13	0.87 ±0.39	23.48 ±2.80
5	1	3	3.63 ±0.65	0.77 ±0.19	20.85 ±1.40
	2	2	3.38 ±0.05	0.78 ±0.05	23.27 ±1.08
	3	3	2.90 ±1.70	0.66 ±0.37	23.92 ±1.54
2.5	1	2	2.72 ±0.01	0.57 ±0.02	20.86 ±0.57
	2	2	2.53 ±0.62	0.67 ±0.12	26.05 ±0.65
	3	4	3.23 ±0.43	0.79 ±0.12	24.35 ±0.93
1	1	3	1.37 ±0.18	0.28 ±0.03	20.40 ±0.60
	2	3	1.50 ±0.61	0.36 ±0.15	23.35 ±0.61
	3	2	2.32 ±1.75	0.54 ±0.41	23.10 ±0.49
Means.					
Treatment.					
10		6.67 ±1.20	3.69 ±0.51	0.74 ±0.14	28.47 ±0.51
5		2.67 ±0.33	3.29 ±0.61	0.73 ±0.14	28.35 ±0.60
2.5		2.67 ±0.67	2.89 ±0.25	0.69 ±0.07	29.20 ±0.62
1		2.67 ±0.33	1.66 ±0.42	0.37 ±0.10	28.08 ±0.42

Table 11.9 - continued.

b. Frond length.

Treatment	Replicate	Number of fronds	Mean length of lamina cms ±S.E.	Mean length of stipe cms ±S.E.	Mean total length cms ±S.E.
10	1	9	15.89 ±1.25	5.11 ±0.44	21.00 ±1.50
	2	5	19.9 ±0.80	6.10 ±0.37	26.00 ±0.72
	3	6	19.25 ±2.78	4.00 ±0.61	23.80 ±3.32
5	1	3	17.17 ±1.88	6.17 ±1.42	23.33 ±1.17
	2	2	18.00 ±1.00	4.75 ±0.75	22.75 ±0.25
	3	3	18.67 ±2.60	4.33 ±0.60	23.00 ±3.17
2.5	1	2	19.5 ±3.00	3.25 ±0.25	22.75 ±2.75
	2	2	21.75 ±5.25	4.75 ±0.25	26.75 ±5.5
	3	4	14.25 ±2.93	5.62 ±1.25	19.87 ±3.96
1	1	3	12.17 ±0.17	2.83 ±0.44	15.00 ±0.50
	2	3	14.00 ±2.02	3.38 ±0.69	17.38 ±2.71
	3	2	14.5 ±3.5	4.00 ±1.00	18.5 ±4.5
Means.					
Treatment.					
10			19.61 ±1.28	5.07 ±0.47	23.60 ±1.85
5			17.94 ±1.09	5.08 ±0.92	23.03 ±1.53
2.5			17.43 ±2.55	4.54 ±0.58	23.12 ±4.07
1			13.45 ±1.01	3.40 ±0.71	16.96 ±2.57

Table 11.10. The physical characteristics of replicate rhizomes under four different nitrogen treatments. The number of long shoot rhizome branches was counted, the length of each branch was measured, and the number of short shoot rhizomes were counted. The number of undeveloped frond buds on each short shoot rhizome was counted. The total fresh weight of the rhizomes were measured, and the rhizomes were then dried in an oven at 80°C until there was no further weight loss to obtain the total dry weight of the rhizome system.

a. The number and length of long shoot rhizome branches.

Treatment	Replicate	Number of rhizome branches	Mean length of rhizome branches cm, ±S.E.	Total length of rhizome system cm
10	1	13	13.77 ±1.74	179
	2	23	13.17 ±2.20	303
	3	18	14.89 ±1.81	268
5	1	9	16.11 ±1.54	145
	2	10	15.00 ±4.28	150
	3	15	13.57 ±1.71	204
2.5	1	8	18.62 ±3.89	149
	2	11	13.09 ±2.53	144
	3	7	15.79 ±5.57	111
1	1	5	15.4 ±6.42	77
	2	5	14.4 ±3.98	72
	3	7	22.14 ±7.17	155
Means.				
10		18.00 ±2.89	13.89 ±1.17	250.00 ±36.91
5		11.33 ±1.86	16.66 ±1.48	166.33 ±18.89
2.5		8.67 ±1.20	15.51 ±2.14	134.67 ±11.92
1		5.67 ±0.67	19.65 ±3.67	101.33 ±26.87

Table 11.10 - continued.

b. Short shoot rhizomes and frond buds.

Treatment	Replicate	Number of short shoots	Short shoots per rhizome branch, ±S.E.	Total frond buds	Frond buds per branch ±S.E.	Frond buds per unit rhizome length
10	1	72	5.54 ±0.59	246	18.9 ±2.3	1.37
	2	105	4.56±0.44	422	18.4 ±2.3	1.39
	3	97	5.39 ±0.59	366	20.3 ±2.6	1.37
5	1	47	5.22 ±0.72	184	20.4 ±3.4	1.27
	2	38	3.80 ±0.77	155	15.5 ±3.4	1.03
	3	51	3.40 ±0.43	160	10.6 ±1.4	0.78
2.5	1	36	4.50 ±0.89	126	15.8 ±3.4	0.85
	2	58	5.27 ±1.10	182	16.5 ±3.6	1.26
	3	36	5.14 ±0.55	133	19.0 ±2.8	1.20
1	1	25	5.00 ±0.84	116	23.2 ±4.2	1.51
	2	25	5.00 ±1.34	93	18.6 ±4.6	1.29
	3	46	6.57 ±1.57	171	24.4 ±7.0	1.10
Means.						
Treatment.						
10		91.3±9.9	5.07±0.31	344.7±51.9	19.3±1.4	1.38±0.01
5		45.3±3.8	4.00±0.37	166.3± 8.9	14.9±1.7	1.03±0.14
2.5		43.3±7.3	5.00±0.55	147.0±17.6	16.6±1.9	1.10±0.13
1		32.0±7.0	5.65±0.78	126.7±23.1	22.3±3.3	1.30±0.12

Table 11.10 – continued.

c. Fresh and dry weight of the rhizome system.

Treatment	Replicate	Total fresh weight, g	Fresh weight per unit length g cm^{-1}	Total dry weight, g	Dry weight per unit length g cm^{-1}	% dry weight
10	1	243.31	1.36	55.60	0.31	22.85
	2	314.67	1.04	67.38	0.22	21.41
	3	279.21	1.04	64.07	0.24	22.95
5	1	123.89	0.85	26.78	0.18	21.62
	2	122.60	0.82	25.93	0.17	21.15
	3	113.42	0.56	28.07	0.14	24.75
2.5	1	85.14	0.57	17.27	0.12	20.28
	2	130.63	0.91	24.07	0.17	18.43
	3	91.3	0.822	17.63	0.16	19.31
1	1	55.63	0.72	11.05	0.14	19.86
	2	58.25	0.81	13.60	0.19	23.34
	3	71.41	0.46	16.61	0.11	23.26
Means, ± S.E. Treatment.						
10		279.06±20.60	1.14±0.11	62.35±3.51	0.26±0.03	28.25±0.34
5		119.97± 3.30	0.74±0.09	26.93±0.62	0.17±0.01	28.31±0.77
2.5		102.36±14.25	0.77±0.10	19.66±2.21	0.15±0.02	26.08±0.39
1		61.76± 4.88	0.66±0.10	13.75±1.61	0.15±0.02	28.06±0.80

Table 11.11. The analysis of variance of the physical characteristics of fronds and replicate rhizomes produced under four different nutrient treatments. The different characteristics are given for each replicate rhizome in Tables 11.9 and 11.10. In all cases the variation is between all four nutrient treatments. Complete analysis of variance tables are given in Appendix 6, Table A6.10 - A6.28.

Characteristic	Variance ratio	Degrees of freedom	Significance
Fronds/replicate	7.579	3,8	*
Frond fresh weight	2.364	3,39	N.S.
Frond dry weight	1.369	3,39	N.S.
Frond dry weight/fresh weight	0.503	3,39	N.S.
Frond lamina length	2.896	3,39	*
Rhizome branches/replicate	8.095	3,8	**
Length of rhizome branches	1.430	3,127	N.S.
Total rhizome length/replicate	6.289	3,8	*
Short shoot rhizomes/replicate	12.713	3,8	**
Short shoots/rhizome branches	2.094	3,127	N.S.
Frond buds/replicate	11.120	3,8	**
Frond buds/rhizome branch	2.459	3,127	N.S.
Frond buds/unit rhizome length	2.131	3,8	N.S.
Total rhizome fresh weight	54.933	3,8	***
Rhizome fr. weight/rhizome length	4.508	3,8	*
Total rhizome dry weight	94.286	3,8	***
Rhizome dry weight/rhizome length	6.370	3,8	*
Rhizome dry weight/fresh weight	3.030	3,8	N.S.

N.S. not significant; * significant at 5% level ($P<0.05$); ** significant at 1% level ($P<0.01$); *** significant at 0.1% level ($P<0.001$).

a percentage of fresh weights of the frond samples were similar (Table 11.9a), and there were no significant differences between treatments (Table 11.11). The frond dimensions were also very variable, the high nitrogen (10) treatment producing high mean values, but not necessarily the highest (Table 11.9b). The analysis of variance of the frond lamina length showed that the differences were significant at 5% ($P<0.05$; Table 11.11).

The physical characteristics of the rhizomes produced by each replicate under the four nitrogen treatments were, in general, similar between treatments (Table 11.10). The number of rhizome branches produced by each replicate was significantly different between treatments (1% level; $P<0.01$. Table 11.11), with the high (10) nitrogen treatment producing most branches (Table 11.10a). The mean length of rhizome branches was similar for each treatment (Table 11.10a), and there were no significant differences due to treatments (Table 11.11). The total length of the rhizome system produced by each replicate under the four nitrogen treatments differed substantially, with the high (10) treatment producing the greatest total rhizome length (Table 11.10a), and there was a significant effect due to the treatments (5% level; $P<0.05$. Table 11.11). The number of short shoot rhizomes produced by each replicate in the four treatments differed considerably between treatments with the high (10) treatment having the highest numbers (Table 11.10b), and the differences were significant (1% level; $P<0.01$. Table 11.11). However, the number of short shoots produced per long shoot rhizome branch for each replicate were similar (Table 11.10b), with no significant differences between treatments (Table 11.11). Similarly, the total number of frond buds produced by each replicate differed considerably between the treatments, the high (10) nitrogen treatment producing most (Table 11.10b), and the differences were significant (1% level; $P<0.01$. Table 11.11). However, the number of frond buds produced per long shoot rhizome branch differed little between treatments (Table 11.10b), with no significant differences (Table 11.11). The number of frond buds produced per unit length of rhizome is also similar between treatments (Table 11.10b), the differences found not being statistically significant (Table 11.11).

The total fresh weight of rhizomes produced under the four nitrogen treatments differed considerably between treatments, the high (10) nitrogen treatment producing the greatest fresh weights as might be expected (Table 11.10c), and these differences are highly significant (0.1% level; $P<0.001$. Table 11.11). The fresh weight of rhizomes per unit length showed smaller differences between treatments, but the dry weight of rhizomes under the high (10) nitrogen treatment was greater (Table 11.10c), and the differences were

significant (5% level; $P<0.05$. Table 11.11). The pattern is similar for the dry weight of rhizomes, both for the total dry weight (Table 11.10c), which showed highly significant differences due to treatments (0.1% level; $P<0.001$. Table 11.11), and the dry weight per unit length of rhizome (Table 11.10c), which also showed significant differences between treatments (5% level; $P<0.05$. Table 11.11). There were however only small differences between the percentage dry weight of the rhizomes under the four different nitrogen treatments (Table 11.10c), which were not significant (Table 11.11).

11.3. Discussion.

11.3.1. The effect of nitrogen availability of HCN released from fronds.

The results presented here show a distinct, but not a proportional, effect of differential nitrogen treatment in both series of trials, the effect being evident in the second series both with respect to cyanogenesis and to other developmental characteristics. This is the response expected given the theoretical evidence presented in chapter 4 for the putative interaction between carbon and nitrogen nutrition.

The first series of trials, involving four consecutive samples taken from fronds, showed a significant difference in the amount of HCN released from fronds, as $\mu g\ g^{-1}$ fresh weight, between treatments (Table 11.3), but no difference between the low nitrogen (1) and nitrogen-free (0) treatments. For each sample, therefore, the significant result is due to the high levels of HCN released by replicates under the high nitrogen (10) treatment. It is therefore possible that both the low nitrogen (1) and the nitrogen-free (0) treatments are similarly stressed with regard to the amount of available nitrogen. The relative amounts of HCN released from fronds do not reflect the differential nitrogen nutrition received by each treatment, which suggests that the partitioning of nitrogen into the cyanogenic glycoside and glycosidase is not in proportion to the available nitrogen. This might be expected since the increase in other nitrogenous compounds, including proteins, is likely to be of foremost importance.

The first series of trials showed a decrease in the amount of HCN released by fronds grown under all three nitrogen treatments (Table 11.2), whilst the significance of the difference between treatments was increased (Table 11.3). The decrease in the amount of HCN released from fronds over the course of the experiment was significant for each of the nitrogen treatments (0.1% level, $P<0.001$, for the high nitrogen (10) treatment; 5% level, $P<0.05$, for both the

low nitrogen (1) and nitrogen-free (0) treatments. Table 11.3e). The decrease in the amount of HCN released is likely to be due to two factors: firstly, the plants were actively growing during the period of the experiment so that there was an increase in the amount of living material in each replicate without an increase in the amount of nitrogen supplied to each replicate; and secondly, each individual frond was aging over the course of the experiment, with the result that there would be a decline of the amount of HCN released by each frond as has been found in the field (chapter 5; Cooper-Driver et al, 1977). It is not possible to separate that part of the decline in cyanogenesis which is attributable to each of these factors.

There is no evidence from the results presented here that repeated sampling of the same fronds has induced increased levels of HCN in either the fronds sampled or fronds subsequently emergent from the same rhizome system, as has been reported for other secondary chemicals in certain plant species (Green and Ryan, 1972; Edwards and Wratten, 1982; Fowler and Lawton, 1985). It is possible that other unidentified biochemical defence mechanisms have been induced by repeated frond sampling and that these have affected the decline noted in the level of HCN released from fronds.

Unlike the first series of trials, the second series, involving four different levels of nitrogen application, showed no significant differences between any treatments at the time of the first sample (Tables 11.4a and 11.5a). All replicates and treatments had large standard errors associated with the mean amount of HCN released from their fronds, showing the large degree of variation found within treatments and within single replicate rhizome systems. Similarly large standard errors were associated with the mean amounts of HCN released from fronds under differential shading treatments (chapter 10). The second set of samples taken released lower levels of HCN (Table 11.4b), but they also have lower standard errors, and there is a highly significant difference between treatments (Table 11.5b). As with the first series of trials, fronds produced by replicate rhizomes under the two lowest nitrogen treatments, 2.5 and 1, release very similar levels of HCN from frond samples; indeed, although the two series of trials cannot be directly compared due to developmental differences, it is interesting to note that the 1 and 0 treatments in the first series and the 2.5 and 1 treatments in the second series have similar mean amounts of HCN released from their fronds. It is not clear why the second set of frond samples taken in the second series of trials should show such little variation compared to the first set of samples. There was no such decrease in the variation found within replicates of the first series of trials.

The mean amounts of HCN released from fronds in the high (10) and medium (5) nitrogen treatments for both sets of samples in the second series of trials are approximately proportional to the level of nitrogen applied to the rhizomes, despite there being no significant difference for the first set of samples. Although no importance can be attributed to this observation, it is possible that these two treatments are showing a direct and proportional response to the amount of nitrogen applied. If this is the case, it would suggest that there is a minimum level of nitrogen, above which additional nitrogen, presumably as amino acids in general and phenylalanine in particular, is channelled to synthesise prunasin and β-glycosidase. This would also explain the similarities between the amounts of HCN released by the 1 and 0 treatments in the first series of trials, and the 2.5 and 1 treatments in the second series, all of which may represent a base level of synthesis of the precursors of HCN in bracken. Although this is an attractive hypothesis, it cannot account for the occurrence of acyanogenic fronds by some replicates of all treatments in both series of trials (Tables 11.2 and 11.4), and it is difficult to assess how important it is.

11.3.2. The effect of nitrogen nutrition on HCN released from glycoside extracts.

All the β-glycoside extracts, taken from fronds of the second series of trials at the same time as the first set of whole tissue samples, released HCN when treated with exogenous β-glycosidase (Table 11.6), showing that although some whole frond samples may not release HCN, they do contain prunasin. It is possible therefore that the acyanogenic fronds, as shown by the effect of toluene on whole tissue samples, lack active, or sufficient quantities of, β-glycosidase. Within each treatment, the amount of HCN released from the whole frond samples was similar to that released from the β-glycoside extracts: thus, in both, the replicates within each nitrogen treatment were ordered similarly according to the amount of HCN released. For instance, fronds of replicate 2 in the high nitrogen (10) treatment released the lowest amounts of HCN of the three replicates in the treatment in both tests, although the amount of HCN released from the β-glycoside extract was greater, in this particular case, than that released from the whole tissue samples. As with the first series of whole tissue samples, the β-glycoside extracts showed no effect of treatment on the amount of HCN released (Table 11.7).

The observation that fronds from each nitrogen treatment released far more HCN when the β-glycoside extracts were used than when the whole tissue samples were used is an indication that there was incomplete hydrolysis

of the β-glycoside within the whole tissue samples. However, there was no statistical significant difference between the two methods of HCN release except for the low nitrogen (2.5) treatment, which showed a significant difference (1% level, $P<0.01$; Table 11.8). The results from the β-glycoside extracts are similar for all four treatments (Table 11.6). This suggests that the differences found with the whole tissue samples are due to differential β-glycosidase activity, even though the differences between the whole tissue samples are not significant. It might have been possible to test this hypothesis at the time the second sample was taken, by preparing β-glycoside extracts from the fronds, since there were significant differences between the amounts of HCN released from whole tissue samples at that time. If the amounts of HCN released from the β-glycoside extracts had remained at similar levels between the samples being taken, it would have demonstrated that there was differential β-glycosidase activity. Alternatively, had it been possible to use β-glycosidase enzyme extracts, these would have settled this question. Unfortunately, neither of these was practical, and it is not known whether the differences found between nitrogen treatments in the first series of trials, and in the second set of samples taken of the second series of trials, are due to differential β-glycoside levels, β-glycosidase activity, or both. It would not be surprising if the differential release of HCN from whole tissue samples under different nitrogen treatments was due to differential β-glycosidase activity, since the additional protein synthesis required for the production of β-glycosidase is likely to represent more of a drain on available resources, particularly nitrogen (as amino acids), than is the production of prunasin.

11.3.3. Variation within treatments.

As has been noted for the field surveys and the shading experiment, there is again substantial variation of cyanogenesis within replicates in both series of trials, notably with replicates producing both acyanogenic and highly cyanogenic fronds. Although this observation adds support to those already reported, it is difficult to explain, particularly since the high nitrogen (10) treatment should have supplied excess nitrogen and nitrogen should therefore have been readily available for the production of cyanogenic compounds.

11.3.4. The effect of nitrogen availability on the physical characteristics of bracken.

It is generally considered that bracken characteristically grows on nutrient-poor soils, although this is likely to reflect land use rather than the ecology of the plant (Fletcher and Kirkwood, 1979). Schwabe (1953) investigated the effect of phosphorus and potassium supply on the physiology of bracken, and he showed that deficiency of both mineral elements can have severe effects on the plant. However, there has apparently been no investigation into the effect of differential nitrogen nutrition on bracken prior to the study reported here.

The physical characteristics of the replicate rhizome systems involved in the second series of trials indicate the effect that differential nitrogen nutrition can have upon clonal material. This is particularly important if developmental effects on cyanogenesis are to be considered. As might be expected, there was a significant effect of nitrogen nutrition on the number of fronds produced by the rhizome systems (Table 11.11). However, only the high nitrogen (10) treatment produced more than a mean of 2.67 fronds per replicate (Table 11.9a). The fronds produced showed no differences between treatments with respect to the fresh weight (Table 11.11), dry weight (Table 11.11), or the percentage dry weight of frond material produced (Table 11.11). Thus the rhizomes have responded to the nitrogen treatment by producing more fronds, each containing approximately the same amount of tissue, rather than by investing in larger fronds with a greater leaf area and hence a higher photosynthetic capacity. It should however be noted that the laminae of fronds produced by the lowest nitrogen (1) treatment were significantly shorter than those from the other three nitrogen treatments (5% level; $P<0.05$. Table 11.11), despite the similarity of the weight of frond material in all treatments. The fronds produced under the nitrogen treatments were very different in stature to fronds produced in the wild. However, mean percentage dry weight is very similar to the value of 23.23% $\pm$1.99 given for several sites throughout Great Britain (from data in Callaghan et al, 1981).

The number of long shoot rhizome branches per replicate increased with nitrogen treatment (Table 11.10a), and differed significantly between treatments (Table 11.11), as did the total length of the rhizome system (Table 11.11). There was no significant difference in the length of the rhizome branches (Table 11.11). Thus there is no intrinsic developmental differences between the rhizome systems under the four different nitrogen treatments. This is also

shown by the number of short shoot rhizomes produced by each replicate, which increased with nitrogen treatment (Table 11.10b). Although there is a significant difference in the total number of short shoots produced by each replicate under the four different nitrogen treatments (Table 11.10b), there is no difference in the number of short shoots produced per long shoot rhizome branch (Table 11.11); thus the difference between treatments in the number of short shoots simply reflects the difference in the total length of the rhizome system, rather than a difference in the production of short shoots on the rhizomes. The length of the rhizome system produced is similar to those studied by other workers. Braid and Conway (1943) and Conway (1949) recorded very rapid rates of growth in juvenile plants raised from spore, of the order of 2m in seven months and 1m in 4 months, respectively. Watt (1940) records annual growth of rhizomes in the field of up to 1m, whilst Oinonen (1967a) recorded growth of 17.9 cm per rhizome apex per year in the wild.

The number of dormant frond buds is very large in all cases (Table 11.10b), considering the length of the rhizome system and the area which had been "invaded" by each rhizome system. The total number of dormant frond buds was greater under high nitrogen and showed a significant difference due to treatment (Table 11.11), but neither the number of frond buds per long shoot rhizome branch (Table 11.11) nor the number of frond buds produced per unit length of the total rhizome system (Table 11.11) showed a significant difference. Again, it therefore seems that the difference in the number of frond buds is accounted for by the difference in the total rhizome length, rather than a difference in the production of dormant frond buds on the rhizomes. The large number of dormant frond buds found in the replicates is similar to that found for the plant in the wild by Watt (1950) in Britain, and Webster and Steeves (1958) in the U.S.A.

The total fresh weight of rhizomes was greater under high nitrogen treatment (Table 11.10c) and showed a highly significant effect of treatment (Table 11.11). There was also a significant effect of treatment on the fresh weight of rhizome per unit length of rhizome (Table 11.11). This shows that the level of nitrogen nutrition had a direct effect on the development of the rhizome, and that the effect of increased nitrogen treatment on the total fresh weight was not due entirely to the increased length of the rhizomes. As would be expected, the total dry weight of the rhizomes (Table 11.10c) also showed a highly significant effect due to the nitrogen treatments (Table 11.11), with the effect on dry weight per unit rhizome length being significant as well (Table 11.11). These results follow those for the fresh weight of rhizomes; no

difference is found between the percentage dry weight of the rhizomes due the level of nitrogen treatment (Table 11.11). It is difficult to compare the measurements taken of rhizomes with those made by other workers, because most previous work has been carried out on the total below ground biomass, giving results in kg m^{-2}. In addition, the size of the rhizomes grown in trays differed greatly from those found in the field and it would seem likely that growth and development of the rhizome system was limited by the trays.

Several of the significant results from the analyses of the physical characteristics of the replicate rhizomes grown under the four nitrogen treatments show a similar pattern. The characteristics of the fronds and rhizomes produced by the high nitrogen (10) treatment tend to differ from those produced by other treatments more than the other treatments do from each other. This can be seen from the means of values given in Table 11.10. Indeed, the only significant results regarding the physical characteristic of the rhizome systems which do not show this pattern are frond lamina lengths (Table 11.11) and the number of long shoot rhizome branches produced by each rhizome system (Table 11.11). The importance of this is difficult to assess, but it would appear that the difference between the high nitrogen (10) and medium nitrogen (5) treatments is greater than the difference between the medium nitrogen (5) and the lowest nitrogen (1) treatments. This could indicate that the nitrogen supplied to the medium nitrogen (5) treatment is below the optimum level.

11.3.5. Nitrogen availability and cyanogenesis.

There have been few studies on the influence of mineral nutrition on cyanogenic plants, possibly because many of the economically important cyanogenic plant species are leguminous and have the ability to fix nitrogen through their symbiosis with Rhizobium bacteria; indeed, these species, in particular Lotus corniculatus and Trifolium repens, have been studied because of their importance as forage plants and their ability to increase the nitrogen available within a system. Boyd et al (1938) reported that cyanogenesis in sudan grass (Sorghum sudanense) is closely related to the level of nitrogen in the soil, the level of HCN released by Sorghum plants increasing after the addition of nitrogen fertilisers to the soil. Other soil nutrients were shown to be unimportant. Although cassava (Manihot esculenta) is commonly tolerant of poor soil conditions (Cooke and Coursey, 1981), Nartey (1981) states that the soil has an important effect in determining cyanogenesis in cassava. Tapper and Reay (1973) and Seigler (1976) both stress the importance of soil nutrients

in determining the level of HCN found in cyanogenic plants.

The relationship found between nitrogen nutrition and cyanogenesis in bracken is interesting since no such relationship was found between the level of total soil nitrogen and cyanogenesis in fieldwork (chapter 4). This may be due to other environmental effects altering cyanogenesis in uncontrolled field conditions. It would be profitable to study the effect of the addition of several different levels of nitrogen fertiliser to field plots, to ascertain whether the pattern was repeated under field conditions. This would allow a degree of control which is lacking in natural sites.

11.4. Conclusions.

The level of nitrogen applied to replicate rhizomes of Pteridium aquilinum had an effect on the amount of HCN released from fronds, but this effect was not in proportion to the amount of nitrogen applied. The level of nitrogen supplied apparently had no effect on the amount of HCN released from β-glycoside extracts treated with exogenous β-glycosidase, fronds from all nitrogen treatments releasing similar amounts of HCN. Presumably differences in the amount of HCN released from whole tissue samples are due to differential synthesis of β-glycosidase.

The level of nitrogen treatment had several effects on the growth of the rhizome system, notably the length and number of long shoot rhizomes. Several other characteristics reflected this effect, such as the number of dormant frond buds and the fresh and dry weight of the rhizome system.

Chapter 12. Cyanogenesis in gametophytes and young sporophytes of **Pteridium aquilinum**.

Unlike most other plant species in which cyanogenesis has been studied in detail, bracken is a pteridophyte; it possesses the pteridophyte life cycle which differs from the spermatophyte life cycle (Figure 1.1). The main difference is that the haploid gametophytes of pteridophyte species are free-living, whilst those of spermatophytes are wholly dependent on the sporophyte generation, and are enclosed in the mega- and microsporangia, the ovules and pollen grains respectively (Sporne, 1975). The ecology of bracken is limited by the ecology of the haploid gametophyte, which represents the establishment phase of the plant, although once colonisation has occurred there can be extensive vegetative spread by the sporophyte rhizome system. In comparison, the establishment of spermatophytes is determined by the seed, which is part of the sporophyte generation.

The gametophytes produce gametes and fertilisation occurs, either with gametes from the same gametophyte (intragametophytic selfing, resulting in a fully homozygous zygote), with gametes from different gametophytes originating from the same sporophyte plant (intergametophytic selfing, analagous to self-fertilisation in spermatophytes), or with gametes from unrelated sporophytes (intergametophytic crossing, analagous to cross-fertilisation in spermatophytes) (Klekowski, 1979). Gametes produced by bracken gametophytes are believed to be self-compatible (Klekowski, 1972b), although there is some evidence for the possession of a weak incompatibility system (Wilkie, 1955). Gametophytes can grow for long periods if fertilisation of the archegonia does not occur, often with the edge of the gametophyte fragmenting, each segment attaining independence (Steeves et al, 1955). This abnormal behaviour of unfertilised gametophytes can be imitated by surgical excision of gametophyte fragments from the main body of the gametophyte, or can be induced by plasmolysing gametophyte tissues (Miller, 1968). In this way, clonal material of gametophytes can be prepared (chapter 2). The gametophytes therefore represent a powerful research tool, since it is possible to obtain haploid clonal, and fully homozygous diploid, experimental material. This allows comparisons to be made between naturally occurring populations of fronds and the gametophytes and juvenile sporophytes produced by spores taken from fronds of those populations.

Establishment of bracken by spore in the field is apparently rare in Britain

(Conway, 1949, 1953; Page, 1976) and Finland (Oinonen, 1967a), although common in New Zealand (Knowles, 1970) and in Costa Rica (Gliessman, 1978). It is possible that selection for polymorphic traits in plants occurs at the time of establishment, since it is at this point in the life cycle of a plant that the plant is most vulnerable (Harper, 1977; Ennos, 1981b). Cyanogenesis at this stage in the life cycle could greatly affect the fitness and survival of the plant, since damage to the tissues of the gametophyte or the juvenile sporophyte would greatly alter the likelihood of successful establishment of the adult sporophyte. If this is the case, it is possible that cyanogenesis in the adult sporophyte plant is a vestige of the gametophyte and/or the juvenile sporophyte being cyanogenic.

Because gametophytes and juvenile sporophytes are so rarely encountered in the field in Britain, it is not possible to investigate the effect of cyanogenesis on the ecology of bracken at the establishment phase. However, the ease of culture of clonal material allows laboratory studies into the biology of cyanogenesis at these stages.

12.1. Methods.

Spores were collected from fronds previously tested for cyanogenesis at the coastal and open sites at location five (chapter 3) in the late summer of 1984. Not all the sampled fronds at these sites produced spores, and no fertile fronds were found at the woodland site at location five in 1984. Only spores from acyanogenic fronds were cultured and tested for cyanogenesis. The spores were washed and sown on nutrient agar medium (chapter 2).

Populations of gametophytes were sampled for cyanogenesis by placing individual gametophytes from the populations in Thunberg tubes and placing toluene in each tube; the HCN released from each gametophyte was collected in 0.5ml of 0.1M NaOH and analysed by the pyridine - pyrazolone test (chapter 2). The amount of NaOH used as an HCN trap was small because of the low levels of HCN likely to be released from the small quantities of tissue available.

Some gametophytes were selected at random from the populations and plasmolysed (chapter 2). Each gametophyte was then placed on nutrient agar in an individual well of a multiwell petri dish. The outgrowths of gametophytic tissue were removed when they were large enough to handle, and they were similarly plated out individually. The cloned gametophytes were grown on until they reached sufficient size to be tested for cyanogenesis; gametophytes weighing less than 2mg were not tested for cyanogenesis since they were unlikely to release sufficient HCN for it to be detected by the pyridine -

pyrazolone test.

Populations of gametophytes produced sporophytes if they were left sufficiently long enough; in general the first sporophyte fronds were produced within two months of the germination of the spores. It is presumed that sporophytes arising from gametophytes grown as a population were the result of intergametophytic selfing. Sporophytes produced from populations of gametophytes were not grown on outside the petri dishes, due to their large numbers. The fronds of juvenile sporophytes were weighed and tested for cyanogenesis using the pyridine - pyrazolone method (chapter 2).

Sporophytes were also produced from cloned gametophytes grown in isolation. It is assumed that such sporophytes were the result of intragametophytic selfing, since they were produced only after the gametophyte had been cloned by plasmolysis, and therefore the antherozoids which fertilised the archegonia must have come from the same gametophyte or its clonal stock. These sporophytes were removed from the petri dishes and were grown up in nutrient agar in 50 X 20mm glass tubes stoppered by corks (chapter 2). This allowed free development of the juvenile sporophytes. Each of the fronds produced by the growing sporophytes was weighed and then sampled for HCN, using the pyridine - pyrazolone method (chapter 2).

12.2. Results.

12.2.1. Populations of gametophytes.

All populations of gametophytes produced material which released HCN which was measurable using the pyridine - pyrazolone method, despite the spores being derived from fronds which were shown to be acyanogenic, according to the picrate test, in the field. Gametophytes grown in populations from spores from the same frond were either cyanogenic or acyanogenic, apparently independently of the frond from which spores were originally taken or of the phenotype of other gametophytes from the population: most population samples contained acyanogenic gametophytes (Table 12.1). The mean amount of HCN released from gametophytes was variable within a population sample, indicated by the large standard errors associated with the means. The amount of HCN released from populations of gametophytes raised from spores taken from fronds at the Blackhill fieldsite were compared by analysis of variance (Table 12.2), which showed that gametophytes raised from spores taken from different fronds in the field differed significantly (5% level, $P<0.05$). However, the two populations raised from spores taken from two

Table 12.1. The mean amount of HCN, µg g^{-1} fresh weight, released from gametophytes sampled from populations raised from spores. The spores were taken from individual fronds which were tested for cyanogenesis and shown to be acyanogenic in the field by the picrate test. The populations were identified by the grid position of the frond from which spores were removed (BH, Blackhill; Gos, Gosford Bay). All the spores were sown on the same day, and the gametophytes were sampled at the same time. The gametophytes were weighed after excess agar and surface moisture were removed. The gametophytes were placed in a Thunberg tube and the HCN released collected in NaOH. The HCN was estimated by the pyridine - pyrazolone method.

Population	Number of gametophytes sampled	Number of cyanogenic gametophytes in population	Mean gametophyte weight, mg ±S.E.	Mean amount of HCN, µg g^{-1}, ±S.E.
BH E9	15	11	15.07 ±3.09	21.24 ±7.17
BH D10	10	7	10.40 ±2.23	12.13 ±3.16
BH C9	15	6	10.19 ±1.33	3.35 ±1.03
BH B10	4	4	11.75 ±3.20	7.36 ±2.00
BH G3	6	3	6.67 ±1.65	2.20 ±1.05
BH F7	9	9	23.78 ±4.67	16.34 ±5.04
Gos A5	3	2	13.00 ±8.50	15.07 ±9.75
Gos E1	5	4	20.40 ±1.60	2.07 ±0.90

Table 12.2. The results of analysis of variance of the amount of HCN released from gametophytes grown in populations. Complete analysis of variance tables are given in Appendix 7, Tables A7.1 - A7.3.

Variation between	Variance ratio	Degrees of freedom	Significance
Blackhill populations	2.467	5,53	*
Gosford populations	3.236	1,6	N.S.
Blackhill and Gosford	0.534	1,65	N.S.

N.S., not significant; *, significant at 5% level ($P<0.05$).

fronds at Gosford Bay did not differ significantly (Table 12.2). The populations of gametophytes from the two habitat sites were compared by analysis of variance (Table 12.2), which showed no significant difference between the gametophytes raised from spores taken from the two sites.

12.2.2. Clonal gametophytes.

On the whole, the clonal gametophytes produced greater amounts of HCN than the gametophytes taken from populations (Table 12.3). There was only one clonal gametophyte which released no measurable HCN (Gos A5/2). Several gametophytes released small amounts of HCN, compared with other members of the same clone, and the standard errors associated with the mean amounts for each clone are large in several cases (Table 12.3). The amount of HCN released from clonal gametophytes grown from spores taken from fronds at the Blackhill fieldsite were compared together by analysis of variance (Table 12.4), which showed that there were highly significant differences between different clones (0.1% level; $P<0.001$). The clones raised from spores taken from the Gosford Bay fieldsite also released significantly different amounts of HCN (1% level, $P<0.01$; Table 12.4). Unlike the gametophytes grown in populations, the clonal gametophytes showed significant differences between the amounts of HCN released due to the habitat site from which the spores were collected (1% level, $P<0.01$; Table 12.4).

12.2.3. Sporophytes raised from populations of gametophytes.

The populations of juvenile sporophytes from which fronds were sampled were variable (Table 12.5), several juvenile sporophytes producing fronds which released no measurable HCN. All but one of the juvenile sporophytes produced at least one cyanogenic frond, the exception being BH H9 3. The amounts of HCN released from the fronds of juvenile sporophytes were highly variable. These results were compared by analysis of variance, which showed that the amount of HCN released from fronds differed significantly between different populations of juvenile sporophytes (0.1% level, $P<0.001$; Table 12.6).

12.2.4. Sporophytes raised from individual clonal gametophytes.

The juvenile sporophytes raised from clonal gametophytes released greater quantities of HCN than the sporophytes raised from populations of gametophytes (Table 12.7). However, some fronds were produced which did not release any measurable HCN, the mean amounts of HCN also being highly variable. The amounts of HCN released by the different sporophytes were

Table 12.3. The mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, released from members of clones of gametophytes. Each clone is identified by the site (BH, Blackhill; Gos, Gosford Bay) and grid position of the frond from which the spore from which it was grown was taken, and a clone number. All the spores were sown at the same time, and each gametophyte was cloned and subcloned at the same time. The gametophytes were weighed after being cleaned of agar and the removal of excess moisture. The HCN was released from each gametophyte by the addition of toluene to the gametophyte placed in a Thunberg tube, and the amount of HCN released was measured by the pyridine - pyrazolone method.

Clone	Number of gametophytes sampled	Number of cyanogenic gametophytes	Mean gametophyte weight, mg ±S.E.	Mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, ±S.E.
BH B8/1	4	4	26.25 ±4.05	17.84 ±4.51
BH B8/2	4	4	21.00 ±1.41	35.83 ±3.87
BH B8/3	4	4	33.50 ±7.80	26.14 ±8.66
BH B10/1	4	4	19.25 ±4.38	29.81 ±5.03
BH B10/2	6	6	17.67 ±4.65	32.77 ±8.72
BH B10/3	2	2	52.50 ±2.50	25.23 ±0.23
BH B10/4	2	2	52.00 ±4.00	32.65 ±3.58
BH D10/1	3	3	7.67 ±4.17	9.13 ±4.36
BH D10/2	4	4	30.25 ±5.51	22.18 ±4.71
BH D10/3	9	9	39.78 ±4.01	5.97 ±1.11
BH E9/2	3	3	41.67 ±10.71	18.47 ±2.45
BH E9/3	5	5	28.60 ±5.12	15.82 ±2.52
BH E9/5	5	5	25.40 ±7.02	16.47 ±7.64
BH E9/6	3	3	25.33 ±14.0	17.06 ±0.87
BH F7/1	3	3	8.00 ±5.01	13.47 ±7.14
BH F7/2	2	2	22.00 ±4.00	21.32 ±16.82
BH F7/3	3	3	4.67 ±2.19	6.01 ±1.87
BH F7/4	4	4	7.25 ±1.03	50.99 ±17.80
BH F7/5	5	5	14.40 ±2.38	17.04 ±6.93
BH G3/1	3	3	19.33 ±5.90	65.71 ±6.83
BH G3/2	2	2	14.00 ±2.00	2.83 ±1.58
Gos A5/1	5	5	14.00 ±3.46	22.11 ±8.86
Gos A5/2	7	6	14.86 ±4.47	8.78 ±3.21
Gos A5/3	4	4	22.50 ±9.74	50.99 ±17.81
Gos E1/1	4	4	8.75 ±2.78	21.16 ±2.70
Gos E1/2	2	2	16.00 ±0.00	15.84 ±4.15
Gos E1/3	3	3	53.00 ±11.93	38.59 ±8.80
Gos E1/4	2	2	31.50 ±1.50	55.74 ±1.13
Gos E1/5	2	2	87.00 ±17.00	22.68 ±4.43
Gos E1/6	2	2	51.00 ±7.00	38.43 ±5.71
Gos E1/7	2	2	77.50 ±3.50	24.54 ±1.11
Gos E1/8	7	7	17.71 ±5.88	96.68 ±22.47

Table 12.4. The results of analysis of variance of the amount of HCN released from clonal gametophytes. Complete analysis of variance tables are given in Appendix 7, Tables A7.4 - A7.6.

Variation between	Variance ratio	Degrees of freedom	Significance
Blackhill clones	4.800	20,59	***
Gosford clones	4.131	10,29	**
Blackhill/Gosford clones	9.322	1,118	**

, significant at 1% level ($P<0.01$); *, significant at 0.1% level ($P<0.001$).

Table 12.5. The mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, released from fronds of juvenile sporophytes raised from gametophytes grown in populations. The sporophytes are identified by the site (BH, Blackhill; Gos, Gosford Bay) and grid position of the frond in the field from which the spores were taken and a number. The fronds were weighed prior to their being placed in a Thunberg tube and being treated with toluene to release the HCN, which was analysed by the pyridine - pyrazolone method.

Sporophyte population	Number of fronds sampled	Number of cyanogenic fronds	Mean frond weight, mg ±S.E.	Mean amount of HCN, $\mu g\ g^{-1}$, ±S.E.
BH B8 1	2	2	4.25 ±0.25	10.58 ±1.91
2	2	2	2.00 ±0.00	8.50 ±5.50
3	2	2	2.75 ±0.25	4.64 ±2.64
4	2	2	4.75 ±2.25	3.82 ±1.42
BH B10 1	1	1	1.00 (n.a)	56.00 (n.a.)
2	1	1	1.00 (n.a.)	57.50 (n.a.)
BH C9 1	4	2	3.63 ±0.69	15.65 ±10.19
2	4	2	3.87 ±0.51	8.97 ±5.69
3	2	1	3.50 ±0.50	2.70 (n.a.)
BH D10 1	2	1	6.00 ±2.00	1.59 (n.a.)
2	2	2	3.50 ±0.50	6.70 ±4.03
3	2	2	2.50 ±0.50	27.05 ±4.08
BH F7 1	2	2	5.50 ±0.50	59.9 ±44.82
2	4	2	3.75 ±0.85	9.30 ±5.47
3	3	1	3.83 ±1.64	10.00 (n.a.)
4	2	2	2.50 ±0.50	11.00 ±6.00
5	2	2	2.00 ±0	5.36 ±2.14
6	2	1	2.25 ±0.75	6.65 (n.a.)
7	3	1	1.17 ±0.17	1.52 (n.a.)
8	2	1	1.00 ±0	2.74 (n.a.)
9	3	2	1.33 ±0.33	22.97 ±16.93
10	3	2	1.83 ±0.17	48.11 ±33.01
BH G3 1	3	3	3.50 ±0.29	18.02 ±4.50
2	3	1	5.00 ±1.15	17.89 (n.a.)
3	3	2	2.33 ±0.33	3.82 ±1.96
4	2	1	7.00 ±4.00	8.73 (n.a.)
5	2	2	4.50 ±0.50	6.80 ±1.40
6	4	4	6.25 ±0.85	114.72 ±44.54
7	3	3	3.83 ±0.44	107.99 ±39.19
8	4	3	2.25 ±0.25	47.68 ±37.88
9	2	1	1.50 ±0.00	8.60 (n.a.)
BH H9 1	4	3	5.00 ±1.22	20.40 ±11.23
2	2	1	3.00 ±1.00	0.62 (n.a.)
3	2	0	2.50 ±0.50	0.00
4	3	3	6.00 ±1.53	17.47 ±8.51
5	3	3	5.00 ±1.00	25.94 ±21.01
6	2	2	4.00 ±1.00	57.10 ±1.10

Table 12.6. The results of analysis of variance of the amount of HCN released from gametophytes grown in populations from spores taken from fronds at the Blackhill fieldsite (Table 12.5). Complete analysis of variance tables are given in Appendix 7, Table A7.7.

Variation between	Variance 'ratio	Degrees of freedom	Significance
Populations	4.860	6,98	***

***, significant at 0.1% level ($P<0.001$).

Table 12.7. The mean amount of HCN, $\mu g\ g^{-1}$ fresh weight, released from fronds of juvenile sporophytes produced by individual gametophytes cloned and grown on in isolation. Each frond produced by these sporophytes was removed and weighed, and placed in a Thunberg tube. Toluene was added to release the HCN, which was analysed by the pyridine - pyrazolone test. Each sporophyte is identified by the grid position of the frond from which the spore from which the gametophyte clone was grown, the number of the clone, and the number of the sporophyte.

Sporophyte clone	Number of fronds sampled	Number of cyanogenic fronds	Mean frond weight, mg ±S.E.	Mean amount of HCN, $\mu g\ g^{-1}$, ±S.E.
BH B8/1 1	5	4	4.90 ±0.84	30.93 ±17.26
BH D10/1 1	3	3	5.17 ±0.83	82.81 ±1.87
2	4	4	9.00 ±1.24	115.43 ±19.30
3	4	4	8.75 ±2.32	101.15 ±14.14
4	3	3	16.33 ±6.98	108.22 ±31.24
BH D10/2 1	6	5	14.00 ±2.52	31.80 ±16.20
BH E9/3 1	3	3	5.33 ±1.45	105.77 ±18.90
2	5	5	11.50 ±1.84	96.79 ±5.78
3	7	7	13.14 ±1.78	128.40 ±12.88
4	4	3	5.50 ±1.55	99.35 ±33.56
5	5	4	13.12 ±2.62	92.29 ±24.45

Table 12.8. The results of the analysis of variance of the amount of HCN released from juvenile sporophytes grown from cloned gametophytes (Table 12.7). Complete analysis of variance tables are given in Appendix 7, Table A7.8.

Variation between	Variance ratio	Degrees of freedom	Significance
Sporophytes	5.777	11,36	***

***, significant at 0.1% level ($P<0.001$).

compared by analysis of variance, which showed that there were highly significant differences between the sporophytes (0.1% level, $P<0.001$; Table 12.8).

12.2.5. Correlations between cyanogenesis and tissue weight in gametophytes and juvenile sporophytes.

The correlation coefficient, r, was calculated for each class of tissue, to establish whether there was a relationship between the weight of tissue sampled and the amount of HCN released by the tissue (Table 12.9). The values of r were calculated for each gametophyte population and all the populations together. Only two populations showed a significant correlation between the weight of gametophytes and the amount of HCN the gametophytes released, BH D10 and BH G3, but overall there was a significant relationship between the weight of gametophytes and the amount of HCN released (5% level, $P<0.05$; Table 12.9a).

Only one gametophyte clone showed a significant and negative correlation between the weight of clonal gametophytes and the amount of HCN released (clone Gos E1/8; 5% level), whilst there was no relationship apparent when all the clonal gametophytes were considered together (Table 12.9b).

The weight of fronds sampled showed no correlations with the amount of HCN released from fronds taken from either populations of juvenile sporophytes or clonal sporophytes (Tables 12.9c and d respectively).

12.3. Discussion.

All the tissues sampled for cyanogenesis in this investigation produced highly variable levels of HCN, both within populations and within clones (Tables 12.1, 12.3, 12.5 and 12.7). These observations are therefore similar to those found throughout this study, showing how unpredictable the variation of cyanogenesis in Pteridium aquilinum can be.

12.3.1. Cyanogenesis in gametophytes.

The fact that all populations of gametophytes raised from spores contain some cyanogenic members (Table 12.1) is very significant, because only spores taken from fronds which were acyanogenic in the field were used. Pteridophyte spores are produced by meiotic divisions within the diploid sporangia. Therefore, since some of the gametophytes were cyanogenic, the fronds producing the spores were all genotypically cyanogenic, even though they were phenotypically acyanogenic when tested by the sodium picrate test.

Table 12.9. The correlation coefficient, r, of the relationship between the tissue fresh weight and the amount of HCN released from the tissue for gametophyte and juvenile sporophyte samples. Because the degrees of freedom is given by df=n-2, any sample size less than three is not applicable for the analysis; however, these samples have been included in the analyses dealing with total samples.

a. Gametophyte populations.

Population	Sample size	Degrees of freedom	Correlation coefficient, r
BH E9	15	13	0.068 N.S.
BH D10	10*	8	0.685 *
BH C9	15	13	0.073 N.S.
BH B10	4	2	0.805 N.S.
BH G3	6	4	0.878 *
BH F7	9	7	0.383 N.S.
Gos A5	3	1	0.924 N.S.
Gos E1	5	3	0.367 N.S.
All samples	67	65	0.289 *

Table 12.9 - continued.

b. Cloned gametophytes.

Clone	Sample size	Degrees of freedom	Correlation coefficient, r
BH B8/1	4	2	-0.423 N.S.
BH B8/2	4	2	-0.692 N.S.
BH B8/3	4	2	-0.685 N.S.
BH B10/1	4	2	0.318 N.S.
BH B10/2	6	4	-0.635 N.S.
BH B10/3	2	not applicable	
BH B10/4	2	not applicable	
BH D10/1	3	1	0.841 N.S.
BH D10/2	4	2	-0.259 N.S.
BH D10/3	9	7	0.510 N.S.
BH E9/2	3	1	0.222 N.S.
BH E9/3	5	3	-0.832 N.S.
BH E9/5	5	3	-0.214 N.S.
BH E9/6	3	1	0.749 N.S.
BH F7/1	3	1	-0.883 N.S.
BH F7/2	2	not applicable	
BH F7/3	3	1	-0.725 N.S.
BH F7/4	4	2	0.809 N.S.
BH F7/5	5	3	0.298 N.S.
BH G3/1	2	not applicable	
BH G3/2	3	1	-0.884 N.S.
Gos A5/1	5	3	-0.632 N.S.
Gos A5/2	7	5	-0.163 N.S.
Gos A5/3	4	2	-0.413 N.S.
Gos E1/1	4	2	0.071 N.S.
Gos E1/2	2	not applicable	
Gos E1/3	3	1	-0.989 N.S.
Gos E1/4	2	not applicable	
Gos E1/5	2	not applicable	
Gos E1/6	2	not applicable	
Gos E1/7	2	not applicable	
Gos E1/8	7	5	-0.843 *
All clones	120	118	-0.152 N.S.

Table 12.9 - continued.

c. Sporophyte populations. Because most of the juvenile sporophytes sampled had two fronds, the samples have been amalgamated into their populations for the analysis.

Population	Sample size	Degrees of freedom	Correlation coefficient, r
BH B8	8	6	0.010 N.S.
BH B10	2	not applicable	
BH C9	10	8	-0.120 N.S.
BH D10	6	4	-0.568 N.S.
BH F7	26	24	0.166 N.S.
BH G3	25	23	0.133 N.S.
BH H9	16	14	-0.189 N.S.
All samples	101	99	0.033 N.S.

d. Juvenile sporophyte clones.

Clone	Sample size	degrees of freedom	correlation coefficient, r
BH B8/1 1	5	3	0.537 N.S
BH D10/1 1	3	1	0.056 N.S.
2	4	2	-0.099 N.S.
3	4	2	-0.927 N.S.
4	3	1	-0.388 N.S.
All D10/1	14	12	-0.384 N.S.
BH D10/2 1	6	4	0.709 N.S.
BH E9/3 1	3	1	0.674 N.S.
2	5	3	0.191 N.S.
3	7	5	-0.253 N.S.
4	4	2	0.104 N.S.
5	5	3	-0.330 N.S.
All E9/3	22	20	0.005 N.S.
All samples	47	45	0.048 N.S.

This supports the observations in previous chapters that all fronds are genetically cyanogenic.

There is some evidence of genetic differentiation between populations of gametophytes grown from spores from the same site. Different populations grown from spores taken from the Blackhill site differed significantly in the amount of HCN released (Table 12.2). From the mean amounts of HCN released from the gametophytes of these populations (Table 12.1), there would appear to be high HCN producing populations (BH E9 and F7), intermediate HCN producing populations (BH D10 and B10) and low HCN producing populations (BH C9 and G3). These types are however far from discrete, and since differences between populations of gametophytes raised from spores from the two highly distant fieldsites do not differ significantly (Table 12.2), it is unlikely that the differentiation found within the Blackhill gametophytes is biologically important.

This is emphasised by the variation shown between individual clones of gametophytes raised from spores taken from the same frond (Table 12.3). Of the clones raised from spores taken from Blackhill, there appear to be both homogeneous parental fronds (BH B10 and E9) and heterogeneous parental fronds (BH B8, D10, F7 and G3). It is difficult to state whether or not the differences found between clones raised from spores taken from the same frond are genetically determined. If they are, it is possible that bracken has two or more sets of genes determining different levels of cyanogenesis, which are separated in meiosis. The individual clones of gametophytes from heterogeneous parental fronds in general have very high standard errors associated with the mean amount of HCN released. It should be emphasised that there is no evidence for genetically acyanogenic bracken sporophytes; thus it remains necessary to explain the differential expression of cyanogenesis in adult sporophytes, whether or not there is segregation in the gametophytes.

There is a highly significant difference between the different gametophyte clones raised from spores taken from Blackhill (0.1% level, $P<0.001$; Table 12.4) and also between the clones raised from spores taken from Gosford Bay (1% level, $P<0.01$; Table 12.4). The clonal gametophytes also show a significant difference between the sites from which the spores were originally taken (1% level, $P<0.01$; Table 12.4). This is in contrast to the results from the gametophytes grown in populations, which showed no difference between the Blackhill and Gosford Bay gametophyte populations (Table 12.2). This is difficult to explain, but it is possible that the gametophytes grown in populations are subject to limited resources due to competition or other interactions between gametophytes within the populations, whilst individually grown gametophytes

are allowed free growth. It can be seen that clonal gametophytes, grown individually rather than in populations, release higher levels of HCN compared to the gametophytes grown in populations, which is likely to be due to the increased availability of nutrients. There is no means of telling to what extent the gametophytes within populations were inhibited in their growth and development. The containers in which the gametophytes were grown were sealed for both the populations and the clonal gametophytes, so it was not possible to determine to what extent resources were limiting with respect to the populations. However, it must be noted that members of the same clone of gametophytes remain highly variable, despite being genetically identical: it is difficult to explain why one member of the clone Gos A5/2 released no measurable HCN whilst other members of the clone released comparatively large amounts of HCN (Table 12.3). This cannot be due to differing nutrient availability, since the nutrient medium was the same throughout. Such variation is analogous to a rhizome system producing both cyanogenic and acyanogenic fronds, since in both cases the clonal gametophytes and the fronds must be genetically identical (allowing for somatic mutations, which are assumed to be rare events).

The fresh weight of a gametophyte may be taken as an indication of its size and, assuming a constant rate of growth for each gametophyte, of its age. Within gametophyte populations, there is evidence that the amount of HCN is related to the weight of gametophytes (Table 12.9a), although the relationship is not particularly close. In contrast, the clonal gametophytes showed no such general relationship, although clone Gos E1/8 showed a significant decrease in the amount of HCN released with an increase in the sample weight (Table 12.9b). The contrast between the two sets of data is difficult to reconcile, but it is possibly due to differences between the growth habits of the populations and clones, since the former are likely to be subject to competition and overcrowding whilst the latter are free to grow unhindered. The importance of the observed relationships between the gametophyte weight and the amount of HCN released is difficult to assess.

12.3.2. Cyanogenesis in juvenile sporophytes.

The sporophytes raised in populations released amounts of HCN similar to the gametophyte populations (Tables 12.5 and 12.1, respectively). The gametophytes are haploid and the sporophytes diploid; it therefore seems possible from these data that there is some form of dosage compensation, perhaps with the sporophytes possessing only one set of active genes for

cyanogenesis. Because of the differences in the development of gametophytes and sporophytes, it was decided that they were not directly comparable statistically.

The sporophytes raised in populations showed a highly significant difference between the populations with respect to the amount of HCN released (0.1% level, $P<0.001$; Table 12.6). All the populations were grown from spores taken from the same field site. Since the populations were grown under the same conditions, this again suggests that the populations may differ genetically. The populations differed with respect to the number of sporophytes produced by gametophytes, all the sporophytes in any one population being sampled (Table 12.5). It is possible that the populations differed with regard to the germination of spores and the growth and fertility of gametophytes, which could account for differences between populations by affecting competition within each population. However, most of the sporophytes sampled produced both cyanogenic and acyanogenic fronds, and were highly variable, which is difficult to explain.

A similar situation is found with the clonal sporophytes (Table 12.7). As these were grown individually, therefore there was no competition between developing sporophytes. Despite the lack of competition, several of the clonal sporophytes had fronds which released no measurable HCN, whilst other fronds released large quantities. It is unlikely that nutrients were limiting to these sporophytes, and this variation cannot be explained. There is however little variation between sporophytes raised from gametophytes belonging to the same clone (Table 12.7), which are therefore genetically identical. There are significant differences between clones of sporophytes, however (0.1% level, $P<0.001$; Table 12.8), which suggests genetic differences within this limited sample. It is possible that developmental differences may account for some of the variation found.

The amount of HCN released from fronds of the individually grown clonal sporophytes is similar to that released from the individually grown clonal gametophytes (Tables 12.7 and 12.3 respectively), once again suggesting that there is dosage compensation within the diploid sporophytes.

There is no relationship between the weight of the sampled fronds and the amount of HCN released by the fronds, for either the sporophyte populations or the sporophyte clones (Tables 12.9c and 12.9d). Within the populations, the sporophytes were sampled before their growth was likely to be limited by the petri dishes in which they were growing, and therefore they consisted of relatively few fronds, with a majority having only two (Table 12.5). It was felt

that in this case the age of the frond might be indicated by its fresh weight, although there is no direct evidence for this, and that the weight might indicate the developmental state of the frond. There is no indication of any relationship between frond weight and cyanogenesis. This suggests that the developmental state of the juvenile sporophyte frond does not affect cyanogenesis, although the developmental state of the whole plant may do so.

12.3.4. The role of cyanogenesis in gametophytes and juvenile sporophytes.

There are no known reports of cyanogenesis in plants with haploid chromosome numbers, either naturally occurring or experimentally prepared. That cyanogenesis occurs within the gametophyte generation as well as the sporophyte generation in Pteridium aquilinum shows that cyanogenesis is independent of any phase change inherent in the alternation of generations. It is possible that cyanogenesis has a function in the gametophyte generation, perhaps related to defence from predation, but there is no direct evidence of this. The amount of HCN released from juvenile sporophytes grown individually under optimal conditions is similar to that found for fully developed mature sporophytes in the field (e.g. chapters 4, 5, 6, 7, and 8) and for sporophytes grown in greenhouse conditions (e.g. chapters 9, 10, and 11), which would suggest that cyanogenesis does not function specifically at this stage in the life cycle, when establishment is taking place. However, it is generally felt that HCN produced by mature bracken is of little significance to insect herbivores (Lawton, 1976; Schreiner, 1980), some of which may be able to detoxify the poison (Beesley et al, 1985). Cooper-Driver et al (1977) provide anecdotal evidence that large mammalian herbivores may browse on mature bracken fronds. It is possible however that the much smaller gametophytes and juvenile sporophytes may be grazed by small and unidentified herbivores. Jones (1962, 1966) presented evidence that acyanogenic forms of Lotus corniculatus were subject to selective grazing by the mollusc herbivores Agriolomax and Arion. It is possible that bracken gametophytes and juvenile sporophytes are similarly predated by molluscs, and that the levels of HCN produced are large enough to deter feeding. It is also possible that cyanogenesis in the gametophytes and juvenile sporophytes protects them from fungal attack, which was thought to have a significant effect on gametophyte and sporeling survival (Conway, 1953). Harborne (1977) suggests that the infection of a cyanogenic plant by a fungal pathogen could lead to the release of HCN when the pathogen damages host cells, with the HCN serving to protect the plant against the pathogen.

12.4. Conclusions.

As previous studies have also shown with mature plants, cyanogenesis in Pteridium aquilinum is very variable within the gametophyte and juvenile sporophyte, stages of the life cycle when it might be expected that the need for defence from predation would be greatest. Cyanogenic gametophytes and sporophytes were grown from spore taken from acyanogenic fronds. Populations of gametophytes and sporophytes raised from spores taken from different acyanogenic fronds at the same site differed in the amount of HCN they released. It is possible that there is a genetic basis for the observed variation. There was no evidence for genetically acyanogenic plants of either generation. Clonal material of both gametophytes and sporophytes was highly variable, with indications that there might be genetic differences between clones. There was no evidence that cyanogenesis was affected by the development of the gametophyte.

13. A Discussion of Cyanogenesis in Bracken.

The results given and discussed in the previous nine chapters have shown that cyanogenesis is a highly variable characteristic in bracken. The variation found occurred at many different levels (Table 13.1) and makes overall conclusions difficult to draw. In all cases it must be assumed that the variation found is due to variation in the amount of HCN released from frond tissues, and that it is from this that the polymorphism originates. There has been little evidence to suggest that there are any fronds which release no HCN at all. Rather, it would appear that the qualitative polymorphism (i.e. either cyanogenic or acyanogenic) found in the field reflects the particular test used in fieldwork, with certain fronds releasing HCN at levels below the sensitivity of the picrate test. The interpretation of the results of studies into the variation found in the amount of HCN released from fronds raises several points, which are discussed in turn below, although it must be noted they are all closely related.

13.1. The control of variation of cyanogenesis in bracken.

No evidence has been found of genotypic control of the amount of HCN released by mature bracken fronds; indeed, there is much evidence from several of the studies reported here that cyanogenesis is under phenotypic control. This is particularly clear from the excavation of rhizomes in the field (chapter 8) and the two series of transplantation experiments (chapter 9). In addition, fronds sampled throughout the season were found to change phenotype, from acyanogenic to cyanogenic early in the season and vice versa later on (chapter 5). Although other authors report isolating four phenotypes of bracken with regard to cyanogenesis (both glycoside and glycosidase present; glycoside only; glycosidase only; neither present) (Cooper-Driver et al, 1977; Lawton, 1976), which can be interpreted as evidence for a genetic polymorphism, results reported in this thesis have only shown variation in the amount of HCN extractable rather than an absolute presence or absence. It would seem likely that the results of other workers in fact show differentiation between the amount of glycoside and glycosidase extracted from tissues.

Although it has been believed that the polymorphism for cyanogenesis in several species, notably Trifolium repens and Lotus corniculatus, was genetically determined (Dawson, 1941; Corkill, 1942; Jones, 1977; Hughes, 1981), there are anomalous reports in the literature. Plants of Lotus corniculatus were found in which the phenotype could be altered by regulating the temperature

Table 13.1. A synopsis of the variation found with respect to cyanogenesis in bracken. The variation is in the amount of HCN released from frond tissues in all cases. The chapters in which each level of variation is discussed are given for reference.

Variation within	between	chapter
geographical region	geographical locations	4
geographical locations	habitats	4
habitats	fronds, over 18m X 18m area	several
habitats	seasons	5
habitats	sites	7
habitats	fronds, over 1m X 1m area	8
rhizomes	fronds	8, 9, 10, 11
rhizome clones	shading treatments	10
rhizome clones	nitrogen treatments	11
fronds	method of HCN extraction	10, 11
juvenile populations	juvenile sporophytes	12
juvenile sporophytes	fronds	12
gametophyte populations	gametophytes	12
gametophyte clones	gametophytes	12

(Ellis et al, 1977a; Ramnani and Jones, 1985). It had been suggested that such unstable individuals might result from heterozygous genotypes, homozygous plants being stable (Ellis et al, 1977a), but this has been discounted (Ramnani and Jones, 1984). Similarly, individuals of L. corniculatus have been reported in which the phenotype of the leaves is different from that of the flowers, although there is a relationship between the two characters (Compton et al, 1983). It has been suggested that these anomalies result from the interaction between a regulatory gene system and environmental factors (Nass, 1972; Professor D.A. Jones, personal communication). Corkill (1942) suggested that modifier genes which operate in concert with the genes for the glycosides and glycosidase could explain inconsistent results in Trifolium repens.

Monomorphic cyanogenic plant species often show HCN production which is highly variable between individuals. Different strains of Sorghum sudanense release different amounts of HCN (Boyd et al, 1938). Nass (1972) suggested that this resulted from multigene inheritance with incomplete dominance, although he does not exclude the possibility that regulatory modifying genes occur. There is a wide variety in the amount of HCN released by different cultivars of cassava (Cooke and Coursey, 1981; Nartey, 1981).

Cyanogenesis in Pteridium aquilinum differs in that no consistent differences between individuals have been found. Under the controlled conditions within the treatments given , to clonal replicate rhizomes in the shading and nutrition experiments described in chapters 10 and 11 respectively, fronds produced by the same rhizome replicate release different amounts of HCN. Similarly, different individual gametophytes originating from the same clone grown under the same controlled conditions released different amounts of HCN. These observations are very difficult to explain, but they show that the differences within an individual plant are as great as those between plants growing at different locations. It must be concluded that there is no evidence for genetic control of cyanogenesis in bracken.

It has been suggested that it is beneficial for plants to maintain variability in the production of secondary chemicals, and that such variation would be selected for (Whitham and Slobodchikoff, 1981; Whitham et al, 1984). This would make adaptation by herbivores to secondary plant compounds more difficult by increasing the variation found in these compounds (Whitham et al, 1984). It has been postulated that somatic mutations, producing genotypically generated variation, could function in this way (Whitham and Slobodchikoff, 1981; Whitham et al, 1984). However, it is also possible that phenotypically generated variation could function in a similar fashion, to the advantage of the

plant. Genetic mechanisms allowing phenotypically generated variation might similarly be selected for. Bracken is renowned for its plastic response to environmental features (Fletcher and Kirkwood, 1979; Page, 1976; Watt, 1976).

13.2. Environmental influences on cyanogenesis in bracken.

Although environmental effects cannot explain the variation found within clones of laboratory cultured gametophytes, results found throughout the field surveys (chapters 4 to 8) indicate that environmental factors play an important role in determining the amount of HCN released from fronds. Thus there are consistent differences between habitat types (chapters 4 to 8), which may reflect environmental differences between habitat types. The consistency of the amounts of HCN released in different years suggests that the factors affecting cyanogenesis at any one site are more or less constant from year to year (chapter 6). Indeed, given such a high degree of phenotypic variability found throughout these investigations, it is surprising that there was no significant variation between different years, a fact which suggests that the frequency of cyanogenic fronds is in some way fixed within a population of fronds (chapter 6).* Although this may appear contradictory to the variation found, it is possible that certain environmental factors involved in determining the variation of cyanogenesis in bracken are on such a scale to be indistinguishable from a fixed genotype in the field. For instance, at any one site environmental factors such as the spring temperature, the amount of rainfall or the amount of available nitrogen are unlikely to change substantially from one year to another. If the level of cyanogenesis is to some degree dependent on factors such as these, cyanogenesis would appear fixed within a population. In addition, the long shoot rhizomes function as long term storage organs for the plant. Thus these rhizomes could store nutrients and intermediary compounds, ameliorating differences between years and buffering the developing fronds against environmental changes. It should however be noted that there would appear to be little evidence of this, since the results from chapter 8 have shown that fronds attached to the same rhizome system can release very different amounts of HCN. This would suggest that long term storage of nutrients or intermediary compounds by the long shoot rhizomes has little effect of cyanogenesis.

* This statement must be reconsidered in the light of the chi-squared analysis of the data in Chapter 6. Please see page 171 and that following page 173.

13.2.1. The interaction between light intensity, nitrogen nutrition and cyanogenesis.

Both the light intensity and the level of available nitrogen have been shown to influence cyanogenesis in greenhouse experiments (chapters 10 and 11 repectively). Throughout the field surveys, bracken fronds growing under woodland conditions released substantially greater amounts of HCN than fronds growing at unshaded sites, implicating tree-cover as a factor affecting cyanogenesis.

Woodland could affect bracken either directly, by altering the light intensity, or indirectly, through the accumulation of litter, the alteration of soil organisms (the rhizosphere) and by altering nutrient cycling. Fletcher and Kirkwood (1979) believed that bracken would benefit from the increased nutrient supply found under woodland conditions. A combination of these direct and indirect effects is likely under woodland conditions.

The morphology of bracken fronds has been shown to be sensitive to light intensity (e.g. Boodle, 1904), and fronds growing under shade have less well developed cuticles, lack a hypoderm, are less thick, possess less than half the number of stomata, and produce fewer hairs than fronds growing in the open (Cook et al, 1979). Bracken is not considered to be tolerant of excessive shading (Burke, 1953; Page, 1976).

Under conditions of low light intensity, photosynthesis is likely to be reduced and carbon assimilation therefore lower than in plants growing in the open (Mooney, 1972; Fitter and Hay, 1983; Schulze, 1982). Carbon is likely to become limiting, resulting in lower production of dry weight and a redistribution of assimilated carbon (Mooney, 1972; Schulze, 1982). Under these conditions, mineral nutrients may be available in excess relative to the available carbon (Bloom et al, 1985). However, the production of NADPH by the light reaction of photosynthesis would also be expected to decrease. NADPH has been shown to be necessary as an energy source for the synthesis of cyanogenic glycosides in some plants (Hosel and Narstedt, 1980). The effect that this would have on bracken is uncertain.

It has been shown experimentally that increased shading (chapter 10) and high levels of nitrogen availability (chapter 11) both result in increased release of HCN from bracken fronds, but not increased prunasin production. The increase in the amount of HCN released with greater shading (from a mean of 69.83 $\pm$ 34.90 $\mu g\ g^{-1}$ fresh weight at 50% ambient light to 188.11 $\pm$ 21.68 $\mu g\ g^{-1}$ at 12.5% ambient light) is similar to the increase due to increased nitrogen availability (from 53.31 $\pm$ 30.11 $\mu g\ g^{-1}$ under treatment 1 of the second series

to 128.91 ± 45.04 μg g^{-1} under treatment 10). The effect of increasing shading is therefore similar to increasing nitrogen availability. It should be noted that the replicate rhizomes in the shading experiment were provided with the same mineral medium used in the treatment 2.5 of the nitrogen experiment. This level of nitrogen was shown in the nitrogen trials to be suboptimal for the production of fresh and dry weight of fronds, and most morphological characteristics. Under shade conditions, this level of nitrogen application is not limiting to HCN production. It would appear that increasing the degree of shading is equivalent to increasing nitrogen availability

For bracken under shaded conditions, an excess of nitrogen resulting from these conditions might lead to increased production of prunasin and β-glycosidase. Large amounts of these metabolites might be accumulated, resulting in highly cyanogenic fronds. Prunasin is produced by a metabolic pathway based on the amino acid phenylalanine (Tapper and Butler, 1971; Hegnauer, 1977; Conn, 1981b), and excess synthesis of this amino acid over and above the needs of protein synthesis could lead to the accumulation of prunasin. Phenylalanine is also involved in the synthesis of aromatic compounds, in particular phenolic acids and the precursors of flavonoids and lignins (Harborne, 1980; Hahlbrock, 1981; Grisebach, 1981), which are dependent on the activity of phenylalanine ammonia lyase (PAL) (Hanson and Havir, 1981) and assimilated carbon metabolites. The synthesis of carbon-based aromatic compounds and their polymers is therefore also limited by photosynthesis, and would utilise less phenylalanine under low levels of illumination than high levels. The alteration of this balance could increase the utilisation of phenyl alanine in secondary metabolism. However, the behaviour and control of the enzymes involved in the synthesis of prunasin is not known.

In addition, a general excess in amino acids could lead to an increase in the production of non-essential proteins. These could include the specific β-glycosidase involved in cyanogenesis. Given the specificity and high rates of activity for the glycosidases involved in cyanogenesis (Hosel, 1981), it is surprising that an increase in the amount of glycosidase, rather than glycoside, appeared to have a large effect on the amount or rate of HCN release. This was seen in both the shading and nitrogen trial (chapters 10 and 11), in which the glycoside extracts showed only small differences between the different treatments.

However, in both the shading and nitrogen trials (chapter 10 and 11), there was a small, but not significant, increase in the amount of HCN released from the glycoside extracts with the increase in shading and nitrogen supplied. This

was insufficient to account for the significance of the increase in HCN released in either case, although it may have resulted in some of the differences in the amount of HCN released. The main increase in the amount of HCN released in both trials must therefore be due to differential glycosidase activity. Under conditions in which nitrogen is limiting, the synthesis of non-essential proteins could represent a significant drain on the availability of nitrogen for other functions, including the synthesis of essential proteins. This could result in a decrease in fitness or productivity and may be deleterious to the plant. Thus under limiting conditions nitrogen is unlikely to be used for the synthesis of non-essential proteins such as those involved in cyanogenesis.

It has been shown both theoretically (McNeill and Southwood, 1978; Mattson, 1980) and in the field (Blair et al, 1983) that shaded plants are more palatable to herbivores. This is due to the relative levels of available proteins and carbohydrates. In general, plants are poor suppliers of nitrogen to herbivores (Mattson, 1980; Rhoades, 1983, 1985), and thus plants with an increased proportion of available protein are more attractive food sources. Thus both the absolute and relative quantities of nitrogen are important in determining the levels of herbivory (McNeill and Southwood, 1978).

It has been suggested that the relative availability of nitrogen and carbon to a plant should determine the nature of secondary plant chemicals used in defence: when nitrogen is relatively abundant, nitrogen-based chemicals acting in a qualitative way (such as alkaloids, glucosinolates and cyanogenic glycosides) would be optimised, whilst when carbon is relatively abundant, carbon-based chemicals acting in a quantitative way (such phenolic acids, terpenoids and tannins) would be most effective (Feeny, 1976; Mattson, 1980; Krischik and Denno, 1983). It is believed that qualitative plant defences are less of a drain on resources than quantitative defences (Rhoades, 1979, 1983), since they are generally present in small amounts. When plants are under stress, including shading or low levels of illumination, they can be expected to decrease the production of carbon-based qualitative allelochemicals in favour of nitrogen-based quantitative allelochemicals (Rhoades, 1983). This would allow a greater allocation of limited carbon resources to growth whilst maintaining defence.

Bracken grown under shaded conditions in the field releases greater amounts of HCN (a quantitative phytochemical) (Cooper-Driver et al, 1977; Lawton, 1976; chapters 4 and 10) and produces lower levels of tannins (Cooper-Driver et al, 1977). The lower production of tannins under shaded conditions by bracken is considered to be due the low availability of carbon

(Tempel, 1981). These observations support the differentiation of plant secondary compounds due to the availability of nutrients (including carbon).

The increased production of tannins by bracken fronds growing in open habitats may alter the release of HCN from the tissues. It has been shown that tannins in plants function by forming complexes with proteins, thereby reducing the efficiency of digestive enzymes and decreasing the availability of nutritive proteins to herbivores (Feeny, 1976; Rhoades, 1979). Tannins have also been shown to complex with plant proteins, both unspecific glycosidases (Goldstein and Swain, 1965) and the specific β-glycosidases involved in cyanogenesis in several plant species (Goldstein and Spencer, 1985). Cyanogenesis has been shown to be inhibited by tannins present in the leaves of cyanogenic plants (Goldstein and Spencer, 1985). Thus under open conditions the high levels of tannins believed to occur could inhibit cyanogenesis in bracken fronds already releasing low levels of HCN. It is also possible that tannins were produced by the fronds sampled in the trials of differential shading and nitrogen availability, and that some of the differential HCN production noted is in fact due to the inhibition of the β-glycosidase by tannins. It would be necessary to study the amounts of HCN released both glycoside extracts and whole tissue preparations under field conditions to estimate the extent of the inhibition of cyanogenesis by tannins in the field.

The level of nitrogen found in the soil samples taken during fieldwork was not correlated with the amount of HCN released from fronds under any habitat conditions. This contrasts with the results found for the nitrogen trials, although there was no direct relationship between HCN released and the relative amount of nitrogen applied in that study. The lack of evidence that the level of nitrogen in the soil is related to cyanogenesis could result in several ways. In both the coastal and open habitats, nitrogen is likely to be limiting. Under such conditions there is unlikely to be excess nitrogen available for the production of secondary metabolites (i.e. over and above the amount of nitrogen required for primary metabolism, such protein synthesis), and therefore a relationship between the level of nitrogen in the soil and cyanogenesis might not be expected. It is possible that the size of soil samples taken was too small for the level of nitrogen present to be representative of the whole area, although in all cases five samples were amalgamated with the intention of producing a sample that was typical of the whole site. Alternatively, it is possible that each soil sample was too large, and that differences which resulted in individual fronds receiving different amounts of nitrogen were obscured. This might be one explanation of how two fronds attached to the

same rhizome system could produce very different levels of HCN (chapter 8). The importance of long distance transport between branches of long shoot rhizomes is not known. It is possible that the roots of the short shoot rhizome closest to a frond are important in the fronds nutrition and water uptake. Since short shoots can be found at different soil depths, the conditions effecting two short shoots close to each other may be very different.

It would be beneficial to study the quantitative changes that occur in the amount of HCN released from fronds in natural stands of bracken under the application of different levels of nitrogen fertiliser. This would enable the effect of known levels of soil nitrogen on cyanogenesis to be studied in the field, particularly if levels of nitrogen which are not limiting could be provided.

13.2.2. Other environmental effects on cyanogenesis in bracken.

Although shading appeared to have the greatest influence on cyanogenesis in bracken in the field (chapter 4), differences were found between sites within both shaded and unshaded (coastal and open) habitat types which cannot be explained by the influence of the relative amounts of carbon and nitrogen available within the habitat. As was shown in chapter 8, cyanogenesis differed between fronds over very small distances and between fronds that were attached to the same rhizome system. These can be explained by environmental differences on a small scale, by developmental differences or epigenetic effects, or by somatic mutations. The latter can be discounted since they are believed to occur comparatively rarely (Whitham and Slobodchikoff, 1984; Whitham et al, 1981), and it is unlikely that such mutations would be able to account for the presence of cyanogenic and acyanogenic fronds, by both the picrate and the pyridine-pyrazolone tests, on the same rhizomes, as was shown to be the case in chapters 8, 9, 10 and 11. Developmental or epigenetic effects, particularly age related effects, may account for some of the differences found between fronds on the same rhizome system, but the large differences found within single rhizome systems at the two 1m squares in the woodland habitat (chapter 8), the nursery plots (chapter 9), and the laboratory experiments (chapters 10 and 11) were not consistent with there being large age or developmental differences between fronds or rhizomes. This is especially true for the transplanted and experimental material. It therefore seems likely that the differences in cyanogenesis between fronds reflect an interaction between cyanogenesis and the environment. For the greenhouse grown material these factors would necessarily be on a very small scale, possibly due to the growth of the rhizomes. For instance, fronds produced by

short shoot rhizomes at different depths within a pot may be under different conditions. Differences of this sort may be superimposed over large scale features, which may explain the correlations between cyanogenesis and various environmental factors (chapter 4).

The correlations shown in chapter 4 (Tables 4.3 - 4.5) were between the percentages of cyanogenic fronds sampled at all sites and both the amount of HCN released, and the mean maximum July temperature. In addition, there were correlations between the percentages of cyanogenic fronds sampled at open habitat sites and both the levels of soil pH and the amounts of Mg in the soil, the latter being a negative correlation. The amounts of HCN released from cyanogenic fronds at all sites were correlated with the mean quarterly spring (April - June) temperatures and the mean maximum July temperatures. In addition, the amounts of HCN released from fronds sampled at open habitat sites was correlated with the levels of soil pH, and the amounts of HCN released from fronds sampled at woodland habitat sites were negatively correlated with the mean amounts of rainfall for each quarter and the annual mean rainfall at each site. When the open site at location two was excluded from the analyses, the amounts of HCN released from fronds sampled at all sites were also negatively correlated with the quarterly and annual rainfall at each site, and the amounts of HCN released from fronds sampled at open sites were positively correlated with the levels of soil pH and negatively correlated with the levels of soil Mg.

The influence of air temperature on cyanogenesis, whether causal or not, is unlikely to be able to explain the differences between fronds found within habitats and at the same sites, over large or small distances. The variation in air temperature within a site is unlikely to be large enough to be related to such large differences between fronds. However, it is possible that frost could have an effect on fronds produced by short shoots at different soil depths within one site. There was no evidence of low winter temperatures affecting cyanogenesis (chapter 4).

Although correlations between soil characteristics and cyanogenesis were only found for the open habitats, this remains as evidence that the soil may be able to influence cyanogenesis. The soil pH and the availability to plants of Mg and several other mineral nutrients are related (Fitter and Hay, 1982). There was no correlation found in this study between soil pH and Mg, however. It is possible that small scale heterogeneity within the soil was responsible for the differences between fronds within a small area.

Bracken has been shown to be sensitive to particularly wet soils (Poel,

No increase in HCN was noted between the different sets of samples, despite the emergence of more fronds in the interim. However, the production of fronds may have results in different levels of water deficit between treatments from the outset of the experiment.

The replicate rhizomes under different treatments in the shading trial did not produce very different numbers of fronds (Table 10.2), and were under conditions of high relative humidity due to the influence of the shade frame. Considerations of differential water deficit between treatments are therefore not relevant.

13.3. Variation of Cyanogenesis within the Growth Season in Bracken.

The results of the survey of cyanogenesis over two growing seasons at location 5 showed an initial increase in both the percentage of cyanogenic fronds, according to the picrate test, and the amount of HCN released from cyanogenic fronds, followed by a decline in both characteristics later in the season (chapter 5). The initial increase in the percentage of cyanogenic fronds was found after the permanent tagging, and thus was due to acyanogenic fronds becoming cyanogenic; this is further evidence that the phenotype of particular fronds is not fixed. It is possible that prunasin was not synthesised until each frond had emerged and begun to photosynthesise. This would be expected if prunasin synthesis relied on NADPH produced by the photosynthetic light reaction as an energy source, as has been shown for taxiphyllin (Hosel and Narstedt, 1980).

It is not possible to separate the decline of the percentage of cyanogenic fronds, and the amount of HCN they released, due to frond age from that produced by external seasonal factors, such as water deficit and available nutrients.

There are theoretical arguments similar to those given in relation to the effect of shading upon the production of secondary plant chemicals (13.2.1) which predict that the production of qualitative nitrogen-based allelochemicals should be high early in the season, and that as the season continues production of these should decrease and be superceded by the formation of quantitative carbon-based allelochemicals (Denno and Raupp, 1983; Krischik and Denno, 1983; Mattson, 1981; Rhoades, 1983, 1985). At the start of the season, carbon will be limiting whilst nitrogen should be available in excess, and thus the most efficient use of secondary plant compound should lead to the production of nitrogen based compounds as allelochemicals. Later in the season, when leaves are exporting photosynthate to other parts of the plant,

carbon is present in excess and therefore the production of carbon-based secondary metabolites is favoured. Feeny (1975) predicts a similar shift in the use of resources on the basis of "apparency": young leaves of a deciduous plant which produces leaves in a single flush are unapparent to herbivores, in that the leaves have been absent prior to flushing; unapparent tissues are likely to contain qualitative antiherbivore compounds acting at low concentrations, since they are likely to be subject to predation mainly from adapted specialist herbivores. Mature leaves, on the other hand, represent apparent tissues, since they have been present long enough to have been detected by non-adapted generalist herbivores, and as such protection would be optimised by the production of quantitative allelochemicals active at comparatively large concentrations, particularly those that act as digestion inhibitors (Feeny, 1975; Raupp and Denno, 1983).

There is evidence that cyanogenic glycosides (Conn, 1980a) and other nitrogen-based secondary plant compounds (Krischik and Denno, 1983) are easily transported around plants, although whether this is the case for bracken or not is not known. It has been suggested that the production of nitrogen-based allelochemicals may be a form of nitrogen storage as well as defensive chemicals, in that such compounds can be metabolised later (when more effective defensive chemicals are available) to release the nitrogen for growth (Krischik and Denno, 1983). Jones (1972a) suggested that cyanogenesis as a defensive mechanism could have evolved from a system of nitrogen storage. Rhizomes of Pteridium aquilinum from greenhouse grown plants which were tested for cyanogenesis and for the presence of prunasin gave no indication of HCN release (chapter 11). It is possible however that bracken in the wild translocates prunasin or its precursors from the fronds to the rhizomes during and at the end of each season, and that the glycoside is stored in the rhizomes during the winter and transferred to the developing fronds at the start of the following season. This would result in a seasonal decrease in the amount of HCN released from fronds.

Cooper-Driver et al (1977) also reported an increase in tannins, carbon-based quantitative herbivore deterrent compounds, as cyanogenesis declines. This is in keeping with the differentiation between qualitative and quantitative plant defences discussed above, and their paper has been quoted in support of these theories (Krischik and Denno, 1983). Tempel (1981) found very variable levels of tannins in bracken during the season. The effect of increased tannins on cyanogenesis cannot be estimated. However, it is possible that the seasonal decrease in cyanogenesis in bracken is in part due

to increased interference from tannins.

It is notable that the laboratory based trials, involving shading and nitrogen treatments, showed a decrease in the amount of HCN released from fronds during the course of the experiments (Tables 10.2, 11.2 and 11.6). Although such a decrease may be due to the partitioning of nitrogen between an increased number of fronds, it is equally likely that the decrease reflects the increasing age of the fronds sampled. The course of the experiment would therefore correspond to the usual seasonal changes, with the major exception that unlike fronds in the wild, there was no simultaneous flush of fronds at the beginning of the 'season'. Thus the fronds in the field would be essentially even aged, whilst those in the greenhouse were of variable age. The conditions within the greenhouse were maintained during the course of the experiments. The replicate rhizomes were not subject to the usual seasonal factors such as variable daylength, temperature, and water deficit, subject to the possibility that replicates producing larger numbers of fronds may have a greater water deficit (13.2.2). Thus in addition to external seasonal effects, bracken fronds show an decline in cyanogenesis with time.

The seasonal decrease in cyanogenesis in the field might have a bearing on the results reported in chapter 4, concerning cyanogenesis in bracken throughout Britain. Out of necessity, the sampling of the 24 sites at nine locations was spread over six weeks from mid June to early August. Although the analysis of the results in chapter 4 showed no effect of location or time of sampling upon cyanogenesis at these sites, it can be seen that if the results reporting a seasonal decline at sites at location 5 (chapter 5) are more widely applicable, it could affect the interpretation of those results referring to Britain as a whole. However, it should be noted that the timing of the start of the growing season in 1983 might have differed considerably from that in 1984, and that the onset of the season in 1983 was two or three weeks later at location 5 than locations 2 and 3 (personal observations; Dr C.N. Page, personal communication). It can be assumed that the onset of the growing season at locations 8 and 9 was later still. It may therefore not be justifiable to extrapolate from the results showing a seasonal decline at one location to locations throughout Britain.

Over the six week period in the middle of the season recorded in 1984, there was a slight increase and then a steady decline in the percentage of cyanogenic fronds in the samples at all three sites at location 5. The rank position of each site was the same throughout the period. Therefore had the variation in the percentage of cyanogenic fronds sampled throughout Britain in

1983 been significant it should have shown up in the analyses. The amount of HCN released by cyanogenic fronds also varied considerably over this mid season period at location 5 in 1984, particularly at the woodland site; throughout the period, however, the fronds sampled at this site released much more HCN than fronds sampled at either the coastal and open sites. This therefore supports the findings discussed in chapter 4 and does not change their interpretation.

13.4. Variation between fronds within a rhizome system.

The results presented in several chapters have shown that the amount of HCN produced by tissues of different fronds belonging to the same rhizome system can release different levels of HCN. This was found to be true in the field (chapter 8), in nursery plots (chapter 9) and under controlled greenhouse conditions (chapters 9, 10 and 11). The possibility was discussed above (13.2.2) that such differences could arise because of heterogeneity within the soil. However, it has been shown that the value of leaves to a plant can differ within a shoot, and that leaves may therefore show differential production of allelochemicals that function as predation deterrents (Krischik and Denno, 1983).

It is possible that within the bracken rhizome system there is a similar differentiation. It might be expected that the earliest emergent fronds would be of most value to the plant, because these fronds represent the use of limited stored resources in the production of photosynthetic tissue prior to the return of photosynthate. These fronds may also be subject to high rates of herbivory, since there will be a limited number of fronds on which herbivores will be able to feed. If this is the case, it may be expected that the first emergent fronds would produce greater quantities of herbivore deterrents than later emergent fronds, and thus may release more HCN than later emergent fronds. However, both "cyanogenic" and "acyanogenic" fronds were found amongst those sampled very early in the season (chapter 5), which shows that no such differentiation occurs over large areas early in the season. It is still possible that such differentiation occurs within an individual rhizome system. However, since short shoots produce only one frond per season (Watt, 1976), control of differentiation between fronds is difficult to envisage.

It is also possible that fronds produced by the same rhizome system, and rhizomes within the same habitat, are competing for limited resources, such as water, mineral nutrients and light (Watt, 1943, 1976; Harper, 1977). Fronds produced by the same rhizome will also be competing for carbohydrates early in the season, prior to becoming exporters of carbohydrate. Watt (1940, 1943,

1976) interpreted several morphological features of bracken to be due to competition both between rhizomes (for 'space', presumably defined by the availability of water and mineral nutrients) and fronds (notably for light). Competing rhizomes could be different genotypes, ramets of the same genotype or intact branches of the same rhizome system.

The density of rhizomes and fronds within each habitat type may be expected to determine the degree of competition at each level. The density of fronds was generally found to be lower at woodland habitat sites than at coastal or open habitat sites (Table 3.3). The excavation of rhizomes in the field (chapter 8) showed that the low density of fronds at the woodland site was coupled with a low density of long shoot rhizomes. Similarly, the high density of fronds at the coastal site was coupled with a high density of rhizomes. It is likely that the open site would have contained a higher density of rhizomes, given its high frond density. This would explain in part the difficulty found when attempts were made to excavate this site.

The density of fronds and presumably rhizomes under woodland conditions is determined largely by the light intensity (Burke, 1953; Tansley, 1953; Dring, 1965). Because shading results in a low frond density, the intraspecific and intraclonal competition will similarly be reduced. The availability of mineral nutrients per frond within the rhizome system may well be greater than in unshaded habitats, although the trees will also be competing for resources. It is possible therefore that the limitations on the growth of bracken brought about by shading increase the nutrient supply to each frond, perhaps leading to an increase in cyanogenesis. Competition between long shoots within the same or different rhizome systems might result in the differential production of HCN.

13.5. The Function of Cyanogenesis in **Pteridium aquilinum**.

The ecology of cyanogenesis in bracken and its control have been discussed from several points of view above. The influence of cyanogenesis on the ecology of bracken has not previously been discussed in this thesis, because it is necessary to understand the control of any feature before considering the role it plays in determining the ecology of the plant.

Cyanogenesis in plant species is believed to function as a herbivore deterrent (Jones, 1972a, 1981; Conn, 1980, 1981b). The effect of cyanogenesis in bracken on insect herbivores has been investigated in two field surveys in cool temperate regions (Lawton, 1976; Schreiner, 1980). Neither study showed that cyanogenesis in bracken functioned as a deterrent to insects.

Cooper-Driver and Swain (1976) showed that the generalist herbivore Schistocerca gregaria preferentially grazed acyanogenic bracken in laboratory tests. Bernays (1977) reported similar results working with Locusta. Cooper-Driver et al (1977) observed that acyanogenic bracken was preferentially grazed by deer under a managed system.

These various papers present conflicting views. Several interpretations are possible: i. bracken in the field may be grazed by specialist insect herbivores that are resistant to HCN, whilst generalist insect herbivores, rarely feeding on the plant, are sensitive to HCN. Compton et al (1985) present data showing that several insect species contain rhodanese, which detoxifies HCN; ii. cyanogenesis in bracken does not affect insects but functions as a deterrent to other groups of herbivores, such as mammals or molluscs; iii. HCN is a redundant antipredation compound in bracken in temperate regions, but may be active under other conditions such as in the tropics or under high rates of herbivory; iv. HCN is redundant in mature bracken but may be active at some other stage in the life cycle, such as gametophytes or establishing juvenile sporophytes; v. cyanogenesis is redundant as a herbivore deterrent at all stages in the life cycle of bracken; vi. cyanogenesis has a function in bracken other than as a herbivore deterrent.

The last of these possibilities (vi) is unlikely, because the large variability found in this research would require such a function to be less necessary to certain fronds than to others.

The other possibilities cannot easily be tested separately. Because gametophytes and juvenile sporophytes are rarely found in the field, it is difficult to test the hypothesis that, under natural conditions, cyanogenesis has a function at these stages of the life cycle. A search for juvenile sporophytes in New Caledonia, in the tropical south west Pacific where it was felt likely that sexual regeneration of bracken would take place, was unsuccessful (unpublished results). Mature stands of bracken growing in New Caledonia did contain cyanogenic fronds. However, the establishment phase within the life cycle of any plant is likely to under the greatest stress and competitive interference (Harper, 1977), and any feature which improves the likelihood of survival will be advantageous to the plant. It is interesting that all the juvenile sporophytes raised individually under controlled conditions were cyanogenic, as were most of those raised in populations (chapter 12). It seems probable that all young sporophytes are cyanogenic to some degree. The ecology of gametophytes and juvenile sporophytes differs greatly from the ecology of the mature bracken plant, particularly with respect to the scale of the plants. It is

interesting to note that the gametophytes, juvenile sporophytes, and mature sporophytes sampled in the field and under experimental conditions all release HCN within similar ranges of amount per fresh weight.

It is possible that cyanogenesis serves to protect the developing fronds from attack by soil organisms. However, if this was the case, it would be expected that emergent fronds would be highly cyanogenic. It was noted in chapter 5 that this was not the case, fronds becoming cyanogenic only after emergence.

The role of cyanogenesis in mature bracken as a herbivore deterrent is difficult to assess. Bracken contains a large number of putative herbivore deterrents (Cooper-Driver, 1976; Jones 1983), and it is possible that any variation in the rates of herbivory of cyanogenic and acyanogenic fronds may be masked by these other compounds. It is possible that specialist herbivores are adapted to most of the chemicals, and thus the array of herbivore deterrents may be effective only against generalist herbivores. However, if this is the case the hypothesis is by definition difficult to test, since it will be unlikely to find generalist herbivores on bracken in the field. Although laboratory experiments have shown selective feeding of bracken by generalist herbivores in the laboratory, it is difficult to extrapolate from these experiments into the situation found in the field, since these insects may never encounter bracken under normal conditions.

It is quite possible that cyanogenesis in bracken is a redundant character under temperate conditions. Bracken insects in Britain (Lawton, 1976) and in the north eastern U.S.A. (Schreiner, 1980) have been shown not to be affected by cyanogenesis. However, bracken in these regions is towards its northerly limits (Tryon, 1941; Page, 1976) and is thus likely to be somewhat stressed. In addition, there are comparatively few known herbivores of the plant in these regions (Lawton, 1976; Schreiner, 1980). It is possible that under warmer conditions, the rate of herbivory is higher and cyanogenesis may act as a herbivore deterrent. It is also possible that other herbivores which previously fed on bracken are not extant in Britain, or are managed, and hence do not feed on bracken to a significant degree. It is believed that the area of bracken-infested land has been increased largely due to the activities of man (Page, 1976, 1982a; Watt, 1976), and perhaps under 'natural' conditions cyanogenesis was more obviously functional.

Bracken possesses other putative defence mechanisms which seem to serve no function. For instance, the production of phytoecdysones by bracken has been shown to have no effect on insects (Jones and Firn, 1978). Similarly,

the production of nectar to attract ants appears to have no effect on herbivory in Britain (Heads and Lawton, 1984; Lawton and Heads, 1984) or in the cool temperate U.S.A. (Tempel, 1983). It is again possible that under greater insect pressure or warmer conditions either of these two systems may be effective as herbivore deterrents.

Cyanogenesis in bracken may have been an active herbivore deterrent in mature fronds under previous climatic conditions. The age of bracken stands is believe to be in excess of 1300 years in extreme cases (Oinonen, 1967a,b). The conditions prevailing at the time of establishment of a stand may well differ substantially from those currently found. In addition, the long generation time, coupled with the apparent rarity of sexual reproduction, could mean that there has not been a sufficient period during which the genes for cyanogenesis could be selected against. It is likely that if cyanogenesis has no function it would be selected against, because the production of the glycoside and glycosidase would represent unnecessary use of resources. It is unlikely that the trait would have neutral selective value.

Alternatively, cyanogenesis may be active elsewhere in the range of bracken, and be present in Britain due to introgression within the species. Again, it would take a considerable period for the genes responsible for cyanogenesis to be lost from the genome of bracken.

Bracken is a plant with a wide ecological tolerance and a particularly plastic response (Page, 1976; Fletcher and Kirkwood, 1979). This plasticity shows itself in the variable morphology of the plant, of both the rhizomes and fronds. It is possible that cyanogenesis is simply another highly plastic response to the prevailing conditions.

13.6. General Conclusions and Further Work Needed.

Throughout this research it has been found that cyanogenesis in bracken is a very variable character. The generation of such variation could arise at several levels. There is no evidence for genetic control of cyanogenesis in bracken, so that the variation found can only be phenotypically generated. A variety of environmental interactions have been implicated as producing variation of cyanogenesis within bracken, although at any one site differences between years are minimal. Cyanogenesis apparently has no function in adult plants of bracken, and it seems possible that cyanogenesis is another example of a plastic response similar to several other characteristics of bracken.

The research which has been described here has raised several points which could be the basis for further research. It would be most beneficial to

examine the effect of both nitrogen availability and light intensity simultaneously, using a blocked experimental design. This should be done in both controlled conditions, such as in a greenhouse, and under field conditions. These experiments would demonstrate whether the effects noted for each were dependent, as has been hypothesised here, or independent. This would require a large amount of replication to obtain workable results.

Several other environmental factors have been implicated as affecting cyanogenesis in mature bracken. It might prove possible to model the environmental determination of cyanogenesis by means of multivariate analysis, possibly using the data presented in chapter 4. Although this would be useful, it would not identify how such environmental control works. Modelling of this sort would also be time consuming, and as such it was felt that more basic ecological data were necessary for the current research, rather than concentrating on a particular aspect of the project.

It has been suggested that differences in the amount of HCN released by fronds under nitrogen and shading treatments were due to differential enzyme activities, due to only small differences resulting from the action of exogenous glycosidase on glycoside extracts taken from fronds grown under different treatments. This could be tested by obtaining active glycosidase extracts from fronds grown under these treatments. Similar work is needed on material grown in the field to show whether the differences between fronds within a site result from differential glycoside or glycosidase production.

It would be possible to investigate the effects of the environmental factors which were shown to be correlated with aspects of cyanogenesis in the field, by means of laboratory experiments. Thus the effect of water availability, soil factors and air temperature could all be studied.

The mode of action of the control of cyanogenesis in bracken needs to be studied for all these systems. This would require investigating the pathways of biosynthesis of prunasin and related compounds. The balance of secondary chemicals in bracken could prove to be very interesting, given the large number of these compounds produced by the plant. This work would need to be done on both laboratory and wild material. Work such as this may explain to some extent how bracken maintains its phenotypic plasticity.

The role of cyanogenesis at different stages of the life cycle of bracken, and in different parts of the world, must also be studied. The role of cyanogenesis in gametophytes and juvenile sporophytes in the field may prove to be especially fruitful. In addition, the importance of cyanogenesis to bracken growing in the tropics, perhaps under conditions of high competitive stress and

herbivory, should be considered.

These problems are similar to those encountered when cyanogenesis in other plants is considered, and relate to our understanding of the process of cyanogenesis and the role of secondary phytochemicals as well as to our knowledge of the ecology of bracken.

Appendix 1. Complete analysis of variance tables for the results presented in Chapter 4.

Table A1.1: The results of blocked analysis of variance on the transformed percentage of cyanogenic fronds sampled at each of three sites at nine location throughout Britain.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Locations	8	8342.8	1042.9	1.27 N.S.
Habitats	2	3278.5	1639.2	1.99 N.S.
Residual	13	10684.6	821.9	
Total	15	13963.1	930.9	
Grand Total	23	22305.9		

Mean transformed percentage of cyanogenic fronds sampled, according to the habitat type, ±S.E.

Coastal	17.4 ±7.2
Open	41.7 ±9.9
Woodland	44.6 ±13.0
Grand mean	35.4 ±10.6

N.S.: not significant.

Table A1.2: The results of blocked analysis of variance on the estimate of the mean amount of HCN released from cyanogenic fronds sampled at each of three sites at nine locations throughout Britain. The amount of HCN released was estimated from the results of the picrate test, scored after 24 hours.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Locations	8	119507	14938	1.68 N.S.
Habitats	2	44973	22487	2.52 N.S.
Residual	13	115824	8910	
Total	15	160789	10720	
Grand Total	23	280303		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
Coastal	45 $\pm$17.4
Open	66 $\pm$29.6
Woodland	142 $\pm$51.5
Grand Mean	85 $\pm$22.0

N.S.: not significant.

Table A1.3. The results of blocked analysis of variance analysis of variance of estimates of the mean amount of HCN released, estimated from the results of the picrate test scored after 24 hours, for all sites excluding site 2, the anomalous open habitat at location 1.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Locations	8	102702	12838	1.847 NS
Habitats	2	83403	28525	4.104 *
Residual	12	83403	6950	
Total	14	140453	10032	
Grand total	22	243155		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
Coastal	42.41 $\pm$17.45
Open	40.15 $\pm$15.35
Woodland	141.87 $\pm$51.49
Grand mean	76.22 $\pm$21.20

* Significant at 5% level ($P<0.05$).

Appendix 2. Complete analysis of variance tables of results presentedin Chapter 6.

Table A2.1. The results of analysis of variance of the transformed percentage of cyanogenic fronds at three sites sampled annually 1983 - 1985.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Years	2	375.26	187.63	3.988 N.S.
Habitats	2	576.16	288.08	6.123 N.S.
Residual	4	188.20	47.05	
Total	6	764.37		
Grand Total	8	1139.63		

Mean transformed percentage of cyanogenic fronds ±S.E.

Coastal	0
Open	11.7 ±6.3
Woodland	19.5 ±6.9
Grand mean	10.4 ±4.0

N.S. Not significant.

Table A2.2. The results of blocked analysis of variance of the estimated mean amounts of HCN released from cyanogenic pinna tips sampled annually at three habitat types, 1983 - 1984. The amount of HCN released was estimated from the results of the picrate test.

Source of variation	Degrees of freedom	Sum of squares	Mean ratio (F)	Variance
Years	2	964.9	482.4	2.752 N.S.
Habitats	2	30704.7	15352.4	87.583 ***
Residual	4	701.2	175.3	
Total	6	31405.9	5234.3	
Grand Total	8	32370.7		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
Coastal	0
Open	15.8 $\pm$8.0
Woodland	131.0 $\pm$14.6
Grand mean	48.9 $\pm$21.2

N.S. Not Significant; *** Significant at 0.1% level ($P<0.001$).

Table A2.3. The results of blocked analysis of variance of the mean fresh weight of representative pinna tips sampled at each of the three habitat sites in 1983 to 1985.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Years	2	4125	2062	1.305 N.S.
Habitats	2	17911	8955	5.669 N.S.
Residual	4	6319	1580	
Total	6	24229	4038	
Grand Total	8	28354		

Mean fresh weight of samples, mg ±S.E.	
Coastal	197.3 ±41.0
Open	169.2 ±6.8
Woodland	91.8 ±3.7
Grand mean	152.8 ±19.8

N.S. Not Significant

Table A2.4. The results of blocked analysis of variance of the mean height of fronds sampled for cyanogenesis at each of the three habitat sites in 1983 to 1985.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Years	2	815.16	407.58	11.78 *
Habitats	2	1747.63	873.81	25.27 **
Residual	4	138.38	34.60	
Total	6	1886.01	314.34	
Grand Total	8	2701.18		

Mean frond height, cm ±S.E.	
Coastal	123.7 ±7.7
Open	106.7 ±7.0
Woodland	140.8 ±7.1
Grand mean	123.7 ±6.1

* Significant at 5% level ($P<0.05$); ** Significant at 1% ($P<0.01$).

Table A2.5. The results of blocked analysis of variance of the transformed percentage of cyanogenic fronds sampled at each of four habitat sites at location six from 1978 to 1984.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Years	5	444.73	88.95	2.84 N.S.
Habitats	3	6750.97	2250.32	71.84 ***
Residual	13	407.22	31.32	
Total	16	7158.19	447.39	
Grand Total	21	7602.92		

Mean transformed percentage of cyanogenic fronds, ±S.E.	
Coastal (exposed)	11.3 ±1.2
Coastal (sheltered)	53.1 ±3.2
Open	50.9 ±4.0
Woodland	37.3 ±1.9
Grand mean	38.7 ±3.8

N.S. Not Significant; *** Significant at 0.1% level ($P<0.001$).

Appendix 3. Complete tables of analysis of variance of results presented in Chapter 7.

Table A3.1. Analysis of variance of the transformed percentage of cyanogenic fronds sampled at each of three sites in three different habitat types at location 6 in 1984.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Between habitats	2	775.9	388.0	1.082 N.S.
Within habitats	6	2151.7	358.6	
Total	8	2927.7		

Mean transformed percentage of cyanogenic fronds, ±S.E.	
coastal	17.6 ±8.9
open	21.1 ±11.4
woodland	38.9 ±12.2
Grand mean	25.9 ±10.9

N.S. Not Significant.

Table A3.2. Analysis of variance of the estimated mean amount of HCN released from cyanogenic fronds sampled at each of three sites in three different habitat types at location 6 in 1984.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Between habitats	2	19634.3	9817.2	41.7 ***
Within habitats	6	1413.8	235.6	
Total	8	21048		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
coastal	22.2 $\pm$11.9
open	29.8 $\pm$6.6
woodland	124.9 $\pm$7.1
Grand mean	58.9 $\pm$8.9

*** Significant at 0.1% ($P < 0.001$).

Appendix 4. Complete tables of analysis of variance of the results presented in Chapter 9.

Table A4.1. The results of analysis of variance of the estimated mean amount of HCN released by cyanogenic fronds from rhizomes which were attached to cyanogenic and acyanogenic fronds in the field prior to their transplantation from four sites at location 6 into experimental garden plots in Edinburgh. The rhizome type is defined by the phenotype of the frond attached to the rhizome sampled in the summer of 1982.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Rhizome	1	839.3	839.3	0.084 N.S.
Residual	12	120490.4	10040.9	
Total	13	121329.7		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
"Acyanogenic" transplants	123.9 $\pm$ 36.2
"Cyanogenic" transplants	140.1 $\pm$ 36.2
Grand mean	129.7 $\pm$ 25.8

N.S. Not significant.

Table A4.2.

a. The results of analysis of variance of the effect of the original habitat type of rhizomes transplanted from four sites at location 6 into garden plots in Edinburgh upon the estimated mean amount of HCN released by cyanogenic fronds.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Habitat	3	33796.9	11265.6	1.287 N.S.
Residual	10	87532.7	8753.3	
Total	13	121329.7		

Mean amount of HCN released, $\mu g\ g^{-1}$	$\pm$S.E.
coastal 1	14.6 $\pm$ 1.7
coastal 2	121.8 $\pm$72.2
open	158.4 $\pm$51.5
woodland	157.4 $\pm$35.0
Grand mean	129.7 $\pm$ 25.8

N.S. Not significant.

Table A4.2 - continued.

b. The results of analysis of variance of the effect of the original habitat type of rhizomes transplanted from four sites at location 6 into garden plots in Edinburgh upon the estimated mean amount of HCN released by cyanogenic fronds, amalgamating the results from coastal sites 1 and 2.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Habitat	2	20019.6	10009.8	1.087 N.S.
Residual	11	101310.1	9210.0	
Total	13	121329.7		

Mean amount of HCN released, $\mu g\ g^{-1}$	$\pm$S.E.
coastal	78.9 $\pm$47.5
open	158.4 $\pm$51.5
woodland	157.4 $\pm$35.0
Grand mean	129.7 $\pm$ 25.8

N.S. Not significant.

Table A4.3. The results of analysis of variance of the effect of the phenotype of fronds attached to the rhizome segments prior to transplantation on the mean fresh weight of pinna tips sampled for cyanogenesis.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Rhizome	1	212.87	212.87	0.492 N.S.
Residual	12	5191.19	432.60	
Total	13	5404.06		

Mean sample fresh weight, mg, ± S.E.	
"acyanogenic"	147.2 ±6.8
"cyanogenic"	155.3 ±9.7
Grand mean	150.1 ±7.9

N.S. Not significant.

Table A4.4. The results of analysis of variance of the effect of the phenotypes of fronds attached to the rhizome segments prior to their transplantation on mean height of fronds produced by rhizomes subsequently in cultivation.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Rhizome	1	2.73	2.73	0.055 N.S.
Residual	12	596.75	49.73	
Total	13	599.48		

Mean frond height, cm, ± S.E.	
"acyanogenic"	39.8 ±2.7
"cyanogenic"	38.9 ±1.6
Grand mean	39.5 ±2.7

N.S. Not significant.

Table A4.5. The results of analysis of variance of the effect of the original habitat from which the rhizome was transplanted on the mean fresh weights of pinna samples removed from fronds of each rhizome segment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Habitat	2	176.56	88.28	0.186 N.S.
Residual	11	5227.50	475.23	
Total	13	5404.06		

Mean sample fresh weight, mg, ± S.E.	
coastal	151.9 ±6.7
open	153.7 ±12.2
woodland	145.4 ±11.2
Grand mean	150.1 ±7.9

N.S. Not significant.

Table A4.6. The analysis of variance of the effect of the original habitat from which the rhizome was removed on the mean height of fronds produced by rhizome transplants.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Habitat	2	97.50	48.75	1.068 N.S.
Residual	11	501.99	45.63	
Total	13	599.48		

Mean frond height, cm, ± S.E.	
coastal	42.9 ±1.7
open	38.6 ±1.9
woodland	36.8 ±4.4
Grand mean	39.8 ±3.1

N.S. Not significant.

Table A4.7. The results of analysis of variance of the effect of the frond phenotype (cyanogenic or acyanogenic, according to the picrate test) on the fresh weights of pinna tips sampled for cyanogenesis.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Phenotype	1	599.0	599.0	0.301 N.S.
Residual	150	298366.0	1989.1	
Total	151	298965.0		

Mean sample fresh weight, mg, ± S.E.	
"acyanogenic"	150.6 ±7.0
"cyanogenic"	146.1 ±4.3
Grand mean	147.2 ±5.2

N.S. Not significant.

Table A4.8. The results of analysis of variance of the effect of the frond phenotype (cyanogenic or acyanogenic, according to the picrate test) on the height of fronds.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Phenotype	1	0.12	0.12	0.001 N.S.
Residual	150	25974.31	173.70	
Total	151	25974.19		

Mean frond height, cm, ± S.E.	
acyanogenic	39.7 ±1.2
cyanogenic	39.7 ±2.1
Grand mean	39.7 ±1.5

N.S. Not significant.

Table A4.9. The analysis of variance of the effect of the original habitat type on the mean amount of HCN released from fronds of rhizomes transplanted from location 8 into pots under greenhouse conditions in Edinburgh.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Habitat	1	18311.25	18311.25	1.502 N.S.
Residual	31	377936.50	12191.50	
Total	32	396247.75		

Mean amount of HCN released, $\mu g\ g^{-1} \pm$ S.E.

coastal	103.5 ±26.1
open	53.2 ±19.5
Grand mean	87.9 ±27.2

N.S. Not significant.

Table A4.10. The analysis of variance of the amount of HCN released by fronds (including acyanogenic) produced by different rhizomes. Only those rhizome segments that produced more than 1 frond have been included in the analysis.

a. coastal habitat.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Rhizome	4	168163.31	42040.83	4.306 *
Residual	18	175748.50	9763.80	
Total	22	343911.81		

Mean amount of HCN released, $\mu g\ g^{-1} \pm$ S.E.

C7	38.0 ±26.9
B1	0.9 ±0.5
A4	269.5 ±67.9
B2	114.0 ±64.3
A8	103.4 ±28.2
Grand mean	39.7 ±1.5

* Significant at 5% ($P<0.05$)

Table A4.10 – continued.

b. open habitat.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Rhizome	2	345.98	172.99	0.040 N.S.
Residual	9	38535.79	4281.75	
Total	11	3881.77		

Mean amount of HCN released, $\mu g\ g^{-1} \pm$ S.E.

C4	49.8 ±21.6
E7	40.8 ±38.9
A1	38.3 ±36.2
Grand mean	43.5 ±32.7

N.S. Not significant.

Appendix 5. Complete tables of analysis of variance of the results presented in Chapter 10.

Table A5.1. Analysis of variance of the amount of HCN released by cyanogenic fronds under each shade treatment, from the first series of samples, 19th May 1985.

a. All treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatment	2	59774.19	29887.09	4.213 *
Residual	33	234089.00	7093.61	
Total	35	293863.19		

Mean amount of HCN released, $\mu g\ g^{-1}$	$\pm$S.E.
50%	69.83 $\pm$34.90
25%	140.05 $\pm$19.65
12.5%	188.11 $\pm$21.68
Grand mean	136.46 $\pm$15.27

* Significant at 5% ($P<0.05$).

b. 50% and 25% treatments only.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatment	1	27761.87	27761.81	3.465 N.S.
Residual	25	200276.00	8011.04	
Total	26	228037.87		

Mean amount of HCN released, $\mu g\ g^{-1}$	$\pm$S.E.
50%	69.83 $\pm$34.90
25%	140.05 $\pm$19.65
Grand mean	119.24 $\pm$18.02

N.S. Not significant.

Table A5.1 - continued.

c. 50% and 12.5% treatments only.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatment	1	59254.75	59254.75	8.711 **
Residual	15	102039.87	6802.66	
Total	16	161294.62		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
50%	69.83 $\pm$34.90
12.5%	188.11 $\pm$21.68
Grand mean	132.45 $\pm$24.35

** Significant at 1% ($P<0.01$).

d. 25% and 12.5% treatments only.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatment	1	14105.44	14105.44	2.211 N.S.
Residual	26	165862.12	6379.31	
Total	27	179967.56		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
25%	140.05 $\pm$19.65
12.5%	188.11 $\pm$21.68
Grand mean	155.50 $\pm$15.43

N.S. Not significant.

Table A5.2. Analysis of variance of the amount of HCN released by cyanogenic fronds under each shade treatment, from the second series of samples, 29th July 1985.

a. All treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatment	2	29170.19	19655.12	3.625 N.S.
Residual	57	288824.56	5067.09	
Total	59	317994.73		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
50%	49.21 $\pm$26.58
25%	97.51 $\pm$11.49
12.5%	116.78 $\pm$16.95
Grand mean	92.83 $\pm$15.92

N.S. Not significant.

b. 50% and 25% treatments only.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatment	1	19655.12	19655.12	3.625 N.S.
Residual	45	243998.24	5422.18	
Total				

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
50%	49.21 $\pm$26.58
25%	97.51 $\pm$11.49
Grand mean	86.21 $\pm$11.04

N.S. Not significant.

Table A5.2 - continued.

c. 50% and 12.5% treatments only.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatment	1	27197.62	27197.62	4.883 *
Residual	22	122528.50	5569.48	
Total	23	263653.37		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
50%	49.21 $\pm$26.58
12.5%	116.78 $\pm$16.95
Grand mean	85.81 $\pm$16.47

* Significant at 5% level ($P<0.05$).

d. 25% and 12.5% treatments only.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatment	1	3544.62	3544.62	0.789 N.S.
Residual	47	211122.37	4491.96	
Total	48	214667.00		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
25%	97.51 $\pm$11.49
12.5%	116.78 $\pm$16.95
Grand mean	102.62 $\pm$9.55

N.S. Not significant.

Table A5.3. Analysis of variance of the amount of HCN released by cyanogenic fronds produced by replicates within each shade treatment for the first series of samples.

a. Replicate rhizomes in the 50% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	2	57386.12	28693.06	13.234 **
Residual	5	10840.69	2168.14	
Total	7	68226.81		

Mean amount of HCN released, $\mu g\ g^{-1}$	$\pm$S.E.
1	2.57 $\pm$1.30
2	6.73 $\pm$4.97
3	179.15 $\pm$42.39
Grand mean	69.83 $\pm$28.51

** Significant at 1% level ($P<0.01$)

b. Replicate rhizomes in the 25% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	5	56472.94	11294.59	1.943 N.S.
Residual	13	75576.25	5813.55	
Total	18	132049.19		

Mean amount of HCN released, $\mu g\ g^{-1}$	$\pm$S.E.
1	204.92 $\pm$30.44
2	88.53 $\pm$33.10
3	84.98 $\pm$45.15
4	200.66 $\pm$18.10
5	108.42 $\pm$60.10
6	106.01 $\pm$91.65
Grand mean	140.05 $\pm$42.85

N.S. Not significant.

Table A5.3 - continued.

c. Replicate rhizomes in the 12.5% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	2	4239.06	2119.53	0.430 N.S.
Residual	6	29573.81	4928.97	
Total	8	33812.87		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
1	228.45 $\pm$44.38
2	174.18 $\pm$43.69
3	179.78 $\pm$21.34
Grand mean	188.11 $\pm$40.53

N.S. Not significant.

Table A5.4. Analysis of variance of the amount of HCN released by cyanogenic fronds produced by replicate rhizomes within each shade treatment for the second sample.

a. Replicate rhizomes in the 50% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	2	20060.34	10030.17	1.392 N.S.
Residual	8	57641.78	7205.23	
Total	10	77702.12		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
1	1.95 $\pm$0.16
2	2.76 $\pm$1.13
3	88.20 $\pm$43.83
Grand mean	49.21 $\pm$44.32

N.S. Not significant.

b. Replicate rhizomes in the 25% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	5	25038.62	5007.61	1.064 N.S.
Residual	30	141258.00	4708.60	
Total	35	166296.06		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
1	66.58 $\pm$18.42
2	97.68 $\pm$34.10
3	84.14 $\pm$27.56
4	115.77 $\pm$21.77
5	150.47 $\pm$37.89
6	81.20 $\pm$18.21
Grand mean	97.51 $\pm$28.01

N.S. Not significant.

Table A5.4 - continued.

c. Replicate rhizomes in the 12.5% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	2	189.25	94.62	0.0212 N.S.
Residual	10	44637.00	4463.70	
Total	12	44826.25		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
1	117.37 $\pm$34.42
2	111.81 $\pm$26.96
3	122.21 $\pm$7.79
Grand mean	116.78 $\pm$32.09

N.S. Not significant.

Table A5.5. Analysis of variance of the amount of HCN released from cyanogenic fronds of the same treatment between the two samples.

a. The 50% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	1	1967.96	1967.96	0.229 N.S.
Residual	17	145929.00	8584.06	
Total	18	147897.00		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
1	69.83 $\pm$34.90
2	49.21 $\pm$26.58
Grand mean	52.89 $\pm$30.06

N.S. Not significant.

b. The 25% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	1	22503.37	22503.37	3.998 N.S.
Residual	53	298345.19	5629.15	
Total	54	320848.56		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
1	140.05 $\pm$19.65
2	97.51 $\pm$11.49
Grand mean	112.21 $\pm$14.31

N.S. Not significant.

Table A5.5 - continued.

c. The 12.5% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	1	27061.25	27061.25	6.882 *
Residual	20	78639.31	3931.97	
Total	21	105700.56		

Mean amount of HCN released, $\mu g\ g^{-1}$ ±S.E.	
1	188.11 ±21.97
2	116.78 ±16.95
Grand mean	145.96 ±18.91

* Significant at 5% level ($P<0.05$).

Table A5.6. Analysis of variance of the amount of HCN released by the enzymatic hydrolysis of β-glycoside extract from replicate rhizomes of bracken grown under three treatments of shading.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	2	17602.00	8801.00	1.727 N.S.
Residual	34	173234.00	5095.12	
Total	36	190836.00		

Mean amount of HCN released, $\mu g\ g^{-1}$ ±S.E.	
1	134.88 ±28.49
2	180.59 ±13.25
3	192.55 ±27.58
Grand mean	172.38 ±20.32

N.S. Not significant.

Table A5.7. Analysis of variance of the amount of HCN released by the enzymatic hydrolysis of β-glycoside extracts from replicate rhizomes in each of three shading treatments.

a. 50% treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	2	52294.31	26147.16	25.599 **
Residual	6	6128.56	1021.43	
Total	8	58422.87		

Mean amount of HCN released, µg g^{-1} ±S.E.	
1	88.37 ±21.26
2	67.13 ± 2.35
3	242.06 ±10.77
Grand mean	134.88 ±28.48

** Significant at 1% ($P<0.01$).

b. 25% treatments

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	5	24562.87	4912.57	1.799 N.S.
Residual	13	35498.25	2730.63	
Total	18	60061.12		

Mean amount of HCN released, µg g^{-1} ±S.E.	
1	197.94 ±19.62
2	134.88 ±19.51
3	164.10 ±24.17
4	166.49 ± 7.60
5	173.29 ±34.40
6	267.19 ±88.86
Grand mean	180.59 ±29.36

N.S. Not significant.

Table A5.7 - continued.

c. 12.5% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Replicates	2	442.87	221.44	0.024 N.S.
Residual	6	54307.50	9051.25	
Total	8	54750.37		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
1	203.99 $\pm$13.40
2	185.91 $\pm$65.30
3	193.79 $\pm$21.52
Grand mean	192.55 $\pm$54.92

Table A5.8. Analysis of variance of the amount of HCN released by two methods from cyanogenic fronds under three shading treatments. Frond samples were treated with toluene to release HCN from whole tissues, and glycoside extracts were prepared followed by the enzymatic hydrolysis of β-glycoside extracts of these fronds.

a. 50% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Method	1	23854.31	23854.31	2.914 N.S.
Residual	16	130984.06	8186.50	
Total	17	154838.37		

Mean amount of HCN released, µg g^{-1} ±S.E.	
Whole tissue	69.83 ±34.90
Glycoside extract	134.88 ±31.75
Grand mean	98.47 ±38.67

N.S. Not significant.

b. 25% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Method	1	15610.44	15610.44	2.925 N.S.
Residual	36	192109.56	5336.37	
Total	37	207720.00		

Mean amount of HCN released, µg g^{-1} ±S.E.	
Whole tissue	140.05 ±19.65
Glycoside extract	180.59 ±13.25
Grand mean	145.96 ±18.91

N.S. Not significant.

Table A5.8 - continued

c. 12.5% treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Method	1	88.69	88.69	0.016 N.S.
Residual	16	88563.44	5535.21	
Total	17	88652.12		

Mean amount of HCN released, $\mu g\ g^{-1}$ $\pm$S.E.	
Whole tissue	188.11 $\pm$21.97
Glycoside extract	192.55 $\pm$27.58
Grand mean	190.33 $\pm$24.80

N.S. Not significant.

Appendix 6. Complete tables of analysis of variance of the results presented to in Chapter 11.

Table A6.1. The analysis of variance of the amount of HCN (µg HCN g^{-1}) released by the action of toluene upon frond samples taken at the first sample of fronds produced by clonal rhizomes under three nutrient treatments. The HCN released was measured by the pyridine - pyrazolone method.

a. All treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	2	419002.00	209501.00	12.210 ***
Residual	40	686303.00	17157.57	
Total	42	1105305.00		

Mean amount of HCN released, µg g^{-1}, ±S.E.

10	298.46 ±31.90
1	99.37 ±38.08
0	102.12 ±31.46

*** Significant at 0.1% level ($P<0.001$).

b. High nitrogen (10) treatment compared with the low nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	249715.00	249715.00	13.146 **
Residual	28	531877.00	18995.60	
Total	29	7815592.00		

Mean amount of HCN released, µg g^{-1}, ±S.E.

10	298.46 ±31.90
1	99.37 ±38.08

** Significant at 1% ($P<0.01$).

Table A6.1 - continued.

c. High nitrogen (10) treatment compared with the nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	309551.00	309551.00	17.024 ***
Residual	32	581872.00	18183.50	
Total	33	891423.00		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.

10	298.46 $\pm$31.90
0	102.12 $\pm$31.46

*** Significant at 0.1% ($P<0.001$).

d. Low nitrogen (1) treatment compared with the nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	40.00	40.00	0.003 N.S.
Residual	20	258857.06	12942.85	
Total	21	258897.06		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.

1	99.37 $\pm$38.08
0	102.12 $\pm$31.46

N.S. Not significant.

Table A6.2. The analysis of variance of the amount of HCN (µg HCN g^{-1}) released by the action of toluene upon frond samples taken at the second sample of fronds produced by clonal rhizomes under three nutrient treatments. The HCN released was measured by the pyridine – pyrazolone method.

a. All treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	2	1042508.00	521254.00	48.600 ***
Residual	45	482646.00	10725.46	
Total	47	1525154.00		

Mean amount of HCN released, µg g^{-1}, ±S.E.

10	343.21 ±26.16
1	41.39 ±16.51
0	54.73 ±17.84

*** Significant at 0.1% level ($P<0.001$).

b. High nitrogen (10) treatment compared with the low nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	695861.00	695861.00	53.693 ***
Residual	34	440643.00	12960.09	
Total	35	1136504.00		

Mean amount of HCN released, µg g^{-1}, ±S.E.

10	343.21 ±26.16
1	41.39 ±16.51

*** Significant at 0.1% ($P<0.001$).

Table A6.2 - continued.

c. High nitrogen (10) treatment compared with the nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	674754.00	674754.00	52.172 ***
Residual	35	452663.00	12933.23	
Total	36	1127417.00		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.

10	343.21 $\pm$26.16
0	54.73 $\pm$17.84

*** Significant at 0.1% ($P<0.001$).

d. Low nitrogen (1) treatment compared with the nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	1021.39	1021.39	0.298 N.S.
Residual	21	71985.56	3427.88	
Total	22	73007.00		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.

1	54.73 $\pm$17.84
0	41.39 $\pm$16.51

N.S. Not significant.

Table A6.3. The analysis of variance of the amount of HCN (µg HCN g^{-1}) released by the action of toluene upon frond samples taken at the third sample of fronds produced by clonal rhizomes under three nutrient treatments. The HCN released was measured by the pyridine - pyrazolone method.

a. All treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	2	380808.62	190404.31	19.802 ***
Residual	56	538460.62	9615.35	
Total	58	919268.62		

Mean amount of HCN released, µg g^{-1}, ±S.E.	
10	198.08 ±18.35
1	23.40 ±10.60
0	43.35 ±26.47

*** Significant at 0.1% level ($P<0.001$).

b. High nitrogen (10) treatment compared with the low nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	237331.00	237331.00	24.833 ***
Residual	43	410956.00	9557.11	
Total	44	649287.00		

Mean amount of HCN released, µg g^{-1}, ±S.E.	
10	198.08 ±18.35
1	23.40 ±10.60

*** Significant at 0.1% ($P<0.001$).

Table A6.3 - continued.

c. High nitrogen (10) treatment compared with the nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	239431.00	239431.00	21.299 ***
Residual	47	528340.00	11241.27	
Total	48	767771.00		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.

10	198.08 $\pm$18.35
0	43.35 $\pm$26.47

*** Significant at 0.1% ($P<0.001$).

d. Low nitrogen (1) treatment compared with the nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	2321.02	2321.02	0.371 N.S.
Residual	22	137623.62	6255.62	
Total	23	139944.69		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.

Nitrogen treatment.

1	23.40 $\pm$10.60
0	43.35 $\pm$26.47

N.S. Not significant.

Table A6.4. The analysis of variance of the amount of HCN (µg HCN g^{-1}) released by the action of toluene upon frond samples taken at the fourth sample of fronds produced by clonal rhizomes under three nutrient treatments. The HCN released was measured by the pyridine - pyrazolone method.

a. All treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	2	589108.25	294554.12	28.002 ***
Residual	61	641654.75	10518.93	
Total	63	1230763.00		

Mean amount of HCN released, µg g^{-1}, ±S.E.

10	200.69 ±22.35
1	7.69 ± 3.85
0	6.99 ± 6.46

*** Significant at 0.1% level ($P<0.001$).

b. High nitrogen (10) treatment compared with the low nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	335234.00	335234.00	24.414 ***
Residual	46	631641.00	13731.32	
Total	47	966875.00		

Mean amount of HCN released, µg g^{-1}, ±S.E.

10	200.69 ±22.35
1	7.69 ± 3.85

*** Significant at 0.1% ($P<0.001$).

Table A6.4 - continued.

c. High nitrogen (10) treatment compared with the nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	415608.94	415608.94	32.485 ***
Residual	50	639698.06	12793.96	
Total	51	1055307.00		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
10	200.69 $\pm$22.35
0	6.99 $\pm$ 6.46

*** Significant at 0.1% ($P<0.001$).

d. Low nitrogen (1) treatment compared with the nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	3.40	3.40	0.007 N.S.
Residual	26	11969.45	460.36	
Total	27	11972.85		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
1	7.69 $\pm$ 3.85
0	6.99 $\pm$ 6.46

N.S. Not significant.

Table A6.5. The analysis of variance of the amount of HCN released from fronds within each of three nitrogen treatments to compare the four different series of samples taken.

a. The high nitrogen (10) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	3	425824.00	141941.31	8.366 ***
Residual	108	1832436.00		16967.00
Total	111	2258260.00		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.

1	296.66 $\pm$39.72
2	343.24 $\pm$26.15
3	199.88 $\pm$18.65
4	200.69 $\pm$22.35

*** Significant at 0.1% level ($P<0.001$).

b. The low nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	3	46984.25	15661.41	4.063 *
Residual	38	146486.31	3854.90	
Total	41	193470.56		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.

1	99.37 $\pm$38.08
2	41.39 $\pm$16.51
3	23.40 $\pm$10.60
4	7.75 $\pm$ 3.85

* Significant at 5% level ($P<0.05$).

Table A6.5 - continued.

c. The nitrogen-free (0) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	3	65225.06	21741.69	3.28 *
Residual	50	331024.69	6620.49	
Total	53	396249.75		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
1	102.12 $\pm$31.46
2	51.44 $\pm$19.20
3	44.85 $\pm$26.31
4	6.99 $\pm$ 6.46

* Significant at 5% level ($P<0.05$).

Table A6.6. The analysis of variance of the amount of HCN ($\mu g\ g^{-1}$) released by the action of toluene upon frond samples taken at the first sample of fronds produced by clonal rhizomes under four nutrient treatments. The HCN released was measured by the pyridine – pyrazolone method.

a. All treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	65949.31	21983.10	1.740 N.S.
Residual	30	378932.50	12631.08	
Total	33	444881.81		

Mean amount of HCN released, $\mu g\ g^{-1}$, ±S.E.	
10	128.91 ±45.04
5	65.70 ±21.87
2.5	10.06 ±6.38
1	53.31 ±30.11

N.S. Not significant.

b. High nitrogen (10) treatment compared with the medium nitrogen (5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	22586.62	22586.62	1.319 N.S.
Residual	21	359571.56	17122.45	
Total	22	382158.19		

Mean amount of HCN released, $\mu g\ g^{-1}$, ±S.E.	
10	128.91 ±45.04
5	65.70 ±21.87

N.S. Not significant.

Table A6.6 - continued.

c. The high nitrogen (10) treatment compared with the low nitrogen (2.5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	57988.50	57988.50	3.102 N.S.
Residual	17	317762.12	18691.89	
Total	18	375750.62		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
10	128.91 $\pm$45.04
2.5	10.06 $\pm$6.38

N.S. Not significant.

d. The high nitrogen (10) treatment compared with the lowest nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	20638.62	20638.62	0.987 N.S.
Residual	16	334677.56	20917.35	
Total	17	355316.19		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
10	128.91 $\pm$45.04
1	53.31 $\pm$30.11

N.S. Not significant.

Table A6.6 - continued.

e. The medium nitrogen (5) treatment compared with the low nitrogen (2.5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	11607.45	11607.45	3.672 N.S.
Residual	14	44254.83	3161.06	
Total	15	55862.28		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
5	65.70 $\pm$21.87
2.5	10.06 $\pm$6.38

N.S. Not significant.

f. The medium nitrogen (5) treatment compared with the lowest nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	511.28	511.28	0.109 N.S.
Residual	13	61170.32	4705.41	
Total	14	61661.60		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
5	65.70 $\pm$21.87
1	53.31 $\pm$30.11

N.S. Not significant.

Table A6.6 - continued.

g. The low nitrogen (2.5) treatment compared with the lowest (1) nitrogen treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	5101.69	5101.69	2.372 N.S.
Residual	9	19360.93	2151.21	
Total	10	24462.61		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
2.5	10.06 $\pm$6.38
1	53.31 $\pm$30.11

N.S. Not significant.

Table A6.7. The analysis of variance of the amount of HCN (µg HCN g^{-1}) released by the action of toluene upon frond samples taken at the second sample of fronds produced by clonal rhizomes under four nutrient treatments. The HCN released was measured by the pyridine - pyrazolone method.

a. All treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	73718.81	245572.94	12.219 ***
Residual	40	80444.56	2011.11	
Total	43	154163.37		

Mean amount of HCN released, µg g^{-1}, ±S.E.

10	108.58 ±13.54
5	51.88 ±11.64
2.5	16.42 ±3.14
1	18.55 ±11.55

*** Significant at 0.1% ($P<0.001$).

b. High nitrogen (10) treatment compared with the medium nitrogen (5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	19633.69	19633.69	7.049 *
Residual	26	72415.81	2785.22	
Total	27	92049.50		

Mean amount of HCN released, µg g^{-1}, ±S.E.

10	108.58 ±13.54
5	51.88 ±11.64

* Significant at 5% ($P<0.05$).

Table A6.7 - continued.

c. The high nitrogen (10) treatment compared with the low nitrogen (2.5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	47817.12	47817.12	18.911 ***
Residual	25	63212.56	2528.50	
Total	26	111029.69		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
10	108.58 $\pm$13.54
2.5	16.42 $\pm$3.14

*** Significant at 0.1% ($P<0.001$).

d. The high nitrogen (10) treatment compared with the lowest nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	45629.87	45629.87	16.264 ***
Residual	25	70138.56	2805.54	
Total	26	115768.44		

Mean amount of HCN released, $\mu g\ g^{-1}$, $\pm$S.E.	
10	108.58 $\pm$13.54
1	18.55 $\pm$11.55

*** Significant at 0.1% ($P<0.001$).

Table A6.7 - continued.

e. The medium nitrogen (5) treatment compared with the low nitrogen (2.5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	5326.16	5326.16	7.752 *
Residual	15	10305.99	687.07	
Total	16	15632.14		

Means.	$\mu g\ HCN\ g^{-1}$, $\pm$S.E.
5	51.88 $\pm$11.64
2.5	16.42 $\pm$3.14

* Significant at 5% ($P<0.05$).

f. The medium nitrogen (5) treatment compared with the lowest nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	4704.84	4704.84	4.095 N.S.
Residual	15	17231.79	1148.80	
Total	16	21936.79		

Means.	$\mu g\ HCN\ g^{-1}$, $\pm$S.E.
5	51.88 $\pm$11.64
1	18.55 $\pm$11.55

N.S. Not significant.

Table A6.7 - continued.

g. The low nitrogen (2.5) treatment compared with the lowest nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	18.19	18.19	0.032 N.S.
Residual	14	8028.68	573.48	
Total	15	8046.88		

Means.	$\mu g\ HCN\ g^{-1}$, $\pm$S.E.
2.5	16.42 $\pm$3.14
1	18.55 $\pm$11.55

N.S. Not significant.

Table A6.8. The comparison of the amount of HCN released by frond samples removed from fronds produced by replicate rhizomes at two sets of samples, within four nitrogen treatments. The HCN was released by the action of toluene upon whole tissues and was analysed by the pyridine - pyrazolone test.

a. The high nitrogen (10) treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	1	3190.69	3190.69	0.252 N.S.
Residual	30	379200.56	12640.02	
Total	31	382391.25		

Means.	$\mu g\ HCN\ g^{-1}$, $\pm$S.E.
1	128.91 $\pm$45.04
2	108.58 $\pm$13.54

N.S. Not significant.

b. The medium nitrogen (5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	1	904.25	904.25	0.291 N.S.
Residual	17	52786.75	3105.10	
Total	18	53691.00		

Means.	$\mu g\ HCN\ g^{-1}$, $\pm$S.E.
1	65.70 $\pm$21.87
2	51.88 $\pm$11.64

N.S. Not significant.

Table A6.8 - continued.

c. The low nitrogen (2.5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	1	138.54	138.54	0.937 N.S.
Residual	12	1774.08	147.84	
Total	13	1912.62		

Means.	$\mu g\ HCN\ g^{-1}$, $\pm$S.E.
1	10.06 $\pm$6.38
2	16.42 $\pm$3.14

N.S. Not significant.

d. The lowest nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Samples	1	3718.03	3718.03	1.597 N.S.
Residual	11	25615.53	2328.68	
Total	12	29333.56		

Means.	$\mu g\ HCN\ g^{-1}$, $\pm$S.E.
1	53.31 $\pm$30.11
2	18.55 $\pm$11.55

N.S. Not significant.

Table A6.9. The analysis of variance of the amount of HCN released under the action of β-glycosidase from β-glycoside extracts taken from fronds from rhizomes grown under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	51223.87	17074.62	2.443 N.S.
Residual	30	209652.12	6988.40	
Total	33	260876.00		

Means.	μg HCN g^{-1}, ±S.E.
10	123.72 ±26.55
5	176.31 ±45.14
2.5	64.41 ±15.54
1	73.38 ±34.49

N.S. Not significant.

Table A6.10. The comparison of the amounts of HCN released from whole tissue frond samples, treated with toluene, and β-glycoside extracts, under the action of β-glycosidase, taken from fronds under four nitrogen treatments. Each nitrogen treatment is analysed separately.

a. High nitrogen (10) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	168.44	168.44	0.009 N.S.
Residual	23	409588.81	17808.20	
Total	24	409757.25		

Means.	µg HCN g^{-1}, ±S.E.
Toluene	128.91 ±45.04
Extract	123.72 ±26.55

N.S. Not significant.

b. The medium nitrogen (5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	36011.87	36011.87	4.216 N.S.
Residual	18	153756.69	8542.03	
Total	19	189768.56		

Means.	µg HCN g^{-1}, ±S.E.
Toluene	65.70 ±21.87
Extract	176.31 ±45.14

N.S. Not significant.

Table A6.10 - continued.

c. The low nitrogen (2.5) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	8861.21	8861.21	10.46 **
Residual	10	8470.44	847.04	
Total	11	17331.64		

Means.	µg HCN g^{-1}, ±S.E.
Toluene	10.06 ±6.38
Extract	64.41 ±15.54

** Significant at 1% ($P<0.01$).

d. The lowest nitrogen (1) treatment.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	1	1006.59	1006.59	0.192 N.S.
Residual	8	41929.15	5241.14	
Total	9	42935.74		

Means.	µg HCN g^{-1}, ±S.E.
Toluene	53.31 ±30.11
Extract	73.38 ±34.49

N.S. Not significant.

Table A6.11. The analysis of variance of the number of fronds produced by each of three replicate rhizome systems grown under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	36.000	12.000	7.579 *
Residual	8	12.667	1.583	
Total	11	48.667		

Mean number of fronds, ±S.E.	
10	6.67 ±1.20
5	2.67 ±0.33
2.5	2.67 ±0.67
1	2.67 ±0.33

* Significant at 5% level ($P<0.05$).

Table A6.12. The analysis of variance of the fresh weight of fronds produced by each of three replicate rhizome systems grown under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	24.19	8.06	2.364 N.S.
Residual	39	133.0	13.41	
Total	42	157.19		

Means fresh weight of fronds, g ±S.E.	
10	3.69 ±0.51
5	3.29 ±0.61
2.5	2.89 ±0.25
1	1.66 ±0.42

N.S. Not significant.

Table A6.13. The analysis of variance of the dry weight of fronds produced by each of three replicate rhizome systems grown under four nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	0.821	0.274	1.369 N.S.
Residual	39	7.197	0.200	
Total	42	8.018		

Mean dry weight of fronds, g ±S.E.	
10	0.745 ±0.140
5	0.734 ±0.138
2.5	0.689 ±0.070
1	0.375 ±0.101

N.S. Not significant.

Table A6.14. The analysis of variance of the angular transformation the dry weight of fronds as a percentage of fresh weight produced by each of three replicate rhizome systems under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	5.094	1.698	0.503 N.S.
Residual	37	124.988	3.378	
Total	40	130.082		

Mean transformed percentage dry weight, ±S.E.	
10	28.47 ±0.51
5	28.35 ±0.60
2.5	29.20 ±0.62
1	28.08 ±0.42

N.S. Not significant.

Table A6.15. The analysis of variance of the lamina length of fronds produced by each of three replicate rhizome systems grown under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	212.05	70.68	2.896 *
Residual	37	902.93	24.40	
Total	40	1114.99		

Mean frond lamina length, cm ±S.E.

10	19.61 ±1.28
5	17.94 ±1.09
2.5	17.43 ±2.55
1	13.45 ±1.01

* Significant at 5%, $P<0.05$.

Table A6.16. The analysis of variance of the number of rhizome branches produced by each rhizome system in each of three replicates grown under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	248.916	82.972	8.095 **
Residual	8	82.000	10.250	
Total	11	330.917		

Mean number of rhizome branches, ±S.E.

10	18.00 ±2.89
5	11.33 ±1.86
2.5	8.67 ±1.20
1	5.67 ±0.67

** Significant at 1% level ($P<0.01$).

Table A6.17. The analysis of variance of the length of long shoot rhizome branches produced by each of three replicate rhizome under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	441.31	147.10	1.430 N.S.
Residual	127	13061.32	102.84	
Total	130	13502.64		

Mean rhizome branch length, cm	±S.E.
10	13.89 ±1.17
5	16.66 ±1.48
2.5	15.51 ±2.14
1	19.65 ±3.67

N.S. Not significant.

Table A6.18. The analysis of variance of the total length of the rhizome system produced by each of three replicates under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	36556.87	12185.62	6.289 *
Residual	8	15500.94	1937.51	
Total	11	52056.94		

Mean total length rhizomes, cm	±S.E.
10	250.00 ±36.91
5	166.33 ±18.89
2.5	134.67 ±11.92
1	101.33 ±26.87

* Significant at 5% level ($P<0.05$).

Table A6.19. The analysis of variance of the total number of short shoot (frond bearing) rhizomes produced by each of three replicates grown under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	6187.99	2062.66	12.713 **
Residual	8	1298.00	162.25	
Total	11	7486.00		

Mean number of short shoots, ±S.E.

10	91.33 ±9.94
5	45.33 ±3.84
2.5	43.33 ±7.33
1	32.00 ±7.00

** Significant at 1% level ($P < 0.01$).

Table A6.20. The analysis of variance of the number of short shoot (frond bearing) rhizomes produced by each long shoot rhizome branch, of each of three replicates under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	38.66	12.89	2.094 N.S.
Residual	127	781.59	6.15	
Total	130	820.24		

Mean number of short shoots per long shoot, ±S.E.

10	5.07 ±0.31
5	4.00 ±0.37
2.5	5.00 ±0.55
1	5.65 ±0.78

N.S. Not significant.

Table A6.21. The analysis of variance of the total number of frond buds produced by each of three replicates under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	90569.62	30189.87	11.12 **
Residual	8	21726.06	2715.76	
Total	11	112295.69		

Mean number of frond buds, ±S.E.

10	344.67 ±51.91
5	166.33 ± 8.95
2.5	147.00 ±17.62
1	126.67 ±23.14

** Significant at 1% level ($P<0.01$).

Table A6.22. The analysis of variance of the number of frond buds produced per long shoot rhizome branch by each of three replicates under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	807.48	269.16	2.459 N.S.
Residual	127	13900.50	109.45	
Total	130	14707.97		

Mean number of frond buds per long shoot, ±S.E.

10	19.33 ±1.36
5	14.85 ±1.69
2.5	16.58 ±1.94
1	22.34 ±3.28

N.S. Not significant.

Table A6.23. The analysis of variance of the total number of frond buds per rhizome unit length produced by each of three replicates under four nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	0.242	0.081	2.131 N.S.
Residual	8	0.303	0.038	
Total	11	0.544		

Mean number of frond buds per rhizome unit length, cm^{-1}	±S.E.
10	1.38 ±0.01
5	1.03 ±0.14
2.5	1.10 ±0.13
1	1.30 ±0.12

N.S. Not significant.

Table A6.24. The analysis of variance of the total rhizome fresh weight of each of three replicates under four different nitrogen

treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	81824.37	27274.79	54.933 ***
Residual	8	3972.06	496.51	
Total	11	85796.44		

Mean rhizome fresh weight, g	±S.E.
10	279.06 ±20.60
5	119.97 ± 3.30
2.5	102.36 ±14.25
1	61.76 ± 4.88

*** Significant at 0.1% level ($P<0.001$).

Table A6.25. The analysis of variance of the total rhizome fresh weight per total rhizome unit length of each of three replicates under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	0.418	0.139	4.508 *
Residual	8	0.247	0.031	
Total	11	0.665		

Mean rhizome fresh weight, g cm^{-1} ±S.E.	
10	1.14 ±0.11
5	0.74 ±0.09
2.5	0.77 ±0.10
1	0.66 ±0.10

* Significant at 5% level ($P<0.05$).

Table A6.26. The analysis of variance of the total rhizome dry weight of each of three replicates under four nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	4275.32	1425.11	94.286 ***
Residual	8	120.92	15.11	
Total	11	4396.25		

Mean rhizome dry weight, g ±S.E.	
10	62.35 ±3.51
5	26.93 ±0.62
2.5	19.66 ±2.21
1	13.75 ±1.61

*** Significant at 0.1% level ($P<0.001$).

Table A6.27. The analysis of variance of the total rhizome dry weight per rhizome unit length.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	0.0252	0.0084	6.370 *
Residual	8	0.0105	0.0013	
Total	11	0.0357		

Mean rhizome dry weight, g cm^{-1}	±S.E.
10	0.257 ±0.027
5	0.165 ±0.014
2.5	0.147 ±0.016
1	0.146 ±0.024

* Significant at 5% level ($P<0.05$).

Table A6.28. The analysis of variance of the angular transformation of the dry weight of rhizomes as a percentage of fresh weight produced by each of three replicates under four different nitrogen treatments.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Treatments	3	10.22	3.41	3.030 N.S.
Residual	8	8.99	1.12	
Total	11	19.21		

Mean angular transformation of percentage dry rhizome weight, ±S.E.	
10	28.25 ±0.34
5	28.31 ±0.77
2.5	26.08 ±0.39
1	28.06 ±0.80

N.S. Not significant.

Appendix 7. Complete tables of analysis of variance of the results presented in chapter 12.

Table A7.1. The analysis of variance of the amount of HCN released by gametophytes sampled from populations grown from spores taken from fronds at the fieldsite at Blackhill. The mean values are given in Table 12.1.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Population	5	3222.90	644.58	2.467 *
Residual	53	13847.08	261.27	
Total	58	17069.98		

* Significant at 5% ($P<0.05$).

Table A7.2. The analysis of variance of the amount of HCN released from gametophytes sampled from populations grown up from spores taken from fronds at the fieldsite at Gosford Bay. The mean values are given in Table 12.1.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Population	1	316.52	316.52	3.236 N.S.
Residual	6	586.88	97.81	
Total	7	903.40		

N.S. Not significant.

Table A7.3. The analysis of variance of the amount of HCN released from gametophytes sampled from populations raised from spores taken from Blackhill and Gosford Bay.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Population	1	147.64	147.64	0.534
Residual	65	17973.40	276.52	
Total	66	18121.04		

Means.	μg HCN g^{-1}, $\pm$S.E.
Blackhill	11.52 $\pm$2.23
Gosford Bay	6.95 $\pm$4.02

N.S. Not significant.

Table A7.4. The analysis of variance of the amount of HCN released from clonal gametophytes produced from spores taken from fronds at the Blackhill fieldsite. The mean values are given in Table 12.3.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Clones	20	12813.73	640.69	4.800 ***
Residual	59	7875.64	133.48	
Total	79	20689.37		

*** Significant at 0.1% level ($P<0.001$).

Table A7.5. The analysis of variance of the amount of HCN released from clonal gametophytes produced from spores taken from the Gosford Bay fieldsite. The mean values are given in Table 12.3.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Clones	10	35009.83	3500.98	4.131 **
Residual	29	24577.06	847.48	
Total	39	59586.89		

** Significant at 1% level ($P<0.01$).

Table A7.6. The analysis of variance of HCN released from clonal gametophytes produced from spores taken from Blackhill and Gosford Bay.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Population	1	6916.81	9616.81	9.322 **
Residual	118	87553.37	741.98	
Total	119	94470.19		

** Significant at 1% level ($P<0.01$).

Mean µg HCN g^{-1}, ± S.E.	
Blackhill	22.27 ±2.10
Gosford Bay	38.38 ±6.18

Table A7.7. The analysis of variance of HCN released from fronds sampled juvenile sporophytes raised from populations of gametophytes grown from spores taken from fronds at the field site at Blackhill.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Population	6	37426.68	6237.78	4.860 ***
Residual	98	12579.82	1283.57	
Total	104	163216.50		

*** Significant at 0.1% level ($P<0.001$).

Means amount of HCN released, $\mu g\ g^{-1}$, $\pm$ S.E.

Gametophyte population	
B8	16.13 $\pm$7.10
B10	56.75 $\pm$0.75
C9	10.39 $\pm$4.57
D10	21.79 $\pm$8.65
F7	17.17 $\pm$5.74
G3	43.88 $\pm$12.50
H9	20.46 $\pm$6.13

Table A7.8. The analysis of variance of the amount of HCN released from fronds of juvenile sporophytes raised from clonal gametophytes. The mean values are given Table 12.7.

Source of variation	Degrees of freedom	Sum of squares	Mean squares	Variance ratio (F)
Sporophyte	11	61010.69	6101.07	5.777 ***
Residual	36	38016.19	1056.00	
Total	46	99026.87		

*** Significant at 0.1% level ($P<0.001$).

Appendix 8.

Dyer and Hadfield (1985). Polymorphism for cyanogenesis in British Bracken (Pteridium aquilinum subsp. aquilinum var. aquilinum).

Proceedings of the Royal Society of Edinburgh, 86B: 462 - 464.

Polymorphism for cyanogenesis in British bracken (*Pteridium aquilinum*, subsp. *aquilinum* var. *aquilinum*)

A. F. Dyer and **P. R. H. Hadfield**

Department of Botany, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JH, U.K.

Cyanogenesis, the release of cyanide from damaged tissue, occurs in many plants (Conn 1980) and in some the character is polymorphic because, on the basis of a simple field test, some individuals are apparently acyanogenic. Bracken is a cyanogenic species (Greshoff 1908) and limited studies at two locations in England have recently revealed it to be polymorphic (Cooper-Driver and Swain 1976; Lawton 1976). Our investigation has been initiated to study the occurrence of cyanogenesis in bracken throughout Britain and to determine the basis for the polymorphism.

In 9 locations throughout mainland Britain, well-grown bracken stands were selected in 3 different habitats: woodland, open field or heath and, where available, coastal sites. At each location, the chosen sites were as close as possible to minimise the effects of other factors. At each site, an 18 m × 18 m grid was marked out and 50 or 100 fronds sampled at regular intervals. The terminal 4 cm of the lowermost pinna of each frond sampled was tested in a 50 × 12 mm stoppered tube by the modified sodium picrate test for HCN (Jones 1966). The bright yellow test paper changes colour in the presence of cyanide, becoming pale orange to chocolate brown

depending on the cyanide concentration. Previous experiments, using known amounts of HCN liberated from KCN solutions by concentrated HCl, had shown that the colour change can be used as a semi-quantitative test.

Most sites were sampled once during 1983, but at one location in SE Scotland, samples were taken at intervals throughout the growing season (Table 1) and at one location in SW Scotland, 4 additional sites were tested annually in July from 1978 to 1982 (Table 2). The picrate test and the more specific Fiegl–Anger test (Fiegl and Anger 1966) gave similar results for these sites.

Table 1. Percentage of cyanogenic fronds in samples from the two sites in SE Scotland tested throughout the summer of 1983. Sample size in brackets; from the original sample of 100 at both sites, several fronds had died or could not be found on subsequent occasions

Site	Date			
	Early June	Mid-July	Mid-August	Early September
Coastal	68 (100)	39 (94)	8 (83)	3 (77)
Open	18 (100)	17 (95)	12 (68)	9 (55)

Table 2. Percentage of cyanogenic fronds in samples from 4 sites in SW Scotland tested in July each year for 5 years. (From 1978 to 1980, the grids were located within about 10 m of the previous year's site. From 1980 to 1982, the sample areas coincided within about 1 m.)

Site	Year					
	1978	1979	1980	1981	1982	Mean
Coastal (exposed dune)	4	3	7	2	4	4
Coastal (partially sheltered)	59	73	79	53	54	64
Open (upland pasture)	34	80	67	62	68	62
Woodland (lowland deciduous)	35	38	51	27	33	37

The results showed bracken to be polymorphic for cyanogenesis throughout Britain. The percentage of cyanogenic fronds in samples from different sites varied from 0 to 100%. Most sites contained both cyanogenic and acyanogenic fronds with a mosaic distribution on the grid. The two phenotypes were otherwise indistinguishable.

At most localities, fronds from woodland sites produced high levels of cyanide, although not always the largest percentage of cyanogenic fronds. The percentage of cyanogenic fronds, and the amount of cyanide they produced, was low at most coastal sites. For woodland sites, samples from the south and east of Britain had a higher percentage of cyanogenic fronds than those from the north and west.

Sites at the location sampled at monthly intervals during the growing season showed a decrease as the season progressed in the percentage of cyanogenic fronds in a sample (Table 1). However, some fronds were acyanogenic from the time of emergence.

The four sites sampled annually showed little change in the percentage of cyanogenic fronds over a period of 5 years (Table 2).

These observations show many parallels with those for *Trifolium repens* (Daday 1954a, b) and *Lotus corniculatus* (Jones 1977), where the existence of a genetically determined polymorphism, sometimes modified by developmental and environmental factors, has been proposed. The stable polymorphic mosaics in bracken are most easily explained in the same way. However, a genetic basis for the polymorphism in bracken has not yet been confirmed, and additional, preliminary, results include the observations that (i) rhizomes producing acyanogenic fronds in the field produced cyanogenic fronds the following season when transplanted into garden plots, and (ii) spores collected from acyanogenic fronds in the field produced cyanogenic sporelings in laboratory cultures. These turned acyanogenic when they became pot bound, as did sporelings raised from spores produced by cyanogenic fronds.

These suggest that the physiological state of the rhizomes or individual frond buds may be important in influencing the expression of the cyanogenic genotype in the fronds which develop from them. Thus, local variations in light, nutrient or water availability may influence the cyanogenic phenotype. It may even be that acyanogenic genotypes do not exist in bracken. The causes of the polymorphism are currently being investigated. An understanding of the underlying mechanism is needed in order to assess the ecological role, if any, of cyanogenesis, and the possibilities of using cyanogenesis as a genetic marker for the study of bracken population structure. This in turn has important implications for strategies of bracken control.

This work is supported by a NERC CASE studentship to P.R.H.H.

Conn, E. E. 1980. Cyanogenic glycosides. In *Encyclopaedia of Plant Physiology*, ed. Bell, E. A. and Charlwood, B. V., Vol. 8, pp. 461–492. Berlin: Springer.

Cooper-Driver, G. and Swain, T. 1976. Cyanogenic polymorphism in bracken in relation to herbivore predation. *Nature* **260**, 604.

Daday, H. 1954a. Gene frequencies in wild populations of *Trifolium repens*. I. Distribution by latitude. *Heredity* **8**, 61–78.

—— 1954b. Gene frequencies in wild populations of *Trifolium repens*. II. Distribution by altitude. *Heredity* **8**, 377–384.

Fiegl, F. and Anger, V. 1966. Replacement of benzidine by copper ethyl acetoacetate and tetrabase as spot test reagent for Hydrogen cyanide and cyanogen. *Analyst* **91**, 282–284.

Greshoff, M. 1908. Transitorische Blausäure in Farnen. *Pharm. Weekbl. Ned.* **45**, 770–773.

Jones, D. A. 1966. On the polymorphism of cyanogenesis in *Lotus corniculatus* I. Selection by animals. *Can. J. Genet.* **8**, 556–567.

—— 1977. On the polymorphism of cyanogenesis in *Lotus corniculatus* VII. The distribution of the cyanogenic form in Western Europe. *Heredity* **39**, 27–44.

Lawton, J. H. 1976. The structure of the arthropod community on bracken. *Bot. J. Linn. Soc.* **73**, 187–216.

References.

Abbott, R.J. (1977). A quantitative association between soil moisture content and the frequency of the cyanogenic form of Lotus corniculatus L. at Birsey, Orkney. Heredity, 38: 397-400.

Abbott, R.J. (1981). Polymorphism for cyanogenesis in Lotus corniculatus L. on links and machair in Orkney and the Outer Hebrides. Transactions of the Botanic Society of Edinburgh, 43: 337-342.

Abrol, Y.P., Conn, E.E., and Stoker, J.R. (1966). Studies on the identification and metabolism of a cyanogenic glucoside in Nandina domestica Thumb. Phytochemistry, 5: 1021-1027.

Alvey, N., Galway, N., and Lane, P. (1982). An Introduction to Genstat. Academic Press, London.

Anderson, D.J. (1961). The structure of upland plant communities in Caernarvonshire. 1. The pattern shown by Pteridium aquilinum. Journal of Ecology, 49: 369-376.

Anderson, M.C. (1964). Light relations of terrestrial plant communities and their measurement. Biological Reviews, 39: 425-486.

Angseesing, J.P.A. (1974). Selective eating of the acyanogenic form of Trifolium repens L. Heredity, 32: 73-83.

Angseesing, J.P.A., and Angseesing, W.J. (1973). Field observations on the cyanogenesis polymorphism in Trifolium repens L. Heredity, 31: 276-282.

Anon. (1966). Colour Chart. Royal Horticultural Society, London.

Anon. (1972). Tables of temperature, relative humidity, precipitation and sunshine for the World. Part III. Europe and the Azores. (MetO856C). H.M.S.O, London.

Armstrong, H.E., Armstrong, E.F., and Horton, E. (1913). Herbage studies II. Variation in Lotus corniculatus and Trifolium repens (cyanophoric plants). Proceedings of the Royal Society, 85: 262-269.

Askew, H.O. (1933). Determination of hydrocyanic acid in white clover. New Zealand Journal of Science and Technology, 15: 227-233.

Balick, M.J., Furth, D.G., and Cooper-Driver, G. (1978). Biochemical and evolutionary aspects of arthropod predation on ferns. Oecologia, 35: 55-89.

Bate-Smith, E.C. (1973). Haemanalysis of tannins: the concept of relative astringency. Phytochemistry, 12: 907-912.

Beck, S.D., and Reese, J.C. (1976). Insect - plant interactions: nutrition and metabolism. Recent Advances in Phytochemistry, 10: 41-92.

Beesley, S.G., Compton, S.G., and Jones, D.A. (1985). Rhodanese in insects. Journal of Chemical Ecology, 11: 45-50.

Beevers, L., and Hageman, R.H. (1980). Nitrate and nitrite reduction. In: The Biochemistry of Plants, Volume 5. The Amino Acids and Derivatives (Ed. Miflin, B.J.). pp. 116-168. Academic Press, London.

Bell, E.A. (1984). Plant - plant interactions. In: The Origins and Development of Adaptation (Ciba Foundation Symposium 102), pp. 40-47. Pitman Books, London.

Bell, P.R. (1958). Variations in germination rate and development of fern spores in culture. Annals of Botany (New Series), 22: 503-511.

Bell, P.R. (1979). The contribution of the ferns to an understanding of the life cycles of vascular plants. In: The Experimental Biology of Ferns (Ed. A.F. Dyer), pp. 58-86. Academic Press, London.

Bell, P.R. (1985). Introduction: the essential role of the Pteridophyta in the study of land plants. Proceedings of the Royal Society of Edinburgh, 86B: 1-4.

Bell, P.R., and Duckett, J.G. (1976). Gametogenesis and fertilisation in Pteridium. Botanical Journal of the Linnean Society, 73: 47-78.

Bennett, W.D. (1968). Isolation of the cyanogenetic glucoside prunasin from bracken fern. Phytochemistry, 7: 151-152.

Bernays, E.A. (1977). Cyanogenic glycosides in plants and their relevance in protection from insect attack. W.R.P.S. Bulletin, 1977 (3): 123-128.

Bishop, J.A., and Korn, M.E., (1969). Natural selection and cyanogenesis in white clover, Trifolium repens L. Heredity, 24: 423-430.

Blaim, H., and Nowacki, E. (1979). Cyanogenesis in Lotus and Trifolium species. Acta Agrabotanica, 22: 19-26.

Blair, R.M., Alcaniz, R., and Harrell, A. (1983). Shade intensity influences the nutrient quality and digestibilty of southern deer browse leaves. Journal of Range Management, 36: 257-264.

Bloom, A.J., Chapin, F.S., and Mooney, H.A. (1985). Resource limitation in plants - an economic analogy. Annual Review of Ecology and Systematics, 16: 363-392.

Boodle, L.A. (1904). Structure of the leaves of bracken (Pteris aquilina) in relation to the environment. Journal of the Linnean Society (Botany), 35: 659-669.

Boersma, P., Kakes, P., and Schram, A.W. (1983). Linamarase and β-glucosidase activity in natural populations of Trifolium repens L. Acta Botanica Neerlandica, 32: 39 -47.

Bower, F.O. (1928). The Ferns, Volume III. Cambridge University Press, Cambridge.

Boyd. F.T., Aamodt, D.S., Bohstedt, G., and Truog, E. (1938). Sudan grass management for control of cyanide poisoning. Journal of American Society of Agronomists, 40: 161-167.

Braid, K.W. (1947). Bracken control - artificial and natural. Journal of the British Grassland Society, 2: 181-189.

Braid, K.W. (1959). Bracken: a review of the literature. Commonwealth Agricultural Bureaux Mimeographed Publication, No.3.

Braid, K.W., and Conway, E. (1943). The rate of growth of bracken. Nature, 152: 750-751.

Briggs, D., and Walters, M., (1984). Plant Variation and Evolution. Second edition. Cambridge University Press, Cambridge.

Brighton, F., and Horne, M.T. (1977). Influence of temperature on cyanogenic polymorphisms. Nature, 265: 437-438.

Burdon, J.J. (1980). Intra-specific diversity in a natural population of Trifolium repens. Journal of Ecology, 68: 717-735.

*

Burke, D.P.T. (1953). A study of the influence of light and soil properties on the growth of bracken (Pteridium aquilinum). Magazine of Blundell's School Science Society, 8: 13-17.

Burns, J.C., Smith, L.H., Moline, W.J., Wedin, W.F., Noller, C.H., and Rhykerd, C.L. (1970). Quantification of hydrocyanic acid in green forage. Crop Science, 10: 578-581.

Cahn, M.G., and Harper, J.L. (1976). The biology of the leaf mark polymorphism in Trifolium repens L. 2. Evidence for the selection of leaf marks by rumen fistulated sheep. Heredity, 37: 327-333.

Callaghan, T.V., Scott, R., and Whittaker, H.A. (1981a). The yield development and chemical composition of some fast-growing indigenous and naturalised British plant species in relation to management as energy crops. ITE project 640. Unpublished report from the Institute of Terrestrial Energy to the Department of Energy, Contract CR20/D4/03.

* Burge, M.N., and Irvine, J.A. (1985). Recent studies on the potential for biological control of bracken using fungi. Proceedings of the Royal Society of Edinburgh, 86B: 187-194.

Callaghan, T.V., Lawson, G.J., and Scott, R. (1981b). Bracken as an energy crop. Solar World Forum, Volume 2 (Eds. D.O. Hall and J. Morton), pp. 1239-1247.

Cates, R.G., and Orians, G.H. (1975). Successional status and the palatability of plants to generalised herbivores. Ecology, 56: 410-418.

Chen, L.Z., and Lindley, D.K. (1981). Primary production, decomposition and nutrient cycling in a bracken grassland ecosystem. Merlewood Research and Development Paper No. 80. Institute of Terrestrial Ecology. Unpublished.

Clapham, A.R., Tutin, T.G. and Warburg, E.F. (1981). Excursion Flora of the British Isles. Third edition. Cambridge University Press, Cambridge.

Clegg, D.O., Conn, E.E., and Janzen, D.H. (1979). Developmental fate of the cyanogenic glycoside linamarin in Costa Rican wild lima bean seeds. Nature, 278: 343-344.

Collinge, D.B., and Hughes, M.A. (1982). Developmental and physiological studies on cyanogenic glycosides of white clover, Trifolium repens L. Journal of Experimental Botany, 33: 154-161.

Compton, S.G., and Jones, D.A. (1985). An investigation of the responses of herbivores to cyanogenesis in Lotus corniculatus L. Biological Journal of the Linnean Society, 26: 21-38.

Compton, S.G., Newsome, D., and Jones, D.A. (1983). Selection for cyanogenesis in the leaves and petals of Lotus corniculatus L. at high latitudes. Oecologia, 60: 353-358.

Conn, E.E. (1979). Cyanide and cyanogenic glycosides. In: Herbivores: their interaction with secondary plant metabolites (Ed. Rosenthal, G.A., and Janzen, D.H.), pp. 387-412. Academic Press, London.

Conn, E.E. (1980). Cyanogenic glycosides. In: The Encyclopaedia of Plant Physiology (New Series), Volume VIII. Secondary Plant Products (Ed. Bell, E.A., and Charlwood, B.V.) pp. 461-492. Springer - Verlag, Berlin.

Conn, E.E. (1981a). Biosynthesis of cyanogenic glycosides. In: Cyanide in Biology (Eds. Vennesland, B, Conn, E.E., Knowles, C.J., Westley, J., and Wissing, F.) pp. 183-196. Academic Press, New York and London.

Conn, E.E. (1981b). Cyanogenic glycosides. In: The Biochemistry of Plants, Volume 7. Secondary Plant Products (Ed. Conn, E.E.) pp. 479-500. Academic Press, London.

Conway, E. (1949). The autecology of bracken (Pteridium aquilinum Kuhn). The germination of the spore, the development of the prothallus and the young sporophyte. Proceedings of the Royal Society of Edinburgh, 63B: 325-343.

Conway, E. (1953). Spore and sporeling survival in bracken (Pteridium aquilinum (L.) Kuhn). Journal of Ecology, 41: 289-294.

Conway, E. (1957). Spore production in bracken. Journal of Ecology, 45: 273-284.

Conway, E., and Stevens, R. (1957). Sporeling establishment in Pteridium aquilinum: effects of mineral nutrients. Journal of Ecology, 45: 389-399.

Cook, G.T., Carr, K.E., and Duncan, H.J. (1979). The influence of morphological differences in bracken pinnules on the foliar uptake of aminotriazole. Annals of Applied Botany, 93: 311-317.

Cooke, R.D., and Coursey, D.G. (1981). Cassava: a major HCN containing crop. In: Cyanide in Biology (Eds. Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J. and Wissing, F.), pp. 93-114. Academic Press, London.

Cooper-Driver, G. (1976). The chemotaxonomy and phytochemical ecology of bracken. Botanical Journal of the Linnean Society, 73: 35-46.

Cooper-Driver, G., and Swain, T. (1976). Cyanogenic polymorphism in bracken in relation to herbivore predation. Nature, 260: 604.

Cooper-Driver, G., Finch, S., Swain, T., and Bernays, E. (1977). Seasonal variation in secondary plant compounds in relation to the palatability of Pteridium aquilinum. Biochemical Systematics and Ecology, 5: 177-183.

Corkill, L. (1942). Cyanogenesis in white clover (Trifolium repens L.), V. The inheritance of cyanogenesis. New Zealand Journal of Science and Technology, 23B: 178-193.

Corkill, L. (1952). Cyanogenesis in white clover (Trifolium repens), VI. Experiments with high glucoside and glucoside free strains. New Zealand Journal of Science and Technology, 34A: 1-16.

Crawford-Sidebotham, T.J. (1972). Role of slugs and snails in the maintenance of the cyanogenesis polymorphism in Lotus corniculatus and Trifolium repens. Heredity, 28: 405-411.

Daday, H. (1954a). Gene frequencies in wild populations of Trifolium repens L. I. Distribution by latitude. Heredity, 8: 61-78.

Daday, H. (1954b). Gene frequencies in wild populations of Trifolium repens L. II. Distribution by altitude. Heredity, 8: 377-384.

Daday, H. (1958). Gene frequencies in wild populations of Trifolium repens L. III. World distribution. Heredity, 12: 169-181.

Daday, H. (1965). Gene frequencies in wild populations of Trifolium repens L. IV: The mechanism of natural selection. Heredity, 20, 355-365.

Darwin, F. (1877). On nectar glands of the common brake, Pteris aquilina. Journal of the Linnean Society (Botany), 15: 398-409.

Dasayanake, M.D. (1960). Aspects of morphogenesis in a dorsivental fern, Pteridium aquilinum (L.) Kuhn. Annals of Botany (New Series), 24: 317-328.

Davidonis, G.H., and Ruddat, M. (1973). Allelopathic compounds thelypterin A and B in the fern Thelypteris normalis. Planta, 111: 23-32.

Davidonis, G.H., and Ruddat, M. (1974). Growth inhibition in gametophytes and oat coleoptiles by thelypterin A and B released from roots of the fern Thelypteris normalis. American Journal of Botany, 61: 925-930.

Davis, R.H. (1981). Cyanide detoxification in common fowl. In: Cyanide in Biology (Eds. Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J. and Wissing, F.), pp. 51-60. Academic Press, London.

Dawson, C.D.R. (1941). Tetrasomic inheritance in Lotus corniculatus L. Journal of Genetics, 42: 49-72.

De Araujo, A.M. (1976). Relationship between altitude and cyanogenesis in white clover (Trifolium repens L.). Heredity, 37: 291-293.

De Silva, B.L.T. (1932). The distribution of calicole and calcifuge species in relation to the content of the soil in $CaCO_3$ and exchangeable calcium, and to soil reaction. Journal of Ecology, 22: 532-553.

Dement, W.A., and Mooney, H.A. (1974). Seasonal variation in the production of tannins and cyanogenic glucosides in the chapparal shrub, Heteromeles arbutifolia. Oecologia, 15: 65-76.

Dewick, P.M. (1984). The biosynthesis of cyanogenic glycosides and glucosinolates. Natural Product Reports, 1: 545-549.

Dring, M.J. (1965). The influence of shaded conditions on the fertility of bracken. British Fern Gazette, 9: 222-227.

Dritschilo, W., Krummel, J., Nafus, D., and Pimentel, D. (1979). Insects colonising cyanogenic and acyanogenic Trifolium repens. Heredity, 42: 49-56.

Duffy, S.S. (1982). Cyanide and arthropods. In: Cyanide in Biology (Eds. Vennesland, B, Conn, E.E., Knowles, C.J., Westley, J., and Wissing, F.) pp. 385-414. Academic Press, London.

Dyer, A.F. (1979). The culture of fern gametophytes for experimental investigation. In: The Experimental Biology of Ferns (Ed. Dyer, A.F.), pp. 253-305. Academic Press, London.

Dyer, A.F., and Hadfield, P.R.H. (1985). Polymorphism for cyanogenesis in British bracken (Pteridium aquilinum). Proceedings of the Royal Society of Edinburgh, 86B: 462-464.

Edwards, P.J., and Wratten, S.D. (1982). Wound-induced changes in palatability in birch (Betula pubescens ssp. pubescens). American Naturalist, 120: 816-818.

Ehrlich, P.R., and Raven, P.H. (1965). Butterflies and plants: a study in coevolution. Evolution, 18: 586-608.

Ellis, W.M., Keymer, R.J., and Jones, D.A. (1976). On the polymorphism of cyanogenesis in Lotus corniculatus L., VI. Ecological studies in the Netherlands. Heredity, 36: 245-251.

Ellis, W.M., Keymer, R.J., and Jones, D.A. (1977a). The effect of temperature on the polymorphism of cyanogenesis in Lotus corniculatus L. Heredity, 38: 339-347.

Ellis, W.M., Keymer, R.J., and Jones, D.A. (1977b). On the polymorphism of cyanogenesis in Lotus corniculatus L. VIII. Ecological studies in Anglesey. Heredity, 39: 45-65.

Ellis, W.M., Keymer, R.J., and Jones, D.A. (1977c). Defensive function of cyanogenesis in natural populations. Experentia, 33: 309-311.

Ennos, R.A. (1981a). Manifold effects of the cyanogenic loci in white clover. Heredity, 46: 127-132.

Ennos, R.A. (1981b). Detection of selection in populations of white clover (Trifolium repens L.). Biological Journal of the Linnean Society, 15: 75-82.

Etherington, J.R. (1982). Environment and Plant Ecology. Wiley, London.

Evans, I.A. (1976). Relationship between bracken and cancer.Botanical Journal of the Linnean Society, 73: 105-112.

Evans, W.C. (1976). Bracken thiamase-mediated neurotoxic syndromes. Botanical Journal of the Linnean Society, 73: 113-132.

Evans, W.C., Patel, M.C., and Koohy, Y. (1982). Acute bracken poisoning in homogastric and ruminant animals. Proceedings of the Royal Society of Edinburgh, 81B: 29-64.

Eyjolfsson, R. (1970). Recent advances in the chemistry of cyanogenic glycosides. Fortschritte Chem. Org. Naturst., 28: 74-108.

Farrow, E.P. (1915). On the ecology of the vegetation of Breckland. Journal of Ecology, 3: 211-228.

Feeny, P.P. (1970). Seasonal changes in oak leaf tannins and nutrients as a cause of spring feeding by winter moth caterpillars. Ecology, 51: 565-581.

Feeny, P.P. (1975). Plant apparency and chemical defence. Recent Advances in Phytochemistry, 10: 1-54.

Fiegl, F. (1960). Spot Tests in Organic Analysis. Elsevier, London.

Fiegl, F., and Anger, V. (1966). Replacement of benzidine by copper ethylacetoacetate and tetrabase as a spot-test reagent for hydrogen cyanide and cyanogen. Analyst, 91: 282-284.

Fitter, A.H., and Hay, R.K.M. (1981). Environmental Physiology of Plants. Academic Press, London.

Fletcher, W.W., and Kirkwood, R.C. (1979). The bracken fern (Pteridium aquilinum (L.) Kuhn); its biology and control. In: The Experimental Biology of Ferns (ed. Dyer, A.F.), pp. 592-636. Academic Press, London.

Fletcher, W.W., and Kirkwood, R.C. (eds.)(1982). Bracken in Scotland. The Proceedings of the Royal Society of Edinburgh, 81B: 1-143.

Foulds, W. (1977). The physiological response to moisture supply of cyanogenic and acyanogenic phenotypes of Trifolium repens and Lotus corniculatus. Heredity, 39: 219-234.

Foulds, W. (1982). Polymorphism for cyanogenesis in Lotus australis populations at Greenough Front Flats, Western Australia. Australian Journal of Botany, 30: 211-217.

Foulds, W., and Grime, J.P. (1972a). The influence of soil moisture on the frequency of cyanogenic plants in populations of Trifolium repens and Lotus corniculatus. Heredity, 28: 143-146.

Foulds, W., and Grime, J.P. (1972b). The response of cyanogenic and acyanogenic phenotypes of Trifolium repens L. to soil moisture supply. Heredity, 28: 181-187.

Foulds, W., and Young, L. (1977). Effect of frosting, water stress, and KCN on the metabolism of cyanogenic and acyanogenic phenotypes of of Lotus corniculatus L. and Trifolium repens L. Heredity, 38: 19-24.

Fowler, S.V., and Lawton, J.H. (1985). Rapidly induced defenses and talking trees: the devil's advocate position. American Naturalist, 126: 181-195.

Frankland, J.C. (1976). Decomposition of bracken litter. Botanical Journal of the Linnean Society, 73: 133-144.

Freeland, W.J., Calcott, P.H., and Anderson, L.R. (1985). Tannins and saponin: interaction in herbivore diets. Biochemical Systematics and Ecology, 13: 189-193.

Gerson, U. (1979). The associations between pteridophytes and arthropods. Fern Gazette, 12: 29-45.

Gibbs, R.D. (1963). History of chemical taxonomy. In: Chemical Plant Taxonomy (Ed. Swain, T.), pp. 43–88. Academic Press, London.

Gibson, P.B., Barnett, O.W., and Gillingham, J.T. (1972). Cyanogenesis in species related to Trifolium repens. Crop Science, 12: 708–720.

Gilchrist, D.G., Leushen, W.E., and Hittle, C.N. (1967). Revised method for the preparation of standards in the sodium picrate assay of HCN. Crop Science, 7: 267–268.

Glass, A.D.M. (1976). Allelopathic potential of phenolic acids associated with the rhizosphere of Pteridium aquilinum. Canadian Journal of Botany, 54: 2440–2444.

Gliessman, S.G. (1976). Allelopathy in a broad spectrum of environments as illustrated by bracken. Botanical Journal of the Linnean Society, 73: 95–104.

Gliessman, S.G. (1978). The establishment of bracken following fire in tropical habitats. American Fern Journal, 68: 41–48.

Gliessman, S.G., and Muller, C.H. (1972). Phytotoxic potential of bracken (Pteridium aquilinum (L.) Kuhn). Madrono, 21: 299–304.

Gliessman, S.G., and Muller, C.H. (1978). The allelopathic mechanism of dominance in bracken in southern California. Journal of Chemical Ecology, 4: 337–362.

Goldstein, J.L., and Swain, T. (1965). The inhibition of enzymes by tannins. Phytochemistry, 4: 185–192.

Goldstein, W.S., and Spencer, K.C. (1985). Inhibition of cyanogenesis by tannins. Journal of Chemical Ecology, 11: 847–858.

Gondwe, A.T.D. (1974). Studies on the hydrocyanic acid contents of some local local varieties of cassava (Manihot esculenta Crantz) and some traditional cassava food products. East African Agricultural and Forestry Journal, 40: 161–167.

Hanson, K.R., and Havir, E.A. (1981). Phenyl alanine ammonia lyase. In: Secondary Plant Products. The Biochemistry of Plants: a comprehensive treatise, volume 8 (ed. Conn, E.E.), pp. 577-626. Academic Press, London.

Harborne, J.B. (1980). Plant Phenolics. In: Secondary Plant Products. The Encyclopaedia of Plant Physiology, new series, volume 8 (eds. Bell, E.A., and Charlwood, B.V.), pp. 329-402. Berlin: Springer-Verlag.

Harbourne, J.B., and Turner, B. (1984). Plant Chemosystematics. Academic Press, London.

Harper, J.L. (1977). The Population Biology of Plants. Academic Press, London.

Harper, N.L, Cooper-Driver, G., Swain, T. (1976). A survey for cyanogenesis in ferns and gymnosperms. Phytochemistry, 15: 1764-1767.

Haukioja, E. (1983). Inducible defences of white birch to a geometrid defoliator, Epirrita autumnata. In: Proceedings of the 5th International Symposium on Insect - Plant Relationships. Pudoc, Wageningen. pp. 199-203.

Haukioja, E., and Niemala, P. (1976). Does birch defend itself actively against herbivores? Report of the Kevo Subarctic Research Station, 13: 44-47.

Heads, P.A., and Lawton, J.H. (1984). Bracken, ants and extrafloral nectaries. II. The effect of ants on the insect herbivores of bracken. Journal of Animal Ecology, 53: 1015-1031.

Hegnauer, R. (1977). Cyanogenic compounds as systematic markers in the Tracheophyta. In: Flowering Plants: Evolution and Classification of Higher Categories (ed. Kubitzki, K.), pp. 191-120. Supplement 1, Plant Systematics and Evolution, Vol. 127.

Hellum, A.K. (1968). Rhizome and frond variation in Pteridium aquilinum in North Michigan. Phytomorphology, 18: 225-231.

Hendrix, S.D. (1977). Resistance of Pteridium aquilinum (L.) Kuhn to insect attack by Trichoplusia ni Hub. Oecologia, 26: 347-361.

Hendrix, S.D., and Maquis, R.J. (1983). Herbivore damage to three tropical ferns. Biotropica, 15: 108-111.

Holttum, R.E. (1968). Flora of Malaya, Volume 2: Ferns. Government Printing Office, Singapore.

Horrill, J.C., and Richards, A.J. (1986). Differential grazing by the mollusc Arion hortensis Fer. on cyanogenic and acyanogenic seedlings of the white clover, Trifolium repens L. Heredity, 56: 277-281.

Hosel, W. (1982). The enzymatic hydrolysis of cyanogenic glycosides. In: Cyanide in Biology (Eds. Vennesland, B, Conn, E.E., Knowles, C.J., Westley, J., and Wissing, F.) pp. 217-232. Academic Press, London.

Hosel, W., and Narstedt, A. (1980). In vitro biosynthesis of the cyanogenic glucoside taxiphyllin in Triglochin maritima. Archives of Biochemistry and Biophysics, 203: 753-757.

Hubbard, C.E. (1968). Grasses. Second edition. Penguin Books, Harmondsworth.

Hughes, A.P. (1966). The importance of light compared with other factors affecting plant growth. In: Light as an Ecological Factor (Eds. Bainbridge, R., Evans, G.C., and Rackham, O.). British Ecological Society Symposium, No. 6. Blackwell, Oxford.

Hughes, M.A. (1968). Studies on β-glucosidase in cultured tissues of Trifolium repens L. Journal of Experimental Botany, 19: 52-63.

Hughes, M.A. (1981). The genetic control of plant cyanogenesis. In: Cyanide in Biology (Eds. Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J. and Wissing, F.), pp. 495-508. Academic Press, London.

Hughes, M.A., and Conn, E.E. (1976). Cyanoglucoside biosynthesis in white clover (Trifolium repens L.). Phytochemistry, 15: 697-701.

Jarrett, F.M., Manton, I., and Roy, S.K. (1968). Cytological and taxonomic notes on a small collection of living ferns from Galapogos. Kew Bulletin, 22: 475-480.

Jermy, A.C., and Walker, T.G. (1974) Lecanopteris spinosa - a new ant-fern from Indonesia. Fern Gazette, 11: 165-176.

Jones, C.G. (1983). Phytochemical variation, colonization, and insect communities: the case of the bracken fern (Pteridium aquilinum). In: Variable Plants and Herbivores in Natural and Managed Systems (R.F. Denno and M.S. McClure, eds.) pp. 513-557. Academic Press, London.

Jones, C.J., and Firn, R.D. (1978). The role of phytoecdysteroids in bracken fern, Pteridium aquilinum (L.) Kuhn, as a defense against phytophagous insect attack. Journal of Chemical Ecology, 4: 117-138.

Jones, C.J., and Firn, R.D. (1979). Resistance of Pteridium aquilinum to attack by non-adapted phytophagous insects. Biochemical Systematics and Ecology, 7: 95-101.

Jones, D.A. (1962). Selective eating of acyanogenic form of the plant Lotus corniculatus L. by various animals. Nature, 193: 1109-1110.

Jones, D.A. (1966). On the polymorphism of cyanogenesis in Lotus corniculatus L., I: selection by animals. Canadian Journal of Genetics and Cytology, 8: 556-567.

Jones, D.A. (1968). On the polymorphism of cyanogenesis in Lotus corniculatus L., II: interaction with Trifolium repens. Heredity, 23: 453-455.

Jones, D.A. (1970). On the Polymorphism of cyanogenesis in Lotus corniculatus L., III: some aspects of selection. Heredity, 25: 633-641.

Jones, D.A. (1971). Chemical defence mechanisms and genetic polymorphism. Science, 143: 945.

Jones, D.A. (1972a). Cyanogenic glycosides and their function. In: Phytochemical Ecology (Ed. Harbourne, J.B.), pp. 103-124. Academic Press, London.

Jones, D.A. (1972b). On the polymorphism of cyanogenesis in Lotus corniculatus L., IV. The Netherlands. Genetica, 43: 394-406.

Jones, D.A. (1973). Co-evolution and cyanogenesis. In: Taxonomy and Ecology (Ed. Harbourne, J.B.), pp. 103-124. Academic Press, London.

Jones, D.A. (1977). On the polymorphism of cyanogenesis in Lotus corniculatus L., VII. The distribution of the cyanogenic form in western Europe. Heredity, 39: 27-44.

Jones, D.A. (1979). Chemical defense: primary or secondary function? American Naturalist, 113: 445-451.

Jones, D.A. (1981). Cyanide and coevolution. In: Cyanide in Biology (Eds. Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J. and Wissing, F.), pp. 509-516. Academic Press, London.

Jones, D.A., Keymer, R.J., and Ellis, W.M. (1977). Cyanogenesis in plants and animal feeding. In: Harbourne, J.B. (ed). Biochemical Aspects of Plant and Animal Coevolution. Academic Press, London. pp. 21-34.

Kaplan, M.A.C., Figueirdo, M.R., and Gottlieb, O.R. (1983). Variation in cyanogenesis in plants with season and with insect pressure. Biochemical Systematics and Ecology, 11: 367-370.

Keymer, R.J., and Ellis, W.M. (1978). Experimental studies on plants of Lotus corniculatus from Anglesey polymorphic for cyanogenesis. Heredity, 40: 189-206.

Kirk, A.A. (1977). The insect fauna of the weed Pteridium aquilinum (L.) Kuhn (Polypodiaceae) in Papua New Guinea: a potential source of biological control agents. Journal of the Australian Entomological Society, 16: 403-409.

Kirk, A.A. (1982). The insects associated with the bracken fern (Pteridium aquilinum (L.) Kuhn) in Papua New Guinea and their possible use in biological control. Acta Oecologia Oecologia Applicata, 3, 343-359.

Kirkwood, R.C., and Hinshalwood, A. (1985). Recent studies on the biology of bracken and the mode of action of herbicides used in its control. Proceedings of the Royal Society of Edinburgh, 86B: 179-186.

Klekowski, E.J. (1972a). Genetical features of ferns as contrasted to seed plants. Annals of the Botanical Garden, Missouri, 59: 138-151.

Klekowski, E.J. (1972b). The evidence against self incompatability in the homosporous fern Pteridium aquilinum. Evolution, 26: 66-73.

Klekowski, E.J. (1979). The genetics and reproductive biology of ferns. In: The Experimental Biology of Ferns (Ed. Dyer, A.F.) pp. 133-170. Academic Press, London.

Knowles, B. (1970). The Autecology of Bracken. New Zealand Forestry Research Institute Indigenous Silviculture Report No. 5 (unpublished).

Kofod, H., and Eyjolfsson, R. (1966). The isolation of of the cyanogenic glycoside prunasin from Pteridium aquilinum (L.) Kuhn. Tetrahedron Letters, 12: 1289-1291.

Kraft, S.J., and Denno, R.F. (1982). Feeding responses of adapted and non-adapted insects to the defensive properties of Baccharis halimifolia L. (Compositae). Oecologia, 52: 156-163.

Krischik, V.A., and Denno, R.F. (1983). Individual, population and geographic patterns in plant defence. In: Variable Plants and Herbivores in Natural and Managed Systems (R.F. Denno and M.S. McClure, eds.) pp. 463-512. Academic Press, London.

Lane, C. (1962). Notes on the Common blue (Polyommatus icarus Rott.). Egg laying and feeding on the cyanogenic strains of Bird's foot trefoil (Lotus corniculatus L.). Entomological Gazette, 13: 112-116.

Lawton, J.H. (1976). The structure of the arthropod community in bracken. Botanical Journal of the Linnean Society, 73, 187-216.

Lawton, J.H. (1982). Vacant niches and unsaturated communities: a comparison of bracken herbivores at sites on two continents. Journal of Animal Ecology, 51: 573-595.

Lawton, J.H. (1984). Herbivore community organisation: general models and specific tests with phytophagous insects. In: A New Ecology: Novel Approaches to Interactive Systems (Eds. Price, P.W., Slobodchikoff, C.N., and Gaud, W.S.), pp. 329-352. Wiley, New York.

Lawton, J.H., and Heads, P.A. (1984). Bracken, ants, and extrafloral nectaries. I. The components of the system. Journal of Animal Ecology, 53: 995-1014.

Lawton, J.H., and MacGarvin, M. (1985). Interaction between bracken and its insect herbivores. Proceedings of the Royal Society of Edinburgh, 86B: 125-132.

Lee, H.C., Cooke, J.A., and Bines, T.J. (1982). Bracken regrowth in upland pasture following asulam treatment. Proceedings 1982 British Crop Protection Conference: Weeds, pp. 961-966.

Louda, S.M., and Rodman, J.E. (1983). Concentration of glucosinolates in relation to habitat and insect herbivory for the native crucifer Cardamine cordifolia. Biochemical Systematics and Ecology, 11: 199-207.

Lousley, J.E. (1939). Pteridium aquilinum in London. Journal of Botany, 74: 200-201.

Lousley, J.E. (1944). Flora of bombed sites in the City of London in 1944. Botanical Exchange Club, 12: 875-883.

Love, A. and Kjellquist, E. (1972). Cytotaxonomy of Spanish plants. 1. Introduction: pteridophytes and gymnosperms. Lagascalia, 2: 23-25.

MacLeod, A. (1982). The bracken problem in the highlands and islands of Scotland. Proceedings of the Royal Society of Edinburgh, 81B: 11-18.

McNaughton, S.J. (1983). Compensatory plant growth as a response to herbivory. Oikos, 40: 329-336.

McNeill, S., and Southwood, T.R.E. (1978). The role of nitrogen in the development of of insect/plant relationships. In: Biochemical aspects of plant and animal coevolution (ed. Harbourne, J.B.), pp. 77-98. Academic Press, London.

Maher, E.P., and Hughes, M.A. (1973). Studies on the nature of the Li locus in Trifolium repens L. II. The effect of genotype on enzyme activity and properties. Biochemical Genetics, 8: 13-26.

Mao, C.H., Blocher, J.P., Anderson, L., and Smith, D.G. (1965). Cyanogenesis in Sorghum vulgare, I: an improved method for the isolation of dhurrin; the physical properties of dhurrin. Phytochemistry, 4: 297-303.

Martin, D.J. (1976). Control of bracken. Botanical Journal of the Linnean Society, 73: 241-246.

Mattson, C.J. (1980). Herbivory in relation to nitrogen content. Annual Review of Ecology and Systematics, 11: 119-161.

Melville, J.D. (1965). Sporeling bracken on Littleworth Common. British Fern Gazette, 9: 228-230.

Melville, J., Coop, I.E., Doak, B.W., and Reifer, I. (1940). Cyanogenesis in white clover (T.repens L.), IV. Methods of determination and general considerations. New Zealand Journal of Science and Technology, 22B: 144-154.

Miller, J.D., Gibson, P.B., Cope, W.A., and Knight, W.E. (1975). Herbivore feeding on cyanogenic and acyanogenic white clover seedlings.. Crop Science, 15: 90-91.

Miller, J.H. (1968). Fern gametophytes as experimental material. Botanical Review, 34: 361-440.

Mirande, M. (1912). Compte Rendus de l'Academie des Sciences, Paris, 155: 651-653.

Mitchell, N.D. (1974). Quantification of the picrate test for cyanide in plant genetic studies. Canadian Journal of Genetics and Cytology, 16: 895-897.

Mitchell, J. (1977). The Effect of Bracken Distribution on Moorland Vegetation and Soils. PhD. thesis, University of Glasgow.

Molesworth-Allen, B. (1968). Pteridium aquilinum on limestone. British Fern Gazette, 10: 34-36.

Moon, F.E., and Rafaat, M.A. (1951). Some biochemical aspects of bracken 'poisoning' in the ruminant animal. II - the significance of the cyanogenetic principle of bracken. Journal of the Science of Food and Agriculture, 2, 327-336.

Mooney, H.A. (1972). The carbon balance of plants. Annual Review of Ecology and Systematics, 3: 315-346.

Mooney, H.A., Ehrlich, P.R., Lincoln, P.E., and Williams, K.S. (1980). Environmental controls on the seasonality of a drought deciduous shrub, Diplacus aurantiacus, and its predator, the checkerspot butterfly, Euphydras chalcedona. Oecologia, 45: 143-146.

Moore, L.B. (1942). Significance of spores in hard fern (Paesia scaberula) infections. New Zealand Journal of Science and Technology, 23B: 113-125.

Nakazawa, S. (1963). The role of protoplasmic connections in the morphogenesis of fern gametophytes. Scientific Reports of Tohoku University (4th series) 29: 247-255.

Narstedt, A. (1981). Isolation and structure elucidation of cyanogenic glycosides. In: Cyanide in Biology (Eds. Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J., and Wissing, F.), pp. 141-181. Academic Press, London.

Nass, H.G. (1972). Cyanogenesis: its inheritance in Sorghum, Lotus, and Trifolium. Crop Science, 12: 503-506.

Nayar, J.K., and Frankel, G. (1963). The chemical basis of host selection in the Mexican bean beetle, Epilachna varivestis. Annals of the Entomological Society of America, 56: 174-178.

O'Brien, T.P. (1963). The morphology and growth of Pteridium. Annals of Botany (New Series), 27: 253-267.

Oinonen, E. (1967a). Sporal regeneration and growth of bracken (Pteridium aquilinum (L.) Kuhn) in Finland in the light of the dimensions and age of its colonies. Acta Forestalia Fennica, 83 (1): 1-96.

Oinonen, E. (1967b). The correlation between the size of Finnish bracken (Pteridium aquilinum (L.) Kuhn) clones and certain periods of site history. Acta Forestalia Fennica, 83 (2): 1-51.

Ottoson, J.G., and Anderson, J.M. (1983). Number, seasonality and feeding habits of insects attacking ferns in Britain: an ecological consideration. Journal of Animal Ecology, 52: 385-406.

Page, C.N. (1976). The taxonomy and phytogeography of bracken - a review. Botanical Journal of the Linnean Society, 73: 1-34.

Page, C.N. (1979). Experimental aspects of fern ecology. In: The Experimental Biology of Ferns (Ed. Dyer, A.F.), pp. 551-589. Academic Press, London.

Page, C.N. (1982a). The history and spread of bracken in Britain. Proceedings of the Royal Society of Edinburgh, 81B: 3-10.

Page, C.N. (1982b). Field observations on the nectaries of bracken in Britain. Fern Gazette, 12: 233-240.

Page, C.N. (1982c). The Ferns of Britain and Ireland. Cambridge University Press, Cambridge.

Palo, R.T. (1984). Distribution of birch (Betula spp.), willow (Salix spp.) and poplar (Populus spp.) secondary metabolites and their potential role as chemical defense against herbivores. Journal of Chemical Ecology, 10: 499-520.

Perring, F.H., and Gardiner, B.G. (Eds.) (1976). The Biology of Bracken. Proceedings of a symposium. Botanical Journal of the Linnean Society, 73: 1-302.

Phillips, R.L. (1968). Cyanogenesis in Lotus species. Crop Science, 8: 123-124.

Poel, L.W. (1951). Soil aeration in relation to Pteridium aquilinum (L.) Kuhn. Journal of Ecology, 39: 182-191.

Poel, L.W. (1961). Soil aeration as a limiting factor in the growth of Pteridium aquilinum. Journal of Ecology, 49: 107-111.

Pusey, J.G. (1963). Cyanogenesis in Trifolium repens. In: Teaching Genetics (eds. Darlington, C.D., and Bradshaw, A.D.), pp. 99-104. Oliver and Boyd, Edinburgh.

Ramnani, A.D., and Jones, D.A. (1984). Genetics of cyanogenesis, cyanoglucoside and linamarase production in leaves of Lotus corniculatus L. Pakistan Journal of Botany, 16: 145-154.

Ramnani, A.D., and Jones, D.A. (1985). Flexibility in cyanogenic phenotype of Lotus corniculatus L. in response to low fluctuating temperatures. Pakistan Journal of Botany, 17: 9-23.

Raupp, M.J., and Denno, R.F. (1983). Leaf age as predictor of herbivore distribution and abundance. In: Variable Plants and Herbivores in Natural and Managed Systems (Eds. Denno, R.F. and McClure, M.S.), pp. 91-124. Academic Press, London.

Raven, J.A. (1985). Physiology and biochemistry of pteridophytes. Proceeding of the Royal Society of Edinburgh, 86B: 37-44.

Rehr, S.S., Feeny, P.P., and Janzen, D.H. (1973). Chemical defense in central American non-ant Acacias. Journal of Animal Ecology, 42: 405-416.

Rhoades, D.F. (1979). Evolution of plant chemical defence against herbivores. In: Herbivores: their Interaction with Secondary Plant Metabolites (Eds. Rosenthal, G.A., and Janzen, D.H.), pp. 3-54. Academic Press, London.

Rhoades, D.F. (1983). Herbivore population dynamics and plant chemistry. In: Variable Plants and Herbivores in Natural and Managed Systems (Eds. Denno, R.F. and McClure, M.S.), pp. 155-219. Academic Press, London.

Rhoades, D.F. (1985). Offensive - defensive interactions between herbivores and plants: their relevance in herbivore population dynamics and ecological theory. American Naturalist, 125: 205-238.

Roberts, J., Pymar, C.F., Wallace, J.S. and Pitman, R.M. (1980). Seasonal changes in leaf area, stomatal conductances and transpiration from bracken below a forest canopy. Journal of Applied Ecology, 17: 409-422.

Roberts, J., Wallace, J.S. and Pitman, R.M. (1984). Factors affecting stomatal conductance of bracken below a forest canopy. Journal of Applied Ecology, 21: 643-655.

Rogers, C.F. and Frykolm, O.C. (1937). Observations on the variation in cyanogenetic power of white clover plants. Journal of Agricultural Research, 55: 533-537.

Rose, F. (1981). The Wild Flower Key. Frederick Warne, London.

Rymer, L. (1976). The history and ethnobotany of bracken. Botanical Journal of the Linnean Society, 73: 151-176.

Saunders, J.A., Conn, E.E., Lin, C.H., and Stocking, C.R. (1977). Subcellular localisation of the cyanogenic glucoside in Sorghum by autoradiography. Plant Physiology, 59: 647-652.

Saupe, S.G., Seigler, D.S., and Escalente-Semerena, J.C. (1982). Bacterial contamination as a cause of spurious cyanide tests. Phytochemistry, 21: 2111-2112.

Schreiner, I.H. (1980). Cyanogenesis and the herbivorous insects of the bracken fern (Pteridium aquilinum). PhD. Thesis, Cornell University, U.S.A.

Schroder, V.N. (1977). Hydrogen cyanide from forage plants. Soil and Crop Science Society of Florida, Proceedings, 36: 569-582.

Schroeder, L.A. (1978). Consumption of black cherry laurel leaves by phytophagous insects. American Midland Naturalist, 100: 294-306.

Schulze, E.D. (1982). Plant life forms and their carbon, water and nutrient relations. In: The Encyclopaedia of Plant Physiology (New Series), Volume 12B. Physiological Plant Ecology II (Eds. Lange, O.L., Nobel, P.S., Osmond, C.B., and Ziegler, H.), pp. 616-676. Springer-Verlag, Berlin.

Schwabe, W.W. (1951). Physiological studies in plant nutrition, XVI. The mineral nutrition of bracken, part 1. Prothallial culture and the effects of phosphates and potassium on leaf production of the sporophyte. Annals of Botany (New Series), 15: 417-446.

Schwabe, W.W. (1953). Physiological studies in plant nutrition, XVI. The mineral nutrition of bracken, part 2. The effects of phosphorous and potassium supply on total dry weights, leaf areas, net assimilation, starch and water water contents in the sporophyte. Annals of Botany (New Series), 17: 225-262.

Siegler, D.S. (1977). The naturally occurring cyanogenic glycosides. Progress in Phytochemistry, Volume IV, pp. 83-120.

Siegler, D.S. (1981). Cyanogenic glycosides and lipids: structural types and distribution. In: Cyanide in Biology (Eds. Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J., and Wissing, F.), pp. 133-144. Academic Press, London.

Smith, A.M., and Fenton, E.W. (1944). The composition of bracken fronds and rhizomes at different times during the growing season. Journal of the Society of Chemical Industries, 63: 218 -219.

Smith, R.T. (1977). Bracken in Britain: I. The background to the problem. Working Paper 189. University of Leeds.

*

Snell, F.D., and Snell, L.T. (1959). Colorimetric Methods of Analysis, Volume 11A. Van Nostrand, New York.

Snyder, F.B. (1950). Inheritance and association of hydrocyanic acid potential, disease reactions and other characters in sudangrass. PhD thesis, University of Wisconsin, U.S.A.

Sokal, R.S., and Rohlf, F.G. (1969). Biometry. (San Francisco: W.H. Freeman).

Sporne, K.R. (1975). The Morphology of Pteridophytes. Fourth edition. Hutchinson, London.

Steeves, T.A., Sussex, I.M., and Partanen, C.R. (1955). In vitro studies on the abnormal growth of prothalli of the bracken fern. American Journal of Botany, 42: 232-245.

Stewart, R.E. (1975). Allelopathic potential of western bracken. Journal of Chemical Ecology, 1: 161-169.

Swain, T., and Cooper-Driver, G. (1973). Biochemical systematics in the Filicopsida. In: The Phylogeny and Classification of the Ferns (Eds. Jermy, A.C., Crabbe, J.A., and Thomas, B.A.), pp. 111-134.

* Smith, R.T., and Taylor, J.A. (Eds.) (1986). Bracken: ecology, land use, and control ecology. Parthenon Press, Carnforth.

Tansley, A.G. (1953). The British Isles and Their Vegetation. Cambridge University Press, Cambridge.

Tapper, B.A., and Butler, G.W. (1971). Oximes, nitriles and 2-hydroxynitriles in the biosynthesis of cyanogenic glycosides. Biochemical Journal, 124: 935-941.

Tapper, B.A., and Reay, P.F. (1973). Cyanogenic glycosides and glucosinolates. In: The Chemistry and Biochemistry of Herbage (Eds. Butler, G.W., and Bailey, R.W.), Volume 1 pp. 447-476.

Taylor, J.A. (1980). Bracken: an increasing problem and a threat to health. Outlines in Agriculture, 10: 298-304.

Tempel, A.S. (1981). Field studies of the relationship between herbivore damage and tannin concentration in bracken (Pteridium aquilinum Kuhn). Oecologia, 51: 97-106.

Tempel, A.S. (1983). Bracken fern and nectar feeding ants: a non-mutualistic interaction. Ecology, 64: 1411-1422.

Tryon, R.M. (1941). A revision of the genus Pteridium. Rhodora, 43: 1-31, 37-67.

Tuomi, J., Niemela, P., Haukioja, E., Siren, S., and Neuvonen, S. (1984). Nutrient stress: an explanation for plant anti-herbivore responses to defoliation. Oecologia, 61: 208-210.

Uribe, E.G., and Conn, E.E. (1966). The metabolism of aromatic compounds in higher plants. VII. The origin of the nitrile nitrogen atom of dhurrin. Journal of Biological Chemistry, 241: 92-94.

Veerasekaran, P., Kirkwood, R.C., and Fletcher, W.W. (1976). The mode of action of asulam in bracken. Botanical Journal of the Linnean Society, 73: 247-268.

Volini, J., and Alexander, K. (1981). Multiple forms and multiple functions of the rhodaneses. In: Cyanide in Biology (Eds. Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J., and Wissing, F.), pp. 77-92. Academic Press, London.

Wagner, W.H. (1972). Solanopteris brunei, a little-known fern epiphyte with dimorphic fronds. American Fern Journal, 62: 33-43.

Ware, W.M. (1925). Experiments and observations on forms and strains of Trifolium repens L. Journal of Agricultural Science, 15: 47-67.

Watt, A.S. (1940). Contributions to the ecology of bracken (Pteridium aquilinum), I: the rhizome. New Phytologist, 39: 401-422.

Watt, A.S. (1943). Contributions to the ecology of bracken (Pteridium aquilinum), II. The frond and the plant. New Phytologist, 42: 103-126.

Watt, A.S. (1945). Contributions to the ecology of bracken (Pteridium aquilinum), III. Frond types and the makeup of the population. New Phytologist, 44: 156-178.

Watt, A.S. (1947). Contributions to the ecology of bracken (Pteridium aquilinum), IV: the structure of the community. New Phytologist, 46: 97-121.

Watt, A.S. (1950). Contributions to the ecology of bracken (Pteridium aquilinum), V: bracken and frost. New Phytologist, 49: 308-327.

Watt, A.S. (1976). The ecological status of bracken. Botanical Journal of the Linnean Society, 73: 217-239.

Webster, B.D., and Steeves, T.A. (1958). Morphogenesis in Pteridium aquilinum. General morphology and growth habit. Phytomorphology, 8: 30-41.

Westley, J. (1981). Cyanide and sulfane sulphur. In: Cyanide in Biology (Eds. Vennesland, B., Conn, E.E., Knowles, C.J., Westley, J., and Wissing, F.), pp. 61-76. Academic Press, London.

White, E.J. and Smith, R.I. (1982). Climatological Maps of Great Britain. I.T.E., Edinburgh.

Whitehead, D.C. (1964). Identification of p-hydroxybenzoic, vanillic, p-coumaric, and ferulic acids in soils. Nature, 202: 417-418.

Whitehead, D.C., Dibb, H., and Hartley, R.D. (1982). Phenolic compounds in soil as influenced by the growth of different plant species. Journal of Applied Ecology, 19: 579-588.

Whitham, T.G. and Slobodchikoff, C.N. (1981). Evolution by individuals, plant - herbivore interactions and mosaics of genetic variability: the adaptive significance of somatic mutations in plants. Oecologia, 49: 287-292.

Whitham, T.G., Williams, A., and Robinson, A.M. (1984). The variation principle: individual plants as temporal and spatial mosaics of resistance to rapidly evolving pests. In: A New Ecology: Novel Approaches to Interacting Systems (P.W. Price, C.N. Slobodchikoff and W.S. Gaud, eds.) pp. 15-51. John Wiley and Sons, New York.

Whitman, R.J. (1973). Herbivore feeding and cyanogenesis in Trifolium repens L. Heredity, 30: 241-245.

Whittaker, R.H., and Feeny, P.P. (1971). Allelochemics: chemical interactions between species. Science, 171: 757-769.

Wilkie, D. (1956). Incompatability in bracken. Heredity, 10: 247-256.

Woodhead, S., and Bernays, E. (1977). Changes in release rates of HCN in relation to palatability of Sorghum to insects. Nature, 270: 235-240.

Yapp, R.H. (1902). Two Malayan myrmecophilous ferns, Polypodium (Lecanopteris) carnossum Blume and Polypodium sinuosum Wall. Annals of Botany, 16: 185-231.

Zitnak, A. (1973). Assay methods for HCN in plant tissues and their application in studies of cyanogenic glycosides in Manihot esculenta. In: Chronic Cassava Toxicity (Eds. Nestel, B., and MacIntyre, R.), pp. 89-96. International Development Research Centre, Ottawa, Canada.